Report

Current Biology

A Rotary Motor Drives *Flavobacterium* Gliding

Highlights

- The gliding motor, a novel rotary motor, spins tethered *F. johnsoniae* cells
- The gliding motor generates high torque
- The gliding motor runs at constant speed rather than at constant torque

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In Brief

Shrivastava et al. describe a novel rotary motor that spins tethered gliding cells. It generates high torque and runs at constant speed. The catalog of biological rotary motors now contains three motors powered by protonmotive force: the F_o ATP synthase, the bacterial flagellar motor, and the gliding motor.

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Report

A Rotary Motor Drives *Flavobacterium* Gliding

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Summary

Cells of *Flavobacterium johnsoniae*, a rod-shaped bacterium devoid of pili or flagella, glide over glass at speeds of 2–4 μm/s [1]. Gliding is powered by a protonmotive force [2], but the machinery required for this motion is not known. Usually, cells move along straight paths, but sometimes they exhibit a reciprocal motion, attach near one pole and flip end over end, or rotate. This behavior is similar to that of a *Cytophaga* species described earlier [3]. Development of genetic tools for *F. johnsoniae* led to discovery of proteins involved in gliding [4]. These include the surface adhesin SprB that forms filaments about 160 nm long by 6 nm in diameter, which, when labeled with a fluorescent antibody [2] or a latex bead [5], are seen to move longitudinally down the length of a cell, occasionally shifting positions to the right or the left. Evidently, interaction of these filaments with a surface produces gliding. To learn more about the gliding motor, we sheared cells to reduce the number and size of SprB filaments and tethered cells to glass by adding anti-SprB antibody. Cells spun about fixed points, mostly counterclockwise, rotating at speeds of 1 Hz or more. The torques required to sustain such speeds were large, comparable to those generated by the flagellar rotary motor. However, we found that a gliding motor runs at constant speed rather than at constant torque. Now, there are three rotary motors powered by protonmotive force: the bacterial flagellar motor, the F_o ATP synthase, and the gliding motor.

Results and Discussion

The Gliding Motor Rotates in Place

We developed a method for tethering *F. johnsoniae* to a glass surface using anti-SprB antibody (Figure 1A). This method is similar to the procedure for shearing and tethering cells of *Escherichia coli* [6, 7], a technique used extensively in studies of chemotaxis of flagellated bacteria. Tethered *F. johnsoniae* cells rotated about a fixed point, as shown in Movie S1. Tracks of their center of mass were circular (Figure 1B). We tracked the tethering point and found its displacement to be within ~5 nm, which is negligible compared to the μm-sized circular trajectory (Figure 1C). In addition, the radius of the circular track remained constant. This argues that the SprB filament is connected to a rotary motor that stays in place. To further test for evidence of rotation, we attached a polystyrene bead to a sheared cell and tracked rotation of the bead (Figures 1D and 1E; Movie S2).

Most Gliding Motors Rotate Counterclockwise

92% of motors rotated counterclockwise, and 8% rotated clockwise (Figure 2A). Changes in the direction of rotation were not observed. Presumably, the direction of rotation of motors observed on tethering determines the direction of translation of SprB filaments when cells glide. Fluorescently labeled SprB has been reported to move along a left-handed closed helical loop [2], whereas labeling with latex beads has shown that SprB molecules move in different directions, often crossing paths while moving on a single cell [5]. We envision that gliding motors are present along multiple looped tracks and that these tracks intersect each other. Analysis of a population of tethered cells showed that the position of the pivot varied from near the pole to near the middle of the cell, but in a majority of cells, the pivot was near the pole (Figure 2B). This suggests that multiple tracks intersect near the pole.

Torque Generated by the Gliding Motor Is Large

Speeds of rotation were calculated from the center-of-mass trajectories using custom MATLAB codes. The cells rotated with an average angular speed of ~1 Hz (Figure 2C). Torque generated by each gliding motor was calculated using a formula described in the Experimental Procedures [8], based upon measurements of angular speed (Figure 2C), cell length, cell width, and trajectory radius (Figure S1). Torque ranged from 200–6,000 pN nm, with most cells running at ~1,000 pN nm (Figure 2D). Torques measured with motors of *E. coli* spinning latex beads (~1 μm diameter) averaged ~1,300 pN nm [9, 10]; therefore, the torques generated by the gliding motor are comparable to those generated by a flagellar motor. Stator elements formed by MotA and MotB proteins act as force-generating units that generate torque for rotation of flagellar motors. It is likely that similar stator elements, albeit made up of different protein subunits, harvest protonmotive force to power rotation of the gliding motor.

The Gliding Motor Runs at Constant Speed

F. johnsoniae cells tethered in a flow cell were exposed to 8% w/v solutions of Ficoll in motility medium (MM). Ficoll is a viscous agent commonly used to alter the load on bacterial flagellar motors [11]. Rotation speeds of single cells were measured. Speeds did not change significantly (Figures 3A and 3B). However, the torque generated by the motors, equal to the viscosity × the viscous drag coefficient × the speed, increased dramatically (Figures 3C and 3D). A gliding cell has multiple moving SprB filaments. It is reasonable for them to move at the same speed rather than at different speeds. Otherwise, if more than one filament adhered to the substratum, the motors would not work synchronously. In our experiment, speed remained constant, but torque increased with increase in load (viscosity). When attached to a bead, which represents a low load compared with that of a tethered cell, the gliding motor rotated the bead at a speed comparable to that of the tethered cell (Figure 1E). We do not know whether speed is an intrinsic property of the motor or whether a cellular mechanism exists that coordinates speeds of different motors.

The Gliding Motor Is Novel

Genome sequencing has shown that *F. johnsoniae* lacks proteins similar to components of the bacterial flagellar motor [12]. GldJ is a putative component of the gliding motor. Presumably GldJ interacts with the Type-IX protein secretion

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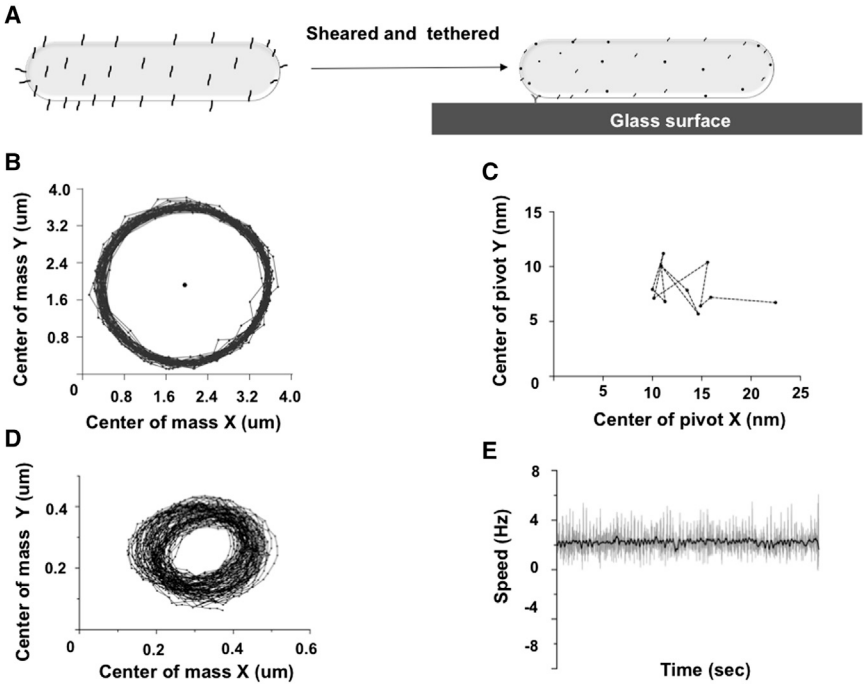

Figure 1. Evidence for a Rotary Motor

(A) The *F. johnsoniae* adhesin SprB is present on the cell surface as ~160-nm-long filaments. SprB was sheared off, and anti-SprB antibody was used to tether *F. johnsoniae* to a glass surface.

(B) The trajectory of the center of mass of a tethered cell plotted over 1,000 frames, with the center of rotation plotted as a black circle.

(C) The position of the center of rotation was averaged over 100 frames and plotted as black circles for a movie spanning 1,200 frames; the drift of the center of rotation shown with a dotted line was negligible (<5 nm).

(D) Center of mass of a 0.5-μm polystyrene bead tethered onto a sheared cell was tracked over 2,192 frames.

(E) Speed of rotation of the bead is plotted in gray, and average speed was calculated every ten frames and plotted in black. The time span was 35 s.

system (T9SS) and is important for the movement of cell-surface adhesins. GldK, GldL, GldM, and GldN are core T9SS proteins, and cells lacking these proteins do not exhibit motility. The macromolecular structure of the gliding motor and its exact interaction with T9SS is unclear. GldL localizes to the cytoplasmic membrane, and it might act as an anchor for the gliding motor [13]. Besides the core T9SS proteins, other Gld and Spr proteins might associate with this motor. The gliding motor appears to associate with T9SS in a manner analogous to the association of the bacterial flagellar motor with the Type-III secretion system (T3SS). In flagellated bacteria, T3SS is required for secretion of axial components of the flagellum. In *F. johnsoniae*, T9SS is required for secretion of the SprB filament and a mobile adhesin, RemA [13, 14].

Model for *Flavobacterium* Gliding

A model for *Flavobacterium* gliding was proposed recently [15] in which rotary motors drive baseplates, to which SprB filaments are attached. The baseplates were visualized by cryoelectron tomography [16]. In our model, gliding motors formed complexes with T9SS, which spanned the inner and outer membranes, harvesting protonmotive force to power SprB rotation. The baseplates moved along the inner surface of the outer membrane (Figure 4). If this is correct, then shearing breaks filaments and fragments baseplates, allowing a motor to spin a fragment together with one or more of its filaments that are adsorbed to the substratum. If SprB is to move the length of cells along tracks, there must be several gliding motors per cell. If there is a molecular rack and pinion that converts rotation to translation, and the pinion rotates, for example, 10 Hz, it would have to be 100 nm in diameter to drive

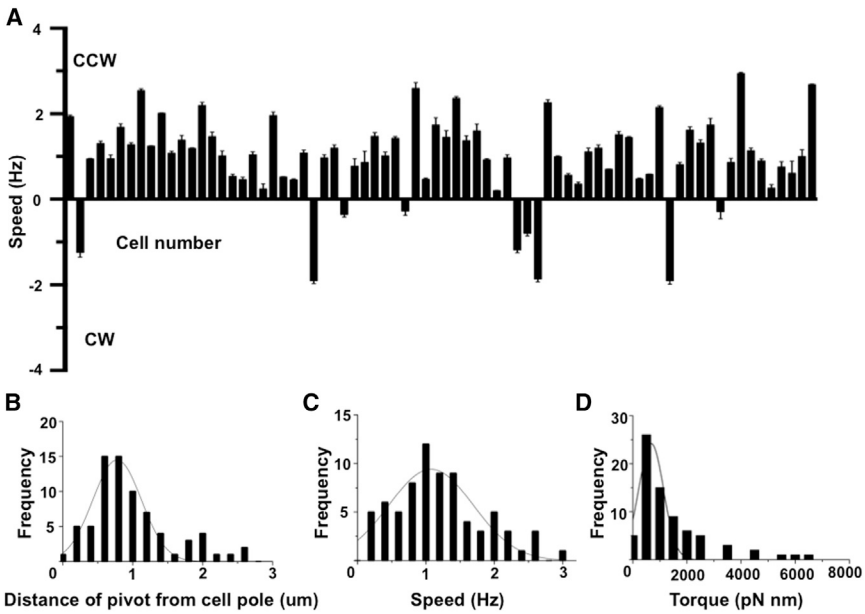

Figure 2. Speeds and Torques Recorded for 74 Tethered Cells

(A) 92% of cells rotated counterclockwise and 8% rotated clockwise. Speed was calculated by recording cell rotation twice for 1-min periods. Average speed for each recording was calculated. Error bars represent SD in speed of the same cell between the two recordings. Changes in direction of rotation were not seen.

(B) Frequency distribution of pivot position for 74 cells normalized for an average cell length of 6 μm. Most cells tethered at a distance of ~1 μm from the cell pole.

(C) Speeds ranged from 0.2–3 Hz, with a majority of cells rotating with a speed ~1 Hz.

(D) The torque varied from ~200 to ~6,000 pN nm, with the majority of cells at a torque ~1,000 pN nm. For torque calculations, see Experimental Procedures.

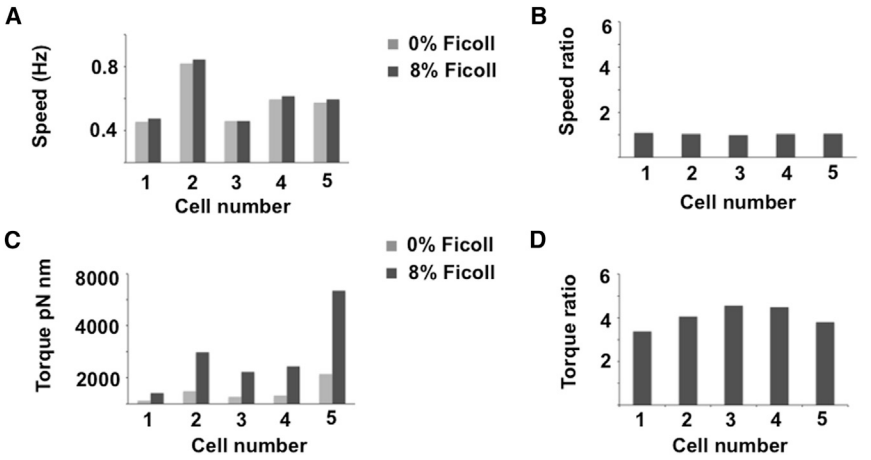

Figure 3. Measured Speeds and Computed Torques for Cells in 0% and 8% Ficoll

(A) Speed at 0% and 8% Ficoll.

(B) The ratio of speeds at 8% and 0% Ficoll is close to 1.

(C) Torque at 0% and 8% Ficoll.

(D) The ratio of torques at 8% and 0% Ficoll approximates the ratio of viscosities, 3.91.

the cell 3 μm/s. Thus, gliding motors could be as large as bacterial flagellar motors. Attempts were made to isolate gliding motors with *Cytophaga* [3], using the methods developed for flagellar motors, but without success. In an alternative model, SprB filaments might be attached to rotary motors directly, with an unknown mechanism that passes filaments from one motor to the next. Advanced microscopic tools might shed light on motor structure and interactions between motors and baseplates. How the gliding motor generates torque and manages to run at a constant speed are interesting questions that beg for answers. We now know of three rotary motors powered by protonmotive force: the bacterial flagellar motor, the F_o ATP synthase, and the gliding motor. The bacterial motors generate about 25 times more torque than the F_1 ATPase.

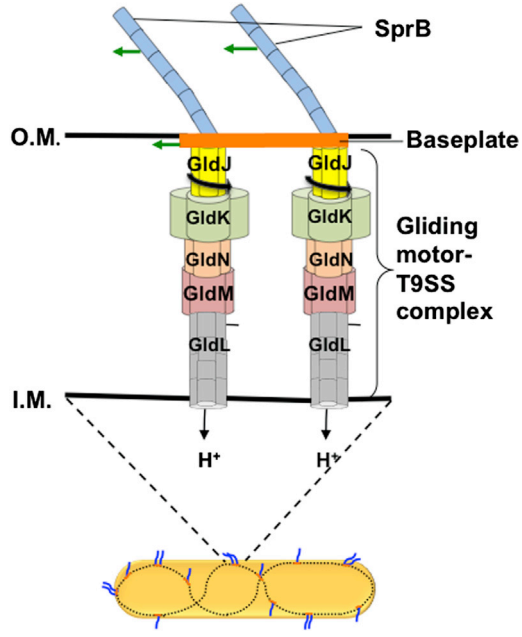

Figure 4. *Flavobacterium* Gliding Model

A *Flavobacterium* cell with two gliding motors attached to a baseplate mounted on a looped track (bottom). Two SprB filaments are attached to the baseplate and move with it. If either of these filaments adheres to the substratum, the cell glides. Shearing shortens the filaments and disrupts the baseplate so that each filament is driven by a different motor. If one filament adheres to the substratum, the cell body spins about the axis of the motor.

Experimental Procedures

Cell Tethering

Cells of wild-type *F. johnsoniae* CJ1827 were grown overnight at 25°C in MM (per liter: 1.1 g Casitone, 0.55 g yeast extract, 1.1 mM Tris [pH 7.5]) with shaking at 50 rpm. These cells were inoculated in fresh MM and were grown in the same way to OD_{600} 0.4. Then, 500 μl of the culture was passed 50 times through polyethylene tubing of inner diameter 0.58 mm between 1 mL syringes equipped with 23G stub adapters, a procedure similar to that used for shearing *E. coli*. The sheared cells were washed with 500 μl MM. Anti-SprB antibody [5] was purified using Melon Gel IgG Spin Purification Kit (product #45206, Thermo Scientific) and preadsorbed against an *F. johnsoniae ΔsprB* mutant. 40 uL of the suspension of sheared cells was incubated for 20 min with the purified antibody diluted 1:10. After incubation, the cells were washed and resuspended in 40 uL MM. The cells were added to a tunnel slide and incubated for 5 min. The slide was washed three times with 200 uL MM.

Imaging and Image Analysis

Movies of tethered cells were recorded using a phase-contrast microscope with a digital camera running at a frame rate of 62 frames per second (Thorlabs, DCC1545M-GL). Custom MATLAB codes were used to analyze cell rotation and bead tracking [17]. The center of mass of the cell was tracked to calculate speed and direction of rotation. Cell length, width, and distance of the center of mass from the center of rotation were calculated.

Attachment of Beads to Sheared Cells and Measurement of Bead Rotation

5 μl polystyrene beads (0.5 μm diameter, Polysciences) with 5 μl anti-SprB antibody were added to 50 μl of cells and incubated for 5 min. If attached to unsheared cells, the beads traveled down the length of a cell, sometimes moving from side to side, indicative of the translation of SprB. If attached to sheared cells, the beads rotated in place, as shown in Figures 1D and 1E. Data were collected for three beads rotating at average speeds of 2.19 Hz, 0.71 Hz, and 0.38 Hz. The rotating beads were imaged using a phase-contrast microscope with a digital camera running at a frame rate of 62 frames per second (Thorlabs, DCC1545M-GL). Movies were analyzed using custom MATLAB codes [17].

Torque Calculations

Torques generated by motors spinning tethered cells were calculated for each cell separately using the formula given in [8], $N_r = (C_r + r^2C_t)2\pi f$, where r = distance between the center of rotation and the center of mass of a cell, f = rotation rate, and C_r and C_t are rotational and translational frictional drag coefficients, respectively. With the cell approximated as a prolate ellipsoid, $C_r = (8\pi\eta a^3/3)/(\ln 2a/b - 0.5)$, $C_t = 8\pi\eta a/(\ln 2a/b + 0.5)$, a = cell length/2, and b = cell width/2.

Ficoll Experiments

F. johnsoniae cells were grown and sheared as described above. Cells were tethered onto a cover glass attached to a flow cell. MM was added at the rate of 50 uL/min using a syringe pump (Harvard Apparatus 22). The rotation of a cell was recorded at 0% Ficoll, and then, a solution at higher concentration was pumped through for a period of 5 min. The rotation rate of the same cell was then recorded. Torque was calculated as described above, using the viscosities measured previously [11]: in cP, 0% 0.986 and 8% 3.86.

Supplemental Information

Supplemental Information includes one figure and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.11.045.

Author Contributions

A.S. and H.C.B. planned the experiments and wrote the paper. A.S. performed the experiments. A.S. and P.P.L. analyzed the data.

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