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Signal Transduction Schemes of Bacteria

Review

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When the Going Gets Tough, the Tough Get Going

Life in the microbial world is no picnic. Hard molecular rain falls incessantly from all sides, nutrients are scarce and unpredictable, experienced competitors lurk at every turn. Faced with continual life and death decisions, survival depends on cunning and quick reflexes. Given strong selective pressures like these, it's no surprise that bacteria have devised sophisticated signaling systems for eliciting adaptative responses to their environment. (For recent reviews see Bourret et al., 1991; Parkinson and Kofoid, 1992; Stock et al., 1991.) The most dramatic of these behaviors are the purposeful movements of cells toward favorable conditions and away from harmful ones. Motile bacteria exhibit locomotor responses to a variety of stimuli, including chemicals, light, osmolarity, temperature, and electric and magnetic fields. Thus, bacteria of many kinds can actively seek optimal living conditions. Even symbiotic and pathogenic organisms follow chemical trails to locate their plant or animal hosts (Long and Staskawicz, 1993 [this issue of *Cell*]).

In addition to outright migration, bacteria exhibit a wide array of regulatory responses to adaptive opportunities. The appearance of novel nutrients and metabolites, for example, triggers production of the transport and metabolic machinery needed to utilize them. Sensory systems also mediate the gene expression changes that follow host invasion. In pathogens, for instance, chemicals, temperatures, or other conditions characteristic of the host environment elicit production of toxins and other virulence factors (Bliska et al., 1993 [this issue of *Cell*]). Bacteria are also prepared for bad times as well as good. Stress conditions of many sorts—such as antibiotics, heavy metals, turgor pressure, or starvation—elicit changes in gene expression that lead to effective coping responses. Starvation countermeasures can be especially elaborate. The gliding Myxococci, for example, deal with nutrient depletion by forming cell aggregates that mature into fruiting bodies with resting spores. Soil Bacilli and some other bacteria also wait out starvation conditions by forming spores. Such developmental responses are truly desperate measures. The cells must have elaborate signaling networks to ensure that they do not make a foolish commitment to differentiate, and, once begun, to usher the process to a successful conclusion (Kaiser and Losick, 1993 [this issue of *Cell*]).

The machinery behind the adaptive responses of bacteria handles signaling tasks fundamental to all cell sensory systems: stimulus detection; signal processing, including amplification and integration of sensory inputs; and production of appropriate output responses. The sensory systems of prokaryotes are not only tractable models for exploring the molecular basis of these events, but also promise to provide general insights into cellular signaling mechanisms. This review focuses on three well-studied sensory systems in Escherichia coli and Salmonella typhimurium to illustrate some of the design features and molecular mechanisms of bacterial signaling proteins. All three systems—control of porin composition in the outer membrane, regulation of glutamine synthetase expression, and chemotaxis—employ communication modules, a pervasive signaling strategy in prokaryotes. Like their eukaryotic counterparts, these versatile devices use the signaling currency of reversible protein phosphorylation. Limited space precludes discussion of other bacterial signaling mechanisms, but many of them are described in the other reviews in this series and in reviews elsewhere that are cited throughout.

Intracellular Signaling via Communication Modules

Many bacterial signaling proteins, from both gram-positive and gram-negative organisms, contain two characteristic primary structure motifs, termed transmitters and receivers (Parkinson and Kofoid, 1992). These so-called communication modules promote signaling transactions within and between proteins. They function in combination with a variety of input and output domains and can be arranged in different configurations to build signaling circuits of many types. The simplest circuits have two protein components: a sensor, often located in the cytoplasmic membrane, that monitors some environmental parameter; and a cytoplasmic response regulator that mediates an adaptive response, usually a change in gene expression (Figure 1). Sensors typically contain a C-terminal transmitter module coupled to an N-terminal input domain. Response regulators typically contain an N-terminal receiver module coupled to one or more C-terminal output domains. Upon detecting a stimulus condition, the input domain of a sensor modulates the signaling activity of its associated transmitter to communicate with its response regulator partner. The receiver domain of the response regulator detects the incoming sensor signal and then alters the activity of its associated output domain to trigger the response.

The only demonstrated mechanism of transmitter–receiver communication involves phosphorylation and dephosphorylation reactions. These enable transmitters to regulate the phosphorylation state of their cognate receivers, which in turn controls response regulator output activity. Although this means of communication has only been explicitly demonstrated in a handful of the many bacterial proteins with communication modules, the high degree of sequence similarity within the transmitter and receiver families and the biological contexts in which they function argue that reversible protein phosphorylation is a widespread signaling strategy in the prokaryotic world. Discussed in this section are the phosphorylation activities of transmitter and receiver modules, structural features important to their signaling capabilities, and general design considerations for their use in building information-

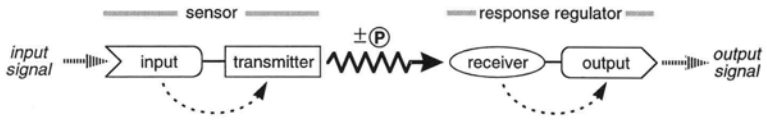

Figure 1. Circuit Elements in Two-Component Bacterial Signaling Systems

Sensory information flows through noncovalent controls exerted by one domain upon another (dashed arrows) and through phosphorylation reactions between communication modules. The convention of representing transmitters by rectangles and receivers by ovals is also used in subsequent figures.

processing networks. Although our current views derive from work on many different systems, the literature cited in support of various points will mainly cover the three signaling systems to be discussed later in more detail.

Phosphorylation Activities of Transmitters and Receivers

Transmitters regulate the phosphorylation state of their receiver partners in two ways (Figure 2). First, they have an autokinase activity that attaches phosphoryl groups from ATP to a histidine residue (Hess et al., 1988a; Ninfa and Bennett, 1991). This autophosphorylation reaction is readily reversible. The product, phosphohistidine, serves as a high energy intermediate for subsequent transfer of the phosphoryl group to an aspartate residue in the receiver (Sanders et al., 1992, 1989). Second, some, perhaps many, transmitter proteins have an apparent phosphatase activity toward their cognate receivers (Aiba et al., 1989b; Igo et al., 1989; Keener and Kustu, 1988; Ninfa and Magasanik, 1986). Sensory stimuli modulate the interplay of these two transmitter activities to regulate the flux of phosphoryl groups to and from target receivers.

Transmitters probably function as dimers, with the catalytic site of one subunit phosphorylating the acceptor site in the other (E. G. Ninfa et al., submitted; Swanson et al., 1993; Wolfe and Stewart, 1993; Yang and Inouye, 1991). Essentially nothing is known about the secondary or tertiary structures of transmitters, but primary structure analyses have provided some insight into their functional architecture (Parkinson and Kofoid, 1992). Over 50 transmitter-containing proteins are known. Sequence comparisons show that transmitters are about 240 amino acids in length, with several blocks of nearly invariant residues (Figure 2). The histidine phosphorylation site is typically located near the N-terminus of the module. Except for this feature, the remainder of the amino half is variable in sequence. This region might contain the specificity determinants needed to identify receiver targets properly, but as yet there is no experimental evidence bearing on this idea. Four blocks of residues in the carboxyl half probably make up the catalytic center. Amino acid replacements at various sites within these conserved regions curtail or eliminate autokinase activity (Liu, 1990; M. R. Atkinson and A. J. Ninfa, submitted; Oosawa et al., 1988; Yang and Inouye, 1991). Two of these segments are glycine rich and resemble nucleotide-binding motifs seen in other proteins.

Receiver-containing proteins can phosphorylate themselves using small molecule donors such as acetyl phosphate or phosphoramidate (Feng et al., 1992; Lukat et al., 1992). Transmitter phosphohistidines probably also serve as substrates for this phosphorylation reaction (Hess et al., 1988a; Sanders et al., 1989). In vivo, receivers presumably acquire their phosphoryl groups by catalyzing phosphotransfers from cognate transmitters and, in some cases, from phosphodonors like acetyl phosphate. Receivers also catalyze hydrolytic loss of their phosphoryl groups (Hess et al., 1988b; Weiss and Magasanik, 1988), with half-lives ranging from a few seconds to many minutes (Figure 2). The characteristic life time of a phosphorylated receiver is evidently crucial to its in vivo signaling role, because response regulator mutants with altered receiver dephosphorylation rates exhibit aberrant regulatory behavior (Aiba et al., 1989b; Bourret et al., 1990). The apparent phosphatase activity of some transmitters might be due to an enhancement of the dephosphorylation ability of their target receivers by an allosteric mechanism. The phosphatase activity of transmitters is ATP dependent, but nonhydrolyzable analogs serve as well, suggesting that ATP functions as a cofactor, possibly as a conforma-

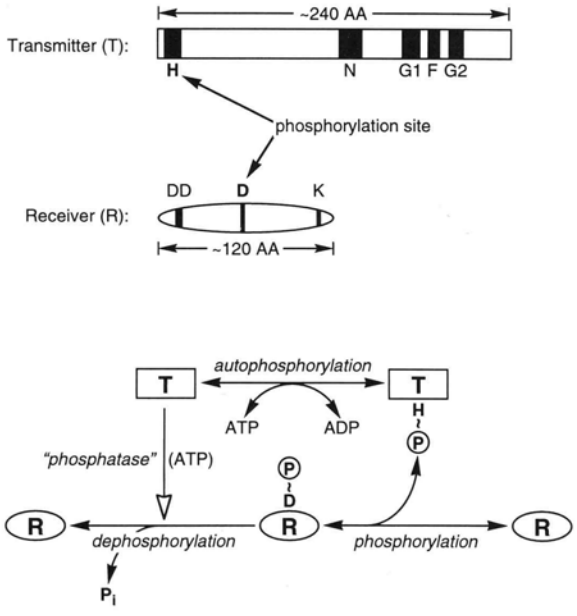

Figure 2. Sequence Features and Phosphorylation Activities of Communication Modules

Sequence tracts most characteristic of transmitters or receivers are indicated by black boxes whose width is roughly proportional to the length of the motif. Each motif is named for its most prominent amino acid. Details of the phosphorylation reactions are discussed in the text.

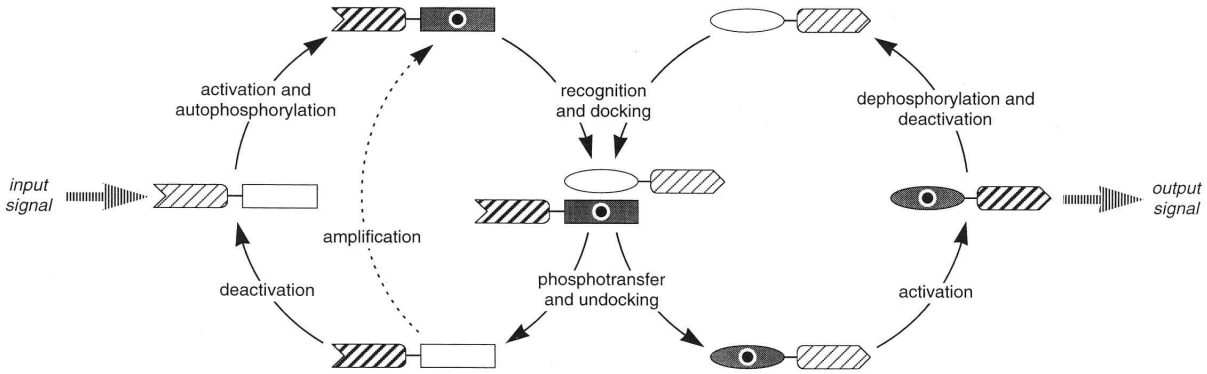

Figure 3. Signaling Transactions of Communication Modules

Although each step is potentially reversible, net information flow occurs only when the cycles proceed in the directions indicated. All steps, but especially the lifetimes of the various activated states, influence the overall signaling characteristics of particular circuits (amplification factor, response latency, response duration, etc.). Activated input and output domains are indicated by dark cross-hatching. Phosphorylated forms of transmitters and receivers are indicated by dark stippling, with the phosphoryl group symbolized by a black circle. These conventions are also used in subsequent figures.

tional effector (Aiba et al., 1989b; Igo et al., 1989; Keener and Kustu, 1988; Ninfa and Magasanik, 1986).

Receiver modules are roughly 120 amino acids in length. Unlike transmitters, their structure is well understood. X-ray studies of the S. typhimurium (Stock et al., 1989a) and E. coli (Volz and Matsumura, 1991) CheY proteins, which correspond to individual receiver modules, revealed a barrel-like arrangement of five sets of alternating β strands and α helices. The β strands align to form a hydrophobic inner core with the α-helical segments wrapped around the outside of the molecule. Residues important for phosphorylation activities are located in an acid pocket at one end of the barrel. These include a pair of aspartates near the N-terminus (Bourret et al., 1990; Brissette et al., 1991; Stewart et al., 1990), a lysine near the C-terminus (Lukat et al., 1991; Stewart, 1993), and a centrally located aspartate that is the site of phosphorylation (Sanders et al., 1992, 1989) (Figure 2). These and other characteristic sequence features are present in over 90 known receiver-containing proteins, suggesting that all receiver modules could be α/β barrels like CheY (Stock et al., 1989a; K. Volz, unpublished data).

Signaling Properties of Transmitters and Receivers

Most transmitter-containing proteins are located in the cytoplasmic membrane with their transmitters projecting into the cell. They typically have two membrane-spanning segments flanking their input domain, which is consequently deployed in the periplasmic space between the inner membrane and cell wall. Sensor input domains differ broadly in structure, reflecting the variety of chemical and physical stimuli they detect. Some have demonstrable ligand binding functions, but most are still poorly characterized, often because the exact nature of the stimulus is unknown. Communication with the cytoplasmic transmitter module involves propagation of sensory information across the cytoplasmic membrane. Mechanisms of transmembrane signaling will be discussed in another section of this review. A few sensor proteins, notably NtrB (see below), are soluble and contain N-terminal domains that may have input roles.

Receiver-containing proteins are invariably cytoplasmic. In most cases, their output domains have DNA binding or other regulatory functions that provide transcriptional control over one or more target genes. The receiver and output domains in response regulators are often joined by flexible linkers (Wootton and Drummond, 1989), suggesting that pliable connections may be important in enabling a receiver module to exert control over its adjoining output domain. Nuclear magnetic resonance studies of CheY indicate that phosphorylation induces substantial conformational changes in receivers (J. Falke, R. Dahlquist, and colleagues, unpublished data; see Hazelbauer et al., 1993), but how this leads to output control is not yet known. On the one hand, phosphorylation might promote association or dissociation of receiver modules, leading to changes in quaternary structure that alter output activity. On the other hand, phosphorylation might modulate direct interactions between receivers and output domains that enhance or inhibit functional activity. Either model could account for the apparent requirement of flexible connectors between the domains.

Because their activities are subject to stimulus control, transmitter and receiver modules are ideally suited as circuit elements for assembling signaling pathways. The signaling characteristics of module-based circuits depend on a number of parameters, including sensitivity to the stimulus, basal and stimulated phosphotransfer rates of the transmitter, and the lifetimes of activated transmitters and receivers (Figure 3). However, specificity in the phosphotransfer process is paramount. E. coli probably contains about 50 transmitter–receiver pairs and nearly as many signaling circuits (Parkinson and Kofoid, 1992; Stock et al., 1990). Inappropriate cross-talk between them is minimal, implying that receivers are precisely matched to their cognate transmitters. Unphosphorylated receivers presumably engage their phosphorylated transmitter partners, transfer the phosphate to their acceptor site, and then

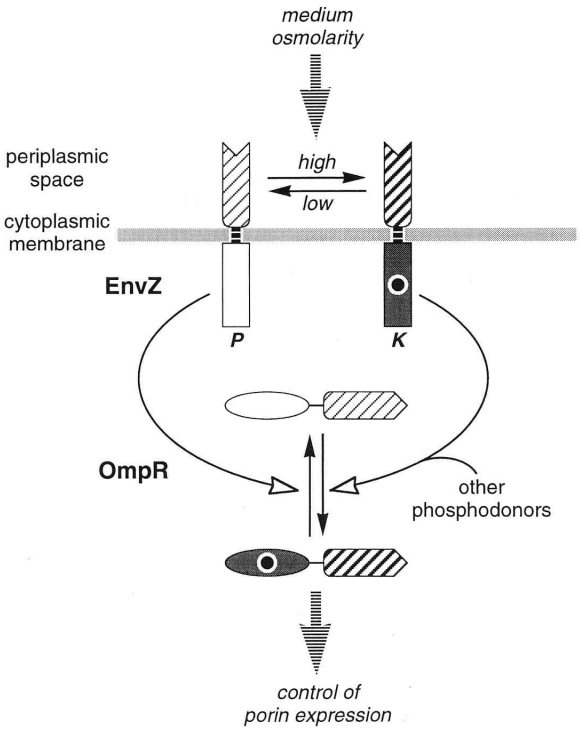

Figure 4. An Osmoregulation Circuit in E. coli

EnvZ and OmpR constitute one of several systems in E. coli for dealing with changes in medium osmolarity. Osmotic stress regulates the ratio of phosphatase (P) to autokinase (K) activities in EnvZ to adjust the phosphorylation state of OmpR, which in turn controls porin expression. Open-headed arrows denote control of the indicated signaling steps. This circuit can be cross-regulated by other phosphodonors.

disengage. Phosphotransfer must involve reversible association of the receiver and the transmitter through specific binding interactions, but the molecular basis for transmitter–receiver recognition is not yet understood.

Osmoregulation

Gram-negative bacteria like E. coli have two cell membranes. The inner, or cytoplasmic, membrane constitutes the main permeability barrier of the cell. The outer membrane excludes macromolecules, but contains numerous pores that permit small molecules to enter the periplasmic space by passive diffusion. OmpF and OmpC, the major porin proteins, are quite similar in structure, but have somewhat different sieving properties: OmpF forms a slightly larger hole than does OmpC. Although the total number of pores in the outer membrane remains fairly constant in different environments, the relative amounts of OmpF and OmpC vary with medium osmolarity. OmpF predominates at low osmolarity, and OmpC predominates at high osmolarity. For more comprehensive reviews of osmoregulation, see those by Igo et al. (1990) and Mizuno and Mizushima (1990).

The signaling system that senses osmolarity and regulates expression of the *ompF* and *ompC* loci typifies the simplest of the sensory pathways based on communication modules (Figure 4). It has two components: EnvZ, an inner membrane sensor, and OmpR, a cytoplasmic response regulator. EnvZ contains a C-terminal transmitter module and an N-terminal periplasmic domain flanked by membrane-spanning segments. The periplasmic domain is needed to detect medium osmolarity changes (Tokishita et al., 1991) and is generally assumed to be the input domain, but how it senses osmolarity is still very much a mystery. Procaine, a local anesthetic that intercalates into membrane bilayers, mimics the effect of high osmolarity (Rampersaud and Inouye, 1991), suggesting that EnvZ may somehow sense membrane curvature or fluidity. In any event, medium osmolarity triggers regulatory responses by controlling the relative rates of two EnvZ-dependent activities (Aiba et al., 1989a; Forst et al., 1989; Igo et al., 1989). High osmolarity promotes autophosphorylation, leading to an increase in OmpR phosphorylation. Low osmolarity promotes dephosphorylation of phospho-OmpR. The kinase and phosphatase activities of EnvZ presumably represent different conformational states of the molecule (designated K and P, respectively, in Figure 4). A number of EnvZ mutants with signaling defects or aberrant OmpF/OmpC expression patterns lack only one of these activities, as though functionally locked in the K or P conformer.

OmpR has an N-terminal receiver module and a C-terminal DNA-binding domain. Multiple OmpR-binding sites lie at the upstream ends of both the *ompF* and *ompC* promoters (Rampersaud et al., 1989). These binding sites must be close to the −35 consensus regions of the *omp* promoters for proper regulation (Maeda and Mizuno, 1990), suggesting that OmpR controls transcription by influencing binding of RNA polymerase to the promoter. Indeed, specific mutations in *rpoA*, which encodes the α subunit of RNA polymerase, influence the OmpR-dependent transcription of *ompF* and *ompC* (Slauch et al., 1991). Both the unphosphorylated and phosphorylated forms of OmpR bind to the target sites, but phospho-OmpR has demonstrably higher affinity (Aiba et al., 1989c). Phosphorylation may promote oligomerization of OmpR, which is normally monomeric, thereby enhancing its ability to bind at tandemly repeated target sites (Nakashima et al., 1991). Genetic studies indicate that in vivo the *ompF* promoter is more efficiently activated at relatively low OmpR phosphorylation states, whereas at high phospho-OmpR levels, the *ompC* promoter is activated and the *ompF* promoter is repressed (Russo and Silhavy, 1991; Slauch and Silhavy, 1989).

The OmpF and OmpC expression patterns of different EnvZ and OmpR mutants are largely consistent with this control model, but reveal additional complexity to the osmoregulation circuitry (Russo and Silhavy, 1991). An EnvZ mutant lacking phosphatase activity makes OmpC, but not OmpF, at all osmolarities, consistent with constitutively high phospho-OmpR levels. An OmpR mutant that cannot be dephosphorylated by EnvZ has a similar $OmpF^-$ $OmpC^+$ phenotype. Conversely, OmpR mutants with autophosphorylation defects have $OmpF^-$ $OmpC^-$ phenotypes, reflecting the inability to phosphorylate OmpR. The surprising part is that EnvZ null mutants lacking both kinase and phosphatase activity do not have this phenotype, but instead are $OmpF^+$ $OmpC^-$, indicative of low levels of

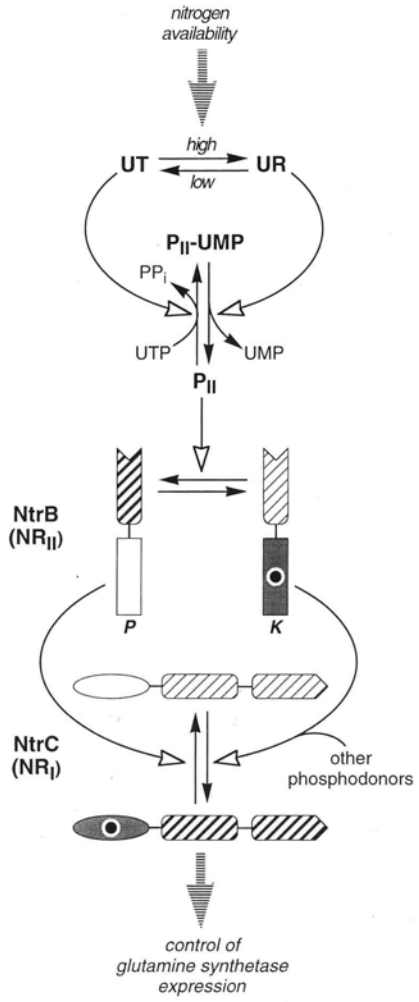

Figure 5. A Nitroregulation Circuit in E. coli

The NtrB–NtrC pathway regulates glutamine synthetase expression in response to changes in nitrogen availability. Signaling machinery for the many other nitrogen-regulated responses in E. coli is not shown. Nitrogen levels modulate the ratio of two enzymatic activities (UT and UR) that reversibly modify the P_{II} protein. P_{II} regulates the ratio of phosphatase (P) to autokinase (K) activities in NtrB to adjust the phosphorylation state of NtrC, which in turn controls glutamine synthetase expression. Open-headed arrows denote control of the indicated signaling steps. This circuit can be cross-regulated by other phosphodonors.

OmpR phosphorylation (Russo and Silhavy, 1991). It seems that OmpR ordinarily can acquire some of its phosphates from other sources (Forst et al., 1990), perhaps small molecules like acetyl phosphate or even other kinases. This low level cross-talk may connect the osmoregulatory circuitry to other physiologically relevant signaling pathways.

Nitrogen Assimilation

Bacteria assimilate nitrogen mainly through synthesis of glutamine from glutamate and ammonia. Depletion of cellular nitrogen reserves activates a variety of remedial responses, including increased expression of *glnA*, the structural gene for glutamine synthetase. The *glnA* gene is transcribed from two promoters: *glnAp1*, an unregulated promoter with relatively modest activity, and *glnAp2*, which is highly activated by nitrogen-limiting conditions. The signaling circuit that regulates transcription from *glnAp2* consists of a transmitter-containing protein (NtrB or NR_{II}), a receiver-containing protein (NtrC or NR_I), and two additional components (a uridylyltransferase, uridylyl-removing [UT/UR] enzyme and its substrate protein P_{II}) with no communication modules (see below and Figure 5). What follows is an abbreviated description of the glutamine synthetase transcriptional control circuit, with many of the nuances omitted. Fuller accounts can be found in reviews elsewhere (Bourret et al., 1991; Stock et al., 1989b).

The response regulator NtrC contains an N-terminal receiver and two additional domains needed to activate transcription from the *glnAp2* promoter, which uses the σ^{54} form of RNA polymerase. The C-terminal domain directs binding of NtrC to short, palindromic repeats located on the upstream (5′) side of the promoter (Ninfa et al., 1987; Sasse-Dwight and Gralla, 1988). When phosphorylated and bound at these sites, NtrC promotes formation of open transcription complexes between RNA polymerase and *glnAp2* (Popham et al., 1989). This reaction is dependent on ATP hydrolysis, most likely catalyzed by the central domain of NtrC (Weiss et al., 1991). The NtrC-binding sites act as transcriptional enhancers. When relocated, they function over long distances in the same DNA molecule (Ninfa et al., 1987) and even from a different molecule that is topologically linked to one with the *glnAp2* promoter (Wedel et al., 1990). At sufficiently high NtrC levels, the DNA-binding sites are not even needed for transcriptional activation (Ninfa et al., 1987). Their purpose is evidently to increase the local concentration of NtrC at the promoter, either by tethering it in the vicinity, or, if the sites are far from the promoter, through DNA looping (Su et al., 1990). Transcriptional activation by NtrC requires phosphorylation of its receiver module (Ninfa and Magasanik, 1986). Unphosphorylated NtrC is dimeric and can bind to single target sites, but phosphorylation greatly enhances cooperative binding to tandem sites by augmenting interactions between NtrC dimers (Weiss et al., 1992). The transcriptionally active form of NtrC is probably tetrameric (Feng et al., 1992; Weiss et al., 1991, 1992). Phosphorylation may promote association between the NtrC receivers, leading to tetramer formation.

NtrB controls the phosphorylation state of NtrC through reciprocal kinase and phosphatase activities (Keener and Kustu, 1988; Ninfa and Magasanik, 1986; Weiss and Magasanik, 1988). However, unlike EnvZ, NtrB is cytoplasmic and responds to nitrogen availability signals from an internal sensing mechanism consisting of the UT/UR and P_{II} proteins. Changes in cellular nitrogen levels modulate UT/UR activity, which in turn controls a covalent modification of P_{II}. Under nitrogen-limiting conditions, UT/UR uridylylates P_{II}; in nitrogen excess, UT/UR removes the modification. The balance between UT and UR activities depends on the cellular ratio of glutamine to 2-ketoglutarate, an indicator of nitrogen availability. Glutamine stimulates the UT reaction, and 2-ketoglutarate stimulates the UR reaction. NtrB in turn senses the cell's P_{II} modification state.

Unmodified P_{II} interacts with NtrB, augmenting its phosphatase activity toward NtrC (Ninfa and Magasanik, 1986; Keener and Kustu, 1988). Autokinase activity predominates when P_{II} is fully modified or absent altogether due to mutation.

Mutants lacking NtrB might be expected to express glutamine synthetase at low levels, but that is not the case. Instead, like OmpR, NtrC can acquire phosphoryl groups from several sources. Acetyl phosphate, in particular, makes significant contributions to in vivo NtrC phosphorylation levels, but other phosphodonors may also (Feng et al., 1992). This could represent physiologically useful signaling (cross-regulation) rather than inappropriate cross-talk (Wanner, 1992). The cell has the option to override cross-regulatory signals through its control of NtrB phosphatase activity.

Chemotaxis

E. coli swims by rotating helical flagellar filaments that function like the propellers on a ship. Individuals can have six or more flagellar motors distributed randomly over the cell surface. Although each motor rotates independently, the filaments are brought together by hydrodynamic forces, form a bundle, and turn in unison at the rear of the moving cell. Rotation in the counterclockwise (CCW) direction propels the cell forward; clockwise (CW) rotation disperses the bundle, causing a turn or tumble. In homogeneous environments, wild-type cells tumble about once a second, and each tumbling episode essentially randomizes the next swimming direction. The result is a three-dimensional random walk, the optimal foraging strategy for finding new food sources. For recent reviews on bacterial motility, see those by Jones and Aizawa (1991) and Macnab (1992).

E. coli is attracted to various sugars and amino acids and repelled by fatty acids, alcohols, and other potentially noxious compounds (reviewed by Eisenbach, 1991; Manson, 1992). These tactic responses are exquisitely sensitive: the cells readily detect chemoeffector changes of 1 part in 1000 in the micromolar concentration range (Segall et al., 1986). Attractant and repellent compounds are sensed directly by means of specific chemoreceptors and not through their beneficial or harmful physiological effects. Recent work indicates that the membrane-bound chemoreceptors are distributed in patches, often at a cell pole (Maddock and Shapiro, 1993; Shapiro, 1993 [this issue of *Cell*]). The functional significance of receptor clustering is unclear, but it cannot be for comparing concentrations at opposite ends of the cell. Large eukaryotic cells can deploy their sensory receptors in patches in order to make spatial discriminations, but this is not a feasible option for bacteria. Their small size and rapid movements essentially preclude sensing strategies based strictly on spatial comparisons (Berg, 1988). Instead, the cells determine their heading in chemical gradients by measuring temporal concentration changes as they move about. Typical E. coli swimming speeds are 10–20 body lengths per second. By comparing current chemoreceptor occupancy with that during the previous few seconds, the cell is able to make measurements over distances of many body lengths (Segall et al., 1986). Favorable stimuli, such as increasing attractant levels, reduce the likelihood of a tumble, thereby prolonging movement in the preferred direction. However, owing to its small size, the cell is constantly buffeted by Brownian motion and cannot swim in straight paths for very long, so chemotactic migration is necessarily a biased random walk process.

The intracellular signaling machinery of chemotaxis is more elaborate than the two regulatory systems described above (Figure 6). In addition to various families of chemoreceptors, six cytoplasmic proteins (CheA, CheB, CheR, CheW, CheY, and CheZ) are needed to process sensory information and to transmit rotational control signals to the flagellar motors. Two special constraints account for this complexity. First, because Brownian motion can reorient them, the cells require short response latencies so they can act on the most up-to-date heading information possible. Chemotactic stimuli trigger motor responses in less than 200 ms (Segall et al., 1982), a much shorter time scale than is needed for regulatory responses. Second, to make temporal comparisons of chemoeffector levels, the cells require a sensory adaptation mechanism that cancels chemoreceptor signal output in static environments, no matter what chemoeffectors may be present. This enables them to reset the threshold sensitivity of the signaling system in order to detect any new change in their chemical environment. The sensory adaptation machinery will be introduced briefly in this section and discussed in more detail after considering mechanisms of transmembrane signaling.

Many bacteria detect chemotactic stimuli with chemoreceptors known as methyl-accepting chemotaxis proteins or MCPs (reviewed by Hazelbauer, 1992; Hazelbauer et al., 1990). E. coli has four different MCPs that mediate responses to serine (Tsr), aspartate and maltose (Tar), ribose and galactose (Trg), and dipeptides (Tap). MCPs are transmembrane proteins, about 550 amino acids in length, with a periplasmic input or sensing domain and a cytoplasmic output or signaling domain. However, the MCP signaling domain does not resemble an orthodox transmitter and, in fact, has no known catalytic function. Rather, it modulates the activity of the transmitter-containing CheA protein to elicit chemotactic responses (Borkovich et al., 1989). CheW couples CheA to chemoreceptor control by promoting formation of ternary complexes containing an MCP dimer, two CheW monomers, and a CheA dimer (Gegner et al., 1992). Once formed, these signaling complexes persist for 10 min or more in vitro (Gegner et al., 1992) and are probably similarly long lived in vivo since most of the CheA and CheW molecules in the cell are associated with receptor patches at the membrane (Maddock and Shapiro, 1993).

CheA has an unusual structural organization (Parkinson and Kofoid, 1992). Its transmitter is centrally located and flanked on each side by additional domains. The C-terminal region functions as an input domain to couple CheA to CheW and the chemoreceptors (Bourret et al., 1993; Liu, 1990). Truncated CheA molecules lacking this segment still have autokinase activity, but their rate of autophosphorylation is no longer subject to sensory control

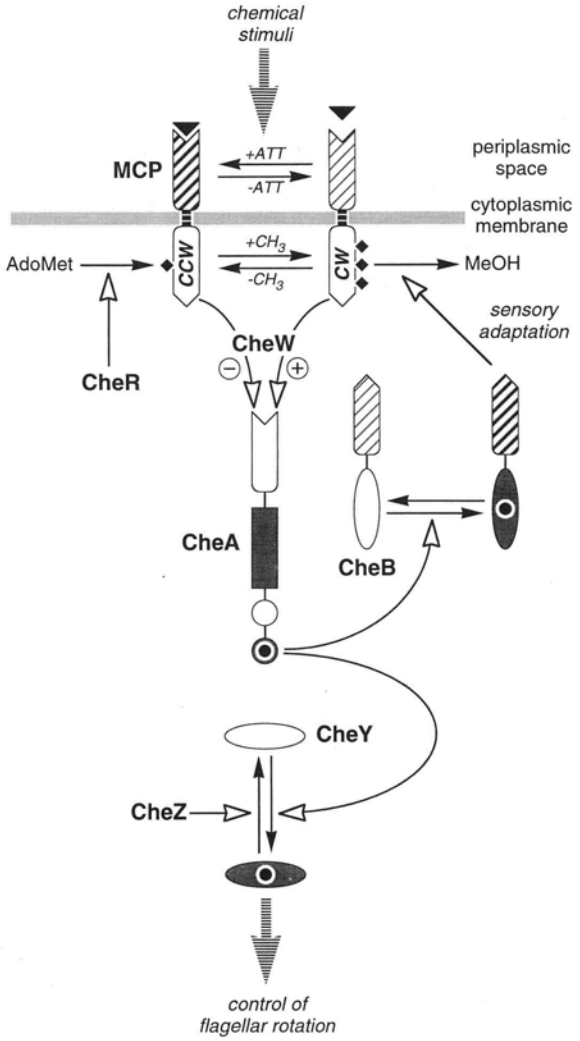

Figure 6. Chemotaxis Circuitry of E. coli

This signaling pathway handles responses to compounds detected by chemoreceptors of the MCP class. Other types of sensory receptors, for glucose, mannitol, or oxygen, for example, probably feed signals into this circuit as well, but their junction points are as yet unknown. Receptor signals control the autophosphorylation rate of CheA to modulate the flux of phosphates to CheY and CheB. Phospho-CheY controls the flagellar motors, and phospho-CheB regulates sensory adaptation through changes in MCP methylation state. The methyl groups are depicted as black diamonds; their number is arbitrary. Open-headed arrows denote control of the indicated signaling steps.

(Bourret et al., 1993). The phospho-accepting histidine in CheA resides outside the transmitter module in a small N-terminal domain (Hess et al., 1988a). Despite this unorthodox arrangement, the CheA autophosphorylation reaction is otherwise like the more typical EnvZ and NtrB cases. The catalytic portion of one subunit attaches a phosphoryl group to the acceptor site in the other subunit of the dimer (Swanson et al., 1993; Wolfe and Stewart, 1993). The unconventional location of the CheA phosphorylation site could be important to the mechanism of receptor coupling control (Parkinson and Kofoid, 1992). Ligand-free receptors stimulate CheA autophosphorylation, whereas attractant-bound receptors inhibit CheA (Borkovich et al., 1989; Ninfa et al., 1991). MCP molecules may control CheA through conformational changes that regulate access of the phosphoacceptor site to the catalytic domain.

CheA donates its phosphates to CheB and CheY (Hess et al., 1988b; Wylie et al., 1988), both of which contain receiver modules. CheY is solely a receiver. Phosphorylation enables it to interact with switch proteins at the base of the flagellar motors to generate CW rotation (Barak and Eisenbach, 1992; Roman et al., 1992). The motors rotate CCW by default, so the relative level of phospho-CheY determines the cell's swimming behavior. Changes in CheY phosphorylation initiate flagellar responses to chemical stimuli. CheB, in contrast, is part of a feedback circuit that terminates motor responses by adjusting the methylation states of MCP molecules, which in turn modulates their signaling properties. As detailed later, two enzymes control MCP methylation levels: CheR, which attaches methyl groups to MCP molecules, and CheB, which removes them. However, the catalytic domain of CheB is only active upon phosphorylation of its N-terminal receiver module (Lupas and Stock, 1989). Thus, by controlling the flux of phosphate through CheA to CheY and CheB, the receptors not only trigger behavioral responses, but also set in motion the sensory adaptation process.

The phosphorylated forms of CheB and CheY have very short half-lives, roughly 10 s under in vitro conditions (Hess et al., 1988b; Wylie et al., 1988). Rapid turnover of these functionally active forms undoubtedly contributes to the short response latency of chemotactic signaling. However, it is difficult to understand how this strategy alone could account for the extraordinary stimulus sensitivity of the chemoreceptors. Attempts to resolve this dilemma have recently focused on CheZ, an enigmatic protein whose only known function is to accelerate the loss of phosphate from CheY (Hess et al., 1988b). Mutants lacking CheZ have high tumbling rates, long response latencies, and are generally nonchemotactic (Segall et al., 1985). Whether CheZ activity is modulated by sensory stimuli, for example, through interaction with MCPs or CheA, remains an open question.

Transmembrane Signaling

How do membrane receptors convert external stimuli into internal signals? The process of transmembrane signaling is fundamental to many sensory systems, but still poorly understood. Owing to their relatively simple structure, MCPs and other bacterial receptors offer good models for exploring transmembrane signaling mechanisms at the molecular level. Extensive study of the MCP system has begun to provide intriguing insights that may well prove generally applicable.

The membrane topology and domain organization of MCP molecules are shown in Figure 7. The periplasmic sensing domain is flanked by transmembrane segments, the second of which connects to the cytoplasmic signaling domain through a linker region. The methylation sites responsible for sensory adaptation are located next to each end of the signaling domain. The cytoplasmic portions of different MCPs, which interact with common signaling

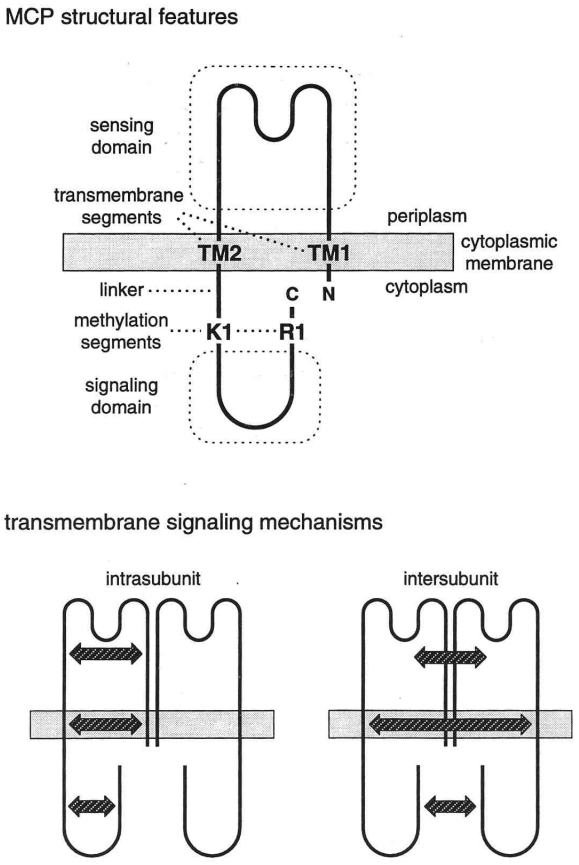

Figure 7. Membrane Topology and Domain Organization of MCP Molecules

Important structural features implicated in MCP signaling activities are shown at the top of the figure, with two general mechanisms of transmembrane signaling shown at the bottom. Since MCPs function as dimers, stimuli could modulate the activity of the cytoplasmic signaling domain by propagating conformational changes either within or between the two subunits.

(CheA and CheW) and methylation (CheR and CheB) components, are quite similar in primary structure. Their periplasmic domains differ substantially in sequence, but certain features are conserved, suggesting that they may also be similar in overall structural organization. To simplify further discussion, it will be assumed that MCPs have similar higher order structures even though few of the experiments described below have been done with more than one MCP species.

MCPs are dimeric, both in the absence and presence of ligand (Milligan and Koshland, 1988). Thus, unlike some eukaryotic receptors, such as the epidermal growth factor receptor, transmembrane signaling by MCPs does not involve ligand-mediated changes in subunit aggregation. This implies that conformational changes induced by ligand binding are propagated from the periplasmic to the cytoplasmic domain through the membrane-spanning segments of the molecule. The critical movements could occur either between the two subunits or within the individual subunits of the dimer (Figure 7). Each mechanism has some experimental support, but on the whole the available evidence implicates a combination of both in transmembrane signaling by MCPs.

MCP Signaling States

Single amino acid replacements throughout the cytoplasmic domain can lock MCP molecules in a CW or CCW signaling mode (Ames and Parkinson, 1988; Mutoh et al., 1986). Receptors locked in either signaling state cause aberrant flagellar rotation and loss of chemotactic ability when expressed in otherwise wild-type cells, implying that both CCW and CW signaling are active processes (Ames and Parkinson, 1988). Both types of locked receptors compete with other MCP molecules for common signaling components, most likely CheA and CheW (P. Ames and J. S. P., unpublished data). In in vitro coupling assays, CW receptors stimulate CheA autophosphorylation, whereas CCW receptors inhibit CheA (Borkovich et al., 1989; Borkovich and Simon, 1990). These effects mimic those produced by ligand-free and ligand-occupied receptors, suggesting that the CW and CCW states represent different signaling conformations of wild-type MCP molecules. Neither signaling mode is well understood. During CW signaling the interplay of structural determinants in the ternary complex leads to an "open" form of CheA with unique catalytic properties, capable of rapid autophosphorylation (Borkovich and Simon, 1990). Upon inhibition by receptors in the CCW signaling mode, CheA assumes a "sequestered" state in which autophosphorylation is blocked, although molecules phosphorylated prior to inhibition can still be dephosphorylated by CheY (Borkovich and Simon, 1990).

Locked output mutations are especially frequent in the middle of the MCP signaling domain (Ames and Parkinson, 1988; Mutoh et al., 1986), a region thought to contain the contact sites for coupling interactions with CheA and CheW (Liu, 1990; Liu and Parkinson, 1991). Soluble polypeptides from this part of the MCP molecule are capable of generating either CCW or CW signals, demonstrating that this region is sufficient for signal production (P. Ames and J. S. P., unpublished data). Cytoplasmic fragments from wild-type MCP molecules, as well as from CW-locked ones, assume the CW signaling mode, implying that it could correspond to a conformationally relaxed state. Corresponding fragments from mutant receptors locked in the CCW mode can produce CCW signals, which may represent a conformationally strained state. In general, CCW fragments tend to oligomerize, whereas CW fragments do not (Long and Weis, 1992). Evidently, intersubunit interactions are more prominent in the CCW conformer. Thus, stimuli that enhance CCW rotation may do so by initiating conformational changes in the sensing domain that ultimately promote tighter interaction between the two subunits of the cytoplasmic signaling domain.

Ligand-Induced Conformational Changes

High resolution structures of the sensing domain of the S. typhimurium aspartate receptor, both with and without ligand, have been determined by X-ray crystallography (Milburn et al., 1991). Each subunit is a bundle, roughly

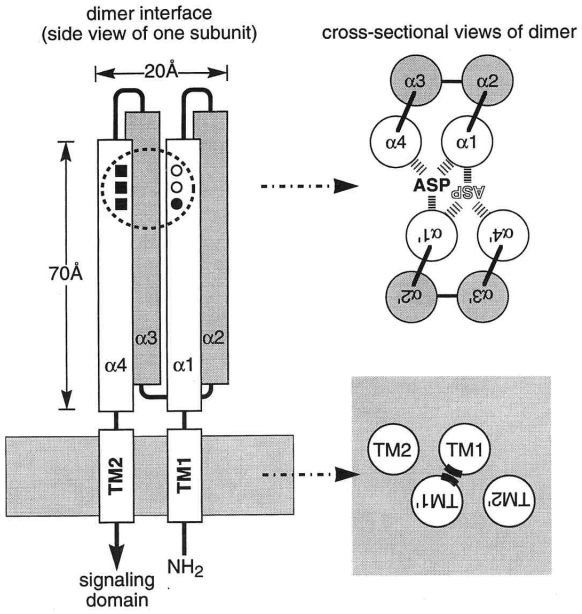

Figure 8. The Periplasmic Sensing Domain of MCP Molecules

This figure is not drawn to scale and only crudely approximates the pertinent structural features gleaned from X-ray studies. Each subunit is a four-helix bundle, one of which is shown in side view on the left. The dashed line circles the residues that form the ligand-binding pocket: those indicated by black squares hydrogen bond to the amino group of aspartate; those indicated by circles hydrogen bond to the α- or γ-carboxyl groups of aspartate. Residues indicated by the open circles come from the other subunit as shown in the cross-sectional view through the ligand-binding pocket on the right. The probable arrangement of transmembrane segments in the dimer, based on computer modeling and on cysteine-directed cross-linking, is shown at the bottom right. The black arcs indicate the putative interaction surface on the TM1 helices. Cysteines at these positions cross-link most readily; other mutational changes at these positions often cause CCW-locked signaling defects.

20 Å wide and 70 Å long, of four α helices (Figure 8). Helices 1 and 4, which connect to the transmembrane segments, contain the residues that form the aspartate-binding site (Lee and Imae, 1990; Wolff and Parkinson, 1988). The amino group of aspartate forms hydrogen bonds to residues in helix 4 (closed squares). The α-carboxyl group hydrogen bonds to a residue in helix 1 of the same subunit (closed circle). In addition, residues in helix 1 of the other subunit (open circles) hydrogen bond to the α- and γ-carboxyl groups. Thus, ligand binding could conceivably cause structural changes both within and between the two subunits. This is most easily appreciated in a planar view of the dimer interface showing the two symmetrical binding pockets between the subunits (Figure 8). Only one of these sites was occupied in the ligand-bound form used in the X-ray studies. Binding studies are consistent with this stoichiometry (Borkovich et al., 1992), but cannot rigorously exclude the possibility that two ligand molecules bind per dimer. In any event, receptor signaling does not seem to be cooperative, so if MCP dimers bind more than one ligand molecule, each subunit must act independently.

The structures of the apo and ligand-bound forms differ mainly by a small change in orientation of the two subunits (Milburn et al., 1991). Since the ligand-binding pocket lies at the membrane-distal end of the domain, this shift could conceivably produce larger displacements by a pivoting or scissors-like movement of the subunits as they traverse the membrane. Whether quaternary changes are the principal mode of transmembrane signaling is not yet clear, however. To facilitate crystallographic analysis of both the apo and aspartate forms, the two subunits were stabilized by a disulfide cross-link between cysteine residues at the periplasmic ends of the first transmembrane segments. Although the cross-linked dimer bound aspartate with normal affinity, it may not have had a fully native structure.

Several receptor studies have used cysteine residues as structural reporters to provide important clues to the conformational changes induced by ligands (Falke et al., 1988; Falke and Koshland, 1987; Milligan and Koshland, 1988). Receptor subunits with single cysteine residues at strategic locations were examined for rates of disulfide bond formation or modification by sulfhydryl reagents in the absence and presence of ligand. The ligand-free receptor molecule was dynamic. Cross-links formed between cysteines at nominally distant sites, either in the same or different subunits, and dimers even exchanged subunits. Addition of aspartate blocked subunit exchanges completely and also influenced the cross-linking rates of cysteines at various locations. These effects imply substantial changes in conformation and overall flexibility of the receptor molecule upon ligand binding, consistent with the notion that attractant ligands stabilize the dimeric state of the receptor. However, this cannot be the only consequence of ligand binding, because receptors with a single transmembrane subunit also undergo signaling-related conformational changes in their cytoplasmic domain. To show this, cysteine cross-links were used to construct hybrid receptor molecules containing one full-length subunit and one subunit with just a ligand-binding domain (Milligan and Koshland, 1991). When reconstituted into lipid vesicles, the chimeric receptors bound aspartate, which in turn altered their substrate properties for the CheR methyltransferase, demonstrating that ligand binding could trigger conformational changes in the cytoplasmic portion of the molecule.

Role of Membrane-Spanning Segments

No matter what the mechanism of transmembrane signaling, the membrane-spanning segments must surely move in the process. Molecular modeling suggests that these segments are α helical (Milburn et al., 1991; Pakula and Simon, 1992), a view supported by genetic and biochemical studies (Chen, 1992; Pakula and Simon, 1992). Cysteine-directed cross-linking has been used to determine the probable spatial arrangement of the transmembrane segments (Burrows, 1991; Lynch and Koshland, 1991; Pakula and Simon, 1992) (Figure 8). The N-terminal segments (TM1 and TM1′) seem to be in close proximity, since cysteines introduced along one face of the TM1 helix (indicated as a black arc) readily form disulfide bridges with their counterparts in TM1′ (Burrows, 1991; Lynch and Koshland, 1991; Pakula and Simon, 1992). Moreover, many

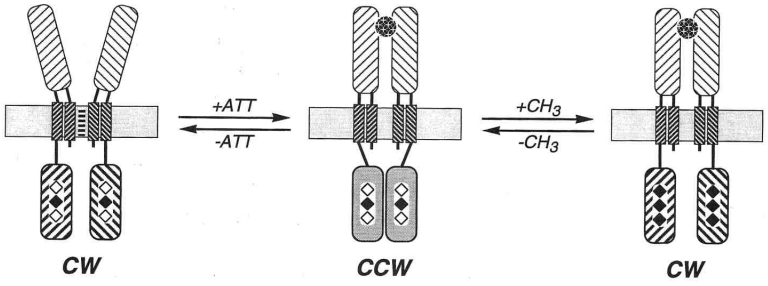

Figure 9. Transmembrane Signaling in MCP Molecules

This model is based on a two-state signaling mechanism. The CCW state is relatively inflexible, and the CW state is more dynamic. Conformational changes induced by binding of an attractant molecule (indicated by the sphere) are transmitted to the cytoplasmic domain through tertiary or quaternary rearrangements. The model is not critically dependent on any particular mechanism, so neither is explicitly indicated in the diagram. Changes in the number of MCP methyl groups (depicted as black diamonds) reverse stimulus effects by altering the mutual affinity of the signaling domain subunits. Again, the mechanistic details are not specified.

other amino acid replacements at these same residue positions generate CCW-locked defects, indicating that this TM1 face is particularly crucial to proper signaling function (Chen, 1992). These features suggest that the TM1 interaction surface could be an important locus for dimerization contacts in MCP molecules. Conceivably, contacts between TM1 and TM1′ could serve as a fulcrum for propagating structural changes to other parts of the molecule, either within or between the subunits.

Aspartate affects the rate of cross-linking between cysteines at either end of TM1, suggesting that transmembrane signaling could involve relative movements of the TM segments (Falke et al., 1988; Stoddard et al., 1992). Virtually any contortion imaginable could serve the purpose, including rotation or lateral motion within the plane of the membrane or piston-like movements perpendicular to the membrane. Unlike the TM1 case, amino acid replacements in TM2, even ones introducing charged residues, rarely interfere with chemotactic ability (Chen, 1992). This high tolerance of structural changes suggests that TM2 may not participate in specific contacts with other transmembrane segments. Nevertheless, it could serve as a conduit for propagating conformational changes between the periplasmic and cytoplasmic domains.

Mechanisms and Models

The model of Figure 9 summarizes available facts and inferences about transmembrane signaling by MCPs. In the absence of ligand, MCP dimers spend much of their time in the CW signaling state, characterized by relatively weak interactions between the subunits in both the periplasmic and cytoplasmic domains. The CW conformer is globally flexible and capable of subunit exchanges. Its dimeric organization may be largely due to association of the TM1 segments. Ligand binding shifts the equilibrium to the CCW signaling state, characterized by tighter association of the subunits in both the periplasmic and cytoplasmic domains. The CCW conformer is incapable of subunit exchange and presumably much less dynamic than the CW form. The conformational changes that occur upon transition from the CW to the CCW conformer may include a weakening of the TM1 interaction, however. This could explain the observation that nearly all amino acid replacements in residues constituting the putative TM1 contact sites lead to CCW-biased signaling behavior (Chen, 1992). It seems more likely that mutational changes would weaken rather than strengthen this interaction.

How does ligand binding favor the transition to the CCW conformer? Since stimulus-dependent conformational changes can propagate between periplasmic and cytoplasmic domains of one subunit, intrasubunit movements may initiate the transmembrane signaling process (Lynch and Koshland, 1992; Milligan and Koshland, 1991). Ligand binding could cause movements of the first and fourth helices in one of the sensing subunits, leading to a relative displacement of the adjoining transmembrane segments. Motions transmitted through the TM2 and linker regions could then weaken intrasubunit contacts within the signaling domain, freeing it to associate more strongly with its counterpart in the other subunit. Dimerization of the signaling domains could in turn propagate conformational changes throughout the second subunit, leading to the stable CCW conformation. When the ligand molecule leaves the binding pocket, these conformational changes would be reversed, favoring transition to the CW state.

Similar mechanisms may operate in more conventional, transmitter-containing sensors like EnvZ. Except for its lack of methylation sites, the domain organization and membrane topology of EnvZ are identical to those of MCPs (Forst et al., 1987). Like MCPs, EnvZ probably functions as a homodimer and has two active signaling states, a kinase mode favored by high osmolarity and a phosphatase mode favored by low osmolarity (Yang and Inouye, 1991). Moreover, amino acid replacements in the transmembrane segments and linker region can lock EnvZ in either signaling mode, indicating the importance of those structural features in regulating transmitter output (Tokishita et al., 1992). Finally, an EnvZ transmitter grafted to the sensing domain of the aspartate chemoreceptor is modulated appropriately by aspartate stimuli, implying that transmembrane signaling in the two systems involves similar conformational controls (Utsumi et al., 1989).

Sensory Adaptation

In the osmotic or nitrogen regulatory signaling circuits, the levels of phosphorylated response regulators vary directly with the magnitude of the stimulus condition, either osmolarity or extent of nitrogen depletion. Subsequent changes

in gene expression lead to adaptive physiological responses—changes in either outer membrane sieving properties or nitrogen assimilation—that eventually reduce stimulus intensity. In the chemotaxis system, sensory adaptation is a continual process that enables the cells to make temporal comparisons as they swim about. The adaptation machinery works to cancel recent stimulus responses, so that the organism is poised to respond to any new changes in chemoeffector concentration. Bacteria and eukaryotes employ a common biochemical strategy for achieving rapid sensory adaptation: alteration of receptor signaling properties through reversible, covalent modification. Instead of phosphorylation, methylation serves this purpose for the MCP family of chemoreceptors. Many bacteria have MCP-like proteins (Morgan et al., 1993), attesting to the popularity of this particular adaptation mechanism in the microbial world. But, despite considerable study, a satisfactory molecular description of the role of MCP methylation in sensory adaptation is not yet at hand.

MCP molecules typically contain four or five methylation sites distributed in two regions flanking the cytoplasmic signaling domain (see Figure 7). The acceptor sites are glutamic acid residues, but several of them start out as glutamines that are later converted to glutamates by post-translational deamidation (Kehry et al., 1983). The methylation sites are embedded in short sequence tracts that probably make up the substrate motif recognized by two MCP-specific modifying enzymes. CheR, the methyltransferase, attaches methyl groups from S-adenosylmethionine (AdoMet) to the side-chain carboxyl of glutamate, forming a glutamyl methyl ester. CheB, the methylesterase, hydrolyzes the methyl groups to liberate methanol and regenerate an unmodified glutamate residue. CheB also catalyzes the irreversible deamidation step that converts certain glutamine residues to glutamic acids capable of accepting methyl groups.

The relative activities of CheR and CheB determine the methylation level of each MCP species. In the absence of chemical stimuli, about half the sites are methylated. In high attractant or low repellent levels, most sites are methylated, whereas in low attractant or high repellent levels, few sites are methylated. These steady-state differences reflect ligand-induced changes in MCP substrate properties. An attractant-occupied receptor is a poor substrate for CheB, but a good substrate for CheR. Thus, MCP methylation levels represent a record of the current chemical environment. Whenever attractant or repellent levels change, the cell initiates a locomotor response and begins to add or remove methyl groups until methylation levels match the new environmental conditions. The final pattern reflects ligand occupancies, which can be different for different receptor classes, but the time course of methylation changes is regulated by a feedback circuit that controls CheB activity globally in response to chemoreceptor signals (Russell et al., 1989; Sanders and Koshland, 1988) (see Figure 6). Since CheR activity remains constant, changes in CheB activity produce transient fluctuations in the methylation level of all MCPs. However, only the chemoreceptor types actively engaged in stimulus detection and signaling sustain net methylation changes.

Changes in MCP methylation state lead to sensory adaptation, but play no role in triggering motor responses. Thus, mutants defective in CheR or CheB function can initiate flagellar responses to sudden changes in attractant or repellent levels. However, they continue to respond until the stimulating chemical is removed, whereas wild-type cells stop responding upon achieving an appropriate MCP methylation state. Amino acid replacements at the methylation sites can produce similar adaptation defects, but under certain conditions these can be phenotypically compensated by methylation changes in other MCP molecules, presumably owing to the global feedback circuitry (Hazelbauer et al., 1989). There is no strict order in which methylation sites in an MCP molecule must be used, and filled sites are randomly distributed over the population of molecules, so the sites seem to be functionally equivalent in their effects on MCP signaling properties (Kehry et al., 1984). Although a full complement of methylation sites is not essential for chemotactic behavior, multiple sites may provide the buffering capacity needed to cope with a wide range of chemoeffector levels (Nowlin et al., 1988).

Simple mechanisms of sensory adaptation can be incorporated into the two-state MCP signaling model (see Figure 9). Assuming that changes in receptor occupancy initiate motor responses by shifting the proportions of molecules in the CCW and CW modes, changes in methylation state could simply shift the equilibrium in the opposite direction to cancel those responses. If the sensing and signaling domains were rigidly coupled, methylation could suppress the effects of attractant binding by reducing the receptor's binding affinity. However, the ligand affinities of fully methylated or unmethylated MCP molecules are not sufficiently different to account for sensory adaptation in this manner (Borkovich et al., 1992; Dunten and Koshland, 1991). Instead, methylation may reverse the effects of ligand binding by mechanically uncoupling the sensing and signaling domains. This could happen if methylation state somehow modulated the interactive forces within and between signaling domain subunits. For example, methylation could promote CW signaling by ligand-bound receptors by weakening the dimerization contacts or by strengthening alternative interactions within each subunit. Sequence analyses suggest that, depending on their methylation state, interactions between α-helical methylation segments could form either two-stranded coiled coils or four-helix bundles (Stock et al., 1991). More detailed structural information about the MCP signaling domain is needed to test these ideas.

Signaling Themes: Bacteria and Beyond

The sensory machinery of microbes is parsimonious in design, yet capable of low noise, high gain signal transmission. What can we learn from these simply elegant systems? Bacteria and higher cells face the same kinds of signaling tasks; might they handle them with the same molecular strategies? It appears they do, even though the mechanistic details sometimes differ. Three themes, pervasive in bacterial signaling systems, may well be universal hallmarks of signal transduction schemes.

Analog Circuits from Digital Components

Most signaling systems behave like analog circuits in that output responses are smoothly graded with stimulus intensity. Yet the underlying circuit elements have primarily digital characteristics. Bacterial protein switches are relatively simple: transmitters have low and high kinase activities; MCPs have CCW and CW signaling modes; receivers have phosphorylated and unphosphorylated states. The analog behavior of circuits built with these two-state devices could arise in several ways. First, some circuits simply use large numbers of switches. The E. coli chemotaxis machinery, for example, contains several thousand chemoreceptor molecules. Integration of many individual MCP outputs would approximate analog behavior. Second, and more importantly, some protein switches are exponential timers. Once turned on, they switch off at characteristic, hard-wired rates. Thus, phosphorylated receivers have half-lives set by their intrinsic dephosphorylation activity. In this regard, the parallels between receiver-containing response regulators and signal-transducing G proteins are quite remarkable (Artymiuk et al., 1990; Chen et al., 1990; Stock et al., 1991).

Two Signals for the Price of One

Bacterial signaling proteins usually have two active signaling modes. Their transitions are not between OFF and ON states, but rather between qualitatively different signals—kinase versus phosphatase, stimulation versus inhibition, repression versus induction. Push–pull signaling should produce faster responses and greater amplification factors than OFF/ON strategies, because stimuli can modulate the ratio of two opposing activities. Push–pull signaling mechanisms probably do not arise de novo. More likely, protein switches begin with simple OFF/ON abilities and acquire a second active signal through subsequent modification of the OFF state. The alterations could be relatively simple ones that exploit a preexisting ability, such as docking with signaling partners. Thus, the phosphatase activity of transmitters and the CCW signaling mode of MCPs could stem directly from target protein binding. The apparent ease with which proteins can generate opposing signaling activities, and the payoffs for doing so, suggests that this might be a common attribute of signaling systems.

The Medium Is Not the Message

Information transfer through reversible, covalent modification of proteins is a ubiquitous signaling strategy. Bacteria make extensive use of phosphorylation and methylation, but in principle other modifications would work as well. The signal is not so much the modification, but its functional consequences to the target protein. Modifications of bacterial signaling proteins invariably seem to modulate their binding contacts, either with another protein or between their own domains and subunits. Signaling modifications must affect protein structure. The nature of the conformational change, how modification triggers it, and how it in turn alters functionality are important questions in any signaling system. Further study of bacterial models will tell us what the answers can be.

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