

Bacterial chemotaxis: **Unsolved mystery of the flagellar switch**

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Impressive progress has been made in understanding the mechanism of bacterial chemotaxis and function of the flagellar motor, but how the direction of rotation is reversed by the ‘flagellar switch’ – a central step in chemotaxis – remains obscure and calls for new experimental approaches.

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Although the phenomenon of bacterial chemotaxis was discovered almost 120 years ago [1], and the modern era of intensive investigation started almost four decades ago with the pioneering studies of Adler [2], the molecular mechanisms underlying bacterial chemotaxis remained a puzzle until some 15 years ago. The mechanisms of chemotactic signal transduction then began gradually to be revealed, and many pieces of the puzzle are now in place. One key step has remained a mystery, however: this is the problem of how the direction of rotation of the flagellar motor is switched, a key event in chemotaxis. Recent results shed new light on the nature of this process, but new approaches are needed to understand in molecular detail how the flagellar motor ‘shifts gear’.

CheY binding and the flagellar switch

Bacteria such as *Escherichia coli* or *Salmonella typhimurium* swim by rotating their flagella. By appropriate modulation of the direction of flagellar rotation, a bacterial cell approaches chemical attractants and avoids repellents (reviewed in [3]). The question of how the chemotaxis system is regulated thus reduces to that of how the direction of flagellar rotation is controlled. The default direction of rotation of the flagellar motor is counterclockwise. A switch to rotation in the clockwise direction occurs in response to a sensory signal, transduced by the chemotaxis protein CheY. CheY is a ‘response regulator’, one part of a ‘two-component’ signal transduction system, and is phosphorylated by the histidine kinase CheA. The phosphorylated form of the protein, CheY~P, binds to the flagellar switch at the base of the flagellar motor [4], and the result is clockwise rotation [5].

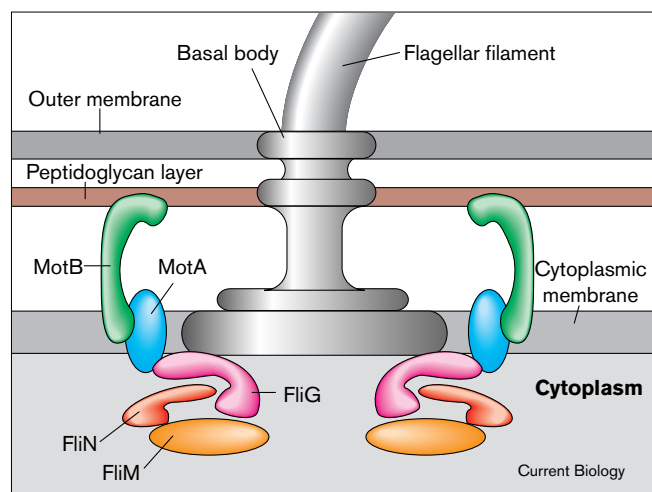
The flagellar switch extends from the base of the flagellar motor into the cytoplasm (Figure 1) [6,7]. The switch is composed of three proteins — FliG, FliM and FliN — which are involved in flagellar assembly, flagellar rotation and controlling the direction of rotation [8]. The amino

terminus of FliM is the CheY docking site [4,9]. Although much is known about how the phosphorylation state of CheY, and thereby its binding to the switch, are regulated (reviewed in [3,10]), the processes that occur within the switch after CheY binding are not known.

As mentioned above, CheY~P binding to the switch elicits clockwise rotation. Turner *et al.* [11], however, found that, at temperatures below about 10°C, motor reversals occur spontaneously even in the absence of CheY. They further showed that, close to 0°C, the clockwise state of the switch has a lower free energy and hence is preferred. Such reversals are highly unlikely at room temperature, where by extrapolation the standard free energy change of switching is about 40 kJ per mole. On the basis of a kinetic model — similar to models proposed earlier by Kuo and Koshland [12] and Macnab [8] — they concluded that, at room temperature, CheY~P binds more tightly to the switch in the clockwise mode than in the counterclockwise mode. It thus appears that CheY binding to the switch is necessary for reversal at room temperature, but not at temperatures close to 0°C.

Several observations appear to question whether binding of CheY to FliM is all that is needed to trigger the sequence of

Figure 1



The switch–motor complex that controls rotation of the bacterial flagellum. FliG, FliM and FliN are switch proteins; MotA and MotB are motor proteins outside of the basal body. There are about 40 molecules each of FliG and FliM per switch–motor complex. FliM is exposed to the cytoplasm and is connected to both FliN and FliG; FliG connects FliM to the basal body of the flagella (reviewed in [8]).

events at the switch that lead to motor reversal at room temperature. When a number of CheY variants generated by single amino-acid substitutions are compared, no correlation is seen between the ability of a variant to bind to the switch protein FliM *in vitro*, and clockwise rotation in cells expressing the variant. One example of this is that, although both phosphorylated and non-phosphorylated forms of the variant CheY95IV bind FliM with twofold higher affinity than the corresponding forms of wild-type CheY, clockwise enhancement is observed only under phosphorylating conditions [13]. This means that the enhanced binding observed with non-phosphorylated CheY95IV is not reflected in enhanced clockwise rotation.

A second example of the lack of correlation between CheY–FliM binding and clockwise rotation is the mutant *cheY106YW*, which exhibits enhanced clockwise rotation *in vivo*, even though, *in vitro*, CheY106YW binds to FliM with no higher affinity than wild-type CheY [14]. And a third example is CheY13DK, which can barely be phosphorylated *in vitro* [15] and binds FliM to a lesser extent than does wild-type CheY~P [16]; nevertheless, cells expressing this mutant protein are clockwise-biased, indicating that the protein is active [15]. Furthermore, although phosphorylation of CheY was shown to increase binding to FliM by an order of magnitude [4], this enhanced binding was found to be insufficient to generate clockwise rotation in cytoplasm-free envelopes, only doing so in the presence of an unidentified cytoplasmic constituent (not a known chemotaxis protein) [5]. All these observations suggest that CheY binding to the switch is not always directly linked to clockwise rotation.

There are a number of possible interpretations of these observations, which are not mutually exclusive. One is that, while bound to FliM *in vivo*, CheY~P might somehow modify the switch; for example, it might phosphorylate a switch or a motor protein. There is no evidence for phosphorylation of switch proteins, but observations have been made that suggest that phosphate groups — phosphate-binding sites or phosphorylation sites — may be involved in the motor function [17,18]. If switch modification is required to change the motor's rotation direction, normal binding of a mutant CheY that cannot modify the switch clearly will not result in enhanced clockwise rotation. Conversely, certain mutations may confer on CheY the ability to induce the clockwise conformation of the switch, even when the affinity of the mutant CheY protein for FliM is normal. This last possibility appears to be true of the active mutant CheY106YW, for example, where the side chain of residue tyrosine 106 is oriented differently than in inactive mutants with substitutions at residue 106 (CheY106YL, for example) [14,19].

A second possible interpretation is that CheY~P might bind to FliM in a sterically different orientation than

non-phosphorylated CheY. And a third possibility is that there could be an effective threshold in CheY–switch binding for the induction of a clockwise signal at the flagellar switch that is not crossed by the enhanced binding of the mutant CheY protein [13]. It thus appears that CheY binding to the switch is not sufficient for reversal, but it is not yet known what else is required.

Is the switch mechanism deterministic or stochastic?

A question that has been raised is whether the transition between the rotational states of the switch is stochastic, involving thermal isomerization, or deterministic, so that the state of rotation is completely determined by the degree of CheY binding to the flagellar switch. Recent work of Scharf *et al.* [20] has addressed this question. They used the double-mutant protein CheY13DK106YW, which is active without phosphorylation and thus eliminates complications resulting from changes in the dynamics of CheY phosphorylation and dephosphorylation. They were thus able to assay cell behaviour as a function of the intracellular concentration of active CheY. Extrapolating the data of Turner *et al.* [11] to room temperature, as discussed above, Scharf *et al.* [20] derived a linear relationship from the kinetic model expressing the variation in the standard free energy difference of switching with the fraction of CheY binding sites occupied. This gave a good fit to their own experimental results.

Scharf *et al.* [20] interpret their data as evidence that switching occurs by a stochastic mechanism, and does not exhibit cooperativity. A contrast was drawn between such a stochastic mechanism and a deterministic mechanism, such as that described by Bray *et al.* [21], where the state of rotation is completely determined by the degree of CheY binding. In our opinion, the distinction between deterministic and stochastic mechanisms is somewhat artificial, as it really involves nothing more than kinetics: a deterministic mechanism would be associated with a very fast transition between CheY binding and the conformational change assumed to mediate the switch in the direction of rotation, so that both the binding and the conformational change appear to be simultaneous. In contrast, in a stochastic mechanism the transition between CheY binding and the conformational change would be relatively slow, so that they are clearly distinct processes; the outcome of CheY binding in this case would be an increased probability of being in the clockwise state.

How does the switch work?

How the switch complex causes the flagellar motor to reverse direction on CheY~P binding, even though the polarity of the proton flux that drives the motor is unchanged, is an intriguing question. Evidently, the symmetry of the switch–motor complex must undergo a transformation to allow this. As the switch proteins form part of the rotor [6,7], it would appear that a conformational

change — for example, from left-handed to right-handed symmetry in a set of interaction sites arranged helically around the rotor [8,22,23] — must occur in at least part of the rotor, perhaps just in the switch complex itself. Such a conformational change should be fast, as rotational reversal is accomplished within less than a millisecond [24]. Unfortunately, no conformational studies have been reported yet, because such *in vitro* measurements require a purified preparation of the switch (or switch–motor) complex, which is not yet available.

In our view, the mechanism of the flagellar motor as a whole needs to be elucidated before the switch process can be completely understood. Furthermore, we may not yet know all of the players in the switching game. For example, fumarate was found to cause reversals in CheY-containing, cytoplasm-free envelopes of *E. coli* and *S. typhimurium* — which otherwise rotate their flagella exclusively in one direction [25,26] — and in intact, CheY-containing gutted cells [27]. Fumarate was shown to interact with the switch–motor complex, leading to a lowering of the free energy of the clockwise state relative to the counterclockwise state [28].

Conclusions

The conformation and steric orientation of CheY appear to be important, not only for binding to the switch complex, but also for triggering subsequent steps necessary to change the direction of the flagellar motor, steps that probably involve conformational changes of the switch proteins. Other factors, such as fumarate, may also be involved. It seems that the mechanism of the switch is far from being elucidated, and new experimental approaches will be required for this purpose.

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