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Gliding Mutants of *Myxococcus xanthus* with High Reversal Frequencies and Small Displacements

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Myxococcus xanthus **cells move on a solid surface by gliding motility. Several genes required for gliding motility have been identified, including those of the A- and S-motility systems as well as the *mgl* and *frz* genes. However, the cellular defects in gliding movement in many of these mutants were unknown. We conducted quantitative, high-resolution single-cell motility assays and found that mutants defective in *mglAB* or in *cglB*, an A-motility gene, reversed the direction of gliding at frequencies which were more than 1 order of magnitude higher than that of wild type cells (2.9 min^{-1} for Δ*mglAB* mutants and 2.7 min^{-1} for *cglB* mutants, compared to 0.17 min^{-1} for wild-type cells). The average gliding speed of Δ*mglAB* mutant cells was 40% of that of wild-type cells (on average 1.9 μm/min for Δ*mglAB* mutants, compared to 4.4 μm/min for wild-type cells). The *mglA*-dependent reversals and gliding speeds were dependent on the level of intracellular MglA protein: *mglB* mutant cells, which contain only 15 to 20% of the wild-type level of MglA protein, glided with an average reversal frequency of about 1.8 min^{-1} and an average speed of 2.6 μm/min. These values range between those exhibited by wild-type cells and by Δ*mglAB* mutant cells. Epistasis analysis of *frz* mutants, which are defective in aggregation and in single-cell reversals, showed that a *frzD* mutation, but not a *frzE* mutation, partially suppressed the *mglA* phenotype. In contrast to *mgl* mutants, *cglB* mutant cells were able to move with wild-type speeds only when in close proximity to each other. However, under those conditions, these mutant cells were found to glide less often with those speeds. By analyzing double mutants, the high reversing movements and gliding speeds of *cglB* cells were found to be strictly dependent on type IV pili, encoded by S-motility genes, whereas the high-reversal pattern of *mglAB* cells was only partially reduced by a *pilR* mutation. These results suggest that the MglA protein is required for both control of reversal frequency and gliding speed and that in the absence of A motility, type IV pilus-dependent cell movement includes reversals at high frequency. Furthermore, *mglAB* mutants behave as if they were severely defective in A motility but only partially defective in S motility.**

Many bacterial species spread over surfaces as expanding swarms (1, 3, 9, 18). The swarming behavior of *Myxococcus xanthus* is due to gliding motility of individual cells; *M. xanthus* cells carry no flagella and cannot swim. Gliding movement consists of translocation along the cell's long axis on a solid surface with one or the other pole leading the way. Swarms of *M. xanthus* expand by coordinating the gliding movements of individual cells (15, 18). Two multigene systems, the A- and S-motility systems, and the *mgl* and *frz* genes have been identified as affecting the ability of vegetative colonies to swarm on agar plates (2, 12, 13, 27).

Among the *M. xanthus* motility mutants isolated so far, *mglA* mutants have the strongest defect in that colonies are rendered nonswarming by a mutation in a single gene (11, 12); their colonies are heaped in the center and have a sharp edge that expands outward due only to cell growth and division. Because the colony shape of *mgl* mutants is indistinguishable from that of A^- S^- double mutants, the *mgl* gene is considered to be required for both A motility and S motility (13). These observations had initially suggested that the MglA protein might be part of the gliding motor (22), but the protein was localized to the cytoplasm and the predicted amino acid sequence resembles those of GDP/GTP binding proteins of the $p21^{Ras}$ type (6, 7). Therefore, it appears to be more likely that the MglA protein is involved in regulating the gliding motor. The *mglA* gene is cotranscribed with an adjacent gene called *mglB*. Deletion of the *mglB* gene reduces the level of *mglA* protein to about 15% of the wild-type level (8). The swarming behavior of *mglB* mutants is intermediate between those of *mglA* mutants and the wild type; *mglB* mutants spread at about 25% of the rate of wild-type swarms and 10 times faster than *mglA* mutant swarms (8).

Wild-type swarming of *M. xanthus* requires both A motility and S motility (13, 15). Previous studies showed that mutants defective in the A-motility system were impaired in swarming, and single cells were found to be unable to glide as individual isolated cells (12, 19b). Molecular analysis of one of the A-motility genes, the *cglB* gene, showed that this gene encodes a lipoprotein with an unusually high cysteine content (19b). All motility retained in this and other A^- mutants is considered to be S motility, because A^- S^- double mutants do not swarm at all (12). Recently, Wu and Kaiser (23–26) discovered that all of the S-motility genes in the *sgl*I region of *M. xanthus* encode proteins required for the synthesis, export, assembly, and regulation of type IV pili. How type IV pili produce S motility is unknown; however, these pili are also required for twitching motility (3, 10).

The *M. xanthus frz* genes were first identified in mutants with an altered aggregation behavior during fruiting-body development (27). The predicted amino acid sequences of the Frz proteins exhibit extensive homology to those of proteins of two-component signal transduction systems, specifically those involved in chemotaxis (*che* genes) in a variety of prokaryotes

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TABLE 1. *M. xanthus* strains used

Strain	Relevant genotype	Motility phenotype or colony morphology	Reference or construction
DK1622	*mgl*$^+$ *frz*$^+$	A$^+$ S$^+$	15
DK3163	*pilR*::Tn*5*(Ω3163)	A$^+$ S$^-$	19a
DK3164	*pilR*::Tn*5*-132(Ω3163)	A$^+$ S$^-$	19a
DK6204	Δ*mglAB*	Nonswarming	7
DK6206	Δ*mglB*	Reduced swarming	8
DK9711	*frzE226*::Tn*5*	"Frizzy"	Mx8 (DZ3377) × DK1622, selected Km^r
DK9712	*frzCD224*::Tn*5*	Smooth edge	Mx8 (DZ4033) × DK1622, selected Km^r
DK9713	Δ*mglAB frzE226*::Tn*5*	Nonswarming	Mx8 (DZ3377) × DK6204, selected Km^r
DK9714	Δ*mglAB frzCD*224::Tn*5*	Nonswarming	Mx8 (DZ4033) × DK6204, selected Km^r
DK9715	Δ*mglAB pilR*::Tn*5*(Ω3163)	Nonswarming	Mx8 (DK3163) × DK6204, selected Km^r
DK9716	*cglB*::Tn*5phoA pilR*::Tn*5*-132(Ω3164)	A$^-$ S$^-$	Mx8 (DK3164) × JZ315, selected Tet^r
DK9717	*cglB*::Tn*5phoA frzE*::Tn*5*(Ω234)	A$^-$ S$^+$	Mx8 (DZ4040) × JZ315, selected Tet^r
DZ3377	*frzE226*::Tn*5 sglA*	"Frizzy"	2
DZ4033	*frzCD224*::Tn*5 sglA*	Smooth edge	2
DZ4040	*frzE*::Tn*5*(Ω234) *sglA*	"Frizzy"	2
JZ315	*cglB*::Tn*5phoA*	A$^-$ S$^+$	16

and in control of twitching motility in *Pseudomonas aeruginosa* (5, 19). Single isolated cells of *frz* mutants are defective in reversing the direction of gliding. With the exception of a *frzD* mutant, *frz* mutants (*frzA*, *-B*, *-C*, *-E*, and *-F*) reverse less frequently than wild-type cells (2). A transposon insertion in the *frzD* region of the *frzCD* gene generated a mutant where the cells reverse more often than wild-type cells (2).

During our motility studies of *mgl* and *cglB* mutants, we recognized that both motility mutants reverse the direction of movement more often than the wild type. We then investigated systematically the movement of individual cells with high optical and temporal resolutions (21). With the assay employed, displacements of as low as 0.03 μm and translocation speeds of as low as 1 μm/min were detectable. Movements of single cells of *mgl*, *cglB*, *frz*, and various double mutants were quantified to examine the relationship between the A- and S-motility genes and the *mgl* and *frz* genes in controlling single-cell motility.

MATERIALS AND METHODS

Bacteria and growth. *M. xanthus* (14) DK1622 and its motility mutant strains were routinely grown on CTT agar plates (11). To assay gliding motility, single colonies were picked, transferred to 5 ml of CTT broth in 50-ml flasks, and grown at 32°C. Cultures were aerated by rotary shaking at 200 rpm. Strains DK9711 (*frzE*226::Tn*5*) and DK9713 (Δ*mglAB frzE*226::Tn*5*) were constructed by Mx8 transduction of kanamycin resistance from strain DZ3377 (*frzE*226::Tn*5*) to DK1622 (*frz*$^+$) and to DK6204 (*frz*$^+$ Δ*mglAB*), respectively. Strains DK9712 (*frzCD*224::Tn*5*) and DK9714 (Δ*mglAB frzCD*224::Tn*5*) were constructed by Mx8 transduction of kanamycin resistance from strain DZ4033 (*frzCD*224::Tn*5*) to DK1622 and to DK6204, respectively. Strain DK9715 was constructed by Mx8 transduction of kanamycin resistance from strain DK3163 [*pilR*::Tn*5*(Ω3163)] to DK6204. Strains DK9716 and DK9717 were constructed by Mx8 transduction of tetracycline resistance from strains DK3164 [*pilR*::Tn*5*-132(Ω3164)] and DZ4040 [*frzE*::Tn*5*(Ω234)], respectively, to strain JZ315. Strains DZ3377, DZ4033, and DZ4040 were kindly provided by D. Zusman, and strain JZ315 was kindly provided by J. Zissler (2, 16, 19). The *M. xanthus* strains used and their relevant genotypes are summarized in Table 1.

Recording and quantification of cell movement by video microscopy. From cultures grown to 80 to 100 Klett density units (4×10^8 to 5×10^8 cells/ml), 10-μl aliquots were withdrawn and spotted on CTT plates (1.5% agar) which had been prepared the day prior to use (21). The droplets were allowed to dry onto the agar for 10 to 15 min and were immediately examined by microscopy. Recording and analyzing of cell movement were conducted by methods described previously (21).

Swarming motility under starvation conditions. *M. xanthus* DK1622, DK9712, DK6204, and DK9714 were grown in CTT broth to a density of 100 Klett units (5×10^8 cells/ml) and concentrated 10 times. Ten-microliter aliquots were spotted on TPM plates (17) containing 0.6% agar and incubated for 24 h at 32°C in the dark. Plates were prepared the day before use.

RESULTS

M. xanthus cells move on solid surfaces by gliding (4, 18, 21). Occasionally, cells reverse the direction of movement, so the leading end of the cell becomes the lagging end. During our video microscopic studies of *M. xanthus*, we noticed that cells of Δ*mglAB* mutants and of A-motility (*cglB*) mutants reversed their direction of gliding more often than wild-type cells. We therefore first studied gliding of *M. xanthus* wild-type cells with respect to reversals and gliding speed in both directions.

Reversals in wild-type cells. The gliding speed of *M. xanthus* wild-type cells was measured, using a motility assay with high optical and temporal resolutions (21). *M. xanthus* wild-type cells (DK1622) that executed at least one reversal during the period of observation were clocked in both their forward and backward directions, and gliding speeds were measured for each. Figure 1 displays the average speed in each direction for 10 cells. No significant difference in gliding speed was evident despite the fourfold variation in average speed among these cells, implying that individual *M. xanthus* cells have no kinetically preferred gliding direction. Speed variations between *M. xanthus* cells are common, and the observed speeds fall within the normal range (21).

A reversal of gliding in a single *M. xanthus* cell is characterized as the following sequence: movement in the forward direction, stop, and movement in the backward direction. The transition from gliding in one direction to gliding in the reverse direction is observed as a transient decrease in gliding speed to less than 1 μm/min (the minimum speed that is measurable [21]) before a cell resumes movement in the opposite direction. During our studies on the reversal behavior, we noticed that gliding movements which are interrupted by stops can be grouped into three classes: (i) forward movement, stop for less than 9 s, and backward movement (we considered this sequence a reversal); (ii) forward movement, stop for more than 9 s, and forward movement (we called this sequence a pause); and (iii) forward movement, stop for more than 9 s, and backward movement. In this study, we analyzed only the first type of reversals.

How often do wild-type cells reverse the direction of gliding? The time interval between two reversals in DK1622 cells was found to be variable. Of 250 cells that all moved during the observation period (Table 2), 13 were found to reverse once or more than once within a period of 20 min. Those cells that reversed performed on average 0.17 reversal per min (Table 2).

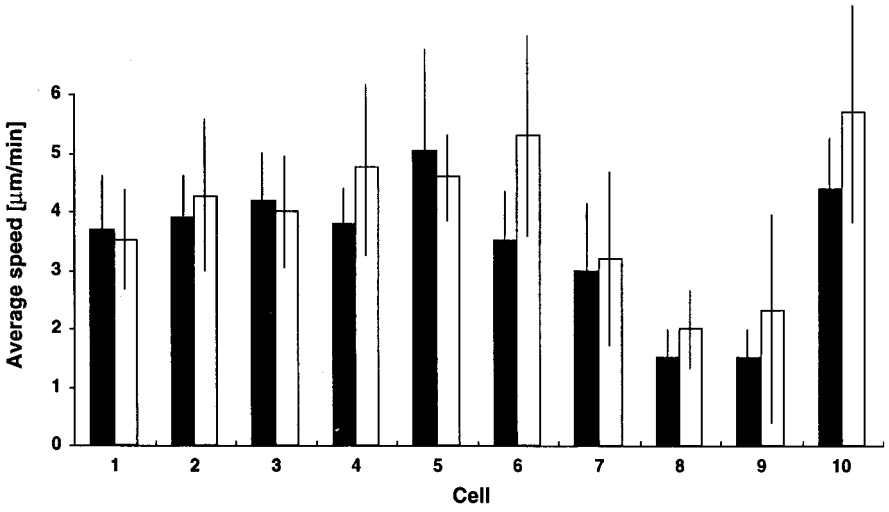

FIG. 1. Forward (solid bars) and backward (open bars) translocation velocities of individual DK1622 cells. Ten cells were measured when moving in the forward and backward directions. For each cell all speed values from each direction were pooled, and the average speed and standard deviation were calculated.

In an independent experiment, 5 of 90 wild-type cells reversed once or more than once within 10 min. Monitoring cells for longer than 20 min proved to be difficult because cells would often associate with other cells in a group and the cell being tracked could not be identified within the group. Since the longer reversal times were difficult to measure, the true average reversal frequency for wild-type cells may be less than the 0.17 reversal per min recorded.

Reversals in *mgl* mutant cells. To investigate the function of the MglA protein in gliding, movement of individual Δ*mglAB* motility mutant cells was quantified. During a 20-min interval, 696 of 792 Δ*mglAB* cells moved: only 12% failed to move. Figure 2 displays the velocity profile of one cell. As illustrated, the movement pattern of this particular Δ*mglAB* cell was characterized by frequent reversals and abrupt, often jerky changes in gliding speed (at $t = 1$ to 140 s and 200 to 310 s) which alternated with intervals where no significant movement was detectable ($t = 140$ to 160 s and 310 to 400 s). During the total observation time of about 474 s, the cell shown in Fig. 2 completed 14 reversals. The net displacements performed were less than 1 μm in 9 min. Figure 3 summarizes the speed measurements for 50 Δ*mglAB* cells. The average speed of an actively gliding Δ*mglAB* mutant cell was 1.9 ± 1.1μm/min. Figure 3 also indicates that 43% of the 2,927 recorded speed values were below 1.0 μm/min, which is the lower limit of reliable measurement (21). Reversal frequencies for 50 Δ*mglAB* mutant cells were quantified. An average reversal frequency of 2.9 min^{-1} (standard deviation, 1.4 min^{-1}) was calculated from 439 reversals (Table 2), indicating that the reversal frequency of Δ*mglAB* mutant cells is at least 1 order of magnitude higher than that of DK1622 cells (Table 2). Cells of Δ*mglAB* mutants traveled on average 0.3 ± 0.4 μm before reversing their directions. These data are compatible with the time-lapse photographs of *mglA* mutant cells published by Hodgkin and Kaiser (13), who reported no net movement over a period of 3 h.

The *mglA* gene is cotranscribed with another gene, called *mglB*. Cells of a Δ*mglB* mutant strain contain only 15% of the wild-type level of the MglA protein (8). This reduction in MglA protein level is not due to transcriptional control. In contrast to *mglA* point mutants and *mglAB* deletion mutants, some swarming of *mglB* colonies is observed on a 1.5% agar surface. However, the swarm expansion rate is one-quarter of that of the wild type (8). We examined 412 individual Δ*mglB* mutant cells by video microscopy. More than 98% of these cells moved during a 20-min time interval. The gliding profile of a single Δ*mglB* mutant cell is displayed in Fig. 4. In contrast to the Δ*mglAB* mutant cell (Fig. 2), this Δ*mglB* cell moved during the entire period of observation. The cell maintained movement in one direction for up to 160 s before reversing. Gliding speeds for 50 Δ*mglB* mutant cells were determined and are represented in Fig. 3. Their average speed is 2.6 ± 1.5 μm/min. Of the 11,169 speed values obtained, 6% were below the minimal detectable speed, indicating that a Δ*mglB* cell is actively gliding for 94% of the time. A particular cell (Fig. 4)

TABLE 2. Reversal frequencies of wild-type *M. xanthus* and *mgl* and *frz* motility mutants

Parameter	Strain:						
	DK1622 (wild type)	DK6204 (Δ*mglAB*)	DK6206 (Δ*mglB*)	DK9712 (*frzD*)	DK9711 (*frzE*)	DK9713 (Δ*mglAB frzE*)	DK9714 (Δ*mglAB frzD*)
Average reversal frequency (min^{-1}) (SD)	0.17 (ND[a])	2.9 (1.4)	1.8 (1.3)	1.5 (1.1)	<0.02 (NA[b])	2.7 (1.4)	3.3 (1.1)
No. of reversals counted	18	439	393	140	0	222	56
Average speed (μm/min) (SD)[c]	4.4 (2.2)	1.9 (1.1)	2.6 (1.5)	4.0 (2.3)	4.3 (2.0)	2.1 (1.4)	1.9 (1.0)
% of cells exhibiting active movement (no. of cells evaluated)	100 (250)	88 (792)	99 (412)	100 (265)	10 (272)	85 (324)	ND

[a] ND, not determined.
[b] NA, not applicable.
[c] For *frzD* mutant cells 3,187 speed values were collected, for *frzE* mutant cells 1,004 speed values were collected, and for Δ*mglAB frzE* double-mutant cells 2,927 speed values were collected. See the text for further description and details.

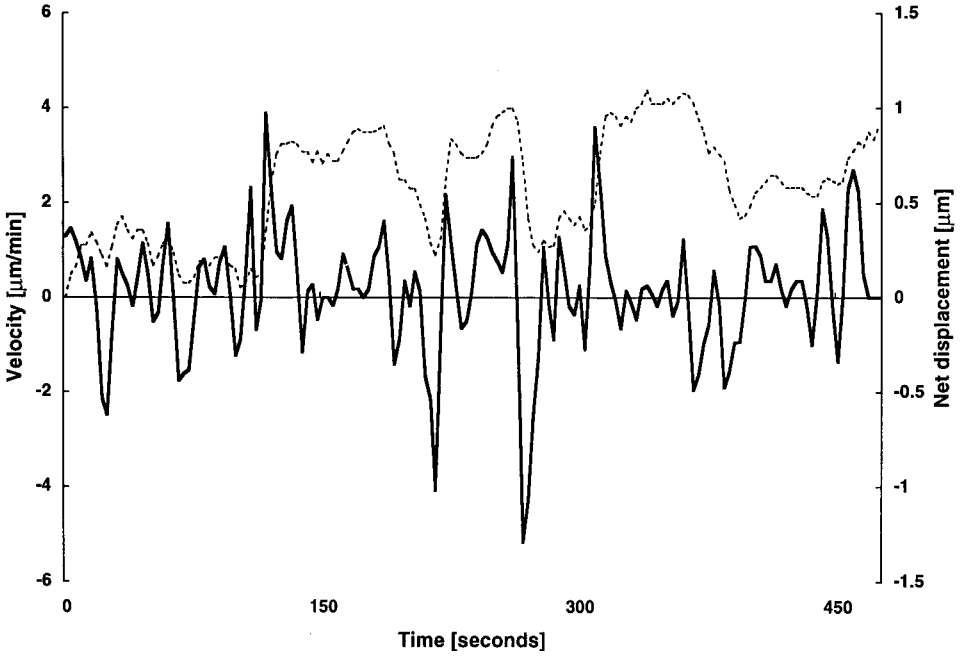

FIG. 2. Velocity profile of an *M. xanthus* Δ*mglAB* cell. Strain DK6204 was grown to a density of 5×10^8 cells/ml in CTT broth. Ten microliters was spotted on a 1-day-old CTT plate (1.5% agar). The lagging end of the cell was tracked for 474 s. Velocity (solid line) and net displacement (dashed line) are displayed. The cell reversed the direction of movement 14 times, at t = 15, 30, 63, 75, 96, 102, 192, 222, 261, 288, 363, 399, 444, and 453 s. Reversal frequencies were calculated from intervals t = 15 to 30, 63 to 75, 96 to 102, 222 to 261, and 261 to 288 s.

accomplished four reversals in 474 s. Reversals for 50 Δ*mglB* mutant cells were quantified, and of 393 reversals counted, the average value was calculated to be 1.8 reversals per min with a standard deviation of 1.3 (Table 2). To test whether the difference in the average reversal frequencies of *mglAB* and *mglB* mutant cells is statistically significant, the two distributions were subjected to a paired Student t test. Taking each measurement as being independent, the probability that the two distributions were derived from one and the same distribution and appeared to be different by chance was found to be less than 10^{-17}. Although the mean values are close (2.9 and 1.8 min^{-1}, respectively), the average reversal frequencies are significantly different. Thus, a decrease in the level of *mglA* protein is reflected by an increase in the frequency of reversal.

Reversals in A$^-$ mutant cells. Colonies of mutants defective in the A-motility system exhibit sharp edges, and no single cells are detectable at the perimeter (12, 19b). Because no *agl* null mutant is available, single-cell analyses were conducted with strain JZ315, which carries a Tn*5pho*A insertion in the *cglB* gene (16). Cells of this strain and *agl* mutant strains are nonmotile when separated from each other by more than 2 μm (12, 19b). However, when in close proximity to each other (<2

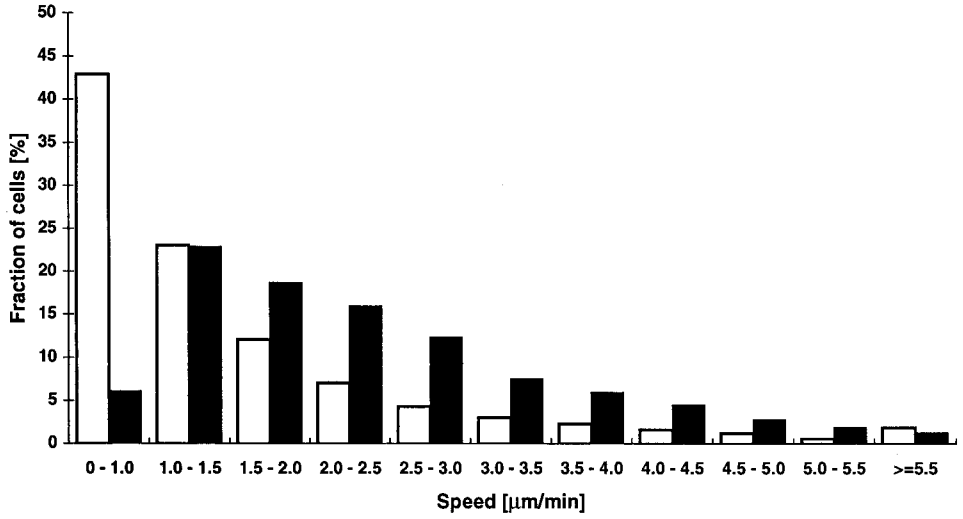

FIG. 3. Distribution of gliding speeds of Δ*mglAB* (DK6204) and Δ*mglB* (DK6206) cells. Gliding speeds from 50 Δ*mglAB* cells (open bars) and 50 Δ*mglB* cells (solid bars) were measured. The speed values for each strain were grouped, and the number of speed values qualifying for each category was determined. For Δ*mglAB* cells 2,927 values were collected, and for Δ*mglB* cells 11,169 values were collected.

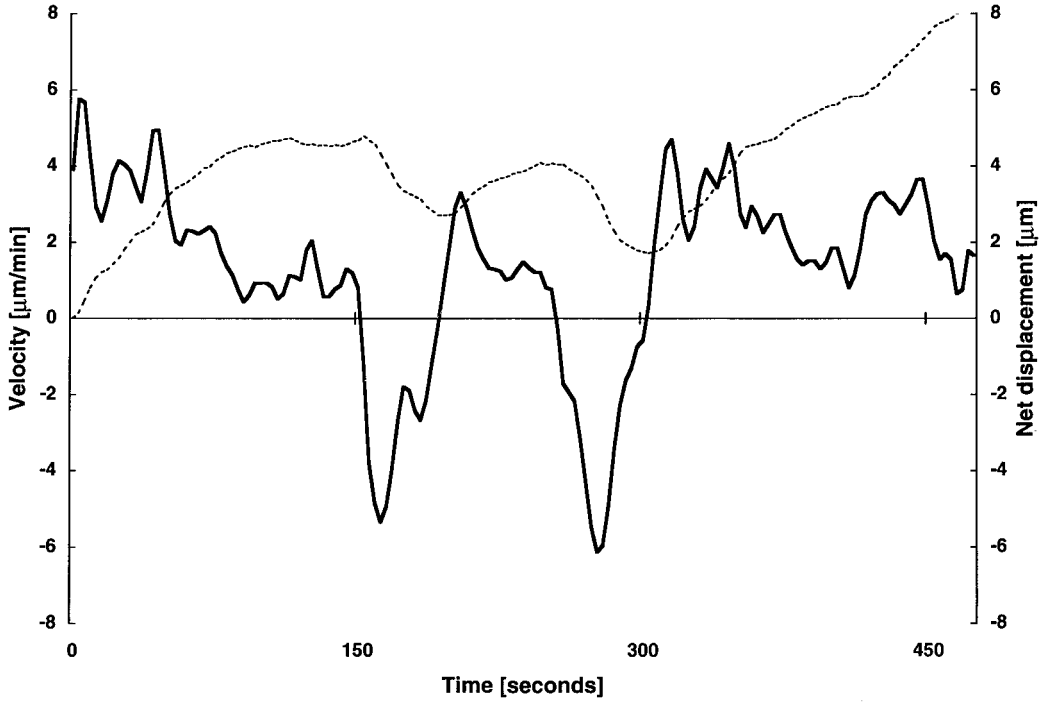

FIG. 4. Velocity profile of an *M. xanthus* Δ*mglB* cell. Strain DK6206 was grown to a density of 5×10^8 cells/ml in CTT broth. Ten microliters was spotted on a 1-day-old CTT plate (1.5% agar). The lagging end of the cell was followed for 474 s. The solid line indicates velocity, and the dashed line indicates net displacement. The cell conducted four reversals (at t = 150, 195, 255, and 303 s).

μm), *cglB* mutant cells showed active movements, presumably reflecting their S motility. These movements included an increased and highly variable pattern of cell reversals. The patterns are illustrated in the velocity profiles of three individual cells (Fig. 5). During the approximately 200-s observation period, the cell whose pattern is shown in Fig. 5A performed abrupt movements that included 15 reversals. If movement of *cglB* cells included such frequent reversals and if most of the runs in one direction lasted for 30 s or less, then we considered these cells to be moving in a high-reversal mode. More than half (54%) of 50 cells that were analyzed in detail moved in the high-reversal mode as illustrated in Fig. 5A. Considering reversals of 50 cells in a high-reversal mode, the average number of reversals was 2.7 (±1.4) min^{-1} (Table 3). Another one-third of the *cglB* cells investigated moved in a pattern that is characterized by the absence of reversals, such as is shown in Fig. 5B. This cell translocated at high speed for a period of up to 150 s without a reversal. This fast forward movement was not smooth but was subject to abrupt changes in translocation speed; after the first reversal, the speed fluctuated between the following approximated values (micrometers per minute): 8, 2, 6, 4, 11, 8, 12, and 8. The apparent bimodal movement pattern (high reversal [Fig. 5A] or low reversal [Fig. 5B]) of *cglB* cells is not due to two subpopulations in the culture, because the same cell can move in both modes, as shown in Fig. 5C. About 16% of the cells investigated were able to move in a pattern where periods of high reversals (Fig. 5C, 30 to 100 s) alternated with intervals of unidirectional, relatively fast movement (Fig. 5C, 100 to 170 s). Thus, for *cglB* mutant cells in close proximity to each other, each cell is able to move in either a high-reversal or a low-reversal mode, with a higher probability of moving in the former mode. A correlation between a particular reversal mode and the cell-to-cell distance or the cell orientation could not be addressed, because these movements were observed for cells in contact with each other.

We examined the average translocation speed of *cglB* cells when in close proximity. For 2,621 speed measurements obtained from 50 cells, the average speed of high-reversal cells was determined to be 2.5 (±1.7) μm/min (Table 3). This value is below the average speed of 5.0 μm/min for wild-type cells when close to each other (21). However, for low-reversal cells the average velocity was 4.7 (±3.0) μm/min, and those *cglB* cells were able to glide with the same high velocities as wild-type cells (Fig. 6). Both wild-type and *cglB* cells utilize the same high speeds at approximately the same frequency (Fig. 6, speed ranges of >4 μm/min). One noticeable difference is that *cglB* cells moved significantly more often at speeds of 1 to 2 μm/min. Because only a small fraction of these cells were conducting unidirectional, high-speed movements and because the average speed value integrates all speed measurements, this average value does not reflect adequately the ability of A^- cells to move with wild-type speeds in close proximity.

Reversals in double-mutant cells containing *frz* mutations. Individual cells of *M. xanthus* mutants defective in the *frz* genes had previously been described to be altered in control of reversal frequency (2). However, the phenotype of a *frz* deletion mutant is opposite from that of a Δ*mgl* mutant; most *frz* mutants have a decreased rather than increased reversal frequency (2) (Table 2). Under our experimental conditions, a *frzE* mutant (DK9711) moved with an average speed of 4.3 ± 2.0 μm/min and an average reversal frequency of <0.02 reversal per min (Table 2).

One exception to the pattern of decreased reversal frequency of *frz* mutants is a Tn*5* insertion in the 3′ end of the *frzCD* gene. Such a *frzD* mutant cell (strain DK9712) glides on average with a speed of 4.0 ± 2.3 μm/min and performs reversals at a rate of 1.5 ± 1.1 per min. This average reversal frequency is comparable to the value determined earlier by Blackhart and Zusman (2). To investigate the possibility of a connection between the *frz*-dependent and the *mgl*-dependent

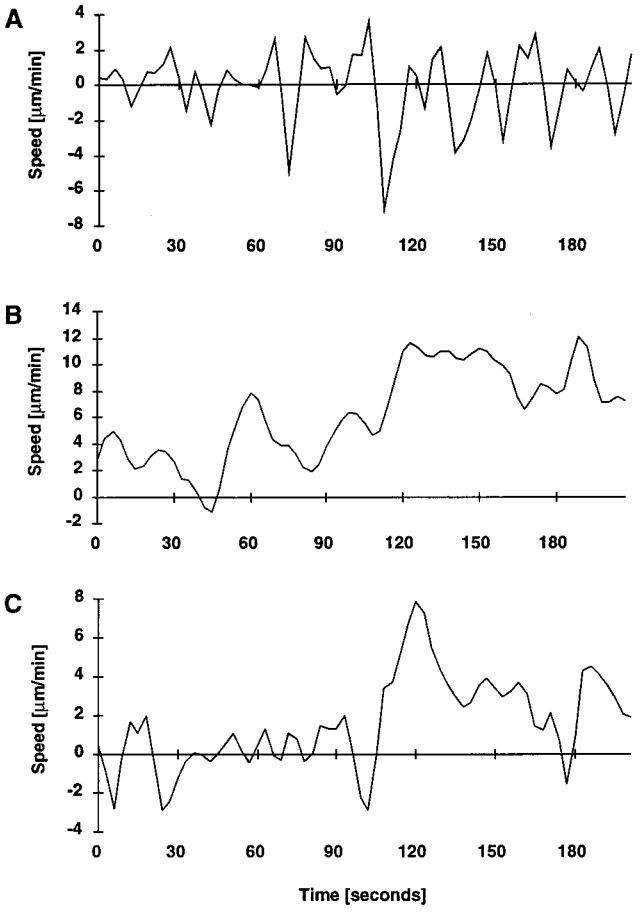

FIG. 5. Movement patterns of *cglB* cells (JZ315) [A^- S^+]) when in close proximity to each other. Panels A, B, and C show results for three different cells. For details, see the text.

control of reversal frequency, two double-mutant strains, a Δ*mglAB frzE* mutant (DK9713) and a Δ*mglAB frzD* mutant (DK9714), were constructed, and the behavior of individual double-mutant cells was examined by high-resolution video microscopy. As shown in Table 2, cells of the Δ*mglAB frzE* double mutant reversed the direction of movement with an average frequency of about 2.7 ± 1.4 min^{-1}. This frequency was very similar to the 2.9 ± 1.4 min^{-1} which was measured for Δ*mglAB* mutants and was clearly different from the frequency found for *frzE* mutant cells (<0.02 min^{-1}) (Table 2). In addition, the average gliding speed of 2.1 ± 1.4 μm/min for Δ*mglAB frzE* double mutants was similar to that for Δ*mglAB* mutants (1.9 ± 1.1 μm/min) but markedly different from that for *frzE* mutant cells (4.3 ± 2.0 μm/min) (Table 2). Single cells of the Δ*mglAB frzD* strain (DK9714) also showed a reversal frequency and average gliding speed that are very similar to those of Δ*mglAB* (Table 2).

We also examined the effect of a *frzE* mutation on the high-reversal phenotype of *cglB* cells by analyzing the single-cell movement of a *cglB frzE* double mutant (Table 3). About 98% of the 240 cells examined were actively moving. Detailed analysis of 50 *cglB frzE* cells revealed that the high-reversal phenotype of *cglB* cells was retained (Table 3). The average number of reversals for the double-mutant cells moving in a high-reversal mode was 2.6 ± 1.4 min^{-1}. Similar to the *cglB* strain, cells were observed to move also in a low-reversal mode with increased speed (3.4 ± 2.8 μm/min [data not shown]). Thus, a *frzE* mutation does not affect the high-reversal behavior of *cglB* cells.

mglA (17) and *frz* (27) are also important for cell movement under starvation conditions that induce fruiting-body development. Swarming motility integrates the gliding movements of individual cells and can be easily detected with the unaided eye. To further investigate the connection between the *frz*-dependent and *mglA*-dependent control of cell movement, the macroscopic swarming behaviors of the *mglA frz* double mutants under growth and starvation conditions were compared. To examine swarming edges under vegetative conditions, CTT plates (1.5% agar) were inoculated with single cells of strains DK1622, DK6204, DK9712, and DK9714 and incubated at 32°C. Swarming edges of single colonies were recorded after 5 days (Fig. 7). To study swarming edges under starvation conditions, cells of these strains were grown in CTT medium, concentrated 10 times, and spotted on petri plates containing TPM starvation medium with 0.6% agar. Edges of swarms were examined after incubation at 32°C for 24 h (Fig. 8). As expected, Δ*mglAB* mutants exhibited a sharp swarming edge (Fig. 7C and 8C) compared to the wild type (Fig. 7A and 8A). Swarms of *frzD* mutants showed an edge containing numerous pronounced flares and projections (Fig. 7B and 8B). Δ*mglAB frzD* double-mutant cells had projections (Fig. 7D and 8D) like those of *frzD* mutant cells (Fig. 7B and 8B), which was qualitatively different from the Δ*mglAB* mutant (Fig. 7C and 8C). The wave-shaped swarming edge of strain DK9714 (Δ*mglAB frzCD*224::Tn*5*), which is clearly different from that of DK6204 (Δ*mglAB*) cells, implies active net cell movement. The swarming pattern of Δ*mglAB frzE* mutant cells under the same conditions was indistinguishable from that of Δ*mglAB* mutant cells; i.e., there was a sharp edge like that in Fig. 7C and 8B

TABLE 3. Reversal frequencies of A^- and S^- motility mutants

Parameter	Strain: JZ315 (*cglB*) (high reversing)[a]	DK9716 (*cglB pilR*)	DK9717 (*cglB frzE*) (high reversing)[a]	DK9715 (Δ*mglAB pilR*)
Average reversal frequency (min^{-1}) (SD)	2.7 (1.4)	0 (NA[c])	2.6 (1.4)	0 [2.0][b] (1.4)
No. of reversals counted	446	0	296	73
Average speed (μm/min) (SD)	2.5 (1.7)	2.0 (1.0)	2.1 (1.1)	1.9 (1.0)
% of cells exhibiting active movement (no. of cells evaluated)[d]	≥98 (373)	~10 (164)	≥98 (240)	30 (310)

[a] Only those cells for which most of the runs between two reversals lasted for 30 s or less were considered. The switching of a single *cglB* cell between the high-reversal and the low-reversal mode is also indicated by the high standard deviation of the average reversal frequency.
[b] Only 30% of the cells moved, and the average reversal frequency of those cells was 2.0 min^{-1}. See the text for more details.
[c] NA, not applicable.
[d] See the text for further description and details.

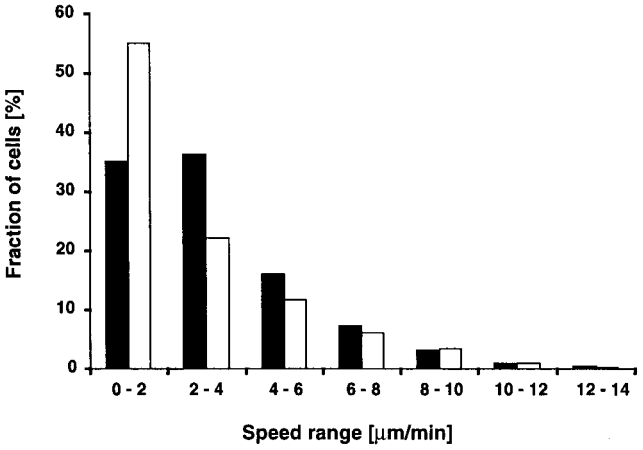

FIG. 6. Distribution of gliding speeds of wild-type (solid bars) and *cglB* (open bars) cells when in close proximity (cell-cell distance of ≤1 μm). The gliding speeds of 50 cells were measured for each strain. Distributions were calculated from 3,446 speed measurements for DK1622 cells and 2,621 measurements for JZ315 cells.

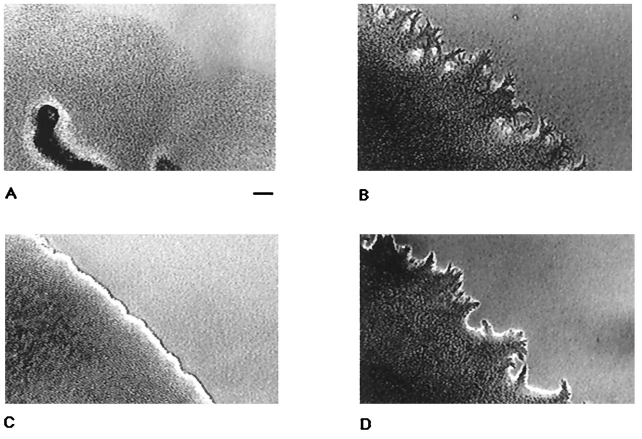

FIG. 8. Swarming patterns of *M. xanthus* motility strains on TPM starvation agar. The agar plates contained 10 mM Tris-HCl (pH 7.6), 1 mM potassium phosphate, 8 mM magnesium sulfate, and 0.6% agar. The plates were prepared on the day prior to use. Cells were grown in CTT broth to a density of 5×10^8 cells/ml and concentrated 10 times, and 10-μl drops were spotted on the agar plates. The plates were incubated at 32°C for 24 h in the dark. (A) DK1622; (B) DK9712; (C) DK6204; (D) DK9714. Bar, 100 μm.

(data not shown). The swarming behavior of the wild type and the Δ*mglAB* and Δ*mglAB frz* mutants on TPM starvation plates containing 1.5% (data not shown) or 0.6% (Fig. 8) agar was observed. The swarming effect of Δ*mglAB frzD* was most pronounced on the support with the lower percentage of agar.

Dependence of cell reversals on S motility. In *M. xanthus*, movement of cells in close proximity is controlled by the S-motility system (12, 15). Recently, Wu and Kaiser (23–26) showed that the S-motility genes in the *sgl*I region encode type IV pili. To examine the effect of S motility on the high-reversal phenotype of *mglAB* mutants, we analyzed 310 cells of an *mglAB pilR* double mutant. In general, the motility of this strain was strongly impaired. Of those cells, only about 30% showed visible movement. In contrast to DK6204, the movement consisted of short translocations, and only 11 of the 20 actively moving cells that were analyzed in detail retained a mode of frequent reversals. In those cells, the average number of reversals was reduced to 2.0 (±1.4) per min (Table 3). This observation indicates that the S-motility defect reduced the extent of motility significantly but only partially affected the high-reversal mode of *mglAB* mutant cells. Interestingly, the average speed of *mglAB pilR* double-mutant cells was 1.9 (±1.0) μm/min, as determined from 1,694 measurements, and thus was similar to speeds of Δ*mglAB* cells.

In order to examine the effect of S motility on the high-reversal pattern of *cglB* mutant cells, we constructed a *cglB pilR* double mutant and analyzed the movement of individual cells by video microscopy. The high-reversal phenotype in these A^- S^- double-mutant cells was completely abolished (Table 3). During the 20-min observation period, 18 of the 164 cells conducted only a short, one-stroke displacement of about 1.2 (±0.9) μm and then stopped. During these short movements, the average speed was 2.0 (±1.0) μm/min (determined from 904 speed measurements). In contrast to the *mglAB pilR* double-mutant phenotype, no reversals were observed. Therefore, type IV pili are required for *cglB* mutant cells to exhibit both the high-reversal mode and the fast movements when in close proximity.

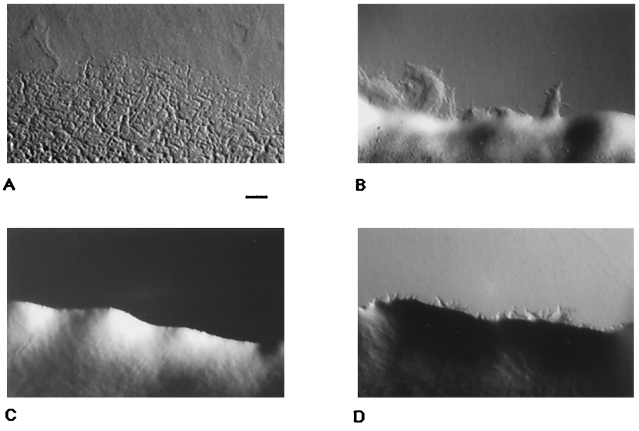

FIG. 7. Swarming patterns of *M. xanthus* motility strains under vegetative conditions on CTT plates (1.5% agar). Cells were streaked on CTT agar plates, which were prepared 1 day before use. The plates were incubated at 32°C for 5 days in the dark. (A) DK1622; (B) DK9712; (C) DK6204; (D) DK9714. Bar, 100 μm.

DISCUSSION

A high-resolution motility assay (21) was used to examined the motility behavior of *mglAB* and *cglB* mutant cells. Quantification of the gliding movement of both strains revealed that individual cells moved in a jerky fashion with a high reversal frequency and a reduced speed compared to DK1622 wild-type cells (Fig. 2 and 5; Table 2). Thus, a decrease in continuous gliding activity, a high reversal frequency, and a reduced speed can explain the absence of net movement observed as the nonswarming phenotype of growing *mgl* colonies (11), as well as the absence of net movement after 3 h (12). We will first elaborate on the phenotype of a *cglB* strain (JZ315) in order to discuss the behavior of *mgl* strains.

S motility in single cells. Recently, it was shown that the *sgl*I locus in *M. xanthus* encodes genes necessary for the structure, export, assembly, function, and regulation of type IV pili (15, 23–26). These extracellular appendages are known to be involved in two types of bacterial surface translocation mechanisms: (i) twitching motility, where cells translocate for a few micrometers by sudden, jerky movements (3, 10), and (ii) S-motility gliding. Currently, details of twitching movement are

unknown, and the mechanistic basis of twitching is obscure. Wild-type cells in close proximity to each other move with high speeds (5.0 ± 2.6 μm/min) compared to speeds of isolated cells (3.8 ± 1.4 μm/min) (21). In the *cglB* strain JZ315 (A^- S^+), movement is detectable only when cells are in close proximity to each other. Cells were observed to translocate either by jerky movement in a high-reversal mode or by unidirectional movement at high, variable velocities. The latter feature is also observed in wild-type cells (Fig. 6; Table 3). Since the *cglB* cells are defective in A motility, both movement patterns are solely due to S motility. Single-cell studies on other A^- S^+ strains, including *agl* mutants, should reveal whether these motility patterns are representative of all A-motility mutants. As determined in preliminary experiments, *cglC* mutant cells (DK1219) exhibited a phenotype similar to the one observed for *cglB* mutants (data not shown).

Considering that movement by S motility depends on type IV pili, it seems plausible that S-motility in *M. xanthus* is related to twitching; both motility forms require type IV pili and are comprised of jerky movements. However, in contrast to twitching, movement by S motility in *M. xanthus* is mostly in the direction of a cell's long axis. S motility is observed only when cells are in close proximity to each other, i.e., when other cells provide part or all of the surface to move on (e.g., during aggregation and fruiting-body formation). It was previously shown that the A- and the S-motility systems provide advantages for *M. xanthus* cells to translocate on different surfaces (20).

Motility defect of *mgl* mutants. It was previously shown that the cellular level of the MglA protein in an *mglB* deletion mutant is reduced to about 15% of the wild-type level (8). Here, we show that the average reversal frequency and the gliding speed in *mgl* mutant cells also correlate with the level of MglA protein; on average, wild-type cells glide with a speed of 4.4 ± 2.2 μm/min and 0.17 reversal per min, Δ*mglB* mutant cells (containing 15% of the wild-type MglA level) glide at 2.6 ± 1.5 μm/min and with 1.8 reversals per min, and Δ*mglAB* mutant cells (containing no MglA) glide at 1.9 ± 1.1 μm/min and with 2.9 reversals/min (Table 2). An alteration in reversal frequency is not necessarily coupled to a change in gliding speed, as is evident from the motility phenotype of *frzD* mutant cells (Table 2). These cells move as isolated cells with about wild-type speed (4.0 ± 2.3 μm/min) but have an increased reversal frequency (1.5 reversals/min). In contrast, Δ*mglB* mutant cells reverse at a frequency (1.8 reversals/min) which is similar to that of *frzD* mutants (1.5 reversals/min) but have a decreased gliding speed (2.6 ± 1.5 μm/min). Thus, *mgl* mutant cells carry two motility defects: one in single-cell gliding speed and one in control of reversal frequency.

In *M. xanthus*, the reversal frequency is controlled not only by *mglA* but also by the *frz* genes. Null mutations in *frz* and *mglAB* have opposite effects; single cells of *frzE*::Tn*5* insertion mutants showed a low number of reversals (<0.02 min^{-1}), whereas cells of Δ*mglAB* mutants exhibited a high number of reversals (2.9 min^{-1}) (Table 2). The single-cell motility assay (Fig. 2, 3, and 4; Table 2) and a swarming assay (Fig. 7 and 8) were used to examine the possible connection between *mgl*-dependent and *frz*-dependent reversals of gliding movements by analyzing *mglAB*, *frz*, and *mgl frz* mutant cells. The observation that *mglAB frzE* double mutants exhibit a high reversal frequency rules out the possibility that reversals in *M. xanthus* are generated independently by MglA and FrzE.

One important question is how a swarming pattern relates to the gliding speed and reversal frequency of single cells. Wild-type cells glide with an average speed of 4.4 μm/min and an average reversal frequency of 0.17 reversal per min (21) (Table 2). The edge of a swarming wild-type colony shows many single cells and cells in loose groups (Fig. 7A). Single cells of *frzD* mutants glide with an average speed of 4.0 μm/min and an average of 1.5 reversals per min (Table 2) (2). This reversal frequency is about 10-fold higher than that of wild-type cells. Edges of growing colonies of *frzD* mutant cells are sharp and coherent, and no single cells are visible (Fig. 7B). Finger-like projections or flares can be observed (Fig. 7B). Colonies of Δ*mglAB* mutants have a similar morphology except that they are smaller and do not have finger-like projections (Fig. 7C). Single-cell analysis of Δ*mglAB* mutant cells revealed a reversal frequency of 2.9 reversals per min, which is about 20-fold above the wild-type reversal frequency. Additionally, the gliding speed of Δ*mglAB* cells was dramatically reduced, to 1.9 μm/min from the 4.4 μm/min for wild-type cells (Table 2). Thus, it seems that a 10-fold increase in reversal frequency to above that found for wild-type cells is sufficient for the formation of a sharp edge of an *M. xanthus* colony. It is also important to notice that reversals in *frz* mutants have been observed for isolated cells, whereas with *cglB* and most *mglAB* mutant cells, reversals were detected for cells in close proximity to each other.

Studies of single-cell movement and of swarming patterns under vegetative and developmental conditions for *mgl*, *frz*, and *mgl frz* mutants revealed an interesting connection between the two sets of genes: the average gliding speed and reversal frequency observed for *mglAB* mutant cells are epistatic in a *frzE* mutant background (Table 2). The motility behavior of *mglAB frzD* double-mutant cells seems to be quite different from that of *mglAB* and *mglAB frzE* mutant cells. Under vegetative (Fig. 7D) and developmental (Fig. 8D) conditions, the flares at the edges of *mglAB frzD* double-mutant swarms resemble those of *frzD* mutants but not those of *mglAB* mutants. Flare formation suggests that movement occurred in *mglAB frzD* cells to an extent that is different from that in *mglAB* cells. Analysis of the average gliding speed and reversal frequency of single *mglAB* and *mglAB frzD* cells did not reveal a significant difference between the two mutant strains (Table 2). Small differences in gliding speed and reversal frequency between different mutant strains may not be detectable by quantitative video microscopy under the conditions employed and may be recognized as a difference in swarming behavior only after prolonged incubation. Flares observed in *mglAB frzD* swarms were noticeable only after incubation for more than 4 days on CTT agar (Fig. 7A). Thus, a *frzD* mutation can partially suppress the S-motility (swarming) defect of *mglAB* mutant cells. Since *mglAB* colonies do not swarm, the suppression of this defect by *frzD* seems to be a gain of function in S motility.

Although colonies of both *mglAB* and A^- S^- mutants exhibit round, sharp edges, the motility patterns of single cells are clearly different (Fig. 2, 5, and 6; Tables 2 and 3). In contrast to A^- S^- cells, Δ*mglAB* cells show a residual movement pattern of high reversals that requires the presence of type IV pili (encoded by S-system genes [Table 3]). Furthermore, a *frzD* mutation partially suppresses the group swarming defect of Δ*mglAB* cells. This effect is more visible under conditions of low percent agar concentration, where *M. xanthus* moves mostly by S motility (20). Comparing the gliding movements of Δ*mglAB* and A^- S^- cells, it seems that the Δ*mglAB* mutant behaves as if it is a strong A-motility system mutant with only partially defective S motility.

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