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The Leucine-Responsive Regulatory Protein Binds to the fim Switch To Control Phase Variation of Type 1 Fimbrial Expression in Escherichia coli K-12

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Phase variation of type 1 fimbriation in Escherichia coli is associated with the site-specific recombination of a 314-bp DNA invertible element. The fim switch directs transcription of fimA, the major fimbrial subunit gene, in one orientation (on) but not the other (off). Switching requires either fimB (on-to-off or off-to-on inversion) or fimE (on-to-off inversion only) and is reduced sharply in strains containing lrp::Tn10 mutations. Both fimE-promoted switching and fimB-promoted switching are stimulated by the amino acids alanine, isoleucine, leucine, and valine, and this regulation requires lrp. Here it is shown that the leucine-responsive regulatory protein (Lrp) binds in and adjacent to the fim switch. Mutations in fim that lower Lrp binding in vitro have corresponding effects on both fimB-promoted switching and fimE-promoted switching in vivo. Lrp initiates binding at one of two sites within the fim switch. Additional cooperative binding results in an extensive region of protection from both DNase I and 1,10-phenanthroline–copper complex-activated DNA cleavage. The region of protection can extend to within 12 bp of the right inverted repeat (switch off) and occupies over one-third of the switch. It is proposed that wrapping of fim DNA around an Lrp complex is required to form a recombination-proficient structure.

Expression of type 1 fimbriae is phase variable and is determined, in part, by the orientation of a short DNA element (switch) that acts in cis to control the transcription of fimA, the major fimbrial subunit gene (1, 15). Switching (DNA inversion) requires a gene situated adjacent to the invertible element, i.e., either fimB (on to off, off to on) or fimE (on to off) (14–16). Furthermore, FimB and FimE share significant homology with the lambda integrase family of site-specific recombinases (7, 14, 20). It is likely that FimB and FimE are the fim recombinases. Normal switching frequencies require the integration host factor (7, 8), H-NS (13), and the leucine-responsive regulatory protein (Lrp) (3, 11). Mutants lacking either hina, hiniD (hip), or lrp show very low frequencies of fimB- and fimE-promoted switching.

The fim switch is subject to environmental control by temperature and independently by the amino acids alanine, isoleucine, leucine, and valine (11). Both fimB-promoted switching and fimE-promoted switching are stimulated by these amino acids, and this stimulation requires lrp. In addition, lrp is involved in the differential control of fimB and fimE activities in response to growth in defined rich medium. Lrp stimulation of the fim switch could be either direct with Lrp participating in fim recombination, or indirect, with Lrp regulating expression of a trans-acting factor. Both fimB transcription and fimE transcription are affected only slightly by mutation of lrp (3). Therefore, it is likely that the marked stimulation of fim by lrp is either direct or occurs through factors other than FimB and FimE.

Lrp, a site-specific DNA-binding protein, controls a regulon of at least 40 genes in Escherichia coli, including fimbrial expression and amino acid transport, degradation, and biosynthesis (3, 6, 9, 10, 18, 19, 22–24). The most extensive studies of Lrp have examined its role in the regulation of ibH transcription (19, 22, 23). Lrp binds to the promoter region of ibH with a high degree of cooperativity, bending the DNA to form a nucleoprotein complex (22, 23). Lrp, also known as Mbf (methylation-blocking factor), participates directly in phase variation of papBA transcription (6, 18). Pap phase variation is associated with alternate dam methylation-protection patterns and requires Lrp (18). Here we show that Lrp binds both within and adjacent to the fim switch and propose that this protein participates directly in the site-specific recombination associated with the phase variation of type 1 fimbriae.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions.
The bacterial strains, bacteriophage, and plasmids used are listed in Table 1. All of the bacterial strains are derivatives of E. coli K-12. The media used included L broth (5 g of sodium chloride, 5 g of yeast extract, and 10 g of tryptone per liter [Difco Laboratories, Detroit, Mich.]) and L agar (L broth containing 1.5% agar [BBL, Cockeysville, Md.]). Sucrose agar, used to select recombinant bacteria (5), is L agar supplemented with 6% sucrose but lacking sodium chloride. MOPS [3-(N-morpholino)propanesulfonic acid] medium supplemented with 10 μM thiamine and 0.4% glucose was prepared as previously described (17). Indicator media were minimal glucose plates supplemented with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyanoside; Bethesda Research Laboratories) at 40 μg/ml (11) or lactose MacConkey plates. Liquid cultures were aerated at 37°C, and the optical densities of the cultures were monitored spectrophotometrically at 420 nm. Inversion of the fim switch was measured following growth in MOPS medium as described previously (11).

Recombinant DNA techniques. Plasmid and chromosomal DNAs were isolated as previously described (2). Restriction
# TABLE 1. Bacterial strains and plasmids used in this study

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<th>Strain, phage, or plasmid</th>
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**Phage P1vir**

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enzymes were purchased from either New England Biolabs (Beverly, Mass.) or Promega (Madison, Wis.). Sequencing was carried out with Sequenase (U.S. Biochemicals). For gel retardation assays, DNA was amplified by PCR with 20 μM each dATP, dGTP, and dTTP; 10 μM dCTP; 2 μl of [α-32P]dCTP (10 μCi/μl; 25 Ci/mmol), and 50 pmol of each primer. Mutant alleles of fimS were constructed by PCR overlap extension (2). Altered sequences were cloned by replacing wild-type fimS in pDG19 by using BsrGI and PstI406I sites. All PCR constructs were sequenced. End-labeled DNA, used in footprinting experiments, was prepared by end-labeling primers with T4 polynucleotide kinase and [γ-32P]ATP (7,000 Ci/mmol) and using them in the appropriate PCR.

**Gel mobility shift assays.** Reaction conditions were essentially as previously described (23) and included the following in a final volume of 10 μl: 1 μl of radiolabeled DNA (1 fmol/μl), 20 mM Tris hydrochloride (pH 8), 0.4 mM EDTA, 0.1 mM dithiothreitol, 50 mM NaCl, 1 mM MgCl2, 12.5% glycerol, 1 ng of bovine serum albumin, and 1 μg of calf thymus DNA or poly(dI-dC) as a nonspecific competitor DNA. Lrp was a generous gift from J. Calvo. Reactions were started by addition of Lrp to the reaction mixture and incubated for 15 min. Binding reactions were separated by electrophoresis through 4% (wt/vol) polyacrylamide (acrylamide-bisacrylamide ratio, 40:1) in TBE (Tris-borate-EDTA) buffer. Gels were run for 105 min at 160 V (constant voltage). Gels were transferred to Whatman 3 MM paper, dried at 80°C, and quantitated on an Ambis Radioanalytic Scanner (Ambis Systems Inc., San Diego, Calif.).

**Footprinting with DNase I.** DNase I footprinting was carried out as described previously for gel retardation assays (2). Reaction mixtures with and without Lrp were incubated for 20 min in a final volume of 25 μl at room temperature. DNase I was diluted in 20 mM MgCl2 and 10 mM CaCl2, and 5 μl was added to the binding reaction (final concentration of DNase I, 67 ng/ml). Approximately 106 dpm of end-labeled DNA was used in each reaction. The reaction was stopped after 1 min by addition of 250 μl of a solution containing 92% ethanol, 0.5 M NH4C2H3O2, and 1 μg of yeast tRNA, and the DNA was collected by centrifugation and washed in 70% ethanol. The dried pellet was resuspended in a sequencing formamide mixture, heated to 80°C for 2 min, and run on a polyacrylamide gel alongside the appropriate sequencing reaction mixture.

**Footprinting with (OP)2Cu2+.** Footprinting with (OP)2Cu2+ (OP is 1,10-phenanthroline) was done with protein-DNA complexes resolved by gel retardation (21). The gel was immersed in 200 ml of Tris-HCl (pH 8). We added 20 ml of solution A (40 mM OP in 100% ethanol–9.0 mM CuSO4 [in water] diluted 1 in 10 with water to 2.0 mM OP–0.45 mM

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**FIG. 1.** Localization of Lrp binding in and adjacent to the fim switch. (A) Nucleotide sequence of the fim switch and adjacent DNA (off orientation). The positions and orientations of all of the primers used in the present study are shown. The primers are referred to by their starting positions (5'). The inverted repeats are shown in bold type. IRL, left inverted repeat. (B) Gel retardation assay of Lrp (130 nM) binding to fim PCR fragments 1 to 4 and 6. The PCR fragments are as designated in panel C. Binding to a PCR fragment (ihIH) encompassing all six ihIH Lrp-binding sites is shown as a control. Assays were performed as described in Materials and Methods. (C) Positions and summary of Lrp binding to fim PCR fragments. The switch is in the off orientation. The coordinates relate to the starts of the various primers used.
CuSO₄) and then 20 ml of solution B (58 mM 3-mercapto- 
propanionic acid in water). The reaction mixture was 
mixed gently for 10 min at room temperature, the reaction was 
stopped by addition of 20 ml of 28 mM 2,9-dimethyl-OP, and the mixture 
was allowed to stand for 2 min. Gels were washed several times 
with distilled water, covered with cling film, and exposed to 
X-ray film for 20 min. Appropriate bands were excised from the gel. DNA was eluted from the gel slice overnight at 37°C in 0.5 M ammonium acetate–1 mM EDTA. Gel debris was 
removed by microcentrifugation, and DNA was precipitated 
with ethanol. The resulting pellet was dried, resuspended in a 
sequencing formamide mixture, heated to 85°C for 2 min, and 
run on a 10% sequencing gel alongside the appropriate 
sequencing reaction mixture.

RESULTS

Lrp binds to the fim switch in vitro. Overlapping DNA 
fragments that included sequences both adjacent to and within 
the fim switch were tested for Lrp binding in gel retardation 
assays (Fig. 1). Lrp at 130 nM, a protein concentration that 
completely shifted an ibIH fragment encompassing all six 
Lrp-binding sites, was used in preliminary experiments. PCR1, 
which includes the end of fimE, the left inverted repeat, and 
161 bp of switch DNA (off orientation), was shifted with loss of 
approximately 70% of free DNA, indicating the presence of at 
least one weak binding site. Incubation of this fragment with a 
lower concentration of Lrp (8 nM) did not produce a retarded 
complex (data not shown). Under the same conditions, PCR2 
and PCR3 were completely shifted to more slowly migrating 
species, producing at least two separate shifted complexes. 
Restriction endonuclease digestion of PCR3 with HaeIII 
produced two fragments of 98 and 137 bp. Whereas the fragment 
within the fim switch was 100% shifted, the second fragment 
was unaffected by the presence of Lrp (data not shown). 
Binding within the fim switch was localized to a 141-bp 
fragment, PCR4 (Fig. 1). PCR5, encompassing the segment 
adjacent to the left inverted repeat within the fim switch, was 
not retarded by Lrp (data not shown).

Titration of PCR4 with increasing Lrp concentrations gave 
two closely associated bands (Fig. 2) with a binding constant of 
0.8 nM⁻¹. Further exposure of some assays with PCR4 showed 
the presence of a weak shift between the double complex and 
free DNA (data not shown). The nucleotide sequence 
encircled by PCR4 contains two sequences that resemble a 
consensus for Lrp binding (23). fim sequences 5'-AGAATTT
TATATT (site 1, positions 2864 to 2852; the reverse comple- 
ment is shown) and 5'-AGAATTTAAGCC (site 2, positions 
2883 to 2895) each differ from the consensus, 5'-AGAATTT 
TATTCT, at four positions (underlined). An additional fragment 
(PCR6) of 80 bp, containing both potential Lrp-binding 
sites, was tested by gel retardation assay. This fragment 
produced a single shift (Fig. 1B), implying that the multiple 
shifts observed with PCR2, PCR3, and PCR4 require the presence of flanking DNA sequences. Lrp binding to PCR6 
was analyzed by (OP)₂Cu²⁺ footprinting (Fig. 3). This fragment 
gave a clear area of protection on both strands that extended 
from the beginning of site 1 to the end of site 2 (45 bp).

Cooperative interactions of Lrp with the fim switch. Gel 
mobility shift assays and DNA footprinting experiments define 
Lrp binding to a 45-bp core region within PCR6. The PCR 
fragments that flank PCR6 (PCR7 and PCR8 [Fig. 1]) pro- 
duced either no shift (PCR7) or a very weak shift (PCR8) 
(binding affinity of less than 1 μM⁻¹). These data imply that 
additional cooperative Lrp interactions are required to pro-
produce the further shifts shown for PCR2, PCR3, and PCR4 (Fig. 1B). To investigate the nature of cooperative Lrp binding to the fim switch, the interaction of Lrp with additional PCR fragments (PCR9, PCR10, and PCR11) was studied.

PCR9 (positions 2834 to 2975), which includes the 45-bp core region and 95 bp towards the right inverted repeat (IRR; switch off), produced three distinct complexes by gel retardation assay (Fig. 4A). Titration of Lrp with PCR9 showed that the more slowly migrating complexes were favored with increasing concentrations of Lrp. (OP)2Cu2+ footprinting of the fastest-migrating complex gave the same area of protection as defined on PCR6 (Fig. 3 and 4B). The intermediate complex had an extended area of protection, including the core footprint (45 bp) plus an additional 25 bp towards the IRR (switch off). Protection was extended a further 15 bp towards the IRR in the most slowly migrating complex (Fig. 4B; for a summary, see Fig. 6). In this complex, the footprint therefore extended for approximately 90 bp and this protection ended only 12 bp from the IRR. The extended protection was weak and was apparent only on the top strand (Fig. 4B).

PCR10 (positions 2774 to 2914), which includes the 45-bp core region and 95 bp away from the IRR (switch off), produced a single retarded complex (data not shown). Incubation of PCR10 with Lrp protected 77 bp from (OP)2Cu2+ activated cleavage (for a summary, see Fig. 6). This region of protection, apparent on both strands, included the core and an additional 30 bp extending away from the IRR (switch off). Therefore, although Lrp initiates binding to the core region (sites 1 and 2), continued Lrp cooperativity permits further Lrp-DNA interactions to occur to the sides flanking these sites.

To analyze the entire region of Lrp binding within the fim switch, DNase I footprinting was carried out with PCR11 (Fig. 1C and 5). The DNase I protection pattern was complex, with alternating regions of protection and enhancement. A summary of all of the footprinting analyses is shown in Fig. 6. Two regions extensively protected on both strands from DNase I cleavage coincide with the A-T-rich central motif of the two consensus-like sites defined above (fim sites 1 and 2). Continued protection (38 bp) was apparent towards the left inverted repeat (switch off) and to a lesser extent (15 bp) towards the IRR (switch off). Furthermore, enhanced regions of DNase I cleavage were detected both between and adjacent to fim sites 1 and 2.

**Mutagenesis of Lrp-binding sites.** By using PCR techniques, fim site 1 was replaced with 5'-AGATGcTcgagTT (fimS1) and site 2 was replaced with 5'-AGAAAATcTcgAGCC (fimS2). We define the fim switch and any elements located adjacent to the fim switch that act in cis to control switching as fimS. When measured by gel retardation assay, Lrp binding was reduced over sixfold (binding constant, 0.12 nM−1) by mutation of site
FIG. 6. Summary of Lrp interactions within the fim switch. (A) Summary of areas protected from (OP)_2Cu^{2+} complex-activated cleavage (dashed line). The two proposed Lrp-binding sites in fim are boxed. The core region is the area of protection found on PCR6 and is bounded by fim sites 1 and 2. The area of protection was extended in both directions by analysis of PCR9 and PCR10. PCR9 formed three distinct complexes by gel retardation analysis. The fastest-migrating complex gave the same area of protection as the core region. The intermediate complex showed extended protection towards the IRR (switch off), and this protection was further extended in the most retarded complex (Fig. 4), ending 12 bp from the IRR. Extension of the region of protection away from the IRR (switch off) was seen with PCR10 and included 30 bp beyond the core region. All binding required the presence of sites 1 and 2. (B) Summary of DNase I analysis of Lrp binding to the fim switch. fim sites 1 and 2 are boxed. Regions of protection are represented on the positive strand by a line above the nucleotide sequence and on the negative strand by a line below the sequence. Nucleotides at which there was enhanced cleavage are marked by a stem and circle.

1 and over threefold (binding constant, 0.22 nM^{-1}) by mutation of site 2 (Fig. 7). When combined, the mutations lowered binding 40-fold (binding constant, 0.02 nM^{-1}).

Additional mutations were introduced into two sequences (both 5'-TTTATT changed to 5'-cTcagag), one between sites 1 and 2 (site 3, fimS3, positions 2873 to 2868) and the other between site 2 and the IRR (site 4, fimS4, positions 2905 to 2900). These alterations did not perturb Lrp binding (data not shown).

Analysis of fim switch mutations on in vivo activity of the fim switch. Allelic exchange was used to transfer mutations in the fim switch (fimS1 to fimS4) into the chromosome at fim (Fig. 8). Intermediate strains were constructed in which the fim switch was replaced by a sacB-Kan cassette in both the wild-type and fimB and fimE mutant backgrounds (5, 15). Allelic exchange between pDG19 (wild-type switch) and the intermediate strains reconstructs the wild-type switch. The fimS alleles were subcloned into pDG19 to replace the wild-type sequence.

The mutations in the fim switch were transferred into the chromosome at fim in both the fimB^- fimE^- and fimB^- fimE^+ backgrounds. fimB-promoted switching (fimB^- fimE^- strains) was determined by growth on defined rich agar, whereas switching promoted by fimE^- was determined in defined rich liquid medium; both were determined at 37°C. The wild-type switching frequencies and frequencies for mutations in sites 1 and 2, as well as sites 3 and 4, are shown in Tables 2 (fimB^+ fimE^+) and 3 (fimB^+ fimE^-). In addition, switching frequencies were measured in the respective lrp mutants of some strains.

Mutations in fim sites 1 and 2 decrease switching frequencies, and both fimB-promoted switching and fimE-promoted switching were affected. fimS1 lowered fimE^-promoted switching 20-fold and fimB^-promoted switching 10-fold. fimS2 had less of an effect, reducing fimE^-promoted on-to-off switching threefold and fimB^-promoted switching, in both directions, twofold. When these two mutations were combined, fimE^- promoted switching was lowered 100-fold and fimB^-promoted switching was lowered 50-fold. In contrast, mutations in sites 3 and 4 had no measurable effect on either fimB^- or fimE^- promoted switching.

The magnitude of the effect of these mutations on switching frequencies shows a good correlation with their effect on in vitro binding of Lrp to the respective fimS alleles. Moreover, the fim switch mutations had little effect on switching frequencies in lrp mutants, with all frequencies lowered to similar values (Table 2 and 3).

DISCUSSION

Site-specific recombination of a 314-bp invertible DNA element is associated with control of phase variation of type 1 fimbriae (1). Although inversion of the fim switch is considered to be controlled by the products of fimB, fimE, fimA, fimD, and hns (7, 8, 13, 14–16), little is known about the mechanism of the site-specific inversion and its potential regulation. We reported previously that rather than being slow and random, the fim switch is capable of high inversion frequencies (>0.7 per cell per generation) and is regulated by environmental conditions, including temperature and the amino acids alanine, leucine, isoleucine, and valine (11). In addition, lrp is required for normal control of fim inversion and for amino acid stimulation of fim (3, 11). Here we show that Lrp binds in and adjacent to the fim switch and suggest that Lrp plays a direct role in fim recombination.

Gel retardation assays identified an 80-bp region within the switch that contains two sites which show agreement with a proposed consensus Lrp-binding site (23). (OP)_2Cu^{2+} foot-printing confirmed Lrp binding to a core region (45 bp) that stretches from fim site 1 to site 2 (Fig. 6). Mutations in these two sequences resulted in a reduction in Lrp binding to fim in vitro. Furthermore, combining the two mutations produced a compound effect, suggesting cooperative interaction between Lrp at fim sites 1 and 2. However, it is not known whether Lrp can bind to both sites independently, and consequently, initial binding to only one of the two sites may be possible.

To test the effect of cis-acting mutations on fim switching, intermediate (Δfim switch and inserted sacB-Kan cassette) strains were constructed to allow replacement of fimS. Mutations were created in fim sites 1 and 2 (fimS1 and fimS2) and moved into the chromosome. Both fimE^- and fimB^-promoted inversion frequencies were lowered in these strains. Furthermore, when the two mutations were combined, there was an enhanced effect on both fimE^- and fimB^-promoted switching frequencies. This supports the hypothesis that Lrp exhibits a degree of cooperative binding to sites 1 and 2. The reduction in inversion frequencies showed an excellent correlation with the reduction in binding affinities in vitro. This implies a direct role of Lrp in fim recombination. The proposed Lrp-binding sites within the fim switch have the same separation as ilvIH sites 1 and 2 (18 bp), although the orientations of the asymmetric sequences within the consensus differ between fim and ilvIH.
FIG. 7. Gel retardation assay of wild-type (wt) and mutant alleles of fimS. fimS (wt) is PCR4 (Fig. 1). fimS1 contains a 5-bp change in the potential Lrp-binding site (fim site 1 in Fig. 6), fimS2 contains a 3-bp change in the potential Lrp-binding site (fim site 2 in Fig. 6). fimS1/2 combines the two mutations. The gel retardation assay was done as described in Materials and Methods with the Lrp concentrations indicated.

Lrp has been shown to bind DNA, organizing it into a nucleoprotein complex (22). Our results are consistent with the notion that Lrp binds at fim sites 1 and 2 and then interacts with the DNA flanking both sides of this region (Fig. 6). Binding to the core region and flanking DNA away from the IRR produced a single complex (77-bp protected region), whereas binding to the core and flanking DNA towards the IRR (in the off orientation) produced three distinct complexes. The formation of these latter complexes was dependent on the concentration of Lrp. Consequently, increasing the concentration of Lrp favors further Lrp-Lrp and/or Lrp-DNA interactions that extend the area of protection. The large regions protected from activated cleavage by (OP)2Cu2+ are likely to reflect distortions in the DNA structure, as well as steric constraints imposed by Lrp.

DNase I footprinting of a region including both the core and flanking sequences showed alternating areas of protection from and enhancement of cleavage. This pattern is consistent with the interaction of Lrp with both pab and ilvIH DNAs (18, 22) and is associated with naked curved DNA or DNA that is wrapped around protein. These data, in combination with the site-directed mutagenesis described above, support a physical role for Lrp in fim recombination. It is likely that Lrp binds within the switch to form a nucleoprotein complex that is prerequisite for recombination. In addition, towards the boundaries defined by footprinting analyses lie two 5-nucleotide dA-dT tracts (positions 2836 to 2840 and 2909 to 2913). Such tracts are known to be intrinsically bent, and these sequences may also be important in the formation of a recombination-proficient structure.

By analogy with other systems, synopsis of the two inverted repeats is necessary for recombination and requires the involvement of DNA-binding proteins that can bend DNA. The present study supports such a physical role for Lrp in fim recombination. In addition, it is known that himA and himD are required for fim recombination. These results support the hypothesis that participation of the integration host factor host has yet to be demonstrated. Two good consensus integration host factor-binding sites exist in and adjacent to the fim switch (7, 8). Consequently, both the integration host factor and Lrp likely induce DNA bending to help align the inverted repeats in a juxtaposition. By comparison with other site-specific recombination systems, the fim recombinases are expected to bind adjacent to the inverted repeats. Thus, since Lrp protection can extend to within 12 bp of the IRR (switch off), it is possible that Lrp interacts directly with the fim recombinases. Lrp is considered to interact with PapI as part of its role in controlling Pap phase variation (18). The area of protection defined by (OP)2Cu2+ footprinting that extended towards the IRR (switch off) was weak and was

![Diagram](image-url)

FIG. 8. Allelic exchange of the fim switch and adjacent sequences (fimS). Intermediate strains were constructed with a sacB-Kan' cassette replacing fimS. Intermediate strains contain two combinations of fimB and fimE alleles (fimB+ fimE+ and fimB+ fimE+) to allow determination of combined and independent switching frequencies (11). The allelic exchanges were carried out with pDG19 or its derivatives containing altered fimS alleles (pBG36, fimS1; pBG37, fimS2; pBG37, fimS1/2; pBG37, fimS3; pBG37, fimS4). Controls included reconstruction of wild-type fimE+ fimB+ and fimB+ fimE strains, in which switching rates were as determined previously (see Tables 2 and 3) (11). S, SphI; P, Psp1406I; B, BsrGI.

**Table 2.** On-to-off switching in fimB+ fimE+ strains containing mutations in Lrp-binding sites

<table>
<thead>
<tr>
<th>fimS allele</th>
<th>fimE+ fimB+</th>
<th>fimB+ fimE+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>3.0 × 10^-4</td>
<td>1.4 × 10^-4</td>
</tr>
<tr>
<td>fimS1</td>
<td>1.4 × 10^-2</td>
<td>2.8 × 10^-4</td>
</tr>
<tr>
<td>fimS2</td>
<td>9.5 × 10^-3</td>
<td>2.4 × 10^-4</td>
</tr>
<tr>
<td>fimS1/2</td>
<td>2.6 × 10^-3</td>
<td>4.3 × 10^-4</td>
</tr>
<tr>
<td>fimS3</td>
<td>3.1 × 10^-1</td>
<td>NT*</td>
</tr>
<tr>
<td>fimS4</td>
<td>2.4 × 10^-1</td>
<td>NT</td>
</tr>
</tbody>
</table>

The switching frequencies were determined either by best fit to a probabilistic model (non-Lrp mutant values) or as the means of the frequencies calculated from at least five separate colonies. These calculations were done as described previously (11).

* Switching frequencies were determined either by best fit to a probabilistic model (non-Lrp mutant values) or as the means of the frequencies calculated from at least five separate colonies. These calculations were done as described previously (11).

**Table 3.** Switching in fimB+ fimE strains containing mutations in putative Lrp-binding sites

<table>
<thead>
<tr>
<th>fimS allele</th>
<th>Off to on (fimB+ fimE)</th>
<th>Off to on (fimB+ fimE)</th>
<th>Off to on (fimB+ fimE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>3.26 × 10^-3</td>
<td>3.59 × 10^-3</td>
<td>1.50 × 10^-3</td>
</tr>
<tr>
<td>fimS1</td>
<td>3.35 × 10^-4</td>
<td>2.56 × 10^-4</td>
<td>1.7 × 10^-4</td>
</tr>
<tr>
<td>fimS2</td>
<td>1.64 × 10^-3</td>
<td>8.3 × 10^-4</td>
<td>1.2 × 10^-3</td>
</tr>
<tr>
<td>fimS1/2</td>
<td>6.3 × 10^-5</td>
<td>2.0 × 10^-5</td>
<td>6.9 × 10^-6</td>
</tr>
<tr>
<td>fimS3</td>
<td>2.36 × 10^-3</td>
<td>3.75 × 10^-3</td>
<td>NT*</td>
</tr>
<tr>
<td>fimS4</td>
<td>2.69 × 10^-3</td>
<td>2.78 × 10^-3</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Switching frequencies were calculated from at least five separate colonies. This calculation was done as described previously (11).

* NT, not tested.
apparent only on the positive strand. It is likely that the extended protection was not strand specific but rather represents low-affinity interactions that were more difficult to detect on the negative strand. There was no evidence of protection in this region by DNase I analysis, but it must be noted that the (OP)$_2$Cu$^{2+}$ method examines distinct complexes from gel retardation assays. Detection of these weak-affinity interactions by DNase I would be difficult because of a background from the higher-affinity complexes. Additional experiments will investigate the possibility that FimB and FimE interact with Lrp.

Even though the mechanisms of type 1 fimbriae and Pap phase variation are quite different, both are controlled by Lrp. Furthermore, whereas Pap phase variation is unresponsive to exogenous leucine levels, inversion of the fim switch is stimulated. Leucine stimulation of fim inversion could result from direct interaction of leucine with Lrp at the fim switch. Therefore, Lrp may play both a structural role and a regulatory role within the fim switch. The molecular basis of this environmental regulation is the subject of our ongoing research.

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