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# Assembly of the Switch Complex onto the MS Ring Complex of *Salmonella typhimurium* Does Not Require Any Other Flagellar Proteins

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**The cytoplasmic portion of the bacterial flagellum is thought to consist of at least two structural components: a switch complex and an export apparatus. These components seem to assemble around the MS ring complex, which is the first flagellar basal body substructure and is located in the cytoplasmic membrane. In order to elucidate the process of assembly of cytoplasmic substructures, the membrane localization of each component of the switch complex (FliG, FliM, and FliN) in various nonflagellated mutants was examined by immunoblotting. It was found that all these switch proteins require the MS ring protein FliF to associate with the cell membrane. FliG does not require FliM and FliN for this association, but FliM and FliN associate cooperatively with the membrane only through FliG. Furthermore, all three switch proteins were detected in membranes isolated from *fliE*, *fliH*, *fliI*, *fliJ*, *fliO*, *fliP*, *fliQ*, *fliR*, *flhA*, *flhB*, and *flgJ* mutants, indicating that the switch complex assembles on the MS ring complex without any other flagellar proteins involved in the early stage of flagellar assembly. The relationship between the switch complex and the export apparatus is discussed.**

Motile bacteria such as *Escherichia coli* or *Salmonella typhimurium* rotate flagella to propel the cell body in liquid. The flagellum consists of three structural parts: filament, hook, and basal body (for reviews, see references 11 and 20). The intact flagellum was defined based on information from structures isolated from membranes (3). In fact, at least three more substructures are believed to be required in vivo to construct a functional flagellum: an export apparatus, a switch complex, and a Mot complex. The Mot complex is not required until the flagellum is completed, whereas the export apparatus and the switch complex are known to be required at the very beginning of construction (for a review, see reference 2). However, the details of the interaction between the two structures are unclear.

The switch complex is composed of three proteins: FliG, FliM, and FliN (29). The physical interactions among these proteins have been shown by genetic, biochemical, and molecular-biological methods. Genetic analyses showed that a function lost by a defect of a given switch protein could be restored by a defect in one of the other proteins, suggesting that all three proteins interact with one another (30). The first decisive evidence of this direct interaction was obtained serendipitously with a switch mutant in which FliG was fused to FliF (a component of the MS ring complex) by a small deletion in the overlapping region of the two genes (5). Oosawa et al. (23) overproduced and purified the three switch proteins and examined the direct interactions among them in vitro. Those authors confirmed the strong interaction between FliG and the MS ring complex (FliF) and showed a weak interaction between FliM and FliG but found no interaction between FliN and any other proteins. The yeast two-hybrid system was employed to detect weak interactions between any combination of pairs of the three proteins and showed that FliG binds to FliF, FliM binds to FliG, and FliN binds to FliM (21). Decisive evidence of the direct interaction between FliM and FliN was obtained by constructing a fusion of the two proteins; the mutant cells could swarm on a semisolid plate (15).

A structure made of switch proteins seems to be fragile, since the hook-basal body complex purified by traditional methods (1) does not contain any of them. Under milder conditions, a ring-shaped appendage called the C ring was found at the cytoplasmic face of the MS ring of the basal body (13). Immunoelectron microscopy showed that all three switch proteins are located on the cytoplasmic surface of the M ring or in the C ring (5, 6). In some switch mutants with the Mot phenotype, the C ring was incomplete or totally missing (31).

Much less is known about the export apparatus. It is obvious that machinery for exporting only flagellar proteins exists, since most external flagellar proteins lack the signal sequence that is recognized by the primary export system (8, 9, 22). However, information about its structure and components is still fragmentary. Vogler et al. (27) demonstrated using temperature-sensitive mutants that several gene products were necessary for the regrowth of filaments. One of them, FliI, has sequence similarity to the catalytic subunit of the $F_0F_1$ ATPase, and functional similarity was supported by a finding that FliI binds ATP in vitro (4) and hydrolyzes it (4a). This may indicate an evolutionary relationship between the flagellar motor and the $F_0F_1$ ATPase.

The export apparatus, if it is a part of the flagellum, is more fragile than the C ring, since even under mild conditions it is missing from the flagellar structure. A quick-freeze method revealed an additional, rod-like structure, which could be a candidate for the export apparatus, at the center of the C ring on the cytoplasmic surface of intact cells (12).

Investigations of the intermediate structures of flagella have revealed the morphological pathway of flagella (10, 16, 24). In this study, we focused on the early steps of flagellar assembly and analyzed the cytoplasmic components of flagella. Using anti-FliG, -FliM, and -FliN antibodies, we studied localization of the three proteins in various flagellar mutants to determine whether a gene of interest is necessary for the switch proteins

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TABLE 1. Strains of *S. typhimurium* used in this study

| Strain | Relevant genotype | Function of product of mutated gene | Reference or source |
|---|---|---|---|
| SJW1103 | None (wild type) | | 24 |
| SJW1684 | Δ*fliF* | MS ring | 16 |
| SJW1611 | Δ*fliG* | Switch | 16 |
| SJW1407 | Δ*fliM* | Switch | This study |
| SJW1408 | Δ*fliM* | Switch | This study |
| SJW2370 | Δ*fliM* | Switch | This study |
| SJW2409 | Δ*fliM* | Switch | This study |
| SJW2343 | *fliN* | Switch | This study |
| SJW1475 | Δ(*fliM-fliN*) | Switch | This study |
| SJW1602 | *fliA* | Sigma F | This study |
| SJW1371 | Δ*fliE* | Putative rod | 16 |
| SJW1381 | Δ*fliE* | Putative rod | 16 |
| SJW2707 | *fliH* | Putative export | 16 |
| SJW2748 | *fliH* | Putative export | This study |
| SJW2726 | Δ*fliI* | Putative export | This study |
| SJW2702 | Δ*fliI* | Putative export | 16 |
| SJW112 | *fliJ* | Unknown | This study |
| SJW1357 | *fliO* | Unknown | This study |
| SJW1628 | Δ*fliP* | Unknown | This study |
| SJW1401 | *fliP* | Unknown | This study |
| SJW180 | Δ*fliQ* | Unknown | This study |
| SJW169 | Δ(*fliQ-fliR*) | Unknown | This study |
| SJW1544 | Δ(*fliP-fliR*) | Unknown | This study |
| SJW192 | Δ(*fliO-fliR*) | Unknown | 16 |
| SJW1616 | Δ*flhA* | Putative export | 16 |
| SJW1520 | Δ*flhA* | Putative export | This study |
| SJW1467 | Δ*flhB* | Unknown | 16 |
| SJW1532 | Δ*flhB* | Unknown | This study |
| SJW1649 | Δ*flhB* | Unknown | This study |
| SJW1160 | Δ*flgE* | Hook | 24 |
| SJW1473 | *flgE* | Hook | This study |
| SJW1437 | Δ*flgJ* | Unknown | This study |

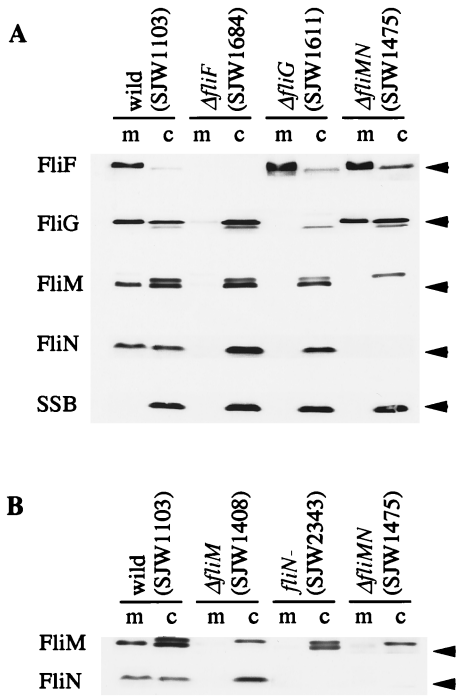


FIG. 1. Immunoblotting of membrane (m) and cytoplasmic (c) fractions prepared from wild-type and nonflagellated mutant strains. The genotypes of the strains are indicated at the top of each lane. Proteins detected with corresponding antibodies are indicated at the left. Arrowheads indicate the region containing major signaling bands cut from polyvinylidene difluoride membranes that were reacted separately with each antibody.

to assemble on the membrane. Interactions among the switch proteins during assembly are discussed.

## MATERIALS AND METHODS

**Bacterial strains and growth condition.** *S. typhimurium* strains used in this study are listed in Table 1. Cells were grown to late exponential phase in 100 ml of Luria broth in a shaking water bath at 37°C.

**Preparation of membrane and cytoplasmic fractions.** Cells were lysed by treatment with 0.1 mg of lysozyme per ml in 10 ml of Tris-EDTA (TE) buffer (10 mM Tris-Cl, 1 mM EDTA [pH 7.9]), stirring at room temperature, and sonication (Branson Sonifier with flat tip; 5 min with 50% duty cycle, 20 s plus 20-s intervals) on ice. Unlysed cells were removed by low-speed centrifugation (7,000 × *g* for 20 min at 4°C). Membranes were separated from cytoplasm by centrifugation at 15,000 × *g* for 15 min at 4°C. One-fiftieth of the preparation of membrane and cytoplasm obtained from 100 ml of culture was applied to each lane of the gels for immunoblotting.

**Electrophoresis and immunoblotting.** Membrane and cytoplasmic fractions were separated in equal amounts by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5 or 10% polyacrylamide). Proteins on sodium dodecyl sulfate gels were electrically transferred to polyvinylidene difluoride membranes in transblotting buffer (25 mM Tris, 192 mM glycine, 20% methanol [pH 8.3]). The membranes were blocked for 30 min with 3% gelatin–TBS (Tris-buffered saline) (20 mM Tris-Cl, 500 mM NaCl [pH 7.5]). They were washed twice with TTBS (0.05% Tween 20, TBS), and were incubated for 1 h with antibodies raised against FliF (26), FliG, FliM, and FliN (23) in 1% gelatin–TTBS with soaking. After being washed twice with TTBS they were incubated for 30 min with goat anti-rabbit immunoglobulin G–horseradish peroxidase (Jackson ImmunoResearch Inc.) in 1% gelatin–TTBS with soaking. The blots were visualized with the Immunostaining HRP kit (Konica, Tokyo, Japan) according to the manufacturer's instructions.

## RESULTS

**FliM and FliN do not interfere with the interaction between FliF and FliG.** Several lines of evidence show that the switch complex binds directly to the MS ring complex composed of FliF protein, and patterns of interaction among the component proteins were partially revealed by in vitro and in vivo assays (6, 21, 23). However, information regarding assembly order among the components is still fragmentary. In particular, although one-to-one correspondence between each component was extensively studied, the influence of other components on their interaction has not been examined (6, 21). In in vitro interaction assays, it was shown that overproduced and purified components interact with one another only at very high protein concentrations (23).

The switch proteins are soluble when present alone and associate with the membrane only when bound to the MS ring complex. We isolated the membrane from nonflagellated mutants and identified flagellar components by immunoblotting.

First, we analyzed FliF, FliG, FliM, and FliN in the membrane fractions isolated from wild-type and switch mutants (Fig. 1A). We used single-stranded DNA binding protein (SSB) as a marker of soluble protein. SSB was detected exclusively in the cytoplasmic fraction, confirming that the membrane fraction prepared by our method was completely devoid of cytoplasmic constituents. FliF was detected predominantly in the membrane fraction of wild-type cells. For *fliG* and *fliM-fliN* deletion mutants, FliF was also detected mostly in the membrane fraction, indicating that FliF can integrate into the membrane without the help of switch proteins, as observed previously (16, 26).

FliG was detected in similar amounts in the membrane and cytoplasmic fractions of wild-type cells (Fig. 1A). The minor band of slightly higher mobility than the FliG band in the cytoplasmic fraction is nonspecific, since the *fliG* deletion mutant retains the band. FliG was detected mostly in the cyto-

plasmic fraction and only slightly in the membrane fraction of the *fliF* mutant, showing that FliF anchors FliG to the membrane. For the *fliM-fliN* mutant, FliG was detected in the membrane fraction as much as in wild-type cells. These results indicate that FliG binds directly to FliF without FliM and FliN.

**FliM and FliN interact cooperatively with the MS ring only through FliG.** Although both FliM and FliN are components of the switch complex, their manner of integration into the complex is still unclear. FliM was detected in similar amounts in the membrane and cytoplasmic fractions of wild-type cells (Fig. 1A). A minor band of slightly lower mobility than the FliM band in the cytoplasmic fraction is nonspecific, since the *fliM-fliN* deletion mutant retains the band. FliN behaves similarly to FliM in wild-type cells; it was found in similar amounts in the membrane and cytoplasmic fractions. For the *fliG* deletion mutant, both FliM and FliN were detected in the cytoplasmic fraction but not in the membrane, indicating that the interaction of these proteins with the MS ring is only through FliG.

For the *fliM* deletion mutant, FliN was detected in the cytoplasmic fraction but not in the membrane fraction (Fig. 1B). Likewise, for the *fliN* mutant, FliM was detected in the cytoplasmic fraction but not in the membrane fraction. Therefore, we conclude that the FliM and FliN proteins require each other for their integration into the switch structure.

**The switch complex forms without the help of any other proteins involved in the early stage of flagellar assembly.** Next we investigated the roles of peripheral proteins in the construction of the switch complex. We examined the location of the switch components in mutants whose genes are thought to be necessary for the formation of other peripheral substructures.

**(i) Effects of component proteins of the export apparatus.** From the results of flagellar regrowth experiments (27), *fliH*, *fliI*, and *flhA* have been determined to be genes coding for components of a putative export apparatus specific for flagellar proteins. We analyzed whether the switch proteins could be detected in the membranes isolated from the *fliH*, *fliI*, and *flhA* mutants (Fig. 2A). For these mutants, FliG, FliM, and FliN were detected in significant amounts in the membrane fraction, indicating that the switch complex forms basically without the *fliH*, *fliI*, and *flhA* products.

**(ii) Effects of other proteins involved in the early stage of flagellar assembly.** Previous studies on flagellar morphogenesis (10, 16) have shown that *fliJ*, *fliO*, *fliP*, *fliQ*, *fliR*, *flhB*, and *flgJ* were necessary in the early stage of flagellar assembly, somewhere in the pathway between MS ring formation and rod formation. Their functions are not known (20). For mutants lacking these genes, FliG, FliM, and FliN were detected in significant amounts in the membrane fraction (Fig. 2B). These results indicate that the switch complex forms basically without the *fliJ*, *fliO*, *fliP*, *fliQ*, *fliR*, *flhB*, and *flgJ* products.

**(iii) Effects of flagellar proteins unrelated to switching or export.** A strain with a mutation in *fliE* which codes for a potential rod component (16) should assemble the MS ring and the switch complex. The *fliA* mutant, which lacks the sigma factor for flagellar late-gene expression, is known to have the complete flagellar hook-basal body structure. The *flgE* deletion mutant lacks the hook protein but has the rod. This mutant should have the export apparatus since rod proteins are exported (8). We used these mutants as positive controls that should have cytoplasmic substructures. For these mutants, FliG was detected in similar amounts in the membrane and cytoplasmic fractions (Fig. 2C). Both FliM and FliN were also detected in significant amounts in the membrane fraction. However, more FliN was detected in the cytoplasmic fraction than in the membrane fraction, as observed for the mutants listed in paragraphs i and ii above (see Discussion).

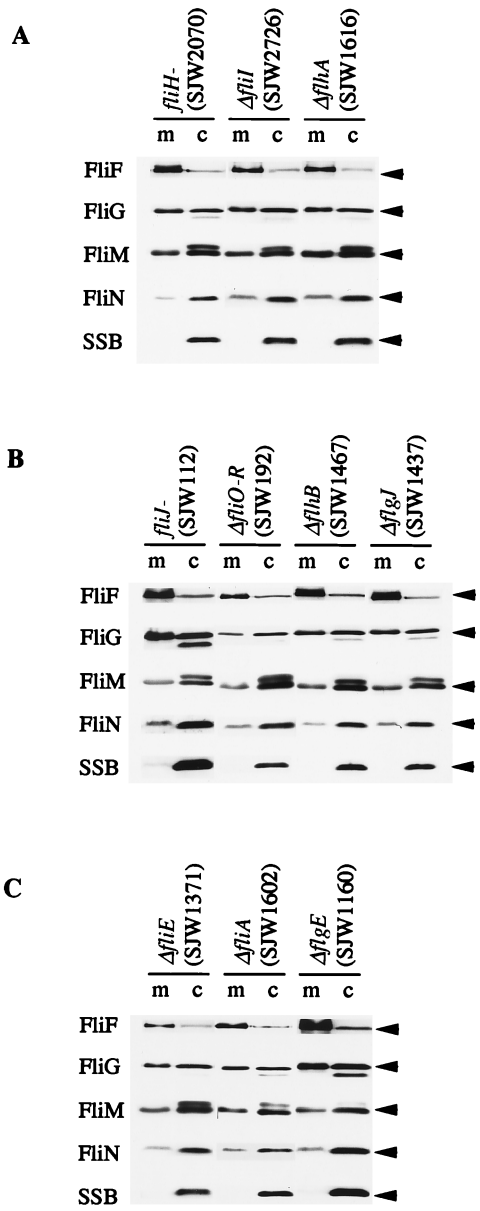


FIG. 2. Immunoblotting of membrane (m) and cytoplasmic (c) fractions prepared from the nonflagellated mutant strains indicated. Other details are as described in the legend to Fig. 1.

The amounts of FliN in the membrane varied from mutant to mutant; for example, they were faint in the membrane fractions from *fliH* and *fliA* mutants. Since FliN does not bind to the membrane at all in the absence of FliM (Fig. 1B), detection of FliN in the membrane fractions of mutants, albeit variable, is enough to prove the integration of FliN into the switch structure. We believe, therefore, that FliN incorporation into the switch complex was not drastically affected by peripheral proteins (see Discussion). We examined several other strains (listed in Table 1) whose mutation sites cover all of the genes for early morphogenesis of a flagellum and got results (data not shown) consistent with those shown in Fig. 1 and 2. The results of the entire set of experiments are summarized in

TABLE 2. Components of MS ring and switch complex detected in membrane fractions from mutant strains

| Mutated gene | Function of its product | Detection of protein[a] | | | |
|---|---|---|---|---|---|
| | | FliF | FliG | FliM | FliN |
| None (wild type) | | + | + | + | + |
| *fliF* | MS ring | − | − | − | − |
| *fliG* | Switch | + | − | − | − |
| *fliM* | Switch | + | + | − | − |
| *fliN* | Switch | + | + | − | − |
| *fliM-fliN* | Switch | + | + | − | − |
| *fliA* | Sigma F | + | + | + | + |
| *fliE* | Putative rod | + | + | + | + |
| *fliH* | Putative export | + | + | + | + |
| *fliI* | Putative export | + | + | + | + |
| *fliJ* | Unknown | + | + | + | + |
| *fliO* | Unknown | + | + | + | + |
| *fliP* | Unknown | + | + | + | + |
| *fliQ* | Unknown | + | + | + | + |
| *fliQ-fliR* | Unknown | + | + | + | + |
| *fliP-fliR* | Unknown | + | + | + | + |
| *fliO-fliR* | Unknown | + | + | + | + |
| *flhA* | Putative export | + | + | + | + |
| *flhB* | Unknown | + | + | + | + |
| *flgE* | Hook | + | + | + | + |
| *flgJ* | Unknown | + | + | + | + |

[a] +, detected; −, not detected.

Table 2. All mutants used here except the *fliF* and switch gene mutants contained the three switch proteins in their membranes. Therefore, we conclude that the switch complex is assembled directly on the MS ring complex without the help of any other flagellar proteins.

## DISCUSSION

We and others have previously revealed the morphological pathway of flagellar construction, focusing on each component of the hook-basal body complex (10, 16, 24). In this study, we have expanded our analysis to include the cytoplasmic substructures of the flagellum by investigating the assembly of the switch complex.

The sole assay in this study is based on detection of switch proteins in the membrane fraction by immunoblotting. The antibodies used reacted with nonspecific proteins. To identify the specifically reacting bands, we confirmed that mutation of the switch genes eliminated them (Fig. 1). FliF was detected predominantly in the membrane, while the switch proteins were detected in the cytoplasmic fraction in amounts equal to or greater than those in the membrane fraction, even in wild-type cells (Fig. 1). This indicates that many molecules of the switch proteins are present in soluble form in cells. To confirm that the detection of switch proteins in the membrane fractions of mutant cells is meaningful, a known soluble protein, SSB, was used as a negative control throughout the experiments.

**Relationship between the MS ring and the switch complex.** Stable FliF-FliG interaction was observed in this and previous studies (5). Physical interactions between FliG and FliM (21, 23) and between FliM and FliN (15) were also observed. These interactions strongly indicate that the switch complex is a component of the rotor part of the motor and not of the stator. FliG is thought to generate torque (18) by interacting with the stator, which is probably composed of the Mot complex (7). Close association of the torque generator and the MS ring, as a rotary base, would be necessary for high-speed rotation. The FliM and FliN proteins may have other roles, for example, mediating outside signals to the FliG protein for switching the rotation through chemotactic systems (28) and maintaining the structural stability of cytoplasmic complexes (see below).

**Relationship among switch proteins in the construction of the switch complex.** Our results show the mutual dependency of the FliM and FliN proteins for assembly, which is consistent with a recent study showing the interaction between the proteins by a cooverexpression system (25), although an in vitro study indicated that purified FliN protein did not interact with the MS ring complex even in the presence of the other switch proteins (23). Protein-protein interaction among switch components was recently demonstrated by the two-hybrid system in yeast (21). More conclusive data about the interaction between FliM and FliN were shown, using a fusion of the two proteins (15). The interactions between FliG and FliM and between FliM and FliN are consistent with our data.

**The switch complex forms prior to other cytoplasmic substructures in flagella.** Among the peripheral structures of the basal body, the Mot complex is excluded from early structures on the assembly pathway. Instead, the Mot proteins are synthesized in a later stage of the transcriptional hierarchy (17) and are believed to assemble surrounding the basal body, visualized as ring particles (14), after the hook-basal body complex is completed.

We show here that formation of the switch complex does not require any components of the export apparatus. This does not exclude the possibility that the switch complex and the export apparatus form independently of each other. However, if the export apparatus can be constructed without the switch complex, it is difficult to explain why null switch mutants are nonflagellate. Vogler et al. (27) showed that a temperature-sensitive *fliN* mutant, as well as *flhA*, *fliH*, and *fliI* mutants, failed to export flagellar proteins at the restrictive temperature. It is very likely that switch formation is necessary for a stable architecture of the export apparatus. In our experiments, integration of FliN into the membrane was somehow affected in most mutants used here, compared with the effect in wild-type cells (Fig. 1 and 2). This may indicate that FliN contributes to the structural stability of the entire cytoplasmic substructure and that mutations of peripheral proteins may also affect FliN stability in the switch complex. Alternatively, it is possible that wild-type cells with a complete flagellar structure maintain FliN more stably than mutants for some other reason, since the *fliA* mutant, for example, which is expected to have a complete cytoplasmic substructure, had a smaller amount of FliN in the membrane fraction (Fig. 2C). The peripheral structures would not be completed without the switch complex if the contribution of FliN to the whole structure is important. We propose, therefore, that the switch complex forms prior to the other cytoplasmic substructures, including the export apparatus. A definite conclusion regarding the dependency of export apparatus formation on the switch structure will have to await future experiments involving the detection of export apparatus proteins.

**Assembly pathway of the early stage of flagellar synthesis.** Figure 3 shows the model of the early assembly pathway revealed by this and previous studies. The master genes *flhC* and *flhD* are needed to express early genes of the flagellar regulon, including the *fliF* gene. After MS ring formation with FliF, FliG binds to the cytoplasmic face of the MS ring to form a partial switch structure. FliM and FliN then associate cooperatively with the FliF-FliG complex to complete the switch complex, probably visualized as the C ring or its main part. The export apparatus is constructed when proteins, including FlhA, FliH, and FliI, interact with the MS ring, probably with the help of the switch complex. The rod proteins FlgB, FlgC, FlgF,

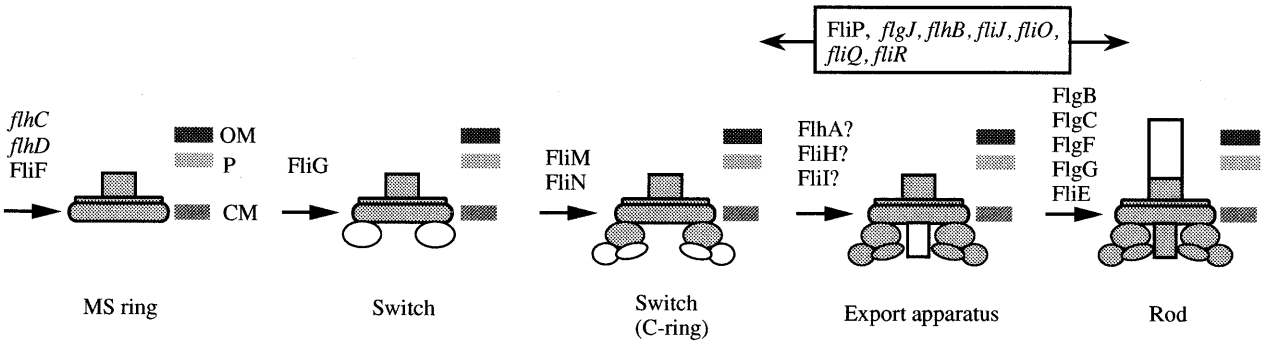


FIG. 3. Early stage of the flagellar morphological assembly pathway, as revealed by this and other studies (modified from reference 19). Where the gene product is known to be incorporated into the flagellar structure, its name is in roman letters; where this is not known or the protein is regulatory, the gene name is in italics. The genes required after switch formation and prior to rod formation are listed in the box. OM, outer membrane; P, periplasmic space and peptidoglycan layer; CM, cytoplasmic membrane.

and FlgG (and perhaps FliE [16]) are transported through the central channel of the MS ring by the action of the export apparatus and form the rod structure. The role of the products of the *flgJ*, *flhB*, *fliJ*, *fliO*, *fliP*, *fliQ*, and *fliR* genes remains to be determined, but the fact that they are needed for rod formation indicates that at least some of them may be involved in export function.

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## REFERENCES

1. **Aizawa, S.-I., G. E. Dean, C. J. Jones, R. M. Macnab, and S. Yamaguchi.** 1985. Purification and characterization of the flagellar hook-basal body complex of *Salmonella typhimurium*. J. Bacteriol. **161:**836–849.
2. **Aizawa, S.-I.** 1996. Flagellar assembly in *Salmonella typhimurium*. Mol. Microbiol. **19:**1–5.
3. **DePamphilis, M. L., and J. Adler.** 1971. Fine structure and isolation of the hook-basal body complex of flagella from *Escherichia coli* and *Bacillus subtilis*. J. Bacteriol. **105:**384–395.
4. **Dreyfus, G., A. W. Williams, I. Kawagishi, and R. M. Macnab.** 1993. Genetic and biochemical analysis of *Salmonella typhimurium* FliI, a flagellar protein related to the catalytic subunit of the $F_0F_1$ ATPase and to virulence proteins of mammalian and plant pathogens. J. Bacteriol. **175:**3131–3138.

4a. **Fan, F., and R. M. Macnab.** Personal communication.

5. **Francis, N. R., V. M. Irikura, S. Yamaguchi, D. J. DeRosier, and R. M. Macnab.** 1992. Localization of the *Salmonella typhimurium* flagellar switch protein FliG to the cytoplasmic M-ring face of the basal body. Proc. Natl. Acad. Sci. USA **89:**6304–6308.
6. **Francis, N. R., G. E. Sosinsky, D. Thomas, and D. J. DeRosier.** 1994. Isolation, characterization and structure of bacterial flagellar motors containing the switch complex. J. Mol. Biol. **235:**1261–1270.
7. **Garza, A. G., L. W. Harris-Haller, R. A. Stoebner, and M. D. Manson.** 1995. Motility protein interactions in the bacterial flagellar motor. Proc. Natl. Acad. Sci. USA **92:**1970–1974.
8. **Homma, M., D. J. DeRosier, and R. M. Macnab.** 1990. Flagellar hook and hook-associated proteins of *Salmonella typhimurium* and their relationship to other axial components of the flagellum. J. Mol. Biol. **213:**819–832.
9. **Homma, M., K. Kutsukake, M. Hasebe, T. Iino, and R. M. Macnab.** 1990. FlgB, FlgC, FlgF, FlgG. A family of structurally related proteins in the flagellar basal body of *Salmonella typhimurium*. J. Mol. Biol. **211:**465–477.
10. **Jones, C. J., and R. M. Macnab.** 1990. Flagellar assembly in *Salmonella typhimurium*: analysis with temperature-sensitive mutants. J. Bacteriol. **172:** 1327–1339.
11. **Jones, C. J., and S.-I. Aizawa.** 1991. The bacterial flagellum and flagellar motor: structure, assembly and function. Adv. Microb. Physiol. **32:**109–172.
12. **Katayama, E., T. Shiraishi, K. Oosawa, N. Baba, and S.-I. Aizawa.** 1996. Geometry of the flagellar motor in the cytoplasmic membrane of *Salmonella typhimurium* as determined by stereo-photogrammetry of quick-freeze deep-etch replica images. J. Mol. Biol. **255:**458–475.
13. **Khan, I. H., T. S. Reese, and S. Khan.** 1992. The cytoplasmic component of the bacterial flagellar motor. Proc. Natl. Acad. Sci. USA **89:**5956–5960.
14. **Khan, S., M. Dapice, and T. S. Reese.** 1988. Effects of *mot* gene expression on the structure of the flagellar motor. J. Mol. Biol. **202:**575–584.
15. **Kihara, M., N. R. Francis, D. J. DeRosier, and R. M. Macnab.** 1996. Analysis of a FliM-FliN flagellar switch fusion mutant of *Salmonella typhimurium*. J. Bacteriol. **178:**4582–4589.
16. **Kubori, T., N. Shimamoto, S. Yamaguchi, K. Namba, and S.-I. Aizawa.** 1992. Morphological pathway of flagellar assembly in *Salmonella typhimurium*. J. Mol. Biol. **226:**433–446.
17. **Kutsukake, K., Y. Ohya, and T. Iino.** 1990. Transcription analysis of the flagellar regulon of *Salmonella typhimurium*. J. Bacteriol. **172:**741–747.
18. **Lloyd, S. A., H. Tang, X. Wang, S. Billings, and D. F. Blair.** 1996. Torque generation in the flagellar motor of *Escherichia coli*: evidence of a direct role for FliG but not for FliM or FliN. J. Bacteriol. **178:**223–231.
19. **Macnab, R. M.** 1995. Flagellar switch, p. 181–199. *In* J. A. Hoch and T. J. Silhavy (ed.), Two-component signal transduction. American Society for Microbiology, Washington, D.C.
20. **Macnab, R. M.** 1996. Flagella and motility, p. 123–145. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, D.C.
21. **Marykwas, D. L., S. A. Schmidt, and H. C. Berg.** 1996. Interacting components of the flagellar motor of *Escherichia coli* revealed by the two-hybrid system in yeast. J. Mol. Biol. **256:**564–576.
22. **Ohnishi, K., Y. Ohto, S.-I. Aizawa, R. M. Macnab, and T. Iino.** 1994. FlgD is a scaffolding protein needed for flagellar hook assembly in *Salmonella typhimurium*. J. Bacteriol. **176:**2272–2281.
23. **Oosawa, K., T. Ueno, and S.-I. Aizawa.** 1994. Overproduction of the bacterial flagellar switch proteins and their interactions with the MS ring complex in vitro. J. Bacteriol. **176:**3683–3691.
24. **Suzuki, T., T. Iino, T. Horiguchi, and S. Yamaguchi.** 1978. Incomplete flagellar structures in nonflagellate mutants of *Salmonella typhimurium*. J. Bacteriol. **133:**904–915.
25. **Tang, H., S. Billings, X. Wang, L. Sharp, and D. F. Blair.** 1995. Regulated underexpression and overexpression of the FliN protein of *Escherichia coli* and evidence for an interaction between FliN and FliM in the flagellar motor. J. Bacteriol. **177:**3496–3503.
26. **Ueno, T., K. Oosawa, and S.-I. Aizawa.** 1992. M ring, S ring and proximal rod of the flagellar basal body of *Salmonella typhimurium* are composed of subunits of a single protein, FliF. J. Mol. Biol. **227:**672–677.
27. **Vogler, A. P., M. Homma, V. M. Irikura, and R. M. Macnab.** 1991. *Salmonella typhimurium* mutants defective in flagellar filament regrowth and sequence similarity of FliI to $F_0F_1$, vacuolar, and archaebacterial ATPase subunits. J. Bacteriol. **173:**3564–3572.
28. **Welch, M., K. Oosawa, S. I. Aizawa, and M. Eisenbach.** 1993. Phosphorylation-dependent binding of a signal molecule to the flagellar switch of bacteria. Proc. Natl. Acad. Sci. USA **90:**8787–8791.
29. **Yamaguchi, S., H. Fijita, T. Taira, K. Kutsukake, M. Homma, and T. Iino.** 1984. Genetic analysis of three additional *fla* genes in *Salmonella typhimurium*. J. Gen. Microbiol. **130:**3339–3342.
30. **Yamaguchi, S., S.-I. Aizawa, M. Kihara, M. Isomura, C. J. Jones, and R. M. Macnab.** 1986. Genetic evidence for a switching and energy-transducing complex in the flagellar motor of *Salmonella typhimurium*. J. Bacteriol. **168:**1172–1179.
31. **Zhao, R., S. C. Schuster, and S. Khan.** 1995. Structure effects of mutations in *Salmonella typhimurium* flagellar switch complex. J. Mol. Biol. **251:**400–412.