Genetic analysis of the bacterial hook-capping protein FlgD responsible for hook assembly

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FlgD of Salmonella enterica is a 232 aa protein that acts as the hook cap to promote assembly of FlgE into the hook structure. The N-terminal 86 residues (FlgD_N) complement flgD mutants, albeit to a small degree. However, little is known about the role of the C-terminal region of FlgD (FlgD_C). Here we isolated pseudorevertants from Salmonella flgE mutants. About half of the extragenic mutations lay within FlgD_C and only one in FlgD_N. These suppressor mutations prevented mutant FlgE subunits from leaking out to some degree. Two weakly motile flgD mutants encoding C-terminally truncated variants, FlgD(1-195) and FlgD(1-138f-s+4aa), secreted larger amounts of FlgE into the culture medium than wild-type cells. Their hooks were shorter, and their length distributions were broader, with significant tailing towards smaller values. These results suggest that FlgD_C contributes to efficient hook polymerization. Therefore, we propose that FlgD_N attaches to the distal end of the hook to promote hook polymerization and that FlgD_C blocks the exit of newly exported FlgE monomers into the culture medium, allowing FlgE to have more time to assemble into the hook.

Flagellar assembly begins with the basal body, followed by the hook, and finally the filament. For construction of the flagellum, most of the flagellar proteins are delivered to the distal end of the growing structure by the flagellar type III protein export apparatus (Minamino & Macnab, 1999). Three capping proteins, Flgl, FlgD and FliD, are required for polymerization of the rod, the hook and the filament, respectively (Ikeda et al., 1985; Ohnishi et al., 1994; Hirano et al., 2001). The FliD cap is made of a pentagonal plate domain as a lid and five axially extended leg-like domains that bind to the filament at the distal end, forming a cavity under the pentagonal plate as a folding chamber for newly exported FliC molecules (Yonekura et al., 2000). There is a symmetry mismatch between the helical subunit array of the filament, with 11 protofilaments forming the tube and the pentameric annular structure of the FliD cap, and this symmetry mismatch is the basis of the mechanism of assembly promotion by the FliD cap (Yonekura et al., 2000).

The hook is a highly curved tubular structure composed of about 120 subunits of FlgE, and assembles with a helical symmetry. FlgE can polymerize in vitro onto the tip of the preexisting hook (Kato et al., 1982). In contrast, FlgD helps the attachment of FlgE monomers to the distal end of the rod and the growing hook (Kubori et al., 1992). When purified FlgD is supplied to flgD mutants, the hook grows.
Genetic analysis of Salmonella FlgD

and hence the mutants recover motility. This suggests that FlgD permits the incorporation of newly exported FlgE monomers to the hook tip in a way similar to the FliD cap (Ohnishi et al., 1994). FlgD is located at the distal end of the hook isolated from flgK mutants but is no longer present at the tip of the hook prepared from flgL mutants, indicating that the FlgD cap is displaced by FlgK prior to filament formation (Ohnishi et al., 1994). Thus, the FlgD cap is only transiently associated with the tip of the hook during hook formation.

The length of the hook is controlled at about 55 nm (Hirano et al., 1994), and FlhB and FliK are involved in hook length control. FlhB is one of the integral membrane proteins of the export apparatus (Minamino & Macnab, 2000). FliK is thought to act as a ruler to measure the hook length (Moriya et al., 2006; Erhardt et al., 2010). When the hook has reached its mature length of 55 nm, temporal association of the N-terminal ruler domain of FliK with the inner surface of the hook during FliK secretion allows the C-terminal domain of FliK to interact with FlhB, thereby terminating the export of proteins required for assembly of the rod and hook, and initiating the export of proteins responsible for filament formation (Minamino et al., 2009).

Salmonella FlgD consists of 232 amino acid residues. FlgD has two domains: a flexible N-terminal domain (FlgD_N) and a compactly folded C-terminal domain (FlgD_C) (Kuo et al., 2008). Kutsukake & Doi (1994) have reported that the N-terminal 86 residues of Salmonella FlgD are functional as the hook cap. A partial atomic model of the hook has been built by the complementary use of X-ray crystallography and electron cryomicroscopy (Samatey et al., 2004; Fujii et al., 2009). Although the crystal structure of the C-terminal domain of FlgD from Xanthomonas campestris has been solved at 2.5 Å (Kuo et al., 2008), how FlgD works is still unknown.

In this study, we carried out genetic analysis of FlgD by isolating suppressor mutants of flgE mutants that cannot form the hook structure at 42 °C, characterized C-terminally truncated variants of FlgD, and showed that FlgD_C contributes to hook assembly. Based on these results, we discuss the role of FlgD_C in hook assembly.

METHODS

Bacterial strains, plasmids, P22-mediated transduction, DNA manipulations and media. Bacterial strains and plasmids used in this study are listed in Table 1. P22-mediated transduction was carried out as described by Yamaguchi et al. (1984). Procedures for DNA manipulation were carried out as described previously (Saijo-Hamano et al., 2004). LB broth (LB) and soft agar plates were prepared as described previously (Minamino & Macnab, 1999). Ampicillin was added at a final concentration of 100 μg ml⁻¹.

Motility assay on soft agar plates. Fresh colonies were inoculated onto soft agar plates and incubated at 30 or 42 °C. At least seven independent experiments were performed.

Secretion assay. Salmonella cells were grown at 30 or 42 °C with shaking until the cell density had reached OD₆₀₀ = 1.2–1.4. After centrifugation, the cellular and culture supernatant fractions were collected separately. Cell pellets were resuspended in SDS-loading buffer and normalized with respect to the cell density to give a constant amount of cells. Proteins in the culture supernatant were precipitated by 10% TCA, suspended in a Tris/SDS loading buffer and heated at 95 °C for 5 min. After SDS-PAGE, immunoblotting with polyclonal anti-FlgD, anti-FlgE, anti-FliK and anti-FliC antibodies was carried out as described previously (Minamino & Macnab, 1999). The polyclonal anti-FlgD antibody efficiently recognizes the C-terminal portion of FlgD, and hence the band of FlgD(1–138E+3–449) was much weaker than those of full-length FlgD and FlgD(1–195) (data not shown). At least three independent secretion assays were performed.

Measurement of hook length. The hook basal bodies were prepared as described by Aizawa et al. (1985), with minor modifications. Samples were negatively stained at 4 °C with 3% phosphotungstic acid (pH 4.5) on carbon-coated copper grids. Micrographs were recorded at a magnification of ×50000 with a JEM-1011 transmission electron microscope (JEOL) operated at 100 kV. Hook length was measured using National Institutes of Health (NIH) Image 1.63 software. More than 200 hook basal bodies prepared from each Salmonella strain were measured.

RESULTS

Isolation of pseudorevertants from hook assembly-deficient flgE mutants

To investigate the hook assembly mechanism in more detail, we carried out genetic analyses of hook assembly-deficient flgE mutants. To do this, we first looked for hook assembly-deficient flgE point mutants from our collections. We previously identified eight weakly motile flgE mutants with lowered efficiency of hook assembly at 30 °C (Moriya et al., 2006). During further characterization of these mutants, we found that motility of the flgE(R96S), flgE(1137S) and flgE(T149I) mutants was much weaker than that of wild-type cells at 42 °C, although slightly better than that of a completely assembly-defective flgE(A9-20) mutant (Fig. 1a). Immunoblotting with polyclonal anti-FlgE antibody revealed that neither protein stability nor secretion of these point mutant FlgE proteins was affected at 42 °C (Fig. 1b, upper panel).

These flgE point mutants produce flagellar filaments at 30 °C (Moriya et al., 2006). Therefore, to test whether these mutants also produce the filaments even at 42 °C, we analysed the cellular and secretion levels of FliC (Fig. 1b, lower panel). A huge amount of FliC was detected in both cell (lane 1) and culture supernatant fractions from wild-type cells (lane 6). However, only very faint FliC bands were seen in the two fractions from the flgE mutants (lanes 2–5 and 7–10). Consistently, the mutants produced the basal bodies, occasionally with very short hooks attached (Fig. 1c). These results suggest that they are severely defective in hook assembly at 42 °C.

The crystal structure of FlgE31, a major proteolytic fragment of FlgE lacking both unfolded N- and C-terminal...
regions, has two domains, D1 and D2, connected by a short stretch of two-stranded anti-parallel β-sheet (Samatey et al., 2004). The R96S and I137S mutations are in domain D1, while the T149I mutation is in domain D2 (Fig. 1d). The side chains of Ile-137 and Thr-149 point to the domain cores of D1 and D2, respectively. The side chain of Arg-96 is exposed on the domain surface, but is not involved in the interaction with the neighbouring subunits. Therefore, we

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**Table 1.** Strains and plasmids used in this study

![Fig. 1.](image-url) Characterization of severely impaired motile *flgE* mutants. (a) Motility assay of SJW1103 (wild-type; WT) and four *flgE* mutants: SJW1353 (∆9-20), SJW2236 (T149I), SJW2248 (R96S) and SJW2252 (I137S). The soft agar plates were incubated at 42 °C for 4 h. (b) Secretion of FlgE and FliC. Immunoblotting using polyclonal anti-FlgE and anti-FliC antibodies of whole-cell proteins (Cell) and culture supernatant fractions (Sup) prepared from SJW1103 (WT), SJW1353 (∆9-20), SJW2236 (T149I), SJW2248 (R96S) and SJW2252 (I137S) grown at 42 °C. The positions of FlgE and FliC are indicated on the right. Molecular mass markers (kDa) are shown on the left. (c) Electron micrograph of flagellar structures isolated from SJW2236 grown at 42 °C. (d) Crystal structure of the 31 kDa fragment of *Salmonella* FlgE, which consists of two domains, D1 and D2 [Protein Data Bank (PDB) ID: 1WLG]. The Cα backbone is colour-coded from blue to red, going through the rainbow colours from the N to the C terminus. Point mutation sites that cause a defect in hook assembly are indicated by a CPK representation.
assume that the effects of the R96S, I137S and T149I mutations may indirectly propagate to the subunit interface at 42 °C.

To investigate the effect of these flgE mutations on hook assembly at 42 °C, we isolated pseudorevertants from these flgE mutants by streaking out overnight cultures on soft agar plates, incubating them at 42 °C for 2 days and looking for motility haloes. Eight, five and 11 motile colonies were isolated from the flgE(R96S), flgE(I137S) and flgE(T149I) mutants, respectively. The motility of these pseudorevertants was clearly stronger than that of their parental flgE strains, although still poorer than that of wild-type cells (Fig. 2a).

DNA sequencing identified 24 missense mutations lying within FlgD, FlgE or FlgG. The flgE(R96S) mutant gave rise to one intragenic suppressor, D63E; four extragenic suppressors in FlgD, K45R and T122R (isolated three times); and three extragenic suppressors in FlgG, Q121L (isolated twice) and G163V. The flgE(I137S) mutant gave rise to two intragenic suppressors, A135V (isolated twice); and three extragenic suppressors in FlgD, G105D, F125S and I153N. The flgE(T149I) mutant gave rise to five intragenic suppressors in FlgD, G100D (isolated twice), T122P, P124Q (isolated twice) and G167C. These second-site flgD mutations were all within FlgDC, except for FlgD(K45R), which lay within FlgDN (Fig. 2b).

We next tested whether these extragenic flgD suppressors display allele specificity. The flgD(K45R) and flgD(T122R) mutations suppressed their parental flgE(R96S) mutation but not the flgE(T149I) mutation. The flgD(G100D) and flgD(T122P) mutations suppressed their parental flgE(T149I) mutation but not the flgE(R96S) mutation. Thus, these second-site flgD mutations did not give the pseudorevertant phenotype to other flgE mutants. These results suggest a possible interaction between FlgD C and FlgE.

To test the effect of each second-site mutation by itself on motility, we isolated the second-site flgD mutants and analysed their motility in soft agar. The motility of all mutants was normal at both 30 and 42 °C (data not shown). As these second-site mutations display no phenotype, they do not appear to affect the function of the wild-type FlgD protein.

**Effect of extragenic flgD suppressors on the secretion level of FlgE**

Large amounts of FlgE were seen in the culture supernatant of the parental flgE mutants compared with the wild-type (Fig. 1). Therefore, we tested whether the extragenic suppressor mutations in FlgD block the leakage of mutant FlgE monomers into the culture medium at 42 °C. The amounts of FlgE secreted by the pseudorevertants were less than those of their parental flgE mutants but still higher than the wild-type level (Fig. 2c). This suggests that the second-site mutations alter the conformation of FlgD to some degree to prevent mutant FlgE monomers from leaking out so that mutant FlgE molecules can have more time to be incorporated into the hook.
**Characterization of weakly motile flgD mutants**

The N-terminal 86 residues of *Salmonella* FlgD are reported to be sufficient to complement flgD mutants (Kutsukake & Doi, 1994). However, we found that most of the extragenic flgE suppressors were in FlgDc, raising the possibility that FlgDc contributes to the hook assembly process. Therefore, we re-examined whether FlgD(1-86) complements a hook assembly-defective flgD mutant strain, SJW156 [flgD(1-72f-s+1aa)], in which a single-base deletion was encountered at A215, resulting in a frame-shift mutation in FlgD (Fig. 2b). Wild-type FlgD restored full motility of the flgD(1-72f-s+1aa) mutant (Fig. 3a). In contrast, FlgD(1-86) restored motility to only a small degree (Fig. 3a). FliC was also detected in the culture supernatants, although at a much lower level than in the wild-type (Fig. 3b, upper panel). Consistently, the number of flagellar filaments was decreased, as judged by dark-field microscopy (data not shown). The amount of FlgE secreted by the cells expressing FlgD(1-86) was much higher than that secreted by the wild-type (Fig. 3b, lower panel). Since flgD null mutants cannot produce the hook due to loss of the hook cap, large amounts of unassembled FlgE subunits are secreted into the periplasm and culture medium (Ohnishi et al., 1994; Minamino & Macnab, 1999). Therefore, we suggest that FlgDN alone cannot exert the hook-capping function efficiently.

![Fig. 3. Effect of C-terminal truncations of FlgD on motility.](image-url)
To further confirm this, we looked for partially or weakly motile flgD mutants with C-terminal truncation. We screened 15 spontaneous flgD mutants of *Salmonella* isolated by the laboratories of S. Yamaguchi and R. M. Macnab (Yale University, New Haven, CT, USA) for motility in soft agar plates in comparison with a wild-type strain and a flgD<sub>1-72f-s+1aa</sub> mutant. We identified two weakly motile flgD mutant strains, MMD623 and MMD651 (Fig. 3c). The mutation identified in MMD623 was a nonsense mutation in the flgD allele that generated an amber (TAG) stop codon at position 196. The other mutation in MMD651 was a single-base insertion between base pairs at A-415 and C-416, causing a frameshift mutation in FlgD. Thus, these mutants produce C-terminally truncated variants of FlgD, FlgD<sub>1-195</sub> and FlgD<sub>1-138f-s+4aa</sub> (Fig. 2b).

We examined whether these C-terminal truncations affected the stability or secretion of FlgD (Fig. 3d, second row). We found that immunodetection of purified FlgD<sub>1-138f-s+4aa</sub> by our polyclonal anti-FlgD antibody was much weaker than that of purified FlgD and FlgD<sub>1-195</sub> (data not shown), indicating that the polyclonal anti-FlgD antibody efficiently recognizes the C-terminal portion of FlgD. Wild-type FlgD, FlgD<sub>1-195</sub> and FlgD<sub>1-138f-s+4aa</sub> were detected in the whole-cell fractions (lanes 1, 3 and 4), whereas FlgD<sub>1-72f-s+1aa</sub> was not (lane 2). These results indicate that the poor motility of the flgD<sub>1-195</sub> and flgD<sub>1-138f-s+4aa</sub> mutants is not due to the instability of the proteins encoded by these mutant flgD alleles. In the culture supernatants, wild-type FlgD, FlgD<sub>1-195</sub> and FlgD<sub>1-138f-s+4aa</sub> were observed (lanes 5, 7 and 8), while FlgD<sub>1-72f-s+1aa</sub> was not (lane 6). The lack of detection of FlgD<sub>1-72f-s+1aa</sub> may be due to protein instability or the lack of epitopes recognized by the polyclonal anti-FlgD antibody.

Like the flgD<sub>1-72f-s+1aa</sub> and flgD<sub>1-86</sub> mutants, the flgD<sub>1-195</sub> and flgD<sub>1-138f-s+4aa</sub> mutants also secreted higher amounts of FlgE into the culture medium than wild-type cells (Fig. 3d, third row). This indicates that these mutant FlgD proteins cannot prevent most FlgE monomers from leaking out. Interestingly, the flgD<sub>1-195</sub> and flgD<sub>1-138f-s+4aa</sub> mutants secreted FlIC, FlgK and FlgL into the culture medium at almost wild-type levels (Fig. 3d, first row). As the export of FlIC, FlgK and FlgL occurs only after completion of hook assembly (Minamino et al., 2008), these mutants produce the hooks and hence switch the export specificity of the export apparatus.

### Hook length and morphology

It has been shown that inefficient hook polymerization considerably affects hook length (Moriya et al., 2006). Therefore, we prepared hook basal bodies from wild-type cells and the flgD<sub>1-195</sub> and flgD<sub>1-138f-s+4aa</sub> mutants, and measured their hook length. The means ± SDs were 38 ± 15 nm for the flgD<sub>1-195</sub> mutant and 42 ± 12 nm for the flgD<sub>1-138f-s+4aa</sub> mutant, compared with 52 ± 8 nm for the wild-type (Fig. 4). While the majority of wild-type hooks had lengths rather sharply distributed within a range from 45 to 60 nm (Fig. 4a), the flgD<sub>1-195</sub> mutant produced many short hooks distributed over a range between 5 and 45 nm, although the peak of the length distribution was still around 45–50 nm (Fig. 4b). Although the broadening of the hook length distribution was less prominent for the flgD<sub>1-138f-s+4aa</sub> mutant, this mutant also produced a significant fraction

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**Fig. 4.** Electron micrographs of the hook basal bodies with the hook length distributions. (a) SJW1103 (wild-type; WT), (b) MMD623 [flgD<sub>1-195</sub>], and (c) MMD651 [flgD<sub>1-138f-s+4aa</sub>].

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of short hooks with a length distributed over a range between 5 and 45 nm, while the peak population was still between 45 and 50 nm (Fig. 4c). These results suggest that the hook polymerization ability of these \textit{flgD} mutants is lower than that of wild-type cells.

It has been shown that any lowering of the efficiency of hook polymerization results in an increase in the secretion level of rod/hook-type export substrates because the rod/hook-type export mode of the flagellar protein export apparatus continues for a longer period (Moriya \textit{et al.}, 2006). The hook length control protein FliK is one of the rod/hook-type substrates (Minamino \textit{et al.}, 1999). Therefore, we tested whether the C-terminal truncations of FlgD also affected the secretion of FliK. Immunoblotting with polyclonal anti-FliK antibody revealed that the secretion level of FliK was much higher than that of the wild-type in the supernatant fractions of the \textit{flgD}(1-195) and \textit{flgD}(1-138f-s+4aa) mutants, and slightly less than that of the \textit{flgD}(1-72+1aa) mutant (Fig. 3d, fourth row), supporting the idea that their hook polymerization ability is significantly reduced.

**Multicopy effect of FlgE on motility of the \textit{flgD} mutants**

We investigated whether overproduction of FlgE could result in partial restoration of motility of a \textit{flgD}(1-138f-s+4aa) mutant. Wild-type FlgD complemented the \textit{flgD}(1-72+1aa) and \textit{flgD}(1-138f-s+4aa) mutants, indicating that these mutant alleles are recessive. Consistently, these alleles did not inhibit wild-type motility (data not shown).

Overexpression of FlgE, which increased both cellular and secretion levels of FlgE (data not shown), restored motility of the \textit{flgD}(1-138f-s+4aa) mutant to some degree, but not that of cells of either the wild-type or the \textit{flgD}(1-72+1aa) mutant (Fig. 5a). We obtained the same results with a \textit{flgD}(1-195) mutant (data not shown).

To investigate how motility of the \textit{flgD}(1-138f-s+4aa) mutant would be improved by overproduction of FlgE, we prepared the hook basal bodies produced by the \textit{flgD}(1-138f-s+4aa) mutant overexpressing FlgE and measured the hook length (Fig. 5b). The mean ± SD of the hook length of the mutant was 52 ± 19 nm. The peak position was nearly recovered to that of the wild-type, indicating that overproduction of FlgE increases the efficiency of hook assembly in the \textit{flgD}(1-138f-s+4aa) mutant. This suggests that the FlgD(1-138f-s+4aa) cap cannot efficiently promote the assembly of newly exported FlgE monomers at the hook tip. In agreement with previous observations that wild-type cells overproducing FlgE produce longer hooks with a broader length distribution than the wild-type (Muramoto \textit{et al.}, 1999; Moriya \textit{et al.}, 2006), the SD of the length distribution was still much larger than the wild-type value (Fig. 5b). This indicates that hook length is not well controlled.

**DISCUSSION**

Most of the component proteins of the flagellar axial structure require capping proteins to assemble at the distal end of the growing structure. The filament cap,
which is formed by the FlfD protein, has five axially extended leg-like domains that bind to the distal end of the filament to promote filament formation by the assembly of FlfC. The leg-like domains of the FlfD cap, which is composed of disordered N- and C-terminal regions of FlfD, play an important role in creating an open assembly site for newly exported FlfC subunits (Yonekura et al., 2000).

FlgD acts as the hook cap to allow newly exported FlgE monomers to polymerize at the distal end of the hook (Ohnishi et al., 1994). The flexible N-terminal domain of FlgD is sufficient for the hook-capping function (Kutsukake & Doi, 1994). Like the filament, the hook is a tubular structure made of 11 protofilaments (Fuji et al., 2009). Therefore, it is plausible that FlgD_N may form flexible leg-like domains that bind to the hook tip in a way similar to the FlfD cap. However, little is known about the role of FlgD_C in hook assembly. To clarify this, we isolated pseudorevertants from flgE point mutants that were severely hook assembly-deficient at 42 °C. Interestingly, most of the second-site mutations were located within FlgD_C (Fig. 2). These second-site flgD mutations blocked leakage of mutant FlgE subunits into the culture medium to some degree (Fig. 2c), thereby making hook assembly relatively more efficient. The parental flgE mutations are not located on the subunit interface in the hook structure (Fig. 1d). The second-site flgD mutations displayed allele specificity. Therefore, we suggest that the second-site flgD mutations compensate for the first-site flgE mutations by altering the physical interface between FlgD and FlgE for hook polymerization. When the second-site mutations are mapped on the crystal structure of FlgD_C from X. campestris, which contains residues 84–221 (Kuo et al., 2008), interestingly, they are localized on one side of the molecular surface of FlgD_C (Fig. 6). This raises the possibility that FlgD_C interacts with newly exported FlgE subunits through this surface, and the interaction between FlgD_C and FlgE may contribute to efficient and proper incorporation of the FlgE subunit into the growing hook.

We next characterized two weakly motile flgD mutants encoding C-terminally truncated variants, FlgD(1-138) and FlgD(1-138f-s + 4aa) (Fig. 3c). The levels of FlgE secreted by these flgD mutants were much higher than those secreted by wild-type cells and were similar to those of an flgD null mutant that does not form the hook (Fig. 3d). Unlike the flgD null mutant, they retained the ability to form the hook to a considerable degree (Fig. 4). However, many of the hooks produced by these mutants had lengths broadly distributed towards ones shorter than those produced by the wild-type cells (Fig. 4). These results indicate that these C-terminally truncated variants of FlgD cannot fully exert the hook-capping function to prevent exported FlgE monomers from leaking out during hook assembly. Therefore, it is very likely that FlgD_C supports or stabilizes the function of FlgD_N.

The crystal structure of Xanthomonas FlgD_C shows two compactly folded domains (Kuo et al., 2008). The remaining N-terminal 83 residues, which are unfolded in solution, bind to the distal end of the rod or the hook to anchor FlgD_C, perhaps to be a half-open lid in a similar manner to the pentagonal plate domain of the FlfD pentamer cap (Yonekura et al., 2000). Interestingly, the sequence alignment of FlgD proteins from S. enterica and X. campestris (Kuo et al., 2008; Supplementary Fig. S1) suggests that the folded domain structure of FlgD_C is completely lost in FlgD(1-86) and FlgD(1-138f-s + 4aa). Considering the results of our genetic and functional analyses, the physical trapping of FlgE monomers under the putative half-open lid formed by FlgD_C perhaps would be one of the roles of the compactly folded domains of FlgD_C. Preliminary X-ray analysis of full-length FlgD derived from Pseudomonas aeruginosa has been carried out (Luo et al., 2009), but its atomic structure is not yet available. In the future, it will be interesting to see extragenic flgE suppressor mutations in the complete FlgD atomic model to understand how these suppressors affect the hook polymerization efficiency. Furthermore, efforts toward high-resolution structures not only of the FlgD cap structure but also of the FlgD-hook complex will be required for building a model of the hook-capping mechanism.
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