Ancient chemoreceptors retain their flexibility

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Bacterial and archaeal microbes possess a wide array of surface-deployed receptors for monitoring their chemical and physical surroundings. Particularly prominent among microbial chemoreceptors are the so-called methyl-accepting chemotaxis proteins (MCPs), which sense changes in the cell's chemical environment and generate intracellular signals that control the organism's pattern of locomotion or gene expression. MCPs have long been known to mediate attractant-seeking and repellent-avoiding behaviors in *Escherichia coli* and other motile microbes, but more recently, MCPs have also been shown to regulate complex developmental programs, such as fruiting body formation (1). The sequenced microbial genomes contain thousands of MCP genes, so it seems likely that evolution has put these versatile chemical sensors to other uses as well. In this issue of PNAS, Alexander and Zhulin (2) describe an insightful structure-guided analysis of MCP sequences that reveals common architectural features conserved over the long evolutionary history of these chemoreceptors. Their work provides important new clues to the molecular signaling mechanism(s) of these remarkable molecules.

Much of our knowledge about MCPs has come from studies of chemotaxis receptors in *E. coli* and *Salmonella* (see refs. 3 and 4 for reviews). Most MCPs are transmembrane proteins with a periplasmic ligand-binding domain for chemical sensing and a cytoplasmic domain for generating and modulating output signals (Fig. 1*A*). Native MCP molecules are homodimers with predominantly α-helical subunits. In the cytoplasmic domain, the subunits form coiled-coil hairpins that intertwine in a four-helix bundle (Fig. 1*B*). The signaling subdomain centered on the hairpin tip of the cytoplasmic domain (Fig. 1, blue) contains highly conserved determinants for interactions with other receptor molecules and with two partner proteins: CheA, a histidine autokinase, and CheW, which couples CheA activity to receptor control. CheA donates its phosphoryl groups to CheY, a response regulator that shuttles between the receptor-signaling complexes and the flagellar motors to control cell movement. Ternary receptor complexes have a kinase-on mode with high autophosphorylation activity and a kinase-off mode with low activity. Ligand occupancy changes drive the signaling equilibrium toward one state or the other to modulate the cell's behavior.

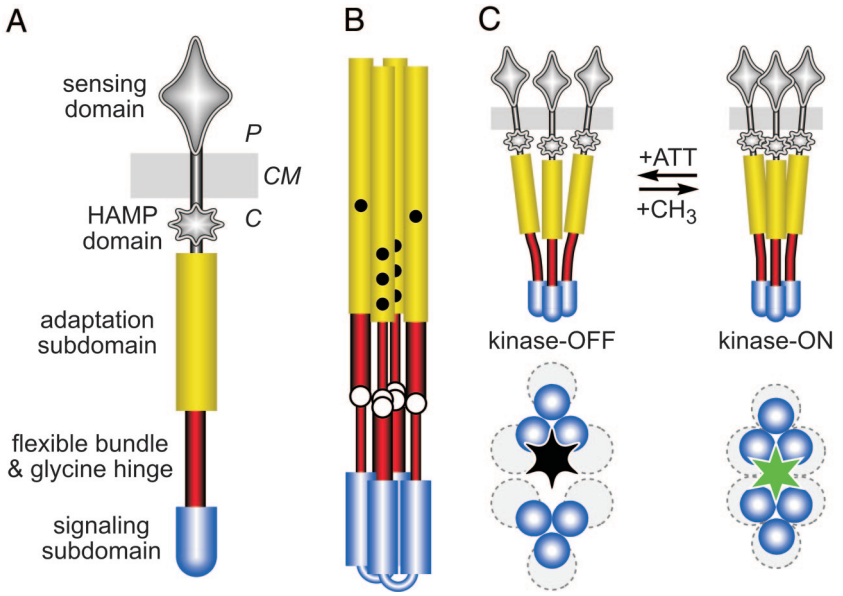

Fig. 1. Structure and function of MCP molecules. (*A*) Schematic of an MCP homodimer; individual subunits are not distinguished. *P*, periplasmic space; *CM*, cytoplasmic membrane; *C*, cytoplasm. (*B*) Schematic of the MCP cytoplasmic domain showing the helical segments in the adaptation and signaling subdomains and the flexible bundle. The thickness of the helices is roughly proportional to their coiled-coil stabilities. Black circles denote the positions of methylation sites in *E. coli* chemoreceptors. White circles denote glycine residues in the glycine hinge between the stable (thick red) and unstable (thin red) helices of the flexible bundle. (*C Upper*) Model of higher-order receptor organization and the role of dimer bending in the kinase-on and -off states of receptor signaling complexes. (*C Lower*) A view from the tips of the trimers of dimers looking toward the cell membrane. The large gray circles denote the boundaries of the periplasmic-sensing domains in each signaling state. The green symbol represents an activated CheA molecule; the black symbol denotes a deactivated CheA.

MCPs sense temporal concentration changes by comparing current ligand occupancy with that averaged over the past few seconds. Chemical history is recorded in the form of reversible methyl ester modifications to specific glutamic acid residues in the cytoplasmic domain. MCP molecules constantly update their methylation record through feedback and substrate-level control of the modification enzymes. Increasing methylation shifts MCP signaling complexes toward the kinase-on state, thereby countering the kinase-off signals elicited by attractant increases. This sensory adaptation mechanism operates over a 5- to 6-log concentration range, enabling MCPs to adjust their window of maximum detection sensitivity to match ambient chemoeffector levels.

The source of the prodigious signal amplification or gain exhibited by MCP molecules remains the outstanding mystery in the field. Measurements of FRET between tagged signaling proteins in living cells have shown that each receptor controls the activity of ≈35 CheA kinase molecules, many more than it could interact with directly (5). The mechanism responsible for this high signal gain appears to involve physical interactions between receptor molecules, which are known to form macroscopic clusters at the cell poles (6). Clustering may allow receptor signaling complexes to form networked arrays that operate in a highly cooperative manner (7).

Alexander and Zhulin (2) looked for common sequence features in MCPs that could provide clues to their high-gain signaling mechanism. They began with all available MCP sequences and devised algorithms for aligning the mol-

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ecules that took into account the known structural features of MCP cytoplasmic domains (8, 9): a central highly conserved helical hairpin flanked by N- and C-terminal arms of comparable length whose coiled-coil structure is based on a 7-aa repeat (two helical turns) with hydrophobic residues in the first (**a**) and fourth (**d**) positions of the heptads. From alignments of nearly 2,000 sequences, Alexander and Zhulin (2) defined seven major length classes of MCP cytoplasmic domains (2). Each class was characterized by symmetric insertions or deletions (indels) of integral numbers of heptad repeat units. Alexander and Zhulin also found that nearly all MCPs contained easily recognizable methylation sites with a conserved sequence signature (2). However, each MCP length class had characteristic numbers and locations of the modification sites. These variations within the adaptation subdomain were accompanied by characteristic variations in the structural features of the neighboring signaling subdomain, implying that sensory adaptation is an ancient capability of these chemoreceptors that has coevolved with the signaling subdomain.

The adaptation subdomain plays a key role in modulating the signaling subdomain of MCP molecules. Ligand binding induces a small (≈2-Å) downward displacement of one of the transmembrane segments in the MCP dimer (10). This asymmetric "piston" motion is received by the HAMP domain and converted into a symmetric conformational change that influences the adaptation subdomain. Its conformational changes are probably subtle ones because MCP molecules can retain signal control after cross-linking the subunits with cysteine-directed disulfides in HAMP or the adaptation subdomain (11). Evidently, small-scale changes in the packing arrangements or dynamic motions of the adaptation subdomain helices are sufficient to regulate the output state of the signaling subdomain. Methylation changes presumably modulate this same conformational parameter, most likely by influencing short-range electrostatic interactions at the subunit interface of the MCP dimer (12).

Alexander and Zhulin's (2) analysis of the MCP region between the adaptation and signaling subdomains provides an important new clue about their control interaction. Two segments in this region exhibited consistent disparities in the side-chain volumes of residues in the **a** and **d** heptad positions, features that are likely to destabilize coiled-coil packing interactions. Their partner segments in the other helical arm exhibited more conventional residue patterns. This combination of less and more stable coiled-coil segments might permit flexing motions. Interestingly, the midpoint of this flexible bundle contains a previously described glycine hinge important for MCP signaling (13). Alexander and Zhulin's new finding that the glycine hinge resides in a flexible bundle subdomain reinforces the idea that "bending is central to the signaling mechanism" (2). What role might it play?

A model that pulls together recent evidence on *E. coli* MCPs is shown in Fig. 1*C*. X-ray structures of soluble MCP signaling fragments exhibit a trimer-of-dimers arrangement of interdimer contacts between highly conserved residues in the signaling subdomain (8). Genetic (14, 15) and cross-linking (16, 17) studies show that *E. coli* receptors form trimers of dimers *in vivo* and signal collaboratively in mixed trimer-based teams. Bending at the glycine hinge could allow MCP signaling subdomains to form trimers of dimers by avoiding clashes between their sensing domains (Fig. 1*C Right*). *In vivo* studies of fluorescently tagged MCPs show that receptor dimers move apart upon attractant stimulation (18). These movements did not occur in trimer-defective receptors, implying that signaling state influences the relative positions of the members of trimer signaling teams. *In vivo* cross-linking studies also reveal stimulus-dependent changes in the relative positions of receptor periplasmic domains, consistent with attractant-induced rotational movements of the dimers in a ternary signaling complex (19). Perhaps attractant stimuli bend or relax the flexible bundle, allowing the individual dimers in a trimer to splay apart and push against neighboring trimers. These movements could conceivably modulate CheA activity. Although the structure of the signaling complex is not known, CheA activation might require interaction with receptor molecules in two different trimers of dimers (20) (Fig. 1*C Lower Right*). If so, then separating the trimers in a signaling team should deactivate their shared CheA (Fig. 1*C Lower Left*).

Trimers of dimers seem to be ideal structural components for assembling 2D receptor signaling arrays (21). However, other MCPs might operate differently (9). Alexander and Zhulin (2) suggest that the variations in length and in trimer contact residues among different MCP classes could reflect different patterns of higher-order organization (2). This is an intriguing prospect; perhaps we are just beginning to learn the signaling secrets of these versatile molecules.

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