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Lrp Stimulates Phase Variation of Type 1 Fimbriation in *Escherichia coli* K-12

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The phase variation of type 1 fimbriation in *Escherichia coli* is associated with the inversion of a short DNA element. This element (switch) acts in *cis* to control transcription of *fimA*, the major fimbrial subunit gene. Thus, *fimA* is transcribed when the switch is in one orientation (the on orientation) but not the other (the off orientation). The *fim* inversion requires either *fimB* (on-to-off or off-to-on inversion) or *fimE* (on-to-off inversion only), as well as integration host factor, and is also influenced by the abundant DNA-binding protein H-NS. Here we report that an additional gene, *lrp*, a factor known to influence the expression of both Pap and K99 fimbriae, is also required for normal activity of the *fim* switch. The frequencies of both *fimB*-promoted and *fimE*-promoted inversions, and consequently the phase variation of type 1 fimbriation, are lower in *lrp* mutants. Lrp affects slightly the transcription of both *fimB* (which is increased) and *fimE* (which is decreased). We believe that these alterations in *fimB* and *fimE* transcription alone are unlikely to account for the sharp reduction in switching found in *lrp* mutants.

Fimbriae, proteinaceous appendages produced by many gram-negative bacteria, promote adherence between bacterial and host cells. Although attachment is probably an important step in pathogenesis, fimbriae are also excellent immunogens (13, 30, 49, 50), and their expression presumably leaves the cell vulnerable to the host's immune defenses. Perhaps to help avoid the immune system, the expression of many fimbrial types is phase variable. Thus, the ability to undergo phase variation, using a range of mechanisms (reviewed in reference 53) that involve changes in DNA methylation (8) and DNA inversion (1, 35), as well as other DNA rearrangements (24, 38, 60), may in itself be a pathogenicity factor (15).

The phase variation of type 1 fimbriation in *Escherichia coli* involves inversion of a 314-bp DNA element (1). This element (switch), which is situated immediately upstream of *fimA*, acts in *cis* to control the transcription of *fimA*, the major fimbrial subunit gene (27, 43). The invertible element determines *fimA* transcription, and hence fimbriation, in one orientation (the on orientation) but not the other (the off orientation). The invertible element presumably contains a promoter (1, 15, 44).

The *fim* inversion requires either *fimB* (on-to-off or off-to-on inversion) or *fimE* (on-to-off inversion), genes that map adjacent to the invertible region (6, 28, 37, 42), and is also influenced by other, unlinked loci. FimB and FimE are small, basic proteins with strong homology to each other (48% amino acid identity) and to the lambda integrase family of site-specific recombinases (14, 17, 28). Although not proven, it seems likely that FimB and FimE are recombinases.

The opposing activities of FimB and FimE have suggested that the *fim* switch is regulated (28, 45), yet the *E. coli* K-12 strains studied by other investigators switched phase at random (15–17, 45). A possible explanation for this paradox has been provided by the recent finding that the strains studied are *fimE* mutants (6).

Under typical laboratory growth conditions (aerated, rich media at 37°C), rapid on-to-off switching promoted by *fimE* predominates, and fimbriation is largely suppressed (6). Indeed, in wild-type (*fimE*$^+$) strains on-to-off inversion is so fast that, irrespective of the starting orientation of the switch, colonies inevitably contain a majority of off-phase cells (6). Thus, the formation of fimbriate and afimbriate phase-variant colonies, long associated with the process of phase variation (11), is in fact a characteristic of slow on-to-off switching in *fimE* mutants.

Two additional proteins, integration host factor (IHF) and H-NS (H1), also influence the *fim* inversion. IHF is required for inversion of the *fim* switch (14, 17), presumably for one of two reasons. IHF may function as an auxiliary factor in the recombination reaction leading to the *fim* inversion, as it does in bacteriophage lambda integration and excision. Alternatively, IHF may be required for the expression of both FimB and FimE. In H-NS (*osmZ* [*pilG*]) mutants, both on-to-off inversion and off-to-on inversion are substantially increased (23, 25, 58). We and others have recently found that the strains used in many of the prior studies of type 1 fimbriation, including those on IHF and on H-NS, are *fimE* mutants (6). We find that IHF is also required for inversion of the *fim* switch in a wild-type (*fimE*$^+$) background (47). The mechanism of H-NS-enhanced inversion in *fimE* mutants is unknown, and the phenotype of *hns* mutants in a wild-type background has yet to be reported.

Here we identify type 1 fimbriation as a member of the leucine-Lrp regulon (18, 32, 46). Using random transposon mutagenesis, we isolated *lrp* mutants as insertions associated with an increased expression of a *fimB-lac* operon fusion. Upon further analysis, we found that both *fimB*-promoted inversion and *fimE*-promoted inversion are sharply reduced in strains lacking *lrp*. Several workers have

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suggested that Lrp helps coordinate the physiological switch that accompanies a shift between nutritionally rich and poor environments (18, 32, 33). We believe that the identification of type 1 Pap and K99 (9, 10) fimbriae as part of the leucine-Lrp regulon supports this idea.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. Bacterial strains, bacteriophages, and plasmids are listed in Table 1; all strains are derivatives of *E. coli* K-12. Media included L broth (5 g of sodium chloride, 5 g of yeast extract, and 10 g of tryptone [Difco Laboratories, Detroit, Mich.] per liter) and L agar (L broth containing 1.5% agar [BBL, Cockeysville, Md.]). Sucrose agar, used to select recombinant bacteria (7), is L agar supplemented with 6% sucrose (J. T. Baker Co., Phillipsburg, N.J.) but lacking sodium chloride. β-Galactosidase assays and measurement of inversion frequencies were done following growth in MOPS [3-(*N*-morpholino)propanesulfonic acid] defined-rich medium (40). MOPS media were supplemented with 10 μM thiamine and either 0.4% glucose (defined-rich and minimal media) or 0.4% glycerol (minimal medium). In some experiments, ammonia was replaced by either 0.2% glycine or 0.2% arginine as the nitrogen source. Where appropriate, media contained 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) (Bethesda Research Laboratories, Inc. [BRL], Gaithersburg, Md.). Inversion of the *fim* switch was measured following growth on MOPS defined-rich medium (40) agar. To determine the proportion of switch-on to switch-off cells within a single colony, colonies were resuspended and spread on indicator media as reported previously (15). Indicator media were lactose-MacConkey agar or minimal glycerol plates supplemented with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (BRL) at 40 μg/ml. Cultures were aerated at 37°C, and growth was monitored spectrophotometrically at 420 nM. β-Galactosidase assays were performed on cells in balanced growth at an optical density at 420 nm of between 0.3 and 0.6. Media were supplemented, when necessary, with ampicillin (50 μg/ml), chloramphenicol (30 μg/ml), tetracycline (15 μg/ml), or kanamycin (25 μg/ml) (Sigma Chemical Co., St. Louis, Mo.) as recommended elsewhere (34).

Genetic techniques. Generalized transduction was carried out as described by Silhavy et al. (54) with P1 *vir*. Allelic exchange was as reported previously except that recombinants were selected at 28 rather than 30°C (7). Transposon mutagenesis, using λ1105, was as described previously (59) except that mutagenized cultures were spread onto lactose-MacConkey indicator media.

Molecular biological techniques and enzyme assays. Chromosomal and plasmid DNAs were isolated as described elsewhere (2, 34). Strain AAEC185 or AAEC189 (5) was used as the host strain for transformations. Conditions for restriction endonuclease digestions were as recommended by the specific manufacturer (BRL or New England Biolabs, Beverly, Mass.). DNA labelling was carried out by using the Multiprime kit (Amersham Co., Arlington Heights, Ill.). Other molecular genetic procedures and recombinant DNA techniques (agarose gel electrophoresis, isolation of restriction fragments, ligation and transformation of plasmid DNA, and Southern hybridization) were conducted as described previously (2, 34). β-Galactosidase assays were as described by Miller (39). Polymerase chain reactions (PCRs) were carried out with *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) for 30 cycles under the following conditions: 94°C (denaturation) for 30 s, 52°C (annealing) for 30 s, and 72°C (extension) for 2 min, except for the final extension, which was for 5 min. PCR buffer included 10 mM Tris (pH 8.3), 1 mM $MgCl_2$, 50 mM KCl, 1-mg/ml gelatin, 20 μM each deoxynucleoside triphosphate, and 50 pM each primer (5′ TAGCGCGTCTTAATAACCAG, which overlaps the 3′ end of *lrp*, and 5′ TGATCATATGACAAGATGTG, a sequence common to both the right and left inverted repeats of transposon Tn*10*).

Construction and characterization of *fimB-lac* and *fimE-lac* operon fusions. Single-copy, chromosomal *lac* operon fusions to *fimB* and *fimE* were constructed by in vitro manipulation of cloned DNA and allelic exchange. Plasmid pIB317, like pIB308 (6), includes the *fimB-fimD′* region of strain MG1655, except that it contains an *Xba*I translation terminator linker (Pharmacia) inserted in the unique *Cla*I site near the 5′ end of *fimB* (28). Similarly, pIB330, a descendant of pIB315 (6) (*fimE-fimD′* region of MG1655), contains an *Xba*I linker in the unique *Eco*O109I site in *fimE* (28). *lac* operon fusions to *fimB* and *fimE* were constructed by subcloning the *lac* genes from pKE005 (6), a descendant of pRS415 (56) in which *lacZYA* is flanked by *Xba*I linkers, into pIB317 and pIB330. The resulting plasmids, pIB341 and pIB335, were used to transfer the fusions into the chromosomes of appropriate intermediate strains by using sucrose counterselection (7). The correct chromosomal structures of the recombinant strains, AAEC261, AAEC261A (*fimB-lac*), and AAEC200 (*fimE-lac*), were confirmed by Southern hybridization (data not shown).

Construction and characterization of a *lac*UV5-*lrp* transcriptional fusion. To complement *lrp*::Tn*10* mutations in *trans*, we constructed a chromosomal *lac-lrp* transcriptional fusion by in vitro manipulation and allelic exchange. A promoterless fragment containing *lrp* was subcloned from pCV180 (22) into pRR2 (7), placing *lrp* downstream of the *lac*UV5 promoter (55). The fusion, carried on pKE20, was transferred to the chromosome of AAEC537 by allelic exchange (7), and the correct chromosomal structure of the resultant strain (AAEC542) was confirmed by Southern hybridization (data not shown). In this strain, *lrp* replaces *lacZYA*.

RESULTS

Transposon mutagenesis of a *fimB-lac* fusion strain. Although *fimB* promotes inversion in both directions, nevertheless favoring the on orientation, *fimE* stimulates recombination from on to off only. Thus, the ratio of FimB to FimE presumably influences the behavior of the *fim* switch (28, 45). To study *fimB* and *fimE* transcription, we constructed single-copy *fimB-lac* and *fimE-lac* operon fusions by allelic exchange (Materials and Methods). Each strain contains the *fim* genes in their native location on the bacterial chromosome in a Δ*lacZYA* mutant of MG1655 (7). Both fusion strains produce pale pink colonies on lactose-MacConkey agar at 37°C, indicating a low level of β-galactosidase expression on this medium.

To identify factors that influence *fimB* transcription, we screened random transposon mini-Tn*10-kan* (mTn*10-kan*) insertions in strain AAEC261 (*fimB-lacZYA*) for alterations in *fimB* transcription on kanamycin lactose-MacConkey indicator plates. Of approximately 8,500 kanamycin-resistant colonies inspected, 18 produced darker red colonies, indicating an increase in β-galactosidase expression. The increases in intensity of color observed ranged from dark red to a level just distinguishable from the wild type. With one exception,

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or phenotype	Reference or construction
E. coli strains		
MG1655	λ⁻ F⁻ Fim⁺	21
CV1008	CV975 [F⁻ *ara thiD* Δ(*lac-pro*) *ilvIH*::MudI1734] *lrp-35*::Tn*10*	46
DL844	MC4100 [F *araD139* Δ(*lacIPOZYA-argF*)*U169 rpsL thi-1*] *mbf* (*lrp*)*-20*::mTn*10*	10
DL845	As DL844 except *mbf* (*lrp*)*-7*::mTn*10*	9
CAG12094	MG1655 *zcb-3059*::Tn*10*	57
CAG18447	MG1655 *proAB81*::Tn*10*	57
CAG18478	MG1655 *zbj-1230*::Tn*10*	57
AAEC090	MG1655 Δ*lacZYA*, insert *sacB*-Neo^r	7
AAEC100	MG1655 Δ*lacZYA*	7
AAEC185	MM294 (λ⁻ F⁻ *supE44 hsdR17 mcrA mcrB endA1 thi-1*) Δ*fimB-fimH* Δ*recA*	5
AAEC187	MG1655 Δ*lacZYA* Δ*fimE-fimA*, insert *sacB*-Neo^r	6
AAEC189	YMC9 (λ⁻ F⁻ *supE44 hsdR17 mcrA mcrB endA1 thi-1* Δ*argF-lac-205*) Δ*fimB-fimH* Δ*recA*	5
AAEC193	MG1655 Δ*lacZYA* Δ*fimB-fimA*, insert *sacB*-Neo^r; lysogenic for unidentified bacteriophage	6
AAEC198	MG1655 Δ*lacZYA fimA-lacZYA*; lysogenic for unidentified bacteriophage	6
AAEC198A	MG1655 Δ*lacZYA fimA-lacZYA*	Exchange of *sacB*-Neo^r of AAEC187 for *fimA-lacZYA* of pIB333 (6)
AAEC200	MG1655 Δ*lacZYA fimE-lacZYA*	Exchange of *sacB*-Neo^r of AAEC187 for *fimE-lacZYA* of pIB335
AAEC261	MG1655 Δ*lacZYA fimB-lacZYA*; lysogenic for unidentified bacteriophage	Exchange of *sacB*-Neo^r of AAEC193 for *fimB-lacZYA* of pIB341
AAEC261A	MG1655 Δ*lacZYA fimB-lacZYA*	Exchange of *sacB*-Neo^r of AAEC520 for *fimB-lacZYA* of pIB341
AAEC268	MG1655 Δ*lacZYA fimB-lacZYA pilG* (*hns*)*-2*-Tet^r; lysogenic for unidentified bacteriophage	P1 transduction of *pilG* (*hns*)*-2*-Tet^r into AAEC261
AAEC280	MG1655 Δ*fimB-fimE*, insert *sacB*-Neo^r	36
AAEC282A	MG1655 Δ*lacZYA fimA-lacZYA* Δ*fimB-fimE*, insert *sacB*-Neo^r; *fim* switch locked on	36
AAEC284A	MG1655 Δ*lacZYA fimA-lacZYA* Δ*fimB-fimE*, insert *sacB*-Neo^r; *fim* switch locked off	36
AAEC338	MG1655 Δ*lacZYA fimB-lacZYA* Δ3(*himD*)::*cat*; lysogenic for unidentified bacteriophage	P1 transduction of Δ3(*himD*)::*cat* into AAEC261
AAEC343	MG1655 Δ*lacZYA fimB-lacZYA himA*Δ*82*; lysogenic for unidentified bacteriophage	P1 transduction of *himA*Δ*82* into AAEC261
AAEC363A	MG1655 Δ*lacZYA fimA-lacZYA*	Exchange of *sacB*-Neo^r of AAEC282A for *fimB*$^+$ *fimE*$^+$ of pMM69
AAEC364A	MG1655 Δ*lacZYA fimA-lacZYA*	Exchange of *sacB*-Neo^r of AAEC284A for *fimB*$^+$ *fimE*$^+$ of pMM69
AAEC366A	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6*	Exchange of *sacB*-Neo^r of AAEC282A for *fimB-am6* *fimE*$^+$ of pMM82
AAEC368A	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6*	Exchange of *sacB*-Neo^r of AAEC284A for *fimB-am6* *fimE*$^+$ of pMM82
AAEC370A	MG1655 Δ*lacZYA fimA-lacZYA fimE-am18*	Exchange of *sacB*-Neo^r of AAEC282A for *fimB*$^+$ *fimE-am18* of pMM86
AAEC372A	MG1655 Δ*lacZYA fimA-lacZYA fimE-am18*	Exchange of *sacB*-Neo^r of AAEC284A for *fimB*$^+$ *fimE-am18* of pMM86
AAEC374A	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6 fimE-am18*	Exchange of *sacB*-Neo^r of AAEC282A for *fimB-am6* *fimE-am18* of pMM87
AAEC376A	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6 fimE-am18*	Exchange of *sacB*-Neo^r of AAEC284A for *fimB-am6* *fimE-am18* of pMM87
AAEC391	MG1655 Δ*lacZYA fimB-lacZYA* Δ3(*himD*)::*cat*, insert 2 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 2 (*lrp*)::mTn*10* into AAEC338
AAEC397	MG1655 Δ*lacZYA fimA-lacZYA*, insert 2 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 2 (*lrp*)::mTn*10* into AAEC198
AAEC406	MG1655 Δ*lacZYA fimB-lacZYA*, insert 4 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 4 (*lrp*)::mTn*10* into AAEC261
AAEC419	MG1655 Δ*lacZYA fimB-lacZYA* insert 22 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 22 (*lrp*)::mTn*10* into AAEC261
AAEC426	MG1655 Δ*lacZYA fimB-lacZYA* insert 30 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 30 (*lrp*)::mTn*10* into AAEC261
AAEC429	MG1655 insert 2 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 2 (*lrp*)::mTn*10* into MG1655

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TABLE 1—*Continued*

Strain or plasmid	Genotype or phenotype	Reference or construction
AAEC429A	MG1655 insert 2 (*lrp*)::mTn*10*	P1 transduction of insert 2 (*lrp*)::mTn*10* into MG1655
AAEC430	MG1655 insert 4 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 4 (*lrp*)::mTn*10* into MG1655
AAEC431	MG1655 insert 22 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 22 (*lrp*)::mTn*10* into MG1655
AAEC431A	MG1655 insert 22 (*lrp*)::mTn*10*	P1 transduction of insert 22 (*lrp*)::mTn*10* into MG1655
AAEC432	MG1655 insert 30 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 30 (*lrp*)::mTn*10* into MG1655
AAEC432A	MG1655 insert 30 (*lrp*)::mTn*10*	P1 transduction of insert 30 (*lrp*)::mTn*10* into MG1655
AAEC434	MG1655 *mbf* (*lrp*)-*20*::mTn*10*	P1 transduction of *mbf* (*lrp*)-*20*::mTn*10* into MG1655
AAEC444A	MG1655 Δ*lacZYA fimB-lacZYA mbf* (*lrp*)-*7*::mTn*10*	P1 transduction of *mbf* (*lrp*)-*7*::mTn*10* into AAEC261A
AAEC466	MG1655 Δ*lacZYA fimA-lacZYA*, insert 4 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 4 (*lrp*)::mTn*10* into AAEC198
AAEC468	MG1655 Δ*lacZYA fimA-lacZYA*, insert 22 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 22 (*lrp*)::mTn*10* into AAEC198
AAEC470	MG1655 Δ*lacZYA fimA-lacZYA*, insert 30 (*lrp*)::mTn*10*; lysogenic for unidentified bacteriophage	P1 transduction of insert 30 (*lrp*)::mTn*10* into AAEC198
AAEC482A	MG1655 Δ*lacZYA fimE-lacZYA mbf* (*lrp*)-*7*::mTn*10*	P1 transduction of *mbf* (*lrp*)-*7*::Tn*10* into AAEC200
AAEC492A	AAEC282A *mbf* (*lrp*)-*7*::mTn*10*	P1 transduction of *mbf* (*lrp*)-*7*::mTn*10* into AAEC284A
AAEC494A	AAEC284A *mbf* (*lrp*)-*7*::mTn*10*	P1 transduction of *mbf* (*lrp*)-*7*::mTn*10* into AAEC284A
AAEC500	MG1655 *lrp-35*::Tn*10*	P1 transduction of *lrp-35*::Tn*10* into MG1655
AAEC506A	MG1655 Δ*lacZYA fimA-lacZYA mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC494A for *fimB*$^+$ *fimE*$^+$ of pMM69
AAEC508A	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6 mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC492A for *fimB-am6* *fimE*$^+$ of pMM82
AAEC511A	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6 mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC494A for *fimB-am6* *fimE*$^+$ of pMM82
AAEC512A	MG1655 Δ*lacZYA fimA-lacZYA fimE-am18 mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC492A for *fimB*$^+$ *fimE-am18* of pMM86
AAEC514A	MG1655 Δ*lacZYA fimA-lacZYA fimE-am18 mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC494A for *fimB*$^+$ *fimE-am18* of pMM86
AAEC520	MG1655 Δ*lacZYA* Δ*fimB-fimE*, insert *sacB*-Neo^r	P1 transduction of *sacB*-Neo^r of AAEC280 into AAEC100 (7)
AAEC527	MG1655 Δ*lacZYA fimA-lacZYA mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC492A for *fimB*$^+$ *fimE*$^+$ of pMM69
AAEC532	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6 fimE-am18 mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC492A for *fimB-am6* *fimE-am18* of pMM87
AAEC533	MG1655 Δ*lacZYA fimA-lacZYA fimB-am6 fimE-am18 mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC494A for *fimB-am6* *fimE-am18* of pMM87
AAEC535	MG1655 Δ*lacZYA*, insert *sacB*-Neo^r, *fimA-lacZYA*	P1 transduction of *sacB*-Neo^r of AAEC090 into AAEC198A
AAEC537	MG1655 Δ*lacZYA*, insert *sacB*-Neo^r, *fimA-lacZYA mbf* (*lrp*)-*7*::mTn*10*	P1 transduction of *mbf* (*lrp*)-*7*::mTn*10* into AAEC535
AAEC542	MG1655 Δ*lacZYA*, insert *lac*UV5-*lrp*, *fimA-lacZYA mbf* (*lrp*)-*7*::mTn*10*	Exchange of *sacB*-Neo^r of AAEC537 for *lac*UV5-*lrp* of pKE20
Bacteriophages		
P1*vir*		Laboratory collection
λ1105	P_{tac} transposase mini-Tn*10-kan* transposon	59
Plasmids		
pCV180	Amp^r PCR-amplified *lrp* open reading frame cloned in pBluescript II SK$^-$	22
pMBF1	Amp^r cosmid pREG153 (29) containing *mbf* (*lrp*) on a 40-kbp insert	9
pRR2	Cm^r pIB300 (7) *'lacI-3' lacA*	7
pMM69	Cm^r pIB307 (7) *fimB*$^+$ *fimE*$^+$	36
pMM82	Cm^r pIB307 (7) *fimB-am6 fimE*$^+$	36
pMM86	Cm^r pIB307 (7) *fimB*$^+$ *fimE-am18*	36
pMM87	Cm^r pIB307 (7) *fimB-am6 fimE-am18*	36
pKE005	Amp^r pRS415 (56) *lacZYA* flanked by *Xba*I translation terminator linkers in *Sma*I (upstream) and *Stu*I (downstream) sites	6
pKE020	Cm^r pRR2, *lrp* open reading frame subcloned into pRR2 on *Hin*dIII (*Bam*HI)-*Hin*dIII fragment of pCV180 (22); *lac*UV5-*lrp* transcriptional fusion	This work
pIB308	Cm^r *Hin*dIII-*Bam*HI *fimA-fimD'* of pIB238 (3) in pIB306	6
pIB315	Cm^r pIB308, Δ*fimB*, insert *Hin*dIII linker	6
pIB317	Cm^r pIB308, *Cla*I site (*fimB*) filled in; *Xba*I translation terminator linker inserted	This work

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TABLE 1—*Continued*

Strain or plasmid	Genotype or phenotype	Reference or construction
pIB330	Cm^r pIB315, *Eco*O109I site (*fimE*) filled in; *Xba*I translation terminator linker inserted	6
pIB333	Cm^r pIB322 (6), *lacZYA* subcloned into *fimA* on *Xba*I fragment of pKE005	6
pIB335	Cm^r pIB330, *lacZYA* subcloned into *fimE* on *Xba*I fragment of pKE005	This work
pIB341	Cm^r pIB317, *lacZYA* subcloned into *fimB* on *Xba*I fragment of pKE005	This work

the altered phenotype cotransduced with the mTn*10* insertion. Twelve colonies that appeared lighter than the wild type were also isolated but were not studied further here.

Influence of mTn*10* insertion mutations on the *fim* inversion. The influence of the insertion mutations on the *fim* switch was assessed by transducing either the wild-type fimbriate strain (MG1655) or a *fimA-lacZYA* fusion derivative of MG1655 (AAEC198) to kanamycin resistance. In these strains, rapid on-to-off inversion ensures that *fimA* transcription is largely repressed, so that colonies look phenotypically off. Thus, we reasoned that we could identify insertions that affected the *fim* switch by screening colonies for alterations in form, such as the appearance of on, off-phase-variant types. Surprisingly, the majority of mTn*10* inserts, isolated by virtue of an increased transcription of *fimB*, had no obvious influence on the *fim* inversion. However, transductants containing inserts 2 (AAEC429 and AAEC397), 4 (AAEC430 and AAEC466), 22 (AAEC431 and AAEC468), and 30 (AAEC432 and AAEC470), like *fimE* mutants (6), produce phase-variant colonies distinguishable by alternate colony morphologies (in the fimbriate strain) or β-galactosidase metabolism (in the *fimA-lac* fusion strain).

Phase switching of type 1 fimbriation is associated with the precise and reversible inversion of the *fimA* promoter region (1, 58). Using Southern hybridization analysis, we confirmed that alterations in phase of one of the insertion mutants (AAEC432; insert 30) coincided with the expected change in orientation of the *fim* switch. Starting with a clone that appeared phenotypically on, off-phase-variant colonies were isolated upon passage on LB agar. Chromosomal DNA was isolated from single phase-variant colonies, and the inversion was followed through switching from on to off and from off to on (data not shown). As with *fimE* mutants, the appearance of phase-on colonies could be attributed to a sharp reduction in the frequency of inversion from on to off.

Genetic and physical map location of mTn*10* inserts 2, 4, 22, and 30. Three genes that are unlinked to *fim* (*hns* [*pilG*], *himD* [*hip*], and *himA*) are known to influence the *fim* inversion. The mTn*10-kan* inserts found here to influence the *fim* inversion (inserts 2, 4, 25, and 30) were tested for their linkage to these genes. Each insert was transduced into a *himA* (AAEC343; Tet^r), *himD* (AAEC338; Cam^r), or *hns* (AAEC268; Tet^r) derivative of AAEC261 (*fimB-lacZYA*), and the transductants were tested for loss of the resident drug resistance marker. All transductants of AAEC343 and AAEC268 remained tetracycline resistant, suggesting that the mTn*10-kan* insertions were not closely linked to either *himA* or *hns*. However, the majority of kanamycin-resistant transductants of AAEC338 (*himD*; Cam^r) became chloramphenicol sensitive, implying close linkage to *himD*. A transductant of AAEC338 that retained the Δ3(*himD*)::*cat* allele was used to map insert 30 relative to *himD*. With the kanamycin-resistant, chloramphenicol-resistant double mutant as the donor, we transduced strains CAG18478 (Tn*10*::20′) and CAG12094 (Tn*10*::21′) to either kanamycin or chloramphenicol resistance and scored for resistance to tetracycline and the second, unselected marker. The frequencies obtained (Fig. 1) support the following clockwise order: insert 30, *zbj-1230*::Tn*10*, *himD*, *zcb-3059*::Tn*10*.

Braaten et al. recently identified a gene, termed *mbf*, that influences the phase variation of *pap* fimbriation (9). *mbf*, which maps at 19.6 min (9), is now known to be allelic to *lrp* (10). Transduction of insert 30 into the *mbf*::mTn*10-tet* insertion mutant DL845 showed the close proximity of insert 30 to *lrp*; all 252 kanamycin-resistant transductants tested became tetracycline sensitive, implying loss of the resident mini transposon.

Southern hybridization and PCR analysis provided physical evidence that mTn*10* insert 30, as well as inserts 2, 4, and 22, lies within *lrp*. Chromosomal DNA was digested with *Ava*I and probed with a 0.5-kbp *Eco*RI-*Hin*dIII fragment of pCV180 (22) which consists of *lrp* (Fig. 2A). Whereas MG1655 produced a single band (Fig. 2A, lane 1), each insertion mutant, as well as the known *lrp*::Tn*10* mutants DL845 and CV1008, produced two distinct bands. Since *Ava*I does not cut within *lrp* (61), we conclude that each mTn*10* insertion lies within *lrp*.

PCR analysis allowed us to estimate the approximate positions of the transposon insertions (Fig. 2B). With a primer that overlaps the 3′ end of *lrp* (5′ TAGCGCGTCT TAATAACCAG), together with a second primer common to both the right and left inverted repeats of transposon Tn*10* (5′ TGATCATATGACAAGATGTG), amplification of chromosomal DNA from CV1008 and DL845 produced single, prominent bands of indistinguishable size (Fig. 2B, lanes 2

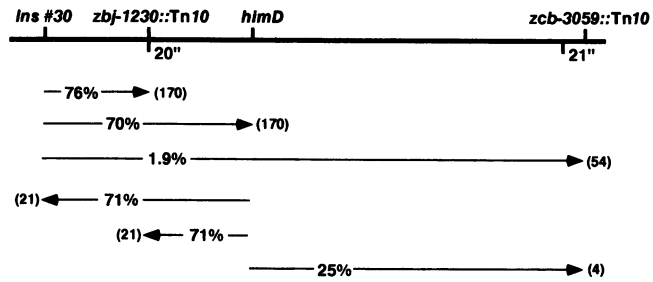

FIG. 1. Genetic organization of mTn*10-kan* insert 30 relative to *zbj-1230*::Tn*10*, *zcb-3059*::Tn*10*, and *himD*. The base of each arrow corresponds to the selected marker on the map above, and the head of each arrow corresponds to the unselected marker. The numbers of transductants scored are indicated in parentheses. The order reflects the cotransduction values and rare double recombinants obtained with *zbj-1230*::Tn*10* as the recipient (Tet^r Cam^r with Kan^r as the selected marker and Tet^r Kan^r with Cam^r as the selected marker).

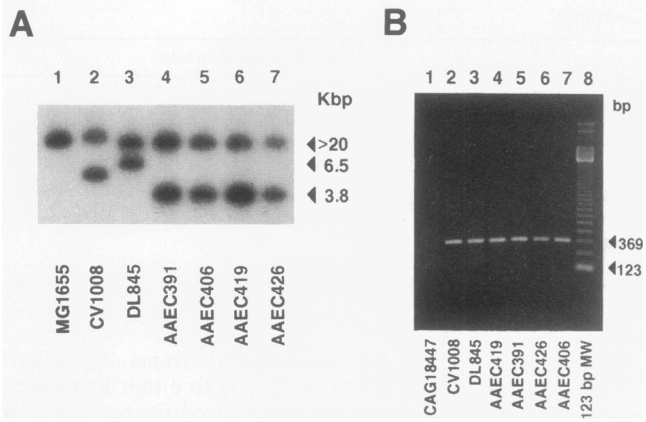

FIG. 2. Southern hybridization and PCR analysis of transposon insertion mutants AAEC391 (insert 2), AAEC406 (insert 4), AAEC419 (insert 22) and AAEC426 (insert 30). The parent strain, MG1655, and known *lrp*::Tn*10* mutants CV1008 (46) and DL845 (9) are included as controls. (A) For Southern hybridizations, chromosomal DNA isolated from the indicated strains was digested with *Ava*I, separated on a 1.2% agarose gel, and blotted to a Zetabind nylon membrane. Hybridizations using the 0.5-kbp *Eco*RI-*Hin*dIII *lrp* fragment of pCV180 (22) were carried out at high stringency. (B) PCR products, amplified by using the primers and conditions described in Materials and Methods, were separated on a 1.3% agarose gel with 123-bp ladder molecular weight markers (BRL).

and 3). No product was detected when chromosomal DNA from CAG18447, a derivative of MG1655 that contains a *proAB81*::Tn*10* allele, was used (Fig. 2B, lane 1). Strains containing mTn*10-kan* inserts 2, 4, 22, and 30 produced bands similar in size to those obtained with DL845 and CV1008 (Fig. 2B, lanes 4 to 7). All the insertions are near the 5′ end of *lrp*, at an estimated position 196 bp downstream of the start. The inserts are in or close to the sequence 5′ GCTTAAC, a site that resembles closely the consensus target for Tn*10* insertion, 5′ GCTNAGC (26).

Transfer of known *lrp* insertion mutations (*lrp-35*::Tn*10* from CV1008, *mbf-7*::mTn*10* from DL845, and *mbf-22*::mTn*10* from DL844) into MG1655 or the *fimA-lac* fusion strains AAEC198 and AAEC198A produced mutants that showed the expected reduction in on-to-off switching. The change in orientation of the *fim* invertible element with phase was monitored for strain AAEC434 (MG1655, *mbf-20*::mTn*10*), using Southern hybridization exactly as described for strain AAEC432, with identical results (data not shown).

As an independent assessment of the *lrp* phenotype, strains containing mTn*10-kan* inserts 2 (AAEC429A), 22 (AAEC431A), and 30 (AAEC432A), together with MG1655 and AAEC500 (MG1655 *lrp-35*::Tn*10*), were tested for growth on minimal medium containing either glycine (33) or arginine (18) as the nitrogen source. Whereas MG1655 formed colonies, growth of the *lrp* mutants was severely reduced.

Complementation of the *lrp*::Tn*10* phenotype. Although transposon insertions within *lrp* repress inversion of the *fim* switch, this effect could reflect the loss of expression of a gene downstream of *lrp* rather than *lrp* itself. To rule out the possibility of a polar effect, we complemented the *lrp*::Tn*10* phenotype with a copy of the gene in *trans*. We used allelic exchange to place *lrp* under the control of the *lac*UV5 promoter, replacing *lacZYA* with the promoterless *lrp* allele of pCV180 (Materials and Methods). As a test of the ability

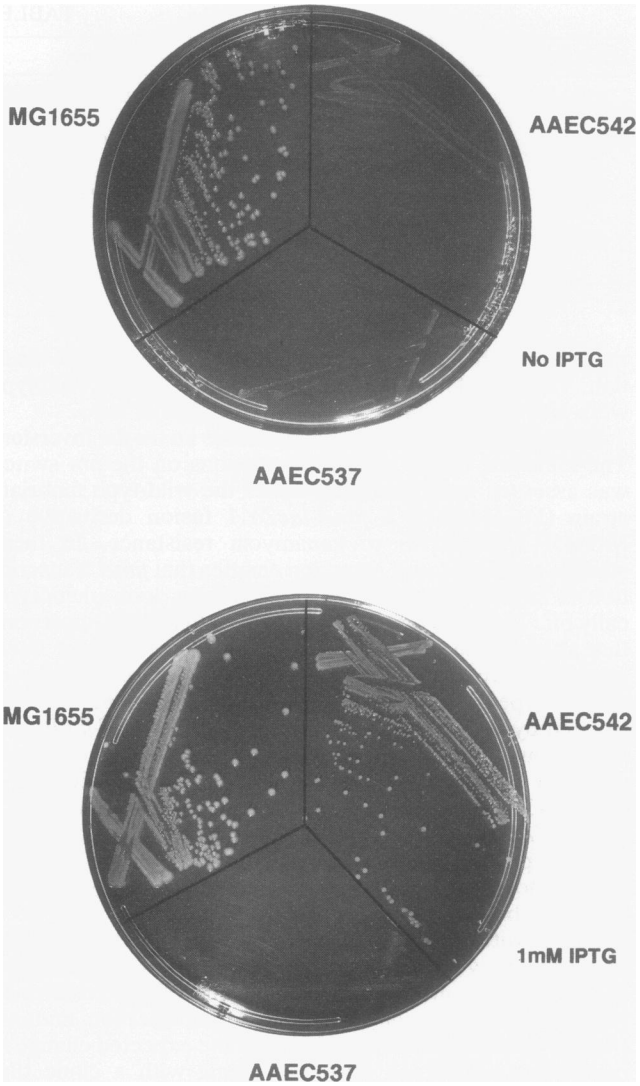

FIG. 3. Growth of strains MG1655 (wild type), AAEC537 [MG1655 *mbf* (*lrp*)-*7*::Tn*10*], and AAEC542 [MG1655 *mbf* (*lrp*)-*7*::Tn*10* Δ*lacZYA*, insert *lac*UV5-*lrp*] on minimal medium with 0.2% glycine as the nitrogen source, with or without IPTG. Plates were incubated for approximately 48 h at 37°C.

of the *lac*UV5-*lrp* allele to complement a *lrp*::Tn*10* insertion, we first examined the growth of strain AAEC542, which contains both *mbf* (*lrp*)-*7*::mTn*10* and *lac*UV5-*lrp* alleles, on minimal media with glycine as the nitrogen source (see above). In the absence of inducer, AAEC542 grew no better than the *lrp*::Tn*10* insertion mutants. With the addition of IPTG to the media, however, AAEC542 grew almost as well as the wild type (Fig. 3).

In the absence of inducer, both AAEC537 and AAEC542 produced on- and off-phase-variant colonies on MOPS media containing X-Gal. Whereas the addition of IPTG to this medium had no effect on the *lac* phenotype of AAEC537, colonies of AAEC542 appeared to be off. This latter result was expected, since wild-type strain MG1655 and its *fimA-lacZYA* fusion derivative, AAEC198 (6), show rapid on-to-off switching on rich agar medium (3, 6). To measure the frequency of on-to-off switching in strains AAEC537 and

TABLE 2. On-to-off inversion of the *fim* switch in *lrp* and *lac*UV5-*lrp lrp* double mutants

Strain	Genotype	Inversion frequency[a]	
		With IPTG	Without IPTG[b]
AAEC537	*lrp*::Tn*10*	NT[c]	9.9×10^{-5}
AAEC542	*lrp*::Tn*10 lac*UV5-*lrp*	$1.26 \times 10^{-2} \pm 0.18 \times 10^{-2}$[d]	9.1×10^{-5}

[a] Inversion frequency per cell per generation.
[b] Calculated by using the Poisson distribution, with a total number of invertants of 8 or fewer.
[c] NT, not tested.
[d] 95% confidence interval calculated by using the *t* distribution.

AAEC542, a single on clone was inoculated onto defined-rich medium either with or without IPTG. Resultant colonies were resuspended and spread onto MOPS media containing X-Gal, but lacking IPTG, to count the proportion of on to off colonies. The frequency of switching of strain AAEC542 from on to off was increased over 100-fold in the presence of IPTG but was indistinguishable from that of its parent (AAEC537) when uninduced (Table 2). Previous estimates (6) for on-to-off switching in wild-type strains ($>10^{-2}$) are comparable to that of AAEC542 in the presence of inducer. Thus, we conclude that the absence of Lrp accounts for the low inversion frequency of the *fim* switch seen in the *lrp* mutants.

Analysis of the *fimB*-promoted and *fimE*-promoted inversions in *lrp* mutants. In contrast to wild-type strains, which inevitably form off-phase colonies on rich agar media, *fimE* mutants produce both on and off colony types (6). FimE promotes on-to-off inversion only (28, 37, 45), and in *fimE* mutants the formation of on-phase-variant colonies reflects a sharp but unidirectional drop in inversion from on to off (6). Since *lrp* mutants also form both on- and off-phase-variant colonies, we considered it likely that Lrp stimulates *fimE*-promoted on-to-off inversion. To confirm this assertion, and to assess the influence of Lrp on the activity of *fimB* (both off-to-on and on-to-off switching), we examined the *fim* inversion in *fimB lrp* and in *fimE lrp* double mutants.

Using allelic exchange, we replaced *fimB* and the 5′ end of *fimE* with a *sacB*-Neo^r cassette (7, 36). As expected (37, 45), the *fim* switch locked either on (AAEC282A) or off (AAEC284A) in these intermediate strains. In a second round of allelic exchange (Fig. 4), we replaced the *sacB*-Neo^r cassettes of strains AAEC282A and AAEC284A, or *lrp* mutants of these strains (AAEC492A and AAEC494A, respectively), with either wild-type or mutant alleles of *fimB* and *fimE*, using sucrose counterselection as described previously (7).

In the *fimB fimE* double mutants, irrespective of the state of *lrp*, the *fim* switch remained locked in the same orientation as in the parent strain (Table 3) (36). The switch also remained locked in *fimB* mutant derivatives of AAEC284A (switch off, *lrp*$^+$) and of AAEC494A (switch off, *lrp*), strains AAEC368A and AAEC511A, respectively. Transfer of the *fimB* mutation into AAEC282A (switch on, *lrp*$^+$) invariably produced strains, such as AAEC366A, that also contain the switch locked off. We attribute this latter result to very rapid on-to-off inversion (frequency, $>10^{-2}$) promoted by *fimE*. All of the above results are consistent with our current view of the activities of *fimB* and *fimE* (6, 36, 37).

As noted above, inversion from on to off in *fimB* mutants was very rapid. However, a *fimB lrp* double mutant (AAEC508A) switched off quite slowly (Table 3). Since our previous estimates (6) show that the frequency of on-to-off inversion in wild-type bacteria is $>10^{-2}$, *fimE*-promoted on-to-off inversion is reduced at least 100-fold in the absence of Lrp. Moreover, in comparison with a *fimE* mutant, both on-to-off inversion and off-to-on inversion were also reduced 100-fold in the *fimE lrp* double mutants (Table 3). Thus, *fimB*-promoted recombination is stimulated by Lrp, also. As expected, the frequency of on-to-off switching was lower in *fimB*$^+$ *fimE*$^+$ bacteria in the absence of Lrp (AAEC527; 1.18×10^{-4}) than our previous estimate of $>10^{-2}$ for wild-type bacteria (6). Likewise, the frequency of off-to-on switching was also lower in the *lrp* mutant (and AAEC506A; 2×10^{-5}) than that found previously (6) in wild-type bacteria (10^{-3}).

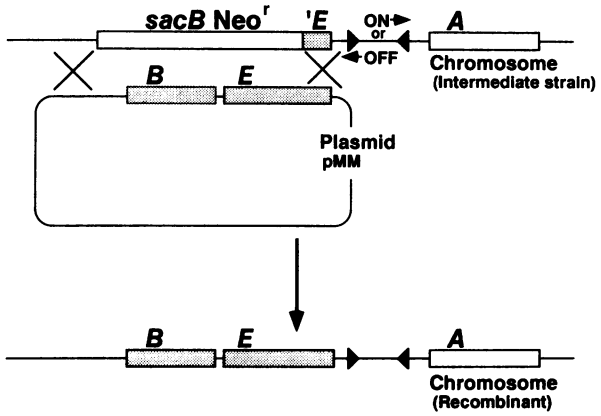

FIG. 4. Construction of *fimB* and *fimE* mutants and *fimB fimE* double mutants in wild-type and *lrp* mutant backgrounds by allelic exchange (7). Recombination between intermediate strains AAEC282A (switch on, *lrp*$^+$), AAEC284A (off, *lrp*$^+$), AAEC482A (on, *lrp*), and AAEC484A (off, *lrp*) and temperature-sensitive plasmids pMM69 (*fimB*$^+$ *fimE*$^+$), pMM82 (*fimB fimE*$^+$), pMM86 (*fimB*$^+$ *fimE*), and pMM87 (*fimB fimE*) generates 16 recombinant strains. The *sacB*-Neo^r cassettes in the intermediate strains replace *fimB* and the 5′ end of *fimE*. The invertible element (opposing arrowheads) is absent from the plasmids and is locked either on or off on the chromosomes of the intermediate strains. Recombination takes place between the two regions of plasmid-chromosome homology shown (×).

Influence of *lrp* on *fimB* and *fimE* transcription. As noted earlier, we first isolated *lrp* mutants as mTn*10* insertions that increased transcription of a *fimB-lacZYA* operon fusion. On lactose-MacConkey agar, these strains appeared only slightly darker than the wild type, suggesting only a modest increase in *fimB* expression. We measured the amount of β-galactosidase produced by *fimB-lac* (AAEC261A and AAEC444A) and *fimE-lac* (AAEC200 and AAEC482A) operon fusion strains in wild-type and *lrp* mutant backgrounds (Table 4). The apparent transcription of both genes was modified by *lrp* under the growth conditions tested. Whereas *fimB* transcription was increased slightly, as expected from the phenotype on indicator medium, *fimE* expression was reduced almost twofold.

DISCUSSION

To assist our analysis of type 1 fimbrial phase variation, we have constructed *fimB-lac* and *fimE-lac* operon fusions, replacing the wild-type *fim* genes on the chromosome. Then, using transposon mutagenesis, we initiated a project aimed at identifying genes that affect *fimB* and *fimE* transcription. In the work presented here, we restricted our analysis to

TABLE 3. Inversion of the *fim* switch in *lrp* mutants on defined-rich medium agar from off to on and from on to off

Type of inversion and genotype[a]	Result or frequency of inversion[b] in:	
	Wild type (strain)	*lrp*::Tn*10* mutant (strain)
Off to on		
fimB$^+$*E*$^+$	NM[c] (AAEC364A)	2×10^{-5}[d] (AAEC506A)
fimBE$^+$	Switch off (AAEC368A)	Switch off (AAEC511A)
fimB$^+$*E*	$2.93 \times 10^{-3} \pm 0.88 \times 10^{-3}$[e] (AAEC372A)	2×10^{-5}[d] (AAEC514A)
fimBE	Switch off (AAEC376A)	Switch off (AAEC532)
On to off		
fimB$^+$*E*$^+$	NM (AAEC363A)	$1.18 \times 10^{-4} \pm 0.88 \times 10^{-4}$[e] (AAEC527)
fimBE$^+$	NM (AAEC366A)	$1.99 \times 10^{-4} \pm 0.94 \times 10^{-4}$[e] (AAEC508A)
fimB$^+$*E*	$2.61 \times 10^{-3} \pm 0.45 \times 10^{-3}$[e] (AAEC370A)	1×10^{-5}[d] (AAEC512A)
fimBE	Switch on (AAEC374A)	Switch on (AAEC376A)

[a] Strains tested are listed in Table 1.
[b] Inversion frequency per cell per generation.
[c] NM, not measured.
[d] Calculated by using the Poisson distribution, with a total number of invertants of 4 or fewer.
[e] 95% confidence interval calculated by using the *t* distribution.

insertions that derepressed *fimB* expression, and that obviously affected the *fim* switch on agar.

Using the approach outlined above, we have isolated insertions in *lrp*, a gene that plays an important role in metabolism (for a review, see reference 41) and that affects both Pap and K99 fimbriation (9, 10). Upon further analysis, we found that the frequency of switching of the *fim* invertible region, and consequently of phase variation, is sharply reduced in *lrp* mutants.

We observed rapid on-to-off switching, a characteristic of wild-type strains, in a mutant that contains both *mbf* (*lrp*)-*7*::mTn*10* and *lac*UV5-*lrp* alleles in the presence of IPTG but not in its absence. Thus, even if the Tn*10* insertions in *lrp* exert a polar affect on *dinH* transcription (the gene immediately downstream of *lrp*) (31), a lack of Lrp, rather than of the *dinH* gene product, accounts for the sharp drop in on-to-off inversion seen in the insertion mutants.

Lrp is a site-specific DNA-binding protein (10, 33, 52) that is capable of protecting extended regions of DNA in vitro from nuclease digestion (33, 52). It affects the transcription of many genes, collectively called the leucine-Lrp regulon (18, 32, 46), and although the expression of some genes is enhanced by *lrp*, others are repressed (see below and reference 41). Lrp influences the phase variation of Pap fimbriation by blocking the methylation of two *dam* sites in the promoter region of *papBA* (9, 10).

Lrp could influence the *fim* inversion either indirectly by altering the expression of other proteins, such as FimB and FimE or IHF, or directly by participating in the *fim* inversion as an auxiliary factor. We find that both *fimB*-promoted (on-to-off and off-to-on) and *fimE*-promoted (on-to-off) recombinations are reduced in *lrp* mutants, even though the transcription of these genes, estimated by using transcriptional fusions, is affected only slightly by *lrp*. Both IHF (*himA* and *himD* [*hip*]) and *lrp* mutants have been isolated by many different selection procedures (reviewed in references 20 and 41), but with the exception of *fim*, no overlaps have been reported. Moreover, whereas IHF is required for type 1 fimbriation in media such as LB (4), Lrp is not (data not shown). These observations suggest that the low switching frequencies observed in *lrp* mutants are not due to a lack of either FimB and FimE or IHF.

We believe that Lrp is required either for the expression of an additional factor that promotes the *fim* inversion or as a direct participant in the recombination reaction. The latter implies that Lrp binds in or near the invertible element. We note the presence of the sequence 5′ TTTATTATCAAT, situated between the 3′ end of *fimE* and the switch. This sequence closely resembles a possible consensus for Lrp binding, 5′ TTTATTCTNAAT (51). Interestingly, the putative Lrp binding site in *fim* overlaps with an IHF binding site (14, 17, 47), suggesting a possible interaction between Lrp and IHF. Irrespective of how Lrp influences the *fim* inversion, there are no *dam* methylation sites either in, or very close to (<280 bp), the *fim* invertible region, and those in the vicinity of *fimB*, *fimE*, and *fimA* appear to be fully methylated (data not shown). Thus, if Lrp influences the *fim* inversion by blocking methylation of GATC sites by *dam* methylase, as it does near the *papBA* promoter to affect Pap fimbriation, then the pertinent sites presumably lie outside of the *fimB-fimA* region.

Lrp appears to play an important role in metabolism, particularly that of nitrogen. Thus, Lrp affects the activities of both glutamine and glutamate synthetases (18), as well as the expression of genes involved in the biosynthesis and degradation of other amino acids (32, 46, 51) and in the transport of oligopeptides (51) and of branched-chain amino acids (22, 48). Lrp also controls the expression of both Pap (9, 10) and K99 (10) fimbriae, as well as *ompF* and *ompC* (18). Since Lrp stimulates genes required for biosynthesis while repressing those involved in amino acid catabolism and transport, Lrp may help control the physiological switch that accompanies a shift between nutritionally rich and poor

TABLE 4. Effect of *lrp* upon the β-galactosidase expression of *fimB-lac* and *fimE-lac* operon fusion strains[a]

Relevant genotype	β-Galactoside expression (strain tested)[b]	
	Wild type	*lrp*
fimB-lac Δ*lacZYA*	30.6 ± 1.2 (AAEC261A)	36.4 ± 0.9 (AAEC444A)
fimE-lac Δ*lacZYA*	23.1 ± 1.7 (AAEC200)	13.3 ± 1.2 (AAEC482A)
Δ*lacZYA*	None detected (AAEC100)	Not tested

[a] Cells were grown in defined-rich medium (40) at 37°C.
[b] The values shown, in Miller units, represent the average of at least eight assays, using cells from two or more cultures.

environments (18, 32, 33). We believe that the identification of type 1, Pap, and K99 fimbriae as part of the leucine-Lrp regulon supports this notion. The expression of fimbriae represents a significant drain on the cell's resources, and we would expect their expression to be tightly controlled in response to nutrient levels. Perhaps significantly, type 1 fimbriation is also influenced by IHF, which, like Lrp, affects nitrogen assimilation (12) and amino acid biosynthesis (20).

Although many members of the leucine-Lrp regulon are influenced by exogenous leucine, others are not (reviewed in reference 41). In those cases investigated, leucine appears to modulate Lrp directly, enhancing or repressing the protein's activity. In turn, Lrp acts as either a positive or a negative regulator of transcription. Thus, through the action of Lrp, the cell responds to the availability of leucine. Clearly, leucine may also influence the *fim* inversion. As others have done in the past, we have used rich, leucine-replete media in the work described here. It is widely accepted that the *fim* switch is random and unresponsive to environmental conditions. However, the demonstration that the *fim* switch is controlled by Lrp suggests that environmental signals (such as leucine) could influence *fim* phase switching.

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