Structure
Previews

The Piston Rises Again

Joseph J. Falke[1,*] and Annette H. Erbse[1]
[1]Department of Chemistry and Biochemistry and the Molecular Biophysics Program, University of Colorado, Boulder, CO 80309-0215, USA
*Correspondence: falke@colorado.edu
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Previous evidence has indicated that the transmembrane signal in bacterial chemoeceptors is carried by the piston displacement of a membrane-spanning signaling helix. Hendrickson and coworkers (Cheung and Hendrickson, 2009; Moore and Hendrickson, 2009) now provide structural evidence that suggests piston transmembrane signaling is widely conserved in bacterial receptors that control ubiquitous two-component signaling pathways.

In bacteria, chemoreceptors and sensor kinases control nearly all cellular responses to extracellular signals and changing environmental conditions (reviewed in Hazelbauer et al. (2008) and Szurmant et al. (2007)). A typical bacterium possesses 10–50 chemosensory signaling pathways, each regulated by a set of chemoreceptors or by a sensor kinase that recognizes different external cues. Typical chemoreceptors and sensor kinases are transmembrane proteins with an external, ligand-specific periplasmic domain, two membrane-spanning helices per subunit, and a cytoplasmic domain consisting of several conserved structural motifs. In the systems characterized thus far, pairs of identical subunits combine to form homodimers, and, in the case of the best-studied chemoreceptors, further combine to form a hexagonal lattice of trimers-of-dimers, yielding an ultrasensitive, ultrastable signaling array (Erbse and Falke, 2009; Hazelbauer et al., 2008; Szurmant et al., 2007). In transmembrane chemoreceptors, the cytoplasmic domain begins with an N-terminal HAMP domain that converts the transmembrane conformational signal into a different type of conformational change, which is transmitted through the three regions of a C-terminal kinase control module: an adaptation region containing adaptive methylation sites, a coupling region, and a protein interaction region that binds and regulates the His kinase CheA (Hazelbauer et al., 2008). In transmembrane sensor kinases, termed His kinase receptors, the cytoplasmic domain begins with an N-terminal HAMP, PAS, GAF, or other domain, followed by a C-terminal His kinase module containing a conserved dimerization histidine-phosphorylation domain (DHp) and a conserved catalytic core (Szurmant et al., 2007; Marina et al., 2005). Both chemoreceptors and His kinase receptors regulate the histidine kinases that control two-component signaling pathways, the predominant type of bacterial signaling circuit.

Bacterial chemoreceptors initiate cellular chemotaxis in response to concentration gradients of chemical attractants and repellents (Hazelbauer et al., 2008). Multiple independent studies have indicated that ligand binding to the periplasmic sensor domain generates a transmembrane signal carried by a piston displacement of a membrane-spanning signaling helix (reviewed in Falke and Hazelbauer (2001)). The first view of this displacement was provided by a distance difference matrix-guided superposition (Chervitz and Falke, 1996) of apo- and attractant-occupied crystal structures solved for the aspartate chemoreceptor periplasmic domain (Milburn et al., 1991), which revealed a 1.6 Å, ligand-induced piston displacement of the C-terminal signaling helix toward the cytoplasm, relative to the N-terminal helix of the same subunit (Figure 1A) (Chervitz and Falke, 1996). In the full-length, membrane-bound receptor, this piston displacement can be trapped in the "up" or "down" state by engineered, signal-locking disulfide bonds (Chervitz and Falke, 1996; Chervitz et al., 1995), and can be detected by changes in disulfide formation rates between engineered pairs of Cys residues in the chemoreceptors of intact bacteria (Hughson and Hazelbauer, 1996). The piston displacement has been detected by EPR (Ottemann et al., 1999), and the piston can be toggled up and down by mutating the electrostatic and hydrophobic anchors of the signaling helix that define its basal position in the lipid bilayer (Draheim et al., 2005; Miller and Falke, 2004). The apo aspartate receptor dimer possesses two symmetric ligand binding sites but, due to negative cooperativity between these sites, only one is occupied (Milburn et al., 1991), thus the piston displacement of the C-terminal signaling helix is triggered in only one subunit yielding an asymmetric transmembrane signal within the homodimer (Figure 1A) (Chervitz and Falke, 1996).

Two new crystallograpic studies of periplasmic sensor domains from the His kinase receptors NarX and TorS, both published this year in *Structure* (Cheung and Hendrickson, 2009; Moore and Hendrickson, 2009), now provide the first structural evidence that His kinase receptors share the same piston transmembrane signaling mechanism as chemoreceptors. The homodimeric NarX receptor binds nitrate and nitrite, and controls the respiratory response to these extracellular ligands. The transmembrane signaling module of NarX exhibits the same domain layout as that of chemoreceptors; the periplasmic domain is a dimer of 4-helix bundles, which sends a conformational signal through the 4 transmembrane helices of the dimer to a cytoplasmic HAMP domain. Structural comparison of apo- and attractant-occupied NarX reveals a ~1 Å ligand-induced piston displacement of the C-terminal helix toward the periplasm relative to the N-terminal helix within the same subunit (Figure 1B) (Cheung and Hendrickson, 2009). NarX possesses a single ligand binding site at the central axis of the dimer such that both subunits of the dimer undergo similar piston displacements, retaining approximate dimer symmetry in both signaling states and generating a symmetric piston signal involving both C-terminal signaling helices of the dimer.

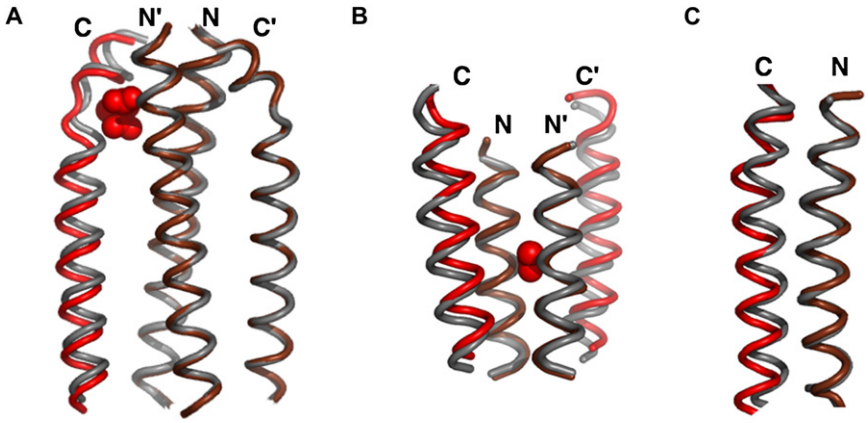

Figure 1. Initiating Transmembrane Signals: Relative Piston Displacements of the N- and C-Terminal Helices in 3 periplasmic Sensor Domains

(A) Aspartate-induced piston displacement of the C-terminal signaling helix in the dimeric periplasmic ligand binding domain of the aspartate receptor stTar. Shown is a distance difference matrix-guided superposition of the apo (gray) and aspartate-occupied (brown and red) crystal structures of the periplasmic domain. Only the N- and C-terminal helices of each subunit, which couple directly to the two transmembrane helices, are depicted. Due to negative cooperativity between the two aspartate binding sites, only a single aspartate molecule (red) binds to the dimer, triggering a piston displacement of the C-terminal signaling helix in one subunit toward the cytoplasm (*down* in this perspective, new position highlighted in red).

(B) Nitrate-induced piston displacement of the C-terminal signaling helix in the dimeric periplasmic ligand binding domain of NarX. Shown is a superposition of the apo (gray) and nitrate-occupied (brown and red) crystal structures of the periplasmic domain. Only the N- and C-terminal helices of each subunit, which couple to the transmembrane helices, are depicted. This image was generated by superimposing the N-terminal helices of the two structures, so as to emphasize the symmetric, ligand-induced piston displacements of both C-terminal signaling helices toward the periplasm (*up* in this perspective, new positions highlighted in red).

(C) Superposition of the apo, monomeric periplasmic ligand binding domains of $vpTorS_S$ and $ecTorS_S$. In the full length receptor, these ligand binding domains are believed to exist as homodimers like those in (A) and (B), but currently crystal structures are only available for each monomer. Shown is an overlay of $vpTorS_S$ (gray) and $ecTorS_S$ (brown and red). Only the N- and C-terminal helices of the subunit, which couple to the transmembrane helices, are depicted. A piston displacement of the C-terminal signaling helix is observed (new position highlighted in red). All images were made in MacPyMol.

The TorS receptor, also believed to be homodimeric, senses the periplasmic binding protein TorT occupied by its ligand trimethylamine-N-oxide (TMAO), thereby regulating the respiratory response to this terminal electron acceptor. Thus far, apo structures have been solved for monomeric TorS periplasmic domains from two different bacterial species. Comparison of these structures suggests they are in different structural states distinguished by a relative piston displacement of the N- and C-terminal helices (Figure 1C). Thus, the TorS periplasmic domain can also generate a piston signal, perhaps slightly larger than the 1-2 Å magnitude observed for the other two periplasmic domains, although the effects of the different sequences on the superposition may also contribute to the larger magnitude (Moore and Hendrickson, 2009).

Overall, the structural studies of periplasmic sensor domains from chemo- and His kinase receptors. Each reveal relative piston displacements of the N- and C-terminal periplasmic helices. Other helices in these periplasmic domains undergo displacements as well, but the N- and C-terminal helices are of special importance because they are believed to be continuous with the two transmembrane helices that anchor each subunit in the membrane and carry transmembrane signals (Chervitz and Falke, 1996; Cheung and Hendrickson, 2009; Moore and Hendrickson, 2009). The simplest model proposes that displacements of the N- and C-terminal helices in the NarX and TorS periplasmic domains drive relative piston movements of the transmembrane helices, thereby sending a conformational signal through the lipid bilayer to the cytoplasmic domain, as already demonstrated for the aspartate and other chemoreceptors (Falke and Hazelbauer, 2001; Hazelbauer et al., 2008). In the aspartate receptor and NarX, the piston displacements of the C-terminal signaling helices carry the signal to a cytoplasmic HAMP domain. For these periplasmic domains, the piston displacements are similar in magnitude but opposite in direction, consistent with their known opposite effects on His kinase activity in chimeric receptors (for example Ward et al. (2002)). The latter chimeras, constructed by swapping modules between chemoreceptors and His kinase receptors, provide additional evidence that the same piston displacements observed in isolated periplasmic domains are responsible for transmembrane kinase regulation in full-length receptors of both classes (Ward et al., 2002).

Another interesting difference between the aspartate receptor and NarX periplasmic domains is the contrasting symmetries of their ligand-induced piston signals; in the aspartate receptor, the signal is carried in only one subunit, while in NarX it is carried in both subunits (Figures 1A and 1B) (Chervitz and Falke, 1996; Cheung and Hendrickson, 2009). This apparent discrepancy does not, however, represent a fundamental mechanistic difference between their transmembrane signaling mechanisms. It has been shown by protein engineering studies of the aspartate receptor that symmetric modifications (disulfide bonds or anchor mutations) designed to simultaneously toggle both C-terminal signaling helices of the dimer up or down yield normal on-off switching (Chervitz et al., 1995; Miller and Falke, 2004; Draheim et al., 2005). Thus, both asymmetric and symmetric piston transmembrane signals can modulate cytoplasmic His kinase activity.

The small-magnitude piston-type helix sliding displacement observed in bacterial sensor domains is ideally suited for transmembrane signaling through a long membrane spanning helices for three reasons (Falke et al., 1997; Falke and Hazelbauer, 2001). First, small molecule binding to a sensor domain provides relatively little binding free energy capable of doing conformational work. A relative helix sliding displacement requires little driving energy as long as its magnitude is less than ~2 Å, so that specific side chain contacts and ridges-grooves packing between adjacent helices are largely maintained. Both the aspartate receptor and NarX piston displacements fall within this low-energy range (Chervitz and Falke, 1996; Cheung and Hendrickson, 2009), as

required by the fact they are regulated by small molecule binding. The somewhat larger displacement proposed for TorS (Moore and Hendrickson, 2009) is not unreasonable since it binds a regulatory protein, TorT, and the resulting protein-protein interaction could perhaps generate enough binding free energy to drive larger changes in side chain and ridges-grooves interactions. Second, transmembrane signals in bacterial receptors must span distances of 150 Å or more from the periplasmic ligand binding site to the cytoplasmic domain, and thus must be transmitted over a remarkably long distance. To a first approximation, the H-bonding framework of an α helix is incompressible along the helix axis, ensuring that a piston force pushing on one end of a helix will be faithfully transmitted throughout the entire helix length. By contrast, helix bends, rotations, or tilts can be more easily damped by long-range helix flexibility over these distances. Third, a small 1-2 Å displacement is large enough to directly regulate the on-off switching of a kinase active site, or trigger a larger structural rearrangement in a signal conversion module such as the HAMP domain. Thus, it appears likely that chemoreceptors and His kinase receptors have retained the same piston mechanism of transmembrane signaling for good biophysical reasons.

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Polyglutamine Dances the Conformational Cha-Cha-Cha

Jason Miller,[1,2,3] Earl Rutenber,[1] and Paul J. Muchowski[1,4,*]
[1]Gladstone Institute of Neurological Disease
[2]Chemistry and Chemical Biology Graduate Program
[3]Medical Scientist Training Program
[4]Departments of Biochemistry and Biophysics, and Neurology
University of California, San Francisco, CA 94158, USA
*Correspondence: pmuchowski@gladstone.ucsf.edu
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While polyglutamine repeats appear in dozens of human proteins, high-resolution structural analysis of these repeats in their native context has eluded researchers. Kim et al. now describe multiple crystal structures and demonstrate that polyglutamine in huntingtin dances through multiple conformations.

There are 66 human proteins with a homopolymeric stretch of five glutamines or more. The overrepresentation of polyglutamine (polyQ)-containing proteins in transcription-related processes suggests a critical function for these repeats (Butland et al., 2007). At least 9 of these 66 proteins have a polyQ stretch that, when expanded beyond a critical threshold, misfold, aggregate, and cause neurodegenerative diseases. Although the structural basis that underlies the toxicity of proteins with expanded polyQ repeats is not clear, numerous laboratories have hypothesized that a variety of misfolded conformers, including monomers, oligomers, and fibrils, are the toxic culprits.

Into this debate enters the heroic crystallography feat of Kim et al. (2009). The authors solved seven independent crystal structures of a Q_{17}-containing exon1 fragment of wild-type huntingtin (Htt^{ex1}), a multifunctional protein that, when mutated in the polyQ stretch ($>Q_{36}$), causes a devastating neurodegenerative disorder called Huntington's chorea (chorea, derived from Greek, describes the involuntary dance-like movements of Huntington's patients). Reminiscent of the dance-like contortions of affected patients, the