

# Quorum Sensing: Cell-to-Cell Communication in Bacteria

Christopher M. Waters and Bonnie L. Bassler

Department of Molecular Biology, Princeton University, Princeton, New Jersey  
08544-1014; emails: cwaters@molbio.princeton.edu, bbassler@molbio.princeton.edu

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## Key Words

autoinducer, quorum quenching, regulon

## Abstract

Bacteria communicate with one another using chemical signal molecules. As in higher organisms, the information supplied by these molecules is critical for synchronizing the activities of large groups of cells. In bacteria, chemical communication involves producing, releasing, detecting, and responding to small hormone-like molecules termed autoinducers. This process, termed quorum sensing, allows bacteria to monitor the environment for other bacteria and to alter behavior on a population-wide scale in response to changes in the number and/or species present in a community. Most quorum-sensing-controlled processes are unproductive when undertaken by an individual bacterium acting alone but become beneficial when carried out simultaneously by a large number of cells. Thus, quorum sensing confuses the distinction between prokaryotes and eukaryotes because it enables bacteria to act as multicellular organisms. This review focuses on the architectures of bacterial chemical communication networks; how chemical information is integrated, processed, and transduced to control gene expression; how intra- and inter-species cell-cell communication is accomplished; and the intriguing possibility of prokaryote-eukaryote cross-communication.

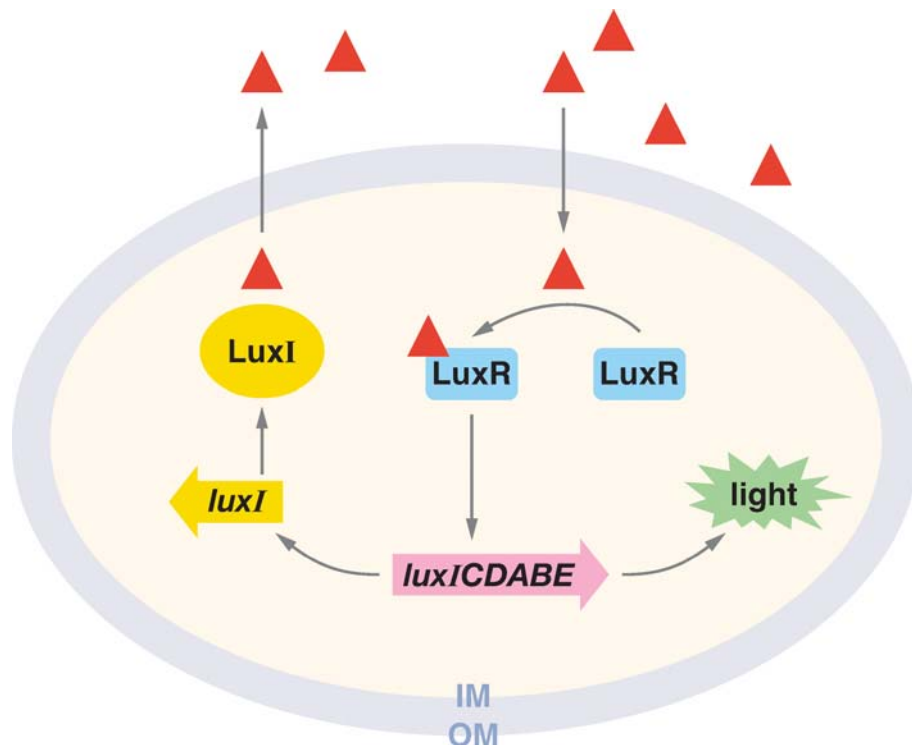
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## QUORUM SENSING

Quorum-sensing bacteria produce and release chemical signal molecules termed autoinducers whose external concentration increases as a function of increasing cell-population density. Bacteria detect the accumulation of a minimal threshold stimulatory concentration of these autoinducers and alter gene expression, and therefore behavior, in response. Using these signal-response systems, bacteria synchronize particular behaviors on a population-wide scale and thus function as multicellular organisms. Here, we describe some well-characterized quorum-sensing systems with the aim of illustrating their similarities and differences. We presume similarities in these systems exist because the ability to communicate is fundamental to bacteria. Differences in the systems likely exist because each system has been optimized to promote survival in the specialized niche in which a particular species of bacteria resides. Thus, the types of signals, receptors, mechanisms of signal transduction, and target outputs of each quorum-sensing system reflect the unique biology carried out by a particular bacterial species.

### Quorum Sensing in Gram-Negative Bacteria

The first described quorum-sensing system is that of the bioluminescent marine bacterium *Vibrio fischeri*, and it is considered the paradigm for quorum sensing in most gram-negative bacteria (Nealson & Hastings 1979). *V. fischeri* colonizes the light organ of the Hawaiian squid *Euprymna scolopes*. In this organ, the bacteria grow to high cell density and induce the expression of genes required for bioluminescence. The squid uses the light provided by the bacteria for counterillumination to mask its shadow and avoid predation (Visick et al. 2000). The bacteria benefit because the light organ is rich in nutrients and allows proliferation in numbers unachievable in seawater. Two



**Figure 1**

Quorum sensing in *Vibrio fischeri*; a LuxIR signaling circuit. Red triangles indicate the autoinducer that is produced by LuxI. OM, outer membrane; IM, inner membrane.

proteins, LuxI and LuxR, control expression of the luciferase operon (*luxICDABE*) required for light production (**Figure 1**). LuxI is the autoinducer synthase, which produces the acyl-homoserine lactone (AHL) autoinducer 3OC6-homoserine lactone (**Figure 2a** and Eberhard et al. 1981, Engebrecht & Silverman 1984), and LuxR is the cytoplasmic autoinducer receptor/DNA-binding transcriptional activator (Engebrecht et al. 1983). Following production, the AHL freely diffuses in and out of the cell and increases in concentration with increasing cell density (Kaplan & Greenberg 1985). When the signal reaches a critical, threshold concentration, it is bound by LuxR and this complex activates transcription of the operon encoding luciferase (Stevens et al. 1994). Importantly, the LuxR-AHL complex also induces expression of *luxI* because it is encoded in the luciferase operon. This regulatory configuration floods the environment with

the signal. This creates a positive feedback loop that causes the entire population to switch into “quorum-sensing mode” and produce light.

A large number of other gram-negative proteobacteria possess LuxIR-type proteins and communicate with AHL signals (Manefield & Turner 2002). These systems are used predominantly for intraspecies communication as extreme specificity exists between the LuxR proteins and their cognate AHL signals. LuxI-type proteins link and lactonize the methionine moiety from S-adenosylmethionine (SAM) to particular fatty acyl chains carried on acyl-acyl carrier proteins (More et al. 1996, Parsek et al. 1999). A diverse set of fatty acyl side chains of varying length, backbone saturation, and side-chain substitutions are incorporated into AHL signals; these differences are crucial for signaling specificity (**Figure 2a** and Fuqua 1999). Structural studies of LuxI-type

**Quorum sensing:** a process of cell-cell communication in bacteria

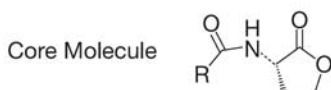
**Autoinducers:** small molecules secreted by bacteria that are used to measure population density

**AHL:** acyl-homoserine lactone

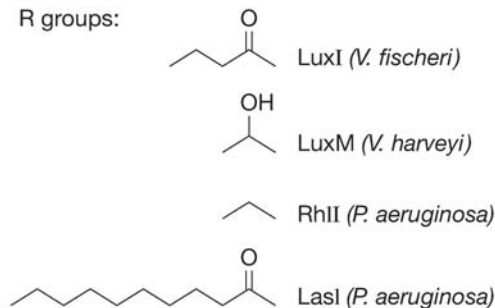
**SAM:** S-adenosyl-methionine

a

### Acyl-homoserine lactones (AHL)

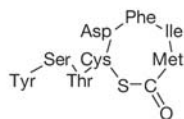


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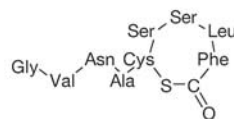


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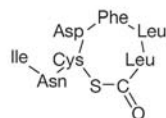
### Oligopeptide autoinducers



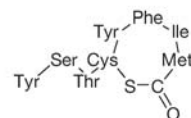
AIP-I (*S. aureus*)



AIP-II (*S. aureus*)



AIP-III (*S. aureus*)



AIP-IV (*S. aureus*)



ComX (*B. subtilis*)



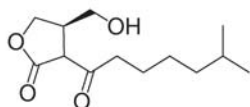
CSF (*B. subtilis*)



CSP (*S. pneumoniae*)

c

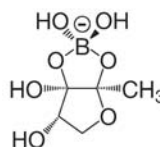
### Streptomyces $\gamma$ -butyrolactones



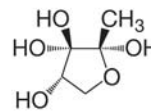
A-factor (*S. griseus*)

d

### AI-2 family



*V. harveyi*



*S. typhimurium*

Figure 2

Representative bacterial autoinducers. The asterisk above the tryptophan in ComX represents an isoprenyl modification.

proteins indicate that each possesses an acyl-binding pocket that precisely fits a particular side-chain moiety (Gould et al. 2004, Watson et al. 2002). This structural feature apparently confers specificity in signal production. Thus, each LuxI protein produces the correct signal molecule with high fidelity. There are some LuxI-type proteins that produce multiple

AHLs, although it is not clear if all are biologically relevant (Marketon et al. 2002). The structures of LuxR proteins suggest that LuxR proteins also possess specific acyl-binding pockets that allow each LuxR to bind and be activated only by its cognate signal (Vannini et al. 2002, Zhang et al. 2002b). Hence, it appears that in mixed-species

environments in which multiple AHL signals are present, each species can distinguish, measure, and respond only to the buildup of its own signal. Importantly, bacteria rarely rely exclusively on one LuxIR quorum-sensing system. Rather, bacteria use one or more LuxIR systems, often in conjunction with other types of quorum-sensing circuits.

Mechanisms must exist to prevent premature activation of LuxIR-type quorum-sensing circuits because both the signal and the detector are synthesized and interact in the cytoplasm (**Figure 1**). One such mechanism, evidenced by the LuxR homologue TraR in the plant pathogen *Agrobacterium tumefaciens*, is the stability of LuxR-type proteins increases upon AI binding. In the absence of autoinducer, TraR has a half-life of a few minutes. However, in the presence of AHL, the half-life of TraR increases to over 30 minutes (Zhu & Winans 1999). The crystal structure of TraR predicts that AHL binding is required for folding of the nascent polypeptide (Zhang et al. 2002b), and indeed radiolabeled TraR was stabilized only when its cognate AHL was added prior to labeling of the protein (Zhu & Winans 2001). Hence, only when AHL accumulates to a significant concentration (both outside and inside the cell) can TraR bind it, fold, and initiate the quorum-sensing cascade. Another mechanism that prevents “short-circuiting” of LuxIR systems is active export of AHL signals (Pearson et al. 1999). When a significant concentration of signal has accumulated, which is indicative of high cell density, diffusion into the cell overwhelms export and thus engages the circuit. AHLs with long acyl side chains are thought to require active export to transverse the bacterial membrane (Pearson et al. 1999).

## Quorum Sensing in Gram-Positive Bacteria

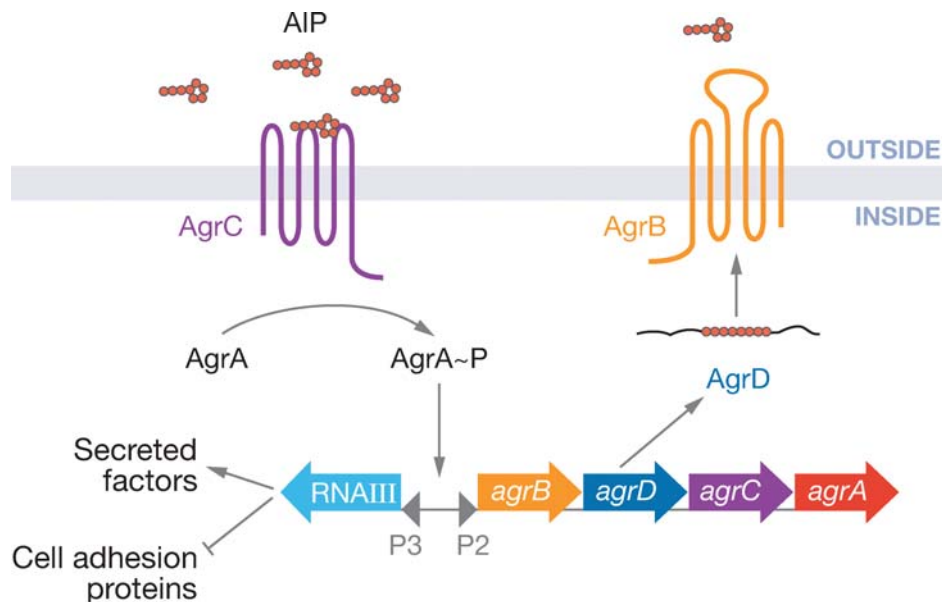
Gram-positive bacteria communicate using modified oligopeptides as signals and “two-component”-type membrane-bound sensor histidine kinases as receptors. Signaling is

mediated by a phosphorylation cascade that influences the activity of a DNA-binding transcriptional regulatory protein termed a response regulator. Similar to the mechanisms by which gram-negative bacteria use LuxIR quorum-sensing systems, each gram-positive bacterium uses a signal different from that used by other bacteria and the cognate receptors are exquisitely sensitive to the signals’ structures. Thus, as in LuxIR systems, peptide quorum-sensing circuits are understood to confer intraspecies communication. Peptide signals are not diffusible across the membrane, hence signal release is mediated by dedicated oligopeptide exporters. In most cases, concomitant with signal release is signal processing and modification. While the biochemistry underlying these events is poorly defined, it is known that most peptide quorum-sensing signals are cleaved from larger precursor peptides, which then are modified to contain lactone and thiolactone rings, lanthionines, and isoprenyl groups (Ansaldi et al. 2002, Booth et al. 1996, Mayville et al. 1999, Nakayama et al. 2001). Many gram-positive bacteria communicate with multiple peptides in combination with other types of quorum-sensing signals.

A fascinating example of peptide quorum sensing exists in *Staphylococcus aureus*, which is normally a benign human commensal but becomes a deadly pathogen upon penetration into host tissues (reviewed in Tenover & Gaynes 2000). *S. aureus* uses a biphasic strategy to cause disease: At low cell density, the bacteria express protein factors that promote attachment and colonization, whereas at high cell density, the bacteria repress these traits and initiate secretion of toxins and proteases that are presumably required for dissemination (reviewed in Lyon & Novick 2004). This switch in gene expression programs is regulated by the Agr quorum-sensing system (**Figure 3**). The system consists of an autoinducing peptide of *Staphylococcus aureus* (AIP) (**Figure 2b**) encoded by *agrD* (Ji et al. 1995) and a two-component sensor kinase-response regulator pair, AgrC and AgrA, respectively

**Figure 3**

Using a two-component response regulatory system, *Staphylococcus aureus* detects and responds to an extracellular peptide. Small red circles indicate the AIP. P2 and P3 designate the promoters for *agrBDCA* and *RNAIII*, respectively.



**AIP:** autoinducing peptide of *Staphylococcus aureus*

(Novick et al. 1995). The AgrB protein exports and adds the thiolactone ring modification to *S. aureus* AIPs (Saenz et al. 2000). Binding of the AIP to AgrC leads to phosphorylation of AgrA. Phospho-AgrA induces the expression of a regulatory RNA termed RNAIII, which represses expression of cell-adhesion factors while inducing expression of secreted factors (Novick et al. 1993). Activated AgrA also induces expression of the *agrBDCA*. This results in increased AIP levels, which ensures that the entire population switches from the low-cell-density to the high-cell-density state (Novick et al. 1995).

*S. aureus* strains are classified on the basis of the sequence of their thiolactone-containing AIP. At present, four different AIPs (Figure 2b and Dufour et al. 2002), and thus four different groups of *S. aureus*, are known. Surprisingly, each AIP specifically activates its cognate AgrC receptor but inhibits activation of all others by competitive binding to the non-cognate receptors (Lyon et al. 2002b). Thus, each AIP inhibits activation of the virulence cascade in the other three groups of *S. aureus* while not affecting the other groups' growth. Coinfection with two different *S. au-*

*reus* groups results in intraspecies competition; the *S. aureus* group that first establishes its quorum-sensing cascade outcompetes the other group. Consistent with this idea, purified AIP II attenuates virulence of a Group I *S. aureus* in a mouse infection model (Mayville et al. 1999). Thus, in *S. aureus*, quorum sensing allows dissemination of closely related progeny while inhibiting the spread of non-kin. Clinical analyses show that each *S. aureus* group is the primary causative agent of a specific type of *S. aureus* disease. This suggests that cell-cell communication has been instrumental in establishing a specific niche for each "strain" (Novick 2003). The codivergence of the signal-receptor pairs occurring in these bacteria may be one molecular mechanism underlying the evolution of new bacterial species.

Streptomycetes are a diverse family of gram-positive soil-dwelling bacteria that are of clinical relevance because they are a major biological reservoir of secondary metabolites, many of which are used as antibiotics (reviewed in Chater & Horinouchi 2003). Streptomycetes use  $\gamma$ -butyrolactones (Figure 2c) as autoinducers and control



morphological differentiation and secondary metabolite production via quorum sensing. Their signals are intriguing because they are structurally related to AHL autoinducers. However, there has not yet been any report describing either cross-communication between or cross-inhibition of streptomycetes and Gram-negative bacteria that communicate with AHLs.

## QUORUM-SENSING NETWORK ARCHITECTURE

Identification of the chemical signals, receptors, target genes, and mechanisms of signal transduction involved in quorum sensing is leading to a comprehensive understanding of cell-cell communication in bacteria. This research is providing insight into the variety of molecular arrangements that enable communication between cells as well as the unique characteristics that the various signaling architectures provide in terms of information dissemination, detection, relay, and response. Below we highlight a few quorum-sensing systems and discuss how each particular network arrangement leads to distinct signaling features.

### Parallel Quorum-Sensing Circuits

The first observation that bacteria could communicate with multiple quorum-sensing signals was in the quorum-sensing system of the Gram-negative, bioluminescent marine bacterium *Vibrio harveyi* (Figure 4). The *V. harveyi* quorum-sensing system consists of three autoinducers and three cognate receptors functioning in parallel to channel information into a shared regulatory pathway. Similar to other Gram-negative bacteria, *V. harveyi* produces an AHL signal termed HAI-1 (3OHC4-homoserine lactone; Figure 2a and Cao & Meighen 1989). Its synthase, LuxM, shares no homology to LuxI-type enzymes but catalyzes the identical biochemical reaction to generate a specific AHL (Bassler et al. 1993, Hanzelka et al. 1999). HAI-1 binds to a membrane-bound sensor histi-

## CHEMICAL COMPLEXITY IN BACTERIAL AUTOINDUCERS

Recent research shows that a rich diversity of chemical molecules is used for communication in the bacterial world. New genetic, biochemical, and imaging techniques have enhanced our ability to identify and measure the readouts of cell-cell communication. These tools have led to the identification of several novel molecules and classes of molecules that are clearly bona fide autoinducers mediating cell-cell communication. A few examples are

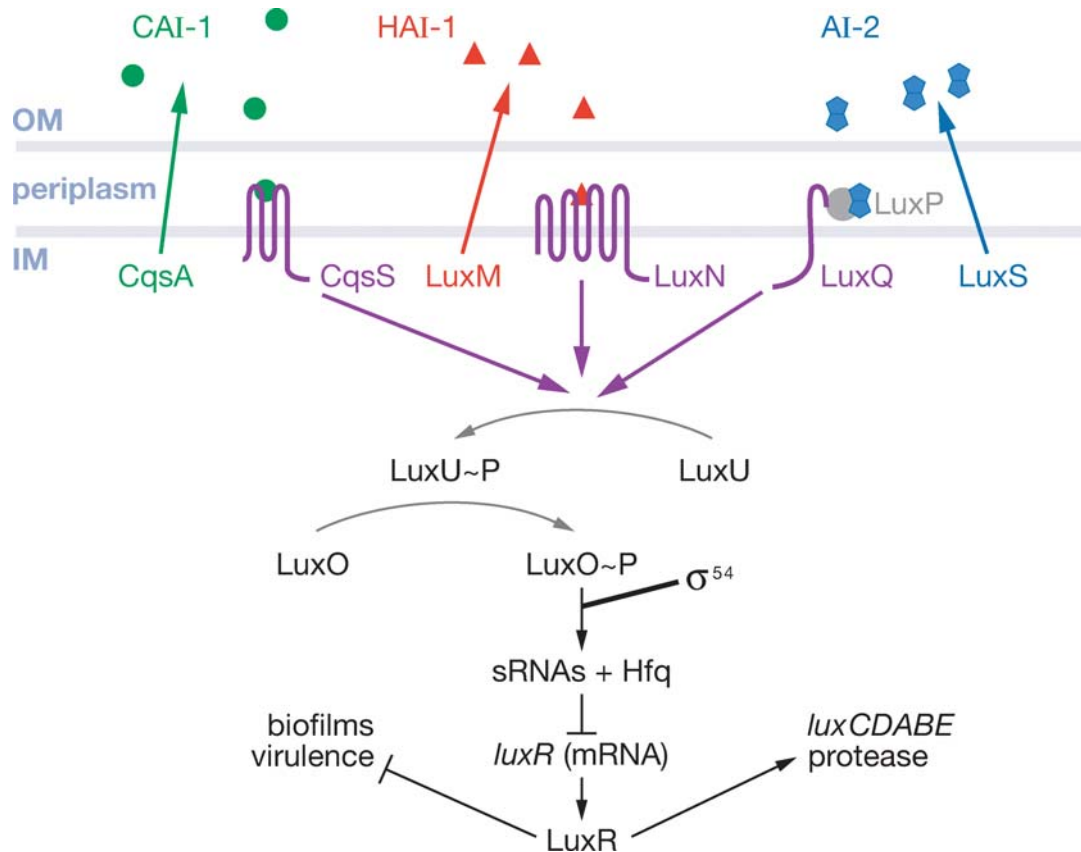
**PQS** The molecule 3,4-dihydroxy-2-heptylquinoline, termed PQS, is a signal that is integral to the *P. aeruginosa* quorum-sensing cascade (Pesci et al. 1999). This signal acts as an additional regulatory link between the Las and Rhl quorum-sensing circuits.

**3OH PAME** 3OH palmitic acid methyl ester (3OH PAME) transmits information via the two-component sensor histidine kinase-response regulator pair, PhcS-PhcR, to cause the plant pathogen *Ralstonia solanacearum* to switch from a motile to an infective state (Flavier et al. 1997).

**CYCLIC DIPEPTIDES** Newly described in a number of gram-negative bacteria, at high concentrations, cyclic-dipeptides antagonize AHL binding to cognate receptors (Holden et al. 1999).

dine kinase (LuxN) similar to sensors in Gram-positive quorum-sensing signaling circuits (Bassler et al. 1993, Freeman et al. 2000). The second *V. harveyi* signal is a furanosyl borate diester known as AI-2 (Figure 2d and Bassler et al. 1994a, Chen et al. 2002), production of which requires the LuxS enzyme (Surette et al. 1999, Xavier & Bassler 2003). AI-2 is bound in the periplasm by the protein LuxP; the LuxP-AI-2 complex interacts with another membrane-bound sensor histidine kinase, LuxQ (Bassler et al. 1994a). The third *V. harveyi* signal, an unidentified molecule termed CAI-1, is produced by the CqsA enzyme, and again, this signal interacts with a membrane-bound sensor histidine kinase, CqsS (Henke & Bassler 2004b).

At low cell density, in the absence of appreciable amounts of autoinducers, the three sensors—LuxN, LuxQ, and CqsA—act as



**Figure 4**

*Vibrio harveyi* produces and responds to three distinct autoinducers. The sensory information is fed into a shared two-component response regulatory pathway. The arrows indicate the direction of phosphate flow in the low-cell-density state. CAI-1, HAI-1, and AI-2 are respectively represented by green circles, red triangles, and blue double pentagons. OM, outer membrane; IM, inner membrane.

kinases, autophosphorylate, and subsequently transfer the phosphate to the cytoplasmic protein LuxU (**Figure 4**). LuxU passes the phosphate to the DNA-binding response regulator protein LuxO (Bassler et al. 1994b, Freeman & Bassler 1999a,b, Freeman et al. 2000). Phospho-LuxO, in conjunction with a transcription factor termed  $\sigma^{54}$ , activates transcription of the genes encoding five regulatory small RNAs (sRNAs) termed Qrr1–5 (for Quorum Regulatory RNA) (Lilley & Bassler 2000, Lenz et al. 2004). The Qrr sRNAs interact with an RNA chaperone termed Hfq, which is a member of the Sm family of eukaryotic RNA chaperones in-

involved in mRNA splicing (Carrington & Ambros 2003). The sRNAs, together with Hfq, bind to and destabilize the mRNA encoding the transcriptional activator termed LuxR (not similar to LuxR of *V. fischeri*) (Lenz et al. 2004). LuxR is required to activate transcription of the luciferase operon *luxCDABE* (Swartzman et al. 1992). Thus, at low cell density, because the *luxR* mRNA is degraded, the bacteria do not express bioluminescence. At high cell density, when the autoinducers accumulate to the level required for detection, the three sensors switch from being kinases to being phosphatases and drain phosphate from LuxO via LuxU. Unphosphorylated LuxO

sRNAs: small RNAs



cannot induce expression of the sRNAs. This allows translation of *luxR* mRNA, production of LuxR, and expression of bioluminescence. This pathway controls many genes in addition to those encoding luciferase (Henke & Bassler 2004a, Mok et al. 2003).

The human pathogen *Vibrio cholerae*, the causative agent of the endemic diarrheal disease cholera, possesses a quorum-sensing network similar to that of *V. harveyi* (Miller et al. 2002). *V. cholerae* has no equivalent to the AI-1/LuxN branch of the system. However, this bacterium does possess the AI-2/LuxPQ and CAI-1/CqsS branches as well as LuxU, LuxO, four Qrr sRNAs, and a *V. harveyi* LuxR-like protein termed HapR. The *V. cholerae* systems function analogously to those of *V. harveyi* but control virulence instead of regulating bioluminescence (Miller et al. 2002, Zhu et al. 2002). Surprisingly, quorum sensing promotes *V. cholerae* virulence factor expression and biofilm formation at low cell density and represses these traits at high cell density (Hammer & Bassler 2003). Quorum sensing commonly controls bacterial virulence factor expression, but typically, induction occurs at high cell density. This opposite regulatory pattern exhibited by *V. cholerae* can be understood in terms of the specific disease that the bacterium causes. Following a successful *V. cholerae* infection, the ensuing diarrhea wash huge numbers of bacteria from the human intestine into the environment. Repression of virulence factor production and biofilm formation genes at high cell density may promote dissemination of *V. cholerae*.

Upon the recent completion of the *V. fischeri* genome sequence, it was revealed that, in addition to LuxIR, homologues of two of the *V. harveyi* quorum-sensing circuits and the shared downstream components are present: LuxMN, LuxSPQ, LuxU, LuxO, and LuxR (referred to as LitR in *V. fischeri*) (Fidopiastis et al. 2002, Lupp & Ruby 2004, Lupp et al. 2003, Miyamoto et al. 2003). In *V. fischeri*, the *V. harveyi*-like quorum-sensing systems activate expression of *litR* at low cell densities. LitR induces expression of *luxR*, which in turn

promotes light production, as described above (Fidopiastis et al. 2002). This latter event occurs at relatively high cell densities, which presumably can be achieved only in the squid and cannot be achieved in the open ocean.

These three vibrio quorum-sensing systems underscore the way in which a common quorum-sensing network can be modified to fit the unique biology of the bacteria. Whereas the two-tiered *V. fischeri* circuit is adapted for two disparate lifestyles, inside and outside of the squid, *V. harveyi* and *V. cholerae* do not possess LuxIR homologues and they are not known to exist in symbiotic relationships. Although *V. harveyi* and *V. cholerae* share many of the same signals and receptors, the relative input from each signal is different in the two species. CqsA/CqsS is the dominant signaling-circuit in *V. cholerae* whereas it is the weakest in *V. harveyi* (Henke & Bassler 2004b). These signaling variations, coupled with their regulation of distinct downstream virulence factors, may be determining factors that allow *V. cholerae*, but not *V. harveyi*, to infect humans.

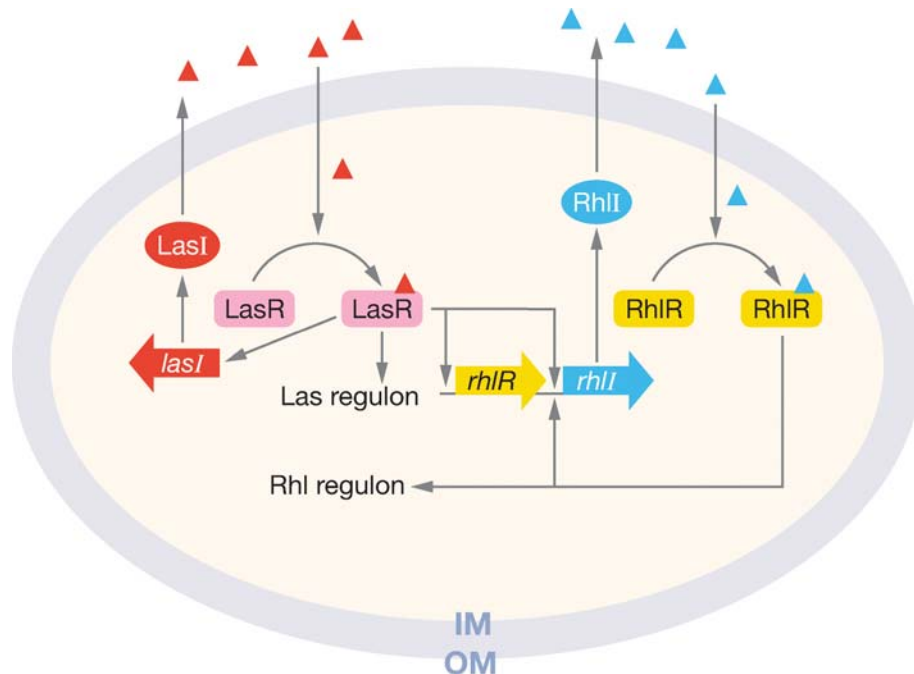
In each vibrio circuit, all signal-receptor pairs channel phosphate to LuxO in the absence of a signal and remove phosphate from LuxO in the presence of a signal. Thus, because all signals lead to a reduction in the level of LuxO-phosphate, each signal reinforces the information encoded in the other signals. This arrangement may allow the network to function as a coincidence detector that significantly activates or represses gene expression only when all signals are simultaneously present or absent (Mok et al. 2003). This signaling architecture may be critical for filtering out noise from molecules in the environment that are related to the true signals and/or noise from signal mimics produced by other bacteria in the vicinity.

## Quorum-Sensing Circuits Arranged in Series

As in the vibrios, the *Pseudomonas aeruginosa* quorum-sensing circuit is responsive to

**Figure 5**

The *Pseudomonas aeruginosa* quorum-sensing circuits operate in series to control a large set of target genes. The LasI autoinducer is represented by red triangles and the RhII autoinducer is shown as blue triangles. OM, outer membrane; IM, inner membrane.



multiple autoinducers, however, unlike those in vibrios, the *P. aeruginosa* regulatory systems are arranged in series rather than in parallel. *P. aeruginosa*, a common soil organism, is also an opportunistic pathogen most notorious for its devastating effects on cystic fibrosis (CF) patients (Eberl & Tummeler 2004). Quorum sensing is essential for chronic *P. aeruginosa* respiratory infection because it controls adhesion, biofilm formation, and virulence factor expression, all of which allow persistence in the lung and are required for disease progression (Smith & Iglewski 2003).

The *P. aeruginosa* quorum-sensing network consists of two LuxIR circuits, termed LasIR and RhIR (Figure 5 and Gambello & Iglewski 1991, Ochsner et al. 1994). LasI, a LuxI homologue, produces an AHL autoinducer (3OC12-homoserine lactone; Figure 2a and Pearson et al. 1994) that binds to LasR. The LasR-autoinducer complex activates a variety of target genes including *lasI*, which sets up the characteristic

positive feedback loop that further activates the system (Figure 5 and Seed et al. 1995). The LasR-autoinducer complex also activates the expression of *rhIR* and *rhII* encoding another quorum-sensing circuit. RhII produces the AHL C4-homoserine lactone (Figure 2a and Pearson et al. 1995). Following accumulation, RhIR binds the RhII-directed signal; this complex activates its own set of target genes. Importantly, because the LasIR system induces both *rhII* and *rhIR*, induction of the genes under RhIR control occurs subsequent to induction of genes under LasIR control.

Microarray analyses of *P. aeruginosa* quorum-sensing-controlled gene expression revealed three classes of genes: genes that respond to only one autoinducer, genes that respond to either autoinducer, and genes that require both autoinducers simultaneously for activation (Hentzer et al. 2003; Schuster et al. 2003; Wagner et al. 2003, 2004). Furthermore, transcriptome analyses showed that these classes of genes are expressed at different

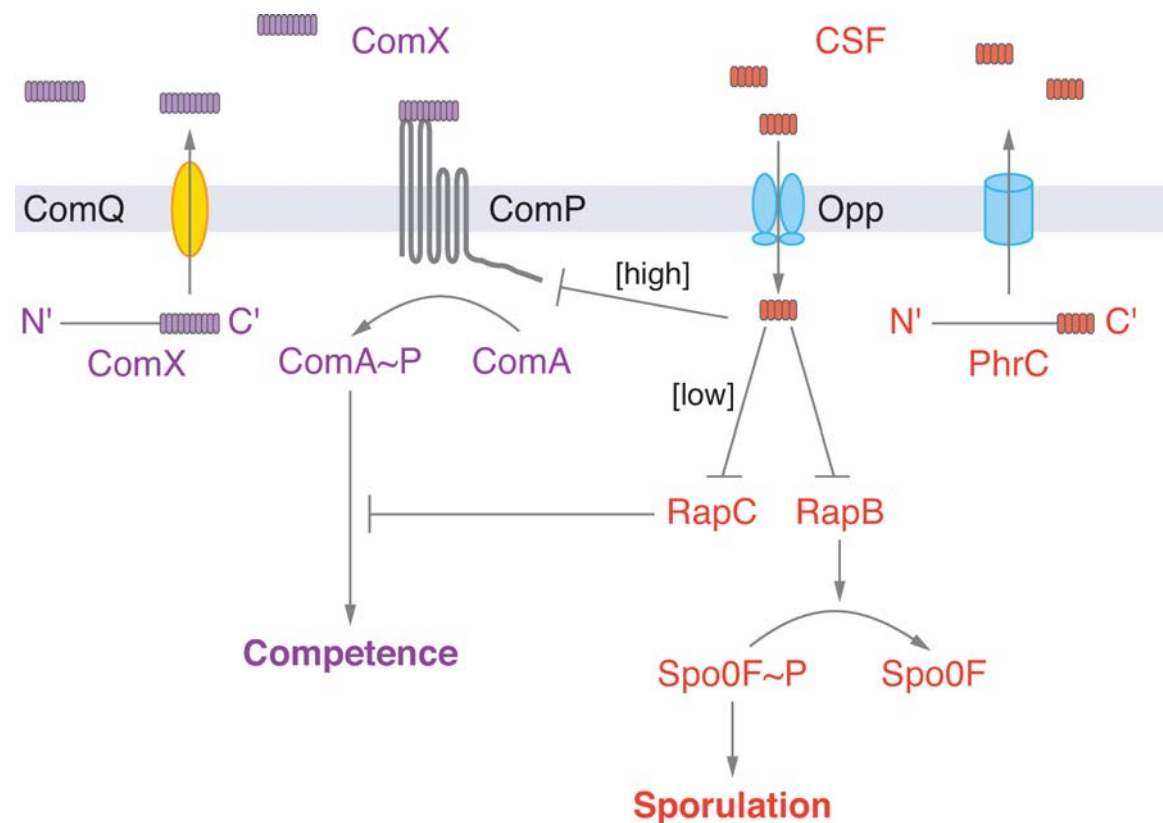
**CF:** cystic fibrosis

times over the growth cycle. This indicates that the tandem network architecture indeed produces a temporally ordered sequence of gene expression that may be critical for the ordering of early and late events in a successful infection (Schuster et al. 2003, Whiteley et al. 1999).

## Competitive Quorum-Sensing Circuits

The above quorum-sensing networks rely on multiple signals acting synergistically. Other quorum-sensing networks are arranged such that the signals antagonize one another. For example, *Bacillus subtilis* has two autoinducing peptides functioning in a network arrange-

ment that allows *B. subtilis* to commit to one of two mutually exclusive lifestyles: competence (the ability to take up exogenous DNA) and sporulation (Figure 6). ComX, a 10-amino acid peptide (Figure 2b and Magnuson et al. 1994, Solomon et al. 1996) that is processed and secreted by ComQ (Bacon Schneider et al. 2002), is detected by the membrane-bound histidine sensor kinase ComP. ComX binding stimulates ComP to autophosphorylate and transfer phosphate to the DNA-binding response regulator ComA (Solomon et al. 1995). Phosphorylated ComA regulates transcription of a variety of genes encoding factors required for competence development (Nakano & Zuber 1991). A second oligopeptide autoinducer, competence and sporulation



**Figure 6**

*Bacillus subtilis* produces two autoinducing peptides that regulate two different developmental pathways: competence and sporulation. ComX is represented as a chain of purple ovals and CSF is shown as a chain of red ovals.

**CSF:** competence and sporulation factor of *B. subtilis*

**CSP:** competence-stimulating peptide of *S. pneumoniae*

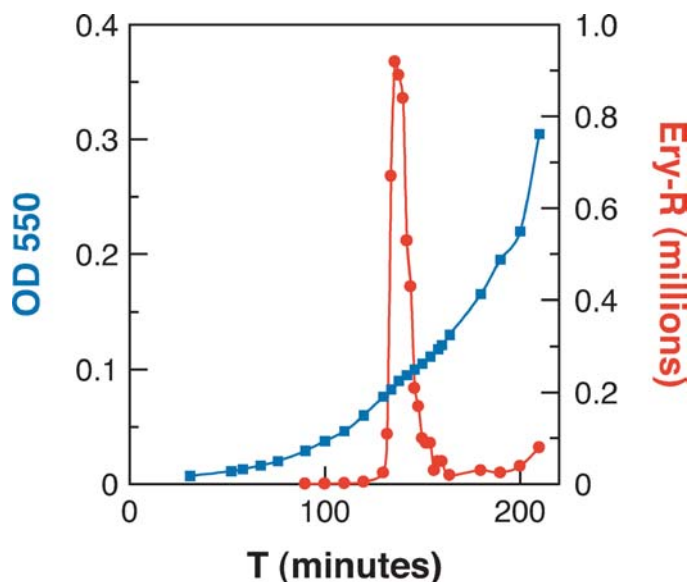
factor of *B. subtilis* (CSF; encoded by the gene *pbrC*), is released via the general secretory apparatus, is re-internalized through the Opp peptide transporter, and acts in the cytoplasm (**Figure 2b**; **Figure 6**; and Lazazzera et al. 1997, Solomon et al. 1996). At low internal concentrations, CSF binds to a protein named RapC and disrupts RapC binding to ComA (Perego 1997, Solomon et al. 1996). RapC binding to ComA inhibits competence development because DNA binding by ComA is prevented. Thus, CSF binding to RapC promotes competence development (Core & Perego 2003). However, at high concentrations, internalized CSF inhibits the ComP-ComA signaling cascade through an unknown mechanism, decreasing competence development and favoring sporulation (Lazazzera et al. 1997, Solomon et al. 1996). CSF also directly promotes sporulation by inhibiting RapB-mediated dephosphorylation of a response regulator named Spo0F, which, in its phosphorylated state, indirectly activates genes required

for sporulation (Grossman 1995, Perego 1997).

## Quorum-Sensing Circuits with On-Off Switches

The above quorum-sensing circuits allow bacteria to transition from a set of low cell density behaviors to a different set of high cell density behaviors. There are, however, quorum-sensing circuits that promote transient expression of particular traits followed by reversion to the original set of behaviors. Such an on-off switch controls competence development in the Gram-positive bacterium *Streptococcus pneumoniae*, which uses an oligopeptide autoinducer named competence-stimulating peptide (CSP) to monitor cell density (**Figure 2b**). CSP is encoded by *comC* (Havarstein et al. 1995, Tomasz & Hotchkiss 1964). The transporter ComAB exports and modifies CSP (Hui et al. 1995). CSP is detected by the membrane-bound sensor histidine kinase ComD, which transfers phosphate to the cytoplasmic response regulator ComE (Pestova et al. 1996). This circuit controls the transcription of gene subsets in a precise temporal order. Early genes are expressed maximally 6–7 min after CSP accumulation; late genes are maximally induced at 9–10 min (Peterson et al. 2000). ComE directly activates transcription of early genes that include *comAB* and *comCDE*; this causes increased signal production and detection (Pestova et al. 1996). This positive feedback loop results in a dramatic, population-wide spike in competence when the bacteria reach the critical cell density (**Figure 7**). ComE also activates transcription of *comX*, a gene encoding an alternate sigma factor (Lee & Morrison 1999), and *comW*, which is required for transcription of late genes encoding proteins essential for DNA uptake (Luo et al. 2004).

A novel feature of *S. pneumoniae* quorum-sensing circuit is the rapidity with which the process of competence development initiates and terminates (**Figure 7** and Tomasz & Hotchkiss 1964). Importantly, competent



**Figure 7**

Competence in *Streptococcus pneumoniae* (red line) rises sharply at a specific growth stage (blue line), followed by a rapid decline. Optical Density (OD550) was used to measure cell number and resistance to erythromycin (Ery-R) was used to assess competence. Figure courtesy of D. Morrison.

*S. pneumoniae* cells are more prone to autolysis than are noncompetent cells (Dagkessamanskaia et al. 2004, Morrison & Baker 1979, Seto & Tomasz 1975, Steinmoen et al. 2003). Thus, the benefit gained from acquiring DNA that can be used as a repository of new genes is maximized by efficiently activating and terminating the process to minimize lethality by autolysis. The events leading to termination have not been defined. It is known that ComX rapidly disappears when competence is terminated, which suggests that regulated proteolysis occurs (Luo et al. 2004).

### Quorum-Sensing Systems Responsive to Host Cues

*Agrobacterium tumefaciens* induces crown gall tumors in plants through transfer and integration of a tumor-inducing (TI) plasmid into plant cells (Zhu et al. 2000). The tumors produce molecules termed opines, which the bacteria use as nutrients (Dessaix et al. 1992). The quorum-sensing circuit of *A. tumefaciens* is especially interesting because it is only activated at the host-bacterial interface owing to a requirement for both plant- and bacteria-produced signals. Mobilization of the TI plasmid is responsive to proximity to the plant because it requires detection of opines by a cytoplasmic receptor termed AccR or OccR (Beck von Bodman et al. 1992, Fuqua & Winans 1994). AccR/OccR-opine binding induces expression of the *V. fischeri*-like *luxR* homologue, *traR* (Fuqua & Winans 1994). TraR responds to an AHL autoinducer produced by the *V. fischeri* LuxI-type enzyme, TraI (Hwang et al. 1994, Zhang et al. 1993). Hence, bacterial number controls TI transfer because TraR bound to its autoinducer induces TI plasmid replication and bacterial-bacterial conjugation, which lead to increased infectivity of the population (Zhu & Winans 1999). Anti-TraR activators exist that limit TraR activity and thus presumably optimize the ratio of bacteria-bacteria to bacterial-plant TI transfer (Chai et al. 2001, Fuqua et al. 1995, Hwang et al. 1995).

### GLOBAL CONTROL: QUORUM-SENSING REGULONS

The advent of genomic profiling has shown that quorum sensing, in many bacteria, controls gene expression in a global manner. Two transcription profiling studies identified over 150 competence-regulated genes in *S. pneumoniae* that were categorized as early, late, delayed-induction, and repressed (Dagkessamanskaia et al. 2004, Peterson et al. 2004). As previously mentioned, early genes are required for signal production, export, and detection whereas some late genes are necessary for DNA internalization. Many of the delayed genes are involved in bacterial stress responses (Dagkessamanskaia et al. 2004, Peterson et al. 2004). Gene-disruption experiments analyzing 124 quorum-sensing-controlled genes found that only 23 are required for competence (Peterson et al. 2004). Quorum-sensing mutants of *S. pneumoniae* and related streptococci show defects in multiple pathways, including biofilm formation, acid tolerance, bacteriocin production, and virulence (reviewed in Suntharalingam & Cvitkovitch 2005). Together, these results suggest that quorum sensing in streptococcus controls the initiation of a global developmental program in which competence development represents only one aspect.

Further evidence that quorum sensing coordinates the control of a large subset of genes comes from transcriptome analyses of *P. aeruginosa* that identify 616 genes as part of the regulon. In one study, addition of autoinducers repressed 222 genes (Wagner et al. 2003). A concurrent study identified 315 quorum-sensing-controlled targets, of which only 38 were repressed (Schuster et al. 2003). Although the two experiments were performed under different growth and autoinducer conditions, the reasons for the discrepancies remain unclear. Importantly, prior to these profiling analyses, quorum-sensing-repressed targets had not been identified in *P. aeruginosa*. Similarly, transcriptional analysis of *V. cholerae* quorum-sensing mutants shows that

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**TI:** tumor-inducing  
**Regulon:** a set of genes under common regulatory control

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**SAH:** S-adenosylhomocysteine

**DPD:** 4,5-dihydroxy-2,3-pentanedione, a molecule generated by LuxS

the entire virulence regulon (>70 genes) is repressed by quorum sensing (Zhu et al. 2002).

These recent whole-genome quorum-sensing studies highlight two important ideas. First, quorum sensing allows bacteria to alternate between distinct genome-wide programs. These findings, along with an enhanced appreciation of the complexity of the quorum-sensing network architectures, have fundamentally changed the perception of bacteria as primitive single-celled organisms. Bacteria now are understood to undergo complex programs of development similar in many respects to eukaryotic organisms. Second, large groups of genes are repressed by quorum sensing. This finding challenges the notion that the primary function of quorum sensing is to initiate activities that are only beneficial to bacterial participation in group activities. Rather, an equally important function of quorum sensing may be to terminate processes that are only beneficial to bacteria living in relative isolation outside of a community structure.

## INTERSPECIES COMMUNICATION AMONG BACTERIA

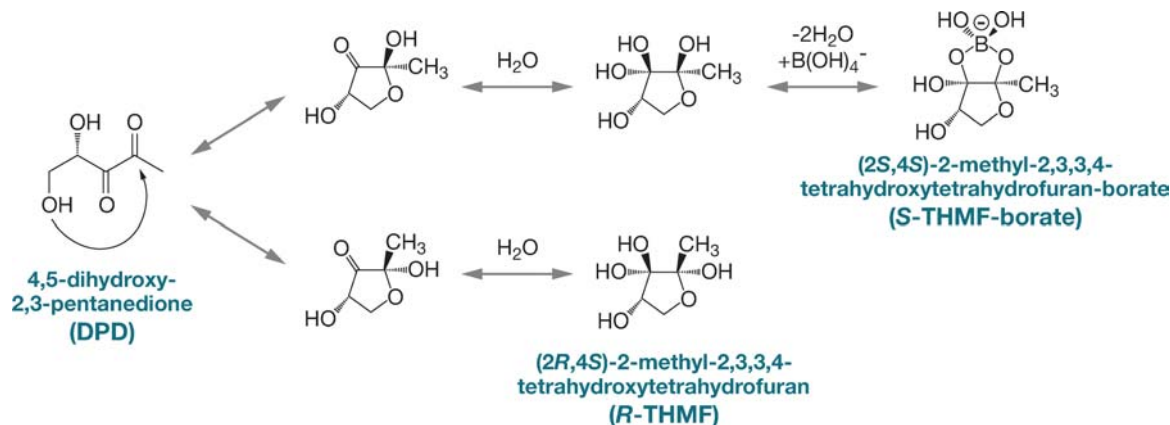
Beyond controlling gene expression on a global scale, quorum sensing allows bacteria to communicate within and between species. This notion arose with the discovery and study of the autoinducer AI-2, which is one of several signals used by *V. harveyi* in quorum sensing (Figure 2d and Figure 4). Specifically, *luxS* encoding the AI-2 synthase is present in roughly half of all sequenced bacterial genomes, AI-2 production has been verified in a large number of these species, and AI-2 controls gene expression in a variety of bacteria. Together, these findings have led to the hypothesis that bacteria use AI-2 to communicate between species (reviewed in Xavier & Bassler 2003).

LuxS functions in the pathway for metabolism of SAM, the major cellular methyl donor. Transfer of the methyl

moiety to various substrates produces the toxic byproduct S-adenosylhomocysteine (SAH) (Schauder et al. 2001). In non-LuxS-containing bacteria and eukaryotes, the enzyme SAH hydrolase metabolizes SAH to adenosine and homocysteine. However, in bacteria containing LuxS, two enzymes, Pfs and LuxS, act sequentially to convert SAH to adenine, homocysteine, and the signaling molecule DPD (Figure 8 and Schauder et al. 2001). DPD is a highly reactive product that can rearrange and undergo additional reactions, which suggests that distinct but related molecules derived from DPD may be the signals that different bacterial species recognize as AI-2. Two distinct DPD-derived signals were identified in *V. harveyi* and *Salmonella typhimurium* by trapping the active molecules in their respective receptors (LuxP for *V. harveyi* and LsrB for *S. typhimurium*), crystallizing the complexes, and solving their structures (Chen et al. 2002, Miller et al. 2004, Taga et al. 2001). In *V. harveyi*, AI-2 is (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (S-THMF borate); in *S. typhimurium*, AI-2 is (2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF) (Figure 2d and Figure 8). Straightforward chemistry links these two molecules, as DPD can cyclize with two equally feasible stereochemistries. Following hydration and borate addition, the upper cyclization pathway in Figure 8 yields the *V. harveyi* AI-2 and the lower cyclization and hydration pathway yields the *S. typhimurium* AI-2.

Identification of boron in *V. harveyi* AI-2 is surprising, as few biological roles for boron are known. However, boron is present in high concentrations (~0.4 mM) in the marine environment, which makes it a reasonable element in the *V. harveyi* AI-2 signal (Bowen 1966). Significantly lower boron concentration is found in terrestrial environments. This makes boron an unlikely component of the *S. typhimurium* AI-2 signal (Fresenius 1988). Importantly, all of the chemical species shown in Figure 8 exist in equilibrium and rapidly





**Figure 8**

AI-2 is a family of interconverting molecules derived from DPD. *Vibrio harveyi* AI-2 is S-THMF-borate and *Salmonella typhimurium* AI-2 is R-THMF. Figure from Miller et al. 2004.

interconvert. Moreover, the concentrations of each molecule can be altered by manipulating the boron concentration. For example, addition of boron to DPD preparations promotes formation of the *V. harveyi* AI-2 molecule at the expense of the *S. typhimurium* signal. This shift is biologically relevant because DPD supplemented with boron causes *V. harveyi* to produce maximal bioluminescence whereas the same mixture inhibits the AI-2 response from *S. typhimurium*. Conversely, DPD preparations depleted for boron promote formation of the *S. typhimurium* signal with the concomitant loss of the *V. harveyi* AI-2. Again, this chemistry is borne out in the effect on AI-2-responsive gene expression in the two bacterial species (Miller et al. 2004).

These initial AI-2 investigations show that bacteria employ a conserved biosynthetic pathway to synthesize chemical signal intermediates whose fates are ultimately defined by the chemistry of the particular environment. Other DPD derivatives may exist and be biologically active. Additionally, some bacteria may possess two or more AI-2 receptors for recognition of different derivatives of DPD and alter particular behaviors in response to the information conveyed by each signal. Because only one enzyme (LuxS) is required to synthesize this family of interconverting sig-

nal molecules, this pathway may represent an especially economical method for evolving a complex bacterial lexicon.

## QUORUM QUENCHING

The fundamental role of quorum sensing appears to be global control of the physiology of bacterial populations. This control is often exerted at the interface of different bacterial populations or at the bacterial-host margin. In niches in which bacterial populations compete for limited resources, the ability to disrupt quorum sensing may give one bacterial species an advantage over another that relies on quorum sensing. Likewise, a host's ability to interfere with bacterial cell-cell communication may be crucial in preventing colonization by pathogenic bacteria that use quorum sensing to coordinate virulence. Thus, it is not surprising that mechanisms have evolved to interfere with bacterial cell-cell communication in processes termed quorum quenching. Analogous mechanisms presumably exist for promoting quorum-sensing-controlled behaviors when such behaviors provide benefits to organisms cohabitating with quorum-sensing bacteria. These latter processes are not yet well defined, so we will focus our discussion primarily on mechanisms of quorum quenching.

## Prokaryote-to-Prokaryote Quorum Quenching

As mentioned, cross-inhibition of AIP-mediated signaling in *S. aureus* represents a clear example of a quorum-quenching mechanism because each of the four AIPs specifically inhibits quorum sensing in competing *S. aureus* groups while not disrupting growth and other cellular functions (Lyon et al. 2002a). Many *Bacillus* species secrete an enzyme, AiiA, that cleaves the lactone rings from the acyl moieties of AHLs and renders the AHLs inactive in signal transduction (Dong et al. 2000). AiiA is extremely nonspecific with regard to the AHL acyl side chain, which suggests that this strategy interferes generically with AHL-mediated communication between gram-negative bacteria (Dong et al. 2001). Significantly, as previously mentioned, *Bacillus* relies on oligopeptide-mediated quorum sensing. Therefore, this tactic, while disrupting gram-negative bacterial communication, leaves *Bacillus* cell-cell communication unperturbed.

The soil bacterium *Variovorax paradoxus* uses a different generalized anti-AHL quorum-quenching tactic (Leadbetter & Greenberg 2000). Like *Bacillus*, *V. paradoxus* also degrades AHLs. However, in this case, AHL destruction occurs via an acylase-mediated lactone ring opening. *V. paradoxus* uses the linearized product of the reaction as a source of carbon and nitrogen. This strategy provides *V. paradoxus* with a double benefit: It terminates competitors' group behaviors and simultaneously increases its own growth potential (Leadbetter & Greenberg 2000). Particular *Ralstonia* isolates contain an AHL acylase encoded by *aiiD*, which suggests a similar anti-quorum-sensing mechanism to that of *V. paradoxus* (Lin et al. 2003). The *Ralstonia* quorum-sensing system is immune because the *Ralstonia* autoinducer, 3OH-PAME (Flavier et al. 1997), is not affected by AiiD activity (described in the sidebar).

In some cases, bacteria may degrade their own autoinducers, which presumably termi-

nates quorum-sensing activities. For example, in stationary phase, *A. tumefaciens* produces the AttM AHL lactonase, which can degrade the *A. tumefaciens* autoinducer (Zhang et al. 2002a). It is hypothesized that it is disadvantageous for *A. tumefaciens* to continue to participate in group activities at this late growth stage and that AttM halts these processes. *Erwinia carotovora* and *Xanthomonas campestris* show a similar loss of AHL in stationary phase growth, which suggests an autoinducer degradative activity (Barber et al. 1997, Holden et al. 1998). *P. aeruginosa* degrades long, but not short, chain AHLs through an AiiD-type acylase named PvdQ (Huang et al. 2003). In this case, the RhII autoinducer, C4-homoserine lactone, is immune, and the LasI autoinducer, 3OC12-homoserine lactone, can be destroyed. Interestingly, *pvdQ* is a member of the LasIR regulon and it is thus under 3OC12-homoserine control (Huang et al. 2003, Whiteley et al. 1999).

Some enteric bacteria, including *S. typhimurium* and *Escherichia coli*, import AI-2 with an AI-2-specific transporter (Surette et al. 1999; Taga et al. 2001, 2003; Xavier & Bassler 2005). Once AI-2 is in the cytoplasm, a series of enzymatic reactions inactivates its signaling activity. This process reduces extracellular AI-2 concentrations to levels indicative of low cell density and—because AI-2 is used for interspecies communication—indicative of monospecies environments. AI-2 internalization is suspected to be another mechanism for interference with chemical communication among bacteria. Because *E. coli* and *S. typhimurium* also produce AI-2 and respond to this signal (Taga et al. 2001, 2003), it is not clear how these bacteria regulate AI-2 import while protecting the fidelity of their own AI-2 signaling cascades.

## Eukaryote-to-Prokaryote Quorum Quenching

Several eukaryotic mechanisms that counteract bacterial quorum sensing have recently

been discovered. The Australian red macroalga *Delisea pulchra* coats its surface with a mixture of halogenated furanones that bear structural similarity to AHLs (Givskov et al. 1996). The furanones are internalized by bacteria, bind to LuxR-type proteins, and cause the degradation of these proteins (Manefield et al. 2002). This strategy prevents bacterial colonization of the algal surface by inhibiting quorum-sensing-controlled biofilm formation.

The legume *Medicago truncatula* controls over 150 proteins in response to AHLs produced by two model quorum-sensing bacteria; *Sinorhizobium meliloti* and *P. aeruginosa* (Mathesius et al. 2003). The plant secretes compounds in response to AHLs. These factors inhibit AI-2 signaling and stimulate AHL signaling in quorum-sensing reporter strains. The plant presumably encourages signaling between AHL-producing bacteria but not AI-2-producing bacteria because only the former are beneficial to the plant. Similarly, *Pisum sativum* (pea) produces AHL mimics that both positively and negatively affect AHL-regulated behaviors in a number of bacterial reporter strains (Teplitski et al. 2000).

Reactive oxygen and nitrogen intermediates generated by NADPH oxidase inactivate the *S. aureus* autoinducing peptide in a mouse air pouch skin model (Rothfork et al. 2004). These findings indicate a novel role for NADPH oxidase, an important component of innate immunity, in protection from bacterial infections. Consistent with this, mice deficient in NADPH oxidase have reduced resistance to infection by *S. aureus*, whereas infection from quorum-sensing mutant *S. aureus* remains unaffected by the loss of NADPH oxidase (Rothfork et al. 2004). This latter result suggests that reactive oxygen species influence infectivity only through quorum quenching. The authors of this study speculate that oxidation of other kinds of quorum-sensing molecules by NADPH oxidase is likely (Rothfork et al. 2004).

Human cells also have quorum-quenching activity. Analysis of primary and immortal-

ized human epithelial cell lines show specific inactivation of the *P. aeruginosa* 3OC12-homoserine lactone autoinducer (the product of LasI) but not of the C4-homoserine lactone autoinducer (the product of RhII) (Chun et al. 2004). Although presently uncharacterized, the quenching activity is membrane associated and heat labile, which suggests that it is a protein. This activity is intriguing in terms of the development of anti-*P. aeruginosa* therapies for treatment of CF.

## Biotechnological Applications of Quorum Quenching

Naturally occurring quorum-quenching processes are being tested as novel antimicrobial therapies. Overexpression of *aiiA* in tobacco and potato plants confers resistance to *E. carotovora*, which requires AHL-controlled virulence factor expression to cause disease (Dong et al. 2001). Likewise, coculture of *Bacillus thuringiensis* decreased *E. carotovora*-mediated plant disease in an *aiiA*-dependent manner (Dong et al. 2004). Mice treated with synthetic antagonists of *S. aureus* AIP show resistance to infection (Mayville et al. 1999). Similarly, purified halogenated furanones appear to attenuate virulence of bacteria in mouse models (Hentzer et al. 2003, Wu et al. 2004). These and other examples predict that inhibition of quorum sensing offers an attractive alternative to traditional antibiotics because these strategies are not bactericidal and the occurrence of bacterial resistance therefore could be reduced. Likewise, approaches aimed at promoting beneficial quorum-sensing associations may enhance industrial-scale production of natural or engineered bacterial products.

## EVOLUTION AND MAINTENANCE OF QUORUM SENSING IN BACTERIA

Quorum sensing presumably provides bacteria benefits from group activities that may be unattainable to an individual bacterium acting

alone. For example, the benefit derived from secretion of antibiotics or proteases may only occur when these exoproducts exceed a particular extracellular concentration, and achieving this concentration is only possible through the synchronous activity of a group of cells. The idea that bacteria cooperate has led to new questions regarding the evolution of cell-cell communication in bacteria, the cost bacteria pay for communicating, how fidelity is maintained in quorum-sensing systems, how cheating is controlled, and if and how “eavesdropping” occurs.

Although these evolutionary questions are new in the context of the molecular mechanisms underlying quorum-sensing-controlled behaviors, there exists an extensive literature dealing with these topics in other social organisms (Bourke 2001, Bradley 1999, Korb & Heinze 2004). For example, some social insects (e.g., ants and bees) have sterile worker castes that promote colony fitness even though the workers have no chance at reproduction (Queller & Strassmann 2002). The predominant explanation for these behaviors rests on Hamilton’s kin-selection theory that predicts that even without directly contributing to reproduction, organisms belonging to a multimember group promote the inheritance of their own genes by increasing the fitness of closely related kin (Hamilton 1964a,b). A key component of kin selection is the ability to recognize another individual as kin. The ability to distinguish between and communicate with specific chemical signal molecules may enable a type of “kin selection” in bacteria. Consistent with this, many higher social organisms rely heavily on chemical signaling to maintain the integrity of the social order (Breed et al. 2004, Holldobler 1995, Queller & Strassmann 2002).

Cases of sacrifice of the individual for the group benefit also exist in microorganisms. For example, both the soil-dwelling bacterium *Myxococcus xanthus* and the slime mold *Dictyostelium discoideum*, in the absence of nutrients, produce resistant spores that survive nonvegetatively for long periods and

can be dispersed to new environments (Dao et al. 2000, Strassmann et al. 2000). Spore development requires a large percentage of the population to undergo a lethal differentiation event that leads to structures whose function is to promote spore generation and dispersal. Chemical communication is required for these developmental events in both *D. discoideum* and *M. xanthus*: cAMP and Differentiation-Inducing Factor initiates development of fruiting bodies in *D. discoideum* (Konijn et al. 1969, Town & Stanford 1979, Town et al. 1976), whereas quorum-sensing communication controls the process in *M. xanthus* (Shimkets 1999).

Two examples exist to date that illustrate a selection for maintenance of quorum sensing. In *V. fischeri*, mutants incapable of luciferase production are outcompeted by the wild-type bacteria in the squid host. This indicates that the squid may possess a policing mechanism to eliminate cheater cells. Interestingly, the defect in these mutants was in luciferase itself, which suggests that the squid somehow distinguishes between cells that can and cannot contribute to light production (Visick et al. 2000). *A. tumefaciens*-induced plant tumors contain a large percentage of plasmid-free bacterial cells (Belanger et al. 1995). These cells have a faster growth rate and may therefore more efficiently grow on the opine nutrients produced in the plant tumors. However, plasmid-free bacteria are unable to initiate new tumor formation. Interestingly, as bacterial density increases and nutrients become limiting, increased bacterial-bacterial conjugation occurs and the TI plasmid is replicated to a higher copy number. Both of these events require quorum sensing and ensure that most of the bacteria acquire copies of the plasmid before they disseminate to a new location. This elegant strategy optimizes growth inside the tumor while maintaining the population’s virulence and at least partially explains why quorum sensing is maintained.

Mechanisms for eavesdropping apparently also exist in quorum-sensing systems.

*P. aeruginosa* does not have *luxS* and therefore does not produce AI-2. However, *P. aeruginosa* detects AI-2 produced by the indigenous nonpathogenic microflora present in CF sputum samples (Duan et al. 2003). In the CF lung, *P. aeruginosa* exists in a complex microbial community composed of a variety of pathogenic and nonpathogenic bacteria. The detection of AI-2 may alert *P. aeruginosa* that *P. aeruginosa* is in the lung and that a program of gene expression that enhances persistence/virulence in the host is required. Consistent with this idea, CF sputum contains high concentrations of AI-2 and AI-2 induces *P. aeruginosa* virulence factor expression (Duan et al. 2003). In another example, *Salmonella enterica*, which has a *V. fischeri*-type LuxR-type protein (SdiA) but no LuxI-type enzyme, intercepts AHLs produced by other LuxI-containing gram-negative bacteria. In response to these signals, *S. enterica* expresses the *rck* operon and other genes that protect *S. enterica* from host defenses in the intestine (Ahmer et al. 1998). This result is interpreted to mean that the AHL signals signify that *S. enterica* is in a dense population of bacteria, which can presumably be attained only inside a host.

The ecological and evolutionary implications of quorum sensing in bacteria are only beginning to be addressed (Travisano & Velicer 2004). However, continued study of such questions hopefully will provide insight into the evolution and maintenance of group dynamics and behavior.

## RHOMBOID: A SHARED PROKARYOTIC AND EUKARYOTIC CHEMICAL COMMUNICATION MECHANISM

New data suggest that some bacterial and eukaryotic signaling mechanisms have a common evolutionary origin. The inner membrane protein AarA of *Providencia stuartii* is required for the release of an extracellular quorum-sensing signal whose structure has

not been defined (Rather et al. 1999). AarA has homology to the *Drosophila melanogaster* RHO (Gallio et al. 2002), which is a serine protease required for intramembrane cleavage, release, and activation of Epidermal Growth Factor receptor ligands (Klambt 2000, 2002). RHO is essential for many developmental processes in *D. melanogaster*, including proper wing vein development and organization of the fly eye (Schweitzer & Shilo 1997). Consistent with the idea that AarA and RHO have a common signaling function, expression of *P. stuartii* *aarA* in a *D. melanogaster* *rho* mutant rescued wing vein development. Likewise, expression of *rho* in a *P. stuartii* *aarA* mutant complemented the quorum-sensing signaling defect (Gallio et al. 2002). Homologues of RHO/AarA are nearly ubiquitous in all three kingdoms of life: bacteria, archaea, and eukaryotes (Koonin et al. 2003). Five of eight tested bacterial Aar/RHO orthologues specifically cleaved RHO substrates, which suggests a widespread conservation of the mechanism of RHO with its bacterial homologues (Urban et al. 2002). These fascinating results show that bacteria and higher eukaryotes share a common cell-cell communication system; however, it has not been determined if any cross-kingdom communication can be mediated by RHO or its homologues.

A recent bioinformatics study suggests that the RHO/AarA finding is not an anomaly but rather that many signaling mechanisms may be shared by prokaryotes and eukaryotes. Enzymes involved in the production of cell-cell signaling molecules in vertebrates have homologues in bacteria but are absent from plants and archaea (Iyer et al. 2004). A few of numerous examples are the enzymes phenylethanolamine N-methyltransferase (which catalyzes the conversion of norepinephrine to epinephrine), histidine decarboxylase (which catalyzes histidine to histamine), and glutamate decarboxylase (which catalyzes glutamate to  $\gamma$ -aminobutyric acid). It is hypothesized that eukaryotes acquired these genes from bacteria through a series of horizontal gene transfer

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**RHO:** rhomboid protein

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events (Iyer et al. 2004). These findings suggest that bacteria and eukaryotes share enzymes responsible for many cell-cell signaling pathways. This points to the exciting possibility that prokaryotic-to-eukaryotic cross-kingdom communication may be more prevalent than is currently appreciated.

## CONCLUSIONS

It is now clear that cell-cell communication is the norm in the bacterial world and that understanding this process is fundamental to all of microbiology, including industrial and clinical microbiology. Our knowledge of quorum sensing may ultimately affect our understanding of higher-organism development. Quorum sensing was, until recently, considered to promote exclusively intraspecies communication and thus enable clonal populations of bacteria to count their cell numbers and alter gene expression in unison. While some autoinducers indeed appear to be extremely species-specific, new research shows that others are

either genus-specific or promote intergenera communication. Further, hints that interkingdom communication occurs are becoming increasingly prevalent. Coincident with these findings are the beginnings of an understanding that prokaryotic and eukaryotic mechanisms that enhance and interfere with bacterial chemical communication also exist in nature. Bacterial quorum-sensing signal detection and relay apparatuses are complex and often consist of multiple circuits organized in a variety of configurations. Because bacteria routinely exist in fluctuating environments containing complex mixtures of chemicals, some of which are signals and some of which presumably do not convey meaningful information, we hypothesize that each quorum-sensing network organization evolved to solve the particular set of communication needs a particular species of bacteria encounters. Elements of these elegant solutions for deciphering complex chemical vocabularies appear to be conserved and used for analogous purposes in eukaryotes.

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## LITERATURE CITED

- Ahmer BM, van Reeuwijk J, Timmers CD, Valentine PJ, Heffron F. 1998. *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. *J. Bacteriol.* 180:1185-93
- Ansaldi M, Marolt D, Stebe T, Mandic-Mulec I, Dubnau D. 2002. Specific activation of the *Bacillus* quorum-sensing systems by isoprenylated pheromone variants. *Mol. Microbiol.* 44:1561-73
- Bacon Schneider K, Palmer TM, Grossman AD. 2002. Characterization of *comQ* and *comX*, two genes required for production of ComX pheromone in *Bacillus subtilis*. *J. Bacteriol.* 184:410-19
- Barber CE, Tang JL, Feng JX, Pan MQ, Wilson TJ, et al. 1997. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol. Microbiol.* 24:555-66
- Bassler BL, Wright M, Showalter RE, Silverman MR. 1993. Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. *Mol. Microbiol.* 9:773-86



- Bassler BL, Wright M, Silverman MR. 1994a. Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. *Mol. Microbiol.* 13:273–86
- Bassler BL, Wright M, Silverman MR. 1994b. Sequence and function of LuxO, a negative regulator of luminescence in *Vibrio harveyi*. *Mol. Microbiol.* 12:403–12
- Beck von Bodman S, Hayman GT, Farrand SK. 1992. Opine catabolism and conjugal transfer of the nopaline Ti plasmid pTiC58 are coordinately regulated by a single repressor. *Proc. Natl. Acad. Sci. USA* 89:643–47
- Belanger C, Canfield ML, Moore LW, Dion P. 1995. Genetic analysis of nonpathogenic *Agrobacterium tumefaciens* mutants arising in crown gall tumors. *J. Bacteriol.* 177:3752–57
- Booth MC, Bogie CP, Sahl HG, Siezen RJ, Hatter KL, Gilmore MS. 1996. Structural analysis and proteolytic activation of *Enterococcus faecalis* cytolysin, a novel lantibiotic. *Mol. Microbiol.* 21:1175–84
- Bourke AF. 2001. Social insects and selfish genes. *Biologist* 48:205–8
- Bowen HJM. 1966. *Trace Elements in Biochemistry*, pp. 9–19. London: Academic
- Bradley BJ. 1999. Levels of selection, altruism, and primate behavior. *Q. Rev. Biol.* 74:171–94
- Breed MD, Guzman-Novoa E, Hunt GJ. 2004. Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Annu. Rev. Entomol.* 49:271–98
- Cao JG, Meighen EA. 1989. Purification and structural identification of an autoinducer for the luminescence system of *Vibrio harveyi*. *J. Biol. Chem.* 264:21670–76
- Carrington JC, Ambros V. 2003. Role of microRNAs in plant and animal development. *Science* 301:336–38
- Chai Y, Zhu J, Winans SC. 2001. TrlR, a defective TraR-like protein of *Agrobacterium tumefaciens*, blocks TraR function in vitro by forming inactive TrlR:TraR dimers. *Mol. Microbiol.* 40:414–21
- Chater KF, Horinouchi S. 2003. Signalling early developmental events in two highly diverged *Streptomyces* species. *Mol. Microbiol.* 48:9–15
- Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczar I, et al. 2002. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 415:545–49**
- Chun CK, Ozer EA, Welsh MJ, Zabner J, Greenberg EP. 2004. Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. *Proc. Natl. Acad. Sci. USA* 101:3587–90
- Core L, Perego M. 2003. TPR-mediated interaction of RapC with ComA inhibits response regulator-DNA binding for competence development in *Bacillus subtilis*. *Mol. Microbiol.* 49:1509–22
- Dagkessamanskaia A, Moscoso M, Henard V, Guiral S, Overweg K, et al. 2004. Interconnection of competence, stress and CiaR regulons in *Streptococcus pneumoniae*: competence triggers stationary phase autolysis of ciaR mutant cells. *Mol. Microbiol.* 51:1071–86
- Dao DN, Kessin RH, Ennis HL. 2000. Developmental cheating and the evolutionary biology of Dictyostelium and Myxococcus. *Microbiology* 146(Pt. 7):1505–12
- Dessaux Y, Petit AS, Tempe J. 1992. Opines in *Agrobacterium* biology. In *The Molecular Signals in Plant-Microbe Interaction*, ed. DPS Verma, pp. 109–36. Boca Raton, FL: CRC Press
- Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH. 2001. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature* 411:813–17**

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This is the first report identifying a small molecule by X-ray crystallography of a ligand bound to its receptor.

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This work highlights the potential for targeting quorum sensing in the development of novel antibacterial therapeutics.

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- Dong YH, Xu JL, Li XZ, Zhang LH. 2000. AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc. Natl. Acad. Sci. USA* 97:3526–31
- Dong YH, Zhang XF, Xu JL, Zhang LH. 2004. Insecticidal *Bacillus thuringiensis* silences *Erwinia carotovora* virulence by a new form of microbial antagonism, signal interference. *Appl. Environ. Microbiol.* 70:954–60
- Duan K, Dammel C, Stein J, Rabin H, Surette MG. 2003. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol. Microbiol.* 50:1477–91
- Dufour P, Jarraud S, Vandenesch F, Greenland T, Novick RP, et al. 2002. High genetic variability of the agr locus in Staphylococcus species. *J. Bacteriol.* 184:1180–86
- Eberhard A, Burlingame AL, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ. 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochemistry* 20:2444–49
- Eberl L, Tummlier B. 2004. *Pseudomonas aeruginosa* and *Burkholderia cepacia* in cystic fibrosis: genome evolution, interactions and adaptation. *Int. J. Med. Microbiol.* 294:123–31
- Engbrecht J, Nealson K, Silverman M. 1983. Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32:773–81
- Engbrecht J, Silverman M. 1984. Identification of genes and gene products necessary for bacterial bioluminescence. *Proc. Natl. Acad. Sci. USA* 81:4154–58
- Fidopiastis PM, Miyamoto CM, Jobling MG, Meighen EA, Ruby EG. 2002. LitR, a new transcriptional activator in *Vibrio fischeri*, regulates luminescence and symbiotic light organ colonization. *Mol. Microbiol.* 45:131–43
- Flavier AB, Clough SJ, Schell MA, Denny TP. 1997. Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol. Microbiol.* 26:251–59
- Freeman JA, Bassler BL. 1999a. A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. *Mol. Microbiol.* 31:665–77
- Freeman JA, Bassler BL. 1999b. Sequence and function of LuxU: a two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. *J. Bacteriol.* 181(3):899–906
- Freeman JA, Lilley BN, Bassler BL. 2000. A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. *Mol. Microbiol.* 35:139–49
- Fresenius W, Quentin KE, Schneider W, eds. 1988. *Water Analysis: A Practical Guide to Physico-chemical, Chemical and Microbiological Water Examination and Quality Assurance*, pp. 420–21. New York: Springer-Verlag. 804 pp.
- Fuqua C, Burbea M, Winans SC. 1995. Activity of the Agrobacterium Ti plasmid conjugal transfer regulator TraR is inhibited by the product of the traM gene. *J. Bacteriol.* 177:1367–73
- Fuqua C, Eberhard A. 1999. Signal generation in autoinduction systems: synthesis of acylated homoserine lactones by LuxI-type proteins. In *Cell-Cell Signaling in Bacteria*, ed. GM Dunny, SC Winans, pp. 211–30. Washington, DC: ASM Press
- Fuqua WC, Winans SC. 1994. A LuxR-LuxI type regulatory system activates Agrobacterium Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. *J. Bacteriol.* 176:2796–806
- Gallio M, Sturgill G, Rather P, Kylsten P. 2002. A conserved mechanism for extracellular signaling in eukaryotes and prokaryotes. *Proc. Natl. Acad. Sci. USA* 99:12208–13

This study's results suggest a conserved Rhomboid-signaling mechanism in eukaryotes and bacteria.

- Gambello MJ, Iglewski BH. 1991. Cloning and characterization of the *Pseudomonas aeruginosa* lasR gene, a transcriptional activator of elastase expression. *J. Bacteriol.* 173:3000–9
- Givskov M, de Nys R, Manefield M, Gram L, Maximilien R, et al. 1996. Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J. Bacteriol.* 178:6618–22
- Gould TA, Schweizer HP, Churchill ME. 2004. Structure of the *Pseudomonas aeruginosa* acyl-homoserine lactone synthase LasI. *Mol. Microbiol.* 53:1135–46
- Grossman AD. 1995. Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis*. *Annu. Rev. Genet.* 29:477–508
- Hamilton WD. 1964a. The genetical evolution of social behaviour. I. *J. Theor. Biol.* 7:1–16
- Hamilton WD. 1964b. The genetical evolution of social behaviour. II. *J. Theor. Biol.* 7:17–52
- Hammer BK, Bassler BL. 2003. Quorum sensing controls biofilm formation in *Vibrio cholerae*. *Mol. Microbiol.* 50:101–4
- Hanzelka BL, Parsek MR, Val DL, Dunlap PV, Cronan JE Jr, Greenber EP. 1999. Acylhomoserine lactone synthase activity of the *Vibrio fischeri* AinS protein. *J. Bacteriol.* 181:5766–70
- Havarstein LS, Coomaraswamy G, Morrison DA. 1995. An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*. *Proc. Natl. Acad. Sci. USA* 92:11140–44
- Henke JM, Bassler BL. 2004a. Quorum sensing regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. *J. Bacteriol.* 186:3794–805
- Henke JM, Bassler BL. 2004b. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *J. Bacteriol.* 186:6902–14
- Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, et al. 2003. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J.* 22:3803–15
- Holden MT, McGowan SJ, Bycroft BW, Stewart GS, Williams P, Salmond GP. 1998. Cryptic carbapenem antibiotic production genes are widespread in *Erwinia carotovora*: facile trans activation by the carR transcriptional regulator. *Microbiology* 144(Pt. 6):1495–508
- Holden MT, Ram Chhabra S, de Nys R, Stead P, Bainton NJ, et al. 1999. Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. *Mol. Microbiol.* 33:1254–66
- Holldobler B. 1995. The chemistry of social regulation: multicomponent signals in ant societies. *Proc. Natl. Acad. Sci. USA* 92:19–22
- Huang JJ, Han JI, Zhang LH, Leadbetter JR. 2003. Utilization of acyl-homoserine lactone quorum signals for growth by a soil pseudomonad and *Pseudomonas aeruginosa* PAO1. *Appl. Environ. Microbiol.* 69:5941–49
- Hui FM, Zhou L, Morrison DA. 1995. Competence for genetic transformation in *Streptococcus pneumoniae*: organization of a regulatory locus with homology to two lactococcal A secretion genes. *Gene* 153:25–31
- Hwang I, Cook DM, Farrand SK. 1995. A new regulatory element modulates homoserine lactone-mediated autoinduction of Ti plasmid conjugal transfer. *J. Bacteriol.* 177:449–58
- Hwang I, Li PL, Zhang L, Piper KR, Cook DM, et al. 1994. TraI, a LuxI homologue, is responsible for production of conjugation factor, the Ti plasmid N-acylhomoserine lactone autoinducer. *Proc. Natl. Acad. Sci. USA* 91:4639–43
- Iyer LM, Aravind L, Coon SL, Klein DC, Koonin EV. 2004. Evolution of cell-cell signaling in animals: did late horizontal gene transfer from bacteria have a role? *Trends Genet.* 20:292–99
- Ji G, Beavis RC, Novick RP. 1995. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. *Proc. Natl. Acad. Sci. USA* 92:12055–59

This study shows that, unlike most gram-positive, bacterial autoinducing peptides that act from the extracellular environment, CSE, a peptide signal in *B. subtilis*, is imported into the cytoplasm.

The regulatory link connecting the quorum-sensing machinery and the genes required for competence is established in this study.

A genetic screen combined with bioinformatics reveals that Hfq and multiple redundant sRNAs control the translation of the master regulators of quorum sensing, LuxR (*V. harveyi*) and HapR (*V. cholerae*).

- Kaplan HB, Greenberg EP. 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. *J. Bacteriol.* 163:1210–14
- Klamt C. 2000. EGF receptor signalling: the importance of presentation. *Curr. Biol.* 10:R388–91
- Klamt C. 2002. EGF receptor signalling: roles of star and rhomboid revealed. *Curr. Biol.* 12:R21–23
- Konijn TM, van deMeene JG, Chang YY, Barkley DS, Bonner JT. 1969. Identification of adenosine-3',5'-monophosphate as the bacterial attractant for myxamoebae of *Dictyostelium discoideum*. *J. Bacteriol.* 99:510–12
- Koonin EV, Makarova KS, Rogozin IB, Davidovic L, Letellier MC, Pellegrini L. 2003. The rhomboids: a nearly ubiquitous family of intramembrane serine proteases that probably evolved by multiple ancient horizontal gene transfers. *Genome Biol.* 4:R19
- Korb J, Heinze J. 2004. Multilevel selection and social evolution of insect societies. *Naturwissenschaften* 91:291–304
- Lazazzera BA, Solomon JM, Grossman AD. 1997. An exported peptide functions intracellularly to contribute to cell density signaling in *B. subtilis*. *Cell* 89:917–25
- Leadbetter JR, Greenberg EP. 2000. Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. *J. Bacteriol.* 182:6921–26
- Lee MS, Morrison DA. 1999. Identification of a new regulator in *Streptococcus pneumoniae* linking quorum sensing to competence for genetic transformation. *J. Bacteriol.* 181:5004–16
- Lenz DH, Mok KC, Lilley BN, Kulkarni RV, Wingreen NS, Bassler BL. 2004. The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in *Vibrio harveyi* and *Vibrio cholerae*. *Cell* 118:69–82
- Lilley BN, Bassler BL. 2000. Regulation of quorum sensing in *Vibrio harveyi* by LuxO and sigma-54. *Mol. Microbiol.* 36:940–54
- Lin YH, Xu JL, Hu J, Wang LH, Ong SL, et al. 2003. Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol. Microbiol.* 47:849–60
- Luo P, Li H, Morrison DA. 2004. Identification of ComW as a new component in the regulation of genetic transformation in *Streptococcus pneumoniae*. *Mol. Microbiol.* 54:172–83
- Lupp C, Ruby EG. 2004. *Vibrio fischeri* LuxS and AinS: comparative study of two signal synthases. *J. Bacteriol.* 186:3873–81
- Lupp C, Urbanowski M, Greenberg EP, Ruby EG. 2003. The *Vibrio fischeri* quorum-sensing systems ain and lux sequentially induce luminescence gene expression and are important for persistence in the squid host. *Mol. Microbiol.* 50:319–31
- Lyon GJ, Novick RP. 2004. Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. *Peptides* 25:1389–403
- Lyon GJ, Wright JS, Christopoulos A, Novick RP, Muir TW. 2002a. Reversible and specific extracellular antagonism of receptor-histidine kinase signaling. *J. Biol. Chem.* 277:6247–53
- Lyon GJ, Wright JS, Muir TW, Novick RP. 2002b. Key determinants of receptor activation in the *agr* autoinducing peptides of *Staphylococcus aureus*. *Biochemistry* 41:10095–104
- Magnuson R, Solomon J, Grossman AD. 1994. Biochemical and genetic characterization of a competence pheromone from *B. subtilis*. *Cell* 77:207–16
- Manefield M, Rasmussen TB, Henzter M, Andersen JB, Steinberg P, et al. 2002. Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. *Microbiology* 148:1119–27

- Manefield M, Turner SL. 2002. Quorum sensing in context: out of molecular biology and into microbial ecology. *Microbiology* 148:3762–64
- Marketon MM, Gronquist MR, Eberhard A, Gonzalez JE. 2002. Characterization of the *Sinorhizobium meliloti* sinR/sinI locus and the production of novel N-acyl homoserine lactones. *J. Bacteriol.* 184:5686–95
- Mathesius U, Mulers S, Gao M, Teplitski M, Caetano-Anolles G, et al. 2003. Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc. Natl. Acad. Sci. USA* 100:1444–49
- Mayville P, Ji G, Beavis R, Yang H, Goger M, et al. 1999. Structure-activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence. *Proc. Natl. Acad. Sci. USA* 96:1218–23
- Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL. 2002. Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. *Cell* 110:303–14
- Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, et al. 2004. A novel form of the bacterial quorum sensing signal AI-2 recognized by the *Salmonella typhimurium* receptor LsrB. *Mol. Cell.* 15:677–87
- Miyamoto CM, Dunlap PV, Ruby EG, Meighen EA. 2003. LuxO controls *luxR* expression in *Vibrio harveyi*: evidence for a common regulatory mechanism in *Vibrio*. *Mol. Microbiol.* 48:537–48
- Mok KC, Wingreen NS, Bassler BL. 2003. *Vibrio harveyi* quorum sensing: a coincidence detector for two autoinducers controls gene expression. *EMBO J.* 22:870–81
- More MI, Finger LD, Stryker JL, Fuqua C, Eberhard A, Winans SC. 1996. Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. *Science* 272:1655–58
- Morrison DA, Baker MF. 1979. Competence for genetic transformation in pneumococcus depends on synthesis of a small set of proteins. *Nature* 282:215–17
- Nakano MM, Zuber P. 1991. The primary role of comA in establishment of the competent state in *Bacillus subtilis* is to activate expression of srfA. *J. Bacteriol.* 173:7269–74
- Nakayama J, Cao Y, Horii T, Sakuda S, Akkermans AD, et al. 2001. Gelatinase biosynthesis-activating pheromone: a peptide lactone that mediates a quorum sensing in *Enterococcus faecalis*. *Mol. Microbiol.* 41:145–54
- Neelson KH, Hastings JW. 1979. Bacterial bioluminescence: its control and ecological significance. *Microbiol. Rev.* 43:496–518
- Novick RP. 2003. Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol. Microbiol.* 48:1429–49
- Novick RP, Projan SJ, Kornblum J, Ross HF, Ji G, et al. 1995. The agr P2 operon: an autocatalytic sensory transduction system in *Staphylococcus aureus*. *Mol. Gen. Genet.* 248:446–58
- Novick RP, Ross HF, Projan SJ, Kornblum J, Kreiswirth B, Moghazeh S. 1993. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. *EMBO J.* 12:3967–75
- Ochsner UA, Koch AK, Fiechter A, Reiser J. 1994. Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *J. Bacteriol.* 176:2044–54
- Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP. 1999. Acyl homoserine-lactone quorum-sensing signal generation. *Proc. Natl. Acad. Sci. USA* 96:4360–65
- Pearson JP, Gray KM, Passador L, Tucker KD, Eberhard A, et al. 1994. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc. Natl. Acad. Sci. USA* 91:197–201



This study, using microarray analysis, shows that the *Pseudomonas aeruginosa* quorum-sensing regulon consists of a large number of genes and defines the temporal order of their expression.

- Pearson JP, Passador L, Iglewski BH, Greenberg EP. 1995. A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92:1490–94
- Pearson JP, Van Delden C, Iglewski BH. 1999. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *J. Bacteriol.* 181:1203–10
- Perego M. 1997. A peptide export-import control circuit modulating bacterial development regulates protein phosphatases of the phosphorelay. *Proc. Natl. Acad. Sci. USA* 94:8612–17
- Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, et al. 1999. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 96:11229–34
- Pestova EV, Havarstein LS, Morrison DA. 1996. Regulation of competence for genetic transformation in *Streptococcus pneumoniae* by an auto-induced peptide pheromone and a two-component regulatory system. *Mol. Microbiol.* 21:853–62
- Peterson S, Cline RT, Tettelin H, Sharov V, Morrison DA. 2000. Gene expression analysis of the *Streptococcus pneumoniae* competence regulons by use of DNA microarrays. *J. Bacteriol.* 182:6192–202
- Peterson SN, Sung CK, Cline R, Desai BV, Snesrud EC, et al. 2004. Identification of competence pheromone responsive genes in *Streptococcus pneumoniae* by use of DNA microarrays. *Mol. Microbiol.* 51:1051–70
- Queller DC, Strassmann JE. 2002. The many selves of social insects. *Science* 296:311–13
- Rather PN, Ding X, Baca-DeLancey RR, Siddiqui S. 1999. *Providencia stuartii* genes activated by cell-to-cell signaling and identification of a gene required for production or activity of an extracellular factor. *J. Bacteriol.* 181:7185–91
- Rothfork JM, Timmins GS, Harris MN, Chen X, Lysis AJ, et al. 2004. Inactivation of a bacterial virulence pheromone by phagocyte-derived oxidants: new role for the NADPH oxidase in host defense. *Proc. Natl. Acad. Sci. USA* 101:13867–72
- Saenz HL, Augsburg V, Vuong C, Jack RW, Gotz F, Otto M. 2000. Inducible expression and cellular location of AgrB, a protein involved in the maturation of the staphylococcal quorum-sensing pheromone. *Arch. Microbiol.* 174:452–55
- Schauder S, Shokat K, Surette MG, Bassler BL. 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol. Microbiol.* 41:463–76
- Schuster M, Lostroh CP, Ogi T, Greenberg EP. 2003. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J. Bacteriol.* 185:2066–79
- Schweitzer R, Shilo BZ. 1997. A thousand and one roles for the Drosophila EGF receptor. *Trends Genet.* 13:191–96
- Seed PC, Passador L, Iglewski BH. 1995. Activation of the *Pseudomonas aeruginosa lasI* gene by LasR and the *Pseudomonas* autoinducer PAI: an autoinduction regulatory hierarchy. *J. Bacteriol.* 177:654–59
- Seto H, Tomasz A. 1975. Protoplast formation and leakage of intramembrane cell components: induction by the competence activator substance of pneumococci. *J. Bacteriol.* 121:344–53
- Shimkets LJ. 1999. Intercellular signaling during fruiting-body development of *Myxococcus xanthus*. *Annu. Rev. Microbiol.* 53:525–49
- Smith RS, Iglewski BH. 2003. *P. aeruginosa* quorum-sensing systems and virulence. *Curr. Opin. Microbiol.* 6:56–60
- Solomon JM, Lazazzera BA, Grossman AD. 1996. Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in *Bacillus subtilis*. *Genes Dev.* 10:2014–24



- Solomon JM, Magnuson R, Srivastava A, Grossman AD. 1995. Convergent sensing pathways mediate response to two extracellular competence factors in *Bacillus subtilis*. *Genes Dev.* 9:547–58
- Steinmoen H, Teigen A, Havarstein LS. 2003. Competence-induced cells of *Streptococcus pneumoniae* lyse competence-deficient cells of the same strain during cocultivation. *J. Bacteriol.* 185:7176–83
- Stevens AM, Dolan KM, Greenberg EP. 1994. Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the lux promoter region. *Proc. Natl. Acad. Sci. USA* 91:12619–23
- Strassmann JE, Zhu Y, Queller DC. 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* 408:965–67
- Suntharalingam P, Cvitkovitch DG. 2005. Quorum sensing in streptococcal biofilm formation. *Trends Microbiol.* 13:3–6
- Surette MG, Miller MB, Bassler BL. 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Natl. Acad. Sci. USA* 96:1639–44
- Swartzman E, Silverman M, Meighen EA. 1992. The luxR gene product of *Vibrio harveyi* is a transcriptional activator of the lux promoter. *J. Bacteriol.* 174:7490–93
- Taga ME, Miller ST, Bassler BL. 2003. Lsr-mediated transport and processing of AI-2 in *Salmonella typhimurium*. *Mol. Microbiol.* 50:1411–27
- Taga ME, Semmelhack JL, Bassler BL. 2001. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. *Mol. Microbiol.* 42:777–93
- Tenover FC, Gaynes RP. 2000. The epidemiology of *Staphylococcus aureus* infections. In *Gram-Positive Pathogens*, ed. VA Fischetti, RP Novick, JJ Ferretti, DA Portnoy, JJ Rood, pp. 414–21. Washington, DC: ASM Press
- Teplitski M, Robinson JB, Bauer WD. 2000. Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Mol. Plant Microbe Interact.* 13:637–48
- Tomasz A, Hotchkiss RD. 1964. Regulation of the transformability of pneumococcal cultures by macromolecular cell products. *Proc. Natl. Acad. Sci. USA* 51:480–87
- Town C, Stanford E. 1979. An oligosaccharide-containing factor that induces cell differentiation in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA* 76:308–12
- Town CD, Gross JD, Kay RR. 1976. Cell differentiation without morphogenesis in *Dictyostelium discoideum*. *Nature* 262:717–19
- Travisano M, Velicer GJ. 2004. Strategies of microbial cheater control. *Trends Microbiol.* 12:72–78
- Urban S, Schlieper D, Freeman M. 2002. Conservation of intramembrane proteolytic activity and substrate specificity in prokaryotic and eukaryotic rhomboids. *Curr. Biol.* 12:1507–12
- Vannini A, Volpari C, Gargioli C, Muraglia E, Cortese R, et al. 2002. The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. *EMBO J.* 21:4393–401
- Visick KL, Foster J, Doino J, McFall-Ngai M, Ruby EG. 2000. *Vibrio fischeri* lux genes play an important role in colonization and development of the host light organ. *J. Bacteriol.* 182:4578–86
- Wagner VE, Bushnell D, Passador L, Brooks AI, Iglewski BH. 2003. Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. *J. Bacteriol.* 185:2080–95

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This study supports the hypothesis that autoinducer binding is required for protein stability of LuxR-like AHL receptors and suggests a mechanism for how bacteria avoid premature activation of their quorum-sensing cascades.

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- Wagner VE, Gillis RJ, Iglewski BH. 2004. Transcriptome analysis of quorum-sensing regulation and virulence factor expression in *Pseudomonas aeruginosa*. *Vaccine* 22(Suppl. 1):S15–20
- Watson WT, Minogue TD, Val DL, von Bodman SB, Churchill ME. 2002. Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. *Mol. Cell* 9:685–94
- Whiteley M, Lee KM, Greenberg EP. 1999. Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 96:13904–9
- Wu H, Song Z, Hentzer M, Andersen JB, Molin S, et al. 2004. Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. *J. Antimicrob. Chemother.* 53:1054–61
- Xavier KB, Bassler BL. 2003. LuxS quorum sensing: more than just a numbers game. *Curr. Opin. Microbiol.* 6:191–17
- Xavier KB, Bassler BL. 2005. Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *J. Bacteriol.* 187:238–48
- Zhang HB, Wang LH, Zhang LH. 2002a. Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. *Proc. Natl. Acad. Sci. USA* 99:4638–43
- Zhang L, Murphy PJ, Kerr A, Tate ME. 1993. *Agrobacterium* conjugation and gene regulation by N-acyl-L-homoserine lactones. *Nature* 362:446–48
- Zhang RG, Pappas T, Brace JL, Miller PC, Oulmassov T, et al. 2002b. Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. *Nature* 417:971–74**
- Zhu J, Miller MB, Vance RE, Dziejman M, Bassler BL, Mekalanos JJ. 2002. Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*. *Proc. Natl. Acad. Sci. USA* 99:3129–34
- Zhu J, Oger PM, Schrammeijer B, Hooykaas PJ, Farrand SK, Winans SC. 2000. The bases of crown gall tumorigenesis. *J. Bacteriol.* 182:3885–95
- Zhu J, Winans SC. 1999. Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters in vitro and decreases TraR turnover rates in whole cells. *Proc. Natl. Acad. Sci. USA* 96:4832–37
- Zhu J, Winans SC. 2001. The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. *Proc. Natl. Acad. Sci. USA* 98:1507–12



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## ERRATA

An online log of corrections to *Annual Review of Cell and Developmental Biology* chapters may be found at <http://cellbio.annualreviews.org/errata.shtml>