Minireview

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Molecular Information Processing: Lessons from Bacterial Chemotaxis*

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Extracellular information is converted into a usable intracellular form via signal transduction. This critically important process occurs in both unicellular and multicellular organisms throughout the living world and is exhibited by prokaryotic and eukaryotic cells alike. The mechanistic details of information processing in living cells vary from case to case, but diverse systems nevertheless display a number of operating principles in common. Thus, the study of one signaling system can yield insights applicable to others. As a practical matter, the investigation of signal transduction mechanisms in bacteria offers significant technical advantages. This minireview focuses on one of the very best characterized examples of information processing in a biological system, that governing chemotaxis in bacteria, and primarily attempts to summarize what has been learned so far that may be of general interest, as well as point out some features that are not yet fully understood.

Many bacteria live in dynamic environments and utilize information processing systems to constantly monitor their surroundings for important changes. Among the appropriate responses to environmental change are alterations in physiology, development, virulence, or location. Although not all bacterial species are motile, many forms of bacterial locomotion exist (often in a single species) including swimming in liquid and gliding, swarming, or twitching over solid surfaces. Organelles known to provide propulsion include flagella and pili; other motors remain unidentified. As a general rule, bacterial species that have invested in a propulsion system are also capable of directed movement, or taxis, to most efficiently translocate to a better environment. Tactic bacteria can respond to a variety of physical stimuli including chemicals, pH, temperature, light, electricity, or magnetism.

Genetic investigations in many bacterial species have identified the elements from which logic circuits controlling chemotaxis are constructed. Genome sequencing has revealed the phylogenetic distribution of these signaling elements even in species where chemotaxis has not been studied. As would be expected for any family of signaling pathways built from common components, there are differences in circuit details (*e.g.* the elements used and the connections between them) among species. However, the central chemotaxis processing pathway appears to be similar across a wide variety of prokaryotic species (both in Bacteria and Archaea), regardless of stimuli or motor type. The most extensive work (>30 years) has been conducted with *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. In these two species, the biochemistry of signaling reactions is well characterized, and atomic resolution structures are available for most individual signaling proteins. Despite immense progress, significant gaps remain in our current state of knowledge about bacterial chemotaxis. What is the nature of higher order interactions that occur between signaling proteins? What is the detailed spatial organization of circuit elements within the cell? What dynamic structural processes occur during signal transmission? What are the structures and functions of those signaling proteins that are phylogenetically widespread but are not present in the best studied model systems?

In this minireview, we discuss (i) the circuit elements that comprise chemotaxis information processing systems in bacteria, (ii) how these signaling elements are connected, both logically and physically, and (iii) how these elements communicate information to one another.

Circuit Elements and Design Logic

Two-component regulatory systems (see Ref. 1 for recent review) are frequently utilized to accomplish signal transduction in microorganisms and plants. In their simplest form, such systems consist of a sensor kinase possessing environmentally regulated autophosphorylation activity and a response regulator with transcription stimulatory activity that is modulated by phosphoryl groups transferred from the kinase. The chemotaxis signal transduction network (see Ref. 2 for recent review) represents a variation on this theme, being built around one sensor kinase (CheA) and two response regulators (CheY and CheB), neither of which regulate transcription. Additional proteins both up- and downstream contribute to the control of phosphoryl group flow through the central two-component system, which in turn dictates locomotive behavior.

The overall layout of the information processing network that controls *E. coli* chemotaxis is displayed in Fig. 1. Several features of circuit design merit comment. The majority of known sensor kinases are transmembrane molecules that combine an external stimulus detection domain and an internal kinase domain into a single molecule, an arrangement that efficiently encodes extracellular information as intracellular phosphoryl groups. In chemotaxis circuitry, however, receptor and kinase functions are separated into different molecules, which permits a single cell to execute an integrated response to multiple, potentially conflicting stimuli (3). Information is transmitted across the cell membrane by chemoreceptor proteins (also termed methylated chemotaxis proteins (MCPs)),[1] each of which contains a unique periplasmic domain to bind ligand or otherwise detect stimuli and a highly conserved cytoplasmic signaling domain to interact with downstream components of the signaling pathway. The number of MCPs and the environmental parameters detected vary from species to species. For example, *E. coli* contains five different types of MCPs, whereas *Vibrio cholerae* has more than 40 (4). In contrast to the prototypical transmembrane topology, some MCPs appear to be entirely cytoplasmic or associated with the cytoplasmic face of the membrane (5), presumably to monitor stimuli impinging on these locations.

Integration of signals from multiple MCPs into a unified response is made possible by CheW, a sort of universal coupling protein that functionally links the chemoreceptors to the CheA kinase. Physical binding of CheW to either MCP (6) or CheA (7) has been demonstrated *in vitro.* However, it is not known whether CheW acts as a scaffolding protein to facilitate a direct connection between MCPs and CheA, or alternatively, if signals are transmitted from the MCPs through CheW to CheA. The meager data available on this point are perhaps more consistent with the possibility of direct communication from MCP to CheA. Notably, genetic experiments suggest one of two signal types can be transmitted from MCPs to CheA in the absence of CheW (8).

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[1] The abbreviations used are: MCP, methylated chemotaxis protein; CheA-P, phosphorylated CheA; CheY-P, phosphorylated CheY; Enzyme I-P, phosphorylated Enzyme I.

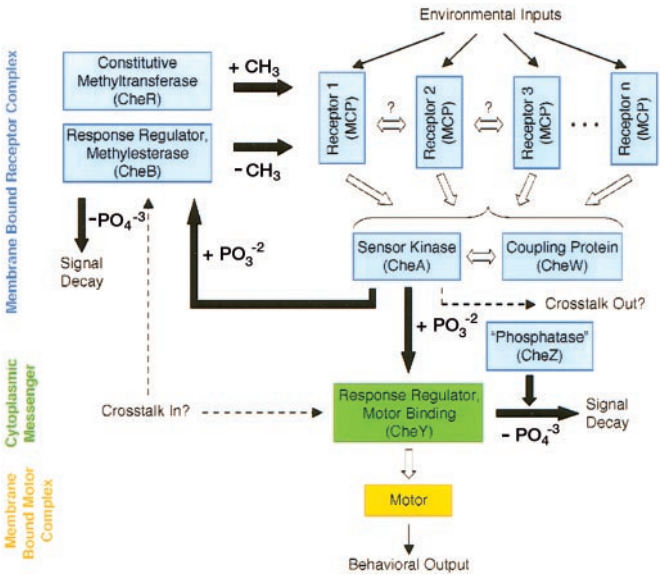

FIG. 1. **Schematic view of the information processing pathway that controls chemotaxis in *E. coli*.** At the poles of the cell, multiprotein receptor complexes (components indicated in *blue*) convert extracellular environmental stimuli into a cytoplasmic signal in the form of fluctuations in phosphorylated CheY concentration. Phosphorylated CheY (*green*), a diffusible signal, carries information to ~6–8 flagellar motor complexes (*yellow*) that are spatially distributed throughout the cell membrane. *Thin arrows* indicate inputs and outputs from the system with hypothetical connections indicated by *dashed arrows*. *Open arrows* indicate communication via protein-protein interactions, and *filled arrows* indicate information flow through covalent modification/demodification reactions.

Once environmental conditions are summarized by the cell in the form of CheA-P concentration, the presence of two response regulators to receive phosphoryl groups from CheA allows the distribution of information to two pathways. In the excitation pathway, CheY physically carries the phosphoryl group signal to the motor. However, the message is not delivered via phosphotransfer from CheY-P to a motor component (9) but rather takes the form of an altered CheY conformation with enhanced motor binding affinity (10). Decay of the CheY-P signal occurs by dephosphorylation, a reaction that is accelerated by CheZ. In the adaptation pathway, CheR constitutively methylates the MCPs, which dramatically increases the ability of the MCPs to stimulate CheA kinase activity (11). Phosphorylation enhances the ability of CheB to demethylate MCPs (12), thus forming a negative feedback loop with delay that turns down CheA kinase activity and mediates a return to prestimulus CheA-P concentration (and hence behavior).

The components of the *E. coli* chemotaxis circuitry described above are largely representative of those of other bacterial and archaeal chemotaxis systems. However, there are some notable differences. The CheZ protein is present only in some proteobacteria such as *E. coli* and *S. enterica*, whereas three other proteins (CheC, CheD, and CheV) that are absent from *E. coli* show wide phylogenetic distribution (13). The functions of CheC, CheV, and CheD are poorly understood. CheV, which has amino acid sequence similarity to CheW and CheY in its N- and C-terminal domains, respectively, functions in both adaptation and receptor coupling (14). Both CheC and CheD appear to function in regulating MCP methylation (15). Although the two proteins have been shown to interact physically, CheD is present in some proteobacterial species that lack CheC, and the two proteins appear to function independently (16). Elucidation of the biochemical activities of these proteins, as well as further characterization of their physiological roles within the many chemotaxis systems in which they occur, will be necessary to understand their significance within chemotaxis signaling circuits.

Circuit Connections

It is possible that the wiring diagram shown in Fig. 1 is not yet complete. In other words, some circuit elements may be connected in ways we do not yet know about. For example, there presently is substantial interest in the possibility that MCPs interact with one another within a "receptor patch" (17). Sensitivity of detection would be dramatically enhanced if stimulus recognition by one MCP affected kinase regulation by neighboring MCPs (*i.e.* binding of a single ligand triggered a disproportionately large response). Furthermore, if the extent of coupling between MCPs was adjustable (*e.g.* diminished as methylation increased), the range of stimulus magnitudes over which a cell could respond would be greatly expanded. Consistent with these ideas, the gain (amplification) of the chemotaxis signal processing network depends in some as yet undetermined manner on the CheB and CheR adaptation enzymes (18).

Additional connections to the chemotaxis signaling network potentially arise from the simultaneous operation of ~30 different two-component regulatory systems inside a single *E. coli* cell, all utilizing proteins with a high degree of structural similarity and the same phosphorylation chemistries. Under such circumstances, phosphotransfer (cross-talk) between noncognate sensor kinase/response regulator pairs could add or remove some phosphoryl groups from the flux through the system. There is a logical need to insulate signaling pathways as distinct entities, but it is conceivable that some degree of interconnectedness between pathways might be desirable, for example to integrate overall cell responses or provide a redundant signaling capability. Selective pressure presumably could strengthen or weaken cross-talk links over the course of evolution. We do not have a clear overall picture of the results of this optimization process.

In addition to the possibility of auxiliary connections within the circuit, there is also the possibility that presently defined connections function in additional ways. Fig. 1 implies that information flow through the chemotaxis pathway is essentially unidirectional from input to output. Might physiologically relevant information also flow in the reverse direction? In the case of the ArcB/ArcA two-component phosphorelay, relatively stable phosphoryl groups can be rapidly removed from the ArcA response regulator by reverse phosphotransfer to the ArcB sensor kinase and thence hydrolyzed to P_i (19). This scheme allows a relatively stable output to be quickly switched following a change in environmental conditions. Rapid switching is critical to successful chemotaxis, with bacteria literally making split-second decisions about whether to continue on their present course or change direction. In *E. coli*, CheZ facilitates the rapid dephosphorylation of CheY-P, thus apparently obviating a need for reverse phosphotransfer. However, most chemotactic species of bacteria do not contain CheZ and therefore must devise another fast means to decrease CheY-P concentration. In *Sinorhizobium meliloti*, which has no CheZ but multiple CheYs, CheY2 interacts with the motor. To terminate the excitation signal, phosphoryl groups are transferred from CheY2 back to CheA and then to CheY1, from which they are lost as P_i (20). Thus, CheY1 acts as a phosphate sink. In *Bacillus subtilis*, which has no CheZ and only one CheY, CheB may fulfill the role of phosphate sink via reverse phosphotransfer from CheY to CheA to CheB (21).

The steady state concentration of a phosphorylated molecule represents the balance between rates of formation and destruction. This dynamic property makes it possible for information to travel "backwards" along a pathway without phosphoryl groups physically doing so. For example, if the rate at which phosphoryl groups exit a pathway is enhanced or diminished, the effects of this change propagate upstream and are reflected as a decrease or increase, respectively, in the new steady state concentration of the first phosphorylated molecule in the pathway. *E. coli* exploits such a change to enable chemotaxis toward some sugars (22). In the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system, phosphoryl groups are transferred from phosphoenolpyruvate through a series of proteins (Enzyme I, HPr, Enzyme IIA, Enzyme IIB) to a sugar molecule as it is transported into the cell. When no sugar is present, phosphate flow backs up and the concentration of Enzyme I-P is high; when sugar is transported, phosphoryl groups drain out of the pathway and the concentration of Enzyme I-P is low. Non-phosphorylated Enzyme I (indicating the presence of sugar) inhibits CheA phosphorylation by an unknown mechanism and thus affects swimming behavior. Reverse information flow via accumulation (as distinct from phosphotransfer) is not currently known to occur in the signaling network depicted in Fig. 1.

Circuit Control Properties

It is striking that most known control (environmental stimuli and methylation/demethylation) of the flow of information through the chemotaxis circuitry is exerted on the first element in the pathway, the MCPs. Although the beginning of a pathway is certainly a logical control point, in principle, control functions could operate on any of several downstream steps. There is precedence in other two-component phosphorelays for multiple checkpoints. In the *B. subtilis* Spo pathway, for example, various phosphatases acting at one of several points each effectively exercises veto power over the decision to sporulate. Only when all phosphatases agree to let the phosphoryl groups pass can sporulation proceed (23). Regulating signal decay in the chemotaxis control network could theoretically serve as an effective means of signal amplification but has been ruled out experimentally because mutant cells lacking CheZ exhibit wild-type signal amplification (18). A more rigorous analysis of network properties is required to determine whether the signaling circuitry depicted in Fig. 1 possesses all control properties necessary to perform chemotaxis or whether additional control features remain to be discovered.

One useful way to assess the performance characteristics of a signaling network is through perturbation analysis, in which component parameters such as protein concentrations or reaction rate constants are altered and the consequences determined. For *E. coli* chemotaxis, such experimental perturbations can have dramatic effects on unstimulated behavior or the time required to adapt following exposure to stimuli (24). However, the ability to precisely restore prestimulus behavior is robust, *i.e.* perfect adaptation is an inherent attribute of the network design that is insensitive to the particular quantitative characteristics of the circuit elements. Thus, individual genetically identical bacteria exhibit different unstimulated behaviors (25), presumably as a consequence of stochastic variations between cells in signaling protein concentrations, but each cell can return precisely to its unique behavior. Theoretical analysis demonstrates that the chemotaxis circuit design incorporates integral feedback control (26), in which the difference between current and basal output is integrated over time and fed back into the circuit. Integral feedback control depends on several specific assumptions about the properties of CheB, CheR, and MCPs, many of which have been experimentally confirmed.

The biochemical output of the chemotaxis signaling network is the concentration of CheY-P, which the motor converts into behavior. The nature of the dose-response curve for CheY-P control of flagellar motor output has been the subject of extensive investigation. Simultaneous measurements of both CheY concentration and behavior obtained from single *E. coli* cells recently yielded a Hill coefficient of ~10 to describe CheY-P/motor interaction (27). This is a much higher degree of cooperativity than was evident from earlier investigations, in which population behavior was related to the average CheY concentration without considering the consequences of the variation in CheY concentration occurring among members of the population. The mechanism controlling the flagellar motor effectively acts as a very sensitive bistable switch with essentially the entire range of motor outputs spanned by deviations in CheY-P concentration of only ~25% around the resting state. To conduct a random search for a better chemical environment, a bacterium must frequently access both motor states. The average concentrations and activities of the chemotaxis proteins collectively set the average CheY-P concentration in the narrow midrange of the dose-response curve where this is possible. In turn, the integral feedback control mechanism described above enables unstimulated cells to keep their CheY-P concentrations locked at the values characteristic of each individual. When the search detects a chemical gradient, the "hair trigger" switching mechanism allows a virtually instantaneous change to one behavioral state to follow the gradient. In molecular terms, the switch structure at the base of the *E. coli* flagellar motor consists of a ring of ~34 FliM proteins to which CheY-P binds with 1:1 stoichiometry (28). Theoretical analysis of the behavior of a ring of proteins, whose individual choices between two conformational states are affected both by ligand binding and by the conformation of neighboring ring members, can satisfactorily account for the experimentally observed switching behavior (29).

In addition to transferring phosphoryl groups from sensor kinases, response regulators can autophosphorylate using small molecules as phosphodonors (30). This property potentially impacts both the control of the chemotaxis circuitry and connections to other circuits. All response regulators tested to date (including CheB and CheY) can utilize the artificial substrate phosphoramidate. In contrast, CheY can utilize the physiologically occurring substrates acetyl phosphate and carbamyl phosphate, whereas CheB cannot. CheB may need to protect itself against receipt of phosphoryl groups from any source other than CheA to preserve the fidelity of its previously described roles in signal amplification and/or integral feedback control. It is also conceivable that the promiscuity of CheY with regard to the phosphoryl group source could allow the overall metabolic state to alter the basal swimming behavior of the cell by contributing to the excitation but not the adaptation pathway of chemotaxis.

Physical Circuit Architecture

As the color scheme in Fig. 1 indicates, the chemotaxis circuitry is largely "hard wired" in the form of a multiprotein complex rather than a network of freely diffusing proteins. This is a common feature of signal transduction systems in general and presumably functions to guard against cross-talk by guiding a signal down a specific route. Such structures may also have the added benefit of accelerating signal transmission. A decade ago the chemotaxis receptor complex was thought to consist of a MCP receptor dimer, two CheW coupling protein monomers, and a CheA kinase dimer (6). The complex is now believed to also include the CheR and CheB enzymes of the adaptation pathway (31, 32) as well as CheZ (33). The relative stoichiometries of the many different proteins in the complex are unknown, but there probably is a molar excess of MCPs (34). How the circuit elements fit together within the membrane-bound receptor complex is unknown. There is good reason to believe that the structure is compact and contains multiple receptor types, because CheR bound to MCP molecules that contain CheR binding sites can methylate MCP molecules that do not possess CheR binding sites (35).

Most of the MCP molecules in a cell cluster together at the cell poles (36). It is not known whether they form one enormous array, which would facilitate conformational coupling with all its previously noted signal processing advantages, or if many smaller receptor complexes simply cluster together in a less organized form. In any case, polar localization appears to be a universal property of MCPs because it occurs in all species of Bacteria and Archaea so far examined (37). Little is known about how polar localization is accomplished beyond a requirement for the conserved cytoplasmic portion of the MCPs (36), and even less is known about the potential function of polar localization.

The other large multiprotein complex in the chemotaxis signaling pathway is the motor. The current view is that CheY acts as a cytoplasmic messenger to shuttle between the two membrane-bound complexes. Phosphorylation simultaneously diminishes the affinity of CheY for CheA (32), which is part of the receptor complex, and enhances the affinity of CheY for FliM (10), which is part of the motor complex. To affect behavior, CheY-P generated at the receptor complex must reach the motor complex before decaying. This is a daunting task because CheZ, which accelerates destruction of CheY-P, is also part of the receptor complex and most probably bound to CheA. To make matters more difficult, phosphorylation enhances the affinity of CheY for CheZ (38). Thus there appears to be a high probability that CheY-P will be dephosphorylated before it travels far from its origin. The co-localization of the enzymes that create and destroy CheY-P may serve as a mechanism to reduce the consequences of random fluctuations in CheY-P synthesis. Perhaps only a sustained signal can produce enough CheY-P to overwhelm CheZ and get through to the motors. The existence of an energetically expensive futile cycle, despite evolutionary selective pressure, may imply that a "gatekeeper" function of CheZ is important for optimal chemotaxis. The distance separating the sites of formation and action of the labile CheY-P messenger also has consequences for locomotive behavior. Receptor complexes are located at the poles of elongated *E. coli* swarmer cells formed by surface-induced differentiation, and such cells exhibit the behavior expected if CheY-P cannot reach motors located randomly around the cell surface before undergoing dephosphorylation

(37). In contrast, both lateral and polar receptor complexes are observed in filamentous *E. coli* cells generated by treatment with the cell division inhibitor cephalexin, and these bacteria can rotate their flagellar motors in either direction (39). Different bacterial species have distinctive spatial patterns of motor location within the cell, which may affect behavioral characteristics. In some spirochetes (very long cells with polar motors) membrane electrical potential is utilized to communicate from one end of the cell to the other (40). How such a mechanism might connect to the chemotaxis signaling network displayed in Fig. 1 is unknown.

Molecular Mechanisms of Information Transmission

Bacterial chemotaxis offers an excellent opportunity to look in great detail at the molecular mechanisms of signal transmission. Coupled protein conformational changes, in a variety of guises, appear to be the primary mechanism utilized to send information through this system. There is clear experimental evidence that intramolecular conformational changes are caused by ligand binding, protein-protein interaction, or covalent modification and strong suspicion that conformational changes are also conveyed intermolecularly between neighboring members of a multiprotein complex.

Tracing the signal through the pathway of Fig. 1 is informative. The process begins with detection when a small molecule or periplasmic binding protein binds to the tip of a MCP. This act of specific recognition is conveyed through the membrane along the ~380-Å length of the MCP molecule as a change in the relative positions of α-helices within a bundle (see Ref. 41 for recent review). The MCPs also act as temperature and pH sensors with the physical effects of heat/cold or protonation/deprotonation apparently resulting in conformations very similar to those achieved upon ligand binding or removal. In addition to an intramolecular change, an MCP directly affected by a stimulus may also influence the conformation of neighboring MCP molecules within an array.

It is not yet known precisely how the receptors control sensor kinase activity over several orders of magnitude, but this regulation could be accomplished by coupling a change in receptor conformation to a change in kinase conformation. Ligand binding and methylation occur at widely separated locations on the receptor, but both events affect kinase activity. The modest (<10-fold) effect of methylation on ligand binding (11) in isolated receptors indicates that the conformations of the ligand binding and methylation sites may be coupled, which could contribute to their linked effects on kinase activity. However, the dramatic ($\sim 10^4$-fold) impact of methylation on the ability of ligand to modulate kinase activity (11, 42) suggests that either methylation and ligand binding are much more tightly coupled in the receptor complex than in isolated receptors, or methylation and ligand binding influence kinase activity through different mechanisms.

The next step in the flow of information is phosphotransfer from the sensor kinase to the response regulators. Phosphorylation demonstrably changes the conformation of response regulators (see Ref. 43 for recent review), which in turn can affect their affinity for other proteins. Conversely, binding of peptides derived from adjacent proteins in the signaling network (CheA, CheZ, FliM) changes the structure of the CheY active site as inferred from changes in the autophosphorylation rate (44). Thus, conformational coupling again occurs in both directions. The detailed means by which CheY-P binding controls motor output is unknown, but a change in the conformation of FliM is certainly a plausible mechanism. The large Hill coefficient characterizing this interaction strongly suggests that the conformations of the multiple FliM molecules present in a single motor are coupled together.

Although the preceding passages have described ligand binding or covalent modification and the associated conformational changes as a linear cause and effect process, it is useful to think about the problem from a different perspective. Protein molecules have dynamic structures and can access multiple conformational states. Ligand binding or covalent modification can alter the energy of different conformations and thus affect how the population is distributed among available conformations. Tight coupling between the conformations of two distinct sites in one protein implies that exactly two overall states predominate in the energy landscape, which is the foundation for the widely observed phenomenon of allostery. In bacterial chemotaxis, which appears to depend on coupling of protein conformations all the way from the receptors to the motors, this property could facilitate the reverse flow of information. One can conceive of flexible structures that transmit information unidirectionally. For example, pulling one end of a string communicates with the other end, whereas pushing does not. However, there is as yet no evidence of devices in the chemotaxis signaling circuit (other than ATP consumption) that restrict the direction in which information is transmitted.

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