Bacterial Chemotaxis: The Early Years of Molecular Studies

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Abstract

This review focuses on the early years of molecular studies of bacterial chemotaxis and motility, beginning in the 1960s with Julius Adler's pioneering work. It describes key observations that established the field and made bacterial chemotaxis a paradigm for the molecular understanding of biological signaling. Consideration of those early years includes aspects of science seldom described in journals: the accidental findings, personal interactions, and scientific culture that often drive scientific progress.

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OVERVIEW

Molecular studies of bacterial chemotaxis and motility began in the period 1965–1969 when Julius Adler and colleagues published pioneering studies with *Escherichia coli* (1–3, 7, 13–15). Over the following two decades, the foundation was laid for current understanding. This review focuses on those early years, when first one and then a few laboratories were involved. It includes aspects of science seldom described in journals: accidental findings, personal interactions, and scientific culture. For these I utilized material from colleagues and from my experiences in chemotaxis, which span the 44 years since I joined Adler's laboratory as a graduate student in January 1968. I thank my colleagues for their contributions and ask their understanding for my editorial decisions as I struggled to stay within the page limit.

Important companions to this review are the recent prefatory chapter by Julius Adler in the *Annual Review of Biochemistry* (6), and John S. (Sandy) Parkinson's article about early years in the Adler laboratory (75). The sidebar provides an overview of *Escherichia coli* chemotaxis.

STUDYING BEHAVIOR WITH BIOCHEMISTRY AND GENETICS

In 1960, new University of Wisconsin Assistant Professor Julius Adler began studying the behavior of *E. coli*. He was inspired by his interest in sensory phenomena, which he traces to observing butterflies as a boy (5), and by the nineteenth-century literature on bacterial taxis he discovered as a young scientist (6). Julius applied biochemical strategies and genetic approaches learned during training with Henry A. Lardy, Arthur Kornberg, and Dale Kaiser to molecular mechanisms of bacterial behavior (6). By the early 1960s, utilizing bacteria, particularly *E. coli*, to identify molecular mechanisms had proven impressively successful for macromolecular synthesis and control of gene expression. Several prominent senior scientists who had been part of these successes were turning their interest to neuroscience. However, for a new assistant professor to begin his independent research career by applying the notion "[a]nything found to be true of *E. coli* must also

A CHEMOTAXIS PRIMER

E. coli traces a random walk of straight runs, generated by counterclockwise rotation of flagellar rotary motors, and tumbling episodes, generated by clockwise rotation, that reorient the cell. The chemosensory system biases random walks by reducing probabilities of clockwise rotation for swims in favorable directions. The system comprises six cytoplasmic proteins and five transmembrane chemoreceptors plus four periplasmic ligand-binding proteins. Chemoreceptors Tsr and Tar mediate responses by binding serine and aspartate, respectively. Tar, Tap, and Trg detect maltose, dipeptides, and galactose or ribose through interaction with ligand-occupied forms of periplasmic binding proteins.

Receptors plus the autophosphorylating histidine kinase CheA and coupling protein CheW form signaling complexes, activating kinase ~1000-fold and placing it under receptor control. Phosphoryl groups are transferred from the kinase to the response regulator CheY. CheY-P binds the flagellar rotary motor, inducing clockwise rotation and thus tumbles. Inherent instability and phosphatase CheZ make CheY-P short-lived. Binding of attractant to receptors inhibits kinase activity, reducing CheY-P and tumble frequency. The system adapts to persistent simulation via receptor methylation by methyltransferase CheR (positive stimuli) and demethylation by methylesterase CheB (negative stimuli). Kinase activities altered by changes in receptor occupancy are restored to prestimulus levels by adjusting receptor methylation. For instance, attractant occupancy enhances methylation and inhibits demethylation, generating increased methylation and thereby restoring kinase activity to prestimulus levels. Ligand-induced changes in signaling complex conformation and kinase activity are rapid, whereas methylation changes are slower. A resulting time disparity of ~4 s creates a molecular memory that provides sensing of temporal gradients. *E. coli* chemotaxis exhibits high sensitivity and wide dynamic range, properties not explained by the simple model outlined above. These properties are thought to reflect interactions in extended arrays of signaling complexes, which vary in size and position but are largest at cell poles (43, 47, 48, 60).

be true of elephants" (64) to something as ephemeral as bacterial behavior was more than a little daring. Yet, Julius' earliest chemotaxis research had a receptive audience. For instance, his first chemotaxis publication was the written version of an invited talk at the 1965 Cold Spring Harbor Symposium (1).

Admiring comments from members of that audience directed students and junior scientists to bacterial chemotaxis. For instance, Howard Berg reports that he started working on a microscope for tracking swimming bacteria in September 1968, after Max Delbruck told him he would work on bacteria to study behavior if only he knew how to "tame" them. For me, in fall 1966 Julius' friend and postdoctoral mentor, Dale Kaiser, remarked to Harriet Ephrussi-Taylor, then a faculty member at Case Western Reserve University, that if he were a beginning graduate student he would join Julius Adler. As a beginning graduate student at Case Western Reserve, I was hoping to work with Dr. Ephrussi-Taylor and was in her office during that conversation. Within a year she became too sick to take students, so after a visit to Madison during Thanksgiving 1967, I began with Julius in January.

ELUCIDATING MOLECULAR MECHANISMS

In his 1969 *Science* paper, Julius (3) demonstrated that chemotactic responses of *E. coli* to galactose or its structural analogs did not require metabolism or transport. Thus, response was not the mechanistic result of benefit provided by the compound, a concept about bacterial behavior common in the literature, but instead was generated by recognizing the compound itself. This implied a specific system for recognition and response. It initiated contemporary studies of bacterial chemotaxis.

Wilhelm Pfeffer: a prominent nineteenth- and early-twentieth-century German botanist and microbiologist who studied microbial chemotaxis and many aspects of botany

The 1969 paper did more. It demonstrated the utility of investigating a sensory phenomenon using a model organism in which mutant derivatives could be generated, genetic manipulations were facile, and a substantial community of scientists could provide genetic and biochemical tools. Julius utilized nonmetabolizable analogs of bacterial attractants and mutant bacteria unable to metabolize or even concentrate an attractant to demonstrate that the chemotactic response was independent of benefit. This seminal study profited from the ready availability of *E. coli* mutants. Subsequent progress was greatly enhanced by generation of many chemotaxis mutants and Julius' practice of providing mutants freely, whether described in publications or not, to interested researchers. This practice became a feature of the field. Particularly notable is Sandy Parkinson, who began mutational analysis of *E. coli* chemotaxis as a postdoc with Julius in 1970. As an independent investigator, Sandy became the primary source of mutant strains, generously making available his vast strain collection and his knowledge and thus enhancing our collective progress.

Choosing *E. coli* as the model organism had an unanticipated benefit. We now know that the number of taxis components varies with species. Five core components are common to almost all systems, but many systems have one or more additional components (95). Also, a species can contain two or more sets of core components. Additional components often provide alternative or partially redundant activities; additional full sets often control responses other than movement (44, 94). Furthermore, the number of chemoreceptors can vary drastically, from a few to more than 50 (95). Serendipitously, *E. coli* contains a single set of the five core components plus only one additional component (a phosphatase) and five receptors. This relative simplicity greatly facilitated the identification of components and their roles, particularly prior to facile gene cloning and sequencing. As is often the case, chance enhanced scientific progress.

THE MAJOR ISSUES

In January 1968 when I joined Julius' laboratory, his group had established the experimental foundations for studying *E. coli* motility and chemotaxis and described the first chemotaxis mutants (1, 2, 7, 9, 13, 15). Julius was preparing what would become the 1969 *Science* paper. Yet we knew almost nothing about machinery or mechanisms. Each member of the lab (**Figure 1**) investigated a major issue: (*a*) attractants and repellents (Marge Dahl, Julius' long-serving and very effective research assistant, plus graduate students Bob Mesibov and me); (*b*) the common machinery (student John Armstrong); (*c*) structure of the motility apparatus (student Melvin Depamphilis) and its mode of function (occasionally by short-term lab members); (*d*) receptors (me and Bob Mesibov); (*e*) the role of methionine (John Armstrong and subsequently others); and (*f*) signaling, the mechanism(s) coupling receptors to flagella (much discussed but not then actively investigated). Here's what happened.

Identifying Attractants and Repellents

Identification of attractants and repellents provided tools for investigating chemotaxis machinery and mechanisms. Over multiple years, Marge Dahl, with some help from others, used the "Pfeffer Assay" to survey innumerable compounds. The assay was essentially Wilhelm Pfeffer's from the 1880s (76). A 1-μL glass capillary tube containing a chemical solution was placed in a bacterial suspension, and cell accumulation in response to the resulting diffusion gradient was determined. Pfeffer assessed accumulation under the microscope. Marge plated capillary contents (4). The data for *E. coli* attractants (8, 63) and repellents (93) remain the most extensive profile of chemotactic sensitivities for any bacterium. In related studies exploring parallels between the behavior of *E. coli* and higher organisms, several neuroactive compounds that Julius obtained from colleagues were

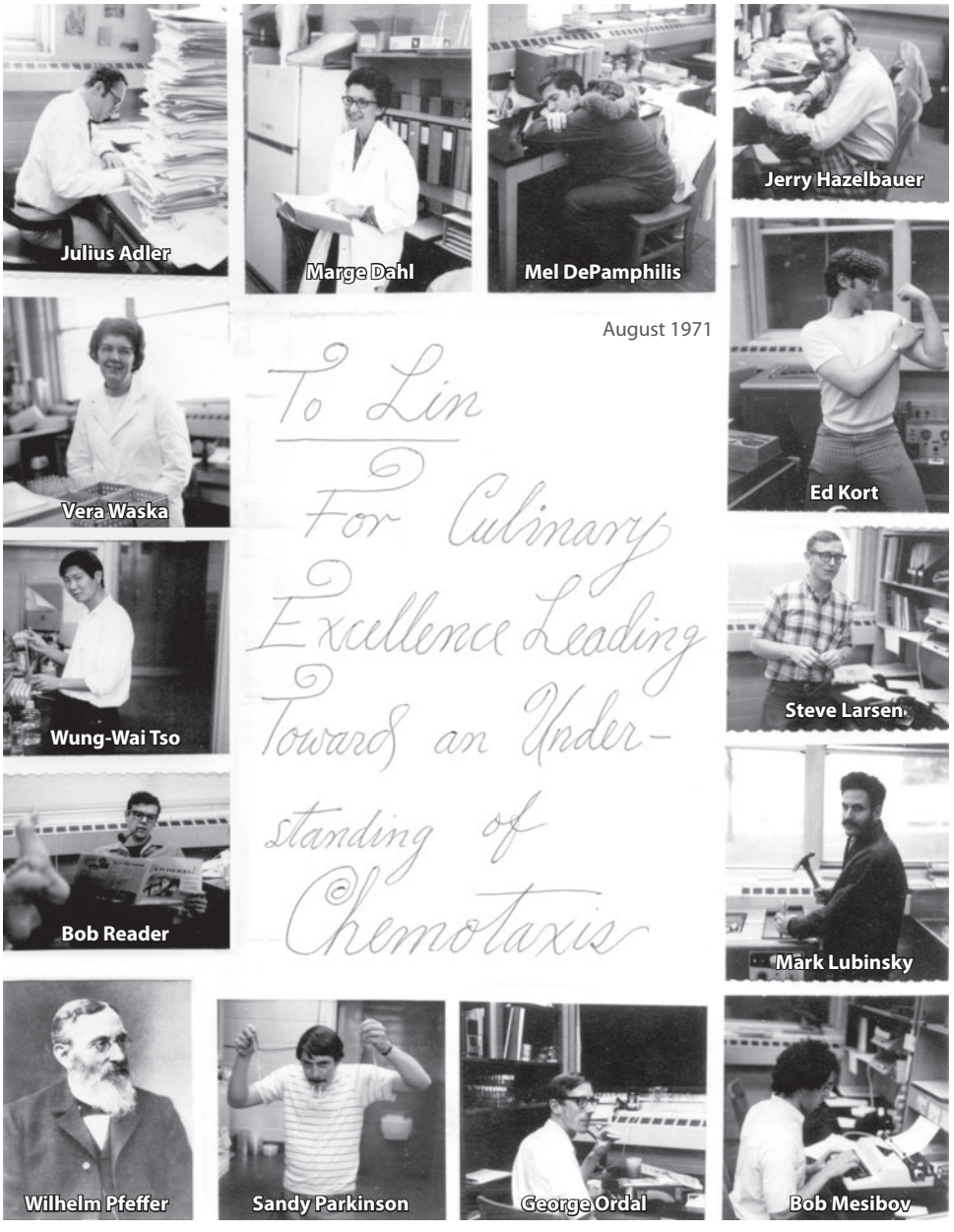

Figure 1

The Adler laboratory, summer 1971. Photos of lab members (plus Wilhelm Pfeffer) arrayed around a certificate of appreciation to Linda Randall, who as Jerry Hazelbauer's spouse had made gourmet desserts for weekly Adler group meetings for the previous year and was being thanked as the two departed for postdocs at the Pasteur Institute in Paris.

surveyed, usually by microscopic observation. These compounds included mescaline and lysergic acid diethylamide (LSD) (obtained from Timothy Leary when still legal). I recall LSD caused *E. coli* to tumble (see also Reference 75).

Generally Nonchemotactic Mutants and the Che Proteins

John Armstrong, Julius' first chemotaxis graduate student, isolated mutants unable to respond to any chemoeffector yet motile and without additional defects. He used semisolid agar plates containing tryptone, a sufficiently low agar concentration such that *E. coli* could swim through water channels in the matrix. As cells multiplied, they consumed amino acids in the tryptone according to their metabolic preferences, serine and then aspartate, creating sequential gradients to which they responded, forming respective rings of cells following those gradients. Using the cells that remained at the point of inoculation to inoculate a new plate and repeating this process multiple times enriched for motility and chemotaxis mutants (15). This yielded mutants defective in each nonreceptor component of the *E. coli* taxis system (14, 15, 33, 71–74). Subsequently, Melvin Simon's laboratory utilized then-new techniques of gene cloning to identify the gene products, the Che (chemotaxis) proteins, which were the common components of the chemotaxis system (80, 81, 83, 85).

Flagellar Structure

Adler's research group investigated motility as well as chemotaxis (7, 9, 13, 79). In the 1960s, little was known about flagellar structure besides what extended from the cell surface. In fall 1964, graduate student Mel DePamphilis began investigations. After many false starts, he isolated intact flagella (see A Breakthrough with No One to Tell). Using a relatively dated electron microscope, he recorded images (34–36) that persisted for 18 years as the best views of the core structure of what we later learned was the flagellar rotary motor.

Flagellar Function

How did the chemosensory system direct motility? In April 1970, Howard Berg got his tracking microscope working. By 1972, he found that cells in buffer traced random walks and that gradients

A BREAKTHROUGH WITH NO ONE TO TELL

In 1966 Mel DePamphilis, an almost third-year graduate student, had little indication his project would be successful. Mel recounts the breakthrough:

> 11 PM one evening I experienced the most exciting moment of my scientific career. I was at Biochemistry's Siemens Elmiskop I electron microscope to check my latest attempt to isolate intact flagella. The microscope required ~40 minutes to load a sample, create a vacuum inside and align the beam. Then I had to work quickly since the sample deteriorated rapidly under the intense beam. Moreover, the camera had only 10 sheets of film and exposures were done manually: opening the shutter, counting three seconds and closing. That evening, I saw the usual spaghetti-like flagellar preparation. Tracing individual strands to search for unusual structures at an end, I froze. Several had them! These were too remarkable and too reproducible to be anything but the long-sought basal body! I was so excited I could not take photos but kept thinking, "My God, I'm the first person to see this." I had to tell someone. After running all over the building, I phoned Julius, sure he would be excited. "Hello," answered a sleepy voice. "Julius, it worked!"; "What worked?"; "The method for isolating intact flagella; it worked!" "That's nice. You can tell me all about it in the morning."

of attractant biased those walks (19). In the same year, Macnab & Koshland (59) published a study using strobe images of swimming bacteria to conclude that gradients were detected by temporal sensing; i.e., there was a "memory" that stored information about the recent past and compared it to the present. The two studies provided the foundation for understanding the strategy by which the chemosensory system guides cells to favorable environments.

Yet we did not understand how flagella generated swimming or how the sensory system biased the random walk. In 1973, three labs provided the answers. First Berg & Anderson (18) concluded from published observations and conceptual arguments that flagella rotate. In 1972, Mike Silverman in Mel Simon's lab saw the consequences of rotation (86). Mike made antiserum to the flagellar hook protein, which makes the universal joint connecting the flagellar filament to the motor. When he added antiserum to a nonmotile mutant strain devoid of filaments but with elongated hooks, he saw cells apparently tethered to the microscope slide rotating first one direction and then the other, and suspended cells tethered to each other and counterrotating. My understanding is that Mike went excitedly to Mel only to hear it could be an artifact. Additional experiments showed that flagella rotation was real (82). Description of the results to Adler and Berg resulted in complementary experiments and three back-to-back *Nature* papers documenting flagellar rotation and identifying the control of rotational direction as the way the sensory system directed motility (16, 58, 82). Thus, the flagellar motor was identified as the first rotary motor in biology. In parallel, its source of energy was identified as protonmotive force (57).

Receptors

Existence of specific chemoreceptors was a fundamental prediction from Julius' 1969 *Science* paper. Potential receptors were identified by a combination of our list of attractants and repellents; the results of "jamming" experiments, which were determinations of response to a gradient of one compound in the presence of a uniformly high concentration of a second; and the information the laboratory had collected about inducible responses, ones greatly enhanced by growth in a specific condition. Because the 1969 paper focused on responses to galactose, the putative galactose

SEEING ISN'T NECESSARILY BELIEVING

Mike Silverman and Mel Simon were convinced they had demonstrated that bacterial flagella rotate (82). Mel reports:

> It fell to me to 'take the story on the road'. I went to a Structural Biology meeting at Lake Tahoe, showed films of rotating bacteria and concluded the flagellum rotated. A very distinguished structural biologist stood up and proclaimed, 'I don't believe it. There is no rotary motion in biology.' I said to the projectionist, 'Run the film again.' He did and our distinguished colleague said, 'It is precessing not rotating.' I had the film run a third time and pointed out that based on the motion, lack of foreshortening, etc., it could not be precession. I showed evidence for rotation of individual flagella and for rotation driven from the basal element. Our questioner ended the discussion by proclaiming, 'I still don't believe it.' While there were such naysayers (29), the chemotaxis community was receptive. After I spoke at Wisconsin, Adler's group used our approach to demonstrate that attractants and repellents controlled the probability clockwise rotation (58) and Howard Berg characterized rotation and provided a conceptual basis for thinking about it (16).

Twenty-three years later ATP synthase became the second documented biological rotary motor (67), and subsequently the subject of a Nobel Prize (28).

receptor was a prime candidate. The sidebars "Isolating" Receptor Mutants from the Strain Collection and Identification of a Protein Component with a Little Help from our Friends describe the unconventional path to its identification.

Identification of the galactose-binding protein as the galactose receptor (more accurately the galactose recognition component) was the first identification of a bacterial sensory protein and one of the first sensory proteins identified for any receptor system, after rhodopsin, as well as the

"ISOLATING" RECEPTOR MUTANTS FROM THE STRAIN COLLECTION

Upon joining Adler's laboratory, my project was to isolate the first chemoreceptor mutant. My interview the previous November had ended with Julius saying something like, "If we're correct, and *E. coli* is attracted to galactose by recognizing its molecular shape, there must be a specific galactose receptor, likely a protein, and thus a gene product. Given a gene, you should be able to isolate specific galactose taxis mutants and use them to identify the receptor. With that, you would earn a Ph.D." I succeeded, but not in the way anticipated. By late spring, neither my initial approach of enrichment using semisolid agar plates nor multiple variations succeeded. Apparently I looked discouraged because Marge Dahl inquired. I explained I had failed to find "galactose-blind mutants," laboratory jargon for galactose receptor mutants, and that such mutants would grow on galactose, not respond to galactose, but respond to other attractants. Marge replied: "We have one!" It was one among many galactose metabolism/transport mutants obtained from colleagues and tested for chemotaxis. Only one, from Esther Lederberg, was galactose taxis negative and derivatives that grew on galactose remained taxis negative. Marge recalled Julius saying save it because it could be useful. It was. I obtained a galactose taxis-positive revertant by selecting for formation of a galactose chemotactic ring; showing that mutant, but not revertant, was defective in galactose transport at submillimolar concentrations; and modifying the enrichment procedure to isolate additional galactose-blind mutants by compensating for the transport defect. That first galactose-blind mutant and a serine-blind mutant isolated by Marge, and discovered in our stain collection by Bob Mesibov soon after I found the galactose-blind mutant, were described in 1969 (49).

IDENTIFICATION OF A PROTEIN COMPONENT WITH A LITTLE HELP FROM OUR FRIENDS

As I began the search for the protein altered in galactose-blind mutants (see "Isolating" Receptor Mutants from the Strain Collection), a daunting task before genes could be cloned, Julius began receiving telephone calls from the distinguished Danish scientist and Harvard professor Herman Kalckar. Kalckar could be difficult to understand because of his strong Danish accent and a tendency to start in the middle of an explanation (53). After each phone call, Julius would emerge from his office unsure of what Kalckar said. Finally, Julius deduced Kalckar thought a galactose transport protein his lab studied was involved in galactose taxis but not why or what he wanted from us. After multiple calls, I was put on the phone with Kalckar's colleague, Winfried Boos. My memory is that Winfried expressed significant doubt about bacterial "behavior" but was extremely helpful in describing the galactose-binding protein and transport defects of its mutants, defects that mimicked those of galactose-blind mutants! Winfried offered to send a mutant and transport-positive parent. Most importantly, he mentioned an osmotic shock procedure that released this periplasmic protein. Off the telephone, I immediately started bacterial cultures and soon was testing osmotic shock fluid from a galactose-blind mutant and taxis-positive parent for radiolabeled galactose binding. When the scintillation counter display showed binding by parent but not mutant extract, I knew we had the galactose receptor. Work remained before publication (45), but the utility of mutants in identifying sensory proteins had been validated, thanks to significant help from our colleagues.

first chemical receptor protein. Importantly, binding proteins could be purified and characterized biochemically. Testing osmotic shock fluids for other predicted receptors revealed previously unknown ribose-binding and maltose-binding activities and indicated these newly discovered periplasmic binding proteins were the respective sugar receptors (45). I was disappointed I didn't find binding activities for anticipated serine or aspartate receptors, but later work demonstrated these should not have been released by osmotic shock (31). Periplasmic binding proteins were water soluble and so could not by themselves signal across the membrane. Thus, we postulated "transducers," transmembrane signaling proteins (45). Transducers were discovered by solving the methionine problem.

MCP: methyl-accepting chemotaxis protein

Taxis toward serine and some repellents (Tsr): a transmembrane chemoreceptor

Taxis toward aspartate and some repellents (Tar): a transmembrane chemoreceptor

Taxis toward ribose and galactose (Trg): a transmembrane chemoreceptor

Methionine and Methylation

Marge and Julius had shown methionine was required for chemotaxis (7). John Armstrong, Julius' first chemotaxis graduate student, pursued the issue in his thesis work (10). He continued these studies after graduating in 1968 and implicated *S*-adenosylmethionine (11, 12). However, its specific role, presumably as a methyl donor, remained unidentified. Seven years and multiple tries after John's departure, graduate student Ed Kort made the pivotal discovery that tritiated-methyl methionine radiolabeled a 60-kDa *E. coli* membrane protein. Ed combined forces with graduate students Michael Goy and Steve Larsen to demonstrate that the 60-kDa protein they named methyl-accepting chemotaxis protein (MCP) was involved in chemotaxis (55). Parallel studies led by postdoc Marty Springer showed methionine was required for tumbling, thus correlating the protein modification with chemotactic behavior (89). Soon thereafter, Ed, Steve, and postdoc Bob Reader moved on, leaving Michael and Marty to pursue methylation. They did so in a very productive partnership, establishing that protein methylation mediated the central process of sensory adaptation (41, 42, 87).

Methyl-Accepting Chemotaxis Proteins

Initial studies of MCP involved the original version of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in individual tube gels, cutting gels into slices and determining radioactivity by scintillation counting. When Michael and Marty turned to the newer technique of thin slab gels and detecting radioactivity by fluorography, the higher resolution immediately revealed that the 60-kDa region contained multiple methyl-labeled bands. In parallel, Mike Silverman in Mel's laboratory used then-new techniques of gene cloning to identify motility and chemotaxis gene products, including an MCP (80, 83–85). Complementary studies from the Adler and Simon laboratories, published as adjacent articles in the *Proceedings of the National Academy of Sciences USA*, identified the multiple bands as belonging to two parallel pathways of signal processing, each mediated by an MCP: Tsr for serine and certain repellents and Tar for aspartate and other repellents (84, 88).

Because the two MCPs handled responses to multiple ligands and a mutant missing both MCPs was generally nonchemotactic, it was thought there was a receptor resembling the galactose-binding protein for each ligand and that MCPs were downstream (88). However, a third MCP, Trg, was identified by my and Julius's labs as the transmembrane component for taxis to galactose and ribose (46, 54), and my lab showed that the three MCPs provided parallel signaling pathways (46). In addition, Tsr and Tar were found by Dan Koshland's lab to bind, respectively, serine and aspartate (31). Thus, the ligands for MCPs could be amino acids or ligand-occupied binding proteins. They were the transmembrane receptors of chemotaxis.

MULTIPLE FORMS OF MCPS

MCPs appear as multiple bands on SDS gels because the proteins are multiply methylated and those modifications increase mobility in SDS-PAGE (27, 30, 32, 37). The laboratories of Dahlquist, Hazelbauer, Koshland, and Simon deduced this essentially simultaneously and all came to the 1980 Gordon Conference on Sensory Transduction in Microorganisms to report. What transpired illustrates a characteristic combination of competition and cooperation. Within 24 hours we realized we had all come to the same conclusion, with different, albeit overlapping, approaches. This was reassuring because methylation added only 14 Da to a 60-kDa protein and separation of methylated forms should have been impossible using SDS-PAGE. We got together the first afternoon, summarized our respective results, designed a sequence of talks to inform the community, and cleared rearrangement of the published program with session chairs. I was to summarize the overall conclusion and describe our results. Alan Boyd from Mel Simon's lab would report theirs and Rick Dahlquist theirs. Dan Koshland indicated it was not necessary for him to talk about their results; I could summarize them. I asked what to say. Dan replied, in his usual witty style, "Tell them we did all that the rest did, only better." In my talk, I said exactly that. Laughter was substantial. This was one of a few times I got the better of Dan in a public intellectual fencing match, an activity at which he was a master.

Phosphorylation

How do receptors communicate with the flagellar motor? In the early 1980s my lab showed that receptors were not clustered around flagellar basal ends (38), indicating signaling could not involve direct physical contact but rather must occur over a distance. In higher organisms such signaling is often accomplished by ion fluxes across membranes. Julius was particularly interested in this possibility. He investigated extensively, first in his own laboratory (77, 90) and then in collaboration with faculty colleague Ching Kung (62). Their collaborative work led to the discovery of mechanosensitive ion channels in bacterial membranes (61, 78). Julius' lab investigated other candidates, including cyclic nucleotides (20, 21) and calcium ions (91, 92). Working on *Bacillus subtilis*, George Ordal found indications of calcium involvement (70). Although findings were never published, several laboratories investigated the possibility that protein phosphorylation was involved, but without success.

The breakthrough came from Mel Simon's laboratory, where the *che* genes had been cloned and placed in vectors producing the respective proteins. As I understand it, new postdoc Fred Hess, familiar with protein purification, proceeded to purify the Che proteins and screen for activities. Adding γ-^{32}P-ATP, he observed autophosphorylation of CheA and correlated it to chemotaxis (52). In rapid succession, publications from the Simon lab (22–24, 50, 51, 68) and one from Jeff Stock's lab (96) defined chemotaxis-linked phosphorylation, demonstrating that it was indeed the signaling mechanism between receptors and the flagellar motor. Beginning with the initial publications (52, 96), it was clear that this phosphotransfer and the proteins involved were members of a family of recently identified two-component regulatory systems (65, 66). These proved to be ubiquitous bacterial signaling elements coupling recognition of the environment and cellular response (25, 26). This meant that information about chemotaxis signaling was relevant to mechanisms of control of gene expression.

THE CULTURE OF THE CHEMOTAXIS COMMUNITY

Intertwined with increased understanding of bacterial chemotaxis was the development of an interactive community. This community continues to flourish, almost 40 years after it began. It is

notable for cooperation and congenial relationships, regular meetings at which most gather, and encouragement of young scientists and those new to the field. I believe the community and its attitudes have contributed much to our collective progress. Our field is not without competition, but cooperation and even friendship persist.

This scientific culture owes much to its initial prominent members. Julius Adler was (and still is) thoughtful, thorough, deliberate, intensely concerned with achieving the highest standards of data and interpretation, interested in considering all explanations and possibilities even those "knowledgeable" scientists might consider unlikely, prone to share, and genuinely encouraging to others. These attributes had substantial positive influences. For a number of years, his was the only chemotaxis laboratory. In the early 1970s, three additional research groups, directed by Howard Berg, Daniel E. Koshland, Jr., and Melvin Simon, respectively, began to publish about bacterial chemotaxis and motility (**Figure 2**). Each is or was (Dan passed away in 2007) a well-respected scientist. At the time he began to study bacterial chemotaxis, Dan, already a member of the National Academy of Sciences, was a major figure in biochemistry and enzymology particularly because of the induced-fit concept of enzyme action. Julius was elected to the Academy in 1978, Berg and Simon in 1984 and 1985, respectively. These four were quite different, but each focused on important issues and aimed to provide convincing conclusions. Thus, chemotaxis and motility publications from their laboratories were relatively few in number but of notable quality and impact. Those of us trained in those laboratories have aimed to emulate this pattern. Publishing few but substantive papers is prevalent in the field to this day.

The first additions were Howard Berg and Dan Koshland. Howard, on his way from Harvard to a faculty position at the University of Colorado, spent the summer of 1970 in the Adler laboratory learning how we handled *E. coli* from Marge Dahl. He was a welcome guest. It was the first time for us that someone else was studying chemotaxis. Moreover, Howard's physics background meant a distinctly different perspective. His ability to explain his insights was invaluable and has continued to be over multiple decades.

In the late 1960s, Dan Koshland became interested in bacterial chemotaxis. As I understand from those who were in his laboratory, Dan was considering several possibilities for molecular approaches to neuroscience. Bacterial chemotaxis was championed by postdoc Frederick (Rick) Dahlquist and research assistant Peter Lovely. Soon thereafter, postdoc Robert (Bob) Macnab, with a chemistry and chemical engineering background, joined the Koshland laboratory. The result was full entry into our field and provision of a distinctly biochemical orientation to chemotaxis. Barry Taylor, a Koshland postdoc a few years later, reports that Dan was not comfortable with genetic analysis but encouraged his people to learn all they could from faculty colleague Bruce Ames. Dan used his prominence and considerable promotional skills to argue that bacterial chemotaxis was a prime system for addressing fundamental questions in neuroscience. We all benefited. He published an entire book, *Bacterial Chemotaxis as a Model Behavioral System* (56), based on a Distinguished Lectureship of the Society of General Physiologists. Activities like these brought substantial visibility to the field and enhanced our respective chances for funding. Dan also brought a style honed by many years in very competitive areas of biochemistry. This style was quite different from Julius' and led to tensions when the styles clashed. Both were professional, so tensions might not have been noticed by outsiders but were quite apparent to those from the respective research groups. Yet those tensions did not create significant extended problems. Communication and cooperation among alumni of the two laboratories have flourished.

The early years conferred not only high standards but also a pattern of encouraging young scientists or those new to the field. Adler, Berg, Koshland, and Simon all gave their students and postdocs not only credit for contributions but also opportunities to present their work. For instance, as a graduate student I spoke at two Gordon Conferences about my work, once in place

Figure 2

Key investigators in the early days of chemotaxis research. (*a*, *left to right*) Howard Berg, Dan Koshland, and Julius Adler at the January 1977 Gordon Conference on Bacterial Cell Surfaces, Miramar Hotel, Santa Barbara, California. The image has been extracted from the participants' photograph and manipulated to show only those three. Photograph reprinted with permission from the Director of the Gordon Research Conferences. (*b*, *clockwise*) Julius Adler, Mel Simon, and Dan Koshland at the September 1978 conference Flagellar Motility in Hakone, Japan. The image has been extracted from the participants' photograph and manipulated to show only those three. (*c*, *clockwise*) George Ordal, Jerry Hazelbauer, Howard Berg, Bob Macnab, Sandy Parkinson, Barry Taylor, and Rick Dahlquist at dinner during the Table Ronde Roussel Uclaf Chemotaxis meeting, March 1983 in Paris, France. The image has been edited to show only researchers studying bacterial chemotaxis.

of Julius and the other because he split his speaking time with me. Similar situations occurred regularly in each group.

Annual Gatherings

A central factor in sustaining and expanding the bacterial chemotaxis community was the development of regular gatherings. By the mid-1970s, the field had expanded from Adler, Berg, Koshland,

and Simon to include laboratories of the initial generation of their chemotaxis offspring: Jerry Hazelbauer (Adler), Sandy Parkinson (Adler), George Ordal (Adler), Rick Dahlquist (Koshland), and Bob Macnab (Koshland). Bob's untimely death in 2003 was a great loss. Various combinations of these nine were regularly speakers in bacterial chemotaxis sessions at meetings such as Gordon Conferences on Bacterial Cell Surfaces or Molecular Pharmacology. This was valuable for us junior scientists, giving exposure to the wider scientific community and contact with the leaders of our growing field. I suspect our invited participation was often suggested by the senior four.

Sensory transduction in microorganisms (STIM): a biennial Gordon Conference

In the early 1980s, the bacterial chemotaxis community found a long-term home in a Gordon Conference. In 1991, our community created, and has since maintained, a companion meeting occurring in alternate years from that Gordon Conference. With one or the other meeting every January for over 20 years, it has become a habit to attend each January meeting (even if we are not invited to speak) and to bring as many members of our research group as space and finances allow.

Sensory Transduction in Microorganisms

The inaugural meeting of the Gordon Conference on Sensory Transduction in Microorganisms (STIM) was in California over New Year's week 1976 (**Figure 3**). It was organized by Bodo Diehn, from the University of Toledo in Ohio. Like Diehn, most speakers and participants studied behavior of eukaryotic microorganisms. Yet there was interest in bacterial chemotaxis and motility: The subjects occupied one day of the four-and-a-half-day conference. Adler, Berg, Koshland, and Parkinson gave major talks; Julius and Dan shared their speaking time with a member of their research groups, graduate student Michael Goy and postdoc Ruth Zukin, respectively. Rick Dahlquist, Bob Macnab, George Ordal, and I gave short presentations. Rick's graduate student Dan Chelsky attended the meeting, so 11 of 73 attendees (15%) worked on bacterial chemotaxis and motility but gave ~25% of the talks.

Unfortunately, the meeting had uncomfortable aspects. One was Diehn's frequently voiced opinion that everyone should use what he considered proper terminology in describing sensory

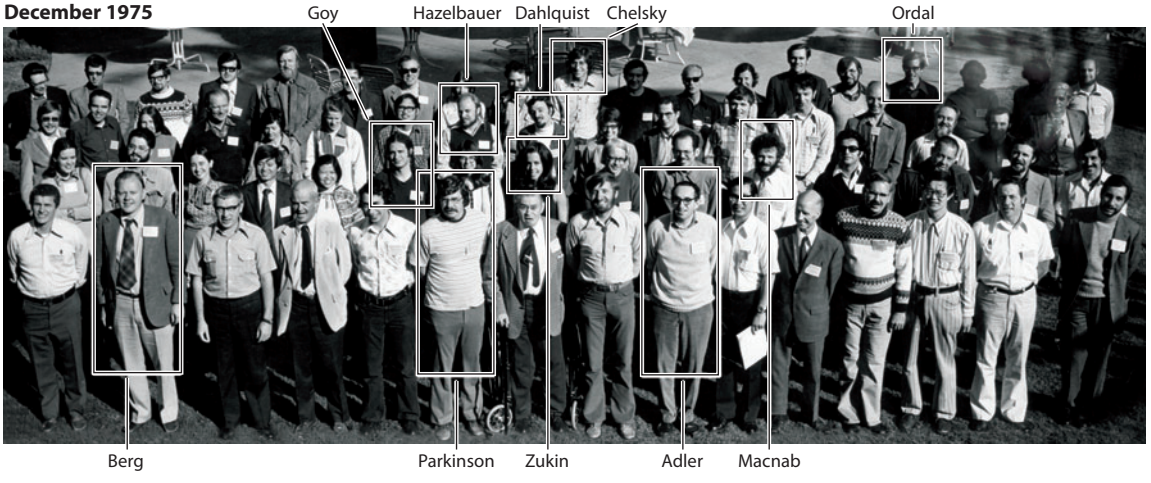

Figure 3

Participant photograph, first Gordon Research Conference on Sensory Transduction in Microorganisms, December 29, 1975 to January 2, 1976, at the Miramar Hotel in Santa Barbara, California. Participants from chemotaxis laboratories are boxed and labeled. Dan Koshland attended the meeting but did not arrive in time to be in the photograph. Photograph reprinted with permission from the Director of the Gordon Research Conferences.

behavior, the terminology of Fraenkel & Gunn (39, 40). He would interrupt speakers if he felt they erred. Perhaps in response, Julius utilized the terminology in his introduction to the session "Chemoaccumulation and Chemotransduction," but with the comic timing of Jack Benny. Most found this hilarious. Julius noted that Wilhelm Pfeffer's use of chemotaxis, a term that Diehn considered inappropriate for the tactic behavior of *E. coli*, had almost 60 years of precedence over Fraenkel and Gunn's term reverse klinokinesis. Those studying bacterial chemotaxis still use Pfeffer's term.

Bacterial locomotion and sensory transduction (BLAST): a biennial independent conference for researchers studying bacterial taxis and motility

In addition, tensions reflected the times. The spectacular successes of molecular biology had resulted in many areas of biology being investigated not only by those who had been trained in an area but also by apparent amateurs. Tensions between traditionalists and molecular biologists occurred in many academic life sciences departments. It was present at this Gordon Conference, even though several working on bacterial chemotaxis, most prominently Julius and Howard, knew and respected the history of studying microbial behavior (6, 17) and several scientists from traditional areas welcomed our participation.

Perhaps as a result of tensions at the 1976 meeting only half of the eight bacterial chemotaxis principal investigators attended the second meeting in 1978 and attendees working on bacteria remained at 11. This changed in 1980, perhaps because Vice-Chair Winslow Briggs particularly valued our community. One-third of the talks were about bacterial motility or taxis and 26 of 110 attendees (24%) were in the field. Importantly, Bob Macnab, who had spoken at all three conferences, was elected vice-chair for 1982. From then on our field has been a central part of this meeting, to our great benefit. 1982 established a pattern of chairs alternating between a scientist studying bacteria and one studying eukaryotic microorganisms, with the vice-chair from the complementary area, and of a program equally distributed between the two areas.

Bacterial Locomotion and Sensory Transduction

In the 1980s, the study of bacterial taxis and motility expanded, as did molecular and mechanistic studies of taxis and motility in the eukaryotic microorganisms, particularly *Dictyostelium discoideum*, making it difficult to fit all desirable topics in one biennial Gordon Conference. The bacterial community began discussing the creation of their own Gordon Conference. The situation came to a head at the 1990 STIM Conference. In planning that meeting, Peter Devreotes and I, chair and vice-chair, respectively, realized coverage of newly developing areas would require fewer talks about topics usually covered. It seemed best to cover some every second meeting, so we identified one prokaryotic and one eukaryotic topic to cover in 1992 but not in 1990. The prokaryotic topic was bacterial flagella. I contacted the major investigators to explain. Bob Macnab was upset. At the meeting, Phil Matsumura mediated at an after-the-evening-session tasting of single malt scotch. Phil posited that having to skip a topic was a positive development because it indicated growth in our areas and thus the basis for creating a second meeting exclusively for bacterial taxis and motility. Bob strongly agreed. Impressively, Phil, in partnership with Sandy Parkinson, proceeded to do so. Thus, the Bacterial Locomotion and Sensory Transduction (BLAST) meeting was born, to be held on alternate years from the STIM Gordon Conference. The field owes this important development to the combination of Sandy's and Phil's visions and initiatives. Sandy recalls spending a morning concocting acronyms for the inaugural meeting. When he posed them to Bob Macnab, Bob's response was, "Let's have a BLAST." So we did.

BLAST is notable because it is a grassroots organization, with no wider affiliation. Phil incorporated BLAST as a nonprofit organization with Board of Directors Phil, Sandy, Mike Manson, and Joseph Falke. It has organized 11 successful meetings over 20 years, maintaining financial stability and greatly enhancing scientific progress. Much is owed to the organizational talents of

Phil and his effective assistant, Peggy O'Neill. Crucial features of BLAST are (*a*) no attendance cap and a sufficiently low cost such that many laboratories can bring multiple members; (*b*) a program for oral presentations constructed from submitted abstracts; and (*c*) a preference for talks by junior scientists, students, postdoctoral researchers, and those new to the field. These features have worked very well. Almost every lab in the field is represented at each meeting. In essence, we all come. The first meeting, 1991 in Austin, Texas, set the tone. We guessed 50 to 60 would attend. Amazingly, 128 registered for a meeting that was designed to pack the most science in the fewest number of days, thus reducing costs. From Thursday evening through Sunday noon, 54 20-minute talks were scheduled: one slot for each attending laboratory. Only a storm disrupting air travel from Europe saved us from complete exhaustion by keeping some European colleagues from attending and thus freeing slots that could be rearranged to create a talk-free Saturday night, when attendees could party or sleep. From that auspicious beginning, the meeting flourished. Some years later, an NSF program officer attending a BLAST meeting remarked that few scientific fields "take care of their young" and that our field reminded him of the special environment of the bacteriophage lambda field in the 1960s.

FINAL THOUGHTS

Studying bacterial chemotaxis and motility has been notably fruitful. *E. coli* chemotaxis became a paradigm for the understanding of molecular mechanisms in biological signaling. The field has expanded to encompass multiple species, each with informative variations on themes identified in *E. coli*. In this regard, George Ordal was a pioneer, beginning molecularly oriented studies of chemotaxis in a distinctly different bacterium, *B. subtilis*, in the 1970s (69). Signaling in chemotaxis is now understood as a prominent example of a much wider phenomenon, two-component signaling, which couples environmental detection to response across bacterial diversity (26). For systems biology, bacterial chemotaxis is providing a tractable example of a sophisticated signaling system for modeling cellular behavior with detailed biochemical parameters.

There is much in the history of our field that could not be included in an article of limited length. Furthermore, the field continues to flourish. Over my 44 years as a participant, each time a major advance generated the worry that everything interesting had been done, new observations revealed additional fundamental questions and wider significance. I am confident this pattern will continue.

SUMMARY POINTS

1. Molecular studies of bacterial chemotaxis and motility began in the 1960s with pioneering work from Julius Adler. His laboratory identified the major areas of investigation pursued in the following two decades, and made multiple seminal observations.
2. In the 1970s, three additional research groups, directed by Howard Berg, Daniel E. Koshland, Jr., and Melvin Simon, began to publish in the area. Their respective expertise enriched the research environment. The four senior scientists contributed not only scientific advances but also an emphasis on quality and impact.
3. As often in science, progress included contributions from accidental findings, personal interactions, and scientific culture.
4. The cooperative and interactive nature of the chemotaxis community contributed much to our collective progress.

5. An important feature of this community has been two alternating biennial meetings at which the field gathers annually.
6. *E. coli* chemotaxis became a paradigm for the understanding of molecular mechanisms in biological signaling.

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The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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