Signal Amplification in Bacterial Chemotaxis through Receptor Teamwork

Chemoreceptors of different detection specificities signal collaboratively in a highly cooperative array, the bacterial equivalent of a neural network

J. S. Parkinson

Motile bacteria move with purpose: their movement machinery, whether for swimming or gliding, is responsive to environmental cues and enables them to seek optimal living habitats. The best studied of these bacterial behaviors is chemotaxis, the movement of an organism toward or away from particular chemicals.

Although first described in the late 1800s, scientists resumed studying chemotaxis in earnest during the 1960s, spurred by Julius Adler at the University of Wisconsin, Madison, who focused on *Escherichia coli*. He and others provided detailed molecular views of the signaling components bacteria use to detect and respond to changes in their chemical environment. More recently, other investigators learned that bacteria possess sophisticated "wetware" devices that can detect minute concentration changes, integrate multiple or conflicting stimuli, and make adaptive decisions based on those inputs. We are just beginning to understand the circuit logic of the chemotaxis signaling pathway and the source of its sophisticated information-processing capabilities. This, then, is the emerging story of the central information-processing unit of bacterial chemotaxis, with *E. coli* in the leading role.

Movement and Behavior in Thermokinetic Hell

The smallness of bacteria consigns them to lives dominated by viscous rather than inertial forces. Moreover, in liquid environments they are constantly buffeted by small molecules that can knock them off course. These physical constraints dictate how they move and behave. Despite these hardships, *E. coli* cells swim at 10–20 body lengths per second by rotating stiff flagellar filaments, which operate much like the propellers on an ocean liner. When the half-dozen or so individual motors on a cell rotate counterclockwise (CCW), the filaments form a bundle and propel the cell (Fig. 1). When one or several motors reverse to rotate clockwise (CW), the bundle flies apart and the cell executes a random directional change or "tumble."

In chemically homogeneous environments, such cells move about in a random walk, with forward swimming punctuated by tumbling. In chemical gradients, the cells bias their random walks to migrate in favorable directions, either toward an attractant or away from a repellent (Fig. 1). Gradient sensing is temporal rather than spatial. Thus, *E. coli* retains a 3- to 4-second memory of its immediate chemical past, which is compared to current conditions to determine if chemoeffector levels have changed. A detectable increase in an attractant, for example, induces the cell to suppress the next random tumble and to continue its current heading. This temporal-sensing strategy is effective only over such short times because Brownian motion also can alter a cell's heading.

The Chemotaxis Signaling Circuitry

E. coli uses a signaling cascade of protein phosphorylation and dephosphorylation reactions to control its flagellar motors (Fig. 1). Some components and reactions (depicted in green, Fig. 1) augment CW rotation; others (in red) suppress

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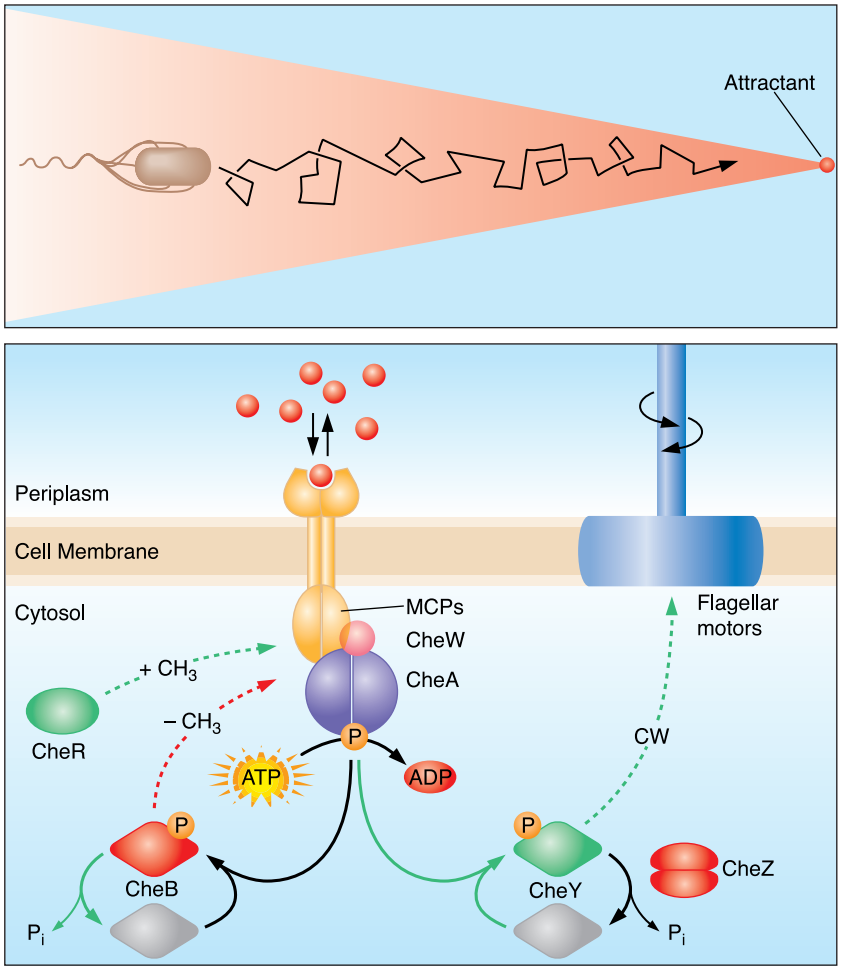
FIGURE 1



(top) The chemotaxis strategy of *E. coli*. Whenever life gets better (e.g., traveling up an attractant gradient), the cell tries to continue its heading by suppressing the next directional change. This plan is often thwarted by unintended course changes, resulting in a random walk biased in the preferred direction. (bottom) The chemotaxis signaling circuit. Green components and reactions enhance the probability of CW flagellar rotation; red components and reactions enhance the probability of CCW rotation, the default behavior. Gray (unphosphorylated) molecules are inactive. Native MCP, CheA, and CheZ molecules are homodimers; CheW, CheB, CheY, and CheR function as monomers.

CW signals to enhance CCW rotation, the default state of the flagellar motors. The CW- and CCW-enhancing elements of the signaling circuit are wired in opposition so that in homogeneous chemical environments the two signals balance, leading the cells to execute random walks.

Transmembrane chemoreceptors known as methyl-accepting chemotaxis proteins (MCPs) govern the flux of phosphates through the signaling cascade. MCP molecules typically have a periplasmic ligand-binding domain for monitoring chemoeffector levels and a cytoplasmic signaling domain that undergoes reversible methylation at 4–6 glutamic acid residues. The methylation state of a receptor encodes the memory of its chemical environment.

Whenever overall ligand occupancy in the population of receptor molecules fails to coincide with their aggregate methylation level, the receptors modulate phosphate fluxes to produce two signals: a feed forward signal that alters motor rotation and a feedback signal that begins updating the methylation record until it reflects the cell's new chemical surroundings. Motor responses occur within a few hundred milliseconds, whereas it takes the sensory adaptation machinery several seconds to update the methylation record. This timing differential creates a comparison window during which the cell is cognizant of differences between its present and past chemical environments.

CheA, a histidine kinase, and CheW, an enigmatic coupling factor, bind to the cytoplasmic domains of MCP molecules to form ternary signaling complexes that oscillate between kinase-on and kinase-off activity states. The proportion of receptor complexes in each signaling state reflects the dynamic interplay between ligand occupancy and methylation state. The signaling cascade begins with CheA, which donates its phosphoryl groups to two competing response regulators, CheB and CheY, thereby activating them. Phospho-CheY binds to the flagellar motors to augment CW rotation. Phospho-CheB, an MCP-specific methylesterase, demethylates MCP molecules. Its counterpart, CheR, an MCP-specific methyltransferase, attaches methyl groups to MCP molecules. Methylation favors the kinase-on signaling state, whereas demethylation favors the kinase-off state.

Signal Gain via Receptor Clustering?

E. coli is extraordinarily sensitive to its chemical environment. Concentration changes as small as

A Tinkering Lifestyle, from Radios and Autos to Chemotaxis in *E. coli*

John S. (Sandy) Parkinson loves to tinker. As a child growing up in a suburb of Wilmington, Del., he took apart all sorts of things, just to see how they worked. "Our basement was littered with bombsights, telephones, radios and other electronic gadgets that had great switches, relays, bulbs, etc., with which I could build new electronic gadgets with switches, relays, bulbs, etc," he recalls. "I was especially proficient at dismantling radios and would compulsively sort and store all of the isolated components. I was less proficient at putting radios back together again."

As an adult, Parkinson took to tinkering with bacteria. Now, at 60, as a professor of biology at the University of Utah in Salt Lake City, he studies the ways in which bacteria sense and respond to changes in their environment. His current focus is chemotaxis in *Escherichia coli*. "The study of the *E. coli* model is directly relevant to the usually more complicated chemotaxis systems of other bacteria and eventually may lead to new therapeutic strategies," he says.

His research, however, is fueled less by the practical applications of his work than by the same curiosity that inspired him during childhood. "Frankly, my interest in this system is not driven by the hope of coming up with a cure for dysentery, but rather by a fascination with the marvelous mechanisms that bacteria have devised for detecting and processing information about their environment," he says.

"Many think of bacteria as 'simple' organisms because their bodies are relatively uncomplicated, but they're really much more sophisticated than we ever suspected," he continues. "They've evolved truly clever strategies for processing sensory information that are based on only a few protein components. They accomplish with a handful of protein molecules in a single cell what higher organisms accomplish with elaborate neuronal networks."

Parkinson grew up in what he describes as an "Ozzie-and-Harriet"-type family life, referring to a popular television program from several decades ago. In his family, he was the oldest among three brothers. His father was a design engineer for the DuPont Co., and his mother primarily a housewife who was very involved in community activities.

When Parkinson became a teenager, he switched from tinkering with radios to internal combustion engines and vehicles powered by them. "I built go-carts and motorbikes, then moved on to cars when I reached driving age," he says. "My circle of high school friends revolved around cars. We spent most of our free time trying to keep our cars running, and trying to improve their performance or appearance."

He began college in 1961 as a pre-med major, but soon realized he enjoyed working in labs and doing experiments more than the prospect of practicing medicine. So he switched to biology, graduated from Haverford College in 1965, and from there went to the California Institute of Technology in Pasadena to pursue a Ph.D. that he completed in genetics and biophysics by 1969.

"Caltech proved to be an absolutely perfect match for my idiosyncratic lifestyle and scientific interests," he says. "I was allowed, even encouraged, to do science of my choice day and night. I could wear jeans and t-shirts seven days a week, and I got to interact with dauntingly intelligent faculty and fellow students. For a lab rat such as me, this was—and still is—a perfect life. And I even get paid for doing it!"

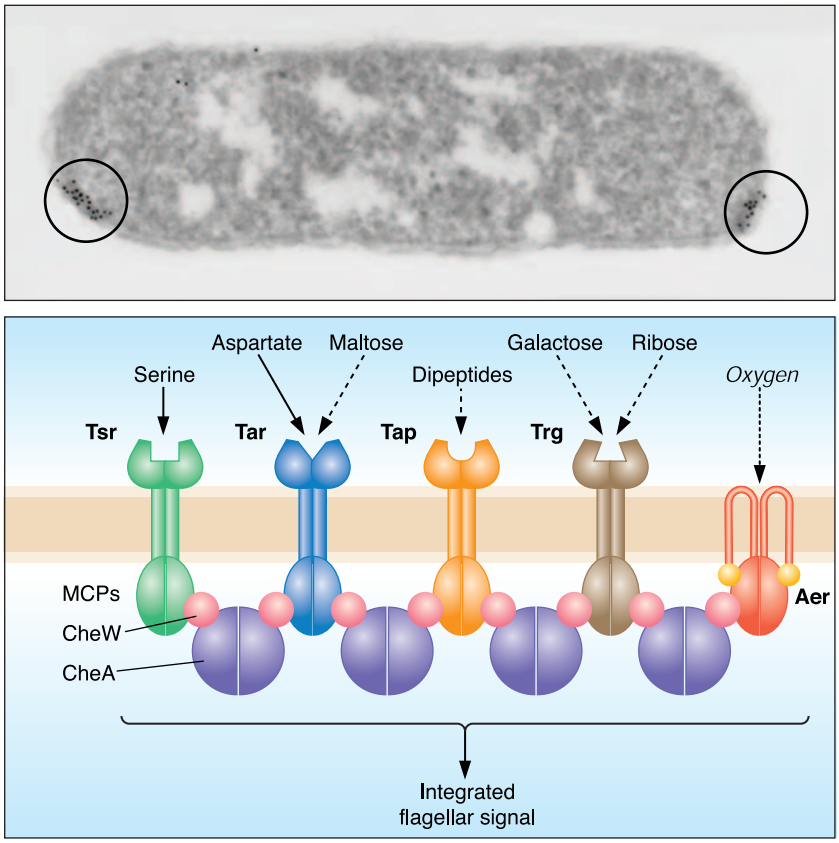
John S. Parkinson

He and his wife Alice, a psychiatric nurse practitioner and clinical instructor at the university's college of nursing, have three children and three grandchildren. He says he practices no "all-consuming" hobbies, but appreciates "good food, museums, travel, and the Utah outdoors." He also devours science fiction novels, particularly "those in the cyberpunk genre." He and his wife take several recreational trips every year. "More than anywhere else, we love hanging out in central Mexico, mainly the Cuernavaca area," he says.

While he finds working with colleagues and undergraduates very rewarding, he calls research "my principal love." The reason should come as no surprise: "I enjoy puzzling out how things might work, and building and testing mechanistic models," he says.

Marlene Cimons
Marlene Cimons is a freelance writer in Bethesda, Md.

FIGURE 2



(top) Thin-section electron micrograph of an *E. coli* cell showing receptor clusters labeled with gold-tagged antibody molecules (figure modified from S. R. Lybarger and J. R. Maddock, J. Bacteriol. **181:**5527–5529, 1999). (bottom) The MCP-family chemoreceptors of *E. coli*. Dashed lines indicate chemoeffectors sensed indirectly, either through periplasmic binding proteins (maltose, dipeptides, galactose, ribose) or through their metabolic effects on the cell (oxygen). All receptor molecules are localized in polar clusters whose integrity depends on bridging connections with the cytoplasmic CheA and CheW proteins. The receptor array generates integrated output signals by summing and amplifying stimulus signals detected by its component receptor types.

1 part per 1,000 can trigger a substantial change in the probability of its next tumbling episode. This high sensitivity prevails over a wide dynamic range, from low nanomolar to nearly millimolar concentrations. The ratio of the fractional change in motor bias to the fractional change in receptor occupancy is defined as the signal gain, a measure of the amplification properties of the sensing-response system. The overall gain factor in the chemotaxis signaling pathway is about 50. Most of this amplification occurs at the stimulus detection stage, according to Victor Sourjik, who is now at the University of Heidelberg in Germany, and Howard Berg at Harvard University in Cambridge, Mass. They devised a sensitive assay based on fluorescence resonance energy transfer (FRET) between the CheY and CheZ signaling proteins to measure stimulus-elicited changes in the CheA kinase activity of living cells.

High signal gain at the stimulus detection step presents a conceptual paradox. How can small concentration changes, which should affect the ligand occupancy of only a few receptor molecules, produce high gain factors, corresponding to the control of many CheA molecules? High gain implies that the sensory information detected by a few receptors is somehow shared with other receptors to produce an amplified output signal. The simplest mechanism of receptor-receptor communication, initially modeled by Dennis Bray and colleagues at Cambridge University in England and subsequently by other theoreticians, would be to propagate conformational changes through direct physical connections between receptors.

Janine Maddock, who is at the University of Michigan in Ann Arbor, and Lucy Shapiro of Stanford University in Stanford, Calif., discovered in 1993 that the chemoreceptors in *E. coli* cluster predominantly at the cell poles (Fig. 2). Perhaps receptor clustering reflects the networking interactions that underlie receptor-receptor communication and signal gain.

E. coli Chemoreceptors

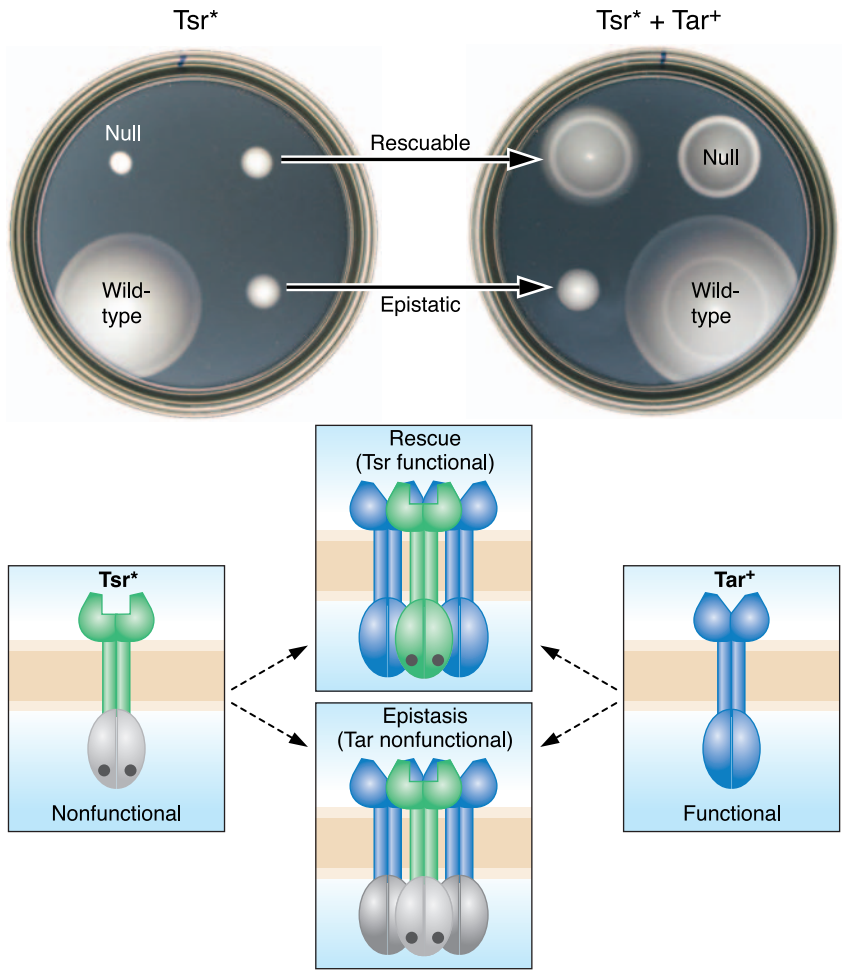
E. coli has five MCP-family chemoreceptors, defined by their highly conserved cytoplasmic signaling domains. Four—Tar, Tap, Trg, Tsr—have conventional membrane topologies with a periplasmic sensing domain that detects ligands either via direct binding or through interactions with ligand-specific periplasmic binding proteins.

The fifth member of the *E. coli* family of chemoreceptors, Aer, is unorthodox in several respects. Aer has no periplasmic sensing domain, but rather has a cytoplasmic domain that

binds a flavin adenine dinucleotide (FAD) cofactor with which it monitors the redox state of the electron transport chain to mediate aerotactic responses to oxygen and other electron acceptors. Aer also has unorthodox methylation sites that are not involved in adapting to aerotactic stimuli.

All five receptor types form ternary signaling complexes with CheA and CheW, and cluster at the cell poles. The integrity of the clusters is largely dependent on CheA and CheW, indicating that these cytoplasmic proteins might form bridging connections or otherwise stabilize direct connections between receptor molecules (Fig. 2). Tsr (the serine chemoreceptor) and Tar (the aspartate/maltose chemoreceptor) are each present at more than 5,000 molecules per cell; Tap, Trg, and Aer are each present at fewer than 500 molecules per cell. Nevertheless, the low-abundance receptors exhibit high signal gain, implying that they can enlist high-abundance receptors to produce strong output signals. Thus, the entire receptor cluster may behave as an integrated signaling unit in which different types of receptors share sensory information to produce amplified output signals.

FIGURE 3

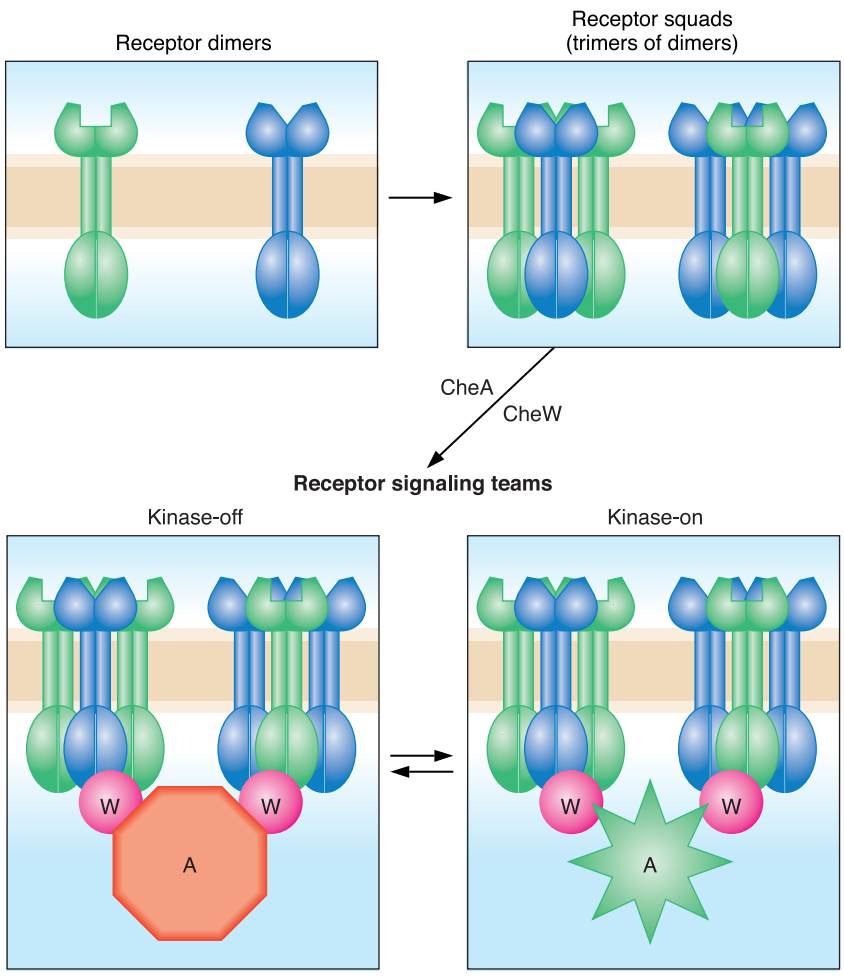


(top) Signaling properties of serine-receptor (Tsr) trimer contact mutants, alone and in the presence of wild-type aspartate receptors (Tar). The morphologies of colonies on tryptone soft agar plates reflect their chemotactic ability. Wild-type Tsr exhibits a ring of cells following a metabolism-generated serine gradient. Wild-type Tar produces a slower ring of cells that follow an aspartate gradient. Wild-type Tsr or a Tsr null mutant have no effect on Tar function, whereas an epistatic Tsr mutant blocks Tar function and a rescuable Tsr mutant regains some serine chemotaxis ability in the presence of Tar. (bottom) A team signaling model to explain rescuable and epistatic receptor mutants. Nonfunctional receptor signaling domains are shown in shades of gray. The model proposes that receptors function in higher-order groups that can be mixed and that members of the same group can influence one another's signaling abilities.

Are Trimers of Dimers the Building Blocks of Receptor Clusters?

Native chemoreceptor molecules are homodimers. However, when Sung-Hou Kim and his fellow crystallographers at the University of California, Berkeley, obtained an X-ray structure of the Tsr signaling domain, they found that the dimers are organized into groups of three, with extensive interdimer contacts at the trimer interface. Notably, the amino acid residues found at the trimer contact sites of Tsr are perfectly conserved in other *E. coli* receptors, suggesting that mixed trimer formation could serve as the structural basis for receptor-receptor communication. Do trimers of receptor dimers form in vivo and, if so, are they structural and functional components of receptor clusters? My research group has taken two experimental approaches to address these questions. We first examined the signaling properties of mutant Tsr receptors, denoted Tsr*, with amino acid replacements at trimer contact residues. We then devised crosslinking assays to detect receptor trimers of dimers in vivo and asked whether trimer contact lesions influence their formation.

FIGURE 4



Organizational levels of receptors and the formation of receptor clusters. Receptor molecules progress from dimers to clusters by assembling into trimers of dimers and then recruiting CheA and CheW proteins to build signaling teams. Teams can contain members with different detection specificities, but all members respond coordinately to input stimuli. Receptor clusters probably represent a collection of interconnected signaling teams, but their architectural links are not yet understood. Some mutations in the trimer-of-dimers contact residues can block receptor clustering at the squad formation step, while other lesions permit cluster formation, but block stimulus-dependent modulation of CheA kinase activity.

Together, these approaches provide compelling evidence that trimers of dimers indeed play an important role in receptor signaling.

Genetic and Crosslinking Evidence for Higher-Order Receptor Signaling Units

Missense mutations at any of the Tsr trimer contact sites nearly always eliminate chemotactic responses to serine, but in several different ways, depending on the nature of the amino acid replacement. Proline replacements, which are expected to disrupt the alpha-helical secondary structure of the trimer contact region, prevent cluster formation and CheA activation. Alanine and tryptophan replacements, which are expected to preserve alpha-helical structure while weakening or distorting the trimer interface, do not prevent the mutant receptors from forming clusters, but destroy their ability to modulate CheA activity. Some mutant receptors fail to activate CheA, whereas others seem to be locked in the kinase-on signaling state.

The signaling interactions between Tsr* receptors and normal receptors in the same cell provide important clues to their functional defects. The proline-containing mutant receptors exert no deleterious effects on the functions of heterologous receptors, such as Tar, consistent with their expected null condition. The alanine and tryptophan mutants, in contrast, exhibit two surprising functional interactions with Tar. Some of the alanine mutants regain serine-signaling ability in the presence of wild-type Tar, an effect we call functional rescue (Fig. 3). Conversely, the tryptophan mutants block the aspartate-signaling ability of wild-type Tar, a genetic effect termed epistasis (Fig. 3).

The rescuable and epistatic properties of Tsr trimer contact mutants can be explained by proposing that receptor molecules normally operate in higher-order groups, or signaling teams that can contain receptors of different types (Fig. 3). In the case of functional rescue, the wild-type members of the team probably impose a normal conformation on the entire signaling unit. In the case of epistasis, the structural distortions caused by the mutant members of the team may prevent function of the entire signaling unit. Conceivably, the bulky side chain in tryptophan mutants hinders stimulus-induced conformational changes in the team, whereas the small side chain in alanine mutants, when teamed with functional receptor molecules, allows such conformational changes.

Although the Tsr* receptors carry mutations at the trimer contact sites, their genetic properties are consistent with any receptor team size greater than a single dimer. Accordingly, we turned to site-directed crosslinking methods to determine more incisively whether receptor signaling teams are based on trimers of dimers. To ask whether MCP molecules form trimers of dimers in vivo, we devised crosslinking assays based on several unique structural features of the trimer. Our general approach was to introduce single cysteine residues into two different receptors and to analyze their crosslinking products when intact cells are subjected to sulfhydryl-targeted crosslinking conditions. We chose reporter sites that were predicted to crosslink in the trimer-of-dimers structure and at which a cysteine replacement was tolerated with no loss of receptor function.

One approach used cysteine reporters at positions in Tsr and Tar that should form disulfide-linked Tsr~Tar products when juxtaposed at the interface of a mixed trimer. Another approach employed a cysteine reporter with trigonal geometry near the trimer contact region and a trifunctional maleimide reagent with a spacer length appropriate for capturing the three axial subunits in a trimer of dimers. Both approaches detect mixed receptor crosslinking products whose formation depends on the relative expression levels of the marked receptors and whether they carry lesions at the trimer contact sites.

Receptors with proline mutations at the trimer contacts fail to form mixed crosslinking products, consistent with a defect in trimer formation. However, receptors with epistatic mutations form mixed crosslinking products, consistent with their team-spoiling behavior.

Trimer-Based Signaling Teams Comprise Receptor Clusters

Taken together, the crosslinking and trimer contact mutant results strongly support our proposal that receptor trimers of dimers are structural and functional building blocks of receptor clusters. According to our current view (Fig. 4), receptor subunits insert into the cytoplasmic membrane, dimerize, and then coalesce into receptor squads consisting of trimers of dimers. Squads can contain receptors of different types; their composition is apparently dictated by the relative numbers of the various receptor molecules in the cell.

Receptor squads form in the absence of CheA and CheW, and so are not yet capable of signaling. They subsequently recruit CheA and CheW proteins to form signaling teams, which might contain several receptor squads and their shared CheA/CheW partners. Trimer contact lesions influence both the assembly and the function of receptor clusters. Mutations, such as proline replacements, that prevent trimer formation are blocked at the squad formation step. Such receptors do not enter higher-order forms and do not build tight clusters. Other trimer contact alterations, such as alanine or tryptophan replacements, allow trimer-of-dimer formation and produce normal-appearing clusters, but the signaling teams in those clusters cannot function properly.

How many receptor molecules comprise a signaling team? Mingshan Li and Jerry Hazelbauer at the University of Missouri in Columbia, Mo., provide an important new clue about the architecture of receptor clusters. They determined that there are about three receptor dimers to one CheA dimer to two CheW monomers. That stoichiometry is consistent with the trimer-of-dimers organization of receptors and could mean that a single trimer of receptor dimers is sufficient to form a functional signaling team.

However, this level of receptor organization cannot easily account for the high gain factors in the chemotaxis signaling system. More likely, the minimal signaling unit contains several trimers of dimers. Alternatively, several signaling teams may cooperate in larger signaling "leagues." Indeed, Sourjik and Berg find that cells with a single type of receptor show highly cooperative responses to their ligands, whereas mixed receptor populations exhibit high gain factors, but lower cooperativity.

These patterns suggest that all of the receptors in a mixed signaling team can respond to a single ligand-binding event by any member of the team. Thus, low-abundance receptors, which probably reside exclusively in mixed teams, have high gain factors because they can influence other members of the team. However, the highly cooperative behavior of homogeneous high-abundance receptor teams, if modeled as an allosteric transition in a multi-protein enzyme complex, would require cooperation between 20 or more receptor molecules.

Deconstructing *E. coli*'s Central-Processing Unit

Much of what is known about bacterial chemoreceptors and their signaling complexes comes from studying overexpressed receptors in membrane vesicles and leaflets. However, these studies may not enable us to fully understand receptor clusters. For example, some CheA, CheW, and chemoreceptor mutants cannot support chemotaxis, yet their proteins have no apparent defects, suggesting that important in vivo signaling activities cannot yet be reproduced in the test tube. Moreover, receptor-receptor interactions, detectable by in vivo crosslinking or genetic suppression studies, have not been seen in broken cells.

The in vivo/in vitro dichotomy could reflect unique aspects of the intracellular environment, such as molecular crowding, cotranslational assembly of protein complexes, or domain-swapping interactions among proteins. Thus, new in vivo experimental approaches that complement existing in vitro methods will be needed to better understand the functional anatomy of bacterial receptor clusters.

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