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Mutants of *Salmonella typhimurium* with an Altered Leucyl-Transfer Ribonucleic Acid Synthetase

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Two trifluoroleucine-resistant mutants of *Salmonella typhimurium*, strains CV69 and CV117, had an altered leucyl-transfer ribonucleic acid (tRNA) synthetase. The mutant enzymes had higher apparent K_m values for leucine (ca. 10-fold) and lower specific activities (ca. twofold) than the parent enzyme when tested in crude extracts. Preparations of synthetase purified ca. 60-fold from the parent and strain CV117 differed sixfold in their leucine K_m values. In addition, the mutant enzyme was inactivated faster than the parent enzyme at 50 C. The growth rates of strains CV69 and CV117 at 37 C were not significantly different from that of the parent, whereas at 42 C strain CV69 grew more slowly than the parent. Leucine-, valine-, and isoleucine-forming enzymes were partially derepressed when the mutants were grown in minimal medium; the addition of leucine repressed these enzymes to wild-type levels. During growth in minimal medium, the proportion of leucine tRNA that was charged in the mutants was about 75% of that in the parent. The properties of strain CV117 were shown to result from a single mutation located near *gal* at minute 18 on the genetic map. These studies suggest that leucyl-tRNA synthetase is involved in repression of the enzymes required for the synthesis of branched-chain amino acids.

In a previous communication (7) we reported the isolation and properties of mutant strains of *Salmonella typhimurium* which were resistant to an analogue of leucine, 5',5',5',-trifluoro-DL-leucine (18. One class of these mutants had high, constitutive levels of enzymes involved in leucine, valine, and isoleucine biosynthesis, and the mutant sites of at least some of them mapped near *gal* on the *Salmonella* genome (1). We subsequently discovered that several fluoroleucine-resistant strains, when grown in a minimal medium, had high levels of leucine-, valine-, and isoleucine-forming enzymes but that these enzyme levels were repressed when the strains were grown in the same medium supplemented with L-leucine. The properties of two of these strains, CV69 and CV117, are described in this paper.

MATERIALS AND METHODS

Bacterial strains. Strains CV19 (*ara-9 flr-19*), CV69 (*ara-9 flr-69*), CV112 (*ara-9 gal-205 leuO2004*), CV117 (*ara-9 gal-205 flr-117*), and CV123 (*ara-9 gal-205 flr-123*) were isolated as fluoroleucine-resistant mutants of *S. typhimurium* LT2 *ara-9* or *ara-9 gal-205* (7). Hfr SU576 (*purC7 strA*) was obtained from H. O. Smith.

Media. Nutrient broth and nutrient agar were used as complete media. A minimal salts solution (SSA) contained per liter of distilled water: K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $(NH_4)_2SO_4$, 1.0 g; sodium citrate dihydrate, 0.97 g; anhydrous $MgSO_4$, 0.05 g. SSA supplemented with 0.2% glucose or 0.2% glucose and 1.5% agar served as liquid and solid minimal media, respectively (M medium, M plates). Arabinose or galactose media contained, respectively, 0.2% L-arabinose or 0.2% D-galactose instead of glucose, and citrate was omitted from the salts solution.

Chemicals. The following chemicals were obtained commercially: bovine serum albumin (Armour Pharmaceutical Inc., Chicago; 9.8 mg N/ml); bulk transfer ribonucleic acid (tRNA) of *Escherichia coli* strain B (General Biochemicals Corp., Chagrin Falls, Ohio); ethylenediaminetetraacetic acid (EDTA, Baker Chemical Co., Phillipsburg, N.J.); L-leucine-*4,5-*^3H, L-leucine-*U-*^{14}C (Schwarz Bioresearch Inc., Orangeburg, N.Y.); adenosine triphosphate (ATP; dipotassium salt), tris(hydroxymethyl)aminomethane (Tris, Mann Research Labs, N.Y., N.Y.).

Determination of growth rates. Erlenmeyer flasks (250 ml) containing 30 ml of M medium and inoculated with a log-phase suspension of cells were shaken in a water bath and the absorbancy determined at 30-min intervals. Absorbancy was converted to cell numbers

(determined by direct microscopic counts) by a standard curve.

Enzyme assays. Cells used for the preparation of extracts were grown aerobically in 2-liter Erlenmeyer flasks containing 300 ml of medium. The procedures for the harvesting of cells and the preparation of extracts have been described previously (4). Enzymes in the pathways leading to valine, isoleucine, and leucine were assayed by the following published procedures: threonine deaminase (11) [L-threonine hydro-lyase (deaminating), EC 4.2.1.16], α-acetohydroxyacid synthetase (23), dihydroxyacid dehydrase (10) (2,3-dihydroxyacid hydrolyase, EC 4.2.1.9), β-isopropylmalate (β-IPM) dehydrogenase (5), and α-isopropylmalate (α-IPM) synthetase (6). For the rapid assay of β-IPM dehydrogenase used in the analysis of recombinants, 1-ml samples of log-phase cultures were treated with toluene (8) and analyzed as usual (5) except that cyclohexane was substituted for toluene in the extraction. Protein was determined by the method of Lowry et al. (15) using bovine serum albumin as standard.

For the assay of leucyl-tRNA synthetase [L-leucine: tRNA ligase (adenosine monophosphate, AMP), EC 6.1.1.4], cells grown in M medium to late-log phase were centrifuged and washed two times with 25 ml of buffer (40 mM Tris-hydrochloride, *p*H 7.4, 5 mM $MgCl_2$, 0.1 mM dithiothreitol). Generally, they were stored frozen prior to use. For assays, cells were resuspended in buffer [20 ml per gram (wet weight) of cells] and disrupted with a Branson S110 sonifier for four 15-sec periods with intermittent cooling. The extract was centrifuged at 17,500 $\times$ *g* for 20 min to remove debris. To remove endogenous leucine, 1 ml of extract was passed through a 10-ml column of G25 Sephadex equilibrated with buffer, and the first milliliter to emerge after the void volume was collected. Each assay contained in a total volume of 1 ml: *E. coli* B bulk tRNA, 0.2 mg (A_{260} equal to 4); Tris-hydrochloride, *p*H 7.4, 40 mM; $MgCl_2$, 5 mM; EDTA, 0.5 mM; K_2H_2ATP, 2.5 mM; dithiothreitol, 0.1 mM; ^{14}C- or ^{3}H-L-leucine (for *ara-9 gal-205* extracts, 10 mCi/mmole ^{14}C or 30 mCi/mmole ^{3}H, 0.001 mM; for CV69 and CV117 extracts, 2.47 mCi/mmole ^{14}C or 30 mCi/mmole ^{3}H, 0.125 mM); extract containing 12 to 20 μg of protein. In experiments in which the ATP concentration was varied, tubes also contained 5 mM NH_4Cl. At least two tubes were run for each assay. Tubes were incubated at 37 C for 3 min and the ^{14}C-leucyl-tRNA was precipitated with 2 ml of cold 8% trichloroacetic acid, *p*H 1.0. Precipitates were collected on type HAWP membrane filters (Millipore Corp., Bedford, Mass.) or type E glass fiber filters (Gelman Instrument Co., Ann Arbor, Mich.). They were washed with 5% trichloroacetic acid and the filters were cemented to planchets, dried, and counted (28% efficiency) in a gas flow counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Tritium-containing samples were counted in toluene containing 0.4% 2,5-bis-[2-(5-*tert*-butylbenzoxazolyl)] thiophene in a scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Assays for valyl-, isoleucyl-, and lysyl-tRNA synthetases [amino acid: tRNA ligase (AMP), EC 6.1.1.9, 6.1.1.5, 6.1.1.6, respectively] were performed in essentially the same manner. For the rapid assay of leucyl-tRNA synthetase employed in the analysis of recombinants, assays contained 0.2 mM ^{3}H-leucine, 30 mCi/mmole, and 0.2 ml of extract (not passed through Sephadex) prepared from 3 ml of stationary-phase cells that had been centrifuged, washed with buffer, and resuspended in 3 ml of buffer prior to disruption by sonic treatment.

Genetic crosses. The media and techniques used in conjugation experiments and the procedure for scoring the "leucine excretion" phenotype were described earlier (1, 7).

Determination of proportion of leucine tRNA that is charged. The preparation of tRNA and the determination of the charging of leucine tRNA in vivo was described previously (24) except that the sodium periodate concentration was changed to 5×10^{-3} M.

RESULTS

Growth rates. The doubling times of strains CV69 and CV117 at 37 C were similar to that of the parent (Table 1). The values for a number of other fluoroleucine-resistant strains are given for comparison. Strain CV112 is a leucine operator constitutive mutant and has high, constitutive levels of the enzymes which form leucine. Strains CV19 and CV123 have high, constitutive levels of leucine- and isoleucine-valine-forming enzymes. At 42 C, the doubling times of the parent and of strain CV117 were essentially the same (ca. 60 min), whereas that of strain CV69 was considerably longer (100 min).

Levels of leucine, valine, and isoleucine enzymes. The pathways leading to the branched-chain amino acids are shown in Fig. 1. The specific activities of five of these enzymes were determined for strains grown in minimal medium and minimal medium supplemented with leucine, valine, and isoleucine (Table 2). α-IPM synthetase, β-IPM dehydrogenase, and α-acetohydroxyacid synthetase from strains CV69 and CV117 were derepressed five to sixfold compared to the wild type when the strains were grown in minimal medium. Threonine deaminase and dihydroxyacid dehydrase, compared in the same way, were derepressed approximately twofold. However, when the same strains were grown in minimal medium supplemented with leucine, valine, and isoleucine, the enzymes were repressed to levels similar to those in the parent grown under the same conditions. For comparison, data are presented for another fluoroleucine-resistant strain, CV19. It had derepressed levels of these enzymes when grown in both minimal and minimal medium supplemented with branched-chain amino acids.

Not all three amino acids were required for the repression effect (Table 3). Leucine by itself repressed these enzymes to near wild-type levels. Valine had little effect in most cases, whereas isoleucine seemed to stimulate rather than repress enzyme levels.

TABLE 1. *Generation times of some fluoroleucine-resistant strains*

Strain	Generation time[a]
Parent	46.4 ± 1.5 (23)[b]
CV69	48.5 ± 1.1 (6)
CV117	48.6 ± 1.6 (7)
CV112	47.2 ± 1.2 (6)
CV19	49.8 ± 1.4 (8)
CV123	58.8 ± 1.9 (9)

[a] Time required (min) for the cells to double in number.

[b] Number of determinations preceded by the standard deviation.

Kinetic parameters of leucyl-tRNA synthetase in crude extracts. Specific activities and apparent K_m values were determined for leucyl-tRNA synthetase in crude extracts of the parent and the mutants, CV69 and CV117. For extracts of all three strains, product formation was linear with time to at least 3 min (<20 μg of protein per assay), and velocity (micromoles product formed in 3 min) was linear with extract protein concentration to at least 20 μg of protein. In the estimation of K_m values, velocity data were discarded if the amount of product formed represented more than 10% of the amount of substrate initially present.

Prior to determining apparent K_m values for ATP, a study was made of the effect of magnesium ion concentration upon velocity using crude extracts from the parent. At a concentration of 2.5 mM ATP, 5 mM Mg^{++} was sufficient to give maximal activity and was used in the study described below. NH_4Cl (5 mM) was added to the assay to compensate for the decreased K^+ resulting from the reduction of K_2H_2ATP at the lower concentrations. No significant difference among the strains was observed for the apparent K_m values for ATP (3.0, 4.3, and 3.6 × 10^{-4} M for parent, CV69, and CV117, respectively).

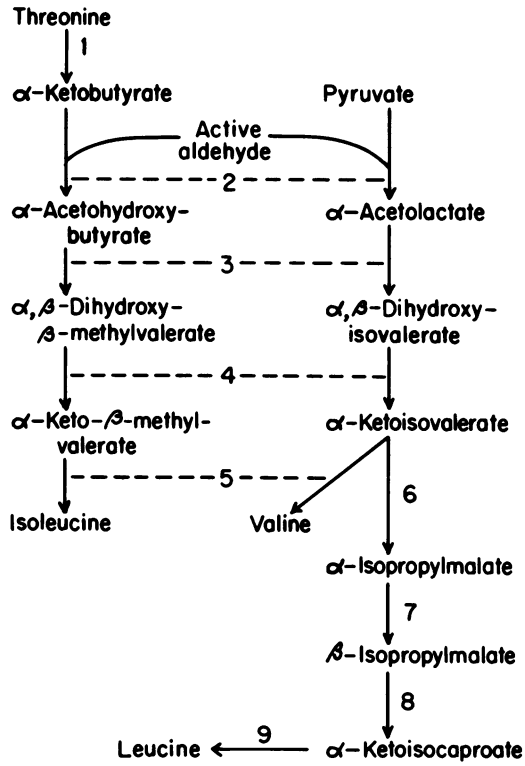

FIG. 1. *Pathways leading to the branched-chain amino acids.*

Estimates were made of K_m values for tRNA

TABLE 2. *Levels of some enzymes involved in leucine, valine, and isoleucine biosynthesis*

Strain	Growth medium[b]	Specific activity[a] of enzymes involved in the biosynthesis of				
		Leucine		Valine and isoleucine		
		α-IPM synthetase	β-IPM dehydrogenase	Threonine deaminase	Dihydroxyacid dehydrase	α-Aceto-hydroxyacid synthetase
Parent	MIVL	0.05	2.0	5.6	2.3	1.1
Parent	M	0.22	5.7	12.4	6.2	2.9
CV69	MIVL	0.05	2.7	6.4	3.1	1.3
CV69	M	1.00	28.0	27.0	11.6	14.1
CV117	MIVL	0.06	5.5	8.4	2.3	1.5
CV117	M	1.30	32.7	26.4	9.6	13.0
CV19	MIVL	0.62	41.7	83.2	43.2	56.2
CV19	M	1.80	42.8	88.1	43.2	61.7

[a] Micromoles product formed per hour per milligram of protein.

[b] Strains were grown in either minimal medium (M) or in minimal medium supplemented with L-leucine and L-isoleucine (each at 50 μg/ml) and L-valine (100 μg/ml). The supplemented medium is termed MIVL above.

TABLE 3. *Effect of exogenous leucine, valine, and isoleucine upon repression*

Strain	Growth medium[b]	Specific activity[a] of enzymes involved in biosynthesis of		
		Leucine	Valine and isoleucine	
		β-IPM dehydrogenase	Threonine deaminase	α-Acetohydroxy-acid synthetase
Parent	MIVL	2.0	5.6	1.1
	MI	7.7	12.6	3.9
	MV	2.7	10.5	2.4
	ML	1.2	9.6	2.3
	M	4.5	12.4	2.9
CV69	MIVL	2.7	6.4	0.8
	MI	46.4	34.2	17.0
	MV	26.3	28.0	12.6
	ML	2.1	9.1	2.3
	M	28.0	27.0	14.1
CV117	MIVL	5.5	8.4	1.5
	MI	36.3	41.1	23.5
	MV	27.4	32.0	14.5
	ML	3.1	13.8	3.3
	M	32.7	26.4	13.0

[a] Micromoles of product formed per hour per milligram of protein.

[b] Abbreviations: M, minimal medium; I, L-isoleucine, 50 μg/ml; V, L-valine, 100 μg/ml; L, L-leucine, 50 μg/ml.

using *E. coli* bulk tRNA. Values for the parent and mutant enzymes were in the range 1 to 4 × 10^{-7} M (leucine tRNA assumed to represent 7% of the total tRNA and average molecular weight assumed to be 26,500). It was difficult to measure such low K_m values, especially for strains CV69 and CV117 where saturating concentrations of leucine were high, and we consider the values we obtained to be rough estimates. There did not appear to be large differences between the enzymes from the parent and mutant strains for K_m values for *E. coli* tRNA.

Some representative curves for the variation in velocity with leucine concentration are shown in Fig. 2A. It is clear that in this experiment the leucine K_m values for the enzymes of strains CV69 (9.0 × 10^{-5} M) and CV117 (1.8 × 10^{-5} M) were significantly higher than the corresponding value for the parent (4.7 × 10^{-6} M). The average values for several determinations are given in Table 4.

The specific activity of leucyl-tRNA synthetase in crude extracts varied somewhat in day to day determinations and depended upon the age of the cells. The data shown in Table 4 are from experiments in which extracts of the three strains prepared from fresh log-phase cells were tested on the same day. Extracts from the mutants had a ca. twofold lower specific activity of leucyl-tRNA synthetase than those from the parent.

That the mutation in strain CV117 did not affect amino acyl-tRNA synthetases in general was shown in other experiments. The specific activities of valyl-tRNA synthetase, isoleucyl-tRNA synthetase, and lysyl-tRNA synthetase did not differ appreciably for the parent and strain CV117. Furthermore, the K_m value for isoleucine was the same in experiments using crude extracts from the two strains (ca. 2 × 10^{-6} M).

Studies with partially purified enzymes. A 60-fold purification of leucyl-tRNA synthetase from the parent and strain CV117 was effected by chromotography on diethylaminoethyl cellulose,

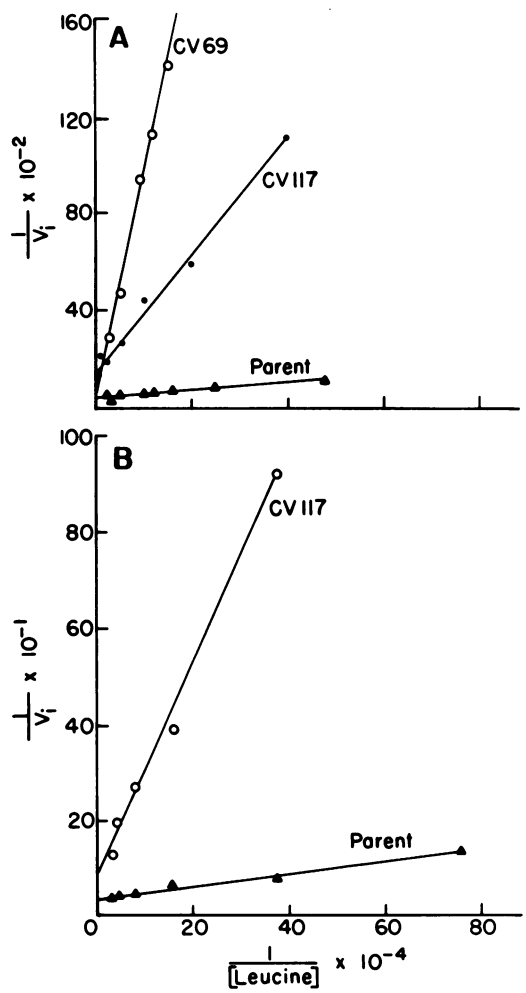

FIG. 2. *Initial velocity (micromoles product per minute per milligram of protein) of leucyl-tRNA synthetase as a function of leucine concentration (Lineweaver-Burk plots). Experiments were carried out with crude extracts (A) or 60-fold purified extracts (B).*

TABLE 4. *Summary of kinetic parameters for leucyl-tRNA synthetase in crude extracts of several strains*

Strain	K_m		Specific activity[b]
	Leucine	ATP[a]	
Parent	$5.3 \pm 4.1 \times 10^{-6}$ (8)[c]	3.0×10^{-4}	$2.2 \pm 0.2 \times 10^{-3}$ (4)
CV69	$5.9 \pm 2.8 \times 10^{-5}$ (4)	4.3×10^{-4}	$1.1 \pm 0.4 \times 10^{-3}$ (4)
CV117	$4.2 \pm 3.8 \times 10^{-5}$ (2)	3.6×10^{-4}	$1.3 \pm 0.6 \times 10^{-3}$ (4)

[a] Adenosine triphosphate.

[b] Micromoles of leucyl-tRNA formed per minute per milligram of protein.

[c] Values in parenthesis refer to the number of determinations. Standard deviations are given for leucine K_m values and specific activities.

G150 Sephadex, and hydroxylapatite (T. Mikulka and J. Calvo, *manuscript in preparation*). The yield of the parent and mutant enzymes was 11 and 6%, respectively. From data on the velocity of the reaction as a function of leucine concentration (e.g., Fig. 2B), the K_m for leucine was estimated to be 6.0×10^{-6} M and 3.8×10^{-5} M for parent and mutant enzymes, respectively (average of two experiments for each). These differences in K_m values, obtained with partially purified enzymes, suggest that the two enzymes are different.

Another indication that the mutation directly affects the structure of leucyl-tRNA synthetase was obtained from studies on the rate of inactivation of the partially purified enzymes at 50.5 C. As can be seen in Fig. 3, the enzyme from the mutant was considerably more labile than was the parent enzyme.

Level of charged leucine tRNA in vivo. The periodate technique of Böck et al. (2) was used to determine the proportion of leucine tRNA ($tRNA^{Leu}$) which was charged (Leu-$tRNA^{Leu}$) in strains grown under different conditions (Table 5). As controls, the same experiments were performed measuring the levels of valine and methionine tRNA. The proportion of $tRNA^{Met}$ that was charged did not vary appreciably as a function of exogenous leucine concentration, whereas the addition of exogenous leucine caused the level of Val-$tRNA^{Val}$ to decrease. The latter might be the result of a decreased endogenous valine pool due to a high concentration of leucine (3). The proportion of $tRNA^{Leu}$ that was charged in strains CV69 and CV117 grown in minimal medium was lower than the wild-type value. The addition of leucine to the medium caused $tRNA^{Leu}$ in CV117 and the parent to become fully charged; in strain CV69, the proportion of $tRNA^{Leu}$ charged increased but not to 100%.

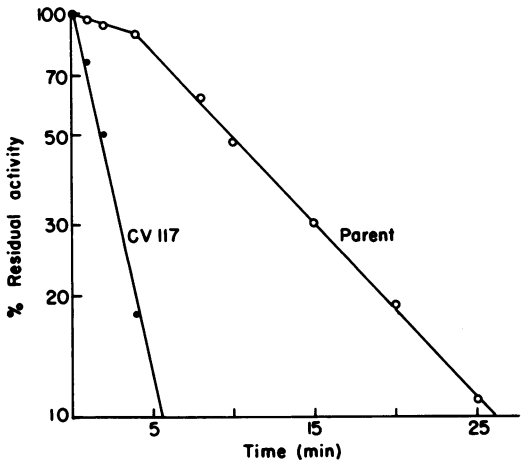

FIG. 3. *Rate of inactivation of partially purified leucyl-tRNA synthetases at 50.5 C. Samples of enzyme from the parent (10 μg of protein/ml) and strain CV117 (22.5 μg of protein/ml) in 20 mM phosphate buffer, pH 7.4, containing 0.1 mM dithiothreitol were incubated at 50.5 C. At the indicated times, duplicate 0.1-ml samples were removed and chilled in an ice bath. After the last sample was taken, tubes were assayed for synthetase activity.*

TABLE 5. *Level of charged leucine tRNA in vivo*

Strain	tRNA	% Total tRNA charged in cells grown in minimal medium containing the following concn of L-leucine (μg/ml)				
		none	12	50	100	200
Parent	Leucine	84	—	—	97	—
	Valine	95	—	—	82	—
	Methionine	81	—	—	90	—
CV117	Leucine	70	—	—	98	—
	Valine	95	—	—	79	—
	Methionine	78	—	—	80	—
CV69	Leucine	60	67	75	74	86
	Valine	100	94	85	—	86
	Methionine	85	82	78	81	81

Are the properties of strain CV117 due to a single mutation? The following conjugation cross (Fig. 4) was carried out: SU576 (*purC7*) × recipient CV117 (*ara-9 gal-205 flr-117*). Samples of donor and recipient were mixed and plated on minimal medium containing galactose as sole carbon source to select for *gal*$^+$ *pur*$^+$ recombinants. Single-colony isolates of recombinants were analyzed for three unselected characters: excretion of leucine, specific activity of β-IPM dehydrogenase, and specific activity of leucyl-tRNA synthetase (Table 6). In all 78 of the *pur*$^+$ *gal*$^+$ recombinants tested, the three characteristics of leucine excretion, high levels of leucine-forming enzymes, and an altered leucyl-tRNA

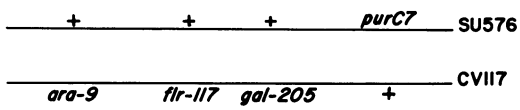

FIG. 4. *Schematic illustration of cross between Hfr SU576 and CV117. Approximate map positions (min): ara, 5; flr and gal, 20; purC, 80.*

synthetase always segregated as a single genetic marker. The data strongly suggest that the three characteristics are the result of a single mutation. Furthermore, the fact that 60% of the *gal*$^+$ recombinants had the phenotype associated with the donor *flr*$^+$ allele indicates that these two markers lie close to one another on the *Salmonella* genome (1).

Location of the mutant locus of strain CV117 on the genetic map of S. typhimurium. The cross shown in Fig. 4 was carried out again, but in this experiment conjugation was interrupted in samples that were removed at suitable time periods and the samples were plated on medium containing arabinose as sole carbon source. *pur*$^+$ *ara*$^+$ recombinants were picked and scored for the unselected markers, *gal* and *flr*. A plot of the number of recombinants as a function of time of interruption is shown in Fig. 5. *ara*$^+$ was observed to enter at ca. 30 min and *flr* and *gal* entered sometime during the interval 42 to 46 min. Since strain SU576 injects clockwise with an origin at 120 min and assuming a time of entry delay of 7 min (20), the map positions are 5 min for *ara* and 17 to 21 min for *flr* and *gal*. This position of *flr-117* close to *gal* coincides with the map location of several leucine regulatory mutations reported earlier (1).

DISCUSSION

The data presented indicate that the mutations in strains CV69 and CV117 are in a locus specifying the structure of leucyl-tRNA synthetase. Thus, the properties of these strains define a new locus designation, *leuS*, and the genotypes of these strains are given as *ara-9 leuS1* and *ara-9 gal-205 leuS2*, respectively. The designations currently in use for loci involved in the synthesis and control of leucine biosynthesis are now *leuA*, *leuB*, *leuC*, *leuD* (structural genes, 16), *leuO* (operator, 8), and *leuS* (leucyl-tRNA synthetase). The locus designated previously as *flr* (1) is probably identical to *leuS*. The designation *flr* will be maintained for mutations conferring resistance to fluoroleucine which have not been shown to be allelic with *leuO* or *leuS*.

Evidence for the involvement of amino acyl-tRNA synthetases in the control of amino acid biosynthetic pathways in bacteria has been obtained in the case of histidine (19), valine (9), and tryptophan (14). On the other hand, similar studies of tyrosyl (21), phenylalanyl (17), methionyl (12), and arginyl-tRNA synthetases (13) have not revealed any role for these enzymes in repression. Our studies with strains CV69 and CV117 indicate that leucyl-tRNA synthetase is involved in the regulation of enzymes that function in branched-chain amino acid biosynthesis. The specific activities of leucine-, valine-, and isoleucine-forming enzymes were elevated two- to

TABLE 6. *Analysis of gal*$^+$ *recombinants from the cross SU576 × CV117 for leucine excretion and the specific activities of β-isopropylmalate (IPM) dehydrogenase and leucyl-tRNA synthetase*

Class	Phenotype			*gal*$^+$ recombinants having indicated phenotypes	
	Leucine excretion[a]	Specific activity			
		β-IPM dehydrogenase[b]	Leucyl-tRNA synthetase[c]	No.	% of total
Donor	none	normal	normal	47	60
Recipient	slight	high	low	31	40
Recombinant[d]				0	0

[a] Measured by an auxanographic test (7).

[b] One portion of a log-phase culture was used to determine cell number by turbidimetry (Klett); a second portion was treated with toluene and assayed for dehydrogenase activity. Specific activity equals activity (absorbancy units) divided by cell number (Klett units). Parent specific activity, 4.0 ± 0.6 (10 samples); mutant specific activity, 11.5 ± 3.4 (11 samples). When values were obtained that did not fall into one of these two ranges (7 samples), the assay was repeated and in each instance, the second determination did fall into one of the above ranges.

[c] Parent specific activity, 26,700 ± 14,700 counts per min per mg of protein (9 samples); mutant specific activity, 4830 ± 1600 counts per min per mg of protein (9 samples). When values were obtained that did not fall into one of these two ranges (20 samples), the assay was repeated and in each instance the second determination did fall into one of the above ranges.

[d] Six possible classes.

fivefold above wild-type levels in extracts of these strains prepared from cells grown in a minimal medium. Furthermore, elevated enzyme levels and an altered leucyl-tRNA synthetase were shown to be the result of a single mutation.

One interpretation of the role of leucyl-tRNA synthetase in the control of branched-chain amino acid biosynthesis is that it is required to form leucyl-tRNA, the latter being more directly involved in control than leucine itself. According to this interpretation, the properties of strains CV69 and CV117 could be explained as follows. The amount of one or more charged $tRNA^{Leu}$ species is reduced when these strains are grown in a minimal medium because of (i) the reduced specific activity of leucyl-tRNA synthetase and (ii) the increased K_m of this enzyme for leucine. The reduced level of charged $tRNA^{Leu}$ acts as a signal to release repression resulting in an increased synthesis of leucine- (and isoleucine-valine-) forming enzymes and hence of leucine (CV117 overproduces and excretes a small amount of leucine). The accumulation of sufficient leucine to overcome the higher K_m and thus cause repression is probably prevented by the effective end-product inhibition control mechanism that is known to operate in *Salmonella* (7). It may be recalled that the synthesis of leucine-, valine-, and isoleucine-forming enzymes was not constitutive in strains CV69 and CV117. Rather, the addition of leucine to the minimal medium repressed the leucine-, valine-, and isoleucine-forming enzymes. Thus, a large excess of exogenous leucine probably overcame the higher K_m and resulted in increased acylation of $tRNA^{Leu}$ and concomitant repression. The properties of strains CV69 and CV117 are similar in many ways to those reported to Roth and Ames (19) for *hisS1520*, a mutant that has an altered histidyl-tRNA synthetase.

If the above interpretation (leucyl tRNA acts as a corepressor) is correct, one would expect to find an inverse relationship between the level of leucyl tRNA and the level of pathway-specific enzymes. For strains grown in a minimal medium, there was such a correlation, *i.e.*, strains CV69 and CV117 had higher enzyme and lower tRNA levels than the wild type (Table 5). On the other hand, other data in Table 5 appear inconsistent with the hypothesis, namely, strain CV117 was derepressed when 70% of the $tRNA^{Leu}$ was charged, whereas strain CV69 was fully repressed when 75% of the tRNA was charged. Possibly, one of the charged $tRNA^{Leu}$ species (there are at least four different $tRNA^{Leu}$ species in *S. typhimurium*) might play a predominant role in repression and the amount of this species might not be accurately reflected in the data in Table 5. The resolution of this question will require experiments in which the levels of individual $tRNA^{Leu}$ species are determined.

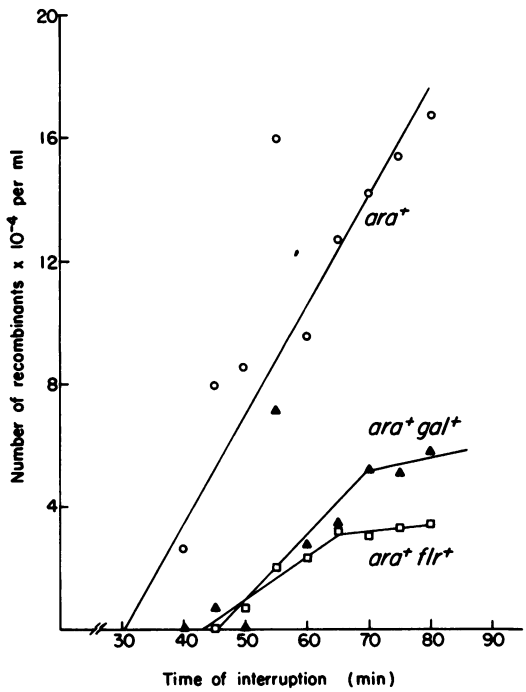

FIG. 5. *Time of entry for ara, gal, and flr for cross: donor SU576 (purC7) × recipient CV117 (ara-9 gal-205 flr-117).*

The characteristics of strains CV69 and CV117 are consistent with a model for the regulation of branched-chain amino acid biosynthesis presented earlier (7). We postulated that species of charged tRNA (rather than the amino acids themselves) interact with a protein aporepressor to form various active repressors and that the latter interact with operators at the *ilv* and *leu* operons to limit expression of those operons. The fact that an alteration of leucyl-tRNA synthetase results in derepression, not only of leucine-forming enzymes, but also of isoleucine- and valine-forming enzymes, is accounted for in the model by postulating that Leu-$tRNA^{Leu}$ is required as a corepressor at both *ilv* and *leu* operators. An alternative to this model is that leucyl-tRNA synthetase itself, or in combination with some modifier (e.g., Leu-$tRNA^{Leu}$) acts as a repressor. None of the data we presented is inconsistent with this possibility. The latter model may or may not involve Leu-$tRNA^{Leu}$, whereas in the former model its formation is a requirement for repression. At present, there is no direct evidence

that Leu-tRNA^{Leu} is involved in repression. However, Williams and Freundlich (24) and Silbert, Fink, and Ames (22) have presented evidence that tRNA^{val} and tRNA^{His} are important in repression of valine- and histidine-forming enzymes, respectively.

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