HHS Public Access

Author manuscript

Mol Microbiol. Author manuscript; available in PMC 2017 July 01.

Published in final edited form as:

Mol Microbiol. 2016 July ; 101(2): 186–193. doi:10.1111/mmi.13389.

Novel mechanisms power bacterial gliding motility

Beiyan Nan[1] and **David. R. Zusman**[2]

[1] Department of Biology, Texas A&M University, College Station, TX 77843, USA.

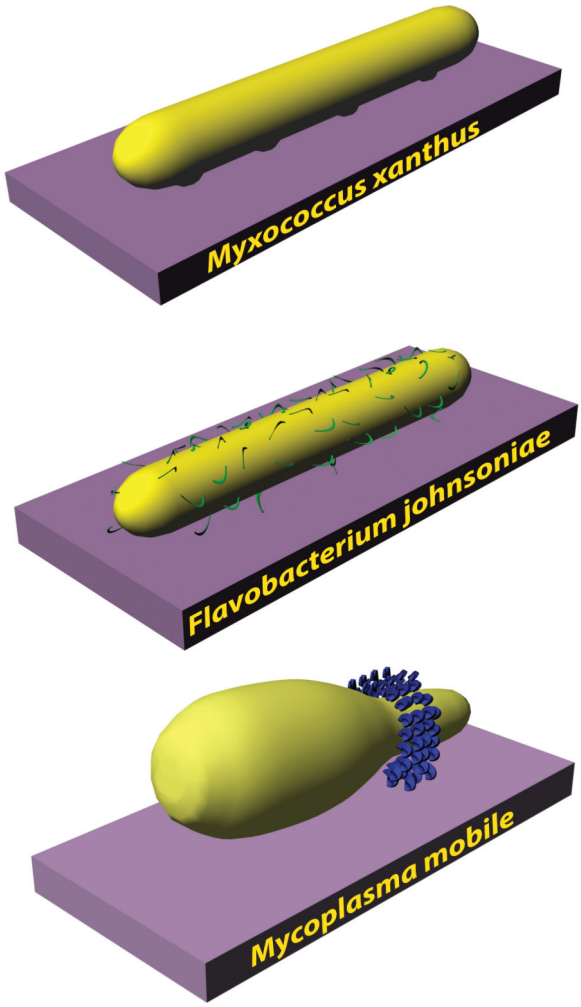
[2] Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

Summary

For many bacteria, motility is essential for survival, growth, virulence, biofilm formation and intra/interspecies interactions. Since natural environments differ, bacteria have evolved remarkable motility systems to adapt, including swimming in aqueous media, and swarming, twitching and gliding on solid and semi-solid surfaces. Although tremendous advances have been achieved in understanding swimming and swarming motilities powered by flagella, and twitching motility powered by Type IV pili, little is known about gliding motility. Bacterial gliders are a heterogeneous group containing diverse bacteria that utilize surface motilities that do not depend on traditional flagella or pili, but are powered by mechanisms that are less well understood. Recently, advances in our understanding of the molecular machineries for several gliding bacteria revealed the roles of modified ion channels, secretion systems and unique machinery for surface movements. These novel mechanisms provide rich source materials for studying the function and evolution of complex microbial nano-machines. In this review, we summarize recent findings made on the gliding mechanisms of the myxobacteria, flavobacteria and mycoplasmas.

Graphical abstract

bnan@tamu.edu and zusman@berkeley.edu



Surfaces are natural habitats for many bacterial species. Some bacteria glide on surfaces without the aid of traditional flagella or pili. The mechanisms of gliding are not well understood. Recent advances revealed that gliding in different bacteria involves diverse motors and a broad spectrum of mechanisms. This review summarizes recent findings on the gliding mechanisms of the myxobacteria, flavobacteria and mycoplasmas.

Introduction

Surfaces are natural habitats for many bacterial species. Surfaces enable bacteria to cooperate with kin, compete with other microorganisms, construct complex biofilms, and interface with humans and other eukaryotic hosts (Persat *et al.*, 2015). To move actively over surfaces, bacteria employ several behaviors including swarming, twitching, gliding, and host tissue interactions (Henrichsen, 1972, Dubreuil *et al.*, 2002). Swarming occurs on soft and moist surfaces and cells are propelled by the rotation of flagella (Kearns, 2010). Twitching motility also functions on soft and moist surfaces and is powered by the extension of type IV pili; pilus retraction can subsequently pull cells forward (Li *et al.*, 2003, Maier *et al.*, 2002, Skerker & Berg, 2001, Chang *et al.*, 2016). Gliding motility, on the other hand, usually

functions on firmer and drier surfaces. Because the motors for gliding were unknown and gliding bacteria have no obvious external structures associated with motility, gliding was traditionally defined by the motors that were not used (flagella and pili) rather than by the motors that were used. This unfortunate definition caused the presumption that the mechanism for gliding in diverse bacteria is likely to be similar.

In the past decade, genomic information has expedited the discovery of motors and motility-related genes in many species. Additionally, the application of advanced electron microscopy (EM) techniques such as cryo-electron tomography (cryo-ET) has enabled the visualization of novel motility-related protein complexes. Furthermore, super-resolution fluorescence microscopy has enabled us to monitor the dynamics of motility-related proteins in live cells. These results revealed that gliding in different bacteria involves diverse motors and a broad spectrum of mechanisms. The aim of this review is to summarize recent findings on gliding in the myxobacteria, flavobacteria and mycoplasmas. Although our knowledge is still limited with many pieces missing, we can already take a peek at the beautiful dynamics of these molecular machineries.

Myxobacterial gliding is powered by modified flagella stator complexes that move rapidly within the membrane

Myxobacteria are Gram-negative δ-proteobacteria. In the order of *Myxococcales*, most species are rod-shaped soil bacteria that feature surface movements and fruiting body formation. *Myxococcus xanthus*, the best studied myxobacterium, is a model organism for studying surface motility, social behaviors, biofilm formation, and interspecies interaction such as predation (Zusman *et al.*, 2007, Keane & Berleman, 2016). *M. xanthus* lacks flagella and is unable to swim in liquid culture. Instead, it employs two distinct mechanisms to move on surfaces: gliding and twitching (Nan & Zusman, 2011). Twitching motility in *M. xanthus* is based on the extension and retraction of type IV pili, similar to that of *Pseudomonas* and *Neisseria* (Wu & Kaiser, 1995, Chang *et al.*, 2016). By contrast, gliding motility in *M. xanthus* appears to be unlike other characterized prokaryotic motility systems. Despite the identification of dozens of gliding-related genes (Hodgkin, 1979, Youderian *et al.*, 2003), the search for the gliding motors lasted for decades. In 2011, two groups reported that a proton channel formed by three proteins AglR, AglQ and AglS is essential for gliding. Importantly, this proton channel/motor complex is homologous to the *Escherichia coli* flagella stator complex MotAB (AglR is a MotA homologue while AglQ and AglS are MotB homologues), suggesting that gliding is powered by proton motive force (PMF) (Nan *et al.*, 2011, Sun *et al.*, 2011). This hypothesis was confirmed by the isolation of a point mutation in the putative proton-binding site in AglQ that completely abolished gliding (Sun *et al.*, 2011).

Since *M. xanthus* gliding does not depend on visible surface appendages, it is still an open question as to how motor proteins in the inner membrane can propagate mechanical force to the cell surface and propel the movement of the cell body. An important clue to this enigma came from a comparison of the MotB homologues from *M. xanthus* with the *E. coli* MotB: both AglQ and AglS from *M. xanthus* lack the C-terminal peptidoglycan attachment motif. Since the *M. xanthus* AglRQS stator complex, unlike its *E. coli* homologue, was untethered,

it could hypothetically be free to move within the membrane. This possibility was confirmed by direct observation of fluorescently tagged AglR and AglQ using super-resolution microscopy (Nan *et al.*, 2013):

Super-resolution microscopy techniques, such as the single-particle tracking photoactivated localization microscopy (sptPALM), are capable of pinpointing the location of individual protein particles with sub-diffraction resolution (<100 nm), and to resolve real time molecular dynamics in live cells (Manley *et al.*, 2008). To study the mechanism by which the AglRQS channel powers gliding, AglR and AglQ were labeled with photoactivatable fluorophores and their molecular dynamics studied at 100-ms time resolution using sptPALM (Nan *et al.*, 2015, Nan *et al.*, 2013). These studies found that single AglRQS channels move in helical trajectories at up to 3-5 μm/s, indicating that rather than being restricted in the membrane, the AglRQS channel functions as the core component in the gliding motor complex. Collectively, the motion of hundreds of motor complexes can appear as rotating helices inside each cell (Nan *et al.*, 2015, Nan *et al.*, 2013). Careful analysis of the molecular behavior of the AglR protein revealed a striking phenomenon: on a firm surface, the fast-moving motor complexes tend to slow down and accumulate at a few "traffic jam" sites on the ventral sides of cells, where the cells contact the gliding surface (Nan *et al.*, 2013). These sites are dynamic as motor complexes continuously enter and leave the clusters. The clusters distribute evenly along the cell body due to helix periodicity and appear to remain near stationary as cells move forward (Nan *et al.*, 2011, Nan *et al.*, 2013) (**Fig. 1A**).

These results help to explain earlier data. By standard resolution microscopy, the fluorescently-labeled proteins, AglR, AglQ and the motor-associated proteins AgmU and AglZ all appeared as blurry fluorescent patches or clusters that change shape and localization constantly. Despite their different cellular localization (AglR and AglQ in the membrane, AgmU in periplasm and AglZ in cytoplasm), when cells were moving on a solid surface all four proteins showed a common feature: they tended to aggregate into a few fluorescent spots that evenly distributed along the long cell axes. Surprisingly, when cells moved forward, these protein clusters did not move along with the cells but remained at fixed positions with respect to the substratum (Mignot *et al.*, 2007, Nan *et al.*, 2013, Nan *et al.*, 2011, Nan *et al.*, 2010, Sun *et al.*, 2011). In other words, the cells appeared to move through these spots, a behavior similar to the eukaryotic motilities that depend on focal adhesions (Smilenov *et al.*, 1999) (**Fig. 1A**). When cells were placed in a liquid broth or in 1% methylcellulose, the labeled proteins appeared to decorate a rotating helix; however, these cells could not move by gliding as they lacked a solid surface (Nan *et al.*, 2013, Nan *et al.*, 2011). Based on the above experimental observations, two models were proposed to interpret the aggregation of motor clusters and to explain the mechanism by which cells transform PMF from the inner membrane into mechanical forces on the cell surface.

The **focal adhesion model** interprets the aggregates of motor complexes as rigid focal adhesion clusters (FACs). According to this model, each locus contains multiple FACs that span the cell envelope and anchor to the substratum. The gliding motor complexes push against FACs, and thus transport these FACs linearly towards the posterior end of the cells. Since FACs and adhesins are proposed to anchor cells to the gliding surface, the backward

translocation of FACs would propel cells forward (Mignot *et al.*, 2007, Sun *et al.*, 2011) (**Fig. 1A**). The exact composition of the putative FACs is still unknown. However, dozens of proteins were found to associate with the gliding complexes, including cytoplasmic, periplasmic and integral membrane proteins and lipoproteins that attach to inner and outer membrane (Luciano *et al.*, 2011, Nan *et al.*, 2010, Jakobczak *et al.*, 2015, Youderian *et al.*, 2003). A possible problem encountered by the focal adhesion model is breaching the cell wall barrier, as the FACs are proposed to repeatedly sever the rigid peptidoglycan layer in order to push the cell body forward. However, it is possible that cells have evolved a novel mechanism to circumvent the cell wall problem, which has not yet been recognized. Over 40 genes have been reported as important for gliding motility in *M. xanthus*, but most of these genes have functions that have not yet been determined.

The **helical rotor model** proposes that the seemingly stationary fluorescence spots seen in gliding cells on surfaces are actually caused by the transient accumulation of motor complexes caught in dynamic “traffic jams.” According to the model, the motor complexes and associated proteins move rapidly in a helical pathway through the membrane, temporarily slowing down when encountering resistance from the gliding substratum. Evidence for these “traffic jams” comes from the movement of motor complexes in cells placed on agar of different composition. On harder agar, clusters of motor complexes appear larger and individual motor complexes slow down significantly; however, on leaving the cluster sites, their maximal velocity is restored (Nan *et al.*, 2013, Nan *et al.*, 2010). The accumulated motor complexes in these traffic jam sites (and their associated proteins) are proposed to exert a force that slightly deforms the cell envelope, generating a backward surface wave as the motor complexes push backward, analogous to a crawling snail. Accordingly, these traffic jam sites would act as force generators to propel the cells forward (Nan *et al.*, 2014, Nan & Zusman, 2011). For detailed computer simulation, see (Nan *et al.*, 2011) (**Fig. 1A**). Indeed, regular spaced surface distortions were visualized using total internal reflection fluorescence microscopy (Nan *et al.*, 2011) and scanning EM (Lunsdorf & Schairer, 2001, Pelling *et al.*, 2005). According to biophysical modeling, this mechanism should provide enough thrust to move the cells forward while avoiding breaching the cell wall (Nan *et al.*, 2011). It is worth noting that the helical rotor model does require adhesion between the cell surface and the gliding substratum. First, adhesive materials such as slime are required to allow the helical waves to transmit the propulsive force to the substrate (Nan *et al.*, 2011, Nan *et al.*, 2014). Second, according to computational modeling, a certain degree of surface adhesion is required for the maintenance of gliding direction (Balagam *et al.*, 2014).

The even distribution of the aggregates of motor proteins and the helical motion of the motor complexes both suggest the involvement of a helical structure in the cell (Mignot *et al.*, 2007, Nan *et al.*, 2013). In fact, MreB, the bacterial actin homologue that has the potential to form helical filaments was found essential for gliding motility in *M. xanthus* (Mauriello *et al.*, 2010, Nan *et al.*, 2013, Nan *et al.*, 2011, Treuner-Lange *et al.*, 2015). The *M. xanthus* MreB filaments appear as fragmented filaments that display helicity when stained with antibody-conjugated fluorescent dyes (Mauriello *et al.*, 2010). MreB filaments from *M. xanthus* are likely to differ from homologues from some other bacteria as helical MreB was

not observed in *Bacillus subtilis* and *E. coli* (Dominguez-Escobar *et al.*, 2011, Garner *et al.*, 2011, van Teeffelen *et al.*, 2011). Insights on MreB, such as its structure, dynamics and interaction with the gliding complex will provide critical information for understanding the mechanism of gliding. Importantly, since MreB is also a central player in cell wall synthesis (Errington, 2015), *M. xanthus* MreB must possess unique versatility to operate on different spatial and temporal scales to orchestrate multiple functions within the same cell.

Flavobacterial gliding couples a rotary motor to a unique secretion system

Many members of the phylum *Bacteroidetes*, including the model organism *Flavobacterium johnsoniae* (previously known as *Cytophaga johnsonae*), move by gliding motility. The shape and size of *F. johnsoniae* cells are similar to that of *M. xanthus*. However, *F. johnsoniae* glides about 50 times faster than *M. xanthus* and can move on a much wider range of surfaces (McBride & Nakane, 2015). *F. johnsoniae* cells, besides gliding along their long axes, sometimes lift one end off a glass surface, rotate their cell bodies around the other end (pivoting) or flip their cell bodies over (Lapidus & Berg, 1982). PMF was determined to be the energy source for flavobacterial gliding (Pate & Chang, 1979). However, the gliding motors in *F. johnsoniae* have still not been identified, in part because the function of the putative gliding motors seems to overlap with a unique protein secretion channel, designated as the type IX secretion system (T9SS) (McBride & Nakane, 2015).

Proteins identified as required for gliding are predicted to form several structural units: a) SprB and RemA, the surface adhesins that move rapidly on cell surfaces, driven by the putative gliding motor (Nakane *et al.*, 2013, Shrivastava *et al.*, 2012), b) an ABC transporter and c) a T9SS that secretes proteins including SprB and RemA (Braun *et al.*, 2005, Nelson *et al.*, 2008, Rhodes *et al.*, 2011, Shrivastava *et al.*, 2012, Shrivastava *et al.*, 2013, McBride & Nakane, 2015). The ABC transporter is not likely to be the motor because it is not conserved in all gliding *Bacteroidetes* (McBride & Zhu, 2013). In contrast, the proteins that have the ability to harvest PMF were predicted to reside in the T9SS (McBride & Nakane, 2015). If this is the case, the gliding motor of *F. johnsoniae* might be analogous to the bacterial flagella motor in which PMF drives both the rotation of flagella and the secretion of flagellar proteins through a type III secretion system (Minamino *et al.*, 2008, Paul *et al.*, 2008).

Although the gliding motors of *F. johnsoniae* have not yet been identified, its function can be monitored through the motion of SprB and RemA. Images obtained using cryo-electron tomography showed that SprB forms 150-nm-long filaments that protrude to the cell surface from a “baseplate” structure underneath the outer membrane (Liu *et al.*, 2007). SprB filaments labeled with fluorescent antibodies or latex beads move along helical trajectories at constant velocity (Nakane *et al.*, 2013). When Shrivastava *et al.* used SprB antibodies to tether *F. johnsoniae* cells onto glass slides through single SprB filaments, the tethered cells spun around a fixed point at a constant angular speed of 1 Hz. These cellular movements presumably reflect the rotation of individual motor units. This observation indicates that *F. johnsoniae* gliding motors rotate in place (Shrivastava *et al.*, 2015). Surprisingly, when the tethered cells were exposed to viscous media, their spinning speed remained unchanged. Thus, *F. johnsoniae* gliding motors appear to generate different torques (200-6,000 pN nm)

at constant speed (Shrivastava *et al.*, 2015), which differs from the flagella motor of *E. coli* that reduces speed to generate higher torque (Chen & Berg, 2000).

How is the *in situ* rotation of the motors transformed into the translational motion of SprB and the forward movement of cells? Several hypotheses have been suggested. The baseplate relay model speculates that one motor unit propels a baseplate on which adhesins such as SprB attach, until the baseplate is engaged by another motor. Thus, if many motors and baseplates line up along a helical track, the rotation of motors will pass adhesins along the track (Nan *et al.*, 2014) (**Fig. 1B**). Another model pictures the adhesion filaments being carried by a continuous conveyor belt, driven by two rotary motors. Several conveyor belts may be arranged in patterns that connect to each other like treads that link sprockets in a snowmobile; this might give the movement of adhesins a helical appearance. Unlike the baseplate relay model, this "snowmobile" model would only require a few motor units (Shrivastava & Berg, 2015) (**Fig. 1B**). Progress in solving the puzzle of *F. johnsoniae* gliding should be forthcoming with the identification of the gliding motors, their number, and the respective functions of the motors and the T9SS.

Mycoplasma mobile gliding utilizes tiny legs marching unitarily

Mollicutes, including *Mycoplasma*, *Spiroplasma* and *Achoreplasma*, are parasitic or commensal bacteria that have very small genomes (Razin *et al.*, 1998). They are related to the Gram-positive *Firmicutes* but lack peptidoglycan. Many *Mollicutes* species glide on sialyated ologisacharides (SO), major components on the surfaces of animal tissues (Kasai *et al.*, 2013), but the mechanisms of gliding are not necessarily conserved across the class (Miyata & Hamaguchi, 2015).

The gliding mechanism of *M. mobile*, a fish pathogen, has been studied in great detail. *M. mobile* forms a membrane protrusion at one cell pole, giving it a unique cell shape similar to a bowling pin. The gliding machinery of *M. mobile* generates a force up to 27 pN, which enables cells to glide smoothly on a broad range of surfaces at a speed of 2.0-4.5 μm/s (Miyata *et al.*, 2002). The *M. mobile* cell surface is covered by membrane-anchored proteins (Wu & Miyata, 2012). Examination of cells by electron microscopy revealed spike-like structures approximately 50 nm in length around the neck area of the bowling-pin-shaped cells (Miyata & Petersen, 2004). These structures are required for gliding motility and appear to function as tiny legs. Three proteins that localize near the neck, Gli123, Gli349 and Gli521 were identified as essential components in the motility machinery (Seto *et al.*, 2005, Uenoyama *et al.*, 2004, Uenoyama & Miyata, 2005b). Among these proteins, Gli349 forms the leg seen under EM, which binds SO directly (Adan-Kubo *et al.*, 2006, Lesoil *et al.*, 2010); Gli521 was proposed to function as a "crank" that connects Gli349 with Gli123 (Uenoyama *et al.*, 2009, Seto *et al.*, 2005, Nonaka *et al.*, 2010); while Gli123 might function as a "mount" that determines the cellular localization of Gli349 and Gli521 (Uenoyama & Miyata, 2005b) (**Fig. 1C**). Each *M. mobile* cell contains around 450 legs in the neck region, which probably localize in a two-dimension matrix (Uenoyama & Miyata, 2005b). In fact, when the membrane was completely stripped by detergent, a cytoskeletal "jellyfish" structure became visible under EM, which contains an oval solid "bell" localized to the small tip of the bowling-pin-shaped cell and dozens of "tentacles" that extend from the bell

to the neck region. Genetic studies suggested that this cytoskeletal structure is connected to the Gli123-Gli349-Gli521 gliding unit (Nakane & Miyata, 2007) (**Fig. 1C**).

M. mobile hydrolyzes ATP as the energy source for gliding. When *M. mobile* cells are treated with detergent and nucleases, they lose most cellular contents. However, these "ghost" cells are able to resume gliding when ATP is added (Uenoyama & Miyata, 2005a). The ATPase that drives *M. mobile* gliding has not yet been identified. Two proteins in the jellyfish cytoskeletal structure are homologous to the α- and β-subunits of the F_1-ATPase, which may function as the motor (Nakane & Miyata, 2007).

Due to the extremely small size of *M. mobile* cells (<1 μm in length), it is technically very difficult to directly observe the gliding units in action. However, indirect observations have provided many clues for the gliding mechanism. For example, in artificially elongated cells, axial variations during gliding were magnified and a repeated pivoting of cell bodies was observed, suggesting that different gliding units function independently (Nakane & Miyata, 2012). Adding excess SO into cell suspensions reduced the number of legs that bind to the surface. Combining this method with high precision co-localization microscopy showed that cells move in unitary 70-nm steps, which might correspond to the strokes of single gliding units (Kinosita *et al.*, 2014).

A centipede model (also called power stroke model) was proposed to explain the gliding mechanism of *M. mobile*. In this model, each Gli123-Gli349-Gli521 gliding unit undergoes a four-stroke mechanical cycle: the Gli349 leg catches SO molecules on the surface, the leg pulls back using ATP hydrolysis as energy, the cell body is dragged forward, then the leg is released from the surface (Miyata & Hamaguchi, 2015) (**Fig. 1C**).

Currently, a bottleneck in the research of gliding mycoplasmas is the lack of a robust method for site-directed genetic manipulations. Once such a method is available, the ATPase that actually powers the gliding units may be identified. Biochemical and biophysical approaches may also reveal additional details of mycoplasma gliding. For example, it may be possible to reconstitute the gliding machinery *in vitro* by assembling purified proteins or by stripping the cellular components that are not required for gliding from the ghost cells. Super-resolution microscopy and optical trapping might also be useful to study the motion of single gliding units.

Conclusion

The gliding mechanisms reviewed here are only a few examples of the diverse ways that bacteria move on surfaces (Jarrell & McBride, 2008). These mechanisms have blurred our definition of motility machineries. On the one hand, novel mechanisms might have evolved through the reconfiguration and repurposing of unrelated structures, such as myxobacterial proton channels and cytoskeletal elements, flavobacterial secretion channels and adhesins, and *M. mobile*'s ATPase and adhesins. On the other hand, common motility structures such as flagella and pili could also be modified for novel functions. For example, new evidence suggests that some filamentous cyanobacteria modify Type IV pili to push instead of pull cells forward (Khayatan et al., 2015). Future studies on surface motility and the associated

motors may inform strategies to control bacterial infections and may yield insights into the design of novel molecular machines.

Acknowledgements

We thank Daisuke Nakane and Abhishek Shrivastava for helpful discussion and critical reading of this manuscript. Our research is supported by the National Institute of Health Grant GM020509 to D.R.Z. and a Texas A&M University Startup funding to B.N.

References

Adan-Kubo J, Uenoyama A, Arata T, Miyata M. Morphology of isolated Gli349, a leg protein responsible for *Mycoplasma mobile* gliding via glass binding, revealed by rotary shadowing electron microscopy. J Bacteriol. 2006; 188:2821–2828. [PubMed: 16585743]

Balagam R, Litwin DB, Czerwinski F, Sun M, Kaplan HB, Shaevitz JW, Igoshin OA. *Myxococcus xanthus* gliding motors are elastically coupled to the substrate as predicted by the focal adhesion model of gliding motility. PLoS Comput Biol. 2014; 10:e1003619. [PubMed: 24810164]

Braun TF, Khubbar MK, Saffarini DA, McBride MJ. *Flavobacterium johnsoniae* gliding motility genes identified by mariner mutagenesis. J Bacteriol. 2005; 187:6943–6952. [PubMed: 16199564]

Chang YW, Rettberg LA, Treuner-Lange A, Iwasa J, Sogaard-Andersen L, Jensen GJ. Architecture of the type IVa pilus machine. Science. 2016; 351:aad2001. [PubMed: 26965631]

Chen X, Berg HC. Torque-speed relationship of the flagellar rotary motor of *Escherichia coli*. Biophys J. 2000; 78:1036–1041. [PubMed: 10653817]

Dominguez-Escobar J, Chastanet A, Crevenna AH, Fromion V, Wedlich-Soldner R, Carballido-Lopez R. Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. Science. 2011; 333:225–228. [PubMed: 21636744]

Dubreuil JD, Giudice GD, Rappuoli R. *Helicobacter pylori* interactions with host serum and extracellular matrix proteins: potential role in the infectious process. Microbiol Mol Biol Rev. 2002; 66:617–629. table of contents. [PubMed: 12456785]

Errington J. Bacterial morphogenesis and the enigmatic MreB helix. Nat Rev Microbiol. 2015; 13:241–248. [PubMed: 25578957]

Garner EC, Bernard R, Wang W, Zhuang X, Rudner DZ, Mitchison T. Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. Science. 2011; 333:222–225. [PubMed: 21636745]

Henrichsen J. Bacterial surface translocation: a survey and a classification. Bacteriol Rev. 1972; 36:478–503. [PubMed: 4631369]

Hodgkin J, Kaiser D. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): Two gene systems control movement. Mol. Gen. Genet. 1979; 171:177–191.

Jakobczak B, Keilberg D, Wuichet K, Sogaard-Andersen L. Contact- and Protein Transfer-Dependent Stimulation of Assembly of the Gliding Motility Machinery in *Myxococcus xanthus*. PLoS Genet. 2015; 11:e1005341. [PubMed: 26132848]

Jarrell KF, McBride MJ. The surprisingly diverse ways that prokaryotes move. Nat Rev Microbiol. 2008; 6:466–476. [PubMed: 18461074]

Kasai T, Nakane D, Ishida H, Ando H, Kiso M, Miyata M. Role of binding in *Mycoplasma mobile* and *Mycoplasma pneumoniae* gliding analyzed through inhibition by synthesized sialylated compounds. J Bacteriol. 2013; 195:429–435. [PubMed: 23123913]

Keane R, Berleman J. The predatory life cycle of *Myxococcus xanthus*. Microbiology. 2016; 162:1–11. [PubMed: 26518442]

Kearns DB. A field guide to bacterial swarming motility. Nat Rev Microbiol. 2010; 8:634–644. [PubMed: 20694026]

Khayatan B, Meeks JC, Risser DD. Evidence that a modified type IV pilus-like system powers gliding motility and polysaccharide secretion in filamentous cyanobacteria. Mol Microbiol. 2015; 98:1021–1036. [PubMed: 26331359]

Kinosita Y, Nakane D, Sugawa M, Masaike T, Mizutani K, Miyata M, Nishizaka T. Unitary step of gliding machinery in *Mycoplasma mobile*. Proc Natl Acad Sci U S A. 2014; 111:8601–8606. [PubMed: 24912194]

Lapidus IR, Berg HC. Gliding motility of *Cytophaga* sp. strain U67. J Bacteriol. 1982; 151:384–398. [PubMed: 7085564]

Lesoil C, Nonaka T, Sekiguchi H, Osada T, Miyata M, Afrin R, Ikai A. Molecular shape and binding force of *Mycoplasma mobile*'s leg protein Gli349 revealed by an AFM study. Biochem Biophys Res Commun. 2010; 391:1312–1317. [PubMed: 20004642]

Li Y, Sun H, Ma X, Lu A, Lux R, Zusman D, Shi W. Extracellular polysaccharides mediate pilus retraction during social motility of *Myxococcus xanthus*. Proc Natl Acad Sci U S A. 2003; 100:5443–5448. [PubMed: 12704238]

Liu J, McBride MJ, Subramaniam S. Cell surface filaments of the gliding bacterium *Flavobacterium johnsoniae* revealed by cryo-electron tomography. J Bacteriol. 2007; 189:7503–7506. [PubMed: 17693495]

Luciano J, Agrebi R, Le Gall AV, Wartel M, Fiegna F, Ducret A, Brochier-Armanet C, Mignot T. Emergence and modular evolution of a novel motility machinery in bacteria. PLoS Genet. 2011; 7:e1002268. [PubMed: 21931562]

Lunsdorf H, Schairer HU. Frozen motion of gliding bacteria outlines inherent features of the motility apparatus. Microbiology. 2001; 147:939–947. [PubMed: 11283289]

Maier B, Potter L, So M, Long CD, Seifert HS, Sheetz MP. Single pilus motor forces exceed 100 pN. Proc Natl Acad Sci U S A. 2002; 99:16012–16017. [PubMed: 12446837]

Manley S, Gillette JM, Patterson GH, Shroff H, Hess HF, Betzig E, Lippincott-Schwartz J. High-density mapping of single-molecule trajectories with photoactivated localization microscopy. Nat Methods. 2008; 5:155–157. [PubMed: 18193054]

Mauriello EM, Mouhamar F, Nan B, Ducret A, Dai D, Zusman DR, Mignot T. Bacterial motility complexes require the actin-like protein, MreB and the Ras homologue, MglA. Embo J. 2010; 29:315–326. [PubMed: 19959988]

McBride MJ, Nakane D. *Flavobacterium* gliding motility and the type IX secretion system. Curr Opin Microbiol. 2015; 28:72–77. [PubMed: 26461123]

McBride MJ, Zhu Y. Gliding motility and Por secretion system genes are widespread among members of the phylum *bacteroidetes*. J Bacteriol. 2013; 195:270–278. [PubMed: 23123910]

Mignot T, Shaevitz JW, Hartzell PL, Zusman DR. Evidence that focal adhesion complexes power bacterial gliding motility. Science. 2007; 315:853–856. [PubMed: 17289998]

Minamino T, Imada K, Namba K. Molecular motors of the bacterial flagella. Curr Opin Struct Biol. 2008; 18:693–701. [PubMed: 18848888]

Miyata M, Hamaguchi T. Prospects for the gliding mechanism of *Mycoplasma mobile*. Curr Opin Microbiol. 2015; 29:15–21. [PubMed: 26500189]

Miyata M, Petersen JD. Spike structure at the interface between gliding *Mycoplasma mobile* cells and glass surfaces visualized by rapid-freeze-and-fracture electron microscopy. J Bacteriol. 2004; 186:4382–4386. [PubMed: 15205441]

Miyata M, Ryu WS, Berg HC. Force and velocity of *Mycoplasma mobile* gliding. J Bacteriol. 2002; 184:1827–1831. [PubMed: 11889087]

Nakane D, Miyata M. Cytoskeletal "jellyfish" structure of *Mycoplasma mobile*. Proc Natl Acad Sci U S A. 2007; 104:19518–19523. [PubMed: 18042728]

Nakane D, Miyata M. *Mycoplasma mobile* cells elongated by detergent and their pivoting movements in gliding. J Bacteriol. 2012; 194:122–130. [PubMed: 22001513]

Nakane D, Sato K, Wada H, McBride MJ, Nakayama K. Helical flow of surface protein required for bacterial gliding motility. Proc Natl Acad Sci U S A. 2013

Nan B, Bandaria JN, Guo KY, Fan X, Moghtaderi A, Yildiz A, Zusman DR. The polarity of myxobacterial gliding is regulated by direct interactions between the gliding motors and the Ras homolog MglA. Proc Natl Acad Sci U S A. 2015; 112:E186–193. [PubMed: 25550521]

Nan B, Bandaria JN, Moghtaderi A, Sun IH, Yildiz A, Zusman DR. Flagella stator homologs function as motors for myxobacterial gliding motility by moving in helical trajectories. Proc Natl Acad Sci U S A. 2013; 110:E1508–1513. [PubMed: 23576734]

Nan B, Chen J, Neu JC, Berry RM, Oster G, Zusman DR. Myxobacteria gliding motility requires cytoskeleton rotation powered by proton motive force. Proc Natl Acad Sci U S A. 2011; 108:2498–2503. [PubMed: 21248229]

Nan B, Mauriello EM, Sun IH, Wong A, Zusman DR. A multi-protein complex from *Myxococcus xanthus* required for bacterial gliding motility. Mol Microbiol. 2010; 76:1539–1554. [PubMed: 20487265]

Nan B, McBride MJ, Chen J, Zusman DR, Oster G. Bacteria that glide with helical tracks. Curr Biol. 2014; 24:R169–R173. [PubMed: 24556443]

Nan B, Zusman DR. Uncovering the mystery of gliding motility in the myxobacteria. Annu Rev Genet. 2011; 45:21–39. [PubMed: 21910630]

Nelson SS, Bollampalli S, McBride MJ. SprB is a cell surface component of the *Flavobacterium johnsoniae* gliding motility machinery. J Bacteriol. 2008; 190:2851–2857. [PubMed: 18281397]

Nonaka T, Adan-Kubo J, Miyata M. Triskelion structure of the Gli521 protein, involved in the gliding mechanism of *Mycoplasma mobile*. J Bacteriol. 2010; 192:636–642. [PubMed: 19915029]

Pate JL, Chang L-YE. Evidence that gliding motility in prokaryotic cells is driven by rotary assemblies in the cell envelopes. Curr. Microbiol. 1979; 2:59–64.

Paul K, Erhardt M, Hirano T, Blair DF, Hughes KT. Energy source of flagellar type III secretion. Nature. 2008; 451:489–492. [PubMed: 18216859]

Pelling AE, Li Y, Shi W, Gimzewski JK. Nanoscale visualization and characterization of *Myxococcus xanthus* cells with atomic force microscopy. Proc Natl Acad Sci U S A. 2005; 102:6484–6489. [PubMed: 15840722]

Persat A, Nadell CD, Kim MK, Ingremeau F, Siryaporn A, Drescher K, Wingreen NS, Bassler BL, Gitai Z, Stone HA. The mechanical world of bacteria. Cell. 2015; 161:988–997. [PubMed: 26000479]

Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. Microbiol Mol Biol Rev. 1998; 62:1094–1156. [PubMed: 9841667]

Rhodes RG, Nelson SS, Pochiraju S, McBride MJ. *Flavobacterium johnsoniae* sprB is part of an operon spanning the additional gliding motility genes sprC, sprD, and sprF. J Bacteriol. 2011; 193:599–610. [PubMed: 21131497]

Seto S, Uenoyama A, Miyata M. Identification of a 521-kilodalton protein (Gli521) involved in force generation or force transmission for *Mycoplasma mobile* gliding. J Bacteriol. 2005; 187:3502–3510. [PubMed: 15866938]

Shrivastava A, Berg HC. Towards a model for Flavobacterium gliding. Curr Opin Microbiol. 2015; 28:93–97. [PubMed: 26476806]

Shrivastava A, Johnston JJ, van Baaren JM, McBride MJ. *Flavobacterium johnsoniae* GldK, GldL, GldM, and SprA are required for secretion of the cell surface gliding motility adhesins SprB and RemA. J Bacteriol. 2013; 195:3201–3212. [PubMed: 23667240]

Shrivastava A, Lele PP, Berg HC. A rotary motor drives Flavobacterium gliding. Curr Biol. 2015; 25:338–341. [PubMed: 25619763]

Shrivastava A, Rhodes RG, Pochiraju S, Nakane D, McBride MJ. *Flavobacterium johnsoniae* RemA is a mobile cell surface lectin involved in gliding. J Bacteriol. 2012; 194:3678–3688. [PubMed: 22582276]

Skerker JM, Berg HC. Direct observation of extension and retraction of type IV pili. Proc Natl Acad Sci U S A. 2001; 98:6901–6904. [PubMed: 11381130]

Smilenov LB, Mikhailov A, Pelham RJ, Marcantonio EE, Gundersen GG. Focal adhesion motility revealed in stationary fibroblasts. Science. 1999; 286:1172–1174. [PubMed: 10550057]

Sun M, Wartel M, Cascales E, Shaevitz JW, Mignot T. Motor-driven intracellular transport powers bacterial gliding motility. Proc Natl Acad Sci U S A. 2011; 108:7559–7564. [PubMed: 21482768]

Treuner-Lange A, Macia E, Guzzo M, Hot E, Faure LM, Jakobczak B, Espinosa L, Alcor D, Ducret A, Keilberg D, Castaing JP, Lacas Gervais S, Franco M, Sogaard-Andersen L, Mignot T. The small G-protein MglA connects to the MreB actin cytoskeleton at bacterial focal adhesions. J Cell Biol. 2015; 210:243–256. [PubMed: 26169353]

Uenoyama A, Kusumoto A, Miyata M. Identification of a 349-kilodalton protein (Gli349) responsible for cytadherence and glass binding during gliding of *Mycoplasma mobile*. J Bacteriol. 2004; 186:1537–1545. [PubMed: 14973017]

Uenoyama A, Miyata M. Gliding ghosts of *Mycoplasma mobile*. Proc Natl Acad Sci U S A. 2005a; 102:12754–12758. [PubMed: 16126895]

Uenoyama A, Miyata M. Identification of a 123-kilodalton protein (Gli123) involved in machinery for gliding motility of *Mycoplasma mobile*. J Bacteriol. 2005b; 187:5578–5584. [PubMed: 16077102]

Uenoyama A, Seto S, Nakane D, Miyata M. Regions on Gli349 and Gli521 protein molecules directly involved in movements of *Mycoplasma mobile* gliding machinery, suggested by use of inhibitory antibodies and mutants. J Bacteriol. 2009; 191:1982–1985. [PubMed: 19124576]

van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, Gitai Z. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. Proc Natl Acad Sci U S A. 2011; 108:15822–15827. [PubMed: 21903929]

Wu HN, Miyata M. Whole surface image of *Mycoplasma mobile*, suggested by protein identification and immunofluorescence microscopy. J Bacteriol. 2012; 194:5848–5855. [PubMed: 22923591]

Wu SS, Kaiser D. Genetic and functional evidence that Type IV pili are required for social gliding motility in *Myxococcus xanthus*. Mol Microbiol. 1995; 18:547–558. [PubMed: 8748037]

Youderian P, Burke N, White DJ, Hartzell PL. Identification of genes required for adventurous gliding motility in *Myxococcus xanthus* with the transposable element mariner. Mol Microbiol. 2003; 49:555–570. [PubMed: 12828649]

Zusman DR, Scott AE, Yang Z, Kirby JR. Chemosensory pathways, motility and development in *Myxococcus xanthus*. Nat Rev Microbiol. 2007; 5:862–872. [PubMed: 17922045]

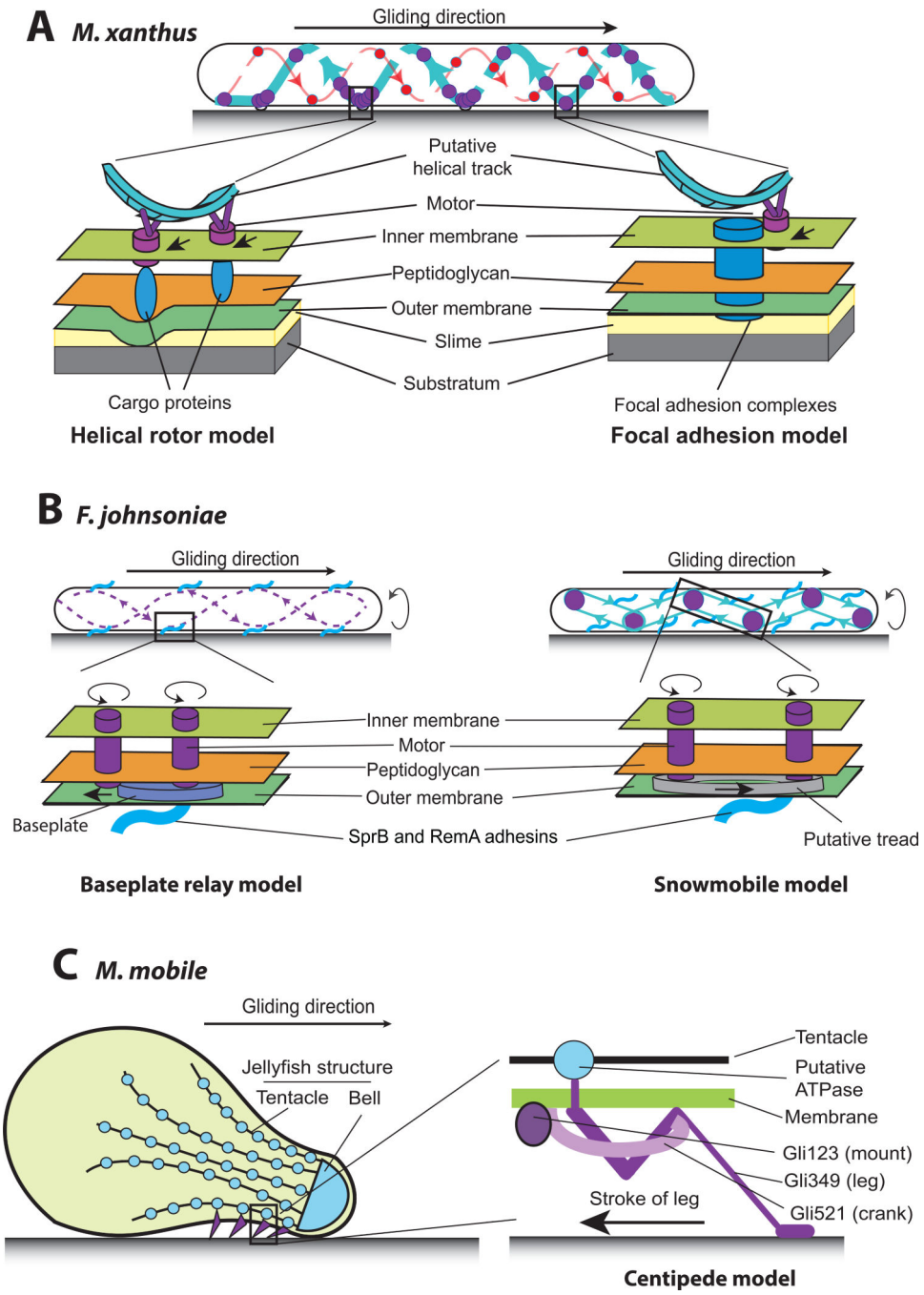


Fig. 1.

Models for gliding motility in *M. xanthus* (A), *F. johnsoniae* (B) and *M. mobile* (C). (**A**) Gliding in *M. xanthus* is powered by MotAB homologues that move along helical tracks in the inner membrane. Two models propose different mechanisms by which cells transform the proton motive force from the inner membrane into a mechanical force on the cell surface. The helical rotor model proposes that due to the increased resistance, the velocity of motor complexes within the membrane slows down at the sites where cells contact the surface. The slowed motor complexes accumulate in dynamic “traffic jams” that deform the

cell envelope, push against the surface, and generate a backward surface wave that propels cells forward. By contrast, the focal adhesion model proposes that focal adhesion complexes (FACs) penetrate the cell envelope and anchor cells to the gliding surface. The motor complexes propel cells forward by pushing the FACs backward. (**B**) Gliding in *F. johnsoniae* is propelled by unknown motors that transport surface adhesins. The baseplate relay model proposes that helically arranged rotary motor units transport surface adhesins on baseplates. The rotation of the motors passes the adhesins along the track. The snowmobile model predicts that motor units are connected to a belt system similar to the treads that link sprockets in a snowmobile. Thus rotation of motor units transports the adhesins along those conveyor belts. (**C**) The “neck” region of the *M. mobile* cell surface is covered by a matrix of protein “legs” that attach to a jellyfish-like cytoskeletal structure. The centipede model proposes that cells are propelled by the strokes of numerous legs, which depend on ATP hydrolysis.