Cell. Mol. Life Sci. 64 (2007) 2733 – 2745
1420-682X/07/212733-13
DOI 10.1007/s00018-007-7176-x

Cellular and Molecular Life Sciences

Review

The elusive engine in *Myxococcus xanthus* gliding motility

T. Mignot

Laboratoire de Chimie Bactérienne, Institut de Biologie Structurale et Microbiologie,
Campus CNRS Joseph Aiguier, 31 Chemin Joseph Aiguier, 13009 Marseille (France), Fax: +33 491718914,
e-mail: tmignot@ibsm.cnrs-mrs.fr

Received 12 April 2007; received after revision 8 June 2007; accepted 27 June 2007
Online First 26 July 2007

Abstract. Bacterial motility is essential for chemotaxis, virulence and complex social interactions leading to biofilm and fruiting body formation. Although bacterial swimming in liquids with a flagellum is well understood, little is known regarding bacterial movements across solid surfaces. Gliding motility, one such mode of locomotion, has remained largely mysterious because cells move smoothly along their long axis in the absence of any visible organelle. In this review, I discuss recent evidence that focal adhesion systems mediate gliding motility in the social bacterium *Myxococcus xanthus* and combine this evidence with previous work to suggest a new working hypothesis inspired from knowledge in apicomplexan parasites. I then propose experimental directions to test the model and compare it to other pre-existing models. Finally, evidence on gliding mechanisms of selected organisms are presented to ask whether some features of the model have precedents in other bacteria and whether this complex biological process could be explained by a single mechanism or involves multiple distinct mechanisms.

Keywords. Gliding motility, bacterial cytoskeleton, focal adhesion, molecular motor, *Myxococcus xanthus*, cell polarity.

Introduction

The ability of cells to move across solid surfaces is central to a broad diversity of biological processes. For example, directed cell migration is essential for development of the embryo, the central nervous system, and basic mechanisms of immunity in higher mammals but it is also the basis for biofilm formation and fruiting body formation in bacteria and unicellular eukaryotes. Indeed, research on cell motility has established the principles of eukaryotic cellular motion in exquisite detail. Although, the identities of the molecules involved can be species and cell type specific, the underlying principles of motility seems to be common. The recurring theme is that cellular motion is powered at focal adhesion sites where cell-surface ligands that provide anchor points with the extracellular matrix are connected intracellularly to the actin cytoskeleton (for a review see [1]). Motion is produced because focal adhesion sites contain motor proteins such as myosin that exert traction on actin fibers and thus "pull" the cell forward relative to the immobilized adhesion complex. Focal adhesions dynamics are highly regulated as sites can mature from sites of active traction to sites of passive adhesion. Thus, focal adhesion sites contain structural proteins but also multiple regulatory proteins that modulate their activity and integrate the action of multiple sites [2]. As the result of this, cell motility proceeds by coordinated cycles of actin polymerization-driven edge protrusions, adhesion and retraction.

In bacteria, microscopy has been very effective in identifying motility mechanisms that involve obvious extracellular structures such as flagella and type-IV

pili. For example, how chemotaxis-dependent signal transduction affects rotation of the flagellar motor to achieve movement towards or away from chemoattractants/repellants in a liquid environment has been characterized in depth (for a review see [3]). It is also known that bacteria can use extracellular appendages to move across solid surfaces. On a moist surface, bacteria may become hyperflagellated and swarm as a group [4]. This surface motility, a case of adapted swimming, is dependent on flagella and the wetness of the surface [5]. How flagella actually power swarming is not understood; however, it is clear that they are the engine involved in this motility (for a review see [6]). Another example is type-IV pili-dependent motility (twitching motility). Early on, Kaiser [7] correlated *Myxococcus xanthus* social motility to the presence of polar pili. Studies in several systems including *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa* and *M. xanthus* directly established that the type-IV pilus can be a motility engine: motion is produced by cycles of pilin fiber extension, binding on the substratum and retraction [8–10].

In many other cases, bacteria are able to translocate smoothly on solid surfaces along their long axis in the absence of visible organelles. Such locomotion, termed gliding motility, is defined by "a translocation along solid bodies […] during which no wriggling, contraction or peristaltic alterations are visible, the change of shape being restricted to bending […]. Gliding movements are not always regular, but intermittent and hesitant, with frequent changes of direction." [11]. Gliding motility is observed in very diverse phylogenetically unrelated bacterial groups among which and non-exhaustively are the myxobacteria, the mollicutes, cyanobacteria and the *Cytophaga/Flavobacterium* group, which comprises bacteria of the bacteroidetes phylum such as *Flavobacterium, Cytophaga* and *Flexibacter sp*, discussed in this article. The mechanism of gliding remains largely mysterious: except for myxobacteria and cyanobacteria for which a common model has been proposed to explain gliding motility [12], studies in various systems have favored multiple mechanisms rather than a universal mechanism for bacterial gliding motility. However, most models lack molecular support and it is possible that apparently different modes of movement may actually use similar but adapted mechanisms.

In this review, I discuss gliding motility in the model organism *M. xanthus* in light of recent findings on periodic adhesion sites. I suggest a model whereby an internal engine pushes against focal adhesions to power movement, as in eukaryotic cell systems, and integrate it to available data and previously published models. Finally, I compare the *M. xanthus* model to other selected examples of bacterial motility in an attempt to extract common principles and differences.

M. xanthus utilizes two independent motility systems

M. xanthus is a Gram-negative rod-shaped bacterium characterized by a complex developmental lifestyle. Starved cells aggregate into distinct mounds that ultimately form dome-shaped fruiting bodies in which the cells differentiate into spores. This developmental cycle depends both on signal-induced differential gene expression and the ability of cells to exhibit surface motility [13, 14]. Hodgkin and Kaiser [15] showed that *M. xanthus* uses two independent motility systems that can be genetically separated. Cells only become completely non-motile when both motility systems are simultaneously inactivated.

Cells lacking social motility (S-motility) do not swarm efficiently at low cell density and show impaired group movements (Fig. 1a). The S-motility system can only power single cell movements under very specific conditions such as when the cells are overlaid with viscous media such as methyl cellulose [10]. S-motility is a form of twitching motility: it depends on polar retractile type-IV pili (Fig. 1b). Coordinated movement of large groups of cells may be produced as extruded pili bind neighboring cells and retract [16]. Accordingly, S-motility depends on cell surface polymers such as the extracellular matrix fibrils and LPS O-antigen [17, 18]. Recently, it was shown conclusively that the polysaccharide portion of the fibrils binds and triggers retraction of the pilus, providing a potential explanation for the contact dependence of S-motility [16].

Adventurous motility (A-motility) can power movement of single cells under most conditions, provided that the substratum is firm enough (Fig. 1a, [19]). The exact nature of the A-engine remains unknown and several models have been proposed. Recently, focal adhesion complexes were suggested to power A-motility (Fig. 1b). Also, moving cells deposit slime trails that resemble mucus laid by snails (Fig. 1b). Slime secretion is thus probably also linked to A-motility. This review discusses the proposed mechanism(s) of A-motility.

Focal adhesion complexes in *M. xanthus* A-motility

Fixed protein complexes in *M. xanthus* moving cells

Genetic approaches to study A-motility have so far failed to reveal its exact mechanism [20, 21]. In fact, genes known to be required for A-motility encode multiple potential functions that are difficult to assign

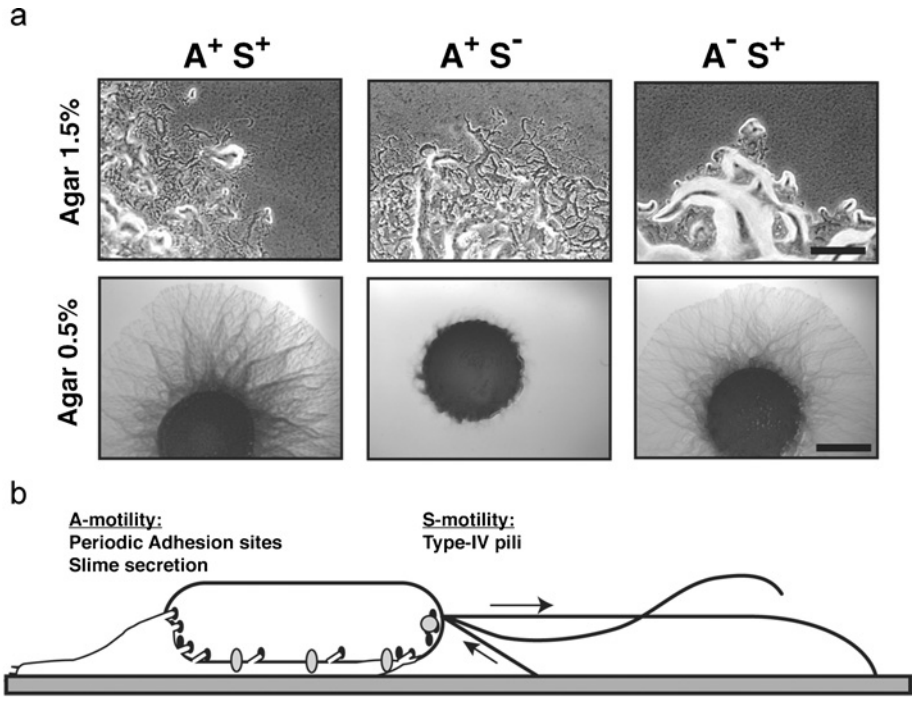

Figure 1. *Myxococcus xanthus* utilizes two independent motility systems. (*a*) Motility phenotypes of strains carrying mutations in either motility systems. The hard (1.5%) and soft (0.5%) agar assays test for adventurous motility (A-motility) and social motility (S-motility), respectively. A wild-type (WT) strain (A^+S^+) moves efficiently on both substrates. An $A^+ S^-$ mutant shows almost normal motility on hard agar but fails to make radial swarms on soft agar. In contrast, an A^-S^+ mutant is proficient of soft agar but shows almost no individual motile cells at the edges of colonies in the hard agar assay. Note the change in scale in the hard agar (scale bar 180 μm) and soft agar assays (scale bar 1 mm). (*b*) Cartoon representation of *M. xanthus* A- and S-motility systems. Type-IV pili (S-motility) located at the leading pole extend and retract to pull the cell body forward. The A-motility system involves periodic focal adhesion sites assembled from the leading pole (gray ellipses). The secretion of slime through pores (black dots) along the cell body and at the rear may also participate in A-motility either by actively powering locomotion, or by providing a substratum to glide on, or both.

to a conspicuous molecular machine. This is in fact only mildly surprising as global genetic screens that investigated *M. xanthus* type-IV pilus-based S-motility did isolate mutations in pilus structural genes but they also revealed a large number of other genes whose relationship with the pili is not obvious [22]. An alternative approach was therefore necessary to investigate the molecular basis of A-motility. Recent cytological studies revealed that the dynamic behavior of a yellow fluorescent protein (YFP)-tagged A-motility protein (AglZ, [23]) could be correlated to movement in a live cell system: AglZ-YFP condensed at the leading cell pole when the cell paused but localized to clusters distributed along the cell body when movement resumed [24]. Remarkably, analysis of AglZ-YFP clusters during movement showed that these clusters remained at fixed positions relative to the substratum as the cell moved forward (Fig. 2a). The clusters were assembled at the leading pole and dispersed when they reached the rear of the cell (Fig. 2a).

The AglZ-YFP clusters display characteristics of eukaryotic focal adhesions

These results suggested that A-motility could be powered at focal adhesion complexes as in eukaryotic cells. Several lines of evidence suggest that both adhesion and traction are likely to occur at the sites where AglZ-YFP accumulates. First, focal adhesions might explain flexing during motility because the AglZ-YFP clusters were localized at the sites where cells bent. Also, when a cell is fortuitously stuck on the substratum at the leading end, it undergoes characteristic "flailing" motions as the A-motor pushes against the flexible cell wall [25, 26]. In these cells, the AglZ-YFP clusters localized between the bends and cell shape transitions were mirrored by dispersal of the complexes suggesting that adhesion occurs at these clusters and that cell shape transitions occurs when the clusters are removed [24]. Second, in artificially elongated cells the clusters were localized in the front half of the cells. In these cells, the number of clusters was not related to cell length but directly proportional to the drag force overcome (*i.e.*, the force necessary to power the motility of a cell of given length and

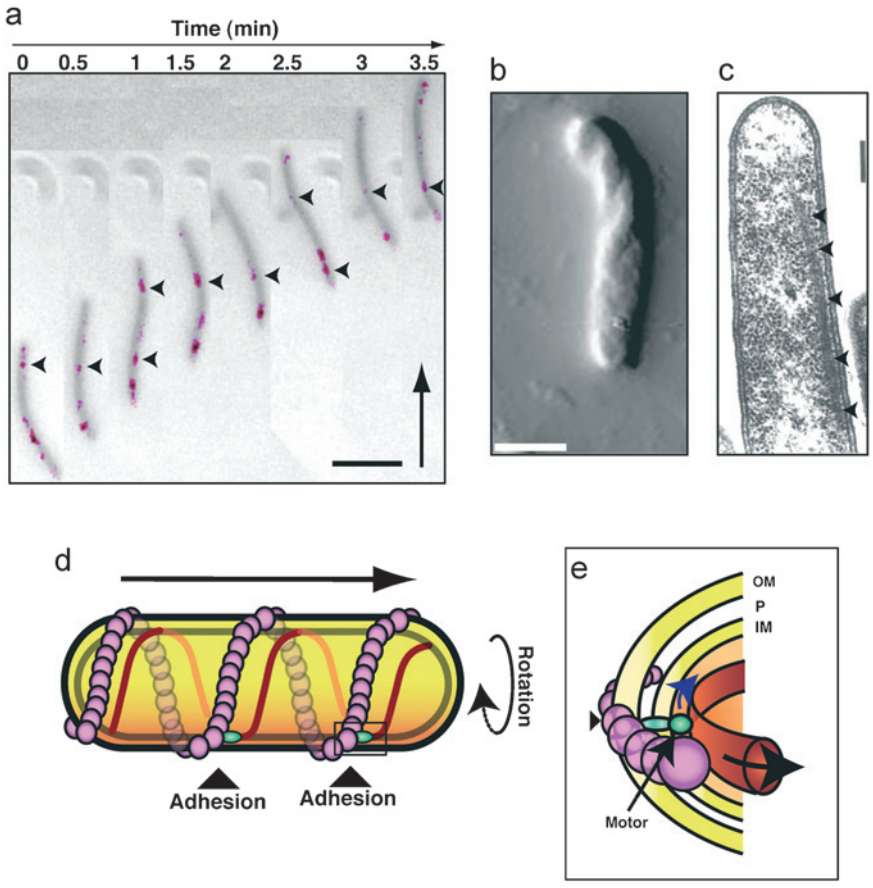

Figure 2. Focal adhesions in *M. xanthus* motility. (*a*) Fixed AglZ-YFP clusters in moving cells. AglZ-YFP localization in a cell moving at constant velocity. Overlay of the phase and YFP (magenta, artificially colored for improved clarity) images captured every 30 s are shown. Scale bar 2 μm. Arrowheads highlight selected bright fluorescence clusters. (*b*) Twisted morphology of *M. xanthus* gliding cells. Helical twists in a single cell observed by atomic force microscopy. Image adapted from [35] with permission (Copyright 2003, National Academy of Sciences, USA). Scale bar 2.5 μm. (*c*) Straight intracellular filament bundles. Longitudinal section of an *M. xanthus* cell showing submembrane filament bundles (arrowheads). Electron microscopy image adapted from [28]. (*d*) Focal adhesion-based model of *M. xanthus* A-motility. Following assembly at the front of the cell, a motor complex (green) connects the intracellular cytoskeleton (red filament) with extracellular adhesion tracks (purple). The motor moves along the cytoskeleton resulting in forward movement and rotation of the cell body. Transient contacts between the motor and the adhesion track ensure that only the motor complex remains fixed relative to the substratum. The motor complex is assembled from the front of the cell and disassembled when it reaches the rear. Based on knowledge of the cytoskeleton in other bacteria the internal cytoskeleton is shown helical, but note that the periodicity of the AglZ-YFP clusters would also be accounted for (through interaction with the external helical bands) if the internal fibers are linear, which was suggested in [28]. (*e*) Front view of assembled A-motility apparatus. A complex (green rod) spanning the inner membrane (IM) and cell wall and periplasmic space (P) links the outer membrane (OM) adhesion sites to the motor (green circle). Physical linkage between the motor and the adhesion track could be regulated at the level of the transmembrane complex (green rod). The arrowhead shows the focal point where adhesion with the substratum is occurring.

velocity), suggesting that locomotion is produced at the AglZ-YFP sites [24]. To remain stationary relative to the substratum the AglZ-YFP clusters must be moving opposite to the direction of the cell and at the same velocity. Such dynamics are what one would expect for a molecular motor complex (AglZ only being one of its components) because the energy cost could certainly be translated into locomotion power. Together, these observations suggest that the AglZ-YFP clusters share characteristics with eukaryotic focal adhesions and could represent A-motility motor units. Also, A-motility is much more efficient on firm and dry surfaces than on soft and wet surfaces [19], a requirement for a focal adhesion-based motility system.

The AglZ-YFP clusters are periodic

The analogy between the AglZ-YFP clusters and eukaryotic focal adhesion complexes suggests that a molecular motor within these clusters exerts traction on a potential *M. xanthus* cytoskeleton. Evidence for such motor is lacking but the existence of a cytoskeleton in *M. xanthus* is very likely: the bacterial actin-like gene *mreB* is present in the *M. xanthus* genome [27] and early electron microscopy (EM) studies revealed intracellular filament bundles close to the

inner membrane in *M. xanthus* cells (Fig. 2c; [28]). The spatial distribution of the AglZ-YFP clusters shows a clear periodicity of ~470 nm [24]. This pattern could be explained by periodic kinetics of assembly at the leading cell pole. However, this is unlikely because the number of clusters per cell is not related to velocity, but dependent on cell length in normal cells (as opposed to artificially elongated cells). Therefore, the distribution of the AglZ-YFP clusters is best explained by proposing that the clusters are anchored along a periodic structure such as an intracellular helix. Consistent with this, the measured pitch of the MreB helix in *E. coli* and *B. subtilis* matches the periodicity of the AglZ-YFP clusters [29, 30]. Further work is needed to determine whether *M. xanthus* MreB has a role in positioning the AglZ-YFP clusters.

A focal adhesion model for A-motility

Based on these results, I suggest a working model whereby A-motility is powered by focal adhesion complexes that link adhesion to the substratum to an internal cytoskeleton *via* a molecular motor (Fig. 2d, e). According to this model, the motor complexes are assembled at the front of the cell and remain fixed as they adhere to the substratum; traction exerted on a continuous cytoskeleton moves the cell forward. The adhesions are removed when they reach the rear of the cell.

The proposed mechanism is analogous to the mechanism of gliding motility in apicomplexan parasites [31, 32]. Apicomplexans use an adhesion-based motility system to invade their host cells. Transmembrane adhesins at the apical end of the parasite ensure a direct connection between the host cell surface and the internal actin cytoskeleton. A complex containing a myosin then translocates the adhesin-actin complex rearward thus propelling the parasite forward. Continuous forward movement results from a proteolytic event that breaks the interaction between the adhesin and the substratum [31, 32]. Likely, the molecular components of *M. xanthus* gliding differ from those of apicomplexans but the principles at work may be very similar. *M. xanthus* outer membrane adhesins may connect the intracellular cytoskeleton through an inner membrane platform where a molecular motor is localized (Fig. 2d, e). As for Apicomplexans, removal of the adhesion sites would be critical for the cell to maintain forward movement and this would be achieved through transient connection with the motor complex (Fig. 2d, e). I also suggest that distinct polar systems, each responsible for assembly and disassembly of the complexes, are present at the leading and lagging poles.

A difficulty with the model is to understand how the multilayered Gram-negative cell envelope would "flow" through fixed adhesion complexes. To explain this, it could be hypothesized that only the cytosolic part of the complex (*i.e.*, the molecular motor where AglZ-YFP localizes) actually remains fixed relative to the substratum by moving opposite to the cell and at the same speed, leading the cytoskeleton and adhesion complexes to "treadmill" as the motor pulls on the first, and establishes transient interactions with the latter (Fig. 2d, e). How might this be organized structurally? Periodic chain-like aggregates were observed in the periplasm of *M. xanthus* cells [33]. These chains may well be organized helically because the cell surface is not smooth but appears twisted along its long axis (Fig. 2b; [34, 35]). Helical surface bands are assembled by an active process that can be blocked by treatment of the cells with sodium azide, suggesting that they are active parts of the motility apparatus [34, 35]. Helices running in the periplasm have been observed in other bacteria [36]. In particular, one such helical structure seems to be an active component of the *Cytophaga/ Flavobacterium* gliding machine [37]. Thus, the intracellular motor could be connected to adhesion surface tracks and pull on a rigid cytoskeleton at the interior of the cell (Fig. 2d, e). Note that the adhesion sites themselves would remain fixed relative to the cell envelope, a fundamental difference with the proposed gliding mechanism in *Cytophaga/Flavobacterium* (discussed below).

Predictions and future perspectives

The proposed model makes several mechanical predictions that can be readily tested experimentally. Direct biophysical measurements are needed to confirm that traction and adhesion are produced at the AglZ-YFP sites. Atomic force microscopy using an appropriate cantilever could address these questions. The proposed connection between the motor complex and helical structures predicts that the cell rotates along its long axis during gliding. Although, rotation of the cell body has been shown in several gliding bacteria [38, 39], there is currently no evidence that *M. xanthus* cells rotate during movement, except for the fact that gliding cells are morphologically twisted [34, 35]; observing such rotation would provide strong mechanical support to models involving continuous helical assemblies.

The model needs to be supported by molecular evidence through characterization of the components involved in adhesion, transmission, power generation and cytoskeleton. Also, how are the adhesion sites assembled at the front of the cell and disassembled at the back? To answer these questions it may be interesting to re-visit the lists of A-motility genes in light of the new hypothesis [20, 21] and search for potential candidates for each component. For exam-

ple, A-motility genes encoding membrane proteins are potentially involved in complexes that connect the adhesion sites to the cytoskeleton [22]. Interestingly, TonB- and Tol-like systems are over-represented [22] and, although such systems may be involved in general envelope metabolism, they could be specific parts of the A-engine. As already mentioned, *mreB* could have a role in A-motility. It is actually not surprising that *mreB* was not found in motility screens because it is likely an essential gene as in other bacteria [40]. However, as bacteria contain several actin-like and non-actin-like filaments, other filaments might be involved [41]. Finally, the model suggests that A-motility is powered by myosin-like proteins. Despite extensive searches, molecular motors that track on the bacterial cytoskeleton have so far not been identified, questioning their existence in prokaryotes. Such motors may exist in *M. xanthus* but are not readily identified with bioinformatics [42]. It is possible that bacteria evolved distinct ways to power cytoskeleton-mediated processes (such as the use of the proton motive force). Clearly, the molecular nature of the A-engine remains completely elusive but studying the localization pattern of A-motility proteins such as AglZ could now provide solid clues towards its elucidation.

Comparison with other A-motility models

In the past, several other models have been proposed to explain A-motility. Can the AglZ-YFP dynamics fit with these models and can we suggest a composite model unifying the different hypotheses?

Surface tension

Surface tension is an effect within the surface layer of a liquid that causes that layer to behave as an elastic sheet. An object suspended in this interface is subjected to an equal "pull" in all directions. Surfactants that tend to accumulate in the interface lower the surface tension. Thus, it was suggested that localized secretion of a surfactant at the rear of the cell could create a gradient of surface tension resulting in net directional forces that would propel the cell forward [43, 44]. Accordingly, addition of molecules with surfactant properties inhibited motility as would be expected if the gradient were disrupted [43]. Also, gliding cells were observed to produce surfactants [43]. This model is probably too simplistic: surface tension may only play an accessory role in A-motility on agar because this motility is very efficient on dry hard surfaces, for example glass, where such effects cannot take place. Also, the surface tension gradient could hardly explain complex flexing motions, the movement of artificially elongated cells and the requirement of periodic adhesions [24, 45].

Inchworm contractions

It was proposed that A-motility could result from the ability of cells to elongate under mechanical contraction (Fig. 3a). This would result in forward propulsion because the slime secreted at the back of the cells would act like a ratchet and prevent them from moving backward [46]. This model was inspired by the previously mentioned helical structures wrapping the *M. xanthus* cell body. The filaments appeared in two conformational states [34], which suggested that they could exert circumferential stress to squeeze the cell body thus making it elongated. This model has not been supported by high-resolution video microscopy, which provided no evidence for changes in cell shape and elongation during motility [25]. Similarly, inchworm contractions have been suggested for other gliders such as *Oscillatoria* and *Archangium* but were also not supported by microscopy [47]. It could be argued that the changes in cell length could be very discrete and thus not captured by optical microscopy. However, in this particular case, the anchor points should be extremely close to one another, which is not supported by the spacing of the AglZ-YFP clusters.

Periplasmic engine

Another model suggested that a molecular motor is anchored to the cell wall and exerts force in the direction of the long axis to move outer membrane adhesion sites (Fig. 3b; [48]). This model would be consistent with a role for TonB- and Tol-like complexes in powering locomotion by harvesting the proton motive force to establish transient contacts with outer membrane adhesions. However, such periplasmic engine supposes stationary sites (relative to the substratum) in the periplasm and not in the cytosol where the fixed AglZ-YFP clusters are located (Fig. 3d). In fact, an analogous periplasmic engine is likely to power gliding in the *Cytophaga/Flavobacterium* group where there is evidence for treadmilling of outer membrane adhesions (discussed below). The focal adhesion hypothesis integrates parts of this model because physical linkage between the putative intracellular motor and the adhesion track could be regulated at the level of the inner membrane complex.

Slime secretion

By far the most discussed model for A-motility is the "slime gun" hypothesis. It was suggested that *M. xanthus* cells are directly propelled by slime secretion through pore complexes (nozzles) observed in the cell envelopes, running along the cell body and seemingly enriched at the cell poles (Fig. 3d; [12]). The diameter of the observed pores was somewhat similar to that of cyanobacterial nozzles, hypothesized to be motor units underlying gliding motility in cyanobacteria

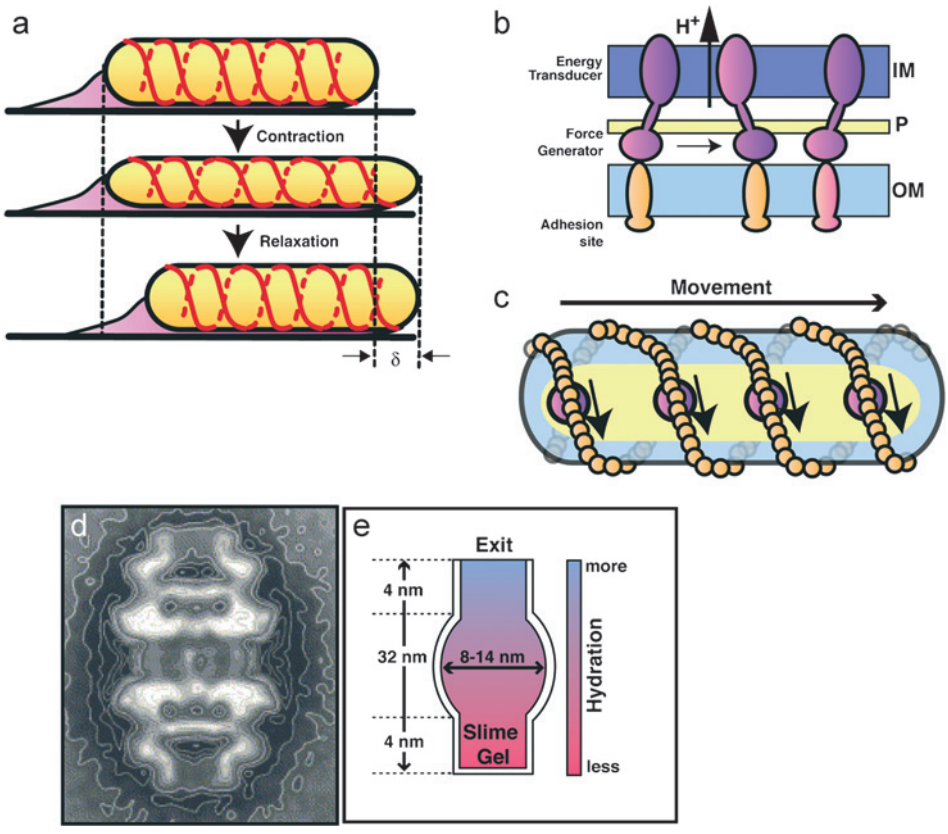

Figure 3. Proposed models for A-motility. (*a*) Inchworm-like contraction model. The propulsive cycle is built on the ability of helically wound filaments to constrict and elongate the cell body. Slime extruded from posterior pores acts like a ratchet and prevents the rear from moving backward, thus filament contraction pushes the front forward. Note that changes in cell dimensions, diameter and length, are expected as a result of the contraction cycle. Upon relaxation of the filaments, the turgor pressure restores the cell's original length and width. Adapted from [46]. (*b*) Periplasmic engine model. Close up of the interface of the surface of the cell and the substratum showing mechanical cycle of a single motor unit. A periplasmic force generator (motor, purple) is anchored to the cell wall (yellow) and binds an outer membrane adhesion complex. An energy transducer (purple) coupled to the motor harvests the proton motive force and leads to a conformational change of the motor corresponding to a stroke moving the adhesion site to the right. Following relaxation the motor engages a new adhesion complex (pink). Compiled from [48, 55]. (*c*) Cartoon of the ventral side of the bacterium depicted in (*b*). Action of engine units anchored in the cell wall results in treadmilling of helical adhesion bands in the outer membrane. The same color code applies. (*d*) Geometry of the slime nozzle. Average from side-view projections of the *Phormidium* nozzle. Similarly, The *M. xanthus* nozzles are proposed as a twofold symmetric open complexes with a central hole of variable diameters ranging between 8 and 14 nm. Reprinted from [12] with permission (Copyright Elsevier, 2002). (*e*) Slime gun model. Slime is a polyelectrolyte gel incorporated in its dehydrated or deswelled state inside the nozzle by an unknown mechanism. The nozzle is an impermeable barrel assumed to hydrate only through the nozzle exit where water flows so that a hydration gradient is established along the nozzle. The swelling of slime is therefore directed toward the nozzle opening. The model computes that this expansion generates sufficient force to propel *M. xanthus* but also cyanobacteria such as *Phormidium* at the observed speeds [12].

(see below, [49]). Assuming that the myxobacterial and cyanobacterial nozzles are the same molecular machine and that slime has the properties of a polyelectrolyte gel, it was computed that the swelling of slime could produce enough force to push the cell body forward at the measured velocities, when hydrated upon secretion (Fig. 3e; [12]). Slime jets powering cell movements are thus theoretically possible, but, although the model is elegant, it still lacks molecular evidence to support it. Electron microscopy of cells gliding directly on grids showed ribbons at the back of the cell suggesting that slime is indeed secreted from the observed pores. Alternatively, it could be argued that these ribbons are formed underneath the cell body (pores are also found along the cell body) as slime is secreted and shaped by surface tracks. As already mentioned, there is evidence for the existence of such tracks at the surface of *M. xanthus* cells and helical arrays of the Oscillin protein were suggested to organize slime ribbons at the cyanobacterial cell surface (discussed below, [50]).

Slime propulsion from the lagging pole is not in agreement with an earlier study by Sun *et al.* [45]: A-motility was not significantly affected when cells were artificially elongated up to ten times their natural length with the antibiotic Cephalexin, which suggested that the motor is evenly distributed along the cell body rather than localized at a cell pole. Accordingly,

the velocity of cells moving with a polar engine (type-IV pili) was strongly and linearly affected by cell elongation [45]. This analysis favors a model predicting distributed motor units such as the focal adhesion model. Thus, if slime propulsion powers gliding, this must be happening along the cell body, possibly at the observed AglZ-YFP adhesion sites. While there are no data against such a model, it is difficult to conceive how force would be applied parallel to the cell body and opposite to the direction of movement. For this, the secretory organelle would have to be somehow "tilted" tangentially to the substratum, which seems difficult to achieve structurally and is not consistent with the described structure of the nozzles [49].

Several composite models involving slime secretion and focal adhesions may be proposed. Any model favoring slime secretion as the active force for gliding motility now has to integrate the critical role of focal adhesions. It could be envisaged that slime secretion powers locomotion, which, combined to distributed adhesion sites, lead to smooth gliding. A prediction, however, would be that mutants lacking the adhesion sites would still display some detectable motility. However, AglZ, which seems to be a component of the focal adhesion complexes is absolutely required for gliding motility [23, 24]. It may also be expected that Cephalexin-elongated cells display aberrant motility patterns resulting from the action of both the back motor and the restriction of the adhesion sites at the front end of the cell (the back of elongated cells is largely depleted in AglZ-YFP clusters). Alternatively, both slime secretion and traction at focal adhesion points could contribute to locomotion. Again, one would expect some motility in an *aglZ* mutant; although the argument is not as strong in this case because detectable locomotion may require both systems to contribute power. Finally, locomotion could be produced solely at the focal adhesion sites and slime secretion would passively facilitate gliding. A definitive discrimination between these possible models will require testing whether or not slime secretion can produce force. So far, this has proven difficult because A-motility mutants that fail to secrete slime have not been isolated. Recently, Yu and Kaiser [21] observed that motility mutants apparently secreted slime from both cell poles and suggested that the complete lack of motility resulted from opposing forces. It is not to be expected that all mutations that inactivate A-motility would necessarily impair proper polarization of the engine. For this, components of the engine itself would have to be essential [21], which appears unlikely. Also, cells with bipolar motor activity would be expected to exhibit small back and forth displacements, a "jerky" behavior that was originally described for a regulatory mutant such as *mglA* [51]. The complete lack of motion supposes that cells exert exactly synchronized even forces at each poles at all times, a possibility that is not suggested by the fact that the bipolar slime trails are curved and not symmetrical [21]. Rather, I suggest that bipolar secretion of slime observed in the A-motility mutants results from entropic organization in curved trails as it "overflows" from beneath the cell body where it is continuously secreted. According to this view, slime secretion would mostly play a passive role in motility. Slime may provide a specific substratum (like ski wax) for cells to glide on. *M. xanthus* cells exhibit motility on a large number of substrata such as agar, plastic and glass possibly because slime provides ligands to the adhesion surface complexes. Thus, *M. xanthus* cells would virtually glide on all surfaces where slime can attach. In support of this view, cells follow preferentially previously laid down slime trails [52].

Multiple mechanisms for bacterial gliding motility

In our current state of knowledge, the focal adhesion model proposed here may only be realistically suggested for *M. xanthus*. Unfortunately, the comparison with other organisms is frustrated by the lack of molecular data, and it is impossible to propose a single mechanism for bacterial gliding motility. In fact, various models were suggested to explain gliding motility including rotating disks, waves of compression, fibrillar bending and moving adhesion tracks (for a review on these see [47]). Thus, the available experimental data argues that there may be multiple mechanisms for gliding motility. However, there may be common "accessories" to the various systems, for example through adapted use of focal adhesions, the potential involvement of internal cytoskeletons and the secretion of slime.

Focal adhesions

Studies from the early 1980 s already suggested that focal adhesion systems are involved in gliding bacteria of the *Cytophaga/Flavobacterium* complex [53, 54]. Pretreatment of the cells with beads inhibited the binding of cells to glass, suggesting that beads titrated specific adhesion sites. These beads were propelled such that a single sphere may move one cell length, proceed around the pole and migrate down the opposite side. Multiple bound spheres may follow the same path but also move in opposite directions. Attached-sphere movements are likely the consequence of the active gliding machinery because spheres were observed to move at speeds comparable to cell velocity and bead movement was disrupted in

the absence of oxygen [54]. Also, in *Flavobacterium johnsoniae* most motility mutants are unable to propel spheres at their surface [55]. Ring-like structures extracted from the envelopes of *F. johnsoniae* and *Flexibacter* were initially proposed to propel bead movements. Such rotary assemblies were absent from a non-gliding mutant [56], which initially led to the suggestion that they may act like wheels [56]; in fact, these structures could be secretion organelles because their dimensions are somewhat similar to those of the *M. xanthus* nozzles [12]. The spheres could instead be moved by helical closed bands of adhesions, the kind of which are suggested here for *M. xanthus*. The cell surface is also twisted helically in *Cytophaga/Flavobacterium* [34] and there is evidence for a periplasmic helix of the gliding lipoprotein GldJ in *F. johnsoniae* [37]. Processive movement of helical bands of adhesion sites could explain cell rotation during gliding and opposite movement of surface beads (Fig. 4; [39, 54]). However, this interpretation is most likely oversimplistic because the beads displayed complex behaviors that cannot be recapitulated by a regular continuous network of adhesion: they could move in opposite directions on the same side of the cell and in other cases, stop at mid-cell before resuming movement in opposite direction [54]. Therefore, several tracks may coexist at the cell surface and these tracks may have irregular topologies (Fig. 4). In fact, complex surface helical patterns is suggested by EM showing that cell parts can be devoid of helical features and that the distance between the nodes resulting from the antiparallel strands can be highly variable [34].

The structural organization of outer membrane adhesion sites may be similar in *Cytophaga/Flavobacterium* and *M. xanthus*. However, for gliding in *Cytophaga/Flavobacterium*, the motor complex is probably exposed in the periplasm and no internal cytoskeleton needs to be invoked for locomotion (here, the cell wall would provide the necessary rigid scaffold). Consistent with this, interference reflection microscopy showed that adhesion zones between *Cytophaga/Flavobacterium* cells and their substrate moved relative to the substratum [39]. The gliding machinery may involve as little as 12 *gld* genes all encoding potential envelope components such as an ABC transport system and exported lipoproteins [57]. The presence of secretory genes suggested that secretion of a polymer material could produce locomotion [55]. However, whereas slime is generally detected in gliding *Cytophaga/Flavobacterium*, *Cytophaga* U67 glides very efficiently in the absence of any detectable slime, favoring a focal adhesion-based model [54]. Only a few *gld* genes have homologues in the *M. xanthus* genome [57], further suggesting that myxobacteria and *Cytophaga/Flavobacterium* evolved distinct mechanisms to power focal adhesion-based motility. Such differences could explain how *Cytophaga/Flavobacterium* cells glide 50–100-fold faster than *M. xanthus* cells (2–4 μm/s vs 1.5–6 μm/min; [25, 58]).

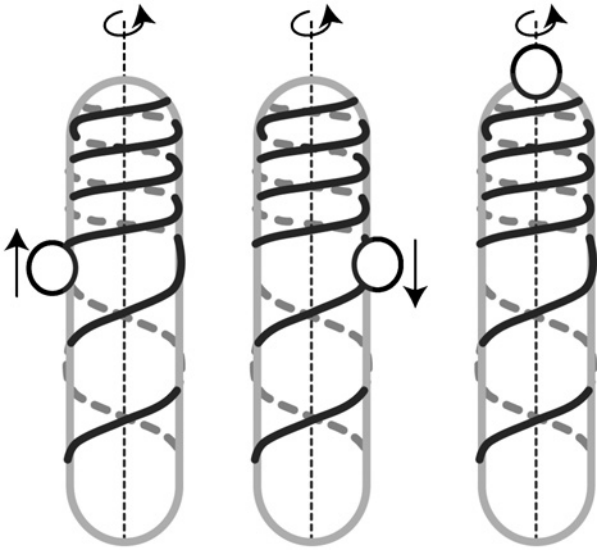

Figure 4. Suggested explanation for observed bead movements in *Flexibacter* and *Cytophaga/Flavobacterium*. Upon encounter with strands of closed helical adhesion tracks that move in the outer membrane, beads may go up and down the sides of a same cell and turn around the cell poles. In an attempt to explain the complex bead motions observed, the distances between the nodes of the helical continuum are pictured as being variable, consistent with EM images [34].

Cytoskeleton

The notion that an internal cytoskeleton may be involved in some cases of bacterial gliding motility was suggested long before the actin-like cytoskeleton was discovered in bacteria [47]. Early searches for organelles involved in gliding motility revealed numerous organized structures reminiscent of cytoskeletal elements: filament bundles in the cytosol of *M. xanthus*, *Anabaena* and *Chondromyces* [28, 59, 60], and 20–30-nm-long tubular structures with a central channel, coined "rhapidosomes", observed in disrupted cells of a variety of gliding bacteria [61]. Experimental evidence showing that these assemblies are involved in gliding is still lacking. It was postulated that they may represent bacterial actomyosin-like complexes but experimental attempts to prove this assumption were not met with success [47]. However, most experiments used F-actin-sensitive approaches now proven to be ineffective of the bacterial actin MreB [62] and, as already mentioned, the existence of myosin-like motors in bacteria is still an open question.

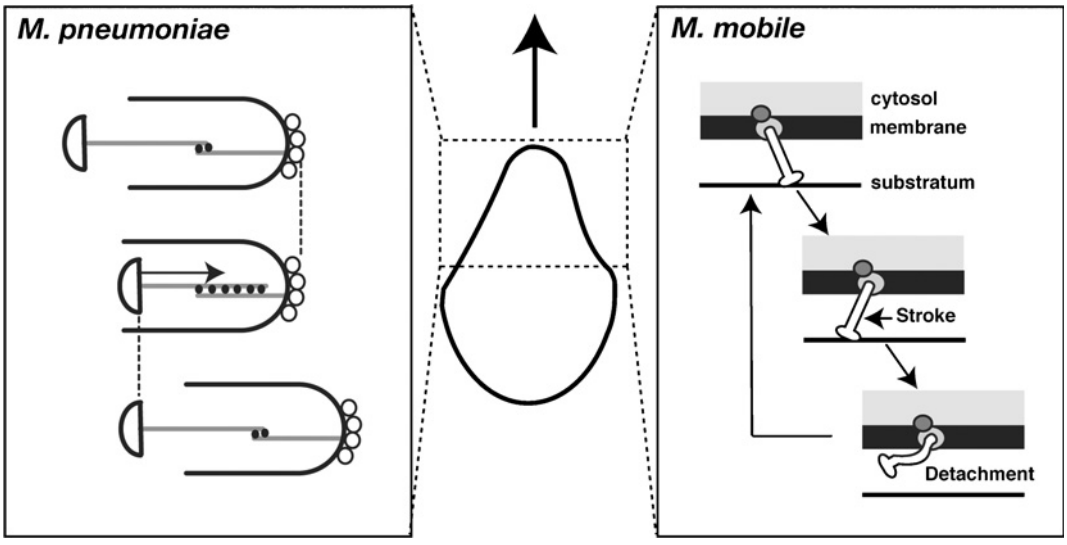

Figure 5. Gliding motility in *Mycoplasma. M. pneumoniae* and *M. mobile* are both pear-shaped cells with a leading terminal organelle. However, studies in these organisms point to fundamentally different mechanisms for gliding motility. In the *M. pneumoniae* terminal organelle, a bowl structure seems connected to envelope adhesion molecules (white circles) by a complex cytoskeletal network (rods). In a process analogous to actomyosin contractions, rods could slide relative to one another through the action of a putative motor (black dots) to bring the bowl complex closer to the tip. As the bowl complex provides resistance against the substratum the systems relaxes back to its fully elongated conformation pushing the adhesive front forward. Model compiled from [66]. In *M. mobile* there is no evidence for a cytoskeletal network in the terminal organelle. Rather, unusually large proteins localized at the "neck" of the terminal organelle are hypnotized to act like legs to power motility. According to that model, the Gli349 protein is the leg that binds to the substratum (white). Connection to the Gli521 protein (light gray) would allow force transmission from the putative motor protein, the ATPase P42 (dark gray). ATP hydrolysis would lead to cycles of attachments and strokes by the Gli349 leg. The action of multiple such machineries localized at the "neck" would thus lead to efficient locomotion. Model compiled from [70, 77–79].

A cytoskeletal organelle for gliding motility has been suggested in *Mycoplasma pneumoniae,* small parasitic bacteria that lack a peptidoglycan [63]. *Mycoplasma* cells show remarkable cell shape complexity: they are pear shaped with a thinner projection that is always the leading end of moving cells. This structure termed the terminal organelle has been linked with adhesion to the respiratory epithelium and gliding motility [64]. EM of *M. pneumoniae* cells revealed a complex macromolecular assembly in the terminal organelle [65]. This finding was recently unambiguously confirmed by cryo-electron tomography of cells in their near native state [66]. Membrane protein layers clustered at the terminal button were found connected to a mysterious "bowl" complex by an electron-dense core. The electron-dense core consisted of two main rods of distinct thickness with multiple contact points. The overall structure appeared segmented and showed extensive conformational flexibility. Henderson et al. [66] suggested that motility is powered by cycles of extensions and retractions of the electron-dense core. According to their model, the cycle would start when adhesion proteins at the tip bind a substrate with the core in a fully relaxed state (Fig. 5). The core would then contract and bring the bowl complex closer to the tip to provide resistance when the core relaxes and pushes the tip further. This would engage new adhesions at the tip and the cycle would resume. Although this model is hypothetical, it is attractive because there is precedence in eukaryotic lamellipodia where cycles of actomyosin contractions produce cell edge protrusion [67]. In the terminal organelle, it is tempting to speculate that the contact points between the rods contain a motor activity allowing the rods to slide relative to one another and thus produce extension-contraction cycles. Note that this model may not apply for another mollicute, *Mycoplasma mobile*, for which there is no evidence for an electron-dense core in the terminal organelle, questioning a cytoskeleton-based process in this organism [68]. In fact, in *M. mobile* motility was suggested to result from repeated binding of surface proteins to the substratum driven by ATPase cycles, a mechanism that would be fundamentally different from that of *M. pneumoniae* (Fig. 5, [69, 70]).

Experimental means of testing the *M. pneumoniae* model may soon be available because it was recently shown that the terminal organelle is indeed the site where the molecular engine is located. In a mutant lacking P41, a protein anchored at the base of the electron-dense core, terminal organelles detached and moved away from the mother cell showing unambiguously that they contain the motility engine [71]. This finding opens exciting perspectives: com-

bined proteomic and tomographic analysis of purified "independent" walking organelles should allow characterization of the individual structural components, the putative cytoskeleton, the enigmatic bowl complex and the molecular motor that fuels motility [72].

Slime

The production of extracellular slime is a characteristic of most gliding bacteria [47]. In fact, the idea that slime secretion propels movement was suggested a long time ago but was revived by EM observations of the potential secretory machine in the gliding cyanobacteria, *Phormidium* and *Anabaena* [49]. In these bacteria, multiple pore complexes (nozzles) were found on both sides of the junctions between cells within a filament ([49]). These nozzles were suggested to be the sites of slime secretion because India ink stained slime originated mostly from the cross walls in the filament, precisely where the pores are enriched. Partial purification of the nozzles revealed a symmetric hollow organelle, confirming their potential role as a secretion apparatus. Two main arguments were put forward to suggest that slime secretion through the nozzle provides the driving force for gliding motility: First, the rate of slime secretion seemed to match the measured gliding velocities. However, technical limitations did not allow precise measurement of the rate of elongation of the slime bands, therefore, this potentially strong argument would need to be strengthened by more quantitative measurements. Second, slime secretion occurred perpendicularly to the long axis of moving *Anabaena* filaments, parallel and opposite to the direction of movement [49]. As discussed for *M. xanthus*, this observation does not prove an active role for slime secretion because passive secretion of slime during movement would also result in slime trails at the back of the cells, parallel to the direction of movement.

The *M. xanthus* "slime gun" model postulates that force is produced as water flows into the nozzles and causes slime to swell upon exit (Fig. 3e; [12]). Slime could also promote motility by pushing the cell as it adheres to the substratum much like pathogenic bacteria polymerizing actin comet-tails to spread inside their host cell [73]. In fact, an earlier finding showed that the surface extrusion of adhering microfibrillar cellulose ribbons could propel an otherwise non-motile *Acetobacter xylinum* [74], suggesting that slime secretion could account for locomotion of the slower gliders (in the μm/min range). However, for bacteria gliding at μm/s speeds such as cyanobacteria and *Cytophaga/Flavobacterium* such a mode of locomotion supposes a tremendous energy cost. The synthesis of such slime would likely involve ATP but experiments with metabolic poisons favor a prominent role of the proton motive force rather than ATP in most studied species [47, 58].

It could also be that slime facilitates motility passively in most systems. In invertebrates, mucous secretions are involved in locomotion but also in other functions such as navigation, defense against predators, resistance to desiccation, food production and structural support [75]. Likewise, multiple roles for bacterial slime have also been suggested [6].

Conclusions

It is now widely accepted that the bacterial cell is a highly organized compartment where proteins are sorted to specific locations to exert their function. Interestingly, early studies on bacterial gliding motility already pointed out that complex cellular structures and cytoskeletons could exist in bacteria, but in the absence of molecular characterization they may have been discarded as specific features of "exotic" bacteria. The *M. xanthus* A-motility periodic adhesion sites suggest an extremely complex underlying architecture because they must be considered in light of the Gram-negative multilayered cell envelope. Thus, whether or not they are connected to an internal cytoskeleton, the *M. xanthus* focal adhesion sites may also contain multiple components undergoing complex spatial regulations. Therefore, investigating this mechanism of gliding motility is also of interest to understand general processes in bacteria. In this review, I have attempted to suggest new directions to investigate A-motility. The focal adhesion model still has numerous black boxes but it makes several predictions that are readily testable. Validation will depend on the characterization of the putative cytoskeleton as well as that of the molecular motor. A role for the cytoskeleton seems reasonable and is suggested by the periodicity of the adhesion sites, EM observations and accumulating evidence that the bacterial cytoskeleton is involved in many essential processes such as the establishment of cell shape, cell division, chromosome segregation, the establishment of cell polarity and recently the positioning of intracellular organelles [40, 76]. Further molecular characterization of A-motility may also reveal whether it evolved as a myxobacterial-specific trait or whether some of its features are general to other gliding bacteria.

Acknowledgements. I would like to thank M. McBride, R. Burchard, J. Viala, J. Merlie, E. Mauriello, D. Zusman, A. Scott and an anonymous reviewer for comments and discussions on this manuscript. Studies by the author presented in this review were supported by a grant from NIH to D. Zusman (GM2059).

1 Wozniak, M. A., Modzelewska, K., Kwong, L. and Keely, P. J. (2004) Focal adhesion regulation of cell behavior. Biochim. Biophys. Acta 1692, 103 – 119.
2 Charest, P. G. and Firtel, R. A. (2007) Big roles for small GTPases in the control of directed cell movement. Biochem. J. 401, 377 – 390.
3 Wadhams, G. H. and Armitage, J. P. (2004) Making sense of it all: bacterial chemotaxis. Nat. Rev. Mol. Cell. Biol. 5, 1024 – 1037.
4 Harshey, R. M. (1994) Bees aren't the only ones: swarming in gram-negative bacteria. Mol. Microbiol. 13, 389 – 394.
5 Wang, Q., Suzuki, A., Mariconda, S., Porwollik, S. and Harshey, R. M. (2005) Sensing wetness: a new role for the bacterial flagellum. EMBO J. 24, 2034 – 2042.
6 Harshey, R. M. (2003) Bacterial motility on a surface: many ways to a common goal. Annu. Rev. Microbiol. 57, 249 – 273.
7 Kaiser, D. (1979) Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA 76, 5952 – 5956.
8 Merz, A. J., So, M. and Sheetz, M. P. (2000) Pilus retraction powers bacterial twitching motility. Nature 407, 98 – 102.
9 Skerker, J. M. and Berg, H. C. (2001) Direct observation of extension and retraction of type IV pili. Proc. Natl. Acad. Sci. USA 98, 6901 – 6904.
10 Sun, H., Zusman, D. R. and Shi, W. Y. (2000) Type IV pilus of *Myxococcus xanthus* is a motility apparatus controlled by the *frz* chemosensory system. Curr. Biol. 10, 1143 – 1146.
11 Pringsheim, E. G. (1951) The *Vitreoscillaceae*; a family of colourless, gliding, filamentous organisms. J. Gen. Microbiol. 5, 124 – 149.
12 Wolgemuth, C., Hoiczyk, E., Kaiser, D. and Oster, G. (2002) How myxobacteria glide. Curr. Biol. 12, 369 – 377.
13 Kaiser, D. (2004) Signaling in myxobacteria. Annu. Rev. Microbiol. 58, 75 – 98.
14 Kaiser, D. (2003) Coupling cell movement to multicellular development in myxobacteria. Nat. Rev. Microbiol. 1, 45 – 54.
15 Hodgkin, J. and Kaiser, D. (1979) Genetics of gliding motility in *Myxococcus xanthus* (*Myxobacterales*). 2. Gene systems control movement. Mol. Gen. Genet. 171, 177 – 191.
16 Li, Y., Sun, H., Ma, X., Lu, A., Lux, R., Zusman, D. and Shi, W. (2003) Extracellular polysaccharides mediate pilus retraction during social motility of *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA 100, 5443 – 5448.
17 Lu, A., Cho, K., Black, W. P., Duan, X. Y., Lux, R., Yang, Z., Kaplan, H. B., Zusman, D. R. and Shi, W. (2005) Exopolysaccharide biosynthesis genes required for social motility in *Myxococcus xanthus*. Mol. Microbiol. 55, 206 – 220.
18 Bowden, M. G. and Kaplan, H. B. (1998) The *Myxococcus xanthus* lipopolysaccharide O-antigen is required for social motility and multicellular development. Mol. Microbiol. 30, 275 – 284.
19 Shi, W. and Zusman, D. R. (1993) The two motility systems of *Myxococcus xanthus* show different selective advantages on various surfaces. Proc. Natl. Acad. Sci. USA 90, 3378 – 3382.
20 Youderian, P., Burke, N., White, D. J. and Hartzell, P. L. (2003) Identification of genes required for adventurous gliding motility in *Myxococcus xanthus* with the transposable element mariner. Mol. Microbiol. 49, 555 – 570.
21 Yu, R. and Kaiser, D. (2007) Gliding motility and polarized slime secretion. Mol. Microbiol. 63, 454 – 467.
22 Youderian, P. and Hartzell, P. L. (2006) Transposon insertions of magellan-4 that impair social gliding motility in *Myxococcus xanthus*. Genetics 172, 1397 – 1410.
23 Yang, R. F., Bartle, S., Otto, R., Stassinopoulos, A., Rogers, M., Plamann, L. and Hartzell, P. (2004) AglZ is a filament-forming coiled-coil protein required for adventurous gliding motility of *Myxococcus xanthus*. J. Bacteriol. 186, 6168 – 6178.
24 Mignot, T., Shaevitz, J. W., Hartzell, P. L. and Zusman, D. R. (2007) Evidence that focal adhesion complexes power bacterial gliding motility. Science 315, 853 – 856.
25 Spormann, A. M. and Kaiser, A. D. (1995) Gliding movements in *Myxococcus xanthus*. J. Bacteriol. 177, 5846 – 5852.
26 Wolgemuth, C. W. (2005) Force and flexibility of flailing myxobacteria. Biophys. J. 89, 945 – 950.
27 Goldman, B. S., Nierman, W. C., Kaiser, D., Slater, S. C., Durkin, A. S., Eisen, J. A., Ronning, C. M., Barbazuk, W. B., Blanchard, M., Field, C., Halling, C., Hinkle, G. et al. (2006) Evolution of sensory complexity recorded in a myxobacterial genome. Proc. Natl. Acad. Sci. USA 103, 15200 – 15205.
28 Burchard, A. C., Burchard, R. P. and Kloetzel, J. A. (1977) Intracellular, periodic structures in the gliding bacterium *Myxococcus xanthus*. J. Bacteriol. 132, 666 – 672.
29 Defeu Soufo, H. J. and Graumann, P. L. (2005) *Bacillus subtilis* actin-like protein MreB influences the positioning of the replication machinery and requires membrane proteins MreC/D and other actin-like proteins for proper localization. BMC Cell. Biol. 6, 10.
30 Kruse, T., Moller-Jensen, J., Lobner-Olesen, A. and Gerdes, K. (2003) Dysfunctional MreB inhibits chromosome segregation in *Escherichia coli*. EMBO J. 22, 5283 – 5292.
31 Sibley, L. D. (2004) Intracellular parasite invasion strategies. Science 304, 248 – 253.
32 Baum, J., Papenfuss, A. T., Baum, B., Speed, T. P. and Cowman, A. F. (2006) Regulation of apicomplexan actin-based motility. Nat. Rev. Microbiol. 4, 621 – 628.
33 Freese, A., Reichenbach, H. and Lunsdorf, H. (1997) Further characterization and *in situ* localization of chain-like aggregates of the gliding bacteria *Myxococcus fulvus* and *Myxococcus xanthus*. J. Bacteriol. 179, 1246 – 1252.
34 Lunsdorf, H. and Schairer, H. U. (2001) Frozen motion of gliding bacteria outlines inherent features of the motility apparatus. Microbiology 147, 939 – 947.
35 Pelling, A. E., Li, Y., Shi, W. and Gimzewski, J. K. (2005) Nanoscale visualization and characterization of *Myxococcus xanthus* cells with atomic force microscopy. Proc. Natl. Acad. Sci. USA 102, 6484 – 6489.
36 Dye, N. A., Pincus, Z., Theriot, J. A., Shapiro, L. and Gitai, Z. (2005) Two independent spiral structures control cell shape in Caulobacter. Proc. Natl. Acad. Sci. USA 102, 18608 – 18613.
37 Braun, T. F. and McBride, M. J. (2005) *Flavobacterium johnsoniae* GldJ is a lipoprotein that is required for gliding motility. J. Bacteriol. 187, 2628 – 2637.
38 Halfen, L. N. and Castenholz, R. W. (1971) Energy expenditure for gliding motility in a blue-green alga. J. Phycol. 7, 133 – 145.
39 Godwin, S. L., Fletcher, M. and Burchard, R. P. (1989) Interference reflection microscopic study of sites of association between gliding bacteria and glass substrata. J. Bacteriol. 171, 4589 – 4594.
40 Carballido-Lopez, R. (2006) The bacterial actin-like cytoskeleton. Microbiol. Mol. Biol. Rev. 70, 888 – 909.
41 Gitai, Z. (2007) Diversification and specialization of the bacterial cytoskeleton. Curr. Opin. Cell. Biol. 19, 5 – 12.
42 Arshinoff, B. I., Suen, G., Just, E. M., Merchant, S. M., Kibbe, W. A., Chisholm, R. L. and Welch, R. D. (2007) Xanthusbase: adapting wikipedia principles to a model organism database. Nucleic Acids Res. 35, D422 – 426.
43 Dworkin, M., Keller, K. H. and Weisberg, D. (1983) Experimental observations consistent with a surface tension model of gliding motility of *Myxococcus xanthus*. J. Bacteriol. 155, 1367 – 1371.
44 Keller, K. H., Grady, M. and Dworkin, M. (1983) Surface tension gradients: feasible model for gliding motility of *Myxococcus xanthus*. J. Bacteriol. 155, 1358 – 1366.
45 Sun, H., Yang, Z. and Shi, W. (1999) Effect of cellular filamentation on adventurous and social gliding motility of *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA 96, 15178 – 15183.
46 Wolgemuth, C. W., Igoshin, O. and Oster, G. (2003) The motility of mollicutes. Biophys. J. 85, 828 – 842.
47 Burchard, R. P. (1981) Gliding motility of prokaryotes: ultrastructure, physiology, and genetics. Annu. Rev. Microbiol. 35, 497 – 529.
48 Spormann, A. M. (1999) Gliding motility in bacteria: insights from studies of *Myxococcus xanthus*. Microbiol. Mol. Biol. Rev. 63, 621 – 641.

49 Hoiczyk, E. and Baumeister, W. (1998) The junctional pore complex, a prokaryotic secretion organelle, is the molecular motor underlying gliding motility in cyanobacteria. Curr. Biol. 8, 1161 – 1168.

50 Hoiczyk, E. and Baumeister, W. (1997) Oscillin, an extracellular, Ca^{2+}-binding glycoprotein essential for the gliding motility of cyanobacteria. Mol. Microbiol. 26, 699 – 708.

51 Spormann, A. M. and Kaiser, D. (1999) Gliding mutants of *Myxococcus xanthus* with high reversal frequencies and small displacements. J. Bacteriol. 181, 2593 – 2601.

52 Burchard, R. P. (1982) Trail following by gliding bacteria. J. Bacteriol. 152, 495 – 501.

53 Ridgway, H. F. and Lewin, R. A. (1988) Characterization of gliding motility in *Flexibacter polymorphus.* Cell. Motil. Cytoskeleton 11, 46 – 63.

54 Lapidus, I. R. and Berg, H. C. (1982) Gliding motility of *Cytophaga* sp. strain U67. J. Bacteriol. 151, 384 – 398.

55 McBride, M. J. (2004) Cytophaga-flavobacterium gliding motility. J. Mol. Microbiol. Biotechnol. 7, 63 – 71.

56 Pate, J. L. and Chang, L.-Y. E. (1979) Evidence that gliding motility in procaryotic cells is driven by rotary assemblies in the cell envelopes. Curr. Microbiol. 2, 59 – 64.

57 Braun, T. F., Khubbar, M. K., Saffarini, D. A. and McBride, M. J. (2005) *Flavobacterium johnsoniae* gliding motility genes identified by mariner mutagenesis. J. Bacteriol. 187, 6943 – 6952.

58 McBride, M. J. (2001) Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. Annu. Rev. Microbiol. 55, 49 – 75.

59 Jensen, T. E. and Ayala, R. P. (1976) The fine structure of a microplate-microtubule array, microfilaments and polyhedral body associated microtubules in several species of *Anabaena.* Arch. Microbiol. 111, 1 – 6.

60 MacRae, T. H. and McCurdy, H. D. (1975) Ultrastructural studies of *Chondromyces crocatus* vegetative cells. Can. J. Microbiol. 21, 1815 – 1826.

61 Lewin, R. A. (1963) Rod-shaped particles in *Saprospira.* Nature 198, 103 – 104.

62 Vollmer, W. (2006) The prokaryotic cytoskeleton: a putative target for inhibitors and antibiotics? Appl. Microbiol. Biotechnol. 73, 37 – 47.

63 Razin, S., Yogev, D. and Naot, Y. (1998) Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. 62, 1094 – 1156.

64 Krause, D. C. and Balish, M. F. (2004) Cellular engineering in a minimal microbe: structure and assembly of the terminal organelle of *Mycoplasma pneumoniae.* Mol. Microbiol. 51, 917 – 924.

65 Balish, M. F. and Krause, D. C. (2006) Mycoplasmas: a distinct cytoskeleton for wall-less bacteria. J. Mol. Microbiol. Biotechnol. 11, 244 – 255.

66 Henderson, G. P. and Jensen, G. J. (2006) Three-dimensional structure of *Mycoplasma pneumoniae*'s attachment organelle and a model for its role in gliding motility. Mol. Microbiol. 60, 376 – 385.

67 Giannone, G., Dubin-Thaler, B. J., Rossier, O., Cai, Y., Chaga, O., Jiang, G., Beaver, W., Dobereiner, H. G., Freund, Y., Borisy, G. and Sheetz, M. P. (2007) Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell 128, 561 – 575.

68 Shimizu, T. and Miyata, M. (2002) Electron microscopic studies of three gliding Mycoplasmas, *Mycoplasma mobile*, *M. pneumoniae*, and *M. gallisepticum*, by using the freeze-substitution technique. Curr. Microbiol. 44, 431 – 434.

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

70 Uenoyama, A. and Miyata, M. (2005) Gliding ghosts of *Mycoplasma mobile.* Proc. Natl. Acad. Sci. USA 102, 12754 – 12758.

71 Hasselbring, B. M. and Krause, D. C. (2007) Cytoskeletal protein P41 is required to anchor the terminal organelle of the wall-less prokaryote *Mycoplasma pneumoniae.* Mol. Microbiol. 63, 44 – 53.

72 Jensen, G. (2007) A nanoengine for gliding motility. Mol. Microbiol. 63, 4 – 6.

73 Stevens, J. M., Galyov, E. E. and Stevens, M. P. (2006) Actin-dependent movement of bacterial pathogens. Nat. Rev. Microbiol. 4, 91 – 101.

74 Brown, R. M., Jr., Willison, J. H. and Richardson, C. L. (1976) Cellulose biosynthesis in *Acetobacter xylinum:* visualization of the site of synthesis and direct measurement of the *in vivo* process. Proc. Natl. Acad. Sci. USA 73, 4565 – 4569.

75 Denny, M. W. (1989) Invertebrate mucous secretions: functional alternatives to vertebrate paradigms. Symp. Soc. Exp. Biol. 43, 337 – 366.

76 Gitai, Z. (2005) The new bacterial cell biology: Moving parts and subcellular architecture. Cell 120, 577 – 586.

77 Ohtani, N. and Miyata, M. (2007) Identification of a novel nucleoside triphosphatase from *Mycoplasma mobile:* a prime candidate motor for gliding motility. Biochem. J. 403, 71 – 77.

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

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