
THE LANGUAGE OF LI
Cells Communicate in Health and Dise
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SMALL TALK

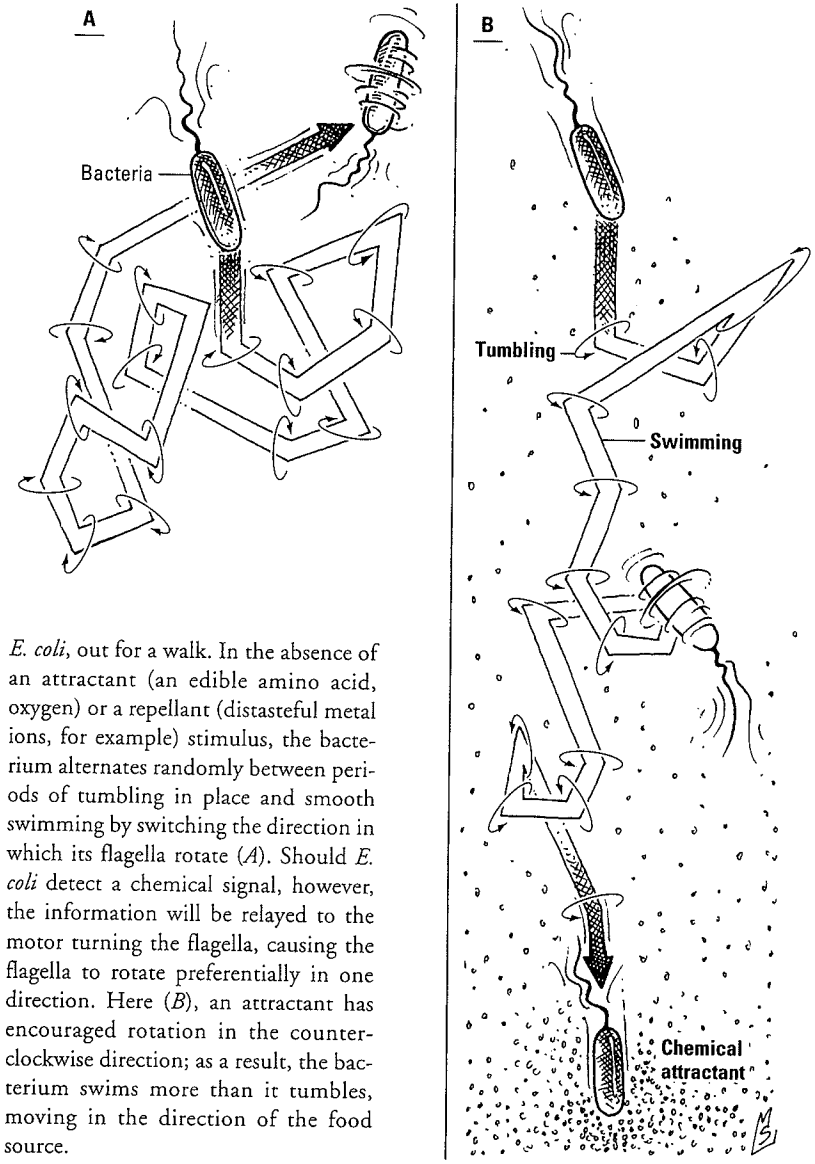
In broad daylight, you are floating in darkness. You swim in silence because you cannot hear. On your right, a cloud of sugar molecules drifts just a few centimeters away—dinner, but how will you find it? On your left, a noxious chemical seeps toward you—but unless you know that you're in danger, how can you escape? If you are the bacterium *Escherichia coli*, sharing space in the human gut with other indigenous flora and fauna, this is your world: unpredictable, at times even inhospitable. But don't dwell on your limitations. Your kind came to life when the earth was still hot and sulfurous and has used the intervening eons to craft all the tools and techniques you need to survive in a capricious environment.

You have many talents. For one thing, you can move. Let the bottom dwellers and the stone huggers ache to be noticed by some passing current; evolution taught you to waltz—chassé, pause, spin, glide, ONE-two-three, ONE-two-three, left, right, zigzag. Your dancing shoes are a skein of protein filaments, or flagella, powered not by muscles and tendons but by a gearbox of proteins that operate as a rotor, twirling the flagella at more than 100 revolutions per second. When the rotor turns counterclockwise, the flagella spiral

into a single tail and you glide in a smooth, straight line. When it spins clockwise, the flagella unfurl and stroke, each to its own beat, and you spin and tumble in place. Reverse again and you resume swimming in a new direction. The rotor switches back and forth as regularly as a metronome, spinning one way for a few seconds, then the other. Spinning and swimming, you meander along to its rhythm, improvising a daydream of a dance that textbooks refer to as the "random walk."

It's a good thing you don't have a mother—she'd surely admonish you to watch where you're going. You can't afford to be so oblivious, she'd scold, or you're liable to waltz right into trouble. As neurobiologist Rodolfo Llinas warns human ramblers, "Active movement is dangerous in the absence of an internal plan subject to sensory modulation. Try walking any distance, even in a well-protected, uncluttered hallway, with your eyes closed. How far can you go before opening your eyes becomes irresistible?" But you could tell her not to worry—you can chart a less haphazard course when you need to. Should an appetizing snack appear on the horizon, the rotor lingers in counterclockwise mode, so that you swim straight ahead, in the direction of the meal, instead of meandering aimlessly. A distasteful substance, on the other hand, shifts the rotor clockwise, and you tumble in search of an escape route—the path you'll follow when it resumes its usual alternating pattern.

By repeatedly adjusting the proportion of clockwise-to-counterclockwise rotation, you can forego your wandering ways in favor of a one-step forward, one-step-sideways shuffle—not exactly what a more advanced creature might think of as purposeful movement but a "biased random walk" that hitches determinedly, if somewhat erratically, toward satisfaction or away from catastrophe. Microbiologist Ann Stock explains: "If the cell finds itself moving in the proper direction, it suppresses tumbling and moves further in that direction. Then it randomly reorients and heads off in a new direction, one that might still be good or may now be bad. If it's going in the



E. coli, out for a walk. In the absence of an attractant (an edible amino acid, oxygen) or a repellant (distasteful metal ions, for example) stimulus, the bacterium alternates randomly between periods of tumbling in place and smooth swimming by switching the direction in which its flagella rotate (*A*). Should *E. coli* detect a chemical signal, however, the information will be relayed to the motor turning the flagella, causing the flagella to rotate preferentially in one direction. Here (*B*), an attractant has encouraged rotation in the counterclockwise direction; as a result, the bacterium swims more than it tumbles, moving in the direction of the food source.

wrong direction, it goes back to tumbling. It seems tortuous, but the same patterns are used by grazing animals to find better pastures. You do a little sampling in all directions and keep going when things are good and reorient when they're not." But how did you determine which direction was the "right direction"? And how did you use that information to change the rotation of your flagella?

I look and listen and touch; if I could swim with you, I would know the human gut as wet and warm, a muffled rush of murky water. But there are other ways to experience life. Your world is vivid with chemicals as well as colors, molecules as well as sounds. They could be your connection to the outside world, the cues that could chart your path. Even this solution presents difficulties, however. Like other cells, you are surrounded by a protective membrane of lipids and proteins isolating your fastidiously composed internal fluids from the unpredictable excesses of your surroundings. Nothing that might upset the delicate balance critical to life can penetrate this barrier—but neither can the chemical signals bearing news about current events. Inside your hermetically sealed bubble, the rotor proteins controlling your flagella are waiting for direction; outside, messengers with the information needed by the rotor proteins mill restlessly in front of closed doors.

Troublesome organism, isn't there any end to your problems? Evolution should have just given up on you, allowed you to starve in silence. Instead it has crafted words to describe your world and rules for combining them, the building blocks of a language that tells you how to move in step with the world around you and gives your crooked walk a glamorous name: "chemotaxis," movement directed by chemicals.

BETTER MOVEMENT THROUGH CHEMISTRY

The ancestors of *E. coli* did not have plastics, but they did have another type of polymer suited to a wide range of applications: proteins. More heterogeneous than polystyrene or polyurethane, nature's

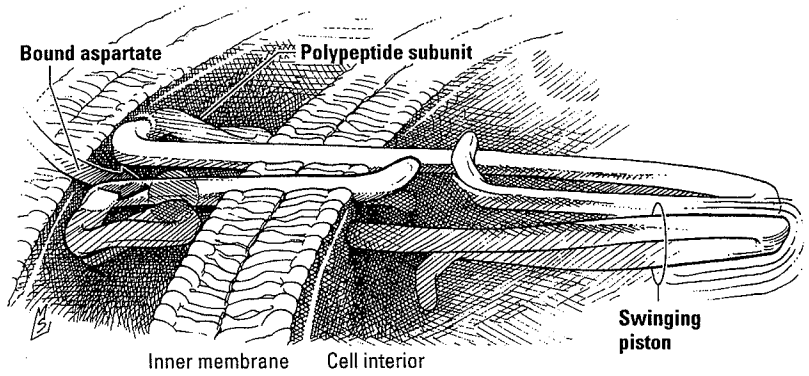
megamolecules are combinations of 20 different building blocks, or amino acids, coupled to each other by the electron-sharing arrangement known as a peptide bond. Man-made plastics can be shaped, molded, or extruded to order, but protein polymers automatically bend themselves into useful shapes, guided only by their affinity for water and the social relationships between their constituent amino acids—no heat or specialized machinery required.* Scores of fragile alliances between amino acids act in concert to stabilize the folded protein, resulting in a three-dimensional configuration as unique as a fingerprint.

Productive only when wedded to another molecule, the protein relies on this structure to play the role of matchmaker, embedding an advertisement for a soul mate in the loops, bulges, and trenches created on its outer surface by the folds:

> **"Are You My Better Half?"**—SFP (single folded protein) with secure position in healthy cell seeks compatible molecule with interest in chemical engineering, architecture, or communication for exclusive short-term relationship. Please reply with details of your chemical structure.

The molecule with the most compatible profile wins the date and enters into a marriage of convenience, arranged by evolution to accomplish a specialized task. Paired with a matching substrate, an enzyme speeds up a vital chemical reaction. A rotor protein and a flagellar protein conspire to move a bacterium. And a protein with a soft spot for a nutrient or a toxin (call the partners "receptor" and "ligand"), as well as a torso rich in water-repelling, or hydrophobic, amino acids on intimate terms with the lipid-rich plasma membrane, is the perfect spy, relentlessly drawn to important information and

*Many proteins do, however, need an assistant—a second protein known, appropriately enough, as a "chaperone"—to complete the job of folding.



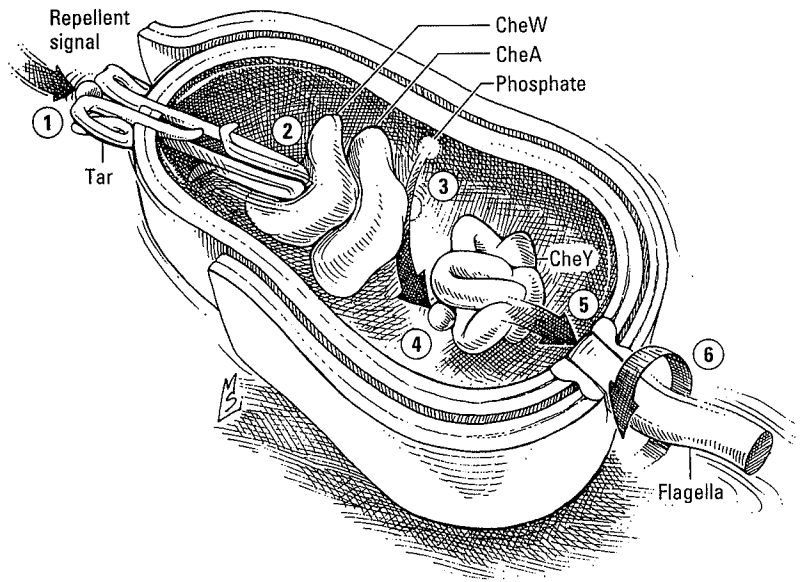
The Tar receptor. The binding site of this sensor protein, which can detect both the amino acid aspartate and repellants such as nickel, projects above the outer surface of the plasma membrane. A mobile helical segment spanning the membrane acts as a "swinging piston" that nudges the first member of an intracellular team of proteins charged with relaying the news to the flagella.

built to share that information with an insider able to put it to good use.

E. coli sponsors five such receptor-ligand partnerships—its five senses. The receptor that biologists call "Tsr" is married to the amino acid serine. The Tar receptor's partner is aspartate. Tap prefers peptides; Trg falls for proteins that bind and transport the sugars ribose and galactose. Aer is devoted to oxygen instead of nutrients. To conserve precious space in a small genome, adultery is not only permissible but encouraged; Tar, for example, has leave to consort with repellants, such as nickel ions, in addition to aspartate.

The architecture of the receptor determines how it will speak as well as to whom it will speak. Look, for example, at the Tar receptor in cross section (*you* can't, of course, but X rays or the magnetic field generated by a nuclear magnetic resonance spectrometer can), and you'll see that it's not a single protein but a dimer (pronounced "DIE-mer")—a pair of identical polypeptide subunits. Note how each rears up out of the membrane like a flower, crowned with a rosette of four helical segments that cradle Tar's preferred ligands. Two of these helices wind out of the binding site and through the membrane, tapping into the rich cytoplasm below. Rooted, Tar is not rigid, however. During the subtle shape-shifting triggered when receptor and ligand embrace, one transmembrane helix is pushed downward and forward. The kick delivered by this "swinging piston" transmits news of the binding of attractant or repellant from outside to inside; in effect, Tar uses its own body to announce the discovery of ligand, much as an electronic sensor might activate a buzzer or a flashing light.

In an organism as small and simple as *E. coli,* one word (or one nudge from a dangling helix) should be enough to tell a rotor "left" or "right." But this bacterium must have had a finicky language



A bacterial "sentence." The binding of a repellant signal to the Tar receptor (*1*) sets the helical piston swinging (*2*), prompting CheW, the "word" heading the intracellular portion of the sentence, to activate the kinase CheA (*3*). This enzyme transfers a phosphate group to CheY (*4*); phosphorylated CheY (*5*), in turn, tells the rotor protein, currently turning counterclockwise (*6*), to change direction.

teacher, for it insists on speaking in complete sentences. The receptor is the subject of this sentence, the predicate a team of intracellular molecules called "Che" (for *che*motaxis) proteins that relay the message from receptor to rotor and add the nuance of context. CheW, the team member that heads this bucket brigade, is merely a go-between. When Tar detects a repellant, CheW takes the kick, then pokes the next protein in line, CheA, the action verb at the core of every chemotactic sentence.

One of a large and influential family of enzymes known as "kinases" (from a Greek word meaning "to move"), CheA, like its kin, responds to a wake-up call with an expletive—"Phosphate!" The chemical group that puts the "P" in ATP, phosphate, bristling with three negative charges, has the power to twist proteins inside out, turn ordinary amino acids into powerful magnets, and disrupt long-standing relationships—a switch flipping switches. "Nickel! Pass it on," whispers CheW, and the excited kinase spits a phosphate group into the face of one of its own histidine residues. The histidine, in turn, spews it at the nearest bystander, an aspartate protruding from the final protein in the relay, CheY. To CheY, phosphate is not an insult but the gift of wings. It soars across the cell, landing on the rotor protein. "No more swimming!" it bellows. The rotor obediently shifts into clockwise mode. Flagella flail, and the bacterium spins like a child on a tilt-a-whirl.

The news of aspartate binding outside the cell travels via the same protein relay but has the opposite effect. Instead of agitating, the receptor tells CheW to tell CheA to keep its mouth shut. The kinase dozes; CheY stays home and keeps to itself. And without phosphorylated CheY meddling with the rotor, the bacterium continues to swim, gliding smoothly toward the food source.

Writers are admonished to be sparing in their use of adjectives, but *E. coli* shamelessly adds two modifiers to every chemotactic sentence. Such verbosity isn't a sin in this language, however—the extra words, the enzymes CheR and CheB, add contextual information

the bacterium needs to fine-tune responses, in the form of methyl groups (a "methyl group" is a chemical "special teams" unit consisting of a carbon atom surrounded by three hydrogen atoms) that they plug in to or pull out of sockets in the tails of chemotactic receptors. Whenever the binding of an attractant stills the receptor and quiets CheA, CheR inserts methyl groups into the sockets; with each addition, the receptor recovers more of its voice. The binding of a repellant, on the other hand, prompts CheA to activate the CheB enzyme along with CheY. Phosphorylated CheB unplugs methyl groups, hushing both receptor and kinase. By adding and subtracting methyl groups, the two enzymes can adjust receptor sensitivity to mitigate the effect of ligand binding. "You can think of chemotaxis receptors as sitting in a balance between the ligand binding state and the methylation state (that is, the number of methyl groups attached to the receptor protein)," explains Ann Stock. "When the two are properly balanced, you have a steady state output. If you add a stimulus, you throw the receptor into a new signaling state, where you get some sort of response. Then, if you change the methylation state to counterbalance the presence of ligand, you restore the initial steady state output." This balancing act, known as *adaptation*—the bacterial equivalent of the adjustment your eyes make when you walk out of a dark room into bright sunlight—links the response to a change in the amount of ligand, rather than concentration per se, extending the range of the chemotaxis system over five orders of magnitude.

Methylation not only keeps bacteria alert but also makes them smarter; in addition to volume control, it serves as a primitive form of memory. A physical record of the bacterium's most recent interaction with the environment, the methylation state of the receptor records the concentration of attractant or repellant. Then, a few strokes later, the organism can reference this value, compare it to the current level of stimulation, and engineer a midcourse correction if necessary. Stock concludes: "The cell is testing out its environment at one point in time. It's swimming, it's moving to a new place,

comparing. And then it makes the decision whether to go forward or tumble based on what has happened over time."

"The modern era begins, characteristically, with a revolution," writes historian Jacques Barzun in *From Dawn to Decadence: 500 Years of Western Cultural Life*, referring to the cultural upheaval that began with Martin Luther's challenge to the Catholic Church. However, as Barzun notes, this cataclysm did not simply erupt out of the blue. The influence of earlier reformers and critics, the invention of the printing press, the introduction of higher-quality paper and ink, and the emergence of skilled printers provided the ideas and tools necessary to transform a local protest into an international spectacle.

Come here. Run away. Just as the Protestant reformation had its roots in the earlier efforts of Wycliff and Gutenberg, the revolution in communication that would one day guide interactions between cells almost certainly had its origins in simple directives like these, governing interactions with the environment. Pioneered by a distant ancestor fortunate enough to stumble upon the virtues of proteins for engineering alliances between molecules, the chemotactic sensory apparatus of bacteria like *E. coli* illustrates fundamental principles common to the transfer of any type of biological information via chemical signals: the exploitation of protein topography to discriminate signals; the use of transmembrane receptors to circumvent the barrier posed by the plasma membrane; the coordination of perception and response, as well as the integration of multiple signals, by means of protein relays featuring kinases; the regulation of receptor sensitivity by chemical modification. Using these principles as a template, evolution would construct a chemical language that would end the isolation of cells, that would allow them to talk to one another, to cooperate, to live as a group yet behave as a single organism—one of the most extraordinary achievements in the history of life.

GROUP DECISIONS

"You want a crab?" A skinny boy of about 10 holds a saucer-sized crustacean by a front claw and shakes it at me.

"That's a big one. You're not taking it home?"

His mother, who is trying to persuade his younger brother to pick up the sand castle paraphernalia, looks up and glares at me. "It already smells," she hisses. Behind her, two hungry seagulls nod in agreement.

"Sure," I reply, and hold out a small plastic bucket.

Back at our beach blanket, my 13-year-old daughter Haley looks up from the latest issue of *Teen People* and peers into the pail. "Why do you have a dead crab?"

"It's not dead; it's just not moving." I prod gingerly at the crab with the end of a pencil, but it has turned peevish and uncooperative. It squats at the bottom of the pail like a sullen garden toad, refusing to budge. I poke more aggressively. Without warning the stalks supporting the crab's beady black eyes shoot straight up at me. "Ahhh! Did you see that? It popped its eyes at me!"

"It's being weird because it's in a bucket of hot water," Haley remarks, turning the page.

"I was going to draw its picture, but I think maybe I'll just let it go. Would you like to help me?" I ask.

We pick a spot near a rocky outcrop, checking first for the gulls. When I'm certain they've moved on in search of other prey, I tip the pail into the surf. Torpid in captivity, the crab dances in the saltwater, energetic as a wind-up toy. It punches at the foaming wavelets, scrambles along the edge of the rocks, cartwheels, hops. It clutches at the slippery rocks. Finally, it darts sideways into a crevasse and disappears, safe at last from hungry gulls and grasping humans.

Dozens of species of marine bacteria also call this tidal flat home—and they don't take any more kindly to deportation than my crab. Relocated to the laboratory, most die, no matter how attentively they're coddled. Microbiologists at Northeastern University