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Collaborative signaling by bacterial chemoreceptors

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Motile bacteria seek optimal living habitats by following gradients of attractant and repellent chemicals in their environment. The signaling machinery for these chemotactic behaviors, although assembled from just a few protein components, has extraordinary information-processing capabilities. *Escherichia coli*, the best-studied model, employs a networked cluster of transmembrane receptors to detect minute chemical stimuli, to integrate multiple and conflicting inputs, and to generate an amplified output signal that controls the cell's flagellar motors. Signal gain arises through cooperative action of chemoreceptors of different types. The signaling-teams within a receptor cluster may be built from trimers of receptor dimers that communicate through shared connections to their partner signaling proteins.

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Introduction

Chemotaxis, the movement of an organism toward or away from chemicals, is an important adaptive behavior of motile bacteria that requires sophisticated information-processing capabilities. The cell must detect attractant and repellent gradients as it moves about, integrate and amplify diverse stimulus inputs, and generate a coherent output signal that elicits an appropriate locomotor response. To accomplish these tasks, bacteria have devised a remarkable molecular mechanism for signal amplification. The chemotaxis machinery of *Escherichia coli* has provided the best molecular views of this novel signaling strategy and will be the focus of this review. Other chemotactic bacteria have similar signaling components, which most likely operate by the same underlying mechanisms.

Chemoreceptor clusters and signal gain

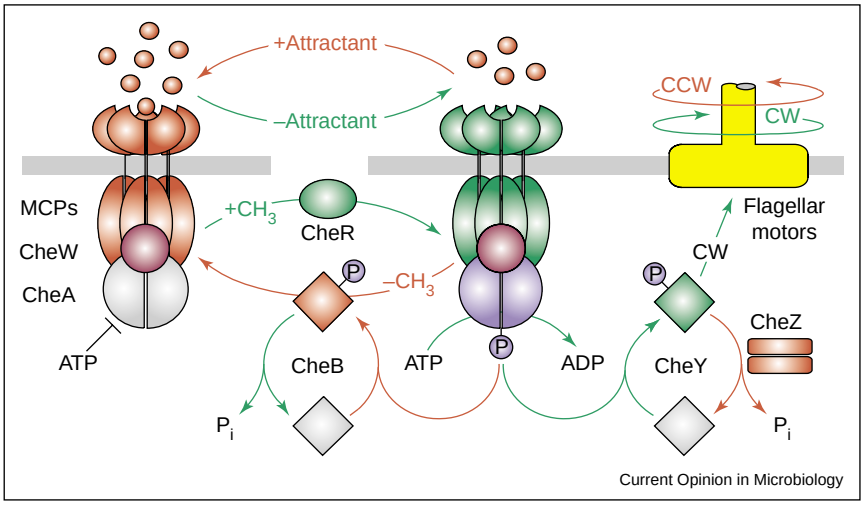
E. coli senses serine, aspartate, and other attractant compounds with transmembrane receptors known as methyl-accepting chemotaxis proteins (MCPs); see [1] for a review. MCPs typically have a periplasmic ligand-binding domain for monitoring chemoeffector levels and a highly conserved cytoplasmic signaling domain that communicates with the flagellar rotary motors via protein phosphorylation reactions (Figure 1); see [2] for a recent review. MCP signaling domains form stable ternary complexes with CheA, which is a histidine autokinase, and CheW, which couples CheA activity to chemoreceptor control [3]. Two response regulators — CheY and CheB — become active upon acquiring a phosphoryl group from CheA. Phospho-CheY interacts with a switch protein at the base of the flagellar motor to augment clockwise rotation, which causes random directional changes while swimming. Counter-clockwise rotation, the default behavior, produces forward swimming. The activated species have intrinsically short half-lives, enabling MCPs to elicit rapid behavioral responses by modulating the supply of CheA phosphoryl groups. Phospho-CheY is also turned over by a specific phosphatase, CheZ, which enhances the speed and coordination of the motor response [4] (see Update).

Phospho-CheB is part of a sensory adaptation system that enables the receptors to function over a wide range of chemoeffector concentrations. MCP signaling domains undergo reversible methylation at 4–6 glutamic acid residues to modulate their ligand-binding and CheA-stimulating properties. The opposing activities of CheR, which is an MCP-specific methyltransferase and CheB, which is an MCP-specific methylesterase that is active when phosphorylated, regulate the MCP methylation state. Feedback control, through CheB, of the receptor methylation state enables the cell to adapt to static chemical environments. Thus, by comparing their ligand occupancy and methylation states, MCPs can recognize and act on temporal concentration changes as the cell swims about in spatial chemoeffector gradients.

Chemoreceptor signaling complexes can detect concentration differences that alter the ligand occupancy of only a few receptor molecules [5•]. Such small stimuli trigger large changes in the rotational behavior of the flagellar motors. The amplification, or gain, of the signaling system, defined as the ratio of the fractional change in motor bias to the fractional change in receptor occupancy, exceeds a factor of 50 [6]. Some gain arises through highly cooperative interaction of phospho-CheY with the motor switch [7], but most of it occurs at the initial detection and signaling steps [5•].

A two-state model of chemoreceptor signaling (Figure 1) accounts for much of their behavior, but cannot explain their prodigious signal gain. The model asserts that

Figure 1



Two-state model of receptor signaling and the chemotaxis phosphorelay pathway. With the exception of the highly schematic rotary flagellar motors, the chemoreceptors (MCPs) and cytosolic signaling proteins (CheA, CheB, CheR, CheW, CheY, CheZ) are depicted in their native subunit organizations. The receptor dimers are further arranged in trimers, which may comprise the active unit for receptor signaling. Colored components represent functionally active states; gray components represent inactive signaling forms. Green components and reaction arrows represent signaling states that enhance clockwise (CW) flagellar rotation; red components and reaction arrows represent signaling states that augment counter-clockwise (CCW) flagellar rotation, the default condition. Binding of an attractant ligand or removal of methyl groups shifts chemoreceptor signaling complexes from the kinase-on (green) to the kinase-off (red) signaling state. Attractant release and methyl group addition shift receptor signaling complexes from the inactive CheA (gray) to the active CheA (blue) state.

chemoreceptor signaling complexes have two CheA activity states — one with a high autophosphorylation rate and one with a very low rate. The flux of phosphoryl groups through the signaling pathway is governed by the fraction of receptor signaling complexes in the kinase-on state, which in turn reflects the interplay between chemoreceptor occupancy and methylation states. However, if each CheA molecule responded to just one receptor, there would be no signal amplification in the system.

To produce the high gain factors in chemotaxis, each CheA molecule might be controlled by an integrated signal from multiple receptors. This could happen if receptors exchanged stimulus information, as demonstrated by modeling studies that invoke mechanistically undefined coupling interactions between chemoreceptors [8–10]. Moreover, we have known for more than 10 years that MCP molecules are clustered at the poles of the cell [11], in *E. coli* and in many other bacteria [12]. Could physical clustering be a mechanism for networking chemoreceptors into a high-gain signaling array? Although the mechanistic and architectural details of receptor networks are still uncertain, there is a growing consensus that this may be the case. (An excellent recent review provides a more extensive discussion of this topic [13].)

Functional interactions between chemoreceptors *in vivo*

E. coli has four canonical MCPs: Tsr (serine detector), Tar (aspartate and maltose detector), Tap (dipeptide detector) and Trg (ribose and galactose detector). A fifth MCP-like receptor, Aer, mediates aerotactic responses, but in a methylation-independent manner [14]. All five receptor types form ternary signaling complexes with CheW and CheA and localize to polar clusters [15]. Tsr and Tar are high-abundance receptors, each present at roughly 3000 molecules per cell. Trg, Tap, and Aer are low-abundance receptors, each present at only a few hundred molecules per cell [16•]. The low-abundance receptors perform well, implying collaborative control over the CheA partners of high-abundance receptors.

An *in vivo* assay based on fluorescence resonance energy transfer (FRET) between tagged CheY and CheZ molecules [5•] has shown that responses mediated by one receptor type are greatly influenced by the presence and relative abundance of other receptors in the cell [17••]. Homogeneous receptor populations respond to ligand occupancy changes in a highly cooperative manner, consistent with an allosteric complex of 10 or more receptor molecules in the cooperative unit. In heterogeneous populations, each receptor type signals with reduced sensitivity and cooperativity, implying functional interactions between different receptors in the same allosteric units.

High-abundance receptors also assist low-abundance receptors in reaching their appropriate methylation levels. Tar and Tsr molecules carry a C-terminal pentapeptide (NWETF) to which the CheR and CheB methy-

lation enzymes bind [18–22]. Low-abundance receptors naturally lack this docking site and methylate and demethylate inefficiently [23–26]. However, they can be assisted in *trans* by receptors that have the docking site. The assistance neighborhood for methylation is about seven receptors; for demethylation, the neighborhood is about five receptors (M Li and GL Hazelbauer, personal communication). Adaptational assistance implies that the interacting molecules are closely packed in the receptor cluster.

Linear polymers with 3–10 galactose moieties triggered prolonged swimming responses from cells containing Trg, the galactose chemoreceptor [27]. Multivalent galactose also extended Tsr and Tar responses in cells containing Trg. These behavioral changes were accompanied by enhanced clustering of the various chemoreceptors, consistent with the idea that receptors of different types work together in a large array. By simultaneously binding to several receptor molecules, multivalent ligands promote receptor packing, which seemingly augments excitatory signaling by the network. Alternatively, the enforced clustering might prolong responses by impairing the sensory adaptation process. This issue could be resolved by measuring responses to multivalent ligands with the *in vivo* FRET signaling assay described above.

Structure of chemoreceptor signaling teams

Native MCP molecules are homodimers of predominantly α-helical subunits (Figure 2). The signaling domain forms a four-helix bundle, with each monomer organized as an anti-parallel coiled coil [28]. The methylation sites reside in juxtaposed helices flanking the membrane-distal tip where CheA and CheW interact [29,30]. The activity of the signaling tip evidently responds to conformational controls from both the ligand-binding domain and the interposed methylation region, which seems to operate as a conformational filter to attenuate or augment the ligand-induced conformational changes. Ligand-binding causes a small vertical displacement of the membrane-spanning segment connected to the signaling domain, but the mechanism of transmembrane signaling, particularly the role of the membrane-proximal HAMP domain, is still poorly understood (see [31] for a review).

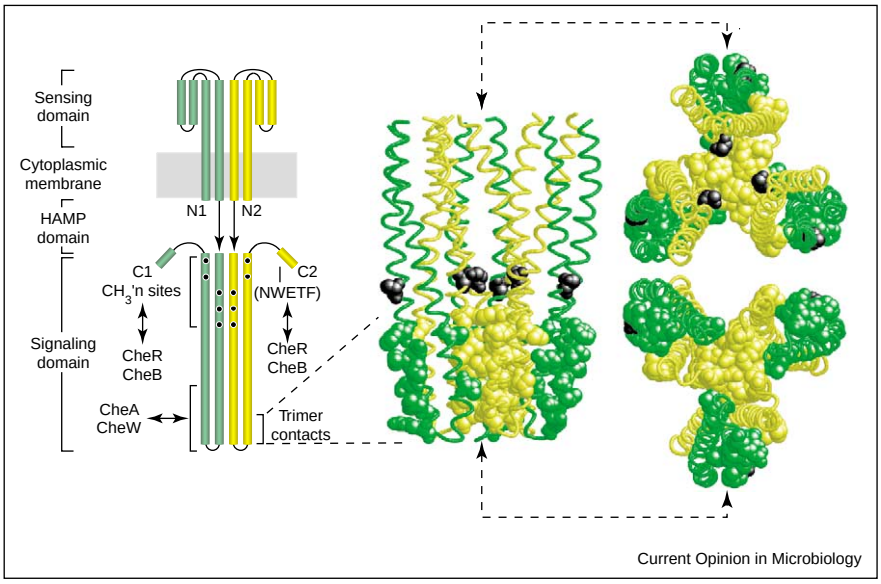
The next level of chemoreceptor organization, which is presumably responsible for inter-receptor communication and cluster formation, may be based on a trimer-of-dimers arrangement seen in X-ray studies of the Tsr signaling domain [28,32••,33•] (Figure 2). The trimer interface lies at the tip of the signaling domain, where CheA and CheW also interact. The 11 principal contact residues are identical in the 5 *E. coli* receptors, raising the intriguing possibility that different receptor molecules might form mixed trimers of dimers *in vivo*. Several recent lines of evidence support this idea, as detailed below.

Amino acid replacements at many of the trimer contact sites of the serine receptor (space-filled residues in Figure 2) not only abrogated Tsr function, but also caused aberrant functional interactions with wild-type receptors of other types [32••]. Some of these Tsr trimer contact mutants (Tsr*) regained serine-sensing ability in the presence of wild-type Tar receptors; others interfered with Tar function. These functional rescue and epistasis effects suggest that receptors of different types normally work together in signaling teams. Accordingly, both types of defective receptors could join trimer-based receptor teams: rescuable receptors would be helped by normal team members, whereas epistatic receptors would spoil the function of the entire team. This model predicts that an epistatic Tsr* defect might be suppressible, in a manner analogous to functional rescue, by a compensatory mutational change in another member of the signaling team. Indeed, the Tar receptor gives rise to many suppressor mutations of this sort (designated Tar^) (P Ames and JS Parkinson, unpublished). The Tar^ mutations cluster in the trimer contact region of Tar, some at the contact sites themselves. Moreover, Tar^-Tsr* suppression patterns are allele-specific, a hallmark of conformational suppression.

Crosslinking assays also suggest that receptors adopt a trimer-of-dimers organization *in vivo* [32••,33•]. The most incisive and versatile crosslinking approach employs a trifunctional maleimide reagent (TMEA) and a cysteine reporter with trigonal symmetry that lies just above the trimer contact region (Figure 2). TMEA treatment of cells bearing receptor molecules with a cysteine at this reporter position generated two- and three-subunit crosslinking products [33•]. Moreover, marked receptors of different types in the same cell formed mixed crosslinking products in amounts and compositions that reflected their relative proportions in the receptor population. Thus, it appears that receptor trimers readily form *in vivo*, following random membership rules. The crosslinking properties of Tsr* mutants support this view [32••,33•] (CA Studdert and JS Parkinson, unpublished). Rescuable and epistatic Tsr* mutants that interact functionally with other receptors readily form trimers and mixed trimers. By contrast, Tsr* mutants that do not interact functionally with other receptors cannot form trimers or mixed trimers [33•]. In sum, these findings indicate that low-abundance receptors reside almost exclusively in mixed signaling teams, which must respond collectively to stimulus inputs from any of their members.

TMEA crosslinking tests showed that receptor trimers form in the absence of CheA, CheW, and other Che proteins [33•], but readily exchange members under these conditions (CA Studdert and JS Parkinson, unpublished). Exchanges are much less frequent in cells containing both CheA and CheW, suggesting that receptor trimers bind

Figure 2



Structural features of chemoreceptors. Left: A schematic view of a receptor homodimer, showing key structural features and interaction sites. Receptor molecules interact directly with four of the six Che components shown in the signaling pathway of Figure 1. CheR/CheB bind to C-terminal docking sites in high-abundance receptors and act on the nearby methylation and demethylation substrate sites. CheA/CheW interact with the tip of the cytoplasmic domain to form ternary signaling complexes. The receptor tip also contains the contact sites for trimer formation, in which mutations produce the rescuable and epistatic Tsr* signaling defects discussed in the text. Right: Atomic structures of receptor trimers based on X-ray studies of the Tsr signaling domain [28]. The identical subunits of each dimer are colored differently to emphasize their non-equivalent positions in the trimer of dimers (yellow subunits at the trimer interface, green subunits oriented away from the interface). The yellow space-filled residues are the principal trimer contact residues. Their counterparts on the perimeter of the trimer (green) might play other roles, for example CheA/CheW-binding, in that orientation. The black space-filled residues identify the trigonal reporter site used for TMEA crosslinking studies. Upper right: A top view showing the close proximity of the reporter sites of the inner subunits. Note that the same residues in the outer subunits, mostly hidden by the green helices, are too far apart to be crosslinked with TMEA. Bottom right: A bottom view of the trimer tip showing the extensive packing of contact residues at the trimer interface and the outside orientation of their counterparts in the green subunits.

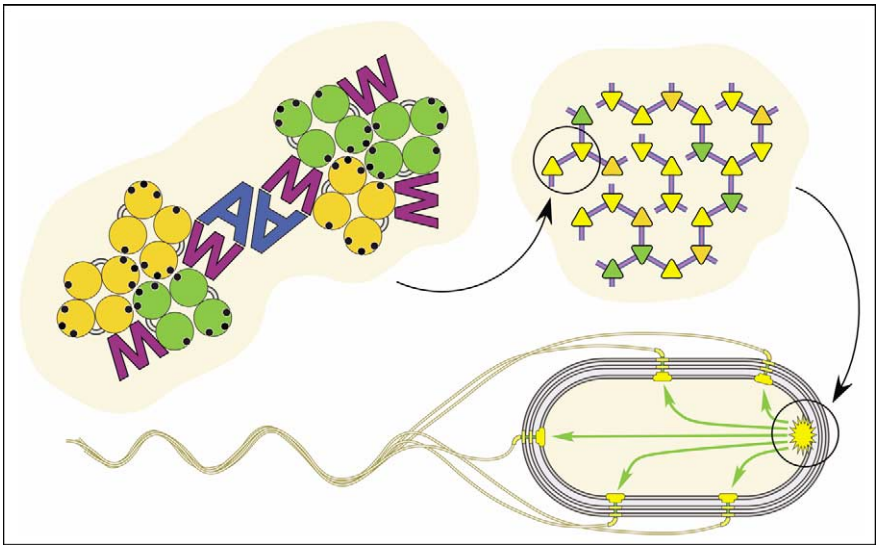
CheA and CheW to form structurally stable signaling teams. The extent of TMEA crosslinking of receptor signaling domains was not obviously influenced by ligand occupancy or methylation state [33•]. By contrast, disulfide crosslinking of reporter sites in the periplasmic domain of the Tar receptor produced a different picture [34]. With this assay, CheA/CheW and aspartate stimuli had a pronounced effect on the extent of inter-dimer crosslink formation. It may be that the periplasmic crosslinks form between receptors in different trimers, whose physical associations depend on ligand occupancy state and shared CheA/CheW connections [35]. Thus, a receptor molecule may at the same time interact with some receptors through cytoplasmic trimer contacts and with the periplasmic domains of different receptor dimers, perhaps through non-specific contacts [36].

Conclusions

Our current view of receptor organization in *E. coli* is summarized in Figure 3. We propose that receptors of different detection specificities form mixed signaling teams based on trimer interactions between the signaling tips of the receptors. Each trimer of dimers binds CheW and CheA signaling partners with an average stoichiometry of two CheW monomers and one CheA dimer [16•]. The CheA dimers in turn create bridging connections to other trimer units, but the number of trimers required to create a functional signaling team is not known. The highly cooperative signaling behavior of homogenous receptor teams suggests that multiple trimers comprise a signaling unit [17••], but smaller units, under the right circumstances, can also account for the observed signal gain [37,38]. Moreover, signal team assembly in a growing cell is expected to be a stochastic process, so the ensemble of receptor teams in a cluster can only approximate a regular array. We assume that the cell's preferred 3:2:1 stoichiometry for the signal team components has evolved to ensure the maximal number of functional teams with the minimal number of broken connections between them.

Even this rather complex architecture may be an oversimplified picture of chemoreceptor signaling networks. Several other dimer–dimer interactions that could conceivably contribute to cluster formation have been documented in electron microscopy studies. Cell membranes containing high levels of Tsr in the absence of CheA and CheW revealed tip-to-tip and antiparallel side-to-side

Figure 3



Proposed structural organization of chemoreceptor clusters. Bottom: receptor clusters typically reside near one pole of the cell and communicate with the randomly distributed flagellar motors through diffusion of CW-eliciting phospho-CheY signals. Top-left: the architectural building blocks of receptor clusters are believed to be trimers of dimers, shown in schematic cross-sectional view, and their CheW/CheA signaling partners. Trimers can contain receptors of different detection specificities (indicated by different colors) because their trimer contact sites (black circles) are identical. CheW monomers bind to trimers, possibly between adjacent dimers [35], and also bind to each subunit of CheA dimers to form inter-trimer connections. Top-right: Schematic view of the trimer array: trimers (indicated by triangles) with different combinations of receptor types have different colors. The three possible CheW/CheA connection points on each trimer lead to formation of a two-dimensional array of receptor signaling teams with highly cooperative signaling properties. The receptor lattice probably contains discontinuities (i.e. trimers with less than three connections to their neighbors), owing to the stochastic nature of the assembly steps. The number and arrangements of trimers, CheW, and CheA that comprise a minimal receptor signaling unit have not been established.

contacts between the receptor molecules [39,40]. The physiological relevance of these interactions is not yet clear because concentrated receptors without their normal binding partners might associate non-specifically, for example, through hydrophobic surface patches. Soluble Tar signaling fragments tagged with leucine zippers form active, structurally stable ternary complexes that contain 2 CheA dimers, 6 CheW monomers, and 12 Tar dimers in both lateral and end-to-end, possibly intercalated, arrangements [41•,42]. Although organized in groups of three, the receptor dimers in these complexes do not make close contacts like those in the trimer-of-dimers crystal structure. These interesting structures are undoubtedly relevant to receptor signaling complexes *in vivo*, but it is difficult to know whether their leucine zipper appendages and lack of membrane-embedding have distorted the normal picture. Clearly, there is still much to learn about the architecture and operation of chemoreceptor signaling clusters in bacteria.

Update

CheZ, the CheY-specific phosphatase, is localized at the receptor signaling complex. A recent FRET study shows that the seemingly paradoxical co-localization of the CheY kinase (CheA) and phosphatase (CheZ) serves to dampen internal gradients of phospho-CheY, producing faster, more coordinated motor responses [42].

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