Sensory Adaptation during Negative Chemotaxis in *Myxococcus xanthus*

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*Myxococcus xanthus* exhibits many tactic movements that require the frz signal transduction system, such as colony swarming and cellular aggregation during fruiting body formation. Previously we demonstrated that the Frz proteins control the chemotactic movements of *M. xanthus* (W. Shi, T. Köhler, and D. R. Zusman, Mol. Microbiol. 9:601–611, 1993). However it was unclear from that study how chemotaxis might be achieved at the cellular level. In this study, we showed that *M. xanthus* cells not only modulate the reversal frequency of cell movement in response to repellent stimuli but also exhibit sensory adaptation in response to the continuous presence of nonsaturating repellent stimuli. The sensory adaptation behavior requires FrzF (a putative methyltransferase) and is correlated with the methylation-demethylation of FrzCD, a methyl-accepting chemotaxis protein. These results indicate that negative chemotaxis in *M. xanthus* is achieved by chemokinesis plus sensory adaptation in a manner analogous to that of the free-swimming enteric bacteria.

*Myxococcus xanthus* is a gram-negative bacterium which exhibits complex social behaviors (6). The bacteria do not contain flagella and cannot swim in a liquid medium. When cells are placed on a solid surface containing nutrients, they move by gliding motility and swarm in groups to colonize new areas which contain additional nutrients and fewer waste products (repellents). When starved, cells aggregate to specific foci, where they form fruiting bodies. The frz mutants are defective in colony swarming and aggregation during fruiting body formation (2, 10, 18), indicating that the frz signal transduction system plays an important role in these tactic movements. When the frz genes were cloned and sequenced, it was found that most of the frz gene products are homologous to chemotaxis gene products from the enteric bacteria (12, 13, 15). Furthermore, many of the Frz proteins were found to have similar biochemical properties to the homologous enteric chemotaxis proteins (13, 14). For example, FrzCD, which is homologous to the cytoplasmic region of the enteric methyl-accepting chemotaxis proteins (MCPs), is modified by methylation in the presence of attractants and demethylation in the presence of repellents; in addition, FrzE, which is homologous to CheA and CheY, is autophosphorylated in a regulated manner (14). Recently, we devised a spatial chemotaxis assay suitable for slowly moving *M. xanthus* (18). By using this assay, we demonstrated that wild-type *M. xanthus* cells spread into compartments with abundant nutrients like yeast extract or Casitone and avoided compartments with no nutrients or repellents (e.g., short-chain alcohols like isoamyl alcohol). The tactic movements were found to be correlated with the methylation-demethylation of FrzCD and to require the frz genes (except frzG). These results, taken together, strongly suggest that the Frz proteins are chemotaxis proteins in *M. xanthus*. However, little is known about how the chemotactic movements are achieved at the cellular level.

In enteric bacteria, chemotaxis is achieved at the cellular level by chemokinesis plus sensory adaptation (4, 7–9, 16, 17). Addition or removal of attractants or repellents changes the tumbling frequency of cell movement: movement up an attractant gradient is extended because of the reduced probability of tumbling, and movement up a repellent gradient is reduced by the increased probability of tumbling (9, 17). Sensory adaptation is essential for chemotaxis because it allows cells to remember past chemical concentrations and to direct their movement. When starved, cells do not exhibit any adaptation behavior in response to 0.15% isoamyl alcohol, even after 24 h (18). These results raised the question of whether *M. xanthus* could adapt to any stimulus and how a possible failure to adapt might affect our understanding of chemotaxis in this organism. We therefore began a more detailed study of the dynamics of cellular behavior in response to various repellents. By using time-lapse video microscopy to monitor the directional movements of *M. xanthus*, we found that although cells never adapted to very high concentrations of isoamyl alcohol (0.15%), they did exhibit sensory adaptation to lower levels of the repellents (less than 0.1% isoamyl alcohol). This sensory adaptation behavior required FrzF and was correlated with the methylation-demethylation of FrzCD. These studies strongly support the hypothesis that chemotaxis in *M. xanthus* is indeed similar to that of the enteric bacteria and is achieved at the cellular level by modulating cell reversals and sensory adaptation.

**Growth of strains and description of assays used.** The bacterial strains used in this study are listed in Table 1. All of the strains are in the D22 background, which contains the wild-type motility systems (3, 19). Cells were grown at 32°C in CMM medium (5 g of Bacto-Casitone per liter, 1 g of MgCl₂ per liter, 10 mM MOPS [morpholinepropanesulfonic acid] [pH 7.6]). Cell cultures were shaken at about 200 to 225 rpm. Cells
were harvested by centrifugation in a microcentrifuge at 1,500 rpm for 10 min and suspended in MOPS buffer (10 mM MOPS [pH 7.6], 8 mM MgCl$_2$, 10 mM sodium pyruvate). Cells subjected to these conditions were found to be fully motile.

For the study of cellular movements in response to chemicals, time-lapse video microscopy was used, since the gliding motility of *M. xanthus* is too slow to be observed in real time. In a typical experiment, 5 ml of a designated medium with 0.7% agar was added to a Falcon tissue culture dish (60 by 15 mm; Becton Dickinson). We used 0.7% agar because it allows cells to move faster and with less lag than on standard 1.5% agar; in addition, the video images were more stable than those obtained with very soft (0.3%) agar (19). After the agar had solidified, a 10-μl volume of bacterial cells (approximately $5 \times 10^6$ cells) was spotted in the center of the plate. After 20 s, the plates were tilted to let the remaining liquid in the drop drain to the edge of the plate, where it could be removed with filter paper. In this way, the cell suspension drop dried very quickly and the cells were ready to be studied immediately. Bacterial movement was recorded with a time-lapse videocassette recorder (JVC BR-9000U). Movement was recorded at a ratio of 120:1 and played back at normal speed. Cellular behavior was determined by studying the recorded video images. It was noted that a small portion of the resuspended wild-type *M. xanthus* cells were present in clumps which contained from a few hundred cells to tens of thousands of cells adhering to each other (6). We found that it was particularly helpful in the studies of sensory adaptation to focus on the behavior of small cell clumps containing 200 to 1,000 cells and to follow their pattern of cellular dispersal in the presence of various chemical stimuli (see below).

**Sensory adaptation to negative chemical stimuli in *M. xanthus***. Previously, we had found that cells grown in rich medium like CYE (10 g of Bacto-Casitone per liter, 5 g of Bacto yeast extract per liter, 1 g of MgCl$_2$ per liter, 10 mM MOPS [pH 7.6]) had poor motility when they were placed on 1.5% agar. To overcome this problem, we tried varying the media and the agar firmness. We found that cells grown in less nutrient medium like CMM (see above) were immediately motile with no detectable lag if they were placed on intermediate-softness 0.7% agar.

Unstimulated wild-type cells reversed the direction of cell movement about once every 5 to 8 min. When they were placed on an agar plate containing repellents like isooamyl alcohol, the frequency of cell reversal was increased to about once every minute (11, 18). As reported previously (18), cells on plates containing 0.15% isooamyl alcohol continued to reverse the direction of cell movement for a long time without showing adaptation (Table 2). However, when we studied the cellular response to lower concentrations of isooamyl alcohol (e.g., 0.03%), we found that cells initially exhibited very frequent reversals; however, after continued incubation, cells returned to the steady-state rate of reversals (Fig. 1). This demonstrates that *M. xanthus* shows sensory adaptation in response to low levels of repellents.

Isoamyl alcohol and most other repellents are volatile chemicals. We were concerned that the observed “adaptation” behavior might be due to evaporation of the isooamyl alcohol in the agar. We therefore performed the following control experiment. Cells were placed on an agar plate containing 0.03% isooamyl alcohol and incubated for 30 min until adaptation was completed. We then added fresh, unstimulated *M. xanthus* cells from MOPS buffer to the same plate and found that the newly added cells initially showed very high reversal frequencies, consistent with the presence of a repellent (Fig. 1). This result indicates that the adaptation behavior observed was not due to loss of isooamyl alcohol from the plate; however, some evapo-

### Table 1. *frz* mutants and their adaptation behavior

<table>
<thead>
<tr>
<th>Strain$^a$</th>
<th>Genotype</th>
<th>Response to 0.05% isooamyl alcohol$^b$</th>
<th>Adaptation to 0.05% isooamyl alcohol$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ2</td>
<td>Wild type</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DZ4147</td>
<td><em>frzA</em></td>
<td>No</td>
<td>Not applicable</td>
</tr>
<tr>
<td>DZ4168</td>
<td><em>frzB</em></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DZ4169</td>
<td><em>frzCD</em></td>
<td>No</td>
<td>Not applicable</td>
</tr>
<tr>
<td>DZ4148</td>
<td><em>frzE</em></td>
<td>No</td>
<td>Not applicable</td>
</tr>
<tr>
<td>DZ4170</td>
<td><em>frzF</em></td>
<td>Yes</td>
<td>Defective</td>
</tr>
<tr>
<td>DZ4146</td>
<td><em>frzG</em></td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

$^a$ All of the strains listed have been previously described (18). The *frz* mutants are in the DZ2 background.

$^b$ Cells responded to 0.05% isooamyl alcohol by increasing their frequency of reversal to about once every minute.

$^c$ Cells adapted to 0.05% isooamyl alcohol in about 30 min.

### Table 2. Correlation between adaptation times and intensity of the chemical stimulus

<table>
<thead>
<tr>
<th>Isoamyl alcohol concn (%)</th>
<th>Avg adaptation time (min) $\pm$ SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>7 $\pm$ 2</td>
</tr>
<tr>
<td>0.03</td>
<td>16 $\pm$ 6</td>
</tr>
<tr>
<td>0.05</td>
<td>27 $\pm$ 7</td>
</tr>
<tr>
<td>0.08</td>
<td>75 $\pm$ 11</td>
</tr>
<tr>
<td>0.15</td>
<td>$\geq$ 1,000</td>
</tr>
</tbody>
</table>

$^a$ The strain used was wild-type DZ2. Cells were transferred from liquid MOPS buffer to 0.7% agar containing MOPS buffer plus various concentrations of isooamyl alcohol. The data presented are averages of two separate experiments.

![FIG. 1. Time course of sensory adaptation of *M. xanthus* to the repellent isooamyl alcohol (0.03%). The first group of DZ2 cells was transferred from liquid MOPS buffer to a 0.7% agar plate containing MOPS buffer plus 0.03% isooamyl alcohol at 0 min. After 30 min (arrow), the second group of DZ2 cells was inoculated onto the same plate. Cell reversal frequencies were roughly estimated from recorded video images. The data presented are the average number of cell reversals per cell per minute in every 5-min interval. The cell reversal frequency of unstimulated cells is about 0.14 reversal per cell per minute.](http://jb.asm.org/)
ration did occur since the adaptation time was shorter for the second group of cells (Fig. 1).

Adaptation times were found to be directly correlated with the intensity of the chemical stimuli. Table 2 shows that as the amount of isomyl alcohol in the agar was increased, it took longer for cells to adapt to the stimuli. When isomyl alcohol concentrations became too high (e.g., 0.15%), cells continued to show high reversal frequencies for a long time and were not able to adapt. It is very likely that these high repellent concentrations are overwhelming stimuli which saturate the adaptation capacity of the signal transduction system.

Cell clump dispersal as a tool for study of cellular behavior in M. xanthus. We found that for routine experiments it is very helpful to study the behavior of cell clumps (which contain 200 to 1,000 cells) because it allows one to study many cells at the same time and the pattern of cellular dispersal for the clump is a good indicator of adaptation. Figure 2 shows one such experiment. When cells were transferred from liquid MOPS buffer to MOPS buffer-0.7% agar (unstimulated cells), they were motile immediately on plating and spread out without a detectable lag. Cells moved outward, forming a dispersal pattern, which on rapid replay of time-lapse videos appears as a “blast” (Fig. 2A). When cells were transferred from liquid MOPS buffer to MOPS buffer-0.7% agar containing 0.03% isomyl alcohol (a negative stimulus), they showed an immediately increase in the frequency of reversal and little net movement. Consequently, cells stayed in the initial clumps and did not disperse (first three frames of Fig. 2B). However, after continued incubation (in this case, after 15 min), cells began to return to the steady-state reversal rate and the clumps showed the blast pattern (last two frames of Fig. 2B). Interestingly, the times needed for initiation of the blast dispersal pattern corresponds to the adaptation times (compare the data in Fig. 1 and 2). Thus, the clump dispersal pattern can serve as a convenient alternative method for the study of adaptation without direct counting of the cellular reversal frequency, which is very time consuming.

The roles of frz genes in sensory adaptation. We tested frz gene mutants for the ability to adapt. As reported previously, frzA, frzCD, and frzE mutants did not show any response to chemical stimuli, indicating their involvement in excitation rather than adaptation (Table 1). frzF (homologous to cheR) mutants were defective in adaptation (Table 1): it took the wild type about 30 min to adapt to 0.05% isomyl alcohol; however, it took more than 60 min for the frzF mutant to adapt to the same stimulus, indicating that FrzF plays a very important role in adaptation. frzG (homologous to cheB) mutants were also expected to be defective in adaptation. However, they exhibited normal adaptation behavior (Table 1). This finding is consistent with our previous results (11, 18) and suggests that frzG is not the only gene involved in the demethylation of FrzCD and that another gene must complement this function. frzB mutants were found to be defective in response to attractants like Casitone and yeast extract but still responsive to repellents like isomyl alcohol (11, 18). We found that frzB mutants were also able to adapt to repellent stimuli (Table 1).
This finding suggests that frzB is involved in positive chemotaxis only.

Previously, we showed that methylation-demethylation of FrzCD was directly correlated with chemotactic movements (11, 18). This finding was confirmed in our studies of adaptation. When M. xanthus cells were transferred from MOPS buffer (a weak repellent) to 0.03% isoyam alcohol (a stronger repellent), they became less methylated as the cell movement reversal frequency was increased (18; Fig. 1 and 2). However, when the cells were pretreated with 0.15% isoyam alcohol (a very strong repellent) and then transferred to 0.03% isoyam alcohol (a weaker repellent), they became more methylated and cell reversal was inhibited (data not shown). These results strongly suggest that the function of FrzCD methylation is similar to that of MCP methylation in enteric bacteria: to serve as a “memory” of past stimulus concentrations, thereby enabling the cells to do temporal comparisons with present levels of the stimulus. Thus, repellent addition is a negative stimulus and repellent removal is an attractant stimulus.

**Effect of Casitone and yeast extract on cellular behavior.** We would like to have been able to show adaptation to attractants. Addition of attractants would be expected to inhibit cell reversal initially, but with continued exposure, cells would adapt to the attractant. Unfortunately, we have not found any chemically defined attractants and were not able to test this hypothesis directly. Spatial chemotaxis assays and methylation studies showed that complex media like Casitone and yeast extract behaved like attractants for M. xanthus cells (18). However, Casitone and yeast extract were the best attractants for M. xanthus only after several hours of incubation (data not shown). Cells plated on Casitone and yeast extract medium showed extremely low rates of motility initially, and it took 1 to 10 h (depending on the cell density) for cells to become motile again. It is not known why cells become nonmotile on CYE medium. However, the reason appears to be unrelated to chemotaxis, since all of the frz mutants also became nonmotile upon transfer to CYE medium (data not shown). We also found that diluted or used CYE medium had reduced inhibitory effects (data not shown), indicating that something in CYE medium is bad for cellular motility.

In summary, we have found that nonsaturating concentrations of repellents, M. xanthus cells show adaptation to changed levels of stimuli. Furthermore, the adaptation behavior is correlated with methylation-demethylation of the FrzCD chemoreceptor. The modification of FrzCD is thought to enable bacteria to do temporal comparisons so that cells move towards more favorable stimuli and away from less favorable ones. The ability of cells to adapt to stimuli explains how M. xanthus could achieve chemotaxis despite its slow movement. In fact, the behavior of M. xanthus is entirely consistent with the theoretical analysis of chemotaxis for enteric bacteria (1). Since the gliding motility is slow, chemotaxis would not be expected for shallow and unstable chemical gradients (5). However, for stable chemical gradients, there should be no theoretical problem for even slowly moving M. xanthus to do temporal comparisons and move towards favorable conditions (1). Such stable gradients would be expected to exist in the natural habitat of M. xanthus, for example, during fruiting body formation or during colony swarming (18).

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**REFERENCES**