




Recent Advances and Future Prospects in Bacterial and Archaeal Locomotion and Signal Transduction

Sonia L. Bardy,^a Ariane Briegel,^b Simon Rainville,^c  Tino Krell^d

University of Wisconsin—Milwaukee, Biological Sciences, Milwaukee, Wisconsin, USA^a; Leiden University, Leiden, Netherlands^b; Laval University, Department of Physics, Engineering Physics and Optics, Quebec City, Québec, Canada^c; Estación Experimental del Zaidín, Granada, Spain^d

ABSTRACT The structure and function of two-component and chemotactic signaling and different aspects related to the motility of bacteria and archaea are key research areas in modern microbiology. *Escherichia coli* is the traditional model organism used to study chemotaxis signaling and motility. However, the recent study of a wide range of bacteria and even some archaea with different lifestyles has provided new insight into the ecophysiology of chemotaxis, which is essential for the establishment of different pathogens or beneficial bacteria in a host. The expanded range of model organisms has also permitted the study of chemosensory pathways unrelated to chemotaxis, multiple chemotaxis pathways within an organism, and new types of chemoreceptors. This research has greatly benefitted from technical advances in the field of cryomicroscopy, which continues to reveal with increasing resolution the complexity and diversity of large protein complexes like the flagellar motor or chemoreceptor arrays. In addition, sensitive instruments now allow an increasing number of experiments to be conducted at the single-cell level, thereby revealing information that is beginning to bridge the gap between individual cells and population behavior. Evidence has also accumulated showing that bacteria have evolved different mechanisms for surface sensing, which appears to be mediated by flagella and possibly type IV pili, and that the downstream signaling involves chemosensory pathways and two-component-system-based processes. Herein, we summarize the recent advances and research tendencies in this field as presented at the latest Bacterial Locomotion and Signal Transduction (BLAST XIV) conference.

KEYWORDS chemotaxis, flagella, flagellar motility, signal transduction, two-component regulatory systems

The capacity to sense and respond to changes in environmental cues is an essential feature of the prokaryotic lifestyle. As a consequence, bacteria and archaea have evolved an array of different molecular mechanisms that permit the detection of signals in order to generate appropriate cellular responses. These responses are mediated primarily by one- and two-component systems, as well as chemosensory signaling pathways (1–4). Whereas the former systems mediate changes primarily at the transcriptional level, chemosensory pathways form the basis for chemotaxis, the directed movement of prokaryotes in compound gradients. The study of signaling processes is not only of fundamental interest but may also contribute to the tackling of one of the central clinical problems, which is the increasing amount of antibiotic-resistant pathogens. There is now a significant amount of data indicating that interference with signal transduction systems, motility, and chemotaxis can be an alternative strategy to weaken or block pathogens (5, 6).

In January 2017, over 130 researchers from around the world met in New Orleans, LA, for edition XIV of the Bacterial Locomotion and Signal Transduction (BLAST)

Accepted manuscript posted online 8 May 2017

Citation Bardy SL, Briegel A, Rainville S, Krell T. 2017. Recent advances and future prospects in bacterial and archaeal locomotion and signal transduction. *J Bacteriol* 199:e00203-17. <https://doi.org/10.1128/JB.00203-17>.

Editor Igor B. Zhulin, University of Tennessee at Knoxville

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Address correspondence to Tino Krell, tino.krell@eez.csic.es.

conference to exchange and discuss exciting advances in their research. This biennial conference is independent of any scientific association and organized by the BLAST board of directors, the members of which are elected by conference attendees. Traditionally, talks at BLAST conferences are selected from abstracts. In this edition, there were, for the first time, two keynote speakers, namely, Sonia-Verena Albers (University of Freiburg, Freiburg, Germany) and George O'Toole (Geisel School of Medicine at Dartmouth College, Hanover, NH). BLAST meetings are focused primarily on two-component and chemotactic signaling, as well as different aspects of the motor and flagellum. Chemosensory pathways represent a particular form of two-component signaling where signal input occurs at chemoreceptors that are associated with the CheA histidine kinase. The BLAST policy is to select preferentially abstracts submitted by young scientists for oral presentation. Together with the high attendance rate of prominent scientists in the field, this has given BLAST meetings a truly intergenerational character. In addition, several awards are given to young investigators. Awardees of BLAST XIV were S. Bhattacharyya (R. M. Macnab poster award for postdoctoral scientists), M. Lynch (R. J. Kadner poster award for graduate students), P. Tohidifar (*Nucleic Acids Research* poster award for young investigators), D. Niketic (*Microbiology* poster award for young investigators), M. Kuehn (BLAST Board of Directors outstanding talk award for graduate students), and C. Diethmaier (BLAST Founders outstanding talk award for postdoctoral scholars). The scientific discussions, whether as part of the formal questions or casual discussions at coffee breaks and posters, are cordial and constructive. Over the years, a BLAST community has established itself and has fostered interactions and exchanges among its diverse members. BLAST meetings are a prime occasion to identify major advances and future prospects in this field of research, which are summarized in this publication.

THE STUDY OF PROKARYOTIC SPECIES OTHER THAN *E. COLI* ENHANCES OUR UNDERSTANDING OF THE MECHANISTIC DIVERSITY AND ECOPHYSIOLOGICAL RELEVANCE OF CHEMOTAXIS

The early days of chemotaxis research were focused primarily on the study of the enterobacteria *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, and much of what we know today is due to the study of these species (7). *E. coli* has four chemoreceptors, all containing a periplasmic four-helix bundle sensor domain, and the Aer receptor, which has a PAS sensor domain with a cytosolic location. These five receptors signal in a single chemosensory cascade that consists of the proteins CheA, CheB, CheR, CheW, CheY, and CheZ, mediating chemotaxis and energy taxis (7). The increased availability of genome sequences showed that the genetic basis enabling chemotaxis is rather different among prokaryotes. There are enormous differences in the number of chemoreceptors, ranging from 1 in the plant pathogen *Xylella fastidiosa* to 88 in the human pathogen *Vibrio parahaemolyticus* (8). In addition, many species contain multiple copies of the cytosolic signaling proteins, and there are species reported to possess more than 10 copies each of CheW and CheY (9). There is also evidence of additional cellular signal transduction proteins like CheD, CheV, CheC, and CheX, whereas other species lack CheZ, CheB, or CheR (2). Significant differences also exist at the level of chemoreceptor topology, as evidenced by the identification of the subfamily of soluble receptors (10), which contrasts with the membrane-bound topology of the *E. coli* proteins. Furthermore, chemoreceptor architecture differs significantly, for example, in the length of the cytoplasmic domain, composed of 24 to 64 seven-amino-acid heptad repeats (11) or the type of sensor domain (12). Another observation is that slightly more than half of the bacterial genomes possess genes encoding chemotactic signaling proteins (2), indicating that about half of the prokaryotic species are chemotactic. Among those species, more than half contain more than one chemotaxis operon in their genome. This issue hence raises the question of what environmental forces are responsible for the evolution and the acquisition and maintenance of the chemotactic machinery in different organisms. In this context, it is generally accepted that

TABLE 1 A selection of bacterial model systems used to study chemotactic signaling

Species	Taxonomic group	Characteristic(s)	No. of chemoreceptors ^a
<i>Agrobacterium tumefaciens</i>	Alphaproteobacteria	Soilborne plant pathogen	20–22
<i>Azospirillum brasilense</i>	Alphaproteobacteria	Plant growth-promoting bacterium	51
<i>Bacillus subtilis</i>	Firmicutes	Soil bacterium, able to form endospores	9–10
<i>Bacillus amyloliquefaciens</i>	Firmicutes	Plant growth-promoting rhizobacterium	4–8
<i>Bdellovibrio bacteriovorus</i>	Deltaproteobacteria	Preys on other bacteria	21
<i>Borrelia burgdorferi</i>	Spirochaetes	Complex life cycle, causative agent of Lyme disease	5
<i>Campylobacter jejuni</i>	Epsilonproteobacteria	Present in the gastrointestinal tract, causes gastroenteritis	5–10
<i>Caulobacter crescentus</i>	Alphaproteobacteria	Present in soil, freshwater, and seawater	19
<i>Comamonas testasteroni</i> CNB-1	Betaproteobacteria	Isolated from activated sludge, degrades aromatic toxic compounds	19–23
<i>Cronobacter sakazakii</i>	Gammaproteobacteria	Opportunistic pathogen, causes life-threatening diseases in infants	23–26
<i>Dickeya dadantii</i>	Gammaproteobacteria	Phytopathogen, causes blight, necrosis, and soft rot disease	42–48
<i>Escherichia coli</i>	Gammaproteobacteria	Enterobacterium of the human gastrointestinal tract	5
<i>Halobacterium salinarum</i>	Archaea	Halophilic bacterium, lives in saline lakes	18
<i>Helicobacter pylori</i>	Epsilonproteobacteria	Human pathogen, colonizes the stomach	3–4
<i>Leptospira interrogans</i>	Spirochaetes	Zoonotic pathogen, causes leptospirosis	12–13
<i>Pseudomonas aeruginosa</i>	Gammaproteobacteria	Omnipresent in nature, universal pathogen	24–26
<i>P. fluorescens</i> Pf0-1	Gammaproteobacteria	Soil bacterium, colonizes plant roots	37
<i>P. putida</i>	Gammaproteobacteria	High metabolic versatility, biodegradation of aromatic compounds	26–27
<i>P. syringae</i> pathovars	Gammaproteobacteria	Plant pathogen, causes multiple bacterial spots and blight diseases	47–51
<i>Ralstonia solanacearum</i>	Betaproteobacteria	Aggressive, soilborne plant pathogen	21–24
<i>Rhizobium leguminosarum</i>	Alphaproteobacteria	Legume symbiont, fixes N ₂	22–30
<i>Rhodobacter sphaeroides</i>	Alphaproteobacteria	Purple, photoheterotrophic bacterium, metabolic diversity	10–11
<i>Salmonella enterica</i> serovar Typhimurium	Gammaproteobacteria	Pathogenic enterobacterium, causes gastroenteritis	9
<i>Shewanella oneidensis</i> MR-1	Gammaproteobacteria	Couples metal reduction with metabolism	26
<i>Sinorhizobium meliloti</i>	Alphaproteobacteria	Legume symbiont, fixes N ₂	8–9
<i>Thermococcus kodakarensis</i> KOD1	Archaea	Thermophile	5
<i>Thermotoga maritima</i>	Thermotogae	Hyperthermophilic bacterium, lives in hot springs	7
<i>Treponema denticola</i>	Spirochaetes	Oral pathogen	18–20
<i>Vibrio alginolyticus</i>	Gammaproteobacteria	Pathogenic marine bacterium	25–26
<i>Vibrio cholerae</i>	Gammaproteobacteria	Lives in aquatic environments, causes cholera	34–44

^aTaken from the MIST2 (8) and SMART (102) databases.

the ability to migrate toward compounds that promote growth is the major ecophysiological reason for chemotaxis. However, there is now a significant body of data showing that chemotaxis is essential for many beneficial and pathogenic bacteria to recognize and attach to different hosts.

To address these issues, the scientific community has turned to studying chemotaxis in a series of alternative model systems, a representative selection of which is shown in Table 1. These species belong to different taxonomic groups, have different lifestyles, and possess various numbers of chemoreceptors. Some of the insight gained and further questions that arose from these studies are summarized here.

Multiple chemotaxis pathways. In contrast to *E. coli*, other bacteria possess multiple chemotaxis pathways. A bioinformatic study has indicated that more than half of chemotaxis pathway-containing genomes contain multiple pathways (2). *Rhodobacter sphaeroides* is the best-studied model bacterium used to investigate such additional chemotaxis pathways. The extensive work of the Armitage laboratory has shown that there is a membrane-bound polar signaling cluster, as well as a cytosolic cluster containing the soluble receptors that are activated by as-yet-unidentified signals. Multiple routes of communication exist between the two pathways because of the action of several CheY and CheB paralogues on both pathways (4). In this respect, several parallels to *Pseudomonas aeruginosa* exist. Two of its four chemotaxis gene clusters are involved in the *che* and *che2* chemotaxis pathways (13). Data suggest that, in analogy to *R. sphaeroides*, the transmembrane receptors signal into the *che* pathway, whereas the soluble Aer-2/McpB chemoreceptor signals into the *che2* pathway. Future studies will show to what extent the differential response to cytosolic or extracytoplasmic signals via different pathways is of general relevance.

Chemotaxis pathways with nonchemotactic functions. Although most chemotaxis pathways appear to mediate chemotaxis, not all do. Other pathways were

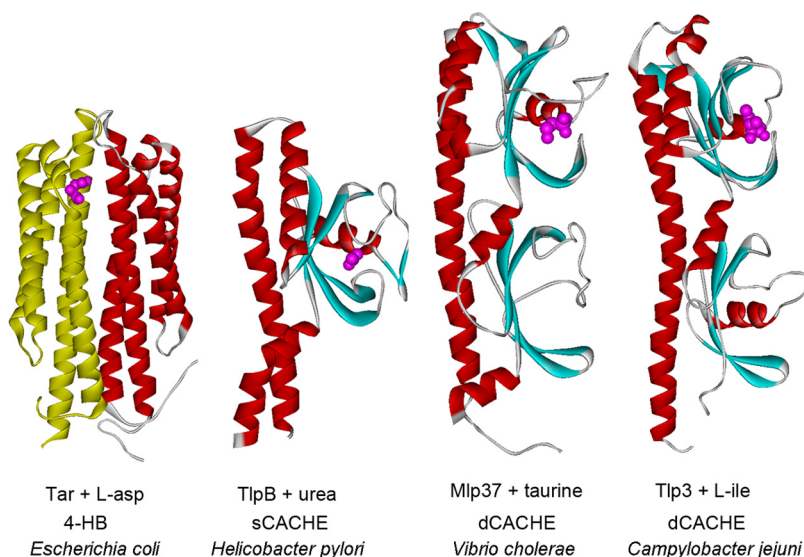


FIG 1 3D structures of different chemoreceptor sensor domains in complex with their respective ligands. Shown are *E. coli* Tar (Protein Data Bank code 4Z9H), *H. pylori* TlpB (3UB6), *V. cholerae* Mlp37 (5AVF), and *C. jejuni* Tlp3 (P4XMR).

shown to possess alternative cellular functions (ACF), like modulation of the levels of the second messengers cyclic di-GMP (c-di-GMP) and cyclic AMP (cAMP), or are related to type IV pilus (TFP)-mediated motility (14–16). This discovery hence raised the question of whether information on pathway function can be obtained by sequence analysis. Bioinformatic studies have classified chemosensory pathways into 19 different groups, of which 17 are associated with chemotaxis, whereas each of the remaining groups is associated with either ACF or TFP motility (2). Single-domain CheY response regulators, composed of a receiver domain, have been associated with chemotaxis (17). However, the CheY homologues with ACF possess additional domains (2) and inspection of such additional domains gives only a glimpse of the underlying complexity of the corresponding signaling processes. In most cases, the CheY receiver domains are fused to multiple domains. Such additional domains include GGDEF and EAL domains for the synthesis and hydrolysis of c-di-GMP, further receiver and histidine autokinase domains, additional sensor domains of the PAS or GAF type, and various combinations thereof (2). The notion that not all chemosensory pathways are associated with flagellum-mediated chemotaxis still needs to be promulgated in the scientific community. For example, there is a significant number of transcriptomic studies that interpret changes in chemosensory signaling genes as changes in bacterial motility without considering the possibility that some chemosensory pathways are not associated with motility. Questions to be addressed in the future are to what degree there is cross talk between the different types of chemosensory pathways and what else these systems might control in cells.

Other chemoreceptors and chemoeffectors. All four *E. coli* chemotaxis receptors contain a four-helix bundle sensor domain. The molecular mechanism by which ligand binding activates this receptor type has been studied extensively by using the Tar receptor. Aspartate binds with high negative cooperativity to the sensor domain dimer, which in turn causes translational and rotational displacements of the final helix of this domain. These displacements are then relayed to the transmembrane region and transmitted to the other end of the receptor, where it modulates CheA activity (18, 19).

However, genome analyses showed that chemoreceptors employ a wide range of different sensor domains. Interestingly, the most abundant domain type in chemoreceptors is not the four-helix bundle but the CACHE domain, in either its monomeric (sCACHE) or its bimodular (dCACHE) conformation (Fig. 1) (20). CACHE domains are

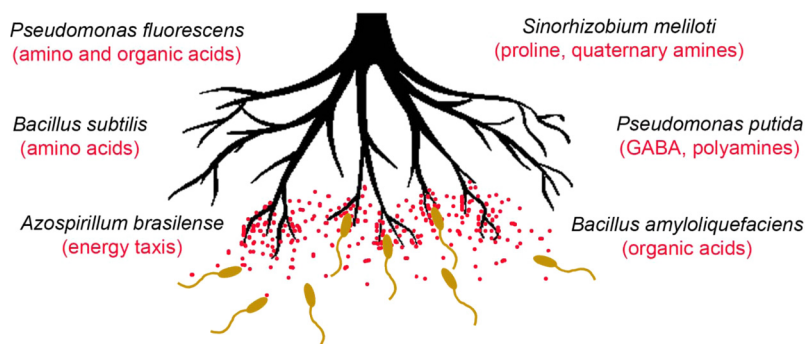


FIG 2 Identification of plant-released compounds that cause chemotaxis to plant roots or germinating seeds.

composed of a long N-terminal helix followed by either one (sCACHE) or two (dCACHE) primarily β -strand-containing globular modules. The abundance of CACHE domains in chemoreceptors agrees with another study demonstrating that dCACHE domains (previously referred to as dPDC domains) are the predominant sensor domains in histidine kinases (21). In recent years, significant progress in the structural biology of different CACHE domains and cocrystal structures with the bound chemoeffector has revealed the determinants of signal recognition. For example, high-resolution structures were reported for the sensor domains of the *H. pylori* TlpB receptor in complex with urea (22), a carboxylic acid sensor of *Pseudomonas syringae* (23) (both sCACHE), the taurine-bound structure of the *Vibrio cholerae* Mlp37 receptor (24), and the amino acid-complexed sensor domain of *Campylobacter jejuni* Tlp3 (25) (Fig. 1). Current investigations are aimed at understanding the molecular mechanism by which ligand binding causes activation of these different receptors. In addition, all of the biochemical and structural data available on dCACHE domains indicate that ligands bind to the membrane-distal module. This raises the question of the role of the membrane-proximal module in signaling, which is a topic that is currently being investigated.

Plant root colonization by beneficial bacteria. The colonization of plant roots by many bacteria is mutually beneficial to both organisms. On the one hand, root colonization can promote plant growth or an induction of systemic plant resistance to pathogens, which are both processes of significant agrobiological interest (26). On the other hand, bacteria gain access to the carbon and nitrogen sources present in root exudates. Chemotaxis to root exudates was found to be essential for efficient root colonization by many rhizobacteria (27). Work on different rhizobacteria has allowed the identification of plant signals that are central to colonization-relevant chemotaxis (Fig. 2). In *Azospirillum brasilense*, an energy taxis receptor was shown to be essential for root colonization (28). The receptor mutant was deficient in chemotaxis to several rapidly oxidizable substrates and terminal electron acceptors like oxygen and nitrate and had a largely reduced capacity to colonize roots. *A. brasilense* has two chemotaxis pathways, one of which is required for efficient root colonization (29). Other studies have assessed the chemotaxis system of the alfalfa symbiont *Sinorhizobium meliloti* to germinating seeds. Chemoreceptor single mutants were screened for chemotaxis to exudates and indicated a dominant role for the McpU chemoreceptor (30). Subsequent studies have shown that this receptor recognizes proline, which is abundantly present in root exudates (31). Quaternary amines such as betaine or choline are also secreted by seeds and roots. Webb et al. have previously shown that *S. meliloti* contains a chemoreceptor that specifically binds such quaternary amines (32). Additional studies underline the central role of amino acid chemotaxis in root colonization. For example, three amino acid-responsive receptors were found to be important in this process in *Bacillus subtilis* (33). In *P. fluorescens*, the deletion of the three genes that encode the amino acid chemoreceptors resulted in a significant decrease in root colonization (34).

In the same species, chemotaxis to Krebs cycle intermediates was identified as another important component of root colonization, and similar observations have also been made for *Bacillus amyloliquefaciens* (35). Furthermore, chemotaxis to GABA and polyamines was associated with root colonization in *Pseudomonas putida* KT2440 (36, 37). Taken together, this knowledge forms the rational basis for attempts to enhance the colonization of plants by beneficial bacteria.

Human pathogens. Chemotaxis is an essential requirement for effective host infection by many animal or human pathogens. *Borrelia burgdorferi*, the causative agent of Lyme disease, has advanced to be an important model organism used to study the relevance of chemotaxis in infection. This spirochete has a complex life cycle that involves both the tick vector and a mammalian host. During its enzootic life cycle, the bacterium migrates from the midgut to the salivary glands within the infected tick, which allows transmission to the next host during tick feeding. Once the bacteria have entered the mammalian host, they disseminate through the skin matrix to reach a multitude of target tissues. Subsequent feeding of ticks on an infected mammalian host allows the bacteria to return to their arthropod host. The spirochete successfully switches between the different hosts by sensing its current environment to determine its next optimal direction and to evade the host's immune system (38, 39). Data demonstrate that chemotaxis is crucial for the dissemination and viability of the spirochete within each host, as well as between mice and ticks (38, 40–42). For example, a *cheA2* mutant was chemotactically unresponsive to attractants and failed to infect mice (40). Another study showed that a *cheY3* mutant was unable to reverse direction and failed to disseminate from the skin matrix to distant tissues or migrate from an infected tick to the murine host (38). The second chemotaxis response regulator, CheY2, does not appear to affect motility or chemotaxis despite having all of the domains/conserved amino acid residues seen in a classical CheY protein, however, the Δ *cheY2* mutant cells were not able to establish persistent infection in mice by needle inoculation or tick bite. CheY2 is therefore thought to be a virulence determinant (43). Studies are under way to characterize the ligand profiles of the six chemoreceptors of *B. burgdorferi* (M. A. Motaleb, unpublished data). These studies will provide crucial insight into the signals that trigger chemotaxis in the different tissues the bacterium encounters during its life cycle.

Recently, further evidence has accumulated showing that pathogenic bacteria have evolved specific chemotactic mechanisms to sense host-derived and niche-specific signals in order to efficiently colonize target tissues. An emerging model organism used to study such mechanisms is *Helicobacter pylori*, which colonizes the human stomach. This organism has three transmembrane chemoreceptors (TlpA, TlpB, and TlpC) and one cytoplasmic chemoreceptor (TlpD) that together feed into a single chemosensory pathway (44). Mounting evidence suggests that signaling through all four chemoreceptors is necessary for efficient colonization of the gastric epithelium (45). It has been shown that *H. pylori* exhibits chemotaxis toward metabolites emanating from the human gastric epithelium and that urea is the primary host-derived metabolite that attracts the bacterium (46). Urea is sensed by TlpB, and its very high affinity enables responses to concentrations as low as 50 nM.

Another model organism for involvement in pathogenicity of the gastrointestinal tract is *C. jejuni*. Li et al. showed that the bacterium exhibits chemotaxis to bile in general, as well as to its major component sodium deoxycholate (SDC) (47). An either Tlp3 or Tlp4 chemoreceptor mutant showed decreased SDC chemotaxis and a reduced ability to colonize the jejunal mucosa. A double mutant deficient in both receptors completely lacked the ability to colonize the mucosa. These data suggest that chemotaxis to bile and SDC is necessary for efficient *C. jejuni* infection (47). Another study has led to the identification of a galactose receptor in invasive *C. jejuni* strains, and receptor inactivation has resulted in a significant decrease in virulence (48). Understanding the specificity of these chemotactic reactions may

provide the basis for the development of therapeutic strategies to reduce host colonization.

EXPANDING THE TOOLBOX

Detailed insights into the structure and function of microbial machineries that are involved in locomotion, sensing of environmental signals, and cellular behavior are increasingly gained by employing new technologies. New tools are also used to determine the characteristic biophysical forces that influence cells in their three-dimensional (3D) environments.

High-speed digital holographic microscopy. Flow fields around cells are now being studied in detail by high-speed digital holographic microscopy (49, 50). This technique is based on the interference patterns around particles that change their size and appearance, depending on the axial (z) position of the object in the imaging field. This allows the accurate localization and tracing of particles in a 3D volume. The presence of tracer beads can make the flow fields around bacteria visible. This technique provides a new tool for the study how biophysical forces influence the formation of nascent biofilms. Comparison with theoretical models reveals that the flow field around attached cells differs from that around swimming cells (51). While the flow field around swimming cells behaves like a dipole and decays rapidly with distance ($1/r^2$), the one around surface-attached cells behaves similarly to a Stokeslet, which decays significantly more gradually ($1/r$). This research provides testable models of how flow fields and shear forces together influence the behavior of as-yet-unattached cells approaching surface-attached cells (N. Farthing, M. Bees, and L. Wilson, unpublished data).

Cryo-EM. New tools also provide different insights into the structure and function of cellular machineries involved in environmental sensing, motility, and surface attachment. Cryoelectron microscopy (cryo-EM) provides the means to study these microbial structures at the molecular level. Here, intact cells are flash frozen without the need for any additional sample preparation or staining procedures. The contrast during imaging in the electron microscope thus originates solely from the biological sample itself. This provides images of nearly native samples with unprecedented detail. One main focus in the field is the study of motility structures in bacteria and archaea, the flagella and archaella, respectively. While rotary motors anchored in the cell envelope propel the cells forward in both motility structures, they are structurally not homologous. The archaellum is a homologue of the bacterial TFP, and in contrast to their bacterial counterparts, archaella rotate to propel cells forward instead of using the extension-and-retraction motion typical of bacterial type IVa pili (T4aP) (52). A homolog of the circadian clock protein KaiC interacts with the base of the archaellar motor and is thought to generate a rotational motion (53).

Single-particle cryo-EM. Electron microscopy of isolated components provides high-resolution maps of individual components of the machinery. The micrographs provide 2D projections of identical particles with different orientations in respect to the electron beam. These different orientations are necessary to computationally generate a 3D, high-resolution density map of the sample. This technique has recently been used to visualize the interaction of the archaellar core protein FlaH inside a ring of FlaX *in vitro* (54).

Cryo-EM can also be used to analyze the structure of filaments with helical symmetry. This approach has been applied to archaellar filaments by using helical reconstruction. It revealed the archaellum filament structure of *Methanospirillum hungatei* to a resolution of 3.4 Å and gave new insight into how its structure is distinct from that of the bacterial TFP; the archaellum is heavily posttranslationally modified by primarily O-linked glycans. Furthermore, the filament lacks a central pore. Instead, the extensive interactions between neighboring archaellins may provide the necessary structural support (55).

Another flavor of cryo-EM is electron cryotomography. This technique is used to image intact molecular complexes inside intact cells. Here, individual cells are rotated

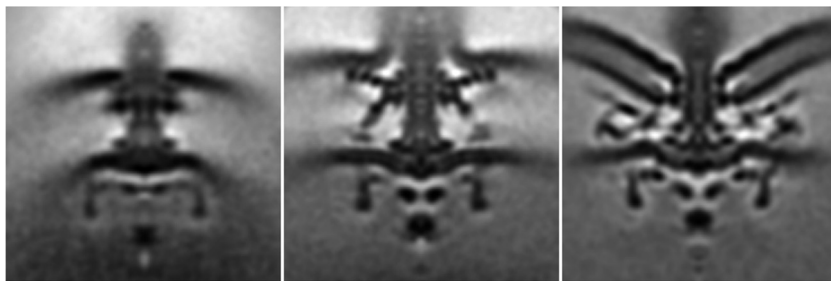


FIG 3 Structures of diverse evolved variants of the bacterial flagellar motor rationalize how different motors produce different torques. Left to right: *S. enterica*, *Vibrio fischeri*, and *C. jejuni* (57). Images kindly provided by M. Beeby.

in the electron microscope while a series of 2D projections is collected. These images are then used to computationally generate a 3D volume of the microbe (56). This method has recently been used to unravel the composition of bacterial flagellar motors in diverse species. To improve the signal-to-noise ratio of the electron density maps, many individual flagellar motors can be computationally averaged together by a method called subvolume averaging. The data sets reveal the *in situ* structure of the motors at molecular (~4-nm) resolution. The comparison of subvolume averages of wild-type and mutant strains helped to identify known components and revealed new structural components, such as the sheath ring characteristic of the motors of *Vibrio alginolyticus* (S. Zhu, T. Nishikino, M. Homma, and J. Liu, unpublished data). The combination of genetic methods, electron cryotomography and subvolume averaging can not only be used for structural studies but also provide functional insight into how differences in torque are related to additional scaffold and stator complexes of different motors (Fig. 3). Altogether, these studies can be used to gain further insight into the evolution of the multiprotein complex of the flagellar motor (57).

Correlative cryo-EM methods. Cryo-EM methods are especially powerful when paired with other techniques. For example, cryo-EM can be paired with light microscopy to correlate localization information from fluorescent light microscopy with the high-resolution information from cryo-EM (58). This method has been used in the past to identify the structures of both membrane-bound and cytoplasmic chemoreceptor arrays (59, 60) and is now being used to study the retraction function of T4bP in *Caulobacter crescentus* (E. R. Wright, unpublished data).

Cryo-EM can also be paired with comparative genomic and bioinformatic methods to gain insights into chemotaxis-related pathways in bacteria (61–63). Most motile bacteria and archaea contain a chemotaxis system that controls the cell's flagellar or archaellar motility, respectively. Additionally, more than half of all chemotactic microbes have additional operons that contain genes with high homology to canonical chemotaxis genes (2). However, these gene products appear to control cell functions unrelated to chemotaxis behavior and, as discussed in further detail above, the structure and function of these systems are still poorly understood. Comparative genomics permits the classification of these additional systems into groups likely to have the same biological function and the determination of which organisms harbor pathways belonging to each group. Electron cryotomography of wild-type and mutant strains of multiple organisms was then used to study the structure of these systems and to determine which genes are indispensable for the formation of the related protein cluster *in vivo*. In addition, this method provides the means to determine a range of growth conditions in which the same system is expressed and assembled in different organisms. Taken together, the combination of results from these techniques allows the generation of testable hypotheses about the biological functions and molecular mechanisms of novel, evolutionarily conserved, chemotaxis-related biological pathways.

Single-cell FRET. Fluorescence resonance energy transfer (FRET) microscopy has been widely used to characterize intracellular kinase activity in bacterial chemotaxis *in*

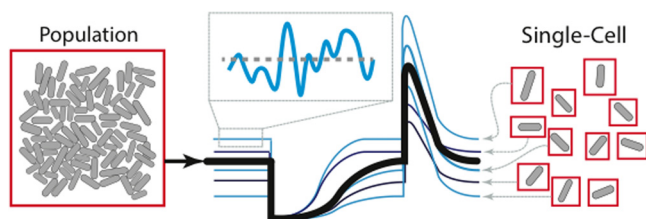


FIG 4 Single-cell FRET over extended times reveals variability in chemotactic signaling dynamics. In contrast to population level FRET, in which one ensemble-averaged FRET response time series (black curve) is obtained by integrating fluorescence signals over many cells (red box on the left), single-cell FRET yields a distinct response time series (blue curves) for every cell by image segmentation in the microscope field of view (red boxes on the right). In addition to revealing cell-to-cell variability, these experiments enable measurements of temporal fluctuations in each single cell (inset) from which kinetic parameters of signaling can be extracted. Image kindly provided by J. M. Keegstra.

in vivo. FRET between fluorophores fused to the response regulator CheY and the phosphatase CheZ gave great insight into chemotactic signaling dynamics at the population level (64). FRET microscopy has been applied at the single-cell level (65), but the quantitative extraction of signaling parameters was limited to population measurements averaging over hundreds of cells, in which effects of fluctuations are lost. This assay has since been optimized for the measurement of signaling dynamics in single cells over extended times (Fig. 4). First results from single-cell FRET revealed large cell-to-cell variability in many signaling parameters, which most likely result from the stochastic expression of chemotaxis genes (66). In addition to cell-to-cell variability, the baseline network activity in a single cell shows slow temporal noise that is augmented in the presence of the methylation-demethylation enzymes CheR and CheB. These fluctuations likely reflect the stochastic enzyme kinetics of the adaptation system and are not detectable in population level FRET because fluctuations uncorrelated across cells are averaged out. Altogether, these results provide a deeper understanding of how molecular noise of multiple origins propagates through chemotaxis signaling to tune the bacterium's mode of environmental exploration (66).

Fluorescent labeling of filaments. When bacterial filaments were labeled with fluorophores for the first time, an entirely new world of possibilities for the study of bacterial motility opened up (67). Real-time visualization of the flagella of swimming bacteria enabled, for example, the study of polymorphic transformations in the filaments of different bacterial species or the interactions between the filaments of swarming cells (68, 69). At the latest BLAST meeting, we heard from two groups who independently used this labeling technique to study how bacteria can back out of a dead end when swimming in a restricted environment (K. Thormann and S. Rainville laboratories, unpublished data). Their observations brought to light new ways in which bacterial filaments can move (a screwing motion and a locked-hook mode). In addition, by sequentially labeling filaments with fluorophores of different colors as they grow outside the cell, it was shown that the rate of flagellum growth decreases with length, as illustrated in Fig. 5 (70). The same collaboration even succeeded in monitoring the growth of flagellar filaments in real time. These results, combined with mathematical modeling, demonstrated that a simple injection-diffusion mechanism controls bacterial flagellar growth outside the cell. Therefore, the previously proposed chain mechanism (71) cannot contribute to filament elongation dynamics because that model predicts a constant growth rate versus length, which is incompatible with new observations.

MD simulations. Oftentimes, experimental techniques provide static snapshots of molecular assemblies. While this information is critical for understanding the structural composition of a biological system, in many cases, this knowledge alone is insufficient to discern function. The combination of such structural data with molecular dynamics (MD) simulation has been shown to provide a powerful approach by which to gain insight into the function of molecular machines (72). Briefly, MD simulation is a computational method that uses an empirically based potential energy function to

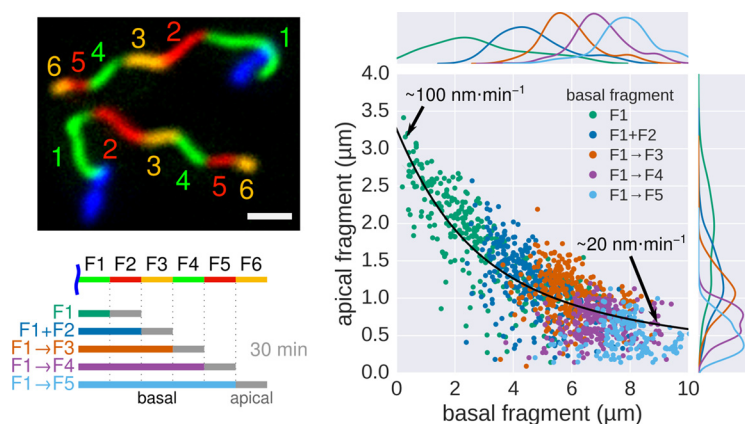


FIG 5 Growth kinetics of individual flagella revealed by *in situ* multicolor labeling. On the left is a representative fluorescence microscopy image of multiple labeling of flagellar filaments with a series of different fluorophores. Scale bar, 2 μm . As shown on the right, basal/apical length coordinates were obtained by varying the duration of basal growth and successive fragments were combined to generate a total of 1,276 basal/apical coordinates from 291 filaments. The growth duration of the apical fragment was 30 min. Average speeds are calculated from the average elongation per 30 min (<1 or >8 μm). The fit represents the injection-diffusion model. The images shown are reproduced from Fig. 3 of reference 70, published under the Creative Commons Attribution 4.0 International license.

characterize the chemical and physical interactions between atoms in a molecule, enabling calculation of the forces between individual atoms and ultimately the molecule's conformation over time.

Recently, a combination of experimental and computational techniques has been applied to chemotaxis arrays (73). Here, crystallographic structures of an individual receptor, CheA, and CheW proteins from *Thermotoga maritima* were assembled and refined according to cryo-EM maps of the extended *E. coli* signaling complex by MD flexible fitting (74), a technique based on MD simulation. A series of subsequent simulations with durations of up to 450 ns allowed investigation of the dynamic behavior of the intact array. Most notably, these simulations revealed a characteristic dipping motion of the kinase domain (P4) of CheA, providing testable predictions that were supported by genetic mutations and behavioral analysis, as well as cross-linking experiments (73; J. S. Parkinson, unpublished data). These results demonstrate the power of all-atom MD simulations but highlight the intense computing power and high-performance software required to investigate large multiprotein complexes such as the chemotaxis array. For example, to achieve a simulated time of 450 ns, the all-atom extended array structure (1.25 million atoms) required the use of the highly scalable NAMD code (75) and ~ 100 graphics processing unit (GPU)-accelerated nodes (16 central processing units plus one GPU per node) on the Blue Waters supercomputer for 360 h. MD simulations are also being used to study the behavior of isolated *E. coli* receptor trimers of dimers *in situ* (I. B. Zhulin, unpublished data), as well as transmembrane signaling in single receptors (K. Schulten, unpublished data).

INDIVIDUAL CELLS VERSUS COLLECTIVE BEHAVIOR

A very exciting and promising area of research is the study of how the behavior of individual cells maps onto collective behaviors at the population level. A good example is the process of fruiting body formation in *Myxococcus xanthus* during the development stage, which can be induced by starvation. It was found that this density-dependent process closely resembles phase separation in passive systems (76). More specifically, it can be described by the phenomena of coarsening, nucleation and growth, and spinodal decomposition observed in material science. Together, these processes can explain the remarkable uniformity in size and distribution of fruiting bodies that is observed in petri dishes. Since speed and reversal frequency are controlled by the genetics of individual cells (77), this realization offers the promising

opportunity to begin to bridge the gap between individual cell behavior and population behavior.

Experimental work on biofilms has also taught us that variability at the cell level has an impact on collective behaviors. It was observed that *E. coli* biofilms containing a mixture of motile and nonmotile cells remained intact longer (many weeks) and contained more biomass than biofilms composed of only motile or only nonmotile bacteria. A heterogeneity in motility, caused by spontaneous mutations in the *flhD* operon, therefore seems to be an advantage for increasing and maintaining a biofilm (78). In studying this relationship between individual and collective behaviors, one is quickly confronted by the notion of noise. Variability is everywhere, in stochastic MD, single-cell response, and collective behavior. How do variations in protein abundance, gene expression, and environmental stimuli affect the behavior of a single cell and its performance? How do these individual actions affect the population? The origin of that variability and how it is controlled and exploited by cells are important areas of research, and many new tools enable their study in exquisite detail.

Monitoring of single motors demonstrated that stochastic fluctuations in (de)methylation reactions in the chemotaxis network generate fluctuations in CheY-P, which in turn cause behavioral variability in a single cell over time (79–81). Single-cell FRET imaging (described above) enabled the measurement of CheY-P activity in individual cells, showing that these differences give rise to phenotypic variations (65, 82). For example, the response to ligand stimuli was found to be highly variable from cell to cell, which can be explained in terms of variability in the Tar/Tsr receptor expression ratio. We have also learned that microbes have evolved effective ways to control that variability. In particular, the CheB phosphorylation feedback loop (which is not needed for perfect adaptation) reduces the cell-cell variability in CheA kinase output (79, 80). Indeed, this feedback loop provides robustness in the chemotaxis pathway by reducing fluctuations in CheY-P levels (83). On the other hand, we know that such variability can also be exploited; the same adaptation system that is responsible for precise adaptation introduces large fluctuations in the time domain that lead to extended runs (Levy flights). These extended runs are found to be beneficial in the absence of any gradient, allowing the population to sample a larger volume more effectively (79). Another clear illustration is the observation of individual trajectories of swimming bacteria, which shows substantial cell-to-cell variability (82) in parameters such as swimming speed, average turning angle, and effective rotational diffusion coefficient. Large data sets obtained by high-throughput 3D tracking (84) reveal that the variations in these parameters are not entirely random but display substantial correlations with each other. Theoretical predictions suggest that this coordinated variation maintains a compromise between high drift velocity and high localization performance in chemoattractant gradients (K. M. Taute, S. Gude, S. J. Tans, and T. S. Shimizu, unpublished data).

We also learned from observations of tens of thousands of individual cells “racing” in a microfluidic device that the shape of the distribution of a given phenotype is also important, since the mapping between phenotype (tumble bias) and chemotactic performance (drift speed in a gradient) is nonlinear. In other words, changing the standard deviation of a distribution can have as large an effect on performances as changing its mean. Both the shape and the mean can be independently affected (82), suggesting that both are under selection pressure from evolution (85).

The emerging conclusion is that variability in individual cell behavior (or protein concentration, for example) should no longer be called noise because it seems to be an important part of the function and might even be selected for (86). Recent decades have seen impressive developments in both theory and experimentation in this field. This has greatly improved our understanding and resulted in a shift in how we think about noise and variability.

SURFACE SENSING AND SIGNAL TRANSDUCTION

Another current area of focus in the community is examining signal transduction and motility outside the traditional test tube and swim plates. For both pathogens and

environmental microbes, the signal transduction that results upon contact with a surface is critical for the transition from the motile to the sessile state and eventual biofilm formation. This developmental process may involve altered levels of the secondary messengers cAMP and c-di-GMP or altered gene expression. Ultimately, this transition results in dramatically different cell physiology, including increased antibiotic resistance (87). Because adherence is the first required step in this transition, understanding how bacteria sense surface contact and the mechanisms driving the subsequent phenotypic changes is critical.

Sensing the surface through flagella and TFP. Some bacteria use their motility organelles, such as flagella or TFP, to recognize interaction with surfaces. The concept of motility organelles as mechanosensors was proposed as early as the late 1980s in *Vibrio parahaemolyticus* (88). Increased external viscosity was found to reduce the rotation of the polar flagellum and thereby increase the expression of lateral flagellar genes (88). More recently, interest in the flagellum as a mechanosensor has expanded to include a breadth of microbes (89, 90). There is an emphasis on the changes in flagellar structure and gene expression as a result of a load increase due to surface contact. In *E. coli*, dramatic load changes lead to an initial reduction in speed, followed by stepwise increases in speed, concomitant with an increase in stator subunits (91). This incorporation of additional stator subunits (MotB) into the preexisting flagellar structure was termed stator remodeling. The stator proteins form the ion channel and work with the rotor protein (FliG) to generate torque. While stator remodeling occurs in response to the mechanical load, it remains unclear how this would contribute to differential gene expression, as seen in *Vibrio* bacteria (92).

Stator remodeling is not unique to *E. coli*. The genome of *P. aeruginosa* encodes two sets of stator proteins, MotAB and MotCD, that generate the torque required for swimming and swarming motility, respectively (93, 94). Current studies are focused on the differential role and possible exchange of these stator complexes in the immediate response of *P. aeruginosa* to load changes resulting from surface contact and adhesion (B. Kazmierczak, unpublished data). The T4aP is another motility organelle found in *P. aeruginosa* and other bacteria that may participate in surface sensing. These thin filaments extend and retract from the poles of cells, mediating attachment and surface translocation (twitching, walking, and slingshot motility) (95). The production and function of T4aP are controlled through the Chp chemosensory system (96, 97). Recently, this chemosensory system was found to have a second function, regulation of intracellular cAMP levels through CyaB (15). Signal transduction through the Chp system is proposed to be mediated through direct PilA-PilJ interactions wherein PilA is the major pilin subunit of the T4aP and PilJ is the sole chemoreceptor of the Chp chemosensory system (16, 97). The increase in cAMP is dependent on surface contact, although the mechanism by which the T4aP senses the surface is still unknown. The O'Toole lab is investigating the role of the motility organelles in surface sensing during the transition to biofilm formation (G. A. O'Toole, unpublished data).

Two-component signaling and surface sensing. In *B. subtilis*, an increased load on the bacterial flagellum activates the two-component signal transduction system DegS-DegU (90). DegU is a transcriptional regulator that, when phosphorylated (DegU~P), controls several different processes, including motility and biofilm formation (Fig. 6). Low levels of DegU~P lead to hyperflagellation in the presence of SwrA. SwrA is the master swarming regulator that accumulates following surface contact. This SwrA accumulation results from a lack of proteolysis by the LonA AAA+ protease/SmiA adaptor (98). While the exact mechanism that relieves proteolysis of SwrA is under investigation (D. Kearns, unpublished data), this surface contact-controlled transcription regulation allows differentiation into swarmer cells. While it is unclear how the signal of an increased load on the flagellum is transduced to the DegS-DegU system, the basal body appears to play an important role. The loss of the flagellar stator (MotB), inhibition of flagellar rotation through overexpression of the clutch protein (EpsE), and tangling of the flagella all resulted in increased phosphorylated DegU through the

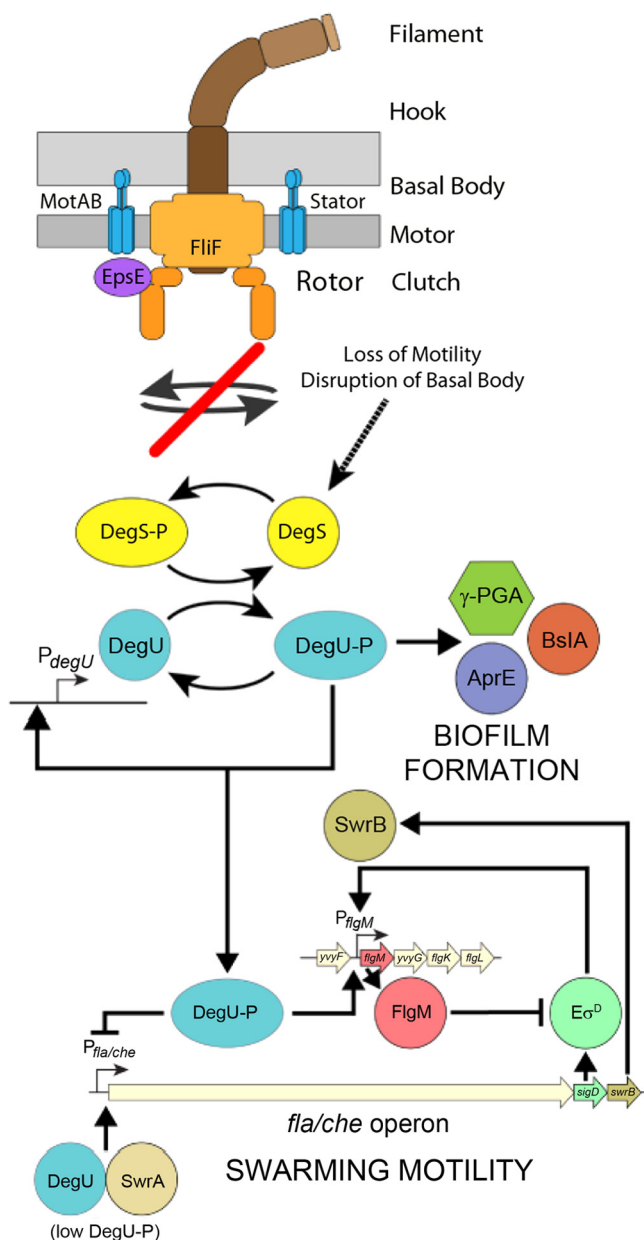


FIG 6 Inhibition of flagellum rotation increases biofilm formation and decreases swarming motility in *B. subtilis*. At the top is a cartoon of a flagellum from a Gram-positive bacterium with, from top to bottom, the MotAB stator in blue, the rotor in orange, and EpsE (the clutch) in purple. Loss of motility or disruption of assembly of the flagellum results in an unknown signal (hashed arrow) that upregulates the DegS-DegU two-component system, leading to an increase in DegU~P, BslA, gamma-polyglutamic acid (γ -PGA), and subtilisin (AprE) and a decrease in *fla/che* operon expression. Conversely, unphosphorylated DegU, along with SwrA and SwrB, increases *fla/che* operon expression, leading to flagellar synthesis and swarming motility. Lines with arrowheads indicate positive effects, while T-end lines indicate inhibitory effects. Promoters are shown as small square-angled arrows. Reprinted from reference 101 with permission from Wiley & Sons.

histidine kinase DegS (90). The consequences of this flagellum-based activation of the DegS-DegU system appear to be far reaching, and the effects on competence are under investigation (D. Dubnau, unpublished data).

The motile-to-sessile transition is also affected by the regulation of two-component signal transduction systems independently of motility/mechanosensors. *Agrobacterium tumefaciens* is a facultative plant pathogen responsible for crown gall disease through the transfer of transfer DNA. In response to acidic conditions, the ChvG-ChvI two-

component signal transduction system is activated. This activity of this system is repressed at neutral pH because of periplasmic interactions between ExoR and the sensor kinase ChvG (99). ExoR is a periplasmic protein containing tetratricopeptide repeats (100). Derepression of ChvG leads to phosphorylation of ChvI and, surprisingly, the reduction of both motility and biofilm formation. As motility and biofilm formation are thought to be mutually exclusive phenotypes, the mechanisms behind this parallel phenotypic pattern are intriguing. These mechanisms are currently being deciphered (C. Fuqua lab, unpublished data).

In this review, we have painted a broad picture of our progress in understanding how microbes sense and respond to their environments by using two-component systems, chemotaxis, and motility organelles. This field greatly benefits from the expanding array of cutting-edge tools used to study in exquisite detail phenomena ranging from collective behaviors to the molecular scale. This level of understanding is also made possible by rich interactions among theory, modeling, and experiments. We have illustrated the general themes of research by citing a few specific examples, but there are obviously countless other exciting and important results that could not be included. In addition, it would be remiss of us not to acknowledge the work that has come before. Years of dedicated study by the founding members of the BLAST community have resulted in a high level of understanding of *E. coli* signal transduction, chemotaxis, and motility. This canonical paradigm forms our baseline of knowledge and allows us to better identify and understand the complex variations that occur throughout the microbial community.

ACKNOWLEDGMENTS

Research in our laboratories is supported by grants from the Spanish Ministry for Economy and Competitiveness (BIO2013-42297 and BIO2016-76779-P to T.K.) and the Canada Foundation for Innovation (S.R.).

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