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Passive Rotation of Flagella on Paralyzed *Salmonella typhimurium* (*mot*) Mutants by External Rotatory Driving Force

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Salmonella typhimurium mot mutants are unable to rotate their flagella. Dark-field light microscopy showed that the flagella could be rotated passively by an external rotatory driving force.

Species of bacteria from common genera such as *Salmonella* or *Escherichia* possess semirigid helical flagella. When a bacterial cell swims forward, the helical waveform of a flagellum is propagated from the proximal to the distal end. The propagation of the waveform is performed by true rotation of an entire filament (3, 5, 18). It has been well accepted that a "flagellar motor," which causes a flagellar filament to rotate, lies at the basal region of each flagellum (1, 3). However, little is known about the mechanism of the flagellar motor. *mot* mutants, which are paralyzed although normally flagellated, have been commonly isolated (2, 6, 19). The nonmotility of *mot* cells suggests that their flagella do not rotate, although the flagella are apparently indistinguishable in shape from those of the wild type. Is a part of the flagellar motor damaged? Do the flagella completely lose their rotatory freedom? In the present study, we show that flagella on the *mot* cells can be rotated passively by an external rotatory driving force generated by a flow of viscous fluid.

The *mot* mutant strains of *Salmonella typhimurium* used in this study are listed in Table 1. The cells were suspended in 150 mM NaCl-50 mM Tris-hydrochloride at pH 7.8. Their individual flagella were observed by a dark-field light microscope with a high-intensity light source (8, 10, 12, 13). The images in the microscope were recorded dynamically by using an ultrasensitive silicon intensifier target video camera (9). All of the *mot* strains in Table 1 were indistinguishable from each other by the criteria used in this study. Flagella of the *mot* cells usually were dispersed about the cell bodies. They appeared to be fairly rigid. None of them rotated. The cell bodies of a small fraction of the cells stuck to a surface of a glass slide spontaneously. The stuck cells allowed us to investigate flagellar movement in more detail. No rotatory Brownian motion was detected with any of the flagella on the stuck *mot* cells. This evidence seemed to indicate that the flagella had completely lost their rotatory freedom as a result of *mot* mutation.

When bacterial cells swim by rotating their flagella, thrust is generated from rotation by virtue of the helicity of the flagella. Conversely, if the flagella of stuck cells were subjected to the flow of fluid, hydrodynamic rotatory torque would be generated on the flagella. The generation of such torque has been demonstrated in the case of an isolated flagellar filament attached on a glass surface at only one end (9). Steady viscous flow in a microscope specimen was made by placing a droplet of suspension medium containing methylcellulose (~50 cps in viscosity) next to the edge of a cover slip. The viscous solution seeped into the specimen by capillary action at a velocity of several micrometers per second. When stuck *mot* cells were exposed to the viscous flow, the flagella on the cells pivoted and pointed in the direction of the flow. Then, they began to rotate spontaneously, that is, each flagellum propagated its helical wave from the proximal to the distal end (Fig. 1). The rotatory frequency actually depended on the velocity of the viscous flow (detailed analyses will be published elsewhere). When the flow was abruptly stopped by applying another drop to the downstream side of the specimen, the flagella immediately stopped rotating. These results indicate that the flagella on the stuck *mot* cells were passively rotated by the viscous flow. This was confirmed by the following observation. When stuck *mot* cells were detached from a glass surface, their flagella stopped rotating. This result was caused by the fact that the flow did not generate any mechanical force on floating cells.

S. typhimurium cells are peritrichously flagellated; that is, they possess several flagella, each of which is attached to a different point of the cell surface. When a cell swims, its flagella tend to form a bundle and rotate coordinately. The

flagella of *mot* cells were also capable of forming bundles; moreover, some of the bundles rotated passively in a viscous flow. Usually, a majority (~⅔) of the stuck *mot* cells formed a flagellar bundle, whereas the remaining cells possessed one or a few flagella. Typically, more than half of these isolated flagella rotated, whereas only a small fraction (~1/10) of the bundles rotated.

The passive rotation is not a peculiar characteristic of the flagella of *mot* cells. Flagella of wild-type cells were also rotated passively. It is well known that bacterial cells stop swimming in the presence of a respiratory inhibitor (4). Wild-type cells (SJW1103) completely stopped rotating their flagella in suspension medium containing 2 mM 2,4-dinitrophenol. The flagella of the 2,4-dinitrophenol-treated cells exhibited the same properties as those of *mot* cells. That is, they were rotated passively by the viscous flow and stopped rotating as soon as the flow stopped.

TABLE 1. *S. typhimurium strains examined in the present study*[a]

Strain	Mutated cistron	Motility	Derivation
SJW1103	Wild type	Motile	A phase 1 stable derivative of *S. typhimurium* TM2
SJW1386	*motA*	Paralyzed	SJW1103
SJW1388	*motA*	Paralyzed	SJW1103
SJW1359	*motB*	Paralyzed	SJW1103
SJW1385	*motB*	Paralyzed	SJW1103
SJW1769	*motC*	Paralyzed	SJW1103
SJW1778	*motC*	Paralyzed	SJW1103

[a] The genetic characterization of these strains will be published in detail elsewhere.

For the cause of nonmotility of the flagella on *mot* cells, one can imagine two kinds of possible models. In model 1, there is some defect in the mechanism which maintains free rotation of a flagellum; consequently, the cell cannot rotate a flagellar filament, even if the rotatory driving force is generated. In model 2, a cell has lost the ability to generate the driving force, although it retains the capacity for free rotation of a flagellum. Our results exclude the possibility of model 1. Instead, they suggest that model 2 is highly probable. Some other models could be imagined. For instance, a flagellar motor may have faulty anchorage to the cytoplasmic membrane; therefore, the entire motor rotates in relation to the cell body. As a result, the flagellar filament exhibits no rotation, although the motor generates the driving force and also retains the capacity for free rotation. This model, however, is unfeasible. According to this model, when *mot* cells are observed without applying external force, it is expected that the flagella would exhibit rotatory motions, even if at very low frequencies, to compensate for the torque generated by the motor. However, such rotatory motions of flagella were not observed at all.

One may postulate that the magnitude of the torque generated by the viscous flow could be so large as to shear some structure between the flagellar filament and the motor. The following preliminary data, however, seem to exclude this possibility. We measured the rotatory frequency and the length of a flagellum and also the flow velocity and the viscosity of the fluid. The magnitude of the torque was obtained, based on the hydrodynamic analyses given by Holwill and Burge (7). In the experimental conditions used

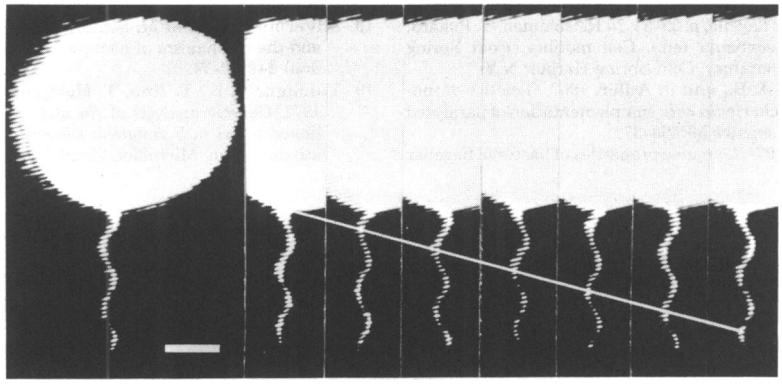

FIG. 1. *Sequential micrographs of a flagellum on a paralyzed mutant cell undergoing passive rotation. The rotation was induced by an external rotatory driving force generated by a flow of viscous fluid. An S. typhimurium motA mutant cell (SJW1386) stuck on a glass slide was observed by a dark-field light microscope with a high-intensity light source. The moving images under the microscope were recorded by an ultrasensitive video camera; then, the images on a video monitor were photographed at intervals of 0.25 s. The white line represents the propagation of the phase of the helical wave form. Bar, 2 μm.*

in this study, the maximum torque generated on a flagellum by the viscous flow was estimated to be approximately 2×10^{-12} dyn·cm. This value is smaller than that of the endogenous driving torque, approximately 6×10^{-12} dyn·cm, that was calculated for each flagellum in an active flagellar bundle (detailed analyses will be published elsewhere).

Recent studies show that the energy source for flagellar rotation is not ATP itself, but rather an intermediate in oxidative phosphorylation, presumably a proton electrochemical potential gradient (proton motive force) across the plasma membrane (11, 14–17). When the endogenous energy sources in *Bacillus subtilis* cells are removed by starvation, the cells lose their motility. Motility can be reactivated by an artificial proton motive force (15). This force, however, does not generate motility in *mot* cells of *B. subtilis* (Matsuura et al., personal communication). This result implies that the defect in *mot* mutants is not simply a loss of proton motive force. Our results show that the flagella of such mutants maintain their rotatory freedom. We conclude that nonmotility of flagella on *mot* cells probably is caused by a defect in the flagellar motor itself, namely, by a failure in the process that converts proton motive force into the rotatory driving force.

Flagella of wild-type and *mot* cells can be rotated passively even when they are not able to generate an endogenous driving force. A successful model for the flagellar motor must take this fact into consideration.

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