Metabolic regulation of *lrp* gene expression in *Escherichia coli* K-12

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Expression of the *lrp* gene is regulated in part by the nutrients available to the cell, and is decreased in rich medium, in glucose minimal media enriched with amino acids, and in minimal medium with alternative carbon sources, such as acetate and succinate. When Lrp production is increased in a given medium, expression of its target genes is also increased. However, when the medium is changed from glucose to acetate, the response of the target genes is governed by many factors.

Keywords: *Escherichia coli*, leucine-responsive regulatory protein, regulation of *lrp* expression, metabolism

INTRODUCTION

The leucine-responsive regulatory protein (Lrp) is a global regulator of *Escherichia coli* metabolism, and is particularly interesting because of the number and metabolic diversity of the reactions it affects (Newman *et al.*, 1992; Newman & Lin, 1995; Calvo & Matthews, 1994). Lrp influences expression of genes whose products are involved in amino acid biosynthesis and degradation, nitrogen metabolism, carbohydrate degradation, synthesis of fimbriae, and transport. Regulation of some of these genes requires leucine as an effector, in which cases Lrp may be thought of as a regulator of variable gene expression like Crp (Lin *et al.*, 1990). For these genes, leucine would act as a regulator just as cAMP does. In other cases, e.g. *gltBDF* (Ernsting *et al.*, 1992) and *gcv* (Lin *et al.*, 1992), leucine has no effect *in vivo*, though Lrp is required for gene expression to the degree that the *lrp* mutant is metabolically deficient for both functions. For these genes, expression is possible as long as Lrp is made at normal levels. Lrp cannot be considered to regulate expression of these genes unless Lrp expression itself varies, or Lrp has effector(s) other than leucine (D'Ari *et al.*, 1993).

Autogenous repression of *lrp* expression by Lrp itself has been previously described (Lin *et al.*, 1992; Wang *et al.*, 1994) and depends on Lrp binding to well-characterized sites upstream of the coding sequence (Wang *et al.*, 1994). However, there is also a strong metabolic effect on Lrp expression which does not depend on the Lrp molecule as shown by the fact that a strain carrying

a *lacZ* insert in the *lrp* gene produces about 1500 Miller units of β -galactosidase in glucose minimal medium (GMM) and only 115 Miller units in Luria broth (LB) (Lin *et al.*, 1992).

In this paper, we consider some of the factors which may regulate expression of the *lrp* gene. We use a strain carrying a chromosomal *lrp::lacZ* fusion to define, in the absence of the Lrp gene product, which factors are responsible for this large reduction in *lrp* expression. We then show that a variation in Lrp concentration *in vivo* can influence expression of the target genes, other things being equal, and that there are other regulators of these target genes.

METHODS

Cultures and growth conditions. The plasmids and the strains used in this study, all derivatives of *E. coli* K-12, are described in Table 1. Carbon sources were added at 0.2% unless otherwise noted. Antibiotics were used as follows: 50 μ g kanamycin ml⁻¹, 30 μ g chloramphenicol ml⁻¹, 100 μ g ampicillin ml⁻¹ and 15 μ g tetracycline ml⁻¹. Minimal medium and growth conditions were as described by Su & Newman (1991).

Genetic methods. Plasmid isolations, DNA manipulations, transductions and transformations were performed as described by Maniatis *et al.* (1982) and Miller (1972).

Enzyme assays. β -Galactosidase was assayed in whole cells according to the method of Miller (1972) and its activity is expressed in Miller units.

Plasmid construction. To construct pMEW100 (pBAD22lrp), we used the *lrp* gene which was previously cloned into pBAD18 with three mutations introduced in its upstream region (data not shown), forming pHE1. To create a

Abbreviation: Lrp, leucine-responsive regulatory protein.

Table 1. Plasmids and *E. coli* K-12 strains used

Strain/plasmid	Genotype and/or relevant characteristics	Source/reference
Strains		
CU1008	<i>ilvA</i>	L. S. Williams
MEW1	CU1008 Δlac	Lin <i>et al.</i> (1990)
MEW22	MEW1 <i>sdaA::</i> λ <i>placMu9</i>	Su <i>et al.</i> (1989)
MEW26	MEW1 <i>lrp::Tn10</i>	Lin <i>et al.</i> (1990)
MEW45	MEW1 <i>lrp::lacZ</i>	Lin <i>et al.</i> (1992)
CP8	MEW1 <i>gltD::lacZ</i>	Tchetina & Newman (1994)
MEW305	<i>serA::</i> λ <i>placMu9</i>	Z. Q. Shao
MEW306	MEW305 <i>lrp::Tn10</i>	This work
MEW307	MEW45 <i>ilvA</i> ⁺	This work
CP67	<i>gcv::lacZ</i>	Lin <i>et al.</i> (1990)
CV975	<i>ilvIH::lacZ</i>	Platko <i>et al.</i> (1990)
Plasmids		
pBAD22		Guzman <i>et al.</i> (1995)
pLRN1	pACYC84 <i>lrp</i> ⁺	Lin <i>et al.</i> (1992)
pMC1871	pBR322 <i>lacZ</i> ⁺	Lin <i>et al.</i> (1992)
pMEW100	pBAD22 <i>lrp</i> ⁺	This work
pMEW101	pBAD22 <i>lacZ</i> ⁺	This work

pBAD::*lrp* protein fusion on pBAD22, the coding region of the *lrp* gene was amplified from pHE1 using primers Lrp1, GGGGTACCCATGGTAGATAGCAAGA and Lrp2, GAAAAGCTTGTAACCTGGAG. The PCR product was cut with *Kpn*I and *Hind*III and ligated to pBAD22 digested with the same restriction enzymes, resulting in pMEW100. This construction method results in the fusion of the complete coding region of the *lrp* gene to the 3rd codon of the *araB* gene, under the control of the *araBAD* promoter.

To construct a pBAD*lrp::lacZ* fusion, a 3 kb *Bam*HI fragment carrying *lacZ* from pMC1871 was purified from a gel using a QIAGEN gel purification kit and ligated to pMEW100 digested with *Bgl*II. The ligation mix was transformed into strain MEW1 and blue colonies were selected on LB plates with 0.2% arabinose and 50 μ g X-Gal ml⁻¹. The resulting plasmid, pMEW101, carries the *lacZ* gene fused in-frame to the 10th codon of *lrp* under the control of the *araBAD* promoter.

RESULTS

Effect of amino acids on *lrp* expression

In strain MEW45, the *Lrp*-deficient mutant used here, growth in GMM gave higher expression of *lrp::lacZ* than we have seen in any conditions tested during exponential phase: 1445 units (Table 2). This was reduced more than 10-fold by growth in LB with glucose, to 98 units, the lowest value we measured. The strong repressive effect of LB was almost equalled by adding Casamino acids (1%) to GMM (170 vs 113 units; Table 2), suggesting that most of the effect of LB is due to amino acids (and not, for instance, to the absence of glucose).

Addition of various amino acids at 50 μ g ml⁻¹ reduced *lrp* expression, but two groups of amino acids produced

a more significant reduction: those derived from oxaloacetate, and those derived from 2-oxoglutarate (Table 2). The diversity of amino acids decreasing *lrp* expression suggests that this may be a general metabolic effect related to general nitrogen and/or carbon nutrition rather than to any specific amino acid. Each of the oxaloacetate-derived amino acids (asparagine, aspartic acid, methionine, lysine and threonine) reduced expression from 1445 to between 1086 and 1193 units (Table 3). However threonine at 50 μ g ml⁻¹ had the most pronounced effect, reducing expression to about 50% (from 1445 to 772 units). A threefold increase in threonine to 150 μ g ml⁻¹ reduced expression only slightly more (to 725 units; data not shown). Adding methionine and lysine with the threonine did not substantially increase inhibition (data not shown). In the 2-oxoglutarate family, no particular amino acid seemed to predominate. Glutamine and glutamate together reduced expression to 1041 units, and arginine and proline to 870. Biosynthesis of serine and leucine limit the growth of an *lrp* mutant, especially at high temperature (Ambartsoumian *et al.*, 1994). We therefore assayed cultures grown with L-serine and L-leucine at 150 μ g ml⁻¹ each, obtaining a value of 1050 units (data not shown). When glycine was also added, a greater decrease was seen (645 units, Table 3).

Threonine also had pronounced and specific effects on the growth of the *lrp* mutant. Addition of threonine (150 μ g ml⁻¹) transiently inhibited growth of the *lrp* mutant. Subculture of cells grown in GMM into medium with 150 μ g threonine ml⁻¹ inhibited growth for about 220 min, after which the cells began to grow again at close to the same rate as cells growing without threonine. This inhibition was not seen in a similar experiment

Table 2. Effect of nutrition on expression from *lrp::lacZ*

Cells of strain MEW45 were grown at 37 °C overnight in liquid media, subcultured and assayed after 3–4 h (mid-exponential phase) and several hours later (late-exponential/stationary phase). Values given are the means $\pm$ SD of at least three determinations. Isoleucine and valine were present in all media. LB, Luria broth. Glucose minimal medium (GMM) includes aspartic acid, asparagine, lysine, methionine and threonine at 50 μ g ml⁻¹. AA family: includes glutamic acid, glutamine, arginine and proline. 2-OG family: includes serine, glycine and cysteine. ND, Not determined.

Growth medium	β -Galactosidase activity	
	Mid-exponential phase	Late-exponential/stationary phase
1. LB	113 $\pm$ 14	304 $\pm$ 104
2. LB + glucose 0.2 %	98 $\pm$ 9	ND
3. GMM	1445 $\pm$ 262	1775 $\pm$ 488
4. GMM + CAA 1 %	170 $\pm$ 21	296 $\pm$ 83
5. GMM + AA family	303 $\pm$ 33	835 $\pm$ 197
6. GMM + 2-OG family	430 $\pm$ 43	842 $\pm$ 210

Table 3. Effects of amino acids on expression from *lrp::lacZ*

Cells of strain MEW45 were grown at 37 °C overnight in liquid GMM with various supplements, each at 50 μ g ml⁻¹, subcultured in the same medium and assayed after 3–4 h. All values are means $\pm$ SD of at least three determinations. Valine and isoleucine were added to all cultures.

Supplement	β -Galactosidase activity
Glutamine, glutamate	1041 $\pm$ 59
Arginine, proline	870 $\pm$ 57
Asparagine, aspartate	1132 $\pm$ 32
Methionine	1086 $\pm$ 26
Lysine	1193 $\pm$ 67
Threonine	772 $\pm$ 93
Serine, glycine, cysteine	645 $\pm$ 76

with the parent strain, MEW1. Since one of the two aspartokinase-homoserine dehydrogenase enzymes, the *thrA* gene product, is inhibited by threonine, the transient stoppage in growth might be due to a requirement for Lrp in transcription of *metL*, the gene encoding the second enzyme. Adding threonine to an Lrp-deficient strain would then block methionine biosynthesis. Dependence on Lrp for activation of the gene encoding the first step in biosynthesis is also seen for leucine and serine (Newman & Lin, 1995; Calvo & Matthews, 1994).

Effect of carbon and energy source on *lrp* expression

Expression of *lrp* also varied a great deal in cultures grown with different carbon sources. Expression was most severely reduced (to about 40% of the glucose-grown level) in cells grown with succinate and acetate

Table 4. Effect of carbon source on expression from *lrp::lacZ*

MEW45 cells were grown at 37 °C overnight in liquid minimal medium with various carbon sources, subcultured in the same medium and assayed after 3–4 h. All values are means $\pm$ SD of at least three determinations.

Carbon source	β -Galactosidase activity
Glucose	1445 $\pm$ 262
Rhamnose	1080 $\pm$ 194
Arabinose	1173 $\pm$ 84
Glycerol	1094 $\pm$ 26
Pyruvate	961 $\pm$ 42
Acetate	558 $\pm$ 34
Succinate	584 $\pm$ 45

(Table 4). Expression was much less reduced in cells grown with glycerol or pyruvate, and with a variety of sugars (maltose, rhamnose and arabinose). Levels were higher in glucose-grown cultures than in any other conditions tested. However, addition of glucose to rich media had little effect, repressing very slightly (Table 2).

Variation in *lrp* expression during growth

All values quoted so far were taken from overnight batch cultures subcultured for several hours and assayed in the exponential phase. These cultures were also assayed several hours later, i.e. in late-exponential or early-stationary phase, and in every case showed a higher level later (Table 2). To see if this effect was due to the cultures becoming anaerobic, we transferred exponential-phase cells to GMM-filled containers and assayed *lrp* after 1.5 h. No increase was seen (data not shown).

Effect of Lrp on *lrp* expression

The experiments quoted above describe the behaviour of the cell in the absence of autogenous regulation. We also tested *lrp::lacZ* expression in strain MEW45 (pLRN1) carrying a functional *lrp* gene on a multicopy plasmid. Cells with several copies of a functional *lrp* gene produced only 244 ± 40 units of β -galactosidase in GMM, whereas cells grown in succinate minimal medium or in LB produced considerably less (182 ± 13 and 122 ± 11 units, respectively). It seems that expression of the *lrp* gene can be strongly repressed either by nutritional changes or by overproduction of Lrp on a multicopy plasmid.

Variation in *serA* expression as influenced by Lrp production

The preceding experiments show that *lrp* expression is altered by a number of factors. This could be of physiological importance only if variations in *lrp* expression within a physiological range influenced the expression of genes regulated by Lrp. We show that variations in Lrp are in fact closely reflected in *serA* expression by measuring *serA* expression in a cell carrying a chromosomal *serA::lacZ* fusion and a plasmid carrying a functional *lrp* gene under the control of the arabinose promoter. We grew strain MEW306 transformed with pBAD*lrp*⁺ in glycerol minimal medium with the arabinose concentrations noted, and found that *lacZ* expression from *serA::lacZ* followed the arabinose concentration from 0 to between 10 and 20 $\mu\text{g arabinose ml}^{-1}$ (Fig. 1a, curve 1). Using strain MEW26 *lrp::Tn10* pBAD*lrp::lacZ*, we showed that *lacZ* expression from *lrp::lacZ* responds similarly over the same range (Fig. 1a, curve 2). These data are replotted in Fig. 1(b) as a percentage of the usual wild-type expression from *serA::lacZ* in glycerol minimal medium (850 units; data not shown). This figure shows that 20 $\mu\text{g arabinose ml}^{-1}$ is enough to achieve expression at a level close to that of a normal Lrp-producing cell. In Fig. 1(c), an estimate of *serA* expression as a function of Lrp is obtained by plotting the curves in Fig. 1(a) against each other. This indicates that at levels of Lrp up to about 600 units β -galactosidase, *serA* expression varies linearly with Lrp.

Effect of growth conditions on expression of Lrp-regulated genes

It is clear that variations in Lrp production may be reflected in target gene expression, all other things being equal. To determine whether this is true in usual growth conditions where all things are not equal, we compared the expression of target genes in GMM with that seen in LB and in succinate minimal medium where the level of *lrp* expression is considerably lower (Table 5).

If Lrp concentration were the major determinant of expression of the genes whose expression it is known to affect, one would expect the level of these to vary in a systematic manner in these media. We tested expression

Fig. 1. Expression of *lrp* under the control of the arabinose promoter pBAD. (a) Cells of strain MEW26 *lrp::Tn10* pMEW101 (pBAD/*lacZ*), curve 1 (●), and strain MEW306 *lrp::Tn10* *serA::lacZ* pMEW100 (pBAD/*lrp*), curve 2 (○), were grown overnight in glycerol minimal medium with various arabinose concentrations, subcultured in the same medium, and assayed for β -galactosidase in mid-exponential phase. L-Serine ($500 \mu\text{g ml}^{-1}$) was added to both cultures because serine is required by MEW306. The basal expression of *serA::lacZ* from a second Lrp-independent promoter (253 units) is subtracted from all values in curve 2. (b) Replotting of (a) curve 1 as a percentage of the *serA::lacZ* expression seen in strain MEW305 grown in glycerol minimal medium with serine $500 \mu\text{g ml}^{-1}$ (850 units from which the same 253 units were subtracted). (c) Replotting of (a): curve 2 is plotted against curve 1, showing *serA* expression as a function of Lrp concentration.

of five genes, *gcv*, *serA*, *ilvIH*, *gltD* and *sdaA*, carrying *lacZ* translational fusions and found that expression of these genes varies in a much more complex way than does *lrp* expression (Table 5). It is obvious that while variations in Lrp production can affect target gene expression, Lrp is only one of the factors that determines the rate of expression of its target genes.

DISCUSSION

In this paper, we demonstrate a number of metabolic factors influencing the expression of the *lrp* gene in *E. coli* K-12. *lrp* expression varied according to both carbon

Table 5. Effect of growth medium on expression of Lrp-regulated genes

For this experiment strains were grown at 37 °C overnight in liquid media as stated, subcultured in the same medium, and assayed after 4 h. All values are means $\pm$ SD of at least two determinations and cited as in Table 2. SMM, succinate minimal medium.

Strain	β -Galactosidase activity in		
	GMM	LB	SMM
CP8	347 $\pm$ 60	20 $\pm$ 2	146 $\pm$ 11
MEW305	472 $\pm$ 31	189 $\pm$ 11	385 $\pm$ 16
CP67	248 $\pm$ 16	166 $\pm$ 12	1421 $\pm$ 61
MEW22	58 $\pm$ 4	156 $\pm$ 10	45 $\pm$ 3
CV975	255 $\pm$ 30	7 $\pm$ 1	199 $\pm$ 29

and nitrogen metabolism. Expression in GMM decreased with the addition of almost any amino acid, particularly those like threonine, which were derived from tricarboxylic acid (TCA) cycle intermediates. Indeed the only amino acids without much effect were leucine and the aromatic amino acids. Expression was not affected by growth on hexoses, glycerol or pyruvate, but was greatly reduced during growth on acetate and succinate. It seems that expression of *lrp* is correlated in some way with the use of the TCA cycle. However the physiological meaning of this correlation is unclear. The mechanism for this metabolic regulation of *lrp* expression is not known. In any case, it does not involve the *lrp* gene product, since these studies were done in an Lrp-deficient strain. The presence of Lrp decreased expression levels in all cases, but metabolic regulation could be shown even when Lrp was present. Autogenous regulation involves binding of Lrp to a site between 32 and 80 bp upstream of the transcription start site of the *lrp* gene, as indicated by gel-mobility shifts, DNA footprinting, and mutation and deletion studies (Wang *et al.*, 1994). In the metabolic regulation system, neither a signalling molecule(s) nor its effector has been identified.

Effects of variation in Lrp concentration on gene expression

The total absence of Lrp, as in the *lrp* mutant, abolishes expression of several genes, including *gcv* and *gltD*, making an *lrp* mutant physiologically deficient in these functions. However a quantitative relationship between Lrp production *in vivo* and target gene expression has not been shown. We show here that gradual variation in Lrp concentration directly affects *serA* expression, as judged by the effect of variations in induction of *lrp* on a variable expression vector such that the Lrp concentration varied with the arabinose concentration provided to the cells. In the experiments shown in Fig. 1, *serA::lacZ* expression was about 250 units without Lrp,

and reached 900 units with 50 μ g arabinose ml⁻¹. We conclude that *serA::lacZ* expression is directly responsive to variations in Lrp concentration within a physiological range. In similar experiments, *lacZ* fusions to *gcv*, *gltD* and *sdaA* behaved similarly (data not shown).

In the preceding experiments, Lrp concentration was the only factor varied. Target gene expression was also studied in two conditions in which *lrp* expression was decreased, but many other metabolic conditions varied: in LB, a rich medium containing a variety of biosynthetic precursors, and in a minimal medium with succinate as carbon source, where the cell relies on gluconeogenesis.

In these experiments, a direct correlation between Lrp concentration and target gene expression could not be seen. This is hardly surprising, since these media vary in so many factors other than Lrp concentration, and each of the target genes has multiple promoters and may respond to several regulators.

For several genes, expression depends absolutely on the presence of Lrp. Above a certain threshold level, the amount of Lrp may not influence expression much, especially for those promoters with high affinity for Lrp. For these genes, Lrp is needed to set the stage, and may thus be involved in determining chromosome structure as suggested earlier by D'Ari *et al.* (1993). A variety of other factors from end-product inhibition to other general transcription factors may be involved in the actual setting of the transcription rate of the target genes.

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