Rotary Nanomotors in the Rear View Mirror

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Rotation is part of our everyday lives. For most of human history, rotation was considered a uniquely human invention, something beyond the anatomical capabilities of organisms. In 1973, Howard Berg made the audacious proposal that the common gut bacterium Escherichia coli swims by rotating helical flagellar filaments. In 1987, Paul Boyer suggested that the FoF1 ATP synthase of E. coli is also a rotary device. Now we know that rotating nanomachines evolved independently at least three times. They power a wide variety of cellular processes. Here, the study of flagellar rotation in E. coli is briefly summarized. In 2020, the Cryo-EM structure of the MotAB stator element of the bacterial flagellum was described. The structure strongly suggests that the MotAB stator rotates to drive flagellar rotation. Similar motors are coupled to other diverse processes. The following articles in this issue review the current knowledge and speculation about rotating biological nanomachines.

Keywords: flagellum, rotation, motor, ion motive force, Howard Berg

INTRODUCTION

This article is intended as an introduction to an issue devoted to biological rotary nanomotors. It provides a historical perspective on the study of rotation of the bacterial flagellum, the first—and for more than 20 years the only—known biological rotary motor. It has recently become clear that rotary motors on a molecular scale are everywhere in nature. It is this realization that inspired the review and perspective articles that are compiled in this issue.

Rotation has some marvelous properties as a form of motion. It can be continuous in one direction as long as a constant force is applied to generate the torque required to balance the resistance. It is reversible, switching from clockwise to counterclockwise and back again (What did they call the two directions of rotation before we had clocks? Perhaps right-handed and left-handed screws). Rotation can be driven from torque applied by a central hub or axle, or it can be driven by an external current that supplies the energy for rotation about a hub or axle. It can generate or be driven by flows of wind, water, or electrical current. It is the ideal motion for conversion of potential energy into kinetic energy, and vice versa.

Rotation has been ubiquitous in our lives since the invention of the wheel, first as an aid to potters and only later as a convenient way to move heavy loads. Until 1973, we can be forgiven for thinking rotation was a uniquely human invention among living organisms. Examples of what superficially looked like rotation were illusions: the helical beat of the long, propulsive cilia of some protozoa; the seemingly circular beat of multiple, much-shorter cilia around the oral opening of a rotifer; the remarkably quick swiveling of an owl's head through nearly 360°.

Everyone agreed there was a good reason that there was no rotation in living organisms. How would connections be made across a rotating joint? Blood vessels, nerves, connective tissue would
all be twisted into an awful snarl, and any communication across the joint would be interrupted. Even at the microscopic level, filaments made of actin, myosin, tubulin, or any other polymerizing protein would become entangled. Another problem was how lubrication would be provided for parts rotating relative to one another.

The problem was that people were thinking too big. There is no need for a filamentous connection between the parts of a molecular motor. There is also no need for a lubricant.

The curtain that blocked our view of rotation in living organisms was raised in 1973. In a paper appearing in *Nature*, Howard Berg and Robert Anderson presented the idea that flagella, thin helical filaments used for motility by bacteria, propel the cells by rotating (Berg and Anderson, 1973). They reasoned from available information. The paper features one hand-drawn figure, thereby extending a precedent set by Watson and Crick 20 years earlier (Watson and Crick, 1953).

Berg and Anderson had two irrefutable pieces of evidence for rotation. A swimming *Escherichia coli* cell is pushed by a bundle of 4–6 flagellar filaments. The first evidence for rotation was provided by the observation that when divalent antibody prepared against flagellar filaments was added to swimming cells, the cells immediately stopped swimming. The interpretation was that their flagellar filaments became crosslinked and could no longer slide past one another as they must in a bundle of rotating filaments. When monovalent antibody raised against the filament was added, motility was retained. The interpretation was that the filaments became thicker when they bound the monovalent antibody but could still slide past one another as they rotated in a bundle.

The second piece of evidence was again provided by divalent antibody, but in a very different way. An extracellular structure called the proximal hook is found at the base of the filament. This hook acts as a universal joint to allow rotation of the flagellum perpendicular to the cell envelope to bend 90° so that the left-handed helical flagellar filaments can come together to form a bundle that pushes the cell. The nucleoid is shown in green, and the chemoreceptor patches at the cell poles are shown in orange. The patch at the old pole is larger. In this image it is at the leading end of the cell, although the old pole can be either at the leading or trailing end (Berg and Turner, 1995).

(B) The three-dimensional random walk. The image at the left shows a two-dimensional projection of a cell that is running and tumbling in three dimensions. The image to the right shows a cell that is swimming in a gradient of an attractant chemical with a higher concentration at the top. The runs in the up-gradient direction are longer than in the image to the left, whereas the runs in the down-gradient direction are of the same length. The shorter runs in the absence of an attractant gradient provide space for Howard Berg to look on approvingly from the upper left.

These discoveries came just at the time that the behavior of swimming cells was being recorded automatically by the tracking microscope invented by Howard Berg (1971). The tracking microscope had been used to show that *E. coli* cells swim in a behavior first called running and twiddling (Figure 1B), with twiddles later being called tumbles (Berg and Brown, 1972). The cells swim on a path (the run) that is as straight as can be given the rotational diffusion of an object as small as an *E. coli* cell. They then go through a brief period of chaotic motion (the twiddle...
or tumble) that reorients them before the onset of the next run. The average change in heading is about 68°, slightly biased in the direction the cell had been heading. Both runs and tumbles show an exponential length distribution, with the mean run lasting about 1 s and the mean tumble about 0.1 s. This behavior was aptly described as a 3-dimensional random walk.

The runs became longer when the cells swam up a concentration gradient of a chemical that was an attractant (Mesibov and Adler, 1972; Adler et al., 1973) or down a concentration gradient of a chemical that was a repellent (Tso and Adler, 1974). The data from the Adler lab with tethered cells made it clear that CCW flagellar rotation (viewed looking down the axis of the flagellar bundle toward the cell) propels runs and implies that CW flagellar rotation leads to tumbles. Cells swimming down an attractant gradient or up a repellent gradient do not change the length of their runs. This led Berg to conclude that *E. coli* is an optimist, happy when things are getting better and soldiering on unresponsively when things are getting worse. This differed from the classical idea that bacteria navigate by responding to negative stimuli with an avoidance response, the Schreckbewegung (shock reaction) described by Engelmann (1883).

The next major advance was the demonstration that flagellar rotation is powered by the ion motive force across the membrane. The Adler lab had shown as early as 1974 that an intermediate in oxidative phosphorylation, not ATP, was the driving force for flagellar rotation (Larsen et al., 1974a). In 1977, motility was induced in a suspension of non-motile de-energized cells of *Streptococcus* strain V4051 with a valinomycin-induced potassium diffusion potential or a transmembrane pH gradient (Manson et al., 1977). Thus, either the electrical or chemical component of the proton motive force (pmf) can drive flagellar rotation. Further confirmation of the role of the pmf in bacterial motility was provided by the Skulachev group (Glagolev and Skulachev, 1978).

Using de-energized tethered cells of the motile *Streptococcus*, it was demonstrated that the rotational speed of the flagellar motor operating at high load is directly proportional to either the electrical or chemical component of the pmf (Manson et al., 1980). In these experiments, the highest value of the pmf was $-80 \text{ mV}$. Rotation was induced even when the sign of the pmf was reversed so that hydrogen ions flowed out of, rather than into, the cells.

A similar result was obtained using voltage-clamp experiments with giant *E. coli* cells (Fung and Berg, 1995). In this study, the linear dependence on pmf was extended to $-150 \text{ mV}$, nearly the $-170 \text{ mV}$ pmf maintained by actively metabolizing...
E. coli cells. It was also shown that an interior positive pmf drove CW rotation of a flagellum that rotated CCW in response to an inwardly directed proton flow. This result confirmed a conclusion reached earlier with tethered cells of a Streptococcus mutant whose flagella were locked in CCW rotation. Their flagella spun CW when an inside-positive potassium diffusion potential was induced with valinomycin in the presence of a high external KCl concentration (Berg et al., 1982).

The motile Streptococcus had one more contribution to make to our understanding of flagellar rotation. It was used to estimate the proton flux through the flagellar motor (Meister et al., 1987). The data suggested that about 1,200 protons were required per rotation of the flagellum. This result led to the conclusion that when the motor operates at high load, as in a tethered cell, it works with close to 100% efficiency. For rapidly spinning flagella, the efficiency is much lower, probably “due to dissipation by processes internal to the motor.”

The bacterial flagellum is a complex machine (Figure 2). In E. coli, the products of about 40 genes (Table 1) are required to construct a fully functional flagellum. In other species, particularly those with very rapidly rotating flagella (e.g., Vibrio species) or internal periplasmic flagella (spirochetes), other

### TABLE 1 | Proteins involved in the regulation, assembly, and structure of the E. col flagellum.

| Protein | Function | Component of mature flagellum | Operon | Map location (min) |
|---------|----------|-------------------------------|--------|-------------------|
| FlgA    | P-ring assembly | No                             | flgA   | 24                |
| FlgB    | Proximal rod    | Yes                           | flgB-J | 24                |
| FlgC    | Proximal rod    | Yes                           | flgB-J | 24                |
| FlgD    | Hook cap        | No                             | flgB-J | 24                |
| FlgE    | Hook protein    | Yes                           | flgB-J | 24                |
| FlgF    | Proximal rod    | Yes                           | flgB-J | 24                |
| FlgG    | Distal rod      | Yes                           | flgB-J | 24                |
| FlgH    | L-ring          | Yes                           | flgB-J | 24                |
| FlgI    | P-ring          | Yes                           | flgB-J | 24                |
| FlgJ    | Muramidase      | No                             | flgB-J | 24                |
| FlgK    | Hook-filament junction (at hook) | Yes | flgKL | 25                |
| FlgL    | Hook-filament junction (at filament) | Yes | flgKL | 25                |
| FlgM    | Inhibitor of $\sigma^F$ | No | flgMN | 24                |
| FlgN    | Chaperone for FlgK and FlgL | No | flgMN | 24                |
| FihA    | Protein export  | Yes                           | fihBAE | 42                |
| FihB    | Protein export specificity | Yes | fihBAE | 42                |
| FihC    | Master transcriptional regulator | No | fihDC | 42                |
| FihD    | Master transcriptional regulator | No | fihDC | 43                |
| FihE    | Regulation of protein exporter | Yes | fihBAE | 43                |
| FiaA    | Sigma factor for late flagellar genes ($\sigma^F$) | No | fiiA | 43                |
| FicC    | Flagellin (polymerizes to form filament) | Yes | fiiC | 43                |
| FicD    | Filament cap (needed for FicC polymerization) | Yes | fiiDST | 43                |
| FicE    | MS-ring rod junction | Yes | fiiE-K | 43                |
| FicF    | MS-ring         | Yes                           | fiiE-K | 43                |
| FicG    | C-ring, interacts with MotA | Yes | fiiE-K | 43                |
| FicH    | Protein export  | Yes                           | fiiE-K | 43                |
| FiiI    | Protein export (ATPase) | Yes | fiiE-K | 43                |
| FiiJ    | Export chaperone | Yes | fiiE-K | 43                |
| FiiK    | Hook-length control | No | fiiE-K | 43                |
| FiiL    | Stability of flagellum | Yes | fii-E-R | 44                |
| FiiM    | C-ring, switch component, binds CheY-P | Yes | fii-E-R | 44                |
| FiiN    | C-ring, switch component | Yes | fii-E-R | 44                |
| FiiO    | Scaffold for FiiPQR assembly | No | fii-E-R | 44                |
| FiiP    | Protein export  | Yes                           | fii-E-R | 44                |
| FiiQ    | Protein export  | Yes                           | fii-E-R | 44                |
| FiiR    | Protein export  | Yes                           | fii-E-R | 44                |
| FiiS    | FiiC chaperone  | No                             | fiiDST | 43                |
| FiiT    | FiiC chaperone  | No                             | fiiDST | 43                |
| MotA    | Stator component, interacts with Flg | Yes | motAB$^a$ | 43                |
| MotB    | Stator component, bind to PG cell wall | Yes | motAB$^a$ | 43                |

$^a$The complete operon is motABcheAW.
The rod, the proximal hook, and the filament extend outward inserted MS-ring. It is composed of 26 copies of the FliF protein. Processes other than flagellar rotation.

Others are required only transiently during the assembly of the flagellum. Two of them, MotA and MotB, are not required to build a flagellum but are required for it to rotate. They are of special interest because they comprise a common element, presumably evolved from a common ancestral gene, that is found in several rotary motors discussed in this issue that are coupled to chemoreceptor patch, to switch the direction of flagellar rotation from CCW to CW. FliG, which is attached firmly to FliF in the MS-ring, interacts with MotA, which supplies the force needed for rotation of the C-ring.

MotAB constitute the ion channel and form the stator component of the flagellar motor. They function as stators because the C-terminal portion of the MotB protein binds tightly to the peptidoglycan (PG) cell wall. Stators can be added to induce the rotation of paralyzed flagellar basal bodies that are already assembled but not functioning (Silverman et al., 1976). When the flagellar motor operates at high load, as in a tethered cell, the addition of each stator causes an equal step increase in rotation speed (Block and Berg, 1984). The original estimate was that up to 8 MotAB units could be added to a motor (Blair and Berg, 1988), but later work raised that estimate to a maximum of at least 11 stator units per motor (Reid et al., 2006). The flagellar motor can operate at low load with a single stator unit, but additional stators are recruited when increased load on the motor requires the production of higher torque (Lele et al., 2013; Tipping et al., 2013).

Charged residues in a cytoplasmic loop of the MotA subunit of the stator make electrostatic contact with charged residues in the C-terminal “motility” domain of the FliG protein to apply torque to the C-ring (Zhou et al., 1998a). In E. coli, the association of stator units with the flagellum is dynamic. There is a rapid exchange between motor-associated stator units and a pool of dissociated stator elements freely diffusing in the membrane (Leake et al., 2006). The dwell time of a stator unit on a motor in that study was about 30 s.

When the stator units are in the membrane and not associated with a flagellar motor, a plug in the MotB subunit blocks the flow of protons (Hosking et al., 2006). A radical refolding of the periplasmic domain of MotB occurs when a stator unit associates with the C-ring (Kojima et al., 2009). This conformational change does two things; it unplugs the ion-conducting channel in the stator, and it extends the PG-binding domain so that it can bind to the PG cell wall. Thus, anchoring of the stator and its activation as an ion channel are coupled events.

The stoichiometry of MotA and MotB was thought to be 4:2 (Kojima and Blair, 2004). It is striking how long that misconception persisted and perhaps delayed the discovery that the stator rotates. An aspartate residue near the cytoplasmic loop of the MotA subunit makes electrostatic contact with charged residues in the C-terminal “motility” domain of the FliG protein to apply torque to the C-ring (Zhou et al., 1998a). In E. coli, the association of stator units with the flagellum is dynamic. There is a rapid exchange between motor-associated stator units and a pool of dissociated stator elements freely diffusing in the membrane (Leake et al., 2006). The dwell time of a stator unit on a motor in that study was about 30 s.

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MotB. Were two of the MotA proteins just loafing some of the time?

The original estimate of A to B subunit stoichiometry in the stator was based on biochemical analysis of purified stator complexes. However, carefully those experiments were carried out, they cannot match the accuracy of actually imaging the stator complexes with cryo electron microscopy. Two groups accomplished that in 2020 (Deme et al., 2020; Santiveri et al., 2020). Both studies agreed that the stoichiometry is 5 MotA to 2 MotB subunits rather than 4:2. The new 5:2 ratio suggested that only one effective ion-conducting channel exists at a time and led to immediate speculation that what must be happening is that the MotA pentamer rotates around the MotB dimer in a temporal series of 10 steps of 36° in a full rotation.

The contacts between MotA and FliG would allow rotation of the stator to drive rotation of the C-ring, and thus of the entire flagellum (Hu et al., 2022). The stators presumably rotate in direction (entire flagellum (Hu et al., 2022). The stators presumably rotate of the stator to drive rotation of the C-ring, and thus of the C-ring. The second is how flagella can rotate bidirectionally (Figure 3). If the C-ring is in an expanded configuration, the CW-rotating stators would make the C-ring rotate CCW. If the C-ring is in a compact configuration, the CW-rotating stators would make the C-ring rotate CW.

This was one of those amazing moments in science when one discovery solves multiple problems. The first is how a flow of ions through the stator into the cell translates into rotation of the stator. The second is how flagella can rotate bidirectionally when the ion flow is normally always from outside the cytoplasm to inside. The third is why a flagellum that normally rotates only CCW reverses to CW rotation when the proton motive force is inside positive; if protons flow outward through the stator, it should rotate CCW and drive CW rotation of the C-ring fixed in the compact conformation.

It was the discovery of the rotating stator that provided the impetus for compiling an issue devoted to rotary nanomachines. Various aspects of flagella are the topics of seven articles. Four articles consider systems that operate with homologs of MotAB that are coupled to functions other than flagellar motility: movement over surfaces by gliding bacteria, transport of large substrates across the outer membrane of gram-negative bacteria, and cell division. Two articles consider a structure that superficially looks like a flagellum but is functionally and evolutionarily completely distinct: the ATP-hydrolysis-driven archaellum of the Archaea. Finally, seven articles discuss the rotary FoF1 ATP synthase. Evidence to support the idea that this enzyme in E. coli is a rotary device was presented by the laboratory of Paul Boyer in 1987 (Kandpal and Boyer, 1987) and demonstrated to be a rotary device by direct observation in 1997 (Noji et al., 1997). The energy for all of these rotary machines other than the archaellum is provided by a transmembrane ion motive force that drives the coupling ion down its potential energy gradient into the cell. This issue celebrates both rotary nanomachines and the life of Howard Berg, who departed us on December 30, 2021. Enjoy.

AUTHOR CONTRIBUTIONS

MM conceived and wrote the article.

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