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A New Locus (*leuK*) Affecting the Regulation of Branched-Chain Amino Acid, Histidine, and Tryptophan Biosynthetic Enzymes

CAROLYN S. BROWN,[1] ROBERT WEST,† RICHARD H. HILDERMAN,[1] FRANK T. BAYLISS,‡ AND ELLIS L. KLINE§*

Department of Biochemistry, Clemson University, Clemson, South Carolina 29631,[1] and Department of Biology, Edinboro State College, Edinboro, Pennsylvania 16444[2]

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A locus (*leuK*) affecting regulation of the leucine operon was selected by isolating a spontaneous Ara$^+$ derivative of an *Escherichia coli* B/r strain carrying an *ara-leu* fusion in which the arabinose operon is under leucine control. Genetic analyses by P1 transduction demonstrated that the lesion is located to the right of the galactose operon. Regulation of the biosynthetic enzymes for leucine, isoleucine-valine, histidine, and tryptophan was altered in a strain carrying *leuK16*. High-level gene expression in the heterozygous merodiploid strain (F′ *leuK*$^+$/*leuK16*) demonstrated the dominance of the mutant allele to the wild-type allele. No apparent effect was observed in the mutant on *N*-acetylornithinase, a biosynthetic enzyme in the arginine pathway, nor on any of the 18 aminoacyl-tRNA synthetases examined. However, compared with that of the parent strain, the extent of the charging of leucyl-, isoleucyl-, valyl-, histidyl-, and arginyl-tRNA was decreased in the mutant.

The synthesis of leucine in *Escherichia coli* is governed by the expression of four structural genes constituting an operon located next to the arabinose gene cluster (32). Analyses of mutant strains of *E. coli* and *Salmonella typhimurium* altered in their control of leucine biosynthesis have revealed some leucine regulatory loci which are linked and others which are unlinked to the leucine operon (1, 4–6, 11, 14, 28). Mutant *S. typhimurium* strains carrying leucine regulatory lesions that are linked to the leucine operon have been shown to contain either operator-constitutive (O^c) mutations (5) or mutations in the *leuA* structural gene which result in a feedback-insensitive α-isopropylmalate synthetase (4, 28). Lesions causing elevated expression of the leucine biosynthetic enzymes which are unlinked to the *leu* operon have been described for the *flrA*, *flrB*, *hisT*, and *leuS* loci (1, 6, 11, 14; B. Ghajar, Ph.D. dissertation, University of California, Davis, 1972). In this paper, we describe another locus (*leuK*) affecting the regulation of the leucine structural genes which is unlinked to the leucine operon or to any other leucine regulatory locus previously described. This lesion also affects the regulation of the isoleucine-valine, the histidine, and the tryptophan biosynthetic pathways.

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† Present address: Department of Genetics, University of California at Davis, Davis, CA 95616.

‡ Present address: Center for Advanced Medical Technology, San Francisco State University, San Francisco, CA 94132.

§ Permanent address: Department of Microbiology, Clemson University, Clemson, SC 29631.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used, their genotypes, and their derivations are given in Table 1. The mutation *leuK16* was formerly designated *leu-16* (32).

Media. Eosin-methylene blue agar, L-broth, and minimal base were described by Sheppard and Englesberg (25). The following supplements were added to minimal medium when required: 0.5% glucose, 0.2% galactose, 0.2% arabinose, 0.4 mM leucine, 1 mM valine, 0.4 mM isoleucine, 0.4 mM histidine, 0.4 mM arginine, and 0.4 mM tryptophan.

Chemicals. Sodium pyruvate, α-ketoisovaleric acid, acetyl coenzyme A (trilithium salt), thiamine pyrophosphate, pyridoxal phosphate, and DL-5-methyl-tryptophan were purchased from Calbiochem. Dihydroxy isovalerate and α-acetohydroxy butyrate were obtained through the Edinboro Foundation. Nicotinamide adenine dinucleotide (grade III), L-histidinol·2HCl, glycine, glutathione, *N*-α-acetyl-L-ornithine were obtained from Sigma Chemical Co. Ninhydrin was purchased from Mallinckrodt Chemical Works. Ethylene glycol monomethyl ether was from Fisher Chemical Co. L-Canavanine sulfate and 1,2,4-triazole-3-alanine were purchased from Nutritional Biochemicals Corp. 5′,5′,5′-Trifluoro-DL-leucine was obtained

TABLE 1. *Bacterial strains used*[a]

Strains	Genotype	Source
F⁻ strains		
B/r UP1007	Wild type	E. Englesberg
B/r SB2074	*ara-leu-1170 + 1238 dau-5 str*	D. Kessler
B/r DC71	*leuB1 mal*⁺ λˢ	D. Kessler
B/r DC74	*ara-leu-1170 + 1238 dau-5 str mal*⁺ λˢ	P1*bt* DC71 × SB2074
B/r SB5031	*ara-leu-1170 + 1238 dau-5 str leuK16*	Spontaneous Ara⁺ derivative of SB2074 (D. Kessler)
B/r DC73	*ara-leu-1170 + 1238 dau-5 str*	P1*bt* DC71 × SB5031
B/r DC501	*ara-leu-1170 + 1238 dau-5 str leuK16 mal*⁺ λˢ *gal-5*	DES-induced Gal⁻ mutant of DC73
B/r DC521	*dau-5 str mal*⁺ λˢ *gal-5*	DES-induced Gal⁻ mutant of DC74
B/r DC937	*dau-5 str leuK16 mal*⁺ λˢ *gal-5*	P1*bt* UP1007 × DC501
B/r DC938	*tolA,B nadA1 aroG dau-5 str leuK16 mal*⁺ λˢ	B. Ghajar
B/r EB145	*dau-5 str mal*⁺ λˢ	P1*bt* UP1007 × DC74
B/r EB146	*dau-5 str mal*⁺ λˢ *leuK16*	P1*bt* UP1007 × DC73
B/r EB209	*ara-leu-1170 + 1238 dau-5 str mal*⁺ λˢ *gal-5*	Segregant from EB2019
F′ strains		
K-12 UC200	*F′ gal*⁺ *pyrD*⁺ *trp*⁺/*gal pyrD trp his*	B. Low
B/r EB2019	*F′ gal*⁺ *pyrD*⁺ *trp*⁺/DC501	UC200 × DC501
B/r EB2020	*F′ gal*⁺ *pyrD*⁺ *trp*⁺/DC521	UC200 × DC521

[a] Auxotrophic requirements: *ara-leu-1170 + 1238*, leucine; *leuB1*, leucine; *nadA1*, nicotinic acid; *trp*, tryptophan; *pyrD*, pyrimidine; *his*, histidine. Abbreviations: *dau-5*, D-arabinose negative; *str*, streptomycin resistant; λˢ, *E. coli* B/r sensitive to lambda infection; DES, diethyl sulfate.

from H. E. Umbarger. All salts and buffers were of reagent grade.

Feeding indicator plates. The procedure used for feeding indicator plates was described previously (14).

Amino acid analog resistance. Resistance to DL-5-methyltryptophan, L-canavanine sulfate, DL-azaleucine, 5′,5′,5′-trifluoro-DL-leucine, and DL-1,2,4-triazole-3-alanine was tested separately by placing 0.3 ml of 0.1 M amino acid analog in the center of an L-agar or glucose-supplemented minimal agar plate that had been streaked with bacteria from the center of the plate to the exterior edge. The wild-type strain, EB145, and the mutant strain, EB146 (*leuK16*), were always placed on the same plate. A resistant character was demonstrated by an increase of growth toward the center relative to that of the isogenic wild-type strain EB145.

Transductions. P1*bt* transduction experiments were performed by the procedure of Gross and Englesberg (12).

Selection of a spontaneous regulatory mutation. The isolation of *leuK16* was accomplished by selecting a spontaneous Ara⁺ derivative of SB2074 which carried the *ara-leu* fusion *1170 + 1238* (Fig. 1), thus placing the arabinose structural genes under the control of the leucine operon (32).

Tests for dominance and segregation. The exogenote F′B3 (14), carrying a wild-type allele for the *leuK* locus, was obtained from UC200. A modification of the Low method (18) for merodiploid construction was used in forming the heterozygous merodiploid. (i) Mating was performed at 50°C (to decrease the restriction nuclease) by mixing equal volumes of donor and recipient strains (1×10^8 to 5×10^8 cells per ml) which were growing in L-broth. (ii) After mating proceeded for 1 hr, 10^{-3}, 10^{-4}, and 10^{-5} dilutions of the mating mixture were shaken vigorously for 1 min. (iii) Samples from each dilution (0.1 ml) were plated on galactose minimal plates, selecting for Gal⁺ progeny. (iv) Colonies were picked from the highest dilution plate after 3 days of incubation at 37°C.

After growth of the heterozygous merodiploid strain (Gal⁺ Ara⁺) for enzymatic assays, approximately 50 Gal⁺ Ara⁺ colonies from streak plates were characterized for their ability to segregate Gal⁻ Ara⁺ and/or Gal⁻ Ara⁻ colonies on eosin-methylene blue-galactose and on eosin-methylene blue-arabinose-galactose media. The Ara⁺ phenotype of a strain carrying the *ara-leu* fusion *1170 + 1238*, in which arabinose is under control of the leucine operon, is indicative of an abnormally derepressed leucine operon. In this situation it is due to the presence of *leuK16*. The segregation of a Gal⁻ Ara⁻ phenotype verifies the presence of the *leuK*⁺ allele on the F′ particle.

Preparation of tRNA. The method of Zubay (33) was used to isolate tRNA from strains EB145 and EB146. Cells were grown with aeration in minimal glucose medium in 13-liter quantities in a New Brunswick Microferm fermentor. The cells were harvested at an absorbance at 660 nm (A_{660}) of 0.55, washed twice with 0.9% sterile saline, and frozen. The cells were then thawed, and 13 g was suspended in 42 ml of sterile saline. To this suspension was added 1.5 volumes of phenol, and the mixture was shaken at room temperature for 30 min. A volume of chloroform equal to 0.1 the volume of the phenol was added, and the mixture was shaken at room temperature for 15 min. The cell suspension was then chilled on ice for 15 min and centrifuged at 10,000 rpm for 25 min at 5°C. The aqueous layer was removed and recentrifuged with a volume of phenol equal to 0.5 the volume of the aqueous layer. After recentrifugation, 4.2 ml of 20% (wt/vol) potassium acetate was added to the aqueous layer. To this solution was added 40 ml of cold ethanol

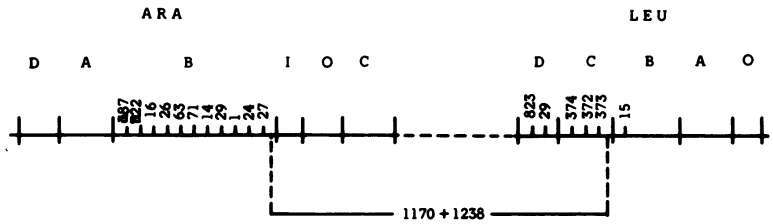

FIG. 1. *Arabinose-leucine gene region in E. coli B/r, showing the ara-leu fusion 1170 + 1238. The arabinose structural genes are under the control of the leucine operator.*

(−18°C). The solution was left at −18°C for 3 h and then centrifuged at 10,000 rpm for 25 min at 5°C. The pellet was suspended in 30 ml of 0.3 M sodium acetate (pH 7.0) at room temperature. After the addition of 15 ml of isopropanol with stirring, the mixture was centrifuged for 25 min at 10,000 rpm at room temperature. To the supernatant was added 15 ml of isopropanol with stirring, and the mixture was again centrifuged at 10,000 rpm for 25 min at room temperature. The pellet was taken up in 2 ml of sterile water containing 5×10^{-4} M magnesium acetate. The A_{260}/A_{280} for these preparations was 1.94, indicating a high degree of purity.

Partial purification of aminoacyl-tRNA synthetases. Aminoacyl-tRNA synthetases from strains EB145 and EB146 were partially purified by diethylaminoethyl column chromatography immediately before assay. Cells were grown with shaking in 500 ml of minimal glucose medium in 1-liter flasks. When the A_{660} reached 0.55, the cells were harvested. The pellet from 1 liter of cells was suspended and sonically oscillated in 4 volumes of 100 mM tris(hydroxymethyl)-aminomethane-hydrochloride (pH 8.0) containing 10 mM magnesium chloride and 10% glycerol. After 15 min of centrifugation at 10,000 rpm and 4°C and 2 h of centrifugation at 198,000 × *g* and 4°C, the supernatant was applied to a diethylaminoethyl column (0.9 by 6 cm) equilibrated with 20 mM potassium phosphate (pH 7.5) containing 10 mM mercaptoethanol, 1 mM magnesium chloride, and 10% glycerol. The column was eluted with 0.25 M potassium phosphate (pH 6.5) containing 10 mM mercaptoethanol, 1 mM magnesium chloride, and 10% glycerol until the A_{280} fell below 0.1.

Preparation of extracts for assay of biosynthetic enzymes. The cultures were grown at 37°C with shaking until they attained an A_{660} of 0.55 on a Gilford spectrophotometer. At this time the flasks were removed and immediately chilled on ice. The cells were harvested at 4°C and washed twice with 10 ml of 0.05 M potassium phosphate buffer (pH 7.5) or tris(hydroxymethyl)aminomethane buffer (pH 7.5). The cells were resuspended in a total of 1.5 ml of buffer and disrupted with a Branson model S-125 Sonifier. Cell debris was removed by centrifugation at 15,000 rpm for 15 min. All steps were carried out at 4°C.

Assay procedures. L-Threonine deaminase (EC 4.2.1.16) (10), α-acetohydroxy acid synthase (EC 1.1.3.12) (27), isomeroreductase (EC 1.1.1.78) (15), dihydroxy acid dehydrase (EC 4.2.1.9) (27), transaminase B (8), α-isopropylmalate synthetase (EC 1.1.1.85) (3, 30), *N*-acetyl ornithinase (EC 3.5.1.16) (29), and aminoacyl-tRNA synthetase (EC subgroup 6.1.1) assays (33) were performed as previously described. Histidinol dehydrogenase (EC 1.1.1.23) was assayed by following the production of reduced nicotinamide adenine dinucleotide in a Gilford spectrophotometer according to method b of Martin et al. (20). A correction for alcohol dehydrogenase activity in the histidinol dehydrogenase assay was made by running a blank of substrate plus extract from strain RW84, which lacks histidinol dehydrogenase (RW84 was kindly provided by Bert Ely). The values obtained with strain RW84 were subtracted from the experimental values. Tryptophan synthase was assayed by the procedure of Yanofsky (personal communication). Protein was determined by the method of Lowry et al. (19).

RESULTS

Effect of *leuK16* on the repression of branched-chain amino acid biosynthesis. The fact that an *ara-leu* fusion strain containing *leuK16* exhibited an Ara^+ phenotype in the presence of leucine initially demonstrated the lack of the proper repression mechanism for the leucine biosynthetic enzymes. Subsequent investigations indicated that EB146 (*leuK16*) excreted leucine as evidenced by its ability to cross-feed a leucine deletion strain (DC455). Excretion of leucine in strains derepressed for the leucine operon has been reported previously (1, 14). In addition, EB146 grown in the presence or absence of branched-chain amino acids had an elevated expression of the *leuA* gene product (α-isopropylmalate synthetase) when compared with the isogenic parental strain EB145 grown under the same conditions (Table 2). The generation times for EB145 and EB146 were virtually identical under these conditions. These findings substantiated the initial observation denoted by the Ara^+ phenotype, i.e., that the arabinose structural genes are under leucine control (Fig. 1).

The effects of *leuK16* on the biosynthesis of the other two branched-chain amino acids (isoleucine and valine) were also analyzed. The activities of each of the five isoleucine-valine biosynthetic enzymes were also highly elevated (Table 2). These results demonstrated that the

component or components derived from the locus *leuK16* are involved in the regulation of the isoleucine-valine structural genes as well as the leucine operon.

Effect of *leuK16* on other amino acid biosynthetic systems. To gain further insight into the effect of *leuK16* on other amino acid biosynthetic systems, a strain carrying *leuK16* was screened for its resistance to various amino acid analogs. Strains carrying the *leuK16* lesion were resistant to analogs for tryptophan and histidine as well as to analogs for isoleucine, valine, and leucine (Table 3). The data also indicated no resistance to L-canavanine sulfate, an analog for arginine.

Since resistance to a specific amino acid analog can be due to elevated production and excretion of the corresponding amino acid, analyses of the gene expression for the histidine, the tryptophan, and the arginine biosynthetic enzymes were conducted in a strain carrying *leuK16*.

The data (Table 4) indicate that *leuK16* had no significant effect on the arginine biosynthetic enzyme *N*-acetylornithinase. However, a comparison of histidinol dehydrogenase and tryptophan synthase activities in EB145 versus EB146 revealed a 12.4-fold increase in the activity of the dehydrogenase and a 7.1-fold increase in the activity of tryptophan synthase in the strain carrying *leuK16*. This pleiotropic effect of a regulatory locus on leucine, isoleucine-valine, histidine, and tryptophan biosynthesis has a precedent in the effects of the *hisT* lesion on branched-chain amino acid, as well as histidine, biosynthesis (6).

Effects of *leuK16* on the aminoacylation of tRNA. Since tRNA's and/or aminoacyl-tRNA synthetases have been implicated in the control of the biosynthesis of leucine, isoleucine, valine, and histidine (1, 2, 6, 9, 17, 23, 24, 26, 31), comparisons of both tRNA's and aminoacyl-tRNA synthetases from strains EB145 and EB146 were made. The data (Fig. 2) demonstrate that the rate and extent of aminoacylation were essentially the same with aminoacyl-tRNA synthetases isolated from the mutant and the parent cells. Differences were observed, however, in the aminoacylation of the tRNA isolated from the two strains. Certain tRNA's from the

TABLE 2. *Effect of leuK16 on the repression of branched-chain amino acid enzymes*

Strain	Pertinent genotype	Generation time (min)	Growth condition[a]	Relative activity[b] of the biosynthetic enzymes for:					
				Isoleucine-valine					Leucine (α-IPM synthetase)
				TD	AHAS	IR	DH	TRB	
EB145	Wild type	65	Excess	1.0	1.0	1.0	1.0	1.0	1.0
		65	Minimal	6.7	4.8	4.1	5.6	7.8	5.1
EB146	*leuK16*	65	Excess	7.1	12.0	2.4	4.8	4.3	33.1
		65	Minimal	13.2	25.2	14.1	30.9	12.1	65.3

[a] Cultures were grown in glucose minimal medium with (excess) or without (minimal) isoleucine, valine, and leucine.

[b] The values are relative to the repressed levels of isoleucine-valine and leucine enzymes for the wild-type control (EB145). The specific activities for EB145 under excess conditions were as follows: threonine deaminase (*ilvA*; TD), 59.9; α-acetohydroxy acid synthase (*ilvB*; AHAS), 9.75; isomeroreductase (*ilvC*; IR), 20.3; dihydroxy acid dehydrase (*ilvD*; DH), 65.7; transaminase B (*ilvE*; TRB), 13.1; α-isopropylmalate synthetase (*leuA*; α-IPM), 0.05. Values are expressed as micromoles of product formed per hour per milligram of protein. All values represent the average of at least three determinations obtained from three independent experiments. The values from the independent determinations agreed within 10%.

TABLE 3. *Resistance to amino acid analogs*[a]

Strain	Genotype	Resistance[b] to:				
		5-MT	AZL	CAN	TFL	TRA
EB146	*leuK16*	+	+	−	+	+
EB145	*leuK*$^+$ (wild type)	−	−	−	−	−
EB2019	F′ *leuK*$^+$/*leuK16*	+	+	−	+	+
EB2020	F′ *leuK*$^+$/*leuK*	−	−	−	−	−

[a] The methodology is described in the text. Abbreviations: 5-MT, 5-methyltryptophan; AZL, azaleucine; CAN, L-canavanine; TFL, trifluoroleucine; TRA, triazolealanine. These compounds are analogs for tryptophan, isoleucine-valine, arginine, leucine, and histidine, respectively. All analyses were made three times.

[b] Resistance (+) or nonresistance (−) to the analog.

TABLE 4. *Effects of leuK16 on repression of other amino acid enzymes*

Strain	Pertinent genotype	Generation time (min)	Growth condition	Relative activity[a] of the biosynthetic enzymes for:		
				Arginine (NAOase)	Histidine (HDH)	Tryptophan (TSase)
EB145	Isogenic wild type	65	Excess[b]	1.0	1.0	1.0
EB146	*leuK16*	65	Excess	0.7	12.4	7.1

[a] Values are relative to repressed levels of histidine, arginine, and tryptophan enzymes for the isogenic wild-type strain EB145. Abbreviations: NAOase, *n*-acetylornithinase (*arg*); HDH, histidinol dehydrogenase (*hisD*); TSase, tryptophan synthase (*trpAB*). Specific activities for EB145 under excess conditions were as follows: NAOase, 14.2; HDH, 0.22; TSase, 12.89. Values are expressed as micromoles of product per hour per milligram of protein. All values represent the average of at least three determinations obtained from three independent experiments. The values from the independent determinations agreed within 10%.

[b] Cultures were grown in glucose minimal medium with excess isoleucine, valine, leucine, histidine, arginine, and tryptophan.

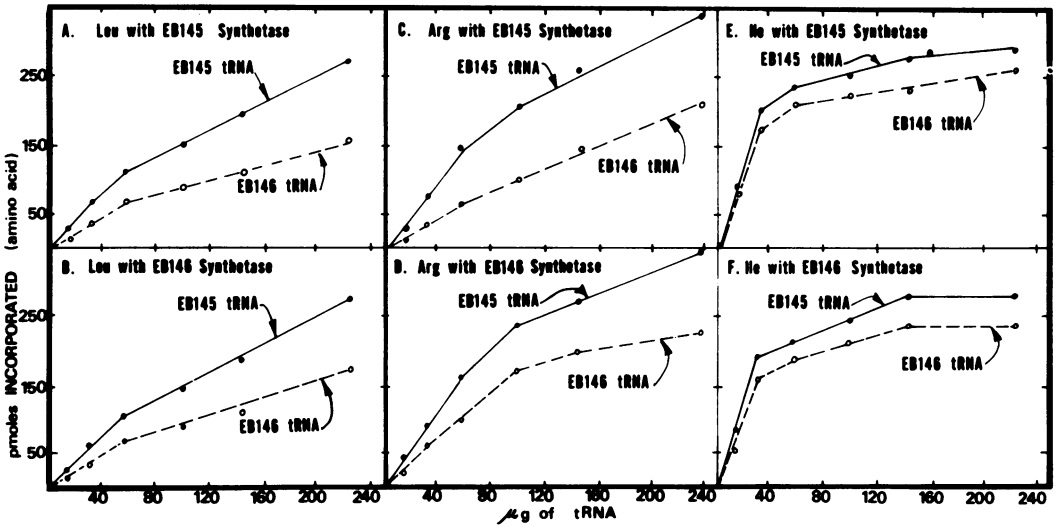

FIG. 2. *Aminoacylation of tRNA from strains EB145 and EB146 (leuK16) by the aminoacyl-tRNA synthetases from strains EB145 and EB146. (A) Incorporation of [^{14}C]leucine into tRNA by the aminoacyl-tRNA synthetase obtained from strain EB145. (B) Incorporation of [^{14}C]leucine into tRNA by the aminoacyl-tRNA synthetase obtained from strain EB146. (C) Incorporation of [^{14}C]arginine into tRNA by the aminoacyl-tRNA synthetase obtained from strain EB145. (D) Incorporation of [^{14}C]arginine into tRNA by the aminoacyl-tRNA synthetase obtained from the strain EB146. (E) Incorporation of [^{14}C]isoleucine into tRNA by the aminoacyl-tRNA synthetase obtained from strain EB145. (F) Incorporation of [^{14}C]isoleucine into tRNA by the aminoacyl-tRNA synthetase obtained from strain EB146. Assays were performed with tRNA from EB145* (●) *and from EB146* (○). *Each point represents the average of at least three independent determinations. The values from the independent determinations agreed within 5%.*

mutant were aminoacylated to a lesser extent than the corresponding tRNA's from the parent strain (Fig. 2). These data indicate that the *leuK16* lesion somehow affected certain tRNA's but had no apparent effect on the aminoacyl-tRNA synthetases.

To determine whether *leuK16* affected all tRNA's or only the tRNA's examined in Fig. 2, aminoacyl-tRNA synthetase assays were performed with 18 different amino acids, using tRNA isolated from both strains EB145 and EB146. Only leucyl-, valyl-, isoleucyl-, histidyl-, and arginyl-tRNA's isolated from the mutant cells showed the decreased ability to be aminoacylated compared with tRNA from the parent cells (Table 5). With the exception of arginine, the defective tRNA's corresponded to the biosynthetic systems which were derepressed in the mutant. As stated above, the tryptophan biosynthetic pathway was also derepressed. Attempts were made to study aminoacylation of tryptophanyl-tRNA, but reproducible results could not

be obtained because of high blank values. The high levels of charging of phenylalanyl-tRNA in EB146 compared with those of EB145 are reproducible. However, because tRNA's have not been invoked in the regulation of the phenylalanine biosynthetic enzymes and because multiple forms of regulatory enzymes in the aromatic pathway add to the complexity of that system, no further investigation of enzymes in the aromatic biosynthetic pathway was made.

It was reasoned that decreased charging in the mutant might be due to the absence of a 3′ CCA terminus on the tRNA. Therefore, attempts were made to demonstrate a difference in the mutant versus the parent strain in the activity of the tRNA-nucleotidyltransferase which transfers a C or an A residue to the 3′ terminus of the tRNA. No differences were found in the activity of this enzyme in the two strains, which meant that the 3′ terminus in the mutant was intact or that the 3′ terminus of certain tRNA's in the mutant had been stripped past the point which could be repaired by the tRNA-nucleotidyltransferase.

TABLE 5. *Aminoacylation of tRNA from strains EB145 and EB146*[a]

^{14}C-amino acid incorporated	Aminoacylation of EB146 tRNA (% aminoacylation obtained with EB145 tRNA)
Leucine	70
Valine	86
Isoleucine	87
Arginine	64
Histidine	83
Methionine	93
Threonine	92
Alanine	104
Glutamate	114
Serine	96
Glycine	102
Lysine	110
Cysteine	105
Aspartate	104
Proline	98
Phenylalanine	130
Tyrosine	99

[a] The aminoacyl-tRNA synthetases were from EB145. All values represent the average of at least three determinations obtained from three independent experiments. Values from the independent determinations agreed within 5%.

Location of *leuK16* relative to the galactose operon. Preliminary P1*bt* transductions indicated that *leuK16* was linked to a lesion in the galactose operon (17 min). Ordering of *leuK16* relative to the galactose operon was determined by demonstrating the linkage of *leuK16* to *gal-5* (a lesion in the *gal* operon) and the absence of linkage to *nadA1* (a lesion which is linked to *gal-5* approximately 89%). P1 transduction analyses demonstrated that *leuK*$^+$ was cotransduced approximately 11% with Gal$^+$ (Table 6). A second experiment was performed in which a strain carrying the lesions *nadA1* and *leuK16* (DC938) was transduced to NadA$^+$. None of the 1,121 NadA$^+$ transductants examined was *leuK*$^+$ (i.e., all NadA$^+$ transductants could cross-feed the leucine deletion strain [DC 455]), indicating that *leuK16* is located on the opposite side of the galactose operon from *nadA*.

Merodiploid genetic analysis of *leuK16*. Heterozygous merodiploid analyses of a strain carrying *leuK16* were performed to test the dominant characteristics of the mutant relative to the wild-type allele. An *E. coli* K-12 strain, UC200, which carries an episome (F′ *gal*$^+$ *pyrD*$^+$ *trp*$^+$; Fig. 2), was mated with two F$^-$ strains, DC501 (*ara-leu-1170* + *1238 leuK16 gal-5*) and DC521 (*ara-leu-1170* + *1238 gal-5*), selecting for Gal$^+$ derivatives. The resulting merodiploid strains, EB2019 and EB2020, were assayed for α-isopropylmalate synthetase (*leuA*), histidinol dehydrogenase (*hisD*), and several isoleucine-valine biosynthetic enzymes (Table 7). The levels of biosynthetic enzymes for the leucine, histidine, isoleucine, and valine pathways in EB2019 (F′ *leuK*$^+$/*leuK16*) were highly elevated compared with those of EB2020, which contains a wild-type allele on both the episome and the bacterial genome. Since a strain carrying *leuK16* gives high-level production of the leucine, histidine, and isoleucine-valine biosynthetic enzymes even in the presence of the wild-type allele, *leuK16* is dominant to the wild-type allele. To substantiate the existence of the heterozygous merodiploid state for the *leuK16* locus in EB2019, a Gal$^-$ Ara$^-$ segregant was isolated (see

TABLE 6. *Ordering of leuK16 by P1bt transduction relative to the galactose operon*

P1*bt* donor strain	Recipient	Selected marker	Nonfeeder/feeder[a]
UP1007 (wild type)	DC 73 (*ara-leu-1170* + *1238 leuK16*)	Leu$^+$	0/1,297
UP1007 (wild type)	DC937 (*gal-5 leuK16*)	Gal$^+$	73/834
UP1007 (wild type)	DC938 (*nadA1 leuK16*)	NadA$^+$[b]	0/1,121

[a] Presence of a nonfeeder indicates that the *leuK16* mutation has been corrected.
[b] Cotransduction of *nadA* with *gal-5* is approximately 89%.

TABLE 7. *Effect of leuK16 on the repression of branched-chain amino acid and histidine enzymes in a heterozygous merodiploid strain (F' leuK⁺/leuK16)*

Strain	Genotype	Growth condition	Relative activity[a] of the biosynthetic enzymes for:					
			Isoleucine-valine				Leucine (α-IPM synthetase)	Histidine (HDH)
			TD	AHAS	IR	DH		
EB2020	F'B3/*gal-6 ara-leu-1170* + *1238*	Excess[b]	1.0	1.0	1.0	1.0	1.0	1.0
EB2019	F'B3/*gal-5 leuK16 ara-leu-1170* + *1238*	Excess	6.8	14.1	3.1	4.8	29.3	28.7
EB209[c]	*gal-5 ara-leu-1170* + *1238*	Excess	0.9	1.2	1.0	1.1	0.8	

[a] The values for isoleucine-valine and leucine biosynthetic enzymes are relative to the repressed levels for the isogenic wild-type control. The specific activities for EB2020 under excess conditions were as follows: threonine deaminase (TD), 60.1; α-acetohydroxy acid synthase (AHAS), 10.2; isomeroreductase (IR), 14.1; dihydroxy acid dehydrase (DH), 59.3; α-isopropylmalate (α-IPM) synthetase, 0.04; histidinol dehydrogenase (HDH), 0.22. Values are expressed as micromoles per hour per milligram of protein. All values represent the average of at least three determinations obtained from three independent investigations. The values from the independent determinations agreed within 10%.

[b] All cultures were grown in glucose minimal medium in the presence of excess branched-chain amino acids.

[c] EB209 is an Ara⁻ Gal⁻ segregant from EB2019.

Materials and Methods) in which recombination from the wild-type locus of the episome had replaced the *leuK16* locus. The resulting strain, EB209, had a gene expression virtually identical to that of EB2020 (F' *leuK⁺/leuK⁺*), which lacks the *leuK16* allele.

DISCUSSION

Previous analyses of lesions unlinked to the *leu* operon which cause increased production of the leucine biosynthetic enzymes have also been shown to cause an elevated expression of the enzymes necessary for the synthesis of isoleucine and valine (1, 4, 6, 11, 14). Such lesions have been described for the *leuS* (leucyl-tRNA synthetase) structural gene in both *S. typhimurium* (1) and *E. coli* B/r (Ghajar, Ph.D. dissertation). Two other leucine regulatory loci, *flrA* (14) and *flrB* (11), isolated by their resistance to trifluoroleucine have also been shown to produce increased gene expression of both the *leu* operon and the *ilv* gene cluster. Common control mechanisms for these pathways might be expected since leucine biosynthesis is a branch of the valine pathway.

In this paper we have described a spontaneous leucine regulatory mutation, *leuK16,* which is unlinked to the *leu* operon or the *ilv* gene cluster. This mutation is linked to the *gal* operon and produces a component which affects the control mechanism for the leucine, the isoleucine-valine, the histidine, and the tryptophan biosynthetic enzymes. To differentiate this locus from previously described unlinked loci affecting the regulation of leucine, we have designated this locus *leuK.*

The elevated leucine, isoleucine, valine, histidine, and tryptophan gene expression exhibited by the *leuK16* lesion could be interpreted as the involvement of a positive factor involved in the expression of branched-chain amino acid, histidine, and tryptophan biosynthetic enzymes, since the product produced by the wild-type allele has no effect on leucine, valine, histidine, or tryptophan gene expression. In other words, the product encoded by a strain carrying the *leuK16* lesion could express itself by allowing enhanced transcription or translation of the genes for the leucine, isoleucine, valine, histidine, and tryptophan biosynthetic systems, even in the presence of the wild-type gene product. A positive control element in the regulation of isoleucine and valine biosynthesis has been previously proposed (16).

That the tRNA's may be involved in the control mechanisms for leucine, isoleucine, valine, and histidine is suggested by the data in Fig. 2 and in Table 5, which show a reproducible decreased extent of charging of these tRNA's from the *leuK16* strain. However, from the information available about transcriptional and translational control of these amino acid systems, it is difficult to incorporate these observations into a positive control model. At present, the possibility that derepression and decreased charging of certain tRNA's in the *leuK16* strain may be two unrelated phenomena cannot be eliminated.

One of several alternative interpretations for the observed derepressions is that the wild-type allele of *leuK* codes for a protein which processes a repressor (or corepressor) for the system to make it functional in repression. If that repressor

(corepressor) is modified in an abnormal manner (as might occur in the *leuK16* strain), repression could not occur. The dominant character of the *leuK16* lesion in the merodiploid could be explained if the mutant-modifying protein has a much higher affinity for the repressor (or corepressor) than does the product of the wild-type allele or if the *leuK16* product can abnormally modify a repressor (or corepressor) even after it is correctly processed by the wild-type product. Such a negative control model would more easily accomodate the decreased tRNA charging observed in the *leuK16* strain compared with that in the *leuK*$^+$ strain. This model would also be consistent with the existing hypotheses of transcriptional control in these systems.

The assays for the extent of charging of tryptophanyl-tRNA in EB145 versus EB146 (*leuK16*) were variable; hence, no meaningful conclusion can be drawn from these data about the involvement of tryptophanyl-tRNA in the regulation of that pathway. Other investigators (7, 22) have suggested that tryptophanyl-tRNA is not involved in the regulation of the *trp* operon; however, there is some evidence that it may be involved in attenuation control (21). The significant decrease in the charging of the arginyl-tRNA from the mutant strain and the apparently normal regulation of the arginine biosynthetic pathway in that strain are in agreement with other data which show that arginyl-tRNA is not involved in the regulation of arginine biosynthesis (13).

Experiments are currently in progress to study further the nature of this *leuK* product and its relationship to mRNA production and tRNA alterations. Investigations of the interrelationships between the controls on the leucine and isoleucine-valine pathways and those on the histidine and the tryptophan are also planned.

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