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Regulation of High-Affinity Leucine Transport in Escherichia coli

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Leucine is transported into E coli by two osmotic shock-sensitive, high-affinity systems (LIV-I and leucine-specific systems) and one membrane bound, low-affinity system (LIV-II). Expression of the high-affinity transport systems is altered by mutations in *livR* and *lstR*, genes for negatively acting regulatory elements, and by mutations in *rho*, the gene for transcription termination. All four genes for high-affinity leucine transport (*livJ*, *livK*, *livH*, and *livG*) are closely linked and have been cloned on a plasmid vector, pOX1. Several subcloned fragments of this plasmid have been prepared and used in complementation and regulation studies. The results of these studies suggest that *livJ* and *livK* are separated by approximately one kilobase and give a gene order of *livJ–livK–livH*. *livJ* and *livK* appear to be regulated in an interdependent fashion; *livK* is expressed maximally when the *livJ* gene is inactivated by mutation or deletion. The results support the existence of separate promoters for the *livJ* and *livK* genes. The effects of mutations in the *rho* and *livR* genes are additive on one another and therefore appear to be involved in independent regulatory mechanisms. Mutations in the *rho* gene affect both the LIV-I and leucine-specific transport systems by increasing the expression of *livJ* and *livK*, genes for the LIV-specific and leucine-specific binding proteins, respectively.

Key words: leucine transport genes, cloning, regulation, *rho* factor

Two distinct leucine transport systems are present in E coli K12. A low-affinity membrane-bound system (LIV-II) is osmotic shock resistant [1–4] and can be observed in membrane vesicle preparations [5]. High-affinity transport systems involving periplasmic leucine-binding proteins (LIV- and leucine-specific binding proteins) are sensitive to osmotic shock treatment [4, 6]. System LIV-I transports L-leucine, L-isoleucine, L-valine, L-threonine, and L-alanine and is absent from strains with mutations in *livJ*, the structural gene for the LIV-binding protein. Mutations in *livK*, the structural gene for the leucine-specific binding protein, affect high-affinity leucine-specific transport [7]. In addition, two other loci, *livH* and *livG*, are essential

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for both the high-affinity leucine transport systems [8]. All four genes are clustered at minute 74 on the revised E coli linkage map [9]. The LIV-II system is weakly repressed (2-fold) by high exogenous L-leucine levels, in contrast to the more pronounced repression (> 10-fold) observed for the high-affinity leucine transport systems [10].

Two mutations resulting in reduced regulation of high-affinity leucine transport have been characterized and map at min 20 on the E coli chromosome [11]. These mutations, *livR* and *lstR*, appear to code for negatively acting regulatory elements. Mutations in several other gene loci alter the activity of the high-affinity transport systems. These mutations include *rho* [12]; *hisT*, which codes for a tRNA modifying enzyme [13]; *leuS*, a gene locus coding for leucyl-tRNA synthetase [14]; and *relA*, the gene coding for guanosine 5′-diphosphate 3′-diphosphate (ppGpp) synthesis [15]. These results suggest that normal leucine transport regulation involves interaction of charged or uncharged leucyl-tRNA and *rho* with transcription and/or translation initiation. The regulation of leucine transport appears similar to that found for the regulation of the biosynthetic operons for tryptophan, histidine, phenylalanine, and leucine [16–19].

Recently we have cloned the entire genetic complex coding for the four known LIV-I transport genes into the plasmid vector pACYC184 [20]. When introduced into a wild-type regulatory background, the genes on this hybrid plasmid, pOX1, are subject to regulation. In this paper we present the results of regulation studies with the cloned leucine transport genes and additional characterization of the effect of *rho* on this regulation.

METHODS

Growth Conditions

Unless otherwise indicated, all strains were grown in MOPS minimal medium [21] with 0.2% glucose, 50 μg/ml thymine, L-arginine, and L-histidine, 10 μg/ml of L-tryptophan, and 1 μg/ml of L-tryptophan, and 1 μg/ml pyridoxine.

Transport and Binding Protein Assays

Leucine transport was assayed as described previously [11]. Binding proteins were assayed by osmotic treatment of cells [6] and subjecting the concentrated shock fluids to equilibrium dialysis as described previously [4].

Restriction Endonuclease Digestion, Preparation, and Ligation of Plasmid DNA

Endonuclease digestions were performed as described by Boyer et al [22]. All enzymes were purchased from Bethesda Research Labs, Inc. (Bethesda, MD). Plasmid DNA was prepared by the cleared lysate procedure of Clewell and Helinski [23] and purified by CsCl gradient ultracentrifugation. Plasmid restriction fragments were isolated by electrophoresis on 0.8% agarose gels as described previously [24]. Plasmid pOX1 was constructed as described [20]. Plasmids pOX5 [29] and pOX6 were prepared by restricting pOX1 with *Bgl* II and *Pst* I, respectively, isolating and religating the fragment containing the pACYC184 vector sequence with T4 DNA ligase. Plasmid pOX7 was prepared by isolating the 2.1 kb *Bgl* II fragment from pOX1 and cloning it into the *Bam* HI site of pBR322 as described [29]. Plasmid pOX13 was similarly prepared by cloning the 4.4 kb *Hind* III-*Bam* HI fragment of pOX1 into pBR322 that had been restricted with *Bam* HI and *Hind* III.

[^{3}H]-Labeling and Analysis of Binding Proteins

Cultures (2 ml) of pOX1-transformed strain AE89 (*livR$^+$ 1stR$^+$ livH*) were grown in MOPS minimal medium plus 0.01 mCi of [^{3}H]-L-alanine. Shock fluids were prepared in 0.2 ml of 0.5 mM $MgCl_2$ as described [6]. Aliquots of shock fluid were electrophoresed in 11% native polyacrylamide gels as described by Laemmli [26]. The binding protein bands were excised from the gel, oxidized in a Packard Sample Oxidizer, and the tritium recovered in scintillation fluid for counting. TCA precipitates were treated similarily, and the levels of binding proteins were calculated as the percent of total TCA precipitable counts in the binding protein bands.

Detection of Binding Proteins by Nitrocellulose Blot and Antibody Reaction

Native gels of shock fluids were prepared as above. The electrophoresed proteins were transferred to nitrocellulose paper as described by Towbin et al [27] in a Savant electrophoretic destainer. The paper was incubated with [^{125}I]-labeled anti-LIV-binding protein antibody at 37°C for 4 h in the presence of 3% BSA and 1% calf serum to prevent nonspecific adsorption. After excess antibody was washed off with PBS, the nitrocellulose paper was dried and autoradiographed.

Transposon tn5 Insertions

Transposon tn5 was transferred to the pOX1 plasmid by infecting a pOX1 plasmid-containing strain with a temperature sensitive lambda phage carrying the tn5 transposon. Infected cells were plated on Luria broth plates containing kanamycin (20 μg/ml) and tetracycline (10 μg/ml) and incubated at 37°C. Resistant colonies were scraped off the plates and used for plasmid DNA preparation. This plasmid preparation was used to transform strain AE205 (*livH livJ*). Cells carrying a plasmid with a tn5 insertion were selected on kanamycin tetracycline Luria broth plates. Those colonies also resistant to 40 μg/ml L-valine (phenotype of LIV-I transport mutant) were selected on minimal media plates and then screened for synthesis of LIV-binding protein and high-affinity LIV-I transport. Colonies that produced LIV-binding protein but were defective in high-affinity L-leucine transport contained plasmids with tn5 insertions in *livH*. Colonies that did not produce LIV-binding protein and showed high-affinity uptake of L-leucine but not L-valine contained plasmids with tn5 insertions in *livJ*.

RESULTS

We have previously reported cloning a 13 kb *Eco* RI fragment into the plasmid vector pACYC184 to form the pOX1 plasmid, which was shown to complement all 4 known classes of high-affinity leucine transport mutations [20]. The leucine-specific binding protein gene has been located, and the direction of transcription was determined to be from left to right in Figure 1. [29].

Structure of the LIV Transport Region

To define the arrangement of genes in the LIV-I transport region of the pOX1 plasmid, we prepared several plasmids containing a subset of the pOX1 DNA sequence. Figure 1 shows the portions of the 13 kb *Eco* RI fragment contained by each of the subcloned plasmids. The deleted plasmids pOX5 and pOX6 contain the entire pACYC184 vector sequence; pOX7 and pOX13 were prepared by cloning the respective restriction fragments shown in Figure 1 into the plasmid vector pBR322 [25]. The ability of these plasmids to complement previously characterized high-affinity leucine transport mutants was determined by transforming the various mutants with each plasmid and measuring

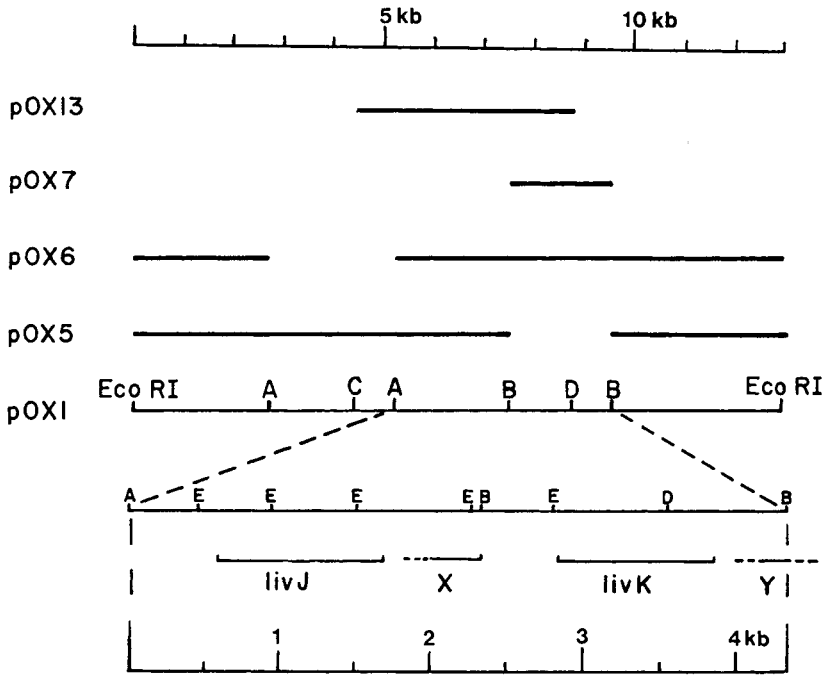

Fig. 1. Plasmids derived from pOX1 were prepared by deleting a small restriction fragment from pOXI and religating the remaining linear strand with T4 DNA ligase (pOX5 and pOX6) or by preparing a defined restriction fragment and subcloning it into a site in pBR322 (*Bam* HI site for pOX7 and the *Bam* HI and *Hind* III sites for pOX13). These plasmids contain the sequences indicated by the solid lines. *livJ* is the structural gene for the LIV-binding protein; *livK* is the structural gene for the leucine-specific binding protein. Restriction sites are indicated by letters. A, *Pst* I; B, *Bgl* II; C, *Hind* III; D, *Bam* HI; and E, *Hinc* II.

leucine transport. Where appropriate an increase in the synthesis of the binding proteins was detected directly by polyacrylamide gel electrophoresis of shock fluids from transformed strains. The results of these complementation tests, together with the previously published pOXI results [20], are presented in Table I. We have previously shown by DNA sequencing that *livK*, the gene for the leucine-specific binding protein, begins 500 bases to the right of the left *Bgl* II site shown in Figure 1 and is transcribed in the rightward direction [29]. Since pOX13 and not pOX7 complements a mutation in *livJ*, the gene for the *LIV*-binding protein, we conclude that *livJ* is upstream from the *livK* gene. We have confirmed this result by isolating a plasmid derived from pOX1 with a transposon tn5 inserted in the *livJ* gene. This plasmid, pOX1::tn5*livJ*24, contains a tn5 insertion approximately 500 bases downstream from the right-hand *Pst* I site, as shown in Figure 2. A *livJ* mutant transformed with pOX1::tn5*livJ*24 produced no protein cross-reactive with antibody to the LIV-binding protein. Therefore this tn5 insertion must occur early in the *livJ* gene. *IlivJ* is over 1 kb in length (the molecular weight of the LIV-binding protein is 37,500) and is intact on the pOX6 plasmid, which is deleted between the *Pst* I sites. Therefore, *livJ* must also be transcribed from left to right, as shown in Figure 1. This position of

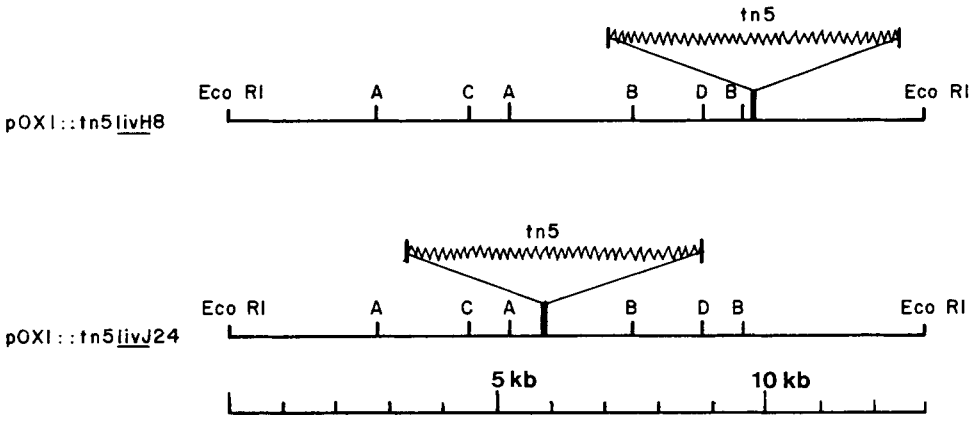

Fig. 2. Tn5 insertions were isolated as described in Methods. The insertions were mapped by restriction enzyme digestion of inserted plasmids and comparison of the products with the known sites in pOX1 and tn5. pOX1::tn5*livH*8 was mapped with restriction enzymes *Hind* III, *Sal* I, and *Bgl* II. pOX1::tn5-*livJ*24 was mapped with restriction enzymes *Hind* III, *Sal* I, and *Pst* I.

the *livJ* gene leaves a minimum of one kilobase from its end to the beginning of the *livK* gene, a distance sufficient to code for an additional protein of up to 35,000 daltons. This region is represented by X in Figure 1.

A comparison of Table I with the plasmid fragments in Figure 1 also reveals that *livH* must lie at least in part to the left of the right *Bgl* II site. A tn5 transposon insertion in the *livH* gene of pOX1 was isolated which confirms this result. As shown in Figure 2, pOX1::tn5*livH8* contains a tn5 insertion approximately 100 bases downstream from the left *Bgl* II site. We conclude that this region, represented by Y in Figure 1, is most likely the *livH* gene.

Recently we have obtained preliminary DNA sequence information which confirms the gene map shown in Figure 1. The *livJ* gene has been located as predicted above. Upstream from *livK* is a 500-base AT-rich region that contains numerous nonsense codons in all three reading frames and is likely to be a spacer or regulatory region for the *livK* gene. Around the right *Bgl* II site we have located a sequence that contains a single reading frame not interrupted by nonsense codons. When translated, this sequence codes for a basic cysteine-rich protein. We expect the *livH* protein to be both basic and cysteine-rich by analogy to the *hisP* protein described by Ames and co-workers for high-affinity histidine transport in S typhimurium [30, 31]. A similar basic cysteine-rich coding region has been found in region X. These DNA sequences will be published elsewhere when completed.

Regulation of Plasmid Transport Genes

We have previously shown that the amplified leucine transport present in pOX1 transformed strains is regulated by the concentration of L-leucine in the medium [20]. To determine if DNA sequences involved in the regulation of both leucine-binding protein genes are present in pOX1 and its derivatives, we have assayed the level of the LIV- and leucine-specific binding proteins produced when transformed strains were grown in the presence and absence of leucine. The results of these experiments are shown in Table II.

TABLE I. Complementation of Known LIV Genes by pOX Plasmids

Plasmid	*livJ*[a]	*livK*[a]	*livH*[b]	*livG*[b]
pOX1	+	+	+	+
pOX5	+	–	–	–
pOX6	+	+	+	X[c]
pOX7	–	+	–	–
pOX13	+	–	–	–

[a]Binding protein gene complementation was assayed by detection of gene products on polyacrylamide gels.
[b]*livH* and *livG* complementation was assayed by leucine transport measurements in plasmid-transformed mutant strains.
[c]X, not determined.

TABLE II. Regulation of Binding Protein Synthesis in Transformed Strains

		Binding protein synthesis[a]			
		LIV		LS	
Strain	Plasmid	No L-leucine	+L-leucine	No L-leucine	+L-leucine
AE89[b]	–	7.9	3.1	2.8	1.0
	pBR322	5.0	3.1	2.0	0.8
	pOX1	80	51	3.0	1.0
	pOX5	74	55	2.0	0.8
	pOX6	89	47	3.5	1.4
	pOX7	5.6	3.1	23	15
	pOX13	86	25	2.7	1.0

[a][^{3}H]-L-alanine counts in binding protein/total[^{3}H]-L-alanine counts in TCA precipitate of osmotic shock fluid × 100. See Methods for experimental protocol.
[b]Strain AE89 is *livR*$^+$ *lstR*$^+$ *livH*$^-$.

Interestingly, while plasmid-bearing strains show an amplification in the level of LIV-binding protein, no concomitant rise in leucine-specific binding protein synthesis is evident. Only when the *livJ* gene is defective or absent, as in the pOX7 plasmid, does the *livK* gene show amplified expression. In the pOX7 plasmid, this amplified expression is still regulated by leucine. In all plasmids examined, significant regulation of the respective leucine-binding protein synthesis was observed. These results suggest that the relevant regulatory sequences are present on these plasmids. In particular, a regulatory region for *livK* must be present in the 500 bases preceding the beginning of the *livK* gene in pOX7. Similarly, a regulatory region for *livJ* must be present in the 500 bases preceding the beginning of the *livJ* gene in pOX6. The lowered efficiency of regulation seen in the transformed strains may be caused by the amplified regulatory sequences, which could saturate the available regulatory components. These results support some degree of interdependent yet separate regulation for *livJ* and *livK* gene expression.

TABLE III. Kinetic Constants for LIV Transport and Leucine-Binding Activities in Binding Protein Mutants

		LIV uptake[a]						L-leucine binding activity pmole/mg protein	
		L-leucine		L-isoleucine		L-valine			
Strain	Genotype	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	*LIV-BP*[b]	*LS-BP*[b]
AE84	*livR*	0.6	8.3	0.25	4.3	0.22	4.2	660	163
AE840102	*livR livJ*	0.8	7.7	ND[c]	ND	ND	ND	34	315
AE84084	*livR livK*	ND	ND	ND	ND	ND	ND	731	0

[a]All strains grown in MOPS minimal medium without leucine and harvested during log phase growth. K_m is given in μM; V_{max} in nM/min per mg dry cell wt.
[b]LS-BP (leucine-specific binding protein) activity was determined by equilibrium dialysis of crude osmotic shock fluids [6] in 2.5 μM [^{3}H]-L-leucine + 200 μM L-isoleucine. LIV-BP activity was calculated as activity in 2.5 μM [^{3}H]-L-leucine minus LS-BP activity.
[c]ND, not detectable.

Synthesis of Leucine-Specific Binding Protein

The interdependent regulation of *livJ* and *livK* expression in the plasmids led us to examine the level of leucine-specific binding protein production in non-transformed strains that were defective in *livJ*, the LIV-binding protein gene. As shown in Table III, the uptakes of L-isoleucine and L-valine, substrates for LIV-binding protein-mediated transport, are not detectable in strain AE840102, which lacks the LIV-binding protein. L-Leucine transport, however, is virtually unaffected. That this is due to an increase in leucine-specific binding protein synthesis resulting in an increase in leucine-specific transport is evident from measurements of the level of the binding proteins by equilibrium dialysis of crude shock fluids. While the amount of LIV-binding protein is 20-fold less in strain AE840102 than that in strain AE84, the leucine-specific binding protein is amplified 2-fold.

Mutation of *livK* in strain AE84084 results in a slight increase in LIV-binding protein activity, which is also consistent with compensatory regulation of the two binding protein genes. The low levels of L-isoleucine and L-valine uptake in strain AE84084 are probably due to a polar effect of a Mu insertion in *livK* on the expression of the downstream *livH* gene.

Rho-Dependent Regulation Is Independent of *livR* Regulation

It is now well established that mutations in the gene for transcription termination, *rho*, cause derepression of high-affinity leucine transport [12]. This phenotype is similar to that produced by the *livR* mutation. We wondered, therefore, whether the two effects were part of the same regulatory mechanism or, alternatively, if the *rho* effect could still be observed in *livR* strains already derepressed for leucine transport. To answer this question, we prepared strain AE141 (*livR ilv*::tn10) by transduction of the *livR* strain AE84 with P1 phage carrying a transposon tn10 insertion in *ilv*. Since the *rho* gene is 85% linked to the *ilv* locus, we were able to introduce a *rho201* mutation into strain AE141 by transducing it with a P1 phage grown on a *rho201 ilv*$^+$ strain (kindly provided by J. Beckwith) and then selecting for *ilv*$^+$ colonies. As shown in Table IV, leucine uptake was increased

over 400% when *rho* was present in this strain background. On the basis of this result, it is unlikely that *rho* exerts its effect on leucine transport by altering expression of the *livR* gene. Thus we conclude that rho-dependent termination is involved in leucine transport regulation in a manner independent of *livR* repression.

Rho Affects the Expression of Both *livJ* and *livK*

We previously suggested that the *rho*-dependent effect on high-affinity leucine transport results from a *rho*-dependent termination in transcription attenuation of LIV-I transport gene expression [12]. It is also possible that derepression of LIV-I genes occurs because a termination upstream from one or more of the genes is relieved by mutations in *rho*. In the latter case, one might expect *livK* expression to be more sensitive to *rho* mutations than the expression of *livJ*, since *livK* is clearly the downstream gene. To assess this possibility, we assayed binding protein synthesis by preparing crude shock fluids of strain X8605 and X8605*rho201*. The strain X8605 background has previously shown a reduced *rho* effect in contrast to the effect found in our own strains (Table IV). We therefore expected strain X8605 shock fluids to show corresponding reduced binding protein levels. These shock fluids were electrophoresed on polyacrylamide gels and transferred to nitrocellulose paper as described in Methods. Table V shows the results of these assays.

TABLE IV. Leucine Transport in *livR rho* Double Mutants

Strain	Genotype	Uptake[a]
AE141	*livR ilv*::tn10	0.27
AE141:P1 (*rho201*)[b]	*livR rho 201*	1.23

[a] Expressed in nM/min per mg dry cell wt at a L-leucine concentration of 0.1 μM. Cells were grown in MOPS minimal medium without leucine.
[b] Strain AE141 was transduced with P1 phage grown on strain X8605 *rho201 ilv*$^+$ and selected for *ilv*$^+$ (85% linked to *rho*). The uptake shown is the average of that obtained for four independent isolates showing elevated transport.

TABLE V. Effect of the *rho201* Mutation on the Synthesis of LIV- and Leucine-Specific Binding Proteins

	LIV-binding protein[a]		LS binding protein[a]	
Strain	−L-Leucine[b]	+L-Leucine	−L-Leucine	+L-Leucine
X8605	100	25	ND[c]	ND
X8605*rho201*	140	120	7	7

[a] LIV- and leucine-specific binding protein synthesis was detected by the antibody nitrocellulose blot method described in Methods. Synthesis was quantitated by determining the amount of [^{125}I]-antibody bound by each band from densitometer tracings of the autoradiographs. It is expressed as the percent relative to LIV-binding protein synthesized by strain X8605 grown without L-leucine after correcting for total protein loaded on the gel.
[b] Cells were grown in MOPS minimal medium as described in Methods with or without 0.1 mM L-leucine (13 μg/ml) and harvested in mid-log phase.
[c] ND, not detectable.

Shock fluid from the *rho* mutant, strain X8605*rho201*, contains an elevated concentration of LIV-binding protein relative to shock fluid from the wild-type strain X8605. The leucine-specific binding protein was not detected in shock fluid from strain X8605 but was clearly present in shock fluid from strain X8605*rho201*. Synthesis of neither binding protein was sensitive to regulation by external L-leucine when the *rho201* lesion was present. These results clearly demonstrate that the expression of both binding protein genes is sensitive to a defective *rho* termination factor.

DISCUSSION

Previous work in several laboratories has led to the general observation that the level of high-affinity transport can be correlated with the concentration of specific periplasmic binding proteins for a given transport system [10]. The regulation of synthesis of the LIV- and leucine-specific binding proteins is a prime determinant, therefore, in controlling the level of high-affinity leucine transport in E coli.

In this paper we have shown that *livJ* and *livK*, genes for the LIV- and leucine-specific binding proteins, respectively, are separated by approximately 1 kb and are capable of independent expression and regulation. Both genes are transcribed in the same direction. It has been shown that transcription and translation of the upstream *livJ* gene have a definite inhibitory effect on expression of the downstream *livK* gene. The magnitude of this inhibition is variable. In the X8605 strain background, no detectable leucine-specific binding protein is produced unless a *rho* lesion is introduced. This result may explain reports by other workers who have not observed leucine-specific uptake in certain E coli strains [32]. The significant levels of leucine-specific binding protein observed in our laboratory may be due to a high basal level of expression of the *livK* gene in our E coli strains.

As shown by the data in Tables II and III, the expression of *livK* may be further stimulated when the *livJ* gene is mutated or deleted. This result is observed whether *livK* is in the chromosome or carried on a plasmid. Several explanations for this phenomenon are possible, but the data presented in this paper suggest that the 500 base pair non-coding region directly in front of the *livK* gene may contain an independent promoter that ordinarily is inactive but that can be activated in response to mutations or other events that reduce *livJ* expression. Several lines of evidence suggest that this explanation is likely. First, it is clear from the results in Table V that the *rho* effect cannot be explained solely on the basis of increased read-through of the downstream *livK* since *livJ* is also affected. Thus it appears that rho can operate in regulatory regions preceding both the *livJ* and *livK* genes. Second, the expression of *livK* is either unaffected or slightly elevated on the pOX1::tn5*livJ*24 plasmid, which contains the 5.5 kb tn5 transposon inserted in the *livJ* gene. It is unlikely that RNA polymerase, which initiates transcription at a promoter in front of *livJ*, would successfully transcribe the entire tn5 sequence and continue through to the *livK* gene. While transcription of the *livK* gene might initiate at a promoter within the tn5 transposon, we consider it more probable that the *livK* gene has a separate promoter. Third, the expression of the *livK* gene on the pOX7 plasmid is still regulated by L-leucine. The effect of the regulatory mutations *livR* and *lstR* also support this interpretation. Anderson and co-workers have shown that *lstR* leads to a dramatic derepression of leucine-specific binding protein synthesis, whereas *livR* leads primarily to an increase in the LIV-binding protein [11]. Both of these lesions map at min 20 on the E coli linkage map. Since these mutations complement each other genetically, they appear to represent

independent negatively acting regulatory elements. The fact that they differentially affect *livJ* and *livK* expression stongly suggests that the *livR* gene product acts as a promoter in front of *livJ*, and that the *lstR* gene product acts as promoter in front of *livK*. Finally, in support of a separate regulatory region for the *livK* gene, the 500 base pair AT-rich region, immediately upstream of the *livK* gene, contains the sequence TGTTTGTCTAT-CAATAAATTCGGAATATTATCTGTTC approximately 250 bases upstream from the *livK* start codon. This sequence corresponds closely to the conserved elements (underlined) or previously identified RNA polymerase promoters [33].

In developing a model for leucine transport regulation, the effects of leucyl-tRNA, *rho* factor, and ppGpp must be considered. With the exception of *rho* factor, we do not know whether the other regulatory elements alter the expression of only one, or both binding protein genes. In addition, the derepressing effects of mutations *hisT* and *leuSI* may be due either to negative regulation by leucyl-tRNA or to a positive effect of uncharged $tRNA^{leu}$.

Our present understanding of the regulation of high-affinity leucine transport is presented in Figure 3. This is represented schematically as a working model, and is not intended to represent precisely the interactions of the various regulatory factors. We suggest that two regulatory loci are involved. The first, in front of the *livJ* gene, ordinarily allows high-efficiency transcription of *livJ*. Regulation at the *livJ* promoter is accomplished by the *livR* gene product, a negative effector for *livJ* gene expression. In addition, transcription termination factor rho and $tRNA^{leu}$ also regulate *livJ* expression [10]. As shown in Figure 3, we suggest that a second regulatory site, most likely including a second promoter, exists directly in front of *livK*. The product of *lstR* apparently acts at the *livK* promoter in a manner similar to the action of *livR* gene product at the *livJ* promoter.

Finally, we wish to point out the evolutionary implications of this model. On the basis of amino acid sequences obtained by Dr. Ovchinnikov and colleagues [34; personal communication] and our own DNA sequencing studies it is clear that *livJ* and *livK* have evolved from a common ancestral gene by gene duplication. The amino acid sequence of the two binding proteins is approximately 80% conserved. Regions X and Y show similar-

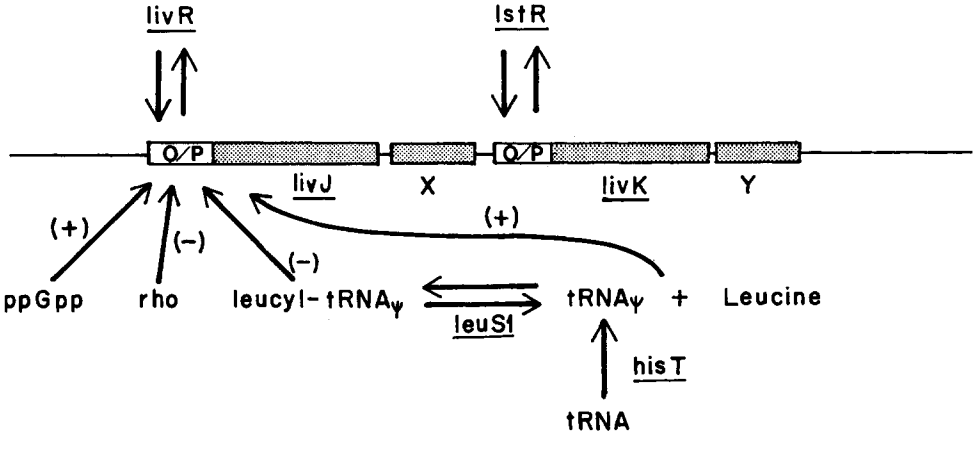

Fig. 3. Model of leucine transport regulation in Escherichia coli.

ities in size and position, suggesting that this duplication involved a larger region than the binding protein gene itself. If the regulatory regions in front of *livK* and *livJ* were included in this duplication, then these regions have diverged from a common ancestor, since the two genes are now regulated differently. Additional sequence analysis will provide more information on the evolutionary relationships of the regulatory regions and mechanisms.

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