Sensory Transduction to the Flagellar Motor of Sinorhizobium meliloti

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Abstract

Molecular mechanisms that govern chemotaxis and motility in the nitrogen-fixing soil bacterium, Sinorhizobium meliloti, are distinguished from the well-studied taxis systems of enterobacteria by new features. (i) In addition to six transmembrane chemotaxis receptors, S. meliloti has two cytoplasmic receptor proteins, McpY (methyl-accepting chemotaxis protein) and IcpA (internal chemotaxis protein). (ii) The tactic response is mediated by two response regulators, CheY1 and CheY2, but no phosphatase, CheZ. Phosphorylated CheY2 (CheY2-P) is the main regulator of motor function, whereas CheY1 assumes the role of a ‘sink’ for phosphate that is shuttled from CheY2-P back to CheA. This phosho-transfer from surplus CheY2-P to CheA to CheY1 replaces CheZ phosphatase. (iii) S. meliloti flagella have a complex structure with three helical ribbons that render the filaments rigid and unable to undergo polymorphic transitions from right- to left-handedness. Flagella rotate only clockwise and their motors can increase and decrease rotary speed. Hence, directional changes of a swimming cell occur during slow-down, when several flagella rotate at different speed. Two novel motility proteins, the periplasmic MotC and the cytoplasmic MotD, are essential for motility and rotary speed variation. A model consistent with these data postulates a MotC-mediated gating of the energizing MotA-MotB proton channels leading to variations in flagellar rotary speed.

Overview

Motile bacteria are propelled by helical flagellar filaments that enable them to swim towards an optimum environment. Mechanisms of swimming, chemoreception, and signal transduction have been most intensely studied in Escherichia coli and Salmonella typhimurium (Macnab, 1996; Stock and Surette, 1996). An increasing interest in non-enteric bacteria in recent years led to new insights into structures and molecular mechanisms that differ from the enterobacterial paradigm (Armitage and Schmitt, 1997). Members of the α-subgroup of bacteria, such as Agrobacterium, Caulobacter, Rhodobacter and Sinorhizobium exhibit variations on the basic chemosensory pathway as a consequence of more than 500 million years separate evolution (Ochman and Wilson, 1987) and as an adaptation to their different environments. The rhizosphere bacterium, S. meliloti, induces nodule formation in the rootlets of Medicago sativa for nitrogen fixation. Motility in viscous soil and detection of attracting root exudates from host plants requires complex patterns of chemosensing, signalling, swimming and motor control (Figure 1). In S. meliloti, all the genes involved in these processes are combined in one large cluster referred to as the “flagellar regulon”. By contrast, the corresponding E. coli genes are dispersed onto in three different chromosomal regions. The flagellar regulon of S. meliloti contains all genes necessary for chemotaxis, biosynthesis, motor rotation, as well as regulatory genes. Gene expression is controlled as a three-level hierarchy and involves transcription factors different from the enterobacterial system (Sourjik et al., 2000).

Chemosensing

Sensing a change in the external concentration of an attractant or repellent by an E. coli cell relies on a conformational change of dimeric membrane-spanning receptors, the MCPs (methyl-accepting chemotaxis proteins; Mowbray and Sandgren, 1998). S. meliloti is similarly capable of sensing and reacting to numerous attractants, including sugars, amino acids, flavonoids, and other stimuli from the host plant (Götz et al., 1982; Malek, 1989). Seven distinct mcp-genes encoding McpT – McpZ serve as transducers for attractants (Figure 1). A deletion of any one of these results in a reduction of chemotaxis towards a set of attractants, whereas deletion of all receptors causes a complete loss of the tactic response. This stepwise reduction of the response may result from “cross-talk” between several receptors. One receptor, McpY, is distinguished by its cytoplasmic location and the possession of two tandem PAS domains, known to bind FAD (Taylor and Zhulin, 1999). We presume that McpY senses the redox state of a cell, thus mediating aerokinesis (acceleration towards oxygen; Zhulin et al., 1995). There is a second soluble sensor, IcpA (internal chemotaxis protein, formerly named Orf1; Greck et al., 1995), present in S. meliloti. This protein is encoded by the first gene in the chemotaxis (che) operon (Sourjik et al., 1998). The encoded 57-kDa protein contains a conserved signaling domain, but neither hydrophobic...
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transmembrane domains nor a periplasmic receptor domain, both features of "classical" MCP's. Overexpressed IcpA interferes with *E. coli* chemotaxis, probably by binding essential components, such as CheW and CheA. IcpA is a good candidate for sensing internal stimuli that reflect the metabolic state of a cell.

**Signaling Pathway**

Unlike in *E. coli*, the signal transduction chain of *S. meliloti* contains two different response regulators, CheY1 and CheY2, but no CheZ phosphatase (Figure 1). This is the basis for a new CheY2-P dephosphorylation mechanism that appears to be ubiquitous among \(\alpha\)-subgroup proteobacteria. Phosphorylation of CheY2 and CheY1 by the CheA autokinase is the crucial step in signal transduction. CheY2-P is the chief regulator of motor function, its action being modulated by CheY1 (Sourjik and Schmitt, 1996). The absence of attractant elicits ATP-dependent autophosphorylation of CheA, which in turn rapidly transfers phosphoryl groups to CheY1 and CheY2; CheY2-P directs the slow-down of motor rotary speed (see below). In the presence of an attractant, CheA is inactive; surplus CheY2-P then retro-transfers its phosphoryl group to CheA, which in turn phosphorylates CheY1 (Sourjik and Schmitt, 1998). CheY1 molecules in a cell are in 10-fold excess over CheA; moreover, CheY1-P autodephosphorylates constantly (\(\tau/2 \sim 10\) sec). CheY1 thus acts as a sink for phosphoryl groups from CheY2-P and emulates the role of a CheZ phosphatase. This mechanism was first detected in *S. meliloti* and now appears as a general feature of the signal transduction chain among members of the \(\alpha\)-subgroup of proteobacteria. In this scheme (Figure 1), CheY2-P and CheY1-P differ in that the former is capable of back-shuttling the phosphoryl group to CheA, whereas the latter is not (Sourjik and Schmitt, 1998). An interpretation compatible with other phospho-relay systems (Stock and Da Re, 2000) predicts that the high free energy (\(\Delta G^{\circ} \sim -14\) kcal mol\(^{-1}\)) of CheA histidyl phosphate is retained by the aspartyl phosphate of the free CheY2-P (E1 state), however, the latter switches to the low-free energy E2 state by a change of conformation when binding to its substrate at the motor (Figure 1). By contrast, CheY1-P may spontaneously assume the conformation that predisposes the low-free energy state (E2), of the aspartyl-phosphate bond (\(\Delta G^{\circ} \sim +2\) kcal mol\(^{-1}\)). The molecular structures of CheY1 and CheY2 and of their phosphorylated derivatives are now being compared by high-resolution NMR, in order to elucidate structural details that may support this interpretation.

![Diagram](image)
Rotary Speed Variation of the Flagellar Motor

Flagellar filaments and their mode of rotation differ greatly between enteric bacteria (γ-proteobacteria) and S. meliloti (α-proteobacteria), due to their long phylogenetic distance (Ochman and Wilson, 1987) and the need of adaptation to different biotopes. The soil bacterium S. meliloti is driven by rigid, right-handed flagella allowing the cell to swim efficiently in viscous media (Götz et al., 1982). Flagellar filaments are assembled from four closely related flagellin subunits (FlaA-FlaD) encoded by linked, but independently transcribed genes (Pleier and Schmitt, 1989; Sourjik et al., 1998). The complex fine structure of S. meliloti filaments is dominated by three helical ribbons that lock the filament in the right-handed conformation thus disabling it from switching hand (Schmitt et al., 1974; Trachtenberg et al., 1987). Concomitantly, the motor of S. meliloti does not reverse its sense of rotation. Rather, a swimming cell is propelled by the unidirectional clockwise (CW) rotation of its right-handed flagellar filaments. The flagellar motors of S. meliloti can increase and decrease their rotary speed (chemokinesis). The decrease of the rotary speed of the five to ten peripherously inserted flagella is asynchronous and thus leads to a change of swimming direction (“caterpillar drive”). The different modes, by which E. coli and S. meliloti cells accomplish directional changes are illustrated in Figure 2. The former briefly reverse the sense of flagellar rotation, which results in a “tumble” motion; the latter reduces the rotary speed of individual flagella without changing the CW sense of rotation, which results in smooth or abrupt “turns” in the swimming path. This speed variation of the S. meliloti flagellar motor is a new feature, which has a molecular corollary in two new motility proteins, MotC and MotD, found in addition to MotA/MotB that form the energizing proton channels (Figure 1). The cytoplasmic MotD may

Figure 2. Comparison of the swimming patterns (top) of E. coli and S. meliloti and the corresponding modes of flagellar filament rotation (bottom). Note that the “biased random walk” in each case alternates between straight “runs” and directional changes. To this end, an E. coli cell reverses the rotation of its left-handed helical flagella from CCW (counterclockwise = run) to CW (clockwise = tumble), whereas the S. meliloti right-handed flagella persist in CW-rotation and increase rotational speed for straight runs (bundle formation) or decrease the rotational speed of individual flagella in an asynchronous fashion, which turns the swimming cell into a new direction (“caterpillar drive”).
function as a relay for tactic signals from the transduction chain to the periplasm. MotC is a periplasmic protein that interacts with the channel protein MotB, as shown by yeast two-hybrid studies. We have postulated that binding of MotC to MotB is an essential prerequisite for proton flow (MotA/B channel open) and that the dissociation of MotC from MotB (or a mutation in either protein) interrupts the proton flow (MotA/B channel closed; Platzer et al., 1997). The dynamics of interaction between MotB and MotC result in a fluctuating proton flow and, hence, in the observed variations of flagellar rotary speed. Systematic deletion analyses of motC led to the conclusion that the N-terminal region of MotC is essential for stability and integration of the protein into the motor complex, whereas the C-terminal region interacts with MotB and regulates the gating of the proton channels that energize the flagellar motor. The path of tactic signals from the cytoplasm to the periplasm that directs the MotC-mediated gating of proton channels is under current study.

References


