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# *flaAII* (*motC*, *cheV*) of *Salmonella typhimurium* Is a Structural Gene Involved in Energization and Switching of the Flagellar Motor

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The *flaAII* gene of *Salmonella typhimurium* has also been termed *motC* and *cheV*, because defective alleles may give rise to a nonflagellate, paralyzed, or nonchemotactic phenotype. We isolated a temperature-sensitive motility mutant (MY1) and have found that the mutation occurs in the *flaAII* gene. In temperature-jump experiments, MY1 could be converted from highly motile to paralyzed within 0.5 s, demonstrating that *flaAII* is a structural gene whose product is immediately essential for motor rotation. The mutant, although chemotactic at permissive temperatures (<36°C), had a higher clockwise rotational bias than did the wild type; it can therefore be regarded simultaneously as *motC*(Ts) and *cheV* (tumbly). The only previously reported *S. typhimurium cheV* mutant was smooth-swimming. A shift toward counterclockwise bias accompanied loss of rotational speed in the restrictive temperature range. This result, by analogy with known proton motive force effects on motor switching, further indicates a central role of the *flaAII* (*motC*, *cheV*) protein in the energy transduction and switching process. Since there is no evidence associating it with the isolable entity known as the basal body, it may reside at the cytoplasmic face of the flagellar motor.

Flagellated bacteria are propelled by rotary motors (5, 25). Both counterclockwise (CCW) and clockwise (CW) senses of rotation occur, the former sense producing swimming (2, 15), and brief periods in the latter sense producing tumbling (17). Modulation of switching between the two senses causes tactic (migrational) responses (13). The subject of bacterial motility and taxis has been reviewed extensively (e.g., references 11, 16, 19, 27).

A large number of genes (ca. 30 [10]) are required for the proper assembly and operation of the bacterial flagellar motor. Several of these genes have been designated *mot*, because mutants have flagella which are unable to rotate despite apparently normal morphology. The *motC* gene of *Salmonella typhimurium* is a particularly interesting one for an understanding of chemotaxis and motility, since mutant alleles can give rise to three distinct phenotypes. The null phenotype is nonflagellate (28, 32), whereas less severe mutations can give rise to either a paralyzed (7) or a nonchemotactic phenotype (28); the gene symbols *flaAII*, *motC*, and *cheV*, respectively, have been assigned, although strictly speaking only *flaAII* (corresponding to the null phenotype) should be used.

Thus, the *flaAII* gene product is needed for the structural integrity of the motor, is in some manner involved in the mechanism of rotation itself, and is at least partially responsible for the switching of the motor between the CCW and CW states. These properties suggest a central structural role (28, 32), but it is possible that participation could be at the level of genetic regulation or organelle assembly. If the protein is indeed a component of the motor, the question arises of whether its role in enabling motor rotation is a direct one involving the actual transduction of proton electrochemical potential energy (proton motive force) into mechanical work or a secondary one involving the application of mechanical force generated elsewhere in the motor.

We have succeeded in isolating a temperature-sensitive strain having a mutation in the *flaAII* gene, and we report the properties of the strain, which are consistent with the *flaAII* gene product being a structural component of the motor, involved directly in chemomechanical energy transduction, and also in switching.

## MATERIALS AND METHODS

**Bacterial strains.** *S. typhimurium* ST1 (4), a motility-selected derivative of wild-type LT2, was used to generate the temperature-sensitive motility mutant (MY1). Bacterial strains used in transductional mapping of MY1 are described in Table 1 and were

obtained from B. A. D. Stocker of Stanford University.

**Mutant selection.** The following selection procedure was employed, with the rationale of successively screening out nonflagellate cells, cells that were motile at 40°C, and cells that were nonmotile even at room temperature or at 30°C. ST1 was mutagenized with ethyl methane sulfonate (Eastman Organic Chemicals, Rochester, N.Y.) by the method of Roth (22) and was grown further for several generations. Cells that were flagellate at 40°C were selected by precipitation with rabbit antiserum (Lee Laboratories, Grayson, Ga.) directed against the two possible flagellar antigens (i and 1,2) for this strain and were grown overnight at 40°C in nutrient broth in the presence of the flagellotropic phage χ (at a multiplicity of infection of 1) to kill cells that were motile at that temperature (24). The surviving cells were washed, and a small inoculum was placed at the center of a semisolid motility plate (0.65% tryptone, 0.35% Bacto-Agar [Difco Laboratories, Detroit, Mich.]) and incubated at room temperature for ca. 12 h, until cells had spread to a diameter of ca. 50 mm. After the cells at the center of the plate were removed, those from the remainder of the plate were collected and grown in nutrient broth at 40°C. A sample of this population was placed on a motility plate and allowed to spread at 40°C, and cells remaining in the center of the plate were collected and grown at 30°C in nutrient broth. Cells were cycled three times between 30 and 40°C in this manner, and those from the center of the final 40°C plate were then streaked on a plate. Single-colony isolates were grown overnight in nutrient broth at 40°C and examined by phase-contrast microscopy in a controlled-temperature room at 40°C. Motile cultures were discarded, and the rest were regrown at 30°C and screened for motility at room temperature. Immotile cultures were discarded, and the temperature dependence of motility in the remainder was examined by phase microscopy, using a variable-temperature stage (model TS-2; Bailey Instruments, Co., Saddle Brook, N.J.). The temperature was raised from 25 to 40°C over a 1-min interval with the cells under continuous observation. Those cultures which lost motility in this temperature range were retained.

**Genetic complementation.** Complementation analysis was carried out by using generalized transducing phage P22 HT *int-3* (21). Phage were passaged twice through the donor. Recipient strains, defective in loci of interest, were grown at 37°C in nutrient broth to a density of $10^9$ ml$^{-1}$ and mixed with phage at a multiplicity of infection of 10, and 50-μl samples were streaked as a single line on motility plates and incubated at either 30 or 42°C for up to 48 h. Plates were then inspected for evidence of trails or swarms deriving from genetic complementation. Samples of cells alone and phage alone were used as controls.

**Motility measurements.** Measurements of swimming speed were made by adding a 5-μl sample of cells in motility medium (10 mM $KPO_4$, 0.1 mM EDTA [pH 7]) to an equal volume of motility medium plus 50 mM serine under a bridged cover slip. The slide-cover slip sample chamber was preequilibrated at the temperature desired for a given measurement by using the variable-temperature stage. Video records taken ca. 1 min after sample mixing were used for data analysis; by this time the sample temperature had stabilized, as judged by the fact that speed was time independent under permissive conditions (up to ca. 55°C for wild-type cells).

For steady-state measurements of cells tethered at 30°C, the experiment was carried out in a controlled-temperature room. For the measurement of terminal rotational behavior at restrictive temperatures, cells were tethered to a cover slip which was then inverted over a flow chamber placed on the variable-temperature stage; because of the experimental geometry, the surface to which the cells were tethered was at a temperature ca. 2 to 4°C lower than that of the stage (estimate based on motility loss).

Flagella were visualized by high-intensity dark-field microscopy as described previously (14).

Recordings of free cells and of rotation of tethered cells were made with a model 650 SIT video camera (Dage/MTI; Michigan City, Ind.) and an Omnisonic II video cassette recorder (Panasonic; Secaucus, N.J.).

**Membrane potential measurement.** Membrane potential of EDTA-permeabilized cells was measured, as described previously (33), by using distribution of [$^3$H] tetraphenylphosphonium ions (specific activity, 0.25 Ci mmol$^{-1}$; a generous gift of H. R. Kaback, Roche Institute of Molecular Biology, Nutley, N.J.).

## RESULTS

Of the temperature-sensitive motility mutants isolated by the procedure outlined in Materials and Methods, one (MY1) possessing vigorous motility at the permissive temperature and a rather abrupt transition to zero motility as temperature was raised was found to carry a mutation in *flaAII* (see below) and was chosen for detailed study.

**Mapping of the mutation conferring temperature-sensitive motility on MY1.** The results of the complementation analysis of MY1 are given in Table 1. With two exceptions, SL2516 (*cheV*) and SJ328 (*flaAIII*), all recipient strains were nonflagellated or flagellated but immotile, and therefore control plates in the absence of phage gave growth but no evidence of spreading; SL2516 gave the slight fuzzy spreading characteristic of smooth-swimming, nonchemotactic mutants, and SJ328 was unstable (28), with single-colony isolates giving rise to a spotty spread on swarm plates and to occasional motile cells in liquid cultures. At 30°C, P22(MY1) and P22(ST1) complemented all the recipient strains tested (as judged by trail and swarm formation). At 42°C, P22(ST1) was still able to complement all strains, whereas P22(MY1) complemented the *motA-motB* deletion mutant SJ629, but failed to complement mutants deleted through *flaAII* (*motC*). By using recipient strains with smaller deletions, it was possible to place the MY1 lesion within subregion ii of *flaAII*. This was of particular interest, since the only motile *flaAII* mutant described previously, SL2516 (*cheV*), also carries a gene that maps in subregion ii (28). P22(MY1) was found to complement SL2516

TABLE 1. Complementation analysis of MY1

| Recipient[a] | Deletion | Donor[b] | | | | |
|---|---|---|---|---|---|---|
| | | P22 (MY1) | | P22 (ST1) | | None |
| | | 30°C | 42°C | 30°C | 42°C | 37°C |
| SJ1727 | *flaD-flaAI*[c] | + | − | + | + | − |
| SJW238 | *flaR-flaAI* | + | − | + | + | − |
| SJ1667 | *flaAII*(i-ix) | + | − | + | + | − |
| SJ1687 | *flaAII*(ii-iv) | + | − | + | + | − |
| SJW383 | *flaAII*(iii)-*flaAI* | + | + | + | + | − |
| SJ1608 | *flaAII*(iv)-*flaAI* | + | + | + | + | − |
| SJ328 | *flaAIII*(iii-iv) | + | +[d] | + | +[d] | −[e] |
| SL2516 | *flaAII*(ii) (*cheV*)[f] | + | + | + | + | −[g] |
| SJ629 | *motA-motB* | + | + | + | + | − |

[a] *S. typhimurium* SJ629, flagellate but paralyzed, is described in reference 7. *S. typhimurium* SL2516, smooth-swimming and nonchemotactic, is described in reference 28 (see also footnotes f and g). All other strains are *Salmonella abortusequi*, nonflagellate, and are described in reference 28.

[b] P22 transducing phage containing a genomic fragment from the donor bacterial strain (in parentheses). ST1 is the wild-type parent of MY1. +, Swarms and trails on semisolid agar; −, growth with no evidence of motility.

[c] The relevant *fla* genes are linked in the order *D-B-P-N-Q-R-S-AIII*(i-iv)-*AII*(i-x)-*AI*. *flaAII* may consist of two genes, *flaAII.2* and *flaAII.1*, linked in that order (Yamaguchi, personal communication; also quoted in reference 12). *motA* and *motB* are linked to each other, but not to the *fla* genes.

[d] Complementation was poor, with only a few swarms.

[e] Slight spotty spread; single-colony isolates revealed a few motile cells in liquid culture.

[f] Not a deletion mutant.

[g] Slight fuzzy spread, as expected from phenotype.

(which was obtained by ethyl methane sulfonate mutagenesis and therefore probably contains a point mutation).

Yamaguchi (personal communication; also quoted in reference 12) has concluded from complementation analysis that *flaAII* is actually two genes, which he has named *flaAII.1* and *flaAII.2*; mutations giving rise to the Che$^-$ or Mot$^-$ phenotype were found by him to occur in *flaAII.2*. Since the mapping in the present paper was based on a presumed single gene, we use throughout the symbol *flaAII*. Both *cheV* and *motC* mutations map in a quite localized region of the genome, indicating that, in any case, they correspond to defects in a single protein. Whether this protein is the sole product of the *flaAII* coding region, or whether there is a distinct additional *flaAII.1* gene product, remains to be established.

**Viability and energetics of MY1.** Although the locus of the mutation in strain MY1 made it seem unlikely, we wished to establish whether the loss of motility of MY1 at the restrictive temperature was just one manifestation of a more central physiological disruption. However, the growth rates of MY1 and its parent ST1 were identical at each of three temperatures tested (30, 37, and 42°C) and in each medium tested (nutrient broth, Vogel-Bonner minimal medium [30] plus glycerol, and Vogel-Bonner medium plus glucose under anaerobic conditions).

To test further whether cellular energetics were normal, the membrane potential of MY1 was measured before and after a shift from 30 to 40°C. The potential increased from −166 to −184 mV, as might be expected from a general increase in metabolic rates. Since these measurements were made at pH 7.5, at which the transmembrane pH difference in bacteria is small (26), this result indicated that there was no gross defect in the proton motive force of MY1 at a temperature at which motility was severely impaired.

**Flagellation.** The inability of strain MY1 to swim at restrictive temperatures was not caused by flagellar loss. Dark-field microscopy indicated a normal complement of 5 to 10 flagella, of normal length but stationary. Flagella sheared (by repeated passage of the cells through a 22-gauge needle) from equal numbers of mutant and parent cells yielded similar amounts of flagellin as judged by Coomassie staining of sodium dodecyl sulfate-polyacrylamide gels.

**Behavioral analysis of MY1.** We examined the behavior of cell populations on swarm plates and of individual free and tethered cells in liquid media.

Figure 1 shows the spreading rate of MY1 and its wild-type parent, ST1, on semisolid motility plates at various temperatures. Whereas the wild type showed an increased rate of spreading with temperature throughout the range examined (26 to 42°C), the rate for MY1 reached a maximum at 36°C and declined abruptly to essentially zero at 42°C. Interestingly, at low temperatures, MY1 spread significantly faster than its parent; this was observed consistently in the triplicate plates of the assay at each of the three permissive temperatures tested.

Swimming speed of free cells was measured at various temperatures by diluting them from motility medium into medium containing an attractant (serine) and recording the resultant smooth-swimming behavior on videotape (Fig. 2). Wild-type cells showed a monotonic increase of swimming speed with temperature, up to approximately 55°C; at higher temperatures, motility deteriorated as a function of time. At 55°C, the mean speed was 50 $\mu m\ s^{-1}$, which is about twice the speed at room temperature. MY1 showed an increase of speed with temperature only as far as 35°C, and at 44°C the cells were immotile. Motility tracks illustrating this

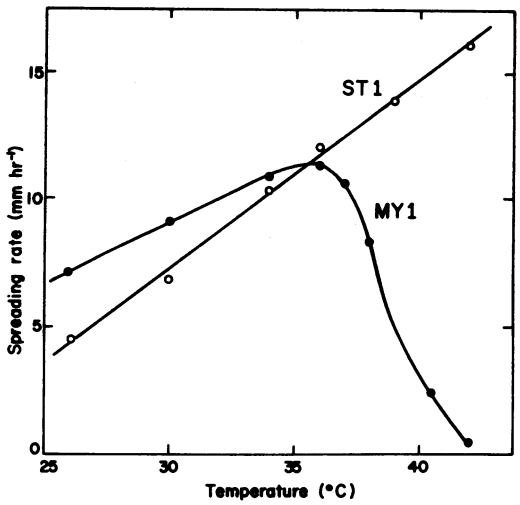


FIG. 1. Spreading rate, as a function of temperature, of the temperature-sensitive motility mutant MY1 (●) and its wild-type parent ST1 (○). A small inoculum was placed at the center of a tryptone semisolid agar plate; after a variable period of stabilization, swarm diameter was found to increase linearly with time. Data are means of the linear spread rates on triplicate plates.

phenomenon are shown in Fig. 3. Thus, the observed loss of chemotactic ability on swarm plates correlated closely with the loss of motility. Even at permissive temperatures, the swimming speed of strain MY1 was slightly lower than that of ST1, whereas, as was noted above, the swarming ability was greater. Possible reasons for this apparent anomaly will be discussed later.

Our observation that speed increased with increasing temperature does not conflict with the recent observation (S. Khan and H. C. Berg, Cell, in press) that the rotation speed of the motor is, at constant load and proton motive force, temperature independent. In the present study, neither proton motive force nor the fluidity of the medium was held constant.

The loss of motility was rapid; when a sample of MY1 was placed under a bridged cover slip that had been preequilibrated at 42°C, most cells were immotile from the earliest possible observation time (2 to 3 s). To improve on the time resolution of these observations, a fine wire (gold-plated tungsten; diameter, 20 μm; length, 20 mm; electrical resistance, 3.8 Ω) was immersed in 8 μl of cell suspension under a bridged coverslip on a microscope slide, and the temperature of the sample was adjusted to 33°C (near the upper limit of the permissive range) by means of the variable-temperature stage. The temperature was abruptly increased by passing electrical current (1.1 W; 2 V, direct current) through the resistance wire. MY1 cells in the vicinity of the wire were totally paralyzed within 0.5 s after the current was switched on (time estimated from video records). Paralysis on this time scale did not result from general physiological damage; in control experiments, ST1 cells underwent an immediate (less than 1-s) acceleration to swimming speeds in excess of 70 μm $s^{-1}$ (indicating that Joule heating had produced a local temperature of over 55°C; Fig. 2). This extremely vigorous motility was sustained for about 20 s, after which it began to deteriorate significantly.

Loss of motility was reversible. When the temperature was lowered from 42 to 35°C, cells began to display 'jiggling" motion after about 30 s, and by 2 min, the majority of the cells were showing translational motion.

The value of the restrictive temperature varied to some extent with the medium in which the cells were placed. For example, in nutrient broth, motility was still fairly good at 42°C and was not totally lost until 48°C. Dense cultures in nutrient broth, placed under a bridged cover slip at 42°C, produced an outwardly migrating aerotactic band, leaving immotile cells in the center; with glucose present, the anaerobic cells in the center were motile, but they were swimming much more slowly than the cells in the aerobic region of the sample. Respiring cells in motility medium at 42°C were immotile, whether or not glucose was present. Since, at permissive temperatures, respiring cells in motility medium were much more motile than were glycolizing cells in nutrient broth, the elevation of the restrictive temperature for cells in nutrient broth cannot be explained by better energization. We modified motility medium in several ways in an

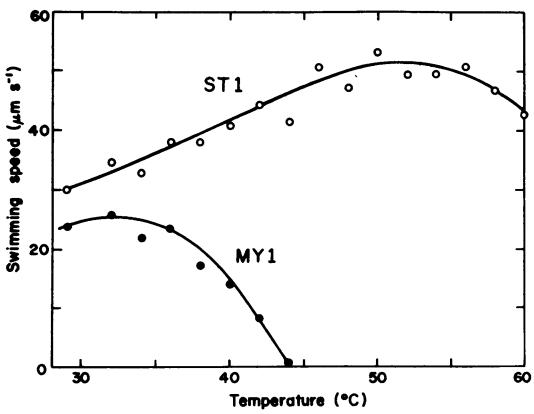


FIG. 2. Swimming speed of MY1 (●) and its parent ST1 (○) as a function of temperature. Data points are means of at least 12 cells, with standard errors of ca. 6%.

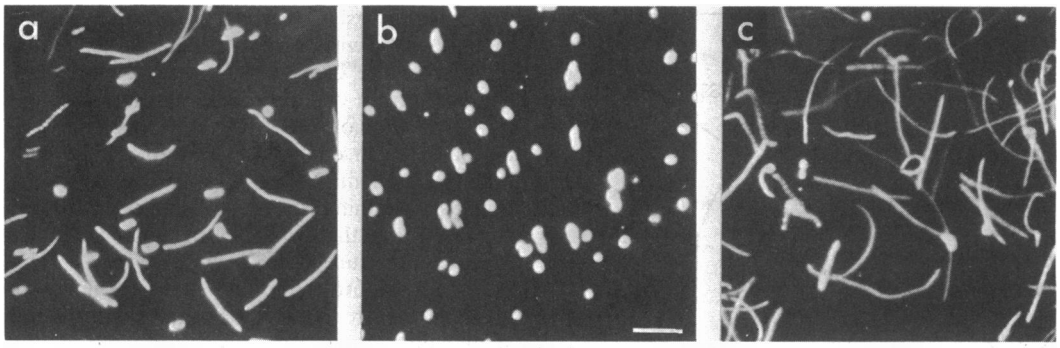


FIG. 3. Motility tracks of MY1 at (a) the highest permissive temperature (35°C) and (b) a fully restrictive temperature (45°C). Motility tracks (c) of the parent ST1 at 45°C are shown for comparison. In all cases, an attractant stimulus (25 mM serine) was given to suppress tumbling. Exposure time, 1 s; bar, 25 μm.

attempt to determine which factors were important for retention of motility. Increased ionic strength (50 mM NaCl), increased osmotic strength (250 mM sucrose), and lowered pH (5.5) each caused a slight elevation of the restrictive temperature. Divalent cations (10 mM $Mg^{2+}$) acted similarly, despite a reduction in motility at permissive temperatures (the latter effect presumably being a consequence of interference with the chelation, by EDTA, of trace amounts of motility-inhibiting divalent cations such as $Cu^{2+}$ [1]). It appears, therefore, that there are a number of factors contributing to the thermal stability of the *flaAII* protein.

Although strain MY1 was chemotactic at permissive temperatures, its unstimulated motility was decidedly more tumbly than that of the wild type. This was confirmed by examining the rotational behavior of tethered cells. At 30°C, MY1 spent 44% (standard error, 5%) of the time in CW rotation, with a reversal frequency of 2.8 $s^{-1}$ (standard error, 0.2 $s^{-1}$). For strain ST1, the corresponding values were 11% and 1.0 $s^{-1}$ (cf. reference 9). Thus, MY1 is intermediate between wild-type and classical tumbly mutants such as SL4041 (*cheB*) (29); the latter spends about 90% of its time in CW rotation (9).

It is conceivable, although unlikely, that the tumbly phenotype derives from a spurious unselected second mutation independent of that conferring temperature-sensitive motility. To examine this possibility, we isolated independent transductants with temperature-sensitive motility by using P22(MY1) as the donor and SJ1687 as the recipient. SJ1687 contains a small deletion in *flaAII* and therefore does not require cotransduction of other flagellar genes for motility. Of 10 independent transductants tested, all had the tumbling motility characteristic of MY1. The same result was obtained with a further 10 independent transductants isolated by using donor phage UV irradiated at a dose (30-W germicidal lamp; 0.3 m, 4 min) that reduced the infective titer to 10%. The UV irradiation causes the transduced DNA to be cleaved into fragments and therefore reduces the probability of corecombination of closely linked mutations (J. S. Parkinson, personal communication). We conclude from these results that the tumbly motility of strain MY1 almost certainly derives from a single mutation in *flaAII*.

Are the motor-switching properties of MY1 temperature dependent? Specifically, does the fraction of time in CW rotation decrease with temperature-dependent loss of motor speed, as has been observed (8) for proton motive force-dependent loss of motor speed? To answer this question, tethered cells were examined, first at a semi-restrictive temperature (estimated to be about 38°C; stage temperature was 41°C) and then at a restrictive temperature (about 42°C; stage temperature 45°C). Under the latter conditions, motility declined to zero within a few minutes (limited by the rate of temperature equilibration). During the terminal period of motility, the CW state was almost never observed. For example, a sample of six cells, whose mean behavior at the lower temperature had been 28% CW (standard error 5%), executed at the upper temperature a mean of 8.9 revolutions in the CCW sense during the last minute for which rotation was observed; only one of these cells exhibited any (a total of 1/4 revolutions) CW rotation. We also used high-intensity dark-field microscopy to visualize individual flagella on cells that were progressively losing motility as the temperature was raised. Since the temperature stage was incompatible with the highly convergent illumination needed for this microscopic technique, we used an infrared lamp (250 W, 25-cm distance) as the heat source. With the microscope stage unit at 35°C or less, both MY1 and ST1 cells displayed vigorous motility, including tumbling and the polymor-

phic transitions characteristic of brief CW rotation (17). As the stage temperature was raised to 45°C over an interval of about 8 min, ST1 cells became increasingly vigorous and continued to display polymorphic transitions. MY1 cells progressively lost motility, and individual flagella were seen to slow down, stop and start intermittently, and then stop permanently. During this period of slow rotation, only the normal, left-handed, helical waveform was seen, and it was invariably travelling from the proximal to the distal end, indicating CCW rotation; this is precisely the behavior exhibited by de-energized cells (8).

We were interested to know whether the *flaAII* lesion in the nonchemotactic mutant SL2516 might also result in temperature-sensitive motility loss. There was a marginal effect, with motility impairment first becoming evident at 50°C (cf. 55°C for wild type) and on a much longer time scale (several minutes) than with MY1.

**Electron microscopy of basal bodies from MY1.** In view of the functional thermolability of the *flaAII* gene product of MY1, we examined by electron microscopy isolated basal bodies (6) that had been subjected to various degrees of heat treatment, in case there might be morphological degradation, either gross or specific, of MY1 basal bodies under conditions in which those from ST1 were intact. No difference was detected between the two strains; in both cases, a general degradation of structure first became evident with samples subjected to a temperature of 65°C for 30 min.

**Electrophoretic properties of MY1 basal body proteins.** Basal bodies from MY1 and ST1 were analyzed on two-dimensional gels (18). No differences were detected in the isoelectric point or electrophoretic mobility of their component proteins.

## DISCUSSION

The isolation of a conditional *flaAII* mutant of *S. typhimurium* has extended our understanding of the role of this gene in several ways. It has allowed the time scale of loss and recovery of *flaAII*-dependent motility to be measured, with a result that places constraints on the role of the gene. It has demonstrated that defects in *flaAII* function can give rise to a CW as well as a CCW bias of the motor. Finally, it has shown that, as motility is reduced by temperature increase, there is a shift toward CCW rotation analogous to the shift induced by lowered proton motive force, suggesting a central role of *flaAII* in energy transduction in the motor.

The loss of motility accompanying a sudden shift in temperature was rapid, with cells going from actively motile to paralyzed in less than 1 s. What type of thermally sensitive process could be responsible for such an abrupt loss of function? Regulation of synthesis of nucleic acid or protein is clearly out of the question. Thermal denaturation of an existing protein that is necessary for motor rotation is therefore indicated; this is the first demonstration that the *flaAII* gene is structural. Since the gene is part of an operon directly responsible for flagellation, we did not expect that the machinery for generating proton motive force would be in any way defective; normal growth rates and normal proton motive force values confirmed this expectation. The *flaAII* protein, then, is specifically involved in motor function. Is it a structural component of the motor? The time scale for motility loss is long enough for exhaustion of a specific metabolite to have occurred (e.g., a 1 mM pool would be abolished in less than 1 s by an enzyme with a turnover number of 1,000 $s^{-1}$, present at 1,000 copies per cell) and also for diffusion of a small molecule, or even a macromolecule, along the length of a bacterial cell (a medium-sized protein would take about 10 ms). We cannot, therefore, eliminate on kinetic grounds a model whereby the *flaAII* protein is in indirect communication with the motor. However, the phenotype of strains having deletions in *flaAII* is invariably $Fla^-$ (32) and not $Mot^-$, as might be expected from an indirect association. We conclude from the combined kinetic and genetic evidence that the *flaAII* protein is probably part of the motor defined as the macromolecular complex at the base of the flagellum. (Another, more remote, possibility would be that the *flaAII* gene codes for a scaffolding protein and that a motor imperfectly assembled from perfectly good subunits is therefore thermally unstable.)

Neither our biochemical nor microscopic studies provided any evidence that the *flaAII* protein is a component of the basal body. However, equally there is no evidence that the basal body comprises the entire flagellar motor. In view of the implication of the *flaAII* protein in the receipt of switch signals (see below), we consider it a strong possibility that the *flaAII* protein resides at the cytoplasmic face of the motor.

Mutants with the $Che^-$ (nonchemotactic) phenotype have abnormal unstimulated switching probabilities, and the phenotype produced by different alleles of a given gene is usually qualitatively the same. Thus, for example, all *cheA* mutants are CCW biased, and all *cheB* mutants are CW biased. The *flaQ* (*cheC*) gene is known to be an exception to this generalization, since examples of both CCW- and CW-biased mutants have been described previously (9, 23, 31). The present study has shown that the *flaAII* gene resembles the *flaQ* gene in this regard; the *flaAII*

mutant MY1 has a greater CW bias than does the wild type, whereas the only other known motile *flaAII* mutant (SL2516) exclusively displays CCW rotation. Parkinson (unpublished data, quoted in reference 19) has found the same plasticity of phenotype among strains with mutations in the homologous genes (*flaB* and *flaA*) in *Escherichia coli*. (Because *flaAII* [*Salmonella* sp.] may consist of two genes there is some uncertainty regarding the exact homology between it and *flaB* [*E. coli*]; Kutsukake et al. [12] have concluded that *flaB* [*E. coli*] is homologous to *flaAII.1* [*Salmonella* sp.] but failed to find a homologous gene to *flaAII.2* [*cheV*], whereas Parkinson [19] has concluded that *cheV* alleles of *E. coli* map in a single gene, *flaB*.) Since proteins synthesized by mutant alleles are generally functionally inferior to the wild-type protein, a simple explanation of the dual phenotype would be that the *flaAII* protein (and similarly the *cheC* protein) has dual functions, with CCW mutants being primarily defective in one function and CW mutants defective in the other. In view of allele-specific interactions demonstrated by Parkinson (quoted in reference 19) for the homologous genes in *E. coli*, a tempting model would be that the *flaAII* protein binds (perhaps alternately) both the *cheY* and *cheZ* proteins. *cheY* mutants are invariably smooth, whereas *cheZ* mutants are invariably tumbly; a CCW-biased *flaAII* mutant would be defective in binding the *cheY* protein, and a CW-biased mutant would be defective in binding the *cheZ* protein. Switch models of this sort have been proposed by Parkinson (19).

The *flaAII* and *flaQ* genes (and the homologous *E. coli* genes, *flaB* and *flaA*) resemble each other in several ways; they can give rise to the $Che^-$ phenotype as well as to the null phenotype ($Fla^-$), the $Che^-$ phenotype can be of the CCW- or the CW-biased type, and the gene products (at least in the case of *E. coli*) interact with the *cheY* and *cheZ* proteins (9, 12, 19, 20, 23, 28, 31, 32; this study). It seems reasonable to suppose that the resemblance may extend to include the mechanism of rotation itself, in which case one expects that some alleles of *flaQ* will, as with *flaAII*, give rise to the $Mot^-$ phenotype; such *Salmonella* mutants have been isolated recently by Yamaguchi (personal communication).

There seems to be an intimate relationship between the mechanism for enabling rotation and the mechanism for determining the sense in which rotation will occur, with a domain of the *flaAII* protein coded for by subregions ii and iii of the gene playing an important role in both mechanisms, as has been suggested previously (28, 32), since (i) a mutant isolated on the basis of conditional motility loss also has abnormal switching probabilities, (ii) the lesion occurs in the same subregion of the gene as a lesion in another mutant (SL2516) isolated on the basis of abnormal switching probabilities, and (iii) other $Mot^-$ *flaAII* mutants described by Yamaguchi et al. (32) carry mutations that map in an immediately adjacent subregion. Since mutations in other regions of *flaAII* have only been observed to give rise to the $Fla^-$ phenotype, the corresponding domains of the protein may be more remote from the energy-transducing and switching mechanisms. Alternatively, if Yamaguchi is correct in concluding (see Results) that there are two distinct genes, *flaAII.1* and *flaAII.2*, then the *flaAII.2* protein may be a component of the energy-transducing and switching mechanism, and the *flaAII.1* protein may play some more general structural role.

It is interesting that although there are numerous example of mutants in which the relative probabilities of rotation in the two senses is abnormal, and of mutants in which rotation in neither sense is possible, no mutants have been described in which rotation in one sense only is impaired. This suggests that the mechanism may be highly symmetrical, so that a sense-specific defect is improbable. Alternatively, if rotation in one sense is impossible, the motor may, instead of stopping, be constrained to remain in the other sense of rotation. There is precedent for the latter type of override mechanism; wild-type cells in high-viscosity media swim indefinitely, presumably because switching to the CW sense of rotation is energetically infeasible (17).

Further evidence for the close relationship between rotation and switching, and the central involvement of *flaAII* in both, comes from the observation that the CCW state becomes progressively favored in strain MY1 as motility is thermally impaired. Khan and Macnab (8) found that whenever motor speed is reduced by reducing the driving force (proton motive force), the CCW sense is favored; in contrast, reduction of motor speed by increasing the frictional load has no effect on switching probabilities. Suppose the *flaAII* protein were part of the bearing structure of the motor and did not participate in any way in the actual generation of torque. Then, a conformation change in the *flaAII* protein, caused by raising the temperature, might make it harder for the motor to rotate, but the energy-transducing mechanism would not be able to distinguish this effect from one produced by increasing the viscosity of the external medium, and therefore switching probabilities should not be altered. The experimental observation, however, is that switching probabilities are altered, indicating that the *flaAII* protein plays an active role in energy transduction in the motor.

The properties of the mutant MY1 emphasize that, although the gene symbol *che* was original-

ly chosen to describe mutants which were chemotactically defective, it more properly refers to mutants which have switching probabilities that differ from those of wild-type cells. It has been known for many years that certain mutants (e.g., *cheB* [3]), although operationally Che$^-$ in swarm or capillary assays, nonetheless display tactic responses in temporal gradient assays. MY1, which has a moderate CW bias (and therefore is by definition a *che* mutant), is in fact superior to the wild type in swarming ability, despite a somewhat lower swimming speed. It appears that there is a range of unstimulated CW bias (perhaps from about 5 to 50%) which permits sufficient modulation by spatial gradient stimuli to yield an effective migrational response and that the bias of wild-type cells is not optimal under all conditions.

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