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MEETING REVIEW

Cell-Cell Communication in Bacteria: United We Stand▽

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PERSPECTIVES OF CELL-CELL SIGNALING

The field of cell-cell signaling and coordinated microbial group behavior arose from two independent discoveries reported about 40 years ago. Tomasz stated in 1965 that a hormone-like extracellular product helped regulate competence in *Streptococcus pneumoniae* (94). The signal was later identified as a peptide—indeed, peptides have emerged as common molecular signals among gram-positive bacteria (31, 56). In 1970, Nealson and colleagues (70) reported that luminescence in the marine bacterium *Vibrio fischeri* (formerly *Photobacterium fischeri*) was produced only at high cell density but could be induced at low density by growth in the spent medium of a high-density culture. They referred to this phenomenon as "autoinduction" (69). The signal factor termed "autoinducer" was later identified as an acylated homoserine lactone (acyl-HSL) molecule. This class of signaling molecules predominates within the proteobacteria (97). The term "quorum sensing" (QS) was introduced to specifically refer to the cell-density-linked, coordinated gene expression in populations that experience threshold signal concentrations to induce a synchronized population response (38). Until then, the community was reluctant to accept the concept that bacterial social behaviors known for *Myxococcus* are the rule, rather than the exception, in the microbial world (84–86). The transformative discoveries that bacteria communicate and exist in nature predominantly as sessile biofilm communities have brought the concept of bacterial multicellularity to the forefront of microbiology.

The continued broad interest in microbial communication and its role in natural populations prompted the American Society for Microbiology (ASM) to sponsor its third conference on Cell-Cell Communication in Bacteria from 7 to 10 October 2007 (CCCB-07) in Austin, TX. The scope was broad, with seven theme-based sessions exploring the mechanisms of microbial cell-cell and cell-host communications, novel signal discovery, signal interference and its potential for clinical intervention, and the theoretical and evolutionary aspects of intercellular communication. The conference was attended by 262 U.S. and foreign scientists who provided 47 oral and 146 poster presentations.

In this conference review, we emphasize the most significant recent advances in the field. We devote particular attention to the social and evolutionary considerations of microbial communication with the intent to shape a broader and deeper appreciation for the driving forces of prokaryotic multicellularity beyond the molecular intricacies of individual network components.

SOCIAL EVOLUTION AND CELL-TO-CELL COMMUNICATION

Social behaviors and the dilemma of cooperation. As our understanding of the molecular mechanisms that govern social behaviors of many different microbes increases, the opportunity arises to view these systems from a social evolution perspective. During the Austin meeting, a number of presentations focused on the evolution and maintenance of bacterial cell-to-cell communication systems. The following section will discuss cooperation from an evolutionary perspective with a particular emphasis on bacterial QS.

From an evolutionary perspective, social behaviors are those that have fitness consequences for both the individual that performs the behavior and a recipient (Table 1). Cooperation has generally been studied in animals; however, the same problems exist at all levels of biological organization (103). Cooperation among microbes often takes the form of a shared investment in a group resource (public good), which is costly for an individual to produce, yet provides a benefit to all the individuals in the local group and population (103–105). It is often assumed that cooperative behaviors between microbes are favored because the population benefits as a whole. However, selection for cooperation is generally not at the population level; rather, selection occurs at the level of the gene or genes responsible for the relevant social behavior (81). Any cooperative behavior is at the risk of invasion by selfish individuals (cheaters), who pay little (or none) of the costs of cooperation but gain all the benefits. When public goods are beneficial, the population grows faster when it consists purely of cooperators. However, in a mixed population social cheaters can outcompete cooperators, thereby gaining a fitness benefit within the population. Cooperation can then break down due to social conflict and can even lead to a population collapse or extinction—natural selection does not act with foresight (103).

Explanations for the evolution of cooperation. Cooperation is widespread in the natural world; thus, mechanisms must exist for its maintenance especially because of the potential spread

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TABLE 1. Classification of social behaviors

Effect on actor	Effect on recipient	
	Positive	Negative
Positive	Mutual benefit	Selfishness
Negative	Altruism	Spiteful

of social cheaters. For the individual that performs the behavior, cooperation provides a direct fitness benefit that outweighs the cost of performing the behavior (81). In addition, cooperation provides indirect fitness benefits to other individuals who carry the cooperative gene. The most common reason for two individuals to share genes in common is for them to be genealogical relatives (kin), which is often termed kin selection (41). By helping a close relative reproduce, an individual transmits genes to the next generation, albeit indirectly; this class of cooperation is altruistic. Kin selection can work in two ways. First, an individual can distinguish kin from nonkin and therefore preferentially direct aid toward them. Second, the population can experience limited dispersal. Here relatives are kept close together, favoring indiscriminate altruism toward neighbors. The limited dispersal mechanism does not require complex cognition, so it could be important in a broad range of organisms, especially microbes. Indirect fitness benefits can also be obtained when cooperation is directed to nonrelatives who share the same cooperative gene (26, 41).

The definition of genetic relatedness (*r*) is the relatedness at the locus/loci of the behavior being considered, not the entire genome. At the Austin conference, Greg Velicer, Indiana University, described the importance of relatedness in *Myxococcus xanthus* at the locus of the social trait when he revealed that a mutation in a single gene restores sociality in a population that is under the threat of extinction. *M. xanthus* is a soil-dwelling bacterium that undergoes multicellular development during periods of starvation, which leads to the development of a wide range of fruiting body types. A cheater genotype termed the "obligate cheater" was identified. In isolation, this strain fails to produce any spores; hence, it is dependent on a social host (34). In competition experiments with cooperators, the cheaters dramatically spread in the population, resulting in population extinction. However, during one such experiment, an obligate cheater reevolved the ability to sporulate in the absence of cooperators, but unlike the wild type, it resisted the future invasion by cheater cells (34). Remarkably, this strain, termed Phoenix after the mythical burning bird that can arise from its own ashes, emerged from just a single mutation that increased the levels of an acetyltransferase (34). Thus, changes in a single genetic locus can enable populations to recover from near-extinction. It also suggests a molecular mechanism whereby cheater cells are suppressed or inhibited so that they cannot take over a population (35).

The complexity of QS. QS is generally assumed to coordinate cooperative behaviors in bacteria. Two complementary talks demonstrated that QS in *Pseudomonas aeruginosa* is a social trait that is exploitable by cheaters (29, 53). Both studies used minimal medium containing carbon sources that required the secretion of QS-dependent proteases (public goods) to support growth. Martin Schuster, Oregon State University (M. Schuster, CCCB-07, abstr. S5:3), demonstrated that after 100 generations, a subpopulation of *lasR* mutants, incapable of responding to QS, developed from the wild type (83). In mixed populations, as the relative size of the *lasR* mutant population grew, population fitness declined, demonstrating that the cheater load can have serious consequences for a population. Interestingly, this reduction in fitness did not occur in the long-term evolution experiments, suggesting that over time populations can adapt to the presence of cheaters (83). Steve Diggle, University of Nottingham, United Kingdom (S. Diggle, CCCB-07, abstr. S5:1), pointed out that the real cost of QS is in the response to the signal, not signal production itself.

Given the cost of QS and the opportunities for cheaters to spread, how can QS be maintained? Brown and Johnstone first proposed a theoretical kin selection model as a mechanism of maintaining QS (14). In support of the Brown and Johnstone model, Jan Kreft, University of Birmingham, United Kingdom (J. U. Kreft et al., CCCB-07, abstr. S6:1), used mathematical modeling to show that clustering of cells has a strong impact on the autoinducer concentration that a cell may sense. Cells within the same cluster can efficiently perceive signals from adjacent cells within that cluster. Importantly, cells within clusters tend to be clonal and so communication among these cells tends to be with kin. This high relatedness within clusters suppresses cheating and may also be a mechanism to prevent confusion from cross talk with neighboring bacterial species. Steve Diggle (S. Diggle, CCCB-07, abstr. S5:1) provided empirical data to show that QS may be maintained by kin selection. In both high-relatedness and low-relatedness treatments, high relatedness favored QS. Perhaps more importantly, QS was not favored in conditions of low relatedness when the wild type and QS *lasR* mutants were mixed (28). It is interesting that many *P. aeruginosa* clinical isolates sampled from the cystic fibrosis (CF) lung are signal-blind *lasR* mutants (82, 90). Cheating provides one explanation for this, and the CF lung may be an environment that is particularly susceptible to this type of behavior. There are, however, alternative explanations. It could be that QS is simply not important for survival in the lung and, as a consequence, is naturally lost. A third possibility is that a mutation in *lasR* confers a growth advantage over a QS-positive strain in the CF environment (25).

A few years ago, the notion of diffusion sensing (DS) was introduced. It was posited that autoinducer functions chiefly to enable individual cells to sense how rapidly secreted molecules diffuse away. Therefore, DS could allow individuals to minimize the loss of costly public goods by extracellular diffusion (78). However, it has now been empirically demonstrated that QS does have social fitness consequences, providing a benefit at the group level that can be exploited by individuals that do not produce signal (28, 83). Burkhard Hense, GSF National Research Centre, Munich, Germany (B. A. Hense et al., CCCB-07, abstr. S6:5), introduced a new concept termed efficiency sensing (ES) (44). ES assumes that low-cost autoinducers are released to test the efficiency of producing costlier exoenzymes, a concept that is similar to DS. However, ES includes the potential for cooperation because microcolonies (clusters) may help protect against interference by other species and cheaters. In this way ES unifies both QS and DS, as it enables cells to sense cell density, diffusion limitation, and cell distribution (clustering) (Fig. 1) (44).

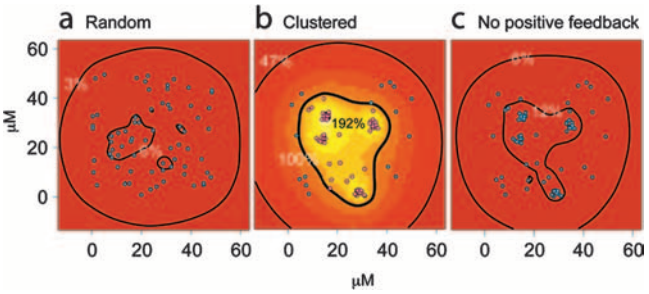

FIG. 1. Effect of spatial clustering on QS signaling. A mathematical model for autoinducer systems with or without positive feedback was developed. The model was used to investigate the effect of the spatial arrangement of autoinducer-producing cells on the accumulation of local autoinducer. Comparing a random (a) with a clustered (b) arrangement of the same number of cells, the threshold concentration for induction is reached only within and near the clusters. The figure shows bacterial cells that are not induced in cyan and those that are induced in purple. Comparing the same clustered pattern with (b) and without (c) positive feedback demonstrates that this characteristic of autoinducer production is critical for reaching sufficient autoinducer concentrations for cells to induce autoinducer production and autoinducer-dependent genes. The autoinducer concentration, as a percentage of the threshold concentration, is indicated by contour lines and background color, for which a linear color map from red (<16%) to white (>200%) was used. In panel b, the thick contour line separates the noninduced cells from the induced cells. The three-dimensional domain is viewed from the top, onto an impermeable surface at the bottom. As the domain is otherwise infinite, the autoinducer can diffuse away.

Clearly consideration of the evolution of microbial social behaviors is a fascinating endeavor that can stimulate everyone in the field to evaluate the utility and maintenance of intercellular communication systems in nature. A challenge for the future will be to combine both mechanistic and evolutionary approaches to further understand microorganisms in their natural habitats.

CELL-CELL SIGNALING IN HOST-MICROBE INTERACTIONS

The paradigm. The marine bacterium *Vibrio fischeri* cycles between a free-living existence and a mutualistic association with its host, the Hawaiian bobtail squid, *Euprymna scolopes*, to which it contributes *lux*-dependent luminescence (97, 98). Luminescence is governed by the *luxI*/*luxR* QS system, which represents the mechanistic paradigm for most acyl-HSL-dependent QS regulatory systems within gram-negative bacteria (33, 61, 65, 109). In these systems, the canonical "I" gene encodes the acyl-HSL synthase, while the "R" gene encodes the regulatory protein, usually an activator. In *V. fischeri*, the *luxI*/*luxR* system is at the bottom of a complex hierarchical regulatory circuit governed synergistically by the AinS *N*-octanoyl HSL (C_8-HSL) and the LuxS autoinducer 2 (AI-2) signal synthases and their cognate sensory histidine kinases LuxN and LuxP/Q, respectively (Fig. 2). LuxN and LuxQ undergo autophosphorylation in the absence of signal input, which leads to phosphorylation of the LuxO response regulator. Phosphorylated LuxO indirectly represses LitR, the central regulator of symbiosis and luminescence, via small RNA (sRNA) negative regulatory elements. Signal input transforms LuxN and LuxQ

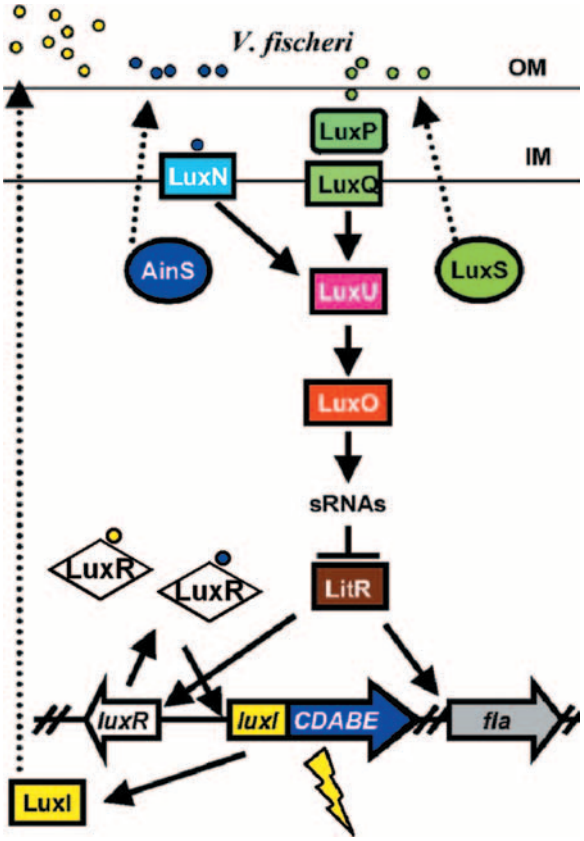

FIG. 2. The *V. fischeri lux* paradigm regulatory cascade. *V. fischeri* produces three QS signals: the LuxI-produced 3-oxo-hexanoyl-HSL (yellow circles), AinS-produced C_8-HSL (blue circles), and a LuxS-produced signal, presumably a furanosyl borate diester (AI-2; green circles). LuxR acts as a receptor for both C_8-HSL and 3-*O*-C_6-HSL (61). C_8-HSL is also a signal for the membrane-bound LuxN sensor kinase, while AI-2 interacts with the LuxP periplasmic binding protein and the LuxQ sensor kinase. At low cell density (low signal concentrations), LuxN and LuxQ autophosphorylate and transfer the phosphate to LuxU, which in turn phosphorylates the LuxO transcriptional activator. Phosphorylated LuxO is predicted to activate the transcription of sRNAs, which inhibit the production of LitR protein. Increasing population density and signal concentrations switch the kinases to phosphatases, leading to dephosphorylated LuxO and production of LitR. LitR activates *luxR* and flagellar genes. In turn, LuxR and inducing levels of the 3-*O*-C_6-HSL stimulate light production. (Note that aspects of this model remain to be tested in *V. fischeri* and are based on the experimentally defined *V. harveyi* model [102].) OM, outer membrane; IM, inner membrane. (Reprinted from reference 96 with permission.)

into phosphatases, leaving LuxO in a largely unphosphorylated, inactive state. Under these conditions, LitR becomes available for the activation of *luxR* and other symbiosis-related functions (Fig. 2). The AinS-specific C_8-HSL input is particularly important for the early establishment and persistence of symbiosis and luminescence in culture. In contrast, luminescence at very high cell densities in the host light organ requires the *luxI*-specific signal system (for an excellent summary of the system see the review by K. L. Visick [96]). Interestingly, Sarah Studer from the Ruby lab, University of Wisconsin (S. Studer and E. Ruby, CCCB-07, abstr. 123B), reported that AinS and LitR appear to control acetate metabolism, which is critical for

bacterial survival within the light organ. Eric Stabb, University of Georgia (A. N. Septer et al., CCCB-07, abstr. 19B), provided evidence that the *V. fischeri* ArcA response regulator of the redox-monitoring ArcAB two-component system represses luminescence under reducing conditions by binding to a site upstream of and proximal to the *luxI* promoter, thereby blocking LuxR activation of bioluminescence. The bacteria encounter oxidative conditions during early colonization of the *Euprymna scolopes* light organ, and the authors hypothesize that this leads to ArcA derepression of the *lux* operon, accounting for the significant induction of luminescence that is seen only during symbiotic infection. These findings are congruent with the hypothesis of Visick and colleagues that bioluminescence benefits the symbiotic bacteria by decreasing ambient oxygen levels to prevent antimicrobial reactive oxygen species production by the host (11, 98). Moreover, the study showed that the autoinducer, produced by LuxI, is also regulated by ArcA and that *arcA* mutants induce luminescence in neighboring wild-type cells. Thus, redox stresses detected by ArcAB in a subpopulation may elicit a population-wide response. This observation has led to the proposal that *V. fischeri* autoinducer is a "redox-responsive alarm signal" with a broader role in *V. fischeri* than its established function as a census-taking molecule.

Computational models presented by Andrew Goryachev, University of Edinburgh, United Kingdom (A. B. Goryachev, CCCB-07, abstr. S6:3), predict that LuxR self-amplification and the presence of sRNA regulatory intermediates contribute significantly to network fitness. In this connection, Josh Williams of the Stevens lab, Virginia Tech (J. W. Williams et al., CCCB-07, abstr. S6:4), showed that LuxR self-amplification buffers against intrinsic acyl-HSL signal variation. This ensures that the switch to inducing conditions is not easily reversed, thereby endowing the system with hysteresis.

The bilingual nature of *Vibrio cholerae*. *V. cholerae* cell-cell communication was the focus of the conference keynote presentation by Bonnie Bassler of the Howard Hughes Medical Institute and Princeton University (B. L. Bassler, CCCB-07, S1:0 Keynote; D. A. Higgins et al., CCCB-07, abstr. 55B). *V. cholerae* exists principally in aquatic biofilms associated with plankton and suspended particulate matter (43). Consumption of contaminated waters provides access to an alternate lifestyle within the intestine of a mammalian host. QS-mediated communication plays a decisive role in the switch between the free-living and host-associated virulent existence. *V. cholerae* senses cell density via the *V. cholerae* autoinducer *1* (CAI-1) and AI-2 autoinducer signals, produced by the CsqA and LuxS autoinducer synthases, respectively (Fig. 3). This organism lacks the classic acyl-HSL signal characteristic of its two marine relatives, *V. fischeri* and *Vibrio harveyi*. The CAI-1 signal system appears to be common within the *Vibrio* genus (46). Bassler revealed the novel chemical structure of CAI-1 as (*S*)-3-hydroxytridecan-4-one (Fig. 3) (46). This molecule represents a unique class of autoinducers, and structure/function studies suggest that the C_{13}-carbon side chain length and the *S* enantiomeric configuration are functionally significant. Interestingly, compounds related to CAI-1 have pheromone-like functions in certain insects (46).

The CAI-1 and AI-2 signals are transduced by the sensor kinases CqsS and LuxP/LuxQ complex, respectively (Fig. 3). CqsS and LuxQ act as kinases in the unliganded state at low

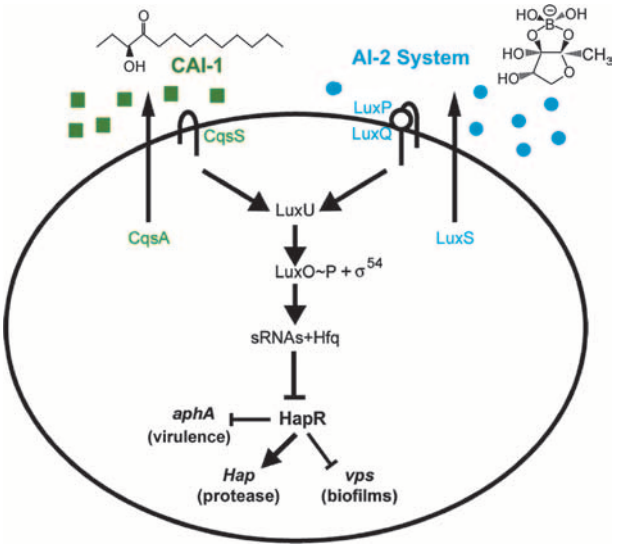

FIG. 3. The *Vibrio cholerae* autoinducer signaling network. The signal synthases CqsA and LuxS produce autoinducers CAI-1 [(*S*)-3-hydroxytridecan-4-one] and AI-2 [(2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate], respectively. Signal inputs are transduced via LuxO to control levels of HapR. At low cell density, in the absence of autoinducers, *hapR* expression is repressed, thereby permitting the expression of virulence factors and biofilm formation. At high cell density and in the presence of autoinducers, LuxO is inactive, permitting HapR production. HapR represses virulence and biofilm formation while activating *hap* protease expression. (Reprinted from reference 42 with permission of the publisher.)

cell density. Both signal inputs are channeled to the common LuxO response regulator via LuxU. LuxO phosphorylation results in the activation of genes encoding the regulatory RNAs Qrr1 to Qrr4. The Qrr RNAs interact with Hfq, the RNA chaperone, and occlude the ribosome binding site of the mRNA encoding the master regulatory factor HapR (a LitR homolog). Low-cell-density conditions are generally experienced by free-living *V. cholerae* in the natural aquatic habitat, where the repression of *hapR* promotes biofilm growth and the expression of virulence factors including the toxin-coregulated pilus. These conditions are thought to prepare free-living bacterial populations for colonization of the mammalian host. Once *V. cholerae* populations in the host gut reach a high density, signal accumulates, and LuxO is dephosphorylated. HapR now represses genes needed for initial host-associated colonization and activates the production of the hemagglutinin protease. This protease promotes bacterial detachment from the intestinal epithelium, thereby stimulating bacterial dissemination so that new infection foci in the intestine can be established or the pathogen can escape into the environment, where it may find a new host (113). Jun Zhu, University of Pennsylvania (Z. Liu et al., CCCB-07, abstr. S4:6), showed that *hapR* repression by σ^{28} plays a critical role in the infection cycle. Specifically, polar flagellum-mediated motility enables bacteria to seek and penetrate the mucosal layer to access the epithelial cells and initiate pathogenesis. Intriguingly, flagella tend to break as the bacteria encounter the viscous intestinal mucosal matrix. This allows the secretion of the FlgM anti-σ^{28} factor, releasing σ^{28} to repress *hapR* expression, thereby priming *V. cholerae* cells for intestinal colonization.

Bonnie Bassler also presented data showing that the Qrr1 to Qrr4 sRNAs regulate the expression of virulence functions in a HapR-independent manner. The rapid synthesis of sRNAs without the need for translation and their inherent lability offer a highly responsive regulatory strategy for the rapid transition between two states. In addition the redundancy of sRNAs exhibited by *V. cholerae* may contribute to the fine-tuning of this regulatory switch. The HapR-independent, sRNA-mediated control of gene expression provides a logical explanation for why classical strains of *V. cholerae* have frameshift mutations in their respective *hapR* genes yet remain toxigenic (43). On the practical side, the suppression of virulence by CAI-1 QS offers a unique opportunity for developing pharmaceuticals based on CAI-1 signal chemistries that may be effective in preventing widespread cholera outbreaks.

The *Legionella* parallel. The gram-negative bacterium *Legionella pneumophila*, which causes a severe pneumonia known as Legionnaires' disease, also alternates between a free-living virulent and a host-associated replicative state. Hosts include protists and alveolar macrophages (68), where the bacteria are contained within a specialized endoplasmic reticulum-derived vacuole. In the replicative phase, the expression of virulence factors and motility are down-regulated, but as cells enter stationary phase, they switch to the transmissive mode in which virulence and motility functions are up-regulated. Thomas Spirig from the Hilbi lab, ETH Zürich, Switzerland (T. Spirig, CCCB-07, abstr. S1:4), reported that *L. pneumophila* harbors a gene system (*lqs*) similar to the *V. cholerae cqsAS* CAI-1 QS system. The functionality of *lqsA* was verified using a CAI-1 reporter strain and by genetic complementation of a *V. cholerae cqsA*-null mutant. *lqsA* encodes a homolog of the BioF 8-amino-7-oxononanoate synthase that catalyzes the condensation of L-alanine and 6-carboxyhexanoyl coenzyme A. This enzyme has a high degree of homology to CqsA. The *lqsR* gene, which is not present in *V. cholerae*, appears to play a key role in governing the transition between the replicative and transmissive (virulent) phase (93). Interestingly, similar gene systems exist in a number of other bacteria including *Burkholderia xenovorans*, an organism that has adapted to complex and diverse niches.

The "ins and outs" of *Yersinia pseudotuberculosis*. *Y. pseudotuberculosis* is a mammalian enteropathogen that causes gastroenteritis. The organism alternates between a free-living aqueous or food-borne and host-associated existence. As described by Hannah Patrick of the Williams group, University of Nottingham, United Kingdom (H. L. Patrick et al., CCCB-07, abstr. S2:3), *Y. pseudotuberculosis* harbors two *luxR*/*I*-type systems, *ypsR*/*I* and *ytbR*/*I*, which together produce a suite of short- and long-chained acyl-HSLs. Detailed genetic studies show that YpsR/I positively activates YtbR/I. These QS systems govern the differential expression of genes related to *Y. pseudotuberculosis* aggregation, motility, and virulence (3). It will be interesting to explore to what degree these regulatory systems contribute to the biphasic life-style of this organism.

An orphan with a mission. The QscR regulator in *P. aeruginosa* is characterized as an "orphan" because it lacks a cognate acyl-HSL synthase gene (22). Pete Greenberg, University of Washington, reported that QscR is critical to the pathogenesis of *P. aeruginosa* and that a *qscR* mutant is hypervirulent due to enhanced expression of RhlR/LasR-controlled phenazine, hydrogen cyanide, and elastase virulence functions. To distinguish between a number of possible QscR regulatory mechanisms, Lequette and colleagues (58) conducted a transcriptome study and showed that the QscR regulon partially overlaps with the LasR and RhlR regulons but also regulates genes not governed by either LasR or RhlR. QscR directly activates genes in a 3-oxo-dodecanoyl-HSL (3-*O*-C_{12}-HSL)-dependent manner, although the protein receptor is more responsive to decanoyl-HSL (C_{10}-HSL) (58), suggesting a possible role for QscR in sensing coexisting, competing neighbors such as *Burkholderia vietnamensis*, a C_{10}-HSL producer. This rationale led to the prediction that QscR might have less-stringent ligand binding characteristics than the highly stable, nearly irreversible ligand binding properties of LasR. Indeed, in *Escherichia coli* reporter assays probing the diffusibility of ligand bound to QscR and LasR, sufficient signal diffused only from QscR. Most recently, Greenberg's group working together with Helen Blackwell's group at the University of Wisconsin identified small molecule inhibitors of LasR that function as agonists for QscR (63). Such compounds could mitigate the antagonistic effects of LasR and QscR on virulence.

"Elaborate Lives," an aria about AidA. *Caenorhabditis elegans* is a useful nonmammalian infection model. Leo Eberl, University of Zürich, Switzerland (J. Wopperer et al., CCCB-07, abstr. S6:2), described the utility of this system to study the role of AidA, a protein required for nematode colonization by strains of the *Burkholderia cepacia* complex. All strains expressing the AiiA acyl-HSL lactonase inhibited the expression of AidA, demonstrating that this protein is QS regulated. AidA is not a virulence factor per se but is required for *B. cepacia* complex strains to persist in *C. elegans*. Furthermore, active QS is essential for nematode pathogenicity by all these strains, leading to the prediction that QS is likely to control other nematocidal determinants (15).

Signaling in microbial-plant interactions. Max Dow, National University of Ireland, Ireland (M. Dow, CCCB-07, abstr. S2-8), described the role of an unusual signaling molecule, *cis*-11-methyl-dodecenoic acid, known as DSF (*d*iffusible *s*ignaling *f*actor), in the plant-pathogenic bacterium *Xanthomonas campestris* pathovar campestris. Biosynthesis of DSF is dependent on the putative enoyl coenzyme A hydratase RpfF (30). DSF signal perception and transduction involve the RpfC hybrid sensor kinase/response regulator and the atypical RpfG response regulator. RpfG features an HD-GYP cyclic-di-GMP phosphodiesterase domain (80) and interacts with a subset of GGDEF domain proteins (2). Thus, DSF exerts its effect through an unusual signal transduction system that blends elements of a two-component system with that of a cyclic-di-GMP-specific second messenger pathway. *X. campestris* encodes 37 proteins with GGDEF, EAL, or HD-GYP domains, some of which are dedicated to the expression of virulence factors while others have a role in motility. One might envisage that these proteins integrate the cell-cell signal and environmental inputs to optimize bacterial development and virulence. In a separate study, Dow and colleagues (R. P. Ryan et al., CCCB-07, abstr. 9B) studied the interaction between *Stenotrophomonas maltophilia* and *P. aeruginosa*. These bacteria frequently share a common niche in the rhizosphere, as well as in the CF lung. *S. maltophilia* also produces DSF and carries *rpf* homolog genes. In coculture, *S. maltophilia* has substantial

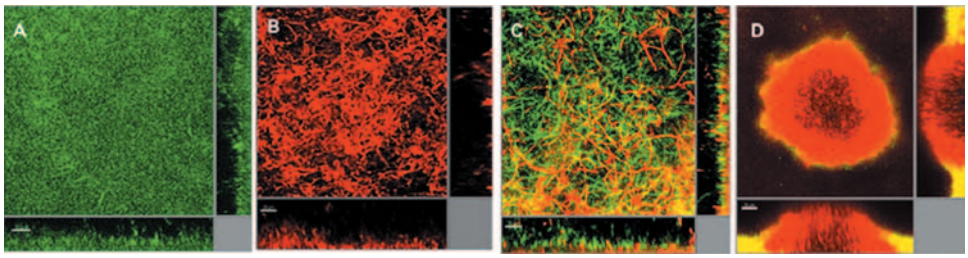

FIG. 4. DSF from *Stenotrophomonas maltophilia* influences biofilm architecture of *Pseudomonas aeruginosa* PAO1, which does not produce the signal. Images are of 4-day-old biofilms in flow cells. (A) *P. aeruginosa* PAO1; (B) *S. maltophilia* K279a; (C) mixed culture of *P. aeruginosa* PAO1 and *S. maltophilia* K279a; (D) mixed culture of *P. aeruginosa* PAO1 and *S. maltophilia* K279a*rpfF* (DSF negative). Bars, 20 μm. (Courtesy of Max Dow, National University of Ireland, Cork; reproduced with permission).

effects on *P. aeruginosa* growth and biofilm formation (Fig. 4). When grown alone, *S. maltophilia* forms biofilms with a filamentous architecture, whereas *P. aeruginosa* forms flat biofilms. In coculture, however, *P. aeruginosa* develops structures with a filamentous architecture within a mixed biofilm. These effects depend on DSF production by *S. maltophilia* and are mediated by a gene designated PA1396 that encodes a receptor protein with a sensory domain related to RpfC. Similar signal systems are present in other plant-associated bacteria, indicating that extensive DSF-specific interspecies communication may occur in nature (36, 79).

The *Pantoea stewartii* paradigm. *Pantoea stewartii* subsp. *stewartii* is a plant pathogen that causes vascular wilt in maize. The bacterium colonizes the xylem as cell-wall-adherent, stewartan exopolysaccharide (EPS)-encased biofilms (55, 99). In this system, the unliganded apo form of EsaR, the LuxR homolog QS regulator of *P. stewartii*, binds DNA and represses *rcsA*. RcsA is a transcription factor of the Rcs phosphorelay system that together with RcsB activates EPS biosynthesis in *P. stewartii* (16, 66). Susanne von Bodman, University of Connecticut (S. B. von Bodman et al., CCCB-07, abstr. S4:5), described two previously unidentified genetic loci essential for stewartan EPS synthesis that are coordinately activated by RcsA/RcsB under acyl-HSL-inducing conditions. Interestingly, an EPS hydrolase is located within the primary EPS biosynthetic cluster. The function of the hydrolase appears to be a stewartan-lipopolysaccharide chain length determinant that protects *P. stewartii* from stewartan-lipopolysaccharide-specific phage infections (C. Roper et al., CCCB-07, abstr. 81B). EsaR is not the only QS regulator that functions as a DNA binding protein in the absence of acyl-HSL ligand; others predicted to operate in this way include various ExpR regulators in *Erwinia* plant pathogens (5, 17, 88) and *Serratia* species (89) plus a new virulence-specific regulator termed VirR in *Erwinia carotovora* subsp. *atroseptica* described by George Salmond, Cambridge University, United Kingdom (R. E. Monson et al., CCCB-07, abstr. 76A).

Steve Winans, Cornell University (C. Tsai and S. C. Winans, CCCB-07, abstr. 143B), showed that the YenI/YenR QS system of *Yersinia enterocolitica* is functionally related to the EsaI/EsaR system of *P. stewartii.* Like EsaR, YenR is DNA binding competent in the ligand-free state, while inducing acyl-HSL concentrations ablate the active apo form and promote protein/DNA dissociation. YenR and EsaR do not activate their cognate "I" signal biosynthetic genes, and the *esaI* gene in *P. stewartii* is constitutively expressed (7). YenR weakly activates its structural gene, while EsaR represses its own expression (67). Intriguingly, regions upstream of both *yenR* and *esaR* each feature an apparent sRNA coding region, termed YenS and EsaS, respectively, which are activated by their cognate regulators (S. B. von Bodman et al., CCCB-07, abstr. S4:5; C. Tsai and S. C. Winans, CCCB-07, abstr. 143B; D. J. Schu et al., CCCB-07, abstr. 13B). Sequence homology suggests that one of the YenS regulatory targets is *yenI*, although a similar homology between EsaS and *esaI* is not apparent. The characterization of a protein product regulated by EsaS is in progress.

What might be the broader functional role of EsaR/YenR-type QS regulators that act as transcription factors in the ligand-free apo form? In *P. stewartii*, QS regulation prevents the premature production of EPS in populations below threshold density. This is important because even small amounts of the EPS interfere with *P. stewartii* cellular adhesion, host colonization, and virulence (55). It follows that a key role of the EsaI/EsaR QS regulatory system is to repress EPS synthesis at low cell density rather than activate genes at high cell density. Moreover, published data (100) and work presented at the conference (S. B. von Bodman et al., CCCB-07, abstr. S4:5; C. Tsai and S. C. Winans, CCCB-07, abstr. 143B; D. J. Schu et al., CCCB-07, abstr. 13B) show that ApoEsaR and ApoYenR also act as transcriptional activators. These findings point to a parallel role for these regulators as activators of specific low-cell-density genes (sRNAs and other putative regulatory functions) and repressors of dedicated high-cell-density functions (EPS synthesis in *P. stewartii*). Although ligand-bound TraR in *Agrobacterium tumefaciens* and LuxR in *V. fischeri* can repress gene systems when their binding sites are artificially overlapped with the RNA polymerase (RNAP) promoter sites (32, 60), their absolute requirement for acyl-HSL to become DNA binding competent suggests that these regulators are programmed to control genes at high cell density.

INTERKINGDOM SIGNALING—A TWO-WAY CONVERSATION

It has become clear that if organisms live in proximity they are likely communicating. Prokaryotic extracellular communication is not confined to conversations between microbes. Some of the most intimate microbial/host relationships exist in the intestine of animals and other organisms including insects and nematodes. These environments are likely shaped by the

dynamic coevolution between microbes and their hosts. The mechanism by which hosts distinguish between "autochthonous" commensals and "allochthonous" intruders is slowly beginning to reveal itself, in part because of the structural and functional parallels of QS signals and hormones. Even more intriguingly, increasing evidence suggests that gut microbial flora can modulate host physiology and immunity (48).

Human intestinal microflora produces a range of QS signals including the aromatic aminated signal AI-3 (91). The structure of AI-3 is not fully characterized, but it derives from tyrosine and possibly other aromatic amino acids and is structurally similar to epinephrine and norepinephrine, which can substitute for AI-3. Indeed, adrenergic antagonists inhibit AI-3-induced gene expression. This cross-functionality is highly suggestive of interkingdom communication (101). Epinephrine and norepinephrine are important in maintaining intestinal homeostasis, suggesting a similar role for AI-3 produced by commensal intestinal microbiota (50).

On the other hand, pathogenic bacteria such as enterohemorrhagic *E. coli* can sense both host adrenergic hormones and AI-3, as these ligands bind directly to the QseC sensor kinase of the QseC/QseB two-component signal transduction system. Although not homologous to adrenergic receptors, QseC is functionally equivalent. Upon AI-3 binding, a complex regulatory cascade ensues, which ultimately promotes enterohemorrhagic *E. coli* growth, motility, cellular adherence, and virulence factor expression. A poster presented by Bradley Bearson, USDA ARS National Animal Disease Center (B. L. Bearson and S. M. Bearson, CCCB-07, abstr. 20A), described the related AI-3/norepinephrine QseBC system in *Salmonella enterica* serovar Typhimurium, in which a *qseC* mutant exhibited diminished motility and reduced colonization of the gastrointestinal tract. Moreover, the α-adrenergic antagonist phentolamine counteracted the norepinepherine-stimulatory effect on *Salmonella* motility (6). Jason Johnston, University of Iowa (J. W. Johnston et al., CCCB-07, abstr. S4:8), described an AI-3-responsive *qseBC* QS system in nontypeable *Haemophilus influenzae* isolates that plays a role in the disease biology of this organism.

Kendra Rumbaugh, Texas Tech University Health Sciences Center (A. K. Bryan et al., CCCB-07, abstr. S4:3), discussed the capacity of the mammalian peroxisome proliferator-activated receptor (PPAR) family of nuclear hormone receptors to function as receptors of the proinflammatory effects of 3-*O*-C_{12}-HSL on mammalian cells. 3-*O*-C_{12}-HSL was an agonist for PPARβ/δ transcriptional activity but antagonized both the transcriptional and DNA binding activities of PPARγ. The proinflammatory effects of 3-*O*-C_{12}-HSL were prevented by rosiglitazone, a well-defined agonist for PPARγ, suggesting that both agonist and 3-*O*-C_{12}-HSL may compete for binding to the ligand binding domain of PPARγ. These results are particularly intriguing because of the potential efficacy of PPARγ agonists to reduce inflammation in *P. aeruginosa* pathologies including CF (52) and because PPARs are the first potential mammalian autoinducer receptors to be described (also see the accompanying article in this issue of the *Journal of Bacteriology*).

Sara Jones from the Versalovic laboratory, Baylor College of Medicine (S. E. Jones and J. Versalovic, CCCB-07, abstr. S4:7), provided new information on the potential immunomodulating and antimicrobial activities of *Lactobacillus reuteri*, a member of the probiotic and commensal microbiota in the mammalian oral cavity and gastrointestinal tract. These bacteria generally inhabit mixed-species biofilms where normal growth and development are governed by *luxS*-dependent signaling between the bacteria and the mammalian host (92). *L. reuteri* secretion of the broad-spectrum reuterin antibiotic and an undefined immunomodulin capable of inhibiting tumor necrosis factor alpha production is also *luxS* dependent. However, exposure of monocytoid cells to spent culture supernatants of the wild-type and *luxS* mutant strains showed no difference in activity. This observation suggests that the probiotic antimicrobial and immunomodulatory effects are not related to LuxS per se but require biofilm development for activity.

Candida albicans is a fungal pathogen that is part of the normal human microflora but can cause systemic candidiasis, particularly in immunocompromised individuals. It is often found in mixed infections with *P. aeruginosa. C. albicans* virulence and invasiveness require the transition from a budding yeast to a hyphal morphology, which occurs within the host environment. Carla Cugini from the Hogan group, Dartmouth Medical School (C. Cugini et al., CCCB-07, abstr. S7:3), described how farnesol produced by *C. albicans* interferes with the quinolone signal-specific QS system in *P. aeruginosa*. The *Pseudomonas* quinolone signal (PQS) binds to the LysR-type PqsR (MvfR) regulator to activate the expression of various virulence factors (27). However, in the presence of farnesol, PqsR interaction with the *pqsA* promoter is nonproductive. Related long-chain isoprenoid compounds, including farnesyl acetate and geranyllinalool, also have an inhibitory effect on PQS production. Treatment with farnesol does not inhibit the growth of *P. aeruginosa*, which places farnesol in the class of QS-inhibitory (QSI) molecules with potential efficacy in controlling *Pseudomonas* infections by specifically blocking virulence factor production (24).

QUORUM QUENCHING—POSSIBILITIES OF BROAD-SPECTRUM THERAPY "JAMMING THE COMMUNICATION PIPELINE"

One of the primary public health concerns is the emergence of antibiotic-resistant bacteria. A breakout session led by Michael Givskov, The Technical University of Denmark, Denmark, and Marvin Whiteley, University of Texas, Austin, focused on the applicability of QS as a target for drug discovery. This is based on the hypothesis that disruption of QS in certain pathogens could diminish signaling-dependent virulence. Most participants agreed that chemical attenuation of bacterial virulence, rather than bactericidal or static strategies, is a highly attractive concept because such antipathogenic agents are unlikely to pose harsh selective pressures for development of resistance mutants. The overall strategy is to control virulence with small chemical molecules that will function as narrow-range signal antagonists, which may enable the immune system to eradicate the biofilm.

The most prominent biofilm-dependent disease caused by *P. aeruginosa* is CF because the pathophysiology of the CF lung creates an environment conducive to bacterial colonization (8). Mike Givskov (M. C. Givskov, CCCB-07, abstr. S2:4 and

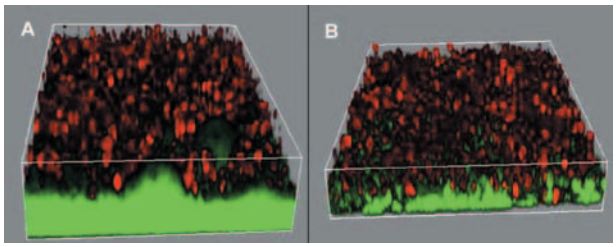

FIG. 5. QS-dependent tolerance of *P. aeruginosa* biofilms toward PMNs. Three-dimensional projection of biofilms (bacteria; green fluorescent) with topical application of PMNs (red fluorescent) on the wild-type strain (A) and the Δ*lasR*/*rhlR* mutant (B). (Reprinted from reference 10 with permission of the publisher.)

118A) provided exciting new insights into the mechanism by which *P. aeruginosa* biofilms impair the host innate immune response. Specifically, he showed that a *lasR rhlR* double mutant strain elicited a pronounced influx of polymorphonuclear leukocytes (PMNs) into the infected tissue compared to the wild-type strain. As a result, the mutant was less virulent and cleared earlier in animal infections (110). An in vitro study applying freshly isolated PMNs on top of biofilms formed by a QS-proficient *P. aeruginosa* strain arrested PMN activity, while biofilms of the QS-deficient strain were readily phagocytosed and penetrated by the PMNs (Fig. 5) (10). Additionally, only the wild-type strain was able to block the oxidative burst associated with the PMN antibacterial response. The extracellular cytotoxic bacterial factor responsible for PMN necrosis was identified as a component of *P. aeruginosa* rhamnolipid B, which is absent in the Δ*lasR*/*rhlR* mutant.

The in vitro PMN necrosis assay is extremely useful to screen for potential QSI compounds (9). For example, prophylactic infiltration of a mouse model lung with garlic extract showed improved clearing and remarkable reduction in mortality. Another compound, furanone C-30, showed little effect on biofilm growth, but treated biofilms were significantly less tolerant to antibiotic treatment (45). Treatment of *Vibrio anguillarum* with furanone C-30 significantly reduced the mortality of rainbow trout (76). Together, these studies show that interference with fundamental virulence processes and/or the ability of bacteria to modulate the host immune system is a promising strategy to manage chronic bacterial infections. Promoting a cautious approach, Givskov emphasized that QSI drugs must be rigorously tested for potential side effects and carcinogenic properties. Because the antibiotic tobramycin has been proven to significantly increase the life expectancy of people with CF, it was suggested that a combination of antibiotics and QSI might be the most effective treatment regimen. Indeed, this approach has shown considerable promise in biofilm flow cell studies (77).

Laurence Rahme, Harvard Medical School (B. Lesic et al., CCCB-07, abstr. S1:2), addressed the QSI theme from the perspective of the *P. aeruginosa* 4-hydroxy-2-alkylquinoline (HAQ) class of signal molecule. The MvfR (also termed PqsR) response regulator responds to two ligands, 4-hydroxy-2-heptylquinoline (HHQ) and 3,4-dihydroxy-2-heptylquinoline (PQS). MvfR regulates a number of virulence factors including the production of pyocyanin, hydrogen cyanide, elastase, and lectins (27). Importantly, a mutation in *mvfR* greatly reduced pathogenicity without compromising bacterial viability in in vivo infection models. Dissection of the biochemical pathway of HAQ synthesis from anthranilic acid and β-keto-fatty acid suggested that halogenated analogs of anthranilic acid inhibit the PqsA-catalyzed step in the HAQ biosynthetic pathway (for details of the putative reaction mechanism see references 39 and 59). A number of synthetic analogs were tested; 2-amino-4-fluorobenzoic acid, 2-amino-6-chlorobenzoic acid, and 2-amino-4-chlorobenzoic acid reduced the synthesis of 4-hydroxy-2-heptylquinoline, PQS, and 4-hydroxy-2-heptylquinoline *N*-oxide and the virulence factors pyocyanin, hydrogen cyanide, chitinase, lectin, and elastase. Interestingly, the analogs also down-regulated QS-independent processes, most notably bacterial osmoprotection. This sensitivity was also apparent in strains that lacked functional copies of *pqsA*, *lasR*, and *rhlR*, suggesting that the impact on osmoprotection is independent of the QS systems. Thermal-injury mouse model studies showed that *P. aeruginosa* infections are significantly reduced by the anthranilic acid analogs, as was the ability of strain PA14 to cause systemic infections. Analog treatment did not affect growth but specifically inhibited the expression of virulence factors, thus satisfying the most critical criterion of a potential QSI treatment strategy.

Staphylococcus spp. produce a variety of extracellular protein virulence factors that enable the organism to attach to host tissue and resist host defenses. These factors are coordinately controlled via the two-component Agr QS system and the cyclic thioester autoinducing peptide (AIP) signals, which are all encoded by the *agrBDCA* gene system (62, 64). AgrC is a peptide signal receptor histidine kinase, and AgrA is the cytoplasmic response regulator. AIP derives from the propeptide encoded by *agrD*. This peptide is processed and secreted by the membrane-spanning AgrB protein, which cleaves segments of the N and C termini to release the active signal. These peptides are highly conserved and between 7 and 10 amino acids long, and most contain a cysteine that interacts with C-terminal carboxyl groups to form the characteristic thiolactone ring. AIPs from different staphylococcal species fall into distinct structural classes. These peptides have cross-interference activity, in that most AIPs inhibit *agr* gene activation in other gram-positive groups. Richard Novick, New York University School of Medicine (R. P. Novick et al., CCCB-07, abstr. S2:6), has taken advantage of this cross talk to define AIP/AgrC interaction specificities. This approach has provided key insights into peptide receptor binding specificity but has also revealed an unexpected level of complexity. AIP signaling is a promising target for therapeutic intervention. The Agr signaling pathway is widespread among *Firmicutes* including *Clostridium* species and bacteria with probiotic activity as described in a poster from the Winzer group, Lancaster University, Lancaster, United Kingdom (K. Winzer et al., CCCB-07, abstr. 131B).

CELL-CELL COMMUNICATION IN BACTERIAL DEVELOPMENT—EXTENDING THE SIGNAL REPERTOIRE

While many bacteria have the ability to develop multicellular communities, some also differentiate into distinct morphotypes. These coordinated developmental dynamics are often

governed by signal-mediated cell-cell communication. *Bacillus subtilis*, a gram-positive soil bacterium, has an enormous capacity to differentiate and direct the morphogenesis of specialized cells within a community. Roberto Kolter, Harvard Medical School (R. Kolter, CCCB-07, abstr. S1:2; P. D. Straight and R. Kolter, CCCB-07, abstr. 110A; D. Lopez-Serrano and R. Kolter, CCCB-07, abstr. 119B), postulated that transition of single nomadic cells to a multicellular biofilm-associated sessile existence may be influenced by signals produced by neighboring bacteria, as exemplified by pellicle formation in static liquid cultures. Pellicles consist of parallel chains of bundled, interconnected cells at the liquid/air interface that ultimately give rise to sporulating fruiting bodies (12). Bundling requires the synthesis of an EPS matrix and the secretion of the extracellular TasA protein (21). A screen of microbially derived compounds using a *tasA*-specific reporter identified the lipopeptide surfactin produced by *B. subtilis*; gramicidin from *Bacillus brevis*; and the *Streptomyces* antibiotics nystatin, amphotericin, and valinomycin, as inducers of *tasA* expression and pellicle formation. These structurally diverse compounds have related activities as K^+-specific ionophores and as such induce K^+ efflux in target microbes. The capacity to sense K^+ flux depends on the KinC sensor kinase as shown by mutant analysis. Given the structural diversity of the compounds described, rather than sensing each compound directly, it may be more efficient for *B. subtilis* to sense and respond to the effect of these ionophores on nearby neighbors and then to organize into protective biofilms.

Anne Tart from Heidi Kaplan's group, the University of Texas Medical School at Houston (A. Tart et al., CCCB-07, abstr. S3:5), focused on QS during the early stages of *M. xanthus* fruiting body development. She described the expression of an early developmental gene, designated 4445 (MXAN_4368). An in vitro assay showed that cell wall perturbations stimulated the expression of a 4445::*lacZ* reporter fusion. Similarly, exposing starving low-density cells to conditioned starvation buffer derived from high-density wild-type cells had the same inducing effect. Fractionation of the conditioned starvation buffer yielded a low-molecular-weight, heat-stable active component whose structural characterization is in progress. The fact that peptidoglycan (PG) hydrolysis products also stimulate 4445::*lacZ* expression suggests that the active component is PG derived. Perhaps extracellular cell wall components accumulated during starvation at high density may act as QS signals to trigger *Myxococcus* fruiting body morphogenesis as a means of cell survival.

Jonathan Dworkin, Columbia University (I. M. Shah and J. Dworkin, CCCB-07, abstr. S3:6), showed that PG fragments stimulated the expression of multiple genes in vegetative *B. subtilis* cells including the production and secretion of YocH murein hydrolase. In addition, the PG fragments induced the germination of *B. subtilis* spores, which depends on the transmembrane serine/threonine kinase PrkC. PrkC features an extracellular PASTA protein domain that appears to mediate PG sensing. The only known kinase target of PrkC is EF-2 elongation factor G (EF-G). The activity of the eukaryotic homolog of EF-G, EF-2, is regulated by phosphorylation, suggesting that EF-G is similarly regulated. Thus, the phosphorylation of EF-G in germination may be the initial step in the reestablishment of cellular metabolism. Interestingly, staurosporine, a kinase inhibitor isolated from *Streptomyces staurosporeus*, inhibits the PrkC-dependent germination triggered by the PG fragments in the wild type. Thus, *B. subtilis* spore germination may be influenced, in part, by cell wall material turnover released by actively growing siblings or other spore-forming bacteria. Additionally, competing organisms such as *Streptomyces* produce signal compounds that may inhibit the molecular switch that leads to spore germination.

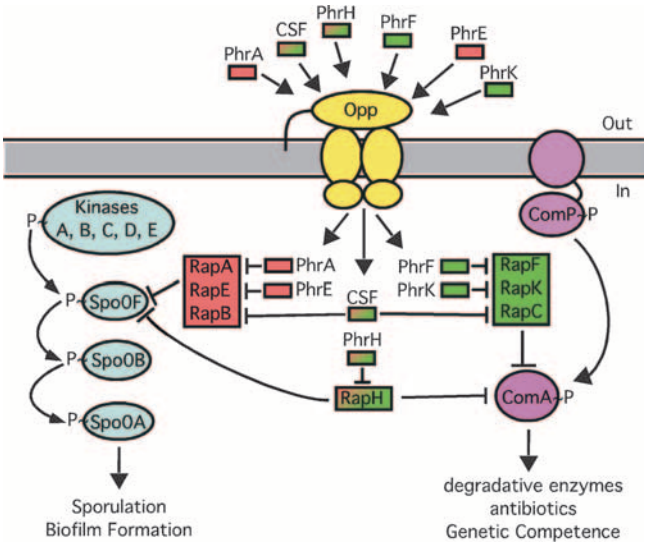

FIG. 6. Response to Phr signaling peptides that regulate *B. subtilis* transcription factors Spo0A and ComA. Phr peptides are secreted pentapeptides that are transported into the cell by an oligopeptide permease (Opp). The peptides interact with cognate Rap proteins to inhibit their phosphatase activity, leading to Spo0F phosphorylation or inhibition of ComA DNA binding activity. Rap/Phr pairs shown in red regulate Spo0A, and Rap/Phr pairs shown in green regulate ComA; red to green shading indicates Phr or Rap interacting with both the Spo0A and ComA pathways. (Reprinted from reference 71 with permission of the publisher.)

INTERCELLULAR COMMUNICATION—COMPETENCE AND HORIZONTAL GENE TRANSFER

Many bacteria utilize cell-cell communication systems to regulate horizontal gene transfer. Gram-positive bacteria characteristically employ small extracellular peptides as cell-cell communication signals to control competence, sporulation, and the production of exoenzymes, polysaccharides, and other secondary metabolites. For example, *B. subtilis* produces three classes of peptide signals, the ComX pheromone, lantibiotic peptides, and the Phr class of pheromone pentapeptide signals that include the *c*ompetence and *s*porulation *f*actor (CSF). ComX interacts with the receptor kinase ComP, leading to autophosphorylation and phosphotransfer to its cognate response regulator, ComA. Phosphorylated ComA is a transcriptional activator of a variety of genes, including those involved in competence (23). When secreted Phr signals reach an external threshold concentration, they enter the cell via the Opp oligopeptide permease complex (Fig. 6). PhrA, CSF, and PhrE inhibit the RapA, RapB, and RapE phosphatase activities, thereby promoting phosphorylation of Spo0F and subsequent sporulation. CSF also acts on RapC, while PhrF and PhrK

interact with the cognate RapF and RapK proteins. These interactions inhibit ComA DNA binding activity, which affects genetic competence, and the expression of the *rapA*, *rapC*, and *rapE* genes in a peptide-specific autoregulatory circuit (56, 71).

B. subtilis harbors eight chromosomally encoded *phr* genes and four that are plasmid encoded. The genes are similar in that they encode oligopeptides of ≈40 amino acids in length, feature a signal sequence, and are generally located next to genes encoding their cognate Rap protein. The serine proteases subtilisin, Vpr, and Epr synergistically process the pre-Phr peptides to generate the mature C-terminal pentapeptides (23). Beth Lazazzera, University of California, Los Angeles (S. Lanigan-Gerdes et al., CCCB-07, abstr. S1:3), described a systematic experimental approach to identify the cleavage site elements recognized by these proteases. While the different proteases have slightly different specificities, conserved amino acids immediately preceding the cleavage site are important for processing. This work represents an important step toward defining the structural features required for peptide signal processing in *B. subtilis* and other bacteria.

Alan Grossman, Massachusetts Institute of Technology (A. D. Grossman et al., CCCB-07, abstr. S3:1), described the function of PhrI peptide and its cognate RapI receptor in controlling the transfer of the integrative conjugative element ICEBs1 in *B. subtilis*. The conjugal transfer of this element can be induced by either the SOS response or RapI. RapI and PhrI are ICEBs1 encoded, which means that populations consisting predominately of ICEBs1 donor cells experience high concentrations of PhrI pentapeptide. PhrI antagonizes the activity of RapI, leading to ImmR-specific repression of *xis* and possibly the activation of the *int*-encoded integrase, thereby favoring ICEBs1 retention (4, 57). In contrast, if the neighboring population is comprised predominately of cells that lack ICEBs1, the PhrI levels are low, allowing normal RapI function and concomitant ImmA-mediated proteolytic reduction of cellular levels of ImmR. This favors *xis* gene expression, ICEBs1 excision, and horizontal transfer. Similar elements containing *rap*/*phr* pairs are found in other *Bacillus* species, suggesting a conserved mechanism of horizontal gene transfer with considerable impact on bacterial evolution and the transfer of desirable and undesirable traits.

Gary Dunny's group, University of Minnesota, provided new insights into the complex regulation of the antibiotic resistance plasmid pCF10 in *Enterococcus faecalis* and a systems biology approach to assay the molecular basis of bistability of the conjugal process (L. C. Case et al., CCCB-07, abstr. S6:6; H. A. Haeming et al., CCCB-07, abstr. 36A). In this system, the central peptide receptor regulator PrgX represses pCF10 conjugation if the concentration of the inhibitory iCF10 peptide (plasmid encoded) accumulates in populations dominated by donors. In contrast, if recipient cells outnumber donors, the peptide mating pheromone cCF10 (chromosome encoded) binds to PrgX to induce plasmid replication and expression of conjugal transfer components including the cell aggregation factor Asc10 (31).

Virulent *Agrobacterium tumefaciens* strains carry Ti plasmids that enable the bacteria to cause crown gall tumors in host plants. The tumors, in turn, produce opine nutrients that are exclusively metabolized by Ti plasmid-carrying bacteria. A subset of opines, the conjugal opines, induce Ti plasmid transfer but only when the Ti plasmid-carrying population reaches a threshold population density that is sufficient to induce Ti plasmid amplification and horizontal transfer (recently reviewed in reference 106). A poster presented by Clay Fuqua's group, Indiana University (T. G. Platt et al., CCCB-07, abstr. 121B), explored the evolutionary pressures that influence the population dynamics of the Ti plasmid. Strains that carry and maintain a Ti plasmid under nutrient-limiting conditions experience a considerable competitive disadvantage in environments lacking opines. However, an ecological model predicts that this cost is alleviated by the benefits of opine catabolism in the disease environment. Thus, QS regulation of Ti plasmid conjugation ensures that it occurs only in environments in which the net effect of the plasmid is beneficial rather than detrimental.

The TraR protein of *A. tumefaciens* is the prototypical acyl-HSL receptor that tightly binds the cognate ligand. In the absence of acyl-HSL, TraR is subjected to rapid proteolysis (112). The crystal structure of DNA-bound TraR provided some important insights about TraR function (95, 111). Steve Winans, Cornell University (E. D. Costa and S. C. Winans, CCCB-07, abstr. 141B), detailed the activation properties of ligand-bound TraR. Residues organized into functional patches within the N-terminal and C-terminal domain of TraR facilitate contact with the α-C-terminal domain of RNAP and are important for transcriptional activation in both class I- and class II-type promoters (for details see also references 107 and 108). Ying Qin from the Farrand group, University of Illinois at Urbana-Champaign (Y. L. Qin and S. K. Farrand, CCCB-07, abstr. 117B), extended these results by identifying a separate N-terminal patch of residues that interface with the σ^{70} subunit of *A. tumefaciens* RNAP.

In addition to the TraR and TraI proteins, the QS system in *A. tumefaciens* requires the TraM antiactivator. This protein is essential for the population to achieve a robust QS switch that is unaffected by intrinsic signal noise as predicted by the computational model presented by Andrew Goryachev, University of Edinburgh (A. B. Goryachev, CCCB-07, abstr. S6:3; for details also see reference 1). The TraM antiactivator is conserved in the QS systems within the *Rhizobiaceae* and *Bradyrhizobiacea*e families. TraM was originally described in *A. tumefaciens* as a modulator of TraR activity (19, 37, 51, 72). Lingling Chen, Indiana University (G. Chen et al., CCCB-07, abstr. S2:2), revealed new structural insights into TraM/TraR associations and inhibition of premature TraR activity based on the more structurally tractable TraM/$TraR_{NGR}$ antiactivator complex of *Rhizobium* sp. strain NGR234 (18). These studies provide evidence for an allosteric mechanism (Fig. 7) in which TraM contacts the exposed linker between the N-terminal domain and C-terminal DNA binding domain and the α10 helix of the first DNA-bound TraR protomer. This interaction induces linker rotation leading to TraR protomer/DNA dissociation. The second TraM monomer accesses the now-exposed linker of the second TraR protomer to destabilize its DNA binding contact. Thus, TraM antiactivation of TraR relies on a sequential allosteric mechanism that repositions the DNA binding module of TraR to block productive association with target promoters. This scenario is distinct from other repressor activities that generally competitively block transcription by steric exclusion of RNAP and excludes simpler models in

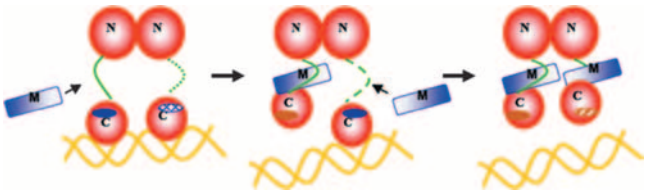

FIG. 7. Mechanism of $TraM_{NGR}$ inhibition of $TraR_{NGR}$, depicting the proposed stepwise dissociation of $TraR_{NGR}$-DNA by $TraM_{NGR}$. The first $TraM_{NGR}$ protomer is thought to interact with the exposed linker (green) and TraR C-terminal binding site (dark blue solid oval), leading to the first $TraR_{NGR}$ protomer/DNA dissociation. This interaction repositions the linker and buried $TraM_{NGR}$ binding site (hatched blue oval) of the second $TraR_{NGR}$ protomer so that the next $TraM_{NGR}$ can bind and induce complete $TraR_{NGR}$/DNA dissociation. (Reprinted from reference 18 with permission of the publisher.)

which the antiactivator directly occludes the DNA binding domain.

Clive Ronson, University of Otago, New Zealand (J. Ramsay et al., CCCB-07, abstr. S3:4), described the existence of an integrative and conjugative element (ICE) in the gram-negative bacterium *Mesorhizobium loti*, which when transferred to nonsymbiotic *M. loti* strains establishes a symbiosis and confers the ability to fix nitrogen. The element, designated ICE*MI*Sym^{R7A}, is 501.8 kb in size and encodes functions required for nodulation, nitrogen fixation, secretion, and metabolism and for ICE*MI*Sym^{R7A} transfer. Excision of the ICE*MI*Sym^{R7A} symbiosis island requires the cell-density-dependent expression of the RlxS relaxase, RdfS excisionase, and an island integrase (75). The island encodes homologs of the *A. tumefaciens* proteins TraR and TraI that promote the synthesis of multiple long- and short-chain acyl-HSLs. Overexpression of *traR* dramatically increases acyl-HSL production and ICE*MI*Sym^{R7A} excision and transfer. A gene designated *msi169* located adjacent to the *traR-traI2* gene system is needed for efficient ICE*MI*Sym^{R7A} excision and acyl-HSL production, while the divergent gene *msi170* appears to repress excision. It is thought that Msi169 represses *msi170*, whose product may be similar to TraM, which is needed for the posttranslational suppression of Ti and Sym plasmid conjugal transfer in bacteria of the family *Rhizobiaceae*.

CONVERSATIONS BETWEEN CELLS—TO TALK OR NOT TO TALK?

Given the complexity of natural systems in which multiple organisms may be "speaking" in competing "languages," individual microbes are faced with the challenge of filtering signal from noise. These filtering mechanisms involve intricate systems by which multiple signal inputs are perceived and appropriately processed. This section provides an overview of fascinating new systems that feature interspecies signaling as it occurs in nature. Jo Handelsman, University of Wisconsin, described how a metagenomics approach advances the understanding of interspecies signaling in complex microbial communities as diverse as Alaskan soil and the midgut of insects (J. Handelsman, CCCB-07, abstr. S5:4). DNA fragments isolated directly from prokaryotes in the gypsy moth gut were cloned into *E. coli* carrying a *luxR*-green fluorescent protein reporter vector. One active clone produced a signal mimic that was identified as a novel indole compound. When semipurified signal was added exogenously to a nonproducing *Enterobacter* sp. strain, a member of the normal flora of the gypsy moth gut, a distinctive change in protein profiles emerged, illustrating that we have much to learn about interspecies communication in nature. Handelsman also provided new insights into the insecticidal biology of *Bacillus thuringiensis*, showing that larval killing requires the resident commensal gut flora in addition to the *B. thuringiensis* toxin (13).

P. aeruginosa is a highly adaptive organism that is found in many environments other than in pathogenic associations with both plant and animal hosts. The success of *P. aeruginosa* in diverse settings suggests that it must be equipped to compete effectively with other bacterial species. Matt Parsek, University of Washington, presented an interesting study (D. An et al., CCCB-07, abstr. 139B) in which green fluorescent protein-labeled *P. aeruginosa* was cocultured with a number of other bacterial species. Interestingly, *P. aeruginosa* outcompeted *A. tumefaciens*, *P. stewartii*, and *B. subtilis* in a LasR/RhlR-dependent manner. Although *Staphylococcus aureus* grew faster than *P. aeruginosa* in coculture, both *B. subtilis* and *S. aureus* were killed by the addition of supernatant collected from wild-type *P. aeruginosa* cultures. Depending upon the microorganism, several different QS-dependent compounds were required for this toxicity, including rhamnolipid, pyocyanin, and cyanide, which had synergistic effects. The growth of bacteria from an aquatic natural sample was also inhibited when they were grown in proximity to *P. aeruginosa*. Moreover, *P. aeruginosa* was effective in excluding other natural microbial isolates when grown in biofilms. These findings demonstrate an important role for QS in bacterial interspecies competition in natural, mixed-species communities.

Allen Decho, University of South Carolina (A. W. Decho et al., CCCB-07, abstr. S5:5), offered an interesting perspective on the complexity of cell-cell communication in microbial mats, which represent a rich microbial ecosystem. Mass spectroscopy of extracted material revealed the presence of many major acyl-HSLs, with octanoyl-HSL and decanoyl-HSL being the most common species. Interestingly, the stability pattern of acyl-HSLs in these ecosystems changes in a diurnal pattern reflecting changes in pH, redox potential, and temperature. Under daylight conditions, short-chain acyl-HSLs have a half-life of less than 8 hours, while the long-chain acyl-HSLs may persist into the night. Decho hypothesized that the differential stability and diffusibility of the acyl-HSLs may provide a mechanism for temporal information processing by the mat microbial community. Specifically, short-chain acyl-HSLs communicate information that requires an immediate response, while the more stable long-chain acyl-HSLs may induce longer-term responses. Certainly, mat communities represent a diverse and ecologically relevant system for assessing the role of QS in situ.

Proteus mirabilis, a gram-negative bacterium that exists in the human gut and in aquatic environments, is an important urinary tract pathogen. Andries Budding, VU Medical Centre, Amsterdam, The Netherlands (A. E. Budding et al., CCCB-07, abstr. S5:6), explored the intricate swarming patterns that occur when two nonidentical *P. mirabilis* strains are grown on an agar surface. They typically develop characteristic concentric

ring patterns with a clear demarcation line, known as the Dienes line, where the two populations meet. This effect is triggered by a small diffusible factor. Microscopic analysis showed that one of the colonies develops large round cells blocked in cell division that undergo lysis. Large-cell formation requires cell-cell contact between the different swarmer cells. In this connection Gibbs and colleagues from the Greenberg lab, University of Washington (K. A. Gibbs et al., CCCB-07, abstr. 53B), reported that this territorial behavior depends on the products of six *ids* genes (for *id*entification of *s*elf). The first two genes may encode elements of a type IV secretion system presumably necessary to secrete Ids gene products. The locus is conserved in different *P. mirabilis* strains with a subset of genes that are nearly identical and others that are variable, suggesting that the latter may impart strain identity.

PARALLELS IN MULTICELLULAR BEHAVIOR AND DEVELOPMENT IN EUKARYOTIC SYSTEMS

Eukaryotic microbes have evolved various degrees of cooperative group behaviors that require cell-cell communication. In addition, intercellular communication is a key factor in metazoan embryological development, which culminates in a complex organization of various cell types that behave coordinately in response to specific messenger molecules. The conference concluded with presentations of representative cell-cell communication systems in eukaryotes that reflected an increasing range of complexities.

Many yeast-type fungi can grow either as unicellular or as filamentous forms depending on the environmental conditions. In *Candida albicans* and *Saccharomyces cerevisiae*, hyphal growth ensues when cells experience conditions of nitrogen starvation. For example, Clair Tseng from the Fink group, the Whitehead Institute (C. C. Tseng and G. R. Fink, CCCB-07, abstr. S7:4), described the involvement of aromatic alcohol signals in triggering the transition from the single budding cell to the multicellular filamentous morphotype when the microbes are at high cell density and in nitrogen-limiting conditions. The synthesis of the alcohol signals is stimulated by a positive feedback regulatory mechanism (20). A second apparent QS regulatory system exists in *S. cerevisiae* in which a small, water-soluble factor is secreted into the medium. This signal appears to control a putative low-affinity amino acid permease. The mechanism of regulation may involve cell-density-dependent changes in RNA stability.

Trypanosoma brucei is a parasitic protist and the causative agent of African sleeping sickness (trypanosomiasis). Motility is central to trypanosome development and pathogenesis in the tsetse fly vector and mammalian host. The parasite has a single flagellum that propels the cell forward in an auger-like spiraling motion (47, 73). Kent Hill and colleagues, University of California, Los Angeles (K. L. Hill and B. T. McLelland, CCCB-07, abstr. S7:5), employed video microscopy to study the motility and behavior of trypanosomes on a semisolid agar. Upon plating, trypanosomes exhibit changes in motility and assemble into multicellular colonies that sense and respond to external stimuli. This behavior results in colony mergers and, ultimately, formation of large megacolonies with projections comprised of highly motile cells. Trypanosome motility is powered by a multisubunit dynein molecular motor. RNA interference against the dynein regulatory protein trypanin demonstrates that directional cell motility is required for colony assembly (74).

Armin Hallmann, Bielefeld University, Germany (A. Hallmann, CCCB-07, abstr. S7:6), provided an excellent overview of a biological system that related evolution to multicellularity. The *Volvocaceae* are a family of biflagellated colonial green algae that include members of various degrees of cellular complexity ranging from the unicellular *Chlamydomonas reinhardtii* to the most advanced member, *Volvox carteri*. The *V. carteri* spheroid consists of two types of cells: the somatic cells, which resemble their unicellular, *Chlamydomonas*-like ancestor, and larger reproductive cells, or gonidia. A complex, transparent, glycoprotein-rich extracellular matrix holds the cells in place and mediates a variety of developmental and physiological responses. Morphogenesis of offspring always occurs within the mother spheroid. *Volvox* germ cells reproduce sexually and asexually. Sexual reproduction requires the sex inducer identified as a glycoprotein that is spontaneously produced by a mutational switch in a single male or when males and females experience elevated temperatures (40, 54). The inducer is extremely potent, functioning at concentrations as low as 10^{-16} M to induce sexual embryogenesis. *Volvox* and its extant relatives represent an ideal model system to explore the molecular basis of cell differentiation and cell fate.

Jane Hubbard, New York University (CCCB-07, abstr. S7:1), offered insights into the critical communication between somatic and germ line cells in the *Caenorhabditis elegans* model system. The decision between germ cell proliferation (mitotic) and differentiation into gametes (meiotic) is dictated by a morphogen signal produced by the distal tip somatic cell (DTC) that interacts with a germ line cell Notch receptor (GLP-1). This interaction blocks meiotic entry in favor of mitosis and undifferentiated germ cell proliferation. The expansion of the proliferation zone as a result of mitotic germ cell division effectively reduces the reach of the DTC signal, thereby permitting meiotic entry and gamete differentiation at the opposite end of the gonad. DTC ablation removes the signal-mediated block of cell differentiation, which causes all germ cells to differentiate (49). Thus, the physical distance between the point of signal generation and signal reception constitutes an important regulatory factor for controlling cell fate within the gonad of *C. elegans*. The same principle applies to bacterial QS particularly in complex natural environments, where signal diffusion, noise due to cross talk, signal mimics and inhibitors, and many other factors influence signal reach. This has been described as "calling distance."

SUMMARY AND PERSPECTIVE

The 2007 ASM conference on Cell-Cell Communication in Bacteria reflected the degree of maturity of the field with considerable emphasis on the broader concepts of microbial social behaviors, interspecies communication, signaling in situ, and the costs/benefits derived from an apparent multicellular existence. Mathematical modeling and simulations are now being employed to test the rigor of signal networks and define components that guarantee network fitness and stability. The conference revealed a number of novel signals distinct from acyl-HSL and AI-2 and peptide signal paradigms, which in-

cluded cell wall components and a range of secondary metabolites. Research in the area of signal chemistry and ligand/target interaction has taken center stage as a potential platform to develop novel chemistries that specifically inhibit virulence mechanisms without restricting bacterial growth. This strategy may hold promise in the control of important human diseases such as CF and cholera pandemics. This approach will no doubt benefit from the enormous amount of recent progress in understanding the molecular basis for the role of bacterial signal/response mechanisms in modulating the host immune systems. Understanding the intricacies of these interactions will facilitate a more streamlined approach to QSI-based drug design. Finally, novel multispecies biological systems were described, showcasing the largely unexplored rich microbial ecology in which cell-cell communication represents an integral part of consortium biology.

The future direction of the field is likely to rely heavily on integrated systems approaches to map global changes in gene/protein expression, metabolism, microbial behavior, and population responses to complex signal inputs in artificial and natural contexts. Increasingly, such studies will be guided by testable mathematical models and predictions based on systematic simulations. The development of sophisticated high-throughput assay techniques combined with the availability of new analytical and high-resolution imaging systems will be critical to realizing a deeper understanding of bacterial developmental and behavioral strategies that enable bacteria to thrive in diverse and fluctuating natural environments. This multidisciplinary approach to studying bacterial systems will take the field to the next exciting level of understanding.

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REFERENCES

1. **Aguda, B. D., and A. B. Goryachev.** 2007. From pathways, databases to network models of switching behavior. PLoS Comput. Biol. **3:**1674–1678.
2. **Andrade, M. O., M. C. Alegria, C. R. Guzzo, C. Docena, M. C. Rosa, C. H. Ramos, and C. S. Farah.** 2006. The HD-GYP domain of RpfG mediates a direct linkage between the Rpf quorum-sensing pathway and a subset of diguanylate cyclase proteins in the phytopathogen *Xanthomonas axonopodis* pv *citri*. Mol. Microbiol. **62:**537–551.
3. **Atkinson, S., T. J. Throup, G. S. Stewart, and P. Williams.** 1999. A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. Mol. Microbiol. **33:**1267–1277.
4. **Auchtung, J. M., C. A. Lee, K. L. Garrison, and A. D. Grossman.** 2007. Identification and characterization of the immunity repressor (ImmR) that controls the mobile genetic element ICEBs1 of *Bacillus subtilis*. Mol. Microbiol. **64:**1515–1528.
5. **Barnard, A. M. L., and G. P. C. Salmond.** 2007. Quorum sensing in *Erwinia* species. Anal. Bioanal. Chem. **387:**415–424.
6. **Bearson, B. L., and S. M. Bearson.** 2008. The role of the QseC quorum-sensing sensor kinase in colonization and norepinephrine-enhanced motility of *Salmonella enterica* serovar *Typhimurium*. Microb. Pathog. **44:**271–278.
7. **Beck von Bodman, S., and S. K. Farrand.** 1995. Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an *N*-acylhomoserine lactone autoinducer. J. Bacteriol. **177:**5000–5008.
8. **Bjarnsholt, T., and M. Givskov.** 2007. Quorum-sensing blockade as a strategy for enhancing host defences against bacterial pathogens. Trans. R. Soc. Lond. B Biol. Sci. **362:**1213–1222.
9. **Bjarnsholt, T., P. Ø. Jensen, T. B. Rasmussen, L. Christophersen, H. Calum, M. Hentzer, H. P. Hougen, J. Rygaard, C. Moser, L. Eberl, N. Høiby, and M. Givskov.** 2005. Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. Microbiology **151:**3873–3889.
10. **Bjarnsholt, T., P. Ø. Jensen, M. Burmølle, M. Hentzer, J. A. J. Haagensen, H. P. Hougen, H. Calum, K. G. Madsen, C. Moser, S. Molin, N. Høiby, and M. Givskov.** 2005. *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. Microbiology **151:**373–383.
11. **Bose, J. L., U. Kim, W. Bartkowski, R. P. Gunsalus, A. M. Overley, N. L. Lyell, K. L. Visick, and E. V. Stabb.** 2007. Bioluminescence in *Vibrio fischeri* is controlled by the redox-responsive regulator ArcA. Mol. Microbiol. **65:**538–553.
12. **Branda, S. S., J. E. Gonzales-Pastor, S. Ben-Yehuda, R. Losick, and R. Kolter.** 2001. Fruiting body formation by *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **98:**11621–11626.
13. **Broderick, N. A., K. F. Raffa, and J. Handelsman.** 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proc. Natl. Acad. Sci. USA **103:**15196–15199.
14. **Brown, S. P., and R. A. Johnstone.** 2001. Cooperation in the dark: signalling and collective action in quorum-sensing bacteria. Philos. Trans. R. Soc. Lond. B Biol. Sci. **268:**961–965.
15. **Cardona, S. T., J. Wopperer, L. Eberl, and M. A. Valvano.** 2005. Diverse pathogenicity of *Burkholderia cepacia* complex strains in the *Caenorhabditis elegans* host model. FEMS Microbiol. Lett. **250:**97–104.
16. **Carlier, A. L., and S. B. von Bodman.** 2006. The *rcsA* promoter of *Pantoea stewartii* subsp. *stewartii* features a low-level constitutive promoter and an EsaR quorum-sensing-regulated promoter. J. Bacteriol. **188:**4581–4584.
17. **Castang, S., S. Reverchon, P. Gouet, and W. Nasser.** 2006. Direct evidence for the modulation of the activity of the *Erwinia chrysanthemi* quorum-sensing regulator ExpR by acylhomoserine lactone pheromone. J. Biol. Chem. **281:**29972–29987.
18. **Chen, G., P. D. Jeffrey, C. Fuqua, Y. Shi, and L. Chen.** 2007. Structural basis for antiactivation in bacterial quorum sensing. Proc. Natl. Acad. Sci. USA **104:**16474–16479.
19. **Chen, G., J. W. Malenkos, M. R. Cha, C. Fuqua, and L. Chen.** 2004. Quorum-sensing antiactivator TraM forms a dimer that dissociates to inhibit TraR. Mol. Microbiol. **52:**1641–1651.
20. **Chen, H., and G. R. Fink.** 2006. Feedback control of morphogenesis in fungi by aromatic alcohols. Genes Dev. **20:**1150–1161.
21. **Chu, F., D. B. Kearns, S. S. Branda, R. Kolter, and R. Losick.** 2006. Targets of the master regulator of biofilm formation in *Bacillus subtilis*. Mol. Microbiol. **59:**1216–1228.
22. **Chugani, S. A., M. Whiteley, Lee, K. M., D. D'Argenio, C. Manoil, and E. P. Greenberg.** 2001. QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **98:**2752–2757.
23. **Comella, N., and A. D. Grossman.** 2005. Conservation of genes and processes controlled by the quorum response in bacteria: characterization of genes controlled by the quorum-sensing transcription factor ComA in *Bacillus subtilis*. Mol. Microbiol. **57:**1159–1174.
24. **Cugini, C., M. Calfee, J. M. Farrow III, D. K. Morales, E. C. Pesci, and D. A. Hogan.** 2007. Farnesol, a common sesquiterpene, inhibits PQS production in *Pseudomonas aeruginosa*. Mol. Microbiol. **65:**896–906.
25. **D'Argenio, D. A., M. Wu, L. R. Hoffman, H. D. Kulasekara, E. Deziel, E. E. Smith, H. Nguyen, R. K. Ernst, T. J. Larson Freeman, D. H. Spencer, M. Brittnacher, H. S. Hayden, S. Selgrade, M. Klausen, D. R. Goodlett, J. L. Burns, B. W. Ramsey, and S. I. Miller.** 2007. Growth phenotypes of *Pseudomonas aeruginosa lasR* mutants adapted to the airways of cystic fibrosis patients. Mol. Microbiol. **64:**512–533.
26. **Dawkins, R.** 1976. The selfish gene. Oxford University Press, Oxford, United Kingdom.
27. **Déziel, E., S. S. Gopalan, A. P. Tampakaki, F. Lépine, K. E. Padfield, M. Saucier, G. Xiao, and L. G. Rahme.** 2005. The contribution of MvfR to *Pseudomonas aeruginosa* pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting *lasRI*, *rhlRI* or the production of *N*-acyl-L-homoserine lactones. Mol. Microbiol. **55:**998–1014.
28. **Diggle, S. P., A. S. Griffin, G. S. Campbell, and S. A. West.** 2007. Cooperation and conflict in quorum-sensing bacterial populations. Nature **450:**411–414.
29. **Diggle, S. P., A. Gardner, S. A. West, and A. S. Griffin.** 2007. Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? Philos. Trans. R. Soc. Lond. B Biol. Sci. **362:**1241–1249.
30. **Dow, J. M., J. X. Feng, C. E. Barber, J. L. Tang, and M. J. Daniels.** 2000.

Novel genes involved in the regulation of pathogenicity factor production within the *rpf* gene cluster of *Xanthomonas campestris*. Microbiology **146:** 885–891.
31. **Dunny, G. M.** 2007. The peptide pheromone-inducible conjugation system of *Enterococcus faecalis* plasmid pCF10: cell-cell signalling, gene transfer, complexity and evolution. Phil. Trans. R. Soc. Lond. B Biol. Sci. **36:**1185–1193.
32. **Egland, K. A., and E. P. Greenberg.** 2000. Conversion of the *Vibrio fischeri* transcriptional activator, LuxR, to a repressor. J. Bacteriol. **182:**805–811.
33. **Fidopiastis, P. M., C. M. Miyamoto, M. G. Jobling, E. A. Meighen, and E. G. Ruby.** 2002. LitR, a new transcriptional activator in *Vibrio fischeri*, regulates luminescence and symbiotic light organ colonization. Mol. Microbiol. **45:**131–143.
34. **Fiegna, F., Y. T. N. Yu, S. V. Kadam, and G. J. Velicer.** 2006. Evolution of an obligate social cheater to a superior cooperator. Nature **441:**310–314.
35. **Foster, K. R.** 2006. Sociobiology: the Phoenix effect. Nature **441:**291–292.
36. **Fouhy, Y., K. Scanlon, K. Schouest, C. Spillane, L. Crossman, M. B. Avison, R. P. Ryan, and J. M. Dow.** 2007. DSF-dependent cell-cell signaling and virulence in the nosocomial pathogen *Stenotrophomonas maltophilia*. J. Bacteriol. **189:**4964–4968.
37. **Fuqua, C., M. Burbea, and S. C. Winans.** 1995. Activity of the *Agrobacterium* Ti plasmid conjugal transfer regulator TraR is inhibited by the product of the *traM* gene. J. Bacteriol. **177:**1367–1373.
38. **Fuqua, W. C., S. C. Winans, and E. P. Greenberg.** 1994. Quorum sensing in bacteria: the LuxR/LuxI family of cell density-responsive transcriptional regulators. J. Bacteriol. **176:**269–275.
39. **Gallagher, L. A., S. L. McKnight, M. S. Kuznetsova, E. C. Pesci, and M. Manoil.** 2002. Functions required for extracellular quinolone signaling by *Pseudomonas aeruginosa*. J. Bacteriol. **184:**6472–6480.
40. **Hallmann, A.** 2003. Extracellular matrix and sex-inducing pheromone in *Volvox*. Int. Rev. Cytol. **227:**131–182.
41. **Hamilton, W. D.** 1964. Genetical evolution of social behaviour, I and II. J. Theor. Biol. **7:**1–52.
42. **Hammer, B. K., and B. L. Bassler.** 2007. Regulatory small RNAs circumvent the conventional quorum sensing pathway in pandemic *Vibrio cholerae*. Proc. Natl. Acad. Sci. USA **104:**11145–11149.
43. **Hammer, B. K., and B. L. Bassler.** 2003. Quorum sensing controls biofilm formation in *Vibrio cholerae*. Mol. Microbiol. **50:**101–104.
44. **Hense, B. A., C. Kuttler, J. Muller, M. Rothballer, A. Hartmann, and J. U. Kreft.** 2007. Does efficiency sensing unify diffusion and quorum sensing? Nat. Rev. Microbiol. **5:**230–239.
45. **Hentzer, M. H., H. Wu, J. B. Andersen, K. Riedel, T. B. Rasmussen, N. Bagge, N. Kumar, M. A. Schembri, Z. Song, P. Kristoffersen, M. Manefield, J. W. Costerton, S. Molin, L. Eberl, P. Steinberg, S. Kjelleberg, N. Hoiby, and M. Givskov.** 2003. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J. **22:**3803–3815.
46. **Higgins, D. A., M. E. Pomianek, C. M. Kraml, R. K. Taylor, M. F. Semmelback, and B. L. Bassler.** 2007. The major *Vibrio cholerae* autoinducer and its role in virulence factor production. Nature **450:**883–886.
47. **Hill, K. L.** 2003. Mechanism and biology of trypanosome cell motility. Eukaryot. Cell **2:**200–208.
48. **Hooper, L. V., and J. I. Gordon.** 2001. Commensal host-bacterial relationships in the gut. Science **292:**1115–1118.
49. **Hubbard, E. J. A.** 2007. *Caenorhabditis elegans* germ line: a model for stem cell biology. Dev. Dyn. **235:**3334–3357.
50. **Hughes, D. T., and V. Sperandio.** 2008. Inter-kingdom signalling: communication between bacteria and their hosts. Nat. Rev. Microbiol. **6:**111–120.
51. **Hwang, I., D. M. Cook, and S. K. Farrand.** 1995. A new regulatory element modulates homoserine lactone-mediated autoinduction of Ti plasmid conjugal transfer. J. Bacteriol. **177:**449–458.
52. **Jahoor, A., R. Patel, A. Bryan, C. Do, J. Krier, C. Watters, W. Wahli, G. Li, S. C. Williams, and K. P. Rumbaugh.** 2008. Peroxisome proliferator activated receptors mediate host cell proinflammatory responses to *P. aeruginosa* autoinducer. J. Bacteriol. **190:**4408–4415.
53. **Keller, L., and M. G. Surette.** 2006. Communication in bacteria: an ecological and evolutionary perspective. Nat. Rev. Microbiol. **4:**249–258.
54. **Kirk, D. L., and M. M. Kirk.** 1986. Heat shock elicits production of sexual inducer in *Volvox*. Science **231:**51–54.
55. **Koutsoudis, M. D., D. Tsaltas, T. D. Minogue, and S. B. von Bodman.** 2006. Quorum-sensing regulation governs bacterial adhesion, biofilm development, and host colonization in *Pantoea stewartii* subspecies *stewartii*. Proc. Natl. Acad. Sci. USA **103:**5983–5988.
56. **Lazazzera, B. A., and A. D. Grossman.** 1998. The ins and outs of peptide signaling. Trends Microbiol. **64:**288–294.
57. **Lee, C. A., J. M. Auchtung, R. E. Monson, and A. D. Grossman.** 2007. Identification and characterization of *int* (integrase), *xis* (excisase) and chromosomal attachment sites of the integrative and conjugative element ICEBs1 of *Bacillus subtilis*. Mol. Microbiol. **66:**1356–1369.
58. **Lequette, Y., J. H. Lee, F. Ledgham, A. Lazdunski, and E. P. Greenberg.** 2006. A distinct QscR regulon in the *Pseudomonas aeruginosa* quorum-sensing circuit. J. Bacteriol. **188:**3365–3370.
59. **Lesic, B., F. Lépine, E. Déziel, J. Zhang, Q. Zhang, K. Padfield, M. H. Castonguay, S. Milot, S. Stachel, A. A. Tzika, R. G. Tompkins, and L. G. Rahme.** 2007. Inhibitor of pathogen intercellular signals as selective anti-infective compounds. PLoS Pathog. **3:**e126.
60. **Luo, Z. Q., and S. K. Farrand.** 1999. Signal-dependent DNA binding and functional domains of the quorum-sensing activator TraR as identified by repressor activity. Proc. Natl. Acad. Sci. USA **96:**9009–9014.
61. **Lupp, C., and E. G. Ruby.** 2005. *Vibrio fischeri* uses two quorum-sensing systems for the regulation of early and late colonization factors. J. Bacteriol. **187:**3620–3629.
62. **Lyon, G. J., P. Mayville, T. W. Muir, and R. P. Novick.** 2000. Rational design of a global inhibitor of the virulence response in *Staphylococcus aureus*, based in part on localization of the site of inhibition to the receptor-histidine kinase, AgrC. Proc. Natl. Acad. Sci. USA **97:**13330–13335.
63. **Mattmann, M. E., G. D. Geske, G. A. Worzalla, J. R. Chandler, K. J. Sappington, E. P. Greenberg, and H. E. Blackwell.** 2008. Synthetic ligands that activate and inhibit a quorum-sensing regulator in *Pseudomonas aeruginosa.* Bioorg. Med. Chem. Lett. **18:**3072–3075.
64. **McDowell, P., Z. Affas, C. Reynolds, M. T. Holden, S. J. Wood, S. Saint, A. Cockayne, P. J. Hill, C. E. Dodd, B. W. Bycroft, W. C. Chan, and P. Williams.** 2001. Structure, activity and evolution of the group I thiolactone peptide quorum-sensing system of *Staphylococcus aureus*. Mol. Microbiol. **41:**503–512.
65. **Miller, M. B., and B. L. Bassler.** 2001. Quorum sensing in bacteria. Annu. Rev. Microbiol. **165:**199.
66. **Minogue, T. D., A. L. Carlier, M. D. Koutsoudis, and S. B. von Bodman.** 2005. The cell density-dependent expression of stewartan exopolysaccharide in *Pantoea stewartii ssp. stewartii* is a function of EsaR-mediated repression of the *rcsA* gene. Mol. Microbiol. **56:**189–203.
67. **Minogue, T. D., M. Wehland-von Trebra, F. Bernhard, and S. B. von Bodman.** 2002. The autoregulatory role of EsaR, a quorum-sensing regulator in Pantoea stewartii ssp. stewartii: evidence for a repressor function. Mol. Microbiol. **44:**1625–1635.
68. **Molofsky, A. B., and M. S. Swanson.** 2004. Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. Mol. Microbiol. **53:**29–40.
69. **Nealson, K. H.** 1977. Autoinduction of bacterial luciferase: occurrence, mechanism, and significance. Arch. Microbiol. **112:**73–79.
70. **Nealson, K. H., T. Platt, and J. W. Hastings.** 1970. Cellular control of the synthesis and activity of bacterial luminescent system. J. Bacteriol. **104:**313–322.
71. **Pottathil, M., and B. A. Lazazzera.** 2003. The extracellular Phr peptide-Rap phosphatase signaling circuit of *Bacillus subtilis*. Front. Biosci. **8:**d32–d45.
72. **Qin, Y., S. Su, and S. K. Farrand.** 2007. Molecular basis of transcriptional antiactivation. TraM disrupts the TraR-DNA complex through stepwise interactions. J. Biol. Chem. **282:**19979–19991.
73. **Ralston, K. S., and K. L. Hill.** 2008. The flagellum of Trypanosoma brucei: new tricks from an old dog. Int. J. Parasitol. **38:**869–884.
74. **Ralston, K. S., and K. L. Hill.** 2006. Trypanin, a component of the flagellar dynein regulatory complex, is essential in bloodstream form African trypanosomes. PLoS Pathog. **2:**101.
75. **Ramsay, J. P., J. T. Sullivan, G. S. Stuart, I. L. Lamont, and C. W. Ronson.** 2006. Excision and transfer of the *Mesorhizobium loti* R7A symbiosis island requires an integrase IntS, a novel recombination directionality factor RdfS, and a putative relaxase RlxS. Mol. Microbiol. **62:**723–734.
76. **Rasch, M., C. Buch, B. Austin, W. J. Slierendrecht, K. S. Ekmann, J. L. Larsen, C. Johansen, K. Riedel, L. Eberl, M. Givskov, and L. Gram.** 2004. An inhibitor of bacterial quorum sensing reduces mortalities caused by vibriosis in rainbow trout (Oncorhynchus mykiss, Walbaum). Syst. Appl. Microbiol. **27:**350–359.
77. **Rasmussen, T. B., T. Bjarnsholt, M. E. Skindersoe, M. Hentzer, P. Kristoffersen, M. Köte, J. Nielsen, L. Eberl, and M. Givskov.** 2005. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. J. Bacteriol. **187:**1799–1814.
78. **Redfield, R. J.** 2002. Is quorum sensing a side effect of diffusion sensing? Trends Microbiol. **10:**365–370.
79. **Ryan, R. P., Y. Fouhy, B. F. Garcia, S. A. Watt, K. Niehaus, L. Yang, T. Tolker-Nielson, and J. M. Dow.** 2008. Interspecies signaling via the *Stenotrophomonas maltophilia* diffusible signal factor influences biofilm formation and polymyxin tolerance in *Pseudomonas aeruginosa*. Mol. Microbiol. **68:**75–86.
80. **Ryan, R. P., Y. Fouhy, J. F. Lucey, L. C. Crossman, S. Spiro, Y. W. He, L. H. Zhang, S. Heeb, M. Camara, P. Williams, and J. M. Dow.** 2006. Cell-cell signaling in *Xanthomonas campestris* involves an HD-GYP domain protein that functions in cyclic di-GMP turnover. Proc. Natl. Acad. Sci. USA **103:** 6712–6717.
81. **Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull.** 2004. The evolution of cooperation. Q. Rev. Biol. **79:**135–160.
82. **Salunkhe, P., C. H. M. Smart, J. A. W. Morgan, S. Panagea, M. J. Walshaw, C. A. Hart, R. Geffers, B. Tummler, and C. Winstanley.** 2005. A cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* displays enhanced virulence and antimicrobial resistance. J. Bacteriol. **187:**4908–4920.
83. **Sandoz, K. M., S. M. Mitzimberg, and M. Schuster.** 2007. Social cheating

in *Pseudomonas aeruginosa* quorum sensing. Proc. Natl. Acad. Sci. USA **104:**15876–15881.
84. **Shapiro, J. A.** 1998. Thinking about bacterial populations as multicellular organisms. Annu. Rev. Microbiol. **52:**81–104.
85. **Shapiro, J. A.** 1988. Bacteria as multicellular organisms. Sci. Am. **258:** 82–89.
86. **Shapiro, J. A., and M. Dworkin.** 1997. Bacteria as multicellular organisms. Oxford University, Oxford, United Kingdom.
87. Reference deleted.
88. **Sjöblom, S., G. Brader, G. Koch, and E. T. Palva.** 2006. Cooperation of two distinct ExpR regulators controls quorum sensing specificity and virulence in the plant pathogen *Erwinia carotovora*. Mol. Microbiol. **60:**1474–1489.
89. **Slater, H., M. Crow, L. Everson, and G. P. Salmond.** 2003. Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. Mol. Microbiol. **47:**303–320.
90. **Smith, E. E., D. G. Buckley, Z. N. Wu, C. Saenphimmachak, L. R. Hoffman, D. A. D'Argenio, S. I. Miller, B. W. Ramsey, D. P. Speert, S. M. Moskowitz, J. L. Burns, R. Kaul, and M. V. Olson.** 2006. Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. Proc. Natl. Acad. Sci. USA **103:**8487–8492.
91. **Sperandio, V., A. G. Torres, B. Jarvis, J. P. Nataro, and J. B. Kaper.** 2003. Bacteria-host communication: the language of hormones. Proc. Natl. Acad. Sci. USA **100:**8951–8956.
92. **Tannock, G. W., S. Ghazally, J. Walter, D. Loach, H. Brooks, G. Cook, M. Surette, C. Simmers, P. Bremer, F. Dal Bello, and C. Hertel.** 2005. Ecological behavior of *Lactobacillus reuteri* 100-23 is affected by mutation of the *luxS* gene. Appl. Environ. Microbiol. **71:**8419–8425.
93. **Tiaden, A., T. Spirig, S. S. Weber, H. Brüggemann, R. Bosshard, C. Buchrieser, and H. Hilbi.** 2007. The *Legionella pneumophila* response regulator LqsR promotes host cell interactions as an element of the virulence regulatory network controlled by RpoS and LetA. Cell. Microbiol. **9:**2902–2920.
94. **Tomasz, A.** 1965. Control of the competent state in *Pneumococcus* by a hormone-like cell product: an example of a new type of regulatory mechanism in bacteria. Nature **208:**155–159.
95. **Vannini, A., C. Volpari, C. Gargioli, E. Muraglia, R. Cortese, R. De Francesco, P. Neddermann, and S. D. Marco.** 2002. The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. EMBO J. **21:**4393–4401.
96. **Visick, K. L.** 2005. Layers of signaling in a bacterium-host association. J. Bacteriol. **187:**3603–3606.
97. **Visick, K. L., and C. Fuqua.** 2005. Decoding microbial chatter: cell-cell communication in bacteria. J. Bacteriol. **187:**5507–5519.
98. **Visick, K. L., J. Foster, J. Doino, M. McFall-Ngai, and E. G. Ruby.** 2000. *Vibrio fischeri lux* genes play an important role in colonization and development of the host light organ. J. Bacteriol. **182:**4578–4586.
99. **von Bodman, S. B., D. R. Majerczak, and D. L. Coplin.** 1998. A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. stewartii. Proc. Natl. Acad. Sci. USA **95:** 7687–7692.
100. **von Bodman, S. B., J. K. Ball, M. A. Faini, C. M. Herrera, T. D. Minogue, M. L. Urbanowski, and A. M. Stevens.** 2003. The quorum sensing negative regulators EsaR and $ExpR_{Ecc}$, homologues within the LuxR family, retain the ability to function as activators of transcription. J. Bacteriol. **185:**7001–7007.
101. **Walters, M., and V. Sperandio.** 2006. Autoinducer 3 and epinephrine signaling in the kinetics of locus of enterocyte effacement gene expression in enterohemorrhagic *Escherichia coli*. Infect. Immun. **74:**5445–5555.
102. **Waters, C. M., and B. L. Bassler.** 2005. Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell Dev. Biol. **21:**329–346.
103. **West, S. A., A. S. Griffin, and A. Gardner.** 2007. Evolutionary explanations for cooperation. Curr. Biol. **17:**R661–R672.
104. **West, S. A., A. S. Griffin, A. Gardner, and S. P. Diggle.** 2006. Social evolution theory for microorganisms. Nat. Rev. Microbiol. **4:**597–607.
105. **West, S. A., S. P. Diggle, A. Buckling, A. Gardner, and A. S. Griffin.** 2007. The social lives of microbes. Annu. Rev. Ecol. Evol. Syst. **38:**53–57.
106. **White, C. E., and S. C. Winans.** 2007. Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **362:**1135–1148.
107. **White, C. E., and S. C. Winans.** 2007. The quorum-sensing transcription factor TraR decodes its DNA binding site by direct contacts with DNA bases and by detection of DNA flexibility. Mol. Microbiol. **64:**245–256.
108. **White, C. E., and S. C. Winans.** 2005. Identification of amino acid residues of the *Agrobacterium tumefaciens* quorum-sensing regulator TraR that are critical for positive control of transcription. Mol. Microbiol. **55:**1473–1486.
109. **Whitehead, N. A., A. M. Barnard, H. Slater, N. J. Simpson, and G. P. Salmond.** 2001. Quorum sensing in Gram-negative bacteria. FEMS Microbiol. Rev. **25:**365–404.
110. **Wu, H., Z. Z. Song, M. Givskov, G. Doring, D. Worlitzsch, K. Mathee, J. Rygaard, and J. N. Høiby.** 2001. *Pseudomonas aeruginosa* mutations in *lasI* and *rhlI* quorum sensing systems result in milder chronic lung infection. Microbiology **147:**1105–1113.
111. **Zhang, R. G., T. T. Pappas, J. L. Brace, P. C. Miller, T. Oulmassov, J. M. Molyneaux, J. C. Anderson, J. K. Bashkin, S. C. Winans, and A. Joachimiak.** 2002. Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. Nature **417:**971–974.
112. **Zhu, J., and S. C. Winans.** 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:**1507–1512.
113. **Zhu, J., M. B. Miller, R. E. Vance, M. Dziejman, B. L. Bassler, and J. J. Mekalanos.** 2002. Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*. Proc. Natl. Acad. Sci. USA **99:**3129–3134.