MOTILE BEHAVIOR OF BACTERIA

E. coli, a self-replicating object only a thousandth of a millimeter in size, can swim 35 diameters a second, taste simple chemicals in its environment, and decide whether life is getting better or worse.

Howard C. Berg

Escherichia coli is a single-celled organism that lives in your gut. It is equipped with a set of rotary motors only 45 nm in diameter. Each motor drives a long, thin, helical filament that extends several cell body lengths out into the external medium. The assemblage of motor and filament is called a flagellum. The concerted motion of several flagella enables a cell to swim. A cell can move toward regions that it deems more favorable by measuring changes in the concentrations of certain chemicals in its environment (mostly nutrients), deciding whether life is getting better or worse, and then modulating the direction of rotation of its flagella. Thus, in addition to rotary engines and propellers, *E. coli*'s standard accessories include particle counters, rate meters, and gear boxes. This microorganism is a nanotechnologist's dream. I will discuss the features that make it so, from the perspectives of several scientific disciplines: anatomy, genetics, chemistry, and physics.

What made work on the behavior of *E. coli* possible? The tale has two geneses. One involves light microscopy and begins in the 17th century, when Antony van Leeuwenhoek first observed swimming bacteria.[1] (See box 1 on page 26.) The other involves molecular genetics and begins in the 20th century, when Joshua Lederberg demonstrated that bacteria have sex, as evidenced by their genetic recombination.[2] (See box 2 on page 28.) Lederberg studied *E. coli* and *Salmonella typhimurium*, two closely related organisms. They are the principal subjects of work now being done on bacterial chemotaxis (the motion of bacteria toward chemical attractants or away from chemical repellents). That work has yielded an important model for understanding the behavior of cells at the molecular level.

Anatomy of *E. coli*

E. coli (like *S. typhimurium*) is a cylindrical organism with hemispherical endcaps (as figure 1 shows). The cell, which weighs only 1 picogram, is about 70% water. Some strains are flagellated and motile; others are nonflagellated and nonmotile. When motile cells are grown in a rich medium (such as salts plus a mixture of amino acids), they swim in the direction of their long axis at a rate of about 35 diameters per second, often changing course but rarely stopping.

The chromosome of *E. coli* consists of a single double-stranded chain of DNA about 700 times longer than the body of the cell. There are 4 639 221 base pairs specifying 4288 genes, most of which encode proteins.[3] The functions of only about 60% of these proteins are known. About 50 different kinds of proteins are required to produce the cell's chemotaxis, roughly half for the assembly of flagella and half for behavioral control.

When *E. coli* grows, it first gets longer and then divides in the middle. In a sense it is immortal, because the mother cell is replaced by two daughters, essentially identical to the daughters of the previous generation. The molecules of DNA in the members of a given set of descendants are identical except for mutations, which occur spontaneously for a given gene, at the rate of about 10^{-7} per generation.

If well fed and held at the temperature of the human gut (37 °C), *E. coli* can synthesize and replicate everything it needs to make a new copy of itself in about 20 minutes. Thus, if we start at noon today with one cell (and lots of food), by noon tomorrow there will be $2^{72} = 4.7 \times 10^{21}$ cells—enough to pack a cube 17 meters on a side! This replication rate explains why single cells dispersed on the surface of hard nutrient agar soon become mounds of cells (colonies) a millimeter or so in diameter and why, in soft agar, the motile progeny of a single cell soon populate the entire plate.

Genetic analysis

A fully functional cell line, or strain, found in the wild is called a wild type. If a mutant cell is found that is missing a particular function, the gene carrying the mutation is named for that missing function. For example, a *che* gene is one encoding a protein (polypeptide) required for **che**motaxis. A cell with such a defect makes flagella and swims, but it does not respond normally to chemical stimuli. The first gene of this type to be identified is called *cheA* (in italics), the second is called *cheB*, and so on through the alphabet. When the protein encoded by the gene is identified, it is called CheA (capitalized and in roman type).

In bacterial chemotaxis, besides the *che* genes, we encounter *fla* genes, so named for their defects in the synthesis of **fla**gella (these genes are now called *flg*, *flh*, *fli*, or *flj*, because there turned out to be more than 26). There are also *mot* genes, named for defects in **mot**ility, or generation of torque. And there are a variety of genes that specify specific chemoreceptors; one, for example, *tar*, is a gene encoding the chemoreceptor Tar, which is so named because it mediates **t**axis toward the amino acid **a**spartate and away from certain **r**epellents. The soft-agar plate shown in box 2 was inoculated with wild-type cells at the top, cells of a *tsr* (the *s* stands for **s**erine) strain at the right, cells of a *tar* strain at the bottom, and cells of a smooth-swimming *che* strain at the left.

The flagellum

The flagellum is an organelle that has three parts (as figure 2 shows). There is a basal body consisting of a

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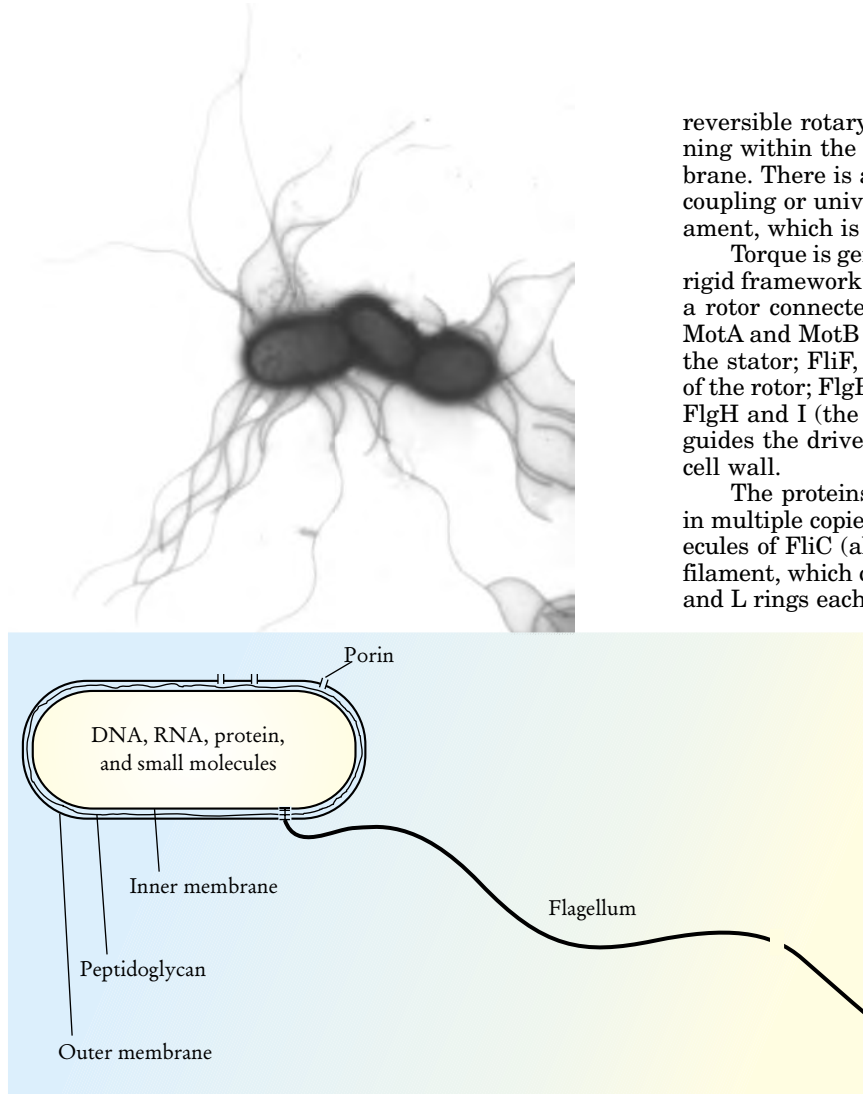
reversible rotary motor embedded in the cell wall, beginning within the cytoplasm and ending at the outer membrane. There is a short proximal hook, which is a flexible coupling or universal joint. And there is a long helical filament, which is a propeller.[4]
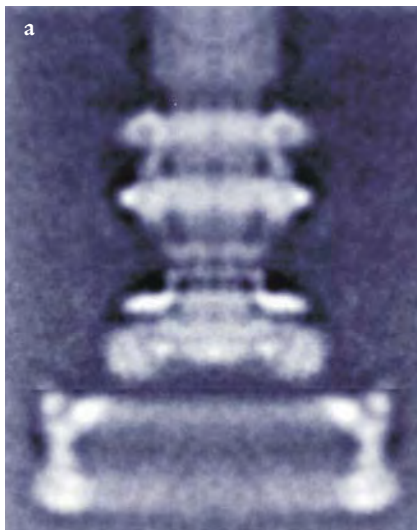
Torque is generated between a stator connected to the rigid framework of the cell wall (to the peptidoglycan) and a rotor connected to the flagellar filament. The proteins MotA and MotB are thought to constitute the elements of the stator; FliF, G, M, and N (the MS and C rings) those of the rotor; FlgB, C, F, and G those of the drive shaft; and FlgH and I (the L and P rings) those of the bushing that guides the driveshaft out through the outer layers of the cell wall.

The proteins that make up the flagellum are present in multiple copies. For example, there are about 5000 molecules of FliC (also called flagellin) per helical turn of the filament, which can have as many as six turns. The MS, P, and L rings each contain about 26 copies of FliF, FlgI, and FlgH, respectively. There appear to be eight stator elements (complexes of MotA and MotB), each of which exerts a similar force.

If one fixes a wild-type cell to a glass slide by one of its flagellar filaments, the motor at the base of that filament spins the cell body at about 10 Hz. This technique, known as tethering, was developed by Mike Silverman and Mel Simon at the University of California, San Diego. If one tethers a paralyzed cell, such as one with defective MotB, the cell body simply executes rotational Brownian movement, like a mirror on a galvanometer fiber. However, if wild-type MotB is made—for example, if a copy of a wild-type gene is added to the cell and expressed—then rotation resumes. The good MotB proteins that are made replace the bad ones, and the cell speeds up. Changes in speed are abrupt, generating a speed–time plot in the form of a staircase with eight steps of equal height.

The flagellum is assembled from the inside out, with the axial components exported through a central channel. The filament grows at the distal end, with molecules of FliC added under the distal cap, which is made of FliD. The growth process is subject to exquisite genetic control. FliC, for example, is not made until the assembly of the basal body is completed. When it is completed, the same apparatus that exports FliC pumps an inhibitor of late-gene transcription out of the cell. This removes the inhibition.

The motor is driven by protons flowing from the outside to the inside of the cell (except for marine bacteria and bacteria that live at high pH, where sodium ions are used instead). The source of energy is a transmembrane electrical potential or pH gradient (or both), generated by respiration for cells grown aerobically. MotA and MotB form a transmembrane channel. Proton translocation is thought to cause the cytoplasmic part of MotA to move or change its shape and exert a force on FliG, thereby driving the rotor. In each cycle of this process, the rotor advances by one or more steps, which are of equal angular increment. From an analysis of fluctuations in the period of rotation (assuming exponentially distributed waiting times between steps), the number of steps is found to be at least 50 per revolution per stator element. As the number of stator elements increases toward eight, the rota-

FIGURE 1. MOTILE BACTERIA. **Top**: Three-cell cluster of *Salmonella typhimurium*. The cell bodies are about 1 μm in diameter and 2 μm long, but they appear fatter because of the electron-dense stain of phosphotungstic acid that was used to prepare the cells for transmission electron microscopy. The flagellar filaments have a wavelength of about 2.3 μm but are distorted by interactions with the substrate. The filaments are 23 nm thick. (Image by Chi Aizawa, Teikyo University.) **Bottom:** Scale drawing of *Escherichia coli*, showing one flagellum, truncated, and three porins (protein channels that allow the entry of water-soluble nutrients). A typical cell has up to six flagella and hundreds of porins. The cell body is 2 μm long; the multilayered wall is about 30 nm thick.[17] The outer membrane is made of polysaccharides and lipids, with the sugar chains pointing outward. The inner membrane's phospholipid bilayer core resembles the membranes that enclose human cells. This membrane is traversed by proteins involved in sensory transduction and in transporting materials and harvesting energy. It constitutes the main permeability barrier that enables the cell to retain the chemicals that make up the cytoplasm—DNA, RNA, proteins, and various water-soluble molecules of lower molecular weight. Between the inner and outer membranes is a porous, gauzelike layer of peptidoglycan (polysaccharide chains cross-linked by peptides), which gives the cell its rigidity and cylindrical shape. When the assembly of this polymer is blocked by an antibiotic such as penicillin, a growing cell cannot cope with the high osmotic pressure of its cytoplasm, and it blows up. The intermembrane space, the periplasm, contains a variety of proteins that either bind molecules that interest the cell (such as sugars) or destroy molecules that pose a threat (such as foreign DNA).

a

b

FIGURE 2. BACTERIAL MOTOR AND DRIVE TRAIN. (**a**) Rotationally averaged reconstruction of electron micrographs of purified hook-basal bodies. The rings seen in the image and labeled in the schematic diagram (**b**) are the L ring, P ring, MS ring, and C ring. (Digital print courtesy of David DeRosier, Brandeis University.)

(Cap) FliD
FlgL
FlgK
(Hook) FlgE
FliC (Filament)
FlgH
FlgI (Bushing)
FlgG
FlgB,C,F (Drive shaft)
L
P
MS
MotB
MotA (Stator)
FliF
C
FliM,N
FliG (Rotor)
FlhA,B FliH,I,O,P,Q,R (Transport apparatus)
45 nm

tion becomes smoother. The number of steps for the intact motor is at least 400 per revolution. Individual steps have yet to be resolved: They are filtered out by the elastic tether.

Detecting chemical gradients

The motor runs either clockwise (CW), as seen by an observer standing on the outside of the cell looking down at the hook, or counterclockwise (CCW), with protons continuing to flow from the outside to the inside of the cell. Switching direction involves the proteins FliG, M, and N.

In a cell wild type for chemotaxis, CW and CCW modes alternate (with exponentially distributed waiting times). When the motors turn CW, the flagellar filaments work independent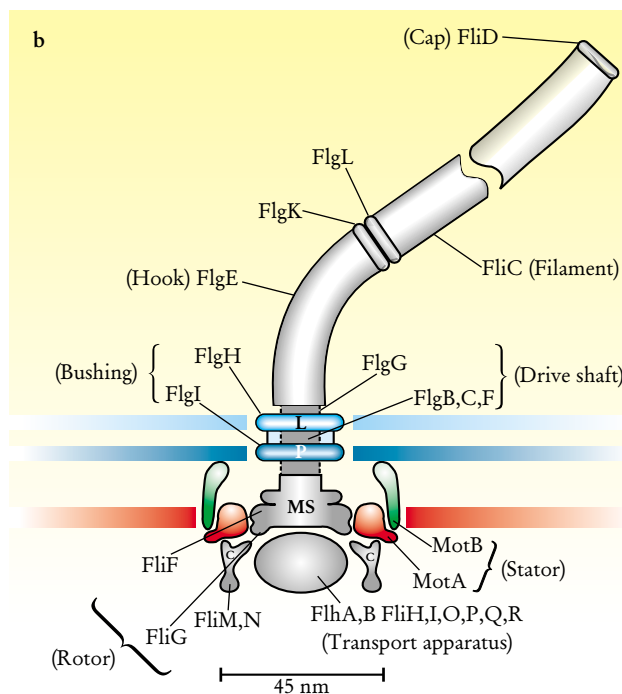ly, and the cell body moves erratically with little net displacement; the cell is then said to "tumble." When the motors turn CCW, the filaments rotate in parallel in a bundle that pushes the cell body steadily forward, and the cell is said to "run." The two modes alternate. The cell runs and tumbles, executing a three-dimensional random walk, as shown in figure 3.

When different flagellar motors in the same cell are studied under conditions in which they cannot interact mechanically, they change directions independently. Yet, when a flagellar bundle drives the cell forward, all of the motors have to rotate CCW. The events that bring about this coordination are not yet understood. The mean run interval

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Box 1. Light microscopy

Antony van Leeuwenhoek, using a single-lens microscope,[14] was intrigued by "animalcules" (little animals) that he saw in his well water. He wanted to know whether they might survive exposure to pepper, so he ground up some and added it to a sample. The number of animalcules waxed and waned until 6 August 1676, when he made a discovery:

I now saw very plainly that these were little eels, or worms, lying all huddled up together and wriggling; just as if you saw, with the naked eye, a whole tubful of very little eels and water, with the eels a-squirming among one another: and the whole water seemed to be alive with these multifarious animalcules. This was for me, among all the marvels that I have discovered in nature, the most marvellous of all; and I must say, for my part, that 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 several creature having its own proper motion.[1]

He was looking at a spirillum, probably *Spirillum volutans*, the large bacterium shown in the accompanying sketch. Leeuwenhoek never saw its flagella. Those organelles of locomotion were first seen on *Chromatium okenii*, another large bacterium, by Christian Ehrenberg in 1836, and later on *S. volutans* by Ferdinand Cohn in 1872.

The subject of bacterial behavior was taken up systematically in the 1880s by the physiologist Theodor Engelmann in Utrecht, and by the botanist Wilhelm Pfeffer in Tübingen. They studied the responses of various species of bacteria to light, oxygen, salts, and a variety of nutrients. Pfeffer, who

thought that bacteria could steer toward or away from a chemical source, coined the term "chemotaxis" to describe their attraction or repulsion. The role that flagella play in that response was examined in detail after dark-field condensers of high numerical aperture were developed, beginning in 1909 with work done by Karl Reichert and culminating in 1920 with the work of Paul Metzner, who described the motion of flagellar bundles of *S. volutans* in stunning detail.[15]

S. volutans has two flagellar bundles (as shown in the sketch), each composed of about 25 flagellar filaments. Here, the cell is swimming from left to right. Its body is helical. The bundle on the left is in the tail configuration; the one on the right is in the head configuration. When the filaments change their directions of rotation, the bundles switch their configurations and the cell moves in the opposite direction.

An *Escherichia coli* bacterium is shown below the *S. volutans* for comparison. As many as six flagellar filaments arise at random from the sides of the *E. coli* cell and form a bundle that appears near one pole. Rotation of the filaments in the bundle pushes the cell forward. When the bundle changes its orientation, the cell goes off in a new direction.
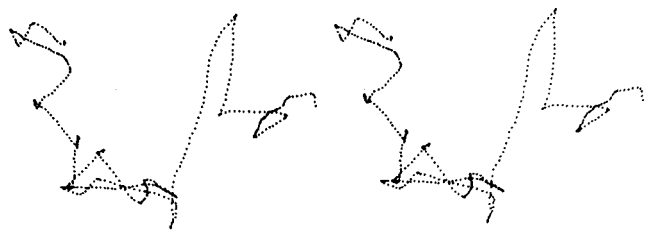
10 micrometers

Spirillum volutans
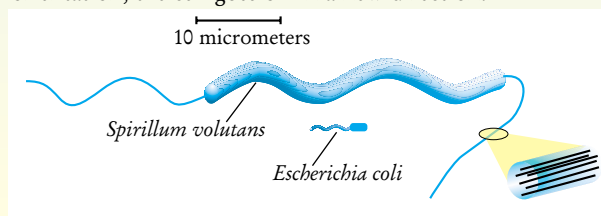
Escherichia coli

FIGURE 3. MOVEMENT. This stereo plot shows about 30 s in the life of one *Escherichia coli* K-12 bacterium swimming in an isotropic homogenous medium.[18] The track spans about 0.1 mm, left to right. The plot shows 26 runs and tumbles, the longest run (nearly vertical) lasting 3.6 s. The mean speed is about 21 μm/s. To see this plot in three dimensions, look at the left image with your left eye and the right image with your right eye, and relax your eye muscles so that the two images overlap. A stereoscope (pair of lenses) helps.

is about 1 s, whereas the mean tumble interval is only about 0.1 s. Both of the times are exponentially distributed.

Although the change in angle generated by a tumble is approximately random, there is a slight forward bias. When, by chance, a cell moves up a spatial gradient of a chemical attractant or down a spatial gradient of a chemical repellent, runs are extended. When, by chance, it moves the other way, runs revert to the length observed in the absence of a gradient. Thus, the bias in the random walk that enables cells to move up or down gradients is positive.

Finally, the behavioral response is temporal, not spatial. *E. coli* does not determine whether there is more attractant, say, in front than behind; rather, it determines whether the concentration increases when it moves in a particular direction. Studies of impulsive stimuli indicate that a cell compares the concentration observed over the past 1 s with the concentration observed over the previous 3 s and responds to the difference.[5]

Constraints imposed by physics

Look again at the runs in figure 3. They are not quite straight. The cell is subject to rotational Brownian movement that causes it to wander off course by about 30° in 1 s. After about 10 s, it drifts off course by more than 90° and "forgets" the direction in which it was going. This makes earlier measurements irrelevant and sets an upper limit on the time that the cell has to decide whether life is getting better or worse. A lower limit is set by the time required for the cell to count enough molecules of attractant or repellent to determine their concentration with adequate precision. The number of receptors required for this task proves to be remarkably small, because diffusion of the molecules to be sensed enables them to be sampled by different points on the surface of the cell with great efficiency.[6]
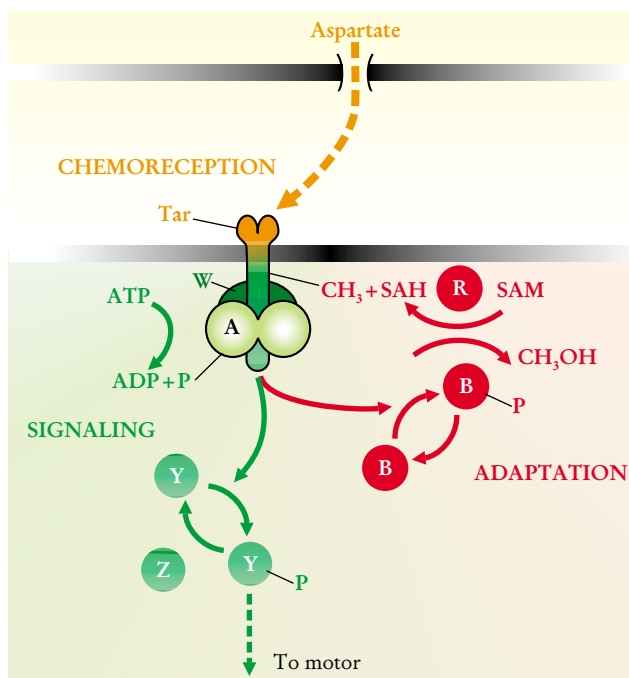
Flagellar mechanics is dominated by viscosity, not inertia: The Reynolds number is low,[7] less than 10^{-4}. So flagella generate thrust by using viscous drag. The viscous drag on a thin rod is about twice as great when the rod moves sideways as when it moves lengthwise. As a result, when oriented slantwise and pulled downward through a viscous medium, a rod moves to one side. (Geoffrey Taylor of the University of Cambridge in the UK showed this in his 1967 demonstration lecture by dropping thin cylinders in syrup.[8]) So, when a cell rotates a helical filament (in effect, a series of slantwise rods), it has to push as well as twist. In reaction the cell body translates and rolls. This scheme may not be very efficient, but it works![9]

Processing chemical signals

The molecular machinery by which *E. coli* modulates the direction of rotation of its flagella is complex, as indicated in figure 4, which shows only the aspartate receptor. (For general reviews of the mechanism, see references 10 and 11.) Three stages of the change-of-direction process can be distinguished:

▷ Chemoreception: Aspartate diffuses to Tar and binds.
▷ Signaling: The aspartate binding reduces the rate at which CheA phosphorylates CheY. Because CheY-P is continuously being dephosphorylated by CheZ, the concentration of CheY-P falls and less CheY-P binds to the base of the motor. When CheY-P binds to the motor, the CW state is stabilized and the CCW state is destabilized; with less CheY-P, the probability that the motor will spin CCW increases, and runs are extended.

FIGURE 4. CHEMOTAXIS MACHINERY. This diagram shows some of the components required for chemotaxis toward the amino acid aspartate. Information flows from the outside of the cell (shown at the top) by way of porins, the periplasmic space, and the cytoplasmic membrane, to the inside of the cell (shown at the bottom), and then to the flagellar motors (not shown). Dashed arrows indicate physical displacement of chemicals by diffusion. Solid arrows indicate chemical modifications of proteins—phosphorylation or methylation. The cytoplasmic components, all Che proteins (CheW, CheA, CheR, CheB, CheY, CheZ), are identified by their fourth letter only. The receptor complex consists of two molecules of Tar, two of W, and two of A, with Tar spanning the cytoplasmic membrane. Chemoreception is depicted in orange, signaling in green (for "go"), adaptation in red (for "stop"). Tar is a protein required for taxis toward aspartate and away from certain repellents. ATP is adenosine triphosphate, the phosphate donor. SAM is S-adenosylmethionine, the methyl donor. The other chemicals shown are ADP, adenosine diphosphate; SAH, S-adenosylhomocysteine; CH$_3$, the methyl group; CH$_3$OH, methanol; and P, inorganic phosphate.

Box 2. Molecular genetics

Joshua Lederberg chose *Escherichia coli* strain K-12 for his experiments on genetic recombination. He did the work at Columbia University and Yale University and published his findings in 1946.[2] Later, at the University of Wisconsin, he manipulated genes for flagella, not of *E. coli* but of a closely related species, *Salmonella typhimurium*, which is encountered in food poisoning. The flagella of *Salmonella* were known to be efficient antigens that could be used to distinguish among pathogenic strains. When genes for flagella were moved into a nonmotile strain, motile recombinants could be readily isolated, because they swarmed through soft agar (as shown at the top of the figure) while the nonmotile variants remained at the point of origin of the inoculation (as shown at the left in the figure). (The use of agar in bacteriology dates from 1882, the swarm technique from 1897.)

In the 1960s, Tetsuo Iino, who had studied with Lederberg and then moved to the National Institute of Genetics in Mishima, Japan, collaborated with Sho Asakura and Goro Eguchi of Nagoya University to publish a series of elegant papers on the assembly of flagellar filaments. The researchers found that the structures could be sheared off of bacteria, dissolved (by heating, for example), and then recrystallized in a test tube. The assembly process could be analyzed by observing the seeds of one antigenic type (or polymorphic form) interacting with monomers of another.

At about the same time, Julius Adler, a biochemist at the University of Wisconsin, chose *E. coli* strain K-12 to use in studies of bacterial chemotaxis. Although he was fascinated by the behavior of butterflies, he sought a simpler system in which to learn about molecular mechanisms. In 1969, he published a now-classic paper in which he showed that bacterial chemotaxis is a matter of esthetics, not material gain.[16] Normal cells are attracted by nutrient analogs that they cannot metabolize, and mutant cells that have lost the ability to take up or metabolize nutrients still find them attractive. While it is clear that taste for a particular chemical evolved because that chemical was useful to the cells, the behavioral decision is made on the basis of taste alone.

The accompanying figure shows a soft-agar plate 8.5 cm in
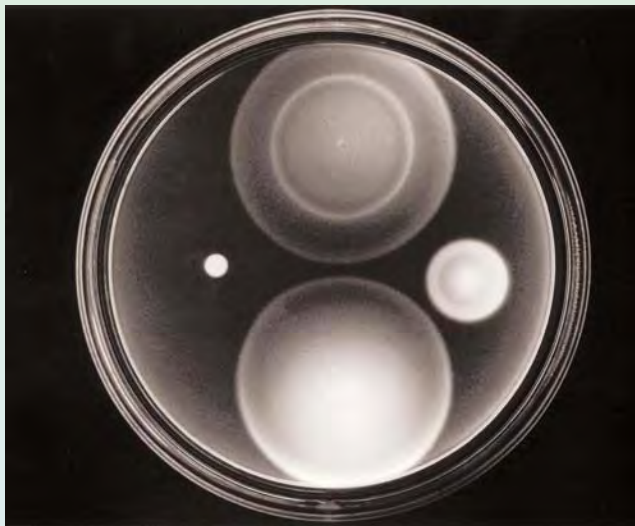
diameter containing a mixture of 20 amino acids. By stabbing the agar with a sterile toothpick dipped in a cell suspension, four *E. coli* strains were inoculated in this plate. The cells were allowed to grow for about 8 h at 30 °C. Then the plate was illuminated slantwise from below and viewed against a dark background. Normal cells (top) first consume serine, generating a spatial gradient of serine that they chase across the plate, forming the outer ring. Then the cells left behind consume aspartate, forming a spatial gradient of aspartate that they chase across the plate, forming the inner ring. Cells of the strain shown at the right have lost their ability to taste the amino acid serine, and so the outer ring is missing. Cells of the strain shown at the bottom have lost their ability to taste the amino acid aspartate, and so the inner ring is missing. Cells of the strain shown at the left can swim but are unable to back up; they become trapped in blind alleys in the agar. Adler perfected the use of this technique for identifying behavioral mutants. (Photo courtesy of Sandy Parkinson, University of Utah.)

▷ Adaptation: The decreased catalytic activity of CheA renders CheA less susceptible to the action of CheB-P and also reduces the concentration of CheB-P, which dephosphorylates spontaneously. Methyl groups are added to Tar by CheR and removed by CheB-P. Hence the methylation level goes up. This tends to restore the catalytic activity of CheA.

When a cell is exposed to a step increment in the concentration of aspartate, it adapts completely: Eventually the CheY-P concentration returns to its initial value and the cell goes back to swimming just as it did before. However, in a cell exposed to a ramp in the concentration of aspartate, adaptation will fail to catch up and the cell will continue to swim more smoothly. In effect, a cell makes temporal analyses by comparing the occupancy of the aspartate binding site to the level of methylation of the receptor's cytoplasmic domain. The occupancy of the binding site reflects current conditions, whereas the level of methylation reflects past conditions. The cell is able to respond to changes in the ambient concentration by comparing these two indicators. Adaptation is a universal feature in sensory physiology; for example, you are not aware of the pressure exerted by your chair on the seat of your pants unless someone calls it to your attention or unless it changes suddenly.

The sensory network shown in figure 4, complex though it is, is relatively well understood, and theorists are beginning to model it. An interesting recent development is the realization that the way in which the network is interconnected may determine which features are robust—that is, which features are relatively insensitive to variations in the concentrations of the proteins that compose the network. In an organism as small as *E. coli*, such variations are inevitable. For example, when a cell divides, soluble proteins are partitioned at random. Because most of the proteins involved in signaling and adaptation are soluble and are present in only a few hundred copies per cell, their concentrations vary. Adaptation back to a particular mean run interval appears to be such a robust feature.[12]

X-ray studies have revealed structures of several of the proteins (or domains of the proteins) involved in chemotaxis, including one of the components of the flagellar motor.[13] Given such structures, we should be able to work out how the proteins function.

Motor mechanics

I am particularly interested in motor mechanics. What makes the motor go? How much torque can it develop at different speeds? How does it change its direction of rotation? What are the mechanical interactions between flagellar filaments that coordinate different motors on the same cell?

Here is a sample experiment. Because shearing a dense cell suspension causes the filaments to tangle and fracture, we can break off most of a cell's flagellar filaments and then cement the cell body to a glass slide. If the cell makes filaments that tend to stick to everything (because of a particular mutation in *fliC*), then it is easy to attach a latex bead to one of the flagellar stubs. If the bead is relatively small—say, 400 nm in diameter—it will spin relatively rapidly, because of its small frictional drag coefficient. We can measure its rotational speed with a weak optical trap by means of back-focal-plane interferometry. The torque required to rotate the bead at angular velocity Ω is just $b\eta\Omega$, where b is a geometrical factor and η is the viscosity of the medium. The viscosity can be changed. Then, by plotting $\eta\Omega$ versus Ω, we can obtain a measure of relative torque versus speed.

At room temperature, the torque–speed curve for the motor of *E. coli* is relatively flat, falling about 10% between stall and 200 Hz. Thereafter it declines linearly, reaching zero torque at about 300 Hz. In the high-torque, low-speed regime, the torque is insensitive to changes in temperature or hydrogen isotope; evidently rates of diffusion of internal components or of proton association–dissociation reactions do not matter. In the low-torque, high-speed regime, on the other hand, the torque falls substantially as the temperature is lowered or as H_2O is displaced by D_2O. Now, rates of proton association–dissociation reactions do matter. What is surprising is that the region of transition between the two regimes is so abrupt.

Other worlds

But I have spoken only of flagellated organisms, of which *E. coli* is the pre-eminent model. There are other bacteria that move without flagella, either through liquids or at liquid–air or liquid–solid interfaces. Their means of locomotion are poorly understood.

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