Introduction to Bacterial Signals and Chemical Communication

Helen Blackwell was born in Ohio and attended Oberlin College for her undergraduate studies, graduating with highest honors in chemistry in 1994. She pursued her graduate studies in organic chemistry at the California Institute of Technology with Robert Grubbs. Helen received her Ph.D. in 1999 and then spent three years as a postdoctoral fellow in the lab of Stuart Schreiber at Harvard University. There, she honed her interests and skills in chemical biology. In 2002, she returned to the Midwest and joined the faculty of the University of Wisconsin—Madison, where she is currently Associate Professor of Chemistry. Helen has established a research program at the very interface of organic chemistry and bacteriology. Her broad goal is to understand the role of chemical signals in host—bacterial interactions and infectious disease, and most recently has focused on quorum sensing pathways in Gram-negative bacteria. Her laboratory has developed a range of synthetic compounds that strongly modulate AHL-based signaling pathways and control multicellular bacterial behaviors.

Clay Fuqua obtained his undergraduate degree in Biology from Old Dominion University in 1986 and his Ph.D. in Microbiology from the University of Maryland in 1991. At Maryland he worked with microbial ecologist Ronald Weiner on the mechanism of a bacterial—invertebrate interaction. During his postdoctoral work with Stephen C. Winans at Cornell University (1991—1995), he discovered, along with several other laboratories over this period, a process of interbacterial communication, now commonly known as quorum sensing. Dr. Fuqua is now a Professor in the Department of Biology, Indiana University—Bloomington, where his diverse research group studies the mechanisms and outcomes of microbial interactions. Current work combines biochemical, molecular genetic, genomic, and ecological approaches to the study of microbial interactions, with the long-term goal of controlling both harmful and beneficial microbial activity.

This thematic issue of *Chemical Reviews* focuses on the surprising and widespread mechanisms of communication discovered among microorganisms. Although microorganisms such as bacteria embody the unicellular mode of life, they can exhibit a striking array of multicellular activities. Underlying many of these behaviors is the ability to communicate through the release of chemical compounds into the environment that are subsequently perceived by other microorganisms in the vicinity and to which they can respond. For the purpose of this issue, we will refer to these compounds as signals. Such chemical communication mechanisms are now known to be prevalent among the bacteria, and several different bacterial languages have been well studied. Bacterial signaling mechanisms are of significant interest, particularly in the areas of human and veterinary health and in agriculture, where they often play important roles in bacterial interactions with their multicellular hosts. It has been known for many years that eukaryotic hosts can perceive bacteria, and in turn some bacteria can perceive their hosts. However, the scientific community has recognized only recently that bacteria often communicate with each other as part of their interaction with host organisms. Bacteria can coordinate their activities via communication mechanisms, exhibiting distributive behaviors that allow them to more effectively colonize and manipulate host organisms, such as in disease and symbiosis. In many cases, the virulence attributes of bacteria are controlled, at least in part via chemical communication. Jamming such bacterial communication systems has therefore become an attractive target for intervention strategies to mitigate and treat infectious disease. Many bacteria are thought to utilize these chemical signaling systems to control cellular behaviors in response to local bacterial population density, a process that has come to be described as "quorum sensing". Quorum sensing mechanisms are often invoked for interbacterial signaling systems, although there are a limited number of bacteria for which a role in population density sensing has been directly proven. Even so, the term quorum sensing is often used synonymously with both signal release and perception.

Bacterial cell—cell signaling is both fundamentally chemical and biological by its very nature. This special issue of *Chemical Reviews* highlights several bacterial communication systems, with review articles contributed by leading chemists and biologists working in this area. We do not attempt to comprehensively span the growing diversity of recognized bacterial signaling systems but instead illustrate the depth of understanding for specific examples and complement this with coverage of several emerging communication systems, some in which the role of the diffusible molecule may blur the distinction between a signal and other extracellular functions. Notably, several reviews herein explore the potential to interfere with and block bacterial communication systems via targeted chemical and biochemical approaches. Such approaches have the potential to provide new insights into bacterial signaling from both fundamental and applied perspectives.

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Among the large and diverse *Proteobacteria* group, a widespread signaling mechanism is based on *N*-acylated L-homoserine lactones (AHLs). The *Proteobacteria* include many of the bacteria historically designated as Gram-negative and have double membrane bilayers (inner and outer). AHLs were originally discovered in species of the marine bacterial genus *Vibrio* as regulators of bioluminescence. These signals are typically generated by LuxI-type synthases and perceived by cognate LuxR-type receptors that behave as transcription factors; AHL:LuxR-type receptor complexes regulate the expression of genes that the bacteria express preferentially under inducing conditions, often at high cell densities. AHL signals have now been identified in a wide range of *Proteobacteria* and have even been reported for Bacteriodetes and cyanobacteria, both of which are outside of the *Proteobacteria*. The response to intercellular, quorum-sensing signals can be quite different among distinct bacterial species, and a significant number of bacteria have multiple, often interlinked, AHL quorum sensing signals.

AHL-based signaling systems have been studied intensely over the past two decades, and a great deal of detail has been revealed, although much remains to be learned. The research on AHLs has been propelled by microbiologists and chemists, and more recently by structural biologists. Microbiologists **Stevens and von Bodman** have collaborated with chemists **Doutheau, Queneau, and Soulère**, combining their expertise to describe the molecular genetic and chemical attributes of AHL-based quorum sensing systems, with an eye toward developing chemical antagonists that can block native AHL action. Their review combines the chemistry and biology of AHL signaling to create an informative example of cross-disciplinary synergy in considering a complex problem.

In contrast, **Spring and co-workers** examine AHL-type quorum sensing from a decidedly more chemical biology perspective and extend similar coverage to the widespread signal compound known as AI-2 (autoinducer 2). AI-2 was discovered by analyzing certain marine *Vibrio* species, the same species in which AHL signals were first discovered. In fact, we now know that a diverse range of bacteria produce AI-2-type compounds, derived from dihydroxypentanedione (DPD). DPD spontaneously forms a furanosyl ester that may or may not be conjugated with boron in its active form. DPD is a byproduct of the activated methyl cycle, and its synthesis is specified by a conserved gene called *luxS*, encoding a *S*-ribosylhomocysteinase that normally functions in the cycle. Along with their description of AHL-based quorum sensing, Spring and co-workers also explore the potential approaches for chemical interference in AI-2 systems.

Structural biologists **Churchill and Chen** provide an in depth examination of the limited amount of structural data on AHL-based signaling systems. Synthesis of AHL signals by LuxI-type enzymes, and the subsequent perception of these signals by LuxR-type transcription factors, is comprehensively described at the structural level, revealing many fundamental attributes of these critical processes. In addition, the authors provide interesting insights into the interactions between LuxR-type proteins and specific antiactivators, proteins that effectively "shackle" these transcription factors into an inactive form.

Decho, Frey, and Ferry outline a very different perspective on AHL-based quorum sensing relative to the reviews introduced above, examining the interactions of these signaling systems with their environment. Many environmental factors impact the stability and activity of AHL signals in the environment, and it is a mistake to consider AHLs as simply signals to inform cells about population density. The review by Decho et al. comprehensively and adeptly considers the impact of the overall environment on signaling via AHLs. Another important environment for many bacteria is that offered by their respective host organisms. **Teplitski, Mathesius, and Rumbaugh** describe several examples where eukaryotic organisms either respond to or degrade bacterial signal molecules, or even produce chemical analogues of these molecules. There is now abundant evidence that mammalian cells can interfere with bacterial signaling pathways and that the bacterial signals in turn can act to modulate the immune response. Likewise, certain plants and other nonbacterial organisms have also evolved ways to combat bacterial signaling systems. This informative review considers many of these examples, their consequences, and their prospects for a variety of applications in both bacterial and eukaryotic contexts.

Five separate reviews in this issue describe the fascinating diversity of signaling and related processes among different bacteria and expand our consideration of bacterial communication beyond the intensely studied AHL and AI-2 systems. Gram-positive bacteria (an important bacterial group with a single membrane bilayer and a thick cell wall) often utilize oligopeptides as signaling molecules, and many of these signaling systems are also well understood. The oligopeptide signals may be cleavage products of larger precursor proteins or may be synthesized de novo. Many contain novel modifications to their constituent amino acids residues. One of the most important and well-studied oligopeptide-based systems is that used by the ubiquitous mammalian pathogen *Staphylococcus aureus*, comprehensively reviewed by **Thoendel, Kavanaugh, Flack, and Horswill**. This review takes a detailed look at the synthesis of the cyclic thiolactone peptide signal (or AIP; autoinducing peptide), its perception, and its profound effect on *S. aureus* virulence. The authors comprehensively outline current knowledge in the field, astutely note areas that are poised for growth, and place these signaling systems within a broader context of the signaling systems in related Gram-positive bacteria.

A different family of bacterial signals, the 4-quinolones, is the focus of the review from **Huse and Whiteley**. Here, the authors draw an intriguing analogy between these bacterial signals and the popular multifunctional "smart phone" communication devices used by humans. The similarity drawn is that both smart phones and certain bacterial signals can serve multiple purposes. Most of the work on the quinolone signals has been performed in the opportunistic pathogen *Pseudomonas aeruginosa,* and thus this bacterium is the primary focus of the review. Many of the *P. aeruginosa* quinolones have roles in physiological processes distinct from signaling, including antibiotic activity, and this review provides several excellent examples of this multifunctionality.

Species of the proteobacterial genus *Xanthomonas* have been known for some time to produce a diffusible signaling factor (aptly named DSF) that regulates many aspects of its virulence on plants. The structure of DSF was determined to be *cis*-11-2-methyl-2-dodecenoic acid (an α,β-unsaturated fatty acid) and is strikingly similar to farnesoic acid, a morphogenetic quorum sensing cue from the single-celled eukaryote *Candida albicans*. **Deng, Wu, Tao, and Zhang** contribute a fascinating review that details the discovery of DSF as a bacterial signal that is capable of cross-kingdom function. More and more bacteria have been found to produce

and respond to DSF-type signals, and their potent modulatory activity on bacterial behaviors such as biofilm formation has been well established. This review provides a valuable synopsis of a rapidly emerging area of the signaling field.

Species of the filamentous fungus-like bacterial genus *Streptomyces* undergo a transition from vegetative filaments (hyphae) that grow through the substrata (e.g., growth medium in the laboratory) to aerial hyphae that extend off the surface and produce environmentally resistant spores. This developmental transition also coincides with the production of potent antibiotics, and the *Streptomyces* represent an important source for many of the antibiotics in use today. The processes of development and antibiotic production both rely on signal molecules that are released into the environment. The review from **Willey and Gaskell** deftly describes these signals and their role in *Streptomyces*. The γ-butyrolactones represent one such class of signaling molecules that regulate antibiotic production along with other aspects of secondary metabolism and appear to function as true quorum sensing cues. In contrast to these lactones is the SapB surfactant peptide. This peptide blurs the distinction between a signal and an extracellular effector, as SapB can clearly be exchanged between cells in a population, but largely seems to function physically in promoting the escape of filaments from the surface during aerial hyphae formation. Many years of work have now shown SapB to be a highly derivatized small peptide that falls into an important class of antibiotic agents, called the lantibiotics, again illustrating the multifunctionality of compounds that also can be classified as bacterial signals.

One role for communication among all types of organisms is self-identification. Determining friend or foe can be a crucial point of information and dictates the mode of interaction between organisms of the same or different species. Members of the genus *Proteus* have a well-evolved sense of self and nonself that was discovered many years ago. *Proteus* moves across wet solid surfaces by a form of flagellar-driven locomotion called swarming, where large rafts of cells drive themselves outward from areas of dense growth. Strikingly, different isolates and subspecies of *Proteus*, when swarming over the same surface, will not converge swarms; each swarm maintaining boundaries of separation in a phenomenon known as the Dienes effect. **Gibbs and Greenberg** recently made major advances in understanding the basis of this territoriality by using a genetic approach. They contribute a review article here that provides a current view of this amazing process. The precise nature of the signal that drives this territorial phenomenon remains unclear, and much more work remains to be done. Interestingly, it is now apparent that *Proteus* species are not the only bacteria that have this ability for self-recognition.

The next two reviews in this issue outline different strategies for manipulating bacterial signaling systems. The first of these, by **Amara, Krom, Kaufmann, and Meijler**, describes recent efforts to modulate signaling pathways with native and non-native macromolecules such as enzymes and antibodies. The enzymatic degradation of quorum sensing signals has been termed quorum "quenching", and there are now multiple examples of naturally occurring enzymes that can function in this capacity. Such enzymes could play myriad roles, and one that is frequently invoked is the ability of one species to quench the signal of a neighboring species and thereby provide the quenching species with an advantage in a given environment. Although the biological significance of quorum quenching is still under debate for specific systems, the potential to engineer enzyme-driven quorum quenching mechanisms is an area of active research. Likewise, the development of antibodies against bacterial signals (both AHLs and peptides) and their use as highly specific inhibitors that tightly bind and thereby sequester bacterial signals has emerged as an exciting new approach for quorum sensing inhibition and virulence control in a variety of contexts.

Another strategy for the inhibition of quorum sensing processes with significant potential is the use of antagonistic, boron-containing signal analogues. **Dembitsky, Al Quntar, and Srebnik** explore the use of a wide variety of boron-containing molecules and also describe natural boronated compounds that play a role in signaling. As introduced above, the structural characterization of the AI-2 signal produced by marine vibrios revealed it to be a furanosyl borate diester and stimulated renewed interest in the potential for boron chemistry in modulating bacterial signaling processes. This insightful review provides an expert description of the potential for this approach and the incentive for continued research in this area.

Finally, **Goryachev** examines bacterial signaling from the perspective of computational modeling. Such modeling approaches are now routinely being used to map out the regulatory architectures of signal transduction pathways. When used in combination with experimentation, these computational approaches are extremely valuable and have already revealed crux points in bacterial signaling pathways that were not recognized as such based on experimentation alone. This review nicely conceptualizes the signaling process and considers how these systems can actually function in the face of a noisy and complex natural environment.

Discerning the meaning of language above the din of the natural world is something that many bacteria clearly do effectively. We are just beginning to understand how this is possible and which languages are the most relevant within a given environmental context. The outstanding collection of reviews in this issue of *Chemical Reviews* provides a small sampling of the range and depth of current research focused on bacterial communication. Understanding these processes provides the opportunity for scientists to intervene in the conversation and guide it in specific directions that potentially mollify the negative activities of microorganisms and promote their beneficial attributes. The challenge is daunting, however, as these communication systems and the environments in which they function are highly complex. Indeed, chemical diplomacy to restrain one microbe could very well instigate the revolt of others. As with most forms of communication, the meaning of the message is largely determined by the perception of those who receive it. We hope you enjoy reading this issue and invite you to join the conversation.

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