A Grand View of the Flagellar Motor
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With apologies to Charles Darwin (4), there is grandeur in the view of the flagellar motor provided by Liu et al. in an article published in this issue (9). They have used cryo-electron tomography (cryo-ET) to reconstruct, in a most elegant manner, the Borrelia burgdorferi version of one of nature’s most exquisite nanomachines—the rotary flagellar motor. Their choice of this skinny spirochete, the causative agent of Lyme disease, for the study enabled them to use the full power of three-dimensional (3D) imaging afforded by tomography to probe the structure of this extraordinary rotary organelle.

Tomography has been in increasing use lately to study intracellular components of bacteria, including the cytoskeleton (2) and chemoreceptor patch (3, 7) of the motile alphaproteobacterium Caulobacter crescentus and, in the article by Liu et al. (9), the flagellar motor of B. burgdorferi. Both bacteria are particularly well suited for cryo-ET studies because of their small diameters. In addition, both possess flagella that arise near the cell pole, making the basal bodies easier to locate. B. burgdorferi is further well suited for this study because it sports a small, linear cluster of adjacent flagellar motors that can be easily visualized.

The principle underlying cryo-ET is that 3D volumes can be reconstructed from the integration of multiple two-dimensional views of thin electron microscopy sections taken at different angles relative to the electron beam. In a typical analysis, successive micrographs of a section are taken as the sample stage is tilted at 1-degree intervals, usually in the range of +70 to −70 degrees normal to the section. The basic idea is illustrated with a simple object in Fig. 1. In this way, not only can a 3D representation be derived but the cross-sectional slice at each level of the structure can be obtained.

Cryo-ET is a relatively recent technique, and its application to cells as tiny as bacteria is even newer. Together with light microscopic imaging of proteins tagged with fluorescent molecules, such as green fluorescent protein, cryo-ET is providing us with unprecedented insights into the in situ ultrastructures of large macromolecular assemblages. Other electron microscopy approaches, such as single-particle analyses, have yielded excellent views of isolated flagellar basal-body structures (11, 12), but these results can be limited by the distortions that arise during the isolation of organelles. For an example, it is unlikely that the vertical displacement of the stators by the highly curved membrane of B. burgdorferi (discussed below) would be observed in in vitro preparations.

Spirochetes have internalized flagella whose filaments rotate within the space between the cell wall and the outer membrane (1). The rotation of the small flagellar bundle in this cell compartment causes counter-rotation of the helical cell body so that the entire bacterium becomes the propeller that generates swimming (6). In B. burgdorferi, several flagellar motors are aligned in a row parallel to the long axis of the cell, as shown in Fig. 2 of the article by Liu et al. (9). Both the basal bodies and the hooks and flagella with which they rise are clearly visible.

One of the accomplishments of Liu et al. was to secure sufficient micrographs of different motors to obtain averaged images without imposing rotational symmetry. Therefore, confidence is high that the subunit stoichiometries of the different components of the motor are inherent properties of those objects rather than artifacts generated during construction of the 3D image. This is no small feat—several of the observations presented would not have been possible had such symmetry been imposed.

The flagellar motor was reconstructed at a remarkable 3.5-nm resolution. The model includes the hook, rod, rotor, stator, C ring, and export apparatus. The hook is poorly resolved because it has a tilted orientation that presumably differs from one motor to the next. The rod shows much better resolution, and its hollow center, which represents the export channel, is clearly visible. The upper part of the motor shows a clear 16-fold symmetry at its periphery in the form of clockwise-tilted radial extensions, or spokes. At the level of the cell membrane, no clear symmetry is evident, but two concentric rings are seen, with the inner probably corresponding to the MS ring and the outer to the C ring. The diameter of the C ring is 57 nm, and the diameter of the MS ring is about half of that. This compares with a C-ring diameter of about 45 nm seen in the Salmonella flagellar motor (5).

The rings show a faint periodicity that suggests that they contain a higher number of subunits. There is electron density between the two rings that may represent the Flg protein, which is predicted to join the two rings. Peripheral to the C ring, there is a blurry 16-fold symmetry that may correspond to stator units that have different positions in the membrane from one motor to the next.

After treatment of cells with the nonionic detergent NP-40, only central elements of the motor remain at the level of the membrane. The presumptive stator elements disappear, as does the C ring. The remaining basal body attached to the cell envelope has a diameter of 45 nm, which may correspond to

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the MS ring with FliG attached. Even though the stator elements are gone, this ring shows a double 16-fold symmetry, with one set of spots on the outside and another located between, and more centrally than, the first.

The side views of the reconstructed motor are even more striking. The extracellular portion of the basal body is quite complex, consisting of a hollow torus that fits closely around the distal rod, a “collar” outside of that, and then more peripheral density that probably corresponds to the stator. The torus is the P ring, since it disappears in the basal bodies from flgI mutants, which nevertheless seem to have normal motility. The collar is a unique feature that may be present only in spirochetes, perhaps an adaptation to their internalized flagellar filaments. At the cytoplasmic face of the MS ring, there is significant, rather well resolved density that must represent the export apparatus, suggesting that it has a defined architecture as well.

One of the most intriguing features of the side views is that the basal body at the membrane level shows a 180° rotational periodicity of about 6 nm in the vertical dimension. A similar periodicity of smaller amplitude exists in the next-higher and -lower levels of the basal body, whereas at the levels of the P ring and C ring, the vertical position of the basal body is not periodic. Since the greatest deviation in height is at the level of the stator proton-conducting transmembrane channels, the implication is that the position of the stators follows the curvature of the membrane, which is quite high in such a skinny bacterium. If all of the stators remain engaged with the rotor, it would seem that it must also exhibit some curvature at the level of the FliG motility domains.

The flagellar motors of spirochetes may differ in some particulars from those of bacteria with external flagella, but certainly there are lessons to be learned from this elegant structure. The strong 16-fold symmetry at the level of the cell wall, if it represents the peptidoglycan-binding domains of stators, certainly suggests a highly ordered assembly. It is not known whether stator units join or leave rotors in B. burgdorferi on a short time scale, as in E. coli (8), but if they do, it becomes difficult to comprehend how this exchange of rotor elements can be so fast and accurate. It will be informative to determine the structure of a B. burgdorferi basal body equipped with subsaturating numbers of stators.

The 16-fold symmetry seen at the level of FliG suggests a one-to-one match of accessible rotor motility domains and stator elements, something not typically incorporated into models for torque generation. For example, in E. coli there are thought to be on the order of 11 functional stator units per motor (10), whereas there are 24 to 26 FliG subunits per motor (13). Could it be that only half of the FliG proteins are engaged in rotation at any given moment or that one set is for clockwise direction and the other half for counterclockwise rotation? If that is generally true, new explanations must be developed to explain how flagella reverse their direction of rotation. The high probability that the position of the stator and rotor elements follows the curvature of the membrane also suggests that the link between the MS-ring-anchoring and motility domains of FliG must be very flexible.

Seldom does one structure raise so many interesting questions. It will be fascinating to discover how generally applicable the B. burgdorferi flagellar motor is to other bacteria. It certainly gives all of us who work on motility a lot to think about.
REFERENCES


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