Cell

Bacterially Speaking

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Bacteria use a variety of means to communicate with one another and with their eukaryotic hosts. In some cases, social interactions allow bacteria to synchronize the behavior of all of the members of the group and thereby act like multicellular organisms. By contrast, some bacterial social engagements promote individuality among members within the group and thereby foster diversity. Here we explore the molecular mechanisms underpinning some recently discovered bacterial communication systems. These include long- and short-range chemical signaling channels; one-way, two-way, and multi-way communication; contact-mediated and contact-inhibited signaling; and the use and spread of misinformation or, more dramatically, even deadly information.

In the 300 years since van Leeuwenhoek's remarkable descriptions of the teeming world of microorganisms, bacteria have been regarded as deaf mutes going about their business without communicating with their neighbors. It was not until the 1960s and 1970s, with the discovery of what is now called quorum sensing, that it became evident that bacteria possess sophisticated systems of communication that enable them to send and receive chemical messages to and from other bacteria. In its simplest form, quorum sensing is a cell-cell communication mechanism by which bacteria count their own numbers by producing and detecting the accumulation of a signaling molecule that they export into their environment. We now know that quorum-sensing-mediated communication is more complicated than originally assumed and, furthermore, is but one of several mechanisms bacteria use to interact with other cells. Here, following a brief review of quorum sensing, we summarize recent developments in the field of cell-cell communication and interaction among bacteria and between bacteria and eukaryotes.

The number of these Animals [bacteria] in the scurf of a man's Teeth are so many that I believe they exceed the number of Men in a kingdom. For upon the examination of a small parcel of it, no thicker than a Horse-hair, I found too many living Animals therein, that I guess there might have been 1000 in a quantity of matter no bigger than the 1/100 part of a sand.

—Antony van Leeuwenhoek, 1684

One for All and All for One

The concept of intercellular communication within a bacterial population originates with the discoveries of Tomasz (Tomasz, 1965) on genetic competence in *Streptococcus pneumoniae* (then known as *Pneumococcus*) and Hastings (Nealson et al., 1970) on bioluminescence in *Vibrio*. Competence is a physiological state in which bacteria are capable of taking up and undergoing genetic transformation by DNA molecules. In 1965, Tomasz reported that entry into the competent state is governed by an extracellular factor that is manufactured by *Streptococcus* itself (Tomasz, 1965). Thus, the competence factor, which was later shown to be a modified peptide (below), was described as a "hormone-like activator" that synchronizes the behavior of the bacterial population. In 1970, Hastings showed that two obscure species of bioluminescent marine bacteria, *Vibrio fischeri* and *Vibrio harveyi*, produced light at high cell density but not in dilute suspensions (Nealson et al., 1970). Light production could be stimulated by the exogenous addition of cell-free culture fluids, and the component responsible, called the autoinducer, was later identified as an acyl-homoserine lactone (AHL; Eberhard et al., 1981) (Figure 1). The combined findings of Tomasz and Hastings suggested that certain bacteria use the production, release, exchange, and detection of signaling molecules to measure their population density and to control their behavior in response to variations in cell numbers. For nearly 20 years, these cell-cell signaling phenomena were considered anomalous occurrences restricted to a few specialized bacteria. It is now clear that intercellular communication is not the exception but, rather, is the norm in the bacterial world and that this process, called quorum sensing, is fundamental to all of microbiology.

How does quorum sensing work? As a population of quorum-sensing bacteria grows, a proportional increase in the extracellular concentration of the signaling molecule occurs. When a threshold concentration is reached, the group detects the signaling molecule and responds to it with a population-wide alteration in gene expression (Figure 2A). Processes controlled by quorum sensing are usually ones that are unproductive when undertaken by an individual bacterium but become effective when undertaken by the group. For example, in addition to competence and bioluminescence, quorum sensing controls virulence factor secretion, biofilm formation, and sporulation. Thus, quorum sensing is a mechanism that allows bacteria to function as multicellular organisms and to reap benefits that they could never obtain if they always acted as loners.

A chemical vocabulary has been established in which Gram-negative quorum-sensing bacteria such as *Vibrio* communicate with AHLs, which are the products of LuxI-type autoinducer synthases. These small molecules are detected by cognate cytoplasmic LuxR proteins that, upon binding their partner autoinducer, bind DNA and activate transcription of target quorum-sensing genes. By contrast, Gram-positive quorum-sensing bacteria, such as *Streptococcus* and *Bacillus*, predominantly communicate with short peptides that often contain chemical modifications. For example, the signaling molecule for genetic competence in *B. subtilis* ComX is a 6 amino acid peptide whose tryptophan residue has been modified by the attachment of a geranyl group (Figure 1; Okada et al., 2005). Signaling peptides such as ComX are recognized by membrane bound two-component sensor histidine kinases. Signal transduction occurs by phosphorylation cascades that ultimately impinge on DNA binding transcription factors responsible for regulation of target genes. In general, bacteria keep their AHL and peptide quorum-sensing conversations private by each speacies of bacteria producing and detecting a unique AHL (AHLs differ in their acyl side-chain moieties), peptide, or combination thereof.

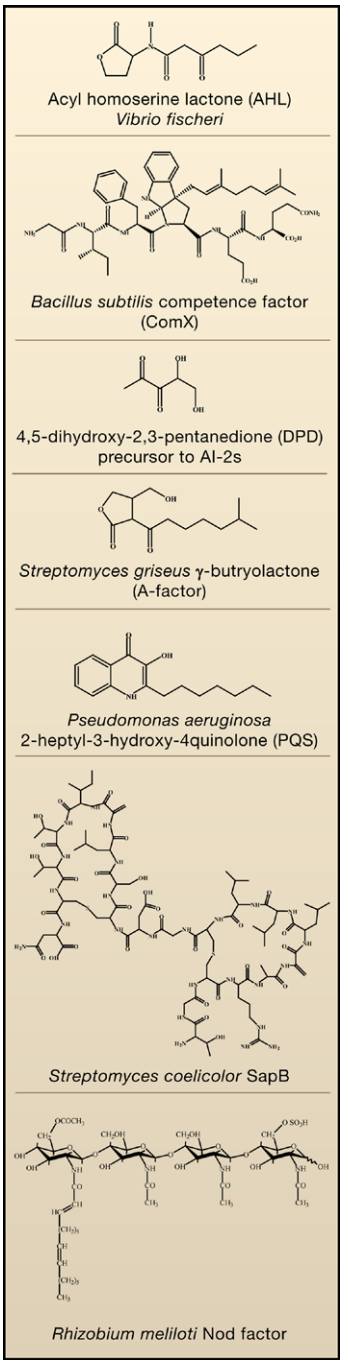

Figure 1. Representative Bacterial Molecules Involved in Intercellular Interactions

A *Vibrio fischeri* AHL autoinducer structure is shown as an example; however, a variety of acyl side chains exist in this family of autoinducers. Likewise, the *Bacillus subtilis* ComX peptide autoinducer is shown as a representative of the variety of peptides used by Gram-positive bacteria as autoinducers. The ComX and SapB structures were redrawn from images kindly provided by D. Dubnau and J. Willey, respectively.

AHLs and peptides represent the two major classes of known bacterial cell-cell signaling molecules. However, our appreciation of the complexity of the chemical lexicon is increasing as new molecules are discovered that convey information between cells. For example, a family of molecules generically termed autoinducer-2 (AI-2) has been found to be widespread in the bacterial world and to facilitate interspecies communication. AI-2s are all derived from a common precursor, 4,5-dihydroxy-2,3 pentanedione (DPD), the product of the LuxS enzyme (Figure 1). DPD undergoes spontaneous rearrangements to produce a collection of interconverting molecules, some (and perhaps all) of which encode information (Xavier and Bassler, 2005). Presumably, AI-2 interconversions allow bacteria to respond to endogenously produced AI-2 and also to AI-2 produced by other bacterial species in the vicinity, giving rise to the idea that AI-2 represents a universal language: a "Bacterial Esperanto." AI-2, often in conjunction with an AHL or oligopeptide autoinducer, controls a variety of traits in different bacteria ranging from bioluminescence in *V. harveyi* to growth in *Bacillus anthracis* to virulence in *Vibrio cholerae* and many other clinically relevant pathogens.

The streptomycetes, common soil-dwelling Gram-positive bacteria, use γ-butyrolactones (Figure 1) to control morphological differentiation and secondary metabolite production. The best studied of these signals, A-factor of *Streptomyces griseus* (one of the earliest recognized signaling molecules in bacteria), antagonizes a DNA binding repressor protein, ArpA, thereby promoting the formation of hair-like projections known as aerial hyphae and the production of the antibiotic streptomycin (Khokhlov et al., 1967; Onaka et al., 1995). Interestingly, γ-butyrolactones are structural analogs of AHLs; however, no crossrecognition of signals has been cited to date. *Myxococcus xanthus*, another soil bacterium, employs a mixture of amino acids derived from extracellular proteolysis as signaling molecules (Kuspa et al., 1992). *M. xanthus* monitors the environment

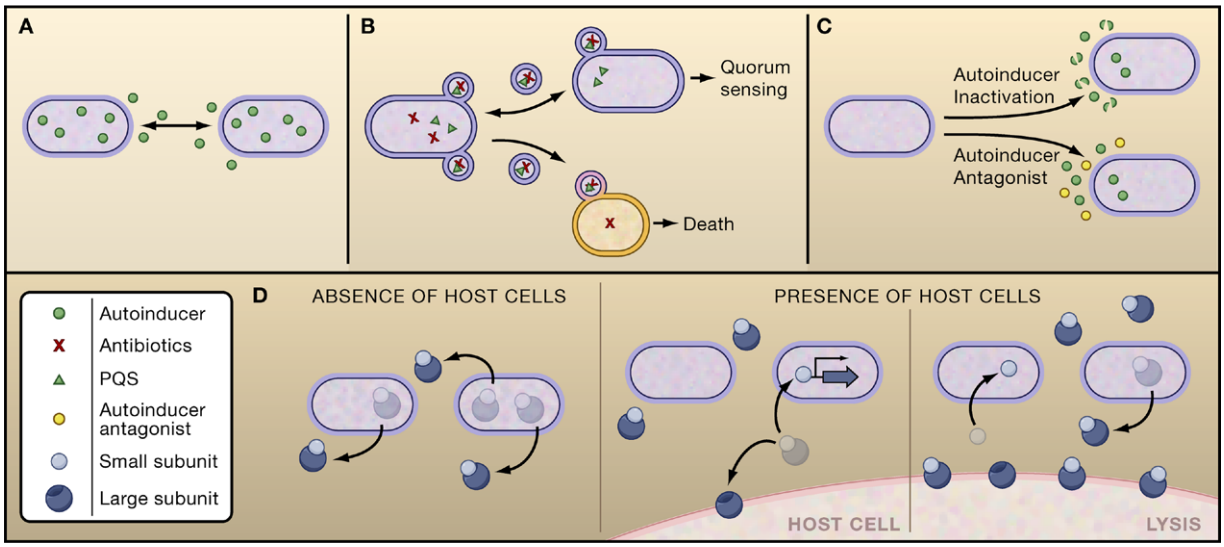

Figure 2. Bacterial Communication over Distances

(A) Quorum sensing. Quorum-sensing bacteria produce and respond to the extracellular accumulation of signal molecules called autoinducers (depicted as green spheres).

(B) Mixed messaging. Membrane vesicles traffic the *Pseudomonas aeruginosa* quinolone signal (PQS; depicted as green triangles) between cells. PQS facilitates group behavior when delivered to other *P. aeruginosa* cells, but other quinolones (X), also contained in the vesicles, are antibiotics that kill other bacterial species.

(C) Quorum quenching. Quorum-sensing bacteria are vulnerable to a variety of quorum-quenching mechanisms such as the enzymatic inactivation of autoinducers or the presence of autoinducer antagonists (yellow spheres), molecules with structure similar to autoinducers that prevent autoinducer detection and response.

(D) Conversations across kingdoms. *Enterococcus faecalis* produces a cytolysin composed of two subunits. The small cytolysin subunit acts as an autoinducer that monitors the environment for other *E. faecalis* cells. The large cytolysin subunit monitors the vicinity for eukaryotic host cells. Left: If no host cells are present, the two subunits form an inactive complex, and production of the subunits is held at a low basal level. Right: If host cells are present, the large subunit binds to the surface of the host cells, leaving the small subunit free to induce high-level production of both subunits, which together bind to target cells and cause them to lyse.

for the simultaneous presence of starvation conditions and trace amounts of certain amino acids (a mixture of tryptophan, proline, tyrosine, phenylalanine, leucine, and isoleucine is especially potent) prior to initiating the quorum-sensing cascade that culminates in a spore-filled fruiting body (discussed further below). Other molecules, including 3-OH palmitic acid methyl ester, cyclic dipeptides, and quinolones (see below), also have roles in bacterial cell-cell signaling (for review, see Waters and Bassler, 2005).

Sending Mixed Messages

Some quorum-sensing molecules, such as the quinolone signal (2-heptyl-3-hydroxy-4-quinolone) of *Pseudomonas aeruginosa* (PQS for *Pseudomonas* quinolone signal, Figure 1), are extremely hydrophobic (Pesci et al., 1999). This is problematic because PQS must travel from cell to cell in an aqueous environment. How does *P. aeruginosa* manage to disperse a water-insoluble signaling molecule in water? A solution to this mystery was recently reported (Mashburn and Whiteley, 2005). In a process reminiscent of eukaryotic packaging of cargo into vesicles that are trafficked between organelles, Gram-negative bacteria, such as *Pseudomonas*, pinch off 0.5 μm-sized vesicles from their outer membranes, and often these vesicles transport various kinds of macromolecules. Mashburn and Whiteley find that *Pseudomonas* packages PQS into vesicles derived from the bacterial membrane and the vesicles deliver the quinolones to neighboring cells (Figure 2B). Remarkably, PQS mediates its own packaging; mutants blocked in quinolone production fail to produce membrane vesicles but regain the capacity to do so when chemically synthesized PQS is supplied exogenously (Mashburn and Whiteley, 2005). In addition to PQS, the *Pseudomonas* vesicles contain other quinolones that function as antibiotics to kill other species of bacteria, such as *Staphylococcus epidermidis*. Thus, *P. aeruginosa* sends mixed messages: To its own kind, it emits signals that foster group behavior, but to other kinds of bacteria, the message delivered is fatal (Figure 2B).

Static in the Line

Recent discoveries of quorum-quenching mechanisms suggest that biological tit-for-tat matches have evolved for counteracting quorum-sensing bacteria. Presumably, anti-quorum-sensing strategies are deployed so that one species of bacteria can outcompete another quorum-sensing species or so that a eukaryote can fend off a quorum-sensing bacterial invader (Figure 2C). One such activity was discovered in soil samples assayed for interference with AHL detection and response in a

promiscuous AHL quorum-sensing-responsive reporter strain. The inhibitory activity was defined as a lactonase enzyme, AiiA, which cleaves the acyl moiety from the lactone rings of AHLs (Dong et al., 2000). AiiA is especially nonspecific with regard to acyl side chains, so it is believed to inactivate many AHL autoinducers. AiiA production is attributed to a variety of *Bacillus* species, which is noteworthy because *Bacillus spp.* are Gram-positive bacteria and use peptide autoinducers to communicate. Thus, *Bacillus* renders the Gram-negative bacterial community mute while managing to continue its own conversations uninterrupted. Analogous strategies have since been discovered. In a particularly insidious scheme, *Variovorax paradoxus* destroys AHLs with an AHL-degrading enzyme that functions by ring opening; after disabling quorum sensing in its foes, *V. paradoxus* consumes the linearized product and uses it to acquire carbon and nitrogen (Leadbetter and Greenberg, 2000). It remains to be established whether AHL degradation has significant consequences for bacterial signaling in the natural environment.

Interference with peptide signaling is also well known. *Staphylococcus aureus* relies on peptide quorum sensing for virulence, and strains of this pathogen are grouped according to the autoinducing peptide that they produce. Each autoinducing peptide, while activating the quorum-sensing cascade of the group that produces it, crossinhibits quorum sensing in the other groups (Figure 2C). The autoinducing peptides are extremely similar in structure, and crossinhibition occurs because the different peptides compete for binding to the autoinducer receptors (Lyon et al., 2002). Presumably, in mixed infections, the *S. aureus* group that is first to successfully establish its quorum-sensing cascade shuts down quorum sensing in the other groups and becomes the predominant group to colonize the host. This has been borne out in in vivo mouse abscess models in which mice injected with a particular *S. aureus* group are susceptible to infection, but not if the *S. aureus* group is coinjected with the autoinducing peptide from another *S. aureus* group (Wright et al., 2005).

Quorum-sensing interference has been reported for AI-2-mediated communication. Some bacteria, such as *Escherichia coli* and *Salmonella typhimurium*, possess AI-2-specific importers that are induced in response to high levels of AI-2 in the environment. AI-2 import eliminates the signal from the extracellular environment. In mixed-species consortia, because AI-2 molecules spontaneously interconvert, bacteria with AI-2 importers can consume both their own AI-2 and that produced by other species in the vicinity. This capability enables AI-2-consuming bacteria to interfere with other species' ability to accurately count the cell density of the population and to appropriately respond to changes in it (Xavier and Bassler, 2005).

Eukaryotes too appear to possess armaments that are specifically aimed at quorum-sensing bacteria. The seaweed *Delisea pulchra* produces halogenated furanones that are structural analogs of AHLs. These molecules bind to LuxR-type transcription factors and cause their proteolysis (Manefield et al., 2002). Reactive oxygen and nitrogen intermediates produced by NADPH oxidase, an important constituent of the mammalian immune system, inactivate *S. aureus* autoinducing peptides in a mouse virulence model system (Rothfork et al., 2004). Paraoxonase enzymes hydrolyze esters and, in humans, are considered to have important antiatherogenic functions. The human paraoxonase (PON) family has three members: PON1, PON2, and PON3. Although their endogenous substrates have not been defined, it is clear that they are lactonases. At least PON2 is capable of inactivating a variety of bacterial AHL autoinducers, suggesting that humans possess anti-quorum-sensing capabilities (Draganov et al., 2005). However, a direct in vivo link between PON2 and AHL inactivation awaits experimental verification.

Intimate Conversations

Some bacteria, as we have discussed, converse over a distance by exchanging diffusible signals that allow members of a community of cells to communicate (quo-

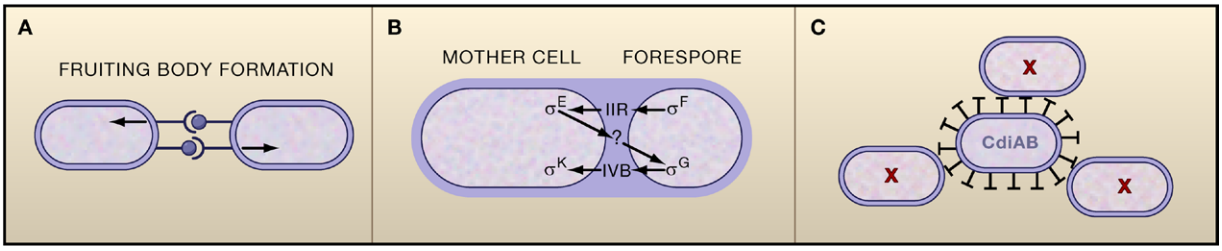

Figure 3. Intimate Bacterial Conversations

(A) Reciprocal C signaling in *Myxococcus xanthus*. The surface-displayed C signal protein interacts with a hypothetical receptor on an adjacent cell to transmit a signal that promotes fruiting-body formation.

(B) Crisscross signaling in *Bacillus subtilis*. Sporulation takes place in a two-compartment sporangium in which three intercellular signals, the secreted signaling proteins SpoIIR (IIR) and SpoIVB (IVB) and an unknown signal (?), are exchanged back and forth. For simplicity, the illustration does not convey that the second and third signaling events take place after the smaller cell has been engulfed by the larger cell.

(C) Contact-dependent inhibition in *Escherichia coli*. Cells producing the proteins CdiAB form aggregates with, and inhibit the growth of, cells lacking the genes for the growth-inhibiting proteins.

rum sensing). At the opposite extreme are short-range signals that require direct contact between individual cells for information exchange. One classic example of this is C signaling, which is critical for multicellular fruiting-body formation by *M. xanthus* (Figure 3A). Cells of this social bacterium move on surfaces by gliding motility in a manner in which individual cells often reverse direction. C signaling promotes a coordinated gliding behavior known as streaming, in which reversals are suppressed. Streaming culminates in the formation of a mound of cells within which spore formation takes place. The C signal is a 17 kDa protein that is derived by proteolysis from a larger precursor (Kim and Kaiser, 1990a, 1990c; Lobedanz and Sogaard-Andersen, 2003). Elegant experiments in which cells were artificially forced into alignment in tiny grooves on an agar plate showed that C signaling requires contact between cells. It has been hypothesized that the function of C signaling is to report on cell alignment, which takes place during the aggregation phase of fruiting-body formation (Julien et al., 2000; Kim and Kaiser, 1990b). C signaling also governs the expression of numerous genes required for spore formation. A cell-surface receptor (perhaps located at the cell poles) is presumed to be required for C signaling, but the putative receptor and the downstream signal-transduction system remain unknown.

The concept that *M. xanthus* cells are capable of intimate interaction is reinforced by the recent dramatic demonstration that particular motility-associated, GFP-tagged outer-membrane lipoproteins readily exchange between *M. xanthus* cells (Nudleman et al., 2005). This finding suggests that *M. xanthus* cells are, at a minimum, capable of at least briefly fusing their outer membranes and sharing outer-membrane proteins.

Exactly how C signaling suppresses gliding reversals remains largely unknown, but an important insight comes from recent cytological studies on the protein FrzS, which is required for directed movement (Mignot et al., 2005). Gliding is mediated by fiber-like pili that extend from one end of the cell and use their tips like a harpoon to latch on to the substratum or another cell. Movement is subsequently achieved by retraction of the pili. The bacteria reverse direction by disassembling pili at one cell pole and reassembling pili at the other pole. FrzS oscillates from pole to pole, evidently traveling along an undefined, cytoskeletal track. Conceivably, FrzS is part of a pilus-assembly complex that alternately governs pilus construction at one pole and then the other. If so, a downstream consequence of C signaling might be suppression of this pole-to-pole oscillation.

An extreme example of intimate cell-to-cell signaling occurs during the process of spore formation in *Bacillus subtilis*. Spores are formed in a two-chamber sporangium that consists of a forespore that ultimately becomes the spore and a mother cell that nurtures the developing spore. Early in sporulation, the forespore and mother cell lie side by side, but later in development, the mother cell wholly engulfs the forespore to create a cell

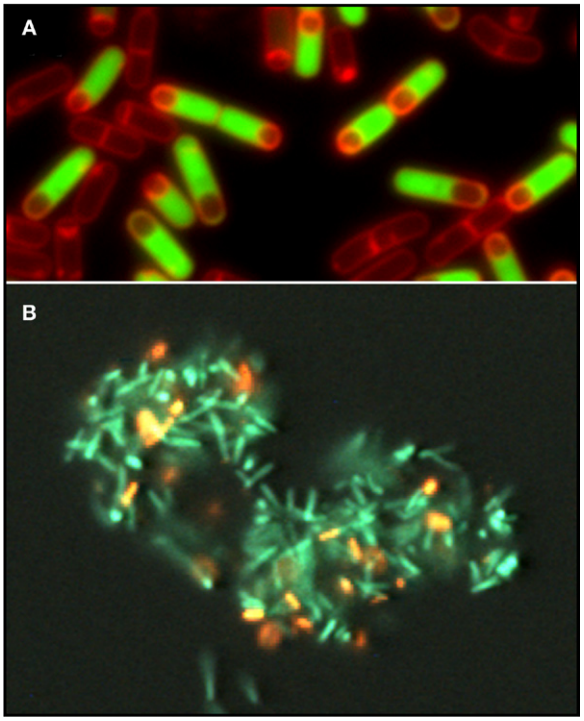

Figure 4. Intercellular Signaling in *B. subtilis* and Contact-Dependent Inhibition by *E. coli*

(A) A fluorescence micrograph of a field of sporulating *B. subtilis* cells harboring *gfp* fused to a promoter controlled by σ^E. Cell membranes were stained with TMA-DPH and were false colored red. Green fluorescence is observed in the mother-cell compartment of sporangia in which σ^E was activated by a signal emanating from the smaller, adjacent forespore compartment (see text and Figure 3B). The experiment was carried out and kindly provided by T. Doan and D. Rudner.

(B) A fluorescence micrograph of an aggregate of *E. coli* inhibitor cells labeled green with GFP and noninhibitor (sensitive) cells labeled red with DsRed. The inhibitor cells produce the contact-dependent inhibitor proteins CdiA and CdiB (see text and Figure 3C). The experiment was carried out and kindly provided by S. Aoki and D. Low.

within a cell. Thus, during sporulation, the forespore and the mother cell are in intimate contact across the membranes where the cells abut each other. Each of these cells follows its own distinctive program of gene expression, but the two lines of gene expression are not independent of one another. Rather, they are linked in criss-cross fashion by three intercellular signaling pathways (Figure 3B and Figure 4A; for reviews, see Losick and Stragier, 1992; Rudner and Losick, 2001). A secreted signaling protein (SpoIIR) produced in the forespore under the control of the forespore transcription factor σ^F triggers the appearance of σ^E in the mother cell. Next, σ^E sets in motion a poorly understood chain of events that activates σ^G in the forespore. Finally, σ^G directs the synthesis of SpoIVB, a secreted signaling protein that triggers the appearance of σ^K in the mother cell.

A case of an "in your face" conversation is contact-mediated growth inhibition in *E. coli* (Aoki et al., 2005). Certain wild strains of *E. coli* inhibit the growth of stan-

dard laboratory strains (*E. coli* K12) by a mechanism known as contact-dependent inhibition. Inhibition is mediated by a pair of proteins called CdiA and CdiB (for contact-dependent inhibitors A and B) that resemble two-partner secretion proteins that are exported to the cell surface by a pathway involving proteolytic processing. When the genes for CdiA and CdiB are introduced into *E. coli* K12, the laboratory strain acquires the capacity to cause growth inhibition in a manner that requires cell-to-cell contact (Figure 3C and Figure 4B). Contact dependence was demonstrated in an elegant experiment in which the inhibitory cells were separated from the sensitive cells by a porous membrane. Pores of 0.4 μm prevented growth inhibition, whereas pores of 0.8 μm (large enough to allow cells to slip through) did not. Furthermore, inhibitory cells were shown to form aggregates with sensitive cells in a manner that depended on the Cdi proteins. Importantly, sensitive cells displaying surface pili are resistant to growth inhibition, presumably because the pili keep the inhibitory cells at a safe distance. The physiological significance of contact-dependent inhibition is unclear, but an attractive possibility is that it functions in the regulation of growth of specific cells in complex communities of bacteria.

Communal Living

Most of the examples we have considered so far involve interactions among large numbers of bacteria in populations existing in liquid environments. Many bacteria are also capable of coordinating their behavior to form sessile communities consisting of large numbers of densely packed cells. These architecturally complex communities, called biofilms, form on surfaces or at air-liquid interfaces. Cells in biofilms are typically held together by an extracellular matrix composed of polysaccharides, protein, and often DNA. As in communes, all of the members of these bacterial communities cooperate in the construction of the biofilm by contributing matrix components.

An interesting twist on communal living has recently been reported in *Vibrio cholerae*, the causative agent of cholera (Meibom et al., 2005). *V. cholerae* exists both free swimming in the ocean and also as a constituent of biofilms, frequently on chitin-containing exoskeletons. *V. cholerae* feeds on this solid polymer of *N*-acetylglucosamine, during which time it acquires DNA by natural transformation (DNA is known to be present in biofilms at concentrations above 100 μg/ml). DNA uptake from the environment is postulated to allow *V. cholerae* to obtain new genes, thereby diversifying its genome. Genes encoding type IV pili and homologs of genes required for genetic competence in Gram-positive bacteria are induced in the presence of chitin and are necessary for *V. cholerae* transformation. An intact quorum-sensing cascade is also a prerequisite for transformation on chitin. These studies demonstrate for the first time that *V. cholerae* has natural competence, that bacterial evolution could be occurring on interfaces in which bacteria are in direct contact with surfaces, and finally that this process is driven by cell-cell signaling.

Another fascinating example of communal living is involved in aerial mycelium formation in streptomycetes, fungus-like bacteria that undergo a complex process of morphological differentiation. During this process, an aerial mycelium is erected that consists of hair-like filaments that project from the surface of the colony. The colony is composed of a branching network of hyphae known as the substrate mycelium. Contributing to the erection of the aerial hyphae are cell-surface proteins and, interestingly in the present context, lantibiotic-like peptides known as SapB (in *Streptomyces coelicolor*; Figure 1) and SapT (in *Streptomyces tendae*). SapB and SapT, which contain the defining thioester bridge of lanthionines, accumulate extracellularly during aerial mycelium formation, serving as surfactants to facilitate the release of nascent aerial hyphae from the substrate mycelium (Kodani et al., 2004; Willey et al., 2006). Thus, as in the case of quorum-sensing molecules, the accumulation of SapB and SapT reflects the cooperative activity of the bacterial community. Unlike quorum-sensing molecules, SapB and SapT are not signals; rather, they are morphogenetic peptides that play a mechanical role in development.

Fratricide

Since the discovery of penicillin by Sir Alexander Fleming in 1928, it has become widely recognized that microorganisms engage in chemical warfare in which one species wards off other species through the production and release of antibiotics and other antimicrobial agents. Sometimes, as in the case of the bacteriocins, bacteria produce agents that kill other strains of the same species. Recently, however, two radical examples of fratricide have come to light in which some members of a genetically identical population of cells kill other members (siblings) of the same population: cannibalism in *Bacillus subtilis* and allolysis in *Streptococcus pneumoniae*.

Spore formation by *B. subtilis*, which is triggered by nutrient limitation, is an elaborate developmental process that takes place over the course of 7 to 10 hours and involves the conversion of a growing cell into a dormant cell type (a spore) that can remain inert for many years. Therefore, it comes as no surprise that the decision to form a spore is a life-or-death one. A *B. subtilis* cell could be at a considerable disadvantage if it commits to spore formation in response to what turns out to be a brief fluctuation in nutrient availability. To guard against this possibility, the bacterium deploys a system of cannibalism in which cells that have entered the sporulation pathway forestall the absolute commitment to spore formation (Gonzalez-Pastor et al., 2003).

At the heart of the cannibalism system is a bistable switch that governs the activation of the master regulator for entry into sporulation, Spo0A. In response to nutrient limitation, about half of the *B. subtilis* cells activate Spo0A and enter the pathway leading to sporulation, and the other (Spo0A-inactive) cells do not. Cells that

have activated Spo0A produce and export a killing factor and a protein toxin that together kill nonsporulating siblings. Their deaths result in the release of nutrients that, in turn, delay or reverse progression into sporulation by the cells that have activated Spo0A. When no siblings remain to be cannibalized and no other sources of nutrients become available, development progresses to the point that spore formation becomes irreversible.

Interestingly, and pertinent to the theme of this review, the response to the cannibalism toxin involves an intercellular chemical signaling system of unusual simplicity (Ellermeier et al., 2006). To avoid suicide, toxin-producing cells (i.e., Spo0A-expressing cells) simultaneously produce a membrane bound immunity protein that neutralizes the toxin in the membrane. The immunity protein dually functions in signal transduction and in protection from the toxin. The immunity protein is encoded by a two-gene operon that also contains the gene for an autorepressor. When bound to the toxin, the immunity protein sequesters the autorepressor, thereby derepressing the operon and initiating synthesis of increased immunity protein and autorepressor. Thus, accumulation of immunity protein that is not bound to the toxin leads to unsequestered repressor, which functions to downregulate expression of the operon and reset the system.

The second example of fratricide is the allolysis behavior of the pathogen *S. pneumoniae* (Guiral et al., 2005; Håvarstein et al., 2006). This Gram-positive bacterium is commonly found in the nasopharynx but also causes a variety of invasive diseases including pneumonia, bacteremia, otitis media, meningitis, and sinusitis. As discussed above, *S. pneumoniae* is known for its ability to enter into a state of genetic competence under conditions of high cell population density in response to a secreted signaling peptide. Analogous to the case of *B. subtilis* sporulation, only a fraction of the *S. pneumoniae* cells in the population become competent in response to the peptide autoinducer. Those that do so elaborate a bacteriocin that causes the lysis of noncompetent cells in the population. What is the purpose of preying on genetically identical siblings? Claverys and coworkers (Guiral et al., 2005; Håvarstein et al., 2006) report that the lysed cells release not only transforming DNA and nutrients but also pneumolysin and other factors important for virulence. Thus, rather than relying on self for secretion of virulence factors, *S. pneumoniae* sacrifices some its relatives for this purpose which facilitates invasion of its host.

Conversations across the Divide

Not only do bacteria sense one another's presence and use chemical signals to communicate, there is new evidence suggesting that some bacteria also detect their eukaryotic hosts. *Enterococcus faecalis* is a Gram-positive bacterium that is a commensal of the human intestine. It is also an opportunistic pathogen involved in urinary tract infections, bacteremia, and infective endocarditis. Pathogenesis depends on a cytolysin composed of two nonidentical peptides (the small subunit, $CylL_S$, and the large subunit, $CylL_L$) that are posttranslationally modified, secreted, and activated. Enterococcal cytolysin is related to lantibiotics, and it is lethal to a broad range of prokaryotic and eukaryotic cells. Interestingly, the smaller of the two peptides ($CylL_S$) making up the cytolysin doubles as a peptide autoinducer in the quorum-sensing induction of the operon encoding the cytolysin and the genes required to modify it (Haas et al., 2002). Two proteins, CylR1 (a predicted membrane protein) and CylR2 (a predicted DNA binding protein) repress transcription of the cytolysin genes, and derepression occurs in response to the cell-density-dependent extracellular accumulation of the $CylL_S$ peptide.

Surprisingly, although the small cytolysin subunit is used to monitor bacterial cell density, the large cytolysin subunit appears to be used to monitor the environment for host cells (Figure 2D). The basis for this discovery was the observation that cytolysin activity is dependent upon the presence of target cells, such as erythrocytes. Gilmore and colleagues report that in the absence of target cells, $CylL_L$ and $CylL_S$ form a stable complex that is inactive for autoinduction and for lysis of target cells (Coburn et al., 2004). Importantly, however, $CylL_L$ has a higher affinity for target cells than for $CylL_S$. It is speculated that in the presence of host cells, $CylL_L$ differentially adsorbs to host cells. Titration of $CylL_L$ by the host cells leaves $CylL_S$ free to accumulate and to act as an autoinducer that promotes high-level synthesis of $CylL_S$ and $CylL_L$, which, in turn, cause the target cells to lyse, presumably by the formation of a pore.

In addition to bacteria detecting host cells, there are examples of interkingdom chemical communication in which the host perceives the presence of the bacterial cells. As discussed above, some eukaryotes perceive hostile quorum-sensing bacteria and take measures to confound them. Another hostile eukaryotic-prokaryotic dialogue occurs between dicotyledonous plants and the pathogenic bacterium *Agrobacterium tumefaciens*. In this case, the bacterium relies on host- and self-produced cues. In response to host-produced signals, the bacterium transfers a fragment of DNA called T-DNA to host cells. The T-DNA forces the host to commence production of opines that, in turn, feed the bacteria. Bacterially produced quorum-sensing signals induce plasmid transfer between the bacterial community, increasing its overall infectivity (Zhu et al., 2000).

Eukaryotes in symbiotic associations with bacteria also participate in the conversation, but they use friendly verbiage. For example, in the initial stages of symbiosis between *Rhizobium meliloti* and its plant host, the earliest signals come from the plant. Plant roots release flavonoid molecules that *Rhizobium* detects in a compartment called the rhizosphere (Peters et al., 1986). In response to these plant signals, *Rhizobium* activates transcription of *nod* genes that are under control of the NodD DNA binding protein. The *nod* genes encode

enzymes that produce Nod factor, a bacterial signal (Figure 1) that induces additional plant processes required for the development of nodules (Mulligan and Long, 1985). Nodules provide the bacteria with a location that has conditions appropriate for nitrogen fixation, which, in turn, benefit the plant. Presumably, similar two-way conversations occur in many other bacterial-eukaryotic encounters, both affable and antagonistic.

I Understand You

The signaling mechanisms we have considered thus far are apparently unique to bacteria. Remarkably, the Gram-negative bacterium *Providencia stuartii* provides an example of an intercellular signaling system that is used by both prokaryotes and eukaryotes. *P. stuartii* is a quorum-sensing pathogen that causes nosocomial infections in humans. The structure of the *P. stuartii* signaling molecule is not known, but it appears to be a peptide. A genetic screen for *P. stuartii* mutants incapable of extracellular autoinducer production revealed the gene *aarA*. AarA has homology to Rhomboid (RHO), which has been primarily studied in *Drosophila melanogaster* (Rather et al., 1999). In flies, RHO, a serine protease, is necessary for the intramembrane cleavage, extracellular release, and activation of ligands for the epidermal growth factor (EGF) receptor. EGF receptor signaling, in turn, is required for specification of cell fate. Consistent with this, *D. melanogaster rho* mutants are defective in several developmental processes including the formation of the correct number of veins and their proper positioning in wings and the organization of the compound eye. Evidence that AarA and RHO have a shared function in extracellular signaling comes from the extraordinary finding that expression of *P. stuartii aarA* in a *D. melanogaster rho* mutant rescued wing-vein development, and, in the reciprocal experiment, introduction of *D. melanogaster rho* into a *P. stuartii aarA* mutant restored quorum-sensing signal production (Gallio et al., 2002). In an extension of the above analysis, eight prokaryotic Rhomboids were tested for the ability to cleave three *D. melanogaster* EGF receptor ligands; five of these trials proved successful. The different rhomboids tested shared only minimal sequence homology; however, all contained the putative serine catalytic triad residues required for activity, and, in every case, amino acid substitutions at these residues destroyed the proteins' function in signal production (Urban et al., 2002). There are over a hundred known rhomboids in archaea, bacteria, and eukaryotes, but none of the microbial functions are known. The above results suggest that these diverse proteins have a conserved enzymatic function, as well as a conserved role in cell-cell communication.

Since the activator—a cell-produced chemical—seems to impose a high degree of physiological homogeneity in a pneumococcal population with respect to competence, one is forced to conclude that in this case [a] bacterial population can behave as a biological unit with considerable... coordination among its members....
One wonders whether this kind of control may not be operative in some other microbial phenomena also.

—Alexander Tomasz, 1965

Conclusions: A Continuing Conversation

Alexander Tomasz' early impressions that bacterial cell-cell signaling could be an organizing principle underlying multicellular behavior were amazingly accurate. What he could not have imagined, and what we are only now beginning to appreciate, is the tremendous complexity in the extracellular chemical milieu that bacteria experience and interpret to garner information about their growth status and potential; their cell numbers; those of their neighbors; and the presence or absence of eukaryotes, both friend and foe. We now understand that there can be one-way, two-way, and multi-way chemical dialogues, as well as the spread of misinformation and the delivery of deadly messages. Chemical conversations span species and kingdoms, and the particular molecules used to convey particular pieces of information appear optimized for their specific purpose: long- versus short-range messaging, contact-mediated or -inhibited signaling, or intra- or extracellular delivery. Increasingly, the molecular mechanisms underpinning bacterial cell-cell signaling begin to resemble those employed by eukaryotes for temporal and spatial patterning, suggesting a shared evolutionary history.

Looking ahead, two considerations lead us to believe that our understanding of the bacterial lexicon is primitive. First, bacteria are known to produce an extraordinarily diverse array of small organic molecules with antibacterial, antifungal, antiviral, and immunomodulatory activities. It is generally believed that these agents serve as armamentaria in the internecine warfare that bacteria wage against each other and against other microbes in the environment. But recent studies show that at subinhibitory doses, antibiotics such as rifampicin and erythromycin cause global changes in gene-expression patterns in bacteria, and many genes of unknown function are affected (Goh et al., 2002; Tsui et al., 2004). Based on this, Davies and colleagues have suggested that many bacterially produced antibiotics may not be released exclusively for purposes of chemical warfare (Goh et al., 2002; Tsui et al., 2004). Rather, these chemicals may act as signals in habitats where their local concentrations are well below those required to inhibit growth. Our

second consideration stems from estimates suggesting that only a minute fraction of the Earth's bacteria have been identified, much less cultivated in the laboratory. Indeed, bacteria and their viruses far and away represent the greatest repository of genetic diversity on the planet. Provocative new approaches, collectively known as metagenomics, in which DNA from various environments is directly cloned into surrogate hosts is providing access to this rich vein of genetic information (Brady et al., 2004; Handelsman et al., 1998; Rondon et al., 2000). With it come completely novel biosynthetic pathways for previously unknown natural products, many of which are small organic molecules. Some molecules discovered through metagenomics exhibit signaling activity. By extrapolation, it seems reasonable to suppose that vast numbers of microbially produced, small organic molecules with intercellular signaling activity await discovery. If so, we may have just begun to eavesdrop on a microbial agora: a bustling polyglot of chemical languages, each with its own biological story to tell.

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