**Isoleucyl-tRNA Synthetase and the RPC-5 Chromatographic Profiles of tRNA\textsuperscript{Ile} and tRNA\textsuperscript{Val}**

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A mutation in the ilvU locus of *Escherichia coli* has led to a complex phenotype that included resistance to thiaisoleucine, a loss of derepressibility of isoleucyl-tRNA synthetase, and an alteration of the RPC-5 chromatographic profile of the branched-chain aminoacyl-tRNA's. The alterations were manifest in an increase in the amount of Species 2 of both tRNA\textsuperscript{Ile} and tRNA\textsuperscript{Val} at the expense of Species 1. A similar alteration, but independent of (and additive to) that caused by the ilvU mutation, was observed upon limitation of either isoleucine or valine. The shift in profile caused by limitation was also independent of the reduced growth rate or the derepression of the isoleucine and valine biosynthetic enzymes that also result from limitation. During chloramphenicol treatment nearly all tRNA\textsuperscript{Ile} and tRNA\textsuperscript{Val} formed appears as species 2. Upon recovery from chloramphenicol, Species 2 of both acceptors are converted to Species 1. It is proposed that the ilvU product not only allows derepression of isoleucyl-tRNA synthetase but also retards the conversion of tRNA\textsuperscript{Ile} to tRNA\textsuperscript{Ile} and that of tRNA\textsuperscript{Val} to tRNA\textsuperscript{Val}. The mutated ilvU loci abolish the derepression and are more efficient in retarding the conversion.

The multivalent repression of the isoleucine and valine biosynthetic enzymes in *Escherichia coli* was first shown to involve some function of the three branched chain aminoacyl tRNA synthetases and, perhaps, the levels of charging of the cognate tRNA's themselves by studies on mutants with altered branched chain aminoacyl-tRNA synthetases (1-6). The mutants with altered isoleucyl-tRNA synthetase activities exhibited derepression only of the enzymes specified by the ilvU gene for isoleucyl-tRNA synthetase (ileS) but also lesions in ilvT and ilvU (7). ilvT is linked 68% to thr and 36% to car (formerly pyrA). ileS is linked 48% to thr and 66% to car. ilvU is linked 55% to proAB and 8.4% to lac. The moderate resistance invoked by the ilvT lesion was independent of the ileS and ilvU mutations which acted synergistically. The ileS lesion alone led to a low resistance to thiaisoleucine and an isoleucyl-tRNA synthetase activity about half that in the wild type strain but not to a derepression of threonine deaminase. The mutation did reduce the affinity of isoleucyl-tRNA synthetase for isoleucine, as had occurred in the original mutant. The ilvU lesion also led to a low resistance to thiaisoleucine but not to a change in isoleucyl-tRNA synthetase activity. However, the ilvU lesion prevented the derepression of the synthetase that is normally observed upon isoleucine limitation (8-10). As a result, strains containing both ileS and ilvU lesions exhibited the low (non-derepressed) level of the high $K_m$ synthetase activity characteristic of the original thiaisoleucine-resistant mutant, a strong resistance to thiaisoleucine, and a strong derepression of the ilvEDA operon. This paper reports some aspects of this peculiar regulatory role of the ilvU locus and describes additional effects of mutations affecting the locus.

**EXPERIMENTAL PROCEDURES**

Organisms and Media—The organisms used in this study were derived from the K-12 strain of *E. coli*. The strains used are listed in Table I. The minimal medium of Davis and Mingioli (13) was used with citrate omitted and glucose at 0.5%. For growth with excess branched chain amino acids, 0.4 mM leucine and isoleucine and 0.8 mM valine were added. Other amino acid supplements, when required, were also supplied at a concentration of 0.4 mM. A 20-fold lower concentration of an amino acid was used for limitation of that amino acid. Thiamin hydrochloride, when required, was supplied at a concentration of 1 $\mu$g/ml.

Episomal Transfer—Transfer of F episomes was accomplished by cross-streaking the F' donor and the recipient on selective media. The resulting F-ductants were purified by single colony isolation. Strains chosen as F-ductants for experiments were examined for capacity to serve as F donors in subsequent crosses.

Preparation of Cell Extracts—Crude cell extracts were prepared as described previously (14). Protein was determined by the method of Lowry et al. (15) with bovine serum albumin as a standard.

Enzyme Assays—Assays of threonine deaminase, dihydroxy acid dehydratase, transaminase B, acetohydroxy acid synthase, and aro-taloxy acid isomerodeeductase were as described previously (14). Aminoacyl-tRNA synthetase activities were assayed as described in the Supplement to this paper.

1 Portions of this paper (including Figs. S-1 to S-17) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 7SM-489, cite authors and include a check or money order for $2.10 per set of photocopies.
Partial Purification of tRNA Synthetases—Crude preparations of aminoacyl-tRNA synthetases, which were as nuclease free as possible, were made by a modification of the method of Kelmers et al. (16) with additional steps indicated by Waters and Novelli (17).

Reversed Phase Chromatography—Separation of [‘H]- and [14C]-aminoacyl-tRNA was performed by the reversed phase chromatographic method (RPC-5) of Pearson et al. (18) as described in the miniprint supplement.

Normalization of Disintegrations per Min—In RPC-5 chromatographic procedures that employed dual labeling with [‘H]- and [14C]-amino acid, it was necessary to account for the differences in counting efficiency between [‘H] and [14C] as well as the relative amount of tRNA indicated by a certain number of disintegrations per minute and the differences, if any, in charging ability of the tRNA’s being compared. The relative amount of tRNA indicated by disintegrations per minute obtained in an RPC-5 chromatogram was determined by the method of Waters and Novelli (17). This method took into account any differences in charging ability of the tRNA’s being compared. Charging of equivalent amounts (usually one to two \( \Delta A_{260} \) units) of tRNA was done with seven or eight different amino acids to give an overall indication of the charging abilities of the tRNA’s.

DBAE cellulose Chromatography—Single families of tRNA isoaccepting species were prepared by the method of McCutchan et al. (19). Dry DBAE cellulose was obtained from Collaborative Research. Columns containing an 8.5-ml bed volume were used.

When the purified family of charged tRNA was to be further separated into isoaccepting species by RPC-5 chromatography, special precautions in handling of the tRNA were taken to prevent unusually strong and irreversible binding of tRNA to the RPC-5 support. This problem was circumvented by resuspending the precipitates from the DBAE-cellulose column in an acetate buffer of pH 5.5 and dialyzing twice against distilled water for 10 h each time. This procedure ensured removal of salts, and aggregation of tRNA’s was eliminated. After the second dialysis, samples were precipitated in a 3-fold volume of 100% ethanol. The precipitates were then stored at \(-20^\circ\text{C}\) and, when desired, resuspended in the starting buffer for RPC-5 chromatography.

**RESULTS**

The Effect of the ilvU Lesion on Isoleucyl-tRNA Synthetase Activity—The effect of the ilvU lesion could be readily demonstrated by comparing the effect of an isoleucine limitation on strains CU426 and its ilvU* parent CU210. Fig. 1 shows that restriction of isoleucine biosynthesis by the addition of valine reduced the growth rate and resulted in a derepression of isoleucyl-tRNA synthetase in strain CU210. The addition of isoleucine restored the growth rate and abolished the derepression. In contrast, there was no derepression of isoleucyl-tRNA synthetase of strain CU426 upon valine addition, although a similar reduction of growth rate occurred. This and other experiments (not shown) led to the idea that the ilvU locus is necessary for derepression of isoleucyl-tRNA synthetase. Experiments were therefore undertaken to determine whether some effector needed for this derepression might be missing in the ilvU strain. Since an isoaccepting species of tRNA\(^{\text{ilv}}\) might be a reasonable candidate for such an effector, the isoacceptor profiles of tRNA\(^{\text{ilv}}\) in thiaisoleucine-sensitive and thiaisoleucine-resistant strains were compared.

Reversed Phase Chromatographic Analysis of Aminoacyl-tRNA from Thiaisoleucine-Sensitive and Thiaisoleucine-Resistant Strains—Strain CU426 (bearing the ilvU459 lesion) and its wild type parent, strain CU210, were grown in the absence of isoleucine and valine. The RNA was prepared from each strain and charged with labeled isoleucine (14C for strain CU210 and \('\text{H}\) for strain CU426). The isoaccepting species of isoleucyl-tRNA were separated by RPC-5 chromatography. Fig. 2 shows that although the two isoaccepting species were separated and eluted in the same fractions for both strains, strain CU210 exhibited more of tRNA\(^{\text{ilv}}\) than did strain CU426. The same two tRNA preparations were also examined for valine acceptance activity. The elution pattern for the tRNA\(^{\text{ilv}}\) isoacceptors of strains CU426 and CU210 are shown in Fig. 3. The difference between the two isoaccepting species of tRNA\(^{\text{ilv}}\) in the two strains was similar to that which had been observed for tRNA\(^{\text{ilv}}\).
ilvU, a Gene Affecting tRNA Isoacceptor Profiles

FIG. 1. Effect of an isoleucine restriction on growth and isoleucyl-tRNA synthetase formation in an ilvU' and an ilvU strain of E. coli. Strain CU210 (ilvU') was grown in minimal medium supplemented with its required amino acids, threonine, leucine, and proline. Its ilvU' derivative, strain CU426, was grown with threonine and leucine. At the time indicated, valine (4 mM) was added to two flasks and incubation was continued. After about one doubling had occurred in the control flasks, isoleucine was added to all flasks. At the times indicated, samples were removed, the turbidities of the cultures were determined, and cell extracts were prepared and assayed for isoleucyl-tRNA synthetase activity.

FIG. 2. Comparison of tRNA^{met} in strains CU210 and CU426. Strain CU210 (•) was labeled with [^{14}C]isoleucine. Strain CU426 (○) was labeled with [^{3}H]isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. [^{14}C] dpm are per 1.00 ml. [^{3}H] dpm are per 1.00 ml. Cells were grown in minimal medium.

and of Valyl-tRNA—Growth of strains CU210 and CU426 under conditions of excess isoleucine, valine, and leucine yielded profiles that are identical to those illustrated for cells grown in the absence of isoleucine and valine (Figs. S-4 and S-5 in the miniprint supplement). (These strains required leucine, but other experiments with prototrophic strains revealed that the addition of leucine was without effect.) In contrast, growth of either strain under conditions of limitation for isoleucine (i.e. under conditions of valine inhibition) yielded profiles in which Species 2 was increased and Species 1 was decreased for both tRNA^{ile} and tRNA^{val}. The effect of the isoleucine limitation in decreasing the species 1/species 2 ratio appeared to be additive over that exerted by the ilvU459 lesion. With another ilvU' strain, auxotrophic for isoleucine and valine, it was shown that the shift in profile was brought about not only by isoleucine limitation but by a valine limitation as well. However, limitation of either proline or leucine had no effect (see miniprint Supplement).

Whereas either isoleucine or valine limitation decreased the Species 1/Species 2 ratios without masking the differences due to the state of the ilvU gene, growth of the ilvU' or the ilvU459 strains in rich medium (L broth) resulted in profiles that were indistinguishable from each other. Furthermore, for both strains, tRNA^{ile} and tRNA^{val} were very much reduced in amount and accounted for only about 5% of the total acceptance activity.

Analysis of the Environmental Signal Affecting the tRNA Profile—Two aspects of the isoleucine and valine limitation might have been of importance in affecting the Species 1/Species 2 ratios. One was the fact that the growth rate is reduced during the period of limitation, whereas it was very fast in L-broth. The second was the fact that limitation of either isoleucine or valine resulted not only in the altered profile but also caused a derepression of the isoleucine and valine biosynthetic enzymes. Both of these aspects were explored with a variety of growth media and a variety of strains in which regulation of the isoleucine and valine biosynthetic enzymes was altered.

It appears that neither the depression in amounts of the Species 2 in rich medium nor their increase with isoleucine or valine restriction are related to growth rates. Except for the shift in profile already described for L-broth-grown cells, no differences were found between profiles of cells grown in minimal salts media containing acetate or glycerol as carbon source or in a minimal salts medium supplemented with an acid-hydrolyzed casein and sources of purines and pyrimi-
dines. There was also no effect of a relA mutation on the profiles.

A survey of several strains in which the function of the ilv gene cluster was derepressed or in which the ilv gene cluster was deleted revealed no correlation between any ilv gene function and the tRNA profiles. Only one strain, CU18, which had been studied earlier (11), was unique in that its tRNA contained no detectable Species 2 of either valine or isoleucine acceptor tRNA (Figs. S-12 and S-13 in miniprint supplement). This mutant will be discussed later. However, since the same kind of profile was observed in a strain in which the ilvU locus was deleted, the deletion strain was studied in more detail. The altered profile exhibited by strain CU18 is independent of the other ilv mutations it carries.

The Effect of Chloramphenicol on the Amounts of Species 1 and 2 of tRNAVal and tRNAIle—Because of the inverse variation exhibited by the two isoacceptors of both tRNAVal and tRNAIle, the possibility was considered that one isoacceptor might be a modified form of the other for each amino acid. In the past, undermodified forms of tRNA have been found to accumulate during exposure of cells to chloramphenicol for several hours (20). The accumulation that occurs under these conditions is presumably due to the lability of one or more of the modifying enzymes, so that in the presence of chloramphenicol, the rapid turnover of such enzymes needed to sustain the modification process no longer occurs. That Species II of both tRNAIle and tRNAVal might be undermodified forms was shown in experiments in which strains CU210 and CU426 were grown in minimal medium to mid-log phase and treated with chloramphenicol at a final concentration of 50 μg/ml for 4.5 h. Chloramphenicol-treated cells yielded profiles of both tRNAIle and tRNAVal with greatly increased Species 2 and decreased Species 1.

The Effect of a pro-lac Deletion on tRNAVal and tRNAIle—If Species 2 of each tRNA were indeed a precursor of corresponding species 1, it would appear that the ilvU strain, CU426, has a reduced capacity to catalyze the modification process. To determine how the ilvU locus was involved in the process, tRNAIle and tRNAVal in a strain bearing a pro-lac deletion which would presumably lack any ilvU function was examined. RPC-5 chromatographic profiles for tRNAIle and tRNAVal from strain CU152 are shown in Fig. 4 and 5. It is evident that, in this strain which carries a chromosomal deletion extending from pro to lac, both Species 2 are completely missing, and all of the chargeable tRNAIle and tRNAVal appear as Species 1. Even upon limitation for isoleucine, which normally results in an increase in Species 2, no second isoacceptor for either tRNAIle or tRNAVal was generated. When an F' episome bearing the region covered by the pro-lac deletion (strain CU154) was transferred to strain CI1159, the RPC-5 chromatographic profiles changed dramatically. When the new strain, strain CU852, was grown in minimal medium, profiles for both tRNAIle and tRNAVal were similar to those encountered in the ilvU+ strains, CU1 and CU210. When strain CU852 was limited for isoleucine, both the tRNAIle and tRNAVal RPC-5 profiles were similar to the respective profiles evident when strain CU210, the ilvU+ parent, was grown in a similar manner. tRNAlb profiles from strain CU1154, another pro-lac deletion strain, and strain CU879 were normalized to indicate equivalent amounts of tRNA per dpm. 14C dpm are per 1.11 ml. 3H dpm are per 1.14 ml.

![Fig. 4. Analysis of tRNAIle in strain CU152. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine ( ) was labeled with [14C]valine. tRNA from cells grown under limitation for isoleucine (○) was labeled with [3H]isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. 14C dpm are per 1.11 ml. 3H dpm are per 1.14 ml.](image)

![Fig. 5. Analysis of tRNAVal in strain CU152. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine ( ) was labeled with [14C]valine. tRNA from cells grown under limitation for isoleucine (○) was labeled with [3H]valine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. 14C dpm are per 1.11 ml. 3H dpm are per 1.16 ml.](image)
The Distribution of Valine and Isoleucine Isoacceptors between Species 1 and 2 during and after Chloramphenicol Treatment—Preliminary experiments had shown that when either an iluU− or an iluU459 strain was treated with chloramphenicol, species 2 of both tRNAile and tRNAval became by far the dominant isoacceptors. Since chloramphenicol has been observed to lead to an accumulation of precursor forms of tRNA’s, it was of interest to determine whether the observed tRNAile and tRNAval isoacceptors 2 might be precursor forms of isoacceptors 1. Therefore, a series of experiments were performed to trace the fate of Species 2 during and following recovery of the cells from chloramphenicol treatment. Cells of strain CU426 that had been labeled with [3H]uridine for a 4.5-h period of exposure to chloramphenicol were allowed to recover for 4½ doublings in the absence of chloramphenicol. Prior to RPC-5 chromatographic analysis, the aminoacylated tRNAile and tRNAval preparations were freed of noncognate, uncharged tRNA’s by chromatography on DBAE cellulose. This step prevented the appearance of tritiated, noncognate, uncharged tRNA’s on the RPC-5 chromatograms. Aminoacylation was, of course, carried out with 14C-labeled amino acid. As shown in Fig. 8 (tRNAile), and Fig. 9 (tRNAval), observations were made on the cells at the end of the chloramphenicol treatment, 2 h after removal of chloramphenicol (a point at which recovery of cell growth had barely begun), after one doubling (3 h after removal), and after approximately 4½ doublings (7 h after removal). It is clear that, for tRNAile, immediately following cessation of chloramphenicol treatment, nearly all of the newly formed tRNA is accounted for by Species 2. After 2 h in minimal medium, at the time growth resumed, about half of the tRNA made during chloramphenicol treatment had already appeared in Species 1, and the overall RPC-5 profile approached the normal pattern. After one doubling, about 90% of the tRNA made during chloramphenicol treatment appeared as Species 1. The overall RPC-5 profile indicated that, of the total tRNAile in the cells at that time, slightly more than the normal amount was found in Species 1 and slightly less than normal was found in Species 2. After 4½ doublings, the overall RPC-5 profiles, representing the total tRNAile in the cell at this point, are normal, while greater than 95% of the tRNA made during the chloramphenicol treatment appeared as Species 1. Similar results were obtained with tRNAval (Fig. 9), except that, during recovery from chloramphenicol treatment, the overall RPC-5 profile steadily approached the normal pattern, and at no time was there a greater than normal amount of tRNAval nor less than normal amount of tRNAile observed.

The simplest model to account for the change in profiles during recovery from chloramphenicol treatment is that tRNAile and tRNAval are converted to tRNAile and tRNAval. Less likely is the alternative possibility that during recovery, both Species 2 are destroyed and both Species 1 are formed by processing of precursors that had been formed during the chloramphenicol treatment but which could not be charged with the cognate amino acids.

tRNA Profiles in Other iluU Mutants—To provide additional evidence that the effects on tRNA profiles observed in iluU459 strains were indeed due to the iluU lesion, additional iluU mutants were examined. These mutants included strain CU418, in which the iluU459 marker from strain CU1018 was transduced a second time into strain CU210 to yield a strain that should have been identical to strain CU426 and strains CU900 and CU901 which contained new iluU lesions. The new lesions were obtained spontaneously by selecting for thiaisooleucine resistance that was linked approximately 50% by transduction to the proA or proB markers. An additional strain (CU899) was chosen for examination in which the level of thiaisooleucine resistance was characteristic of the iluU strains but in which the lesion was not linked to proA or proB. Presumably one or more of the lesions conferring thiaisooleucine resistance in strain CU1018 is present in strain CU899. Examination of tRNAile and tRNAval profiles from strain CU418 grown in minimal medium revealed profiles analogous to those observed in the other iluU459 strain, CU1426. Examination of new iluU− strains, CU900 and CU901, grown in minimal medium supplemented with excess leucine, isoleucine, and valine revealed profiles for tRNAile and tRNAval similar to those observed in the iluU459 mutants, strains CU426 and CU1426. Strain CU901 revealed a tRNAile profile in which the increase in species 2 was slightly greater than that in strain CU900. The tRNAval profile from strain CU901.

FIG. 6. Comparison of tRNAile in strains CU176 and CU879. Strain CU176 (○) was labeled with [14C]isoleucine. Strain CU879 (□) was labeled with [3H]valine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. [14C] dpm are per 1.00 ml. [3H] dpm are per 0.97 ml. Cells were grown in minimal medium.

FIG. 7. Comparison of tRNAval in strains CU176 and CU879. Strain CU176 (○) was labeled with [14C]valine. Strain CU879 (□) was labeled with [3H]isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. [14C] dpm are per 1.00 ml. [3H] dpm are per 0.97 ml. Cells were grown in minimal medium.
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Fig. 8 (left). Recovery from the effect of chloramphenicol on the distribution of tRNA\(^{\text{Leu}}\) between Species 1 and 2. Strain CU426 growing in minimal medium was treated with chloramphenicol in the presence of \([^{3}H]\)uridine. Recovery was in the presence of cold uridine. In all cases, the total tRNA isolated was charged with \([^{14}C]\)isoleucine. \(^{3}H\) radioactivity indicated total Species 1 (■) or 2 (○) as indicated. \(^{14}C\) radioactivity indicated the tRNA\(^{\text{Leu}}\) formed during chloramphenicol treatment (Species 1, □; Species 2, ○). For other details, see text. (Replot of areas under curves in Fig. S-16 in miniprint supplement.)

Fig. 9 (right). Recovery from the effect of chloramphenicol on the distribution of tRNA\(^{\text{Val}}\) between Species 1 and 2. Cells growing in minimal medium were treated with chloramphenicol in the presence of \([^{3}H]\)uridine. Recovery was in the presence of cold uridine. In all cases, the total tRNA isolated was charged with \([^{14}C]\)valine. \(^{3}H\) radioactivity indicated total Species 1 (■) or 2 (○) as indicated. \(^{14}C\) radioactivity indicated the tRNA\(^{\text{Val}}\) formed during chloramphenicol treatment (Species 1, □; Species 2, ○). For other details, see text. (Replot of areas under curves in Fig. S-17 in miniprint supplement.)

did, however, resemble that observed in strain CU900 and the other ilvU mutants that had been analyzed. The tRNA\(^{\text{Leu}}\) and tRNA\(^{\text{Val}}\) profiles from the strain that was not ilvU\(^{+}\), but, nevertheless, thiaisoleucine-resistant, CU899, grown with excess leucine, isoleucine, and valine, were characteristic of those observed in the thiaisoleucine-sensitive strains CU1 and CU210. Thus, thiaisoleucine resistance by itself is not a cause of altered tRNA profiles.

The Effect of ilvU and Thiaisoleucine Resistance on Enzyme Levels—The data in Table II illustrate activities of the isoleucine and valine biosynthetic enzymes and isoleucyl- and valyl-tRNA synthetases in the ilvU\(^{+}\) strain CU210, and several ilvU\(^{-}\) derivatives of it that had received the ilvU459 marker or in which the new ilvU lesions had been selected. The ilvU459 strains differed from strain CU210 in that they exhibited a nonrepressible ilvEDA operon and valyl- and isoleucyl-tRNA synthetases that were not derepressed upon limiting isoleucine. Strains CU900 and CU901 exhibited isoleucyl- and valyl-tRNA synthetase levels that are characteristic of the ilvU459 strains, but their isoleucine and valine biosynthetic enzyme levels are characteristic of the thiaisoleucine-sensitive strains, CU1 and CU210. All of the enzymes assayed in strain CU899 exhibited wild type levels under all conditions employed. Thus it would appear that the ilvU459 lesion differs from the two more recently selected lesions or there is some as yet unidentified lesion that has been cotransduced with the ilvU459 lesion and which is responsible for the less effective repression of the ilvU459 strains. It should also be noted that when strain CU426 was first studied, it exhibited a low level of derepression of the ilvU459 strains.

The data in Table III show that transfer of ilvU\(^{+}\) and ilvU\(^{-}\) episomes to various recipient strains serves to alter the derepression characteristics of isoleucyl-tRNA synthetase (but not valyl-tRNA synthetase) while exerting no effect on the isoleucine and valine biosynthetic enzyme levels of the recipient. The strains in which ilvU is deleted or completely inactive (strains CU152, CU176, CU18, and CU892) have an isoleucyl-tRNA synthetase that can be derepressed to only half the extent that it can in the wild type upon limitation for isoleucine. Upon transfer of an ilvU\(^{+}\) carrying episome, the resulting strains (strains CU852 and CU897) exhibited normal derepression of isoleucyl-tRNA synthetase upon isoleucine limitation, while the isoleucine and valine biosynthetic enzyme levels were not affected by this episomal transfer. Upon transfer of an episome carrying ilvU\(^{-}\), the resulting strains (strains CU879 and CU896) exhibited no elevation of isoleucyl-tRNA synthetase upon limitation for isoleucine, while their isoleucine and valine biosynthetic enzyme levels remained unaffected by the episomal transfer. Thus it appears that the ilvU gene product is involved in regulation of isoleucyl-tRNA synthetase in such a manner that the wild type genotype (ilvU\(^{+}\)) is essential for normal derepression. When the ilvU gene product is deleted, there is still some derepression of isoleucyl-tRNA synthetase, but when the ilvU gene...
### Table II

The effect of ilvU lesions on the specific activities of the isoleucine and valine biosynthetic enzymes and the branched chain aminocetyl-tRNA synthetases

<table>
<thead>
<tr>
<th>Strain (Pertinent Genotype)</th>
<th>Growth Medium</th>
<th>Acetoxyhydroxy Acid Synthase Assayed with 1 mM L-valine</th>
<th>Isoleucyl tRNA Synthetase Assayed with 1 mM L-isoleucine</th>
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<td></td>
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<tr>
<td>CUD10 (ilvU)</td>
<td>LPT</td>
<td>32.6</td>
<td>24.8</td>
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<td></td>
<td>Repressing</td>
<td>15.1</td>
<td>7.5</td>
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<tr>
<td></td>
<td>Limiting Leucine</td>
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<td>67.8</td>
</tr>
<tr>
<td></td>
<td>Limiting Isoleucine</td>
<td>114.2</td>
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<td>L-Broth</td>
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<td></td>
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<tr>
<td>CUD426 (ilvU4469)</td>
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<td>L-Broth</td>
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<td></td>
<td>Repressing</td>
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<td>Limiting Isoleucine</td>
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<td></td>
<td>Repressing</td>
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<td>Limiting Isoleucine</td>
<td>118.6</td>
<td>50.6</td>
</tr>
<tr>
<td></td>
<td>Repressing</td>
<td>16.2</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Limiting Isoleucine</td>
<td>109.3</td>
<td>62.8</td>
</tr>
</tbody>
</table>

a. LPT medium was minimal medium supplemented with excess leucine, proline and threonine; repressing medium was the same plus isoleucine and valine; limiting media were like repressing except for the limiting amino acid. For concentrations, see experimental.

b. Enzyme assayed in the presence of 1 mM valine.

### Table III

Effect of the ilvU locus on enzyme levels

<table>
<thead>
<tr>
<th>Strain (Pertinent Genotype)</th>
<th>Growth Conditions</th>
<th>Acetoxyhydroxy Acid Synthase Assayed with 1 mM L-valine</th>
<th>Isoleucyl tRNA Synthetase Assayed with 1 mM L-isoleucine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>---</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>CUD18 (ilvU2741)</td>
<td>Repressing</td>
<td>16.89</td>
<td>15.51</td>
</tr>
<tr>
<td></td>
<td>Limiting</td>
<td>183.30</td>
<td>122.47</td>
</tr>
<tr>
<td>CUD82 (ilvU2741 lae)</td>
<td>Repressing</td>
<td>19.30</td>
<td>12.98</td>
</tr>
<tr>
<td></td>
<td>Limiting</td>
<td>232.99</td>
<td>171.78</td>
</tr>
<tr>
<td>CUB94 (F ilvU569 lae-ilvU2741 lae)</td>
<td>Limiting</td>
<td>16.30</td>
<td>11.00</td>
</tr>
<tr>
<td>CUB97 (F ilvU58 lae-ilvU2741 lae)</td>
<td>Repressing</td>
<td>20.48</td>
<td>16.62</td>
</tr>
<tr>
<td>CUD76 (ilvU2821 lae)</td>
<td>Repressing</td>
<td>19.66</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Limiting</td>
<td>12.83</td>
<td>15.33</td>
</tr>
<tr>
<td>CUB52 (F ilvU58 lae-ilvU2821 lae)</td>
<td>Repressing</td>
<td>15.15</td>
<td>17.77</td>
</tr>
<tr>
<td>CUB79 (F ilvU58 lae-ilvU2821 lae)</td>
<td>Repressing</td>
<td>18.69</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td>Limiting</td>
<td>19.89</td>
<td>9.21</td>
</tr>
<tr>
<td></td>
<td>Repressing</td>
<td>14.08</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>Limiting</td>
<td>101.44</td>
<td>11.46</td>
</tr>
</tbody>
</table>

a. Repressing conditions: cells grown in excess branched-chain amino acids; derepressing conditions: cells grown in excess valine and leucine, limiting isoleucine. Note: The acetoxyhydroxy acid synthase and isomeroeductase activities do not undergo derepression under conditions of limiting isoleucine.

b. Threonine deaminase assayed in the presence and absence of 1 mM L-isoleucine.

c. Acetoxyhydroxy acid synthase assayed in the presence and absence of 1 mM L-valine.
product is present in the altered form that causes thiaisoleucine resistance (e.g. ilvU459), no derepression can be achieved.

**DISCUSSION**

The ilvU459 lesion, which was first recognized in strain CU1018 along with two other lesions conferring thiaisoleucine resistance, accounts for two of the several differences between strain CU1018 and its thiaisoleucine-sensitive parent. One effect of ilvU459 is that it prevents derepression of isoleucyl-tRNA synthetase upon an isoleucine restriction. In the experiments reported here, the ilvU459 lesion was shown to lead to tRNA\textsuperscript{ilvU}\textsubscript{leu}, tRNA\textsuperscript{ilvU}\textsubscript{val}, and tRNA\textsuperscript{ilvU}\textsubscript{ala} profiles that were altered relatively to those of the *E. coli* wild type, strain CU1. Two additional ilvU lesions (ilvU1216 and ilvU1217) resulted in tRNA profiles similar to those carried by ilvU459.

That the ilvU gene product might be involved in regulation was indicated by the dominance of ilvU\textsuperscript{+} over ilvU\textsuperscript{−}. Furthermore, when an episome carrying either an ilvU\textsuperscript{+} or ilvU\textsuperscript{−} marker was transferred to ilvU deletion strains, the deletion phenotype (a missing Species 2 of tRNA\textsuperscript{ilvU}\textsubscript{leu} and tRNA\textsuperscript{ilvU}\textsubscript{ala}) was suppressed.

It is likely that, for both tRNA\textsuperscript{ilvU}\textsubscript{leu} and tRNA\textsuperscript{ilvU}\textsubscript{val}, Species 2 is an undermodified form of Species 1, since the isoacceptors for both amino acids made during chloramphenicol treatment appeared as Species 2 and, during recovery, when modification enzymes could again be made, were converted into Species 1.

A model that accommodates these observations is that the ilvU\textsuperscript{−} locus impedes the modification of Species 2 into Species 1. This restriction of modification might be due to inhibition of or repression of one or more modification enzymes that might require continued resynthesis. The ilvU\textsuperscript{−} allele presumably permits even less modification of Species 2 into Species 1 than does the ilvU\textsuperscript{+} allele. Upon deletion of ilvU, this regulation of modification is lost, and the conversion of Species 2 into Species 1 is both rapid and complete.

If a single locus impedes some process in the conversion of both tRNA\textsuperscript{ilvU}\textsubscript{leu} and tRNA\textsuperscript{ilvU}\textsubscript{val} to their respective Species 1, a question arises of whether the two isoacceptors could undergo any change that would be the same for both. Sequencing of *E. coli* B tRNA\textsuperscript{ilvU}\textsubscript{leu} (21, 22) and *E. coli* D and *K-12* tRNA\textsuperscript{ilvU}\textsubscript{val} (23–27) revealed that the differences between two forms of tRNA\textsuperscript{ilvU}\textsubscript{leu} separated by MAK column chromatography are not the same as those between the two forms of tRNA\textsuperscript{ilvU}\textsubscript{val}. For tRNA\textsuperscript{ilvU}\textsubscript{leu}, one form contained one less dihydroxyridine residue than did the other. For tRNA\textsuperscript{ilvU}\textsubscript{val}, there were a number of modification differences between the two species but not the same differences as those between the two forms of tRNA\textsuperscript{ilvU}\textsubscript{leu}. Furthermore, there was some difference between the primary sequences of the two forms of tRNA\textsuperscript{ilvU}\textsubscript{val}.

It may be that the basis for separation of isoacceptors by RPC-5 chromatography is different from that for the separations studied earlier, and that, for both amino acids, the Species 2 fractions contained more than one species which upon modification were eluted as Species 1 which were also heterogeneous. Recently, genes for tRNA\textsuperscript{ilvU}\textsubscript{leu} have been found in three separate ribosomal RNA operons (28). All three could have identical sequences, but, if not, they might be similar enough that the products of all three tRNA\textsuperscript{ilvU}\textsubscript{leu} genes would be eluted together from the RPC-5 columns used in this study provided all three products had been modified to the same extent. Whether these modifications are the same or different for tRNA\textsuperscript{ilvU}\textsubscript{leu}, tRNA\textsuperscript{ilvU}\textsubscript{val}, and tRNA\textsuperscript{ilvU}\textsubscript{ala}, it is all three modifications that the ilvU product retards.

It seems unlikely that the ilvU459 mutation is an “up-promoter” mutation, for the ilvU\textsuperscript{+} phenotype was neither mimicked in ilvU\textsuperscript{+}/ilvU\textsuperscript{−} merodiploids, nor further enhanced in ilvU\textsuperscript{+}/ilvU\textsuperscript{−} merodiploids. The ilvU product may be under autogenous control and the difference between the ilvU\textsuperscript{+} and ilvU\textsuperscript{−} strains may be due not to the amount of ilvU product but to the kind. The retardation may be specific for the branched amino acid acceptors, since the ilvU459 lesion had no effect on the tRNA\textsuperscript{a}’s for histidine, phenylalanine, and lysine.

Recently, Thomale and Nass (29) reported isoleucyl-tRNA profiles different from those reported here in that as many as seven isoacceptors were separated. Their procedure, involving an elaborate “sizing” of the RPC-5 matrix particles and different starting materials, may have separated isoacceptors that were eluted together in our experiments. However, in agreement with our findings, these workers found no alteration in profile when growth rate was altered by changing the carbon source. With the same column materials, they found tRNA\textsuperscript{leu} profiles like those we describe (Fig. S-3).

The property of the ilvU459 allele of enhancing the physiological effect exerted by the ilvU\textsuperscript{−} allele probably accounts for the dominance of the mutant allele over the wild type. An analogous situation may be found in *lac* mutations that lead to a “super repressor” phenotype which is due to a repressor protein unable to bind inducer or binding the operator more tightly (30). The dominance of ilvU459 over ilvU\textsuperscript{−} or the ilvU deletion with respect to tRNA profiles was also exhibited with respect to the derepressibility of isoleucyl-tRNA synthetase. The failure of the synthetase to be derepressed is probably due to the nature of the ilvU459 product rather than to the amount of Species 2 of either tRNA\textsuperscript{ilvU}\textsubscript{leu} or tRNA\textsuperscript{ilvU}\textsubscript{ala}. Thus, the ilvU deletion strain (CU152), with no Species 2, exhibited about half as much derepression of the synthetase upon isoleucine limitation as did the ilvU\textsuperscript{+} strain, whereas the ilvU\textsuperscript{−} strain, with excess Species 2, exhibited none.

A precise definition of the role played by the ilvU locus in retarding tRNA modification will be dependent upon development of a system that allows the in vitro conversion of Species 2 of tRNA\textsuperscript{ilvU}\textsubscript{leu} and tRNA\textsuperscript{ilvU}\textsubscript{ala} to the corresponding Species 1. In that way it will be possible to distinguish between inhibition of the process and repression of the synthesis of the necessary enzyme(s). Definition of the presently obscure role played by the locus in derepression of the isoleucyl-tRNA synthetase may be more difficult and may await the development of an in vitro enzyme-forming system. Whether the inability of isoleucyl-tRNA synthetase to undergo derepression in the ilvU mutants is the basis for thiaisoleucine resistance may also be revealed by the additional studies.

**REFERENCES**

null
Figure 5A shows that the 3^{rd} strain C210 exhibited a profile of isoleucine acceptors when grown in excess branched-chain amino acids, much like that exhibited by cells of strain C7 (Figure 3), except that the absence of the branched-chain amino acids. When an isoleucine restriction was imposed by adding excess valine, however, the profile exhibited a shift in which isoleucyl tRNA \(^{3}_{\text{C210}}\) appeared to have been increased. In this respect, isoleucine limitation has appeared to select the \(3^{rd}\) strain.

Fig. 5B. Effect of Isoleucine Limitation on tRNA. tRNA from cells grown in minimal medium with excess isoleucine, isoleucine, and valine (B) was labeled with \(^{35}\)S-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per cell. %DFM's are per 1.02 ml.

Fig. 5C. Effect of Isoleucine Limitation on tRNA. tRNA from cells grown in minimal medium with excess isoleucine, isoleucine, and valine (B) was labeled with \(^{35}\)S-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per cell. %DFM's are per 1.02 ml.

Since a valine limitation cannot be readily induced in a strain prototrophic for isoleucine and valine, at isoleucine and valine auxotroph, C210, was used. As Figures 5B and 5C show, isoleucine limitation of amino acids led to increases in the amounts of the second isoleucine acceptors for both amino acids relative to the amounts in cells grown under repressing conditions. For this strain as well as for other isoleucine and valine auxotrophs examined, the increase in the second isoleucine acceptor was greater when the non-acceptor amino acid was limiting.

The effect of growth in a rich medium on the RNE-5 profile. Although there was no difference between the tRNA profiles of cells grown in the presence of the branched-chain amino acids, and those of cells grown in excess branched-chain amino acids, for either the isoleucine-sensitive cells or the isoleucine-resistant strain, strain C210, was used. As Figures 5B and 5C show, isoleucine limitation of amino acids led to increases in the amounts of the second isoleucine acceptors for both amino acids relative to the amounts in cells grown under repressing conditions. In this case, the increase in the second isoleucine acceptor was greater when the non-acceptor amino acid was limiting.

Fig. 5D. Comparison of tRNA. tRNA from cells grown in a medium containing isoleucine, and valine (B) was labeled with \(^{35}\)S-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per cell. %DFM's arc per 1.02 ml. %DFM's are per 1.02 ml.
ilvU, a Gene Affecting tRNA Isoacceptor Profiles

The isoaacceptor profile in Strain C230B during the course of screening many strains altered in control of the Met-tRNA and valine elongation enzymes for correlation with an L-iso- or L-iso type of tRNA isoacceptor profile, one strain was found in which the second species for both tRNA Met and tRNA Val was completely absent. The profiles for this strain, C209, are shown in Figure 5-13 and 5-14. When tRNA was isolated from the strain, no tRNA Met was detected, but large amounts of tRNA Val were detected. This strain was retested for the presence of tRNA Met, and tRNA Val were detected, indicating that the strain was not truly L-iso, but that the tRNA Met present in the strain was selectively inhibited.

Figures 5-15 and 5-16 show the profiles of tRNA Met and tRNA Val, respectively, in the L-iso and L-iso strains. Although the tRNA Met was selectively inhibited, the tRNA Val was not affected. This suggests that the L-iso strain may have a selective advantage in certain environments. In addition, the tRNA Met was found to be preferentially used in the L-iso strain, indicating that the strain may have a selective advantage in certain environments.

The effect of Chloramphenicol (Cm) on the formation of valine and isoleucine isoacceptor profiles in Strain C198 was studied. Recovery from Chloramphenicol treatment in Figure 5-17 shows that the profile changes observed with Chloramphenicol treatment are reversible. The profiles obtained with and without Chloramphenicol treatment are shown in Figure 5-17. The profile obtained with Chloramphenicol treatment is identical to the profile obtained without Chloramphenicol treatment, indicating that the effects observed with Chloramphenicol treatment are reversible.
ilvU, a locus in Escherichia coli affecting the derepression of isoleucyl-tRNA synthetase and the RPC-5 chromatographic profiles of tRNAIle and tRNAVal.

J T Fayerman, M C Vann, L S Williams and H E Umbarger


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