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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 *himA*, *himD* (*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 biosyn-

thesis (3, 6, 9, 10, 18, 19, 22–24). The most extensive studies of Lrp have examined its role in the regulation of *ilvIH* transcription (19, 22, 23). Lrp binds to the promoter region of *ilvIH* 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.

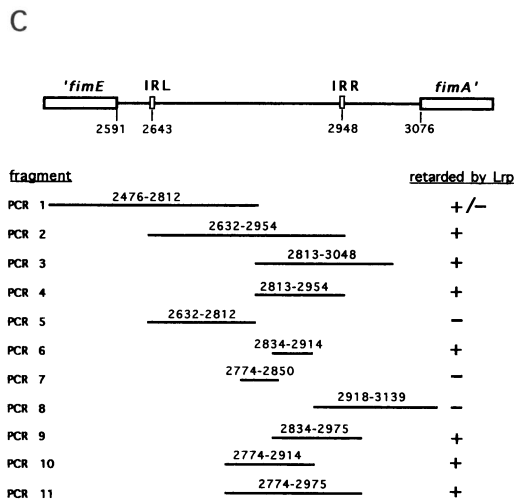
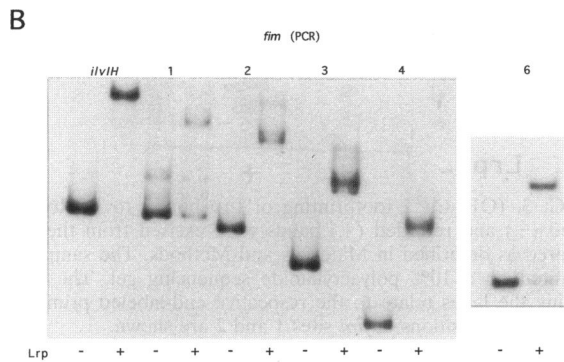
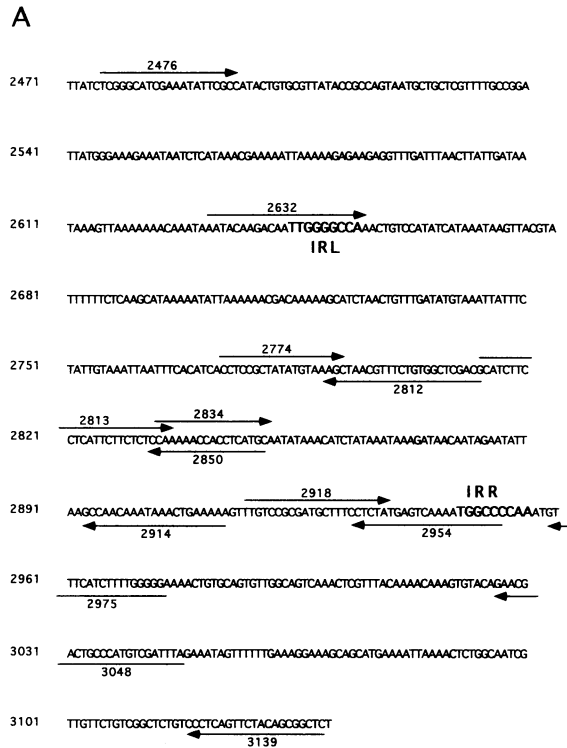
The bacterial strains, bacteriophage, and plasmids used are listed in Table 1. All of the bacterial strains are derivatives of *E. coli* K-12. The media used included L broth (5 g of sodium chloride, 5 g of yeast extract, and 10 g of tryptone per liter [Difco Laboratories, Detroit, Mich.]) and L agar (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 μ M 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-galactopyranoside; Bethesda Research Laboratories) at 40 μ g/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

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TABLE 1. Bacterial strains and plasmids used in this study

Strain, phage, or plasmid	Genotype or phenotype	Source, reference(s), or construction
<i>E. coli</i> strains		
MG1655	λ^- F ⁻ Fim ⁺	Our stocks (4, 12)
AAEC198A	MG1655 Δ lacZYA <i>fimA-lacZYA</i>	5
AAEC370A	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18</i>	5
BGEC 132	MG1655 Δ lacZYA Δ <i>fimE-fimA</i> Ω (<i>sacB-Kan</i> ^r) <i>fimA-lacZYA</i>	Exchange of <i>fimE-fimA</i> (including <i>fimS</i>) of AAEC198A with <i>sacB-Kan</i> ^r from pDG28
BGEC 144	MG1655 Δ lacZYA Δ <i>fimE-fimA</i> Ω (<i>sacB-Kan</i> ^r) <i>fimA-lacZYA fimE-am18</i>	Exchange of <i>fimE-fimA</i> (including <i>fimS</i>) of AAEC370A with <i>sacB-Kan</i> ^r from pDG28
BGEC154	MG1655 Δ lacZYA <i>fimA-lacZYA</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 132 for <i>fimE-fimA</i> (including <i>fimS</i>) from pDG19
BGEC 156	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 144 for <i>fimE-fimA</i> (including <i>fimS</i>) from pDG19
BGEC 158	MG1655 Δ lacZYA <i>fimA-lacZYA fimS2</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 132 for <i>fimE-fimA</i> (including <i>fimS2</i>) from pIB366
BGEC 160	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS2</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 144 for <i>fimE-fimA</i> (including <i>fimS2</i>) from pIB366
BGEC 162	MG1655 Δ lacZYA <i>fimA-lacZYA fimS1</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 132 for <i>fimE-fimA</i> (including <i>fimS1</i>) from pIB369
BGEC 164	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS1</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 144 for <i>fimE-fimA</i> (including <i>fimS1</i>) from pIB369
BGEC 166	MG1655 Δ lacZYA <i>fimA-lacZYA fimS1/2</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 132 for <i>fimE-fimA</i> (including <i>fimS1/2</i>) from pIB372
BGEC 168	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS1/2</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 144 for <i>fimE-fimA</i> (including <i>fimS1/2</i>) from pIB372
BGEC 170	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 (lrp)::mTn10 (Kan</i> ^r)	P1 transduction of insert 2 [(<i>lrp</i>)::mTn10] (3) from AAEC429A into BGEC 156
BGEC 172	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS2 (lrp)::mTn10 (Kan</i> ^r)	P1 transduction of insert 2 [(<i>lrp</i>)::mTn10] from AAEC429A into BGEC 160
BGEC 174	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS1 (lrp)::mTn10 (Kan</i> ^r)	P1 transduction of insert 2 [(<i>lrp</i>)::mTn10] from AAEC429A into BGEC 164
BGEC 176	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS1/2 (lrp)::mTn10 (Kan</i> ^r)	P1 transduction of insert 2 [(<i>lrp</i>)::mTn10] from AAEC429A into BGEC 168
BGEC 178	MG1655 Δ lacZYA <i>fimA-lacZYA fimS3</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 132 for <i>fimE-fimA</i> (including <i>fimS3</i>) from pIB374
BGEC 180	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS3</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 144 for <i>fimE-fimA</i> (including <i>fimS3</i>) from pIB374
BGEC 182	MG1655 Δ lacZYA <i>fimA-lacZYA fimS4</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 132 for <i>fimE-fimA</i> (including <i>fimS4</i>) from pIB376
BGEC 184	MG1655 Δ lacZYA <i>fimA-lacZYA fimE-am18 fimS4</i>	Exchange of <i>sacB-Kan</i> ^r from BGEC 144 for <i>fimE-fimA</i> (including <i>fimS4</i>) from pIB376
BGEC 190	MG1655 Δ lacZYA <i>fimA-lacZYA fimS2 (lrp)::mTn10 (Kan</i> ^r)	P1 transduction of insert 2 [(<i>lrp</i>)::mTn10] from AAEC429A into BGEC 158
BGEC 192	MG1655 Δ lacZYA <i>fimA-lacZYA fimS1 (lrp)::mTn10 (Kan</i> ^r)	P1 transduction of insert 2 [(<i>lrp</i>)::mTn10] from AAEC429A into BGEC 162
BGEC 194	MG1655 Δ lacZYA <i>fimA-lacZYA fimS1/2 (lrp)::mTn10 (Kan</i> ^r)	P1 transduction of insert 2 [(<i>lrp</i>)::mTn10] from AAEC429A into BGEC 166
Phage P1vir		Laboratory collection
Plasmids		
pDG17	Cm ^r pIB333 (4) Δ <i>ScaI (lacZ)</i> to <i>ScaI</i> (vector)	This work
pDG19	Cm ^r pDG17; subcloning of <i>NheI</i> (partial fill in)- <i>SphI</i> fragment from pMM86 (15) into pDG17 <i>HindIII</i> (partial fill in)- <i>SphI</i> site	This work
pDG21	Cm ^r pDG19; <i>BamHI</i> linker insertion at <i>SphI</i> in <i>fimE</i> Δ <i>BamHI (SphI)-BamHI (lacZ)</i> site	This work
pDG28	Cm ^r pDG21; subcloning of <i>sacB-Kan</i> ^r (<i>BamHI</i>) cassette from pIB279 (5) into pDG21 cut with <i>BamHI</i>	This work
pIB366	Cm ^r pDG19; replacement of <i>fimS</i> with <i>fimS2 BsrGI-Psp1406I</i> fragment	This work
pIB369	Cm ^r pDG19; replacement of <i>fimS</i> with <i>fimS1 BsrGI-Psp1406I</i> fragment	This work
pIB372	Cm ^r pDG19; replacement of <i>fimS</i> with <i>fimS1/2 BsrGI-Psp1406I</i> fragment	This work
pIB374	Cm ^r pDG19; replacement of <i>fimS</i> with <i>fimS3 BsrGI-Psp1406I</i> fragment	This work
pIB376	Cm ^r pDG19; replacement of <i>fimS</i> with <i>fimS4 BsrGI-Psp1406I</i> fragment	This work



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 μ M (each) dATP, dGTP, and dTTP; 10 μ M dCTP; 2 μ l of [α -³²P]dCTP (10 μ Ci/ μ l; 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 *Bsr*GI and *Psp*1406I 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 μ l: 1 μ l of radiolabeled DNA (1 fmol/ μ l), 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 μ g 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 μ l at room temperature. DNase I was diluted in 20 mM MgCl₂ and 10 mM CaCl₂, and 5 μ l was added to the binding reaction (final concentration of DNase I, 67 ng/ml). Approximately 10⁵ dpm of end-labeled DNA was used in each reaction. The reaction was stopped after 1 min by addition of 250 μ l of a solution containing 92% ethanol, 0.5 M NH₄C₂H₃O₂, and 1 μ g 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

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 (*ilvIH*) encompassing all six *ilvIH* 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.

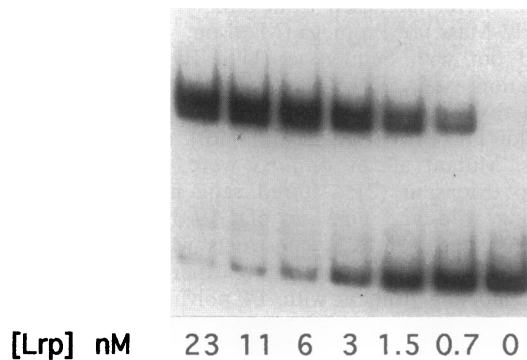


FIG. 2. Gel retardation assay of PCR4 titrated with Lrp (at the concentrations indicated). The assay was carried out as described in Materials and Methods, and the dried gel was scanned and analyzed on an Ambis Radioanalytic Scanner.

CuSO₄) and then 20 ml of solution B (58 mM 3-mercaptopropionic 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 *ilvIH* 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 *Hae*III 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 encompassed by PCR4 contains two sequences that resemble a consensus for Lrp binding (23). *fim* sequences 5'-AGAIGT

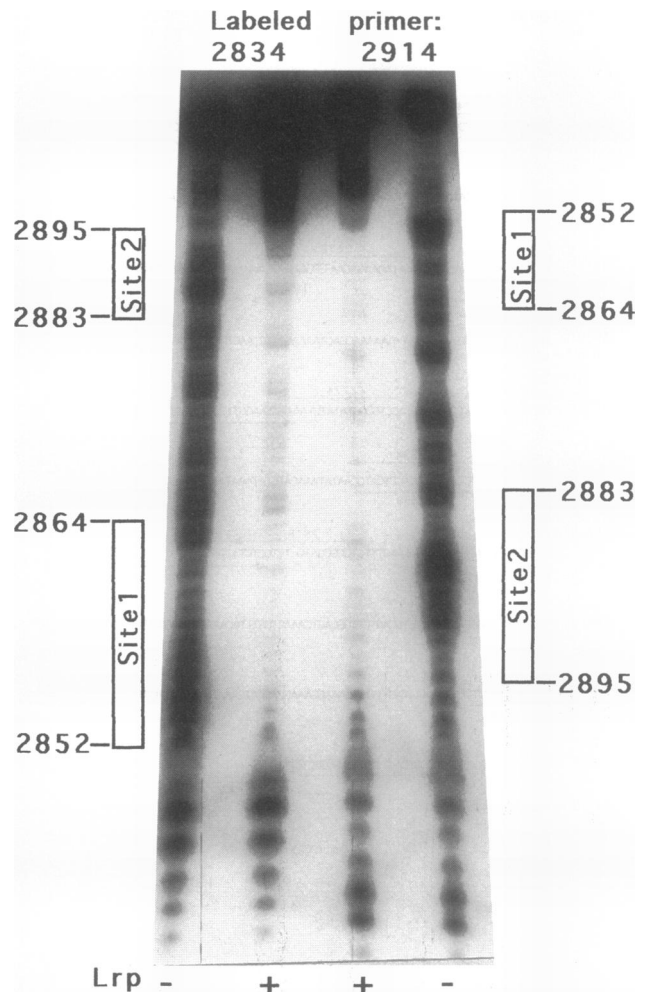


FIG. 3. (OP)₂Cu²⁺ footprinting of Lrp bound to PCR6. Nonretarded (–) and retarded (+) bands were excised from the gel and prepared as described in Materials and Methods. The samples were separated on a 10% polyacrylamide sequencing gel. The numbers heading the lanes relate to the respective end-labeled primers from Fig. 1A. The positions of *fim* sites 1 and 2 are shown.

TATATT (site 1, positions 2864 to 2852; the reverse complement is shown) and 5'-AGAATATTAAGCC (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]) produced 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-

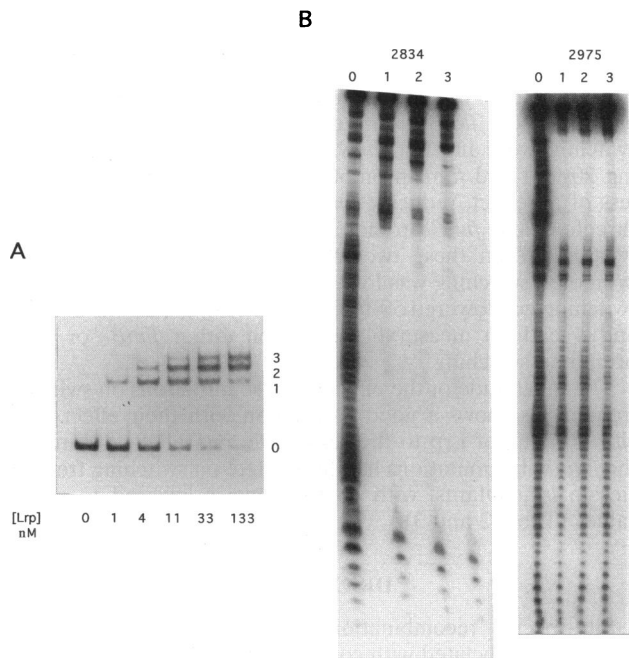


FIG. 4. Characterization of Lrp binding to the *fim* switch. (A) Gel retardation assay of PCR9 (includes PCR6 [Fig. 1] and 60 bp towards the IRR [switch off]). The Lrp concentrations used are shown. (B) $(OP)_2Cu^{2+}$ footprinting of retarded (lanes 1, 2, and 3) and nonretarded (lane 0) bands from panel A. The samples were prepared as described in Materials and Methods and separated by electrophoresis through a 10% sequencing gel. The top number relates to the coordinate of the end-labeled primer used (Fig. 1A).

duce 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. $(OP)_2Cu^{2+}$ 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 $(OP)_2Cu^{2+}$ -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*

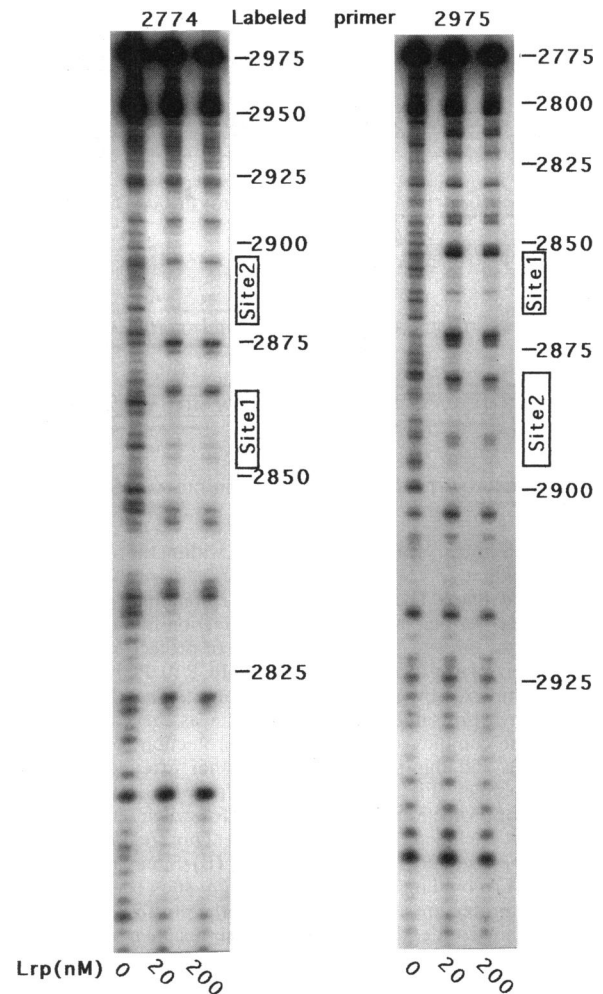


FIG. 5. DNase I analysis of Lrp binding within the *fim* switch. DNase I footprinting was done with PCR11 as described in Materials and Methods. The end-labeled primers used are shown above the relevant lanes. The samples were separated on an 8% sequencing gel alongside the respective dideoxy sequencing reaction. The positions of *fim* sites 1 and 2 are shown, as are the nucleotide positions for reference to either Fig. 1A or 6.

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'-AGAATcTcgAGCC (*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

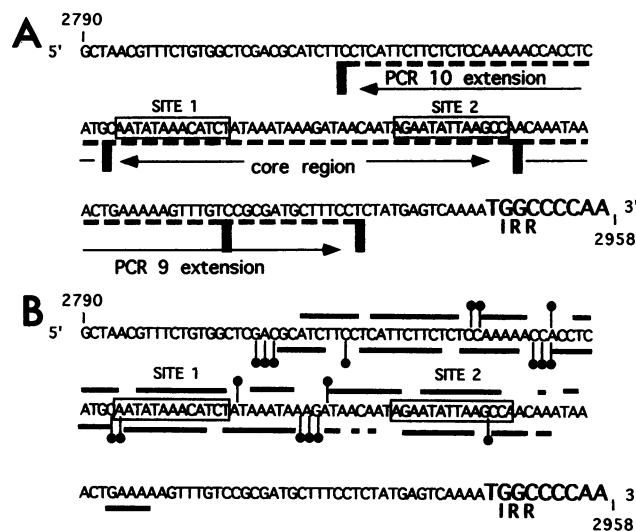


FIG. 6. Summary of Lrp interactions within the *fim* switch. (A) Summary of areas protected from $(OP)_2Cu^{2+}$ complex-activated cleavage (dashed line). The two proposed Lrp-binding sites in *fim* are boxed. The core region is the area of protection found on PCR6 and is bounded by *fim* sites 1 and 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'-cTcgag), 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^r* 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*, *himA*, *himD*, 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). $(OP)_2Cu^{2+}$ footprinting 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 (Δ *fim* switch and inserted *sacB-Kan^r* 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*.

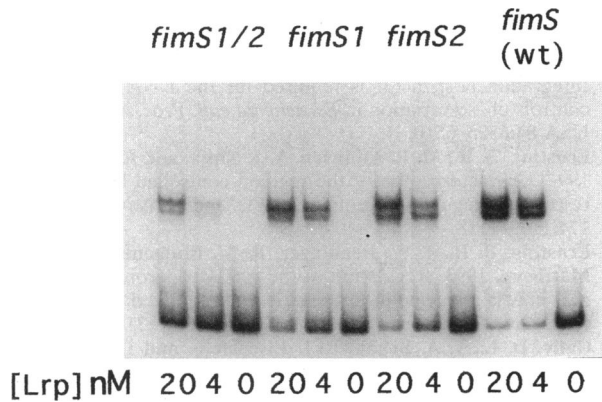


FIG. 7. Gel retardation assay of wild-type (wt) and mutant alleles of *fimS*. *fimS* (wt) is PCR4 (Fig. 1). *fimS1* contains a 5-bp change in the potential Lrp-binding site (*fim* site 1 in Fig. 6). *fimS2* contains a 3-bp change in the potential Lrp-binding site (*fim* site 2 in Fig. 6). *fimS1/2* combines 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 bend 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 $(OP)_2Cu^{2+}$ 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 *pap* and *ilvIH* DNAs (18, 22) and is associated with naked curved DNA or DNA that is

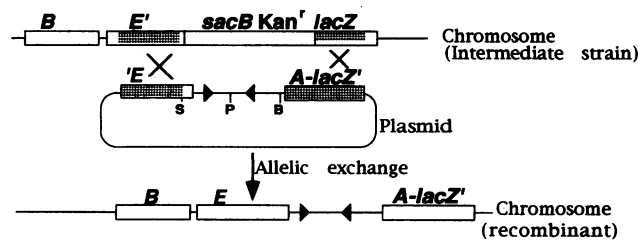


FIG. 8. Allelic exchange of the *fim* switch and adjacent sequences (*fimS*). Intermediate strains were constructed with a *sacB*-*Kan*^r cassette replacing *fimS*. Intermediate strains contain two combinations of *fimB* and *fimE* alleles (*fimB*⁺ *fimE*⁺ and *fimB*⁺ *fimE*) to allow determination of combined and independent switching frequencies (11). The allelic exchanges were carried out with pDG19 or its derivatives containing altered *fimS* alleles (pIB366, *fimS1*; pIB369, *fimS2*; pIB372, *fimS1/2*; pIB374, *fimS3*; pIB376, *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, *Sph*I; P, *Psp*1406I; B, *Bsr*GI.

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

<i>fimS</i> allele	Switching frequency ^a /cell/generation	
	<i>fimE</i> ⁺ <i>fimB</i> ⁺	<i>fimB</i> ⁺ <i>fimE</i> ⁺ <i>lrp2::mTn10</i>
Wild type	3.0×10^{-1}	1.4×10^{-4}
<i>fimS1</i>	1.4×10^{-2}	2.8×10^{-4}
<i>fimS2</i>	9.5×10^{-2}	2.4×10^{-4}
<i>fimS1/2</i>	2.6×10^{-3}	4.3×10^{-4}
<i>fimS3</i>	3.1×10^{-1}	NT ^b
<i>fimS4</i>	2.4×10^{-1}	NT

^a 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 (*lrp* mutant values). These calculations were done as described previously (11).

^b NT, not tested.

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, although direct participation of the integration host factor 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 $(OP)_2Cu^{2+}$ footprinting that extended towards the IRR (switch off) was weak and was

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

<i>fimS</i> allele	Switching frequency ^a		
	Off to on (<i>fimB</i> ⁺ <i>fimE</i>)	On to off (<i>fimB</i> ⁺ <i>fimE</i>)	Off to on (<i>fimB</i> ⁺ <i>fimE</i> <i>lrp2::mTn10</i>)
Wild type	3.26×10^{-3}	1.50×10^{-3}	2×10^{-5}
<i>fimS1</i>	3.35×10^{-4}	2.56×10^{-4}	1.7×10^{-5}
<i>fimS2</i>	1.64×10^{-3}	8.3×10^{-4}	1.2×10^{-5}
<i>fimS1/2</i>	6.3×10^{-5}	2.0×10^{-5}	6.9×10^{-6}
<i>fimS3</i>	2.36×10^{-3}	3.75×10^{-3}	NT ^b
<i>fimS4</i>	2.69×10^{-3}	2.78×10^{-3}	NT

^a Switching frequencies were determined as the means of the frequencies calculated from at least five separate colonies. This calculation was done as described previously (11).

^b 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 $(OP)_2Cu^{2+}$ 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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REFERENCES

- Abraham, J. M., C. S. Freitag, J. R. Clements, and B. I. Eisenstein. 1985. An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:5724-5727.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, and K. Struhl. 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
- Blomfield, I. C., P. J. Calie, K. J. Eberhardt, M. S. McClain, and B. I. Eisenstein. 1993. Lrp stimulates the phase variation of type 1 fimbriation in *Escherichia coli* K-12. J. Bacteriol. **175**:27-36.
- Blomfield, I. C., M. S. McClain, J. A. Princ, P. J. Calie, and B. I. Eisenstein. 1991. Type 1 fimbriation and *fimE* mutants of *Escherichia coli* K-12. J. Bacteriol. **173**:5298-5307.
- Blomfield, I. C., V. Vaughn, R. F. Rest, and B. I. Eisenstein. 1991. Allelic exchange in *Escherichia coli* using the *Bacillus subtilis* *sacB* gene and a temperature-sensitive pSC101 replicon. Mol. Microbiol. **5**:1447-1457.
- Braaten, B. A., J. V. Platko, M. W. van der Woude, B. H. Simons, F. K. de Graaf, J. M. Calvo, and D. A. Low. 1992. Leucine-responsive regulatory protein controls the expression of both *pap* and *fan* pili operons in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **89**:4250-4254.
- Dorman, C. J., and C. F. Higgins. 1987. Fimbrial phase variation in *Escherichia coli*: dependence on integration host factor and homologies with other site-specific recombinases. J. Bacteriol. **169**:3840-3843.
- Eisenstein, B. I., D. S. Sweet, V. Vaughn, and D. I. Friedman. 1987. Integration host factor is required for the DNA inversion that controls phase variation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:6506-6510.
- Ernsting, B. R., M. R. Atkinson, A. J. Ninfa, and R. G. Matthews. 1992. Characterization of the regulon controlled by the leucine-responsive regulatory protein in *Escherichia coli*. J. Bacteriol. **174**:1109-1118.
- Ernsting, B. R., J. W. Denninger, R. M. Blumenthal, and R. G. Matthews. 1993. Regulation of the *gltBDF* operon of *Escherichia coli*: how is a leucine-insensitive operon regulated by the leucine-responsive regulatory protein? J. Bacteriol. **175**:7160-7169.
- Gally, D. L., J. A. Bogan, B. I. Eisenstein, and I. C. Blomfield. 1993. Environmental regulation of the *fim* switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media. J. Bacteriol. **175**:6186-6193.
- Guyer, M. S., R. R. Reed, J. A. Steitz, and K. B. Low. 1981. Identification of a sex-factor-affinity site in *E. coli* as gamma delta. Cold Spring Harbor Symp. Quant. Biol. **45**:135-140.
- Kawula, T. H., and P. E. Orndorff. 1991. Rapid site-specific DNA inversion in *Escherichia coli* mutants lacking the histone-like protein H-NS. J. Bacteriol. **173**:4116-4123.
- Klemm, P. 1986. Two regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type 1 fimbriae in *Escherichia coli*. EMBO J. **5**:1389-1393.
- McClain, M. S., I. C. Blomfield, K. J. Eberhardt, and B. I. Eisenstein. 1993. Inversion-independent phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **175**:4335-4344.
- McClain, M. S., I. C. Blomfield, and B. I. Eisenstein. 1991. Roles of *fimB* and *fimE* in site-specific inversion associated with phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **173**:5308-5314.
- Neidhardt, F. C., P. L. Bloch, and D. F. Smith. 1974. Culture media for enterobacteria. J. Bacteriol. **119**:736-747.
- Nou, X., B. Skinner, B. Braaten, L. Blyn, D. Hirsch, and D. Low. 1993. Regulation of pyelonephritis-associated pili phase variation in *Escherichia coli*: binding of the PapI and the Lrp regulatory proteins is controlled by DNA methylation. Mol. Microbiol. **7**:545-553.
- Platko, J. V., D. A. Willins, and J. M. Calvo. 1990. The *ilvIH* operon of *Escherichia coli* is positively regulated. J. Bacteriol. **172**:4563-4570.
- Sherratt, D. J. 1993. Site-specific recombination and the segregation of circular chromosomes. Nucleic Acids Mol. Biol. **7**:202-216.
- Sigman, D. S., M. D. Kuwabara, C.-H. B. Chen, and T. W. Bruce. 1991. Nuclease activity of 1,10-phenanthroline-copper in study of protein-DNA interactions. Methods Enzymol. **208**:414-433.
- Wang, Q., and J. M. Calvo. 1993. Lrp, a major regulatory protein in *Escherichia coli*, bends DNA and can organize the assembly of a higher-order nucleoprotein structure. EMBO J. **12**:2495-2501.
- Wang, Q., and J. M. Calvo. 1993. Lrp, a global regulatory protein of *Escherichia coli*, binds co-operatively to multiple sites and activates transcription of *ilvIH*. J. Mol. Biol. **229**:306-318.
- Willins, A. K., C. W. Ryan, J. V. Platko, and J. M. Calvo. 1991. Characterization of Lrp, an *Escherichia coli* regulatory protein that mediates a global response to leucine. J. Biol. Chem. **266**:10768-10774.