A Social Medium: ASM’s 5th Cell-Cell Communication in Bacteria Meeting in Review

Karine A. Gibbs, Michael J. Federle
Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, USA; University of Illinois at Chicago, Center for Pharmaceutical Biotechnology, Chicago, Illinois, USA

The 5th American Society for Microbiology Conference on Cell-Cell Communication in Bacteria (CCCB-5), which convened from 18 to 21 October 2014 in San Antonio, TX, highlighted recent advances in our understanding of microbial intercellular signaling. While the CCCB meetings arose from interests in pheromone signaling and quorum sensing, it was evident at CCCB-5 that the cell-cell communication field is continuing to mature, expanding into new areas and integrating cutting-edge technologies. In this minireview, we recap some of the research discussed at CCCB-5 and the questions that have arisen from it.

The 5th American Society for Microbiology Conference on Cell-Cell Communication in Bacteria (CCCB-5), which convened from 18 to 21 October 2014 in San Antonio, TX, and was organized by Marvin Whiteley and Eric Stabb, highlighted recent advances in our understanding of microbial intercellular signaling. (For reviews of past meetings, see references 1 to 4.) Traditionally, cell-cell communication has mainly focused on quorum sensing (QS), in which bacteria release small diffusible molecules that can alter a neighboring cell’s behavior when present at threshold levels, therefore permitting for the measurement of local cell density. It was evident at CCCB-5 that the cell-cell communication field is continuing to mature, moving from identifying new QS molecules to understanding environmental inputs affecting QS, deconstructing cross talk between QS systems both within and between bacteria, and developing tools to “eavesdrop” and ideally interrupt or inhibit QS-based conversations. Moreover, enough of the basic parameters for QS are known such that multiple models, ranging from evolutionary to synthetic, can be formed. The field is also moving into understanding the environmental and sociological impacts of cell-cell communication, whether via QS or other communication methods such as cell contact-dependent self-recognition, by examining the roles of kin selection/survival and the formation and maintenance of complex communities. The diversity of topics presented at CCCB-5 reveals the continued expansion of the cell-cell communication field and begs the question: where does the field go from here?

CCCB-5 began with a moving tribute to J. Woodland Hastings, one of the founders of the cell-cell communication field, by E. Peter Greenberg. Briefly, Kenneth Nealson, Terry Platt, and J. W. Hastings showed that the production of light by a marine Vibrio species was due to the accumulation of a secreted, cell-produced molecule. Their seminal paper in the Journal of Bacteriology, “Cellular control of the synthesis and activity of the bacterial luminescent system,” still stands as a simple, elegant proof of autoinduction (5) and remains the basis for many QS assays performed today. An expansive reflection of the life and research of J. W. Hastings, a member of the National Academy of Sciences, was recently published in the Proceedings of the National Academy of Sciences (6). Beyond his own research, J. W. Hastings was an advocate for other scientists in the field, and as E. P. Greenberg pointed out, J. W. Hastings emphasized that we should have fun in science and enjoy the simple pleasures of discovery. With that in mind, we have highlighted some of the cutting-edge research presented, separated by overall topic areas, to whet one’s appetite for the exciting discoveries discussed at CCCB-5 and will end with emerging questions.

PERCEPTION, INTERPRETATION, AND HANDLING CROSS TALK

Central to bacterial communications and interactions with the environment are signals, and as such, correct interpretation of signals is crucial. Many bacteria, such as the QS model systems of Pseudomonas aeruginosa, Vibrio harveyi, and Vibrio cholerae, can respond to multiple QS signals via the binding of cell-permeable signals to different regulatory proteins. A challenge for each bacterial cell is how to avoid cross talk between, or interference due to, multiple QS signals. At CCCB-5, researchers addressed how signal specificity is encoded within a protein, why multiple QS systems might be present, and the benefits and disadvantages of cross talk between signaling systems.

Kicking off the scientific portion of the meeting, Bonnie Bassler presented work from her research group that asked the simple question: how does the protein that binds a QS signal recognize its specific ligand? At high cell densities, binding of a diffusible QS signal to the response regulator LuxN induces light production by V. harveyi cells. The Bassler research group examined where the specificity for binding the cognate ligand N-[(R)-3-hydroxybutanoyl]-1-homoserine lactone (3OH-C4 HSL) is found within LuxN. LuxN exhibits a high binding affinity for 3OH-C4 HSL compared to QS signaling molecules produced by other bacteria, such as N-3-hydroxy-hexanoyl-HSL (3OH-C6 HSL) or N-butanoyl-l-homoserine (C4 HSL) (7). By using mutational analysis,
the Bassler group found that a specific subdomain called an autoinducer-specific domain determines the specificity for the signal ligand (7). Interestingly, in the context of the entire protein, single amino acids act to validate the C3 modification and the length of the side chain on an acyl-HSL QS molecule. Therefore, individual amino acids within the receptor protein are sufficient to determine ligand-binding specificity. These findings are consistent with published reports indicating that molecular recognition between two proteins can depend on a minimal number of specific residues (8–10). Unknown are how frequently mutations in these specific amino acid sites within LuxN arise within a population and whether a single cell could tolerate multiple LuxN-derived proteins that each bind a different QS molecule.

Rather than inquire how QS signals are differentiated, Sarah Jung, a graduate student in Wei-Leung Ng’s research group, addressed why V. cholerae genes encode four different QS receptors (the previously described CqsS and LuxO proteins and the newly described VC1831 and VpsS proteins), all of which act upstream of a single regulator protein, LuxO. At low cell densities of V. cholerae, phosphorylated LuxO induces transcription of four regulatory RNAs, resulting in the expression of genes important for virulence and biofilm formation; in contrast, at high cell densities, these virulence and biofilm formation genes are repressed, while competence genes are induced (reviewed by Nge and Bassler [11]). Binding of QS signals to any of the four receptors is sufficient to induce this lifestyle change. Jung and colleagues demonstrated that individual strains with a single or two mutations in these QS receptors displayed cell density-dependent behaviors similar to that of the wild-type parent and that all four QS receptors must be removed for a V. cholerae mutant strain to become QS deficient. To probe why there might be redundancy in signal interpretation, Jung and colleagues employed the fact that the addition of excess amounts of the QS signal CAI-1, a V. cholerae QS autoinducer that binds CqsS, inhibits LuxO activation. They added excess CAI to strains with single, double, and triple mutations of the QS receptors and examined the expression of LuxO-dependent genes. Surprisingly, the only strain that exhibited a response to the excess CAI-1 was a triple mutant strain, leading Jung and colleagues to put forward the hypothesis that the apparent redundancy in V. cholerae QS receptors may permit temporal regulation of the QS response and may bolster the bacterium from intrinsic fluctuations in the environment. Whether redundancy in QS systems provides a fitness advantage to V. cholerae remains to be seen. It stands to reason that redundancy in QS pathways might be beneficial for other bacterial systems, but this likely depends on whether the QS pathways intersect via receptors acting upstream of a single regulator, as is the case for V. cholerae, or are independent or parallel.

In contrast, Maria Hadijefrangiskou presented work from her research group tackling what happens when signaling systems overlap. In uropathogenic Escherichia coli (UPEC), the PmrAB and QseBC two-component systems are known to have intersecting functions, as deletion of qseC causes a deregulation of PmrA-regulated genes (12). Hadijefrangiskou and colleagues found that both QseB and PmrA directly control the qseBC operon in a protein-protein ratio-dependent manner; PmrA enhances QseB binding to the qseBC operon at equimolar concentrations. However, QseB is prevented from binding the qseBC operon when PmrA is at greater concentrations, resulting in the downregulation of QseB and QseC, and interestingly, the kinase activity of QseC is essentially vestigial. In the case of UPEC, these two signaling pathways intersect for transcriptional regulation, raising the question of how many other known two-component systems overlap in a similar manner. Other open questions are how did these two systems emerge within UPEC and why are both maintained.

Potential cross talk in bacterial communication might also come from animals and plants. Amy Schaefer from Caroline Hardwood and E. P. Greenberg’s research groups at the University of Washington presented data that the endophyte Pseudomonas sp. strain GM79, which was isolated from the roots of the Eastern cottonwood tree, carries a gene encoding an orphan QS response regulator, OryR, that likely responds to a plant-produced signal. The bacteria might actively transport this signal into the cell, thereby providing a way for the bacterium to sample its environment and for the plant to communicate with the bacterium.

SOCIAL BEHAVIORS OF COMMUNICATION
A continuing frontier is the role that cell-cell communication plays in population behaviors such as self-recognition, quorum sensing, and biofilm formation. Luke McNally from Sam Brown’s research group at the University of Edinburgh addressed the foundational question of what is the genesis of quorum sensing. Using phylogenetic comparative analysis, McNally and colleagues have proposed that QS likely emerged via the via the pre-existing public (secreted) goods to regulate (through signaling) social traits. Such social adaptation suggests that there likely exist environments and/or circumstances in which sociality is beneficial, e.g., in quorum sensing, where taking a census of population density is advantageous. Dan Wall probed this tension between cooperation and competition during his talk on the role of TraA in cell-cell recognition for myxobacteria. The surface protein TraA mediates the strain-specific transfer of outer membrane (OM) proteins in the social bacterium Myxococcus xanthus through homotypic, single-protein interactions, and this can subsequently modulate motility (13–15). Interestingly, Wall presented data in which mixing nonmotile and motile cells leads to the inhibition and subsequent death of the motile cells; this competitive behavior was dependent on transfer of OM components. In contrast, Wall also presented results showing that exchange of OM components can lead to beneficial shared resources within a population, which was demonstrated when a mixed population of cells with a damaged cell envelope was revived only by wild-type cells capable of OM component transfer (and not by those cells incapable of transfer). Therefore, cooperative behaviors could lead to a population homeostasis and rejuvenation, not to mention highly restricted communication through physically touching. Indeed, a balance between competition and solitary lifestyles versus cooperative and social behaviors seems to be a recurrent theme in the bacterial self-recognition field.

Polonca Stefanic from Ines Mandic Mulec’s research group at the University of Ljubljana, presented evidence that Bacillus subtilis can also exhibit self-recognition behavior: visible boundaries can form between swarms of nonrelated B. subtilis strains (Fig. 1). Stefanic and colleagues also used matrix-assisted laser desorption ionization (MALDI) imaging to determine unique compounds found in these boundaries and are currently pursuing their identification and regulation. Focusing on the biochemical examination of known self-recognition proteins, Karine Gibbs presented work from her research group demonstrating that two proteins,
which were genetically shown to encode determinants of self-identity in Proteus mirabilis, bind in vitro in an allele-restricted interaction and that the binding specificity for each protein is found in a subdomain of a variable region. Further, conversion of binding affinities in vitro correlates with conversion of self-identity in vivo, suggesting that this molecular recognition might help to drive population behaviors (Fig. 2).

An underlying question remains, however: do cells exhibit self-recognition behaviors and employ kin discrimination as a mechanism to hedge against the emergence of cheaters or other population “bad” actors? The emergence and policing of cheaters were beautifully examined by multiple research groups, including those of Steve Diggle at the University of Nottingham, E. P. Greenberg at the University of Washington, Martin Schuster at Oregon State University, and Avigdor Eldar at Tel Aviv University.

In a similar vein, the regulation of social behaviors in mixed-species populations is a burgeoning area of inquiry. Lucy McCully, a graduate student in Mark Silby’s research group at the University of Massachusetts Dartmouth, and colleagues addressed this by examining motility that is socially induced in the combined presence of Pseudomonas fluorescens Pf0-1 and Pedobacter sp. strain V48 (Fig. 3). By using transcriptomic and genetic analyses, McCully and colleagues have demonstrated that this induced social motility is dependent on contact, likely through a conserved secretion system, and requires communication from both members of this bacterial consortium, raising the issue of how many other bacteria are fraternizing with organisms outside their species to encourage sociality.

Speaking of motility, an initial phase of biofilm formation in P. aeruginosa is attachment to a surface, followed by a cell-cell coordinated motion termed “twitching.” In oral and poster presentations, Cynthia Whitchurch’s research group examined the nature of this P. aeruginosa surface-based twitching motility, employing impressive microscopy to probe how bacteria communicate and how they alter their environment. Through a combination of cell division and active migration, P. aeruginosa cells move outwards along a surface from an initial area of inoculation. Coating these cells and their paths is an extracellular “slime,” which contains extracellular DNA (eDNA) (Fig. 4). Lynne Turnbull from the

FIG 2 Concurrent exchanges of the variable regions within two self-recognition proteins are sufficient for self-recognition in vivo. Self-recognition behaviors in P. mirabilis can be visualized as the formation of a macroscopically visible boundary between the two approaching populations: populations that merge are considered to have the same identity, while populations separated by a boundary are considered to have different identities. Here we performed self-identity assays on populations that are genetically identical except for the plasmid-expressed loci depicted above. Notations are as follows: oval represent the swarm below with the chromosomal ids locus at the top and the plasmid-encoded ids locus at the bottom; rectangles show six ids genes in sequence with alleles from strain BB2000 (orange), strain HI4320 (blue), or missing (white). Blue boxes within an orange box indicate a variable-region exchange. Image courtesy of Lia Cardarelli, Christina Saak, and Karine Gibbs.
Whitchurch group presented data indicating that eDNA is sticky and as such can act as cellular glue that provides long-range alignment for cells and promotes cell-cell communication (16). Indeed most cells under conditions containing DNase I did not move. Questions remain as to how the DNA is produced and whether it is actively secreted. Also, could eDNA be a target for antibiofilm agents? Further research from the Whitchurch group presented by a graduate student, Erin Gloag, suggests that surface etching by migrating *P. aeruginosa* cells might also contribute to cell-cell coordination during initial biofilm formation. Briefly, Gloag and colleagues demonstrated that during twitching, groups of *P. aeruginosa* cells can furrow beneath an agar surface, creating tracks (or troughs) into which subsequent cells from the population move (17). These natural, bacterium-made microfluidic channels may also be present in the migratory patterns of other bacteria. It is fun to consider what would happen when opposing channels collide or whether another bacterium can invade pre-existing channels. Similarly, do the channels contribute to temporal fluxes in the composition of biofilms from the early colonizers to the late colonizers, such as the case for catheter encrustation in the bladder?

QS IN PATHOGENIC ORGANISMS

Contentious interactions among and between microbes and their human or animal hosts remains an obvious current quest to identify mechanisms of signaling as a component of pathogenesis. The incidence of the nosocomial pathogen *Acinetobacter baumannii* in clinical settings has been growing in recent years, coincident with emerging multidrug-resistant infections. *A. baumannii* is especially resistant to desiccation and is capable of survival under non-favorable conditions, due in part to its ability to generate biofilms, especially in hospital settings. Philip Rather’s laboratory has focused on the expression levels of the homoserine lactone synthase AbaI that produces 3OH-C_{12} HSL and its receptor, AbaR (18). N-Acyl homoserine lactone (AHL) signaling was demonstrated to contribute to biofilm development and swarming motility in these organisms. The Rather research group had the novel observation that two distinct colony morphologies, opaque and translucent, are apparent upon close examination of colonies of *A. baumannii* grown on agar plates and display a low frequency (~0.2 to 0.3%) of reversible switching between morphology types (Fig. 5). Interestingly, the opaque variants, but not the translucent ones, were found to generate high levels of AHLS. Thus, a global switching
A mechanism is in place that apparently provides a population the option to participate in intercellular communication or abstain from it and may offer a bet-hedging strategy for lineages to elaborate subpopulations with distinct physiological states that offer different fitness advantages under fluctuating environmental conditions. A Galleria mellonella (wax worm) insect model of infection was used to demonstrate that opaque variants were more virulent and better able to disseminate through tissues of the insect larvae, while translucent morphotypes were more adept at biofilm development.

Adaptation to changing environments is also possible through horizontal gene acquisition, and high-frequency transfer of antibiotic resistance determinants on plasmids was recognized to be stimulated by pheromones in Enterococcus more than 35 years ago (19). Modern multiple-drug-resistant (MDR) strains have become a leading health threat in hospital-acquired infections and contribute to dispersion of genetic elements of resistance to other pathogenic species (20). However, drug resistance often comes at a cost. In the process of examining whether commensal Enterococcus faecalis strains could occupy the same nutritional niche as that of MDR strains, with the anticipation that commensals could, if selected, compete with MDR strains in patients and hospital environments, an unanticipated finding was discovered. Michael Gilmore explained that a prominent commensal strain of Enterococcus faecalis, SUS, was able to suppress growth of an MDR strain V538 in bacterial culture. Susceptibility to strain SUS was dependent on strain V538 carrying an antibiotic resistance plasmid, pKIV2. Interestingly, transfer of pKIV2 to strain SUS suppressed V538 sensitivity, and SUS mutations in the pheromone transporter pptA rendered it unable to suppress growth of strain V538, indicating a role for pheromone-induced gene responses in the cell harboring pKIV2. Though the pheromones inducing growth suppression were identified, the mechanism by which growth of V538 was suppressed has not yet been elucidated, but it was likely due to the expression of an excision recombinase used in plasmid or phage excision that would result in DNA lesions. Therefore, in acquiring plasmid-borne drug resistance, V538 swallowed a poison pill whose effect is potentiated by pheromones of nonresistant commensals.

An exciting new discovery of toxin regulation by quorum sensing was presented in the poster session for work completed by Charles Darkoh, Heidi Kaplan, and coworkers. The toxin pathogenicity locus of Clostridium difficile encodes toxins A and B, together with regulators controlling their expression (21, 22). Consistent with the classic gene expression pattern of quorum sensing-regulated genes (as described for luminescence activity in Vibrio fischeri by K. Nealon, T. Platt, and J. W. Hastings in their seminal work [5]), toxin expression in C. difficile cultures was found to correlate with culture cell density. These observations led to identification of an accumulating toxin-inducing (TI) factor in culture supernatants. Hypervirulent strains of C. difficile, known to produce elevated levels of toxins, were also found to produce elevated levels of TI activity. Significantly, patients with C. difficile infections (CDI) that were toxin positive had TI activity in their stool samples, whereas toxin-negative CDI patients did not. Transposon mutagenesis studies produced some toxin-negative variants with lesions in genes orthologous to the Agr quorum-sensing pathway described in staphylococcal species (23). With these strong indications that toxin expression is mediated through cell-to-cell signaling, there is great anticipation that the toxin-mediated disease caused by C. difficile may be susceptible to therapeutics directed at signaling disruption.
independently initiated equally important experimentation leading to the paradigm that Gram-positive bacteria utilize peptides for communication (24–26). Over the last 40 years, discovery of numerous variations and derivatives of these chemical families have been elucidated. More recently, new families of chemical signals have stimulated the cumulative imagination of the field to wonder just how diverse and expansive the chemical space encompassing microbial chemical signaling might be. An exciting theme to emerge from CCCB-5 was the expanding use of direct imaging to characterize chemical signals produced by microbes. Mass spectrometry (MS) imaging, particularly matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) imaging, has in recent years undergone massive expansion and automation in the research group of Pieter Dorrestein. A wide variety of surfaces inhabited by complex microbial communities have undergone imaging analysis to survey production of secondary metabolites. It is anticipated that the release of compounds from microbes manipulates the behavior of surrounding cells, whether they are nearby competing or cooperating microbes, or possibly the hosts on which they exist. Not surprisingly, the number of small molecules detected by this highly sensitive methodology is extensive, and analysis of the data generates extremely large data sets. Compilations of data files have undoubtedly driven the need for repositories, which thanks to the Dorrestein research group, has culminated in development of a web-based, open-access platform onto which data sets can be submitted and accessed by members of the research community (http://gnps.ucsd.edu/). One of the most exciting ongoing projects in the Dorrestein laboratory has been mapping chemical signals across the surface of the human body, providing metabolomic profiles that can now be correlated to skin microbiota. For more information, see the 2011 article of Watrous and Dorrestein (27).

Direct imaging of multispecies cocultures provides a new tool to assess signals contributing to cellular interactions by asking when and where they are produced. While the functions of such compounds may not be immediately apparent, temporal and spatial patterns could provide unique evidence of directionality and order during a dialog. Though it may be difficult to predict if in \textit{vitro} coculturing will bring about physiologically meaningful interactions between bacteria, discovery of new molecules, along with the genes accounting for their production, provides a starting point from which function can be elucidated. Charlotte Michelsen presented evidence that mutualistic interactions occur between examples of host-adapted \textit{P. aeruginosa} and \textit{Staphylococcus aureus} isolates that coincide among the polymicrobial community of cystic fibrosis (CF) patient lungs. The \textit{P. aeruginosa} DK2 isolate (a prominent clone among CF patients in Denmark) showed enhanced growth properties when cocultured with \textit{Staphylococcus}. Interestingly, improved growth was lost if the staphylococcal strain used in coculturing was an \textit{agr}, \textit{clp}, or \textit{sarA} mutant, suggesting that extracellular products were influencing the interaction phenotype. MS imaging indicated production of interaction-dependent compounds and led to the identification of at least one \textit{P. aeruginosa} compound produced when the \textit{agr} system was intact.

Two-dimensional (2D) MS imaging has proven to be a powerful technique in monitoring secondary metabolite production during multispecies culturing, and by combining this technique with fluorescent reporter strains, gene expression activities of one strain can be monitored to indicate coincident differential expression patterns in response to small molecules produced by another. Elizabeth Shank demonstrated her research group’s current interest in monitoring metabolite production and gene expression levels of soil microbes using artificial soil materials that facilitate growth and imaging technology. Additionally, the spatial orientation of bacterial cells also impacts QS behaviors, and new technologies can help probe these interactions. Jodi Connell from Marvin Whiteley’s research group at the University of Texas, Austin, demonstrated how micro-three-dimensional (micro-3D) printing and scanning electrochemical microscopy (SECM) could be combined to develop a spatial profile of molecules such as pyocyanin that is QS regulated in \textit{P. aeruginosa} (Fig. 6). Connell and colleagues were able to examine the production of this QS product in groups as small as 500 cells, opening the door for scaling spatial examination of multiple QS-capable populations (or replicates) simultaneously when applied in combination with other currently available chip technologies.
A long-held anticipated application of cell-cell signaling studies is the notion that disrupting bacterial communication networks will allow for the harnessing of bacterial behaviors. If signaling networks control genetic pathways whose expression results in phenotypes that are harmful to health, manufacturing, or agriculture, then it is reasonable to predict that such behaviors can be altered, if not inhibited, through signaling interference. Likewise, the same reasoning can be applied to promoting beneficial behaviors. Helen Blackwell discussed several lines of study her research group has initiated to develop effective inhibitors (as well as activators) of quorum-sensing pathways. Taking a rational design approach to synthesize signaling compound variants and correlating their structure with receptor affinity and activation potential has advanced a fundamental understanding by which ligand-based signal transduction occurs. Importantly, testing synthetic ligand variants of AHLs on several receptor types (i.e., TraR, LasR, and LuxR) has identified degenerate ligands that can engage more than one receptor, even in diverse bacterial species that display strict signaling specificity. Conversely, mutagenesis of receptors has identified variants of LasR that have opposite signaling responses when bound to cognate ligands, resulting in antagonists that function as agonists and vice versa. By studying mechanisms underlying signaling, it is anticipated that new varieties of molecules will have the capacity to either work broadly on diverse receptors across numerous bacteria or work narrowly to pinpoint a species among a conglomerate (for more, see the 2015 article by Welsh et al. [28]). B. Bassler provided additional perspective on signaling networks control genetic pathways whose expression results from a high-throughput compound library screen that associated with lysozyme resistance. Michael Federle described responses resulting in aggregation and biofilm-like development with the sequence reversed (rev-SHP) was added to cultures at time zero. Time-lapse images (0 to 150 minutes) indicate cellular changes leading to aggregation. Aggregation is coincident with lysozyme resistance, and both proteins are blocked by the inhibitor cyclosporine. Image credit, Juan Cristobal Jimenez; image courtesy of Michael Federle.

FIG 7 Cell aggregation of Streptococcus pyogenes is induced by SHP peptide pheromones. A 50 nM concentration of synthetic SHP peptide or a peptide with the sequence reversed (rev-SHP) was added to cultures at time zero. Time-lapse images (0 to 150 minutes) indicate cellular changes leading to aggregation. Aggregation is coincident with lysozyme resistance, and both proteins are blocked by the inhibitor cyclosporine. Image credit, Juan Cristobal Jimenez; image courtesy of Michael Federle.

orthologs of other streptococcal species, including Streptococcus agalactiae and Streptococcus dysgalactiae, which suggests that, like AHL receptors, the Rgg family of proteins will be effective targets of therapeutic compounds.

CONCLUDING REMARKS

With the 5th CCCB behind us, we eagerly await the next exciting developments for the field. As next-generation sequencing and meta-omic technologies provide the newest and most powerful tools to study complex microbial communities in the context of a host or in the environment, how will cell-cell communication studies contribute to this burgeoning area of research? What role does communication play in establishing, maintaining, and diversifying healthy, balanced ecosystems? Before the next CCCB conference, how many new chemical entities constituting communication signals will be identified? How will they be detected? As large data sets are generated, there is a clear need to foster interactions between microbiologists and statisticians, mathematical modelers, and information technologists. There is also a clear need to engage ecologists and other experts in system dynamics. We anticipate that imaging platforms will continue to accelerate advances, providing information on chemical masses, stabilities, diffusion rates, and delivery mechanisms. Last, what new discoveries will be described in elucidating the effects that communication networks have on microbial behaviors? How will this information affect the way we understand microbial interactions and their interactions with our bodies, our environment, and our world? Studies in cell-cell communication continue to provide vibrant avenues forward in discoveries of the microbial world—and we hope many new ones will be shared at the next CCCB conference.

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