Influence of L-Leucine and L-Alanine on Lrp Regulation of *foo*, Coding for F165$_1$, a Pap Homologue

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The *foo* operon encodes F165$_1$ fimbriae that belong to the P-regulatory family and are synthesized by septicemic *Escherichia coli*. Using an Lrp-deficient host and the *lrp* gene cloned under the arabinose pBAD promoter, we demonstrated that *foo* was transcribed proportionally to the amount of Lrp synthesized. L-Leucine and L-alanine decreased drastically the steady-state transcription of *foo* and modified phase variation, independently of the presence of FooI. Specific mutations in the C-terminal region of Lrp reduced or abolished the repressive effect of these amino acids, indicating that they modulate F165$_1$ by affecting Lrp.

Virulence of *Escherichia coli* O115:KV165 strains associated with septicemia in young pigs involves synthesis of F165$_1$ fimbriae, encoded by the *foo* gene cluster and belonging to the P fimbrial family (15). The regulatory region of the *foo* cluster contains two divergent open reading frames coding for the *fooI* and *fooB* genes, an arrangement similar to that of the *pap* operon (1, 14) (Fig. 1). The intercistronic region between the two promoters in both operons contains two GATC sites (GATC-I and GATC-II) separated by 102 bp (2).

As is seen with many other virulence determinants, fimbriae are not expressed constitutively, and their synthesis is tightly regulated (20). Expression of F165$_1$ is controlled at the transcriptional level by two mechanisms which modulate, on one hand, the percentage of cells producing fimbriae (ON cells) and not producing fimbriae (OFF cells) in a single colony (phase variation) and, on the other hand, the amount of antigen at the surface of a single cell (basal transcription) (8, 14). These two types of regulation are superimposed so that transcription in ON cells may be more or less active, leading to more or less fimbrial synthesis. Pap phase variation is regulated by a complex epigenetic mechanism that specifies the formation of specific DNA methylation patterns of two GATC sites (2, 16). Regulation of F165$_1$ expression is equally complex, being mediated by Lrp (leucine-responsive regulatory protein), by Dam methylation, and by FooI and FooB, homologues of PapI and PapB, respectively (8).

The presence of Lrp is required for expression of F165$_1$ and Pap (2, 8). It was shown that for some operons regulated by Lrp, exogenous leucine and alanine antagonize the effect of Lrp (4, 24). F165$_1$ synthesis is strongly repressed by the presence of exogenous L-leucine or L-alanine (8), whereas the addition of amino acids doesn't affect the expression of Pap (2). However, expression of three other fimbriae is controlled by a mechanism involving Lrp and is regulated by exogenous leucine or alanine. Among them, CS31A belongs to the P-regulatory family (21), whereas K99 and type 1 fimbriae do not (2, 11).

Lrp was first identified as a global regulator that regulates the expression of 35 to 75 genes in *E. coli* (including many operons involved in the metabolism of amino acids and in the formation of fimbriae) by binding to specific DNA sequences and affecting DNA conformation (22). A microarray analysis has shown that more than 400 genes are significantly Lrp responsive and that most of them are involved in stationary-phase metabolism (26). The mechanism by which Lrp acts is now thought to involve different associations of Lrp monomers. Recently, it was shown that at micromolar concentrations Lrp self-associates to hexadecamers and to octamers instead of the dimer conformation previously suggested (5). The presence of leucine induces a transition of the hexadecamers or the octamers into leucine-bound octamers (5). The C-terminal domain of Lrp is primordial for this autoassociation (5). Whatever the mechanism, L-leucine modulates the effect of Lrp, antagonizing it or intensifying it, or in some cases not affecting it at all (2, 4, 11, 13, 24). There is much less knowledge about alanine as a regulator of gene expression, and such an effect might be mediated through Lrp (28). Mutations in Lrp have been isolated on the basis of their effect on *ilvIH*, an operon positively regulated by Lrp (24). Analysis of these mutations suggested the existence of three functional domains: an N-terminal domain containing a helix-turn-helix motif implicated in DNA binding, a middle domain responsible for transcriptional activation, and a C-terminal domain that is required for the response to leucine. The objective of this study was to determine, by introducing specific mutations in the C-terminal region of Lrp, whether leucine or alanine affects the interaction of Lrp with the regulatory region of *foo*.

Influence of leucine and alanine on Lrp-induced *foo* expression. The *lrp* gene was introduced into pBAD22CM under the control of the *araBAD* promoter, which is inducible by exogenous arabinose (4). This vector was introduced into an *lrp*-

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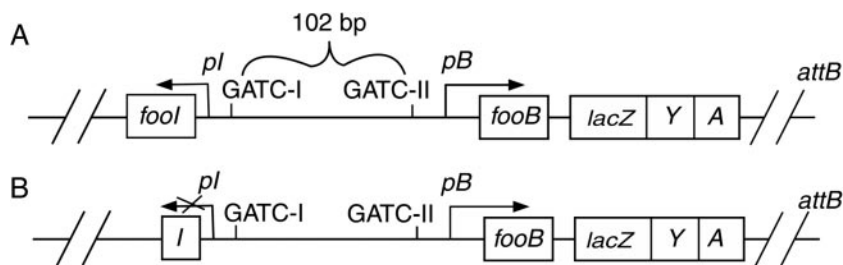
FIG. 1. Schematic representations of *fooIB-lacZYA* and *fooB-lacZYA* fusions. (A) Strain FB1001 and derivatives bearing the *fooIB-lacZYA* fusion, including the *fooI* gene. (B) Strain FB2001 and derivatives bearing the *fooB-lacZYA* fusion, without the complete *fooI* gene. These fusions have been inserted at the *attB* site into the chromosome of *E. coli* CT4A by using the λRS45 derivative phages (λMT01 and λMT02). Both transcriptional fusions contained the *foo* regulatory region, including the two GATC sites. The arrows show the orientation of transcription, and *p* indicates *foo* promoters.

deficient host strain (FB1001) containing a single chromosomal *fooIB*p-*lacZ* fusion with intact *fooI* and *fooB* genes and the whole *foo* intercistronic region, leading to strain FB1002 (Fig. 1; Table 1). The activity of the *fooB* promoter was monitored by assaying the β-galactosidase activity of FB1002 cells grown in NIV minimal medium with arabinose concentrations ranging from 0 to 60 μg/ml (4). The NIV medium is composed of 0.01 M potassium phosphate (pH 6.4), 0.2% ammonium sulfate, 0.001% CaCl$_2$, and 0.02% MgSO$_4$ neutralized with NaOH (pH 7.0) (23). Supplements were used at the following concentrations: 0.2% (wt/vol) glycerol, 50 μg of L-isoleucine/ml, 50 μg of valine/ml, and 2 mg of serine/ml. The *fooB* promoter activity increased proportionally to external arabinose concentrations up to a saturation level of 45 μg/ml (Fig. 2A). In contrast, *foo* expression was abolished in the Lrp-deficient strain FB1001 grown under the same conditions (Fig. 2A).

Some residual β-galactosidase activity was detected in the absence of arabinose, probably due to the leakiness of the arabinose promoter (12).

In contrast to P fimbriae, the expression of *foo* in wild-type strains is repressed by the presence of exogenous leucine or alanine at 1.5 mM (14). At this concentration, both leucine and alanine repressed *foo* expression at all the arabinose concentrations tested (Fig. 2A). Leucine at 0.75 mM has been reported to repress expression of *ilvIH* (24), but alanine was not tested until now. Using the same concentration of leucine as Platko and Calvo, we observed a significant reduction of the *ilvIH* promoter activity of strain FB3002 (*ilvI'-lacZ*, pBAD-*lrp*) grown with arabinose at 50 μg/ml (Table 2). However, addition of alanine at 0.75 or 1.5 mM did not lower significantly the *ilvIH* expression (Table 2). Thus, *ilvIH* expression appears to be alanine insensitive, in contrast to *foo* expression.

TABLE 1. Bacterial strains and bacteriophages used in this study

| Strain or phage	Description	Amino acids changed in Lrp	References
E. coli strains			
CU1008	*ilvA*		27
MEW1	CU1008 Δ*lac*		23
CT4A	*ilvA lac ara lrp*::Tn*10*		27
CV1008	*ilvI'-lacZ lrp-35*::Tn*10*		25
FB1001	CT4A *fooIB-lacZ*		This study
FB1002	FB1001 pBAD22CM-*lrp*		This study
FB1003	FB1001 pBAD22CM-*lrp-1*	Lrp^{S41P}	This study
FB1004	FB1001 pBAD22CM-*lrp-2*	Lrp^{L131R}	This study
FB1005	FB1001 pBAD22CM-*lrp-3*	Lrp^{L131A}	This study
FB1006	FB1001 pBAD22CM-*lrp-4*	Lrp^{L136R}	This study
FB1007	FB1001 pBAD22CM-*lrp-5*	Lrp^{V149A}	This study
FB2001	CT4A *fooB-lacZ*		This study
FB2002	FB2001 pBAD22CM-*lrp*		This study
FB2006	FB2001 pBAD22CM-*lrp-4*	Lrp^{L136R}	This study
FB3002	CV1008 pBAD22CM-*lrp*		This study
FB3003	CV1008 pBAD22CM-*lrp-1*	Lrp^{S41P}	This study
FB3004	CV1008 pBAD22CM-*lrp-2*	Lrp^{L131R}	This study
FB3005	CV1008 pBAD22CM-*lrp-3*	Lrp^{L131A}	This study
FB3006	CV1008 pBAD22CM-*lrp-4*	Lrp^{L136R}	This study
FB3007	CV1008 pBAD22CM-*lrp-5*	Lrp^{V149A}	This study
Bacteriophages			
λMT01	λRS45 containing *fooIB-lacZ*		M. C. Tessier, C. Martin, and J. Harel (unpublished results)
λMT02	λRS45 containing *fooB-lacZ*		M. C. Tessier, C. Martin, and J. Harel (unpublished results)
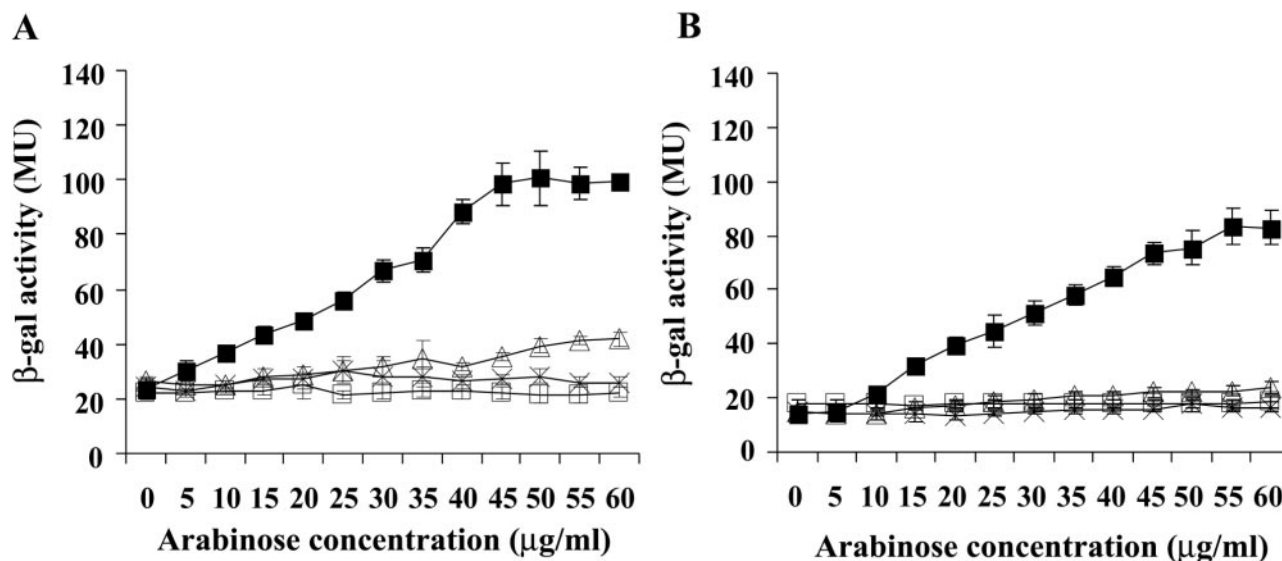
FIG. 2. Influence of Lrp mutations and effects of leucine and alanine at 1.5 mM on the expression of *fooB*p. (A) *fooIB-lacZ* derivatives strain FB1002, containing wild-type Lrp (solid lines, black squares), and strain FB1001, deficient in Lrp (dotted line, empty squares). (B) *fooB-lacZ* derivatives FB2002, containing wild-type Lrp (solid lines, black squares), and FB2001, deficient in Lrp (dotted line, empty squares). The curves obtained in minimal medium without the addition of amino acids are represented by black squares, with the addition of alanine shown by empty triangles and the addition of leucine indicated by an X. The Durnett test was used to compare the effect of different Lrp concentrations on the *foo* operon (9). MU, Miller units.

Effects of specific Lrp mutations on the repression of *foo* by leucine and alanine. A study realized with the *ilvIH* promoter showed that the C-terminal third of Lrp is responsible for the response to L-leucine (24). In addition, in a recent study Ettema et al. (10) surveyed the structure of all known prokaryote Lrp-like molecules. They found a ligand-binding domain, RAM (regulation of amino acid metabolism) fused to the DNA-binding domain of Lrps. In the *E. coli* Lrp, this domain is located between residues 73 and 149. Following the procedure described by Labrie et al. (19) and by using the USE mutagenesis kit purchased from Amersham Pharmacia Biotech, we created mutations in the RAM domain of Lrp at those sites known to result in leucine insensitivity on the *ilvIH* promoter (L136R and V149A) and at an additional site in its C-terminal region based on the properties of some residues (L131R and L131A) (Table 1). As a negative control, a mutation in the N-terminal region was also introduced (S41P). This latter substitution abolished the *fooIB-lacZ* expression, whatever the arabinose concentration added (Table 3).

Strain FB1006 (L136R) and FB1004 (L131R) grown in glycerol minimal medium showed a similar *fooIB-lacZ* expression in response to arabinose as the parental strain carrying wild-type Lrp. However, neither leucine nor alanine repressed *foo* expression in these strains (Table 3). Thus, the L136R and L131R mutations, which caused leucine insensitivity at the *ilvIH* promoter, caused both leucine and alanine insensitivity at *fooB*p. A weak level of repression of *fooB-lacZ* expression due to leucine or alanine was also observed for strain FB1007 (V149A) at each arabinose concentration tested (Table 3). If these observations demonstrated an intrinsic characteristic of Lrp, they should be seen at other Lrp-regulated promoters. We therefore introduced the Lrp mutations in strains harboring an *ilvI'-lacZ* fusion. Transcription of *ilvIH* was shown to be leucine independent by using Lrp^{L136R} and Lrp^{V149A}, as also observed by Platko and Calvo, and by using Lrp^{L131R} (Table

TABLE 2. β-Galactosidase activity of the different CV1008 derivatives (*ilvI'-lacZ*)[a]

Strain	β-Galactosidase activity (%)	
	Without amino acid	+Leucine, 1.5 mM
FB3002 (Lrp)	100.0 ± 19.2	17.4 ± 4.2
FB3003 (Lrp^{S41P})	20.4 ± 5.9	18.0 ± 3.6
FB3004 (Lrp^{L131R})	218.5 ± 37.7	174.3 ± 29.3
FB3005 (Lrp^{L131A})	126.9 ± 2.4	161.6 ± 27.5
FB3006 (Lrp^{L136R})	102.4 ± 18.6	83.8 ± 16.2
FB3007 (Lrp^{V149A})	107.2 ± 15.6	48.0 ± 7.2

[a] The arabinose concentration used was 50 μg/ml. Specific activities obtained were normalized to that of FB3002.

TABLE 3. β-Galactosidase activity of the different FB1001 and FB2001 derivatives[a]

Strain	β-Galactosidase activity (Miller units)		
	Without amino acids	+Leucine, 1.5 mM	+Alanine, 1.5 mM
FB1002 (Lrp)	107.7 ± 9.8	28.3 ± 2.4	34.2 ± 2.7
FB1003 (Lrp^{S41P})	12.2 ± 3.2	14.0 ± 3.7	14.8 ± 3.3
FB1004 (Lrp^{L131R})	132.5 ± 2.8	147.2 ± 5.2	135.9 ± 2.6
FB1005 (Lrp^{L131A})	109.0 ± 13.7	92.4 ± 8.5	121.6 ± 19.6
FB1006 (Lrp^{L136R})	89.4 ± 2.2	105.1 ± 2.0	98.9 ± 4.0
FB1007 (Lrp^{V149A})	93.8 ± 2.1	66.0 ± 5.3	74.3 ± 2.9
FB1008 (Lrp^{E133G})	17.4 ± 3.8	14.1 ± 1.2	17.6 ± 4.1
FB2001 (Lrp)	75.5 ± 6.6	17.6 ± 2.7	22.0 ± 0.9
FB2006 (Lrp^{L136R})	61.7 ± 8.7	62.5 ± 6.3	63.5 ± 5.7

[a] The arabinose concentration used was 50 μg/ml. The post-hoc test of Tukey was used to analyze the effect of the medium on the expression of *foo* (9).

TABLE 4. *foo* phase variation in strain FB1002

Medium	Starting colony phenotype	% Lac⁺ colonies[a]	Avg % Lac⁺ colonies	Switch frequency[b]		
				ON to OFF	OFF to ON	Avg
NIV	1st blue	82.6 ± 3.6	78.05	6.9×10^{-3}		8.45×10^{-3}
	2nd blue	73.5 ± 5.9		1×10^{-2}		
	1st white	45.3 ± 1.5	23.65		1.8×10^{-2}	9.4×10^{-3}
	2nd white	2 ± 0.5			8×10^{-4}	
NIV-leu	1st blue	1.5 ± 1	2.1	3.9×10^{-2}		3.85×10^{-2}
	2nd blue	2.7 ± 0.7		3.8×10^{-2}		
	1st white	0.027 ± 0.02	0.013		1×10^{-5}	5×10^{-6}
	2nd white	0			0	
NIV-ala	1st blue	5 ± 2	4.25	3.7×10^{-2}		3.75×10^{-2}
	2nd blue	3.5 ± 0.3		3.8×10^{-2}		
	1st white	0.2 ± 0.2	0.2		8.5×10^{-5}	7.4×10^{-5}
	2nd white	0.2 ± 0.1			6.3×10^{-5}	

[a] Mean of at least two experiments.

[b] Strains were inoculated onto NIV–glycerol–arabinose–X-Gal plates supplemented or not with L-leucine or L-alanine. Colonies showing a uniform phenotype were suspended in NIV salts. Appropriate dilutions were spread onto the same medium as the parent colony. After growth, the colonies were scored for a white or a blue phenotype. The switch frequencies were calculated using the formula $(M/N)/g$, where M is the number of cells that underwent phase transition, N is the total number of cells evaluated, and g is the total number of generations, estimated to be 27; that gave rise to the colony (7). A minimum of 2,000 colonies were screened for each test, and three independent tests were done to calculate the percentage of Lac⁺ colonies.

2). Therefore, these results indicate that the residues at positions 131, 136, and 149 in Lrp are essential for the L-leucine and L-alanine responses. Because the Lrp mutants presented similar responses towards both amino acids, the mechanism of repression by alanine on *foo* appears to be similar to that of leucine. Among the operons regulated by Lrp and leucine, only a few are also regulated by alanine. Why alanine represses only a subset of leucine-responsive regulated operons remains to be elucidated. This might imply that another protein(s) could interact with Lrp to control the promoter activity, as was suggested for the Lrp-like LysM regulator in *Sulfolobus solfataricus* (3).

The Lrp leucine residues 131 and 136 were shown to be essential for the amino acid response. This hydrophobic residue was replaced at these positions by a hydrophilic one, arginine, to investigate whether the amino acid binding to Lrp involves a hydrophobic interaction. These Lrp mutants were unresponsive to leucine and alanine (Table 3). However, the lack of response did not seem to be the consequence of the interruption of a hydrophobic bond, since a hydrophobic residue conversion (Lrp^L131A) did not restore the repression.

Interestingly, Lrp carrying the L131R mutation differed in function from wild-type Lrp even in the absence of leucine and alanine. FB1004, which harbors this Lrp mutation, produced a significantly higher *foo* activity (about 1.2- to 1.7-fold) in minimal glycerol medium compared to FB1002 containing wild-type Lrp (Table 3). Thus, this mutation seems to intensify the activation by Lrp of *foo* expression in addition to causing a leucine- and alanine-nonresponsive phenotype. As described by Chen and Calvo (5), at micromolar concentrations Lrp self-associates primarily to hexadecamers, a combination of two octamers. The L136 residue, located in proximity to the L131 residue, is important for this autoassociation. Thus, the L131R mutation could be responsible for the formation of more-stable octamers. In this way, a higher number of Lrp^L131R octamers could bind the regulatory region of *foo* and intensify the activation of the operon. In contrast, this amplification of expression was not observed with the L131A substitution, indicating that the two Lrp molecules were quite different in terms of stability.

In addition to the C-terminal region of Lrp, it was already shown that residues A126, E133, and Thr134 are necessary for the activation of the *pap* operon in opposition to the *ilvIH* operon (18). The region containing these residues was considered *pap* specific. In our study, the same E133G mutation was introduced into Lrp to obtain strain FB1008. As observed for the *pap* operon, the expression of *foo* was abolished with this Lrp mutant.

Influence of FooI on leucine- or alanine-mediated *foo* repression. Since FooI is involved in *foo* expression and phase variation (6), we wondered if the effect of leucine and/or alanine might be mediated by FooI. The *fooB*p activity was significantly lower in the absence of *fooI* than in its presence, at all the concentrations of arabinose tested (Fig. 1 and 2B). Thus, like PapI (17), FooI appears to be an activator of the *foo* operon. Moreover, we clearly demonstrated that the sensitivity of *foo* to leucine and alanine is independent of FooI, since the same degree of repression was obtained in the presence or in the absence of the *fooI* gene (Fig. 2A versus B; Table 3, FB1002 and FB2002). In addition, the mutant strains producing Lrp^L136R presented the same leucine- and alanine-nonresponsive phenotype independently of the presence of FooI (Table 3, FB1006 versus FB2006). Taken together, these results showed that FooI is not necessary for the repressive effect exerted by the amino acids on *foo* expression.

Effects of leucine and alanine on phase variation and on basal transcription of the *foo* operon. To investigate whether leucine and/or alanine affected *foo* phase variation, the Lac phenotype of *fooB-lacZ* cultures was analyzed. A single FB1002 colony in the OFF or the ON state was picked from a Luria-Bertani plate and cultured overnight in Luria-Bertani broth at 37°C. This culture was diluted 50-fold in NIV broth containing 0.1% glycerol and 0.1% D-glucose and incubated overnight at 37°C. This culture was diluted in NIV-glycerol containing 50 μg of arabinose/ml with or without L-leucine or L-alanine and incubated at 37°C until an optical density at 600

nm between 0.4 and 0.5 was reached. Then, the phase variation assay was performed as previously described (7) on NIV 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside–agar plates containing 50 μg of arabinose/ml with or without amino acids by counting the number of blue and white colonies (Table 4). Phase variation occurred in strain FB1002, leading to a majority of colonies in the on phase using ON inoculating colonies, whereas a majority of colonies in the OFF phase was observed using OFF inoculating colonies (Table 4). When using ON inoculating colonies, without amino acids, 78.05% of cells were in the ON phase. In the presence of leucine or alanine, 2.1 and 4.35% of cells, respectively, were in the ON phase. A decrease in the number of ON colonies was also observed in the presence of both amino acids when using OFF inoculating colonies. Thus, the phase variation favored the OFF phenotype when alanine or leucine were added to the medium. Moreover, in the absence of amino acids, the blue colonies were dark blue or very dark blue. The very dark blue and dark blue colonies were excised from the plates and suspended separately in 1 ml of phosphate-buffered saline buffer for direct β-galactosidase activity measurement. The β-galactosidase activity representing the average of three very dark blue and dark blue colonies was 1,619 and 611 Miller units, respectively. In contrast, the pale blue cells grown with alanine or leucine demonstrated low β-galactosidase activities (305 and 381 Miller units, respectively). These results indicated that the amino acids had a drastic effect on the basal transcription of *foo* and on the phase variation control. For the strain expressing the mutated Lrp^{L136R}, neither the phase variation nor the basal transcription of *foo* was affected by the presence of the amino acids (data not shown). Taken together, our results suggest that the interaction between the C-terminal region of Lrp and the amino acids is mainly responsible for the reduction of *foo* basal transcription and phase variation.

Conclusions. In summary, this study demonstrates that the *foo* operon is transcribed proportionally to the amount of Lrp synthesized. We showed that the repressive effect of exogenous leucine and alanine on *foo* basal transcription and phase variation is mediated by Lrp, since specific mutations in the C-terminal region of Lrp totally abolished their action. In addition, we showed that FooI is not required for the repressive effect of both amino acids.

REFERENCES

1. **Blyn, L. B., B. A. Braaten, and D. A. Low.** 1990. Regulation of *pap* pilin phase variation by a mechanism involving differential dam methylation states. EMBO J. **9:**4045–4054.
2. **Braaten, B. A., J. V. Platko, M. W. van der Woude, B. H. Simons, F. K. de Graaf, J. M. Calvo, and D. A. Low.** 1992. Leucine-responsive regulatory protein controls the expression of both the *pap* and *fan* pili operons in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **89:**4250–4254.
3. **Brinkman, A. B., S. D. Bell, R. J. Lebbink, W. M. de Vos, and J. van der Oost.** 2002. The *Sulfolobus solfataricus* Lrp-like protein LysM regulates lysine biosynthesis in response to lysine availability. J. Biol. Chem. **277:**29537–29549.
4. **Chen, C., and E. B. Newman.** 1998. Comparison of the sensitivities of two *Escherichia coli* genes to in vivo variation of Lrp concentration. J. Bacteriol. **180:**655–659.
5. **Chen, S., and J. M. Calvo.** 2002. Leucine-induced dissociation of *Escherichia coli* Lrp hexadecamers to octamers. J. Mol. Biol. **318:**1031–1042.
6. **Crost, C., J. Harel, F. Berthiaume, A. Garrivier, M. C. Tessier, H. Harivony Rakotoarivonina, and C. Martin.** 2004. Influence of environmental cues on the transcriptional regulation of *foo* and *clp* coding for F1651 and CS31A adhesins in *Escherichia coli*. Res. Microbiol. **155:**475–482.
7. **Crost, C., A. Garrivier, J. Harel, and C. Martin.** 2003. Leucine-responsive regulatory protein-mediated repression of *clp* (encoding CS31A) expression by L-leucine and L-alanine in *Escherichia coli*. J. Bacteriol. **185:**1886–1894. (Erratum, **185:**2972.)
8. **Daigle, F., C. Forget, C. Martin, M. Drolet, M. C. Tessier, H. Dezfulian, and J. Harel.** 2000. Effects of global regulatory proteins and environmental conditions on fimbrial gene expression of F165₁ and F165₂ produced by *Escherichia coli* causing septicaemia in pigs. Res. Microbiol. **151:**563–574.
9. **Dawson, B., and R. G. Trapp.** 2001. Basic and clinical biostatistics. Lange Medical Books, New York, N.Y.
10. **Ettema, T. J., A. B. Brinkman, T. H. Tani, J. B. Rafferty, and J. Van Der Oost.** 2002. A novel ligand-binding domain involved in regulation of amino acid metabolism in prokaryotes. J. Biol. Chem. **277:**37464–37468.
11. **Gally, D. L., J. A. Bogan, B. I. Eisenstein, and I. C. Blomfield.** 1993. Environmental regulation of the *fim* switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media. J. Bacteriol. **175:**6186–6193.
12. **Guzman, L. M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose pBAD promoter. J. Bacteriol. **177:**4121–4130.
13. **Haney, S. A., J. V. Platko, D. L. Oxender, and J. M. Calvo.** 1992. Lrp, a leucine-responsive protein, regulates branched-chain amino acid transport genes in *Escherichia coli*. J. Bacteriol. **174:**108–115.
14. **Harel, J., F. Daigle, C. Forget, M. C. Tessier, C. Crost, and C. Martin.** 2000. Phase variation of F165₁ (Prs-like) fimbriae from *Escherichia coli* causing septicaemia in animals. Can. J. Microbiol. **46:**1101–1107.
15. **Harel, J., F. Daigle, S. Maiti, C. Desautels, A. Labigne, and J. M. Fairbrother.** 1991. Occurrence of *pap-*, *sfa-*, and *afa*-related sequences among F165-positive *Escherichia coli* from diseased animals. FEMS Microbiol. Lett. **66:**177–182.
16. **Hernday, A., M. Krabbe, B. Braaten, and D. Low.** 2002. Self-perpetuating epigenetic pili switches in bacteria. Proc. Natl. Acad. Sci. USA **99**(Suppl. 4):16470–16476.
17. **Hernday, A. D., B. A. Braaten, and D. A. Low.** 2003. The mechanism by which DNA adenine methylase and PapI activate the *pap* epigenetic switch. Mol. Cell **12:**947–957.
18. **Kaltenbach, L., B. Braaten, J. Tucker, M. Krabbe, and D. Low.** 1998. Use of a two-color genetic screen to identify a domain of the global regulator Lrp that is specifically required for *pap* phase variation. J. Bacteriol. **180:**1224–1231.
19. **Labrie, V., H. E. Beausoleil, J. Harel, and J. D. Dubreuil.** 2001. Binding to sulfatide and enterotoxicity of various *Escherichia coli* STb mutants. Microbiology **147:**3141–3148.
20. **Low, D., E. N. Robinson, Jr., Z. A. McGee, and S. Falkow.** 1987. The frequency of expression of pyelonephritis-associated pili is under regulatory control. Mol. Microbiol. **1:**335–346.
21. **Martin, C.** 1996. The *clp* (CS31A) operon is negatively controlled by Lrp, ClpB, and L-alanine at the transcriptional level. Mol. Microbiol. **21:**281–292.
22. **Newman, E. B., R. D'Ari, and R. T. Lin.** 1992. The leucine-Lrp regulon in *E. coli*: a global response in search of a raison d'etre. Cell **68:**617–619.
23. **Newman, E. B., B. Miller, L. D. Colebrook, and C. Walker.** 1985. A mutation in *Escherichia coli* K-12 results in a requirement for thiamine and a decrease in L-serine deaminase activity. J. Bacteriol. **161:**272–276.
24. **Platko, J. V., and J. M. Calvo.** 1993. Mutations affecting the ability of *Escherichia coli* Lrp to bind DNA, activate transcription, or respond to leucine. J. Bacteriol. **175:**1110–1117.
25. **Platko, J. V., D. A. Willins, and J. M. Calvo.** 1990. The *ilvIH* operon of *Escherichia coli* is positively regulated. J. Bacteriol. **172:**4563–4570.
26. **Tani, T. H., A. Khodursky, R. M. Blumenthal, P. O. Brown, and R. G. Matthews.** 2002. Adaptation to famine: a family of stationary-phase genes revealed by microarray analysis. Proc. Natl. Acad. Sci. USA **99:**13471–13476.
27. **Tuan, L. R., R. D'Ari, and E. B. Newman.** 1990. The leucine regulon of *Escherichia coli* K-12: a mutation in *rblA* alters expression of L-leucine-dependent metabolic operons. J. Bacteriol. **172:**4529–4535.
28. **Zhi, J., E. Mathew, and M. Freundlich.** 1999. Lrp binds to two regions in the *dadAX* promoter region of *Escherichia coli* to repress and activate transcription directly. Mol. Microbiol. **32:**29–40.