

# The Turn of the Screw: The Bacterial Flagellar Motor

## Minireview

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Peritrichously flagellated bacteria, especially the two we will discuss, *Salmonella typhimurium* and its kissing cousin *Escherichia coli*, assemble about 10 flagella over their cell surfaces. Bacterial flagella differ in every aspect but name from eukaryotic flagella (undulipodia). In the eukaryotic flagellum, dynein motors powered by ATP generate moving bends by stepping along linear microtubular tracks. The eukaryotic flagellum undulates like a snake inside its cellular membrane. In contrast the bacterial flagellum works like a power boat with a rotary motor turning a rigid, helical propeller. The source of energy is not ATP but rather the electromotive gradient of protons or sodium ions across the cell's membrane. A working bacterial flagellum contains about 20 proteins, over an order of magnitude fewer than the number of protein species comprising the eukaryotic undulipodia, and not surprisingly it is about an order of magnitude smaller in its dimensions. (For a review of the bacterial flagellum, see Macnab, 1996).

In *E. coli* and *S. typhimurium*, flagella turning at speeds of 18,000 rpm push cells at 30  $\mu\text{m/s}$ , but the speed records are set by motors in other bacteria that turn at rates exceeding 100,000 rpm and push cells at hundreds of micrometers per second. What is all the more remarkable is that unlike kinesin, *ncd*, and myosin, flagellar motors can run in both directions, i.e., clockwise (CW) and counterclockwise (CCW). These motors appear to be more powerful than myosin, kinesin, and *ncd* motors (Table 1), and they also deliver constant torque of 4500 pN nm (Berry and Berg, 1997) at speeds over 6000 rpm, whereas force falls off with velocity for myosin, kinesin, and *ncd*. Only at high speeds does torque in flagellar motors decrease linearly with speed (Berry et al., 1995).

### Flagellar Architecture

More so than other motors, the flagellum resembles a machine designed by a human (Figure 1a). The flagellar filament (propeller) is a 10  $\mu\text{m}$ -long, thin, rigid, cork-screw-shaped structure, with a helical period of about 2  $\mu\text{m}$ . The filament is connected to the hook by two junctional proteins. Named according to its shape, the flexible hook acts as a universal joint permitting the filament and motor to rotate about different axes. The filament, junction, hook, and drive shaft all appear to have a common helical design. The remaining flagellar parts are rings. The L and P rings are believed to act as a bushing through which the rotating drive shaft passes. These two rings are anchored in the outer membrane and peptidoglycan, respectively. (Gram-positive bacteria, which do not have these layers, do not have L and P rings.) In the periplasm, the drive shaft inserts in a socket just above the S ring. The M ring is a 25 nm disk,

which traverses the cell's inner membrane. Extending into the cytoplasm from the extended M ring is the C ring, a 45 nm annulus. A ring of about 10 membrane particles known as studs surround each flagellar motor. These probably sit in the L-shaped shelf made by the M and C rings.

### Motor Mechanisms

Dozens of mechanisms have been proposed for the flagellar motor over the years (Caplan and Kara-Ivanov, 1993), but there is still too little information to decide among them. Over the last five years, however, there have been genetic, biochemical, and structural studies that give insights into which components are responsible for torque generation, assembly, and switching.

### The Components of the Motor: *MotA*, *MotB*, *FliG*, *FliM*, and *FliN*

About 50 genes are particular to the flagellum and its chemosensory machinery. These genes are named by their null phenotype: *Fla<sup>-</sup>* (occurring at three loci denoted *flg*, *flh*, or *flj*) if they make an incomplete flagellar structure; *Mot<sup>-</sup>* if the flagellum is completed but doesn't rotate; and *Che<sup>-</sup>* if the flagellum is made and rotates but doesn't reverse with appropriate frequency so that chemotaxis is impaired. There are  $\sim 40$  *fla* genes, which code for structural proteins, regulatory proteins, and proteins involved in assembly of the flagellum. There are two *mot* genes (*motA* and *motB*). There are 6 *che* genes and about an equal number of receptor genes, which make up the chemical computer that senses the environment and computes the response.

Of the 50 genes, only three, *fliG*, *fliM* and *fliN*, give rise to mutants that have all three phenotypes: *Fla<sup>-</sup>*, *Mot<sup>-</sup>*, and *Che<sup>-</sup>*. The products of these three, along with *MotA* and *MotB*, are likely to be involved in torque generation and motor reversal.

### *FliG*, *FliM*, and *FliN* Form a Complex

*FliG*, *FliM*, and *FliN* are cytoplasmic proteins, which form the switch complex, a structure important for torque generation and reversal of rotation direction. The switch is bistable; that is, it switches between two stable conformations. Under constant environmental conditions, a motor rotating CCW (i.e., the switch complex is in the CCW conformation) has a constant probability per unit of time of switching to CW rotation (i.e., to the CW conformation). Conversely, a motor rotating CW has a (different) constant probability of switching to CCW rotation. Phosphorylated *CheY*, the response regulator, alters the probability of switching by binding to the switch

Table 1. Statistics for Flagellar Motors of *S. typhimurium/E. coli* versus Myosin, Kinesin, and *ncd*

Rotational speed	300 rps vs. NA
Linear speed ( $r = 15$ nm)	30 $\mu\text{m/s}$ vs. $< 10$ $\mu\text{m/s}$
Torque/generator	$\sim 550$ pN nm vs. NA
Force ( $r = 15$ nm)	$\sim 35$ pN vs. 5–10 pN
Efficiency	unknown but could be $\sim 100\%$ vs. $\sim 50\%$

NA, not applicable.

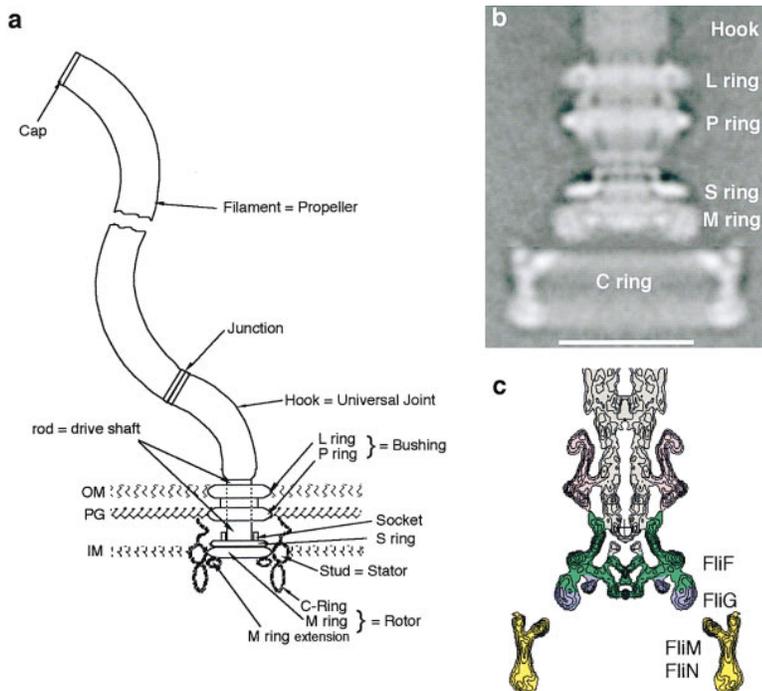


Figure 1. The Bacterial Flagellum

(a) Schematic of the bacterial flagellum as a mechanical device. The parts thought to be responsible for torque generation are shown in thicker lines. They lie inside the cell except for the studs, which cross the membrane and are thought to be anchored to the peptidoglycan layer (PG). The filament or propeller lies outside the cell's inner (IM) and outer (OM) membranes and is about 10  $\mu\text{m}$  long.

(b) Image of the flagellum. The image is an average of about 100 individual electron micrographic images of a frozen-hydrated preparation of flagella. The bar corresponds to 25 nm.

(c) A color-coded copy of a slice through the cylindrically averaged flagellar structure. FliF, which forms the M ring, S ring, and socket, is shown in green. FliG, shown in blue, is attached to the cytoplasmic face of the M ring. FliM and FliN, shown in yellow, are thought to make up the C ring. The L and P rings are shown in pink, and the rod and the short stretch of hook are gray.

complex. The cell's chemotactic capability depends on the correct modulation of switching probabilities. Mutations that affect switching probabilities will have a  $\text{Che}^-$  phenotype.

#### ***FliN May Have No Direct Involvement in Torque Generation***

Changes that produce the  $\text{Che}^-$  and  $\text{Mot}^-$  phenotypes in FliN map in the C-terminal 87 residues (Figure 2a). Overexpression of the nonmotile alleles of FliN restores some motor function, which calls into question FliN's role in torque generation. The effect of overexpression has been interpreted as a reduced binding of a mutant FliN to the flagellum, which is overcome by increasing its concentration (Lloyd et al., 1996). Thus FliN may stabilize the structure rather than participate directly in torque generation. Moreover, these same C-terminal 87 residues of FliN have sequence homology to Spa33, a protein involved in transmembrane transport in *Shigella flexneri* (Tang et al., 1995), and FliN has been directly implicated in the flagellar protein export. Thus, although FliN is part of the switch complex, it may not be directly involved in rotation or perhaps even in switching.

#### ***FliM Is Important in Switching but May Not Be Directly Involved in Torque Generation***

FliM is the protein to which phospho-CheY binds (Welch et al., 1993), and it has  $\sim 90$   $\text{Che}^-$  (or switch) mutations (Figure 2a) compared to 8  $\text{Mot}^-$  mutations (Irikura et al., 1993; Toker et al., 1996). Thus, FliM appears to be directly involved in switching. As is the case with FliN, overexpression of nonmotile alleles of FliM results in restoration of some motor function (Lloyd et al., 1996). Thus FliM is unlikely to be involved in torque generation directly but rather in switching.

#### ***FliG Is Directly Involved in Torque Generation and Probably in Switching***

$\text{Che}^-$  alleles of FliG (Figure 2a) map to the middle third of the 331 residues whereas the  $\text{Mot}^-$  alleles map to the

C-terminal half (Irikura et al., 1993). The N-terminal  $\sim 56$  residues are not essential for rotation, assembly, or switching. Most of the substitutions in the C-terminal region, which affect torque generation, involve replacement of nonpolar side chains with polar ones or proline (Irikura et al., 1993). These appear to destabilize the protein rather than being directly involved in torque generation (Lloyd and Blair, 1997). Three charged residues (R279, D286, and D287), which are conserved among the FliGs of different species, are directly involved in torque generation but are probably not involved directly in proton transfer since any one of them can be replaced by a neutral amino acid without total loss of function (Lloyd and Blair, 1997). In contrast to FliM and FliN, overexpression of  $\text{Mot}^-$  alleles of FliG does not restore motor function (Lloyd et al., 1996). Thus, FliG is likely to be involved directly in torque generation and, along with FliM, in switching (Marykwas and Berg, 1996; Togashi et al., 1997).

#### ***The mot Genes Anchor the Motor, Transport and Transfer Protons, and Position the Torque Generators***

MotA and MotB behave differently from the switch proteins. No mutations in *motA* or *motB* in otherwise wild-type cells result in  $\text{Fla}^-$  or  $\text{Che}^-$  phenotypes. When either MotA or MotB is missing in a cell, the flagella are paralyzed. If the missing protein is gradually restored, the motors regain torque in up to eight equal steps. Thus, the Mot proteins comprise or activate eight independent torque generators. In contrast, when FliM or FliN are underexpressed, motor speeds vary erratically rather than in discrete steps (Tang and Blair, 1995; Tang et al., 1995).

MotA (Figure 2b) is a transmembrane proton channel, which appears to make four membrane crossings. The two cytoplasmic parts of MotA contain about 190 of the 295 residues whereas the periplasmic loops are short,

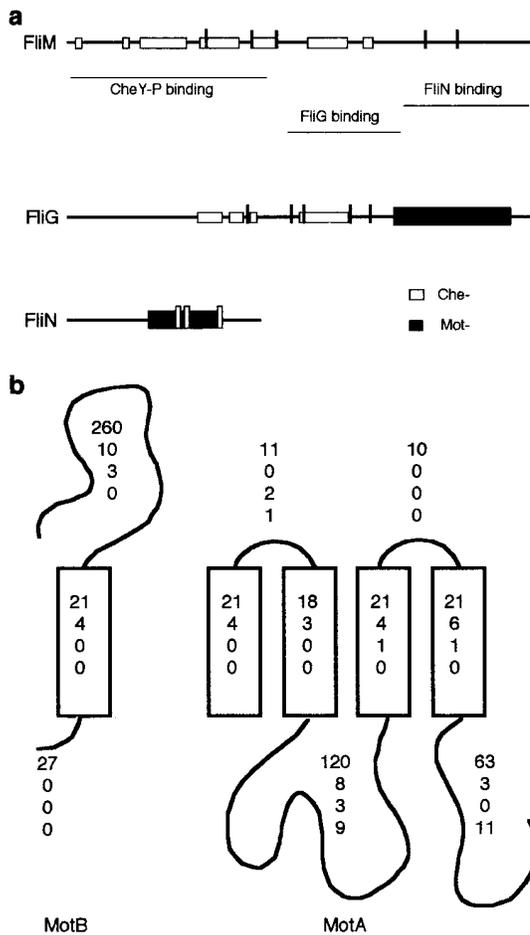


Figure 2. The Five Key Motor Proteins

(a) Schematics for the switch proteins showing regions that produce Che<sup>-</sup> and Mot<sup>-</sup> phenotypes and, in the case of FliM, regions associated with binding of phospho-CheY, FliN, and FliG.

(b) Schematic of the Mot proteins showing the disposition of the sequence to the membrane, periplasm (top) or cytoplasm (bottom). The columns of numbers associated with each segment give the numbers of residues in the segment (top), and the number of mutations that result in nonmotile phenotypes, severe phenotypes (twitch but don't swim), and (bottom) mild phenotypes (swim but less well than wild type).

about 10 residues each. Mutations in the transmembrane region severely impair or abolish function and affect proton conduction (Zhou and Blair, 1997). The mutations identified in the cytoplasmic domain vary in their phenotypic effects from mild effects of slow swarming rates to complete loss of function (Mot<sup>-</sup>). However, the bulk of these mutations do not seem to affect transmembrane proton transport. The slow swimming alleles of MotA generate normal torque at very slow motor speeds but less torque at high motor speeds and thus swim more slowly. Slow swimming alleles seem to affect the utilization of protons rather than their delivery across the membrane. These slow mutations may affect the conformation of the cytoplasmic domain or alter its binding to other motor parts. Thus, MotA appears to have two functional roles: delivery of protons across the

Table 2. Motor Numerology

Number of steps/revolution	~400
Number of protons/revolution	~1200
Number of torque generators	8
Number of studs	10
Number of copies of FliG	25–45
Number of copies of FliM	~35
Number of copies of FliN	~110

membrane and utilization of the protons to generate torque.

MotB (Figure 2b) is also part of the transmembrane proton channel but appears to make only one membrane crossing. Unlike MotA, the bulk of MotB is periplasmic and is thought to anchor the motor to the peptidoglycan layer. Mutations in *motB* lie in the periplasmic and transmembrane domains. Compared to MotA, they generally have a severe phenotype, usually resulting in no motor function. Suppressors of some of the *motB* mutations map to *motA*, *fliG*, and *fliM* (Garza et al., 1996); hence, these mutations reveal additional functions besides MotB's putative interaction with the peptidoglycan. Two of the mutants of *motB*, in which cells had partial motor function, gave rise to motors with only one or sometimes two torque generators rather than the usual 8. Each torque generator in the mutant functioned at wild-type levels. Thus, the mutations in *motB* don't interfere with the mechanism but rather with the presentation of the MotA/MotB complex to the motor. Thus, MotB serves three functions: anchoring of the motor to the cell, positioning of the motor's parts, and, with MotA, formation of the proton channel.

#### The Composition of the Motor

Of the flagellar mass of about 1,000,000,000 daltons, only ~1% is due to the motor (most of the mass is in the propeller). The copy numbers of the switch proteins (Table 2) (Zhao et al. 1996a, 1996b) are not yet agreed upon. These estimates correspond to a mass for the switch complex of about 5,000,000 daltons or 0.5% of the flagellum. The switch complex has been visualized in electron micrographs of isolated flagella (Figures 1b and 1c), but further work is needed to make detailed assignments of the switch proteins to features seen in images. It appears that FliG forms the cytoplasmic face of the extended M ring whereas FliM and FliN are part of the C ring (Francis et al., 1994). MotA and MotB appear to be components of the 10 studs (Figure 1a). The studs are not present in isolated flagella but have been seen in electron micrographs of freeze-fractured cells. There are no estimates of how many copies of MotA or MotB are in each stud.

#### Assignment of Parts to Stator and Rotor

Since MotB and MotA form a complex and the former appears to bind to the peptidoglycan, most agree that this pair are part of the stator. Because it connects the switch proteins to the drive shaft, FliF (an M-ring protein) must be part of the rotor. Mutants of *fliF* and *fliG* that produce a FliF–FliG fusion protein assemble rotating motors. If FliF moved as part of the rotor while FliG remained stationary as part of the stator, then a motor assembled from a fusion of the two proteins should not rotate. However, since it does rotate, FliG must also be part of the rotor.

The assignment of FliM and FliN is less certain. There is a mutation that produces a fusion of FliN and FliM. That the resulting motors still turn provides support for the idea that if either is part of the rotor, then both are. When either FliM or FliN is underexpressed in cells, the motors behave erratically. In contrast, when MotA or MotB are substoichiometric, the motors turn smoothly but generate less torque. Thus, units of MotA and MotB appear to function independently, with each unit adding an equal increment of torque. FliM and FliN, in contrast, do not assemble into independent torque generators and are more likely to be part of the rotor.

### **Numerology**

Numerology (Table 2) is important because it can provide clues to the motor's mechanism. A successful model must reconcile the numerology, but first we need accurate measurements of the numbers. We would like to know why are there eight torque generators and yet one sees ten studs? What limits the number of torque generators? Does the number of steps per revolution equal the number of torque generators times the number of generator-binding sites in the rotor? Are there really three protons used per step? Which residues bind these protons? Does each proton bind to the same set of residues or are there three sets of residues, one for each proton?

The mechanism of the flagellar motor remains a mystery. It is not clear if there are any similarities of its mechanism to those used in eukaryotic cells. The differences are apparent but we may not know about similarities until we have more detailed structures for the motor parts; we look forward to the day when we do.

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