Mycoplasma takes a walk

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The mechanism of movement of individual cells shows extensive diversity. In the Eukarya, ATP is the driving force for cell motility (1). In contrast, in the Bacteria and Archaea, the chemiosmotic pathway of energy transduction powers flagellar rotation. Depending on the species, either hydrogen or sodium ion flux results in cell movement. ATP does not directly participate in providing the energy needed for this type of motility (2). But some species of bacteria move by means other than flagellar-based motility. Do hydrogen or sodium gradients propel these organisms as well? In a recent issue of PNAS, Uenoyama and Miyata (3) directly showed that for a wall-less bacterial species, *Mycoplasma mobile*, intracellular ATP is the driving force for its relatively fast gliding along a glass surface. This is the second type of motility powered by ATP identified in bacteria, the first being the retraction of type IV pili as seen, for example, in *Myxobacteria* and *Pseudomonas* (4, 5).

Uenoyama and Miyata (3) used a multistep approach to reach this conclusion, but the essential experimental design was borrowed from eukaryotic cell biology. First, they isolated a spontaneously occurring mutant of *M. mobile* that can attach more efficiently to a glass surface than the wild type does. This mutant enabled them to assay the motility of a large number of cells. Second, using a procedure similar to that used by Gibbons and Gibbons (6) on sea urchin sperm, they rendered the membrane of gliding *M. mobile* cells permeable with a low concentration of the detergent Triton X-100. What remained were nonmotile ghosts that lacked DNA but still adhered to the glass. Finally, when incubated with ATP, the ghosts regained motility with speeds similar to untreated cells (≈2 μm/s). The results are striking, and the reader is urged to view the accompanying movie in their supporting information. Uenoyama and Miyata tested other nucleotide triphosphates, but ATP worked most efficiently. These results, plus other published experiments on intact cells, including one that showed that arsenate inhibited gliding of *M. mobile* (7), conclusively point toward ATP as the driving force.

How do these bacteria glide, and how does ATP drive cell motion? Although much has yet to be worked out, over the past several years, significant progress has been made along these lines (8). *M. mobile* attaches to a glass surface, and it then aligns itself such that it tapers at one cell pole; the cell resembles a flask with a head-like structure, neck, and cell body (ref. 9 and Fig. 1 *Upper*). Its dimensions are quite small, with a long axis of only 1.0 μm and a short axis of 0.6 μm (10). Attachment to the glass and cell displacement occur at the neck, with the head serving as the leading end of the cell (11). The cell does not reverse directions, and it exerts a substantial force of 27 pN during gliding (12). Three major approaches have been used to identify cellular proteins involved in cell motility and attachment. First, because there is no known genetic exchange system for *M. mobile*, and thus no way to target specific genes, UV-induced mutants were isolated, screened, and characterized for ones that were deficient in either motility or attachment to a surface (10). Second, genes adjacent to others previously identified as being involved in gliding, and part of the same operon, were hypothesized to participate in motility. These genes, and the proteins that they encode, were analyzed in detail (13). Third, monoclonal antibodies were screened for their ability to inhibit mo-

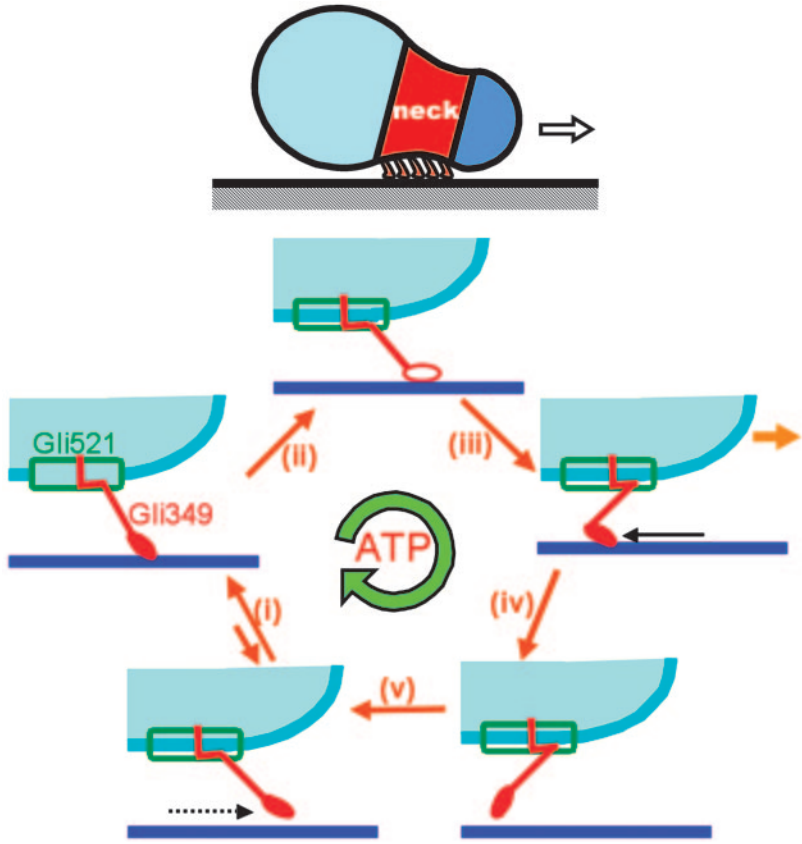

Fig. 1. Diagram of *M. mobile* (*Upper*) and proposed gliding mechanism (*Lower*). (*Upper*) Schematic illustration of three subcellar regions of *M. mobile* (9). The direction of gliding is indicated by an open arrow. The neck region is specialized for gliding and contains the spike structure observed by electron microscopy. (*Lower*) Hypothetical mechanical walking cycle. A *Mycoplasma* cell is represented by the light-blue color. A spike (Gli349) is represented as a rod protruding from the cell and supported by a force generator (Gli521) represented as a rectangular box. The glass surface is represented by a solid line. The cycle of transition of the spike occurs in five steps: (*i*) initial binding, (*ii*) tight binding, (*iii*) stroke, (*iv*) release, and (*v*) return. The cycle is powered by ATP. The figure was modified from ref. 8.

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tility and/or attachment. Proteins that react with the monoclonal antibodies were identified, and comparisons were made to the previously isolated mutants. These antibodies were used not only to sort out at which step in motility inhibition occurs, e.g., cell attachment, gliding, or release (see below), but were also used to identify the region of the cell where these proteins were localized (8, 13–15).

Three proteins have been identified from their analysis. Gli349 (gliding protein of 349 kDa) is a large protein involved in attachment of *M. mobile* to the glass surface, and it localizes at the neck of the cell. Antibodies to this protein inhibit cell attachment, and a mutant lacking this protein fails to attach (14). The second is Gli521. This huge protein is also located preferentially at the neck. Monoclonal antibodies directed to Gli521 still allow cells to attach, but gliding is inhibited. Thus, Gli521 likely participates in either generating the force needed for cell gliding or transmitting that force to the gliding machinery (15). A third protein, Gli123, was also recently identified as being localized at the neck. Mutants lacking this protein fail to properly localize Gli349 and Gli521at the neck. Thus, Gli123 is believed to be involved in localization of motility proteins within the cell (13). Other proteins will likely emerge that are involved in *M. mobile* motility. For example, there is one other ORF of unidentified function in the same operon with *gli123*, *gli349*, and *gli521* (13). In addition, genomic and proteomic analysis of *M. mobile* points toward additional proteins as possible candidates involved in motility (16).

What is known about the gliding machinery of *M. mobile?* Freeze-fracture electron microscopy indicates that many spikes that are 51 nm long and 4.3 nm wide emerge from the outer leaflet of the cell membrane predominately at the neck region. These spikes are involved in cell attachment, as a mutant lacking these spikes fails to bind to glass. Furthermore, this mutant also lacks Gli349 (17). These results, plus evidence that Gli349 localizes at this region of the cell, indicate that these spikes likely contain or are composed of Gli349. In addition, the estimated volume that one Gli349 protein occupies is consistent with this protein serving as the spike (8). Because there are ≈400 Gli349 molecules per cell, there is likely to be an equivalent number of spikes (8).

The results of these and several other studies have led to a model of *M. mobile* gliding (Fig. 1 *Lower*). First, the spike attaches to the glass surface loosely and then more tightly. A stroke toward the back of the cell allows it to glide forward. The spike then detaches from the glass and reorients, and there follows another cycle of attachment, stroke, detachment, and reorientation; the cell walks! Previous results (7) and those presented by Uenoyama and Miyata (3) indicate that ATP powers the cycle, although the putative cycle of ATP binding, dephosphorylation, and release has not yet been worked out. This type of movement resembles that of crawling eukaryotic cells (1), but the volume of an *M. mobile* cell is ≈2,000 times smaller. With a tiny genome of only 777 kb, *M. mobile* has been found to lack the genes that encode eukaryotic motor proteins involved in cell movement (16).

Do other gliding *Mycoplasma* species use the same motility machinery as found in *M. mobile*? Several other *Mycoplasma* species glide and have a flask-like structure similar to that of *M. mobile*, but are many times slower. Genomic analysis indicates that several of the gliding genes of *M. mobile* (*gli123*, *gli349*, and *gli521*) are well conserved in the closely related gliding species *Mycoplasma pulmonis*, but orthologues are not evident in several other gliding species, including *Mycoplasma pneumoniae* (8, 18). These results suggest significant variation in the gliding machinery among *Mycoplasma* species, and it may be that gliding evolved more than once in this group of bacteria (16). However, if only a few proteins participate in gliding, wide variation in amino acid sequence may be more likely than if there were many proteins involved in forming the apparatus, as is the case for flagella-based motility (8).

The relationship between virulence and motility is unclear (8). *M. mobile* is a pathogen that infects fish. Several other *Mycoplasma* species, including *M. pneumoniae* and *M. pulmonis*, cause disease in mammals. The connection between motility and virulence has not been studied in detail in any of these species. Because mutants deficient in gliding are most often inhibited in attachment, drawing a definitive connection between gliding and virulence is difficult. Clearly, such experiments await the development of a gene transfer system.

What is the significance of the findings presented? This "*in vitro*" ghost model of gliding should certainly help in determining how the machinery works. Along these lines, none of the proteins so far identified in gliding resemble classical ATPases (3), so there may well be a novel ATP utilization mechanism. In a recent publication (19), *M. mobile* has been shown to move attached latex beads in an ordered and continuous manner along preformed grooved paths. *M. mobile* may play a significant future role in microtechnological applications as microtransporters. We anxiously await the results of future studies on this tiny, but quite intriguing, bacterium.

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