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DNA Supercoiling and the Lrp Protein Determine the Directionality of *fim* Switch DNA Inversion in *Escherichia coli* K-12

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Site-specific recombinases of the integrase family usually require cofactors to impart directionality in the recombination reactions that they catalyze. The FimB integrase inverts the *Escherichia coli fim* switch (*fimS*) in the on-to-off and off-to-on directions with approximately equal efficiency. Inhibiting DNA gyrase with novobiocin caused inversion to become biased in the off-to-on direction. This directionality was not due to differential DNA topological distortion of *fimS* in the on and off phases by the activity of its resident P_{fimA} promoter. Instead, the leucine-responsive regulatory (Lrp) protein was found to determine switching outcomes. Knocking out the *lrp* gene or abolishing Lrp binding sites 1 and 2 within *fimS* completely reversed the response of the switch to DNA relaxation. Inactivation of either Lrp site alone resulted in mild on-to-off bias, showing that they act together to influence the response of the switch to changes in DNA supercoiling. Thus, Lrp is not merely an architectural element organizing the *fim* invertasome, it collaborates with DNA supercoiling to determine the directionality of the DNA inversion event.

Site-specific recombinases of the integrase family are usually associated with the integration and excision of DNA sequences such as bacteriophage genomes from bacterial chromosomes or other replicons. The best-studied integrase is Int, the prototypic member of the family that catalyzes the integration and excision of bacteriophage lambda from the chromosome of *Escherichia coli* (33, 40). Although the integration and excision reactions both require Int, an additional phage-encoded factor called the excisionase (Xis) confers directionality by being specific for the excision reaction. Despite its name, the excisionase has no enzymatic activity. Instead, it is an architectural element that helps to organize the local structure of lambda DNA in a way that favors the excision reaction. The Xis protein has been classified as a recombination directionality factor (RDF), and several other proteins have been identified that provide, or may provide, an analogous function in other integrase-dependent site-specific recombination reactions (23–25). The requirement for the RDFs arises due to the similarities of the DNA substrates and products of the integration and excision reactions. The RDF confers directionality by stimulating one reaction while inhibiting the other.

An integrase-mediated site-specific recombination event controls the phase-variable expression of type 1 fimbriae in *E. coli*. A key difference between the fimbrial and phage recombination mechanisms is that the fimbrial system involves DNA inversion and not integration/excision. The promoter for *fim* operon transcription (P_{fimA}) is carried on a 314-bp invertible DNA element called the *fim* switch (*fimS*), and expression of the *fim* structural genes depends on its orientation (1, 15). With P_{fimA} directed toward the *fim* operon, the genes are transcribed, and when it is inverted to the opposite orientation, the *fim* operon is silent (Fig. 1A). The FimB and FimE site-specific recombinases catalyze inversion of *fimS*. FimB inverts the switch in the on-to-off and off-to-on directions with approximately equal efficiency, while FimE shows a strong preference for switching in the on-to-off direction (16, 27, 37). The activity of FimE dominates that of FimB, making on-to-off switching predominant under many growth conditions (4, 27). The FimB protein binds equally well in vitro and in vivo to its target sites located at the functionally equivalent left (IRL) and right (IRR) inverted repeats that flank the switch (7, 12). Several widely studied laboratory strains of *E. coli* K-12 harbor knockout mutations in the *fimE* gene and exhibit a two-way inversion of *fimS* that is catalyzed by FimB alone (4). This form of the switch is reminiscent of the phage integration/excision systems in being catalyzed by a single integrase protein.

Inversion of *fimS* requires the Lrp accessory protein in addition to the recombinases. Lrp binds to two sites within the switch, where it acts positively on DNA inversion (5, 17, 22, 34) (Fig. 1a). It is thought that Lrp alters the trajectory of the *fimS* DNA to enhance the formation of a synaptic complex for recombination. Similarly, DNA supercoiling might be expected to influence recombination efficiency through its effects on the topology of the switch DNA by analogy with other site-specific recombination systems (3, 21, 31). In the absence of FimE, bacteria growing in LB medium exhibit FimB-mediated on-to-off and off-to-on switching at approximately equal rates of $\sim 10^{-3}$ per cell per generation (18, 27, 37). This pattern is disturbed following inhibition of DNA gyrase, the type II topoisomerase that introduces negative supercoils into DNA in an ATP-dependent manner. If phase-off bacteria are treated with the DNA gyrase-inhibiting antibiotic novobiocin, DNA becomes relaxed (Fig. 1B), switching becomes biased in the off-to-on direction, and the bias becomes more pronounced as the dosage of the drug is increased (12).

The most straightforward explanation for these observations is that the phase-off switch and the phase-on switch become distinct as substrates for FimB following gyrase inhibition: relaxed phase-on switches are poor substrates for FimB, whereas relaxed phase-off switches make suitable substrates. Thus,

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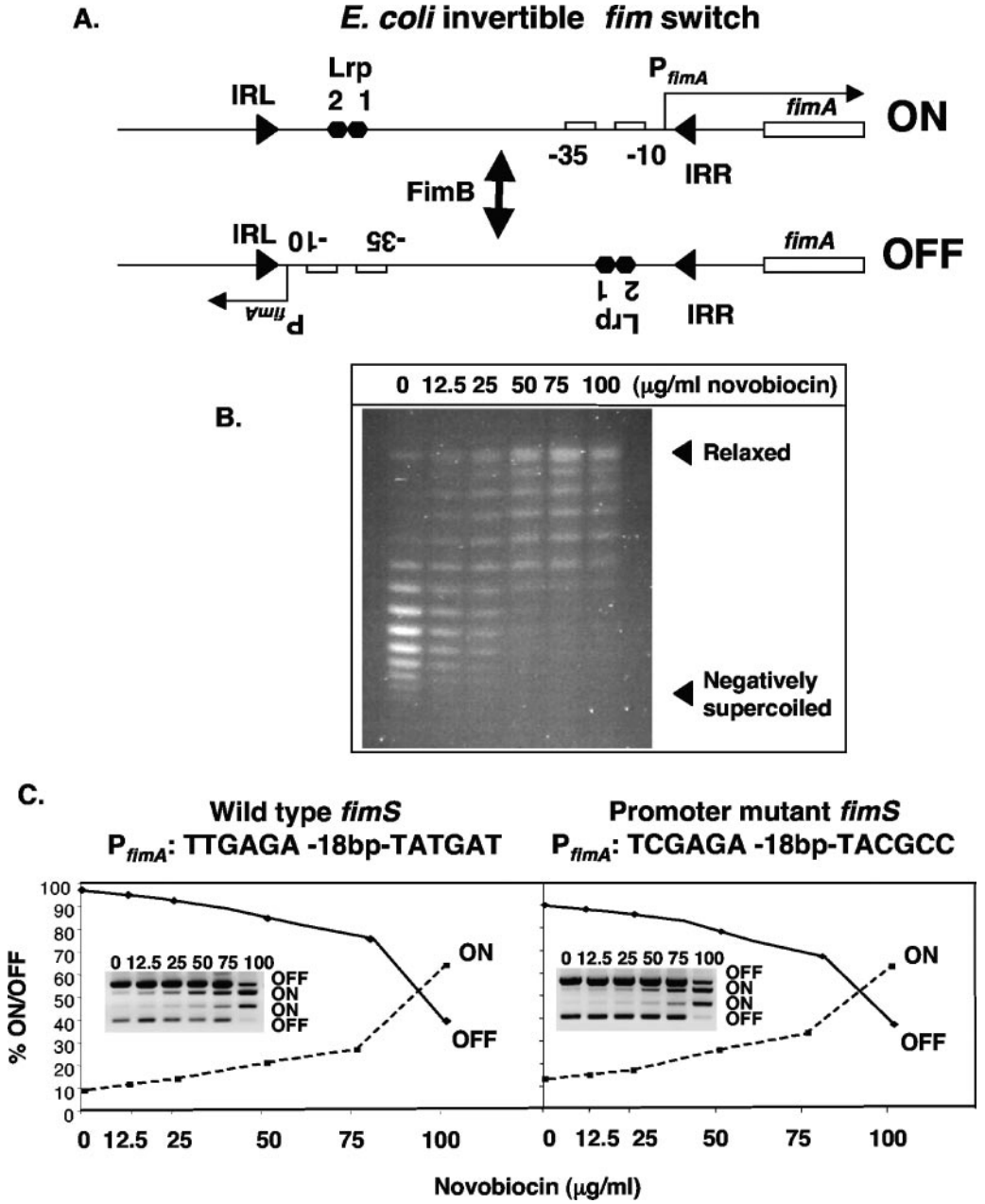

FIG. 1. DNA relaxation and *fim* switch inversion preferences. (A) Phase-on and -off *fim* switches, showing inverted repeats IRL and IRR, the P_{fimA} promoter (−10, −35), the *fimA* transcription start site, and Lrp binding sites 1 and 2. (B) Topoisomers of plasmid pUC18 isolated from *E. coli* K-12 strain VL386 treated with novobiocin at the concentrations shown. (C) Switch inversion preferences with and without a functional P_{fimA} promoter (structures summarized above each panel) at increasing concentrations of novobiocin. Densitometric data from PCR switch assay gels (insets) were used to plot the graphs. Numbers above each gel lane are μg/ml of novobiocin. Bands corresponding to phase-on or -off are labeled.

when the switch inverts to the ON phase, it is difficult for FimB to catalyze the reverse reaction if the DNA is too relaxed, and *fimS* becomes trapped in phase-on. To account for the observed difference in inversion preferences when gyrase is inhibited, one must consider local features within or close to *fimS* that might collaborate with DNA relaxation to influence inversion bias. Such features would have to adopt phase-specific configurations to enable on and off switch orientations to be distinguished.

The effect of transcription initiated from the promoter within *fimS* is an attractive candidate for the role of a phase-specific feature capable of imparting directionality to the inversion reaction. Transcription alters local DNA topology by creating differentially supercoiled domains that flank the moving RNA polymerase (10, 14, 26, 32, 43). The *fim* promoter is active in on and off switches, the same transcription start site is used in both phases, and the promoter is active to a similar level regardless of switch orientation or the degree of negative supercoiling of the DNA (11, 29, 30). Moreover, the domains of relaxed (or even positively supercoiled) DNA that are cre-

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or description	Reference or source
E. coli strains		
VL386	ϕ(*fimA-lacZ*)λ pL(209)*fimE*::IS*1*	1
VL386 *recD*	VL386 *recD*::Tn*10*	37
VL386 *lrp*	VL386 *lrp*::Tn*10*	This work
AK13	VL386 *recD*::Tn*10* Δ*fimB*::*kan*	This work
AK14	VL386 *recD*::Tn*10* Δ*fimB*::*kan* Lrp binding site 1 mutation	This work
AK15	VL386 *recD*::Tn*10* Δ*fimB*::*kan* Lrp binding site 2 mutation	This work
AK16	VL386 *recD*::Tn*10* Δ*fimB*::*kan* Lrp binding sites 1 and 2 mutation	This work
BL21(DE3)	F^- *dcm ompT hsdS gal*	39
Plasmids		
pSLD203	*fimB* gene cloned in pUC18	13
pKMC102	*lrp* gene cloned in pZC320	K. McFarland
pSGS501	*fimB*::*kan fimE*::IS*1* ϕ(*fimA-lacZ*) cloned in pAYC184, switch phase off	37
pSGS501Lrp-1	Lrp binding site 1 mutated in pSGS501	This work
pSGS501Lrp-2	Lrp binding site 2 mutated in pSGS501	This work
pSGS501Lrp-1/2	Lrp binding sites 1 and 2 mutated in pSGS501	This work
pKMC301	*lrp* gene cloned in pET22b	K. McFarland
pMMC108	*fimS* cloned as 550-bp fragment in the PstI site of pMMC106	M. McCusker
pMMC108Lrp-1	Lrp binding site 1 mutated in pMMC108	This work
pMMC108Lrp-2	Lrp binding site 2 mutated in pMMC108	This work
pMMC108Lrp-1&2	Lrp binding sites 1 and 2 mutated in pMMC108	This work
pUC18	Cloning vector, Ap^r	45

ated by the movement of RNA polymerase may not be constant in both orientations of *fimS*. This is because in on and off switches the supercoiled domains are propagated at opposite ends of *fimS* where the facility with which they can dissipate by rotational diffusion may be distinct. Any such inequality would be exacerbated by the inhibition of DNA gyrase because this topoisomerase would be less able to supercoil relaxed DNA negatively or to relax positively supercoiled DNA. Since transcription from the *fim* promoter always reads across one of the inverted repeats of *fimS* (Fig. 1A), the associated topological disturbance could interfere with the ability of FimB to utilize these DNA sequences.

Alternatively, a DNA-binding protein could sustain a phase-specific nucleoprotein complex at the switch that disfavors FimB-mediated inversion in phase-on but not phase-off following gyrase inhibition. Obviously, this second possibility does not exclude the first. Here we demonstrate that the leucine-responsive regulatory protein (Lrp), a DNA binding and bending protein (42), plays such a role and is a directionality determinant in the *fim* site-specific recombination system.

MATERIALS AND METHODS

Media, growth conditions, and genetic techniques. All strains were derivatives of *E. coli* K-12 (Table 1). VL386 *lrp-201*::Tn*10* was constructed by P1*vir*-mediated phage transduction (28, 38) using a CSH50 *lrp-201*::Tn*10* lysate (16). Complementation was carried out with plasmid pKMC102 (lrp^+), a single-copy plasmid derived from pZC320 (36). Bacteria were cultured in L broth (Difco) or L agar (containing agar at 1.5% wt/vol). MacConkey-lactose agar plates (28) were used for Lac phenotype determination. Unless otherwise stated, liquid cultures were grown overnight at 37°C with aeration. Antibiotics were used at the following concentrations: carbenicillin, 100 μg ml^{-1}; kanamycin, 20 μg ml^{-1}; chloramphenicol, 25 μg ml^{-1}; tetracycline, 12.5 μg ml^{-1}. Plasmid DNA was introduced to bacterial cells by $CaCl_2$ transformation (35) or electroporation (19) using a Bio-Rad Gene Pulser.

Molecular biological techniques. Plasmid DNA was isolated using QIAGEN Midi columns or Wizard mini prep columns (Promega). DNA fragments were purified from agarose gels using the High Pure PCR product purification kit (Roche Applied Science). Restriction enzymes were purchased from New England Biolabs and used according to the manufacturer's directions. Automated sequencing was carried out at GATC Biotech. Oligonucleotide synthesis was by MWG Biotech. Plasmid topoisomer distributions were analyzed by agarose-chloroquine gel electrophoresis as described previously (20). At the concentration of chloroquine used (2.5 μg/ml), the more negatively supercoiled topoisomers ran fastest in the gel.

Determination of *fim* switch orientation. The orientation of the *fim* switch on the chromosome was determined as previously described (37). This method exploited a unique BstUI restriction site in the *fim* switch that results in restriction fragment length dimorphism among BstUI-digested PCR products that are characteristic of phase-on and -off switches.

Fifty microliters of culture was boiled following overnight incubation at 37°C. Oligonucleotides OL4 and OL20 (Table 2) were used with *Taq* polymerase (New England Biolabs) to amplify the switch region as a 726-bp DNA product. The PCR cycle involved denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, followed by a final extension for 10 min at 72°C. Samples were cooled to 60°C, and 10 units of BstUI were added and incubated at 60°C for 3 h. Digested PCR products were electrophoresed on 2% agarose gels. Phase-off switches yielded DNA fragments of 539 and 187 bp, while phase-on switches gave fragments of 433 and 293 bp. The propor-

TABLE 2. Oligonucleotides

Primer name	Sequence (5′–3′)[a]
OL4	GACAGAACAACGATTGCCAG
OL20	CCGTAACGCAGACTCATCCTC
AKBSFORBIO	CTCCAAAAACCACCTCATGC
BSREVBIO	CCCCCAAAAGATGAAACATTT
SDMlrp1fw	CCAAAAACCACCTCATGCAACTCGAGCATCTA TAAATAAAGATAAC
SDMlrp1rv	GTTATCTTTATTTATAGATGCTCGAGTTGCAT GAGGTGGTTTTTGG
SDMlrp2fw	GATACCAATAGAATCTCGAGCCAACAAAT AAAC
SDMlrp2rv	GTTTATTTGTTGGCTCGAGATTCTATTGTTATC
ARLCLOfw[b]	AAGACAATTGGGGCCAAACTGTCC
ARLCLOrv[c]	GGGCAGTCGTTCTGTACACTTT
FimBfw	GCGCGTCTGTAATTATAAGGG
FimBrv	CCCTGGTATCTCAACTAT

[a] Restriction enzyme cleavage sites are underlined.
[b] Primer tailed with restriction site MfeI.
[c] Primer tailed with restriction site BsrGI.

tions of on- and off-specific fragments in the population was determined using QUANTITY ONE image analysis software.

Site-directed mutagenesis. Site-directed mutagenesis was performed using the Quikchange II (Stratagene) kit according to the manufacturer's recommendations. Oligonucleotide pairs (MWG Biotech) used to mutate the Lrp binding sites Lrp-1 (SDMlrp1fw and SDMlrp1rv) and Lrp-2 (SDMlrp2fw and SDMlrp2rv), individually and in combination, are described in Table 2. Plasmid pMMC108 was used as the substrate to generate plasmids pMMC108Lrp-1, pMMC108Lrp-2, and pMMC108Lrp-1+2. Lrp binding site 1 was replaced with 5′-AACTCGAGCATCT-3′ and site 2 was replaced with 5′-AGAATCTCGAG CC-3′ as previously described (17). The presence of either site 1 or 2 mutations introduced an XhoI restriction site. Binding site alterations were confirmed by sequencing, PCR analysis using oligonucleotides OL4 and OL20, and XhoI digestion of PCR products.

Purification of Lrp. Expression of N-terminally His-tagged Lrp was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) in exponentially growing 100-ml BL21(DE3) cultures harboring pKMC301. After 2 h, the cells were harvested and lysed by sonication. Lysates (~15 ml) were applied to a His-Bind Quick column (Novagen) (2) preequilibrated with 10 ml binding buffer (4 M NaCl, 160 mM Tris-HCl, 40 mM imidazole, pH 7.9). The column was then washed with 10 ml wash buffer (4 M NaCl, 480 mM imidazole, 160 mM Tris-HCl, pH 7.9) and the protein eluted in 5,001-μl fractions of 5 ml of elution buffer (4 M imidazole, 2 M NaCl, 80 mM Tris-HCl, pH 7.9). Glycerol was added to a final concentration of 10% to each fraction. Fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and those containing Lrp were pooled and dialyzed against 1 liter of 100 mM phosphate buffer, pH 8.0, 10% glycerol, 1 mM EDTA, pH 8.0, 300 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol. Lrp was estimated to be approximately 95% pure.

DNA binding and gel retardation. Binding of purified Lrp protein to the wild-type and Lrp binding site mutant alleles of the switch was tested by electrophoretic mobility shift assay. Probes (144-bp) were amplified by PCR with *Pfu* polymerase (Stratagene) using the primer pair AKBSFORBIO and BSREVBIO (Table 2) and using pMMC108, pMMC108Lrp-1, pMMC108Lrp-2, and pMMC108Lrp-1+2 as templates. Amplified probes were gel purified (Roche Applied Science). The oligonucleotide AKBSFORBIO had a 5′ biotinylated end allowing detection of protein-DNA complexes. Amplified probe was incubated with increasing concentrations (0 to 50 nM) of purified Lrp protein for 15 min at room temperature as recommended by manufacturers of the electrophoretic mobility shift assay kit (Pierce). Protein-DNA complexes were resolved by electrophoresis through a 7.5% polyacrylamide gel for 1 h at room temperature. The gel was then electrophoretically blotted and developed.

Allele replacement. The allele replacement method used to eliminate Lrp sites 1 and 2 was based on one described previously (37). A DNA fragment containing the Lrp binding site(s) mutation(s) (PCR amplified using the primer pair Arlclofw and Arlclorv and the plasmids pMMC108Lrp-1, pMMC108Lrp-2, and pMMC108Lrp-1+2 as template DNA) was cloned into pSGS501 following digestion with MfeI and BsrGI, to give pSGS501Lrp-1, pSGS501Lrp-2, and pSGS501Lrp-1+2. pSGS501, pSGS501Lrp-1, pSGS501Lrp-2, and pSGS501Lrp-1+2 were subsequently digested with EcoRV. An 8.5-kb fragment that included the *fimB* gene interrupted by a Kan^r cassette with or without the Lrp site mutations was gel extracted. Two micrograms of this fragment was electroporated into strain VL386*recD*, generating strains AK13 to AK16 (Table 1). Transformants were selected on L agar containing kanamycin. Oligonucleotides FimBfw and FimBrv were used to verify *fimB*::*kan* by PCR analysis and DNA sequencing.

RESULTS

Eliminating the *fimA* promoter does not rescue biased *fimS* inversion. The movement of RNA polymerase during transcription creates differentially supercoiled domains in double-stranded DNA (10, 14, 26, 32, 43). This suggests an attractive explanation for the directional biasing seen in FimB-mediated inversion of *fimS* when DNA gyrase activity is inhibited by novobiocin treatment (11). The transcription start site of the *fimA* promoter (P_{fimA}) has been mapped previously, is constant in both phase-on and phase-off switches, and is unaffected by changes in DNA supercoiling (8, 11, 29, 30). Transcription originating at P_{fimA} is directed toward distinct regions of the chromosome in phase-on and phase-off switches (Fig. 1A). These different regions of the chromosome may have distinct abilities to absorb positive supercoils emanating from the switch. Normally, gyrase eliminates these transcriptionally generated positive supercoils, but when the topoisomerase is inhibited, the supercoils may persist to different extents in phase-on and phase-off orientations of the switch, making the two forms of the switch distinguishable as suitable recombination substrates for FimB.

To test this hypothesis, we introduced base pair substitution mutations into the chromosomal copy of *fimS* at both the −10 and −35 motifs of P_{fimA} that completely abolished promoter activity without altering the length of the *fim* switch. The effect of novobiocin on DNA supercoiling was confirmed by high-resolution agarose gel electrophoresis of reporter plasmid topoisomers. The plasmid was seen to be increasingly relaxed as the dose of novobiocin increased (Fig. 1B). We then compared *fim* switch inversion in the wild type and its P_{fimA} promoter knockout derivative for sensitivity to novobiocin treatment. In both cases, the phase-off switch was seen to invert progressively in the off-to-on direction when gyrase activity was inhibited (Fig. 1C). Had transcription from P_{fimA} been the generator of distinct substrates for FimB through an effect on local DNA supercoiling, one would have anticipated that the promoter knockout mutant would have shown either no bias or altered bias when gyrase activity was inhibited. Our findings showed that P_{fimA} promoter activity was not responsible for the biasing of *fimS* inversion outcomes in relaxed DNA, so we sought an alternative explanation.

The switching bias of *fimS* is reversed in an *lrp* mutant. Genetic analysis revealed a role for the Lrp protein in determining *fimS* inversion outcomes. Lrp is essential for *fimS* inversion when the *fimB* gene is present in single copy but is dispensable in the presence of multicopy *fimB* (13). An *lrp* knockout mutation was introduced into strain VL386 by bacteriophage P1 transduction. Inversion of *fimS* was restored in this mutant following introduction of a multicopy plasmid encoding the FimB recombinase. When DNA gyrase activity in this strain was inhibited progressively with increasing concentrations of novobiocin, the orientation of *fimS* became biased in the off orientation (Fig. 2A). The culture was 63% on and 37% off in the absence of novobiocin, and this shifted to 10% on and 90% off at the highest concentration of the antibiotic used. This was the reverse of the situation seen in the wild-type strain that expressed Lrp, where novobiocin treatment resulted in biasing in favor of the on orientation (Fig. 1C). Complementation of the *lrp*::Tn*10* mutation with a plasmid-borne *lrp* gene restored the wild-type inversion pattern of *fimS* (biased off-to-on) in response to gyrase inhibition (Fig. 2B). These data showed that the Lrp protein played a pivotal role in determining the outcome of FimB-mediated *fimS* site-specific recombination with relaxed DNA templates.

The Lrp binding sites determine the response of *fimS* to DNA relaxation. The Lrp binding sites 1 and 2 were removed from the switch by base substitution without altering the switch length to yield derivatives lacking just site 1 or just site 2 or lacking both sites. The altered sequences were tested in vitro for binding of purified Lrp protein. Binding of Lrp to sites 1 and 2 is known to be cooperative (34). An electrophoretic mobility shift assay showed that removal of either site strongly reduced Lrp-mediated

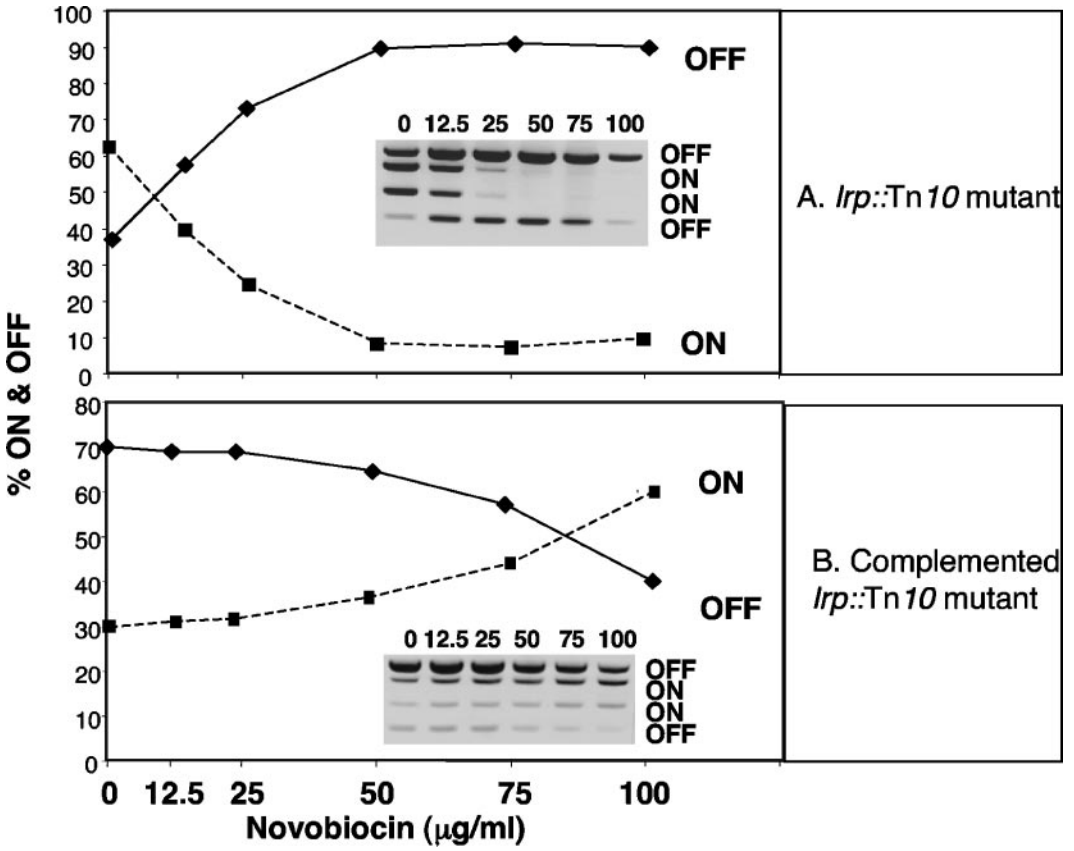

FIG. 2. Inactivation of the *lrp* gene reverses the response of the *fim* switch to DNA relaxation. (A) Treatment with increasing concentrations of novobiocin results in a strong on-to-off bias in an *lrp*::Tn*10* knockout mutant, the opposite of the pattern seen in the wild type. (B) Complementation of the *lrp* knockout mutation with a functional copy of the *lrp* gene restores the wild-type pattern of response to novobiocin treatment. Densitometric data from PCR switch assay gels (insets) were used to plot the graphs. Numbers above each gel lane are μg/ml of novobiocin. Bands corresponding to phase-on or -off are labeled.

shifting of a labeled switch probe while removal of both sites completely abrogated Lrp binding (Fig. 3).

The individual Lrp site mutations and the double mutation were placed within the *fimS* element on the chromosome by homologous recombination. When a multicopy plasmid carrying the *fimB* gene was introduced, *fimS* inversion was restored in each strain, in agreement with previous data (13). The strains were then treated with increasing concentrations of novobiocin. In the mutants lacking just site 1 or site 2, the off-to-on biasing that is characteristic of the wild-type switch was no longer seen. In fact, a very slight shift in favor of the off orientation was observed as the concentration of novobiocin increased (Fig. 4). For example, cultures that began as ~20% phase-off in the absence of novobiocin had become ~30% phase-off at the highest dose of antibiotic used (Fig. 4B and C). These results indicated that the Lrp binding sites were required for the switch to respond to DNA relaxation. In the switch lacking both sites 1 and 2, the effect of the mutations on DNA inversion preferences was much more dramatic. Here, a very strong bias in favor of the off phase was clearly seen (Fig. 4D), a pattern that mimicked precisely that observed in the *lrp* knockout mutant (Fig. 2A). A culture with approximately equal numbers of on and off switches was shifted to ~20% on and ~80% off at the highest concentration of antibiotic. These results showed that Lrp, acting through sites 1 and 2, is responsible for determining the directionality of the response of the *fimS* invertible element to DNA relaxation.

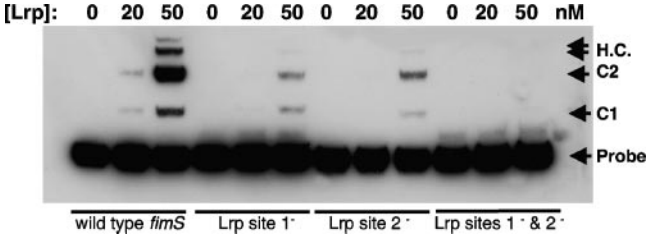

FIG. 3. Inactivation of the Lrp binding sites 1 and 2 within the *fim* switch. Data are shown from an electrophoretic mobility shift assay monitoring interaction of purified Lrp protein with the wild-type *fim* switch and mutants deficient in site 1, site 2, or sites 1 and 2. The protein concentrations used are given at the top of each lane. Lrp-*fimS* complexes are labeled at the right: complexes C1 and C2 and higher-order complexes (H.C.).

DISCUSSION

When DNA gyrase activity is unperturbed by novobiocin treatment, FimB inverts the *fim* switch in either direction with

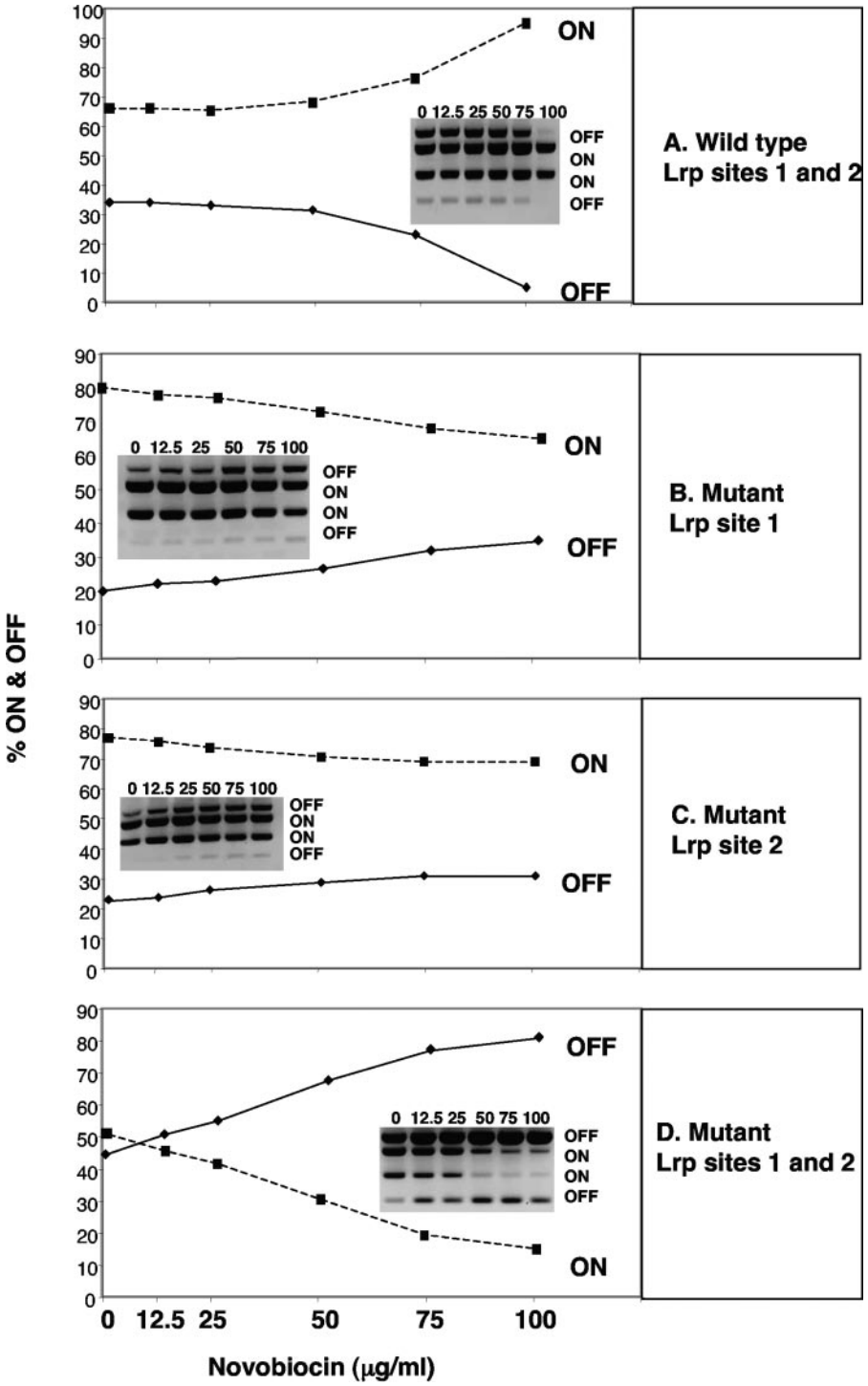

FIG. 4. Removal of the Lrp binding sites alters the response of the *fim* switch to DNA relaxation. With sites 1 and 2 intact, *fimS* becomes biased in favor of phase-on in response to increasing concentrations of novobiocin (A). Elimination of site 1 (B) or 2 (C) prevents on-biasing in response to novobiocin. When Lrp sites 1 and 2 are both disrupted, the response of the switch to novobiocin treatment is reversed (D) and resembles that seen in the *lrp*::Tn*10* mutant (Fig. 2). The *y* axes of the graphs report the percentage of on and off switches in the population. The dosage of novobiocin is reported on the *x* axes. Densitometric data from PCR switch assay gels (insets) were used to plot the graphs. Numbers above each gel lane are μg/ml of novobiocin. Bands corresponding to phase-on or -off are labeled.

approximately equal efficiency. As increasing concentrations of novobiocin progressively inhibit gyrase activity with a concomitant relaxation of DNA supercoiling, *fimS* biasing in the off-to-on direction becomes apparent (Fig. 1). Thus, DNA relaxation creates a situation in which the on form of the switch becomes a trap that is difficult to escape. An attractive hypothesis proposed previously to account for this observation (12) envisaged a role for the P_{fimA} promoter within the *fim* switch as

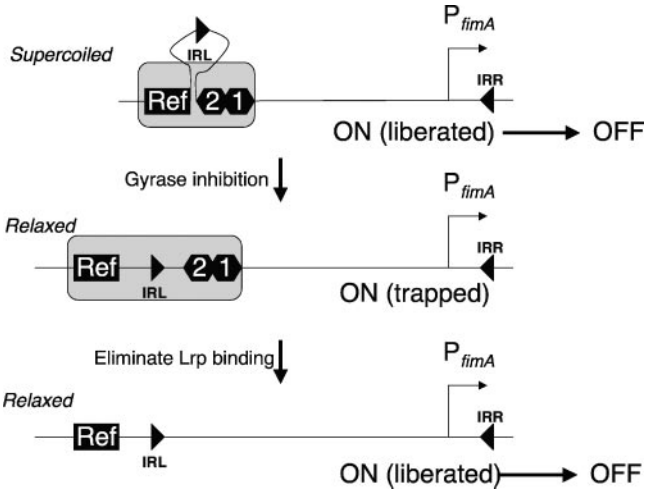

FIG. 5. A model to account for trapping of the switch in the on orientation following DNA relaxation. The relevant features of the *fim* switch are shown: inverted repeats IRL and IRR, Lrp sites 1 and 2 (numbered hexagons), the P_{fimA} promoter, and a putative reference point (Ref) located in the noninvertible region of the chromosome adjacent to IRL. Communication between Ref and the occupied Lrp binding sites facilitates the formation of an inhibitory complex (gray box). Negative supercoiling of the DNA assists extrusion of IRL from the inhibitory complex (top). Removal of negative superhelicity following gyrase inhibition abrogates extrusion of IRL, and the switch is trapped in the on orientation (middle). Elimination of Lrp binding disrupts the inhibitory complex, and rapid on-to-off switching occurs (bottom).

a generator of topologically distinct substrates through the well-established ability of RNA polymerase movement on a DNA substrate to create differentially supercoiled domains (10, 14, 26, 32, 43). In this model, differential diffusion of supercoils created by P_{fimA} activity in phase-on and phase-off would result in distinct substrates for FimB. However, we show here that inactivation of the P_{fimA} promoter has no influence on the off-to-on-biased response of the switch to DNA relaxation.

On the other hand, we have now established cooperative roles for the Lrp DNA-binding protein and DNA supercoiling in determining the directionality of FimB-mediated *fim* switch inversion. In particular, we have shown that Lrp protein is required to maintain the phase-on trap. When this protein is removed from the cell by inactivation of the *lrp* gene, or when the sites within *fimS* to which it binds are disrupted, the phase-on trap is relieved. Thus, Lrp performs a role that is analogous to the RDFs that have been described in integrase-mediated integration and excision events (23–25). It allows two otherwise similar recombination substrates to become distinguishable, and like an RDF of the excisionase type, it probably does so by acting as a DNA-bending architectural element.

What is the nature of the phase-on trap? It seems clear that the recombination machinery can distinguish between phase-on and -off switches and that the Lrp protein bound to sites 1 and 2 plays a role in this discriminatory mechanism. The most straightforward model envisages a fixed reference point (Ref), currently of unknown molecular composition, in a constant (i.e., noninverting) region of the chromosome (Fig. 5). Bioinformatic analysis reveals no evidence of another Lrp binding site to the left of IRL where it might contribute the Ref function. However, a previously characterized integration host factor (IHF) binding site in this area (6), with the anticipated DNA bend that is a characteristic of occupied IHF sites, might fulfill the role of Ref. Communication between Ref and the mobile (i.e., inverting) and fully occupied Lrp binding sites 1 and 2 constitutes the on/off discriminatory mechanism. This communication influences the quality of the recombination substrate (the inverted repeats) and determines the efficiency with which it can be processed by the FimB recombinase. Further modulation of recombination is imposed by supercoiling or relaxation of the DNA.

The proposed communication between Ref and the occupied Lrp binding sites 1 and 2 has the potential to inhibit the ability of IRL to participate with IRR in recombination (Fig. 5). Negative supercoiling facilitates the presentation of the IRL sequence, allowing it to interact with IRR in the FimB-mediated DNA inversion reaction. Relaxation of the DNA due to attenuation of gyrase activity inhibits IRL presentation, and the on orientation of the switch becomes increasingly disfavored as a recombination substrate. This results in the experimentally observed build-up in the population of bacteria with phase-on switches following novobiocin treatment.

The trapping of IRL in an inert complex within the relaxed phase-on switch requires sustained communication between Ref and the Lrp proteins at sites 1 and 2. When the Lrp binding sites are disrupted (or expression of Lrp protein is eliminated by inactivation of the *lrp* gene), the trap is relieved. Partial relief is seen when only one site (either 1 or 2) is disrupted. In the complete absence of Lrp protein binding, the relaxed switch is an excellent substrate for FimB-mediated recombination and shows a strong on-to-off inversion preference.

DNA supercoiling and nucleoid-associated proteins collaborate in many transposition and site-specific recombination systems (3, 21, 31). In transposon Tn*10*, supercoiling and the IHF protein cooperate to guide the transposition event down one of two possible pathways (9). The role of IHF has been described as that of a molecular spring that helps to modulate transposition by influencing the proficiency of the transposon ends for interaction with the transposase (9). This mechanism has some similarities to the role proposed here for the Lrp protein in the presentation of recombination substrates in *fim* DNA inversion (Fig. 5).

Why has the *fim* switch evolved such an elaborate mechanism for determining the directionality of DNA inversion? The *fim* operon can acquire *fimE* knockout mutations quite readily due to insertion of insertion elements and via other mechanisms (4). The resulting mutants express type 1 fimbriae in an apparently random on-off manner due to the action of the FimB recombinase on the switch. Perhaps a completely stochastic switching mechanism might not meet the requirements of the bacterial population under all circumstances, making it advantageous to bias switching in one direction or the other under certain conditions. DNA relaxation is a prerequisite for on-biased *fimS* inversion, and relaxation is associated with low rates of metabolic flux in the cell (41). Moreover, novobiocin treatment mimics specifically the effect of an unfavorable [ATP]/[ADP] ratio on DNA gyrase (44). This may represent a signal that the bacterium lacks energy, and a transition from a planktonic to an attached lifestyle might be advantageous. An

enhanced tendency to express type 1 fimbriae would offer an attractive solution and is consistent with the known contributions of these cell surface appendages to niche colonization and the formation of biofilms (7).

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