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The Leucine-Responsive Regulatory Protein of *Escherichia coli* Negatively Regulates Transcription of *ompC* and *micF* and Positively Regulates Translation of *ompF*

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The two major porins of *Escherichia coli* K-12 strains, OmpC and OmpF, are inversely regulated with respect to one another. The expression of OmpC and OmpF has been shown to be influenced by the leucine-responsive regulatory protein (Lrp): two-dimensional gel electrophoresis of proteins from strains with and strains without a functional Lrp protein revealed that OmpC expression is increased in an *lrp* strain, while OmpF expression is decreased. In agreement with these findings, we now present evidence that transcriptional (operon) fusions of *lacZ*$^+$ to *ompC* and *micF* are negatively regulated by Lrp. Lrp binds specifically to the intergenic region between *micF* and *ompC*, as indicated by mobility shift assays and by DNase I footprinting. The expression of an *ompF′-lacZ*$^+$ gene (translational) fusion is increased 3.7-fold in an *lrp*$^+$ background compared with an *lrp* background, but expression of an *ompF-lacZ*$^+$ operon fusion is not. Studies of in vivo expression of the outer membrane porins during growth on glucose minimal medium showed that the OmpF/OmpC ratio is higher in *lrp*$^+$ strains than it is in isogenic *lrp* strains. The effect of Lrp was not seen in a strain containing a deletion of *micF*. Our studies suggest that the positive effect of Lrp on OmpF expression stems from a negative effect of Lrp on the expression of *micF*, an antisense RNA that inhibits *ompF* translation.

The *ompC* and *ompF* genes code for outer membrane proteins that determine the permeability of the outer membrane (38), and they map at 48 and 21 min, respectively, in the *Escherichia coli* K-12 chromosome (4). The OmpF protein forms a pore of 1.16 nm in diameter that allows for rapid diffusion of nutrients across the outer membrane of the cell (40, 41). OmpF expression is repressed by high osmolarity and high temperature, and under these conditions, the expression of the OmpC porin is derepressed (29). The OmpC protein forms a slightly smaller pore (1.08-nm diameter) that excludes bile salts and generally slows the rate of diffusion of nutrients across the membrane (40, 41). The porins represent a major investment of protein synthetic capacity, and switching between OmpF and OmpC may be an important part of the adaptations made by enteric bacteria as they enter a mammalian host by ingestion and travel down the gastrointestinal tract to the intestines (41).

Regulation of porin expression in response to osmolarity involves the EnvZ and OmpR proteins (15). These two proteins form a two-component regulatory system, in which EnvZ is the sensor and OmpR is the response regulator (reviewed in reference 54). EnvZ is embedded in the inner membrane, with domains extending into the periplasm and the cytoplasm. In response to environmental signals, EnvZ autophosphorylates a histidyl residue in the cytoplasmic domain and transfers this phosphoryl group to an aspartyl residue on OmpR. EnvZ also acts as a phosphatase, converting phosphorylated OmpR to its dephosphorylated form. In media of low osmolarity, levels of phosphorylated OmpR are low and *ompF* expression is activated (52, 53). When the osmolarity of the medium is high, levels of phosphorylated OmpR are increased, *ompC* expression is stimulated, and *ompF* expression is depressed (14). Phosphorylated OmpR positively regulates *ompC* transcription, and it negatively regulates *ompF* transcription (47).

ompC and *ompF* are also regulated in response to the temperature of the growth medium, and the thermal regulation of *ompF* involves the *micF* regulatory gene (3, 10). The *micF* gene is divergently transcribed with respect to *ompC*, and it encodes a small RNA product, the 5′ end of which is complementary to the 5′ end of *ompF* mRNA (35). Formation of an mRNA-*micF* RNA duplex would be expected to interfere with translation of *ompF*, and strains harboring high-copy-number *micF*$^+$ plasmids in fact lack OmpF and have decreased levels of *ompF* mRNA (35). Takayanagi and coworkers (55) have recently performed a deletion analysis to identify *cis*-acting elements responsible for control of *micF* transcription. In addition to the three OmpR binding sites located in the *ompC-micF* intergenic region and required for transcriptional activation of *micF* by OmpR, they identified a region between −148 and −131 bp relative to the start site for *micF* transcription that was responsible for OmpR-independent repression of *micF* expression. The protein(s) responsible for repression by binding to this site has not yet been identified. Recent studies (17) have identified two proteins in crude extracts that compete for binding to an additional site between −97 and −59 bp upstream of *micF*.

Coyer and coworkers (10) have shown that thermoregulation of *micF* RNA levels is still seen in mutant strains lacking a functional OmpR protein, and they have also demonstrated that a shift from M-9 minimal medium to Luria broth (LB) results in increased expression of *micF* in both *ompR* and *ompR*$^+$ backgrounds. The *cis*-acting elements responsible for thermal regulation of *micF* expression were shown to lie within a fragment containing *micF* and extending 144 bp upstream of the transcription start site. One of the two proteins in crude extracts identified by Gidrol and Farr (17) is heat resistant, and it may be involved in thermal regulation of *micF*.

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OmpF expression is also regulated posttranscriptionally by SoxRS-dependent repression in response to redox stress (8), and this repression requires a functional *micF* gene. While one of the two *micF*-binding proteins identified by Gidrol and Farr, the redox-sensitive binding factor, appears to be involved in responses to oxidative stress, this protein is isolated from crude extracts of Δ*soxRS* mutants and is thus not a component of the *soxRS* regulon.

The leucine-responsive regulatory protein (Lrp) of *E. coli* K-12 strains is known to regulate the expression of over 40 polypeptides, increasing the expression of some and decreasing the expression of others (reviewed in references 6 and 39). A two-dimensional gel electrophoretic analysis of protein expression in *lrp*$^+$ and *lrp*::Tn*10* strains showed that the expression of the outer membrane proteins OmpC and OmpF is influenced by Lrp (11). The expression of OmpC is increased and the expression of OmpF is decreased in *lrp*::Tn*10* strains, compared with their expression in *lrp*$^+$ strains. These results suggest that Lrp may be a negative regulator of OmpC expression and a positive regulator of OmpF expression, but indirect effects of Lrp on the expression of these genes are also possible. Some Lrp-regulated operons (e.g., *ilvIH* and *gltBD*) have been shown to be directly regulated by Lrp, with Lrp binding upstream of these genes to bring about changes in transcription (12, 58). In contrast, the proteins of the Ntr regulon are indirectly regulated by Lrp, through effects on glutamate synthase expression (11).

The effect of an *lrp* mutation on the expression of OmpC and OmpF proteins is similar to that elicited either by an increase in osmotic strength or by an increase in the temperature of the medium. An increase in temperature or osmolarity leads to increased expression of *ompC* and decreased expression of *ompF* (24), as does an *lrp* mutation. Furthermore, studies performed in our laboratory (12) and in the laboratories of Elaine Newman (27) and Joseph Calvo (6) have demonstrated that Lrp levels are lower during growth in rich media like LB than during growth in minimal media. These observations led us to investigate the relationship between regulation of porin expression by osmolarity, by *micF*, and by Lrp.

This work demonstrates that Lrp and osmolarity exert their effects independently on *ompC* and *ompF* expression during growth in glucose minimal MOPS [3-(*N*-morpholino) propanesulfonic acid] medium. We also show that Lrp is a negative regulator of *ompC* and *micF* transcription and that it has a significant effect on the OmpF/OmpC ratios in outer membrane preparations. *ompF-lacZ*$^+$ transcriptional fusions are not positively regulated by Lrp, while *ompF′-lacZ*$^+$ translational fusions are. The positive effect on OmpF expression exerted by Lrp is probably due to the negative effect of Lrp on the expression of *micF*. We have demonstrated binding of purified Lrp to the *ompC-micF* intergenic region and have identified nine protected regions by DNase I protection analysis. We suggest that the effect of Lrp on the expression of both OmpC and OmpF results from interaction of Lrp with the *ompC-micF* intergenic region.

MATERIALS AND METHODS

Media and growth conditions. All cultures were grown aerobically in rotary action shakers at 37°C. Cell growth was monitored spectrophotometrically with a Perkin-Elmer spectrophotometer at 420 nm after fixation with 0.9% formaldehyde. Glucose minimal medium was MOPS minimal medium (37) supplemented with 0.4% glucose as the carbon source and with 10 μM thiamine, while lactose minimal MOPS medium contained 0.4% lactose instead of glucose. MOPS medium contained 9.52 mM ammonium chloride unless stated otherwise. Luria broth (LB) (49) was used as a rich medium. Where indicated, media contained the antibiotic ampicillin (100 μg/ml), kanamycin (50 μg/ml), or tetracycline (20 μg/ml). Cultures were maintained on LB agar plates supplemented with appropriate antibiotics or, where noted, on lactose minimal MOPS plates.

Bacterial strains and their construction. The *E. coli* K-12 strains used in this work are described in Table 1. Strains MF66 (*micF*$^+$ *micF-lacZ*$^+$ *lrp*::Tn*10*) and MF67 (*micF*$^+$ *lrp*$^+$ *micF-lacZ*$^+$) were constructed by transformation of strains BE10.2 and PS2209, respectively, with plasmid pmicB21, which contains a *micF-lacZ*$^+$ fusion (34). Cells were made competent for transformation by the method of Chung et al. (9).

Generalized transduction mediated by P1*vir* was carried out as described by Miller (32). Strains MF5.4 and MF8.1 were derived from strain PS2209, and they were isolated by selection for growth on lactose following transduction with P1*vir* lysates of strains MH225 and MH513, respectively. Strain MH225 contains an *ompC-lacZ*$^+$ operon fusion upstream of *ompC*::Tn*5* (18), while strain MH513 carries an *ompF-lacZ*$^+$ operon fusion upstream of *ompF*::Tn*5* (19). Transduction with these strains as donors can have two consequences, depending on the site of the downstream crossover. If this crossover occurs upstream of Tn*5* on the incoming DNA, the results will be *omp(C,F)-lacZYA omp(C,F)*$^+$. If the crossover is downstream of Tn*5*, the resulting strain will be *omp(C,F)-lacZYA omp(C,F)*::Tn*5*. Strains MF6.4 and MF10.1 (both *lrp-35*::Tn*10*) were derived from strains MF5.4 and MF8.1, respectively, and they were isolated by selection for tetracycline resistance following transduction with a P1*vir* lysate of strain CV1008, which carries a Tn*10* insertion in *lrp* (44).

Strains DBMC and DBSM were derived from strains MC4100 and SM3001, respectively, and they were isolated by selection for tetracycline resistance following transduction with a P1*vir* lysate of strain BE2, which carries a Tn*10* insertion in *lrp* (11).

Strain MH621, which contains Φ(*ompF′-lacZ*)hyb.16-21 (see Table 1, footnote *a*) adjacent to a λ prophage (19), was grown overnight in 10 ml of LB, and the cells were harvested by centrifugation and resuspended in 1 ml of 10 mM $MgSO_4$. Resuspended cells (100 μl) were exposed to short-wave UV light for 60 s to promote excision of the λ phage adjacent to the *ompF′-lacZ*$^+$ translational fusion. The cells were diluted with 5 ml of LB, and the culture was grown at 37°C for 3 h, whereupon a drop of chloroform was added and cell debris was removed. The resulting λ phage lysate was used to transduce strain PS2209. Lac$^+$ transductants were selected on lactose minimal MOPS plates. This transduction, by an aberrantly excised defective λ phage, involves integration of the circularized phage via homologous recombination at *ompF* and necessarily results in an *ompF′-lacZYA ompF*$^+$ merodiploid. The resulting strain, BE99.5 (*lrp*$^+$), was transduced with a P1 lysate from strain BE2, and cells were selected for tetracycline resistance to obtain an isogenic *lrp*::Tn*10* strain (BE100.5). Because strains BE99.5 and BE100.5 are *recA*$^+$ and contain a gene duplication, they are somewhat unstable and tend to lose the ability to grow on lactose. Therefore, these strains were maintained on lactose minimal MOPS plates and overnight cultures were grown in lactose minimal MOPS medium.

Subcloning of the intergenic region between *ompC* and *micF*. The sequences of the complete *ompC* (33) and *micF* (10) genes have been determined. *ompC* and *micF* are divergently transcribed, with 253 bp of DNA between their transcription start sites (3, 10). Plasmid pMF1 carries a subclone of the intergenic region between *ompC* and *micF*, derived from plasmid pKI0041 (3). Plasmid pKI0041 was subjected to the PCR by denaturation at 94°C for 1 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, all followed by a final extension step at 72°C for 7 min. The following primers were used for the PCR: primer 1, 5′ CGG GAT CCC *GAC TTT CAT GTT ATT AAC CCT C* 3′; and primer 2, 5′ CCC AAG CTT GGG *ATA GTT TTT CTG TGG TAG CA* 3′ (sequence complementary to *ompC-micF* shown in italic type). Primer 1 contains sequence beginning at position +89 of the *ompC* coding region and extending to position +69, as well as an added *Bam*HI site (underlined), while primer 2 contains sequence beginning at position +143 of the *micF* coding region and extending to position +124, as well as an added *Hin*dIII site (underlined). The numbering system for the *ompC-micF* intergenic region is that of Coyer et al. (10). The 507-bp PCR product was cleaved with *Bam*HI and *Hin*dIII and ligated into the *Bam*HI and *Hin*dIII sites of plasmid pGEM-3Z (Promega) to create pMF1. Ligation products containing inserts were identified by using blue-white screening on 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) plates.

Gel mobility shift assays. The assay for binding of Lrp protein to the *Bam*HI-*Hin*dIII fragment from plasmid pMF1, containing the *ompC-micF* intergenic region, was carried out essentially as described by Ricca et al. (48). Plasmid pMF1 DNA was cut with *Hin*dIII and *Bam*HI and diluted to 0.052 μg/μl in labeling reaction buffer (50 mM Tris-Cl [pH 8], 50 mM $MgCl_2$, 5 mM dithiothreitol) in the presence of 0.5 μl of [α-^{32}P]dATP (10 μCi/μl; 25 Ci/mmol; ICN no. 33002) and 2 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). The mobility shift assays were then performed, and the results were analyzed by PhosphorImager scanning (Molecular Dynamics) as described by Ernsting et al. (12). Curve-fitting to the Hill equation (20) was displayed by using the Kaleidagraph program (Abelbeck Software) and optimized by manual adjustment of the cooperativity coefficient α to minimize deviations between the data and the theoretical curve.

β-Galactosidase assay. The β-galactosidase assay used measures the absorbance changes at 420 nm associated with *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolysis, and it is similar to that described by Miller (32), as modified by Platko et al. (44) and Ernsting et al. (12). The units of β-galactosidase activity

TABLE 1. *E. coli* strains and plasmids used in this work

Strain or plasmid	Description[a]	Source or reference
Strains		
MC4100	F$^-$ Δ(*argF-lac*)*U169 flbB thiA rpsL150 araD139 relA ptsF25 deoC1*	7
MH20	F$^-$ Δ(*argF-lac*)*U169 rpsL150 flbB relA thiA*	19
W3110	F$^-$ λ$^-$ IN(*rrnD-rrnE*)*1 fimE rph*	F. Neidhardt
PS2209	W3110 Δ(*argF-lac*)*U169*	B. Wanner
CAG12112	MG1655 *zbi-3109*::Tn*10kan*	51
CV1008	F$^-$ *ara thi* Δ(*argF-lac*)*U169 ilvIH*::MudI1734 *lrp-35*::Tn*10*	44
BE2	W3100 *lrp-35*::Tn*10*	11
BE10.2	PS2209 *lrp-35*::Tn*10*	This work
BE99.5	PS2209 Φ(*ompF′-lacZ*$^+$)hyb.16-21 *ompF*$^+$	This work
BE100.5	BE99.5 *lrp-35*::Tn*10*	This work
DBMC	MC4100 *lrp-35*::Tn*10*	This work
DBSM	SM3001 *lrp-35*::Tn*10*	This work
MH621	MH20 Φ(*ompF′-lacZ*$^+$)hyb.16-21 *ompF*::Tn*5*	19
MH225	MC4100 *malQ7* Φ(*ompC-lacZ*$^+$)10-25 *ompC*::Tn*5*	18
MH513	MH20 Φ(*ompF-lacZ*$^+$)16-13 *ompF*::Tn*5*	19
MF5.4	PS2209 Φ(*ompC-lacZ*$^+$)10-25	This work
MF6.4	MF5.4 *lrp-35*::Tn*10*	This work
MF8.1	PS2209 Φ(*ompF-lacZ*$^+$)16-13 *ompF*$^+$	This work
MF10.1	MF8.1 *lrp-35*::Tn*10*	This work
MF66	BE10.2 (*micF*$^+$) pmicB21 (*micF-lacZ*$^+$)	This work
MF67	PS2209 (*micF*$^+$) pmicB21 (*micF-lacZ*$^+$)	This work
SM3001	MC4100 Δ*micF1*	31
Plasmids		
pKI0041	Ap^r; vector pBR322; *ompC-micF* intergenic region	3
pmicB21	Ap^r; vector pKM005; *micF-lacZ*$^+$	34
pMF1	pGEM-3Z (Promega) with the *ompC-micF* intergenic region inserted between the *Bam*HI and *Hin*dIII sites	This work

[a] Φ designates an operon or protein fusion (19). Each fusion is designated by an allele number, indicating the particular site at which the fusion joint is located. Protein fusions are designated by the suffix hyb. before the allele number.

are the arbitrary activity units originally defined by Miller (32): $1{,}000 \times \Delta A_{420}$ min^{-1} ml^{-1}. However, because the optical densities of the cell cultures shown in Fig. 1 through 4 were measured at 420 nm, rather than at 600 nm, the slopes given in the figure legends must be multiplied by 2.0 to obtain values in Miller units.

Preparation and analysis of outer membrane proteins. Isolation of outer membranes and polyacrylamide gel electrophoresis in the presence of urea and sodium dodecyl sulfate (SDS) were carried out by a modification of previously described procedures (36, 46). Bacteria were grown for ~10 h at 37°C with shaking in 50 ml of glucose minimal MOPS medium. The pellet from 50 ml of cells was washed once in 20 mM sodium phosphate buffer, pH 7.2, and resuspended in 1 ml of the same buffer. The cells were disrupted by sonication, and cellular debris was removed by centrifugation. The inner membranes were solubilized by incubation of the lysate with 0.1 volume of 5% *N*-lauroylsarcosine (sodium salt; Sigma) for 30 min at room temperature. The sarcosyl-insoluble fraction containing the outer membranes was recovered by centrifugation at 100,000 × *g* for 1 h. The membranes were resuspended in 100 μl of sodium phosphate buffer. Equivalent amounts of the outer membrane preparations based on the A_{420}s of the cultures at the time of harvest were solubilized by being boiled for 5 min in 2× Laemmli sample buffer (25). The outer membrane proteins were separated on SDS-polyacryamide gels (16 by 18 cm) that contained 8 M urea. The ratio of acrylamide monomer to bisacrylamide (10%:0.275%) in the separating gel was found to be critical for good separation of the outer membrane proteins. Gels were stained with Coomassie brilliant blue, and bands were quantitated by using an XRS Scanner (Millipore Corp.) with Visage version 4.6Q software (BioImage, Inc.).

DNase I protection. Footprinting analyses with DNase I were performed essentially as described by Brenowitz et al. (5). A plasmid preparation of pMF1 DNA (13 μg) was digested with *Pvu*II and either *Bam*HI or *Hin*dIII. The digests were electrophoresed through a 1.66% agarose gel, and the bands containing the *ompC-micF* intergenic region were excised. The insert in pMF1 extends from +89 bp relative to the transcription start site directed by promoter P1 for *ompC* (21, 42) to +143 bp relative to the P2 transcription start site for *micF* (2), and it includes 253 bp of DNA between the two transcription start sites. After digestion with *Pvu*II and *Bam*HI, this region is contained in a 709-bp fragment, and after digestion with *Pvu*II and *Hin*dIII, it is contained in a 636-bp fragment. Each band was purified by using a Geneclean kit (BIO 101, Inc.). The recovered DNA was labeled with Klenow DNA polymerase (Boehringer Mannheim), [α-^{32}P]dATP (48 μCi; 3,000 Ci/mmol), and [α-^{32}P]dGTP (48 μCi; 3,000 Ci/mmol). Impurities and unincorporated nucleotides were removed by passing the labeled DNA through a Bio-spin 6 chromatography column (Bio-Rad) and an Elutip-d column (Schleicher & Schuell).

Lrp, 0 to 100 nM, was incubated with labeled DNA for 30 min in a 200-μl reaction mixture containing 10 mM Tris chloride buffer (pH 8.1), 5 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM dithiothreitol, 10 μg of bovine serum albumin, 0.4 μg of calf thymus DNA, and 100 mM KCl. DNase I (Gibco BRL) (0.02 U) was added to each mixture and allowed to react for exactly 2 min at room temperature. Reactions were quenched by the addition of 700 μl of stop solution (645 μl of 100% ethanol, 5 μl of tRNA [1 mg/ml], and 50 μl of saturated ammonium acetate) previously chilled in an ethanol-dry ice bath. The resulting DNA fragments were precipitated, dried, and dissolved in 5 μl of formamide loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF). Samples were heated at 90°C for 10 min, chilled on ice, and run on a 6% acrylamide sequencing gel at 70 W with the appropriate sequencing ladders. The ladders were generated by the dideoxynucleotide chain termination method (50), using 20-mer primers extending towards the intergenic region from +96 nucleotides upstream of the P1 promoter-directed transcription start site of *ompC* (GATCCCGACTTTCATGTTAT) or +151 nucleotides upstream of the P2 promoter-directed transcription start site of *micF* (AGCTTGGGATAGT TTTTCTG). After electrophoresis, gels were dried onto Whatman 3MM filter paper and subjected to autoradiography.

RESULTS

Characterization of strains containing *ompC-lacZ*$^+$ and *ompF-lacZ*$^+$ operon fusions. Kawaji et al. (24) and Forst et al. (14) have shown that growth in medium containing high concentrations of sugars or dextrans leads to effects on the expression of the outer membrane porin genes *ompC* and *ompF*. An increase in the osmotic strength of the medium was shown to increase transcription of *ompC* and to decrease transcription of *ompF*. A series of *ompC-lacZ*$^+$ and *ompF-lacZ*$^+$ operon fusions constructed in Silhavy's laboratory were reported to be regulated by sucrose in a similar manner, although quantitative data concerning the effect of sucrose on these fusion strains were not given (19). We have moved operon fusions Φ(*ompF-*

TABLE 2. Effect of sucrose on β-galactosidase expression in *ompC-lacZ*$^+$ and *ompF-lacZ*$^+$ operon fusion strains

Sucrose concn (M)	β-Galactosidase sp act (U/A_{420} of culture)[a] of strain:	
	MF5.4 (*ompC-lacZ*$^+$)	MF8.1 (*ompF-lacZ*$^+$)
0	47	111
0.1	68	ND[b]
0.2	100	77
0.5	133	ND[b]

[a] Activity measurements were made by using samples of cultures growing exponentially in glucose minimal MOPS medium. Each value represents the slope of a graph of activity versus culture A_{420} and is based on at least five data points.
[b] ND, not determined.

lacZ$^+$)16-13 and Φ(*ompC-lacZ*$^+$)10-25 into *E. coli* K-12 strain PS2209 (Δ*lac*), and we have shown that the effects of sucrose on expression of β-galactosidase by these fusion strains are qualitatively in agreement with those observed in previous studies. The results of these experiments are shown in Table 2. Sucrose increases the expression of β-galactosidase in strain MF5.4 (*ompC-lacZ*$^+$) and decreases enzyme expression in strain MF8.1 (*ompF-lacZ*$^+$). Further experiments examining the effect of Lrp on the sucrose response of these fusions were performed in the presence of 0.2 M sucrose.

As discussed in Materials and Methods, introduction of the *omp(C,F)*::*lacZ*$^+$ fusion into an *omp(C,F)*$^+$ strain by P1 transduction can result in either an *omp(C,F-lacZ*$^+$*) omp(C,F)*$^+$ merodiploid or an *omp(C,F-lacZ*$^+$*) omp(C,F)*::Tn*5* strain, depending upon the position of the downstream crossover during integration into the chromosome. Strains MF5.4, MF6.4, MF8.1, and MF10.1 were characterized by determination of their sensitivity to kanamycin and by electrophoretically analyzing preparations of outer membrane proteins of these strains for the expression of OmpC and OmpF. Strains MF5.4 and MF6.4 were found to lack intact OmpC and to be kanamycin resistant, while strains MF8.1 and MF10.1 were found to express OmpF and to be kanamycin sensitive.

Lrp decreases β-galactosidase expression from an *ompC-lacZ*$^+$ operon fusion. Figure 1 shows the effect of Lrp on β-galactosidase activity expressed from an *ompC-lacZ*$^+$ operon (transcriptional) fusion. β-Galactosidase activities were compared for strains MF5.4 (*ompC-lacZ*$^+$ *lrp*$^+$) and MF6.4 (*ompC-lacZ*$^+$ *lrp*::Tn*10*), each grown in steady state in the absence or presence of 0.2 M sucrose. In both the absence and presence of 0.2 M sucrose, the *lrp* mutant strain showed a significant (1.7-fold) increase in *ompC-lacZ*$^+$ expression compared with its isogenic *lrp*$^+$ parent. Thus, Lrp does not modulate the effect of sucrose on expression of a transcriptional *ompC-lacZ*$^+$ fusion.

Leucine is known to modulate the effect of Lrp as an activator (12, 27, 44) or repressor (11, 26–28) of many genes in the Lrp regulon, and so we examined the effect of leucine on expression of the *ompC-lacZ*$^+$ operon fusion in *lrp*$^+$ and *lrp* backgrounds. The results of such an experiment are summarized in Table 3. High levels of leucine in the medium lead to decreased growth rates of K-12 strains because of isoleucine restriction (45), and so we compared expression of the *ompC-lacZ*$^+$ fusion in the presence of isoleucine and valine with expression in the presence of leucine, isoleucine, and valine. In an *lrp*$^+$ background, the presence of leucine in the medium resulted in a 1.2-fold increase in β-galactosidase expression, while in an *lrp* background no effect of leucine was seen. While the effects of leucine on *ompC* expression were small, this result strengthens the conclusion that *ompC* is regulated by Lrp, in that the effect of leucine depends on the presence of a functional Lrp protein. The effects of leucine on an *ompC-lacZ*$^+$ operon fusion are also in full agreement with measurements of the effect of leucine on the expression of OmpC and OmpF in vivo by two-dimensional electrophoretic analysis, which led to the classification of these proteins as leucine insensitive (11).

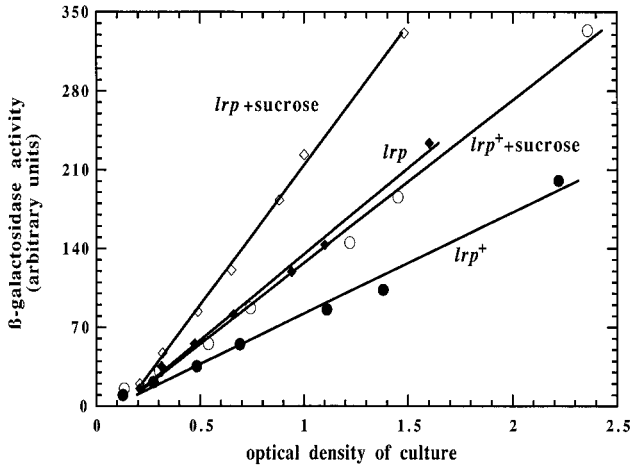


FIG. 1. Lrp decreases expression of β-galactosidase from an *ompC-lacZ*$^+$ operon (transcriptional) fusion. Strains were grown in glucose minimal MOPS medium at 37°C in the absence (filled symbols) or presence (open symbols) of 0.2 M sucrose, and the optical density of the culture was monitored at 420 nm. Strain MF5.4 (*ompC-lacZ*$^+$ *lrp*$^+$) showed slopes of 88 U/A_{420} of culture in the absence of sucrose (filled circles) and 142 U/A_{420} of culture in the presence of sucrose (open circles). Strain MF6.4 (*ompC-lacZ*$^+$ *lrp*::Tn*10*) showed a slope of 152 U/A_{420} of culture in the absence of sucrose (filled diamonds) and a slope of 250 U/A_{420} of culture in the presence of 0.2 M sucrose (open diamonds).

Expression from an *ompF-lacZ*$^+$ operon fusion is not positively regulated by Lrp. As described above (Table 2), increasing osmolarity of the growth medium leads to a decrease in transcription of *ompF* (14). In Fig. 2, the results of measurements of β-galactosidase activity from an *ompF-lacZ*$^+$ operon (transcriptional) fusion are shown. Strain MF10.1 (*ompF-lacZ*$^+$ *lrp*::Tn*10*) displayed a small increase (1.3-fold) in β-galactosidase activity compared with MF8.1 (*ompF-lacZ*$^+$ *lrp*$^+$) both in the presence and in the absence of 0.2 M sucrose. The effect of Lrp on *ompF-lacZ*$^+$ expression in this experiment was not the same as the effect of Lrp on expression of the OmpF protein previously seen by the two-dimensional gel electrophoretic analysis (11). Lrp had very little effect on sucrose-mediated repression of *ompF-lacZ*$^+$ expression. Sucrose de-

TABLE 3. Effect of leucine on β-galactosidase expression in an *ompC-lacZ*$^+$ operon fusion strain

Additions	β-Galactosidase sp act (U/A_{420} of culture)[a] of strain:	
	MF5.4 (*lrp*$^+$)	MF6.4 (*lrp*::Tn*10*)
None	45	82
Ile + Val	45	81
Leu + Ile + Val	55	83

[a] Activity measurements were made by using samples of cultures growing exponentially in glucose minimal MOPS medium. Each value represents the slope of a graph of activity versus culture A_{420} and is based on at least five data points. The concentrations of added amino acids were as follows: Ile, 0.4 mM; Val, 0.6 mM; and Leu, 20 mM.

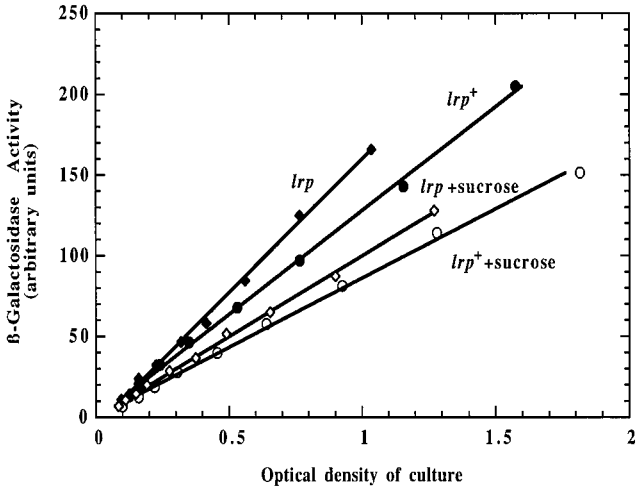


FIG. 2. Lrp decreases rather than stimulates β-galactosidase expression from an *ompF-lacZ*$^+$ operon (transcriptional) fusion. Strains were grown in glucose minimal MOPS medium at 37°C in the absence (filled symbols) or presence (open symbols) of 0.2 M sucrose, and the optical density of the culture was monitored at 420 nm. Strain MF8.1 (*ompF-lacZ*$^+$ *lrp*$^+$) showed a slope of 132 U/A_{420} of culture in the absence of sucrose (filled circles) and a slope of 91 U/A_{420} of culture in the presence of sucrose (open circles). Strain MF10.1 (*ompF-lacZ*$^+$ *lrp*::Tn*10*) showed a slope of 175 U/A_{420} of culture in the absence of sucrose (filled diamonds) and a slope of 100 U/A_{420} of culture in the presence of sucrose (open diamonds).

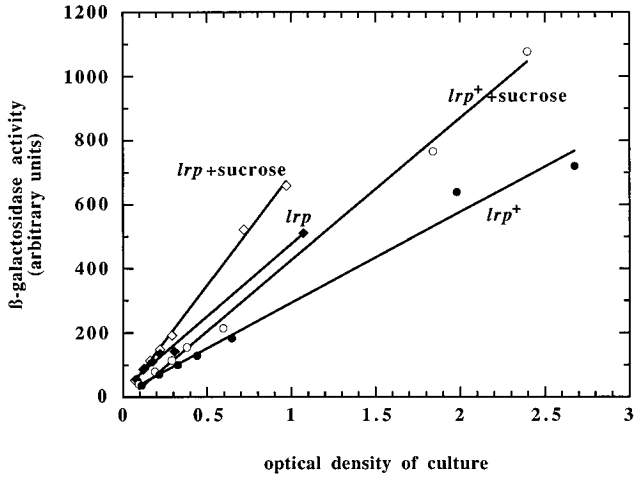


FIG. 3. Lrp decreases expression of β-galactosidase from a plasmid-borne *micF-lacZ*$^+$ operon (transcriptional) fusion. Strains were grown in glucose minimal MOPS medium at 37°C in the absence (filled symbols) or presence (open symbols) of 0.2 M sucrose, and the optical density of the culture was monitored at 420 nm. Strain MF67 (*micF*$^+$ *micF-lacZ*$^+$) showed a slope of 250 U/A_{420} of culture in the absence of sucrose (filled circles) and a slope of 318 U/A_{420} of culture in the presence of sucrose (open circles). Strain MF66 (*micF*$^+$ *micF-lacZ*$^+$ *lrp*::Tn*10*) showed a slope of 500 U/A_{420} of culture in the absence of sucrose (filled diamonds) and a slope of 700 U/A_{420} of culture in the presence of sucrose (open diamonds).

creased the expression of this transcriptional fusion by 1.8-fold in an *lrp* background and by 1.5-fold in an *lrp*$^+$ background. These results are again in agreement with osmotic regulation of *ompF* being mediated directly by OmpR (22, 23, 42). The apparent discrepancy between the effect of Lrp shown here (leading to a 1.1- to 1.3-fold decrease in *ompF-lacZ*$^+$ expression) and the effect of Lrp on OmpF expression as analyzed by two-dimensional gel electrophoresis (an ~2-fold increase in expression) led us to investigate other factors involved in regulation of *ompF* expression.

The expression of a plasmid-borne *micF-lacZ*$^+$ operon fusion is inhibited by Lrp. The *micF* gene specifies an antisense RNA that is complementary to the 5′ end of *ompF* mRNA, and the *micF-ompF* hybrid RNA is poorly translated (2, 35). *micF* was shown to be involved in the repression of OmpF translation in response to increased temperature and osmolarity (1, 3). We hypothesized that the effect of Lrp on the expression of OmpF might also involve *micF*. The *micF* gene maps immediately upstream of, and is divergently transcribed with respect to, *ompC*: only 253 bp separate their transcription start sites (3, 10). Thus, there is a possibility that binding of Lrp to the intergenic region could negatively regulate the transcription of both of these genes, leading to a direct negative effect on *ompC* transcription and an indirect positive effect on *ompF* translation. The availability of a plasmid containing a *lacZ* operon fusion to the *micF* promoter (34) allowed us to measure the effect of Lrp on the expression of the *micF-lacZ*$^+$ fusion (Fig. 3). When levels of expression of this fusion in isogenic *lrp*$^+$ and *lrp* strains were compared, Lrp was found to decrease *micF-lacZ*$^+$ expression 2.0-fold in the absence of sucrose and 2.2-fold in the presence of sucrose. Thus, the effects of Lrp on expression of a chromosomal *ompC-lacZ*$^+$ operon fusion and on a plasmid-encoded *micF-lacZ*$^+$ operon fusion were quantitatively very similar, and they lend support to the idea that Lrp binding to the region between these two promoters may be directly responsible for both effects. As with both the *ompC-lacZ*$^+$ and *ompF-lacZ*$^+$ transcriptional fusions, the absence of functional Lrp protein did not significantly affect regulation of *micF-lacZ*$^+$ expression by sucrose.

Expression of an *ompF′-lacZ*$^+$ translational fusion is increased by Lrp. One would expect that the observed increase in *micF* transcription in an *lrp* mutant strain would lead to a decrease in *ompF* translation. The small increase in transcription of *ompF-lacZ*$^+$ in *lrp* strains would be countered by an increase in antisense RNA from the negatively regulated *micF* promoter. If this were the case, we would expect that expression of an *ompF′-lacZ*$^+$ translational (protein) fusion, containing the *ompF* promoter and ribosome-binding site as well as the *micF* binding site (10), would be increased in an *lrp*$^+$ strain compared with an *lrp* strain. Figure 4 shows the effects of Lrp and sucrose on the expression of an *ompF′-lacZ*$^+$ translational fusion derived from a strain, MH621, constructed in Silhavy's laboratory (18). Inclusion of 0.2 M sucrose in the medium decreased β-galactosidase activity by 2.1-fold in cells of strain BE99.5 (*lrp*$^+$) and by 1.8-fold in cells of strain BE100.5 (*lrp*::Tn*10*). Thus, the effect of sucrose on the expression of β-galactosidase driven by an *ompF′-lacZ*$^+$ translational fusion is very similar to the sucrose effect on an *ompF-lacZ*$^+$ transcriptional fusion. In contrast, while expression of the *ompF-lacZ*$^+$ transcriptional fusion was decreased 1.3-fold by Lrp during growth in the absence of sucrose, expression of the *ompF′-lacZ*$^+$ translational fusion was increased by Lrp 3.7-fold in the absence of sucrose and 3.2-fold in the presence of 0.2 M sucrose. Thus, *ompF* expression is positively regulated by Lrp at the level of translation, and this effect of Lrp is exerted independently of the effect of sucrose. These results are consistent with those of earlier studies in which the expression of OmpF was monitored by two-dimensional gel electrophoresis (11).

Effect of Lrp and *micF* on porin expression in outer membrane preparations. Table 4 summarizes the results of a series of analyses of porin expression in outer membrane preparations of isogenic wild-type (MC4100), *lrp* (DBMC), Δ*micF* (SM3001), and Δ*micF lrp* (DBSM) strains. The porin profiles

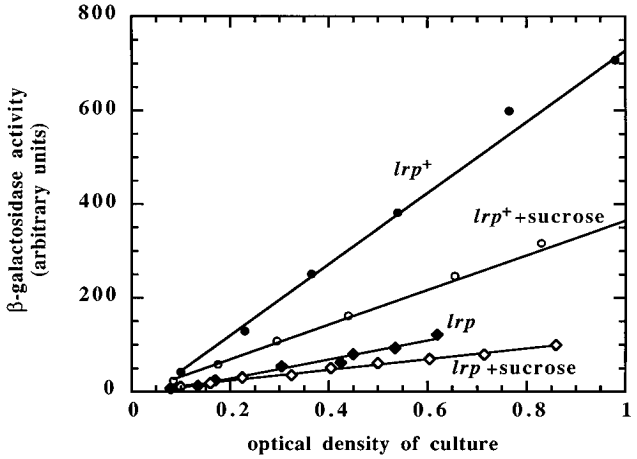


FIG. 4. Lrp increases expression of β-galactosidase from an *ompF-lacZ*$^+$ translational (protein) fusion. Strains were grown in glucose minimal MOPS medium at 37°C in the absence (filled symbols) or presence (open symbols) of 0.2 M sucrose, and the optical density of the culture was monitored at 420 nm. Strain BE99.5 (*ompF-lacZ*$^+$ *lrp*$^+$) showed a slope of 760 U/A_{420} of culture in the absence of sucrose (filled circles) and a slope of 369 U/A_{420} of culture in the presence of sucrose (open circles). Strain BE100.5 (*ompF-lacZ*$^+$ *lrp*::Tn*10*) showed a slope of 204 U/A_{420} of culture in the absence of sucrose (filled diamonds) and a slope of 114 U/A_{420} of culture in the presence of sucrose (open diamonds).

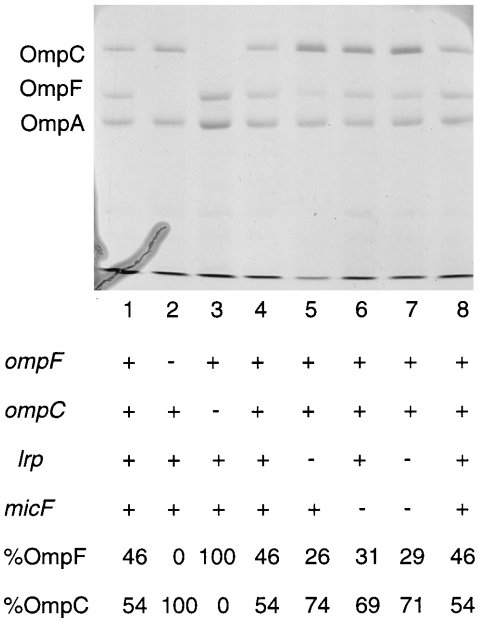


FIG. 5. Effects of *lrp* and *micF* on expression of outer membrane porins. Strains were grown in glucose minimal MOPS medium, and outer membrane proteins were isolated and analyzed by electrophoresis in the presence of SDS and 8 M urea as described in Materials and Methods. The positions of OmpC, OmpF, and OmpA are shown, and they were verified by preparation of outer membrane proteins from strains MH621 (*ompF*::Tn*5*; lane 2) and MH225 (*ompC*::Tn*5*; lane 3). Equivalent amounts of outer membrane proteins, based on the cell density of the culture at the time of harvest, were analyzed for the wild-type parent strain, MC4100 (lanes 1, 4, and 8); its *lrp* derivative, strain DBMC (lane 5); strain SM3001 (MC4100 Δ*micF*; lane 6); and strain DBSM (SM3001 lrp; lane 7). The + and − symbols below the lanes indicate the presence or absence of a functional product of the listed genes in the strain from which the outer membranes were prepared. The outer membrane porins were quantitated by scanning as described in Materials and Methods, and a summary of the results is presented below the figure. %OmpF and %OmpC were calculated by dividing the amounts of OmpF and OmpC, respectively, by the amount of OmpC plus OmpF (Table 4).

of outer membrane preparations were analyzed several times for each strain, by using at least two separate preparations. The averaged data are summarized in Table 4. Surprisingly, during the quantitation of the profiles, we realized that the absolute levels of OmpA appeared to be lower in Δ*micF* strains than in their *micF*$^+$ counterparts, although the combined levels of protein for all three porins were approximately constant. For this reason, we have normalized OmpC and OmpF levels to total porin rather than to OmpA. Figure 5 shows the results of a representative experiment by which the porin profiles of these different strains can be compared. Comparison of lanes 4 (wild type) and 5 (*lrp*) indicates that the *lrp* mutation resulted in a significant decrease in the OmpF/OmpC ratio, in agreement with the results of two-dimensional electrophoretic analyses of the whole-cell protein profiles of isogenic *lrp*$^+$ and *lrp* strains in a W3110 background (11). A *micF* deletion in the *lrp*$^+$ strain SM3001 (lane 6) also resulted in a decrease in the OmpF/OmpC ratio compared with the profile of the wild-type strain MC4100. In a *micF* deletion strain, introduction of an *lrp* mutation did not lead to a further change in the OmpF/OmpC ratio compared with that of the isogenic *lrp*$^+$ strain. Thus, the *lrp* mutation and the deletion of *micF* independently and non-additively lead to an increase in the percentage of total porin that is OmpC (~1.6-fold) and a decrease in the percentage of total porin that is OmpF (~1.5-fold) compared with the values for these proteins obtained with strain MC4100 (Table 4). These results are consistent with a role for *micF* in the regulation of *ompF* translation during growth in glucose minimal MOPS medium. They suggest that Lrp-dependent repression of *micF* contributes to the high OmpF/OmpC ratio of the wild-type strain compared with the *lrp* mutant during growth in this medium.

The Lrp protein binds the intergenic region between *ompC* and *micF*. If the effect of Lrp on the expression of OmpC and OmpF results from binding in the *ompC-micF* intergenic region, we would expect to be able to see the binding in vitro. A fragment of DNA containing the intergenic region (253 bp) as well as the entire *micF* gene and the upstream portion of the *ompC* gene was subcloned into plasmid pGEM-3Z to create plasmid pMF1, and restriction digests of this plasmid were used for mobility shift assays. The DNA mobility shift assays whose results are shown in Fig. 6 indicate that Lrp binds to the

TABLE 4. Profiles of outer membrane protein expression in *E. coli*

Strain	% OmpC[a]	% OmpF[a]	% OmpA[a]	% OmpF/% Omp (C+F)	No. of determinations
MC4100 (*lrp*$^+$ *micF*$^+$)	32 ± 2	27 ± 3	41 ± 4	0.46	6
DBMC (*lrp micF*$^+$)	50 ± 12	18 ± 8	33 ± 2	0.26	4
SM3001 (Δ*micF*)	48 ± 5	22 ± 2	30 ± 5	0.31	3
DBSM (*lrp* Δ*micF*)	51 ± 6	21 ± 4	29 ± 4	0.29	3

[a] Values were calculated as percentages of the total of OmpC plus OmpF plus OmpA porins. Data are means ± standard deviations.

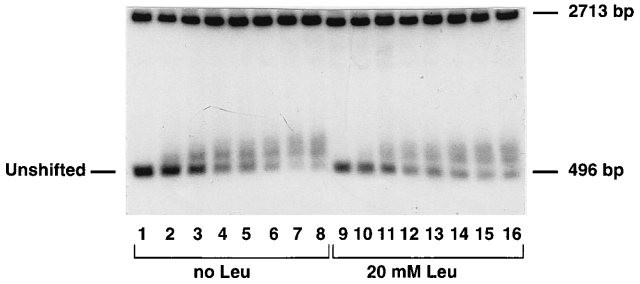


FIG. 6. Lrp binds to DNA containing the *ompC-micF* intergenic region. Each mobility shift assay contained 240 pM DNA from an end-labeled *Bam*HI and *Hin*dIII digest of plasmid pMF1 carrying the intergenic region between *ompC* and *micF*, as well as 0.15 μg of unlabeled sonicated calf thymus DNA per μl. The binding solutions for lanes 1 through 8 did not contain leucine, while the binding solutions for lanes 9 through 16 contained 20 mM leucine. Lrp concentrations were as follows: lanes 1 and 9, no Lrp dimer; lanes 2 and 10, 25 nM Lrp dimer; lanes 3 and 11, 38 nM Lrp dimer; lanes 4 and 12, 44 nM Lrp dimer; lanes 5 and 13, 50 nM Lrp dimer; lanes 6 and 14, 63 nM Lrp dimer; lanes 7 and 15, 69 nM Lrp dimer; and lanes 8 and 16, 75 nM Lrp dimer. Lrp binds to the 496-bp fragment containing the *ompC-micF* intergenic region, but it does not affect the mobility of the 2,713-bp fragment derived from the vector.

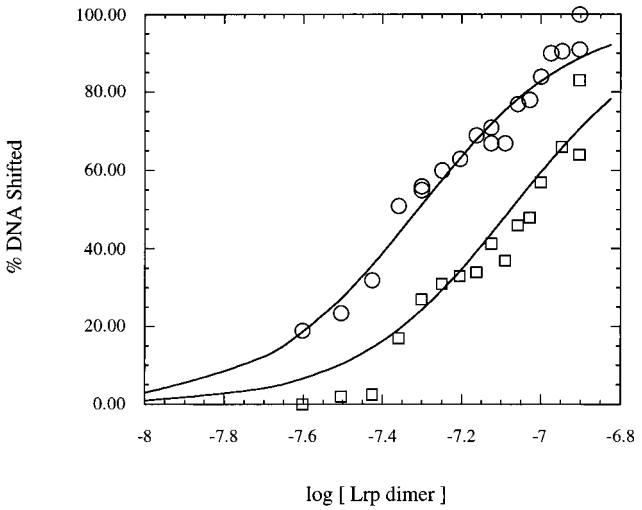


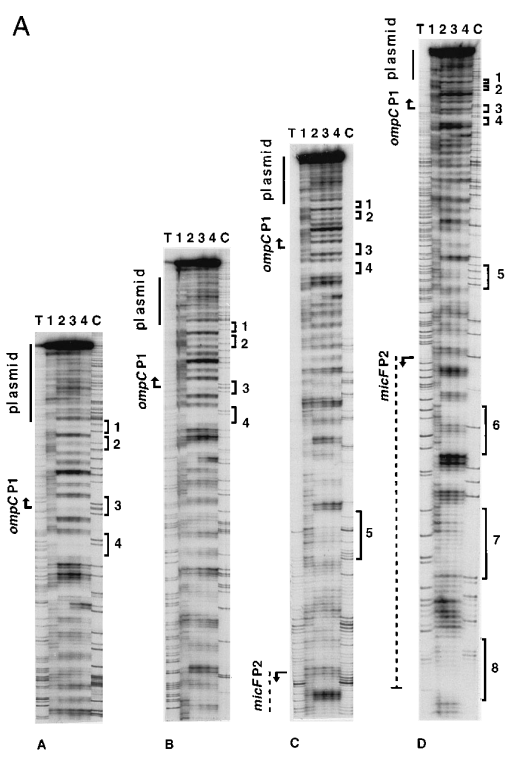
FIG. 7. Effect of Lrp dimer concentration on binding of Lrp to the *ompC-micF* intergenic region. Data from gel mobility shift assays carried out in the presence of a ramp of Lrp dimer concentrations from 0 to 125 nM were quantified by PhosphorImager scanning and plotted as the percent DNA bound versus the concentration of Lrp. The circles represent total DNA shifted by Lrp into either complex divided by total DNA, and they reflect the binding of Lrp to the higher-affinity site(s) in the *ompC-micF* intergenic region. Data obtained in the absence of leucine from the experiment whose results are shown in Fig. 6 have also been plotted on this graph. The squares represent DNA shifted into the second (upper) complex divided by total DNA, and they reflect the binding of Lrp to the lower-affinity sites in this intergenic region. No such data from the experiment whose results are shown in Fig. 6 were included, because the separation between the complexes was too small to permit resolution on the PhosphorImager. Theoretical Hill binding curves (20) were fitted to the data (see the text). The higher-affinity band showed an apparent dissociation constant of 49 nM and a Hill coefficient (α) of 2.2. The lower-affinity band (the more highly retarded band) showed an apparent dissociation constant of 87 nM, and a Hill coefficient of 2.2. α is a measure of the cooperativity of binding of Lrp to the DNA.

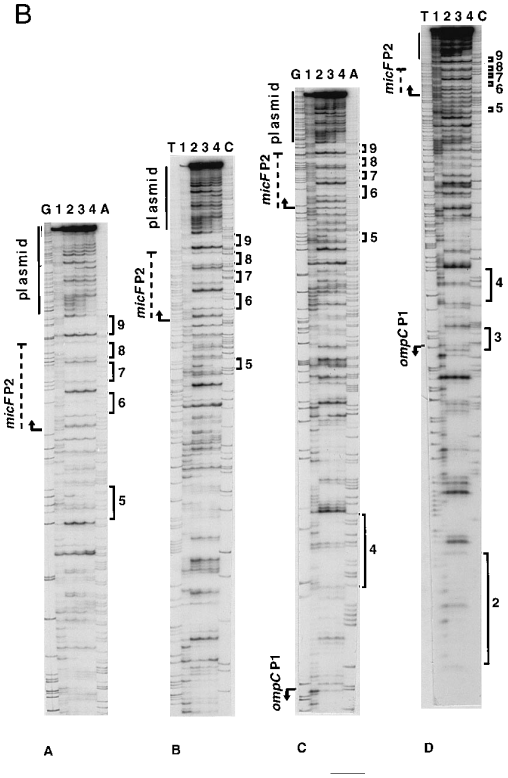
496-bp DNA fragment containing the intergenic region (lanes 1 through 8). As the concentration of Lrp in the binding reaction is increased, the DNA fragment containing the *ompC* and *micF* promoters is shifted into at least two bound complexes with different mobilities. Leucine, which has been shown to antagonize the binding of Lrp to the DNA of the *ilvIH* (48), *lysU* (28, 30), and *gltBDF* promoter regions (12), has a minimal effect on the affinity of Lrp for the DNA fragment containing the *ompC-micF* intergenic region. Lanes 9 through 16 show Lrp binding to the same DNA fragment over the same range of Lrp concentrations as were used for lanes 1 through 8 but in the presence of 20 mM leucine. While leucine did not lead to dissociation of Lrp from this fragment, its presence did lead to subtle alterations in the mobilities of the shifted fragments. In particular, the resolution of the shifted DNA into fragments with two different mobilities is less evident when leucine is present in the mobility shift assay. Such findings are consistent with earlier evidence that the binding of leucine to Lrp does not abolish DNA binding by the ligated Lrp but that it may alter the affinity of Lrp for specific sites on DNA (12).

We have performed mobility shift experiments similar to those whose results are shown in Fig. 6, but using 16 different concentrations of Lrp dimer, ranging from 0 to 125 nM (data not shown). When the gels from these experiments were subjected to quantitative PhosphorImager scanning, the results shown in Fig. 7 were obtained. The circles represent the percentages of DNA shifted by Lrp into either complex, and they reflect the binding of Lrp to the higher-affinity site(s) in the *ompC-micF* intergenic region (the less retarded band). The squares represent the percentages of DNA shifted into the second (more retarded) complex, and they reflect the binding of Lrp to the lower-affinity site(s) in this intergenic region. For these analyses, the data shown in Fig. 7 were fitted to the Hill equation (20), assuming n binding sites. In this equation, $y = ([\mathrm{Lrp}]^{\alpha}/K^{\alpha})/(1 + [\mathrm{Lrp}]^{\alpha}/K^{\alpha})$, K is the apparent dissociation constant for the Lrp-DNA complex, y is the fractional saturation of a site or sites with ligand, and $1 \leq \alpha \leq n$ is a measure of the apparent degree of cooperativity of binding. As shown in Fig. 6, binding of Lrp to the fragment containing the *ompC-micF* intergenic region occurred in two phases. Hill binding curves fitted to the plotted data showed that the first mobility shift was associated with an apparent K_d for Lrp of 49 nM, and a Hill coefficient of 2.2, while occupancy of the second site or sites by Lrp was associated with a K_d of 84 nM and a Hill coefficient of ~2.2. A Hill coefficient significantly greater than one indicates that cooperative binding of Lrp is associated with complex formation leading to a particular mobility shift. Such cooperativity can arise because there are two or more binding sites for Lrp on the DNA, and binding of Lrp to one site enhances occupancy of the other site(s) as well. Alternatively, if Lrp were monomeric in solution at the concentrations used for these mobility shift assays, but it bound to DNA as a dimer, Hill coefficients of up to 2 could be observed. The DNase I protection assays described below suggest that cooperativity arises from interactions of Lrp molecules bound at multiple sites.

The data from Fig. 6 have also been analyzed by using the Hill equation. In the absence of leucine, a K_d of 58 nM and a Hill coefficient of 2.1 were obtained for the first mobility shift (data not shown). In the presence of leucine, the two shifts were not quite as clearly separated, and it was possible to measure only the apparent affinity of binding of Lrp to either site or both sites (the total percentage of DNA shifted). This apparent K_d was 67 nM, and again the Hill coefficient was 2.1. Thus, the K_d measured for Lrp binding in the presence of 20 mM leucine was only 1.2-fold higher than the K_d measured in the absence of leucine. These results are in excellent agreement with the earlier observation that leucine leads to a 1.2-fold increase in the level of expression of β-galactosidase in strain MF5.4 (*ompC-lacZ*$^{+}$).

DNase I footprinting analysis of the binding of Lrp to the *ompC-micF* intergenic region. Figure 8 shows the results of





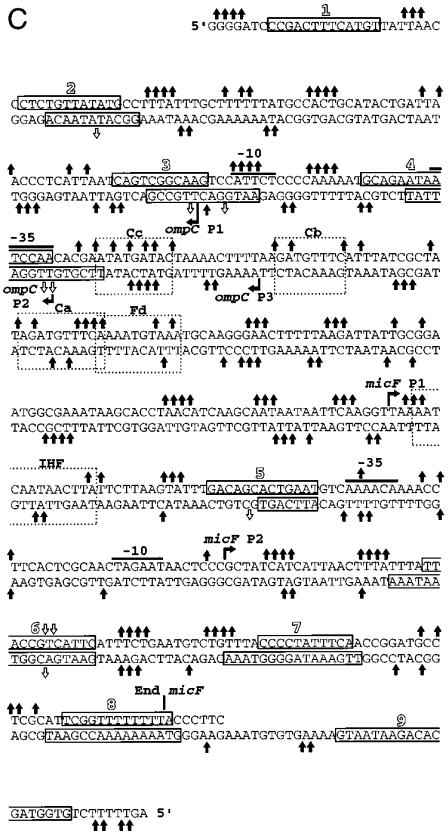


FIG. 8. DNase I footprint assays of Lrp binding to the *ompC-micF* intergenic region. Results of DNase I protection assays for the *ompC* coding strand (A) and noncoding strand (B) are shown. DNA fragments containing the intergenic region were labeled at their 5′ ends, and then they were incubated for 30 min in 200 μl of assay buffer in the presence of 0, 25, 50, 100 nM purified Lrp (lanes 1, 2, 3, and 4, respectively) before being digested by DNase I. Lanes labeled G, A, T, and C indicate chain termination sequencing ladders generated with the indicated dideoxynucleotides. Sets A, B, C, and D represent four identical assays loaded on the gel at ~0, 60, 120, and 180 min after the initiation of electrophoresis. Numbered brackets indicate sequences protected from DNase I digestion by Lrp. Bent arrows indicate the transcription start sites associated with promoter P1 for *ompC* (21, 42) and promoter P2 for *micF* (2). The dashed line corresponds to the entire *micF* coding region transcribed from the P2 promoter. Solid bars indicate plasmid DNA from the cloning vector. (C) Summary of DNase I footprinting results for the *ompC-micF* intergenic region. The DNA sequence shown is that determined by Esterling and Delihas (13), and it has been confirmed in our footprint analysis. The three transcription start sites for *ompC* (21, 42) are indicated on the diagram, as are the two transcription start sites for *micF* (2), where P1 is a minor promoter and P2 is the major promoter for *micF* transcription. The −10 and −35 regions of P2 are shown for *micF*, and those of P1 are shown for *ompC*. Sequences enclosed by solid boxes were protected from DNase I cleavage in the presence of Lrp. Sites that become hypersensitive to DNase I cleavage in the presence of Lrp have been indicated by black arrows, while outlined arrows indicate sites where DNase I cleavage is partially but not fully protected against by Lrp. Also shown are the area in the intergenic region protected from DNase I cleavage by integration host factor (21) and the four binding sites for OmpR (Ca, Cb, Cc, and Fd) detected by in vivo methylation protection (56).

DNase I footprinting analysis of DNA fragments containing the *ompC-micF* intergenic region, the *micF* gene, and the upstream portion of the *ompC* gene. Lrp binding leads to the induction of phased hypersensitivity that extends from the end of the *micF* gene through the intergenic region and into the upstream portion of the *ompC* gene. Such extensive phased hypersensitivity is associated with the DNase I protection patterns of several other Lrp-regulated operons, e.g., *ilvIH* (57), *pap* (43), *lysU* (16, 28), and *lrp* itself (59). Regions that were protected by Lrp from DNase I cleavage were indicated on the footprints of each strand, and they are also shown in Fig. 8C. Regions 3 and 4 overlap the start site of transcription and the −35 region of P1 of the *ompC* gene, respectively. Region 4 overlaps the transcription start site for P2, and it lies within the transcribed region of *ompC* from promoter P3. Regions 1 and 2 are located within the transcribed region of *ompC* starting from all three promoters. The positioning of these four regions is consistent with direct repression of *ompC* transcription by Lrp. Region 5 is centered 47 bp upstream of the major (P2-directed) start site of transcription of *micF*. Regions 6, 7, and 8 are located within the *micF* gene. The positioning of these regions is also consistent with repression of *micF* transcription being mediated by Lrp.

DISCUSSION

Regulation of the OmpC-OmpF switch. In this communication, we provide evidence that Lrp negatively regulates the transcription of *ompC* and *micF*. We have shown that Lrp binds to multiple regions in the *ompC-micF* intergenic region and that the positions of these regions as determined by DNase I protection assays are consistent with a role for Lrp as a transcriptional repressor of both *ompC* and *micF*. As shown in Fig. 8C, the nine protected regions show minimal overlap with the known binding sites for integration host factor (21), and OmpR (56), in agreement with the absence of an effect of Lrp on the increase in *ompC* transcription caused by sucrose. Our results are consistent with the assumption that Lrp and OmpR bind independently and that binding of Lrp to a promoter containing bound OmpR represses transcription of *ompC* even in the presence of OmpR.

Our results also suggest that a direct effect of Lrp on *micF* transcription may be responsible for the observed effects of Lrp on OmpF expression. In the presence of a *micF* deletion, Lrp has no effect on the expression of OmpF (Table 4). It should be noted that the *micF* deletion also abolishes the repression of OmpC expression exerted by Lrp. The *micF* strain SM3001, constructed by Matsuyama and Mizushima (31), contains a deletion that extends from 41 bp upstream of the start site of *micF* transcription directed by P2 to position +69 in the *micF* gene. This deletion removes Lrp-protected regions 6 and 7. The increased expression of OmpC in strain SM3001 that has been noted both by us and by Matsuyama and Mizushima (31) may indicate that Lrp no longer binds to the *ompC* promoter in this strain.

As summarized in the introduction, a number of *cis*-acting factors that affect *micF* transcription or bind upstream of the *micF* gene have been identified, although Lrp was not identified as one of these factors. The *cis*-acting factors that affect *micF* expression can be divided into two groups: those that affect both *ompC* and *micF* expression, such as OmpR and Lrp, and those that affect only *micF* expression, such as SoxRS (8) and the factor(s) mediating thermal activation of *micF* (3). It seems clear that the 253-bp region between *micF* and *ompC* is rich in binding sites for regulatory proteins, as befits a switch region controlling the synthesis of highly expressed proteins as well as an important physiological function.

Preliminary studies of the effect of Lrp on *micF* and *ompF* RNA levels suggest that control of *micF* and *ompF* expression by temperature is not affected by an *lrp* mutation (13a). Previous studies have shown that *micF* expression is increased when cells are shifted from a glucose minimal medium to LB and that increased *micF* transcription does not require a functional OmpR protein (10). Since the levels of Lrp in LB are known to be decreased compared with those in glucose minimal media (6, 12, 27), it will be interesting to determine whether the effect of LB on *micF* expression requires a functional Lrp protein.

Interaction between Lrp and the *ompC-micF* intergenic region. The affinity of Lrp for the *ompC-micF* intergenic region seen in vitro (apparent K_d values of ~50 nM and 90 nM) is considerably lower than the in vitro affinities of Lrp for *ilvIH* (apparent K_d values of 2.2 and 6.9 nM for the high- and low-affinity complexes, respectively) and for *gltBD* (an apparent K_d value of 2.0 nM) (12). The nine regions that are protected from DNase I cleavage by Lrp in the *ompC-micF* intergenic region show only weak homology to the reported consensus sequence for Lrp, AGAATTTTATTCT (58). The two regions with the closest matches are 1 and 3, which match the consensus at only 8 of 13 positions. The relatively poor matches with the Lrp consensus sequence are consistent with the low affinity of Lrp for the *ompC-micF* intergenic region, compared with its affinities for the promoter regions of other target genes.

The footprinting data suggest that sufficiently high concentrations of Lrp might completely block transcription of *ompC* and *micF*. It is, of course, possible that other DNA-binding proteins in the cell interact with Lrp and/or with the *ompC-micF* intergenic region. These putative proteins could enhance repression of *ompC* and *micF* transcription by Lrp during growth in glucose minimal MOPS medium at 37°C.

We have presented evidence that at least two different complexes are formed when Lrp binds to DNA containing the *ompC-micF* intergenic region and that binding to each of these complexes appears to be cooperative, with a Hill coefficient of 2.2 for each complex formation. These results are consistent with our observation of multiple regions protected from DNase I cleavage by Lrp in this region.

The very minor effect of leucine on binding of Lrp to the *ompC-micF* intergenic region is also in agreement with the results of earlier studies using two-dimensional gel electrophoretic analysis (11). In those studies, very little effect of leucine on OmpC and OmpF expression was seen during exponential growth at 37°C in glucose minimal MOPS medium, and these proteins were classified as leucine-insensitive members of the Lrp regulon.

Physiological role of the Lrp regulon. The finding that expression of the OmpC and OmpF porins is regulated by Lrp provides useful insight into the physiological role of the Lrp regulon. We, and others, have previously postulated that Lrp modulates metabolic pathways in *E. coli* in response to the availability of nutrients in the medium (6, 12, 27) and that it may mediate changes necessary for survival outside an animal host. Genes and proteins that are involved in catabolism of amino acids and oligopeptide transport are negatively regulated by Lrp, while genes and proteins involved in amino acid biosynthesis and the assimilation of ammonia are positively regulated by Lrp. The pattern of regulation of *ompC* and *ompF* by Lrp is in general accord with this model. OmpF is maximally expressed in minimal media, at low temperatures and low osmotic strength. OmpC is maximally expressed in rich media, at high temperatures and high osmotic strength, and it appears

to be induced during bacterial growth in the gastrointestinal tract, where its smaller pore size excludes bile salts (41).

The experiments described in this paper were carried out under conditions of steady-state growth. During a growth shift, for example, from outside-the-host to inside-the-host conditions, the roles of OmpR and Lrp may be temporally different as well as functionally different. OmpR can rapidly be interconverted between active (phosphorylated) and inactive forms. In contrast, Lrp is present in cells at high concentrations (60), and decreased concentrations of Lrp on entrance into a rich medium probably require either dilution by cell division or degradation of existing protein (12). This two-tiered response to a changing environment may serve to damp fluctuations in expression of the OmpF and OmpC porins. Continued study of the contribution of Lrp to control of the OmpC-OmpF switch should be useful both for understanding the switch and for elucidating the physiological role of Lrp.

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