JOURNAL OF BACTERIOLOGY, Apr. 1994, p. 2308–2311
0021-9193/94/$04.00+0

Vol. 176, No. 8

Characterization of the *fliL* Gene in the Flagellar Regulon of *Escherichia coli* and *Salmonella typhimurium*

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Received 29 November 1993/Accepted 15 February 1994

***fliL* is a small gene of unknown function that lies within the beginning of a large flagellar operon of *Salmonella typhimurium* and *Escherichia coli*. A spontaneous *fliL* mutant of *S. typhimurium*, containing a frameshift mutation about 40% from the 3′ end of the gene, was moderately motile but swarmed poorly, suggesting that FliL might be a component of the flagellar motor or switch. However, in-frame deletions of the *E. coli* gene, including an essentially total deletion, had little or no effect on motility or chemotaxis. Thus, FliL does not appear to have a major role in flagellar structure or function and is therefore unlikely to be a component of the motor or switch; the effect on motility caused by truncation of the gene is probably an indirect one.**

The flagellar regulons of *Escherichia coli* and *Salmonella typhimurium* contain exclusively genes concerned with flagellation, motility, and chemotaxis (10). *fliL* is the first gene in one of the flagellar operons (3, 7) and is immediately followed by *fliM* and *fliN*, two of the three genes responsible for flagellar rotation and switching between the counterclockwise and clockwise directions (13); it was discovered as a result of cloning the adjacent gene, *fliM* (3, 7), having escaped attention in searches for mutants. FliL is found in the membrane fraction in minicell experiments (3, 11), and on the basis of its sequence, it would be expected to span the membrane just once and have a C-terminal domain in the periplasm. Its role in the flagellar system is unknown.

Malakooti et al. (11) found that an *E. coli* strain, JM7623 (Fig. 1b), whose *fliL* gene had been disrupted by a kanamycin resistance gene (*Km*^{r}) cassette, was nonflagellate; they concluded that the mutation was not polar on downstream genes and that the null phenotype for *fliL* was nonflagellate.

We subsequently encountered in *S. typhimurium* the first example of a spontaneous *fliL* mutant, SJW2295 (12). This strain carried a severe truncation of the gene yet was motile. Its properties have led us to examine more closely the role of the *fliL* gene.

MATERIALS AND METHODS

Bacterial strains and plasmids. GM2163 (New England Biolabs, Beverly, Mass.) was used for transformations with DNA containing *Cla*I sites, to avoid *dam* methylation. UH869 (4) was used as a minicell-producing strain. AW330 (1) and EKK9 (8) are *E. coli* strains that are wild type for motility and chemotaxis; we used AW330 initially but later changed to EKK9 because we found it had better motility and a higher level of transformation efficiency. *E. coli* JM7623 (11) and *S. typhimurium* SJW2295 (*fliL*) are described in Results.

pJM4 contains an insert of *E. coli* DNA in the vicinity of *fliL* (11). pMAK705, which has a temperature-sensitive origin of replication (2), was a gift from S. Kushner. Other plasmids are described below.

Chemicals. Ampicillin, kanamycin, and chloramphenicol were from Sigma (St. Louis, Mo.). The immunoblotting kit was from Amersham (Arlington Heights, Ill.). Restriction enzymes, Klenow fragment, and T4 DNA ligase were from New England Biolabs. AmpliTaq DNA polymerase was from Perkin-Elmer Cetus (Norwalk, Conn.).

Transfer of *fliL* deletion mutant alleles onto the chromosome. Transfer was accomplished by the method of Hamilton et al. (2), with slight modification in the case of the *Bam*HI-*Cla*I deletion allele of *fliL* in plasmid pMR14 (see Results), with which transformation was accomplished by electroporation, and the transformed cells (after 1 h of growth at 30°C to recover) were diluted into 5 ml of Luria broth plus 10 μg of chloramphenicol per ml and grown overnight at 44°C to enrich for recombinants before plating.

RESULTS

Properties of a *S. typhimurium* mutant with a truncated *fliL* gene. Strain SJW2295 (13), a poorly swarming derivative of wild-type strain SJW1103 (14) (Fig. 2), was found by DNA sequencing to have a single-base insertion at codon 90 of *fliL*, resulting in the loss of over 40% of the wild-type sequence (Fig. 1b). In liquid medium it was less vigorously motile than the wild type, and when subjected to a temporal stimulus of serine it did not show any significant increase in smooth swimming. High-intensity dark-field light microscopy (9) revealed that it had fewer flagella (typically one to three) than the wild type (typically five to eight). When tethered, it rotated more slowly than the wild type, mostly in the counterclockwise direction but with some reversals to clockwise rotation. Thus, the mutant was capable of assembling flagella and of rotating and switching them but was somewhat defective in all of these functions. Nonetheless, the fact that it was flagellate and motile at all was surprising in view of the severity of the mutation.

Reexamination of the *Km*^{r}-inactivated *fliL* *E. coli* mutant, JM7623. We then reexamined the issue of polarity in strain JM7623. When it was transformed with the *fliL*-containing plasmid pJM4 and spotted onto semisolid tryptone agar plates containing ampicillin, swarming was not observed until after 36 h at 30°C, which is much longer than usual (ca. 16 h) for true complementation. When cells from a swarm were cured of the

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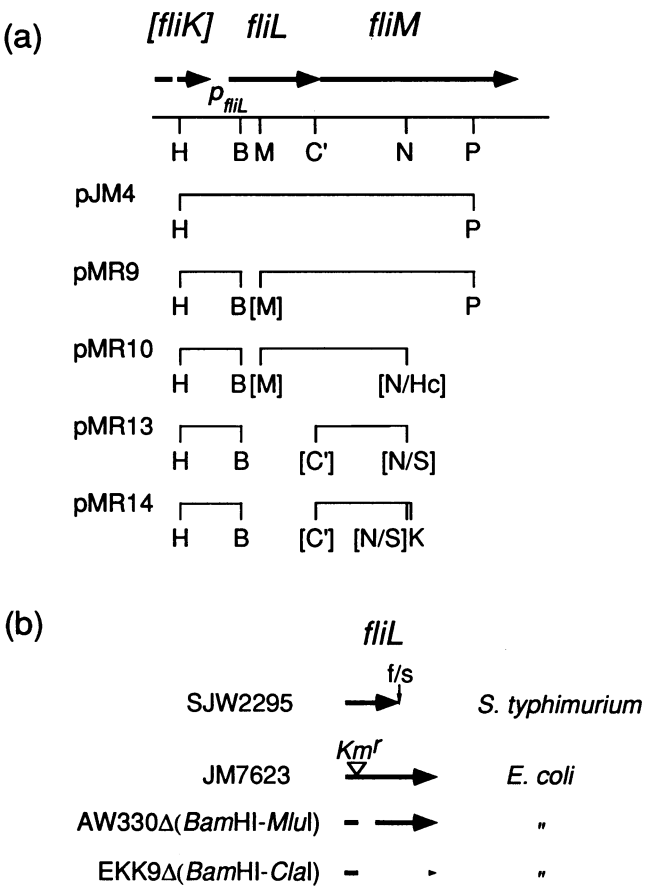

FIG. 1. (a) Plasmids used in this study containing wild-type or mutant alleles of *E. coli fliL*. pJM4 contains the wild-type promoter (p_{fliL}) of the *fliL* operon as well as the *fliM* gene truncated at its *Pvu*II site. pMR9 contains the same insert except for an in-frame deletion between the *Bam*HI and *Mlu*I sites of *fliL*. pMR10 contains the same insert as pMR9, except that *fliM* is now truncated at its *Nru*I site, and the insert has been placed in the vector pMAK705. pMR13 has an in-frame deletion between the *Bam*HI site of *fliL* and an artificially introduced *Cla*I site (C') at its 3′ end and uses pUC18 as the vector. pMR14 has the same insert but in the vector pMAK705. B, *Bam*HI; C, *Cla*I; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn*I; M, *Mlu*I; N, *Nru*I; P, *Pvu*II; S, *Sma*I. Restriction sites that have been lost in the digestion and religation process are in brackets. (b) Schematic representations of the *fliL* gene in various chromosomal mutants. The wild-type allele is 154 and 155 codons in length in *E. coli* and *S. typhimurium*, respectively. *Km^r*, insertion of the kanamycin resistance gene cassette; f/s, frameshift mutation.

plasmid, they remained motile but were now kanamycin sensitive, unlike the original host strain. We conclude that restoration of motility upon transformation with pJM4 is a consequence of recombination (the genotype of the host strain JM7623, *recBC sbcB*, permits recombination by the *recF* pathway) and that the absence of complementation indicates that the *Km^r* insertion mutation is polar. Since downstream genes have a nonflagellate null phenotype, the phenotype of *fliL* itself cannot be deduced from these experiments.

Construction and properties of an *E. coli* mutant with an in-frame *Bam*HI-*Mlu*I deletion in *fliL*. We next proceeded to construct in-frame deletions in *fliL*. Plasmid pJM4 (Fig. 1a) (11) was digested with *Bam*HI and *Mlu*I, and the larger fragment was filled with Klenow fragment, religated (regenerating the *Bam*HI site), and introduced by transformation into XL1-Blue to give plasmid pMR9 (Fig. 1a); the construction was verified by sequencing. The deletion is in frame and results in the loss of codons 17 to 48, with a product molecular mass of 13 kDa in contrast to the wild-type mass of 17 kDa. In minicell experiments, both the internally deleted FliL protein, FliL Δ(*Bam*HI-*Mlu*I), and the truncated FliM protein, FliM*, were seen (Fig. 3, lane 2); immunoblotting confirmed the identity of FliM* (data not shown). Thus, as expected, the in-frame *fliL* deletion had no major polar effects.

The deletion mutation was then transferred onto the chromosome of wild-type *E. coli* AW330 to give strain AW330 Δ(*Bam*HI-*Mlu*I) (Fig. 1b). DNA hybridization (Fig. 4, lane 4) and sequence analysis confirmed that the internally deleted *fliL* sequence had been successfully transferred, yet the mutant swarmed at essentially the wild-type rate (Fig. 2).

Construction and properties of an *E. coli* mutant with an essentially total *fliL* deletion. The sequence deleted in strain AW330 Δ(*Bam*HI-*Mlu*I) is poorly conserved between *E. coli* and *S. typhimurium* (6), so to be certain of the null phenotype, we decided to create a much more drastic mutation, extending from the *Bam*HI site to a *Cla*I site introduced at the end of the gene. The resulting plasmid, pMR13 (Fig. 1a), retains only codons 1 to 17 and the last codon (codon 154) of *fliL*. Minicell analysis with pMR13-transformed cells revealed the predicted truncated FliM product, FliM** (Fig. 3, lane 5), whose identity was further confirmed by immunoblotting (data not shown); thus, the *fliL* deletion is not significantly polar.

This large deletion in *fliL* was then transferred onto the chromosome of wild-type *E. coli* EKK9 to give strain EKK9

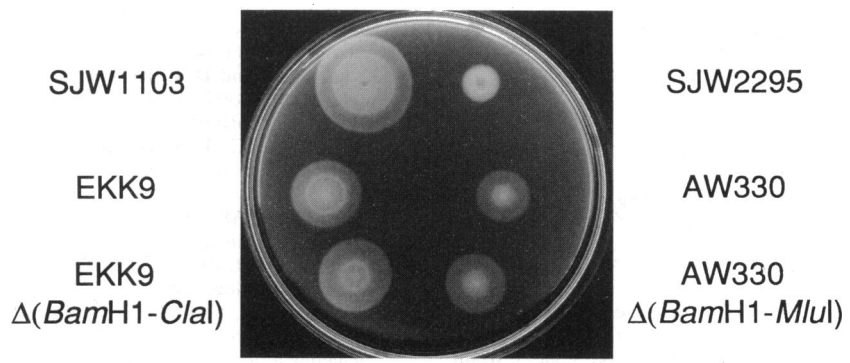

FIG. 2. Swarming of wild-type and *fliL* mutants of *E. coli* and *S. typhimurium* on tryptone-agar (0.375% agar), shown after 18 h at 30°C. The petri dish is 9 cm in diameter.

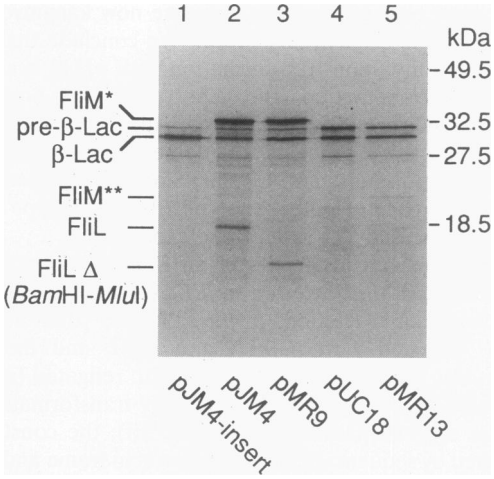

FIG. 3. Analysis of radiolabeled products from minicells transformed with various plasmids. Lane 1, pJM4 after removal of its *Hin*dIII-*Pvu*II insert and religation. Lane 2, pJM4, producing the full-size FliL protein as well as the *Pvu*II-truncated version of FliM (FliM*). Lane 3, pMR9, producing the *Bam*HI-*Mlu*I-deleted FliL protein FliL Δ(*Bam*HI-*Mlu*I) and FliM*. Lane 4, pUC18, the vector for pMR13. Lane 5, pMR13, producing *Bam*HI-*Cla*I-deleted FliL (which is too small to be seen) plus the *Nru*I-truncated version of FliM (FliM**). Molecular mass markers are shown to the right.

Δ(*Bam*HI-*Cla*I) (Fig. 1b). The chromosomal replacement was verified by DNA hybridization (Fig. 4, lane 5) and sequencing.

On swarm plates (Fig. 2) the deletion mutant EKK9 Δ(*Bam*HI-*Cla*I), just like the mutant with the smaller deletion, spread as rapidly as its parent and exhibited chemotactic rings within the swarm; the motility of free-swimming cells and the rotational behavior of tethered cells were also similar to those of the wild type (data not shown).

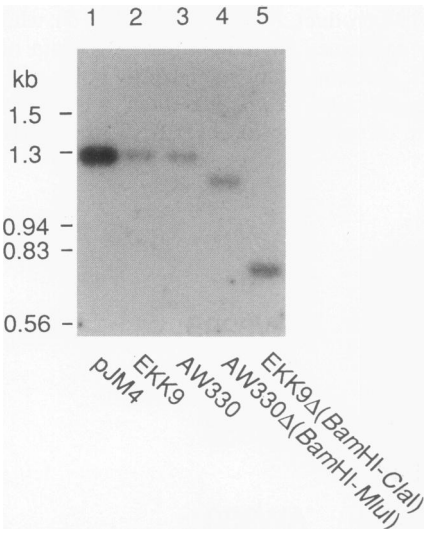

FIG. 4. DNA hybridization of *Bam*HI-*Pvu*II-digested *E. coli* DNA, probed with an oligomer corresponding to the start of the *fliM* gene. Lane 1, plasmid pJM4. Lane 2, strain EKK9, wild type. Lane 3, strain AW330, wild type. Lane 4, strain AW330 Δ(*Bam*HI-*Mlu*I). Lane 5, strain EKK9 Δ(*Bam*HI-*Cla*I). Size markers are shown to the left.

DISCUSSION

We started out with the presumption, based on an earlier study (11), that *fliL* was necessary for flagellar assembly or structure. Then we found that a severe truncation of the gene, which might have been expected to generate the null phenotype, still sustained flagellation and motility (although not at wild-type levels). We have now established that the nonflagellate phenotype of strain JM7623 (11) was a result of polarity, and we suspect that the impaired motility associated with truncation of the *fliL* allele in strain SJW2295 may be due to partial polarity or some other indirect effect.

When we tested the null phenotype rigorously, by making an in-frame deletion of virtually the entire *fliL* gene, we found that the cells exhibited essentially wild-type chemotactic swarming. (Interestingly, in *Caulobacter crescentus* the null phenotype of *fliL* has been found to be flagellate but paralyzed [5]. The reason for this species difference is unclear, but it should be noted that the *C. crescentus* sequence is significantly different in several regards [5, 15], the most notable one being a region of extremely high charge density that is lacking in the enteric bacteria; conceivably *fliL* in *C. crescentus* encodes additional functions.)

What then is the role of FliL in *E. coli* and *S. typhimurium*? At this point there are still no real clues, but in view of the fact that null mutants were capable of rotation and switching, we do not think it likely that FliL is a direct component of the motor or switch in the sense that FliG, FliM, and FliN are.

ACKNOWLEDGMENTS

We thank P. Matsumura, S. Yamaguchi, S. Kushner, and L. Rost for gifts of strains and plasmids; K. Oosawa for the gift of anti-FliM antibody; and M. Kihara for technical assistance.

This work has been supported by USPHS grants AI12202 and GM40335.

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