How 34 Pegs Fit into 26 + 8 Holes in the Flagellar Motor

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The article by Brown et al. (6) in this issue of Journal of Bacteriology makes it possible to integrate a series of investigations that have given us a high-resolution picture of the rotational flagellar motors of Escherichia coli and Salmonella enterica. Although details will surely differ, it should also serve as a model for the flagellar architectures found within a wide spectrum of prokaryotes.

The beauty of the bacterial flagellum is captured in reconstructions of the basal body (21) and the external rod, hook, and filament, which comprise the propeller (18, 24). The output of this device is also impressive: E. coli flagella spin at hundreds of rotations per second, either clockwise (CW) or counterclockwise (CCW), and rotation is driven by a transmembrane proton current that brings up to a million H+ ions per motor cell per second. (For reviews of bacterial flagella and motility, see references 2, 11, and 13.) The reversibility of the motor generates alternating straight runs (CCW rotation) and reorienting tumbles (CW rotation) to create a three-dimensional random walk. During chemotaxis, the random walk is biased so that runs become longer when a cell happens to run up a gradient of an attractant chemical or down a gradient of a repellent chemical. (For reviews of bacterial chemotaxis, see references 1, 15, and 23.) The CheY protein, activated by phosphorylation at the chemoreceptor patch, binds to the motor to promote CW rotation and therefore tumbling.

Much of the bacterial flagellum can be extracted from the cell envelope as an intact basal body/hook/filament complex (8). This portion of the flagellum consists of three rings that encompass a central rod attached to a flexible hook that in turn is attached to a long, left-handed helical filament. The rings are, from the cell-proximal to cell-distal end of the basal body, MS, P, and L. Each is a polymer of one polypeptide. The rings associate with the cell membrane, the peptidoglycan wall, and the cytoplasmic membrane proton flow. The partial crystal structure of FliG from Thermotoga maritima (5, 12) strongly suggests that two distinct and rather distant domains are responsible for interactions with FliF and MotA, respectively. A long helix and a flexible linker connect these two domains. The bulk of the C ring is made up of the FliF protein, which associates at its cytoplasmic face with the FliG protein. FliG connects the MS ring to the C ring, and it also interacts with the cytoplasmic loops of MotA to generate rotation in response to transmembrane proton flow. The partial crystal structure of FliG from Thermotoga maritima (5, 12) strongly suggests that two distinct and rather distant domains are responsible for interactions with FliF and MotA, respectively. A long helix and a flexible linker connect these two domains. The bulk of the C ring is made up of the FliF and FliN proteins, with 32 to 36 FliM monomers, most of which probably bind a FliN tetramer (16). (FliN exists in ~100 copies per basal body.)

The structure of the large middle domain of FliM was recently solved (14). It is rather compact, with dimensions of 5 by 3.5 by 3 Å. At one end of the long axis there is a poorly resolved flexible GGXG-containing loop that joins the two pseudosymmetric domains of the folded polypeptide. At the other end is the C-terminal region that binds the FliN tetramer. The N-terminal sequence that binds to phospho-CheY is also not resolved. The C ring is ~44 nm in diameter and can accommodate 32 to 36 FliM subunits if the long axis of FliM is perpendicular to the ring and the intermediate axis is parallel to the circumference of the ring. Cross-linking studies using introduced cysteine residues are consistent with that organization (14).

The work of Brown et al. (6) ties all this information together. Tryptophan-scanning mutagenesis of FliG implicates two regions, which flank the connecting helix, as being important for flagellar assembly, motility, and directional control. The region in the domain closer to the MS ring contains an EHPQ...R sequence that is conserved in FliG proteins from a wide range of bacteria. The second region includes a hydrophobic patch that is on the opposite side of the motility domain from the ridge of charged residues that interact with the cyto-

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* Published ahead of print on 3 November 2006.
plasmic loop of MotA to drive flagellar rotation (25). So far, so good. But what about the numerical mismatch between the MS and C rings, and how can one region on a FliM subunit simultaneously interact with two distinct and rather distant sites on FliG?

The solution offered by Brown et al. (6) is ingenious and compelling. If, for example, the MS ring of a particular basal body has 26-fold symmetry, implying that 26 FliF subunits attach to 26 FliG subunits, then 26 FliM subunits can contact the hydrophobic patches on the motility domains of FliG. The remaining 6 to 10 FliM subunits could face inward to interact with the EHPQ...R motif, which is close to the N-terminal region of FliG that attaches to FliF. Indeed, Thomas et al. (21) found that the most proximal part of the C ring has the same symmetry as the MS ring. Thomas et al. (21) also found that, at the level of FliM, there was a lower electron density inside the high-density outer wall of the C ring, a feature consistent with a 26 + 8 arrangement of FliM subunits.

Figure 1 presents several views of a model for how the C ring may be configured in a CW-rotating motor, based on the most recent data. Phospho-CheY is not shown. It may bind only to the outward-facing FliM subunits that contact the FliG motility domain. CheY binding presumably generates a change in the conformation of FliM (and FliN) that is transmitted to FliG to reposition the motility domain with respect to the Mot protein complexes. The inward-facing FliM subunits might serve to stabilize the association of the MS and C rings.

This model raises a number of questions. How can FliM within the C ring accommodate two orientations of FliM monomers, which must lead to nonequivalent subunit contacts? Does phospho-CheY bind only to the outward-facing or inward-facing FliM subunit or to both, and does CheY binding change the distribution of FliM between the two conformations? How does phospho-CheY binding modify the way that FliM interacts with the FliG motility domain? What coordinates the movements of FliM subunits within the ring (7, 9) to give essentially instantaneous switching from CCW to CW rotation and back? How do interactions between MotA and FliG change to produce the two different directions of rotation, and how are Mot complexes recruited to, and distributed around, the MS and C rings (10, 22)? The answers to these questions must be found before we can say that we have attained an in-depth understanding of the relationship between flagellar structure and flagellar function.

I thank David Blair and David DeRosier for extensive and very helpful discussion during the preparation of this commentary.

REFERENCES

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.