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λ *plac*Mu Insertions in Genes of the Leucine Regulon: Extension of the Regulon to Genes Not Regulated by Leucine

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The leucine regulon coordinates the expression of several *Escherichia coli* genes according to the presence of exogenous leucine, which interacts with the *lrp* gene product, Lrp. We isolated and characterized 22 strains with λ *plac*Mu insertions in Lrp-regulated genes. Lrp and leucine influenced gene expression in a surprising variety of ways. We identified two genes that are regulated by Lrp and not affected by L-leucine. We therefore rename this the leucine-*lrp* regulon. Genes coding for glycine cleavage and leucine biosynthesis enzymes have been identified as members of the leucine-*lrp* regulon. We suggest that the *lrp* gene product activates genes needed for growth in minimal medium, and we show that the gene is repressed by its own product and is highly repressed during growth in rich medium.

We recently showed that in *Escherichia coli* the branched-chain amino acid L-leucine is the effector of a global response that governs a group of genes known as the leucine regulon (11). Regulation of the synthesis of a number of enzymes from different metabolic pathways is affected by the presence of L-leucine in the growth medium (11, 16). This response to exogenous L-leucine was altered in an *E. coli* mutant carrying an insertion in a gene at 20 min (11). This gene, referred to originally as *rbl*, was renamed *lrp* by consensus among interested investigators (11, 20).

The *lrp* gene codes for a 38-kDa dimer, the leucine-responsive regulatory protein (Lrp), which has been purified extensively (31). Under the name of the *ilvIH* binding protein, it was shown to bind to two sites upstream of *ilvIH* (22), for which it is a positive regulator. A mutation in *lrp* made the strain constitutive for *ilvIH* expression (20) and changed only one nucleotide, resulting in a substitution of Glu for Asp. The *lrp* gene was shown to be identical to *oppI* (2) and is also thought to be identical to *livR* (12, 20).

Until now, the genes of the leucine regulon have been identified by guesswork or by the accident of a previously known regulatory gene proving to be identical to *lrp*. As a result of a somewhat different accident, *lysU* has now been added to the regulon. We present here the results of a screening among random λ *plac*Mu9 insertions for leucine- and Lrp-regulated expression of *lacZ*. We used a system based on that used for the isolation of *din* genes (8). We also show that some genes are regulated by Lrp without leucine, and therefore we rename the regulon as the leucine-Lrp regulon.

MATERIALS AND METHODS

Cultures. The strains used in this study—all derivatives of *E. coli* K-12—are described in Table 1. The plasmids used are also listed in Table 1. Cultures were grown as described previously (11) with L-isoleucine and L-valine (each at 50 μg/ml) added to the minimal medium used to grow strain CU1008 and all its derivatives to compensate for the *ilvA* mutation carried by these strains.

Map locations. Map locations were determined first by conjugation and then by transductions with strains with conveniently placed Tn*10* insertions as devised by Singer et al. (24).

Other genetic techniques. Plasmid isolations, DNA manipulations, transductions, and transformations were performed as described previously (13, 14).

Enzyme assays. L-Serine deaminase was assayed in toluene-treated whole cells as previously described (11). β-Galactosidase activity, assayed in whole cells by the method of Miller, is expressed in Miller units (14).

Strain construction. (i) Isolation of strains with mutations in genes whose expression is controlled by leucine and the *lrp* gene product. From a random pool of λ *plac*Mu inserts, we selected those whose expression was affected by leucine. We then transduced an *lrp* mutation into the strains and kept for further study those that were affected by Lrp. Strain MEW1 was infected with λ *plac*Mu9 and helper phage λ pMu507 (3), incubated for 1 h in tryptone broth, centrifuged, washed, and plated on minimal medium with lactose and kanamycin (each at 80 μg/ml) with and without leucine (300 μg/ml). The resultant colonies were streaked on LB medium containing kanamycin and on minimal lactose-kanamycin medium with and without L-leucine. β-Galactosidase assays were made on strains in which the growth rate seemed to be altered by the presence of L-leucine (100 μg/ml). The *lrp*::Tn*10* mutation was transduced into relevant strains, and β-galactosidase was again assayed. Mutations in genes affected by Lrp and L-leucine were transduced by antibiotic resistance into strain MEW1, and the *lrp* double mutants were created by transduction as described above. The strains with target mutations transduced into strain MEW1 (CP1 through CP66) and the corresponding *lrp* derivatives were used for the studies described herein.

(ii) Isolation of strains with mutations in the glycine cleavage operon. Strain MEW85 *serA* was infected with λ *plac*Mu9 as described above and incubated in minimal glucose-kanamycin medium with glycine; ampicillin was added after 60 min. After about 4 h, the cells were washed and spread on minimal lactose-kanamycin plates with L-serine. Colonies that grew with glucose and L-serine but not with glucose and glycine were retained as possible glycine cleavage mutants (18). The glycine cleavage mutations were confirmed by mapping with the Singer mapping kit (24) and by a further

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TABLE 1. Bacterial strains, plasmids, and phages

Strain, plasmid, or phage	Genotype or relevant characteristics	Source or reference
E. coli		
CU1008	*E. coli* K-12 Δ*ilvA*	L. S. Williams
MEW1	Δ *lac* derivative of strain CU1008	11
MEW26	MEW1 *lrp*::Tn*10*	This work
MEW28	*sdaA*::Cm^r	28
MEW36	MEW22 *lrp*::Tn*10*	11
CP1-68	MEW1 carrying λp*lac*Mu9 inserts in various genes	This work
MEW45	MEW1 *lrp*::*lacZ*	This work
MEW85	MEW1 *serA*	This work
Plasmids		
pACYC184		5
pGS146	pACYC184 carrying *gcv*	26
pBluescript		Stratagene Co.
pWB6	pBluescript carrying *sdaB*	Z. Q. Shao
pLRN1	pACYC184*lrp*$^+$	This work
pLRN2	p*lrp*::*lacZ*	This work
pMC1871	pBR322 carrying *lacZ*	R. K. Storms
Phages		
λ 1F10		9
λ p*lac*Mu9	λ p*lac*Mu1, Kan^r	3
λ pMu507	λ *c*I ts857 *S*am7 MuA$^+$B$^+$	3

transduction into MEW85*serA* to verify the phenotype. For physiological studies, two independently isolated glycine cleavage mutations were transduced into strain MEW1, creating strains CP67 and CP68.

(iii) Isolation of a strain carrying an insertion in the *lrp* gene. Strain MEW85 *serA* was infected with λ p*lac*Mu9, treated as described above, and then spread on plates with serine as the carbon source to select for *lrp* mutants. Strains able to grow on glucose-minimal medium supplemented with L-serine but not in the same medium supplemented with glycine were retained as possible *lrp* mutants. We identified the strains that carried insertions in *lrp* by transducing tetracycline resistance from an *lrp*::Tn*10* (Tet^r) mutant into the putative *lrp*::λ p*lac*Mu (Kan^r), identifying as *lrp* mutants those in which 100% of the 50 tetracycline-resistant transductants tested were sensitive to kanamycin.

(iv) Isolation of a serine-requiring mutant. Strain MEW85 *serA* was isolated from strain MEW1 by penicillin selection in a UV-treated population, selecting for a strain that required either L-serine or glycine. That the mutation was in *serA* was confirmed by demonstrating 20% linkage to *metK* and by cotransduction with a Tn*10* element in Singer strain 18604.

Construction of plasmids carrying *lrp* and *lrp*::*lacZ*. We subcloned the *lrp* gene from Kohara phage λ 1F10 onto pACYC184 by using a *serA lrp* double mutant, which cannot use glycine as a source of L-serine, and selecting for transformants that could grow on glucose-minimal medium with glycine, thus creating pLRN1 *lrp*$^+$. We then inserted *lacZ* from pMC1871 into the *Bgl*II site of *lrp*, thus making an in-frame fusion of *lacZ* to *lrp*, forming plasmid pLRN2 *lrp*::*lacZ*.

TABLE 2. Activity of β-galactosidase transcribed from Lrp-regulated promoters[a]

Class[b]	Strain	β-Galactosidase activity (U) in medium with:	
		No addition	Leucine
1	CP4	525	250
	CP4 *lrp*	15	15
	CP5	325	20
	CP5 *lrp*	15	15
2	CP61	30	75
	CP61 *lrp*	1,250	1,150
	CP60	20	100
	CP60 *lrp*	160	160
3	CP52	25	125
	CP52 *lrp*	30	25
4	CP36	525	5
	CP36 *lrp*	525	425

[a] Typical strains from each class were grown in glucose-minimal medium with and without leucine (100 μg/ml) and subcultured in the same medium, all at 37°C; then β-galactosidase activity was assayed. For classes 1 and 2, two strains were chosen, the first with the largest effect of the *lrp* mutation, and the second with the largest effect of leucine. The results given are the averages of three experiments. Leucine reduced expression to from 10 to 50% in various class 1 mutants and increased it from 1.7- to 4.5-fold in class 2 mutants. The *lrp* mutation reduced expression to from 4 to 20% in class 1 mutants and increased it 2- to 42-fold in class 2 mutants. Leucine and Lrp together stimulated expression two- to fivefold in class 3 mutants. No range can be given for class 4 mutants, since only one mutant of this type was isolated.

[b] In class 1 mutants (11 isolates), Lrp activates and leucine decreases its effect. In class 2 mutants (6 isolates), Lrp represses and leucine decreases its effect. In class 3 mutants (4 isolates), Lrp and leucine together activate β-galactosidase activity. In the class 4 mutant, Lrp and leucine together repress β-galactosidase activity.

RESULTS

Isolation and preliminary characterization of λ p*lac*Mu inserts in genes controlled by leucine and the *lrp* gene product. We isolated a number of mutants in which λ p*lac*Mu was inserted into unidentified genes whose expression was affected by L-leucine and the *lrp* gene product (see Materials and Methods). To characterize these, β-galactosidase was assayed in these strains and in the corresponding *lrp* derivatives grown in minimal medium with and without L-leucine (100 μg/ml).

The expression of the various promoters was affected by L-leucine and by the *lrp* gene product in different ways (Table 2). In some cases (class 1), Lrp activated gene expression and L-leucine decreased activity, a pattern described previously for *ilvIH* (20) and *serA* (11). In others (class 2), Lrp repressed and L-leucine increased activity, as in *lysU* and *sdaA* (11, 12). In class 3, both the *lrp* gene product and L-leucine were needed to induce activity; in class 4, both were needed to repress activity.

In the first 22 isolates studied, we found 11 of class 1, 6 of class 2, 4 of class 3, and only 1 of class 4. Where possible, the results for two strains of each class are given in Table 2; the strain in which an *lrp* mutation gives the largest effect is cited first, and then the strain with the largest L-leucine effect is given. Ranges for the entire class are cited in footnote *a* to Table 2. Most of the genes affected have not yet been identified, nor is it known how many different genes are represented. However, all were assayed for L-serine deaminase and proved normal for that activity.

In class 1 mutants, the effect of the absence of Lrp on gene

TABLE 3. Regulation of gene expression in *gcv* mutants[a]

Strain	Relevant genotype	β-Galactosidase level in medium with:	
		No addition	Leucine (100 μg/ml)
CP67	*gcv*	1,675	1,450
CP67	*gcv lrp*	75	75
CP68	*gcv*	1,475	1,250
CP68	*gcv lrp*	75	75

[a] Results are expressed as described in footnote *a* of Table 2. Each experiment is the average of two or three determinations.

TABLE 4. Regulation of gene expression in leucine auxotrophs[a]

Strain	β-Galactosidase activity (U) in medium with leucine at:		Effect of leucine starvation[b]
	50 μg/ml	5 μg/ml	
CP55	850	4,450	7
CP55 *lrp*	75	1,700	23
CP66	775	400	0.5
CP66 *lrp*	300	50	0.17

[a] Results are expressed as described in footnote *a* of Table 2. Each experiment is the average of two or three determinations. Strain CP55 is representative of three other isolates giving very similar results.

[b] Ratio of β-galactosidase activity with 5 μg of leucine per ml to that with 50 μg of leucine per ml.

expression was much greater than that of the addition of L-leucine to the wild type; this was observed previously for *serA* and *ilvIH* functions (11). These results suggest that the *lrp* gene product is needed for expression of these genes and that L-leucine modulates expression of the activated gene, probably by interacting with Lrp. However, leucine probably does not totally reverse Lrp action, as by removing Lrp from the DNA. The repressive effect of Lrp in class 2 mutants is also greater than the effect of L-leucine for most isolates, as is the case with class 2 prototype genes, *sdaA* and *lysU* (11, 12). On the other hand, L-leucine had no effect on gene expression in *lrp* mutants of classes 1 through 4.

Regulation of the glycine cleavage operon by Lrp but not by leucine. We showed above that Lrp can interact with L-leucine and the promoters it regulates in at least four ways. Study of the glycine cleavage (*gcv*) genes shows that Lrp can also have drastic effects on expression of genes that are not affected by L-leucine.

Since Lrp controls synthesis of several enzymes in related areas of metabolism (L-serine deaminase, phosphoglycerate dehydrogenase, and L-threonine dehydrogenase), we thought that enzymes of glycine metabolism might also be affected by Lrp. The following experiment shows that Lrp, in fact, regulates expression of the *gcv* genes.

We isolated strains that carried λ p*lac*Mu insertions in the *gcv* operon as described previously (18) by screening a random pool of inserts in a *serA* strain for those that could use serine but not glycine to fulfill their auxotrophic requirement. The mutations were then transduced to strain MEW1 to form strains CP67 and CP68, which carried the *gcv* mutation but not *serA* (see Materials and Methods).

That strain CP67 indeed carried a *gcv* mutation was confirmed by the map position of the insertion, with 40% linkage to *serA* (63.0 min), consistent with the location of the *gcv* operon at 62.6 min. Strain CP68 was mapped in the same way with similar results. To be sure that these inserts were really in the *gcv* genes, we transformed pGS146 *gcv*$^+$ into the *serA gcv* parent of CP67 and found that the plasmid complemented the *gcv* mutation and allowed the strain to grow with glucose and glycine.

We then assayed strains CP67 and CP68 and their respective *lrp* derivatives for β-galactosidase activity (Table 3). The absence of Lrp decreased transcription of the *lacZ* insert to a very low level, 4% of the parent strain, regardless of whether L-leucine was present. The addition of L-leucine to either the parent or the *lrp* mutant had no significant effect (the β-galactosidase level with L-leucine was 85% of the level without L-leucine). We conclude that Lrp is absolutely required for *gcv* transcription. This is the first operon identified in which Lrp regulation is unaffected by leucine.

Physiological deficiency in glycine cleavage in the *lrp* mutant. If the requirement for Lrp in the transcription of the *gcv* operon is indeed close to absolute, an *lrp* mutant (e.g., MEW26 *lrp*::Tn*10*) should have the same physiological deficiency as a *gcv* mutant. The *lrp* mutant would be unable to cleave glycine to C_1-tetrahydrofolic acid and would therefore be unable to derive either L-serine or nitrogen from glycine. In fact, the *lrp* mutant was deficient in both of these functions.

Although the parent strain MEW1 could grow in glucose minimal medium with glycine as nitrogen source, strain MEW26 *lrp*::Tn*10* could not. Further, whereas a *serA* mutant can use either L-serine or glycine provided exogenously as a source of cellular serine, the *serA lrp* double mutant we constructed could only use L-serine. This is presumably due to the inability of the *serA lrp* mutant to derive C_1 units from glycine, resulting in a serine deficiency in cells incubated in glucose-glycine medium. It is clear, then, that the *lrp* mutation reduces *gcv* expression to a physiologically insignificant level.

A glycine cleavage deficiency was further confirmed by showing similar growth problems in an *lrp glyA* double mutant. *E. coli* derives its C_1 units mainly from the conversion of serine to glycine via the *glyA* gene product, serine hydroxymethyltransferase, and from glycine cleavage. An *lrp glyA* mutant should have neither serine hydroxymethyltransferase nor glycine cleavage enzymes and so should have great difficulty in generating C_1 units.

To test this, we made an *lrp glyA* mutant by transducing *lrp*::Tn*10* into MEW1 *glyA*. This strain had severe growth problems consistent with a loss of glycine cleavage activity. The strain could not grow in glucose-minimal medium. It grew well on LB plates supplemented with purines, L-methionine, L-histidine, and thymidine, which require C_1 units for synthesis, but grew slowly on LB plates without such supplements. The addition of these compounds to glucose-minimal medium did not support growth. It is clear, therefore, that Lrp is a major physiological effector of synthesis of glycine cleavage enzymes.

Identification of other Lrp-regulated genes: insertions in some Lrp-regulated genes leads to leucine auxotrophy. The system we used for isolating insertion mutants in Lrp-regulated genes would not allow us to isolate auxotrophs, with the exception of those requiring leucine. In fact, five of our insert-carrying strains required L-leucine. In all of them, the *lrp* mutation reduced expression from *lacZ* in cells grown with sufficient leucine for growth (50 μg/ml) (Table 4).

All were mapped by using the Singer kit and showed linkage to a Tn*10* element at 2 min (strain CAG12095) of 82% for strain CP66 and 97 to 100% for the other four strains. It

TABLE 5. Regulation of *lrp* expression[a]

Strain	Relevant genotype	β-galactosidase activity (U) in:		
		Minimal medium with:		LB
		No addition	Leucine	
MEW45(pACYC184)	*lrp*::*lacZ* pACYC184	1,550	1,475	125
MEW45(pLRN1)	*lrp*::*lacZ* pACYC184*lrp*	150	200	75
MEW1(pLRN2)	*lrp*$^+$ p*lrp*::*lacZ*	4,550	5,325	900
MEW26(pLRN2)	*lrp* p*lrp*::*lacZ*	13,600	11,875	900

[a] The strains indicated were each grown at 37°C in glucose-minimal medium with and without L-leucine (100 μg/ml), with antibiotics appropriate to the particular plasmids, and subcultured in the same medium; then β-galactosidase activity was assayed. The values given are the averages of three determinations.

seems likely that the four strains, and probably all five, carry insertions in the 1.8-min L-leucine biosynthesis operon.

Because strain MEW1 *ilvA* requires L-isoleucine for growth, we transduced each of these insertion mutations into an *ilvA*$^+$ derivative of MEW1, allowing us to test for growth in the absence of isoleucine. Four strains (*ilvA*$^+$ derivatives of CP53, CP54, CP55, and CP64) grew with L-leucine only; but one (CP66 *ilvA*$^+$) grew much more slowly than the others in this medium.

If (some of) the insertions were in L-leucine biosynthesis genes, as these results suggest, one would expect them to be derepressed in leucine-starved cells. We therefore grew the cells overnight with either 5 or 50 μg of L-leucine per ml, subcultured them in the same medium, and then assayed the β-galactosidase. In four similar mutants (representative data for one mutant are shown in Table 4), L-leucine starvation caused a fivefold increase in β-galactosidase activity; it did so in both the *lrp* mutant and the wild-type. We conclude that these mutants do carry insertions in one or more of the L-leucine biosynthesis genes.

Expression of the L-leucine-biosynthesis gene(s) is clearly activated by Lrp and is also controlled independently by L-leucine. The effects of L-leucine starvation and the *lrp* mutation were additive. This is the first case of L-leucine affecting expression in an *lrp* mutant and probably reflects the attenuation mechanism by which the operon is regulated (30).

The case of strain CP66 was very different. The β-galactosidase activity of strain CP66 was actually repressed by L-leucine starvation in both the parent strain and the *lrp* mutant. The *lrp* gene product increased expression in the presence of excess L-leucine and mitigated the repressive effect of L-leucine considerably during leucine starvation.

Identification of Lrp-regulated genes: location of some inserts near the *sdaB* gene. Three of our class 3 inserts, including strain CP52, mapped very close to the insert in strain 12079, *fuc*::Tn*10*, showing 79, 94, and 85% linkage. This strongly suggests that the inserts are in or near *sdaB*, which codes for the second L-serine deaminase, which is expressed mainly in cells grown on rich medium (28).

To see whether the CP52 insert might be in *sdaB*, we assayed β-galactosidase activity in cells grown in three media: in glucose-minimal medium, where *sdaB* is repressed; in glucose-minimal medium with L-leucine, where *sdaB* is slightly induced; and in LB, where *sdaB* is expressed at its highest level (28). β-Galactosidase activity followed exactly that pattern: 150, 350, and 2,000 U, respectively (35, 125, and 350 U in another of the three insert strains).

These data suggest that *sdaB* expression is regulated by Lrp. However, recent evidence suggests that this regulation may be indirect (data not shown). In any case, the major influence on *sdaB* is not Lrp. Lrp affects expression of *sdaB* in glucose-minimal medium with L-leucine (Table 2). However, the *lrp* mutation had almost no effect on the high level of *sdaB* expression in LB. We conclude that the relatively slight effects of leucine are mediated by Lrp, directly or indirectly, but that other effectors cause major changes in the regulation of transcription of *sdaB* in LB.

Possible identification of an insert in *livJ/K*. In the only class 4 mutant isolated so far, strain CP36, Lrp together with leucine repressed transcription profoundly, to 1% or less of the level seen in cells grown without leucine. We mapped this mutation by using the Singer kit and found the mutation to be 73.5% linked to an insertion at 75.5 min (144 of 196 transductants) and 29.6% linked to one at 76.5 min (61 of 206 transductants). This is consistent with the insertion being located in *livJ/K*, which is known to be regulated by Lrp, and coding for the branched-chain amino acid transport system (21).

Further metabolic screening of the *lrp* mutant and the strains carrying Lrp-regulated insertions. To try to identify the function of the remaining unidentified genes of our Lrp-regulated set, we screened strains MEW1 and MEW26 *lrp*::Tn*10* and all the insertion strains for the ability to use various carbon and nitrogen sources. The *lrp* mutant could not use glycine as a nitrogen source, and neither could several of our insert strains. However, several insert mutants also had difficulty using a whole group of nitrogen sources, L-serine, L-arginine, L-alanine, and proline, that MEW1 used easily. This suggests that the strains are deficient in reactions that transfer ammonia and/or amino groups and that Lrp regulates synthesis of one or more of the enzymes for handling nitrogen obtained at a low level from these amino acids. These insertion strains are found in classes 1 and 2 and thus represent at least two different genes and perhaps more. One of these may be *gltB*, the gene coding for glutamate synthase, since strains with mutations in *gltB* were originally described as being unable to use L-arginine, proline, or glycine as a nitrogen source (19).

Similarly, strain CP8, in which activation by Lrp is lessened by leucine (class 1), was unable to use several carbon sources, D-xylose, D-ribose, and rhamnose, that both the *lrp* mutant and its parent are able to use. This suggests that Lrp target genes code for products involved in a much larger array of metabolic reactions than was previously thought.

Transcription from *lrp* is decreased by Lrp and by growth in rich medium. We wondered what else besides Lrp might control *lrp* transcription. We therefore transformed plasmid pLRN2 *lrp*::*lacZ* into our parent strain and the *lrp* mutant and measured the β-galactosidase activities of cells grown with and without L-leucine (Table 5). β-Galactosidase activity was greatly increased in the *lrp* mutant. A chromosomal

lrp::*lacZ* insertion was similarly regulated. This showed a β-galactosidase level of 1,550 U in a strain carrying pACYC184 itself but only 150 U in a strain carrying pLRN1 (pACYC184 *lrp*$^+$).

It is clear from these experiments that *lrp* transcription is autogenously regulated. On the other hand, it is affected very little by leucine. This, then, is the first representative we have identified of the sixth class of Lrp-leucine interactions, those promoters which are repressed by Lrp but unaffected by leucine.

A major decrease in *lrp* transcription was seen in LB medium. Both the chromosomal and plasmid-carried *lrp*:: *lacZ* were repressed over 10-fold by growth in LB. We conclude that the concentration of Lrp in LB-grown cells is considerably lower than that of cells grown in minimal medium and that genes requiring elevated concentrations of Lrp may be regulated by it only in minimal medium. Even though *lrp* transcription is autoregulated, some other regulation must override this in order to result in this decrease in Lrp in LB.

Other factors regulating *gcv* expression. During our assays of *gcv* mutants, we noticed a great variation in the results from experiment to experiment. We found that the β-galactosidase level varied for any strain as a function of the growth phase. We found a gradual progression from 1,150 U at an optical density of 0.155 to 2,275 U at an optical density of 1.089 in strain CP67 (Fig. 1). This same progression was seen in the presence of leucine or glycine (Fig. 1). However, in the *lrp* mutant, there was so little activity that no such pattern could be seen (Fig. 1A). It is clear, then, that *gcv* expression is influenced by some factor related to the growth phase and that it may be difficult to compare *gcv* values in different growth media without controlling for this.

Another factor regulating expression of the *gcv* genes is the availability of C_1 units from other sources and the compounds in whose synthesis they are involved (17, 18). However, as noted by Stauffer in his review (25), there is a good deal of confusion as to the effect of C_1 units on *gcv* expression. In our experiments, the addition of the end products greatly decreased *gcv* expression. When we grew strain CP67 with the end products of C_1 metabolism (adenine, guanine, methionine, histidine, and thymidine), the level of β-galactosidase fell to 35 to 75 U throughout the growth cycle (Fig. 1B). Most of this was due to the addition of purines, as indicated by β-galactosidase levels from 475 to 150 U at different stages of the culture with purines (Fig. 1B).

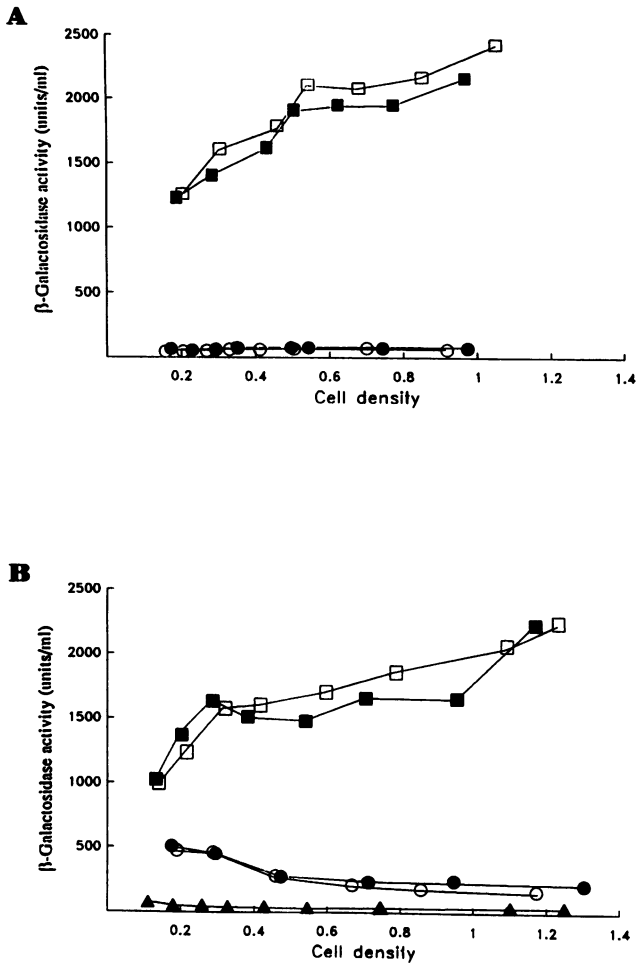

FIG. 1. Regulation of *gcv* expression. Cells were grown in glucose-minimal medium at 37°C with the additions noted below and subcultured in the same medium. β-Galactosidase activity was assayed at different points in the growth curve. Cell density is plotted in units of optical density at 600 nm. (A) Additions to strain CP67: □, none; ■, L-leucine. Additions to strain CP67 *lrp*: ○, none; ●, L-leucine. (B) Additions to strain CP67: □, none; ■, glycine; ○, adenine; ●, guanine; ▲, adenine, guanine, L-histidine, methionine, and thymidine. L-Leucine, adenine, and guanine were provided at 100 μg/ml each, glycine was added at 400 μg/ml, and all others were added at 50 μg/ml.

DISCUSSION

In this paper, we identify several genes that are regulated by the *lrp* gene, and we demonstrate a very complex interaction between the *lrp* gene product and the genes that it regulates. We show that Lrp regulates some genes on which L-leucine has no effect, and we suggest that the regulon should now be known as the leucine-*lrp* regulon. Among the newly identified genes are *gcv*, a regulator of *sdaB* (28), and one or more genes involved in leucine biosynthesis. The genes currently known to be regulated by Lrp are listed in Table 6.

The leucine regulon may control adaptation to growth in minimal medium. Lrp regulates expression of genes whose products are involved in amino acid biosynthesis, amino acid transport, amino acid degradation, and amino acyl-tRNA synthesis. As judged by the effect of an insertion in the *lrp* gene, Lrp activates synthesis from *serA*, *ilvIH*, and *gcv*. It represses synthesis from *sdaA*, *tdh*, *lysU*, and *oppA-D*. L-Leucine lessens the effect of Lrp at all of these except the *gcv* gene.

One could summarize this with the idea that Lrp increases biosynthetic functions and decreases degradative ones, a tendency that is partly reversed with exogenous L-leucine. This would be even more evident in LB medium, where there would be a considerable concentration of L-leucine and, as shown here, a very great decrease in Lrp concentration, so that degradative functions would be even more favored in LB than in minimal medium with L-leucine.

We suggest that Lrp is a mechanism for controlling a switch from rich environments like those in the intestines to the poor environments of the outside world and the laboratory minimal medium. Lrp would repress genes involved in the degradation of exogenous compounds and activate those involved in the biosynthesis of compounds starting from simple intermediates such as pyruvate.

TABLE 6. Identified genes known to be regulated by Lrp[a]

Gene	Position (min) on genome	Activity encoded	Effect of:		
			Lrp	Leucine	Lrp + leucine
ilvIH	1.85	Acetolactate synthetase III	Activation	−	
	2	Leucine biosynthesis	Activation	NT	
lrp	20	Leucine-responsive regulatory protein	Repression	None	
oppA-D	27	Oligopeptide permease	Repression	+	
sdaA	40.2	L-Serine deaminase I	Repression	+	
	60.5	Gene regulating *sdaB*			Activation
serA	63	Phosphoglycerate dehydrogenase	Activation	−	
gcv	63	Glycine cleavage enzymes	Activation	None	
livJ/K	76	Branched-chain amino acid transport	None	None	Repression
tdh	91	Threonine dehydrogenase	Repression	+	
lysU	94	Lysyl-tRNA synthetase II	Repression	+	

[a] +, increased activity; −, decreased activity; None, no effect; NT, not testable.

Because Lrp is apparently made at lower concentration in LB, it is likely to affect transcription of only genes with a higher affinity for it. In minimal medium, Lrp might act on those and other genes, repressing degradative genes and activating biosynthesis genes. The Lrp mutant can grow in minimal medium, but its enzyme content is not optimized for this culture condition. One of its most severe problems comes from the fact that Lrp is needed to activate genes involved in L-leucine biosynthesis. This probably accounts for the fact that the *lrp* mutant grows slowly because of leucine starvation (11). The mutant also makes much more L-serine deaminase and threonine dehydrogenase, and probably other enzymes as well, than it needs. However, this may not interfere much, because the substrate requirement of these enzymes is high and they may not function much in minimal medium (15, 16).

Lrp as a major regulator of C_1 metabolism. Whatever its other and possibly more general metabolic roles may be, Lrp has a profound effect on C_1 metabolism. In *E. coli* grown in glucose-minimal medium, C_1 units are derived mainly from L-serine, either by serine hydroxymethyltransferase with the concomitant synthesis of glycine or from glycine by the glycine cleavage enzymes (17). These routes are balanced according to the ratio of C_1 to glycine required by the cell.

Lrp is clearly involved in maintaining this balance. The addition of L-leucine would decrease the amount of L-serine available, both by increasing deamination of L-serine and by decreasing its biosynthesis. Despite the decrease in L-serine, a supply of glycine could still be assured, both from threonine via threonine dehydrogenase, which is induced by L-leucine, and from serine hydroxymethyltransferase. C_1 compounds would then be made from both L-serine and glycine.

In the extreme conditions caused by Lrp deficiency, in the mutant and perhaps also in LB, the cell must become entirely dependent on serine hydroxymethyltransferase for C_1 production, unless it has some third pathway for C_1 production. The fact that an *lrp glyA* mutant grows extremely poorly suggests that no other pathway contributes much C_1 in an *lrp* background. This would present little problem for the *lrp* mutant itself in LB, where the requirement for C_1 is not great.

Lrp activity without L-leucine as an effector: autoregulation of *lrp*. We have shown one case where Lrp is absolutely required for physiologically significant levels of expression and L-leucine has no effect: *gcv* expression. Although there may be some residual synthesis in the *lrp* mutant, it was less than 4% of the parental activity; the *lrp* mutant was physiologically deficient in *gcv* function. This regulation depends on Lrp only and not on L-leucine. However another, as yet unidentified, effector could also be involved.

We have also shown one case where Lrp decreases transcription of a gene, also without a significant effect of L-leucine; this is the *lrp* gene itself. This cannot be the only control on *lrp* transcription. In LB-grown cells, *lrp* expression was very much decreased, suggesting that autoregulation by Lrp must be overridden by other controls.

The fact that Lrp is required for expression of at least one gene and decreases expression at another suggests, by extension, that it may be used to activate and silence genes and that Lrp is independent of L-leucine. However, genes regulated in this way would not have been identified by the screen we used, which was based on regulation by L-leucine. We are therefore now undertaking a different and more general screening procedure to find loci regulated by Lrp without leucine.

Interaction of Lrp with its promoters. By studying regulation of *lacZ* insertions in target genes of Lrp, we showed that binding of Lrp can either activate or repress these genes. Regulation by Lrp is influenced by L-leucine in most but not all of the cases studied so far. In some cases, L-leucine lessens the effect of Lrp binding; in others, regulation requires both Lrp and L-leucine. In two cases, leucine has little or no effect; Lrp is absolutely required for transcription of *gcv* and represses its own expression. These interactions are summarized in Table 7.

Several regulatory proteins act as both repressors and

TABLE 7. Regulatory interactions at Lrp target genes

Action	Prototype gene
Lrp activates	
Leucine reduces effect	*ilvIH*
Leucine is needed for Lrp effect	*sdaB*[a]
Leucine has no effect	*gcv*
Lrp represses	
Leucine reduces effect	*sdaA*
Leucine is needed for Lrp effect	*livJK*
Leucine has no effect	*lrp*

[a] Or gene regulating *sdaB*.

activators (29). OmpR can activate or repress at a single promoter, *ompF*, depending on its phosphorylation state, the site at which it binds, and the property of the promoter itself (1, 29). We suggest that the conformation of Lrp may change according to whether L-leucine is bound. The effect of Lrp in any particular case may depend on the design of the promoter, the affinity of Lrp for L-leucine, and the relative affinity of free Lrp and leucine-Lrp for particular sequences. L-Leucine may not be the only small molecule with which Lrp interacts. In vitro studies have shown that L-alanine also influences Lrp binding, although the L-alanine must be present at concentrations higher than those required for L-leucine.

Many regulatory proteins act in concert with other proteins, like catabolite gene activator protein (CAP) with the *lac* repressor. We suggest that Lrp may also interact in this way, perhaps with CAP, integration host factor, and others. We are examining the metabolism of double mutants.

More general effects of Lrp. Although the *lrp* gene product integrates all of the reactions of serine and glycine metabolism, it also affects a very diverse group of other reactions. This suggests that it acts as a regulator of this local area of metabolism (and possibly others) but that it also has very broad effects on metabolism. The effects identified so far have largely been involved in amino acid metabolism, but this bias is probably partly a result of the interests of the persons investigating Lrp.

There are a relatively large number of Lrp molecules in the cell, about 3,000 38-kDa dimers, as judged by using Western blots (31). This is much lower than the number of histonelike protein molecules in the cell (about 50,000 molecules [6]), similar to the number of CAP molecules, and much higher than the number of local regulators like AraC (10). This is consistent with a broader role for Lrp in cell metabolism. We suggest that Lrp is similar to RAP-1 and ABF-1 (4, 7) as a general regulator, in spite of its drastic effects on specific areas.

This is made likely by the fact that Lrp regulates a large number of genes. An analysis of two-dimensional gels suggested that Lrp regulates about 30 genes (12). However, in the present study of random inserts in Lrp-regulated genes, we found several genes that are regulated only two- to fourfold and might be missed on two-dimensional gels. On the other hand, we do not know how many of our first 30 isolates represent isolates with inserts in the same gene. However, we do know that none of the inserts is in *sdaA* (data not shown), and we estimate therefore that Lrp binds to between 50 and 75 genes.

What would be the effect of scattering 100 or more dimers of Lrp across the genome, a fortiori if Lrp turns out to be a bending protein like CAP (23)? We suggest that Lrp acts as a major DNA organizer of *E. coli*, probably together with other binding proteins like CAP and IHF (6). Perhaps bacteria accumulated these local binding proteins as they evolved and then capitalized on their presence in the cell and used them as hooks by which to fold DNA. Specificity would be conferred by the adaptation of Lrp to specific sites; less specific proteins like HU would then facilitate folding into a pattern determined by specific binding proteins.

Precedent for the use of a specific regulatory protein for other purposes is seen in the case of ArgR, which is both the regulator of arginine biosynthesis and part of the *cer* recombination system of ColE1 (27). One could further speculate that DNA folding itself might vary in different environmental conditions and might itself affect gene expression.

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