GUEST COMMENTARIES

Fine Structure of a Fine Machine

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Bacteria can move in several ways, but probably the most common means of locomotion is by the rotary organelles called flagella. Flagella are evolutionarily ancient and may be the most widespread machines of propulsion in biology: genes for flagellar components are found in almost half of the more than 500 bacterial genomes presently on the NCBI website, in diverse, deeply branched lineages. The essentials of flagellar function have been understood for more than 30 years. A transmembrane ion current, commonly protons but sometimes sodium ions, is harnessed to drive rotation of a membrane-embedded motor linked to a slender helical filament that functions as propeller (4, 13, 17). By regulating the direction and/or speed of flagellar rotation in response to various sensory cues, cells can bias their movement towards nutrients, temperatures, or other conditions that favor survival (1, 2, 18). The physiology of the flagellar motor has been studied extensively, and many aspects of flagellar motor function are well understood (reviewed in reference 3). While ultrastructural studies have also been useful, providing an overall view of the shape of the flagellar basal body, until recently such studies have lacked the resolution needed to illuminate molecular mechanisms. Our structural understanding of the flagellum has taken a great leap forward with the electron microscopic reconstructions reported in this issue by Thomas et al. (25), which reveal the flagellar basal body in stunning new detail.

The flagellum has three main parts: a rigid, helical filament that is the propeller; a short, flexible hook that functions as a universal joint; and the basal body, which is embedded in the cell membrane(s). Early electron micrographs showed that the basal body consists of a set of rings mounted on a central rod (Fig. 1A) (8). The rings were named according to their locations relative to layers of the cell envelope: L ring, lipopolysaccharide; P ring, peptidoglycan; S ring, supramembrane; and M ring, inner membrane (Fig. 1A). The M and S rings were subsequently found to be composed of a single protein (27) and are now recognized as a single entity termed the MS ring. By use of gentler isolation procedures, Francis et al. (10) and Khan et al. (14) prepared more-intact basal bodies from *Salmonella* that contain another large ring at the inner end, termed the C ring (for cytoplasmic). Micrographs of this more-intact structure in vitreous ice provided the basis for single-particle reconstructions at approximately 22-Å resolution, which for several years were the most detailed images available (10, 24). The reconstruction shows a fairly ornate structure

with a silhouette reminiscent of a chess piece (Fig. 1B). Because the reconstruction procedure relied on axial averaging (i.e., blurring the map by computationally rotating it about its long axis), azimuthal variations in density—and thus most subunit structure within the rings—were effectively washed out.

Subunit structure in the C ring was subsequently seen in bottom views (26, 30). Importantly, these images showed that the size of the C ring, and thus the number of subunits present, is variable. Rings with 34 subunits are most abundant, but 33- and 35-subunit versions are also seen. To complicate matters further, a reconstruction of the MS ring by Suzuki et al. showed that its symmetry is quite different from that of the C ring, with a value closer to 25 subunits per ring (23). Recognition of this size variability and implementation of a suitable reconstruction procedure that can take it into account were key factors in the resolution improvement achieved here by Thomas et al. (25). By classifying particles according to size (and thus subunit number) and applying just the averaging procedures appropriate for each symmetry class, they obtained separate reconstructions for each size of particle, all of which reveal the structure more clearly than the previous one-size-fits-all reconstruction.

The resulting images are spectacular. One representative close-up suffices to illustrate the magnitude of the resolution improvement. Where the previous best image showed a rounded blob at the bottom of the C ring, the new images show a clearly resolved band of density winding around the bottom of the ring like a spiral whisk (Fig. 1C). Other parts of the structure are similarly rich in new detail. Of particular note, one feature in the C ring—the more inboard lobe of electron density at the top—is found to have a symmetry that matches that of the MS ring (i.e., about 25-fold), whereas other features of the C ring display the ~34-fold symmetry first seen in bottom views. And as it happens, the MS ring also shows variable symmetry, in this case ranging between about 24- and 26-fold.

What can these new views of the structure tell us about the function of the motor? The basal body seen in the electron micrographs is thought to be just the rotating part of the motor. The MotA/MotB protein complexes that form the stator (the part that remains stationary relative to the cell body) are believed to be in the membrane just above the C ring (Fig. 1B). These stator complexes conduct the ions that energize the motor, probably harnessing ion flow to drive conformational power strokes that push on the C ring to drive rotation (16). The C ring is thus the element of the rotor most important for rotation and switching. It is formed from the proteins FliG, FliM, and FliN, each present in many copies (29, 31). Crystal structures have been solved for most of the FliG and FliN proteins (5, 6) and will soon be available for most of FliM (21).

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FIG. 1. Ultrastructure of the flagellar basal body. (A) An early electron micrograph of the basal body from *Escherichia coli* (reprinted from reference 8 with permission of the publisher). One-letter names of some of the rings are given. The thicker arrow indicates the junction between the hook and filament. (B) Single-particle reconstruction of the flagellar basal body of *Salmonella* (reprinted from reference 24 with permission of the publisher). This structure includes the large drum-shaped feature called the C ring (located in the cytoplasm in the intact motor). The structures purified for electron microscopy are believed to represent only the rotor. The stator is formed by the proteins MotA and MotB, which are positioned somewhere in the membrane above the C ring. The approximate positions of the membrane (yellow) and the stator complexes (red) are indicated. (C) Detail from the new basal body reconstruction reported in this issue by Thomas et al. (25). The region shown corresponds approximately to the dashed box in panel B. (D) Two hypotheses for the arrangement of FliG, FliM, and FliN in the motor.

The resolution of the new electron microscopic images is almost sufficient to allow crystal structures to be docked into the map. Thus, remarkably, we may soon have a near-atomic structural model for most of the C ring.

Certain features of subunit organization already seem clear. FliN has been suggested to form the rounded feature at the bottom of the C ring, on the basis of its crystal structure and cross-linking studies indicating that it forms a roughly donut-shaped tetramer (22). This placement of FliN appears to be supported by the new images, because FliN tetramers distorted only slightly from the hypothesized donut shape (into the shape of a spiral lock washer) would fit well in the spiral density at the bottom of the C ring. Also, the inboard lobe of electron density at the top of the C ring, which exhibits symmetry matching that of the MS ring, is very likely due to a domain of FliG. FliG is known to be present in the same number of copies as FliF, the protein that forms the MS ring (9), and a variety of mutational, binding, and cross-linking studies place FliG near the top of the C ring (11, 15, 19, 20, 32). As discussed by Thomas et al. (25) and Lowder et al. (19), the present data are consistent with either of two FliG arrangements, both with FliG at the top but one having FliG in a more outboard position than the other (Fig. 1D). Whichever of these placements proves correct, it seems that FliM must form the middle, relatively thin part of the C ring wall. A forthcoming crystal structure of FliM should provide a critical test of this model (21). Can FliM subunits be fitted into this part of the C ring, or is a different arrangement required?

In the cell, switching of motor direction is controlled by the signaling molecule phospho-CheY, which promotes clockwise rotation (in the enteric species typically used in chemotaxis studies) (12, 28). Motor switching is the culminating event in chemotaxis, yet its molecular mechanism remains very poorly understood. The new images of the basal body, and the structural models they will inform, should provide a concrete framework for addressing this question. The present images are of basal bodies locked by mutation in the clockwise rotational state. When similarly detailed images are

obtained for motors in the counterclockwise state, what differences will emerge? The flagellar switch is highly cooperative, responding to increasing phospho-CheY with a Hill coefficient greater than 10 (7). This sharp switching is credited with much of the sensitivity of the chemotactic response. A fuller structural understanding of the C ring should illuminate this aspect of switch function also.

Symmetry provides a large advantage for image reconstruction because it allows the use of averaging procedures that reinforce symmetry-related features in the map relative to the noise. However, in the case of the *Salmonella* basal body, the occurrence of two different symmetries means that most basal bodies have, strictly speaking, no rotational symmetry at all. In a basal body with a 25-subunit MS ring and a 34-subunit C ring, for example, none of the subunits can be in strictly equivalent environments. Parts of the structure can and apparently do conform approximately to one or another rotational symmetry, but at the interfaces where neither symmetry can fully apply, rotational averaging procedures are as likely to blur as to enhance the structural details. These interfacial regions could be some of the most interesting and important places in the structure. How can they be visualized accurately? To see the structure in high detail and without benefit of any symmetry-based averaging will require much larger numbers of images and possibly the use of target models that utilize the crystal structures. Electron tomography might also make an important contribution here, as it could in principle allow in situ visualization of the fully intact motor, including the stator complexes responsible for force generation.

While the flagella of diverse species appear to be built from basically the same parts, certain parts, most notably the C-ring protein FliN, show some variation between species. FliN is present in more than 100 copies (in the *Salmonella* motor) and is thus a major component of the basal body. In most bacilli and in *Spirochaetes*, FliN is replaced by the protein FliY, which is about twice as large. Some species contain both FliN and FliY. What do FliY-containing basal bodies look like? We do not know, because such basal bodies have not yet been isolated

with their C rings intact. As efforts to push the resolution envelope continue with *Salmonella*, it seems a good time also to look more closely at other species. The advanced state of knowledge for *Salmonella* is largely due to efforts invested in biochemical characterization of the basal body, particularly the development of gentle but effective purification procedures (10, 14). Similar efforts with other species should be fruitful, allowing comparative studies of flagellar structure and function in the larger world.

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