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New Amino Acid Regulatory Locus Having Unusual Properties in Heterozygous Merodiploids

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Spontaneous mutants of *Escherichia coli* B/r resistant to 5',5',5',-trifluoro-DL-leucine contain defects in a gene which maps to the left of the threonine region. Low-level constitutive expression of the isoleucine-valine and leucine operons is caused by this mutation in haploid strains. This is in contrast to extremely high levels of gene expression in the heterozygous merodiploids (F' wild type/mutant allele). The properties of these mutants define a new locus and suggest that it encodes a subunit protein which is involved in the repression of the structural genes for the branched-chain amino acid pathways.

The synthesis of leucine by *Salmonella typhimurium* (4, 26) and *Escherichia coli* B/r (H. Yang, *unpublished data*) is governed by four structural genes which comprise an operon near the arabinose gene cluster. Isoleucine and valine biosynthetic enzymes in these bacteria are encoded by the genes in two or three operons located near *metE* (2, 31-33, 36, 43). Analysis of mutants which are resistant to the leucine analogue, 5',5',5',-trifluoro-DL-leucine (TFL) has revealed that the expression of these systems is dependent on elements both linked and unlinked to their respective operons (1, 5). One class of mutants resistant to TFL overproduces leucine and has been characterized as being a consequence of operator constitutive mutations (6). A second class of mutants is insensitive to feedback inhibition by leucine owing to an alteration in the product of the *leuA* gene (5, 41). A third class comprises those mutations which are not linked to the leucine operon. These latter mutations cause constitutive production of enzymes for isoleucine, valine and leucine biosynthesis. One representative of the latter class has been characterized as a leucyl-transfer ribonucleic acid (tRNA) synthetase mutant (1). In this paper, we identify, with the use of two TFL-resistant mutations (*flr-6* and *flr-7*), a new locus affecting the regulation of branched-chain amino acid biosynthesis. These mutations are unlinked to the *ilv* or *leu* operons. We further demonstrate a greater derepression of the *ilv* and *leu* operons in the heterozygous merodiploids than is exhibited by the mutant haploid strain.

MATERIALS AND METHODS

Bacterial strains. A list of the bacterial strains used, their sources, and pertinent markers are given in Table 1. The mutation *flr-6* was formerly designated *leu-6* (22); as a consequence of this study, this designation has been changed from *leu* to *flr*.

Media. Eosin methylene blue agar (EMB), L-broth, and minimal base have been described (37). The following supplements were added to minimal medium when required: 0.2% glucose, 0.2% galactose, 0.4% glycerol, 0.004% L-arginine, 0.004% L-leucine, 0.004% L-isoleucine, 0.004% L-valine, 0.004% L-threonine, 0.004% uracil, 10^{-3} M TFL, and 1.5% Difco agar. A glucose minimal agar plate containing 0.0004% arginine and 0.0004% uracil was used to detect Pyr^- (arginine and uracil auxotroph) segregants which were detectable by their small colony size.

Growth rate determination. One-liter Erlenmeyer flasks containing 200 ml of minimal medium were inoculated with 0.05 ml of cultures which had been grown overnight in minimal medium. Absorbancy at 660 nm was determined at 30-min intervals.

Transduction. P1bt transduction experiments were performed by the procedure described by Gross and Englesberg (13).

Isolation of mutants. Fluoroleucine-resistant (FLR) mutants were isolated as previously described (5).

Feeding plates. Two strains, DC455 (containing an arabinose-leucine deletion) and AB1203 (possessing isoleucine and valine requirements), were grown in 200 ml of supplemented minimal medium with glucose used as the carbon source. The cells were harvested at an optical density (OD) of 0.5 at 660 nm, washed twice with 100 ml of minimal salts,

TABLE 1. *Bacterial strains*[a]

Strains	Pertinent markers[b]	Source
F⁻ strains		
B/r DC74	*ara-leu* 1170 + 1238	D. P. Kessler
B/r SB5111	*pyrA1*	P1bt (SB5013) × SB5003
B/r DC500	*pyrA1 ara-leu* 1170 + 1238 *gal-4*	D. P. Kessler
B/r SB5003	*thr-1*	D. P. Kessler
B/r ELK 1	*pyrA1*	P1bt (SB5111) × SB5003
B/r ELK 2	*pyrA1 flr-6*	P1bt (SB5111) × SB5021
B/r ELK 3	*pyrA1 flr-7*	P1bt (SB5111) × SB 5022
B/r ELK 19	*thr-1 gal-4*	P1bt (SB5010) × DC500
B/r ELK 9	*pyrA1 flr-6 gal-4*	P1bt (ELK 5) × ELK 19
B/r ELK 10	*pyrA1 flr-7 gal-4*	P1bt (ELK 6) × ELK 19
B/r SB5021	*thr-1 flr-6*	Spontaneous from SB5003
B/r SB5022	*thr-1 flr-7*	Spontaneous from SB5003
B/r DC455	*ara-leu* 1101	D. P. Kessler
K-12 W3110	Wild type	C. Yanofsky
K-12 AB1203	*ilv-7*	E. A. Adelberg
K-12 UC201	$trpR_2$	C. Yanofsky
F′ strains		
K-12 UC200	F′ *gal*⁺ *pyrD*⁺ *trp*⁺/*gal*⁻ *pyrD*⁻ *trp*⁻ *his*⁻	B. Low
B/r DC2026	F′ *leuA28/leuA28 arg-2*	H. L. Yang
B/r ELK 1000	F′ *leuA28*/SB5111	DC2026[c] × SB5111
B/r ELK 1001	F′ *leuA28*/SB5003	DC2026 × SB5003
B/r ELK 1002	F′ *leuA28*/ELK 2	DC2026 × ELK 2
B/r ELK 1003	F′ *leuA28*/ELK 3	DC2026 × ELK 3
B/r ELK 1004	F′ *gal*⁺/ELK 10	UC200 × ELK 10
B/r ELK 1005	F′ *gal*⁺/ELK 9	UC200 × ELK 9
B/r ELK 1006	F′ *flr-6 ara-leu* 1109/DC74	B. Ghajar
B/r ELK 1008	F′ *flr-6 ara-leu* 1109/ELK 3	ELK 1006 × ELK 3
B/r ELK 1009	F′ *flr-6 ara-leu* 1109/ELK 2	ELK 1006 × ELK 2

[a] Auxotrophic requirements: *pyrA*, arginine and pyrimidine; *leuA*, leucine; *ara-leu*, leucine; *thr*, threonine; *arg*, arginine, *ilv*, isoleucine-valine; *gal*⁺ or *gal*⁻, ability or inability to use galactose as a carbon source.

[b] Abbreviations: *flr*, genotype conferring trifluoro-DL-leucine resistance; ELK, bacterial strains constructed at Univ. of California, Davis, by the author.

[c] Merodiploids used to construct F′ strains.

and resuspended in 20 ml of minimal salts. A 10-ml sample of the suspended cultures was pipetted into 1 liter of glucose minimal agar (*p*H 6.8) containing all requirements except isoleucine or valine in the case of AB1203 and leucine in the case of DC455. This seeded agar was then poured into petri dishes to be used as feeder test plates. Strains to be tested for the excretion of leucine, isoleucine, and valine were spotted on the appropriately seeded plates. After incubation at 37 C each resulting colony exhibited a surrounding halo of diffuse growth if it excreted the amino acid required by the seeded bacteria (Fig. 1). The halo was detectable after 8 hr when heterozygous merodiploids were tested and after 20 hr with mutant haploid strains.

Tests for dominance and segregation. Exogenotes from "F′B2" (Fig. 2) carrying a mutation in *leuA* and a wild-type allele for this *flr* locus, or from F′ *flr-6* (Fig. 2) carrying a deletion extending from *leuA* into the arabinose operon, were obtained from DC2026 or ELK1006. The construction of merodiploids for the *flr* locus was by a modification of Low's method (23). (i) Matings were performed at 37 C by mixing equal volumes of donor (10^8 to 5×10^8 cells/ml) and recipient (10^8 to 5×10^8 cells/ml) strains which were growing in L-broth. (ii) After mating for 1 hr, 10^{-3}, 10^{-4}, and 10^{-5} dilutions of the mating mixtures were interrupted by vigorous shaking for 1 min. (iii) Samples (0.1 ml) from each dilution were plated on minimal glucose plates, selecting for Pyr⁺ (arginine and uracil prototrophy) Leu⁺ (leucine prototrophy) progeny. (iv) Colonies were picked, after 2 days of incubation at 37 C, from the highest dilution plate on which they appeared and checked for excretion of valine, isoleucine, and leucine. Verification that a diploid state existed for the locus conferring fluoroleucine resistance was established by isolating Pyr⁻ (arginine and uracil auxotroph) segregants which had lost their leucine-excreting character. These Pyr⁻ (arginine and uracil auxotroph) segregants were detected as small colonies after 72 hr of growth at 37 C on glucose minimal plates containing one-tenth the normal amount of arginine and uracil.

A control merodiploid for the galactose gene cluster was constructed by using UC200 as the

"F'*gal*" (F'B3) donor, with *gal-4* derivatives of the strains possessing *flr-6* and *flr-7* mutations as the recipient. The diploid state was verified by isolation of Gal⁻ segregants on EMB-galactose plates.

Preparation of extracts. Cultures grown overnight in supplemented minimal medium were used to inoculate 200 ml of minimal medium containing glycerol as the carbon source with the following supplements when required: L-arginine, L-valine, L-leucine, L-isoleucine, and uracil. The cultures were grown with shaking at 37 C and chilled with ice when an OD of 0.5 at 660 nm was attained. Bacteria from the ice-chilled cultures were pelleted by centrifugation at 4 C, washed twice with either 100 ml of 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride containing 0.001 M KCl (*p*H 7.2) for the α-isopropylmalate synthetase assay or with 50 ml of 0.1 M potassium phosphate buffer (*p*H 7.0) for the threonine deaminase and α-acetohydroxy acid synthetase assays. The cells were washed again, pelleted, and resuspended in a total of 1.5 ml of the appropriate buffer and disrupted with a Bronwill Biosonick sonifier. Bacterial debris was removed by centrifugation at 30,000 × *g*. All steps were carried out at 4 C.

Assay procedures. L-Threonine deaminase (11), α-acetohydroxy acid synthetase (38), and α-isopropylmalate synthetase (4, 42) assays were performed as previously described. Protein was determined by the method of Lowry et al. (25). Enzyme assay results are expressed as micromoles of product formed per hour per milligram of protein in the extract.

Chemicals. Sodium pyruvate, thiamine pyrophosphate, flavine adenine dinucleotide, α-ketoisovaleric acid, trilithium salt of acetyl coenzyme A, and pyridoxal phosphate were purchased from Calbiochem. TFL was a gift from H. E. Umbarger.

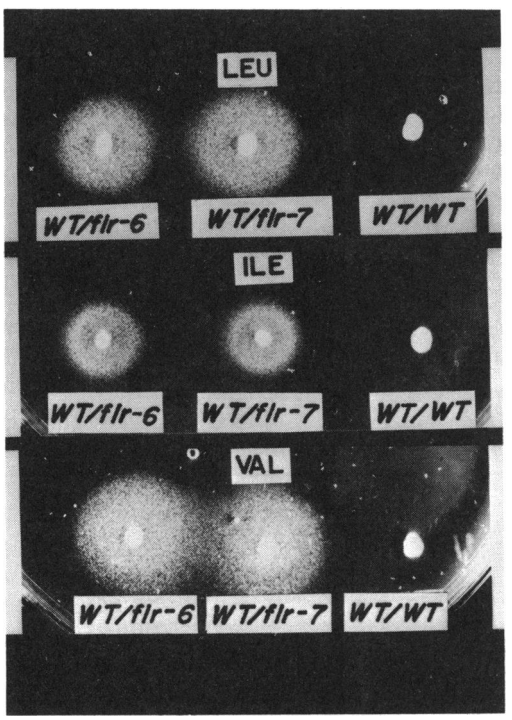

FIG. 1. *Feeding indicator plates showing leucine, isoleucine, and valine excretion after 16 hr of incubation at 37 C of fluoroleucine-resistant merodiploids ELK 1002 (F' WT/flr-6) and ELK 1003 (F' WT/flr-7), with ELK 1000 (F' WT/WT) used as the wild-type control.*

RESULTS

Phenotypes of fluoroleucine-resistant mutants. Two spontaneous mutants [SB5021 (*flr-6*) and SB5022 (*flr-7*)] were isolated independently from SB5003 on the basis of their resistance to TFL (5). These mutants and their derivatives, which carry the *flr-6* or *flr-7* mutation, excrete leucine, isoleucine, and valine. When the fluoroleucine-resistant phenotype was eliminated by recombination, excretion of leucine was eliminated as determined by testing the Thr⁺ (threonine prototrophy) transductants (see Table 3) for these two phenotypes. The growth rates of haploid (ELK 2 and ELK 3) and merodiploid (ELK 1002 and ELK 1003) FLR strains are virtually identical to the parent (Table 2).

Mapping of flr-6 and flr-7. Transductional crosses were performed in order to determine the location of *flr-6* and *flr-7* with respect to *thr* and *pyrA* (40). Results in Table 3 and Table 4 show that both *flr-6* and *flr-7* are more closely linked to *thr* than to *pyrA*. The order of the *flr* locus with respect to *thr* and *pyrA* (Table 4) was determined by transducing an

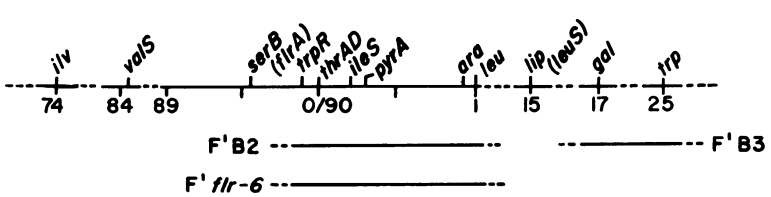

FIG. 2. *Map positions of genetic loci pertinent in this study. The location of these loci were obtained from references 14, 21, and 36. All genetic symbols, except the flrA locus described in this study, are those given by Taylor (36). The relative position of (flrA) to trpR and (leuS) to lip have not been determined.*

analogue-sensitive, *pyrA1* recipient (SB5111) with phage grown on SB5021 (*flr-6, thr-1*) and SB5022 (*flr-7, thr-1*), selecting Pyr$^+$ (arginine and uracil prototrophy) recombinants, and scoring the number of Thr$^-$ (threonine auxotroph) Pyr$^+$ (arginine and uracil prototrophy) transductants which inherited the excreting phenotype. High inheritance of leucine excretion by the Thr$^-$ (threonine auxotroph) Pyr$^+$ (arginine and uracil prototrophy) transductants would indicate that the mutant locus is between *pyrA* and *thr*. Low inheritance of leucine excretion by the Thr$^-$ (threonine auxotroph) Pyr$^+$ (arginine and uracil prototrophy) transductants would place the mutant site on the *serB* side (Fig. 2) of threonine (40). The results in Table 4 show 17% of the Thr$^-$ (threonine auxotroph) Pyr$^+$ (arginine and uracil prototrophy) transductants acquired the *flr-6* locus and 20% acquired the *flr-7* locus, while none of the Thr$^+$ (threonine prototrophy) Pyr$^+$ (arginine and uracil protrotrophy) transductants inherited the *flr* locus. Therefore, we conclude that *flr-6* and *flr-7* are located on the *serB* side of threonine.

TABLE 2. *Generation times for haploid and merodiploid fluoroleucine-resistant strains*

Strains	Pertinent markers	Generation time[a] (min)
F strains		
SB5111	*pyrA1*	60
ELK 1	*pyrA1*	60
ELK 2	*pyrA1 flr-6*	61
ELK 3	*pyrA1 flr-7*	60
F' strains		
ELK 1001	F'B2/ELK 1	70
ELK 1002	F'B2/ELK 2	71
ELK 1003	F'B2/ELK 3	69

[a] Time required for the doubling of exponentially growing bacterial cells when glycerol minimal medium was used for the F' strains and a glycerol, arginine, uracil, minimal medium for the haploid strains.

Heterozygous merodiploid character for the flr locus. The construction of merodiploids is described in Materials and Methods. Figure 1 demonstrates that the heterozygous merodiploid strains excrete high levels of isoleucine, valine, and leucine into the medium (the strains in Fig. 1 carry the *flr* allele in the chromosome). The mutant haploids, on the other hand, exhibit barely detectable excretion of branched-chain amino acids. High-level excretion (identical to that depicted in Fig. 1) also occurs in a heterozygous merodiploid strain that carries the *flr-6* mutation on the episome (F' *flr-6/flr*$^+$). However, this merodip-

TABLE 3. *Linkage of flr-6 and flr-7 with thr and pyrA by P1bt transduction*[a]

Recombinant class	Cross I:[b] P1bt Bact	+ + *pyrA*$^-$		Recombinant class	Class II:[c] P1bt Bact	+ + *pyrA*$^+$	
		flr-6 thr +				*flr-7 thr* +	
flr-6 thr$^-$ *pyrA*$^+$		423/1,728	24%	*flr-7 thr*$^+$ *pyrA*$^+$		297/1,242	24%
flr-6 thr$^+$ *pyrA*$^-$		238/1,728	14%	*flr-7 thr*$^+$ *pyrA*$^-$		216/1,242	17%
flr^{+d} *thr*$^+$ *pyrA*$^+$		863/1,728	50%	*flr*$^+$ *thr*$^+$ *pyrA*$^+$		585/1,242	47%
flr$^-$ *thr*$^+$ *pyrA*$^-$		214/1,728	13%	*flr*$^+$ *thr*$^+$ *pyrA*$^-$		144/1,242	12%

[a] In both cases Thr$^+$ recombinants were selected on minimal medium containing arginine and uracil.
[b] Cross I, recipient strain SB5021 × P1bt donor strain SB5111.
[c] Cross II, recipient strain SB5022 × P1bt donor strain SB5111.
[d] flr$^-$ = no leucine excretion.

TABLE 4. *Ordering flr-6 and flr-7 with respect to thr and pyrA by P1bt transductions*

Recombinant class	Cross I:[a] P1bt Bact	*flr-6 thr-1* +		Recombinant class	Cross II:[b] P1bt Bact	*flr-7 thr-1* +	
		+ + *pyrA1*				+ + *pyrA1*	
flr-6 thr$^-$ *pyrA*$^+$		0/1,148	0%	*flr-7 thr*$^+$ *pyrA*$^+$		0/844	0%
flr-6 thr pyrA$^+$		56/1,148	5%	*flr-7 thr*$^-$ *pyrA*$^+$		60/844	7%
flr^{+c} *thr*$^+$ *pyrA*$^+$		830/1,148	71%	*flr*$^+$ *thr*$^+$ *pyrA*$^+$		584/844	69%
flr$^-$ *thr pyrA*$^+$		272/1,148	24%	*flr*$^+$*thr*$^-$ *pyrA*$^+$		200/844	24%

[a] Cross I, recipient strain SB5111 × P1bt donor strain SB5021.
[b] Cross II, recipient strain SB5111 × P1bt donor strain SB5022.
[c] *flr*$^-$ = no leucine excretion.

loid strain is highly unstable, giving rise to low-level leucine excretors at a high frequency. In contrast to the high excretion by the F′ *flr-6/flr*$^+$ heterogenote, a *flr-6* homogenote excretes barely detectable levels of branched-chain amino acids. This suggests that the strains carrying *flr-6* and *flr-7* are more highly derepressed for the enzymes in the *ilv* and *leu* operons when the wild-type allele is also present. To confirm this, assays for enzymes involved in the synthesis of branched-chain amino acids were performed. Table 5 shows that the two haploid mutants (lines 5 and 8) and the *flr-6* homozygous merodiploid (line 12) have slightly higher levels of enzymes for branched-chain amino acid biosynthesis than their parent (line 1) or wild-type (line 3) strain when grown under nonrepressing or repressing conditions. When the wild-type allele is introduced on the exogenote, the two mutants exhibit extremely high levels of α-isopropylmalate synthetase, threonine deaminase and α-acetohydroxy acid synthetase (lines 6 and 9).

The enzyme levels of an "F′*gal*" derivative of each mutant were analyzed to determine whether the high enzyme levels of the mutants in the merodiploid state were due to some type of influence by the fertility factor. The results (Table 5) demonstrate that the high levels of enzymes in the mutant "F′B2" merodiploids were not caused by the fertility factor (lines 7 and 10).

The following experimental results suggest that *flr-6* and *flr-7* are located within the same gene. Both of these mutants conferring fluoroleucine resistance are located on the same side of threonine (Table 4); they cotransduce with threonine at about the same frequency and have similar phenotypic characteristics (fluoroleucine resistance, high derepression of the *ilv* and *leu* operons by the heterozygous merodiploid strains, and branched-chain amino acid excretion). Finally, to test whether *flr-6* and *flr-7* were allelic mutations, an F′ carrying *flr-6* was introduced by mating into a strain carrying *flr-7*. It was assumed (in light of the properties of the heterozygous merodiploids) that, if a wild-type allele, corresponding to either *flr-6* or *flr-7*, was present trans to either *flr* mutation, high-level gene expression would have resulted. The levels of enzymes involved in isoleucine, valine, and leucine biosynthesis in this merodiploid (Table 5, line 11) were characteristic of those found in the mutant haploid strains (Table 5, lines 5 and 8). These low levels of enzyme activity seem most readily explained if these mutations are in one gene. However, the possibility still remains

TABLE 5. *Levels in fluoroleucine-resistant strains of some enzymes involved in leucine, isoleucine, and valine biosynthesis*

Line no.	Strains	Pertinent markers[b]	Specific activity[a] of enzymes involved in biosynthesis of					
			Leucine		Isoleucine-valine			
			α-Isopropylmalate[c] synthetase		Threonine deaminase		α-Acetohydroxy acid synthetase	
			−[d]	+	−	+	−	+
1	ELK 1	*pyrA1*	0.13	0.03	15.3	9.6	10.4	8.2
2	ELK 1001	F′B2/ELK 1	0.22	0.04	16.4	8.7	11.4	8.1
3	SB5111	*pyrA1*	0.16	0.04	11.5	7.2	7.0	4.5
4	ELK 1000	F′B2/SB5111	0.24	0.04	11.5	7.5	7.0	5.0
5	ELK 3	*pyrA1 flr-7*	0.30 (1.9)[e]	0.10 (2.5)	26.65 (2.5)	18.00 (2.5)	14.20 (2.0)	10.00 (2.2)
6	ELK 1003	F′B2/ELK 3 (*flr-7*)	3.80 (17.0)	2.88 (72.0)	160.00 (14.0)	159.00 (21.0)	206.00 (29.0)	208.00 (42.0)
7	ELK 1004	F′B3/ELK 10 (*flr-7*)	0.28 (1.7)	0.09 (2.3)	27.30 (2.4)	20.00 (2.8)	14.50 (2.1)	9.80 (2.2)
8	ELK 2	*pyrA1 flr-6*	0.45 (2.8)	0.38 (9.5)	26.10 (2.3)	22.41 (3.1)	15.10 (2.2)	11.40 (2.5)
9	ELK 1002	F′B2/ELK 2 (*flr-6*)	4.10 (19.0)	2.10 (53.0)	150.00 (13.0)	130.01 (17.0)	227.13 (32.4)	233.11 (47.0)
10	ELK 1005	F′B3/ELK 9 (*flr-6*)	0.47 (2.1)	0.33 (8.3)	28.31 (2.5)	20.51 (2.8)	14.12 (2.0)	10.91 (2.4)
11	ELK 1008	F′*flr-6 ara-leu* 1109/ ELK 3 (*flr-7*)	0.37 (2.3)	0.13 (3.3)	24.85 (2.2)	19.61 (2.7)	16.00 (2.3)	11.10 (2.5)
12	ELK 1009	F′*flr-6 ara-leu* 1109/ ELK 2 (*flr-6*)	0.49 (2.0)	0.41 (10.2)	29.01 (2.52)	21.69 (3.0)	17.01 (2.4)	12.10 (2.4)

[a] Product formed (micromoles) per hour per milligram of protein.

[b] F′B2: exogenote which carries the wild type allele of the *flrA* locus, a mutation in *leuA* gene, begins to the left of the *trpR* gene and ends on the right side of the leucine operon. F′B3: an exogenote which carries the genes from galactose to tryptophan.

[c] Assay for α-isopropylmalate synthetase was done with the use of a Beckman Kintrac VII spectrophotometer.

[d] + and − designate whether a bacterial strain was grown in 0.5% glycerol minimal medium in the presence or absence of the branched-chain amino acids.

[e] Values in parentheses show the fold increase in specific activity above SB5111 and ELK 1000.

that they could be in two separate but closely linked genes which together encode for products with a single function.

The involvement of *flr-6* and *flr-7* in the regulation of amino acid biosynthesis and their close proximity to a locus (*trpR*) that is involved in the regulation of tryptophan (28) warranted an initial investigation of an *E. coli* K-12 strain with a known *trpR* mutation to determine its affect on the *ilv* or *leu* operons. The results in Table 6 demonstrate that a *trpR* mutation does affect regulation of the *ilv* and *leu* operons.

DISCUSSION

Mutations conferring fluoroleucine resistance that are unlinked to the leucine operon and are not allelic to the *leuS* locus are characteristic of class III mutant types (1, 5). Strains containing such mutations produce derepressed levels of enzymes involved in branched-chain amino acid biosynthesis and excrete leucine and isoleucine. The low level of gene expression in the F′ *flr-6/flr-7* merodiploid (Table 5) and the map position of these two mutations (Tables 3 and 4) suggest that the two FLR mutants described here (*flr-6* and *flr-7*) are located within the same gene. Results show (Fig. 1, Tables 3 and 4) this locus to map near threonine and verify that mutations in this gene have all the phenotypic and enzymatic characteristics of FLR mutants (1, 5). To distinguish this locus from previously described *flr* loci (J. M. Calvo; B. N. Ames, *unpublished data*), we have designated it as *flrA*. The location of *flrA* was defined by P1bt transductions to be on the left side of threonine (Table 4). This clearly separates it from the *ileS* locus which maps between *thr* and *pyrA* (15). Identification of such a locus supports the idea that there exists a common element in the repression of all three pathways (5, 10).

Mechanisms for the repression of amino acid biosynthesis in some cases have been shown to involve in some way a derivative of the amino acids (8, 30). The association of aminoacyl-tRNA synthetases or possibly charged tRNA with the repression of gene expression for the synthesis of amino acids has been shown and is evidenced in the cases of tryptophan (17), histidine (35), isoleucine (39), valine (9, 44), and leucine (1) regulation. However, precise mechanisms of repression for amino acid biosynthesis have not yet been elucidated.

A negative control model for the repression of branched-chain amino acid gene expression proposed by Calvo et al. involves the complexing of charged leucyl-, isoleucyl-, and valyl-tRNA with a specific protein designated as an aporepressor (5). If this model for repression is correct, one group of mutations causing elevated expression of the *ilv* and *leu* operons would be in the gene or genes encoding the aporepressor. The near-normal enzyme levels in the mutant haploid (*flr-6* and *flr-7*) and *flr-6* homozygous merodiploid (F′ *flr-6/flr-6*) strains (Table 5) indicates that the product encoded by *flr-6* and *flr-7* is still effective in its regulatory function. The contrasting results (Table 5, Fig. 1) for the heterozygous merodiploids demonstrates that there is interaction between the mutant and wild-type alleles leading to a product which is now ineffective in its regulatory function. If we assume that the *flrA* locus codes for an aporepressor which is composed of several subunits, then it is possible to suggest a model explaining the elevated gene expression in the heterozygous merodiploids. Dominant regulatory mutants in *E. coli* K-12 for β-galactosidase (7), alkaline phosphatase (12), and capsular polysaccharide (27) biosynthesis have been attibuted to the formation of a

TABLE 6. *Levels in trpR2 of some enzymes involved in branched-chain amino acid biosynthesis*

Strain	Pertinent markers	Specific activity[a] of enzymes involved in the biosynthesis of					
		Leucine		Isoleucine-valine			
		α-Isopropylmalate[b] synthetase		Threonine deaminase		α-Acethydroxy acid synthetase	
		−[c]	+	−	+	−	+
UC201	*trpR2 thr*⁻	2.49	2.65	1.53	1.53	5.40	4.44
W3110	Wild type	2.41	1.25	0.94	0.64	9.75	2.54

[a] Product formed (micromoles) per hour per milligram of protein.

[b] Assay for α-isopropylmalate synthetase was done by the use of a Klett-Summerson photoelectric colorimeter.

[c] + and − designate whether a bacterial strain was grown in 0.5% glycerol minimal medium in the presence or absence of the branched-chain amino acids.

defective regulator protein due to the complexing of protein subunits from the mutant and wild-type allele. Proteins involved in the repression of amino acids have also been well documented (16, 18, 20). Therefore the unusually high derepression of the *ilv* and *leu* operons in the heterozygous merodiploid strains could be explained by the formation of an inactive aporepressor, due to the mutant subunit having a higher affinity for the wild-type subunit than does the wild-type subunit itself.

Assuming that charged tRNA is involved in repression, a mutant with alterations in an enzyme necessary for modification of the tRNA molecules for branched-chain amino acids could also cause derepression of these three biosynthetic pathways. Investigation by Ames et al. (*unpublished data*) on *hisT* regulatory mutants has shown that they cause derepressed enzyme levels of other amino acid-synthesizing systems in addition to histidine and has revealed that this locus encodes a tRNA modifying enzyme. An alteration in the *flrA* locus is shown by enzymatic data (Table 5) and excreting responses (Fig. 1) to be similar to the histidine mutant described in that they both derepress the leucine, isoleucine, and valine operons.

If one applies the in vitro results of Hatfield and Bruns (14) (showing that a charged leucyl-tRNA affects the conformation of threonine deaminase) and the aporepressor-charged-tRNA model for repression, it is reasonable to assume that a leucine-tRNA that is not properly modified may not be able to inhibit threonine deaminase formation or provide the normal mechanism for repression. If these assumptions are correct, the lack of threonine deaminase inhibition and the unusually high levels of enzymes in the heterozygous merodiploids (Table 5) grown in the presence of branched-chain amino acids could be explained by an improper modification of leucyl-tRNA. The dominance of mutations in a nucleotide modifying enzyme has been documented (3). Several models could explain the unusual effect of this locus in the heterozygous condition if *flrA* encodes a tRNA modifying enzyme. If the enzyme is composed of subunits, this effect could be explained by the mixing of mutant and wild-type subunits causing the formation of an inactive modifying enzyme or an enzyme that modifies tRNA at a site not normally modified, either of which could directly interfere with the mechanism of repression. An alternative explanation could be that the modifying enzyme synthesized in strains carrying *flr-6* or *flr-7* slightly affects the repression mechanism by modifying the tRNA at a site different from the wild-type modifying enzyme. However if both the wild-type and the altered enzyme are present at the same time, the tRNA is modified in such a way that the repression mechanism is very ineffective.

The location of *flr-6* and *flr-7* relative to threonine and their proximity to the *trpR* locus (40) warranted an investigation to determine whether there is any direct association between these loci. Evidence has established (Table 6) that a *trpR* mutant does, indeed, affect the regulation of the *ilv* and *leu* operons. Additional studies are in progress in an attempt to define further the relationship between *flrA* and *trpR*, and to survey possible types of "metabolic interlock" (19, 21) that may occur between branched-chain amino acid and tryptophan biosynthesis.

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