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Towards a model for *Flavobacterium* gliding

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Abstract

Cells of *Flavobacterium johnsoniae*, a rod-shaped bacterium about 6 μm long, do not have flagella or pili, yet they move over surfaces at speeds of about 2 μm/s. This motion is called gliding. Recent advances in *F johnsoniae* research include the discovery of mobile cell-surface adhesins and rotary motors. The puzzle is how rotary motion leads to linear motion. We suggest a possible mechanism, inspired by the snowmobile.

Keywords

Flavobacterium johnsoniae; rotary motor; SprB filament

Introduction

Flavobacterium (phylum bacteroidetes), *Myxococcus* (phylum proteobacteria) and *Mycoplasma* (phylum firmicutes) are three bacterial genera that provide model systems for gliding that can be manipulated genetically. *Flavobacterium johnsoniae* and *Myxococcus xanthus* contain peptidoglycan and are rod shaped, whereas *Mycoplasma mobile* is devoid of peptidoglycan and is flask shaped. *M. mobile* gliding machinery localizes around the neck region of the cell and utilizes ATP hydrolysis to power movement. A protein present at the neck anchored by a cytoskeletal network acts as a ‘leg’ that adheres to a surface and enables gliding by a centipede-like mechanism [1]. The *M. mobile* model cannot explain gliding of the other bacteria, such as *F. johnsoniae* and *M xanthus*, which are powered by a protonmotive force [2,3]. Several models have been proposed to explain gliding of *M xanthus* [4,5], which occurs at about $1/60^{th}$ the speed of gliding in *F johnsoniae*. Of these models, the helical rotor model appears to be the most widely accepted [6]. According to this model, the gliding motor of *M. xanthus* moves along a helical intracellular track, and this motion somehow causes movement of cell-surface components. In contrast, *F johnsoniae* has rotary motors that stay in place and power gliding [7]. This solves the ‘peptidoglycan problem’ [5] associated with the helical rotor model for *M. xanthus*. The *F. johnsoniae* motor drive shafts pierce the peptidoglycan layer; they don't move laterally through the peptidoglycan. The cell-surface and motor components implicated in *F.*

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johnsoniae gliding, discussed below, are genetically unique, different from those identified in other bacteria. The mechanism of *F. johnsoniae* gliding is different from that of both *M. xanthus* and *M. mobile*.

Bacteria of the phylum bacteroidetes are present in diverse ecological niches ranging from aquatic and terrestrial environments to the gut microflora. The family *Flavobacteriaceae*, which is a part of this phylum, comprises commensal bacteria and opportunistic pathogens. Some of these bacteria infect fish and are causative agents of columnaris disease, bacterial coldwater disease and bacterial gill disease. They also secrete a large array of enzymes that help degrade and utilize extracellular proteins and polysaccharides. They are distinctive in being able to move rapidly over surfaces, even glass, without flagella or pili, organelles that enable other bacteria to swim, swarm, or twitch [8].

For large flying objects, such as birds, airplanes, or humans in gliders, gliding refers to a smooth continuous motion that occurs without the use of engines. A glider falls through the air, buoyed by its wings. It moves at high Reynolds number, carried forward by inertia, resisted by drag. A microscopic organism immersed in water moves at low Reynolds number, overwhelmed by drag (whether on a surface or not). It cannot coast [9]. Thus, bacterial gliding is an active process that requires constant influx of energy and must be caused by movement of proteins that carry cells over a surface. We think the machinery used for bacterial gliding might be analogous to the machinery that powers a snowmobile. How does one build such a machine on a microscopic scale?

Cells of *Flavobacterium johnsoniae* are amongst the fastest known gliders and have emerged as a powerful model system to study bacteroidetes-specific gliding. They have mobile cell-surface adhesins that move in a looped fashion and interact with another surface on which the bacteria glide. Recent advances in genetic manipulation of *F. johnsoniae* (reviewed by McBride and Nakane in this issue) led to discovery of proteins that are required for gliding. Some of these proteins form filaments that project from the surface of the cell and move along its length [10,11], while others form motors that can rotate these filaments [7]. How rotation leads to linear displacement is an open question. Are the cell-surface filaments carried by treads driven by rotary motors, in the way that chains are driven by sprockets powered by rotary motors? Clearly, more experimental evidence is required. In any event, the filaments interact with a surface and enable motion of the cell.

The mechanism for propulsion

The movement of cell-surface adhesins was first reported by Pate and Chang [2] for *Flavobacterium columnare* (previously known as *Flexibacter columnaris*) and *Flavobacterium johnsoniae* (previously known as *Cytophaga johnsoniae*). Movement was detected by attaching polystyrene latex beads to a cell and tracking their displacement. Similar work was done with *Cytophaga U67*, which is a close relative of *F. johnsoniae* [12]. Electron microscopy data show that one *F. johnsoniae* cell has many cell-surface filaments. More recently, it was discovered that the filaments are composed of a protein, SprB. Motion of anti-SprB antibody conjugated polystyrene beads, and of anti-SprB antibodies labeled with a fluorescent dye, was recorded and tracked [11,13]. Some reports suggest that

filaments move along looped tracks [12-14] while one report suggests that they move helically [11].

Recording of a cell gliding over a stationary polystyrene bead (Fig.1 and MovieS1) provides useful insights on the motion of a SprB filament. These recordings suggest (i) the presence of a continuous track and (ii) that once a filament is attached to a surface, it does not need to detach for a cell to glide. In Fig.1 and MovieS1, one or more SprB filaments are attached to a bead at their distal ends. This end of the filament does not move relative to the bead and the glass surface to which the bead is adsorbed. The filament however, is loaded onto a component (call it a tread) that moves along a track fixed to the rigid framework of the cell, presumably, the peptidoglycan layer. The filament and tread are in motion relative to the cell-surface, while the track is fixed to that surface. The filament and tread are pushed or pulled along the track, which results in motion of the cell body relative to the end of the filament that is attached to the bead. The track loops around the end of the cell, so when the cell pole reaches the bead, the cell flips over, i.e., the lagging pole becomes the leading pole, while the direction of cell motion in the laboratory frame remains the same. On the other hand, if the distal end of SprB filament is free, it is pulled along the surface of the cell. The motion of SprB can be visualized by attachment of a free anti-sprB coated latex bead or of fluorescent anti-SprB antibody. The identity of the filament, SprB, is known, but the identities of the tread and track are not. Structures visualized by cryo-em tomography as patches that are connected to SprB filaments and are present at the base of the outer membrane [10] might be sections of tread.

The average length of an *F. johnsoniae* cell is 6 μm, and the average gliding speed is 2 μm/s. So, one SprB filament can propel a cell for 3 s. After that, if the cell is to continue to glide in the same direction, the filament must detach from the glass surface and another SprB filament must take its place. One *F. johnsoniae* cell has many filaments that move at the speed of ∼2 μm/s, which is also the speed of a gliding cell. So, the filaments work in parallel rather than in tandem. The *F. johnsoniae* rotary motor is a constant-speed device [7], which is convenient if different SprB filaments are to move in parallel. As long as all filaments are moving along the same track or on parallel tracks, the number of points of adhesion increases the propulsive force that can be exerted but not the speed of cell movement.

Surface adhesins for gliding

Sticking to a surface is the first step a cell takes towards gliding. So, it is important that the surface filament has regions that are adhesive. *F. johnsoniae* has two surface adhesins, SprB and RemA. RemA has a lectin domain that binds exo-polysaccharides, and it's over-expression results in cell aggregation [14]. SprB is a protein of molecular weight 669 kDa, four times larger than RemA. Both SprB and RemA are involved in sticking a cell to glass. The adhesive domains of SprB are not known. *Flavobacteria* are present in most aquatic and terrestrial environments. Filament-like structures were recently observed for *Flavobacterium columnare* [15]. Sequence alignment of *F. johnsoniae* and *F. columnare* SprB shows that while the two proteins have regions of similarity, they also have large regions that are not conserved. Large regions of *F. johnsoniae* SprB, spread all over the amino-acid sequence, are absent in *F. columnare* SprB. Despite missing these regions, *F. columnare* glides. *F.*

columnare is a fish pathogen and is the causative agent of columnaris disease. It has the ability to adhere to fish and form biofilms [16], it is likely that surface-adhesion domains of *F. columnare* SprB might be required for both surface colonization and gliding.

From rotation to gliding

Our work demonstrated that a rotary motor is involved in *F. johnsoniae* gliding [7]. One possible mechanism for converting rotation to longitudinal motion is that of a protein tread connecting two rotary motors in a manner similar to that by which a chain connects two sprockets, or by which treads in a snowmobile are linked to a gasoline engine. In *F. johnsoniae* this chain would be the moving tread carrying SprB. Gld proteins probably form the rotary motor, but the identity of other components is not known. To identify the components, more information about mutants that are defective in gliding is needed. Besides being defective in gliding, many such mutants lack the ability to secrete an array of proteins, which include the cell-surface adhesins SprB and RemA, via the Type IX protein secretion system (T9SS) [17-24]. It is unclear if mutants lacking genes that encode T9SS proteins are defective in gliding because they lack the secretion apparatus or the gliding motor. Differentiating between proteins that play exclusive roles in either gliding or secretion via T9SS appears to be crucial. While most structural information for the Gld proteins is lacking, recently, the preliminary X-ray analysis for the carboxy-terminal fragment of GldM (PorM) was reported [25]. With increasing knowledge about gliding proteins, the next step forward might be to study their interactions and to visualize the structure of the gliding apparatus.

It is instructive to recall how the basal body of the flagellar rotary motor was visualized. The iconic images made by DeRosier [26] were of motors dissolved out of *Salmonella* with neutral detergents, by the method of Aizawa et al. (1985) [27], who followed the procedure of DePamphilis and Adler (1971) [28] but worked at higher pH. Cell walls were weakened by treatment with lysozyme-EDTA and the spheroplasts were dissolved with Triton X-100. The viscosity of the lysate was lowered by treatment with DNase, and insoluble components were separated by differential centrifugation. The basal bodies could be found because they were at the proximal ends of flagellar filaments. Might one covalently link beads to SprB filaments and fish for components isolated by a similar procedure? More recent work by cryo-em tomography [29,30] began with spirochetes, because their cell bodies were thin [31]. Might cryo-em be used to find gliding motor components in *F. johnsoniae* minicells?

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

1. Bacterial gliding, i.e. movement over a surface, of bacteria that have neither flagella nor pili, requires constant propulsion.
2. Gliding *Flavobacterium* have a rotary motor and mobile cell-surface filaments.
3. The machinery required for gliding could be analogous to the components that power and drive a snowmobile.

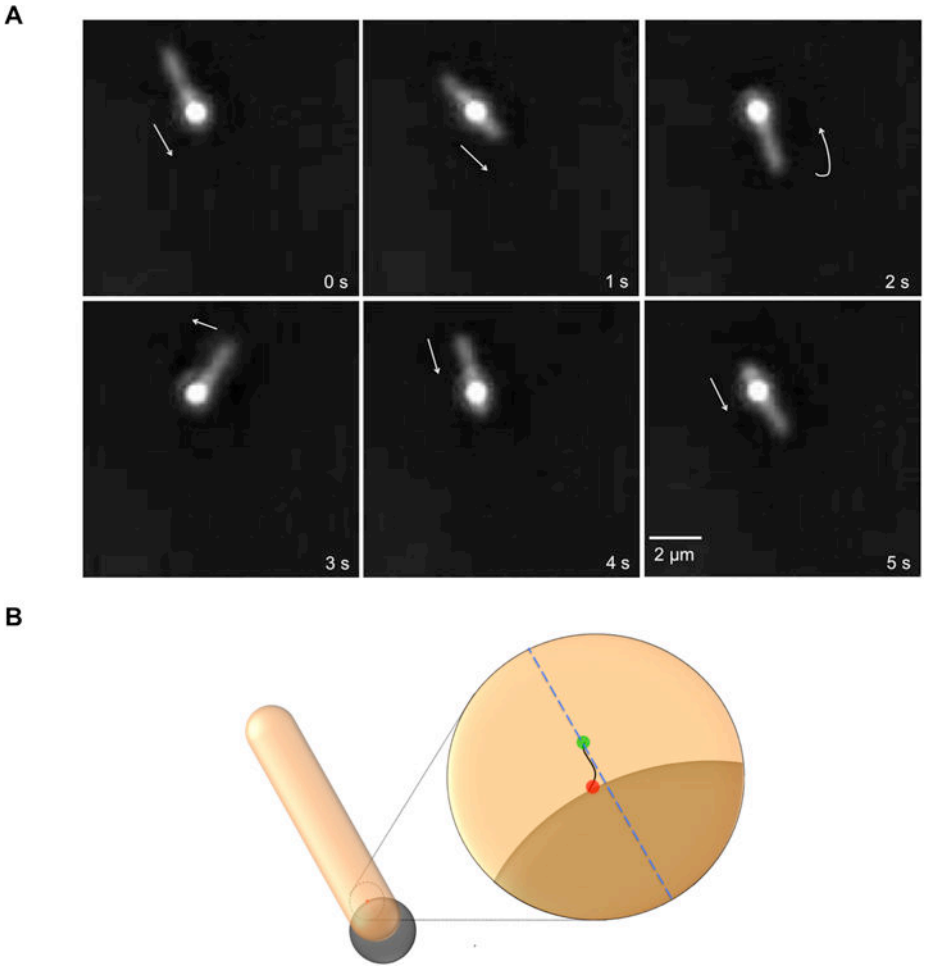

Figure 1.

Movement of *F. johnsoniae* on a stationary polystyrene bead. (A) Images at 1 s time intervals from Movie S1, taken using a phase contrast microscope with a bright-phase objective (Nikon Plan 40× BM), showing a rod-shaped *F. johnsoniae* cell (slightly out of focus) moving over a spherical bead (0.5 μm). Time is shown at the bottom right of each frame and a scale bar is shown at bottom left of the last frame. Arrows depict direction of motion of the leading pole (0-3 s), which later becomes the lagging pole (4 s). (B) Diagrammatic representation of a cell moving over a stationary bead, with a magnified view in the inset. In the magnified view, a SprB filament (black), which is underneath the cell, is bound to the bead at an attachment site (red dot) that remains fixed. The SprB filament is loaded on a tread guided by a track (blue dotted line), and the SprB filament-to-tread attachment site (green dot) is in motion relative to the bead.