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Novel Conserved Assembly Factor of the Bacterial Flagellum▽

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TP0658 (FliW) and its orthologs, conserved proteins of unknown function in *Treponema pallidum* and other species, interact with a C-terminal region of flagellin (FlaB1-3 in *T. pallidum*; FliC in most other species). Mutants of orthologs in *Bacillus subtilis* and *Campylobacter jejuni* (*yviF*, CJ1075) showed strongly reduced motility. TP0658 stabilizes flagellin in a way similar to FliS, suggesting that TP0658 is a conserved assembly factor for the bacterial flagellum.

The motility of most bacterial species depends on the proper function of the flagellar apparatus. At least 50 proteins are required for its assembly and function (15).

In order to identify new components of the bacterial flagellar apparatus and their relationships, we tested all ~1,000 open reading frames (ORFs) of *Treponema pallidum* for protein-protein interactions with the FliC homologs FlaB1, FlaB2, and FlaB3 (4) by systematic yeast two-hybrid assays. *T. pallidum* was chosen as a model for this systematic protein interaction analysis because of its limited genome size (8) and the availability of an ORFeome, that is, a complete set of cloned ORFs (8, 16).

TP0658 interacts with flagellin proteins. First, all *T. pallidum* ORFs were cloned into yeast two-hybrid prey and bait vectors (pLP-GADT7 [Clontech] and pLP-GBKT7Amp, a derivative of pLP-GBKT7 [Clontech] with kanamycin resistance replaced by ampicillin resistance; pUniD entry and prey clones were kindly provided by T. Palzkill (16). The prey plasmids were converted into an array and screened with FliC homologs FlaB1, FlaB2, and FlaB3, as described by Cagney et al. (3). Surprisingly, screens with the three *T. pallidum* FliC homologs, FlaB1-3 (TP0868, TP0792, and TP0870), resulted in 24 different positives, among which one protein, TP0658 (FliW), was a widely conserved but uncharacterized protein of unknown function (Table 1). The TP0658-flagellin interactions were highly specific because TP0658 was found only with the three flagellin baits but with none of several hundred other bait proteins we have screened (B. Titz et al., unpublished data). The TP0658-flagellin interaction could be also verified by using an overlay assay: flagellins of *T. pallidum* were tagged with a hemagglutinin (HA) peptide (YPYDVPDYA) and expressed in *Escherichia coli* (Tables 2 and 3), and lysates were gel separated and blotted onto a polyvinylidene difluoride membrane. The membrane was blocked and then incubated with 25 nM purified glutathione *S*-transferase (GST)-TP0658 fusion protein or 25 nM GST control protein, and the bound proteins were detected with either anti-GST or anti-HA antibodies (G1160

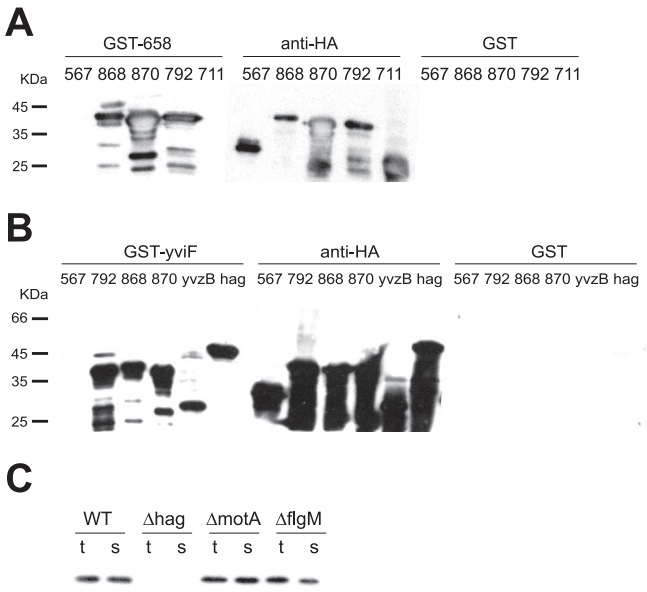

FIG. 1. *T. pallidum* protein TP0658 and its *B. subtilis* ortholog YviF interact with flagellin proteins. (A) HA-tagged flagellin proteins of *T. pallidum*—TP0868 (FlaB1), TP0792 (FlaB2), and TP0870 (FlaB3)—were expressed in *E. coli* BL21(DE3) cells and the total lysate was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane. The membrane was blocked and then incubated with 25 nM purified GST-TP0658 fusion protein (left panel) or 25 nM GST control protein (right panel). Detection of bound GST-protein (anti-GST antibody G1160; Sigma-Aldrich) and HA-tagged proteins (anti-HA antibody HA.11; Covance Research Products) was done by using standard Western blotting procedures. The proteins TP0567 and TP0711 are randomly chosen negative controls to exclude the nonspecific binding of TP0658. Probing with anti-HA (middle panel) and anti-GST antibodies (right panel) served as controls for expression levels and nonspecific binding, respectively. (B) HA-tagged flagellin proteins of *T. pallidum* (TP0792, TP0868, and TP0870) and *B. subtilis* (Hag and YvzB) were expressed in *E. coli* and tested for protein interactions with GST-tagged YviF (GST-yviF) in an overlay assay. The protein TP0567 was included as negative control. Probing with anti-HA and GST alone served as controls for expression levels and nonspecific binding, respectively. (C) Lysates of a *B. subtilis* wild-type strain (168) and of *hag*, *motA*, and *flgM* mutants (Table 2) were tested for proteins interacting with GST-yviF in an overlay assay. The molecular mass of the bands shown corresponds to Hag (~31 kDa); total (t) and soluble (s) extracts are shown.

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TABLE 1. Yeast two-hybrid interactions of flagellin proteins (sorted by prey)

Bait[a]	Prey[b]	Bait count[c]	Prey description[d]
TP0870	TP0005	10	DNA gyrase, subunit A (*gyrA*) (*Bacillus subtilis*)
TP0870	TP0046	13	Hypothetical protein (*Treponema pallidum*)
TP0868	TP0050	10	Conserved hypothetical protein (*Borrelia burgdorferi*)
TP0792	TP0064	8	Hypothetical protein
TP0870	TP0066	11	Hypothetical protein
TP0792	TP0160	13	Prolyl-tRNA synthetase (*proS*) (*Escherichia coli*)
TP0870	TP0174	1	Hypothetical protein
TP0870	(TP0258)	90	Conserved hypothetical protein (*Borrelia burgdorferi*)
TP0870	(TP0288)	94	Spore coat polysaccharide biosynthesis protein (*spsF*)
TP0870	TP0396	14	Flagellar basal-body rod protein (*flgB*) (*Treponema denticola*)
TP0870	(TP0455)	57	Conserved hypothetical protein (*Borrelia burgdorferi*)
TP0870	(TP0563)	78	Hypothetical protein
TP0870	TP0629	6	Hypothetical protein
TP0792	**TP0658**	3	Transmembrane protein, putative (*Bacillus subtilis*)*
TP0868	**TP0658**	3	Transmembrane protein, putative (*Bacillus subtilis*)*
TP0870	**TP0658**	3	Transmembrane protein, putative (*Bacillus subtilis*)*
TP0868	(**TP0661**)	57	Hypothetical protein
TP0870	(**TP0661**)	57	Hypothetical protein
TP0870	TP0702	2	Conserved hypothetical protein (*Borrelia burgdorferi*)
TP0792	(**TP0764**)	87	Conserved hypothetical protein (*Borrelia burgdorferi*)
TP0870	(**TP0764**)	87	Conserved hypothetical protein (*Borrelia burgdorferi*)
TP0870	(TP0788)	63	Hypothetical protein
TP0792	(**TP0832**)	41	Hypothetical protein
TP0868	(**TP0832**)	41	Hypothetical protein
TP0870	(**TP0832**)	41	Hypothetical protein
TP0870	(TP0870)	59	Flagellar filament 31-kDa core protein (*flaB3*)
TP0870	TP0873	1	Hypothetical protein
TP0870	(TP0907)	77	Conserved hypothetical protein (*Borrelia burgdorferi*)
TP0870	(TP0989)	107	P26 (*Borrelia burgdorferi*)
TP0870	(TP0993)	167	Rare lipoprotein A, putative (*Borrelia burgdorferi*)

[a] Flagellin proteins tested as baits against the whole-genome prey library of *T. pallidum*. FlaB1 = TP0868, FlaB2 = TP792, FlaB3 = TP0870.
[b] Preys identified with more than one flagellin are marked in boldface. Preys not reliably identified due to unspecific binding (binding to more than 5% of the tested baits) are shown in parentheses.
[c] The bait count represents the number of unique baits a prey has been found with and thereby indicates nonspecific binding (numbers are based on 450 baits screened). For example, TP0005 has been found with 10 different baits, including TP0870.
[d] Species names given after the prey description indicate the source of annotation for these proteins. *, TP0658 is most likely not a membrane protein since it is fairly well soluble (the misleading annotation appears to stem from a hydrophobic sequence in the middle of the protein).

[Sigma-Aldrich, Germany], HA.11 [Covance Research Products, California]). Indeed, all three flagellin interactions of TP0658 could be verified in vitro by using this overlay assay (Fig. 1A).

The interaction is conserved in *Bacillus subtilis*. To test whether the protein interactions of TP0658 are conserved, we tested its ortholog in *B. subtilis*, YviF, with the two flagellin proteins of *B. subtilis*, Hag and YvzB. Hag is the full-length flagellin protein of *B. subtilis*, whereas YvzB represents an N terminally truncated form of unknown function. We PCR amplified the *yviF* gene from *B. subtilis* genomic DNA and expressed it as a GST fusion. The *hag* and *yvzB* genes of *Bacillus subtilis* were HA tagged by cloning them into the pHB-HA3 vector. The interactions between pHB-HA3-hag, pHB-HA3-yvzB, and GST-yviF were tested as described previously using an overlay assay that clearly showed that YviF interacts with both *B. subtilis* flagellin proteins (Fig. 1B). In addition, YviF binds to all three flagellin proteins of *T. pallidum*, suggesting that the interaction epitope is highly conserved even among distantly related species (Fig. 1B). Moreover, the interaction between YviF and Hag could be confirmed in an overlay assay using different *B. subtilis* deletion strains (Fig. 1C).

TP0658 and FliS bind to similar epitopes of flagellin. Since the structure of flagellin is known (19), we mapped the TP0658 interaction epitope of the flagellin protein to get structural insight into the function of this interaction. First, we tested the systematic truncations of TP0868 (FlaB1) for the binding of GST-TP0658 in an overlay assay. Specific fragments of TP0868 were expressed as HA fusions (in vector pHB-HA3) and tested for GST-TP0658 binding. This overlay assay showed that TP0658 interacts with an epitope within the C-terminal 55 amino acids of TP0868 (L_{231}-C terminus) (Fig. 2A and B). An interaction with the C-terminal half of flagellin is also supported by the interaction of *yviF* with the N-terminally truncated flagellin, *yvzB* (Fig. 1B), which is naturally lacking the region homologous to the first 110 amino acids of TP0868 (Fig. 2A). Strikingly, the TP0658 interaction epitope of FlaB1 is similar to the FliS-binding site in FliC: FliS binds to the C-terminal 40 amino acids of *Salmonella enterica* serovar Typhimurium flagellin—the region implicated in the polymerization of flagellin (17). For a more detailed characterization of the interaction epitope, we synthesized the amino acid sequence of the C-terminal binding domain of TP0868 as overlapping 15-mer peptides on a cellulose membrane by using an automated spot synthesizer (7) (MultiPep, Intavis, Germany) and then probed them with GST-TP0658 (using the same conditions as in the overlay experiments). GST-TP0658 interacted with peptides that correspond to the sequence between L_{231} and D_{247}

TABLE 2. Strains and plasmids

B. subtilis strain or plasmid	Description and/or genotype	Source or reference
Strains		
168	*trpC2*	BGSC[a]
Δ*hag* mutant	*argF4 flaC51 hag-1 hisA1 ura*	BGSC
Δ*mot* mutant	(SPbc2) *motA*::Tn*917 trpC2*	BGSC
Δ*flgM* mutant	*flgM*Δ80 *pheA1 trpC2*	BGSC
Δ*upp* mutant	Strain used for *yviF* deletion	6
Δ*yviF* mutant	*yviF* deletion by phleomycin-upp cassette integration (6)	This study
Plasmids		
pDG148-Stu	P_{spac} regulated protein expression in *B. subtilis*	11
pDG-yviF	HA-tagged *yviF* cloned into pDG148-Stu	This study
pUniD/V5-His-TOPO	Entry vector for UPS system (14)	Invitrogen
pUniD-TP0561, -TP0658, -TP0711, -TP0792, -TP0868, and -TP0870	*T. pallidum* ORFs cloned into pUniD/V5-His-TOPO	16
pUniD-yviF, -hag, and -yvzB	*B. subtilis* ORFs cloned into pUniD/V5-His-TOPO	This study
pUniD-hag-HA and pUniD-hag-N255A	Targeted mutations of the TP0658 binding site in Hag	This study
pMM110	GST fusion protein expression in *E. coli*	16
GST-TP0658 and GST-yviF	pUniD constructs recombined with pMM110	This study
pHB-HA3	HA-tagged protein expression in *E. coli*	14
pHB-HA3-TP0561, -TP0711, -TP0792, -TP0868, -TP0870, -hag, -yvzB, -hag-HA, and -hag-N255A	pUniD constructs recombined with pHB-HA3	This study
pAC28 and pEGST	Coexpression of proteins in *E. coli*	12

[a] BGSC, *Bacillus* Genetic Stock Center.

of TP0868 (Fig. 2A and C). A peptide comprising the interaction epitope sequence (VGL_{231}DIAAENLQAAESRIRD$_{247}$) was also able to inhibit the binding of TP0658 to all three *T. pallidum* flagellin proteins (Fig. 2D). In order to identify amino acids crucial for binding, we then systematically replaced each position of the previously identified peptide (V_{229}GLDIAAE NLQAAESRIRD$_{247}$) with alanine and tested the resulting peptides for binding, showing that I_{233} and N_{237} of the previously identified interaction epitope are crucial for binding in this assay (Fig. 2E). For additional verification, the corre-

TABLE 3. Primers used in this study

Primer	Sequence
hagHAforward	TACGACGTCCCAGACTACGCTGTTGACATGGCTAAAGAGATGAG
hagHAreverse	AGCGTAGTCTGGGACGTCGTATGGGTAAATTGTGTGCTCTAGACGATTTTGTAC
hagN255Aforward	AGCGCTTCTGGTGAAGCTTTGACAGCTGCTGAG
hagN255Areverse	CTCAGCAGCTGTCAAAGCTTCACCAGAAGCGCT
yviF_forward	AATTCTCGAGGAATGATCATTCATACGAAGTA
yviF_reverse	AATTGAGCTCCTAGCATGATTCTCCTCCAA
hag_forward	AATTCTCGAGGAATGAGAATTAACCACAATAT
hag_reverse	AATTGAGCTCTTAACGTAATAATTGAAGTA
yvzB_forward	AATTCTCGAGGAATGGATGCGCTTATTGAGGA
yvzB_reverse	AATTGAGCTCTTAACGTAACAATTGAAGCA
pUniD_forward	CTATCAACAGGTTGAACTG
pUniD_reverse	CAGTCGAGGCTGATAGCGAGCT
L_{59}reverse	GGTTGAGCTCAGAGGCCGCGGATTTGGCTGC
N_{60}forward	TTGGCTCGAGGAAACCAGGCATCCACCAATGC
S_{141}reverse	GGTTGAGCTCAGGAGAAGCGGCCCGTGAGCA
V_{118}forward	TTGGCTCGAGGAGTGGCAGAGGTAGACCGCAT
S_{141}reverse	GGTTGAGCTCAGGAGAAGCGGCCCGTGAGCA
I_{200}forward	TTGGCTCGAGGAATCGGCACCATCGATGCTGC
L_{231}forward	TTGGCTCGAGGACTTGACATCGCTGCGGAGAA
S_{199}reverse	GGTTGAGCTCAGCTCTTGTTGGCCGAGTCTG
yviF_p1	CATGGGTGTTGGAGGAAGG
yviF_p2	AGTCGACCTGCAGGCATGCAAGCTGTTCTTCTTTTATGTTCATTTGGCC
yviF_p3	CGAGCTCGAATTCACTGGCCGTCGTACCATGGCCAAATGAACATAAAAGAAGA AACAAAGCATCCGATTGGAGG
yviF_p4	ATCGTTTATATCGACTAAGTCG
pHB-HA3_pDGforward	AAGGAGGAAGCAGGTATGGCAGGTTACCCATACGAC
pHB-HA3_pDGreverse	GACACGCACGAGGTCAGTCGAGGCTGATAGCGAGCT
TP0658_pEGSTforward	AATTGGATCCATGGAGATTCAGACGAAGACGC
TP0658_pEGSTreverse	AATTCTCGAGTCAACATTGTTCCTGCGCCCTTC
TP0868_pAC28forward	AATTGGATCCATGATTATCAATCACAACATGAG
TP0868_pAC28reverse	AATTGAGCTCCGGAGAATTGAGAGAATCGAC

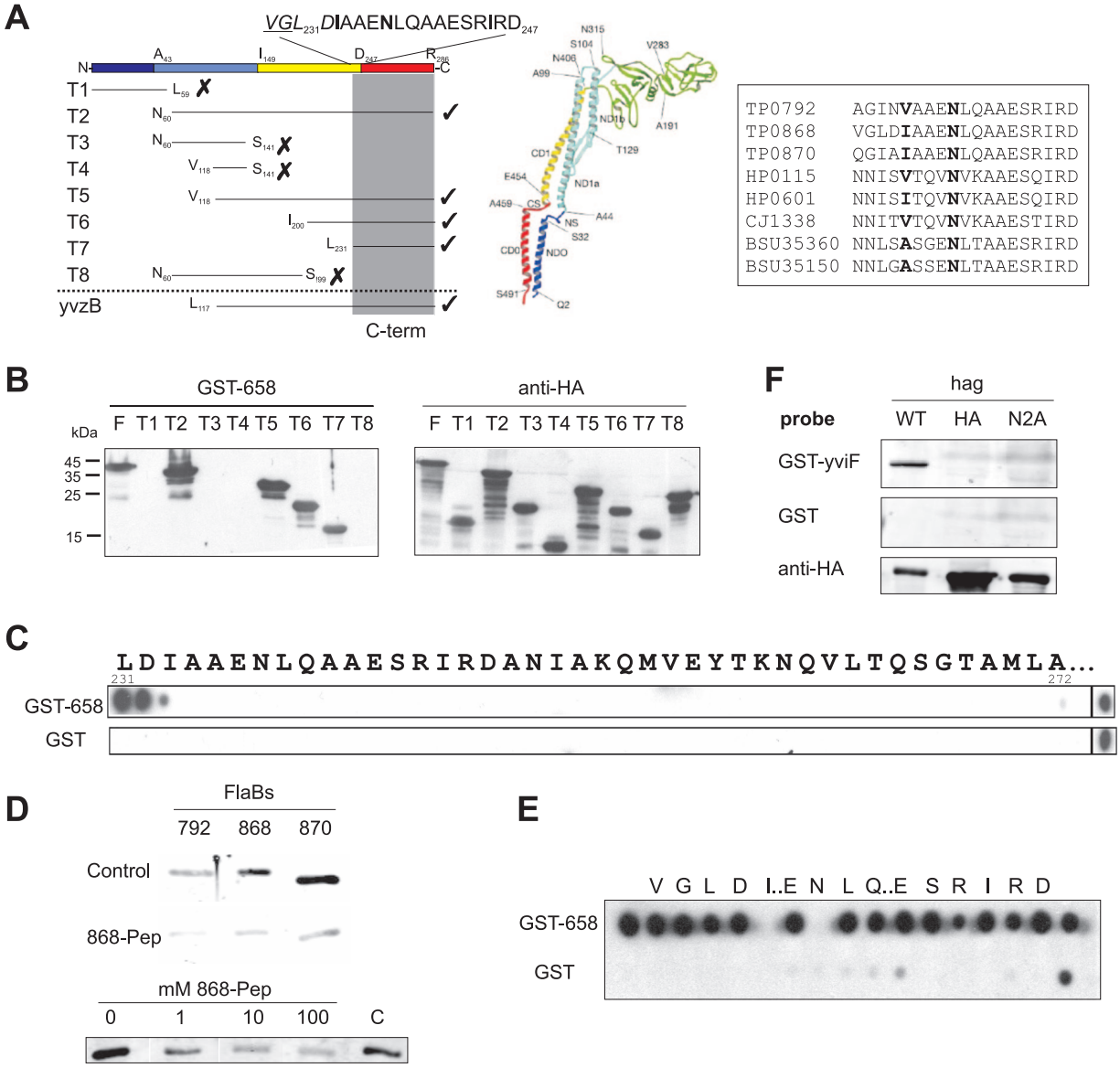

FIG. 2. TP0658/yviF binds to the C-terminal loop region of flagellin. (A) Schematic representation of TP0868 (FlaB1) fragments and results of interaction epitope mapping. The *S. enterica* serovar Typhimurium flagellin structure is shown (19); the primary sequence of TP0868 is color coded accordingly (not drawn to scale). (B) Eight fragments of TP0868 (T1 to T8) were tested for interactions with GST-658 in an overlay assay; positive and negative results for the fragments are indicated by ticks and crosses in panel A. The interaction region is indicated by a shaded box in panel A. (C) Corresponding sequence (L_{231}-C terminus) as tested by peptide spotting. The sequence was synthesized as overlapping peptides of 15 amino acids with 1-amino-acid shifts directly onto a cellulose membrane. These peptides were tested for interactions with GST-658 or GST as a control. The first amino acid of each 15-mer peptide is shown above its spot. The last peptide is a positive control recognized by the anti-GST antibody. Only the first three peptides starting from L_{231} interacted, indicating an interaction epitope between L_{231} to D_{247}. (D) In the upper panel, a 100 mM concentration of synthetic peptide of this epitope region (868-Pep) but not of a control peptide (DRRLADHFCGKIHCG) was able to inhibit the binding of GST-658 to all three flagellin proteins—FlaB1 (TP0868), FlaB2 (TP0792), and FlaB3 (TP0870)—in an overlay assay. The lower panel shows that the inhibitory effect of 868-Pep was concentration dependent (shown for TP0868, FlaB1). (E) An extended interaction peptide (V_{229}-D_{247}) was tested in an alanine scan showing that I_{233} and N_{237} are crucial for binding. The first and the last spots of the row show wild-type and antibody control sequences, respectively. The remaining peptide spots have the indicated amino acid replaced by alanine. Double dots ("..") indicate positions not synthesized because of naturally occurring alanine residues at these positions (these peptides correspond to the wild-type sequence in position 1). (F) Mutants of the *B. subtilis* Hag binding epitope (i.e., *B. subtilis* flagellin) were tested for binding to YviF in an overlay assay. The construct hag-HA has the interaction epitope replaced by an HA tag. hag-N255A (N2A) has the crucial Asn_{255} residue (Asn_{237} in FlaB1/TP0868) replaced by alanine. Probing with GST protein and anti-HA antibodies served as controls. The interacting region in different flagellin orthologs is shown in the alignment in panel A, right panel: *Treponema pallidum* flagellins TP0792 (FlaB2), TP0868 (FlaB1), and TP0870 (FlaB3); *Helicobacter pylori* flagellins HP0115 (flagellin B) and HP0601 (flagellin A); *Campylobacter jejuni* flagellin CJ1338 (flagellin B); and *Bacillus subtilis* flagellins BSU35360 (Hag) and BSU35150 (YvzB).

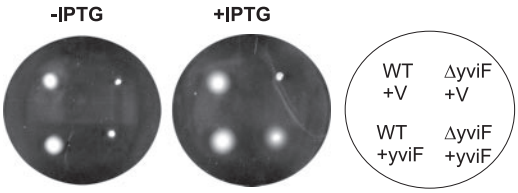

FIG. 3. A *yviF* deletion mutant shows impaired motility. The *B. subtilis* Δ*yviF* mutant was tested in a swarming assay without (−IPTG) or with (+IPTG) induction of *yviF* expression from a plasmid (+yviF). *B. subtilis* cells transformed with the empty vector, pDG148-Stu, served as controls (+V). The Δ*yviF* mutant has a clear swarming defect that can be rescued by *yviF* expression.

sponding interaction epitope (Fig. 2A), N_{247}NLSASGENLTA AESRIRD$_{265}$ in *B. subtilis* Hag was replaced by an HA tag sequence. In a second construct, pUniD-hag-N255A, the asparagine residue at position 255 of Hag was replaced by alanine. This residue corresponds to N_{237} in TP0868 and was found to be crucial for TP0658 binding in the SPOT analysis; the second residue crucial for binding in the SPOT analysis, I_{233}, is naturally exchanged by alanine in Hag, and it is assumed to have either a species-specific relevance or an indirect effect on binding by influencing the peptide's conformation in the spot analysis. Strikingly, an overlay of the two mutant proteins (in pHB-HA3) with GST-yviF showed the complete loss of binding (Fig. 2F), confirming the essentiality of Asn$_{255}$ (Asn$_{237}$ in TP0868) and demonstrating the evolutionary conservation of the interaction epitope in both *T. pallidum* and *B. subtilis* proteins.

***yviF* is required for motility.** A functional involvement of *yviF* in bacterial motility was tested by using a *B. subtilis yviF* deletion mutant. The Δ*yviF* strain was created by specific integration of a phleomycin-upp cassette into the *yviF* locus as described by Fabret et al. (6). The Δ*yviF* mutant showed a strong reduction of motility in a swarming assay (on LB plates with 0.25% agar) (Fig. 3). As expected, only IPTG (isopropyl-β-D-thiogalactopyranoside)-induced expression of *yviF* from a plasmid (pDG-yviF [11]) could rescue the motility phenotype, clearly proving that the reduced motility is due to the lack of the *yviF* gene in the Δ*yviF* mutant.

A FliS mutant in *E. coli* could not be rescued by expressing TP0658 (data not shown). This suggests that the functions of the two proteins are not identical or that species-specific differences prevented a successful rescue.

TP0658 and its orthologs are new assembly factors of the flagellum. Based on the similar interaction pattern of FliS and TP0658/yviF, both of which interact with the C terminus of flagellin (the region involved in the polymerization of flagellin), we hypothesized that TP0658/yviF might be a novel assembly factor for flagellin proteins. In fact, the deletion of *yviF* leads to a strong reduction in the amount of flagellin protein detected in *B. subtilis* cells (Fig. 4A); this is probably due to an inhibition of flagellin incorporation into the nascent filament and subsequent degradation. Strikingly, TP0658 appears to stabilize FlaB1 (TP0868) when coexpressed in *E. coli* from the vectors pEGST and pAC28 (12), respectively (Fig. 4B). Notably, the stabilization does not lead to solubilization of flagellin; possible explanations are a protective effect of TP0658 against proteases (as seen for FliS (17) or an involvement of TP0658 in

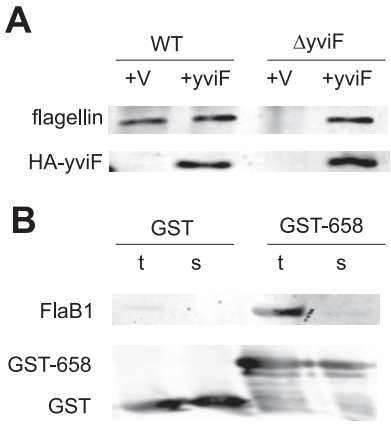

FIG. 4. TP0658 and *yviF* stabilize flagellin. (A) *B. subtilis* strains were tested for flagellin (Hag) and YviF expression from a plasmid. Hag and HA-yviF were detected by a GST-yviF overlay and anti-HA antibodies, respectively. Wild-type (WT) and Δ*yviF* cells carrying an empty plasmid, pDG148-Stu (+V) or the *yviF* expression plasmid, pDG-yviF (+yviF), are compared. Flagellin can only be detected when yviF is present. (B) His-tagged TP0868 (FlaB1) and GST-tagged TP0658 (or GST) were coexpressed in *E. coli*. The expression was done overnight, relying on the basal expression levels of both constructs to obtain physiological more relevant expression levels. Total (t) and soluble (s) lysates were tested for the presence of protein. TP0868 (FlaB1) can only be detected when TP0658 is present; coexpression of TP0658 thus leads to TP0868 stabilization. Please note that stabilized TP0868 is not found in the soluble fraction, e.g., supporting a stabilization at the membrane (see the text).

a membrane (basal body complex)-associated assembly mechanism as proposed for the secretin pilot protein MxiM (see below) (13).

Recently, the structure of BH3618, the *Bacillus halodurans* ortholog of TP0658, was determined by a Structural Genomics Project (PDB code 2AJ7). A type three secretion system (TTSS) component related to BH3618 by its somewhat similar structure is MxiM (PDB code LY9T), a secretin pilot protein functioning as an assembly factor in the periplasmic space. MxiM is thought to support the assembly of the MxiD polymer directly at the outer membrane by means of its interaction with the C-terminal part of its substrate MxiD. However, the evolutionary and functional relationships between BH3618 and MxiM need to be studied in more detail.

Conclusions. Although two previous screens (10, 18) indicated a role of the TP0658 family of proteins in motility, neither was sufficient on its own to justify a reannotation of the protein. Here we provide additional evidence and synthesize all available information into a model that supports the classification of a whole cluster of orthologous genes (COG1699) as a novel family of flagellar assembly factors.

In summary, (i) TP0658/CJ1075 mutants have a motility defect (Fig. 3) (10), (ii) TP0658/yviF are located in flagellar operons, (iii) TP0658/yviF/CJ1075/HP1154/HP1377 bind to flagellin (18; R. Finley, unpublished data), (iv) both TP0658 and FliS bind to the C-terminal part of flagellin which is implicated in polymerization, and (v) TP0658/yviF stabilizes flagellin. TP0658 and its function thus seems to be widely conserved in bacteria (Fig. 5).

The molecular details of TP0658 activity remain unclear but are reminiscent of type III secretion chaperones such as

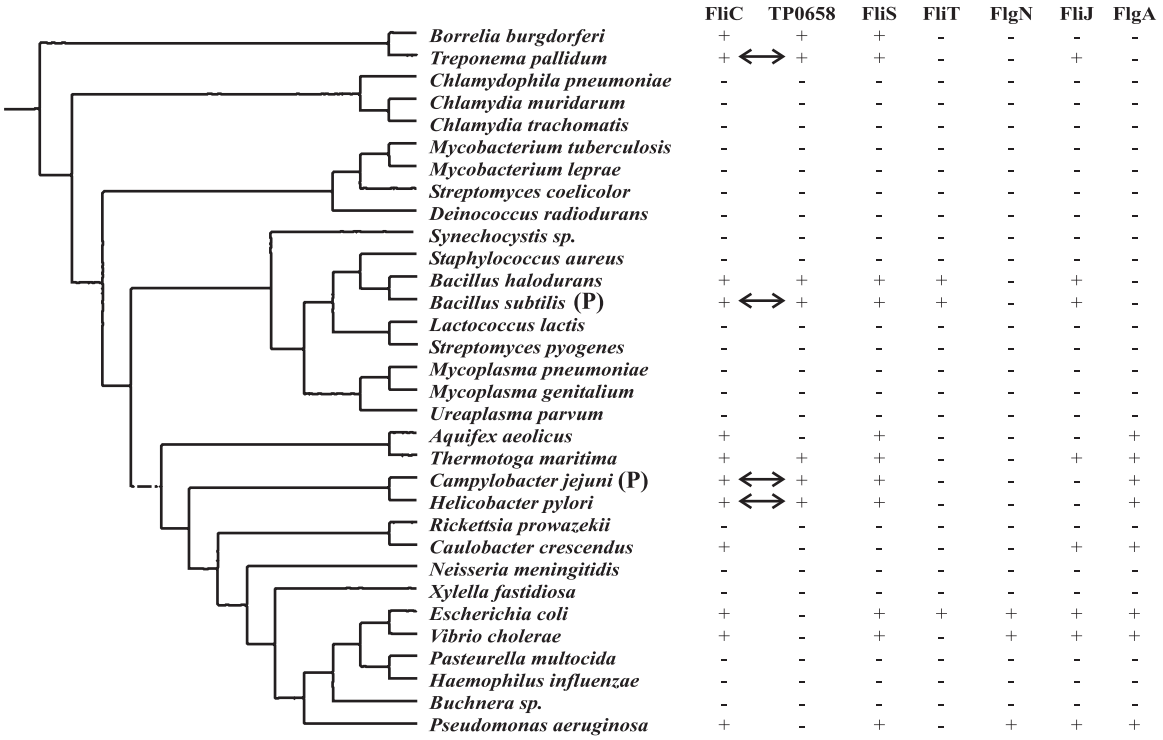

FIG. 5. Conservation of TP0658 and functional relatives in bacteria. A phylogenetic tree (5) shows the presence of TP0658 and other flagellum-related chaperones: FliS is the main chaperone for flagellin (FliC); FliT is a chaperone for the filament capping protein FliD; FlgN is a chaperone for the hook-filament junction proteins FlgK and FlgL; and FliJ is a chaperone for rod and hook proteins (1, 2, 9, 20). FlgA is an assembly factor for the P-ring. TP0658 and flagellum-related chaperones are only found to simultaneously occur with FliC. Experimentally shown interactions between FliC and TP0658 are indicated by double-headed arrows (*C. jejuni* [R. Finley, unpublished data]; *H. pylori* [18]). Mutations of TP0658 homologs in *B. subtilis* and *C. jejuni* are known to have a motility phenotype (P) (*C. jejuni* [10]).

the export chaperone FliS in *S. enterica* serovar Typhimurium (1, 17). FliS binds to flagellin at the same epitope as TP0658 and appears to stabilize flagellin. However, since TP0658 also occurs in genomes with FliS (Fig. 5), it remains to be investigated whether these assembly factors act cooperatively or independently.

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ADDENDUM IN PROOF

After consulting various experts we suggest renaming TP0658 and its orthologs FliW, reflecting its close genomic association with other *fli* genes as well as its association with the flagellar filament. Note that "W" appears to be the last available letter for the *fli* genes.

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