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"Frizzy" genes of *Myxococcus xanthus* are involved in control of frequency of reversal of gliding motility

(development/aggregation/chemotaxis)

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ABSTRACT ***Myxococcus xanthus*, a Gram-negative bacterium, has a complex life cycle that includes fruiting body formation. Frizzy (*frz*) mutants are unable to aggregate normally, instead forming frizzy filamentous aggregates. We have found that these mutants are defective in the control of cell reversal during gliding motility. Wild-type cells reverse their direction of gliding about every 6.8 min; net movement occurs since the interval between reversals can vary widely. The *frzA–C*, *-E* and *-F* mutants reverse their direction of movement very rarely, about once every 2 hr. These mutants cannot aggregate normally and give rise to frizzy filamentous colonies on fruiting agar or motility agar. In contrast, *frzD* mutants reverse their direction of movement very frequently, about once every 2.2 min; individual cells show little net movement and form smooth-edged "nonmotile" type colonies. Genetic analysis of the *frzD* locus shows that mutations in this locus can be dominant to the wild-type allele and that its gene product(s) must interact with the other *frz* gene products. Our results suggest that the *frz* genes are part of a system responsible for directed movement of this organism.**

Myxococcus xanthus is a Gram-negative rod-shaped bacterium that exhibits a complex life cycle consisting of a vegetative phase and a developmental phase (1–3). During the vegetative phase, cells move as large units called "swarms" or "hunting groups," which prey on other microorganisms or utilize organic substrates in their path. The mechanism of movement used by these bacteria is termed "gliding motility," which is usually described as movement in the direction of the long axis of the cells at a solid–liquid or an air–liquid interface (4). Since bacteria that exhibit gliding motility do not produce flagella or any other obvious motility organelle, several novel mechanisms have been proposed to explain this mechanism of movement (4–7). When *M. xanthus* cells are starved on a solid surface at high cell density, the developmental phase is initiated. Cells glide towards specific sites to form fruiting bodies, large aggregates or raised mounds in which sporulation later occurs. In *M. xanthus*, the mounds of myxospores are called fruiting bodies.

As part of our studies of development in *M. xanthus*, we isolated a large number of mutants that were defective in fruiting body formation (8). One class of nonfruiting mutants were called "frizzy" since these cells, when plated on fruiting agar, formed frizzy filament-like aggregates in contrast to discrete mounds (9). The cells within the filaments sporulated normally. Since nonfruiting mutants of *M. xanthus* show only a limited number of phenotypes (8), we became interested in these relatively infrequent but distinctive aggregation-defective mutants. Transposon Tn*5* insertions linked to a frizzy (*frz*) mutation were isolated (10) and used to map the various mutants in our collection (9). A search through 36 mutants exhibiting the frizzy phenotype showed that all were linked to the same Tn*5* insertion sites. Three-factor cross analysis of 22 of these mutants allowed us to establish the relative positions of the mutations. The region of DNA carrying the *frz* genes was then cloned in *Escherichia coli* by selecting for the kanamycin resistance element present on a Tn*5* insertion linked to the *frz* genes (11). The cloned DNA was analyzed by isolating and characterizing new Tn*5* insertions at frequent intervals within the *M. xanthus* DNA and transducing the mutated DNA into *M. xanthus* for recombinational and complementation analysis (12). These studies identified five or six *frz* complementation groups on 7.5 kilobases (kb) of cloned DNA (see Fig. 1). All of the complementation groups are contiguous except the last, *frzF*, which is separated from the others by 1.4 kb of DNA. The different *frz* loci behave as separate transcriptional units, and mutations in all the loci are recessive, except for those in *frzD*, which are *trans*-dominant (11).

MATERIALS AND METHODS

Cells and Growth Conditions. *M. xanthus* FB (DZF1) is derived from DK101. DZF3390, DZF3387, DZF3319, and DZF3408 are described in ref. 11. DZF1313 is described in ref. 9. DZF3386 contains the Tn*5* insert Ω224. The strain was constructed by transducing pBB224 into DZF1 and screening for a DNA replacement with the Tn*5* as described previously (11). Vegetative cultures were grown on Casitone/yeast extract (CYE) broth (13) and maintained on CYE agar plates. Transduction experiments, DNA purification and analysis, plasmid construction, and bacteriophage P1 transduction experiments were performed as described in ref. 11.

Photo- and Video Microscopy. A liquid culture of the appropriate *M. xanthus* strain was grown overnight to a density of about 5×10^8 cells per ml. The culture was diluted 10- to 20-fold and 3 μl was spotted onto fresh ½ CTT agar (14) in a 50 × 12 mm Petri plate. The drop of cell suspension was then allowed to dry at room temperature. The cell movements were observed and recorded by using a Zeiss microscope attached to a Panasonic video camera and Panasonic time-lapse video recorder. Drying of the agar during the course of the experiments was reduced by the use of a heat filter and by the periodic addition of water to the plate. The photomicroscopy was accomplished using an Olympus phase-contrast microscope with an attached Olympus 35-mm camera. The cells were prepared as described above and photographed at 2-min intervals. The temperature of the room was about 22°C.

RESULTS

Frizzy mutants were originally identified by their aberrant developmental phenotype: On rich media—e.g., CYE agar

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Abbreviation: kb, kilobase(s).
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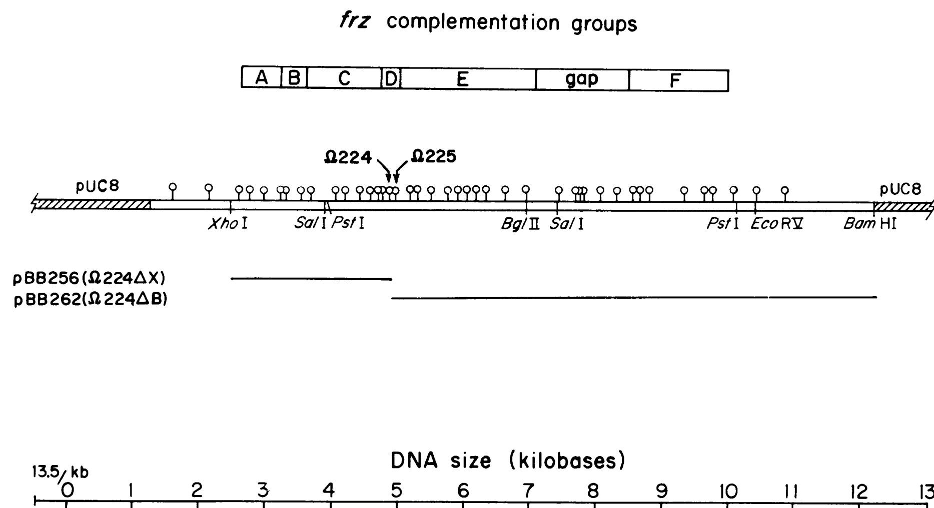

FIG. 1. Physical and genetic map of the *frz* region of the *M. xanthus* chromosome. Circles indicate mutation sites. The physical and complementation data summarized here were published previously (11). The positions of the deletions in plasmids pBB256 and pBB262 are indicated by solid lines.

(13)—the morphology of the colonies appeared to be normal, while on fruiting agar the colony morphology was frizzy. We recently observed that on motility agar (14) (½ CTT), a medium with reduced nutrients that promotes motility but still inhibits fruiting, frizzy mutants showed the frizzy colony phenotype (Fig. 2). Thus, the *frz* gene products are not developmentally specific, although they are necessary for normal developmental aggregation. These results also suggest that the *frz* gene products may be involved in cell motility. We therefore followed the movement of individual cells by time-lapse microscopy. Fig. 3 shows the movement of the parental "wild-type" strain DZF1 (strain FB) and a *frzE* mutant, DZF3390. Careful observation of at least 20 individual cells of both wild-type and mutant strains revealed that both groups moved at the same rate, about 2 μm/min. However, cells of the *frz* strain rarely reversed their direction of movement. The same results were observed for representative strains of the *frzA*, *frzB*, *frzC*, *frzE*, and *frzF* complementation groups. Table 1 shows some quantitative data on directional movements obtained from following 100 cells of DZF1 and 60 cells of DZF3390 (*frzE*) by time-lapse video microscopy. The wild-type strain DZF1 was observed

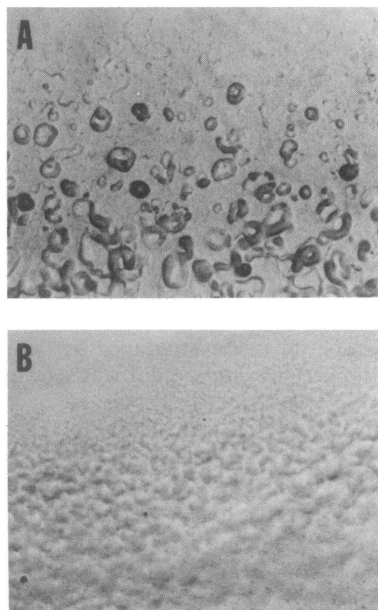

FIG. 2. Morphology of colonies of *M. xanthus* on motility agar. (*A*) Frizzy mutant (*frzE*) (*A*) and wild-type (DZF1) (*B*) were plated on ½ CTT agar and incubated at 30°C for several days. The edges of the colonies were photographed at about ×20.

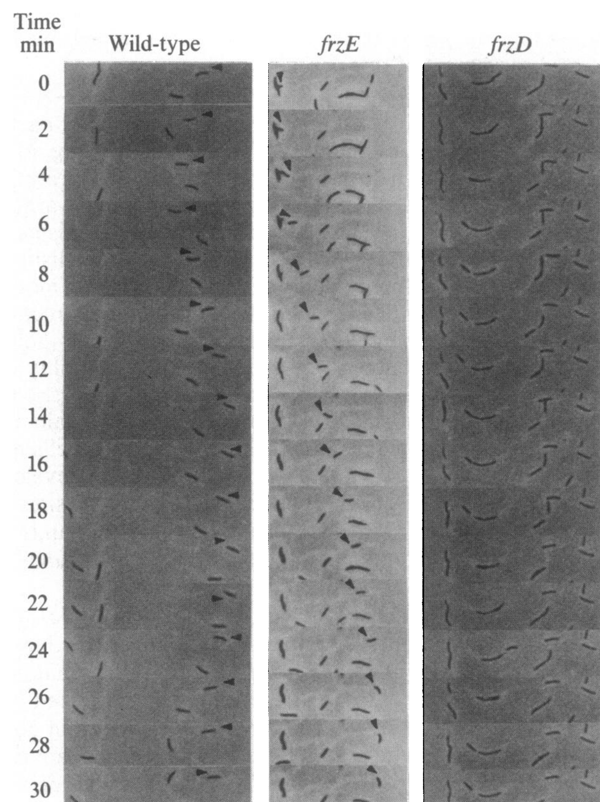

FIG. 3. Pattern of cell movement on motility agar. The wild-type strain, DZF1, a *frzE* mutant, and a *frzD* mutant were plated on ½ CTT motility agar and incubated at 22–24°C. The cells were photographed at 2-min intervals, using a Zeiss phase-contrast microscope. The cells are 4–6 μm in length and 0.5 μm in diameter. The arrowheads indicate direction of movement of selected cells during a 30-min interval; no arrowheads are indicated for the *frzD* mutant because the cells reversed direction so frequently that the 2-min time lapse used in this experiment was too lengthy to show directional changes.

Table 1. Frequency of cell reversal in wild-type and frizzy mutants

Strain	Genotype	Mean observation time per cell, min	No. of cells observed	No. of reversals per cell	Time between reversals, min
DZF1	Wild-type	52	100	8.3 ± 3.2	6.8 ± 2.5
DZF3390	*frzE*	50	60	0.4 ± 0.7	124 ± 77
DZF3386	*frzD*	82	25	38 ± 9.5	2.2 ± 0.3

A liquid culture was grown overnight in CYE broth to about 5×10^8 cells per ml. The cells were then diluted 10- to 20-fold and 3 μl was spotted onto ½ CTT agar in a 50 × 12 mm Petri plate. The drop of cell suspension was then allowed to dry at room temperature, and the cell movements were observed and recorded. Results in the last two columns are presented as mean ± SD.

to reverse its direction about every 6.8 min; in contrast, the *frzE* mutant reversed its direction very infrequently, on the average of about once every 2 hr. Thus mutants of *M. xanthus* that show the frizzy phenotype appear to be defective in the control of the ability of cells to reverse direction. It should be noted that these experiments were performed with strain DZF1 as our wild-type parental strain. DZF1 is known to have a defect in the S-motility system (*sglA*) (15). We therefore repeated most of these experiments with the fully motile strain DZ2 (*sglA*$^+$). This strain gave results very similar to those reported here for DZF1.

Another type of directional control mutant was also found (see Fig. 1). Two Tn*5* insertions about 100 base pairs apart (Ω224 and Ω225) that lie between *frzC* and *frzE* led to a different phenotype than that described for the other *frz* mutants. These *frzD* mutants form smooth-edged colonies on both fruiting medium and motility medium. The colony morphology resembles that of the nonmotile mutants described by Hodgkin and Kaiser (14). However, examination of individual cells by time-lapse microscopy (Fig. 3) showed that they are in fact as motile as the wild-type parental strain (i.e., they moved at about 2 μm/min). However, the *frzD* mutants very frequently reverse their direction of movement; in addition, they showed small variations in the intervals between reversals, resulting in little net movement of individual cells. Table 1 shows the reversal rate for 25 cells followed for 82 min by time-lapse video microscopy. These cells were found to reverse their direction approximately once every 2.2 min compared to 6.8 min for wild type. Thus, like the other *frz* mutants, the *frzD* mutants are defective in the control of cell reversal, but, unlike the other *frz* mutants, they change their direction more frequently than wild type.

The *frzD* mutant alleles are dominant to the wild-type allele (11): merodiploids that contain a wild-type allele and a mutant allele show the *frzD* "nonmotile" colony phenotype. One possible explanation of this dominance is that the Tn*5* insertions in *frzD* cause polypeptide chain termination within the Tn*5*, resulting in a defective *frzD* polypeptide that prevents the wild-type polypeptide from functioning properly. This hypothesis predicts that deleting DNA to one side of the Tn*5* insertion should have no effect because chain termination prevents that DNA from being expressed, whereas deleting DNA to the other side of the transposon should eliminate the dominant "nonmotile" phenotype. We therefore constructed plasmids with deletions between the *Xho* I site and Ω224 (pBB256) and between Ω224 and the *Bam*HI site (pBB262) (Fig. 1). These plasmids were then transduced into *M. xanthus* DZF1, where the phenotype of the kanamycin-resistant transductants could be ascertained (12) (Table 2). Transductants containing pBB256 did not exhibit the "nonmotile" *frzD* phenotype. On the other hand, pBB262 was able to confer the "nonmotile" phenotype on DZF1. These results support our hypothesis that a defective polypeptide (an amber fragment) is responsible for the genetic dominance shown by the *frzD* mutations. These results also indicate that the direction of transcription of *frzD* is from left to right as shown in Fig. 1.

The genetic experiments presented in Table 2 and elsewhere (11) indicate that *frzD* is functionally related to the other *frz* loci, even though the *frzD* mutants isolated do not confer the frizzy phenotype to the cells. For example, when pBB225 (a plasmid containing Ω225 in *frzD*) is transduced into some *frzE* mutant recipients, 17–64% of the transductants are Fru$^+$ (i.e., they have normal motility and colony morphology). This suggests that the defective *frzE* gene product(s) may interact with the *frzD* gene product(s), preventing the dominant "nonmotile" phenotype. Similar results have been obtained when *frzA* Tn*5* insertion mutants were used as recipients (unpublished data). In addition, when pBB262 is present in a strain that is unable to express *frzE*, the colonies appear frizzy rather than "nonmotile" (Table 2). This result supports the hypothesis that the *frzD* gene product(s) interact with the *frzE* gene product(s).

DISCUSSION

A common property of the *frz* mutants is that they are all affected in the frequency with which cells reverse their direction of gliding: the *frzD* mutants reverse direction too frequently, whereas the *frzA–C*, *-E* and *-F* mutants rarely

Table 2. Complementation analysis using deletion plasmids originating within *frzD*

		Frequency (%) of phenotypes among Kan^R transductants					
		pBB256			pBB262		
Strain	Genotype	Fruiting	"Nonmotile"	Frizzy	Fruiting	"Nonmotile"	Frizzy
DZF1	Wild-type	100	0	0	0	100	0
DZF3387	*frzA*	0	0	100	0	100	0
DZF3319	*frzB*	0	0	100	0	100	0
DZF3408	*frzC*	0	0	100	0	100	0
DZF3390	*frzE*	100	0	0	0	0	100
DZF1313	*frzF*	100	0	0	0	100	0

All transductions were performed as described (11, 12). Donor plasmids pBB256 and pBB262 were transduced into wild type (DZF1) and representative mutants of *frzA*, *frzB*, *frzC*, *frzE*, and *frzF*. All the kanamycin-resistant (Kan^R) transductants were merodiploids. Over 200 transductants of each cross were scored for their fruiting and motility phenotype on fruiting agar (CF agar).

reverse direction. These observations suggest that the *frz* gene products are part of the regulatory mechanism controlling the frequency of cell reversals during gliding. What is the function of this regulatory mechanism in *M. xanthus*? We propose that it is part of a chemotactic system, possibly analogous to the systems found in flagellated organisms such as *Escherichia coli*, *Salmonella typhimurium*, and *Caulobacter crescentus* (16, 17). *E. coli* and *S. typhimurium* regulate their chemotaxis activity by changing the direction of flagellar rotation from counterclockwise, which causes directed movement (running), to clockwise, which causes tumbling (16). The net directional movement of the bacterium is then determined by the frequency of tumbling and running in response to a chemotactic gradient. In *C. crescentus*, which contains only a single polar flagellum (17), the reversal of flagellar rotation from counterclockwise to clockwise results in the bacteria swimming in reverse, rather than tumbling. *M. xanthus* DZF1 reverses its direction of gliding about once every 7 min on average. Net movement is observed since the interval between reversals can vary widely. This statement is not strictly correct, since cells can change direction after a reversal. However, *M. xanthus*, unlike flagellated organisms that resume running in a random direction after an episode of tumbling, usually moves within the confines of a preexisting slime trail in an essentially two-dimensional plane. We think that the regulation of cell reversal in *M. xanthus* is therefore extremely important for the directed movement of this organism.

The motility phenotypes of the *frz* mutants are similar to those of some well-characterized mutants of *E. coli* and *C. crescentus*. *frzA–C*, *-E* and *-F* mutations cause *M. xanthus* cells to reverse direction very infrequently. This phenotype is analogous to mutations in *cheA*, *cheW*, *cheY*, *cheR*, and *cheD* of *E. coli* (18) and *cheC* and *cheG* of *C. crescentus* (19). Mutations in these genes cause the cells to swim smoothly for long periods of time because they tumble much less frequently than wild-type cells, or, as in the case of *C. crescentus*, they fail to reverse the direction of swimming. In contrast, mutations in *cheB* or *cheZ* of *E. coli* can cause cells to tumble much more frequently than wild-type cells; this phenotype is analogous to that found in the *frzD* mutants of *M. xanthus*.

Dworkin and Eide (20), in a comprehensive study, were unable to find any chemotactic substances for *M. xanthus*. Furthermore, they raised the question of whether the slow movement of the individual cells is consistent with a sensory system for detecting small molecules, which diffuse at a relatively rapid rate. We think it is possible that *M. xanthus* may chemorespond to macromolecular signals with low rates of diffusion or to cell-bound signals. Thus it is not inconceivable that *M. xanthus* may have a modified chemotactic system adapted to its slow movement within a network of slime trails.

Methyl-accepting chemotaxis proteins (MCPs) are thought to play an important role in the chemotactic response of *E. coli*, *S. typhimurium*, and *C. crescentus* (18, 19). The degree to which these proteins are methylated reflects the presence of chemotactic stimuli in the cell's environment, and these proteins transmit the information to the motility system of the cell. We labeled wild type and representative *frz* mutants with [*methyl*-^{3}H]methionine or *S*-adenosyl[*methyl*-^{3}H]methionine (21) and analyzed cell extracts on sodium dodecyl sulfate/polyacrylamide gels for the presence of possible MCPs in *M. xanthus*. Several methylated proteins were observed. However, none contained alkali-labile methyl groups, and no obvious differences were observed between the wild-type and *frz* mutant strains. It is possible that MCPs are present in *M. xanthus* but are not abundant. We hope that purification of the *frz* gene products will allow us to study the biochemical basis of this motilty control system in *M. xanthus*.

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