Marvels of Bacterial Behavior

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THE SUBJECT of bacterial behavior has a wonderful history that I can only touch upon here. Bacteria were discovered in 1676 by Antony van Leeuwenhoek, who was captivated by their motility: ". . . no more pleasant sight has ever yet come before my eye than these many thousands of living creatures seen all alive in a little drop of water moving among one another each separate creature having its own proper motion . . ." ("Letter 18, Pepper Water," p. 144, Dobell 1932). The frontispiece and title page of a book about van Leeuwenhoek's "little animals" are shown in figure 1. This book was published on the three hundredth anniversary of van Leeuwenhoek's birth by Clifford Dobell, a British microbiologist, who learned archaic Dutch and translated the letters that van Leeuwenhoek had sent to the Royal Society. Van Leeuwenhoek did his work with single lens microscopes, one of which is shown in the mezzotint in his left hand. The paper on the table announces his appointment as a fellow of the Royal Society, which occurred in 1680.

Two hundred years went by before serious work on bacterial motility was taken up again by Theodor Engelmann in Utrecht and Wilhelm Pfeffer in Tübingen. Modern studies were begun by Julius Adler, a biochemist at the University of Wisconsin in Madison, who published his first paper on the subject, "Chemotaxis in *Escherichia coli*," in 1965 (Adler 1965). *E. coli* was the bacterium of choice, because more was known about this organism than any other free-living thing. An electron micrograph of *E. coli* included in this paper is shown in figure 2. This particular cell has 5 flagella with long thin helical filaments that extend well beyond the area shown; their pitch is about 2.3 micrometers. Flagellar filaments are several micrometers long but only about

[1] Read 29 April 2005. The figures in the article are black and white copies of slides or initial frames of movies shown in the lecture.

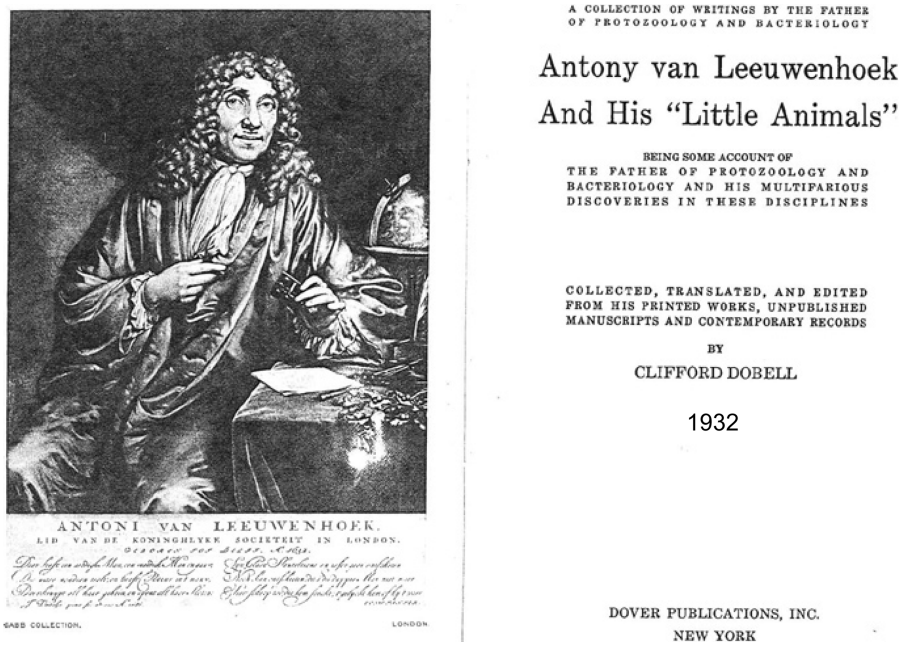


FIGURE 1. A book on Antony van Leeuwenhoek's little animals (animalcules) published by Clifford Dobell (Dobell 1932). The frontispiece is a mezzotint made from a painting by Jan Verkolje (1686) that hangs in the Boerhaave Museum in Leiden. This museum also houses a small collection of van Leeuwenhoek's single lens microscopes; he made several hundred, but fewer than ten survive.

a twentieth of the wavelength of light in diameter. A schematic drawing of *E. coli* is shown in figure 3. The cell is shaped like a cocktail sausage, about 1 micrometer in diameter by 2 micrometers long. Ten thousand such cells laid side by side would span the width of your finger. If fed well, cells grow longer and divide in the middle, once every 20 minutes. A saturated culture in the laboratory contains about a billion cells per cubic centimeter: the population of India in a spoonful. Given that *E. coli* lives in your gut, it outnumbers all of the other motile organisms in attendance at this lecture by a vast margin. The particular strain that most of us study is called K12; it was isolated from the feces of a diphtheria patient at Berkeley in 1922. Its DNA has been sequenced and encodes 4,286 genes. Only about 1% of these are required for motility.

The cell has a 3-layered wall (fig. 3). The outermost layer is penetrated by portholes, called porins, that allow the passage of water-soluble molecules of small molecular weight (the size of sucrose or less). The peptidoglycan layer is a quasi-rigid network that gives the cell its non-spherical shape. The inner membrane is a lipid bilayer, like the

Modern work was begun by Adler (1965): here is an electron micrograph of *E. coli* shown in his first paper.

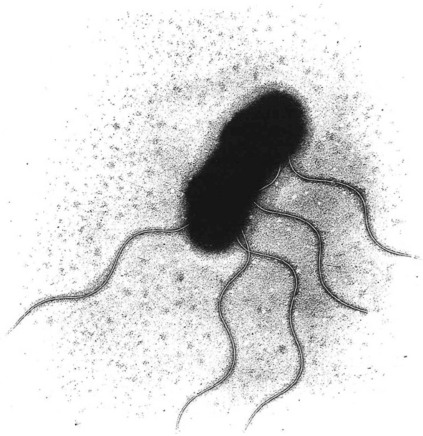


FIGURE 2. An electron micrograph of *E. coli* published by Julius Adler (Adler 1965). This cell is negatively stained with the salt of an element of high atomic number, which has spread only a few micrometers out from the cell body. As a result, the flagella appear truncated.

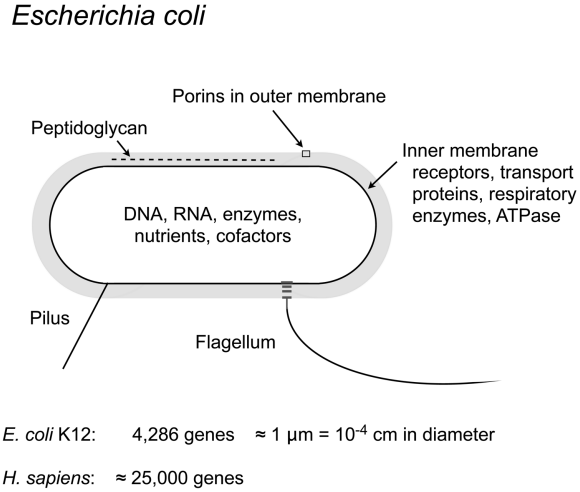


FIGURE 3. *E. coli* is a Gram-negative bacterium with a 3-layered cell wall, comprising an outer membrane (with porins), a peptidoglycan layer, and an inner membrane. There are cytoskeletal elements attached to the inner membrane (not shown) but none within the cytoplasm itself, which is devoid of membranes, ropes, girders, and other structural elements present in eukaryotic cells. The external organelles shown include a pilus and a flagellum. The pilus is straight, the flagellum helical.

plasma membrane of a human cell. There are two kinds of external organelles, pili, involved in adhesion, e.g., to cells of the urogenital tract, and flagella, that enable cells to swim. Several flagella (about 4 to 6, on average) arise at random points on the sides of the cell. Each is driven at its base by a reversible rotary motor, embedded in the cell wall (Berg and Anderson 1973).

In 1969, Adler published a paper showing that cells of *E. coli* have specific chemoreceptors (Adler 1969). He perfected an assay introduced by Pfeffer, shown in figure 4. A U-shaped tube about 1 mm in diameter is placed on a microscope slide and covered with a square cover slip. A suspension of *E. coli* is pipetted into the space between the cover slip and the slide. Then a fine capillary tube is inserted containing an aliquot of a solution of a chemical attractant (e.g., an amino acid or sugar). Diffusion of the attractant from the tip of the capillary tube generates a spatial gradient to which the bacteria respond. They swim up the gradient, accumulating near the tip of the capillary tube and then swimming inside. Adler withdrew such tubes after about an hour, spreading their contents on nutrient agar plates, counting colonies the next day (with each colony derived from a single cell). By varying the concentration of attractant in the capillary tubes he was able to obtain dose-response curves. For the best attractants, e.g., the amino acids aspartate and serine or the sugars galactose and glucose, responses were detectable at about 1 micromolar but peaked at about 1 millimolar, where several hundred thousand cells swam into the tubes. Adler obtained a striking result: cells could respond to chemicals that they could neither take up from the external medium nor metabolize. So chemotaxis is

Adler perfected a capillary assay.

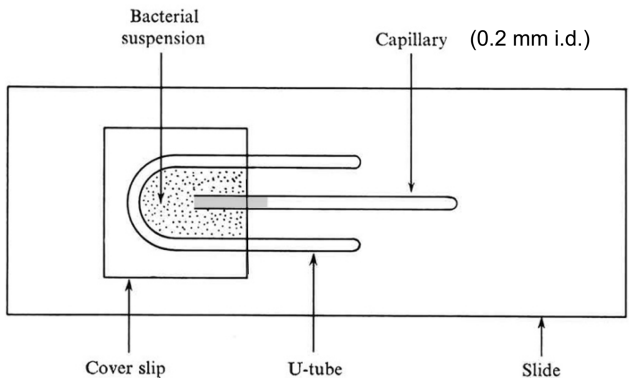


FIGURE 4. The capillary assay, as perfected by Adler (1973) and described in the text

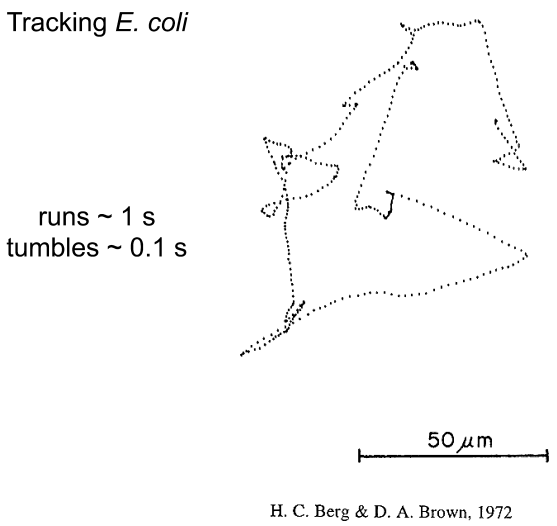


FIGURE 5. Thirty seconds in the life of one wildtype *E. coli* cell, tracked in a homogeneous, isotropic medium, in the absence of any chemical attractants (Berg and Brown 1972)

a matter of aesthetics rather than material gain. Clearly, chemotaxis developed so that cells could seek out and grow on various nutrients, but the seeking requires only that the cell is able to taste the nutrient.

I started working on bacterial behavior in 1968, curious to know how cells might find their way into Adler's capillary tubes. So I built a microscope that tracks individual cells in three dimensions (Berg and Brown 1972). A 2-dimensional projection of one 3-dimensional track is shown in figure 5. This is 30 seconds in the life of a wildtype *E. coli* cell swimming in the absence of any chemotactic stimulus. The track is digitized, with about 12 data points per second. The cell picks a direction at random and swims steadily in a nearly straight line, and then abruptly changes its mind and swims in a new direction. The motion while the cell is changing its mind is rather erratic, so these events are called tumbles. Tumbles are relatively short, about 0.1 second, on average. The intervals between tumbles, called runs, are much longer, about 1 second, on average. Some of the dots in the track are close together, but these represent runs in a direction in or out of the plane of the figure. The cell executes what a physicist calls a random walk. If one places one of Adler's capillary tubes, say, at the right edge of the figure, runs to the right get longer; however, runs to the left stay about as long as they are in the absence of a stimulus. So *E. coli* is an optimist: if life gets better, enjoy it more; if it gets worse, don't worry about it! It wasn't long before we learned that bacteria swim by rotating their flagellar filaments (Berg and Anderson 1973). If the direction of rotation is coun-

terclockwise (CCW) as viewed from behind the cell, the several filaments form a bundle that pushes the cell forward (Macnab and Ornston 1977). If one or more filaments turn clockwise (CW), the bundle fragments, and the cell moves erratically with little net displacement. In my lecture, these events were demonstrated with a pair of spiral wires. So bacterial behavior depends upon the direction of flagellar rotation (Larsen et al. 1974). When runs get longer, as during a chemotactic response, cells spend more time rotating their flagella CCW.

A movie was shown of *E. coli* swimming, the first frame of which appears in figure 6. These cells were labeled with a fluorescent dye and imaged in fluorescence by strobed laser illumination (Turner et al. 2000). Short exposures are required, since the flagella spin about 100 revolutions per second. The cells swim about 30 diameters a second, crossing the field of view in about 2 seconds. This movie can be viewed and/or downloaded from the Web, as described in the figure legend.

A marvelous experiment confirming that bacterial flagella rotate was carried out by Mike Silverman and Mel Simon (Silverman and Simon 1974). Break most of the flagella off of cells by viscous shear and attach flagellar stubs to a glass slide using an anti-filament antibody. Now, the flagellar motor, rather than driving the filament, turns the cell body. These so-called tethered cells were shown in a movie, the first frame of which appears in figure 7. Approximately one-fourth of the cells in the

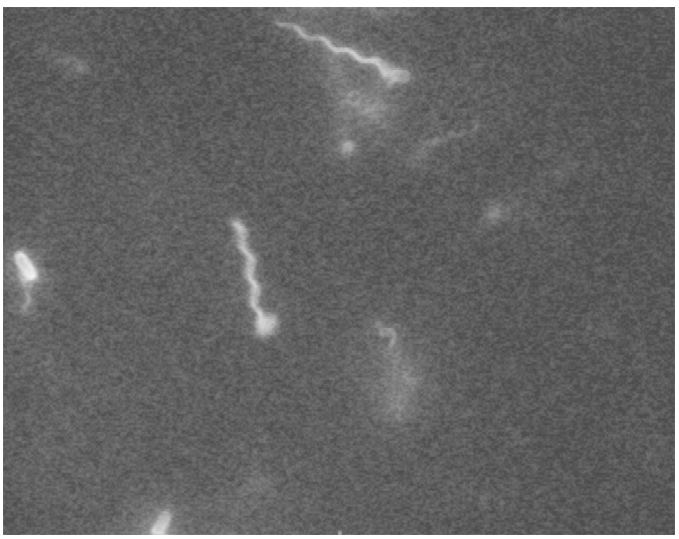


FIGURE 6. The first frame of a movie showing swimming *E. coli*. The images are of fluorescent cells. Two of the cells in this field show flagellar bundles. To see this film, go to Berg (Web site), click on "Movies," and then on "Swimming *E. coli*." This clip is entitled "Fluorescent filament leaving bundle."

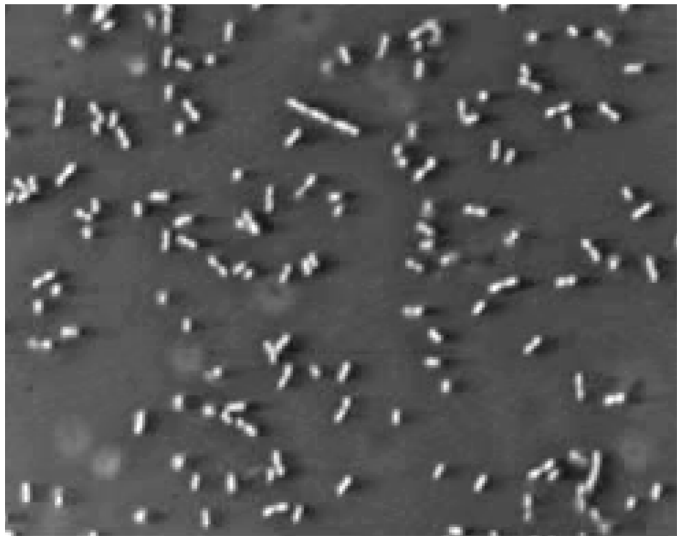


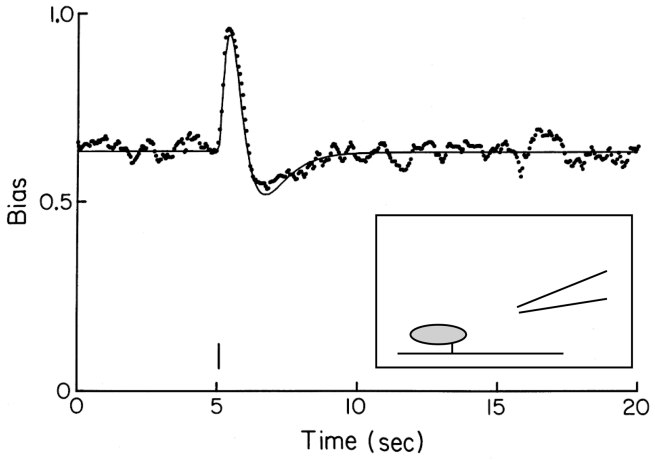
FIGURE 7. The first frame of a movie showing tethered cells of a motile *Streptococcus*. The cells pinwheel, spinning alternately clockwise (CW) and counterclockwise (CCW). To see this film, proceed as described in the legend to figure 6 and select "Tethered bacteria." This clip is entitled "Tethered *Streptococcus*."

field of view pinwheel, now counterclockwise, now clockwise, the larger cells more slowly, the smaller ones more rapidly (on average, about 10 revolutions per second). This movie also can be viewed and/or downloaded from the Web, as described in the figure legend.

If one looks at the runs in figure 5, it is evident that cells cannot swim in a straight line. They are subject to rotational Brownian motion. This problem was discussed in 1905 by Einstein, who found that the root-mean-square angular displacement of a small particle (here, the cell) increases as the square root of the time. If one puts in numbers, one finds that an *E. coli* cell will wander by a root-mean-square angle of 90° in about 10 seconds. Therefore, after 10 seconds, it has forgotten where it has been. So measurements of concentrations made more than 10 seconds ago are no longer relevant. This sets an absolute limit on the time that cells have to decide whether life is getting better or worse.

We found that *E. coli* understands this problem by studying the chemotactic response of *E. coli* at the level of a single flagellar motor, as shown in figure 8 (Block et al. 1982; Segall et al. 1986). A micropipette containing a negatively charged chemical attractant (the amino acid aspartate) was brought to within a few micrometers of a tethered cell, as shown in the inset. Electrodes in the pipette and in the pond allowed us to eject aspartate, which is negatively charged, in a controlled manner. The plot shows the result obtained when tethered cells

The impulse response tells us that cells make temporal comparisons over a time span of 4 s.



The cell compares counts made over the past 1 s with counts made over the previous 3 s and responds to the difference (Block, Segall, Berg, 1982).

FIGURE 8. The response of *E. coli* to a short pulse of attractant, delivered at 5.06 seconds. Tethered cells were stimulated by iontophoretic pipettes, as shown in the inset. See Block et al. (1982); Segall et al. (1986).

were exposed to a short pulse applied at 5.06 seconds. The ordinate is the bias of the cell, i.e., the fraction of time that it spins CCW. The effect of the short pulse is a biphasic response lasting about 4 seconds, called an impulse response. The bias approaches 1 within about 1/2 second, returns to the baseline within about 1 second, and remains below the baseline for the following 3 seconds. The areas of the two lobes of the response are nearly the same. The argument is rather elaborate, but what this experiment shows is that cells compare counts of attractant molecules made over the past second with counts made over the previous 3 seconds and respond to the difference. So, cells make temporal comparisons over a time span substantially less than 10 seconds: they have a short-term memory spanning 4 seconds.

Another bit of physics that *E. coli* understands is viscous drag. There is a dimensionless parameter in the equations of motion of fluids called the Reynolds number, R. For a swimming cell, this is the cell size, times its speed, times the density of the medium, divided by the viscosity of the medium. For *E. coli*, this works out to be about 10^{-5}. The Reynolds number indicates the relative importance of terms in the equations of motion of fluids that involve inertia (acceleration of masses) compared with those that involve viscous shear. Given a Reynolds number of 10^{-5}, *E. coli*'s behavior is completely dominated by viscosity. For

example, if a cell swims at top speed and then puts in the clutch, it coasts to a stop within a tenth of the diameter of a hydrogen atom! So, if flagella are to generate thrust, they must do so by using viscous drag. The secret is the asymmetry of the drag on a thin rod (or segment of the flagellar filament), about twice as large when it moves sideways as when it moves lengthwise; see figures 6.2 and 6.3 of Berg (1993). A wonderful article about this world was written by Ed Purcell (Purcell 1977).

The main quest of most of the people studying bacterial chemotaxis is to understand the signal transduction pathway. What is the machinery that enables cells to swim in a purposeful manner? Signal processing involves interactions between different kinds of proteins, beginning with receptors that bind specific chemical attractants, shown in figure 9. The receptors are long α-helical molecules sensitive to serine (Tsr), to aspartate or maltose (Tar), to ribose or galactose (Trg), or to certain dipeptides (Tap). These ligands bind to a domain in the periplasm, outside the cell's inner membrane, or they bind to a small binding protein that, in turn, binds to this receptor domain.

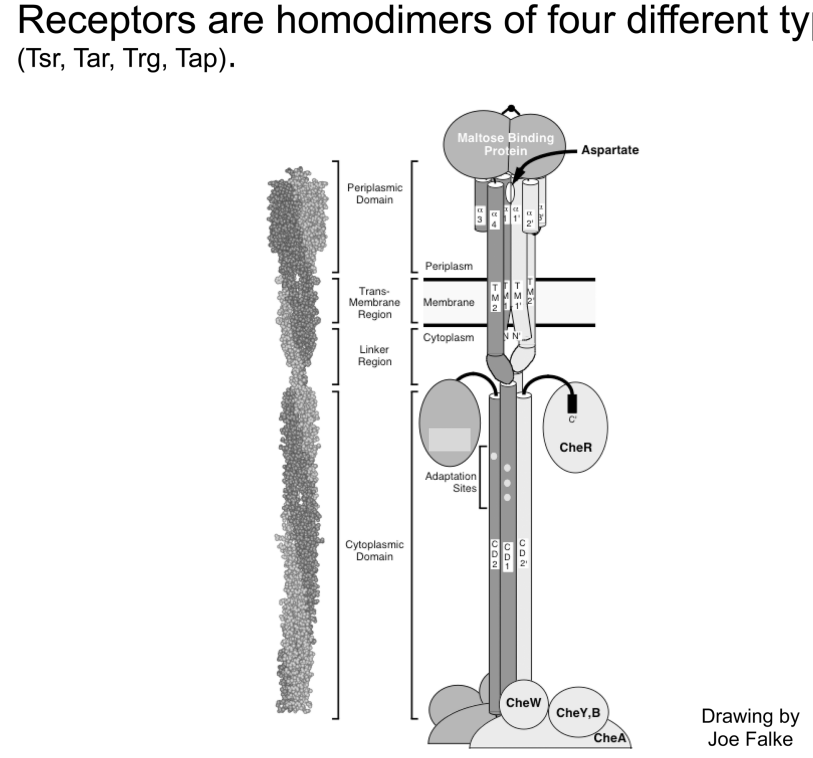


FIGURE 9. The structure of a single receptor homodimer (left) shown schematically with other key proteins (right). For a description of these additional proteins, see figure 14. Figure courtesy of Joe Falke.

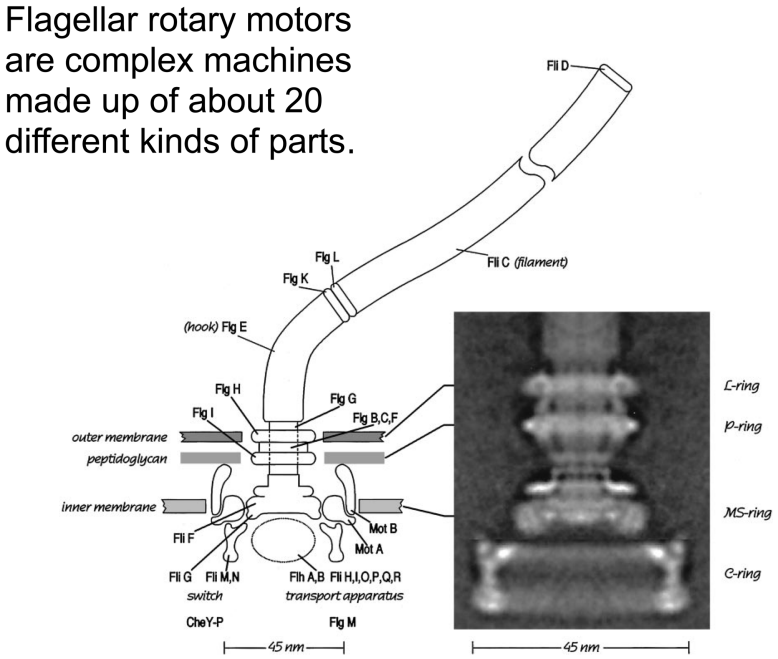


FIGURE 10. The flagellar rotary motor. The inset is a reconstruction of cryo-electron micrographs assembled by David DeRosier. It is what one would see looking through a spinning rotor. Most proteins are labeled Flg, Flh, or Fli, depending upon where their genes are located on the *E. coli* chromosome. Null mutants of any of these genes result in cells without flagellar filaments. Mutations in *mot* genes result in paralyzed flagella.

Ultimately, the receptors control the direction of rotation of the flagellar motors, shown in figure 10. A motor is made of about 20 different kinds of parts. There is a ring in the cytoplasm called the C-ring, a set of rings in the inner membrane called the MS-ring, a drive shaft that extends from the MS-ring to a flexible coupling (or universal joint) called the hook, a bushing that gets the drive-shaft through the outer layers of the cell wall, called P and L rings, two adapter proteins, and finally a long helical filament terminated by a cap. The motor is driven by sets of proteins that span the inner membrane but are bolted down to the peptidoglycan layer, called Mot proteins. MotA (4 copies) and MotB (2 copies) comprise force-generating units (at least 8) powered by a transmembrane proton flux. This is an ion-driven machine, not an ATP-driven machine. The motor is assembled from the inside out in a highly regulated manner. Surprisingly, the filament grows at the distal end rather than at the proximal end. For more about this remarkable machine, see Berg (2003).

The remaining signal-transduction machinery is shown schematically

Receptors are coupled to motors by diffusion of CheY-P.

(CheY is phosphorylated by the receptor kinase CheA and dephosphorylated by the phosphatase CheZ. CheY-P binds to the flagellar motors.)

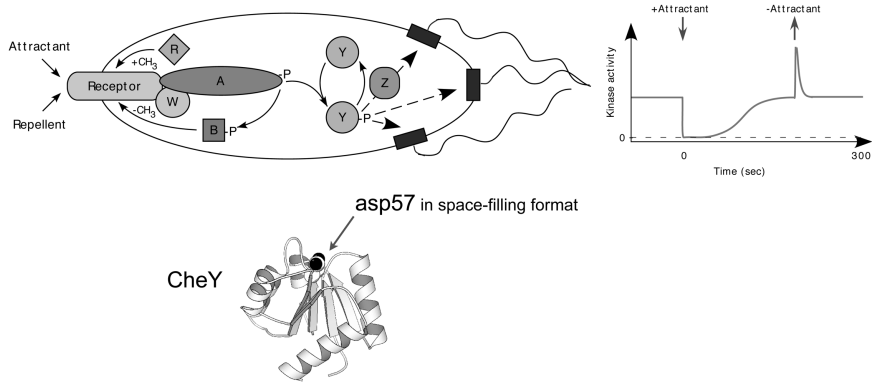


FIGURE 11. *E. coli's* signal transduction pathway. Receptors in clusters activate a kinase, CheA. When activated, the kinase phosphorylates a small signaling protein called CheY, shown at the bottom in a ribbon diagram. Copies of CheY-P diffuse to the base of each flagellar motor, where they bind, increasing the likelihood of CW rotation. The phosphate is removed by a phosphatase, CheZ. The kinase is linked to the receptor via a coupling factor, CheW. The cytoplasmic domain of the receptor is methylated by a methyltransferase, CheR, and demethylated by the methylesterase CheB, which is also activated by the kinase. The diagram at the right shows changes in kinase activity generated by the sudden addition of a large amount of attractant.

in figure 11. The receptors control the activity of a kinase, CheA, a molecule that phosphorylates (adds phosphate to) a small signaling molecule called CheY. CheY-P diffuses to the flagellar motors, where it binds and increases the probability of CW rotation. If one adds an attractant, say aspartate, the kinase activity goes down. However, there is a problem, because the lifetime of CheY-P is too long. So the cell has another molecule, a phosphatase called CheZ, that removes the phosphate. Thus, when the cell is suddenly exposed to aspartate, its motors respond within a few tenths of a second.

The rapid change in the kinase activity following a large step-addition of an attractant is shown in figure 11 in the diagram at the right. After a few minutes, the cells adapt, i.e., the kinase activity returns to its initial value, even though the attractant is still there. This process involves the methylation of the cytoplasmic domain of the receptor, catalyzed by a methyltransferase, CheR. The methylation sites (adaptation sites)

are shown by the 4 light gray dots in figure 9. When the attractant is removed, these methyl groups are clipped off by a methylesterase, CheB, which is activated by the kinase. Adaptation allows cells to sense changes in their environment without worrying about ambient concentrations. This increases the range of concentrations over which they can respond.

We have studied interactions of these proteins by fluorescence resonance energy transfer (FRET), as illustrated in figure 12. An interesting result is shown in figure 13, in which the change in kinase activity is plotted as a function of the amount of attractant added. On the left is shown a set of dose-response curves obtained by addition of the non-metabolizable aspartate analog, α-methylaspartate. The four sets of curves were obtained at different ambient concentrations of α-methylaspartate, as noted in the figure legend. All of these kinds of data can be condensed into one figure, as shown on the right, if the fractional change in kinase activity is plotted as a function of the fractional change in receptor occupancy. The receptors show prodigious gain: when their occupancy changes by 1%, the kinase activity changes by 35%. This amplification occurs via the interactions of adjacent receptors, which are arranged in tight clusters, as shown in figure 14. Most of the chemotaxis proteins enumerated in figure 11 turn out to be concentrated in

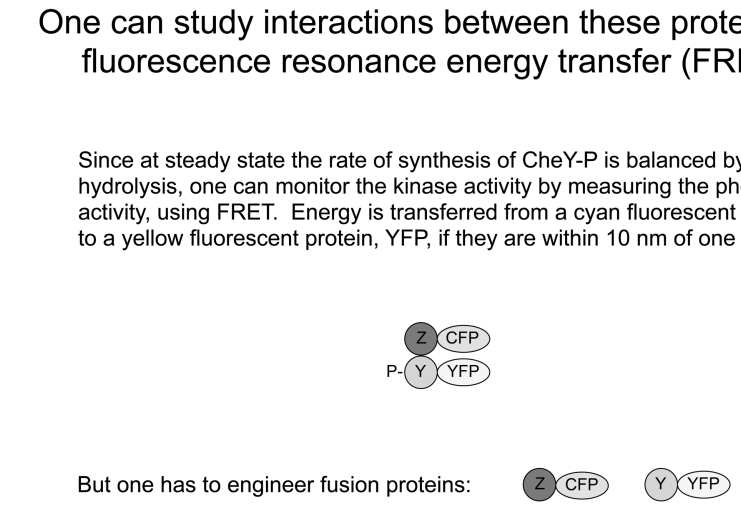


FIGURE 12. An illustration of how protein-protein interactions can be monitored by fluorescence resonance energy transfer (FRET). A fusion of one protein is constructed with a cyan fluorescent protein, and a fusion of a second protein is constructed with a yellow fluorescent protein. When the two proteins interact (bind to one another), fluorescence energy is transferred from the cyan to the yellow fluorescent protein. So the cyan fluorescence goes down and the yellow fluorescence goes up. These changes can be measured.

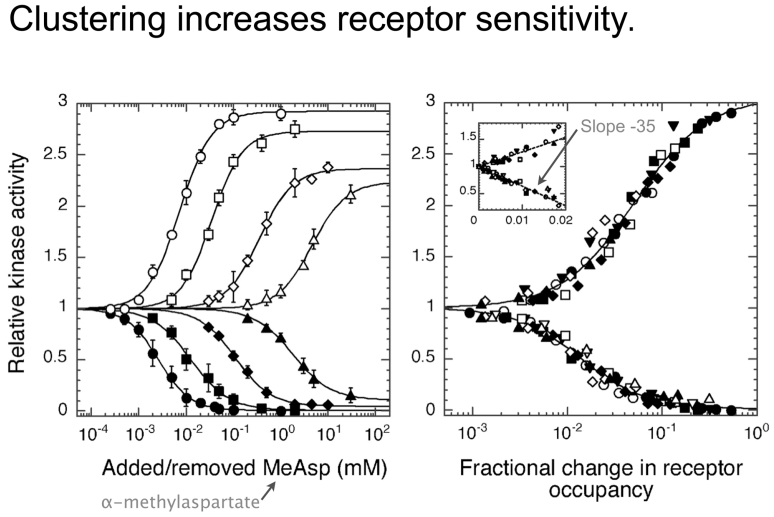


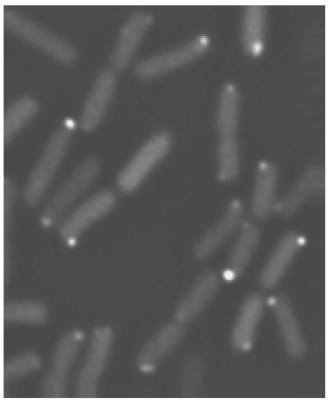
FIGURE 13. Dose-response curves obtained by the FRET technique. The curves on the left were obtained with cells at different ambient concentrations of the non-metabolizable attractant α-methylaspartate (0, 0.1, 0.5, and 5 mM, respectively). More α-methylaspartate was added, and the cells were allowed to adapt. The curves shown with the open symbols were obtained when the concentration of α-methylaspartate was returned to the ambient level. When the fractional change in kinase activity is plotted as a function of the fractional change in receptor occupancy, all of such data fall on two curves, as shown on the right (Sourjik and Berg 2002). The fractional change in kinase activity is 35 times larger than the fractional change in receptor occupancy. This amplification occurs via receptor-receptor interactions.

these clusters, and current work focuses on attempts to understand how clusters are assembled and how they function; see the reviews by Sourjik (2004) and Parkinson et al. (2005).

Given this state of affairs, it is fair to say that *E. coli* has a very small brain, a brain designed to process information generated by small changes in the concentrations of chemical attractants encountered by the cell as it moves through its environment. To learn more, see Berg (2000, 2004).

H. G. Wells, in *The War of the Worlds* (Wells 1898) marveled at the fighting machines brought to earth by his Martians: "And of their appliances, perhaps nothing is more wonderful to a man than the curious fact that what is the dominant feature of almost all human devices in mechanism is absent—the *wheel* is absent; amongst all the things they brought to earth there is no trace or suggestion of their use of wheels. One would

Receptors and other chemotaxis proteins are clustered.



E. coli has a brain, but it is very small (cells labeled with YFP-CheR).

FIGURE 14. Receptor clusters tagged by YFP-CheR and visualized by fluorescence. The clusters appear as diffraction-limited spots about 0.2 μm in diameter near the cell poles. Photograph courtesy of Victor Sourjik.

have at least expected it in locomotion. And in this connexion it is curious to remark that even on this earth Nature has never hit upon the wheel, or has preferred other expedients to its development" (bk. 2, chap. 2); ". . . stark and silent and laid in a row, were the Martians—*dead!*—slain by the putrefactive and disease bacteria against which their systems were unprepared . . . by the humblest things that God, in His wisdom, has put upon this earth" (bk. 2, chap. 8). Wells died in 1946. I think he would have been amused to know that the bacteria that destroyed his Martians were propelled by rotary engines.

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