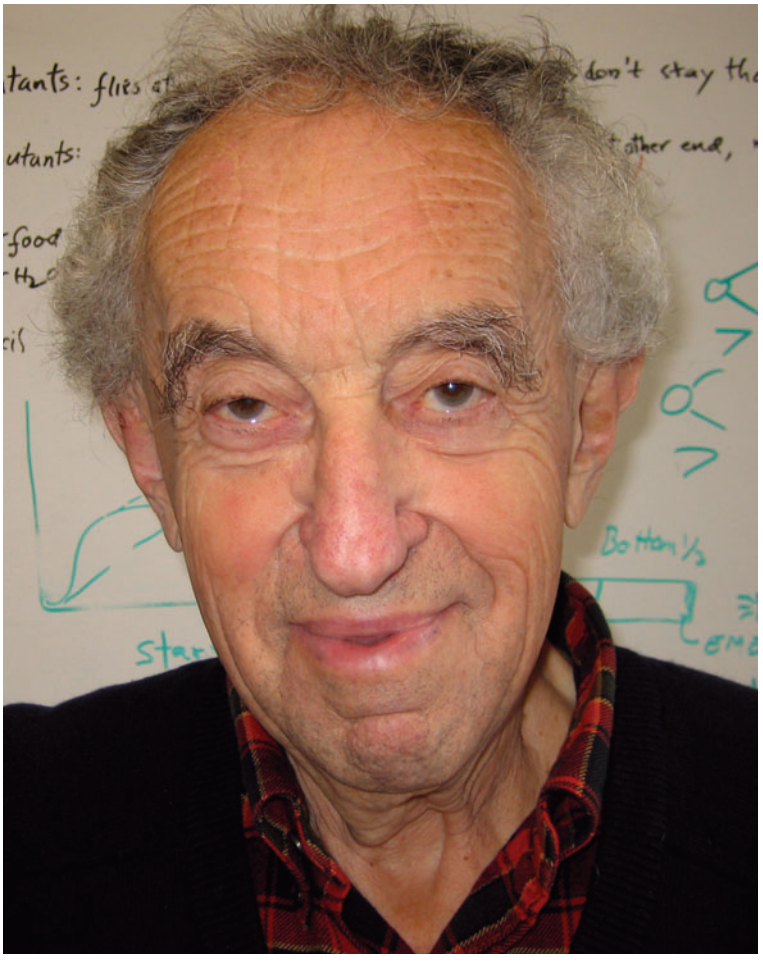

Julius Adler

My Life with Nature

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Annu. Rev. Biochem. 2011. 80:42–70

First published online as a Review in Advance on March 10, 2011

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

This article's doi: 10.1146/annurev-biochem-121609-100316

0066-4154/11/0707-0042$20.00

Keywords

bacteria, behavior, chemotaxis, *Drosophila*, molecular biology

Abstract

After a childhood in Germany and being a youth in Grand Forks, North Dakota, I went to Harvard University, then to graduate school in biochemistry at the University of Wisconsin. Then to Washington University and Stanford University for postdoctoral training in biochemistry and genetics. Then at the University of Wisconsin, as a professor in the Department of Biochemistry and the Department of Genetics, I initiated research on bacterial chemotaxis. Here, I review this research by me and by many, many others up to the present moment. During the past few years, I have been studying chemotaxis and related behavior in animals, namely in *Drosophila* fruit flies, and some of these results are presented here. My current thinking is described.

Contents

These are my teachers:
the red-bellied woodpecker
and the muskrat
when it's 10 below zero,
the red-admiral butterfly
and the jewel weed
when it's 96

1. BEGINNING YEARS

To me, nature has always been an inspiration. Both the beauty of nature and the science of nature are the things that have dominated my life.

When I was three years old (my mother told me), I said in a baby-talk southern German dialect, "Mama has beautiful flowers in her blanket." Every Sunday, my first grade class took a hike in the woods. During one of these walks, I remember a fox staring at us from its burrow and a European swallowtail gracefully gliding over the branches. My good friend Justin shared his interest in butterflies with me.

My father had a small butcher shop, which was handed down through generations, and he was also a cattle dealer. We lived in a little village, Edelfingen, where our ancestors had lived for 300 years. In the fifteenth century, the Jews of the German lands were forcibly expelled from nearly all the cities (for example Vienna 1421, Cologne 1426, Augsburg 1439). They escaped to the villages that would accept them. In those villages they were not allowed to take part in husbandry, or to participate in guilds, or to own land, so that little remained for them but petty trades.

By 1850, emancipation of the Jews in Germany had started to take place, and by 1930, about half of them had moved back into the cities. In the larger cities, many blossomed into professionals: businessmen, artists, musicians, and scientists. For a description of some of this, see David Nachmansohn's *German-Jewish Pioneers in Science 1900–1933*, *Highlights in Atomic Physics*, *Chemistry*, *and Biochemistry* (1). That book has influenced me because in a small way I am a part of it.

Beginning in 1933, the Nazis made life for German Jews intolerable. In 1938, it was time for my parents, my sister, and me, now eight years old, to leave Germany. Not so fortunate were my father's sisters, Zilli, who had always lived with us, and Paula, who had escaped to Holland with her husband and two daughters; they were all killed.

On the *George Washington*, all of the ship's passengers were jubilant as the harbor of New York City came into view. After a two-day train ride, we arrived in Grand Forks, North Dakota, where my mother's relatives had settled in 1880. My father and mother opened a little neighborhood grocery store, which they kept up for 30 years.

When not helping out in the store, I attended school. Ambition together with ability led to achievement: editor of the school's newspaper, debater at the state finals, and valedictorian of my class of 203 students. My greatest interest, stimulated by my teacher Hazel McMaster and her science club, was biology. Every week in the summer, Winston Jensen and I went out into the country to identify prairie flowers. I put together perhaps the largest collection found anywhere of butterflies of North Dakota and Minnesota. My friends Art Herman, Russell Rhode, and I met monthly to study texts by philosophers, including Nietzsche and others. Here is an example of my

determination as a youth: There was a contest to make as many words as possible out of the letters in "Pepsi Cola hits the spot." I hunted for three weeks through the unabridged dictionary in the public library, came up with 3,789 words, took them to the post office at 11:45 PM of the day due for postmarking, but the mail box didn't get emptied until 12:14 AM, and I never heard.

"We've had a grand time," sang the class of '48, with an eye to an even grander future.

Then came Harvard University. I majored in biochemical sciences, which included a mixture of courses in biology, chemistry, and physics. The most influential teachers were George Wald (of rhodopsin fame), who lectured on biochemistry, Louis Fieser on organic chemistry, and Clyde Kluckhohn, who taught anthropology of the American Indians of the Southwest. The most important experience was provided by my biochemistry tutor David Novelli; I was allowed to do research in his and Fritz Lipmann's laboratory at the Massachussetts General Hospital. (I never did talk to Lipmann, but I was inspired by standing near him on the elevator.) My research there resulted in my first scientific product, "Nicotinic Acid and Derivatives as Growth Stimulants for *Acetobacter suboxydans*." At Harvard, I made many new friends, including Tom Eisner, now studying insect behavior at Cornell, and Eric Kandel, now a professor of brain science at Columbia University College of Physicians and Surgeons.

During the summers between college academic years, I worked in my parents' store, but in addition, I got to know William Cornatzer, chaiman of the Department of Biochemistry at the University of North Dakota in Grand Forks. Pursuing my interest in butterflies and putting that together with my newly discovered knowledge of organic chemistry, I decided I would try, with help from Dr. Cornatzer, to identify the orange pigment on the wings of the orange-sulfur butterfly. I spent much of several summers catching thousands of these butterflies but never got enough to isolate the pigment. Nevertheless, I think it was a good project for just an undergraduate. (The desperate appeals by the "cat lady"—the lady who loved butterflies and had dozens of cats in her home—finally convinced me to stop collecting butterflies.)

I decided to go to graduate school in biochemistry at the University of Wisconsin, where Henry Lardy kindly accepted me into his laboratory. It was a thrilling time for me. Hank discovered that the carboxylation of propionate forms succinate in animals, and I took on this project for a year, working with rat liver mitochondria (2, 3). Hank's discovery, however, was taken over by Severo Ochoa's gigantic group. At that point, in response, I decided to work on the most obscure problem I could possibly find, the mechanism of itaconic acid metabolism in rat liver mitochondria, the subject of my PhD thesis in 1957 (4, 5).

While in Hank's laboratory, I surreptitiously did my first study of behavior. We were raising biotin-deficient rats to find out if the carboxylation of propionate requires biotin, which it turned out it did. I faithfully raised such deficient rats but on the side I gave one of them a choice of eating from a cup that contained food lacking biotin and a cup with food containing biotin. Almost immediately, the rat ate exclusively from the biotin-containing cup (6). That made me think about possible mechanisms.

From Hank, I learned the value of broad interests, tolerance of individual differences, and the glory of freedom in research.

Between receiving my PhD and starting postdoctoral studies, I spent the summer of 1957 taking Cornelis van Niel's course in microbiology at the Hopkins Marine Station in Pacific Grove, California. Unforgettable inspiration was generated by Van Niel, as well as by the beauty of the local seashore with its abundance of fabulous inhabitants. Also attending this class was Tetsuo Iino, who had just gotten his PhD with Joshua Lederberg in Madison and was to become a founder of the Japanese school of study of bacterial flagella. Tetsuo and I had great times together collecting butterflies in Pacific Grove. But my greatest accomplishment there was to make a decision on my research subject as an independent scientist. I wanted to study behavior, but which behavior of which organism? The Marine Station has a grand

library with journals dating back to nearly the beginning. There I found the "ancient" (1880s) publications of Wilhelm Pfeffer on the chemotactic behavior of bacteria. I decided that was it!

My first postdoctoral study was with Arthur Kornberg at Washington University from 1957 to 1959. I had heard a seminar by Arthur at the Massachusetts Institute of Technology when I was an undergraduate at Harvard, and I decided then and there that I would like to learn from him. I worked with Arthur, his wife Sylvy, Ernie Simms, Bob Lehman, and Maurice Bessman to discover more about DNA synthesis in *Escherichia coli* (7–10). Bob remains my close friend and advisor.

From Arthur, I learned the value of narrow interests, intense dedication to only one subject, and the need for objective and simple assays. Between Henry Lardy's liberalism and Arthur's conservatism, I decided on a middle-of-the-road policy.

Fred Bergmann was my inspiration and companion at the University of Wisconsin's Department of Biochemistry, where he studied plants with Robert Burris; he also was at Washington University, where he worked on amino acid–activating enzymes with Paul Berg. Fred and I were particularly close because he also came from a little German village, very near to mine. Beyond all that, Fred introduced me to the girl who would soon become my wife, Hilde Wohl, who came to the United States from Nuremberg in 1941. Hilde has been my wife for over 45 years. She is my greatest admirer and my best critic. We have a musician son, David, and an artist daughter, Jeane.

Between this postdoctoral study and my next one, in the summer of 1959, I took two courses at Cold Spring Harbor that influenced my life. One was on bacterial genetics, and the other was on phage genetics. Among our inspiring teachers were Milislav Demerec, Evelyn Witkin, George Streissinger, Frank Stahl, and Salvador Luria. There, I started many lasting friendships with others just beginning their scientific careers: Masayasu Nomura with wife Junko, Marshall Nirenberg, and more.

I moved to Stanford with the Kornberg group in the fall of 1959 to learn genetics from Dale Kaiser. He taught me how to study the genetics of galactose metabolism in *E. coli* (11). Dale is a marvelous teacher, a great experimentalist, and one of the kindest people. In recent years, he has been studying how bacteria form a fruiting body. Also at Stanford, I learned much from the enthusiastic Esther Lederberg, who was continuing her research on bacterial genetics.

My best companion at Stanford was Buzz Baldwin. He and I went for a hike nearly every weekend to discuss science, people, and nature. Although a physical biochemist, Buzz has over the years kindly encouraged and criticized all that I have done in the biological sciences.

2. MIDDLE YEARS

Now, I had reached independence! The University of Wisconsin's Department of Biochemistry, joined by the Department of Genetics, had called me back to become an assistant professor.

It was September 15, 1960, on Picnic Point, just after my arrival in Madison that Gobind Khorana and I met for the first time, each hiking separately and expecting to meet no one. Gobind had arrived recently from the University of British Columbia to become director of part of the Enzyme Institute, and I had just arrived from Stanford. Arthur had told both Gobind and me that we must get together because of similar interests. It was "love" at first sight. We had so much in common in terms of science, philosophy, and personality.

Gobind and I did research together for about a year. A catalyst for this was the visit of Arturo Falaschi from Italy to be a postdoctoral fellow with me for a brief period until space opened up at Stanford in Arthur's laboratory. Arturo, Gobind, and I then worked together on the chemical synthesis of polynucleotides (12). However, my greatest interest was not there. Gobind and I discussed this often. Gobind kindly encouraged me to work more

fully on what I really dreamed about—the behavior of bacteria.

A part of my job was teaching courses. This not only informed and inspired students, but it also kept me up to date on the latest developments. For 15 years, I taught one of the world's first molecular genetics courses, together with Hatch Echols, also a new assistant professor in the Biochemistry Department (and a close friend of mine, here until 1969 and then in Berkeley until his untimely death in 1993), and with Ernst Freese, also a new assistant professor in the Genetics Department (here until 1963, then he went to NIH to become chief of the Laboratory of Molecular Biology and director of the Basic Neurosciences Program at the National Institute of Neurological Diseases and Stroke; he died prematurely in 1990). We were joined occasionally by Gobind, Tom RajBhandary, and Masayasu Nomura. After that, I taught a course on molecular neurobiology together with David Nelson (my good friend, who is here still today) and with Ching Kung for 12 years; we had occasional lectures by Barry Ganetzky on flies and by Tony Stretton (my friend in the Zoology Department, who is here yet) on worms. At Woods Hole, I helped Howard Schachman teach a course on DNA, and several times at Cold Spring Harbor, I taught a course on behavior by Barry Ganetzky (on flies), Eric Kandel (on the mollusc *Aplysia*), and me (on bacteria).

2.1. Early Research on Chemotaxis

When I arrived in Madison, 50 years ago, I started behavioral work on *E. coli*. It was first necessary to develop an objective assay for bacterial motility (13); to devise a simple, chemically defined medium for motility; and to find a growth medium that was best for the synthesis of flagella (**Figure 1**) (14). Then I needed to devise an objective assay for chemotaxis (15). (I had deserted enzymology, Arthur Kornberg's only love, and I am sure he must have felt abandoned by my study of the wildest of subjects, behavior. Yet, I feel he must have approved of my quantitative, simple assay of chemotaxis, which I had carefully modeled after his presumed demands.) Bacteria are attracted by a chemical coming out of a capillary (**Figure 2**), and then, given more time, they are attracted into the capillary. In this assay for chemotaxis (15), the number of bacteria attracted inside the capillary was measured (**Figure 3**). After that, I learned how to just simply observe chemotaxis on an agar plate (**Figure 4**). (In a brilliant and methodical way, the fantastic Marge Dahl, now retired in allergy-free Santa Barbara, was the technician who managed all this and supervised all further work in my laboratory for 17 years.)

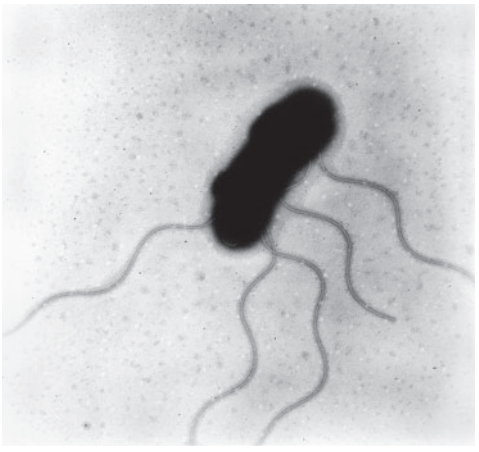

Figure 1

Electron micrograph of *Escherichia coli* showing flagella (41).

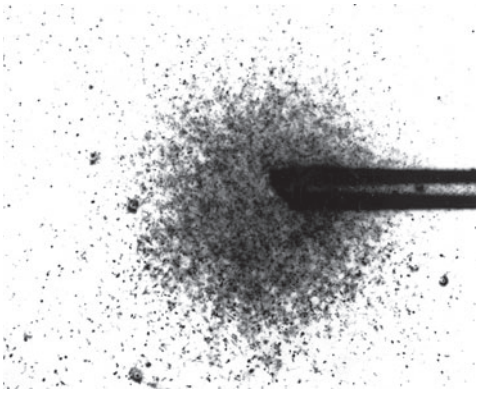

Figure 2

Escherichia coli attracted by tryptone coming out of a capillary (46).

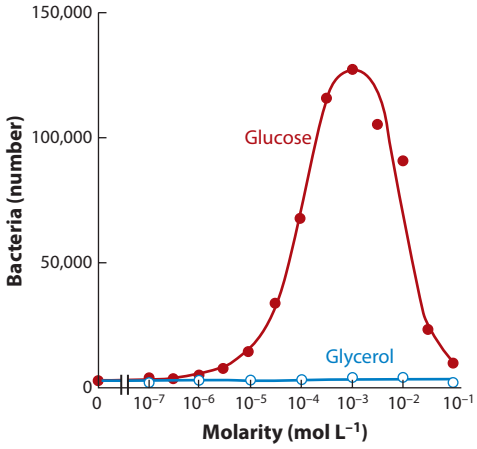

Figure 3

Escherichia coli attracted into a capillary containing D-glucose but not attracted by glycerol (46).

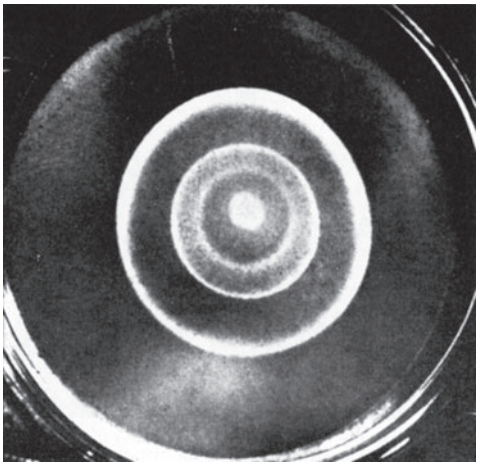

Figure 4

Escherichia coli are deposited in the center of a semisolid agar plate containing tryptone, where they grow, use up the local L-serine, and search for L-serine, moving to the outermost, L-serine, ring. Then some of those bacteria left behind in the center use up the local L-aspartate, then search for L-aspartate, moving to the second, L-aspartate, ring. And some bacteria left behind use up the local L-threonine, search for L-threonine, and move into the third, L-threonine, ring (42).

Every year the Department of Biochemistry accepted ~20 talented graduate students, and right away, I had some of these assigned to me: John Armstrong, Mel DePamphilis, and

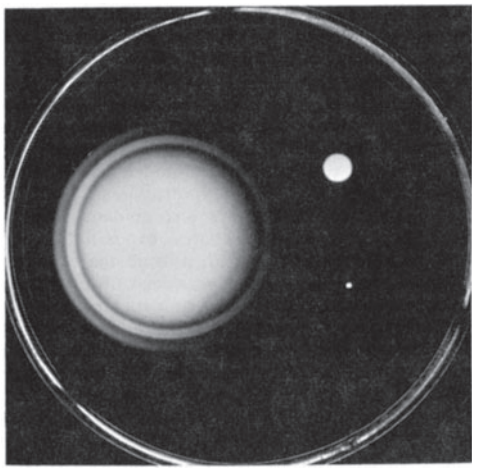

Figure 5

Normal *Escherichia coli* will spread on a semisolid agar plate containing tryptone (*left*), but generally nonchemotactic mutants spread little (*top right*), and nonmotile mutants do not spread at all (*lower right*) (18).

Sylvia Zottu Schade. John (now deceased, became professor in the Department of Biology, University of Ottawa) discovered the first mutants that failed in chemotaxis ("generally nonchemotactic mutants") (16) but were still motile (**Figure 5**). He found three such genes needed for chemotaxis, the *che* genes (*cheA*, *cheB*, and *cheC*) (17), and then we mapped these genes on the *E. coli* chromosome (18). Sandy Parkinson, who was here as a postdoctoral fellow from 1970 to 1972 and then went on to become a professor in the Department of Biology at the University of Utah, brought this genetic research much further (19–21), and he continues the use of genetics to discover molecular mechanisms of behavior (22). Mel (now section chief, National Institute of Child Health & Human Development, NIH) succeeded for the first time in purifying intact flagella from *E. coli* and also from *Bacillus subtilis* (23); then he determined their structure by means of the electron microscope (24). Subsequently, he found out how the flagellar hook–basal body complex is attached to the various parts of the cell envelope (25). At the same time at the University of California, San Diego, Mel Simon's

laboratory reported the purification and study of intact flagella (26) and hooks (27) from *B. subtilis*. This pioneering work was continued later in *E. coli* and *Salmonella* by others, so that we now have a good understanding of the structure of the flagellum and what each part of it does (see below). Sylvia (who went on to work at the University of Illinois Medical Center at Chicago) purified bacteriophage chi for the first time (28), and she found that it requires bacterial motility to attach to moving flagella and that chi-resistant bacteria were not motile, so we were able to isolate motility mutants in that way (29).

After we found the mutants that failed chemotaxis totally (i.e., John's *che* mutants, the generally nonchemotactic mutants), Jerry Hazelbauer & Bob Mesibov (30) discovered "specifically nonchemotactic mutants," those that failed chemotaxis to only a single attractant and its close relatives, namely one mutant for L-serine and one for D-galactose.

Which of all the chemicals attract *E. coli* and which repel? That was one of the first things we investigated. Bob Mesibov tested attraction to all the common amino acids and related chemicals, altogether 53 of them (31). The best of these were L-serine and L-aspartate. Two different chemoreceptors were involved, one for L-serine represented by a mutant we isolated that failed in L-serine taxis, and another for L-aspartate represented by a mutant we isolated that failed in L-aspartate taxis (31, 32). (Soon after getting his PhD with me in 1971, Bob took off for Tasmania to become an apple picker and eventually to compile "Millipedes of Australia" and "Tasmanian Centipedes.")

Jerry Hazelbauer & Marge Dahl (33) tested all the common sugars and related chemicals, altogether 85 of them. The best attractants were D-ribose, D-galactose, D-glucose, D-mannitol, D-sorbitol, maltose, and trehalose. Nine different chemoreceptors were involved. Mutants lacking the D-galactose receptor, or the D-mannitol receptor, or the maltose receptor were isolated (33). (Jerry is now chairman of the Department of Biochemistry at the University of Missouri and has continued all these years to make many discoveries in bacterial chemotaxis.) Wolfgang Epstein and I (34) then found out that phosphotransferase sugars were attractants and that mutants lacking their enzymes II failed to be attracted by them.

Wung-Wai Tso studied repellents of *E. coli* by testing 164 chemicals (35). These were grouped into at least nine classes, presumably each for a different chemoreceptor. The most potent repellents were short-chain fatty acids, hydrophobic amino acids, indole, skatol, benzoate, salicylate, Co^{2+}, Ni^{2+}, H^+, OH^-. Mutants lacking hydrophobic amino acid taxis and a mutant lacking salicylate taxis were isolated and described (35). (Wung-Wai is now a professor of biochemistry at the Chinese University of Hong Kong, where he studies the motility of spermatozoa.) In addition, the mutant for attraction to L-serine (see above) was found to not be repulsed by a number of these repellents such as acetate and L-leucine (32, 35), and the mutant for attraction to L-aspartate (see above) was found to not be repulsed by Co^{2+} and Ni^{2+}, according to Bob Reader et al. (32). (Bob is now a physician in Bolton, Ontario.) In contrast, some inorganic salts were found by Youlin Qi to be attractants (36). (Youlin now does research on monkeys at the University of Chicago.) Louis Tisa and coworkers (37–39) explored the role of Ca^{2+} ions in bacterial chemotaxis. (Lou is now a professor of microbiology and genetics at the University of New Hampshire, where he works on microbial physiology.) David Repaske found that certain attractants and repellents are not detected by specific chemoreceptors but rather by proton movement across the cytoplasmic membrane, causing a change in the internal pH (40). (Dave is now director in the Department of Pediatrics at the Nationwide Children's Hospital of Ohio State University.)

The news of our studies of chemotaxis spread fast, and it was Max Delbrück who perhaps appreciated these the most. Once he had completed his work on *E. coli* bacteriophage, he switched to the behavior of "simple" organisms: He worked on phototropism in the mold *Phycomyces*, and his student Rod Clayton studied phototaxis in purple bacteria. That his own

E. coli could itself be used to study behavior blew his mind.

Max invited me to speak at the 1965 Cold Spring Harbor symposium on "Sensory Receptors," where the "orthodox" neurobiologists described their results on the behavior of animals. I addressed the meeting (41):

> Very little is known about the mechanism of behavior in bacteria. One would like to know how the bacteria sense the gradients of chemicals that stimulate a chemotactic response. Are there sensory receptors in bacteria? In addition one would like to know how the sensed data are translated into action. Is there a coordinating system that directs the flagella? To try to get answers to these questions, a study of chemotaxis in *Escherichia coli* was undertaken in this laboratory. This organism was chosen because the vast knowledge of its biochemistry and genetics could be brought to bear on the problem.

My work was well received by some, and contested by others. Soon after, I published what we knew at that time in "Chemotaxis in Bacteria" (42).

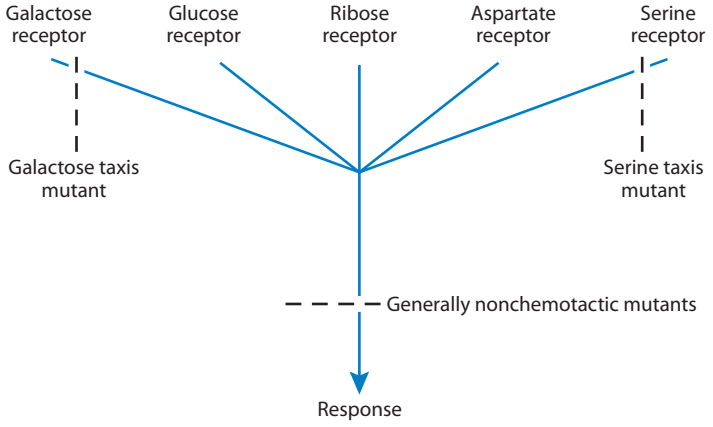

Figure 6

Specifically nonchemotactic mutants fail in a specific response owing to a receptor defect; for example, the galactose taxis mutant is deficient in the galactose receptor, and the serine taxis mutant is deficient in the serine receptor. By contrast, generally nonchemotactic mutants fail in all of the chemotactic responses owing to a defect in the final common pathway that tells flagella how to respond (46).

At Harvard, Howard Berg invented a tracking microscope to follow the behavior of bacteria (43). In 1970 he, together with Mary and children, came to Madison for four months to learn about *E. coli* and about our assays for chemotaxis. Then, he and Douglas Brown (44) described how to track bacteria that carry out chemotaxis. Howard went on, and continues, to make many advances in the study of the behavior of bacteria with his colleagues (45).

How do bacteria sense the chemicals that attract them? One way would be by metabolizing them so that energy (ATP) is produced, and then that energy tells the flagella what to do. An alternative way might be that bacteria have chemoreceptors, which instruct the flagella without any metabolism and without any energy production. The second way is how animals do it. I decided early that, if the first way were correct, I would stop the work on chemotaxis and go on to a different subject. It turned out that the second way was correct.

I published this pivotal conclusion in 1969 in "Chemoreceptors in Bacteria" (46). Many experiments presented there tell that, even in the absence of any metabolism of a chemical, the bacteria can still be attracted to it and that, even if a chemical can be metabolized and could thus yield ATP, it would not necessarily be an attractant. **Figure 6** was presented in that paper (46), and it is really the basis of all subsequent research on bacterial chemotaxis.

As to other early students of chemotaxis, there were two. Mel Simon with his group was highly instrumental in working out the mechanism of *E. coli* chemotaxis, especially, but not exclusively, using genetic approaches; he and Michael Silverman (47) started reporting this work in 1972 with a study of *E. coli* flagella. Dan Koshland, with his group of largely but not exclusively biochemists, was also highly instrumental. He started reporting research on chemotaxis in 1972 with Rick Dahlquist and Peter Lovely (48), Bob Macnab (49) and Robert Aksamit (50); they studied the closely related *Salmonella typhimurium* (now called *Salmonella enterica* serovar Typhimurium); and later, they also studied *E. coli*.

2.2. The Receptors for Chemotaxis

What are the chemoreceptors? When I arrived in Madison in 1960, I asked Hatch Echols to give me a strain of *E. coli* to study. Hatch, by just good luck for me, gave me B275. It turned out that this strain required L-methionine for growth. I found that it would do chemotaxis only when provided with L-methionine, but not when deprived of it (13). Somehow L-methionine was required for chemotaxis. But how?

It was the team of Ed Kort, Michael Goy, Steve Larsen, and me that discovered the answer. A graduate student in the Genetics Department, Ed was highly resistant to doing any biochemistry, so he studied the genetics of the methionine requirement of chemotaxis (51). Eventually, I convinced him to use radioactive methionine to learn how methionine functions in chemotaxis. (Ed is perhaps the most brilliant person I have ever met, but after he received his PhD here and did a short postoctoral study elsewhere, Ed decided to become a house painter and has now built a huge painting enterprise.) Michael, a great guitarist and a graduate student in neuroscience, had the highest enthusiasm for this study. (He is now a professor in the Department of Cell and Molecular Physiology of the University of North Carolina at Chapel Hill and works on an endocrine peptide that coordinates electrolyte balance in animals.) Steve was a graduate student in Biochemistry. (He is now a professor in the Department of Microbiology and Immunology at Indiana University School of Medicine and works on the genetics of diseases caused by *Chlamydia*.) In 1975, we discovered that the methyl group of methionine is incorporated into a novel protein, the methyl-accepting chemotaxis protein (MCP), located in the cytoplasmic membrane, that attractants such as L-serine bring about its methylation, and that some of the chemotaxis mutants we isolated were not methylated (**Figure 7**) (52). In short, MCP was found to be the receptor for bacterial chemotaxis. This represented the first time that a sensory chemoreceptor was ever chemically identified; previously, George

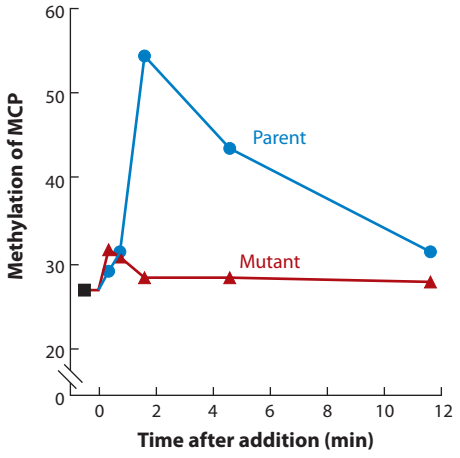

Figure 7

Effect of addition of L-serine on the methylation of methyl-accepting chemotaxis protein (MCP). The mutant is defective in L-serine taxis, but otherwise like the parent (52).

Wald had discovered rhodopsin as the sensory photoreceptor.

This MCP became known as MCPI (or as the serine receptor or Tsr), and its gene was named *tsr* (taxis to serine and certain repellents) (53). Then in 1977, MCPII was discovered in the cytoplasmic membrane by Marty Springer (now executive director of research at Merck Research Laboratories), Michael Goy, and me (53) and by Michael Silverman & Mel Simon (54). MCPII is also known as the aspartate receptor or Tar, and its gene was named *tar* (taxis to aspartate and other repellents) (53). Another cytoplasmic membrane protein MCPIII, serving D-ribose and D-galactose, was discovered by Hisato Kondoh, Carl Ball, and me (55). (Hisato is now project director of the Institute for Molecular and Cellular Biology at Osaka University, where he works on the development of fish. Carl is chair of the Department of Biology at Alverno College and studies yeast physiology.) The gene is called *trg* (taxis to ribose and galactose); mutants defective in *trg* were already known owing to the work of George Ordal and me (56), Strange & Koshland (57), and Hazelbauer & Harayama (58). (George is now a professor in the

Department of Biochemistry at the University of Illinois at Urbana-Champaign, where he studies chemotaxis in *B. subtilis*.) MCPIV, another cytoplasmic membrane protein, was described without any known function by Mel Simon's group (59), so they called its gene *tap* (taxis-associated protein), and later, Mike Manson and coworkers discovered that it is the receptor for dipeptides (60). Finally, another cytoplasmic membrane protein, the MCP-like receptor Aer for oxygen taxis (aerotaxis), was discovered by Sandy Parkinson's group (61) and by Barry Taylor's group (62). It has little or no extracellular part, although it does go through the cytoplasmic membrane, and inside, it contains flavine adenine dinucleotide, which interacts with an inside protein. So putting it all together, *E. coli* has five MCP-like proteins.

Dan Koshland's laboratory (63), Kimiko Yamamoto & Yasuo Imae (64), and others have found that receptors in *Salmonella* are similar to those in *E. coli*, including MCPI, MCPII, MCPIII, and Aer; except that MCPII, although it mediates aspartate taxis, does not respond to maltose, and MCPIV has not been found. A different MCP, Tcp for taxis toward citrate and repulsion by phenol, is present (64). Going through the sequenced genomes, Kelley Hughes and colleagues (65) found that *Salmonella* has in addition three putative MCPs, which *E. coli* lacks.

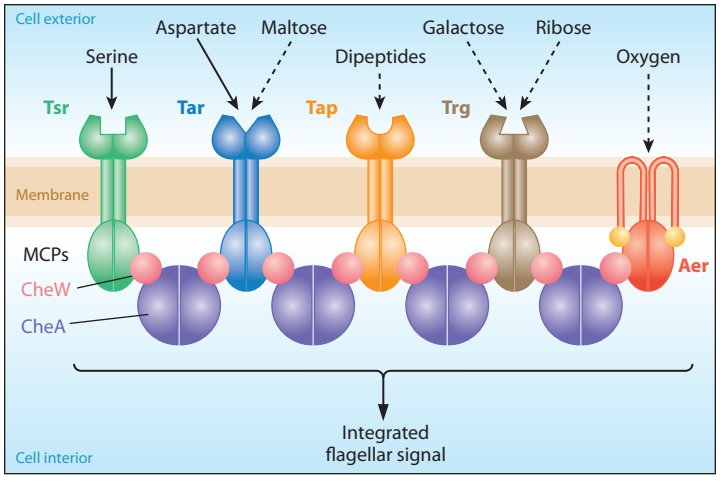

Figure 8

The methyl-accepting chemotaxis protein (MCP) family of chemoreceptors of *Escherichia coli*. L-serine and L-aspartate interact directly with MCPs (*solid arrows*), whereas maltose, dipeptides, galactose, ribose, and oxygen (*dashed arrows*) each require a protein that interacts with the chemotaxis protein. Reproduced from Parkinson (74).

In 1970, Herman Kalckar suggested to us that the receptor for D-galactose might be the galactose-binding protein (66), discovered by Yasuhiro Anraku (67) and studied by Winfried Boos (68). Jerry Hazelbauer and I showed in 1971 that this was indeed correct and that mutants lacking D-galactose taxis were lacking this periplasmic protein (69); the galactose-binding protein ultimately turned out to interact with the cytoplasmic-membrane protein MCPIII (55). A periplasmic ribose-binding protein of *Salmonella* was purified by Robert Aksamit & Dan Koshand (50). It was shown by them to be the D-ribose receptor for taxis, and mutants missing it were missing ribose taxis (70). The ribose-binding protein interacts also with MCPIII (55). The periplasmic maltose-binding protein was discovered by Odile Kellerman & Sevec Szmelcman (71). Jerry Hazelbauer established that it is the receptor for maltose taxis (72). Mike Manson and collaborators demonstrated that it interacts with *E. coli* MCPII (73). Mike and his colleagues showed that the dipeptide-binding protein, also periplasmic, interacts with MCPIV (60). In contrast to all these, MCPI interacts directly with L-serine (52), and MCPII interacts directly with L-aspartate (53, 54). All this is summarized here in **Figure 8**, thanks to Sandy Parkinson (74). The detailed structure of MCP is shown in **Figure 9**, thanks to Joe Falke & Sung-Hou Kim (75).

The genes for the MCP's in *E. coli* and in *Salmonella* have been mapped. Furthermore, the sequence of deoxynucleotides in these genes has been determined, and thus, the presumed sequence of amino acids in their proteins is now known. (I did not play any part in that.) This was done with the MCP genes by the laboratories of Mel Simon, Dan Koshland, and Jerry Hazelbauer—*tsr* (76), *tar* (77, 78), *trg* (79), and *tap* (78). The proteins made by some of the MCP genes have been purified and

characterized (77, 80). Then the three-dimensional structures of the MCPs were determined to learn the molecular mechanisms involved (81–88).

MCPs have been found in many other bacteria and archaea [see the review by David Zusman et al. (89)]: 10 in *Sinorhizobium* (formerly *Rhizobium*) *meliloti*, 10 in *B. subtilis*, 11 in *Rhodobacter sphaeroides*, 18 in *Caulobacter crescentus*, 18 in *Halobacterium salinarum*, 21 in *Myxococcus xanthus*, and 25 in *Pseudomonas aeruginosa*. Michio Homma and coworkers (90) reported that there are 45 in *Vibrio cholera*. George Wadhams & Judith Armitage (91) wrote that there are more than 60 in *Magnetospirillum magnetotacticum*. So we were lucky that *E. coli* has only five, which made everything much easier to study. Roger Alexander & Igor Zhulin (92) found that MCPs occur in 152 genomes of bacteria and archaea, and they have compared these MCPs.

Janine Maddock and Lucy Shapiro discovered that the MCPs are located at the cell's termini, both in *Caulobacter crescentus*, where this was first detected (93), and also in *E. coli* (94). Laura Kiessling and coworkers (95) found that this localization of MCP in *E. coli* was stabilized by a polymer of repellent and destabilized by a polymer of attractant.

Already in 1976, my laboratory had begun subcellular studies of chemotaxis, i.e., in vitro biochemical experiments. After Steve Kleene (who now works on olfaction in frog and mouse in the Department of Cancer and Cell Biology at the University of Cincinnati) and Myron Toews (who now works on adrenergic receptors in the Department of Pharmacology at the University of Nebraska Medical Center) showed that glutamic acid 5-methyl ester is the methylated amino acid of MCP (96) and that in vivo it gives rise to methanol (97), Steve Kleene & Ann Hobson (now in the Department of Laboratory Medicine, University of Washington) showed that attractants and repellents work in a cell-free extract to give rise to methylation and demethylation (98). Mary Hedblom (now at Abbott Laboratories in Evanston) found that L-serine could bind to vesicles of wild-type but not *tsr E. coli* (99). At about this time, subcellular biochemical work on chemotaxis was initiated also by Dan Koshland and his group; they began this with a study of the enzyme that methylates MCP (100).

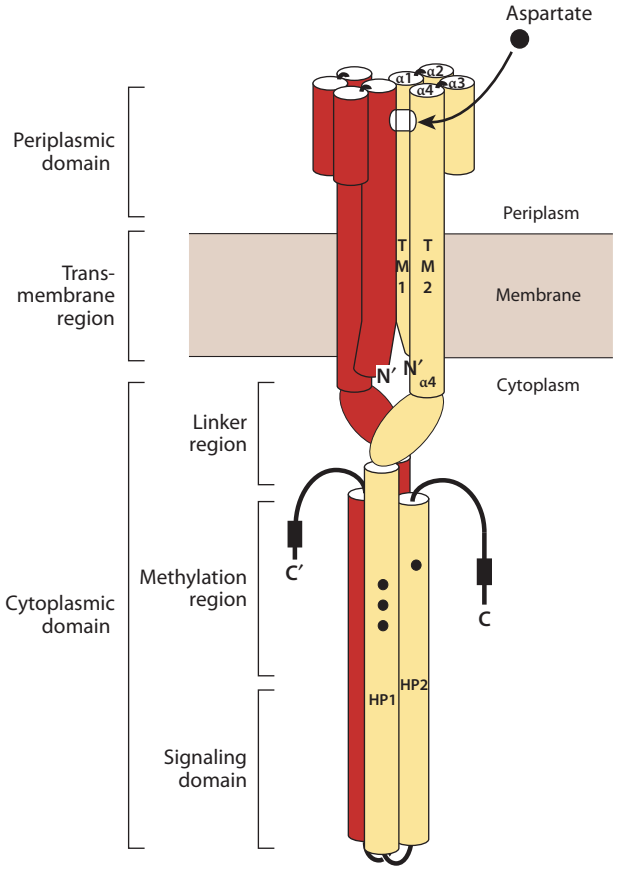

Figure 9

Structure of the methyl-accepting chemotaxis protein (MCP). The two subunits of the dimer are indicated in red and yellow. Four methylation sites are shown for this aspartate receptor (*filled circles*); the serine receptor has five. TM, transmembrane helix; HP, helical hairpin; N, N terminus; C, C terminus. Reproduced from Falke & Kim (75).

2.3. The Part Between Receptors and Flagella

The middle components of the chemotaxis mechanism, that is, the parts after the MCP receptors and before the flagella, are the Che proteins. These are made by the *che* genes, and they carry out "excitation" and "adaptation." As stated above, John Armstrong and I discoved

these; we found three of them (17, 18). Now six are known: CheW, CheA, CheY, CheZ, CheR, and CheB. We owe this knowledge to Sandy Parkinson (19–21), Dana Aswad & Dan Koshland (101), Michael Silverman & Mel Simon (102), and Anthony DeFranco et al. (103). By responding to the MCP's interaction with an attractant or repellent, CheA, CheW, CheY, and CheZ tell the flagella what to do: namely to carry out excitation, see below. Then, CheB and CheR carry out adaptation to the attractant or repellent by methylating MCP or demethylating it, respectively, as described below.

The genes for the Che proteins in *E. coli* and in *Salmonella* have been mapped, and the sequence of deoxynucleotides in these genes has been determined, so the presumed sequence of amino acids in their proteins is now known. (I played no part in any of that.) This was done by the laboratories of Mel Simon, Ann Stock, Jeff Stock, Sandy Parkinson, and Phil Matsumura—*cheW* (104, 105), *cheA* (104, 106, 107), *cheY* (104, 108), *cheZ* (104, 109), *cheB* (104, 110), and *cheR* (104, 111). To study the molecular mechanisms, the three-dimensional structures of these Che proteins were then determined: CheW (112), CheA (113–121), CheY (122, 123), CheZ (124), CheR (125), and CheB (126, 127).

The *che* genes have, by now, been found universally among bacteria (both the ones that move by flagella and those that move by pili) and in the archaea as well. See the summary of the *che* genes by Hendrik Szurmant & George Ordal (128), the summary of archaeal *che* genes by Ken Jarrell et al. (129), and the article by John Spudich (130). In addition, see the report of archaeal *che* genes by Johannes Rudolph & Dieter Oesterhelt (131), and the examples of *che* genes in the nonflagellated *M. xanthus* by Wenyuan Shi & David Zusman (132) and *P. aeruginosa* by John Mattick (133).

2.4. The Flagella

The energy source for *E. coli* flagella is not ATP, but rather it is a proton-motive force (the gradient of H^+ ions); this was discovered by Steve Larsen, me, Jay Gargus, and Bob Hogg (Jay and Bob were at Case Western Reserve University) (134). For some other bacteria, a gradient of Na^+ ions instead of H^+ ions is used by certain flagella, as reported by Tatsuo Atsumi, Linda McCarter, & Yasuo Imae (135). The idea that bacterial flagella work by rotating we owe to Howard Berg & Robert Anderson (136), who presented evidence in favor of that. Michael Silverman & Mel Simon (137) proved this experimentally and showed that rotation can be in either direction. Then Steve Larsen et al. (138) demonstrated that bacteria rotate their flagella counterclockwise to produce running to attractant and clockwise to produce tumbling to repellent. In place of flagella, type IV pili bring about motion, termed twitching motility, that works by means of extension, tethering, then retraction of the polar pili, for example, in *M. xanthus* (132) and in *P. aeruginosa* (133). In the archaea, flagella have a chemistry that is different from that of bacterial flagella according to a summary by Ken Jarrel et al. (129), and the energy source is ATP instead of a proton flux, as found by Dieter Oesterhelt's group (139).

Michael Eisenbach, a postdoctoral fellow from the Weizmann Institute of Science from 1978 to 1980 (he returned to the Weizmann Institute as professor), discovered that even cell envelopes of *E. coli* would rotate their flagella, but only counterclockwise if a source of proton motive force is added (140). Michael has continued this work at the Weizmann and learned, together with Shoshana Ravid and Phil Matsumura, that addition of CheY is necessary for clockwise rotation (141).

Progress in determining the structure of the flagellum is summarized here. Mel DePamphilis and I were the first to isolate and characterize flagella from *E. coli* (23–25); see David Blair's **Figure 10*a*** (142). For *Salmonella*, Shin-ichi Aizawa, Bob Macnab, and their colleagues (143) confirmed this structure but made it apparent that the base of the flagellum is much more complex. The currently known structure is shown in **Figure 10*b*** and in

Figure 11. Mutants in *fliG*, *fliM*, and *fliN* fail in reversing the direction of rotation, so FliG, FliM, and FliN are the switch complex that is central for response to stimuli, according to Aizawa et al. (143) and Macnab and coworkers (144, 145). Then it was discovered by Adam Driks & David DeRosier (146), Shahid Khan's group (147), and David DeRosier and colleagues (148) that the base of the flagellum contains a C ring (see **Figures 10*b*** and **11**). A few years later, Khan and coworkers found FliG, FliM, and FliN in the C ring (149, 150). The binding of CheY-P to FliM, which generates clockwise rotation of the flagella, was discovered by Martin Welch, Kenji Oosawa, Shin-ichi Aizawa, and Michael Eisenbach (151). Further advances were made by use of electron microscopy by David DeRosier and his group (152), by use of NMR by Rick Dahlquist and colleagues (153), as well as by crystallization and coprecipitation by David Blair and his group (154, 155). Daniela Stock and colleagues have characterized the FliG monomer and FliG polymer that are involved in rotational switching (156). Cyclic diguanylate, one factor that controls motility, was shown to interact directly with the base of the flagellum by David Blair, Rasika Harshey, and coworkers (157) and by Victor Sourjik's group (158). For the remainder of the flagellar genes, see the review by Bob Macnab & Sandy Parkinson (159) and those by Bob Macnab (160–162). More recent reviews of flagellar action are available (163–166).

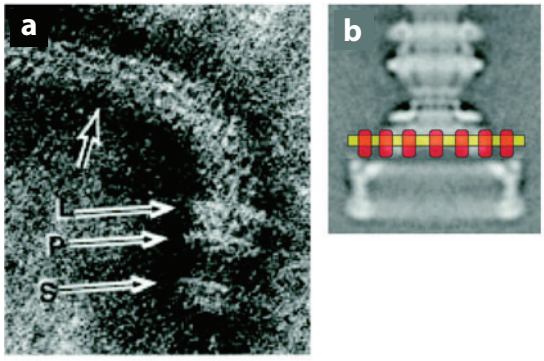

Figure 10

(*a*) A 1971 electron micrograph of the basal body of *Escherichia coli* from DePamphilis & Adler (24) as presented by David Blair (142). The top arrow indicates the junction between hook and filament. The other arrows indicate the L ring (L), named for its attachment to the outer (lipopolysaccharide) membrane of the cell wall; the P ring (P), named for its association with the peptidoglycan layer of the cell wall; and the S ring (S), named "supramembrane" for its location just above the cytoplasmic membrane. Below the S ring is the M ring (not labeled) named for its location at the cytoplasmic membrane. (The S ring and the M ring are now known to be a single ring named the MS ring.) (*b*) A 2006 image of the flagellar basal body of *Salmonella* as presented by David Blair (142). The figure indicates the approximate positions of the cytoplasmic membrane (*yellow*) and the stator complexes (*red*). The stator complexes are composed of MotA and MotB, remain stationary relative to the cell body, and conduct protons that energize the motor. The region below the cytoplasmic membrane is the C ring.

2.5. Two-Component Systems

An amazing development appeared in 1985 and 1986. Ann Stock, Dan Koshland, and Jeff Stock (167) discovered that CheY and CheB are closely related to three other transcriptional regulators that are not involved with chemotaxis. In addition, Norihiro Mutoh & Mel Simon (104) found that CheA is related to a transcriptional regulator not involved with chemotaxis. At about the same time, other investigators outside the chemotaxis field found other cases of such protein similarities (168–172). Fred Ausubel and collaborators named these proteins "two-component systems" (173).

But the most fantastic was yet to come: There is phosphorylation in the two-component system. The father of this was Boris Magasanik, who, with Alexander Ninfa in 1986, demonstrated for glutamine synthesis in *E. coli* that ATP was used by the first component to phosphorylate the second component (174). Soon thereafter, phosphorylation was discovered by a number of laboratories in various two-component systems. These earliest discoveries for chemotaxis were reported in 1987 and 1988 by Mel Simon's group (175, 176) and by David Wylie, Ann Stock, and Jeff Stock (177). Subsequent reviews of phosphorylation in chemotaxis and in other systems have been presented (178–183).

These findings led to a general model, i.e., the concept of a phosphorylated

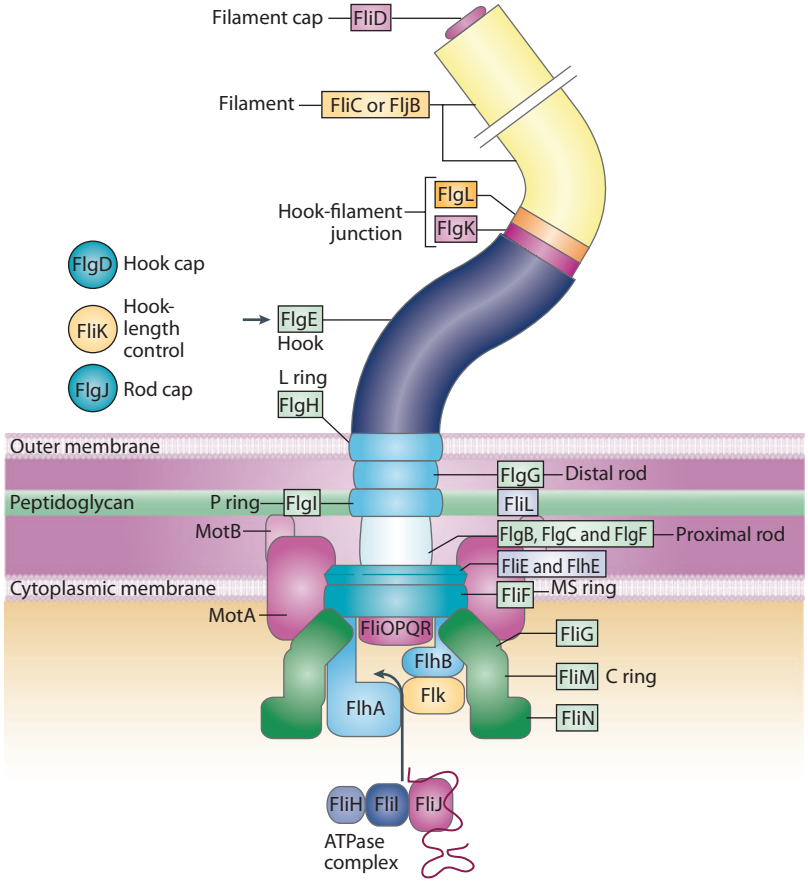

Figure 11

Diagram of the bacterial flagellar motor as presented by Fabienne Chevance & Kelly Hughes (251). FliG, FliM, and FliN make up the rotor, also called the "switch." MotA and MotB make up the stator, through which protons (or in some cases sodium ions) flow.

two-component system for sensing a large variety of stimuli to perform a variety of functions. By phosphorylation with ATP at a histidine residue of the first component, and then transferring the phosphate to an aspartate residue of the second component, this system causes varied effects like changing the direction of the rotation of flagella or controlling a gene that is needed for synthesis of a protein. The two-component system is found in bacteria, archaea, single-cell eukaryotes such as protozoa, fungi, algae, and in green plants, but not, it seems, in animals, according to Igor Zhulin's group (184). Jeff Stock and colleagues (185, p. 3013) wrote, "No histidine protein kinase (or response regulator) genes are present in the completed genome sequences of *Caenorhabditis elegans*, *Drosophila melanogaster*, or *Homo sapiens*, and it is thought that these enzymes are absent from the animal kingdom as a whole."

Taking into account the role of phosphorylated intermediates, scientists have created a cell-free system that reconstitutes chemotaxis: Each of the purified components—Tar, CheW, CheA, and CheY—was added together with ATP to yield the expected response to the attractant aspartate, namely dephosphorylation of CheY. This was done by Katherine Borkovich, Nachum Kaplan, Fred Hess, and

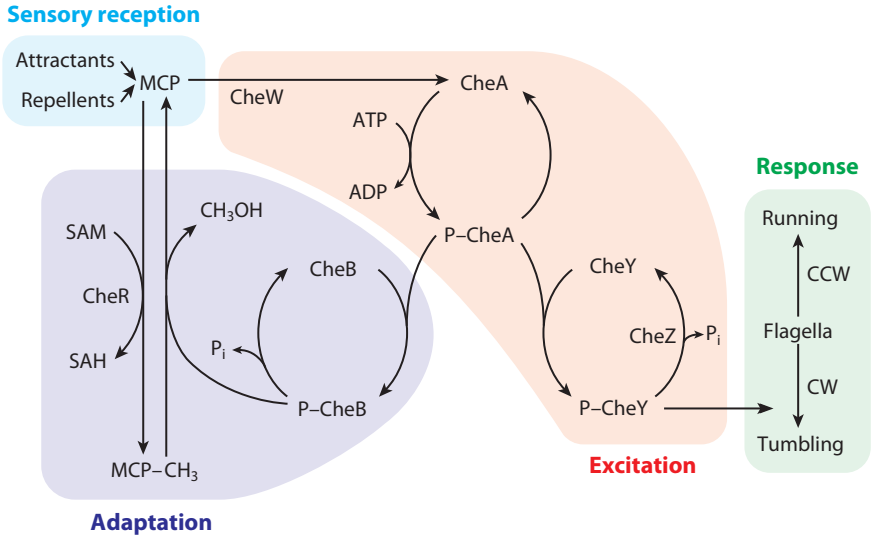

Figure 12

Current view of the mechanism of chemotaxis in *Escherichia coli* and *Salmonella*. Repellents promote the phosphorylation of CheA and consequently the phosphorylation of CheY, which brings about tumbling. Attractants block the phosphorylation of CheA and of CheY, so tumbling is not promoted, and instead, running predominates. These processes are called excitation. Then adaptation follows: Repellents cause demethylation of the methylated methyl-accepting chemotaxis protein (MCP), and attractants cause its methylation. CCW, counterclockwise rotation; CW, clockwise rotation; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

Mel Simon (186) and by Elizabeth Ninfa, Ann Stock, Sherry Mobray, and Jeff Stock (187).

Putting it all together, *E. coli* has five cytoplasmic-membrane chemotaxis receptors, which are the MCPs (namely Tsr, Tar, Tap, Trg, and Aer), and four periplasmic chemotaxis receptors, which combine with the MCPs (namely those for maltose, D-ribose, D-galactose, and dipeptides) (**Figure 8**). Then in *E. coli* and *Salmonella*, the MCPs interact with four excitation components, CheW, CheA, CheY, and CheZ. Then CheY-P interacts with the flagellar switch (the rotor), containing FliM, FliN, and FliG, to determine that the rotation of the flagellum will be clockwise (for repellents), and CheY remains unphosphorylated to determine that the rotation will be counterclockwise (for attractants). The rotation is brought about by MotA and MotB (the stator). Then adaptation is brought about by methylation or demethylation of MCP by CheB and CheR, respectively.

In **Figure 12,** I present the chemotaxis mechanism as it is known today in *E. coli* and *Salmonella*.

As to prokaryotic species other than *E. coli* and *Salmonella*, a search of 450 genomes by Kristin Wuichet & Igor Zhulin has identified chemotaxis genes in genomes of 245 species (188). Reviews of chemotaxis in bacteria and archaea from 2004 to 2010 are found in References 189–199. For additional recent articles on two-component systems, including chemotaxis, see the five volumes dedicated to this subject (200–204).

2.6. Responses to Other Stimuli

My laboratory has, in addition, studied *E. coli's* responses to sensory stimuli other than chemicals. Hanjing Yang (205) discovered that *E. coli* are repelled by blue light (phototaxis). (Hanjing now works on bacterial DNA repair and mutagenesis at the University of

California, Los Angeles.) I found that *E. coli* are repelled by cold and attracted by warmth (thermotaxis) (206). Yasuo Imae studied thermotaxis extensively; it was one of his life-long goals to understand this. With collaborators, he discovered that all four of the MCPs of *E. coli* can function as thermoreceptors, as reported in a paper dedicated to him by the rest of the authors with deep sorrow, respect, and affection (207). Wenyuan Shi and Michael Lentz then studied this attraction to warmth by raising the temperature with use of an electric shock (208). (Wenyuan is a professor of dentistry and molecular biology at the University of California, Los Angeles, and continues to study the behavior of bacteria; Mike roams the countryside of southern Wisconsin to enjoy its caves and its beauty.) Wenyuan discovered that *E. coli* swims to the anode but that *Salmonella* swims to the cathode, and he and coworkers worked out the mechanism for these activities (galvanotaxis) (209, 210). Congyi Li and Youlin Qi with others in my lab showed that *E. coli* are repelled by both low and high osmolarity, i.e., they seek an optimum osmolarity (osmotaxis) (211–213). (Congyi, now called Richard, studies the use of DNA for crime analysis as a professor of forensics at Indiana University.) Factors causing the lack of flagella were studied by Wenyuan Shi, Congy Li, Charles Louise, and coworkers (214–216).

In addition to chemotaxis, now often referred to as "swimming," there is another form of motion referred to as "swarming." (I didn't study this.) Swimming takes place in water or in dilute agar (about 0.25% weight/volume) in response to attractants and repellents. With more concentrated agar, (0.45% weight/volume) bacteria, such as *E. coli*, swarm instead; they elongate, become multinucleate, produce more flagella, and spread outward in a thin, highly motile layer, as presented in "Dynamics of Bacterial Swarming" by Howard Berg and collaborators (217). Chemotaxis is not required for swarming in *E. coli* or in *Salmonella* according to a discovery by Rasika Harshey and colleagues, but CheY/CheY-P is required because the ability to reverse the motor direction is important for swarming (218). Matthew Copeland, Douglas Weibel, and colleagues (219) found that the bundles of flagella on swarmer cells remain cohesive during frequent collisions with neighboring cells.

2.7. Electrophysiology

In an animal's nervous system, there are action potentials. Are there action potentials in bacteria carrying out behavior? This question intrigued me from the very start, but I say right away that there is no evidence for this in *E. coli*. With action potentials in mind, I was happy to accept an invitation from Seymour Benzer to use electrophysiology on *E. coli* with him at the Salk Institute in the summer of 1969. Our conclusion was that there were no positive results.

In 1985, Hans Ruthe (a postdoctoral fellow from Germany) and I succeeded in making giant *E. coli* spheroplasts (5 to 10 μm in diameter, ten times the size of ordinary *E. coli*) by using cephalexin with lysozyme (220). [We didn't know that giant spheroplasts were described earlier (221, 222).] Then these giants were used for patch-clamp recording by Boris Martinac, Matthew Buechner (my always-wearing a tie graduate student, now at the University of Kansas, where he works on the behavior of *C. elegans*), Anne Delcour (my postdoctoral fellow from Cornell University and Belgium, now at the University of Houston, where she continues to study electrophysiology of bacteria), me, and Ching Kung. We discovered that there are electrical changes in *E. coli* when it responds to mechanical stimuli, even though there seem to be no action potentials (223). Out of 11 additional publications written jointly by my lab with Kung's lab, 4 more are listed here (224–226), and 2 more papers are by Changhai Cui (my graduate student from Beijing and now the program director of the Division of Neuroscience and Behavior at NIH), Dean Smith, and me (227, 228). (Kung had been studying electrophysiology on *Paramecium* for many years before this, and after our joint publications, he carried *E. coli* research much further. He has recently been studying electrophysiology in

yeast, which has resulted in discoveries on the mechanism of touch.)

The role that ion fluxes play in the chemotaxis mechanism is still not clear. Sevec Szmelcman (a postdoctoral fellow with me from the Pasteur Institute) showed that attractants and repellents both produce a hyperpolarization, judged by use of a radioactive permeable cation as an indicator of the membrane potential rather than judged by direct electrophysiology (229). The role of outer membrane permeability in chemotaxis was studied by Colin Ingham, Matthew Buechner, and me (230). (Colin is now at Wageningen University, the Netherlands, where he uses interdisciplinary approaches to study microbial growth.)

3. LATER YEARS

At the start, I had imagined that the mechanism of behavior by bacteria and the mechanism of behavior by animals would be basically the same and also that by studying one it would be possible to know the other. That was indeed the lesson learned from Lipmann's study of metabolism in bacteria and from Kornberg's study of DNA synthesis in bacteria, and from other research. But it was not the case here. In 1991, Linda Buck & Richard Axel (231) discovered that the receptors for odor recognition in mammals are seven-transmembrane proteins, not MCP, and this turned out to be the case also for taste receptors according to Charles Zuker and coworkers (232, 233) and Linda Buck's group (234, 235), as reviewed by Bob Margolskee (236). Then, from the receptors, the sensed information of course goes to the brain for analysis before action can be taken by the muscles. Although basic similarities certainly remain between bacterial behavior and animal behavior, the mechanisms of the two are different. This was apparent to me by 1995.

So in 1997, I began to study the behavior of animals. Which one to choose? People have about 100,000,000,000 neurons, mice about 10,000,000,000, zebrafish about 1,000,000, flies like *Drosphila* about 100,000, and the roundworm *C. elegans* 302. I chose *Drosophila*, after trying mice, because of its relative simplicity and because the doubling time was short (two weeks). Barry Ganetzky and Bob Kreber in the Department of Genetics here provided enormous help. My research on the behavior of *Drosphila* was made practical by many talented undergraduates.

As with mammals, the gustatory and olfactory receptors of *Drosophila* are also seven-transmembrane proteins according to Qian Gao & Andrew Chess (237), John Carlson and coworkers (238, 239) and Charles Zuker and colleagues (240). For a recent review of taste and smell in *Drosophila*, see the reports by Leslie Vosshall & Reinhard Stocker (241), by Richard Benton (242), and by Ana Silbering & Richard Benton (243). For the mechanism of sensing light in *Drosophila*, see the recent review by Ben Katz & Baruch Minke (244).

To me, one of the most interesting questions of behavior is how an organism can make a decision about what to do when it encounters conflicting stimuli. A study of this would lead into the mechanism that is in control of the organism. I had already approached this in *E. coli* that were confronted with an attractant together with a repellent (245; see also Reference 246). Now, I began to study the mechanism of decision making in *Drosophila*. Opposing stimuli—an attractant (light) and a repellent (methyl eugenol)—are placed at one end of a tube and flies at the other end. Repellent slightly exceeds attractant, so the flies remain where placed, as far away as they can get. Mutants do not remain there. Undergraduates in my laboratory have isolated and studied nine such mutants (247). We are now identifying the genes involved in that mechanism, and we will determine how the proteins normally made by those genes declare a decision. This may apply not only to flies but perhaps also to other organisms including humans. It may be that indecisive people and those making wrong decisions, especially even criminals, could be aided by this knowledge.

We are now getting further into that mechanism that is in control of behavior. Two different attractants (light and water) are placed

at one end of a tube, and two different repellents (heat and benzaldehyde) are placed at the other end. Then, flies are started near the repellent end. They quickly go to the attractant end. Mutants are unable to do this and wander randomly. In this way, we (Lar Vang, Alex Kleven, Drew Winter, and I) have isolated mutant flies (seven at this writing) that are unresponsive to all kinds of stimuli, though they are motile (unpublished results). We will study these mutants to discover the mechanism that is defective in these mutants, namely the mechanism that dictates a response.

Recently, I conceived a new idea. I entertain the following in "In Search of The Boss, The Thing in Each Organism That Is in Charge of It" (248):

> The Boss is the thing inside every organism—humans, other animals, plants, microorganisms—that is in charge of the organism. I don't mean this in any mystical or spiritual or religious sense, but rather I mean it in terms of chemistry and physics. You may think that The Boss is a wild idea, and certainly the evidence for is it is poor, but I think it's true, and at least it's a hypothesis to be tested.

All the things that an organism does are controlled by The Boss, but this control is not always direct: Many aspects are delegated to managers, who delegate to foremen, who delegate to workers. So far it is largely the workers that have been studied, and sometimes the foremen are revealed, and rarely the managers, but The Boss has remained largely hidden. By itself, or by delegating, The Boss controls metabolism, behavior, reproduction, and other functions.

How can we get experimental evidence for testing this idea? One approach is to study much further the *Drosophila* mutants described above to discover the mechanism that controls the organism, and we are doing that. Another approach is to return to *E. coli* (since The Boss would be universal, it would be found there, too) to learn if there is a mechanism that overrules all other mechanisms. Dick Burgess and coworkers (249) and James Lupski et al. (250) discovered that *E. coli* has an operon that controls all three of the most basic processes—DNA synthesis, RNA synthesis, and protein synthesis. How is that operon turned on and off? Is it by The Boss? We are pursuing that.

"Bossology" aims to discover the mechanism that is in control of an organism. Does bossology really exist? Is it true or false? Time will tell.

This journey of 50 years has led me to the following model of behavior (**Figure 13**). Its mechanism will be worked out further by continued use of biochemistry and genetics.

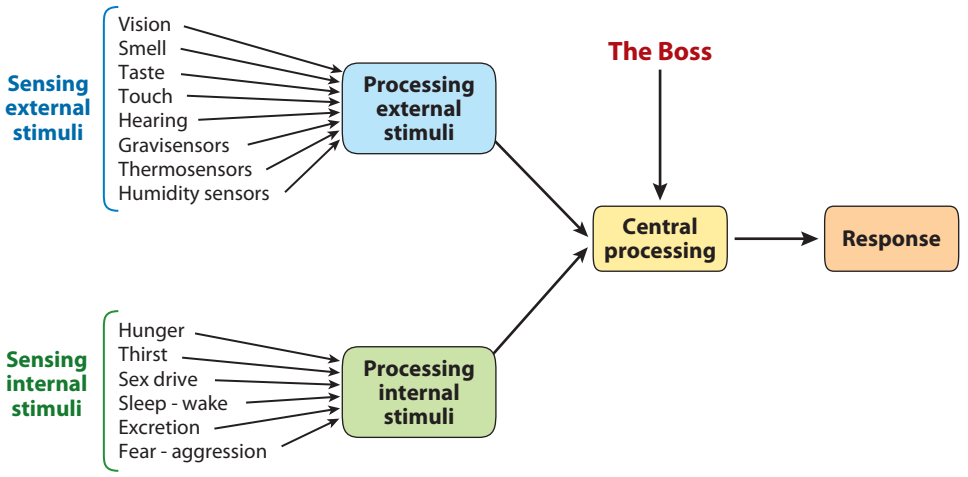

Figure 13

The mechanism of behavior. This applies to all organisms: microorganisms, plants, animals including humans.

DISCLOSURE STATEMENT

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

ACKNOWLEDGMENTS

I am most highly indebted to the Camille & Henry Dreyfus Foundation, Inc., for funding the past six years of research by undergraduate students. For support over many years, I thank the National Science Foundation, the National Institutes of Health, the Muscular Dystrophy Association, the Lucille P. Markey Charitable Trust, and the Graduate School at the University of Wisconsin-Madison. My thanks to Hilde Adler for the cover photo.

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