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Escherichia coli Lrp (Leucine-Responsive Regulatory Protein) Does Not Directly Regulate Expression of the *leu* Operon Promoter

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Studies by R. Lin et al. (J. Bacteriol. 174:1948–1955, 1992) suggested that the *Escherichia coli leu* operon might be a member of the Lrp regulon. Their results were obtained with a leucine auxotroph; in leucine prototrophs grown in a medium lacking leucine, there was little difference in *leu* operon expression between *lrp*$^+$ and *lrp* strains. Furthermore, when *leuP-lacZ* transcriptional fusions that lacked the *leu* attenuator were used, expression from the *leu* promoter varied less than twofold between *lrp*$^+$ and *lrp* strains, irrespective of whether or not excess leucine was added to the medium. The simplest explanation of the observations of Lin et al. is that the known elevated leucine transport capacity of *lrp* strains (S. A. Haney et al., J. Bacteriol. 174:108–115, 1992) leads to very high intracellular levels of leucine for strains grown with leucine, resulting in the superattenuation of *leu* operon expression.

Lrp (leucine-responsive regulatory protein) controls the expression of a number of operons in *Escherichia coli* involved in amino acid biosynthesis and degradation, the transport of amino acids, and one carbon metabolism (for a review, see references 4, 5, and 16). In general, anabolic target genes are positively regulated and catabolic genes are negatively regulated by Lrp. It is for these reasons that Lrp has been considered to be a global regulator of metabolism in *E. coli*, playing an especially important role when cells make transitions between rich nutritional conditions and lean conditions in which they must synthesize most of their building blocks from simple carbon sources and salts.

Another interesting feature of Lrp is that its mode of action is sometimes but not always affected by elevated levels of the amino acid leucine. Altogether, six different patterns of regulation by Lrp have been recognized, depending upon whether Lrp acts negatively or positively and upon the way in which leucine affects expression. For those cases in which Lrp acts positively as an activator, leucine sometimes overcomes the effect of Lrp (thus causing reduced expression), sometimes potentiates the effect of Lrp, and sometimes has no effect on Lrp-mediated activation. Similarly, for cases in which Lrp acts negatively, there are examples in which leucine overcomes the effect, is required for the effect, or has no effect upon Lrp-mediated repression.

The *leuPABCD* operon of *E. coli* is known to be regulated by a transcription attenuation mechanism (8, 25). Some work by Lin et al. suggested that the *leu* operon may also be controlled by Lrp (14). Among *E. coli* strains containing p*lac*Mu9 insertions that Lin et al. isolated, several had insertions within the *leu* operon, including strain CP55 with an insertion in *leuB* (14, 22). For strain CP55 [Φ(*leuB-lacZ*)] grown with excess leucine, β-galactosidase levels were more than 10-fold higher than those in an isogenic strain containing an inactive *lrp* gene, suggesting that Lrp acts positively on *leu* operon expression (14). Furthermore, exogenous leucine increases the growth rate of strains lacking a functional *lrp* gene (2, 4), leading some to conclude that leucine synthesis in such strains is impaired (2, 16). If Lrp is an activator of *leu* operon expression, then strains lacking Lrp might be impaired in leucine biosynthesis and thus exhibit a partial leucine requirement (2, 16).

Here we investigate in more detail the role that Lrp plays in regulating expression of the *leu* operon and the question of whether leucine synthesis is indeed impaired in *lrp*-containing strains. We found that mutations in Lrp affect *leu* operon expression to a limited extent, but only indirectly, and that leucine synthesis is likely not impaired in a strain lacking functional Lrp.

We repeated some of the experiments reported by Lin et al. (14) and observed, as they did, a more than 10-fold-lower level of β-galactosidase activity in strain CP55 (*lrp*$^+$) than in an isogenic *lrp* strain that we created (Tables 1 and 2). Similar results were obtained with another isogenic set of strains derived from strain P90C to which we transferred the *leuB*::p*lac*Mu9 allele (Tables 1 and 2).

The results of Lin et al. (14) and the results shown in Table 2 were obtained with strains that were leucine auxotrophs and therefore the effect of *lrp* mutations could only be tested for these strains grown with excess leucine or under conditions of leucine limitation. To determine whether an *lrp*::Tn*10* allele affects *leu* operon expression in cells grown in a minimal medium without excess or limited leucine, we measured the specific activity of the *leuB* gene product, β-isopropylmalate (β-IPM) dehydrogenase, in a strain having a wild-type *leu* operon. The results (Table 3) show that a null mutation in *lrp* had only a small effect upon *leuB* expression in cells grown in a minimal medium in the absence of leucine. It may be noted that Lin et al. found only a 2.6-fold difference in reporter gene expression between strain CP55 [Φ(*leuB-lacZ*)] and an isogenic strain carrying an *lrp* null allele when the two strains were grown to the point where they had depleted their supply of leucine (14). Therefore, a null mutation in *lrp* has a relatively small effect upon *leu* operon expression when the intracellular leucine concentration is either undisturbed (our results) or limiting for growth (14).

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TABLE 1. Bacterial strains of *E. coli* used in this study[a]

Strain	Genotype	Source or reference
P90C	*ara thi* Δ*lac-pro*	20
CP55	Δ*lac* Δ*ilvA leuB*::p*lac*Mu9	E. Newman
CV1008	P90C *ilvIH*::Mu dI 1734 *lrp-35*::Tn*10*	18
CV1216	P90C *lrp-35*::Tn*10*	This laboratory
CV1512	CP55 *lrp-35*::Tn*10*	This study
CV1513	P90C *leuB*::p*lac*Mu9	This study
CV1514	CV1513 *lrp-35*::Tn*10*	This study
CV1517	P90C λ *Pleu att-lacZ*	This study
CV1518	CV1517 *lrp-35*::Tn*10*	This study
CV1520	P90C λ *Pleu-lacZ*	This study
CV1521	CV1520 *lrp-35*::Tn*10*	This study
CV1543	P90C λ *PilvIH Pleu att-lacZ*	This study
CV1544	CV1543 *lrp-35*::Tn*10*	This study
CV1547	P90C λ *PilvIH Pleu-lacZ*	This study
CV1548	CV1547 *lrp-35*::Tn*10*	This study
CV1549	P90C λ *P(−)ilvIH Pleu-lacZ*	This study
CV1550	CV1549 *lrp-35*::Tn*10*	This study

[a] Strains CV1216, CV1512, CV1514, CV1518, CV1521, CV1544, CV1548, and CV1550 were created by transduction with P1 phage grown on strain CV1008, with selection for growth in the presence of 15 μg of tetracycline per ml. Similarly, strain CV1513 was formed with phage grown on strain CP55 and with 50 μg of kanamycin per ml. Strain CV1517 was constructed by amplifying region −247 to +230 from the *leu* operon of *E. coli* by PCR and cloning into plasmid pRS415 upstream of the *lacZ* gene (20). After transferring the *leuP-lacZ* fusion to λ phage by homologous recombination (20), single lysogens were identified as described earlier (24). Strains CV1520, CV1543, CV1547, and CV1549 were constructed similarly and contained the following: CV1520, 265 bp (position −247 to +18 from the *leu* operon); CV1543, 2,147 bp (from +50 downstream of the *ilvIH* promoter to +230 downstream from the *leu* promoter); CV1547, 1,927 bp (from position +50 downstream of the *ilvIH* promoter to +18 downstream from the *leu* promoter); CV1549, 1,840 bp (from −37 upstream of the *ilvIH* promoter to +18 downstream from the *leu* promoter).

For the experiments described in the paragraph above, cells were also grown in the presence of leucine. For each strain, the addition of leucine to the medium resulted in reduced expression of the operon, a result expected because of the known control of this operon by a leucine-dependent transcription attenuation mechanism (8, 25). Under this condition of leucine excess, there was a difference in β-IPM dehydrogenase specific activity in *lrp*$^+$ and *lrp* strains, but it was only about 3-fold, rather than the 10-fold difference observed with strains containing *leuB-lac* translational fusions. However, β-IPM dehydrogenase assays (Table 3) are not as sensitive as β-galactosidase assays (Table 2), and the β-IPM dehydrogenase values shown in Table 3 for strain CV1216 were at the limits of detection.

Any effects of an *lrp* mutation upon *leu* operon expression could be upon initiation of transcription or upon attenuation of transcription. In an attempt to distinguish between these possibilities, two sets of strains were prepared, each having a wild-type *leu* operon at its normal location in the chromosome and a *leu* promoter-*lacZ* transcriptional fusion in single copy at the phage lambda attachment site. One set of strains contained the *leu* promoter without the attenuator (position −247 to +18) directly attached to *lacZ* (strains CV1520 *lrp*$^+$ and CV1521 *lrp-35*::Tn*10*), whereas the second set contained the *leu* promoter and attenuator (−247 to +230) attached to *lacZ* (strains CV1517 *lrp*$^+$ and CV1518 *lrp-35*::Tn*10*) (Fig. 1). For constructs having the *leu* promoter without the attenuator, there was little or no difference in β-galactosidase specific activity between *lrp*$^+$ and *lrp* strains grown in minimal medium with or without leucine (Table 4). These results suggest that the *leu* promoter is not regulated by Lrp, either directly or indirectly. For strains with constructs having both the *leu* promoter and attenuator and grown in the absence of exogenous leucine, an *lrp* null allele led to an approximately fourfold-higher level of reporter gene expression, a surprising result that will be discussed later. For the same strains, growth in the presence of leucine resulted in a substantial reduction in reporter gene expression, as expected for a system under attenuation control (23) (Table 4). However, the extent of the repression was about fivefold higher for the strain containing the *lrp* null allele than for the *lrp*$^+$ strain, a result reminiscent of the original finding of Lin et al. (Table 2 and reference 14). Taken together, the results shown in Table 4 strongly suggest that any effects that Lrp might have on *leu* operon expression are not due to effects on transcription initiation but rather on some subsequent process such as transcription attenuation.

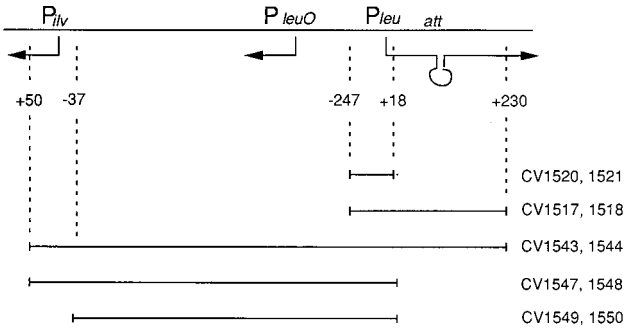

FIG. 1. Schematic representation of constructs used in this study.

We return to the surprising result presented in Table 4 involving constructs having a *leu* promoter-attenuator–*lacZ* transcriptional fusion in single copy at the phage λ attachment site. For strains grown in the absence of leucine, reporter gene expression was about fourfold higher in an *lrp* strain than in an *lrp*$^+$ strain. The sequence of the construct in the region of the promoter and attenuator was verified by sequencing, but out of concern that a mutation might have occurred during the transfer of *leuP att-lacZ* to phage lambda, we repeated these experiments with the plasmid-containing strains from which the single copy λ lysogens were prepared, normalizing specific activities for plasmid copy number (estimated by measuring β-lactamase activity) (9). Reporter gene specific activities were higher, as expected for plasmid-containing strains, but the pattern was similar, i.e., there was threefold-higher expression in an *lrp* strain than in an *lrp*$^+$ strain (data not shown). In addition, in order to explore any possible effects of Lrp on translation, we cloned this same *leu* promoter-attenuator fragment

TABLE 2. Specific activity of β-galactosidase in *lrp*$^+$ and *lrp* strains having a *leuPAB′-lacZ* translational fusion[a]

Strain	*lrp* allele	Sp. act. of β-galactosidase[b]
CP55	*lrp*$^+$	334 ± 46
CV1512	*lrp-35*::Tn*10*	19.5 ± 2.4
CV1513	*lrp*$^+$	166 ± 11
CV1514	*lrp-35*::Tn*10*	16.2 ± 0.6

[a] Strains were grown at 37°C with shaking in SSA minimal salts (11) containing 0.2% glucose; 50 μg (each) of isoleucine, valine, leucine, and proline per ml; and 5 μg of thiamine per ml. Cultures were grown overnight and diluted to an A_{550} of 0.01, and samples were taken at different times in the log phase for assay.

[b] Specific activity is in Miller units (15). β-Galactosidase assays were performed as described previously (12, 13). Values represent the means and standard deviations for multiple samples taken throughout the log growth phase from at least two independent experiments.

TABLE 3. Specific activity of β-IPM dehydrogenase in *lrp*$^+$ and *lrp* strains[a]

Strain[b]	*lrp* allele	Doubling time (min) in medium		Sp. act. of β-IPM dehydrogenase[b]	
		Without Leu	With Leu	Without Leu	With Leu
P90C	*lrp*$^+$	57	67	2.0 ± 0.2 (3)	0.2 ± 0.05 (3)
CV1216	*lrp-35*::Tn*10*	82	62	1.5 ± 0.03 (3)	0.07 ± 0.03 (3)

[a] Strains were grown in minimal glucose medium containing 50 μg (each) of isoleucine, valine, and proline per ml and 5 μg of thiamine per ml in the presence or absence of 100 μg of leucine per ml. Cultures were grown overnight, diluted to an A_{550} of 0.01, and samples were taken at different times in the log phase for assay.
[b] Assays were performed as described previously (19), except that cells were broken by sonication; 2,4-dinitrophenylhydrazones were extracted into toluene and then aqueous sodium carbonate as described by Stieglitz and Calvo (21), except that 1.5 ml of Na_2CO_3 and 0.25 ml of KOH were used and A_{540} was determined. Specific activity is expressed as micromoles per hour per milligram of total protein. Values in parentheses represent the number of samples analyzed. Each sample was assayed in duplicate. A repetition of this experiment gave similar results.

(position −247 to +230) into plasmid pRS414 (20) creating a *leuA′-lacZ* translational fusion. This construct also showed a similar pattern of expression, being about sixfold higher in an *lrp* strain than in an *lrp*$^+$ strain (data not shown).

The results in Table 3 (with the *leu* operon at its normal position in the chromosome) and Table 4 (with the *leu* promoter and attenuator located at the phage lambda attachment site) seem at odds in the case of cells grown in the absence of leucine, with the effect of an *lrp* allele being to reduce expression slightly in one case and increase expression in the other. We considered the possibility of this result being related to the long-range interaction between the *leu-500* and *ilvIH* promoters described by M. Fang and H.-Y. Wu (6, 7). They demonstrated that transcription from the *leu-500* promoter of *Salmonella typhimurium* is affected by transcription initiated at the *ilvIH* promoter, located 1.9 kb away. They postulate a promoter relay mechanism involving another gene, *leuO*, located between the *leu* and *ilvIH* operons. To determine whether similar long-range effects might be at play in the *E. coli* strains that we analyzed here, we prepared *lacZ* fusion constructs containing the *leu* promoter (with or without the attenuator) together with upstream DNA that did or did not include the *ilvIH* promoter (Fig. 1). For strains possessing the longest construct (upstream DNA including the *ilvIH* promoter and DNA downstream of the *leu* promoter, including the attenuator), there was little difference in reporter gene expression between *lrp*$^+$ and *lrp* strains when cells were grown in either the absence or presence of leucine (Table 5; Fig. 1). For strains having similar constructs lacking the *leu* attenuator, reporter gene expression was higher as expected, but again, there were only modest differences (less than twofold) between *lrp*$^+$ and *lrp* strains (Table 5; Fig. 1). Finally, to determine whether transcription from the *ilvIH* promoter had an effect upon *leu* promoter expression, we analyzed strains having constructs containing nearly the same amount of upstream DNA but lacking the *ilvIH* promoter. For these strains and conditions of growth, deleting the *ilvIH* promoter had no discernable effect (Table 5; Fig. 1). As with the shorter *leu* promoter construct (Table 4), we found only modest differences in reporter gene expression due to a mutation in *lrp*. Furthermore, comparison of the constructs shows that the upstream DNA containing the *ilvIH* promoter has no significant effect on expression from the *leu* promoter alone (Table 5). Taken as a whole, these results again suggest that Lrp has little effect on *leu* expression, except perhaps for cells grown with excess leucine. There was no hint that Lrp might *decrease* expression from the *leu* promoter, as was suggested from results with strains CV1517 and CV1518 (Table 4). These latter results we assume to be some sort of artifact, although we were unable to establish that in our experiments.

We return to the original observation of Lin et al. (14) that *leu* operon expression is reduced in an *lrp* strain grown with excess leucine. We considered the possibility that Lrp indirectly affects *leu* transcription attenuation by affecting intracellular concentrations of leucine or of leucyl-tRNA. If Lrp were a repressor of *leuS* (encodes leucyl-tRNA synthetase), then increased levels of leucyl-tRNA synthetase in an *lrp-35*::Tn*10* strain could result in higher levels of leucyl-tRNA and lower levels of *leu* operon expression through additional attenuation. We tested this possibility by preparing constructs containing *leuS-lacZ* transcriptional fusions and measuring β-galactosidase levels in *lrp*$^+$ and *lrp* strains containing these constructs. No differences in reporter gene expression between the two strains were found (data not shown).

We also explored the potential effects of Lrp upon the transport of leucine that might result in altered intracellular levels of leucine. *livJ* and *livKHMGF*, operons involved in transporting leucine into *E. coli*, are negatively controlled by Lrp, both as measured by transport assays and by levels of reporter gene activity (10). It should be noted that for some strains having

TABLE 4. Specific activity of β-galactosidase in *lrp*$^+$ and *lrp* strains having *leuP-lacZ* transcriptional fusions at the λ attachment site[a]

Strain	*lrp* allele	*leu* attenuator[b]	Sp. act. of β-galactosidase[c] in medium		Ratio of sp. act. (without Leu/with Leu)
			Without Leu	With Leu	
CV1520	*lrp*$^+$	Absent	1,870 ± 425	2,780 ± 243	0.67
CV1521	*lrp-35*::Tn*10*	Absent	2,020 ± 240	1,960 ± 293	1.0
CV1517	*lrp*$^+$	Present	432 ± 110	80.7 ± 10.4	5.5
CV1518	*lrp-35*::Tn*10*	Present	1,950 ± 518	64.0 ± 8.9	30

[a] Culture conditions as in legend to Table 3.
[b] Strains CV1520 and CV1521 have region −247 to +18 from the *leu* operon (contains *leu* promoter but not attenuator) attached to a *lacZ* gene on phage λ in single copy at the λ attachment site (Fig. 1). Strains CV1517 and CV1518 are similar except that they have region −247 to +230 from the *leu* operon which contains both the *leu* promoter and attenuator.
[c] Specific activity in Miller units. Values represent the means and standard deviations for multiple samples taken throughout the log growth phase from at least two independent experiments.

TABLE 5. Specific activity of β-galactosidase in *lrp*$^+$ and *lrp* strains having *leuP-lacZ* transcriptional fusions with upstream DNA at the λ attachment site[a]

Strain	*lrp* allele	P*ilvIH*[b]	*leu* attenuator[b]	Sp. act. of β-galactosidase[c]		Ratio of sp. act. (without Leu/ with Leu)
				Without Leu	With Leu	
CV1543	*lrp*$^+$	+	+	2,500 ± 250	96 ± 6.4	26
CV1544	*lrp-35*::Tn*10*	+	+	2,890 ± 189	36 ± 2.3	79
CV1547	*lrp*$^+$	+	−	5,380 ± 473	3,370 ± 136	1.6
CV1548	*lrp-35*::Tn*10*	+	−	3,120 ± 338	2,510 ± 246	1.2
CV1549	*lrp*$^+$	−	−	5,370 ± 454	3,340 ± 143	1.6
CV1550	*lrp-35*::Tn*10*	−	−	3,110 ± 267	2,430 ± 278	1.3

[a] Strains were grown as described in Table 3.
[b] +, present; −, absent.
[c] Specific activity is in Miller units. Values represent the means and standard deviations for multiple samples taken throughout log growth phase from at least two independent experiments. Similar results were obtained for cells grown to late log and early stationary phases of growth.

lacZ insertions within *liv* genes, the phenotypes suggest that *liv* genes are positively controlled by Lrp (3, 14, 22). The discrepancy in results obtained with different fusion constructs is not understood, but for the analysis that follows, we assume that Lrp negatively affects *liv* expression because this conclusion is also supported by direct measurement of transport activity (10). In an *lrp*$^+$ strain, exogenous leucine causes extensive repression of the two *liv* operons (3, 10, 14, 22). By contrast, an *lrp* strain has high constitutive levels of transport activity and therefore is expected to have high intracellular levels of leucine when grown in the presence of exogenous leucine. Thus, the reduced expression of the leucine operon that is observed in an *lrp* strain grown in the presence of leucine (14) (Table 2) may be due to superattenuation caused by high intracellular leucine concentrations. To test this idea, we repeated the experiment described in Table 2, sometimes supplementing the medium with leucine-containing dipeptides instead of leucine. In *E. coli*, dipeptides are transported primarily by the dipeptide permease, encoded by the *dppABCDF* operon (1, 17). As shown in Table 6, exogenous glycyl leucine and, to a lesser extent, alanyl leucine caused severe repression of *leu* operon expression in the *lrp*$^+$ strain while the *lrp* strain showed low values under all three conditions. This result is most easily explained by assuming that dipeptide transport is not affected by Lrp, and that the dipeptide permease system has a high rate of transport of leucine-containing dipeptides, resulting in high intracellular leucine concentrations and superattenuation of *leu* operon transcription. By this view, superattenuation of *leu* transcription is a consequence of very high intracellular leucine concentrations, concentrations that can be achieved by the growth of a wild-type strain with exogenous leucine-containing dipeptides or by the growth of an *lrp* strain in the presence of leucine.

To summarize, we confirmed the original observation of Lin et al. that *leu* operon expression is extremely low in a strain having an *lrp* null allele (14), but found that this result is only seen in cells grown in the presence of excess leucine. For a prototrophic strain grown without excess leucine, an *lrp* null allele had little effect upon *leu* operon expression (Table 3). Furthermore, an *lrp* null allele had little effect upon *leu* promoter expression in strains having just the *leu* promoter fused to a *lacZ* reporter gene (Table 4 and 5). With our *E. coli* strains grown under our defined conditions, we did not find that the *leu* promoter was affected by transcription initiated almost 2 kb away at the *ilvIH* promoter, as has been suggested by Fang and Wu for the *leu-500* promoter in *S. typhimurium* (6, 7). The effects of Lrp upon *leu* operon expression, originally observed by Lin et al. (14) and confirmed by us, must be related to events secondary to transcription initiation, likely either transcription attenuation at *leu att* or translation initiation at the beginning of structural genes. The simplest explanation of all of our results is that leucine transport capacity is elevated in a strain lacking Lrp (10) and that growth of such a strain in the presence of leucine causes high intracellular levels of leucine, which in turn cause very low levels of *leu* operon expression through the transcription attenuation mechanism. The results shown in Table 6 involving growth in media supplemented with leucine-containing dipeptides are consistent with this interpretation. The overall conclusion of these studies is that any effects of Lrp on *leu* operon expression are indirect.

While this overall conclusion seems justified, it must be noted that several aspects of our data are not readily explained. For example, in the presence of leucine and the *leu* attenuator, the ratio of *leu*::*lacZ* expression in *lrp*$^+$ to that in *lrp* is more than 10 when the fusion is in *leuB* and all upstream DNA is present (Table 2), 2.7 when DNA stretching upstream to the *ilvIH* promoter is included (Table 5), and 1.3 when upstream DNA goes only to position −247 (Table 4). Or consider that for *lrp*$^+$ strains grown in the absence of leucine, *leu*::*lacZ* expression was about threefold higher in constructs containing about 1,500 bp of upstream DNA than for those containing only 250 bp (Table 4 and 5). These comparisons suggest that there may be some long-distance effects of upstream sequences upon expression from the *leu* promoter.

Finally, the conclusion of previous studies that *lrp* strains grow slowly because they are starved for leucine (2, 16) needs to be evaluated in the context of our results. *lrp* strains do grow more slowly than isogenic *lrp*$^+$ strains (Table 3) and the growth

TABLE 6. Specific activity of β-galactosidase in *lrp*$^+$ and *lrp* strains having a *leuPAB′-lacZ* translational fusion and grown in media supplemented with dipeptides[a]

Strain	*lrp* allele	Sp. act. of β-galactosidase[b]		
		Leu	Gly Leu	Ala Leu
CP55	*lrp*$^+$	470 ± 50	31 ± 6	124 ± 40
CV1512	*lrp-35*::Tn*10*	30 ± 7	20 ± 3	51 ± 17

[a] As in legend to Table 2, except that cultures were sometimes supplemented with leucine at 50 μg/ml (Leu), glycyl leucine at 75 μg/m; (Gly Leu) or alanyl leucine at 77 μg/ml (Ala Leu).
[b] Specific activity is in Miller units. Values represent the means and standard deviations for multiple samples taken throughout log growth phase from at least two independent experiments.

rate of *lrp* strains is increased by inclusion of leucine in the medium (Table 3), but *lrp* strains do not appear to be defective for *leu* operon expression (Table 3). In fact, if *lrp* strains were starved for leucine, the expected (though not observed) result is elevated *leu* operon expression caused by relief of transcription attenuation. The transcription attenuation mechanism is functional in *lrp* strains because Lin et al. showed in their original work that a leucine limitation imposed upon a *lrp* strain with a *leu* mutation resulted in elevated expression of the *leu* operon (14). The underlying basis for the slow growth of *lrp* strains and for leucine-mediated stimulation of growth remains unclear.

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