

The ATPase FliI Can Interact with the Type III Flagellar Protein Export Apparatus in the Absence of Its Regulator, FliH

Tohru Minamino,^{1,2} Bertha González-Pedrajo,³ May Kihara,³ Keiichi Namba,^{1,2,4}
and Robert M. Macnab^{3*}

*Protonic NanoMachine Project, ERATO,¹ and Dynamic NanoMachine Project, ICORP,² JST, and
Graduate School of Frontier Biosciences, Osaka University,⁴ Seika, Kyoto 619-0237, Japan,
and Department of Molecular Biophysics and Biochemistry, Yale University,
New Haven, Connecticut, 06520-8114³*

Received 5 February 2003/Accepted 21 April 2003

***Salmonella* FliI is the ATPase that drives flagellar protein export. It normally exists as a complex together with the regulatory protein FliH. A *fliH* null mutant was slightly motile, with overproduction of FliI resulting in substantial improvement of its motility. Mutations in the cytoplasmic domains of FlhA and FlhB, which are integral membrane components of the type III flagellar export apparatus, also resulted in substantially improved motility, even at normal FliI levels. Thus, FliH, though undoubtedly important, is not essential.**

Bacterial flagellar assembly begins with the basal body, followed by the hook and finally the filament (11). Almost all of the external components are translocated into a central channel in the nascent structure by an export apparatus that is believed to be located within an annular pore in the basal body MS ring (5, 8, 21). The export apparatus consists of six integral membrane components (FlhA, FlhB, FliO, FliP, FliQ, and FliR) and three cytoplasmic components (FliH, FliI, and FliJ) (15, 16). Export of bacterial flagellar proteins has characteristics in common with type III secretion of virulence factors by pathogenic bacteria (1, 7, 10, 11).

FliI is a flagellum-specific ATPase, which converts the energy of ATP hydrolysis into the energy for flagellar protein export (4, 16–18, 20, 23). FliH is a regulatory protein that is thought to prevent FliI from hydrolyzing ATP until the energy can be used for export (2, 6, 13, 16). FliJ functions as a general chaperone that prevents export substrates from premature aggregation in the cytoplasm (12).

A tentative model for the flagellar export process has been proposed (16, 17, 26) in which a (FliH)₂/FliI heterotrimeric complex (16), FliJ, and substrate diffuse to the cytoplasmic domains of FlhA and FlhB and form a complex. ATP hydrolysis by FliI drives export substrate translocation through the export apparatus, placing the substrate in the channel from whence it diffuses to its final assembly destination.

In the present study, we have found that overproduction of FliI and also certain second-site mutations in *flhA* and *flhB* are capable of conferring considerable export capability and motility to a *fliH* null mutant.

Bacterial strains and plasmids used in this study are listed in Table 1 and shown graphically in Fig. 1. Luria-Bertani broth, soft tryptone-agar plates, and M9-Casamino Acids medium plus 1% glycerol were prepared as previously described (15).

Effects of overproduction of FliI and FliJ on motility and

flagellar protein export of a *fliH* null mutant. A *Salmonella fliH* null mutant MKM11 (6) has the sequence MSNEL-Δ226-PGVL, i.e., it contains only nine amino acid residues of FliH (Fig. 1A). It displays a leaky motile phenotype, indicating that substrate export still occurs with low probability in the absence of FliH. We transformed the null mutant with plasmids encoding the soluble export components FliH, FliI, and FliJ and examined the transformants for swarming ability (Fig. 2A). FliH complemented the mutant. FliI overproduced from a pTrc99A-based plasmid restored motility to a considerable degree. When produced from a pET19b-based plasmid, which has low expression levels in a non-T7 host, FliI did not restore motility. Plasmids encoding FliJ did not restore motility. Both FliI and FliJ were detected at similar cellular levels (measured as described previously [17]) when produced from pTrc99A-based plasmids (Fig. 2B), indicating that the failure of FliJ to bypass the *fliH* null mutation was not a result of failure of expression.

The improvement in motility of the null mutant upon overproduction of FliI was, as expected, a consequence of restoration of flagellar protein export. We used the hook-capping protein FlgD as an example (Fig. 2C) (17). Small amounts of FlgD were detected with vector controls, presumably because of the leaky motile phenotype of the null mutant.

Effects of overproduction of point mutant variants of FliI on the motility and export capability of the *fliH* null mutant.

FliI(K188I) and FliI(Y363S) (3, 4) (Fig. 1B), whose mutations lie within the ATPase catalytic domain, have 1% and 2% of wild-type ATPase activity, respectively, and retain the ability to form a FliH/FliI complex, while FliI(R7C/L12P) has an ATPase activity twice that of wild-type but does not form the complex (16). FliI(R7C/L12P) produced from pTrc99A was able to significantly bypass the motility defect of the *fliH* null mutant (Fig. 3A). Plasmids encoding catalytic variants of FliI failed to bypass the motility defect of the *fliH* null mutant. Consistent with the motility data, FlgD was strongly detected in the culture supernatants of the *fliH* null mutant transformed with pTrc99A-based plasmids producing wild-type FliI or

* Corresponding author. Mailing address: Yale University, Department of Molecular Biophysics and Biochemistry 0734, 266 Whitney Ave., P.O. Box 208114, New Haven, CT 06520-8114. Phone: (203) 432-5590. Fax: (203) 432-9782. E-mail: robert.macnab@yale.edu.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Source or reference
<i>Escherichia coli</i> strains		
NovaBlue	Recipient for cloning experiments	Novagen
BL21(DE3)/pLysS	Overproduction of proteins	Novagen
HMS174(DE3)/pLysS	Overproduction of proteins	Novagen
<i>Salmonella</i> strains		
JR501	For converting <i>E. coli</i> plasmids to <i>Salmonella</i> compatibility	19
KK1013	Deletion of region III	9
MKM11	$\Delta fliH$	6
MKM11gK	$\Delta fliH fliG::Tn10$; defective in region I	This study
MKM11hB	$\Delta fliH fliB::Tn10$; defective in region II	This study
MMA10xx series	$\Delta fliH fliA$ pseudorevertants from MKM11	This study
MMA11xx series	<i>fliA</i> second-site mutants from corresponding MMA10xx strain	This study
MMB10xx series	$\Delta fliH fliB$ pseudorevertants from MKM11	This study
MMB11xx series	<i>fliB</i> second-site mutants from corresponding MMB10xx strain	This study
SJW1103	Wild-type strain for motility and chemotaxis	25
SJW2936	Deletion of region II	22
SJW1429	<i>fliH</i>	15
Plasmids		
pET19b	Vector	Novagen
pTrc99A	Vector	Pharmacia
pMM310	pET19b/His-FliH ^a	17
pMM306	pTrc99A/His-FliH	17
pMM405	pET19b/His-FliJ ^a	17
pMM406	pTrc99A/His-FliJ	17
pMM1701	pET19b/His-FliI ^a	17
pMM1702	pTrc99A/His-FliI	17
pMM1704	pTrc99A/His-FliI(R7C/L12P)	16
pMM1706	pTrc99A/His-FliI(K188I)	16
pMM1708	pTrc99A/His-FliI(Y363S)	16
pBGtI(Δ 1-7)	pTrc99A/His-FliI(Δ 1-7) ^b	This study
pBGtI _{CAT}	pTrc99A/His-FliI _{CAT}	This study
pIFF700	pTrc99A/His-FLAG-FliI	4
pIFF7001, etc.	pTrc99A/His-FLAG-FliI Δ 1, etc.	Fan Fan, unpublished

^a Although in many cases we made pET19b-based versions of plasmids, we include in the table only those encoding the wild-type alleles of FliH, FliI, and FliJ.

^b FliI(Δ 1-7) was obtained in a previous study (18) as a clostripain proteolysis fragment with an apparent mass of 48 kDa and was given the name FliI_{CL48} in that paper. The N-His-tagged version of FliI_{CL48} in the present paper has been given the more descriptive name His-FliI(Δ 1-7).

FliI(R7C/L12P) but was detected weakly or not at all with the plasmids encoding catalytic variants of FliI (Fig. 3B). Failure to bypass was not a consequence of protein instability, since all mutant derivatives of FliI were detected at the same level as the wild type (data not shown) (using anti-His [Qiagen] antibody [15]).

Effects of overproduction of truncated variants of FliI on the motility of the *fliH* null mutant. In a previous study (18), it was demonstrated that a proteolytic fragment of FliI lacking the first seven amino acids failed to bind FliH. To examine the ability of N-terminally truncated versions of FliI to restore the motility of the *fliH* null mutant, we constructed His-FliI(Δ 1-7) (lacking the first seven amino acids) and also His-FliI_{CAT} (lacking the first 110 amino acids and so consisting solely of the catalytic domain) (Fig. 1B). Neither could form a stable FliH/FliI complex (data not shown). However, FliI(Δ 1-7) was able to substantially bypass the motility defect of the *fliH* null mutant, whereas FliI_{CAT} was not (Fig. 4A). Likewise, FliI(Δ 1-7) restored the export of FlgD, whereas FliI_{CAT} did not (data not shown). Immunoblotting with anti-His antibody revealed that both truncated forms of FliI were present at wild-type levels (Fig. 4B). We purified His-FliI, His-FliI(Δ 1-7), and His-FliI_{CAT} by nickel-nitrilotriacetic acid chromatography as previously described (17) and measured their ATPase activities

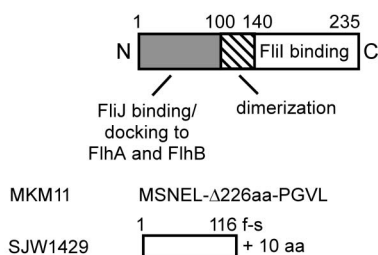
using a malachite green assay (16). The ATPase activities of FliI(Δ 1-7) and FliI_{CAT} were 6 and 29% of that of wild-type FliI. Thus, even though FliI(Δ 1-7) was less catalytically active than FliI_{CAT}, this difference was less important for the bypass phenomenon than the presence of an almost intact N-terminal region.

Effects of overproduction of scanning amino acid deletion variants of FliI. A series of plasmids encoding N-terminally His-FLAG-tagged FliI variants with scanning 10-amino-acid deletions throughout its N-terminal region had been constructed (4) (F. Fan, unpublished data) (Fig. 1B). None were able to bypass the motility defect of the *fliH* null mutant (Fig. 5A). All of the deletion variants were stable (Fig. 5B). Thus, the conclusion based on the truncated variants (Fig. 4) remains valid, namely, that the N-terminal region of FliI is essential for the bypass effect.

ATPase activity measurements were made on purified His-FLAG-FliI Δ 1 and His-FLAG-FliI Δ 2 (the other deletion variants were too insoluble to permit them to be assayed). Their activities were about 3 and 50%, respectively, of that of wild-type His-FLAG-FliI.

Effect of overproduction of FliH and FliI on the motility of a *fliH* truncation mutant strain, SJW1429. Strain SJW1429 (Fig. 1A) is a *fliH* mutant that is totally immotile and so is

A. FliH



B. FliI

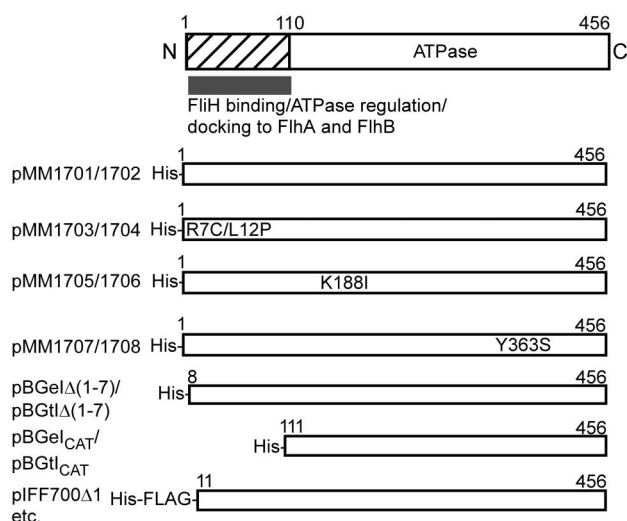


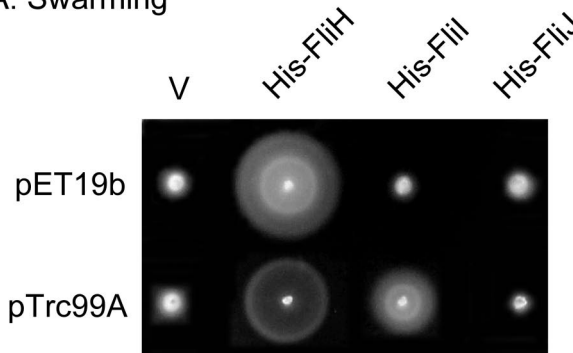
FIG. 1. (A) The primary structure of the ATPase regulatory protein FliH and schematic representation of mutant FliH proteins produced by MKM11 and SJW1429. FliH is a 235-amino-acid protein that can be roughly divided into three regions: an N-terminal region consisting of the first 100 amino acids, a middle region containing amino acid residues 101 to 140, and a C-terminal region comprising the remainder of the protein. The N-terminal region, middle, and C-terminal regions are essential for interaction with FliJ, for dimerization, and for interaction with FliI, respectively. (B) The primary structure of the flagellum-specific ATPase FliI and schematic representations of the FliI products produced by the plasmids listed in Table 1.

phenotypically more defective than the genotypically null strain MKM11. From previous studies (6), we can conclude that the mutant FliH in SJW1429 retains the FliJ binding domain but lacks about half of its dimerization domain and the entire FliI binding domain.

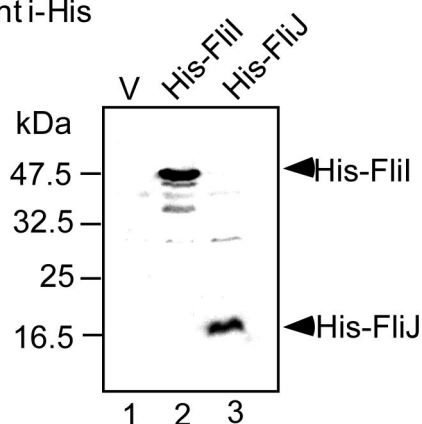
SJW1429 cannot be complemented by overproduction of FliH (Fig. 6), suggesting that the truncated form is dominant negative. Overproduction of FliI also does not result in motility; thus, the C-terminally truncated FliH protein appears to interfere with the bypass effect of overproducing FliI, possibly by interfering with FliI binding to FliA or FliB. If both FliH and FliI are overproduced, however, SJW1429 becomes motile, indicating that the wild-type FliH/FliI complex is functional even in the presence of the truncated form. We suggest that this latter phenomenon represents complementation and not bypass.

Isolation of pseudorevertants of the *fliH* null strain. When the *fliH* null mutant was streaked from an overnight culture onto soft tryptone-agar plates and incubated at 30°C for 2 days, small swarms were found emerging from the broad streak generated by the weak motility of MKM11. Thirteen motile colonies were purified from such swarms. These pseudorever-

A. Swarming



B. Cell, anti-His



C. Supernatant, anti-FlgD

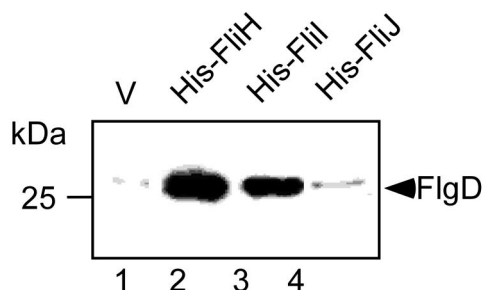


FIG. 2. (A) Swarming of the *fliH* null mutant with pET19b-based and pTrc99A-based plasmids encoding the proteins shown. V, vector. Plates were incubated for 4.5 h at 30°C. (B) Immunoblot, using anti-His antibody, of whole cells of the *fliH* null mutant transformed with plasmids encoding the proteins shown. (C) Immunoblot, using polyclonal anti-FlgD antibody, of the hook-capping protein FlgD in culture supernatants of the *fliH* mutant transformed with plasmids encoding the proteins indicated. Cellular levels of FlgD were the same in all cases (data not shown).

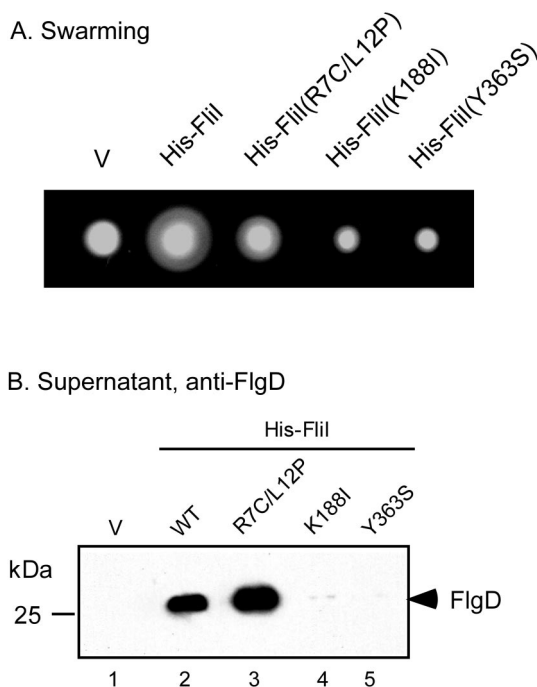


FIG. 3. (A) Swarming of the *fliH* null mutant transformed with plasmids encoding the point mutant FliI variants shown. Plates were incubated for 4.5 h at 30°C. (B) Immunoblot, using anti-FlgD antibody, of the culture supernatant fractions of the *fliH* null mutant transformed with plasmids encoding the point mutant FliI variants shown. V, vector.

tants, illustrated by MMA1002 and MMB1005, swarmed considerably better than the null mutant (Fig. 7A), although not as well as the wild-type strain SJW1103 (Fig. 7B). Consistent with this, FlgD was detected in the culture supernatant from the pseudorevertants, although in much smaller amounts than with the wild-type strain (data not shown).

To determine the location of the mutations in terms of the major flagellar regions I, II, and IIIa,b (11), transductional crosses were carried out with phage P22HT int as described previously (24). P22 lysates were propagated on 13 pseudorevertants and transduced into strains defective in each of the major regions (Table 1). A large number of abortive transductants (trails) appeared when MKM11hB was used as a recipient; in contrast, no trails were produced when MKM11gK or KK1013 was used (data not shown). Thus, the mutations apparently lay in region II and might be closely linked to the *flhB* operon, which consists of 3 genes, *flhB*, *flhA*, and *flhE* (14). FlhB and FlhA are integral membrane components of the export apparatus; the role of FlhE is not known.

DNA sequence analysis established that the mutations were in *flhA* and *flhB*. They were all missense mutations: I21T, L22F (seen four times), and V404M in FlhA and P13T, A21V, A21T (seen twice), and I27N in FlhB. All of these except FlhA (V404M) lie near the N termini of the two proteins, close to the interface with the first membrane span. FlhA(V404M) lies in the cytoplasmic C-terminal domain of the protein.

The second-site mutations from a set of the pseudorevertants were transferred by P22 phage-mediated transduction into SJW2936 (deletion of region II) to construct strains containing only the second-site mutations. These strains,

illustrated by MMA1102 and MMB1105 (Fig. 7B), swarmed less well than the wild-type strain, although much better than the pseudorevertants (Fig. 7A). In agreement with this, the level of FlgD was found to be slightly lower in the culture supernatants of second-site *flhA* and *flhB* mutants than in the culture supernatants of wild-type cells (data not shown). Thus, in the presence of wild-type FliH, the second-site *flhA* and *flhB* mutations impair flagellar protein export to a small degree.

Effects of overproduction of FliI on the motility of pseudorevertants and the second-site *flhA* and *flhB* mutants. We examined whether overproduction of FliI enhanced the motility of the pseudorevertants and the second-site mutants. The swarm sizes of pseudorevertants overproducing wild-type FliI were substantially larger than those of cells harboring the pTrc99A vector alone (data not shown). Thus, the bypass effect still operates with the pseudorevertants. In contrast, overproduction of His-FliI had no effect on the swarm sizes of the *flhA* and *flhB* second-site mutants.

Bypass of FliH. In this study, we have investigated effects of overproduction of soluble components of the type III flagellar export apparatus on both the motility and flagellar protein export properties of a *Salmonella fliH* null mutant, MKM11. Overproduction of FliI, but not of FliJ, resulted in bypass of the *fliH* null mutation with respect to both export and motility. Both the catalytic domain and the flagellum-specific N-terminal domain of FliI were necessary for this effect. The motility defect of the *fliH* null mutant could also be bypassed by certain

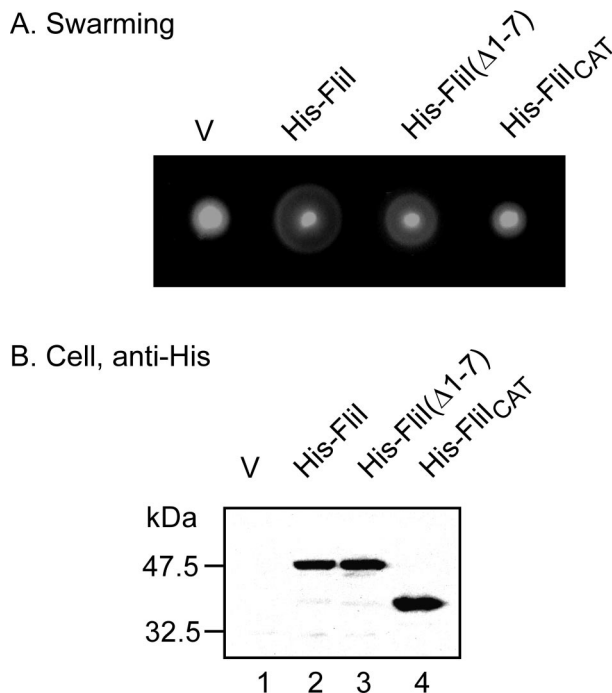


FIG. 4. (A) Swarming of the *fliH* null mutant transformed with pTrc99A-based plasmids encoding the truncation variants of FliI shown (see Fig. 1B). Plates were incubated for 4.5 h at 30°C. (B) Immunoblot, using anti-His antibody, of whole-cell proteins of the *fliH* null mutant transformed with the same plasmids as in panel A. V, vector.

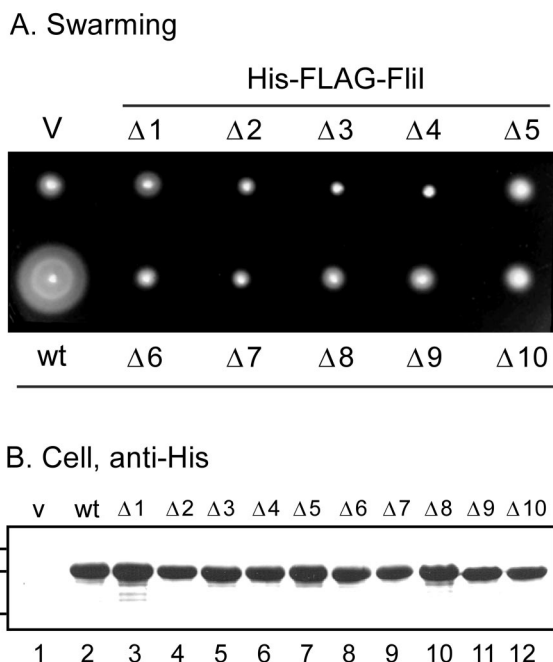


FIG. 5. (A) Swarming of the *fliH* null mutant transformed with pTrc99A-based plasmids encoding 10-amino-acid deletion derivatives of FliI. Plates were incubated for 5 h at 30°C. V, vector. (B) Immunoblot, using anti-His antibody, of whole-cell proteins of HMS174(DE3)pLysS transformed with the same plasmids as in panel A.

mutations in the *flhA* and *flhB* genes, whose gene products are integral membrane components of the type III flagellar export apparatus. The simplest explanation for these results is that excess FliI avoids the need for subtle regulation by FliH and that FlhA and FlhB can be altered in certain ways that permit tighter binding of FliI. However, we cannot rule out the possibility that the two bypass processes operate by different mechanisms.

Based on the absence of a bypass effect with FliJ, it seems unlikely that overproduction of FliI results in an increase in formation of a putative FliI/FliJ complex. Therefore, we hypothesize that overproduction of FliI increases the probability of its productive interaction with the integral membrane components of the export apparatus.

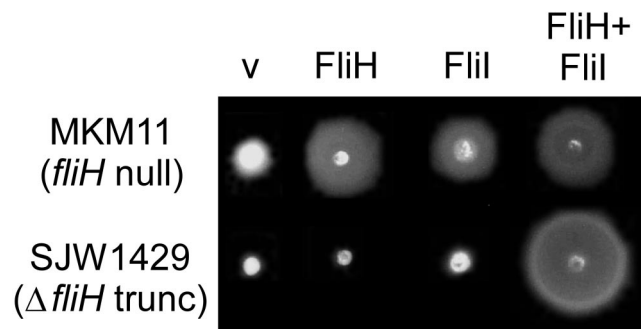


FIG. 6. Swarming of the *fliH* null mutant MKM11 and the *fliH* frameshift mutant SJW1429 (see Fig. 1B) transformed with pTrc99A-based plasmids encoding His-FliH, His-FliI, and His-FliH plus His-FliI. v, vector. Plates were incubated for 4.5 h at 30°C.

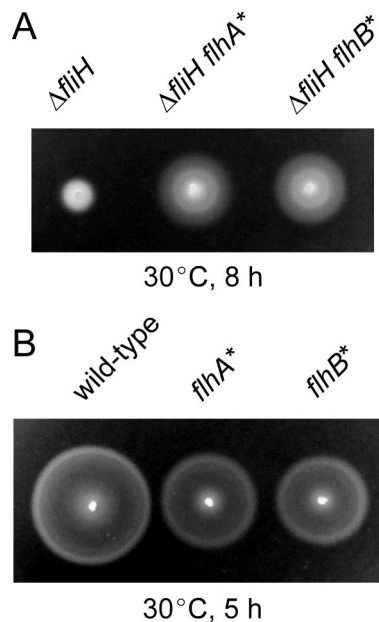


FIG. 7. (A) Swarming of MKM11 ($\Delta fliH$) and pseudorevertants MMA1002 ($\Delta fliH flhA^*$) and MMB1005 ($\Delta fliH flhB^*$). (B) Swarming of SJW1103 (wild type) and second-site mutants MMA1102 (*flhA**) and MMB1105 (*flhB**). The plates were incubated at 30°C for the times indicated.

The *fliH* null mutant displays a leaky motile phenotype, indicating that FliH is not absolutely essential for flagellar protein export (6). However, since FliI alone, even when overproduced, does not support motility as well as the FliH/FliI complex, we propose that FliH plays an important role in the effective docking of FliI to the export apparatus to enable the energy of ATP hydrolysis to be usefully coupled to the translocation of export substrates.

Unlike wild-type FliI, FliI(R7C/L12P) and FliI with truncation of only the first seven amino acids at the N terminus fail to make a complex with FliH (16, 18). However, overproduction of His-FliI(R7C/L12P) and His-FliI($\Delta 1-7$) still overcame the absence of FliH in MKM11 (Fig. 3 and 4), and pTrc99A-based plasmids encoding them complemented a *fliI* mutant to some degree (data not shown). This suggests that even though they cannot make a stable complex with FliH when it is present, both of them retain the ability to interact with the export apparatus when FliH is absent. We propose that the N-terminal flagellum-specific region of FliI is responsible for the docking to the export apparatus (Fig. 1B) as well as for its interaction with FliH (16).

Overproduction of FliI fails to enhance the motility of SJW1429, which produces the first 116 amino acids of FliH along with some frame-shifted residues, indicating that C-terminally truncated FliH interferes with the bypass ability of FliI. We have shown previously that FliH proteins containing 10-amino-acid deletions in the middle and C-terminal regions cannot form a stable complex with FliI and exert a strong dominant-negative effect on the motility of the wild-type strain (6). Therefore, we propose that the N-terminal region of FliH is responsible for association with the export apparatus as well as binding to FliJ (6).

Bypass of the *fliH* null mutation by mutations in *flhA* and *flhB* and the importance of their N-terminal cytoplasmic sequences. Bypass mutations lie within *flhA* and *flhB*, whose gene products are integral membrane components of the type III flagellar export apparatus. Therefore, it is likely that of the integral membrane components of this apparatus, FlhA and FlhB are the ones that interact most directly with FliI. Bypass of the FliH defect occurs even at chromosomal levels of expression of *fliI*, suggesting that conformational changes have occurred within FlhA and FlhB that result in an increase in their binding affinity for FliI.

FlhA and FlhB are integral membrane proteins consisting of 692 and 383 amino acid residues, respectively (14), and both of them have large cytoplasmic C-terminal domains. None of the bypass mutations lie deep within their transmembrane domains. In fact, all but one lie in the short N-terminal cytoplasmic sequences of the two proteins, at or close to the beginning of the first transmembrane span. These N-terminal cytoplasmic sequences must therefore be involved in the interaction with FliI.

The second-site mutant strains swarm better than the pseudorevertants. However, they do not swarm as well as the wild type (Fig. 7). This suggests that these N-terminal mutations in FlhA and FlhB, while beneficial in terms of binding of FliI in the absence of FliH, actually reduce binding to the wild-type FliH/FliI complex to some degree.

We acknowledge Shigeru Yamaguchi for providing SJW2936 and phage P22HTint, Kouhei Ohnishi for providing polyclonal anti-FlgD antibody, and Fan Fan for construction of the FliI deletion plasmids.

This work has been supported by USPHS grant AI12202 to R.M.M. and by a postdoctoral fellowship from Consejo Nacional de Ciencia y Tecnología (CONACYT) (to B.G.P.).

REFERENCES

- Aizawa, S.-I. 2001. Bacterial flagella and type III secretion systems. *FEMS Microbiol. Lett.* **202**:157–164.
- Auvray, F., A. J. Ozin, L. Claret, and C. Hughes. 2002. Intrinsic membrane targeting of the flagellar export ATPase FliI: interaction with acidic phospholipids and FliH. *J. Mol. Biol.* **318**:941–950.
- 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 F_0F_1 ATPase and to virulence proteins of mammalian and plant pathogens. *J. Bacteriol.* **175**:3131–3138.
- Fan, F., and R. M. Macnab. 1996. Enzymatic characterization of FliI: an ATPase involved in flagellar assembly in *Salmonella typhimurium*. *J. Biol. Chem.* **271**:31981–31988.
- Fan, F., K. Ohnishi, N. R. Francis, and R. M. Macnab. 1997. The FliP and FliR proteins of *Salmonella typhimurium*, putative components of the type III flagellar export apparatus, are located in the flagellar basal body. *Mol. Microbiol.* **26**:1035–1046.
- González-Pedrajo, B., G. M. Fraser, T. Minamino, and R. M. Macnab. 2002. Molecular dissection of *Salmonella* FliH, a regulator of the ATPase FliI and the type III flagellar protein export pathway. *Mol. Microbiol.* **45**:967–982.
- Hueck, C. J. 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.* **62**:379–433.
- Kihara, M., T. Minamino, S. Yamaguchi, and R. M. Macnab. 2001. Inter-genic suppression between the flagellar MS ring protein FliF of *Salmonella* and FlhA, a membrane component of its export apparatus. *J. Bacteriol.* **183**:1655–1662.
- Kutsukake, K., Y. Ohya, S. Yamaguchi, and T. Iino. 1988. Operon structure of flagellar genes in *Salmonella typhimurium*. *Mol. Gen. Genet.* **214**:11–15.
- Macnab, R. M. 1999. The bacterial flagellum: reversible rotary propeller and type III export apparatus. *J. Bacteriol.* **181**:7149–7153.
- 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. ASM Press, Washington, D.C.
- Minamino, T., R. Chu, S. Yamaguchi, and R. M. Macnab. 2000. Role of FliI in flagellar protein export in *Salmonella*. *J. Bacteriol.* **182**:4207–4215.
- Minamino, T., B. González-Pedrajo, K. Oosawa, K. Namba, and R. M. Macnab. 2002. Structural properties of FliH, an ATPase regulatory component of the *Salmonella* type III flagellar export apparatus. *J. Mol. Biol.* **322**:281–290.
- Minamino, T., T. Iino, and K. Kutsukake. 1994. Molecular characterization of the *Salmonella typhimurium* *flhB* operon and its protein products. *J. Bacteriol.* **176**:7630–7637.
- Minamino, T., and R. M. Macnab. 1999. Components of the *Salmonella* flagellar export apparatus and classification of export substrates. *J. Bacteriol.* **181**:1388–1394.
- Minamino, T., and R. M. Macnab. 2000. FliH, a soluble component of the type III flagellar export apparatus of *Salmonella*, forms a complex with FliI and inhibits its ATPase activity. *Mol. Microbiol.* **37**:1494–1503.
- Minamino, T., and R. M. Macnab. 2000. Interactions among components of the *Salmonella* flagellar export apparatus and its substrates. *Mol. Microbiol.* **35**:1052–1064.
- Minamino, T., J. R. H. Tame, K. Namba, and R. M. Macnab. 2001. Proteolytic analysis of the FliH/FliI complex, the ATPase component of the type III flagellar export apparatus of *Salmonella*. *J. Mol. Biol.* **312**:1027–1036.
- Ryu, J., and R. J. Hartin. 1990. Quick transformation in *Salmonella typhimurium* LT2. *BioTechniques* **8**:43–44.
- Silva-Herzog, E., and G. Dreyfus. 1999. Interaction of FliI, a component of the flagellar export apparatus, with flagellin and hook protein. *Biochim. Biophys. Acta* **1431**:374–383.
- Suzuki, H., K. Yonekura, K. Murata, T. Hirai, K. Oosawa, and K. Namba. 1998. A structural feature in the central channel of the bacterial flagellar FliF ring complex is implicated in type III protein export. *J. Struct. Biol.* **124**:104–114.
- Togashi, F., S. Yamaguchi, M. Kihara, S.-I. Aizawa, and R. M. Macnab. 1997. An extreme clockwise switch bias mutation in *fliG* of *Salmonella typhimurium* and its suppression by slow-motile mutations in *motA* and *motB*. *J. Bacteriol.* **179**:2994–3003.
- 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 archaeobacterial ATPase subunits. *J. Bacteriol.* **173**:3564–3572.
- 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.
- Yamaguchi, S., H. Fujita, K. Sugata, T. Taira, and T. Iino. 1984. Genetic analysis of *H2*, the structural gene for phase-2 flagellin in *Salmonella*. *J. Gen. Microbiol.* **130**:255–265.
- Zhu, K., B. González-Pedrajo, and R. M. Macnab. 2002. Interactions among membrane and soluble components of the flagellar export apparatus of *Salmonella*. *Biochemistry* **41**:9516–9524.