Genetic and biochemical studies of \textit{livS} mutation affecting the regulation of branched-chain amino acid transport in \textit{Salmonella typhimurium}

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ABSTRACT

A mutation \textit{livS} (\textit{livS}1 in KA231) that occurred in a \textit{Salmonella typhimurium} LT2 mutant strain CE5 (\textit{ilvC}8 \textit{brnQ}4) expressed pleiotropic effects on cellular characteristics. The mutation not only resulted in derepression of the transport of branched-chain amino acids (Ohnishi et al. 1980), but also altered the properties of the ribosome. Ribosomes of KA2313 (\textit{brnQ}4 \textit{livS}1), an \textit{Ilv}+ transductant of KA231 from the wild-type donor, appeared to be more unstable than those of the ancestral strain KA204 (\textit{brnQ}4) in a Tris-HCl buffer without MgCl2. When ribosomes of KA2313 were suspended in the buffer, they released substantial amounts of smaller proteins of less than 20,000 daltons into the buffer, and ribonuclease I was activated. Ribosomes of KA204 liberated a little but much lesser amount of such proteins under the same condition, and ribonuclease I stayed in an inactive state. The \textit{livS} gene was found to be closely linked to \textit{aroA} located at 19 min on the \textit{Salmonella} genetic map. Co-transduction frequency of \textit{livS} with \textit{aroA}, and \textit{vice versa}, ranged from 12 to 55%.

1. INTRODUCTION

\textit{Salmonella typhimurium} is known to have several, active transport systems for branched-chain amino acids (\textit{L}-isoleucine, \textit{L}-leucine and \textit{L}-valine). By now, biochemical and genetic studies of these systems have revealed that three general systems (high-affinity (LIV–I), low-affinity–(1) (LIV–II) and low-affinity–(2) (LIV–III)) and a leucine-specific (LS) system are responsible for transport of these amino acids (Kiritani and Ohnishi 1978; Ohnishi and Kiritani 1983). The LIV–I system is characterized by a low \(K_m\) of transport for them and by association with a periplasmic leucine-isoleucine-valine-threonine-binding-protein (LIVT–BP). The LS system also has a low \(K_m\) for leucine transport comparable to LIV–I and is associated with a leucine-specific binding protein (L–BP). The LIV–II and LIV–III systems appear to transport these three amino acids through membrane-bound carrier proteins.

Previously, we reported the isolation of two regulatory mutant strains, KA231 and KA600, that have derepressed levels of LIV–I and LIV–II (Ohnishi \textit{et al.} 1980, 1983). These two strains possessed not only high transport...
activities for branched-chain amino acids, but also increased amounts of LIVT-BP and L-BP. These two binding proteins purified from KA231 were proved to be identical with those of the wild-type strain in immunological and biochemical properties (Ohnishi and Kiritani 1983). The mutant locus livR responsible for derepression of branched-chain amino acid transport in KA600 has been mapped in the region of 75–77 min on the Salmonella genetic map (Ohnishi et al. 1983). The mutant locus affecting regulation of the transport in KA231, however, has not been found in the livR region. We have designated the mutant locus in KA231 as livS, which has previously and tentatively been referred to as liv-231 (Ohnishi et al. 1980).

The present investigation aimed at determining the genetic locus of livS mutation and elucidating relevant properties accompanying with the mutation. The results show that the livS gene resides in a region near aroA locus and that the livS mutant exhibits somewhat unusual stability in isolated ribosomes, together with a derepressed state in the transport of branched-chain amino acids.

2. MATERIALS AND METHODS

**Bacterial strains.** The strains used in this study were all derivatives of *Salmonella typhimurium* LT2 and are listed in Table 1. MA533 was isolated after successive treatments of KA231 with a mutagen, ethylmethanesulfonate (EMS), and subsequent penicillin-screening. The pyrD character of OH514 was determined by following tests; 1) growth of cells was stimulated by addition of orotic acid or uracil but not by dihydroorotic acid (Yan and Demerec 1965), 2) in a conjugation cross, entry time of pyr+ marker from the donor Hfr SA534 to the recipient OH514 was 36 min, while that of pyrC+ to KA574 (pyrC73) was 42 min, and 3) many Pyr+ recombinants were formed in a transductional cross from KA574 to OH514, comparable to the cross from the wild-type to OH514.

**Growth media.** Minimal medium (Kiritani 1974) was used in most experiments. The medium was supplemented, when required, with L-isoleucine (10 μg/ml), L-leucine (10 μg/ml), L-valine (20 μg/ml), adenine (35 μg/ml), uracil (50 μg/ml) and Ca-pantothenate (0.1 μg/ml). To grow mutants carrying aroA gene, minimal medium was supplemented with L-tryptophan (20 μg/ml), L-tyrosine (20 μg/ml), L-phenylalanine (50 μg/ml), L-methionine (45 μg/ml) and p-aminobenzoic acid (1 μg/ml). It should be pointed out that 0.001% Casamino Acids could stimulate growth of aroA mutants. When galactose was used as a sole carbon source for bacterial growth, 5 g per liter of it was substituted for glucose in the minimal medium. Nutrient Broth (Difco Lab.) or L-broth (Ohnishi et al. 1983) was used for conjugation and transduction experiments.
Streptomycin (200 μg/ml) was added in selection medium of the conjugational crosses between Hfr SA534 and MA533 to counterselect the parent Hfr.

**Genetic crosses.** Conjugations were carried out by the method described previously (Kiritani 1974). In crosses between Hfr SA534 and a recipient F- strain (MA533 or OH204), genetic markers for the transport of branched-chain amino acids and the requirement of isoleucine-valine (i.e., ilvC brnQ livS) were used as unselected markers. Pur+, Gal+, Pyr+ and Aro+ recombinants were selected on minimal agar medium supplemented with large amounts of isoleucine (100 μg/ml) and valine (200 μg/ml) together with the other required growth factors. Frequency of livS+ gene from the donor among these recombinants was then determined by examining capability of their growth on supplemented medium containing regular amounts of isoleucine and valine plus excess glycyl-L-leucine (400 μg/ml). As seen in Table 2, recombinant types I and III (LivS−) carrying ilvC and livS genes can, while types II and IV (LivS+) can not, grow on the medium containing excess glycyleucine, regardless of the presence or absence of brnQ. P22-mediated transduction was carried out according to the method described previously (Kiritani 1974). Since OH204 was a P22-lysogenic strain, lysates of P22 phage from the strain

<table>
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<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<td>Wild-type</td>
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<td>Ohnishi et al. 1980</td>
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<td>ilvC8 brnQ4</td>
<td>Ohnishi et al. 1980</td>
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<td>Ohnishi et al. 1980</td>
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<td>Ohnishi et al. 1980</td>
</tr>
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<td>SA2013</td>
<td>aroA67</td>
<td>SGSC*</td>
</tr>
<tr>
<td>OH204</td>
<td>ilvC8 brnQ4 livS1 aroA67</td>
<td>aroA transductant of KA231 from SA2301</td>
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<td>gal mutant of KA231 mutagenized with EMS</td>
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<tr>
<td>OH514</td>
<td>ilvC8 brnQ4 livS1 gal−2001 pyrD1984</td>
<td>ppyr mutant of MA505 mutagenized with EMS</td>
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<td>MA522</td>
<td>ilvC8 brnQ4 livS1 gal−2001 pyrD1984 str−r</td>
<td>str−r spontaneous mutant of OH514</td>
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<td>ilvC8 brnQ4 livS1 gal−2001 pyrD1984 pur−2008 str−r</td>
<td>pur−2008 mutant of MA522 mutagenized with EMS</td>
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<td>serA rfa−8058 HfrK4</td>
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<td>CV117</td>
<td>ara−9 gal−205 leuS2</td>
<td>SGSC*</td>
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</table>

* SGSC: Salmonella Genetic Stock Centre.
were prepared according to the method of Hoppe and Roth (1974) by inducing the lysogen with UV light. Exponentially growing cells of OH204 in L-broth (200 ml) were centrifuged, washed once with glucose-depleted minimal medium (MMG) and suspended in 40 ml of MMG. The suspension was equally distributed in 8 petri-dishes (9 cm in diameter) and subjected to UV-irradiation for 1 min at a distance of 75 cm under a 10 watt-germicidal lamp (Matsushita Elec. Co.). It was then added into 160 ml of L-broth, and the induced P22-phages were allowed to multiply aerobically at 37°C for 90 min. After the culture was treated with an aliquot of chloroform, cell debris were removed by centrifugation. Then phage particles in the supernatant were sedimented by ultracentrifugation at 80,000 g for 30 min and suspended in 2 ml of MMG. The phage suspension was stored at 4°C with chloroform.

Preparation of ribosome-free extracts and ribosomes. Bacterial cells were grown aerobically at 37°C overnight in minimal medium and the culture was diluted by the ratio of 1:25 in the same medium. Cells in the diluted culture were aerated at 37°C to an exponential phase (5x10⁸ cells/ml), collected by centrifugation, washed twice with 30 mM Tris-HCl buffer (pH 7.8) and suspended in the same buffer but containing 10 mM MgCl₂ so as to give a density of 0.1 g of cells (wet weight) per ml. They were disrupted by 2 min-sonication and cell debris were removed by centrifugation at 12,000 g for 10 min. The supernatant was then centrifuged for 2 h at 100,000 g to sediment ribosome particles. The resulting supernatant was used as a ribosome-free extract. The pellet was resuspended in the same volume of 30 mM Tris–HCl buffer (pH 7.8) as the supernatant and used as a ribosome preparation. Since Mg²⁺ inhibited activity of RNase I, MgCl₂ was not added in the buffer. These procedures were carried out in the cold.

Assay of RNase I activity. For assay of RNase I activity, the method of
Chakraburty and Burma (1968) was modified. The reaction mixtures contained, in a total volume of 0.4 ml, 2 μmoles of 2-mercaptoethanol, 0.4 mg of tRNA (Sigma Chem. Co.), 4 μmoles of ethylenediaminetetraacetic acid (EDTA), an appropriate amount of a ribosome-free extract or a ribosome preparation corresponding to 70 to 120 μg of protein and 40 μmoles of Tris-HCl (pH 7.4). Assays were also performed in the absence of EDTA. The reaction mixtures were incubated at 37°C for various periods and the reaction was stopped by addition of 2 ml of 10% trichloroacetic acid (TCA). Controls containing everything in the reaction mixture were chilled in ice for the corresponding assay periods and then TCA was added. After thorough mixing and centrifugation, extinction by each supernatant was measured at 260 nm in a Hitachi Spectrophotometer 139. The amount of released nucleotides (μmoles/mg of protein) was calculated assuming a molar extinction coefficient of 10,000 for them. Protein was determined according to Lowry et al. (1951) with crystalline bovine serum albumin as a standard.

Assay of leucyl-tRNA synthetase activity. To minimize loss of leucyl-tRNA synthetase (EC 6.1.1.4) activity in crude extracts of cells, extracts were prepared by the method of Hayashi et al. (1970). Bacterial cells grown to an exponential phase in 250 ml of minimal medium containing isoleucine, valine and pantothenate were collected by centrifugation, washed once with 30 mM Tris-HCl buffer (pH 7.8) and suspended in 10 mM Tris-HCl buffer (pH 7.8) containing 10 mM MgCl₂, 20 mM NH₄Cl and 20 mM 2-mercaptoethanol so as to give a cell density of 0.1 g per ml. They were disrupted by sonication and centrifuged at 12,000 g for 30 min. The supernatant was used as a crude extract.

To determine leucyl-tRNA synthetase activity, the method of Rouget and Chapeville (1970) was slightly modified. The reaction mixtures contained, in a total volume of 0.4 ml, 5 mM MgCl₂, 5 mM 2-mercaptoethanol, 2 mM ATP, 10 μM L-(¹⁴C)-leucine (3.3 × 10⁸ cpm/μmole), 0.4 mg of tRNA (Sigma Chem. Co.), 100 mM Tris-HCl (pH 7.4) and crude extract equivalent to 60 μg of protein. They were incubated at 37°C for 5 min and the reaction was stopped by addition of 2 ml of 10% TCA. Control values were obtained with the same reaction mixtures but without ATP. The reaction mixtures were then filtered through pieces of glass microfibre paper (Whatman GF/B), and each piece was washed twice with 5 ml each of 10% TCA. Radioactivity retained on each piece of paper was measured with a Beckmann liquid scintillation counter.

Assay of leucine transport activity. Assay of leucine transport activity of bacterial cells was carried out by the method described previously (Ohnishi et al. 1983).
Polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was carried out according to the method described previously (Ohnishi and Kiritani 1983). For the electrophoresis, ribosome preparations and ribosome-free extracts that were stored at $-80^\circ C$ for 7 days were used. Portions of the ribosome preparations were centrifuged again for 2 h at 100,000 g, respectively, and the precipitate was suspended in the same volume of Tris–HCl buffer (pH 7.8) as the original portion. These ribosome fractions and supernatants were also subjected to the electrophoresis.

3. RESULTS

Genetic mapping. To determine the livS locus on the chromosome, strains MA533 and OH204 were crossed with an Hfr strain SA534 and the mating was interrupted at 90 min. Recombinants formed on selective media were isolated and tested for frequencies of unselected donor markers. As shown in Table 3, pur, gal and pyrD loci can be arranged in this order with no discrepancy with the order described by Sanderson and Roth (1983) in the Salmonella genetic map. The livS locus may be placed between aroA and pyrD, or on the distal side of pyrD. To ascertain its location, detailed interrupted conjugation experiments were carried out. As seen in Figs. 1 and 2, Pur+, Gal+, Pyr+ and Aro+ recombinants first appeared on selective media at 24, 29, 36 and 36 min, respectively, after recipients were mixed with the donor SA534. To estimate the entry time of livS+ into the recipients, many of the recombinants obtained in each selection at various mating interval were isolated at random and tested for their capability to grow on the medium containing excess glycylleucine. As presented in Table 4, LivS+ clones, non-grower in the presence of excess glycylleucine, were found among Pur+, Gal+, Pyr+ and Aro+ recombinants. Their estimated numbers were plotted in Fig. 3. With no exception, the entry time of livS+ marker extrapolated in Gal+, Aro+ and Pyr+ recombinants was 36 min, which corresponded to the time of pyr+ and aro+ entrance. These results suggest that the livS mutational site is located.

Table 3. Frequencies of the donor alleles among recombinants derived from crosses between Hfr SA534 and recipient strains*

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Selected marker</th>
<th>No. of recombinants tested</th>
<th>Percent of unselected markers</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>pur+</td>
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<td>MA533</td>
<td>pur+</td>
<td>370</td>
<td>100</td>
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<tr>
<td></td>
<td>gal+</td>
<td>241</td>
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<td></td>
<td>pyrD+</td>
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<td>30.9</td>
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<tr>
<td>OH204</td>
<td>aroA+</td>
<td>222</td>
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</table>

* Matings were interrupted at 90 min.
Fig. 1. Time of entry of pur+, gal+ and pyrD+ loci. Conjugation cross between donor Hfr SA534 and recipient MA533 was interrupted at intervals, and Pur+ (●), Gal+ (○) and Pyr+ (△) recombinants in 0.1 ml of the mating mixture were scored. Average numbers of recombinants on duplicated plates were plotted.

Fig. 2. Time of entry of aroA+ locus. Conjugation cross between donor Hfr SA534 and recipient OH204 was interrupted at intervals, and Aro+ recombinants were scored. Average numbers of recombinants on duplicated plates were plotted.
Table 4. Frequency of \textit{livS}^{+} marker among \textit{Pur}^{+}, \textit{Gal}^{+}, \textit{Pyr}^{+}, or \textit{Aro}^{+} recombinants derived from crosses between \textit{Hfr} SA534 and recipient strains

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Marker selected</th>
<th>Interrupted time (min)</th>
<th>Average no. of recombinants per plate*</th>
<th>No. of recombinants tested</th>
<th>No. of \textit{LivS}^{-} clones**</th>
<th>No. of \textit{LivS}^{+} clones**</th>
<th>Frequency of \textit{LivS}^{+} clones (%)</th>
<th>Estimated no. of \textit{LivS}^{+} clones per plate</th>
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* Average numbers of recombinants on duplicated plates are listed.

** Genotypes of LivS^{-} and LivS^{+} are indicated in Table 2.
Regulatory livS mutation

Experiments were then performed to check joint transduction of the livS mutation with aroA and pyrD markers. No joint transduction of livS was observed with pyrD. However, in crosses between OH204 (recipient) and CE5 (donor), LivS+ clones were found among AroA+ transductants at a frequency of 55.1% (Table 5). Since both CE5 and OH204 carry ilvC and brnQ genes, identification of LivS- clones among AroA+ transductants has been made on minimal medium containing isoleucine, valine and pantothenate (see Table 2). The livS+ character in some of these transductants was verified by measuring

![Fig. 3. Time of entry of livS+ locus. As indicated in Table 4, numbers of LivS+ recombinants among Gal+ (○), Pyr+ (△) and Aro+ (▲) recombinants were estimated and plotted.](image)

Table 5. Joint transduction of livS+ and pyrD+ or aroA+ genes mediated by P22 phage grown on CE5*

<table>
<thead>
<tr>
<th>Recipient</th>
<th>No. of transductants tested</th>
<th>No. of LivS- clones</th>
<th>No. of LivS+ clones</th>
<th>Frequency of LivS+ clones (%)</th>
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<tbody>
<tr>
<td>MA533</td>
<td>536</td>
<td>536</td>
<td>0</td>
<td>0</td>
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<tr>
<td>OH204</td>
<td>287</td>
<td>129</td>
<td>158</td>
<td>55.1</td>
</tr>
</tbody>
</table>

* PyrD+ or AroA+ transductants were selected and their growth response on minimal medium containing isoleucine, valine and pantothenate was tested (see Table 2).

near the aroA and pyrD loci on the chromosome.

Experiments were then performed to check joint transduction of the livS mutation with aroA and pyrD markers. No joint transduction of livS was observed with pyrD. However, in crosses between OH204 (recipient) and CE5 (donor), LivS+ clones were found among AroA+ transductants at a frequency of 55.1% (Table 5). Since both CE5 and OH204 carry ilvC and brnQ genes, identification of LivS- clones among AroA+ transductants has been made on minimal medium containing isoleucine, valine and pantothenate (see Table 2). The livS+ character in some of these transductants was verified by measu-
ring their ability to uptake (14C)-isoleucine as shown in Table 6. The uptake by LivS+ transductants was comparable to that by donor CE5 and the growth of these clones was inhibited by excess glycylleucine. On the contrary, LivS- transductants took up isoleucine as efficiently as OH204 or KA231 and grew well even in the presence of excess glycylleucine. In a reciprocal transductional cross, LivS- transductants harbored the aroA marker at a frequency of 12.0% (Table 7). It is concluded that the livS mutational site in KA231 is closely linked to aroA.

**Lencyl-tRNA synthetase activity and leucine transport.** We have attempted to examine whether, or not, leucyl-tRNA or its synthetase is involved in

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**Table 6. Transport activity of LivS- and LivS+ transductants from a cross between donor CE5 and recipient OH204**

<table>
<thead>
<tr>
<th>Transductant or strain</th>
<th>Relevant genotype</th>
<th>Growth on glycyl-L-leucine (400 μg/ml)**</th>
<th>No. of transductants tested</th>
<th>Uptake of isoleucine*** (μmoles/min/g cells)</th>
<th>Relative transport activity****</th>
</tr>
</thead>
<tbody>
<tr>
<td>LivS-</td>
<td>ilvC brnQ livS</td>
<td>+</td>
<td>33</td>
<td>2.7-7.1</td>
<td>0.7-1.9</td>
</tr>
<tr>
<td>LivS+</td>
<td>ilvC brnQ lvs+</td>
<td>-</td>
<td>34</td>
<td>0.7-2.0</td>
<td>0.2-0.5</td>
</tr>
<tr>
<td>KA231</td>
<td>ilvC brnQ livS</td>
<td>+</td>
<td>-</td>
<td>3.7</td>
<td>1.0</td>
</tr>
<tr>
<td>OH204</td>
<td>ilvC brnQ livS arnA</td>
<td>+</td>
<td>-</td>
<td>3.3</td>
<td>0.9</td>
</tr>
<tr>
<td>CE5</td>
<td>ilvC brnQ</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>KA931</td>
<td>ilvC</td>
<td>-</td>
<td>-</td>
<td>4.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* All transductants and strains, except OH204, were grown in minimal medium supplemented with isoleucine (100 μg/ml) and valine (200 μg/ml); additional supplements for OH204 were tryptophan, tyrosine, phenylalanine, p-aminobenzoic acid and methionine.

** Growth of transductants and strains on glycyl-L-leucine was determined on minimal medium containing isoleucine, valine and excess glycyl-L-leucine. For OH204, the medium also contained other required substances as indicated above.

*** Initial uptake of L-(14C)-isoleucine in bacterial cells for 20 sec was measured; concentration of L-(14C)-isoleucine was 7.4 μM, and the specific activity was 2.6×10⁶ cpm per μmole.

**** These values were calculated for each taking the value of isoleucine uptake by KA231 as 1.0.

**Table 7. Joint transduction of livS and aroA genes mediated by P22 phage grown on OH204**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>No. of transductants tested</th>
<th>No. of AroA+ clones</th>
<th>No. of AroA− clones</th>
<th>Frequency of AroA− clones (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE5</td>
<td>200</td>
<td>176</td>
<td>24</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* LivS- (ilvC brnQ livS) transductants were first selected on minimal medium containing isoleucine, valine, pantothenate, tryptophan, tyrosine, phenylalanine, methionine, p-aminobenzoic acid and Casamino Acids (0.001%). Then 200 of them randomly isolated were tested for requirement of aromatic amino acids by checking growth on the medium containing only isoleucine, valine and pantothenate.
Regulatory $livos$ mutation

The above findings suggest that the extract from KA2313 may contain tRNA degrading enzymes, such as RNase, at high levels. To confirm this possibility, the activity of RNase I in the extract was measured. Although RNase I is localized in the periplasmic space (Neu and Heppel 1964), it is known that, when cells are disrupted, the enzyme migrates and binds to ribosomes and that the ribosome-bound RNase I is activated in the presence of EDTA (Spahr and Hollingworth 1961). As expected, RNase I in ribosome preparations from the wild-type and KA204 could be activated by EDTA and only very low activity was found in the absence of EDTA (Fig. 4A and B).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Activity of leucyl-tRNA synthetase*</th>
<th>Leucine transport** unpressed</th>
<th>Leucine transport** repressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td></td>
<td>0.76</td>
<td>4.44</td>
<td>2.83</td>
</tr>
<tr>
<td>CV117</td>
<td>leuS</td>
<td>0.04</td>
<td>5.00</td>
<td>2.64</td>
</tr>
<tr>
<td>KA204</td>
<td>brnQ</td>
<td>0.81</td>
<td>1.48</td>
<td>—</td>
</tr>
<tr>
<td>KA2313</td>
<td>$brnQ\ livos$</td>
<td>0.93</td>
<td>4.65</td>
<td>—</td>
</tr>
</tbody>
</table>

* Specific activity of $L^{(14C)}$-leucine was 3.3 $\times$ 10$^8$ cpm per $\mu$ mole. Activity of the enzyme is expressed in nmoles of leucine incorporated in leucyl-tRNA per min per mg of protein.

** Initial uptake of $L^{(14C)}$-leucine by intact cells was measured for 20 sec. Concentration of $L^{(14C)}$-leucine was 8.1 $\mu$ M. To repress the leucine transport system, 1.2 mM glycyl-L-leucine was added to the culture medium. The transport activity is expressed by nmoles of leucine incorporated per min per g of cells.
On the contrary, RNase I in a ribosome preparation from KA2313 was in the activated state even without EDTA; the activity in the absence of EDTA was almost equivalent to that in its presence (Fig. 4C). Activity of RNase I in ribosome-free extracts prepared from all these strains was quite low; amounts of solubilized nucleotides, regardless of the presence or absence of EDTA, were less than 0.8 μmoles per mg of protein at 10 min incubation.

**SDS-polyacrylamide gel electrophoresis of ribosomal proteins.** From above experiments, we suspected that degradation of ribosome might be easier in KA2313 than in the wild-type in Tris-HCl buffer without Mg²⁺. As presented in Fig. 5A, the ribosome preparation and ribosome-free extract prepared from KA204 in the presence of MgCl₂ showed similar figures of protein bands to those from KA2313, respectively, on a SDS-polyacrylamide gel after electrophoresis. When the ribosome preparations of KA204 and KA2313 that were suspended in Mg²⁺-free Tris-HCl buffer were centrifuged again and analyzed by the electrophoresis, proteins from their resultant ribosome fractions also
exhibited similar figures of protein bands on a gel (Fig. 5B, lanes 1 and 2). However, these supernatants differed markedly from each other in the composition of proteins, especially in those of low molecular weights. Amounts of small proteins whose molecular weights were less than 20,000 daltons were a little in KA204 (Fig. 5B, lane 3), but abundant in KA2313 (Fig. 5B, lane 4). Thus it may be concluded that binding of smaller proteins to ribosomes is looser in KA2313 than in KA204. It should be pointed out that ribosomes of LivS⁺ AroA⁺ transductants restored characteristics of those of the wild-type (data not shown).
The *livS* gene apparently governs regulation of branched-chain amino acid transport (Ohnishi et al. 1980). The *livS* mutation provokes derepressed transport activities for isoleucine, leucine and valine, mediated by high-affinity (LIV-I) and low-affinity-(1) (LIV-II) systems as well as by leucine-specific (LS) system. Two binding proteins participated in the LIV-I and LS systems are also increased about three-fold in KA2313 (*brnQJ livSl*) over their levels in the wild-type strain (Ohnishi and Kiritani 1983). By conjugational and transductional analyses, *livS* gene was found to be closely linked to *aroA* gene located at 19 min on the Salmonella genetic map (Sanderson and Roth 1983). The *livS* gene is clearly different from *livR* gene located in a region of 75 to 77 min on the map (Ohnishi et al. 1983), though these two genes appear to regulate branched-chain amino acid transport in a similar fashion. Reciprocal co-transduction studies between *aroA* and *livS* loci revealed that the frequency of LivS+ AroA+ clones among AroA+ transductants (55.1%) was significantly higher than that of LivS− AroA− clones among LivS− transductants (12.0%) (Tables 5 and 7). At present, the reason for this difference is not known. The *livS* mutation of *S. typhimurium* corresponds well with *livR* and *lstR* mutations of *E. coli* (Anderson et al. 1976) in respect of its linkage relationship with *aroA* and the mode of regulation for branched-chain amino acid transport. A genetic region involving *livR* and *lstR* of *E. coli* is linked with *aroA* at a frequency of 50 to 74% and with *pyrD* at a frequency of about 3%. In *S. typhimurium*, no linkage relationship between *livS* and *pyrD* has been detected by P22-mediated transduction. This might be due to the fact that P22 phage can transduce a much shorter DNA fragment than P1 phage of *E. coli*. *E. coli* mutants carrying *livR* and *lstR* alleles have derepressed levels of the LIV–I and LS systems, though, unlike *livS* mutant of *S. typhimurium*, the LIV–II system appears to remain normal.

It has been reported in *E. coli* that leucyl-tRNA or its synthetase is required for repression of isoleucine, leucine and valine transport (Quay et al. 1975, Quay and Oxender 1976). When a mutation occurred in the *leuS* gene controlling the leucyl-tRNA synthetase activity in an *E. coli* cell, leucine-transport activity in the cell under growth-restricted condition was considerably derepressed. Upon examining whether, or not, *livS* mutation affects leucyl-tRNA synthetase activity, we found that an active RNase I existing in cell-free extract of KA2313 disturbed assay of the leucyl-tRNA synthetase activity. The RNase I bound to ribosomes in extracts of the wild-type, however, stays as an inactive form unless EDTA is added (Fig. 4). We also found that, when ribosomes were suspended in Mg²⁺-free Tris–HCl buffer, association of smaller ribosomal proteins to a ribosome is loose in KA2313 (Fig. 5). At present, this phenomenon may be explained in terms of 1) an alteration of
the ribosome configuration, 2) activation of RNase I by its conformational alteration, or 3) the activation of the enzyme induced by an unknown factor; the active RNase I would induce alteration of ribosome indirectly. Experiments to clarify these points are in progress. It is also unknown whether the $\text{livS}^+$ gene controls the regulation of branched-chain amino acid transport directly or indirectly. It is noteworthy that deficiency in leucyl-tRNA synthetase in CV117 neither restricts growth of cells, nor derepresses the transport systems of branched-chain amino acids.

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REFERENCES


