

FimZ Is a Molecular Link between Sticking and Swimming in *Salmonella enterica* Serovar Typhimurium

Steven Clegg^{1*} and Kelly T. Hughes²

Department of Microbiology, College of Medicine, University of Iowa, Iowa City, Iowa 52242,¹ and
Department of Microbiology, University of Washington, Seattle, Washington 98195²

Received 3 August 2001/Accepted 26 November 2001

***Salmonella enterica* serovar Typhimurium produces two types of filamentous appendages on its surface. Fimbriae mediate adherence to tissues and cells via receptor-specific interactions, and flagella are the organelles of motility. These appendages play a role in colonization and dissemination, respectively, from infected surfaces and may be important components of bacterial survival. Increased expression of FimZ in serovar Typhimurium resulted in bacteria which were hyperfimbriated but were nonmotile in soft agar. This lack of motility was associated with down regulation of the *flhDC* master flagellar operon. Therefore, FimZ represents a molecular connection between flagella and fimbrial formation in serovar Typhimurium, indicating that the synthesis of flagella and fimbriae are oppositely controlled.**

Motile strains of the Enterobacteriaceae swim using flagella and adhere to mannose-containing glycoconjugates of eucaryotic cells by means of type 1 fimbriae. Both of these phenomena are consequences of the production of proteinaceous appendages on the cell surface. The genetic systems of *Salmonella enterica* serovar Typhimurium involved in flagellar and type 1 fimbrial production, assembly, and regulation have been independently described (4, 5). The expression of flagellar genes is regulated by a complex process involving numerous regulatory proteins that control the production of components necessary for correct assembly of the flagellum structure (4). The molecular biology of bacterial locomotion has been extensively investigated and involves a complex signal transduction pathway (1).

The type 1 fimbrial genes of *S. enterica* serovar Typhimurium have been described previously (5). The synthesis of these appendages occurs via the chaperone-usher pathway, and the assembly components of the system are related to those described for *Escherichia coli* fimbrial biogenesis (10, 15). However, the regulation of *fim* gene expression in *S. enterica* serovar Typhimurium is different to that described in other bacteria. Three genes, *fimZ*, *fimY*, and *fimW*, of the *fim* gene cluster have been shown to affect the expression of *fimA*, encoding the major fimbrial subunit (16–18). Both FimZ and FimY are necessary for the expression of *fimA* in vivo although FimZ alone can bind in vitro to the *fimA* promoter region (14, 18). A comparison of the amino acid sequence of FimZ indicates that it is a member of the family of response regulators (9). However, a contiguous gene encoding a sensor kinase is not located adjacent to *fimZ* since the gene product of *fimY* does not exhibit relatedness to this class of proteins (17).

In the studies described below, we present evidence that the expression of FimZ from a strong heterologous promoter results in a reduced ability of *S. enterica* serovar Typhimurium to

move through soft agar (Swim). This effect on motility is independent of the presence of surface-associated fimbriae and not due to a physical interference with flagellum formation by fimbrial structures. Therefore, FimZ provides a molecular connection between the Fim and Swim phenotypes in serovar Typhimurium.

Strains and growth conditions. The strains of *S. enterica* serovar Typhimurium used in this study are shown in Table 1. To test for the production of type 1 fimbriae, bacteria were cultured at 37°C for 48 h in static, liquid broth as previously described and examined for the presence of surface-associated fimbriae by hemagglutination and reactivity with specific antiserum (12). To determine the Swim phenotype, strains were inoculated into soft agar (1% tryptone, 0.7% NaCl, 0.35% agar) and incubated overnight at ambient temperature. When appropriate, tetracycline was included at a final concentration of 20 µg/ml. The ability to move through the agar was recorded using a Kodak DC120 digital camera system.

Isolation and characterization of the conditional *fimz* mutant *S. enterica* serovar Typhimurium *fimz2::Tn10(T-POP)*. The conditional *fimZ* mutant of *S. enterica* serovar Typhimurium LT2 was isolated following transposon mutagenesis using the Tn10dTc[del-25](T-POP) system that has previously been described to isolate conditional (tetracycline-dependent) phenotypes (13). The Tn10dTc[del-25] T-POP will transcribe from the *tetA* promoter within the T-POP transposon into adjacent chromosomal DNA. The mutant was isolated by random T-POP mutagenesis of a strain carrying the *fliC5050::Mu d-lac* transcriptional fusion selecting for T-POP encoded tetracycline resistance as described by Rappeleye and Roth (13). The T-POP insertion in *fimZ* was identified as an insertion that resulted in a Lac⁻ phenotype in the presence of tetracycline and a Lac⁺ phenotype in the absence of tetracycline (Tc-induced inhibition of *fliC* transcription).

The precise site of the Tn10d(T-POP) insertion was determined by sequence analysis using a primer specific for the left and right ends of the T-POP element. PCR amplification with these primers under decreasing stringency resulted in the amplification of a DNA fragment that was shown by DNA se-

* Corresponding author. Mailing address: Department of Microbiology, College of Medicine, University of Iowa, Iowa City, IA 52242. Phone: (319) 335-7778. Fax: (319) 335-9006. E-mail: stevenclegg@uiowa.edu.

TABLE 1. Strains and plasmids used in this study

Strain	Genotype	Source or reference
Serovar Typhimurium LT2		
TT18796	<i>proAB47/F'128(pro lac) zcf-3833::Tn10dTc[del25](T-POP)</i>	13
TH1077	<i>fliC5050::MudJ</i>	7
TH4038	<i>fimZ2::Tn10dTc[del-25](T-POP)</i>	This work
LB5010	Wild-type LT2 derivative, fimbriate	3
LBH4	<i>fimH::Km^r</i> of LB5010, nonfimbriate	8
LBH4(T-POP)	<i>fimZ2::Tn10(T-POP)</i> transductant of LBH4	This work
TH714	<i>fliB5001::MudJ</i>	7
TH4054	<i>flhC5456::MudJ</i>	This work
TH4313	DUP4102[<i>fliC5050</i>] <i>MudA(flhC5213)</i>]	This work
Serovar Typhimurium SL1344(T-POP)	<i>fimZ2::Tn10(T-POP)</i> transductant of SL1344	This work
Plasmids		
pISF182	pACYC184 carrying <i>fimZ</i>	18
pISF187	<i>Km^r</i> cassette inserted into <i>fimZ</i> on pISF182	18
pISF239	<i>fimZ-lacZ</i> reporter fusion	16

quence analysis to contain the *fimZ* gene sequence. This was confirmed by further DNA sequence analysis and revealed that the transposon had inserted within the *fimY-fimZ* intergenic region of the *S. enterica* serovar Typhimurium LT2 *fim* gene cluster at a site that is 265 and 63 nucleotides upstream of the previously determined *fimZ* transcription and translation initiation sites, respectively (14, 18). The Tn10dTc[del-25] T-POP has a deletion of the normal transcriptional terminator of the *tetA* transcript and, in the presence of tetracycline, will transcribe genes adjacent to the site of insertion from the *tetA* promoter. As previously reported (5, 17), all the *fim* genes downstream of *fimZ* are transcribed in the opposite orientation to that of *fimZ*. Therefore, in the presence of tetracycline the expression of *fimZ* is the only gene of the cluster that is directly affected by the transposon insertion.

Following growth in the presence of tetracycline *S. enterica* serovar Typhimurium *fimZ2::Tn10(T-POP)* was found to be strongly fimbriate and characterized by the ability to mediate mannose-sensitive agglutination of guinea pig erythrocytes, react with specific fimbrial antiserum, and adhere to eucaryotic cells (Table 2). A comparison of fimbrial expression by this strain to the parental LT2 isolate indicates that many more

TABLE 2. Expression of the Fim and Swim phenotype by serovar Typhimurium strains

Strain	Presence (+) or absence (-) of Tet during incubation	Fim ^a	Swim ^b	β -Galactosidase activity of <i>fimZ-lacZ</i> fusion ^c
TH4038	+	+++	-	293
TH4038	-	-	+	19
LBH4(T-POP)	+	-	-	179
LBH4(T-POP)	-	-	+	28
SL1344(T-POP)	+	+++	-	ND ^d
SL1344(T-POP)	-	-	-	ND

^a Type 1 fimbriae were detected using specific antiserum and the ability to agglutinate guinea pig erythrocytes only in the absence of mannose (12).

^b Expansion through soft agar after incubation for 18 to 24 h (see Fig. 1).

^c β -Galactosidase activity of the *fimZ-lacZ* fusion reported as previously described (17).

^d ND, not determined.

appendages are produced by the transposon mutant following growth in the presence of tetracycline. This hyperexpression of type 1 fimbriae has a phenotype identical to that previously described by our group following overproduction of FimZ in *S. enterica* serovar Typhimurium (17, 18). Because FimZ is a transcriptional activator of *fimA* (14), the hyperfimbriate phenotype is consistent with a relatively high level of expression of the FimZ polypeptide.

In the absence of tetracycline, no hemagglutination was observed and type 1 fimbriae were not detected on the surface of *S. enterica* serovar Typhimurium *fimZ2::Tn10(T-POP)*. The lack of fimbrial expression is similar to observations that have been made using a *fimZ* mutant of serovar Typhimurium (18). Disruption of *fimZ* transcription by its homologous promoter following insertion of the Tn10(T-POP) transposon would be expected to result in relatively poor expression of *fimZ* in the absence of tetracycline.

Fimz production affects the ability of *S. enterica* serovar Typhimurium *fimZ2::Tn10(T-POP)* to move through soft agar. The ability of *S. enterica* serovar Typhimurium *fimZ2::Tn10(T-POP)* to move through soft agar is shown in Fig. 1A. In the presence of tetracycline, the bacteria remained localized to the point of the inoculum and were unable to swim, over an 18- to 24-h period, from this site. However, in the absence of tetracycline, the bacteria expanded from the inoculum and rapidly migrate through the agar.

In order to demonstrate that the physical presence of fimbriae on the surface of the bacteria was not responsible for the lack of expansion through the agar in the presence of tetracycline, we examined the motility of a nonfimbriate mutant. *S. enterica* serovar Typhimurium LBH4 carries a mutation of the *fimH* gene and is nonfimbriate. The construction and characterization of this mutant has been described (8), and bacteria have never been observed to express type 1 fimbriae. Following transfer, by transduction, of the *fimZ2::Tn10(T-POP)* allele to this strain, the ability of bacteria to migrate through soft agar was observed (Fig. 1B). As for *S. enterica* serovar Typhimurium *fimZ2::Tn10(T-POP)*, the double mutant rapidly expanded from the site of the inoculum when grown in the absence of

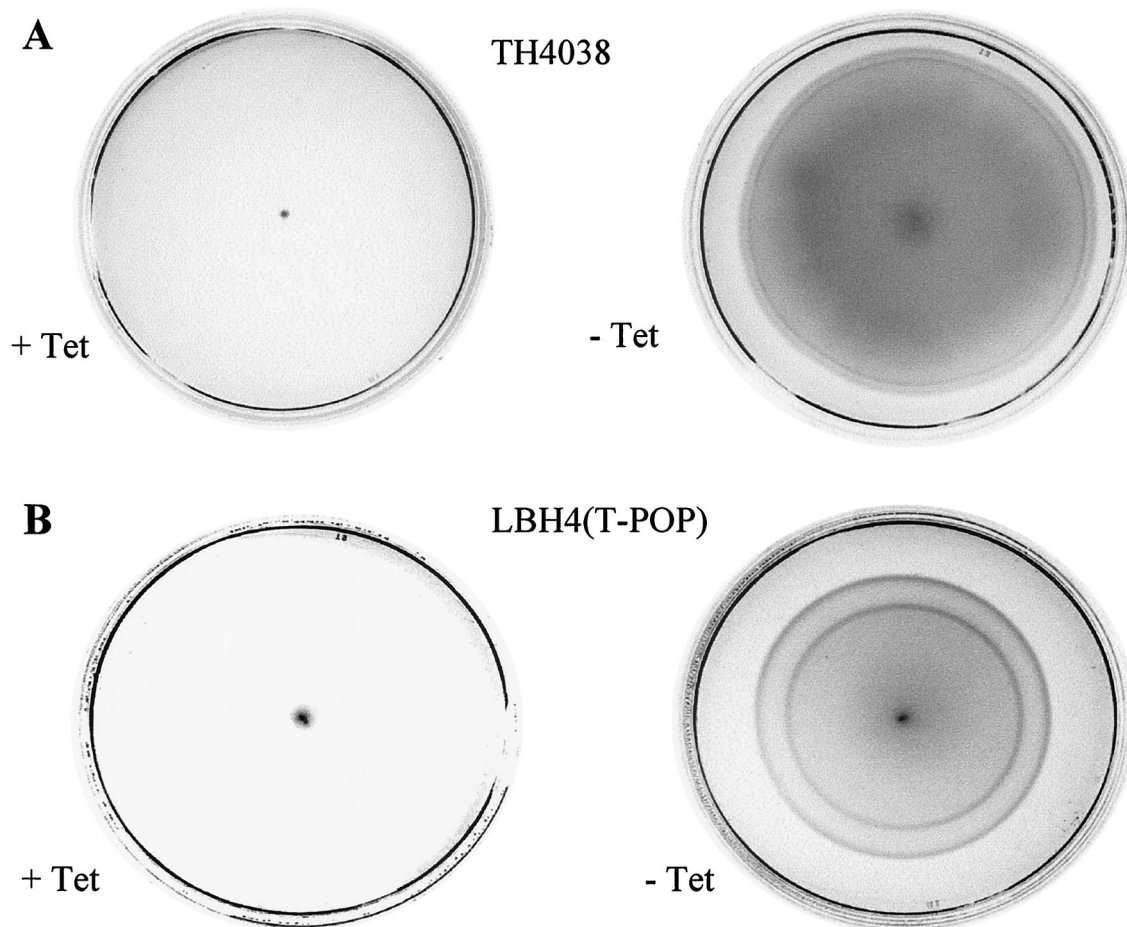


FIG. 1. (A) Movement of the fimbriate LT2 derivative (TH4038) through soft agar in the presence and absence of tetracycline. (B) Movement of the nonfimbriate fimH LT2 derivative (LBH4) through soft agar in the presence and absence of tetracycline. All cultures were incubated at ambient temperature for 24 h.

tetracycline but remained localized if grown in the presence of the antibiotic. As expected, this double mutant did not produce fimbriae under either condition. Consequently, the inability of the *S. enterica* serovar Typhimurium strains to rapidly expand through the soft agar in the presence of tetracycline cannot be explained by effects of fimbrial appendage formation on the surface of the bacteria. Also, a *fimA* mutant of the strain carrying *fimZ*::Tn10(T-POP) exhibited the same motility phenotype as *S. enterica* serovar Typhimurium LBH4. This mutant was constructed using the technique of Datsenko and Wanner (6) and carries a deletion of the *fimA* gene producing no FimA subunits. Therefore, the motility phenotype cannot be due to intracellular accumulation of high levels of FimA polypeptide in the presence of tetracycline.

Since the differences associated with movement by *S. enterica* serovar Typhimurium through soft agar were observed due to the presence or absence of tetracycline, we determined whether the presence of the antibiotic itself was responsible for the difference in bacterial movement. *S. enterica* serovar Typhimurium LT(pISF182) is a transformant possessing a recombinant plasmid expressing the *fimZ* gene, and these transformants are strongly fimbriate due to the overexpression of both *fimZ* and *fimY* (17, 18). The presence of the plasmid confers

chloramphenicol resistance on the bacteria that are tetracycline sensitive. *S. enterica* serovar Typhimurium LT2(pISF182) was strongly fimbriate and did not rapidly expand through soft agar (Fig. 2). Similarly, the nonfimbriate mutant, *S. enterica* serovar Typhimurium LBH4, transformed with pISF182 did not spread through the agar and is, as expected, nonfimbriate. Therefore, in all serovar Typhimurium LT2 derivatives examined, the lack of movement through soft agar was associated with overexpression of *fimZ* regardless of the antibiotic resistance phenotype. To confirm that FimZ production plays a role in altering bacterial movement through agar, *S. enterica* serovar Typhimurium LT2 transformants possessing plasmid pISF187 were examined. This plasmid is derived from pISF182 and carries a nonpolar mutation in *fimZ* (18). Bacteria carrying this plasmid were as motile as *S. enterica* serovar Typhimurium LT2.

In order to determine whether increased expression of *fimZ* correlated with a decreased movement in a highly virulent strain of *S. enterica* serovar Typhimurium, the *fimZ*::Tn10(T-POP) allele was transferred, by transduction, to *S. enterica* serovar Typhimurium SL1344. This isolate is a mouse-virulent strain that has extensively been used to investigate serovar Typhimurium pathogenesis. An *S. enterica* serovar Typhimurium

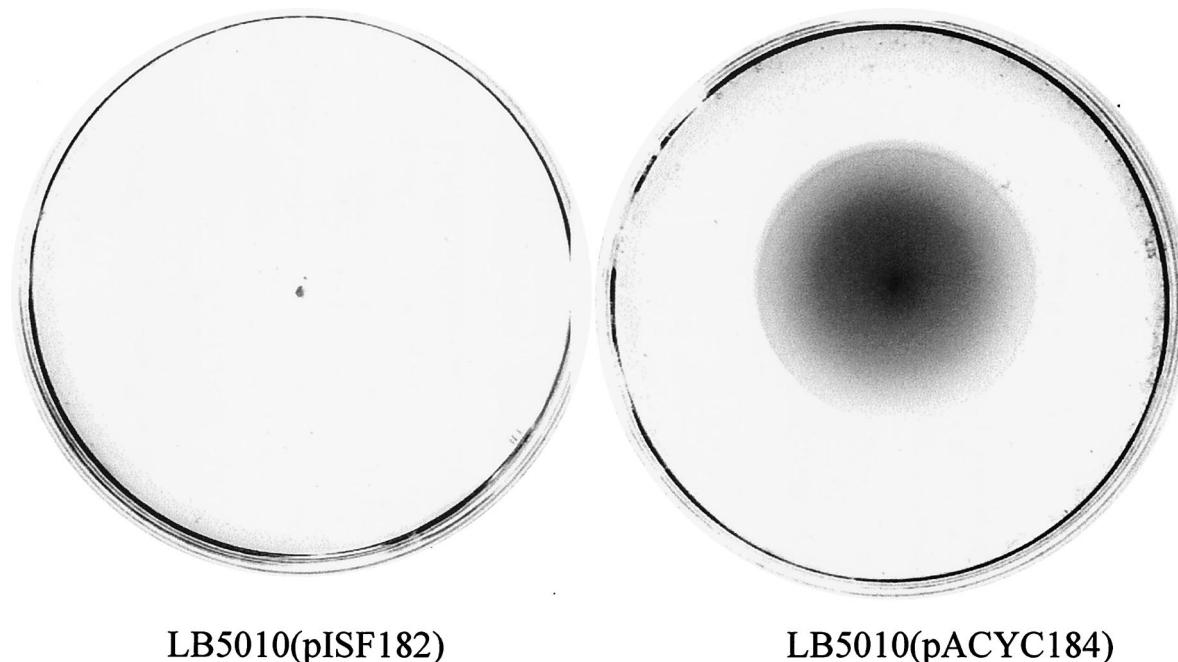


FIG. 2. Movement of LT2 (LB5010) transformants through soft agar. The plasmid pISF182 possesses a functional *fimZ* determinant and pACYC184 is the vector alone. Plates were incubated at ambient temperature for 18 h.

SL1344 transductant carrying the *fimZ*::Tn10(T-POP) mutation exhibited fimbriation and motility phenotypes in the presence or absence of tetracycline identical to those of the LT2 strain (Table 2). Therefore, the observed differences in movement were not strain specific but were found in two distinct isolates of *S. enterica* serovar Typhimurium when *fimZ* was overexpressed.

Expression of *fimZ* by *S. enterica* serovar Typhimurium *fimZ*::Tn10(T-POP). Since we have previously demonstrated that FimZ is an autoregulatory protein (18), a plasmid-based *fimZ-lacZ* reporter construct was used to detect the level of *fimZ* expression in serovar Typhimurium *fimZ*::Tn10(T-POP). Table 2 shows the level of gene expression following growth in either the presence or absence of tetracycline. The level of *fimZ-lacZ* expression was 6 to 15 times greater following growth in the presence of tetracycline than following growth in its absence. Also, following growth in the presence of tetracycline, the level of flagellar antigen (Hi) expression, as detected by specific serum, was significantly decreased (Table 3).

TABLE 3. Flagellum gene expression and antigen (Hi) production in serovar Typhimurium possessing *fimZ*::T-POP allele

Presence (+) or absence (-) of Tet	β-Galactosidase expression in:			Serum titer using anti-H(i) ^b
	<i>fliC-lacZ</i>	<i>fliB-lacZ</i>	<i>flhC-lacZ</i> ^a	
+	110	8	20	80
-	1,100	620	200	640

^a The β-galactosidase levels were the same for *flhC-lacZ* in either a haploid strain (TH4054) that is disrupted for the *flhC* gene or in a tandem duplication strain (TH4313) carrying a functional *flhDC* operon in addition to the *flhC-lacZ* fusion.

^b Titer represents the reciprocal of the highest dilution of serum causing visible agglutination of a bacterial suspension.

Expression of *flhdc* by *S. enterica* serovar Typhimurium *fimZ*::T-POP. *fimZ*::T-POP was isolated as a strain with the tetracycline-induced Lac⁻ phenotype of a strain carrying a *fliC-lacZ* fusion, demonstrating that the overexpression of *fimZ* resulted in decreased *fliC* transcription, consistent with decreased production of the FimH(i) flagellin. Expression of the two flagellin genes was measured using *fliC-lac* and *fliB-lac* transcriptional fusions in strains carrying the *fimZ*::T-POP insertion. Induction of *fimZ* with tetracycline resulted in a 10-fold reduction in *fliC* transcription and a greater than 50-fold reduction in *fliB* transcription (Table 3). The *fliC* and *fliB* genes are transcribed from class 3 flagellar promoters. The flagellar regulon of serovar Typhimurium is a transcriptional hierarchy of three promoter classes (4). At the top of the transcriptional hierarchy is the class 1 *flhDC* operon. The FlhDC proteins are transcriptional activators for class 2 flagellar promoters. The *fliA* gene is transcribed from a class 2 promoter and encodes a flagellum-specific sigma factor, σ²⁸, which specifically transcribes class 3 flagellum promoters, including the *fliC* promoter. In order to determine if the *fimZ*::T-POP insertion exerted its regulatory effect at the level of class 1 *flhDC* promoter transcription, the expression of an *flhDC-lac* transcriptional fusion was measured in strains carrying the *fimZ*::T-POP insertion in the presence and absence of tetracycline. The *flhDC-lac* fusion exhibited tetracycline-dependent repression of Lac expression in the presence of the *fimZ*::T-POP insertion, indicating that the effect of *fimZ* overexpression on the flagellar regulon was at the level of transcription of the class 1 *flhDC* promoter (Table 3).

Summary and conclusion. The ability of bacteria to adhere to solid surfaces under certain environmental conditions and also migrate to ecological niches favorable to growth are fun-

damental events in colonization and dissemination during bacterial survival. In the case of *S. enterica* serovar Typhimurium, these events would facilitate infection and transmission between hosts. Consequently, coordination between the machinery for production of both adherence factors and movement would facilitate coordination between these two systems. We have previously demonstrated that FimZ is a positive transcriptional activator of type 1 fimbrial expression in serovar Typhimurium and mediates *fimA* expression by binding to the *fimA* promoter region (18). In the studies described above, we have shown that a conditional (tetracycline-dependent) *fimZ* mutant of serovar Typhimurium is hyperfimbriate in the presence of tetracycline but nonfimbriate in its absence as expected. However, the overexpression of FimZ was also associated with a decrease in motility. This phenotype was not a result of large numbers of fimbriae expressed on the bacterial surface or due to the accumulation of FimA subunits intracellularly leading to a stress response, since the loss of movement was also demonstrated in nonfimbriate mutants. Overexpression of FimZ as a result of the presence of the *fimZ* gene carried on a multicopy plasmid also results in the inability of bacteria to migrate through the agar. The decrease in motility in the presence of increased amounts of FimZ was not due to the complete absence of flagella on the bacterial surface since electron microscopy indicated a few flagellum filaments on the surfaces of bacteria grown in the presence of tetracycline. However, under these conditions the amount of flagellar antigen produced was significantly reduced and could account for the decreased motility. In wild-type strains of serovar Typhimurium, the intracellular concentration of FimZ may be critical to producing opposite effects on flagellar and fimbrial gene expression. Consequently, relatively large amounts of FimZ are likely to favor type 1 fimbrial expression with decreasing flagellum production. Since we have previously demonstrated that FimZ binds to the *fimA* promoter region (18), we examined the *flhDC* promoter for nucleotide sequences similar to those found for *fimZ*. No regions of similarity were observed, and it is currently unknown whether FimZ binds directly to the *flhDC* promoter.

FimZ may represent a molecule that facilitates communication between type 1 fimbrial expression by the bacteria and the motility phenotype and could be part of a signal pathway facilitating coordination between localization by adherence and dissemination by locomotion. Several fimbrial gene clusters have been reported for *S. enterica* serovar Typhimurium, but their coordination with flagellar expression is unknown (2). Recently, the ability of a fimbrial gene product, MrpJ, to repress the flagellar regulon in *Proteus mirabilis* was reported

(11). The molecular function of MrpJ in fimbrial expression is unknown, but it is believed to be part of the *P. mirabilis* *mrp* gene cluster. Our observations using *S. enterica* serovar Typhimurium demonstrate that a positive activator of *fimA* expression can down regulate flagellar gene expression. Therefore, communication at the molecular level between fimbrial and flagellar expression in enterobacteria may be a common phenomenon in motile organisms.

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