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Roles of *fimB* and *fimE* in Site-Specific DNA Inversion Associated with Phase Variation of Type 1 Fimbriae in *Escherichia coli*

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Evidence obtained with an improved in vivo assay of fimbrial phase variation in *Escherichia coli* supported a revised understanding of the roles of *fimB* and *fimE* in the site-specific DNA rearrangement with which they are associated. A previously proposed model argued that *fimB* and *fimE* play antagonistic, unidirectional roles in regulating the orientation of the invertible DNA element located immediately upstream of *fimA*, the gene encoding the major subunit of type 1 fimbriae. This conclusion, though, is based on an in vivo DNA inversion assay using recombinant plasmid substrates under conditions that, among other things, were incapable of detecting recombination of the *fim* invertible element from the on to the off orientation. Using a modified system that overcame this and several additional technical problems, we confirmed that *fimB* acts independently of *fimE* on the invertible element and that the additional presence of *fimE* results in the preferential rearrangement of the element to the off orientation. It is now demonstrated that *fimE* can act in the absence of *fimB* in this recombination to promote inversion primarily from on to off. In contrast to the previous studies, the effect of *fimB* on a substrate carrying the invertible element in the on orientation could be examined. It was found that *fimB* mediates DNA inversion from on to off, as well as from off to on, and that, contrary to prior interpretations, the *fimB*-associated inversion occurs with only minimal orientational preference to the on phase.

Type 1 fimbriae, produced by many members of the family *Enterobacteriaceae*, are multisubunit proteinaceous appendages that mediate mannose-sensitive bacterial adherence to eukaryotic cells. In *Escherichia coli*, these organelles have been implicated in promoting colonization and subsequent infection. The genes encoding type 1 fimbriae in *E. coli* are positioned at 98 minutes on the chromosomal map (8). The oscillating on-and-off expression of the organelles, a process known as phase variation, is correlated with the inversion of a 314-bp DNA sequence immediately upstream of *fimA* (1, 5). Type 1 fimbriae are produced when the invertible element is in one orientation (on) and are not produced when the invertible element is in the alternate orientation (off), presumably because of the presence of a promoter within the invertible element.

A number of genes have been identified whose products affect the invertible element in *trans*, several mapping at sites distant from *fim*. One such gene, *pilG* (*osmZ*) (20, 32), recently shown to encode the histonelike protein H1 (21, 25), alters the frequency of inversion by an unknown mechanism. Two other genes, *himA* and *himD*/*hip*, which together encode integration host factor (IHF), are required for efficient recombination of the *fim* invertible element (11, 15). IHF is a heterodimeric DNA-binding and -bending protein which was initially identified for its role in bacteriophage lambda integration and excision (reviewed in reference 17). Two additional genes required for the inversion event, *fimB* and *fimE* (*hyp*), map immediately adjacent to the *fim* invertible element (23, 24, 27). It has been suggested that the relative concentrations of the FimB and FimE proteins determine the orientation of the *fim* invertible element as well as its frequency of inversion (28). The predicted amino acid sequences of the FimB and FimE proteins show significant homology to members of the integrase family of site-specific DNA recombinases (11, 15), suggesting that FimB and FimE are themselves recombinases that act in the context of IHF.

An in vivo inversion assay based on simultaneous carriage of two compatible plasmids, each carrying either the invertible element or the *trans*-active genes (*fimB* and/or *fimE*), was previously used to study the roles of *fimB* and *fimE* in recombination (23). Results led to a model in which *fimB* mediates DNA inversion from the off to the on orientation and *fimE* mediates inversion from on to off. The assay, however, did not examine the ability of *fimB* to mediate inversion from on to off; nor did it directly demonstrate that *fimE* can act in the absence of *fimB* in promoting the on-to-off inversion. In addition, the model does not agree with observations of the recombination event as it occurs on the chromosome of *fimE* mutants (5, 27). The current model suggests that a *fimE* mutant strain should be in the on phase and incapable of switching to the off phase. The invertible element in strains carrying mutations in *fimE*, however, oscillates bidirectionally and with little orientational bias. To reconcile these paradoxical results, we used a new plasmid-based recombination system that eliminated some of the problems associated with previous studies. Results from this plasmid-based assay enabled us to synthesize a new model of the roles of *fimB* and *fimE* in recombination.

MATERIALS AND METHODS

Bacterial and bacteriophage strains and media. The bacterial strains are all derivatives of *E. coli* K-12; all those with the AAEC designation used in this study are derivatives of MG1655, which is F^- λ^-. Construction of strains AAEC072 (MG1655 Δ*fim*) and AAEC078 (MG1655 Δ*fim* Δ*recA*) has been previously described (4). Strain AAEC088 (Δ*fim* Δ*himA* Δ*recA*) was constructed from AAEC072 as follows. Strain AAEC072 was made *himA* by P1 transduction from

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strain K1299 *himA82*::Tn*10* (18), selecting for Tet^r and screening for resistance to infection by bacteriophage Mu to give AAEC080. A Tet^s derivative of AAEC080, AAEC086, was selected on fusaric acid (7). AAEC086 was made *recA* by P1 transduction from JC10240 (10); transductants were screened for sensitivity to UV irradiation. One UV^s transductant was saved as AAEC088. Strain VL821 (Δ*fim* Δ*himA* Δ*recA*) was constructed from VL584 (16) by these techniques.

P1 transductions were done with P1 *vir* by standard techniques (31). Bacteria were grown in LB medium (tryptone and yeast extract were purchased from Difco Laboratories, Detroit, Mich.) at 37°C with aeration unless otherwise indicated. For plate media, agar (BBL, Cockeysville, Md.) was added to 1.5%. Media were supplemented with ampicillin (40 μg/ml), chloramphenicol (20 μg/ml), or tetracycline (15 μg/ml) when appropriate (Sigma Chemical Co., St. Louis, Mo.).

Recombinant DNA techniques. Restriction endonucleases (Bethesda Research Laboratories, Inc., Gaithersburg, Md.; Boehringer Mannheim Biochemicals, Indianapolis, Ind.; or New England BioLabs, Inc., Beverly, Mass.), T4 DNA ligase, T4 DNA polymerase, and Klenow enzyme (Bethesda Research Laboratories) were used according to the manufacturers' recommendations. Synthetic phosphorylated DNA linkers were purchased from Pharmacia (Piscataway, N.J.). Oligonucleotides were synthesized at the University of Michigan's Biomedical Research Core Facilities. Polymerase chain reaction was performed with *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). DNA sequencing was performed with Sequenase II (United States Biochemical Corporation, Cleveland, Ohio). DNA was labeled with the Multiprime kit purchased from Amersham Corp. (Arlington Heights, Ill.). All electrophoresis was performed with SeaKem GTG agarose (FMC Corp., Rockland, Maine). All other molecular genetic procedures were performed essentially as described previously (2).

Plasmid constructions. The plasmids used in this study were constructed as outlined below. The ~8.7-kbp *Nru*I-*Sal*I *fim* fragment from pIB254, a recombinant plasmid that contains all the known *fim* genes cloned from MG1655 (3), was subcloned into *Sal*I-*Hin*cII-digested pACYC184 to generate pMM17.

Plasmid pKK223-3 (9) was digested with *Hin*dIII, treated with Klenow enzyme and deoxynucleoside triphosphates to generate blunt ends, and recircularized. This treatment destroys the *Hin*dIII site while generating an *Nhe*I site, generating plasmid pMM26. The ~270-bp *Sph*I-*Sma*I fragment containing the T4 gene 32 transcription terminator from pHP45Ω (29) was subcloned into the *Sph*I-*Pvu*II sites of pMM26 to generate pMM28. Plasmid pMM28 was digested with *Eco*RI and *Sph*I, treated with T4 DNA polymerase and deoxynucleoside triphosphates to make both ends blunt, and recircularized to retain the *Eco*RI site. The resulting plasmid is pMM30. The *Hin*dIII-*Ssp*I fragment from pMM30 was subcloned into *Hin*dIII-*Nru*I-cut pACYC184 to generate pMM32. The plasmids pMM30 and pMM32 contain both the *rrnB*T1T2 terminators from pKK223-3 and the T4 gene 32 terminator from pHP45Ω. These terminators flank a polylinker sequence and are oriented to terminate transcripts that may start from sequences cloned into the polylinker. The 834-bp *Nru*I-*Hpa*I fragment carrying the *fim* invertible element from pIB238 (6) was ligated to 8-bp *Bam*HI linkers and cloned into the *Bam*HI site of pMM32. The resulting plasmids are pMM34 and pMM36. Plasmid pIB238 is a derivative of pIB233. Plasmid pIB233 is a derivative of pBR322 that lacks the *Aat*II-*Eco*RI fragment.

Two oligonucleotides (3221 [5′-GGGAGTCGGCTTTCTCGCCA]; 3222 [5′-GGGGATCCTCAAACCTCTTCTCTTTTA]) were synthesized. Sequences of the oligonucleotides that are homologous to *fim* sequences are underlined. Oligomer 3221 hybridizes to a sequence immediately upstream from a *Stu*I site located 253 bp from the 3′ end of *fimE*. Oligomer 3222 hybridizes at the 3′ end of *fimE*, including the sequence corresponding to the stop codon, and includes eight additional nucleotides that include a *Bam*HI site but that are not found in the *fim* sequence. These two oligomers were used in a polymerase chain reaction with pIB238 as a template. The polymerase chain reaction product was digested with *Stu*I and *Bam*HI and cloned into *Stu*I-*Bam*HI-cut pIB238. The resulting plasmid, pMM39, contains no *fim* DNA sequences 3′ from the end of the *fimE* coding region. Oligomers 3221 and 3222 were used to sequence the amplified region of pMM39 as well as the corresponding region of pIB238 to confirm that no errors were introduced during the polymerase chain reaction. Plasmid pMM39 was cut with *Bgl*II and recircularized to delete the ~970-bp *Bgl*II fragment (23). The resulting plasmid is pMM61.

Plasmid pMM61 was digested with *Hpa*I and *Bam*HI and treated with Klenow enzyme and deoxynucleoside triphosphates, and the large fragment was recircularized. The resulting plasmid is pMM63. Plasmid pMM61 was also digested with *Bgl*II and *Hpa*I and treated with Klenow enzyme and deoxynucleoside triphosphates, and the large fragment was recircularized. The resulting plasmid is pMM65.

Detecting type 1 fimbriae production. Bacteria were screened for production of type 1 fimbriae by assaying for mannose-sensitive agglutination of yeast cells (13).

RESULTS

Confirmation of roles of *fimB* and *fimE* in a noncomplementing Δ*fim* strain. The genetic analysis of cloned (plasmid-based) systems in *E. coli* is confounded by several problems, including background expression from native genes, interplasmid recombination between homologous sequences, and selection bias resulting from multicopy expression of the products of the cloned genes. In the initial study of the roles of *fimB* and *fimE*, these genes were expressed from one plasmid, the *fim* invertible element (the substrate) was cloned on another, compatible plasmid, and the host was *E. coli* HB101 (Fim$^-$) (23). Unfortunately, it has recently been found that this host strain not only contains *fim* sequences (4) but also supports inversion of the cloned 314-bp *fim* element (4, 28). Therefore, we chose a new host, *E. coli* AAEC078, which contains a verified deletion of all known *fim* genes, including *fimB* and *fimE*, and does not support recombination of a cloned copy of the *fim* invertible element (4).

Even in this improved host, we observed recombination between plasmids carrying the *fim* invertible element and plasmids carrying *fimB*, *fimE*, and the invertible element's left inverted repeat sequence (IRL), which resides 49 bp from the 3′ end of *fimE*. This intermolecular recombination is *recA* independent, occurs between the left inverted repeat sequences of the invertible element on the two plasmids, and is not observed in the absence of *fimB* and *fimE* (data not shown). To avoid this additional confounding problem, we constructed a series of *fim* plasmids whose cloned *fim* sequences terminate no further downstream than the 3′ end

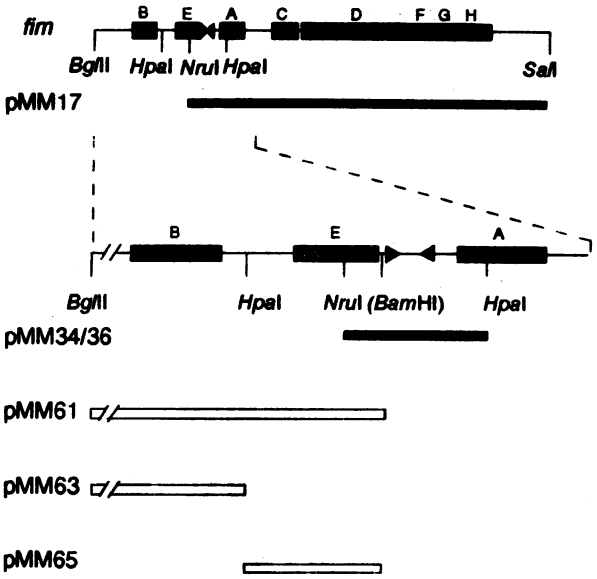

FIG. 1. *fim* locus and the portion of *fim* DNA sequences included on each of the plasmids used in this study. The *fim* invertible element, located between *fimE* and *fimA*, is indicated by the opposing arrowheads. The inversion substrate plasmids pMM17, pMM34, and pMM36 (indicated by the closed bars) are each derivatives of pACYC184 and confer resistance to chloramphenicol. The complementing plasmids pMM61, pMM63, and pMM65 (indicated by the open bars) are each derivatives of pIB233 (Materials and Methods) and confer resistance to ampicillin. The *Bam*HI site located in parentheses is present only on the complementing plasmids.

of the *fimE* coding region, thereby eliminating the left inverted repeat sequence of the invertible element. These three plasmids, pMM61 (*fimB fimE*), pMM63 (*fimB*), and pMM65 (*fimE*), are diagrammed in Fig. 1. We found no evidence of intermolecular recombination between any of these plasmids and any of the inversion substrate plasmids described below.

Our initial recombination studies used plasmid pMM17 (Fig. 1) as the inversion substrate. Plasmid pMM17 carries all the known *fim* genes except for *fimB* and *fimE*; it is identical to the substrate used in previous studies (23) in all respects, except for the source of the cloned *fim* genes. Plasmid pMM17 can exist in either of two isomeric forms, depending on the orientation of the *fim* invertible element (i.e., either pMM17-off or pMM17-on), which can easily be distinguished by restriction analysis with an enzyme that cuts asymmetrically within the invertible segment.

Strain AAEC078 carrying pMM17 alone was Fim$^-$; restriction analysis of pMM17 isolated from this strain revealed that the invertible element was exclusively in the off orientation (Fig. 2B, lane 2). When AAEC078 pMM17-off was transformed with the compatible plasmid, pMM61 (*fimB fimE*), the resulting bacteria were Fim$^+$. Restriction analysis of plasmid DNA isolated from these bacteria indicated that the off isomer of pMM17 predominated (Fig. 2B, lane 3), as expected from previous studies (23). Results obtained when AAEC078 pMM17-off was transformed with either pMM63 (*fimB*) or pMM65 (*fimE*) (Fig. 2B, lanes 4 and 5) were also essentially the same as the results of previous studies (23). That is, roughly equal amounts of pMM17-off and pMM17-on were observed in the presence of *fimB* alone, whereas predominantly pMM17-off was observed in the presence of *fimE* alone.

Neither the above experiments nor previous studies (23) ruled out the possibility that *fimB*, in addition to a role in recombination of the *fim* invertible element from the off to the on orientation, is also involved in recombination from on to off. These experiments also failed to demonstrate the *fimB*-independent role of *fimE* in recombination of the *fim* invertible element from the on to the off orientation. To address these concerns, we felt it was necessary to observe the effects of *fimB* and *fimE* on a recombination substrate

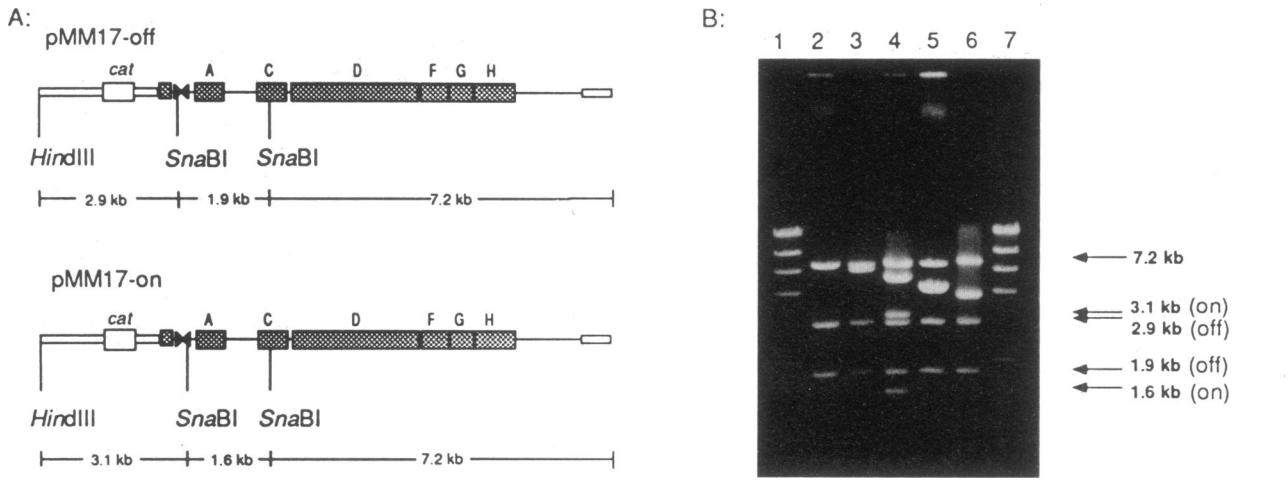

FIG. 2. (A) Relevant restriction sites and fragment sizes of the inversion substrate plasmid pMM17 (both isomers are shown although only pMM17-off could be used as the inversion substrate; see text). Each of the complementing plasmids contains a single *Hin*dIII site and no *Sna*BI site. The extent of *fim* DNA sequences is indicated by the hatched region; the *fim* invertible element is indicated by the opposing arrowheads. Vector sequences, including the *cat* gene, are represented by the open boxes. (B) Agarose gel (1%) of *Hin*dIII-*Sna*BI digests of plasmid DNA isolated from AAEC078. Lanes 1 and 7 are molecular weight markers. Lane 2, pMM17; lane 3, pMM17 plus pMM61; lane 4, pMM17 plus pMM63; lane 5, pMM17 plus pMM65; and lane 6, pMM17 plus pIB233 (vector control). The positions of bands representing the on (1.6 kb, 3.1 kb) and the off (1.9 kb, 2.9 kb) orientations of the *fim* invertible element on pMM17 are indicated.

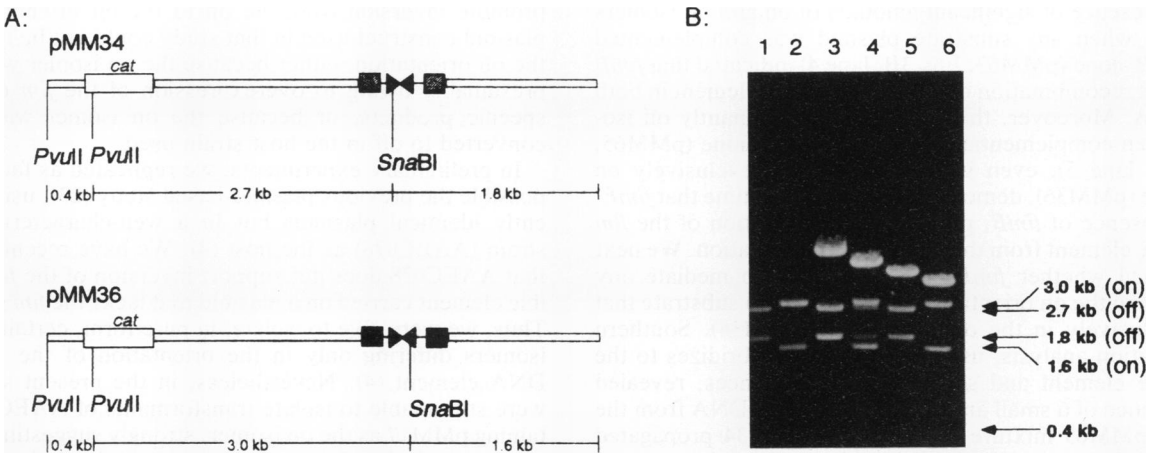

FIG. 3. (A) Relevant restriction sites and fragment sizes of the inversion substrate plasmids pMM34 (the off isomer) and pMM36 (the on isomer). Each of the complementing plasmids has a single *Pvu*II site and no *Sna*BI site. The extent of *fim* DNA sequences is indicated by the hatched region; the *fim* invertible element is indicated by the opposing arrowheads. Vector sequences, including the *cat* gene, are represented by the open boxes. (B) Agarose gel (1%) of *Pvu*II-*Sna*BI digests of plasmid DNA isolated from AAEC078. Lanes: 1, pMM34; 2, pMM36; 3, pMM36 plus pMM61; 4, pMM36 plus pMM63; 5, pMM36 plus pMM65; 6, pMM36 plus pIB233 (vector control). The positions of bands representing the on (1.6 kb, 3.0 kb) and the off (1.8 kb, 2.7 kb) orientations of the inversion substrate plasmids are indicated.

with the *fim* invertible element starting exclusively in the on orientation.

Use of *himA* mutants to isolate pMM17-on. Previous studies attempted to purify the on isomer of an inversion substrate plasmid similar to pMM17 but were unable to do so with strain HB101 as the host (23). It was concluded that cells harboring the substrate in the on orientation were nonviable. In light of recent reports that HB101 supports inversion of a plasmid-borne copy of the *fim* invertible element (28), we attempted to isolate pMM17-on using the noncomplementing strain AAEC078 as the host. Although we could recover mixed isomers of pMM17 from a host also carrying pMM63 (*fimB*), none of the pMM63$^-$ pMM17$^+$ transformants of AAEC078 that were recovered subsequently by using this mix of plasmids carried exclusively pMM17-on. We postulate that this failure to isolate pMM17-on was due to the expected lethal effect of the high-level expression of fimbriae (or fimbrial subunits) from the multicopy pMM17-on. To investigate this possibility, we repeated the mixed-plasmid transformation in the Δ*fim* Δ*himA* strain VL821. We have recently found that fimbrial expression is markedly reduced in the absence of IHF (30), which is encoded in part by *himA* (17). Transformants carrying only pMM17-on were readily obtained when the same mixture of pMM17 and pMM63 was used to transform VL821. These transformants were always Fim$^+$, presumably because of the counterbalancing effects of increased *fim* expression from the multicopy plasmid and decreased *fim* expression resulting from the absence of IHF. Purified pMM17-on could efficiently transform the Δ*fim* Δ*himA* strain AAEC088 (as well as VL821); however, this isomer was still unable to transform the isogenic strain AAEC078.

Construction of substrate plasmids resistant to orientational bias. Although we were able to isolate pMM17-on in AAEC088 and VL821, we could not then study the effects of *fimB* and *fimE* on the DNA inversion in these genetic backgrounds, since IHF is needed for this process (11, 15). We therefore constructed a new set of substrate plasmids that would be resistant to the orientational bias observed with pMM17. Plasmids pMM34 and pMM36, which each contain the *fim* invertible element, lack the rest of the *fim* genes; they are identical except for the orientation of the invertible element (Fig. 1). To minimize orientation-specific transcriptional effects on the replication efficiency of these plasmids, the invertible element was bounded by a pair of transcription terminators positioned to block transcripts initiating from within the cloned segment of *fim* DNA (33; data not shown). These measures markedly reduced or eliminated the orientational bias observed with pMM17; both pMM34 (isolated as the off isomer) and pMM36 (isolated as the on isomer) were stable in AAEC078.

Analysis of recombination in the absence of orientational bias of the invertible element. DNA inversion results obtained with pMM34 (off) as the substrate, in conjunction with pMM61, pMM63, or pMM65, were qualitatively similar to those observed with pMM17 (data not shown). These results indicated that all the substrate sequences necessary for DNA inversion on a plasmid substrate are contained on the short sequence of *fim* DNA carried by pMM34. Orientation results obtained with pMM34 were quantitatively similar to those seen when the on-oriented plasmid pMM36 (Fig. 3) was used as the recombinational substrate. The equivalence of isomer proportions at the time of analysis, regardless of the initial orientation of the invertible element, suggests that the recombination reactions utilizing either pMM34 or pMM36 had already reached equilibrium by the time of plasmid harvesting.

Inversion results obtained either with pMM34 or with pMM36 in conjunction with pMM61 (*fimB fimE*; Fig. 3B, lane 4) or pMM65 (*fimE*; Fig. 3B, lane 6) were also quantitatively similar to those observed with pMM17. However, results obtained when either pMM34 or pMM36 was complemented with pMM63 (*fimB*; Fig. 3B, lane 5) revealed that a majority of inversion substrates were present as on isomers; importantly, a significant amount of the off isomer was also observed. These results are quantitatively dissimilar to results obtained when pMM17 was complemented with pMM63 (Fig. 2B, lane 4), which may represent the off orientational bias observed with inversion substrate plasmids capable of expressing type 1 fimbriae.

The presence of significant amounts of on and off isomers obtained when any substrate plasmid was complemented with *fimB* alone (pMM63; Fig. 3B, lane 4) indicated that *fimB* promotes recombination of the *fim* invertible element in both directions. Moreover, the finding of predominantly off isomers when complementation was with *fimE* alone (pMM65; Fig. 3B, lane 5), even when starting with exclusively on substrate (pMM36), demonstrates for the first time that *fimE*, in the absence of *fimB*, promotes recombination of the *fim* invertible element from the on to the off orientation. We next determined whether *fimE* alone was able to mediate any inversion to the on orientation by starting with substrate that was exclusively in the off orientation (pMM34). Southern hybridization analysis, using a probe that hybridizes to the invertible element and some flanking sequences, revealed the presence of a small amount of pMM34-on DNA from the pMM34-pMM65 mixture but not from pMM34 propagated alone (data not shown). Thus, *fimE* is capable of promoting a small amount of off-to-on inversion, but its major effect is to mediate on-to-off inversion.

Environmental effects on orientation of plasmid-borne invertible element. Cultures of *E. coli* produce more type 1 fimbriae when grown in static broth than cultures grown either in shaking broth or as colonies on agar (5, 26). Although this differential expression has been ascribed mostly, if not entirely, to selection of fimbriate cells in static broth culture, the possibility of environmental regulation of the invertible element has not been excluded. Since the phase-variable expression of type 1 fimbriae correlates with the orientation of the *fim* invertible element, we reasoned that our in vivo assay system might detect such environmental regulation. To do so, it would have to demonstrate differences in the orientation of the invertible element in nonselectable (afimbriate) bacteria grown under different environmental conditions. Therefore, we determined the orientation of the invertible element, as carried by an inversion substrate plasmid (pMM17, pMM34, or pMM36), in AAEC078 cells containing pMM61 (*fimB fimE*) that were grown under various conditions. We found no difference in the equilibrium orientation of the DNA element among bacteria grown in static broth, in well-aerated broth, or in pooled colonies (data not shown).

DISCUSSION

Results from a recent study using recombinant plasmids have provided a model of the roles of *fimB* and *fimE* in the site-specific recombination event associated with the phase variation of type 1 fimbriae (23). Namely, *fimB* and *fimE* act antagonistically in orienting the DNA invertible element; *fimB* promotes inversion from off to on, whereas *fimE* promotes the reverse. Although this model has gained wide acceptance (19, 22), observations by us (4, 5) and others (27, 28) have compelled a reexamination for two important reasons. First, the host strain used in the previous studies, HB101, reported previously to be Δ*fim* (24), is now known to contain a significant amount of *fim* DNA (4). Moreover, one report indicates that this strain is capable of supporting recombination of the cloned invertible element even in the absence of plasmid-borne copies of *fimB* and *fimE* (28). Second, analysis of inversion on the chromosome (i.e., the native state of the system) suggests that *fimB* is capable of promoting rearrangement of the phase variation switch in both directions (5, 27).

In addition to these problems, the previous study (23) was incapable of determining the ability of either *fimB* or *fimE* to promote inversion from the on to the off orientation. The plasmid construct used in that study could not be isolated in the on orientation, either because the on isomer was lethal, presumably owing to overexpression of the *fim* organelle-specific products, or because the on isomer was rapidly converted to off in the host strain used.

In preliminary experiments, we replicated as faithfully as possible the previous plasmid-based study (23), using apparently identical plasmids but in a well-characterized Δ*fim* strain (AAEC078) as the host (4). We have recently shown that AAEC078 does not support inversion of the *fim* invertible element carried on a plasmid that lacks the *fim* genes (4). Thus, we were able to isolate, in pure form, certain plasmid isomers differing only in the orientation of the invertible DNA element (4). Nevertheless, in the present study, we were still unable to isolate transformants of AAEC078 containing pMM17 as the on isomer, strongly suggesting that the presence of this form of the plasmid is, indeed, lethal, presumably from overexpression of fimbriae.

We believe that the results of previous studies (23) and the results from our preliminary studies with pMM17 (while both are consistent with the current model) do not fully support the concept that *fimB* and *fimE* play antagonistic, unidirectional roles (23). We feel that there are other explanations for these results, given the inability of this system to detect recombination of the *fim* invertible element from the on to the off orientation. It could not be determined whether *fimB* promotes recombination in both directions or whether *fimE* has a role independent of *fimB* in promoting recombination from the on to the off orientation. To address these concerns, it was necessary to isolate plasmid isomers, pMM34 and pMM36, that were identical except for the orientation of the *fim* invertible element. Unlike pMM17, these plasmids lacked all *fim* genes. Perhaps consequently, they also failed to exhibit the orientational bias seen with plasmid pMM17.

Using pMM34 (initially isolated as the off isomer) and pMM36 (initially isolated as the on isomer), we found that *fimB* promotes recombination in both directions. Contrary to our results with pMM17, a majority of the plasmid substrates pMM34 and pMM36 were present in the on orientation. These results indicate that *fimB*, in the absence of orientational bias on the part of the substrate plasmid, promotes inversion in either direction, with a moderate preference for inversion from the off to the on orientation. These results do not fully support the current model (23) but rather are consistent with observations of recombination on the chromosome of *fimE* mutant strains; *fimE* mutants exhibit recombination in both directions, with a slight preference in favor of the on orientation (5, 12). It seems likely, based on the results reported here as well as the recent observation that strain CSH50 carries a mutation in *fimE* (5), that *fimB* is equivalent to the uncharacterized *trans*-active factor previously found in strain CSH50 to map near *fimA* (16).

The inversion-substrate plasmids pMM34 and pMM36 also provided a clear demonstration of the *fimB*-independent role of *fimE*. An inversion-substrate plasmid that initiated in the on orientation (pMM36) was isolated almost entirely in the off orientation when complemented with *fimE* (pMM65). This indicates that *fimE* in the absence of *fimB* promotes recombination of the *fim* invertible element from the on to the off orientation. A Southern analysis, used as a more sensitive assay of the activity of *fimE*, demonstrated that *fimE* also has a small amount of activity in promoting recombination of the *fim* invertible element from the off to the on orientation. Whether this activity is observed in recombination of the *fim* invertible element on the chromo-

some of *fimB* mutant strains remains to be seen. It is also unclear what, if any, its biological significance might be.

Taken together, the results reported here support a new model for the roles of *fimB* and *fimE* in the recombination event associated with the phase variation of type 1 fimbriae expression: *fimB* promotes recombination of the *fim* invertible element in both directions with only moderate orientational preference, whereas *fimE* promotes recombination primarily from the on to the off orientation. The fact that *fimB* promotes recombination of the *fim* invertible element in both directions might seem to make the function of *fimE* superfluous. We would now argue, however, that the regulation of type 1 fimbriae expression is more complex than previously believed. In fact, we have recently shown that *fimE* plays an important, perhaps primary, regulatory role in phase variation (5).

Despite the apparent reconciliation of results observed from recombination on a plasmid-based substrate with those from the native recombination process on the chromosome, we do note some differences. In the plasmid system, changing the growth conditions had no effect on the proportion of invertible elements in the on versus the off orientation. In contrast, growth conditions are known to markedly influence the orientation of the chromosomally located invertible element (5, 26). Part, if not all, of this growth effect is likely due to the variable selection of fimbriate or nonfimbriate bacteria (14). Alternatively, it is possible that the plasmid substrates used in this study lack certain *cis*-acting sites needed for these growth effects.

Although plasmid-based studies are powerful probes of certain aspects of recombination systems, they all suffer the generic problems involving gene dosage and, perhaps, topology. We are currently conducting a complementary analysis of the roles of *fimB* and *fimE* on recombination of the *fim* invertible element on the chromosome. To do so, we will be using a recently developed allelic exchange system (6) to construct isogenic mutants. It is hoped that studying the *fim* recombination in its native location, organization, stoichiometry, and topology will permit insights otherwise unavailable.

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