Type IV pilus of *Myxococcus xanthus* is a motility apparatus controlled by the *frz* chemosensory system

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Although flagella are the best-understood means of locomotion in bacteria [1], other bacterial motility mechanisms must exist as many diverse groups of bacteria move without the aid of flagella [2–4]. One unusual structure that may contribute to motility is the type IV pilus [5,6]. Genetic evidence indicates that type IV pili are required for social gliding motility (S-motility) in *Myxococcus*, and twitching motility in *Pseudomonas* and *Neisseria* [6,7]. It is thought that type IV pili may retract or rotate to bring about cellular motility [6,8], but there is no direct evidence for the role of pili in cell movements. Here, using a tethering assay, we obtained evidence that the type IV pilus of *Myxococcus xanthus* functions as a motility apparatus. Pili were required for *M. xanthus* cells to adhere to solid surfaces and to generate cellular movement using S-motility. Tethered cells were released from the surface at intervals corresponding to the reversal frequency of wild-type cells when gliding on a solid surface. Mutants defective in the control of directional movements and cellular reversals (*frz* mutants) showed altered patterns of adherence that correlate reversal frequencies with tethering. The behavior of the tethered cells was consistent with a model in which the pili are extruded from one cell pole, adhere to a surface, and then retract, pulling the cell in the direction of the adhering pili. Cellular reversals would result from the sites of pili extrusion switching from one cell pole to another and are controlled by the *frz* chemosensory system.

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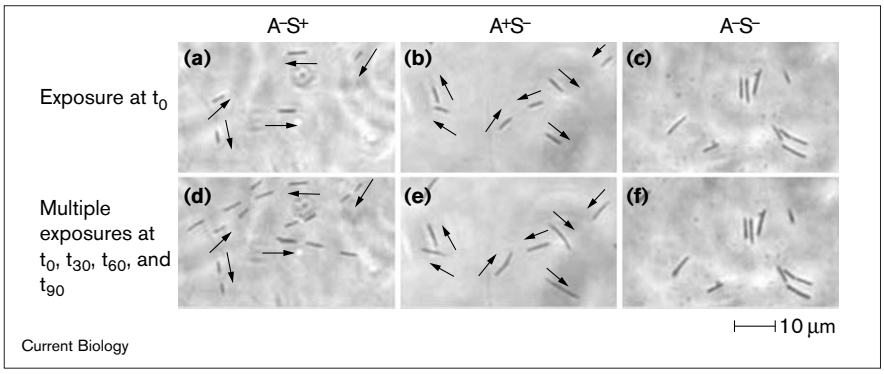
Results and discussion

Silverman and Simon [9] developed a useful assay for studying flagellar motion by tethering a flagellum filament from *Escherichia coli* to a solid surface using antisera to flagellin. Under these conditions, the tethered flagellum cannot rotate; the flagellar motor, however, continues to rotate, causing the bacterial body to spin in the opposite direction [9]. We had initially intended to use a similar tethering assay to study S-motility associated with the type IV pilus in *M. xanthus*: we planned to tether the pilus and then determine the impact of this immobilization on cellular movements. However, unlike flagella-based motility in *E. coli*, which moves cells up to 25 μm/second, gliding motility associated with the type IV pilus in *M. xanthus* is very slow (about 0.1 μm/second) and difficult to distinguish from the random cell movement caused by Brownian motion. To address this problem, we placed *M. xanthus* cells in a highly viscous medium, 1% methylcellulose, and examined the cells by microscopy. We found that in this medium, the Brownian motion of the cells was much smaller, and the cells exhibited unusually rapid gliding motility (up to 0.4 μm/second) on glass or polystyrene surfaces.

M. xanthus has two different motility systems to control their movements: S-motility and A-motility [10,11]. Figure 1 shows that both A^-S^+ and A^+S^- cells were able to move as single cells on the solid surface covered with 1% methylcellulose; in this medium, S-motility is the most rapid. As we were primarily interested in cell movements that were associated with S-motility and type IV pili, most strains used in this study contained mutations in an A-motility gene so that movements observed were due to S-motility alone. It is worthwhile to mention that S-motility was originally observed as cell-group movement on a 1.5% agar surface; under this condition, isolated A^-S^+ cells are not motile [10,11]. Nevertheless, Sun *et al.* recently found that isolated A^-S^+ cells do move on a 0.4% agar surface [12]. This study further confirmed that A^-S^+ cells can move well as individual cells. On the basis of these findings, we conclude that under certain environmental conditions (such as wet surfaces), S-motility associated with the type IV pilus in *M. xanthus* does function in isolated, individual cells. This finding is consistent with twitching motility associated with the type IV pilus in other bacteria, which also occurs with individual cells under certain environmental conditions [6,7].

As shown in Figure 2, most (about 85%) of the wild-type *M. xanthus* cells were observed to settle to the bottom of the solid surface and glide forward or backward in the direction of the long axis of the cells. Nevertheless, some cells were perpendicular to the surface and showed jiggling movements (Figures 2 and 3). These cells appeared to have one of their cell ends tethered to the solid surface while the cell bodies moved in place. We note that spontaneous tethering

Figure 1

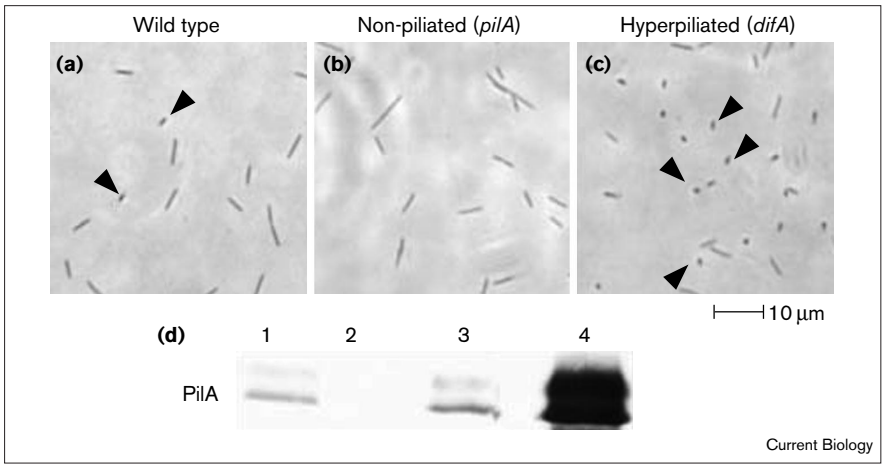


M. xanthus cells gliding on a polystyrene surface covered with 1% methylcellulose. *M. xanthus* cells were placed in a 24-well cell-culture plate containing 1% methylcellulose in MOPS buffer (10 mM MOPS, 8 mM $MgSO_4$, pH 7.6). After the cells settled to the bottom of the wells, gliding motility was observed using an inverted microscope (Leica) with a 32× objective lens. Serial digital images were taken at 30 sec intervals using a Spot camera (Diagnostic Instruments Inc.). **(a–c)** Cell positions at time zero (t_0). **(d–f)** An overlay of four consecutive pictures taken at 0, 30, 60 and 90 sec. Arrows indicate the directions of cellular movement. (a,d) Strain MxH1216 (A^-S^+) [17]. Single cells moved at approximately 0.4 μm/sec, and thus replicate images are seen in (d) because of motility during the 90 sec interval. (b,e) Strain DK10407 (*pilA*; A^+S^-). Single cells moved at approximately 0.05 μm/sec. Therefore, cells in (e) appear twice as long as cells in (b). (c,f) Strain SW538 (A^-S^-) [12]; no motility was observed for these cells.

of cells was dependent on the presence of functional type IV pili as: first, wild-type cells treated with strong shear forces that are known to remove pili (Figure 2d) showed very few tethered cells (data not shown); second, *pilA* mutants, which are defective in pilus biogenesis, did not show any tethered cells (Figure 2); third, *pilT* mutants, which contain paralyzed pili, could still be tethered (Figure 3); and, fourth, mutants that are hyperpiliated (Figure 2d) showed a much higher percentage of tethered cells (Figure 2c).

Time-lapse analysis of tethered cells (Figure 3a–d) showed that these cells were not just adhering to the solid surface but were actually producing some motion, which we describe as a jiggling movement. These results are consistent with the previous findings that type IV pili are involved in motility. In contrast, the *pilT* mutant cells, which contain non-functional (paralyzed) pili, were also tethered but did not show any motion (Figure 3e–h), indicating that the movements observed in wild-type cells were not the result of simple Brownian motion or an artifact of photography. On further analysis of individual tethered cells, we focused the microscope on the tip of the untethered end, and then followed the movement of the untethered cell tip by readjusting the focus of the microscope. We found that after an interval, the cells that were

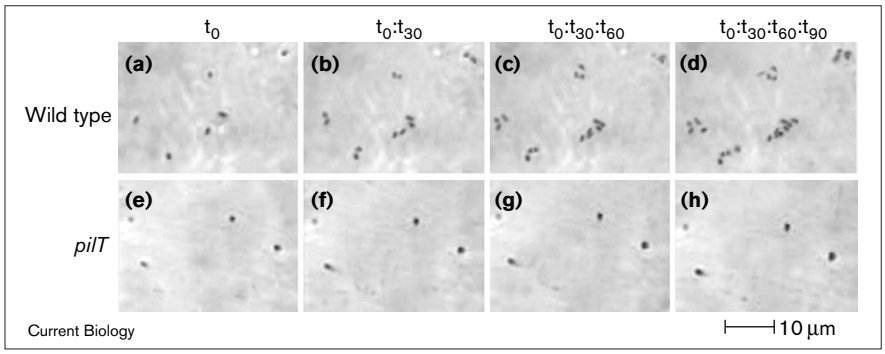
Figure 2



Tethering of *M. xanthus* cells is pilus dependent. *M. xanthus* cells, prepared as described in Figure 1, were examined by microscopy 10 min after settling to the bottom of the wells. The cells that are perpendicular to the surface are the tethered cells (arrowheads). **(a)** Wild-type $PilA^+$ strain (DK1622); about 15% of the cells were tethered to the polystyrene surface. **(b)** Strain DK10407, a *pilA* mutant (defective in pilus biogenesis); no cells were tethered. **(c)** Strain SW504 [18], a *difA* mutant (hyperpiliated); about 60% of the cells were tethered. The *difA* mutant is defective in fibril production [12]. Similar results were observed with other fibril-minus mutants (data not shown). **(d)** *M. xanthus* cells were agitated vigorously with a vortex mixer and the sheared pili precipitated using 100 mM $CaCl_2$ [19]. The precipitate was then analyzed by western immunoblotting using anti-PilA antibody [19]. Lane 1, DK1622 (wild type); lane 2, DK10407 (*pilA*); lane 3, DK10409 (*pilT*); lane 4, SW504 (*difA*).

Figure 3

Tethered *M. xanthus* cells show a jiggling motion that is *pilT* dependent. *M. xanthus* cells were prepared as described in Figure 1. Tethered cells were observed with an inverted microscope and serial digital images taken at 30 sec intervals. These images were then overlaid to show the motion of the tethered cells. **(a–d)** Wild type (DK1622). **(e–h)** DK10409 (*pilT* mutant). (a,e) Time zero (t_0). (b,f) Overlay of images taken at t_0 and after 30 sec. (c,g) Overlay of images taken at t_0, and after 30 and 60 sec. (d,h) Overlay of images taken at t_0, and after 30, 60 and 90 sec.



end-up would retreat closer to the surface and then 'lie down' parallel to the surface (Figure 4a) and move away from the previous attachment site (Figure 4b). Conversely, some gliding cells would 'stand up' on their ends and begin jiggling movements. We also tracked the movement of single cells for a long time and found that these cells could be tethered from either end or move forward from either end, suggesting that any pili-associated movement can occur at either cell end. These observations are consistent with the hypothesis that the motion generated by pili involves the shortening of pilus filaments and that this process occurs from alternating cell ends.

We also followed the movement of *M. xanthus* cells as they went through the transition from being tethered (one end up) to being parallel to the surface. Among 200 cells observed undergoing this transition, every cell moved forward away from the tethered end (as shown in Figure 4b and illustrated in Figure 5). A previous study found that most *M. xanthus* cells are piliated at only one pole even though they can be piliated at both poles [13]. Our observations suggest that pilus assembly and retraction switches from one pole to another and that only one pole is active at one particular time. Based on these findings, it is logical to assume that, when a cell going through the transition from being tethered to being parallel, the active pilus filaments should be switched from the tethered end to the non-tethered end. As we observed that these cells always moved forward away from the tethered ends (Figure 4b), it is very likely that the pili are located in the front of the cell bodies during gliding, unlike flagella that push cells from the rear.

Gliding cells of *M. xanthus* periodically reverse their direction of movement; the control of this reversal process is required for directed motility and chemotaxis [14]. If pilus action were responsible for generating movement during S-motility, reversals of gliding direction should be associated with pilus assembly and retraction switching from one cell pole to another. We tested this hypothesis by analyzing several chemotaxis mutants of *M. xanthus* that exhibit either reduced (*frzE*) or enhanced (*frzD*) reversal frequencies [14–16]. As shown in Table 1, there was a correlation between cellular reversal intervals and tethering times. The average time for cellular reversal of S-motile gliding cells (the ones that

Figure 4

Behavior of tethered *M. xanthus* cells. **(a)** Three tethered *M. xanthus* cells (located in the middle of the images) retreating closer to the surface. The images were observed with a 100× objective lens. The tips of the untethered ends were in focus initially (first frame). In two minutes, the image was out of focus (second frame). The image was back in focus when the objective lens was moved closer to the surface (third frame), indicating the tethered cells had retreated closer to the surface. After two more minutes, the tethered cells had retreated further and were lying down on the surface (fourth frame). **(b)** A tethered cell moving away from the tethered end. Serial digital images were taken at 15 sec intervals and then overlaid to show the process. The cell was initially tethered (first frame), then lay down and moved away (frames 2–4). The untethered end is indicated with an arrowhead. The same behavior was observed with 200 other tethered cells that went through the same transition.

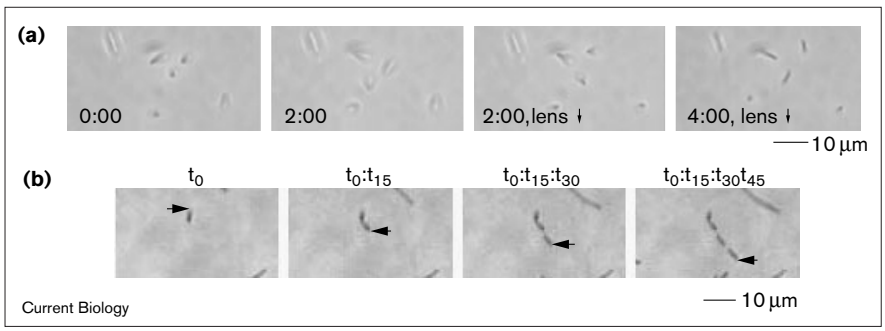
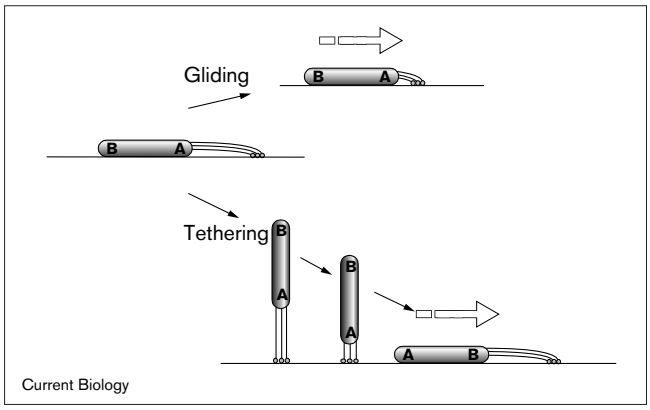


Figure 5



Model of motility mediated by the type IV pilus. The ends of two cells are labeled A and B. See text for explanation.

were moving parallel to the surface) was around 8 minutes and the average tethering time for S-motile cells (the ones that were tethered at one end and perpendicular to the surface) was also around 8 minutes. In the *frzD* mutation background, gliding cells reversed much more frequently (about once every 1.8 minutes), and the average tethering time of the *frzD* mutants was similar. In the *frzE* background, gliding cells rarely reversed and the tethered *frzE* cells remained tethered for over 60 minutes. The association between the control of directional movements and type IV pilus localization and action supports a role for the *frz* chemosensory system in controlling pilus-mediated S-motility in *M. xanthus*.

Table 1

Correlation between reversal interval and tethering time.

Genotype	Reversal interval on solid agar (min)	Tethering time in 1% methylcellulose (min)
frz$^+$	7.89 ± 5.32	7.97 ± 4.23
frzD	1.81 ± 0.47	1.56 ± 0.56
frzE	> 60 min	> 60 min

M. xanthus cells were placed on an agar surface [12] or in polystyrene culture dishes as described in Figure 1 and then analyzed by video microscopy. The reversal interval is defined as the interval between the time when a cell just finishes one reversal to the time when it begins the next reversal. It was determined by following the movement of many cells over many hours on an agar surface. The tethering time is defined as the interval between the time when a cell initially stands up to the time when it lies down. It was determined by following the length of time cells were observed to be perpendicular to the substrate in 1% methylcellulose. The data presented are the averages of 50 cells studied. The strains used in this experiment contained a *difA* allele, which was introduced into the cells by Mx4-mediated generalized transduction. This was done to increase the percentage of tethered cells (see Figure 2). The strains used were: SW504 (*frz*$^+$, *difA*), SW522 (*frzD*, *difA*) and SW520 (*frzE*, *difA*). Similar results were observed with strains lacking the *difA* mutation (data not shown). Analysis of variance showed that the differences between *frz*$^+$, *frzD* and *frzE* were statistically significant and that there was a correlation between reversal interval on solid surface and tethering time in 1% methylcellulose.

We propose the following model to explain pili-mediated S-motility in *M. xanthus* (see Figure 5). We suggest that the pilus filaments (or some adhesins associated with the ends of the pili) bind the pili to a solid surface and that force is generated by pilus retraction. As pili are thought to be located in front of the cell body during movement, and cells lie down following tethering, we suggest that a gliding bacterium may extrude the pilus filaments forward, allowing it to attach to a solid surface, then retracts the pili filaments to move closer to the adherence site(s). Most interestingly, our study showed that cellular reversals may involve switching active pili bundles from one cell pole to another and that this process is controlled by the *frz* signal transduction system.

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