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The Tie That Binds the Dynamic Duo: the Connector between AS1 and AS2 in the HAMP Domain of the *Escherichia coli* Tsr Chemoreceptor▽

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A phospholipid bilayer membrane provides a selective permeability barrier that separates a cell's world into "me" and "not me." A large fraction of a bacterial genome is devoted to encoding proteins that allow only the desired molecules to breach this barrier and become concentrated in, or excluded from, the intracellular space. The membrane also filters information about the environment using transmembrane receptors to communicate what is important to the cell. Transmembrane signaling is therefore one of the key biological activities to understand. In this issue, Ames et al. (1) present a high-resolution genetic analysis of one critical 14-residue segment of the *Escherichia coli* Tsr chemoreceptor. Their results suggest that a detailed mechanistic understanding of one type of transmembrane signaling is within reach.

Bacterial chemotaxis remains the system of choice for studying a number of fundamental questions about sensory transduction, partly because of the simplicity of the behavior and partly because of the tractability of bacteria as experimental subjects. *E. coli* cells swim in a three-dimensional random walk that consists of several seconds of swimming in gentle curves, called runs, which are punctuated by brief intervals (~0.1 s) of rapid, undirected reorientation, called tumbles. Runs correspond to the counterclockwise (CCW) rotation of all four to six left-handed helical flagellar filaments. Tumbles occur when at least one flagellum in the bundle rotates clockwise (CW). Runs toward higher attractant concentrations are extended because tumbles are suppressed, and the random walk is thus biased in a favorable direction (for a review of chemotaxis, see reference 17).

Bacterial chemoreceptors can be thought of as transmembrane allosteric enzymes. In Tsr, the regulatory site at which serine binds is near the apex of the extracellular (periplasmic) domain, and the catalytic subunit is the CheA kinase bound to the membrane-distal tip of the cytoplasmic domain (Fig. 1). These two regions are separated by a phospholipid bilayer and about 350 Å, a large distance on the molecular scale. Tsr is a homodimer, and serine binds at the dimer interface. The critical conformational change associated with serine binding is probably a downward, 1- to 2-Å piston-like displacement of the second transmembrane helix (TM2) into the cytoplasm. This event translates into a thousandfold decrease in the receptor-stimulated activity of CheA, which phosphorylates the response regulator CheY. The binding of phospho-CheY to flagellar motors promotes CW rotation, and decreased CheA activity inhibits tumbling and lengthens up-gradient runs (for a review of chemoreceptor function, see reference 8).

To migrate in an attractant gradient, a cell must continually adjust its sensitivity. Serine binding increases the rate of methylation of certain glutamate residues in the adaptation domain, whose negative charge is thereby neutralized. As a result, the stimulation of CheA is restored, the serine-induced displacement of TM2 is reversed (12), and the affinity of the regulatory site for serine (13) decreases, so the cells can now respond to further increases in serine concentration.

How can serine binding, manifested as a modest displacement of TM2, lead to drastic changes in the output activity of the receptor? A large part of the answer lies within the HAMP domain (hereafter simply HAMP), which connects TM2 to the rest of the cytoplasmic domain. HAMP contains two amphipathic helices of about 18 residues (6) joined by a flexible connector of 14 residues (Fig. 2A). HAMPs are widely distributed linkers of functional domains of homodimeric proteins in many organisms (4, 9), and they are often found immediately after a membrane-spanning helix. In Tsr, HAMP serves as a two-way conduit for information passed between the extracellular and intracellular domains.

A nuclear magnetic resonance structure of the Af1503 HAMP from the thermophilic archaeon *Archeoglobus fulgidis* (11) reveals a parallel four-helix bundle in which the connector nestles up against the bundle. The helices show knob-on-knob (x-da) packing rather than the more usual knob-in-hole (a-d-a) packing. Tsr HAMP has been modeled based on the Af1503 structure (Fig. 2B). Interactions of the connector with the bundle may stabilize knob-on-knob packing and serve as a regulator of receptor activity. Ames et al. (1) thus focused their attention on the connector.

They began with the premise that Tsr behaves as a two-state device, existing either in an "on" (CheA-stimulating) or "off" (CheA-inhibiting) form (2, 5). The equilibrium between these two states, both within an individual receptor and within the receptor patch (10, 14), determines the overall receptor activity and, thus, the intracellular level of phospho-CheY, to which the motors are exquisitely sensitive (7). If the connector stabilizes one or more conformations of HAMP, rather subtle changes could have profound effects on receptor function. Accordingly, each of the 14 residues in the connector (positions 234 through 247) was randomized by degenerate codon mutagenesis, and 179 of the 266 possible single-residue substitutions were identified. All of the mutant receptors were then tested for their ability to mediate serine taxis.

Only four connector residues appear to be specifically important for TSR connector function (Fig. 2C). All four residues are similar or identical between Tsr and the closely related Tar (aspartate) receptor (Fig. 2A). One, Gly-245, is part

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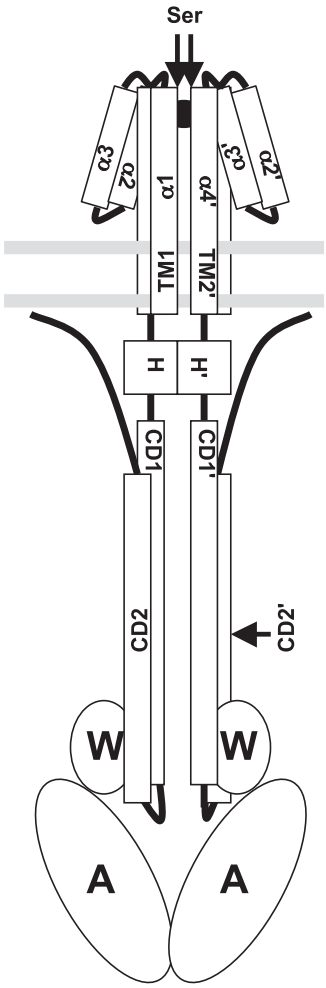

FIG. 1. Schematic diagram of the Tsr homodimer. One of the two subunits is designated as prime (′). The positions of the polar headgroups of the cytoplasmic membrane are indicated in gray. The four helices of the periplasmic domain are labeled α1 through α4, and the two transmembrane helices are shown as TM1 and TM2. HAMP is indicated by H, and the two long helices that participate in the extended four-helix bundle of the cytoplasmic domain are labeled CD1 and CD2. The curved black line represents the unstructured C-terminal region. Serine is depicted as a black oval bound at the periplasmic dimer interface. The two arrows indicate that serine can bind at either of two rotationally symmetrical sites.

of a flexible region that can be shortened by single-codon deletions or extended by the addition of up to 10 extra Gly residues. Only bulky, hydrophobic replacements at Gly-245 impair Tsr function seriously. This segment of the connector may simply have to be long and pliable enough to allow the four-helix bundle to form.

Position 235, also normally occupied by Gly, is less permissive. Some replacements there lock Tsr in the "on" signaling state, and others lock it "off." Gly-235 is in the middle of a tight turn leading from AS1 into the rest of the connector (Fig. 2C), suggesting that the conformation of this turn can favor either the "on" or "off" signaling state. Some "locked-off" Tsr proteins were dominant in heterodimers, suggesting that certain residues at position 235 stabilize the CCW-signaling state sufficiently to impose it upon the entire four-helix bundle. Substitutions that lead to locked phenotypes were previously found in other parts of Tsr (2).

Leu-237 and Ile-241 pack up against the bundle and might be expected to interact with AS1 or AS2 (Fig. 2C). Substitutions at these positions generate a plethora of signaling phenotypes. Nonfunctional replacements at Ile-241 lead to CW-biased, CW-locked, or, in one case (I241S), bipolar signal outputs. (A bipolar receptor is locked "on" in the absence of adaptive methylation and "off" in its absence.) These results are consistent with a role for Ile-241 in stabilizing the "off" conformation of HAMP.

At Leu-237, different replacements generate CW-biased, CCW-biased, CW-locked, CCW-locked, bipolar, or inverted responses. (An inverted receptor, like L237Q, is locked "off" in the absence of adaptive methylation/demethylation and locked "on" in its presence.) Different substitutions might stabilize, or destabilize, either the "on" or "off" conformation. Thus, Leu-237 may function as a "toggle" or "ratchet" between the two signaling states (1). None of the mutations targeting Leu-237 or Ile-241 are dominant in heterodimers, so their effects are mild enough be overcome by a wild-type HAMP subunit.

An ultimate understanding of an allosteric mechanism requires knowing how the binding of the regulatory ligand initiates the conformational change that alters the activity of the catalytic site. In Tsr, HAMP is clearly crucial to this process, so what new insights does the current study offer? Most obviously, it shows that there is a very delicate balance between the two signaling states. Chemically conservative substitutions at either Leu-237 or Leu-241 can drastically shift the equilibrium between the "on" and "off" states. Thus, precise steric interactions between the connector and the four-helix AS1-AS2 bundle must modulate HAMP function.

Does this knowledge allow us to correlate a signal output with a particular conformation of HAMP? Not yet, but perhaps soon. There is no direct evidence that the modeled Tsr HAMP structure corresponds to either of the two signaling states in the intact protein. However, cysteine- and disulfide-scanning analyses demonstrate that a four-helix HAMP bundle exists in the intact Tar receptor (15) and the intact Aer redox receptor (19).

Two models have been proposed to explain HAMP function (Fig. 3). Williams and Stewart (20) suggested that AS1 interacts either with the cytoplasmic face of the cell membrane or with AS2. The amphipathic nature of AS1 should allow it to align parallel to the membrane, with its hydrophobic face in contact with the hydrophobic interior of the phospholipid bilayer and its hydrophilic face in contact with the polar headgroups. Extending TM2 into the cytoplasm could destabilize AS1-membrane interactions, so in this model, the four-helix bundle would correspond to the "off" conformation of HAMP. However, no one has shown that AS1 interacts with the membrane in an intact receptor. Furthermore, many HAMP-containing proteins, including Aer, have no known transmembrane signal.

Alternatively, Hulko et al. (11) proposed that signaling involves a 26° rotation of AS1 relative to AS2 that converts knob-on-knob packing to knob-in-hole packing. It is not clear which conformation would correspond to which signaling state, although interactions with the connector should be crucial for stabilizing either one. However, it is not obvious how a piston-

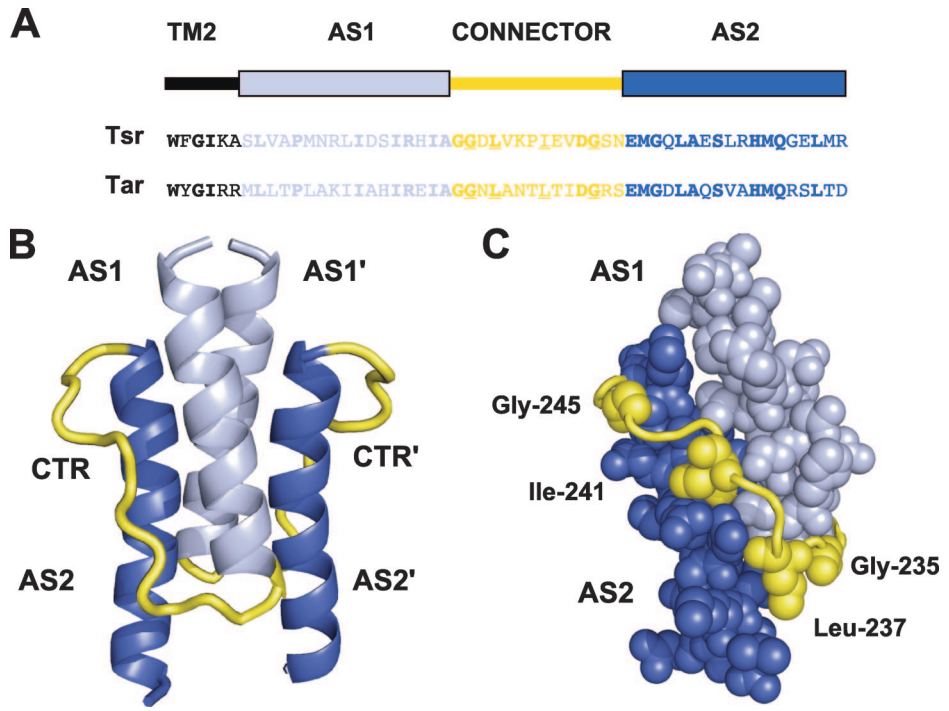

FIG. 2. HAMP structure. (A) The top drawing shows a linear representation of HAMP. The precise boundary between TM2 and AS1 is not known; the boundary shown is based on the Af1503 HAMP structure (11). Beneath that, the Tsr and Tar amino acid sequences are shown, using the same color scheme as in the upper diagram. Identical residues are shown in boldface type. Residues in the Tsr connector that are susceptible to replacements that destroy or impair receptor function are underlined. (B) Ribbon diagram of the four-helix bundle of the Tsr HAMP domain modeled on the nuclear magnetic resonance structure from Af1503. The color scheme is the same as in panel A. (C) Space-filling model of one Tsr HAMP subunit. In the connector, only the residues at which substitutions conferred strong chemotaxis phenotypes are labeled. The color scheme is the same as in panel A. (Images in B and C are courtesy of J. S. Parkinson.)

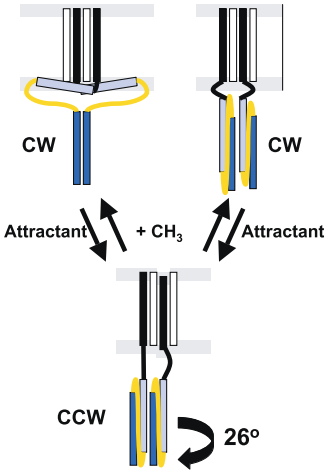

FIG. 3. Two models for Tsr HAMP function. The diagram at the upper left is based on data reported by Williams and Stewart (20). In the "on" (CW) conformation of the receptor, the AS1 and AS1′ helices are oriented nearly parallel to, and embedded in, the inner face of the cell membrane, with the hydrophobic face of the helix in contact with the hydrophobic interior of the membrane and the hydrophilic face in contact with the cytoplasm or the phospholipid head groups (indicated in gray). The diagram at the upper right is based on the data described previously by Hulko et al. (11). In the "on" conformation, the four-helix bundle is in one of its structurally stable arrangements, with either knob-on-knob or knob-in-hole packing. The bottom diagram shows the receptor in an "off" (CCW) configuration. One of the TM2 helices is pushed down slightly, and HAMP is in the "off" conformation. The four-helix bundle has rotated 26° from the hypothetical "on" state shown at the upper right.

like movement of TM2 would apply torque to the four-helix bundle.

The phenotypes of the Tsr connector mutations can be reconciled with either model. CW-biased or locked mutants could destabilize the "off" conformation regardless of what the "on" conformation might be. Similarly, substitutions that lock the receptor CCW could stabilize the "off" state or destabilize the "on" state.

The inverted phenotype of L237Q is difficult to explain, although inverted responses were seen previously in chemoreceptors (16). The Tsr cytoplasmic domain expressed by itself is in an "on" signaling mode (3). If attractant binding places HAMP in a strained conformation in which it inhibits CheA, methylation could reverse the attractant-induced change to restore the relaxed "on" state. If the L237Q substitution stabilizes a different strained conformation of HAMP, CheA activity also might be inhibited. This conformation could stimulate demethylation instead of methylation, which would push the signaling state even more toward "off." (Two signaling states do not necessarily imply only two HAMP conformations.)

A final thought is that HAMP may not always function in the same way. Some receptors, like Tsr and NarX (18), clearly transmit signals across the membrane. Others, like Aer, have no known transmembrane signal. HAMP may be a highly adaptable structure that can be utilized in various ways to achieve interdomain communication in a wide variety of pro-

teins. The multiple aspects of bacterial chemoreceptors that can be monitored, including the observation of both the positive and negative signaling states and adaptive methylation, provide very sensitive assays for teasing apart the multiple roles that HAMP may play in transmembrane signaling.

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