COMMENTARY

Transmembrane Signaling Is Anything but Rigid[▽]

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It is an anthropomorphic fallacy to think of molecular mechanisms in terms of mechanical equivalents. My biochemist friends shudder when I use Archimedes's six basic machines to describe how bacterial chemoreceptors function during transmembrane signaling. They prefer ΔG , ΔH , ΔS , and rate constants with lots of k 's in them. How right they have been becomes apparent upon reading "Mutational Analysis of the Control Cable That Mediates Transmembrane Signaling in the *Escherichia coli* Serine Chemoreceptor" by Kitanovic et al. (6) in this issue.

Recent models for transmembrane signaling have resorted to descriptions of pistons (1, 8), scissors (10), and gear boxes (5), with tuned springs thrown in for good measure (4). Now it appears that a rope or, more specifically, a control cable may be the operative analogy. The mutational analysis presented in the work of Kitanovic et al. (6) suggests that five residues between the aromatic anchor of the second transmembrane helix and the HAMP domain of the serine chemoreceptor Tsr (the control cable) tolerate most amino acid replacements. This observation argues against the existence of a rigid, highly structured connection between the periplasmic ligand-sensing domain and the cytoplasmic signal output domain of the receptor.

A fortuitously timed theoretical paper by Park et al. (9) describes molecular modeling of the behavior of the Tar chemoreceptor upon binding aspartate. The starting point was the structures of the ligand-free and ligand-bound periplasmic domains of Tar (16), the Af1503 HAMP structure (5), and a best-fit modeling of the first transmembrane (TM1) and second transmembrane (TM2) helices that flank the periplasmic domain. The TM domains were modeled in the context of a phosphatidyl-choline bilayer membrane. Phosphatidyl choline is not present in the membranes of *E. coli*, but this discrepancy probably has no significant effect on the outcome. The starting structure used for modeling is shown in Fig. 1A.

The essential conclusion from the modeling is that the 1.4-Å piston-like inward displacement of one helix 4 of the periplasmic domain induced by aspartate binding (9, 16) is converted into a bowing of TM2 (Fig. 1B). (Simultaneous inward displacements of both of the helix 4s should initiate similar, but more extreme, responses.) Trp-209 of the aromatic anchor (3) remains at the hydrophobic-polar interface on the cytoplasmic side of the membrane. To accommodate the bowing, Trp-209

moves several angstroms laterally along the membrane interface (Fig. 1B), thus displacing the control cables that are the focus of the mutational analysis of Kitanovic et al. (6). The molecular modeling predicts that this lateral movement increases the helicity and, hence, stiffness, of the control cable, which in turn would drive the N termini of the two AS1 helices closer together. A consequence of this movement is the splaying of the C termini of the AS2 helices of the HAMP domain (Fig. 1B), which communicate directly with the crucial adaptation region of the cytoplasmic coiled-coil four-helix bundle.

The results of Kitanovic et al. are in keeping with this scenario. Of the 19 possible replacements at each of the five residues (GIKAS) in the control cable, only six completely eliminate Tsr function. Another three severely compromise Tsr function, and a further nine partially disrupt function. Thus, only 18 out of 95 substitutions have noticeable effects. Four of these are Pro replacements, with each residue other than G213 being susceptible. In fact, G213Y was the only change at that position that gave a detectable phenotype. Several other substitutions introduced charge changes at I214 and K215, which might alter interactions with the polar head groups at the membrane surface; the I214E and K215I changes produced the only repellent-mimic (CheA kinase-stimulating, turn-on) phenotypes. Finally, introduction of aliphatic residues in place of S217 produced modest attractant-mimic (CheA kinase-inhibiting, turn-off) phenotypes.

The basic conclusion is that the control cable has helical properties but is unlikely to be a continuous, rigid helix connecting TM2 to AS1. This conclusion is supported by mutational analyses that show that the immediate downstream region at the beginning of AS1 also has some helical character but is unlikely to function as a rigid helix connecting the control cable and AS1 (14). The G213 residue, in particular, may act as a flexion joint between TM2 and the control cable; even the G213P Tsr variant had near-normal function. The helix-promoting aliphatic substitutions at S217, which generate an attractant-mimic phenotype, are consistent with the idea that attractant binding increases helicity of the control cable.

Changes that presumably destabilize helicity, however, like the Pro substitutions at residues 214 to 217, did not invariably produce repellent-mimic phenotypes. The complexity of the situation is compounded by evidence that the Tsr HAMP domain shows biphasic behavior (18), producing kinase-inhibiting output at the extremes of tight and loose packing, with kinase-stimulating behavior associated with intermediate degrees of packing. Thus, it is hard to predict what phenotype to expect from helix-destabilizing replacements in the control cable. What becomes clear, however, is that models that invoke a

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FIG. 1. Conformational changes induced by attractant (ATT) binding. (A) The periplasmic domain (light blue), TM helices (pink), and the HAMP domain (light green) of the unstimulated receptor dimer are shown in a pseudohelical representation. The membrane is shown as a lollipop cartoon, and the AS1-AS2 connectors in the HAMP domain are shown in red. The control cables are depicted by the short black lines connecting TM2 and AS1, and the connection of AS2 to the adaptation domain is shown in blue. (B) Stick diagram of the attractant-bound receptor. Binding of an attractant ligand (orange oval) at the apex of the periplasmic domain induces a 1- to 2-Å inward piston-like movement of helix 4 (downward-pointing arrow) of one subunit that is directly transmitted to TM2. The result is a bowing of that TM2 helix and a lateral movement of its C terminus (short horizontal arrow) by several angstroms at the hydrophobic-polar interface on the cytoplasmic side of the membrane. The resulting conformational change forced on the HAMP domain leads to an outward splaying of the C-terminal ends of AS2.

rigidly rotating or tilting helical connection between TM2 and the HAMP domain are unlikely to be correct, at least for *E. coli* chemoreceptors.

All of the recent genetic and biochemical analyses of intact chemoreceptors can be organized into a unified model for transmembrane signal transduction in which the HAMP domain acts as a dynamic bundle (17). Despite the speculative nature of some of the eight proposed steps, the model lends itself to experimental rebuttal or support. The first five steps are shown in the transition from Fig. 1A to Fig. 1B, and the remaining three steps are outlined in Fig. 2.

Step 1. Binding of aspartate (Tar) or serine (Tsr) near the membrane-distal apex of the periplasmic domain induces a 1- to 2-Å inward movement of helix 4.

Step 2. This inward movement pushes the N terminus of TM2 farther into the hydrophobic phase of the membrane. This event is consistent with the observation that W192, located at the hydrophobic-polar interface on the periplasmic side of the membrane, is not essential for Tar function (3). W192 may help position TM2 and the periplasmic domain properly in the ligand-free state but still be able to be displaced from the interface for signaling to occur.

Step 3. Because the cytoplasmic aromatic anchor (WY in *E. coli* Tar, WF in *E. coli* Tsr) clings stubbornly to the membrane interface, it is not displaced vertically but rather moves laterally. The length of TM2 is accommodated, according to the computer simulations, by a bowing of TM2 that displaces the aromatic anchor laterally in the membrane. This phenomenon may explain why Trp, the most energetically favored residue at

FIG. 2. Conformational changes in the cytoplasmic domain induced by attractant binding and adaptive methylation. (A) The HAMP domain (light green), adaptation domain (blue), flexible regions (orange), and kinase control region (magenta) of an unstimulated receptor. The C-terminal flexible region and the NWETF motif (black box) are also shown. The control cables are shown as the short black lines at the top. A single methylated glutamyl residue is indicated with a yellow circle in each subunit. The receptor is in a kinase-stimulating state. (B) After attractant binding, the HAMP domain undergoes a conformational change that leads to a splaying of the C termini of the AS2 helices. As a result, the adaptation domain becomes less tightly packed, and the kinase control domain becomes more tightly packed, leading to inhibition of receptor-stimulated CheA kinase activity. (C) Increased methylation of glutamyl residues in the adaptation domain, indicated by the three yellow circles per subunit, neutralizes repulsive negative charges and allows tighter packing of the adaptation domain. As a result, both the HAMP and kinase control domains return to their original conformations, and the receptor regains kinase-stimulating activity.

hydrophobic-polar interfaces within membranes (15), is found at this position in other chemoreceptors (3) and the histidine kinase NarX, which functions much like a chemoreceptor in transmembrane signaling (2, 12, 13).

Step 4. The lateral displacement of the aromatic anchor enhances the probability of a helical conformation in the control cable that may continue into the N terminus of helix AS1 of the HAMP domain.

Step 5. The formation of a continuous helix forces the two N-terminal ends of AS1 within a dimer closer together at the input end of the HAMP domain. In compensation, the C termini of the two AS2 helices are pushed farther apart.

Step 6. The splaying of the C termini of the AS2 helices disrupts the orderly packing of the four-helix bundle of the dimer in the adaptation region.

Step 7. According to the yin-yang model for interaction of the adaptation and signal output (CheAW interaction) domains (11), looser packing of the adaptation domain leads to tighter packing of the signal output domain and inhibition of CheA kinase activity.

Step 8. The loose packing of the adaptation domain stimulates the methylation of specific glutamyl residues in that region. This neutralization of repulsive negative charges favors tighter packing of the adaptation domain and restores the original packing of the HAMP and signal output domains. Thus, TM2 would go back to its original conformation (7), and the signal output domain would loosen up and again be able to stimulate CheA activity.

This scenario is comfortably consistent with the rigorous

genetic and biochemical experimental data on native chemoreceptors. Those salubrious attributes obviously do not guarantee its accuracy. It is also important to emphasize that other proteins with HAMP domains may have very different ways of coupling the input signal to the control of kinase/phosphatase activity. The delicate balance of protein conformation and communication between protein subdomains must always be kept in mind. It seems unlikely that artificial, chimeric constructs retain all of the properties of their intact progenitors. It is therefore advisable to be cautious about overinterpreting results from experiments utilizing such unnatural constructs.

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