REVIEW

"Frozen" dynamic dimer model for transmembrane signaling in bacterial chemotaxis receptors

SUNG-HOU KIM

Department of Chemistry and Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

Abstract

The crystal structures of the ligand binding domain of a bacterial aspartate receptor suggest a simple mechanism for transmembrane signaling by the dimer of the receptor. On ligand binding, one domain rotates with respect to the other, and this rotational motion is proposed to be transmitted through the membrane to the cytoplasmic domains of the receptor.

Keywords: chemotaxis; dynamic receptor; receptors; signal transduction; transmembrane signal

All living organisms have evolved, as essential traits for survival, mechanisms to recognize nutrients and toxic substances in their environment. Even the simplest organisms, such as bacteria, can detect increased concentrations of nutrients or attractants and toxic substances or repellents. Furthermore, many microorganisms, such as *Escherichia coli* and *Salmonella typhimurium*, move (chemotax) toward attractants or away from repellents by changing the ratio between swimming and tumbling motions, and take up nutrients and exclude toxic substances. When each of the helical flagella on the cell surface of these organisms rotates in a counterclockwise direction, the flagella form a bundle and the bacterium appears to move in a swimming motion; when the flagella rotate in a clockwise direction, the organism tumbles (Fig. 1; Berg & Brown, 1972; Macnab & Koshland, 1972; Adler, 1975; Koshland, 1988). The initial step in sensing changes in the environment and triggering signal transduction for chemotaxis is achieved by the receptors present in the periplasmic membrane. There is a family of receptors that can sense a wide variety of chemicals such as amino acids, small peptides, sugars, metal ions, phenolic compounds, and numerous other compounds. The signals received by these receptors are converted into a single, integrated signal and passed through a common intracellular signaling pathway, ultimately dictating the direction of the rotation of the flagellar "motors."

All bacterial chemotaxis receptors identified so far in *E. coli* and *S. typhimurium* have considerable homologies in amino acid sequences, and they are predicted to have the same overall tertiary structures. Like many mammalian transmembrane receptors, such as receptors for insulin and various growth factors,

they are composed of 3 domains per monomer: a ligand binding domain, a transmembrane domain (consisting of 2 presumed transmembrane helices, TM1 and TM2, instead of one in the mammalian receptors), and a cytoplasmic domain. The functional forms of the bacterial chemotaxis receptors are organized as dimers (Milligan & Koshland, 1988).

Analogous to the process in mammalian cells, signaling within bacterial cytoplasm is accomplished by a rapid process of successive phosphorylation of specific cytoplasmic proteins (Borkovich et al., 1989; Bourret et al., 1989) counterbalanced by dephosphorylation, finally activating the motor of each flagellum. Current understanding of the signaling process for bacterial chemotaxis (Berg & Brown, 1972; Parkinson, 1978; Koshland, 1988; Bourret et al., 1989, 1991; Stock et al., 1991; Hazelbauer et al., 1993) is summarized herein.

In the absence of any stimulants in the environment, a bacterium moves in a random walk by alternating swimming and tumbling motions (Fig. 1B). In a concentration gradient of increasing repellents or decreasing attractants, the receptors, with the help of chemotaxis protein W (Che W), trigger autophosphorylation of Che A proteins at a histidinyl residue, which in turn transfer the phosphate groups to Che Y proteins on an aspartyl residue (Fig. 2). The phosphorylated Che Y proteins cause each helical flagellum to rotate clockwise (CW; when viewed from the inside of a bacterium), which triggers the bundled flagella to unbundle, interrupting the swimming motion and making the bacterium tumble. As Che Y proteins become dephosphorylated by Che Z proteins, the flagella return to counterclockwise (CCW) rotation, and the bacterium swims along in a direction different from the previous direction, giving the receptors another chance to sample a different environment.

This fast signaling process in bacteria is further modulated by a slower adaption process by methylation and demethylation of

A

No concentration
gradient

Increasing concentration
gradient of attractants

B
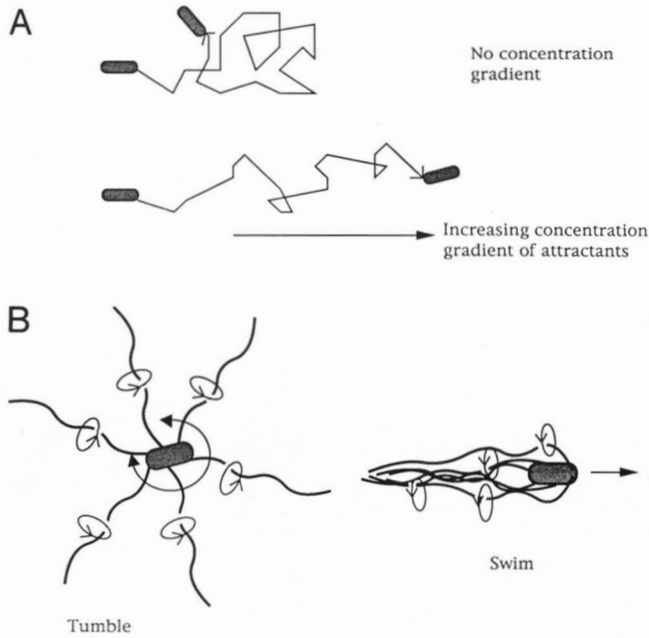
Swim

Tumble

Fig. 1. A: Random walk of a bacterium in the absence of stimulants and biased random walk along the increased concentration of attractants. **B:** Counterclockwise rotation of flagella causes a bacterium to swim and clockwise rotation to tumble.

the cytoplasmic domains of the receptors (Russo & Koshland, 1983; Stock et al., 1985; Dunten & Koshland, 1991): Che R proteins methylate and Che B proteins demethylate the cytoplasmic domains of the bacterial receptors (Fig. 2), resulting in sensitization and desensitization of the receptors. For example, in the presence of persistent high concentrations of attractants or repellents, the tumble signal is gradually increased or decreased, respectively, to reach the level at random walk.

Almost all of the proteins in the signaling and adaptation pathways have been identified, cloned, and expressed, making the bacterial chemotaxis system one of the simplest and best characterized signal transduction systems. Although the biochemistry and regulation of signaling by these proteins have been extensively studied, the mechanism of transmembrane signaling is not known. The currently prevailing monomeric "piston"

model is based on amino acid sequence data and biochemical studies. Recent crystallographic studies on the ligand domain of aspartate receptor with and without aspartate contradict this model. A new dynamic dimer model based on the crystallographic studies and other biochemical data is described.

The monomeric (piston) model

The currently prevailing model for transmembrane signaling in bacterial chemotaxis receptors is that, when a stimulant binds to the ligand binding domain of a receptor, one transmembrane helix moves relative to the other *within a receptor monomer*, and this movement is detected by the cytoplasmic domain of the receptor (Fig. 3A; for a review, see Koshland, 1988). This model was initially conceived from the predicted secondary structures derived from the amino acid sequences of the receptors. The primary structures contain 2 highly hydrophobic regions, each of which could span the membrane thickness when rolled into an α-helix (Boyd et al., 1983; Krikos et al., 1983; Russo & Koshland, 1983; Bollinger et al., 1984). The model was also thought to be consistent with the finding that 1 aspartate molecule binds to each monomer of the aspartate receptor from *S. typhimurium* (Foster et al., 1985). Subsequently, it was found that this family of receptors functions as dimers (Milligan & Koshland, 1988). Even when the receptor is in a dimeric form, no cooperativity was observed in Scatchard analysis of aspartate binding to the receptor, leading Mowbray and Koshland (1990) to conclude that the monomeric units probably act independently in the dimeric receptor.

Additional evidence supporting this model was presented in a set of experiments (Milligan & Koshland, 1991) in which a site-directed mutant, N36C (residue 36 asparagine mutated to cysteine), of *S. typhimurium* aspartate receptor was used to construct stable dimers by covalently crosslinking (between α1 and α1′ in Fig. 4B) one intact mutant receptor monomer to another with or without the cytoplasmic domain. The crosslinked dimers with varying amounts of cytoplasmic domain missing showed substantially diminished yet preferential methylation of the cytoplasmic domain in the intact monomer when the aspartate was bound to the hybrid dimers. (In this study, the methylation rate of the cytoplasmic domain, which measures the extent of the slow adaptation process, was taken as an indirect measure of fast signal transmission by phosphoryl transfer pathways.) This
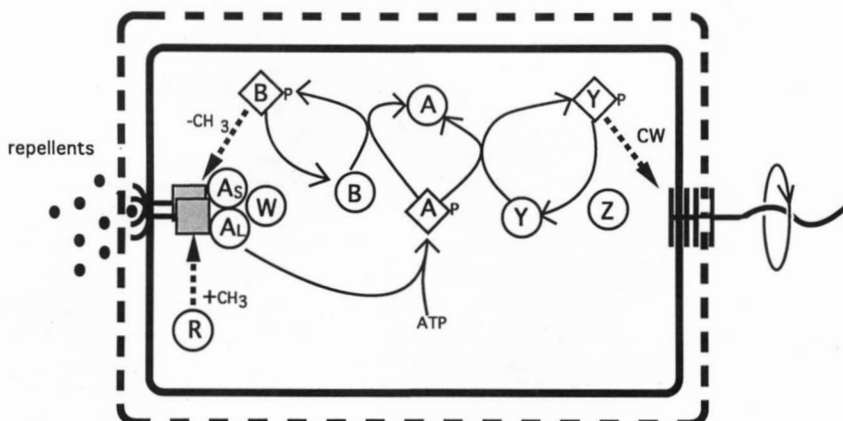
Fig. 2. Schematic drawing of the signaling pathway in bacterial chemotaxis. Outer- and inner-membranes are shown as broken and continuous thick lines respectively. Phosphorylated forms of Che B, Che A, and Che Y are shown as diamonds and nonphosphorylated forms of them, as well as all other proteins (Che R, Che W, and Che Z), are shown as circles. Che R and Che B methylate and demethylate the receptor respectively, and Che Z is a phosphatase. Che A is known to exist in a short form (A_S) and a long form (A_L).
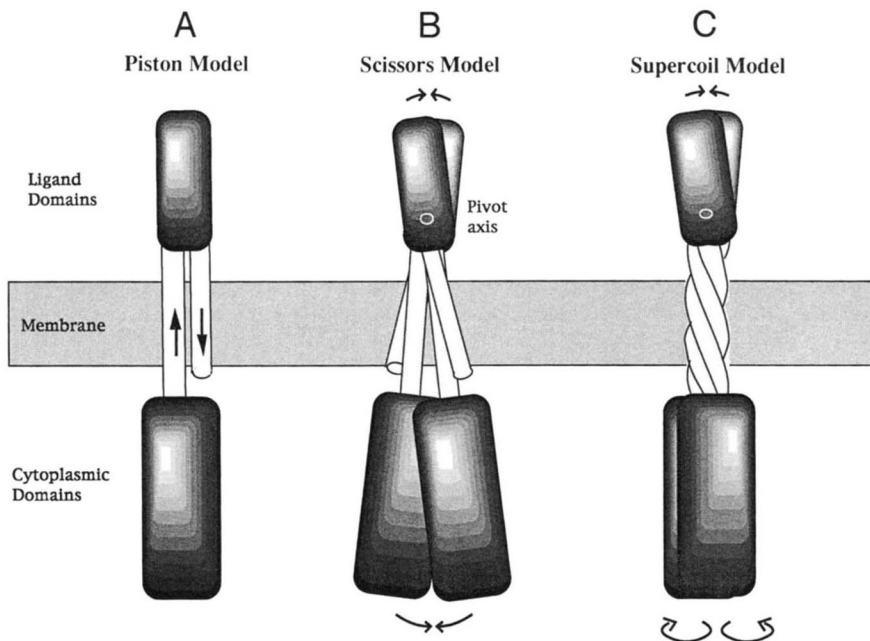
Fig. 3. Models for the mechanism of transmembrane signal transmission in the aspartate chemotaxis receptor of *S. typhimurium*. **A:** Piston model, in which ligand binding alters the relative position/orientation of 2 transmembrane helices (TM1 and TM2) within a monomer. **B:** Scissors model, in which attractant binding brings 2 ligand domains closer by rotation of one monomer with respect to the other around a pivot axis, resulting in decreased distance between 2 cytoplasmic domains. **C:** Supercoil model, in which attractant binding unwinds the negatively supercoiled, central quasi-4-helical bundle of the dimer, resulting in a change of the relative orientation of the 2 cytoplasmic domains.

differential methylation rate of the crosslinked dimer, even in the absence of a second cytoplasmic domain, was presented as support for the monomeric "piston" model of transmembrane signal transmission.

The strongest evidence against this model comes from the crystal structures of the ligand domains of the same crosslinked

N36C mutant with and without bound aspartate (Milburn et al., 1991). A significant change in the 2 helices (to which 2 transmembrane helices, TM1 and TM2, are connected) within a ligand domain monomer is the key predicted feature of the piston model, but no such change was observed in the crystal structures. The expected structural change was also not found in the
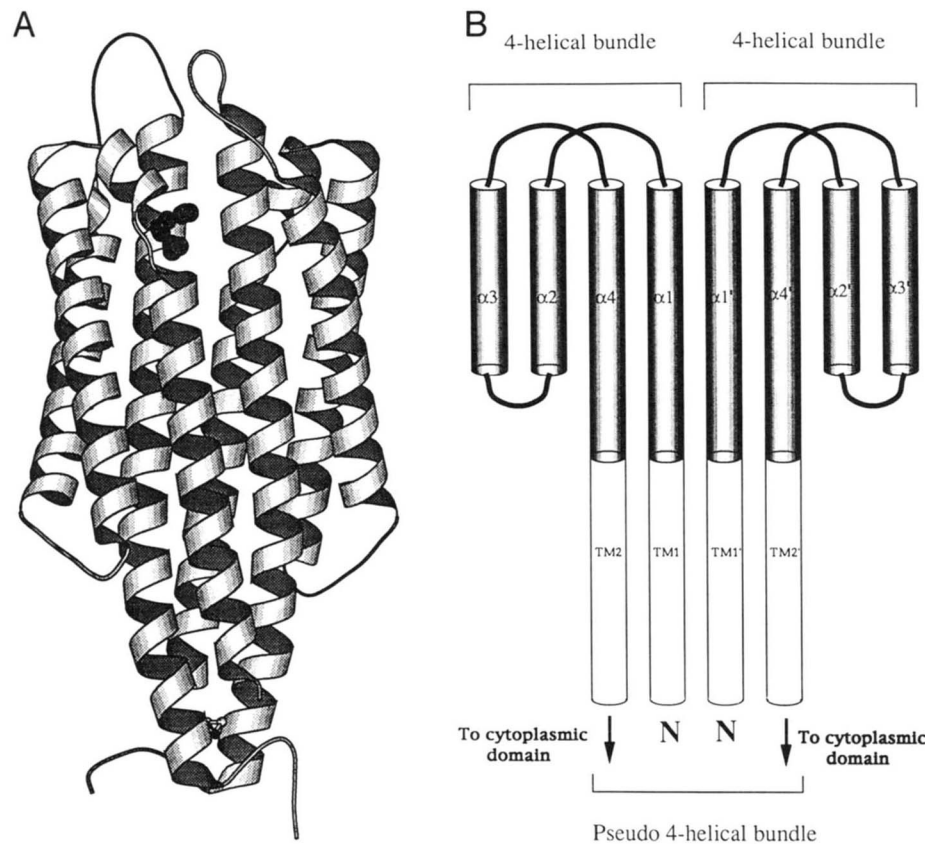
Fig. 4. **A:** Backbone structure of the disulfide-bonded ligand-domain dimer of the aspartate chemotaxis receptor of *S. typhimurium*. One bound aspartate is shown. The crystal structure of the apo protein without the bound ligand is very similar to this except the inter-monomer angle. **B:** The topological structure of the ligand-domain dimer based on its crystal structure is shown shaded. Two transmembrane helices per monomer are shown as unshaded cylinders. Two types of 4-helical bundles are indicated.

crystal structures of the wild-type ligand domain monomer with and without the bound aspartate (Yeh et al., 1993). There are 2 additional pieces of evidence against this model: (1) In both crystal structures of the aspartate bound dimers, only 1 aspartate per dimer was bound at the dimer interface. This is in contrast to the earlier observation of 1 aspartate per monomer (Foster et al., 1985), but is consistent with a recent study that showed a negative cooperativity of the second aspartate binding per dimer (Milligan, 1991) and another study showing 1 aspartate per receptor dimer stoichiometry (J.I.T. Yeh et al., unpubl. results). (2) The crosslinking between α1 and α4, which should restrict the relative movement of TM1 and TM2 within each receptor monomer, has the same effect on the methylation pattern (reduced yet preferential methylation) on the cytoplasmic domains (Falke et al., 1988) as the crosslinking between α1 and α1' of the N36C mutant (Milligan & Koshland, 1991), which should restrict the relative movement of TM1 and TM1' in the receptor dimer. These results suggest that such single crosslinking may leave some transmembrane signaling (as assayed by the methylation rate) intact, and thus is not a sensitive method to distinguish between the monomeric and dimeric mechanisms of transmembrane signaling. In addition, the slow adaptation process by methylation used in the hybrid mutant dimer experiments of Milligan and Koshland (1991) may not be an appropriate measure of the fast signaling process.

Subunit rotation in the dimeric receptor

In the crystal structure of the ligand domain dimer of the aspartate receptor of *Salmonella typhimurium* (Fig. 4A; Milburn et al., 1991; Scott et al., 1993; Yeh et al., 1993), each monomer is a 4-helix bundle in which the 2 longest helices (α1 and α4) presumably extend to 2 transmembrane helices (TM1 and TM2; Fig. 4B). The 2 long helices from one monomer and those from the other form a quasi-4-helix bundle in the middle of the dimer, suggesting that the 4 transmembrane helices also form a 4-helix bundle (Milburn et al., 1991; Pakula & Simon, 1992). A 3-dimensional model of the receptor without the cytoplasmic domain is shown in Figure 5A. Notice that only 1 ligand molecule was bound at the dimer interface (Milburn et al., 1991).

When the 2-Å-resolution crystal structures of the dimer with and without bound aspartate were compared, there was little evidence for conformational changes that could lead to a shift of TM1 relative to TM2 within the monomer structures (Milburn et al., 1991; Scott et al., 1993; Yeh et al., 1993). However, when the intersubunit angles of the apo dimer (with no bound aspartate) and the aspartate-complexed dimer were compared, it was found that the latter has an approximately 4° smaller angle, suggesting that aspartate binding induces a rotation of one monomer with respect to the other, making the aspartate-bound dimer more compact. This 4° intersubunit rotation was observed in the crosslinked N36C mutant ligand domains (Milburn et al., 1991) as well as in noncrosslinked wild-type ligand domains (Yeh et al., 1993). Such intersubunit change is consistent with the results of crosslinking experiments on transmembrane helices (Lynch & Koshland, 1991) and the cytoplasmic extension of the transmembrane helices (Stoddard et al., 1992). The importance of the interface between 2 monomers for signaling is also implicated from mutational studies of *E. coli* aspartate receptor, where all but 1 constitutively signaling mutant have mutations at or near the interface (Gardina et al., 1992). Similar observations were

made in ribose/galactose receptor (Yaghmai & Hazelbauer, 1992).

Extrapolating the observations described above for the ligand domains to the intact receptor dimers, one can imagine the rotational motion of the ligand domains transmitted mechanically to the cytoplasmic domains via conformational changes in the transmembrane 4-helix bundle. There may be many ways such mechanical transmission can occur. Depending on the absence or presence of attractive interactions among the transmembrane helices, 2 extreme cases can be considered: the scissors model and the supercoil model, respectively (Kim et al., 1993; Fig. 3B,C). In the scissors model, the closing motion of the 2 ligand domains caused by ligand binding would bring 2 cytoplasmic domains close to each other. In the supercoil model, the same closing motion of 2 ligand domains would unwind the transmembrane 4-helix bundle, causing one cytoplasmic domain to change its orientation with respect to the other. One can generalize this concept to receptors with multiple nonidentical transmembrane helices such as common receptors with seven or more transmembrane helices. Transmembrane helices in these receptors may be divided into two or more "tectonic plates," each consisting of two or more strongly interacting helices, and the extracellular signal triggers the rotation or sliding of one or more "plate(s)" with respect to the rest, thus changing the conformation of the cytoplasmic domain of the receptors.

Two aspects of the dimeric model require special explanation. (1) The dimeric model, such as the scissors or supercoil model, is based on the crystal structures of a soluble ligand domain in the absence of membrane. Consequently, the effect, if any, of membrane and/or of the transmembrane domain on the structure of the ligand domain and its motion is not predictable and thus is ignored. (2) The observed 4° intersubunit rotation causes a clear but small conformational difference between the apo and the aspartate-bound ligand–domain dimer, but under physiological conditions the change may be much greater. The probable reason for the small observed rotation angle is that the negatively charged SO_4^{2-} ion, present in high concentration in the crystallization conditions of the apo protein, simulates the negatively charged aspartate ion and binds to the aspartate binding site (see below). The apo structure with the bound sulfate probably has a conformation partially similar to that of the aspartate-bound form, thus resulting in the small difference between intersubunit rotation angles of the 2 forms.

The dynamic dimer model

It is conceivable that the "open" apo conformation and the "closed" aspartate complex conformation described above may simply be 2 static conformations stabilized by crystal lattice forces among many conformations available for the dimeric receptor in solution. In fact, there is physical and biochemical evidence suggesting that each receptor dimer in the apo form is in a dynamic state. Such a dynamic state for the apo form of the receptors is implicated by the disulfide crosslinking experiment of Falke and Koshland (1987), where 2 residues as far as 30 Å apart (in the crystal structure) could come close to form a detectable amount of crosslinked product, but much less in aspartate-bound receptor dimer.

One of the physical reasons for the dynamic behavior is suggested by the crystal structure of the apo ligand domain. In the
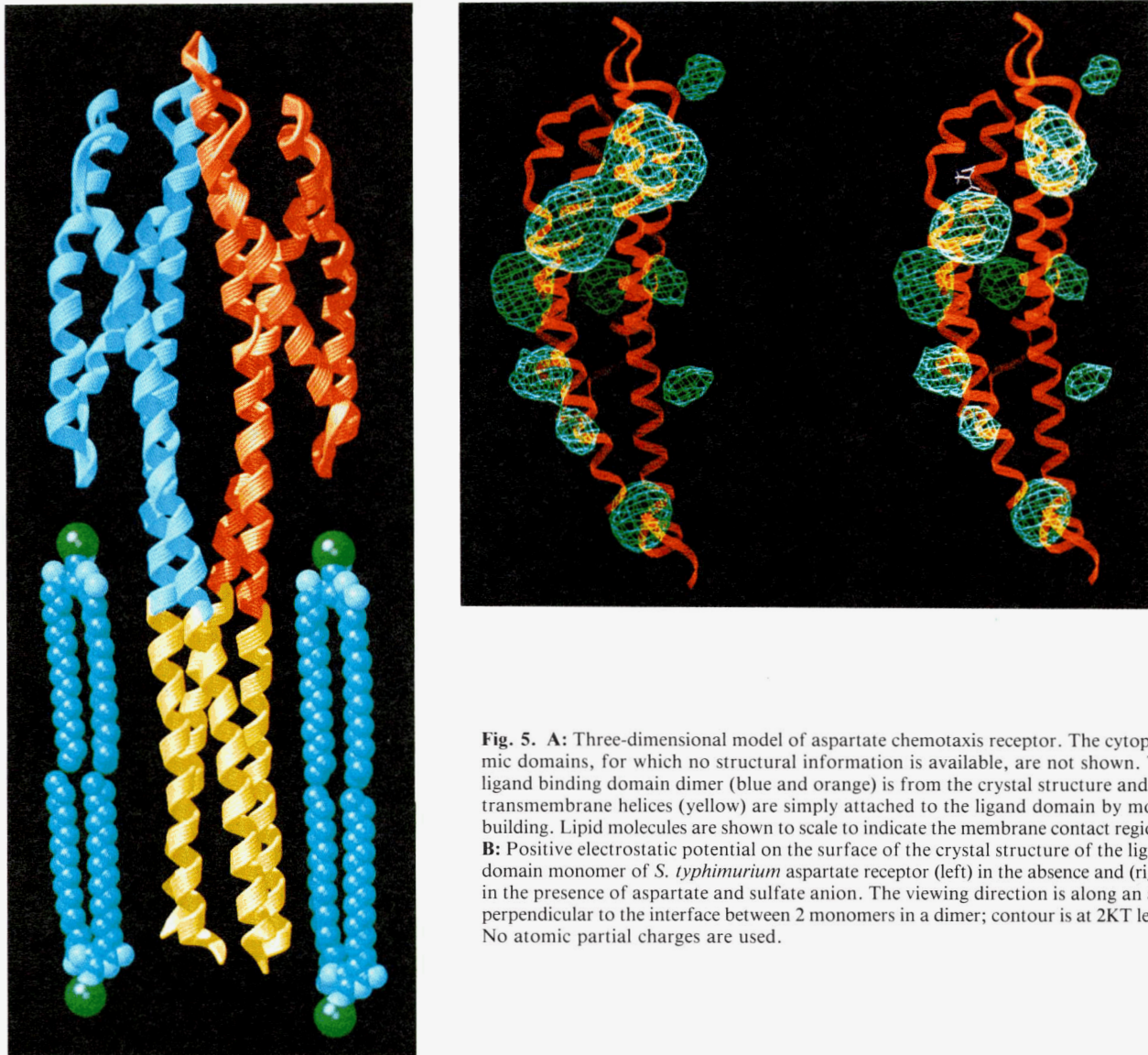
Fig. 5. A: Three-dimensional model of aspartate chemotaxis receptor. The cytoplasmic domains, for which no structural information is available, are not shown. The ligand binding domain dimer (blue and orange) is from the crystal structure and the transmembrane helices (yellow) are simply attached to the ligand domain by model building. Lipid molecules are shown to scale to indicate the membrane contact regions. **B:** Positive electrostatic potential on the surface of the crystal structure of the ligand domain monomer of *S. typhimurium* aspartate receptor (left) in the absence and (right) in the presence of aspartate and sulfate anion. The viewing direction is along an axis perpendicular to the interface between 2 monomers in a dimer; contour is at 2KT level. No atomic partial charges are used.

absence of aspartate, each monomer in the crystal structure has a large surface of strong positive electrostatic potential at the ligand binding site located at the dimer interface (Fig. 5B), suggesting that the 2 monomers in the dimer would repel each other, keeping them in a dynamic open state if the potential is not neutralized. (In the apo crystal structure, these positive charges are neutralized by a high concentration of negatively charged sulfate ions present in the crystallization solution.)

When aspartate binds to the ligand binding site, most of the repulsive force is neutralized (Fig. 5B) by the negative charges of aspartate, and dimerization is further stabilized by many hydrogen bonds between the aspartate and residues from both monomers (Milburn et al., 1991), thus "freezing" the dynamic dimer into a closed dimer (Kim et al., 1993). There is biochemical evidence for such freezing: there is a decrease in subunit exchange rate among receptor dimers when aspartate binds to wild-type receptor dimers (Milligan & Koshland, 1988).

Three conformational populations of receptors

In the dynamic dimer model, 3 conformational populations are considered for the receptors: a dynamic apo receptor dimer can assume many conformational states, whereas an attractant-bound or a repellent-bound receptor can each occupy a smaller conformational space (as shown schematically in Fig. 6). It is assumed that in the absence of any signals delivered to the flagellar motors, each helical flagellum rotates constitutively CCW, causing all flagella to bundle, resulting in a swimming motion of the bacterium (Fig. 1B; Clegg & Koshland, 1984) and that only the receptors in R conformation can produce the tumble signals. Based on these assumptions, one can imagine the following scenario.
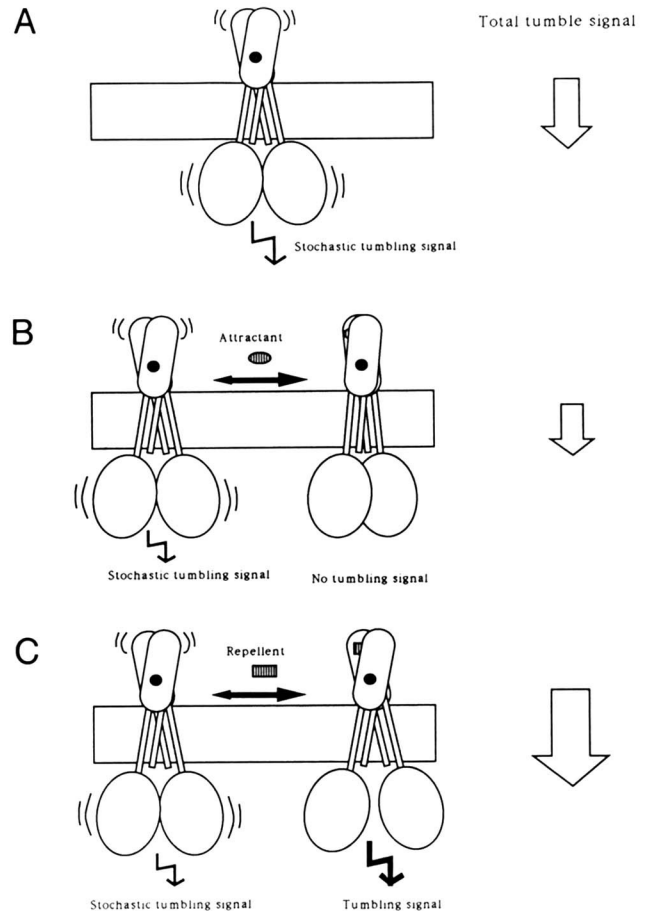
In the absence of attractants or repellents, because a small fraction (approximately corresponding to the ratio of (area under **R**)/(shaded area + areas under **R** and **A**) in Fig. 6) of the
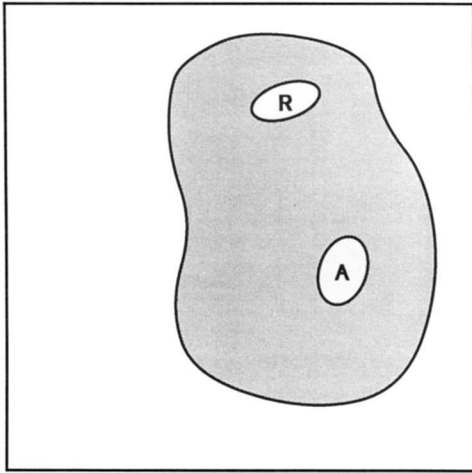
Fig. 6. A schematic representation of the conformational space available to a dimeric receptor of chemotaxis is shown as a large shaded area. The conformational area that corresponds to a "tumble signal" in response to repellants is marked by **R**, and that assumed by the receptors bound to attractants is marked by **A**. In the absence of any stimulants, a receptor can assume any conformation within the boundary, but an attractant-bound receptor or a repellant-bound receptor can assume only those conformations represented by **A** or **R**, respectively.

Fig. 7. "Frozen" dynamic dimer model of signaling. Three proposed states of signaling in aspartate receptor are shown schematically using the scissors model as an example. Similar analogy can be made for the supercoil model. **A:** In the absence of stimulants. **B:** In the presence of attractants. **C:** In the presence of repellents.

dimeric apo receptor population would be found in the conformational area corresponding to the repellent-bound conformation (**R** in Fig. 6) that results in the phosphorylation of Che A protein (tumble signal), one would expect a moderate increase in the relative concentration of phosphorylated Che Y protein (tumble signal; Fig. 7) with respect to that of unphosphorylated Che Y. When the relative concentration reaches a critical value, a cooperative interaction of the proteins with the flagellar motor turns 1 or more flagella CW, causing the flagellum bundle to unbundle and resulting in tumbling motion of the bacterium. This process then activates Che Z proteins to start accelerating dephosphorylation of the Che Y proteins, thus reversing the cooperative interaction and bringing down the local relative concentration of the phosphorylated Che Y proteins. This reverses the flagellum rotation to the CCW direction, resulting in a swimming motion of the bacterium in a direction different from the previous swimming direction, thus completing the first cycle (tumbling followed by swimming) of a random walk of the bacterium and allowing the receptors to sample the state of a new environment. This scenario is compatible with the observed behavior of bacteria in the absence of stimulants, where long swimming and short tumbling motions alternate.

When a bacterium swims in a direction of increasing repellent concentration, the dynamic dimer model assumes an increase of the frozen receptor population in an open conformation (corresponding to the area **R** in Fig. 6) due to bound repellent and a decrease of the conformationally dynamic population with no bound repellent. Since the **R** conformation of the receptors causes phosphorylation of Che A, and therefore of Che Y, this situation would result in an additional increase in the phosphorylated Che Y concentration, thus more tumbling motion of bacteria (Fig. 7C). On the other hand, when the bacterium swims in a direction of increasing attractant concentration, there will be an increase of a "frozen" population in the "closed" conformation (corresponding to area **A** in Fig. 6) due to the bound

attractants and a decrease of a conformationally dynamic population with no bound attractants. Because now a smaller population of the receptors is in a dynamic state, the population that falls into conformational area **R** is expected to be smaller than when in the absence of attractants. This situation would result in a lower concentration of phosphorylated Che Y, thus causing a decrease in tumbling motion (Fig. 7B) and resulting in a biased random motion of the bacterium toward the attractants. The signals initiated at the receptor level can then be amplified by, for example, multiple turnover of 1 or more downstream signal transfer steps. In the presence of persistent stimulants, the sensitivity of receptors to the environment is reduced by methylation of the cytoplasmic domain by Che R (Russo & Koshland, 1983; Koshland, 1988).

The dynamic dimer model of the receptor provides a structural basis for understanding the chemotaxis signaling at a molecular level and presents a viewpoint different from the steady-state model (Koshland, 1980), where the microfluctuation of the phosphorylated Che Y concentration from an average steady-state value is proposed as the cause for the random walk in the absence of any stimulant. However, much more structural information is needed for all other participating mol-

ecules and their complexes as well as kinetic information for the multiple steps involved in the entire process of chemotaxis signaling. A structural viewpoint such as that presented here may complement recent kinetic analysis of the phosphorylation cascade in bacterial chemotaxis by computer simulation (Bray et al., 1993).

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