Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5956–5960, July 1992
Cell Biology

The cytoplasmic component of the bacterial flagellar motor

(molecular motors/chemiosmotic coupling/bacterial motility)

IMRAN HUMAYUN KHAN*, THOMAS S. REESE†, AND SHAHID KHAN*

*Departments of Anatomy & Structural Biology and Physiology & Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461; and †Laboratory of Neurobiology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Contributed by Thomas S. Reese, April 2, 1992

ABSTRACT **We have used electron microscopy to examine freshly isolated *Salmonella typhimurium* and *Escherichia coli* basal flagellar fragments, purified without resort to extremes of pH or ionic strength. Such fragments contain the large bell-like basal structures visualized recently in freeze-substituted or fixed preparations. We have found *mot* (non-motile) mutants produced by lesions in *fli* genes (*G, M, N*) in which the bell structures do not coisolate with the flagellar basal body. The coisolation of the bell with the flagellar basal body was unaffected in strains lacking the genes for the motility-associated Mot proteins or for the Che family of proteins, which are necessary for chemotaxis. Proper assembly and interaction of the cytoplasmically located bell with the membrane-associated flagellar basal structures appears to be necessary for motor function. The FliG, FliM, and FliN proteins are thought to form a structural complex responsible for energization and switching of the flagellar motor. Our findings are consistent with the existence of such a complex and imply that it forms part of the flagellar bell.**

The rotation of bacterial flagella is energized by electrochemical gradients across the cytoplasmic membrane (1). The flagellar base embedded in the cell envelope presumably contains the motor components responsible for energizing rotation of the flagellum, but the structures responsible for flagellar rotation have not been completely determined.

Flagellate but nonmotile mutant strains, *mot* mutants, of the enteric bacteria *Salmonella typhimurium* and *Escherichia coli* provide a key to determination of flagellar motor structure and, thus, to unraveling its mechanism of operation. Genetic analyses of the motility and chemotaxis apparatus in these bacteria have identified five genes that when mutated give rise to the Mot phenotype (2). For instance, *mot* mutants are produced upon deletion of either the *motA* or *motB* gene, both of which code for transmembrane proteins (3, 4). Small lesions in any one of the other three genes (*fliG, fliM*, and *fliN*) can produce *mot* or *che* (motile but nonchemotactic) mutant bacteria, though deletion results in nonflagellate bacteria. Analysis of suppresser mutations suggests that the FliG, FliM, and FliN proteins form a structural complex (5, 6). It has also been postulated that this complex may interact with CheY and CheZ, two cytoplasmic proteins involved in switching of the rotation sense (5, 7).

We have shown (8) that the *motA* and *motB* gene products in *E. coli* are responsible for the formation of the intramembrane particle rings that surround flagellar bases. Subsequently, we identified (9) bell-like cytoplasmic structures continuous with the flagellum in rapidly frozen *S. typhimurium* cell envelope preparations, but technical limitations on sample size made it impossible to determine which features of these structures are typical. Earlier literature documented cytoplasmic structures contiguous with flagella in whole bacteria (for review, see refs. 9 and 10), but again it was not clear whether such structures were a typical feature of flagellar bases or composed of material representing, for instance, a transient stage in flagellar morphogenesis. Only the work on *Spirillum volutans* is free from such criticism because of the uniformity of the basal structures in the characteristic polar flagellar clusters (see figure 4 in ref. 10).

We focused initially on developing methods for isolation and purification of the intramembrane and cytoplasmic flagellar structures, starting from currently employed protocols (11, 12), and found, to our surprise, that flagellar bases isolated by these protocols contained the large bell-like structures associated with the basal bodies of glutaraldehyde-fixed flagella (13). However, these structures rapidly degraded during the subsequent purification procedure. They also degraded with time, albeit more gradually.

An alternative protocol for purification of flagellar bases was developed in which the bell-like structures appeared routinely as part of the basal flagellar complexes. Since isolated flagellar preparations cannot be directly assayed for motor rotation or flagellar assembly, structure–function relationships were probed by studying flagellar bases isolated from mutant strains. Flagellar bases of strains carrying *mot* lesions of the *fliG, fliM*, and *fliN* genes lacked the bell but the coisolation of the bell with the flagellum was unaffected by absence of the Mot (A and B) or the Che (A, B, R, W, Y, and Z) proteins. On the basis of these data and previous work (8, 14), we propose that the energy-transducing complex for flagellar rotation is composed of two structural modules: (*i*) a membrane module responsible for ion transport and anchorage to the cell wall and (*ii*) a soluble cytoplasmic module responsible for controlling access of the energizing protons to the cytoplasm.

MATERIALS AND METHODS

Strains. *S. typhimurium* SJW1759, SJW2818, and SJW2817 carried *mot* alleles of *fliG*; SJW1764, SJW1796, and SJW1813 carried *mot* alleles of *fliM*; and SJW1784, SJW1775, and SJW1794 carried *mot* alleles of *fliN* (6). *S. typhimurium* SJW2960 carried a *motA–motB* deletion. Parent strain *S. typhimurium* SJW1103 was wild-type for motility and chemotaxis. The *S. typhimurium* strains were isolated by S. Yamaguchi and obtained from R. M. Macnab (Yale University). Other parent strains used were *S. typhimurium* ST1 (9) and *E. coli* RP437 (15). *E. coli* HCB627 (16), derived from RP437 and with the deletion of *cheY, cheZ, cheA, cheW, cheB*, and *cheR*, was a gift from A. Wolfe.

Isolation of Basal Flagellar Fragments. The protocol we used to isolate basal flagellar fragments containing the bell structures is based on the protocol of Aizawa *et al.* (12) but differs from it in important ways (Fig. 1). We replaced steps involving changes in pH (to dissolve cellular debris and the flagellar filament) with mechanical manipulations. The bac-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: HBB, hook basal body.

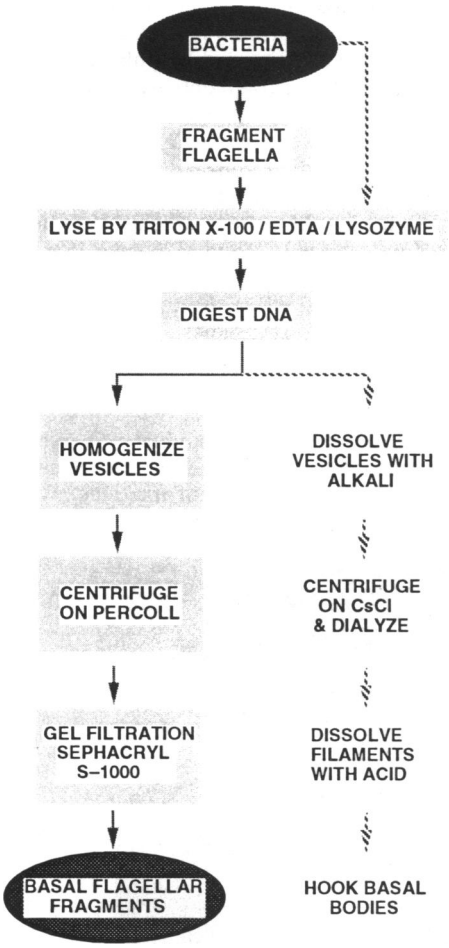

FIG. 1. Isolation of flagellar bases. Solid arrows indicate the steps for isolation of basal flagellar fragments. Hatched arrows indicate previously published steps for isolation of hook basal bodies (HBBs) (12).

teria were grown at 35°C, checked for motility, and harvested at late exponential phase. After resuspension of the cells in buffer A (50 mM NaCl/10 mM Tris·HCl, pH 8.0), the flagella were fragmented by blending (commercial Osterizer blender set at 17,900 rpm). The bacteria, retaining only basal flagellar stubs, were centrifuged, washed thrice in buffer A, and then resuspended in buffer B [0.1 M Tris·HCl (pH 8.0)]. Buffer B also contained the following protease inhibitors: soybean trypsin inhibitor (100 μg/ml), leupeptin (10 μg/ml), and *p*-tosyl-L-arginine methyl ester (TAME; 10 μg/ml, ref. 17). Cells were lysed by addition of 1% Triton X-100, 10 mM EDTA, and freshly made lysozyme (100 μg/ml; Sigma). The lysate typically cleared within 30 min at 4°C; $MgSO_4$ (final concentration, 10 mM) was then added, and the lysate was incubated at 30°C for 20 min to get rid of the endogenous DNA. Unlysed cells were centrifuged (12,000 × *g* for 20 min), and the flagellar fragments in the supernatant were pelleted (60,000 × *g* for 1 hr) and resuspended in 1–5 ml of buffer C (10 mM Tris·HCl, pH 8.0/5 mM EDTA/1% Triton X-100). Initially, we examined the basal flagellar fragments in negatively stained preparations by electron microscopy (diluting them appropriately in buffer), even though large areas of the grid needed to be scanned to count statistically significant numbers of samples. The wild-type populations predominantly contained the large bell-like basal structures, but these structures disappeared with time, even at 4°C. In one experiment, 67% of a wild-type *S. typhimurium* population contained these structures at this stage; by 80 hr, this fraction had decreased to 36%. The bell structures were rapidly degraded within 20 min at room temperature upon exposure to high pH (pH 11.0), low pH (pH 2.5), or high salt (2.0 M CsCl or NaCl). Heat treatment (50°C) for 5 min at pH 7.8 in 10 mM Tris/5 mM EDTA/0.1% Triton X-100 (TET buffer; ref. 12) had a similar effect. The bell structures were unaffected in 50% Ficoll or Percoll solutions. These factors guided further purification of the basal flagellar fragments.

Shearing of the buffer C suspension, by passage back and forth between two 5-ml syringes (21-gauge needles), mechanically disrupted outer membrane debris. EDTA at 5 mM was also required but greater EDTA concentrations degraded the flagella. The effectiveness of the shearing procedure was monitored by measuring the decrease in the ratio of porins to flagellin with conventional SDS/PAGE (data not shown). Sometimes the reduction in debris permitted electron microscopic examination of the flagellar fragments after washing and appropriate dilution in buffer. It was usually desirable to further purify the fragments by Percoll density gradient centrifugation and Sephacryl S-1000 (Pharmacia LKB) column gel filtration (18). The lead fractions, free of Percoll, were pooled, and the basal flagellar fragments were centrifuged and resuspended in 0.1 ml of TET buffer (pH 8.0) for electron microscopic examination.

Electron Microscopy of Negatively Stained Preparations. The samples were stained with 2% (wt/vol) aqueous uranyl acetate prefiltered with Whatman 2 filter paper. A 5-μl aliquot of sample was applied to glow-discharged Formvar- and carbon-coated 200-mesh grids and incubated for 2 min, and then excess liquid was daubed off with filter paper. Stain (10 μl) was immediately applied and the excess was similarly removed after 20 sec. The grids were dried and viewed at 80 kV on a JEOL-100-CX at ×10,000–50,000.

RESULTS

An improved method for purification of flagellar basal structures yielded fields of view of flagellar fragments that were free of cellular debris. In contrast to the results with previous methods (9), the homogeneity of the basal structures could be readily assessed by electron microscopic examination. Flagellar fragments isolated from wild-type strains, could be

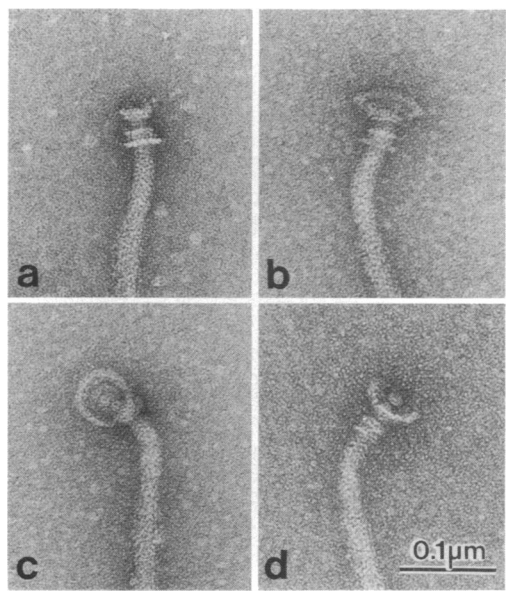

FIG. 2. Flagellar basal structure (negatively stained preparation) viewed by electron microscopy. (*a*) Standard HBB structure. (*b–d*) Aspects of the bell basal structure. (*b* and *d*) Side views. (*c*) *en face* view. (*c* and *d*) Rod-like extension of the hook into the bell interior.

classified into two major groups (Fig. 2). One group contained the standard basal structure typical of HBB preparations (Fig. 2*a*): two sets of basal rings (L/P and M/S) were coaxially arranged around a cylindrical rod contiguous with the thicker hook and filament (19). The other group had a large basal bell structure contiguous with the basal body; a cytoplasmic location for this structure could be inferred from the fact that the M/S ring of the basal body is known to be associated with the cytoplasmic membrane, lying within or at the cytoplasmic face of this membrane (10). Various aspects of this structure, including *en face* views, were apparent (Fig. 2*b–d*). There was a reassuring resemblance of the *en face* views of the isolated structure to the cytoplasmic flagellar structure visualized in *S. typhimurium* cell envelope preparations (see figure 3 in ref. 9) and, indeed, the cytoplasmic structures found in other bacteria (e.g., see figure 4 of ref. 10). Tilted views of the bell plus interactions with the support film and stain generated the "caped" (Fig. 2*b*) and "wingnut" (Fig. 2*d*) structures reported by others (13, 20). A central feature of the bell structure (Fig. 2*d*) could correspond to the "dome" feature noted in ref. 13 or the rod seen in rapidly frozen cell envelope preparations (9). A detailed description of the complex bell structure will be published separately.

The majority of flagellar fragments isolated from wild-type *S. typhimurium* ST1 and SJW1103 and *E. coli* RP437 contained the bell structure (Fig. 3*a* and Table 1). In some instances, dome-shaped or rod-like protrusions that were both more irregular and smaller than the bell extended out from the M/S ring; in others the fragments were broken at the rod junction between the L/P and M/S rings. Structures that could not be categorized as either standard HBB or bell basal complexes made up 5–15% of the total population derived from wild-type structures (Table 1).

It was apparent from the data on the wild-type structures, particularly for *S. typhimurium*, that basal flagellar fragments including the bell could be isolated without glutaraldehyde fixation (13). However, the bell module was either labile or had a labile association with the M/S ring because it disappeared with time in the absence of fixatives. We therefore looked for mutants in which this lability might be accentuated.

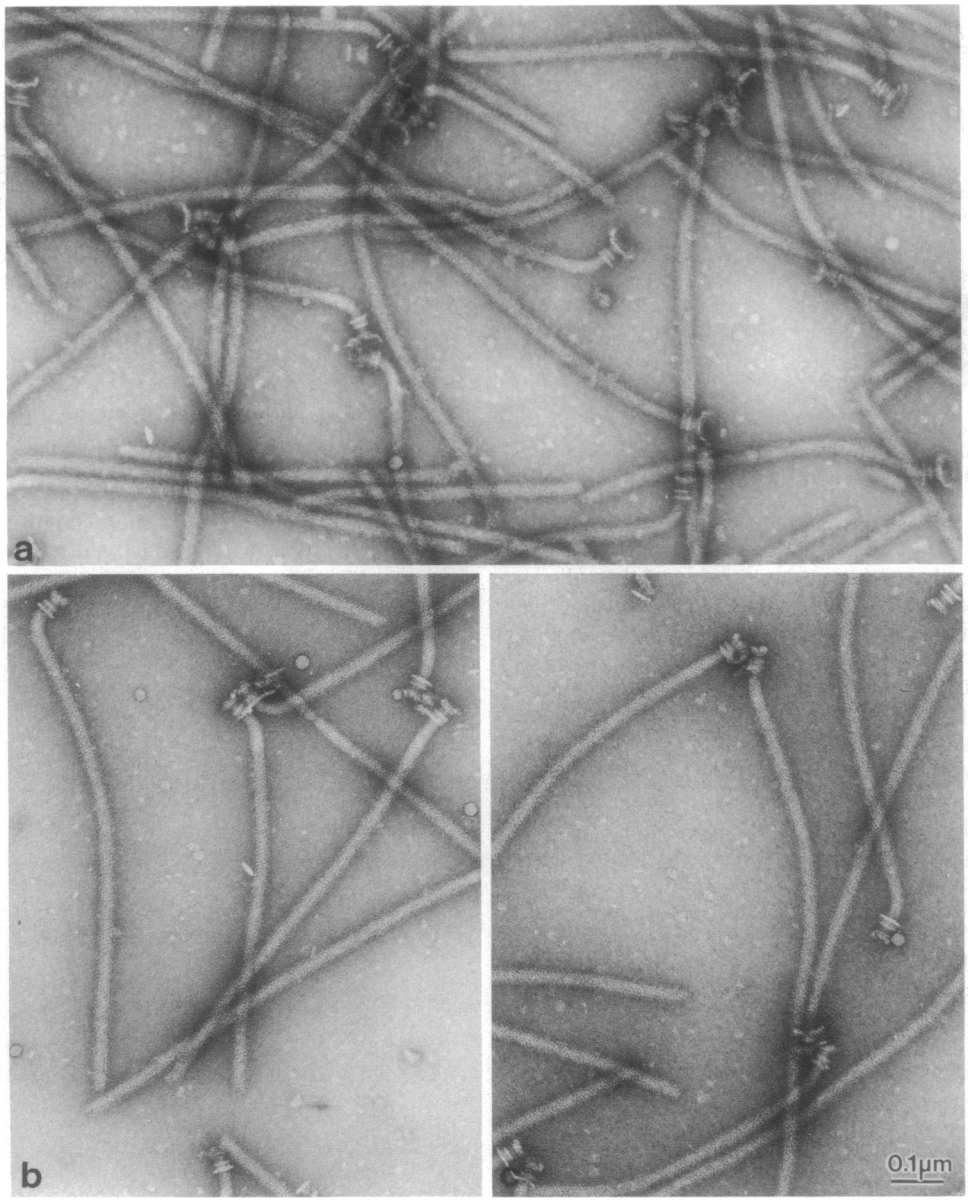

FIG. 3. Low magnification overviews of preparations of basal flagellar fragments prepared to preserve the bell structures. (*a*) *S. typhimurium* ST1, wild-type for motility and chemotaxis, showing the presence of the bell structure. (*b*) *S. typhimurium* SJW1759 carrying a *fliG mot* allele. (*c*) *S. typhimurium* SJW1784 carrying a *fliN mot* allele. Both of these *fli* mutants lack the bell structure.

Table 1. Characteristics of flagellar bases in various mutants

Strain	Genotype	Type of base, % of total			N
		Belled	Standard	Intermediate	
S. typhimurium SJW1103	Wild-type	80	16	4	622
E. coli RP437	Wild-type	44	43	13	663
S. typhimurium SJW1759	*fliG mot*	2	87	11	448
S. typhimurium SJW2818	*fliG mot*	0	56	44	772
S. typhimurium SJW2817	*fliG mot*	0	74	26	543
S. typhimurium SJW1764	*fliM mot*	0	93	7	429
S. typhimurium SJW1796	*fliM mot*	0	51	49	808
S. typhimurium SJW1813	*fliM mot*	0	49	51	810
S. typhimurium SJW1784	*fliN mot*	0	82	18	490
S. typhimurium SJW1794	*fliN mot*	0	78	22	514
S. typhimurium SJW1775	*fliN mot*	0	49	51	809
S. typhimurium SJW2960	Δ(*motA–B*)	73	21	6	433
E. coli HCB627	Δ(*cheA–Z*)	45	44	11	451

N, number of flagellar bases counted for each genotype. Duplicate preparations of basal flagellar fragments were made for each strain.

An extensive library of mutant strains carrying *fliG*, *fliM*, and *fliN mot* and *che* alleles is now available in *S. typhimurium*. We examined three *mot* mutant strains for each gene, chosen on the basis of intragenic complementation (6) and sequence data (ref. 7; R. M. Macnab, personal communication). Each mutant carried an alteration in the primary amino acid sequence distinct from that carried by the other two in the set. Basal flagellar fragment populations obtained from these strains overwhelmingly lacked the bell structures (Table 1 and Fig. 3 *b* and *c*). The flagellar fragments contained the standard HBB structure, morphologically identical to comparable structures found in the wild-type parents (Fig. 2*a*). Table 1 summarizes the distributions of the two major types of structures found in basal fragments isolated from wild-type populations: the standard HBB and the bell. These forms represent two extremes in terms of the extent of cytoplasmic basal structure. As in wild-type populations, a fraction of the fragments in these mutant populations contained basal complexes intermediate in size between the standard and belled type, with irregular-dome or rod-shaped extensions from the cytoplasmic face of the HBB M/S ring. Flagellar bases with such extra structural features occurred with a frequency comparable to that found for wild-type populations in some mutants (SJW1759, SJW1764, SJW1784, and SJW1794) but accounted for about half of the population in others (SJW2818, SJW1796, SJW1813, and SJW1775). The fact that the percentage of these structures increased in mutant populations lacking the bell suggests that they could be partially degraded bell structures. An analysis of images of these intermediate basal structures visualized by methods less disruptive than negative stain (21) will be published separately.

Analysis of the mutant strains shows that small *mot* lesions of the *fliG*, *fliM*, or *fliN* genes can have a striking effect on the coisolation of the bell with the rest of the flagellar base. The remarkable difference between wild-type bacteria and these mutants was underscored by examination of basal flagellar fragments from strains deleted for *motA* and *motB* genes or all of the *che* genes. The association of the bell with the flagellum was unaffected in the deleted strains (Table 1).

DISCUSSION

A cytoplasmic component of the bacterial flagellum, the bell structure, is here analyzed in several different mutants in which flagellar motility is affected. A modification of existing protocols for isolating and purifying flagellar bases had to be developed to reproducibly show the bell structure in negatively stained preparations by electron microscopy. We initially examined *mot* mutants where we hoped to find examples of defective interactions of the bell with the membrane-associated basal structures because the Mot phenotype results from defective transmembrane transport of the energizing protons or its uncoupling from force production (1). We found that deletion of the *mot* or *che* genes left the bell structure unaffected. Mutants deleted for *fliG*, *fliM*, or *fliN* genes could not be used because they are nonflagellate. We reasoned, however, that if the FliG, FliM, and FliN proteins form a major part of the bell, they would be present at high copy numbers consistent with the large size of the bell. Small perturbations of their structure might significantly affect their interactions with each other and with other flagellar components, such as the basal body M/S ring. Indeed, the bell structure was dramatically affected in strains carrying *mot* alleles of the *fliG*, *fliM*, and *fliN* genes. These observations indicate that the cytoplasmically located bell module, or a part of it, is a structural component of the flagellar motor. However, these data do not address whether the bell is needed for flagellar assembly because the bell could be lost during isolation.

Geneticists have long postulated (5) that a complex of the FliG, FliM, and FliN proteins, the "switch complex" (6), might be a part of the bacterial motor machinery. The switch complex could just as well be named the "motor complex," since mutations in all three *fli* genes give rise to *mot* and to *che* alleles. The fact that very similar structural defects are found in the three *mot* mutant groups, corresponding to these three genes, is consistent with this hypothesis and implies that such a complex forms part of the bell and governs its interaction with the basal body. The data presented in this study are most easily explained in terms of the rationale, outlined above, that led us to initiate the mutant work and this hypothesis is also consistent with independent evidence that the FliG protein is part of the flagellar base. Temperature-sensitive *fliG* mutants undergo rapid loss of flagellar rotation upon shift to restrictive temperature (22) and immunolabeling of HBBs isolated by conventional methods from motile mutant strains carrying *fliF–fliG* fusions suggest a basal cytoplasmic location for FliG (23). We cannot rule out the possibility that the FliG, and FliN proteins help with the assembly of, rather than form, the bell structure. If so, these proteins would constitute rather remarkable examples of assembly proteins in which mutant forms disrupt assembly in a graded fashion and allow assembly of imperfect basal cytoplasmic structures. These structures would have to support flagellar formation but would confer a lack of motility (*mot* mutants) or altered motility (*che* mutants).

The *fliG*, *fliM*, and *fliN mot* mutant strains examined here constitute a significant fraction, ≈31% (ref. 7; R. M. Macnab, personal communication), of the *mot* mutants isolated for these three genes in *S. typhimurium*. While we had hoped to find examples of *mot* lesions caused by destabilization of the Fli proteins, which would then be amplified to alter the quaternary structure of the multisubunit assembly, it seems remarkable that all of the mutants examined apparently fall into this category. For instance, mutant forms with impaired function but conserved tertiary structure, resulting from single residue changes at the active site, are common in T4 lysozyme (24). Perhaps protein domains responsible for proton transport or force generation require simultaneous alteration of many residues for disruption of function.

It is also striking that many of the mutants examined here carry subtle alterations at the level of primary structure. The *fliM mot* mutant strain SJW1764, for instance, arises from a single Phe → Leu substitution (7). Such data are consistent with the proposal that the net free energy of stabilization of proteins is small and may be significantly perturbed by changes in single amino acids (25, 26).

The nature and location of the elastic deformations of motor proteins responsible for force production remain unknown. It has been postulated that elastic-force-producing domains are located in the periplasm, forming linkages with the cell wall (27, 28). The MotB protein, thought to form the flagellar intramembrane ring particles together with MotA (8), has a large periplasmic domain (29) that may form such an elastic linkage. An alternative possibility is that the motor proteins reside in the cytoplasm, with the flagellar intramembrane ring particles simply serving for anchorage and ion transport. Analogous construction of other motile (30, 31) and chemiosmotic (32) energy-transducing systems make such a possibility attractive. In particular, the involvement of cytoplasmic structure in the energization of the flagellar motor is consistent with a kinetic analysis that showed that the sites in the proton transport pathway responsible for control of the energizing flux lie outside the transmembrane electric field, toward the cytoplasm (14). Force-producing configurational transitions within the flagellar bell could control the energizing ion flux analogous to the control exerted by ATP synthase reactions occurring within the cytoplasmic modules of the membrane ATPases (32).

Almost certainly, other components of the flagellar base await isolation. These include the intramembrane particle rings and, possibly, additional cytoplasmic or periplasmic structures (see figure 4 of ref. 9 and figure 2 of ref. 13). Nevertheless, the copurification of the bell module with the HBB complex of the flagellar base should enable detailed analysis of the physicochemical properties of this structure and, therefore, enhance understanding of mechanochemical coupling in the flagellar motor.

We thank Dr. Robert M. Macnab for the *S. typhimurium* strains used in this study and for communication of results prior to publication, Dr. Stephan Schuster for suggesting fragmentation of flagella prior to detergent extraction, John Chludzinski for expert photography, and Cheng Huang for help in examination of some mutants.

This work was supported by Public Health Service Grant GM36936 (to S.K.) from the National Institutes of Health. I.H.K. was supported by Biomedical Training Grant GM07491 from the National Institutes of Health.

1. Khan, S. (1990) in *The Bacteria: A Treatise on Structure and Function*, ed. Krulwich, T. A. (Academic, New York), Vol. 12. pp. 301–343.
2. Iino, T., Komeda, Y., Kutsukake, K., Macnab, R. M., Matsumura, P., Parkinson, J. S., Simon, M. I. & Yamaguchi, S. (1988) *Microbiol. Rev.* **52**, 533–535.
3. Wilson, L. & Macnab, R. M. (1988) *J. Bacteriol.* **170**, 588–597.
4. Wilson, L. & Macnab, R. M. (1990) *J. Bacteriol.* **172**, 3932–3939.
5. Parkinson, J. S., Parker, S. R., Talbert, P. B. & Houts, S. E. (1983) *J. Bacteriol.* **155**, 265–274.
6. Yamaguchi, S., Aizawa, S.-I., Kihara, M., Isomura, M., Jones, C. J. & Macnab, R. M. (1986) *J. Bacteriol.* **168**, 1172–1179.
7. Sockett, H., Yamaguchi, S., Kihara, M., Irikura, V. M. & Macnab, R. M. (1992) *J. Bacteriol.* **174**, 793–806.
8. Khan, S., Dapice, M. & Reese, T. S. (1988) *J. Mol. Biol.* **202**, 575–584.
9. Khan, S., Khan, I. H. & Reese, T. S. (1991) *J. Bacteriol.* **173**, 2888–2896.
10. Swan, M. A. (1985) *J. Bacteriol.* **161**, 1137–1145.
11. DePamphilis, M. & Adler, J. (1971) *J. Bacteriol.* **105**, 376–383.
12. Aizawa, S.-I., Dean, G. E., Jones, C. J., Macnab, R. M. & Yamaguchi, S. (1985) *J. Bacteriol.* **161**, 836–849.
13. Driks, A. & DeRosier, D. J. (1990) *J. Mol. Biol.* **211**, 669–672.
14. Khan, S., Dapice, M. & Humayun, I. (1990) *Biophys. J.* **57**, 779–796.
15. Parkinson, J. S. (1978) *J. Bacteriol.* **135**, 45–53.
16. Wolfe, A. J., Conley, M. P. & Berg, H.C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6711–6715.
17. Vale, R. D., Reese, T. S. & Sheetz, M. P. (1985) *Cell* **41**, 39–50.
18. Hjorth, R. & Pertoft, H. (1982) *Biochim. Biophys. Acta* **688**, 1–4.
19. Sosinsky, G. E., Francis, N. R., Stallmeyer, M. J. B. & DeRosier, D. J. (1992) *J. Mol. Biol.* **223**, 171–184.
20. Schuster, S. C. & Bauerlein, E. (1992) *J. Bacteriol.* **174**, 263–268.
21. Serwer, P. (1977) *J. Ultrastruct. Res.* **58**, 235–243.
22. Dean, G. E., Aizawa, S.-I. & Macnab, R. M. (1983) *J. Bacteriol.* **154**, 84–91.
23. Francis, N., Irikura, V. M., Yamaguchi, S., DeRosier, D. J. & Macnab, R. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6304–6308.
24. Grutter, M. G., Hawkes, R. B. & Matthews, B. W. (1979) *Nature (London)* **277**, 667–669.
25. Matsumura, M., Becktel, W. J. & Matthews, B. W. (1988) *Nature (London)* **334**, 406–410.
26. Shortle, D. (1989) *J. Biol. Chem.* **264**, 5315–5318.
27. Meister, M., Caplan, S. R. & Berg, H. C. (1989) *Biophys. J.* **55**, 905–914.
28. Stolz, B. & Berg, H. C. (1991) *J. Bacteriol.* **173**, 7033–7037.
29. Chun, S.-Y. & Parkinson, J. S. (1988) *Science* **239**, 276–278.
30. Sheetz, M. P., Steur, E. R. & Schroer, T. A. (1989) *Trends Neurosci.* **12**, 474–478.
31. Schroer, T. A. & Sheetz, M. P. (1991) *J. Cell Biol.* **115**, 1309–1318.
32. Maloney, P. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington), Vol. 1, pp. 222–243.