

Bacterial Chemosensing: Cooperative Molecular Logic Dispatch

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Bacterial chemotaxis is mediated by transmembrane receptors that bind attractant and repellent chemicals and control an intracellular protein kinase. Each cell contains thousands of receptor subunits that form a tightly packed array at one pole. Recent studies of bacterial behavior have begun to reveal the molecular logic of this sensory architecture.

The components of the chemotaxis sensory system are highly conserved throughout the Bacterial and Archeal kingdoms. The chemotaxis system of *Escherichia coli* has been studied in the greatest detail (reviewed in [1]), and it is without a doubt the best understood signal transduction system in biology. Research in this field has broad relevance for understanding the molecular mechanisms of membrane receptor-mediated information processing. Mechanistic similarities between bacterial chemoreceptors and mammalian type I receptors — such as those for insulin, growth hormone and cytokines — have been demonstrated by fusing the ligand-binding domain of a bacterial chemotaxis receptor to the cytoplasmic domain of the insulin receptor to produce a hybrid receptor tyrosine kinase that is regulated by binding of the chemoattractant ligand [2].

A typical *E. coli* cell has several thousand transmembrane chemotaxis receptors encoded by a family of five homologous genes. These transmembrane proteins interact with homologous SH3-like domains in two auxiliary cytoplasmic proteins, CheW and CheA. In CheA, a histidine protein kinase domain is fused to the amino-terminus of the SH3 region. Protein kinase activity is inhibited when attractants such as aspartate or serine bind to extracytoplasmic sensory domains of the receptors. CheA kinase activity results in phosphorylation of a monomeric response regulator protein, CheY. Phosphorylated CheY readily diffuses away from CheA and subsequently binds to flagellar motors in the cell envelope, where it promotes a change in the direction of motion.

Immuno-electron microscopy established that *E. coli* chemoreceptors, together with CheA and CheW, tightly cluster together at one pole of the cell [3]. Subsequent investigations using immuno-electron microscopy, immuno-fluorescence microscopy and fluorescence microscopy with green fluorescent protein fusions have confirmed and extended this observation to the point that it is now reasonably established that chemotaxis receptors generally associate with CheA and CheW to form large assemblies in all Bacterial and Archeal chemotaxis systems [4–6].

What are the implications of these higher-order receptor assemblies for chemotaxis signal transduction? It had previously been assumed that transmembrane signaling involved stimulus-induced changes within receptor homodimers. Various mechanisms for this were advanced, including rotation of one subunit with respect to the other, a scissors motion between subunits, and a piston-like motion between transmembrane helices [7]. The realization that signaling is mediated by a higher-order structure has opened the possibility that inter-dimer interactions could play a critical role [8,9]. Such higher-order cooperative mechanisms would help explain the ultrasensitive responses that have been difficult to understand in the context of intra-dimer signal transduction mechanisms.

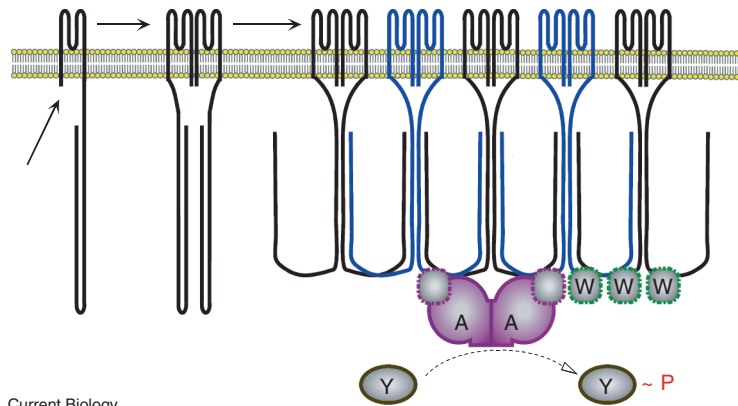
In order to clarify the protein–protein interactions and response of the chemotaxis system *in vivo*, Sourjik and Berg [10] developed a technique for assaying CheA kinase activity using fluorescence energy transfer between fusions to the cyan and yellow fluorescent proteins. Their recently reported findings using this fluorescent reporter technology confirm the idea that inter-dimer interactions play an important role [11]. The results indicate that the degree of cooperativity and sensitivity exhibited by a particular type of chemoreceptor depends upon its level of expression relative to other chemoreceptors, as well as its state of adaptive modification. The Hill coefficients for the response of the system to attractant ligands were observed to vary between one and ten, depending on the conditions. Wild-type cells that have an intact chemoreceptor modification–adaptation system exhibited a high gain in their response to attractant stimuli without a high cooperativity (a Hill coefficient of about two). With the adaptation system present, the relative sensitivity of the system to a particular ligand varied depending upon the relative expression levels of the chemoreceptors, but the cooperativity did not change.

These data were fit with a modified Monod-Wyman-Changeux two-state model [12] that involves coupling between multiple two-state systems [11], extending previous formulations for two-state signaling for chemoreceptor dimers [13]. In general, the number of interacting chemoreceptor subunits in such formulations is always greater than the Hill coefficient. Thus, Sourjik and Berg's [11] results imply that interacting clusters with a size of at least ten dimers can respond in a coordinated way to ligand binding events. Several other recent studies [14–16] have probed inter-dimer interactions for chemoreceptors *in vivo* using genetic approaches and chemical cross-linking. The results of these studies are also consistent with functional interactions among multiple chemoreceptor dimers.

These findings raise as many questions about the functional interactions between chemoreceptors as they answer. Each receptor subunit is composed of parallel and antiparallel alpha-helices. The amino-terminal extracytoplasmic sensory domain is an

Figure 1. Receptor complex formation.

Receptor monomers are inserted into the membrane, dimerize and then associate together in an interconnected array through domain swapping. CheW and CheA binding to the hairpin turn region facilitates this assembly process. CheA is activated by the receptors and CheW and phosphorylates CheY.



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up-down-up-down four helix bundle. This is connected via a hydrophobic membrane-spanning helix to a long hairpin-like antiparallel coiled coil that extends into the cytoplasm (Figure 1). The CheW and CheA proteins bind near the hairpin loop region. How do the chemoreceptor subunits interact literally to form the higher order structures that are seen *in vivo*? The amino-terminal extracytoplasmic domains have long been known to form stable dimers, but these portions of the receptors do not appear to participate in the assembly of the membrane receptor complexes.

Most of the models of higher-order chemoreceptor interactions to date have focused on the crystal structure of the antiparallel coiled coil cytoplasmic domain of the serine chemoreceptor Tsr [16]. The packing of alpha-helical elements in this crystal has been characterized as a trimer of dimers. There is considerable evidence, however, that this form of the receptor does not interact with CheA and CheW to form active signaling complexes. For example, there does not appear to be room in this structure to bind CheW at the 1:1 stoichiometry that can be achieved *in vitro* [8]. Thus, at best, the trimer of dimers represents an intermediate in the self-assembly process that is required to generate polar sensory receptor arrays. A likely mechanism for the formation of extended inter-dimer interactions is domain swapping [18] — the second helical segment (after the hairpin) from one chemoreceptor could readily interact with the core coiled coil from a different dimer (Figure 1) [19].

The cooperative behavior and ultrasensitivity of the bacterial chemosensory system have inspired a broad range of efforts to understand the molecular logic of this sensory system. The recent confirmation of functional inter-dimer chemoreceptor interactions is an important advance in understanding the role of the higher-order chemoreceptor architecture in transmembrane signaling. Domain swapping among different types of chemoreceptor provides a natural mechanism for the integration of different signals. In the polar arrays of tightly packed receptors, the density of antiparallel helical partners in the cytoplasm would inevitably lead to dynamic coiled coil interactions causing homodimers to lose their functional identity within a forest of fluctuating affiliations [8,9,20]. Thus, sensing is not a process that occurs via one chemoreceptor at a time, but rather through the combined responses of many receptors to process information from the environment.

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