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Fimbrial Phase Variation in *Escherichia coli*: Dependence on Integration Host Factor and Homologies with Other Site-Specific Recombinases

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Expression of *fimA*, the structural gene for type 1 fimbriae of *Escherichia coli*, is phase variable. Significant homologies were identified between the recombinases which control fimbrial phase variation, FimB and FimE, and the integrase class of site-specific recombinases. Normal expression of *fimA* was shown to require the integration host factor (IHF). Mutations in either the *himA* or the *himD* (*hip*) gene, which encode the α and β subunits of IHF, respectively, prevented phase variation and locked expression of *fimA* in either the "on" or "off" phase. In addition, both *himA* and *himD* lesions caused a sevenfold reduction in expression of a Φ(*fimA-lacZ*) operon fusion in strains in which *fimA* was locked in the on phase. Thus, IHF plays a dual role in controlling *fimA* expression: it is required both for inversion of the *fimA* control region and for efficient expression from the *fimA* promoter. A mechanism by which IHF may exert control over *fimA* expression is discussed.

Type 1 fimbriae of *Escherichia coli* are surface proteins which mediate adhesion to eucaryotic cells. The structural gene encoding the fimbrial subunit, *fimA*, is transcriptionally regulated by a phase-variable mechanism involving inversion of a 314-base-pair chromosomal DNA segment carrying the *fimA* promoter (1, 10). In the "on" phase, the promoter is in the appropriate orientation to initiate transcription of *fimA*; in the inverted ("off") orientation, *fimA* cannot be transcribed and fimbriae are not synthesized. Inversion of this regulatory DNA segment is directional and is controlled by the products of the *fimB* and *fimE* genes, located immediately upstream from *fimA*. The *fimB* gene product switches the invertible segment to the on orientation, and the *fimE* gene product switches the *fimA* gene off (10). Such directional, site-specific recombination is reminiscent of the integration and excision of phage λ (4). The FimB and FimE proteins were therefore compared with the integrase class of site-specific recombinases (Fig. 1); significant homologies were identified with precisely those regions of the integrases thought to be involved in forming their active sites (3). However, FimB and FimE showed no homology with the other class of site-specific recombinases, the invertases which mediate DNA inversions such as those involved in *Salmonella typhimurium* phase variation and phage Mu G-loop inversion (5). This finding implies that FimB and FimE may function by a mechanism similar to that of the phage λ Int-Xis system.

The λ Int protein depends upon the integration host factor (IHF) for efficient function (6). IHF is a heterodimeric protein composed of two subunits: IFH-α is encoded by the *himA* gene (13), while IHF-β is encoded by *himD* (also called *hip*; 8). Analysis of the DNA sequences to which IHF binds in phage λ has revealed a consensus sequence for IHF binding: PyrAANNNNTTGAT (6, 11). In addition to its role in certain site-specific recombinations, IHF has also been implicated more directly in the control of gene expression, for example, the regulation of the *cII* gene of phage λ (6). Such regulation is not by site-specific recombination, and, indeed, IHF has not previously been shown to be involved in the control of gene expression via phase variation. Because of the homologies between FimB, FimE, and the λ integrase, we examined the nucleotide sequence around the *fim* invertible region and identified two sequences which are perfect matches to the consensus IHF-binding site (Fig. 2). We therefore investigated the possible role of IHF in *fimA* expression.

E. coli K-12 VL386 (Table 1) carries a transcriptional *lac* fusion to the *fimA* gene (this gene was previously designated *fimD*; 9). Expression of this fusion is phase variable (Table 2). The level of expression of *fimA*, in either the on state or the off state, was analyzed by β-galactosidase assay (Table 2). Cells in the on phase were found to produce three- to fivefold more units of β-galactosidase than those in the off state.

To examine the role of IHF in *fimA* expression, the *himA82*::Tn*10* insertion was introduced by P1-mediated transduction. The transduction procedure has been described elsewhere (14). Cultures of strain VL386 to be used as recipients for transduction were grown both from colonies which were in the on configuration and from colonies in the off configuration for Φ(*fimA-lacZ*) expression (Table 1). When the recipient was in the on state, >80% of the Tet^r transductants were Lac^+. Presumably, the Lac^- derivatives arose as a result of a phase switch during growth of the culture, prior to transductional introduction of the *himA* lesion. Similarly, when the recipient was in the off state, >80% of the transductants were Lac^-. Both Lac^+ and Lac^- derivatives were selected for further analysis. All the Lac^+ *himA82*::Tn*10* transductants (e.g., strain CH1480) were found to be locked in the on state and did not switch to Lac^- at any detectable frequency ($<10^{-6}$; Table 2). Similarly, the *himA82*::Tn*10* insertion locked Lac^- derivatives in the off state (e.g., strain CH1481). Identical results were obtained

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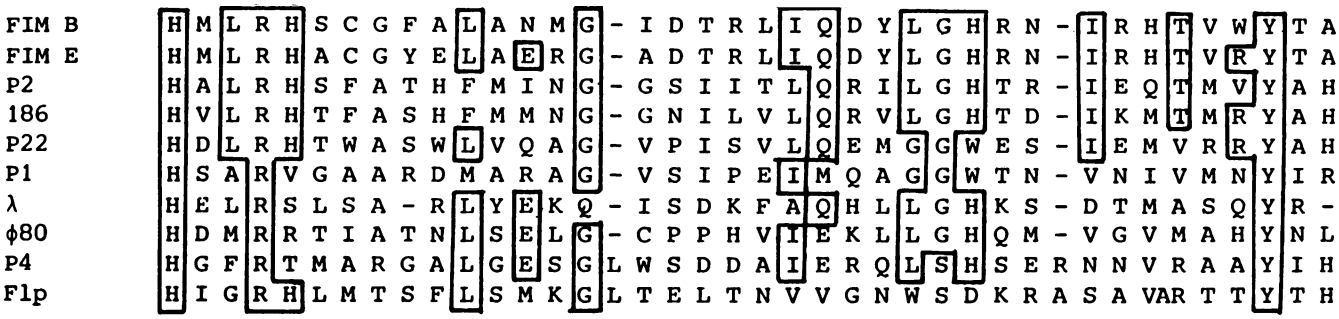

FIG. 1. Homologies between the C-terminal regions of the Fim regulatory proteins, FimB and FimE, and other site-specific recombinases. Residues 140 to 178 from FimB and residues 135 to 173 from FimE are shown; the sequences were taken from the results of Klemm (10). The sequences of the P2, 186, P22, P1, λ, ϕ80, P4, and Flp recombinase proteins are taken from the data of Argos et al. (3). Residues that are conserved in one or both of the Fim proteins and in at least two of the other proteins are boxed. Only exact matches are included.

using an independently isolated *himA*::Tn*10* insertion (e.g., strains CH1482 [Lac$^+$] and CH1483 [Lac$^-$]; Table 2). Thus, it is clear that efficient inversion of the 314-base-pair DNA fragment required for phase variation depends upon an intact *himA* gene and that FimB and FimE alone are not sufficient. Since cells lacking a functional *himA* gene are deficient in IHF, these results imply that fimbrial phase variation is IHF dependent.

In addition to its effect of phase switching, the *himA* mutation also reduced the absolute level of β-galactosidase expression from Φ(*fimA-lacZ*) fusions (Table 2). For example, β-galactosidase expression in the *himA* derivative CH1480 (phase locked in the on position) was about sevenfold lower than that of the parental strain, VL386 (also in the on state). *himA* strains locked in the off state showed no detectable expression from the *fimA-lacZ* fusion (Table 2). Thus, *himA* mutations have two distinct effects on *fimA* expression: they not only inhibit phase switching but they also reduce the absolute level of expression from the *fimA* promoter.

To confirm that the *himA* lesion is responsible for the phenotypic effects observed, strains CH1480 and CH1481 were transformed (12) with a plasmid carrying a functional *himA* gene to give strains CH1486 and CH1487, respectively. The plasmid, pBR322-*himA*$^+$, has been described previously (13). Phase variation of the Φ(*fimA-lacZ*) fusion was restored by introduction of the *himA* plasmid (Table 2). Furthermore, the level of β-galactosidase expression from the Φ(*fimA-lacZ*) fusion in strains CH1486 and CH1487 was restored to that of their parent VL386 (Table 2). As a control, the vector alone (pBR322) was transformed into CH1480 and CH1481. This plasmid did not restore phase switching and had no effect on Φ(*fimA-lacZ*) expression (strains CH1484 and CH1485; Table 2). It should also be pointed out that neither plasmid had any effect on Φ(*fimA-lacZ*) expression when introduced into the HimA$^+$ parental strain, VL386. Thus, expression of the Φ(*fimA-lacZ*) fusion in VL386 is not affected by the presence of *himA* in multicopy (Table 2).

As mutations in the structural gene for IHFα clearly affected expression of Φ(*fimA-lacZ*), we anticipated that mutations in *himD* (also known as *hip*), which encodes the β subunit of IHF, would have a similar effect. Strain E444 carries a chromosomal deletion of *himD* into which the gene encoding chloramphenicol acetyltransferase has been inserted (Table 1). This mutation was transduced into the Φ(*fimA-lacZ*) fusion strain VL386. As with the *himA* muta-

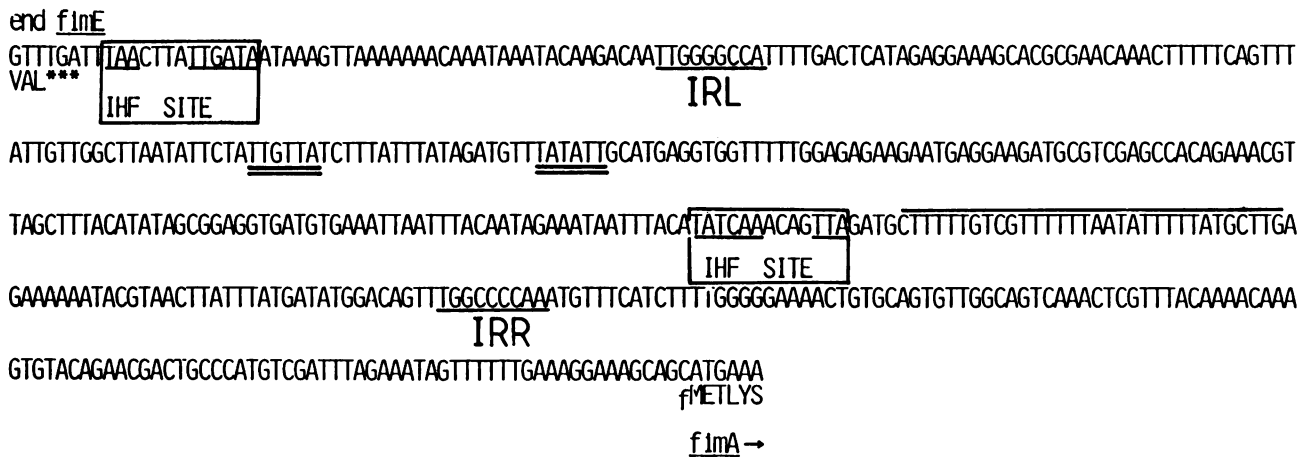

FIG. 2. The phase-variable region of the *fim* operon. The entire *fimE-fimA* intergenic region is shown, taken from references 1 and 10. The sequence extends from the translational termination codon (***) of the regulatory gene, *fimE*, to the initiation codon of the fimbrial subunit gene, *fimA*. The invertible region involved in the control of *fimA* expression is entirely contained within this sequence; the inverted repeats which flank the invertible region are underlined. The sequence is drawn with the invertible region in the on orientation. A possible promoter sequence is doubly underlined. The two potential IHF-binding sites discussed in the text are boxed. The sequence which may form a DNA bend is overlined.

TABLE 1. Bacterial strains

Strain	Genotype	Source reference
VL386	*ara* Δ(*lac-pro*) *rpsL thi* Φ(*fimA-lacZ*) λp1(209)	C. S. Freitag and B. I. Eisenstein (9)
DS936	*galK rpsL himA*::Tn*10* (derived from *himA*$^+$ parent, K37)	D. J. Sherratt
DS940	*galK rpsL himAΔ82*::Tn*10* (has Tn*10* closely linked to *himA* deletion)	D. J. Sherratt (13)
E444	Δ*himD*::Cm^r	E. Flamm
CH1478	VL386(pBR322)	This work
CH1479	VL386(pBR322-*himA*$^+$)	This work
CH1480	VL386 *himAΔ82*::Tn*10* (transduced from DS940; phase-locked Lac$^+$)	This work
CH1481	VL386 *himAΔ82*::Tn*10* (transduced from DS940; phase-locked Lac$^-$)	This work
CH1482	VL386 *himA*::Tn*10* (transduced from DS936; phase-locked Lac$^+$)	This work
CH1483	VL386 *himA*::Tn*10* (transduced from DS936; phase-locked Lac$^-$)	This work
CH1484	CH1480(pBR322)	This work
CH1485	CH1481(pBR322)	This work
CH1486	CH1480(pBR322-*himA*$^+$)	This work
CH1487	CH1481(pBR322-*himA*$^+$)	This work
CH1569	VL386 Δ*himD*::Cm^r (phase-locked Lac$^+$)	This work
CH1570	VL386 Δ*himD*::Cm^r (phase-locked Lac$^-$)	This work

tion, Cm^r transductants were locked in either the on or the off configuration (e.g., strains CH1569 and CH1570, respectively; Table 1). Moreover, expression of the Φ(*fimA-lacZ*) fusion locked in the on (Lac$^+$) state by the *himD* mutation (CH1569) was reduced compared with that of the *himD*$^+$ derivative. Like *himA*, a functional *himD* gene is required both for phase switching and for high-level expression from the *fimA* promoter. Thus, mutations in the genes encoding either subuit of IHF have similar effects on *fimA* expression.

It is clear from the results presented in this paper that expression from the *fimA* promoter is reduced by about sevenfold in the absence of IHF. As Φ(*fimA-lacZ*) operon fusions were used to monitor expression, it seems probable that this regulation is at the transcriptional level. IHF has previously been shown to be required for the expression of the phage λ *c*II gene. In this case the mechanism of IHF action is not known although, in view of the fact that IHF binds to DNA, it seems probable that IHF facilitates the adoption of the optimum DNA conformation for RNA polymerase binding and transcription initiation. In addition to its effect on transcription from the *fimA* promoter, IHF is also shown to be required for fimbrial phase variation itself. The site-specific recombination catalyzed by the λ Int protein is also an IHF-dependent process (6). Moreover, the proteins involved in catalyzing fimbrial phase variation (FimB and FimE) are found to share significant homology with the λ Int protein and other members of the integrase class of site-specific recombinases (Fig. 1). This implies that the mechanisms of recombination will be similar, either intermolecular, as in the case of the λ Int protein, or intramolecular, as for the fimbrial inversion system. For recombination to proceed and invert the fimbrial promoter segment of DNA, the recombinases must bring together the crossover sites which are located at either end of the 314-base-pair invertible region. This is a relatively short sequence for such an event, and IHF presumably facilitates the adoption of a DNA conformation favorable for recombination. This is certainly consistent with the finding that IHF is a DNA-binding protein with strong homology to the histonelike Hu proteins (13). The conformational change induced by IHF probably involves DNA bending (6). Significantly, a sequence motif which is similar to that found in bent DNA (2) can be found immediately adjacent to the potential IHF-binding site within the *fim* invertible region (Fig. 2). Furthermore, an analogous DNA-bending sequence occurs close to the IHF-binding site *H2* in *attP* of phage λ (15). The possibility that this sequence contributes to the looping of the fimbrial control region is currently being investigated.

TABLE 2. Analysis of Φ(*fimA-lacZ*) expression in IHF-deficient strains[a]

Strain	Relevant genotype	β-Galactosidase activity (U)	Switching frequency
VL386 on	*himA*$^+$ *fimA*, on	1,512	4×10^{-2}
VL386 off	*himA*$^+$ *fimA*, off	502	2.6×10^{-2}
CH1478	*himA*$^+$ *fimA*, on; pBR322	1,335	3×10^{-2}
CH1479	*himA*$^+$ *fimA*, on; pBR322-*himA*$^+$	1,547	3.2×10^{-2}
CH1480	*himA*::Tn*10 fimA* on	229	$<10^{-6}$
CH1481	*himA*::Tn*10 fimA* off	0	$<10^{-6}$
CH1484	CH1480(pBR322)	204	$<10^{-6}$
CH1485	CH1481(pBR322)	0	$<10^{-6}$
CH1486	CH1480(pBR322-*himA*$^+$)	1,574	3×10^{-2}
CH1487	CH1481(pBR322-*himA*$^+$)	1,336	3×10^{-2}
CH1569	Δ*himD*::Cm^r *fimA* on	207	$<10^{-6}$
CH1570	Δ*himD*::Cm^r *fimA* off	0	$<10^{-6}$

[a] VL386 colonies used to grow cultures for assay were designated "on" when deep red on MacConkey lactose plates and "off" when pale red. However, because of the high frequency of phase switching (1 in 1,000 per generation), a proportion of cells in each colony will have switched to the opposite phase. Therefore the absolute value of β-galactosidase activity differed somewhat from assay to assay depending on the point during growth at which phase switching occurred. However, sufficient assays were performed such that the standard deviation on the figures presented is less than 10%. β-Galactosidase assays were performed as previously described (14). Details of strains and their construction are given in Table 1. The switching frequency was determined by counting colonies on MacConkey-lactose indicator plates. Since switching can occur during the development of a colony on a plate, the apparent switching frequency in phase-variable strains was higher than the calculated frequency per generation (7).

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