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ilvU, a Locus in *Escherichia Coli* Affecting the Derepression of Isoleucyl-tRNA Synthetase and the RPC-5 Chromatographic Profiles of $tRNA^{Ile}$ and $tRNA^{Val}$*

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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 $tRNA^{Ile}$ and $tRNA^{Val}$ 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 $tRNA^{Ile}$ and $tRNA^{Val}$ 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 $tRNA_2^{Ile}$ to $tRNA_1^{Ile}$ and that of $tRNA_2^{Val}$ to $tRNA_1^{Val}$. 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 *ilvEDA* operon. (Similarly, isoleucine limitation results in derepression only of the *ilvEDA* products.)

Genetic analysis of one thiaisoleucine-resistant mutant revealed that it contained not only a lesion in the structural 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 *ilvS* 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[1]

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 μg/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 acetohydroxy acid isomeroreductase were as described previously (14). Aminoacyl-tRNA synthetase activities were assayed as described in the Supplement to this paper.

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[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. 79M-489, cite authors and include a check or money order for $2.10 per set of photocopies

TABLE I
Strains used

Strain	Genotype	Source or reference
21	F⁻ *thr leu*	R. H. Pritchard
CU1	F⁺ λ⁺	Strain K-12
CU18	F⁻ *ilvA466 ilvC2004 ilvU2141 metE200 galT12* λ⁻	Pledger and Umbarger (11)
CU152	F⁻ *ara* Δ*(pro-lac) thi*	Originally called CSH26 (12)
CU154	F *ilvU⁺ lac⁺ pro⁺*/Δ*(pro-lac) his trp thi strA supF*	Originally called CSH28 (12)
CU176	F⁻ *ara* Δ*(pro-lac) thi strA*	Originally called CSH50 (12)
CU210	F⁻ *thr leu pro*	Selection of a derivative of strain 21 following treatment with 4-nitropyridine *N*-oxide
CU418	F⁻ *ilvU459 thr leu*	P1 mediated transduction of CU210 with CU1018 as donor by T. Newman, Purdue University
CU426	F⁻ *ilvU459 thr leu*	P1 mediated transduction of CU210 with CU1018 as donor
CU852	F *ilvU⁺ lac⁺ pro⁺*/*ara* Δ*(pro-lac) thi*	Episomal transfer to CU152 from CU154
CU877	F *ilvU⁺ lac⁺ pro⁺*/*ilvU459 thr leu*	Episomal transfer to CU426 from CU154
CU878	F *ilvU⁺ lac⁺ pro*/*ara* Δ*(pro-lac) thi*	Diethyl sulfate (DES) mutagenesis of CU852
CU879	F *ilvU459 lac⁺ pro⁺*/*ara* Δ*(pro-lac) thi*	P1 mediated transduction of CU878 with CU426 as donor
CU882	F⁻ *ilvA466 ilvC2002 ilvU2141 metE200 galT12 lac* λ⁻	DES mutagenesis of CU18
CU896	F *ilvU459 lac⁺ pro⁺*/*ilvA466 ilvC2002 ilvU2141 metE200 galT12 lac* λ⁻	CU882 from CU879
CU897	F *ilvU⁺ lac⁺ pro*/*ilvA466 ilvC2002 ilvU2141 metE200 galT12 lac* λ⁻	Episomal transfer to CU882 from CU878
CU899	F⁻ *ilv-2142 thr leu pro*	Spontaneous thiaisoleucine-resistant mutant of CU210
CU900	F⁻ *ilvU2126 thr leu pro*	Spontaneous thiaisoleucine-resistant mutant of CU210
CU901	F⁻ *ilvU2127 thr leu pro*	Spontaneous thiaisoleucine-resistant mutant of CU210
CU1018	*ilvS453 ilvT458 ilvU459*	Formerly TIR-8, Szentirmai *et al.* (3)

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).

tRNA Preparation—Transfer RNA was prepared as described in the miniprint supplement.

Reversed Phase Chromatography—Separation of [^{3}H]- and [^{14}C]-aminoacyl-tRNA was performed by the reversed phase chromatographic method (RPC-5) of Pearson *et al.* (18) as described in the Supplement.

Normalization of Disintegrations per Min—In RPC-5 chromatographic procedures that employed dual labeling with ^{3}H- and ^{14}C-amino acids, it was necessary to account for the differences in counting efficiency between ^{3}H and ^{14}C 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 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[2] 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°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 $tRNA^{Ile}$ might be a reasonable candidate for such an effector, the isoacceptor profiles of $tRNA^{Ile}$ 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 (^{14}C for strain CU210 and ^{3}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 CU426 exhibited more of $tRNA_2^{Ile}$ and correspondingly less of $tRNA_1^{Ile}$ than did strain CU210.

The same two tRNA preparations were also examined for valine acceptance activity. The elution pattern for the $tRNA^{Val}$ isoacceptors of strains CU210 and CU426 are shown in Fig. 3. The difference between the relative amounts of Species 1 and Species 2 of $tRNA^{Val}$ in the two strains was similar to that which had been observed for $tRNA^{Ile}$.

Growth Medium Effects on the Profiles of Isoleucyl-tRNA

[2] The abbreviation used is: DBAE, *N*-[*N'*-(*m*-dihydroxyborylphenyl)succinamyl]aminoethyl.

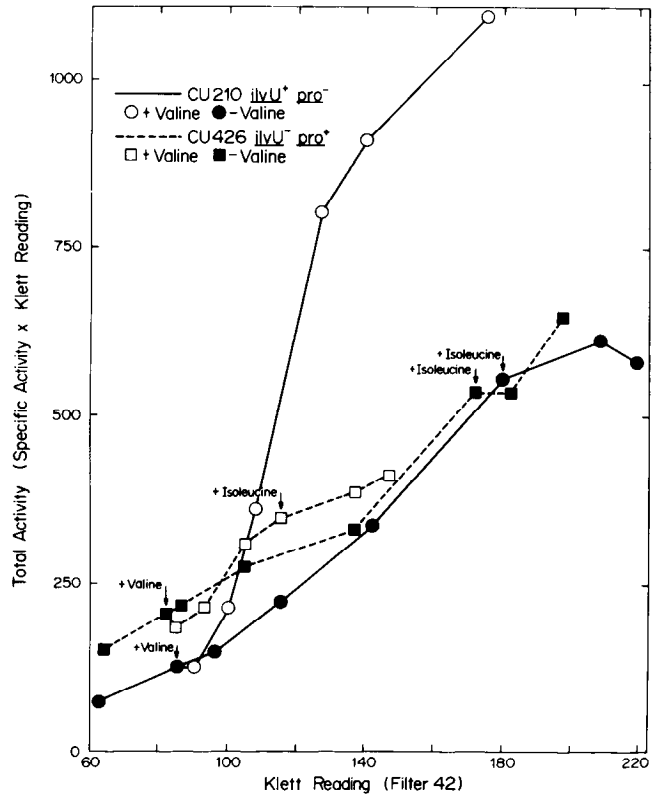

FIG. 1. **Effect of an isoleucine restriction on growth and isoleucyl-tRNA synthetase formation in an *ilvU*$^+$ and an *ilvU*$^-$ strain of *E. coli*.** Strain CU210 (*ilvU*$^+$) was grown in minimal medium supplemented with its required amino acids, threonine, leucine, and proline. Its *ilvU*$^-$ derivative, 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.

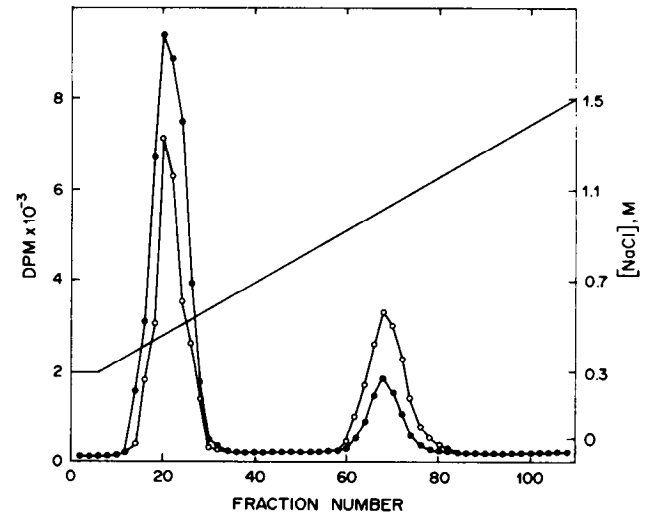

FIG. 2. **Comparison of tRNA^{Ile} in strains CU210 and CU426.** Strain CU210 (●) was labeled with [^{14}C]isoleucine. Strain CU426 (○) was labeled with [^{3}H]isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. ^{14}C dpm are per 1.00 ml. ^{3}H dpm are per 1.00 ml. Cells 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$_2^{Ile}$ and tRNA$_2^{Val}$ 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 causes 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-

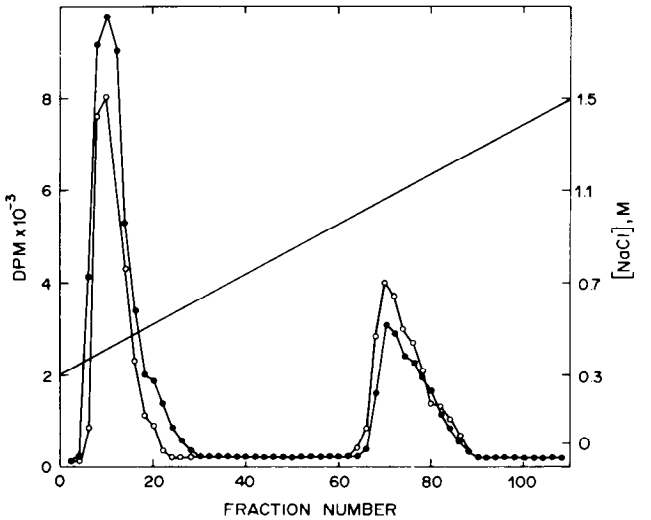

FIG. 3. **Comparison of tRNA^{Val} in strains CU210 and CU426** Strain CU210 (●) was labeled with [^{14}C]valine. Strain CU426 (○) wa labeled with [^{3}H]valine. Samples were normalized to indicate equiv alent amounts of tRNA per dpm. ^{14}C dpm are per 1.00 ml. ^{3}H dpn are per 1.00 ml. Cells were grown in minimal medium.

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 tRNA^{Val} and tRNA^{Ile}—Because of the inverse variation exhibited by the two isoacceptors of both tRNA^{Val} and tRNA^{Ile}, 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 tRNA^{Ile} and tRNA^{Val} 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 tRNA^{Ile} and tRNA^{Val} with greatly increased Species 2 and decreased Species I.

The Effect of a pro-lac Deletion on tRNA^{Val} and tRNA^{Ile}—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, tRNA^{Ile} and tRNA^{Val} in a strain bearing a *pro-lac* deletion which would presumably lack any *ilvU* function was examined. RPC-5 chromatographic profiles for tRNA^{Ile} and tRNA^{Val} 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 tRNA^{Ile} and tRNA^{Val} appear as Species 1. Even upon limitation for isoleucine, which normally results in an increase in Species 2, no second isoacceptor for either tRNA^{Ile} or tRNA^{Val} was generated. When an F′ episome bearing the region covered by the *pro-lac* deletion (strain CU154) was transferred to strain CU152, the RPC-5 chromatographic profiles changed dramatically. When the new strain, strain CU852, was grown in minimal medium, profiles for both tRNA^{Ile} and tRNA^{Val} were similar to those encountered in the *ilvU*$^+$ strains, CU1 and CU210. When strain CU852 was limited for isoleucine, both the tRNA^{Ile} and tRNA^{Val} RPC-5 profiles were similar to the respective profiles evident when strain CU210, the *ilvU*$^+$ parent, was grown in a similar manner. tRNA^{Ile} profiles from strain CU176, another *pro-lac* deletion strain, and strain CU879, a *pro-lac* deletion strain containing a *pro-lac* episome carrying the *ilvU459* marker, are compared in Fig. 6 and those for tRNA^{Val} are compared in Fig. 7. While in strain CU176 no Species 2 of either tRNA^{Ile} or tRNA^{Val} was found, profiles characteristic of those encountered in the *ilvU*$^-$ strain, CU426, were obtained from strain CU879.

Dominance of the ilvU459 Mutation—That the *ilvU* locus was carried on the F *pro-lac* episome made it possible to determine whether *ilvU*$^-$ or *ilvU*$^+$ was dominant. Episomal transfer from strain CU154 to strain CU426 was performed on a threonine-leucine medium upon which only the recipient could grow. Several isolated colonies appearing where the two strains were mixed were then examined for thiaisoleucine resistance and for capacity to transfer the episome to strain CU152. It was found that all of the episome-containing clones were thiaisoleucine-resistant. Thus, the *ilvU459* lesion carried by strain CU426 was dominant over the *ilvU*$^+$ gene carried on the F *pro-lac* episome.

One of the merodiploid clones was saved as strain CU877. The isoleucyl-tRNA and valyl-tRNA profiles of strain CU877 were examined and were found to be similar to those of its haploid *ilvU459* parent, strain CU426.

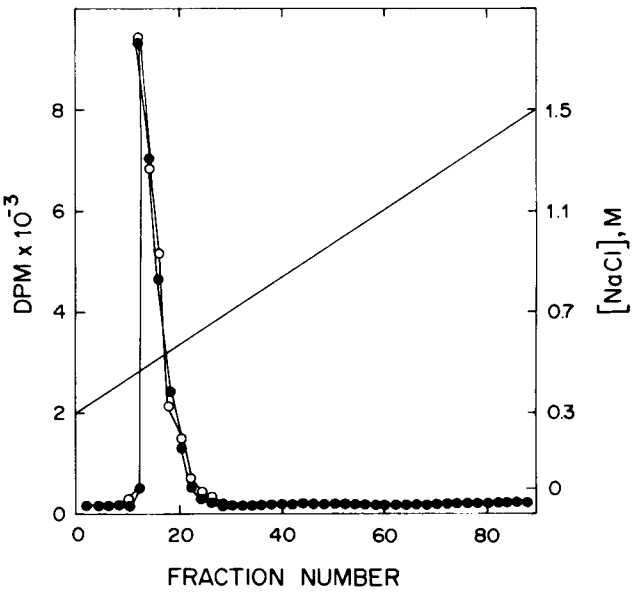

FIG. 4. **Analysis of tRNA^{Ile} in strain CU152.** tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labeled with [^{14}C]isoleucine. tRNA from cells grown under limitation for isoleucine (○) was labeled with [^{3}H]isoleucine. Samples were normalized to indicate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

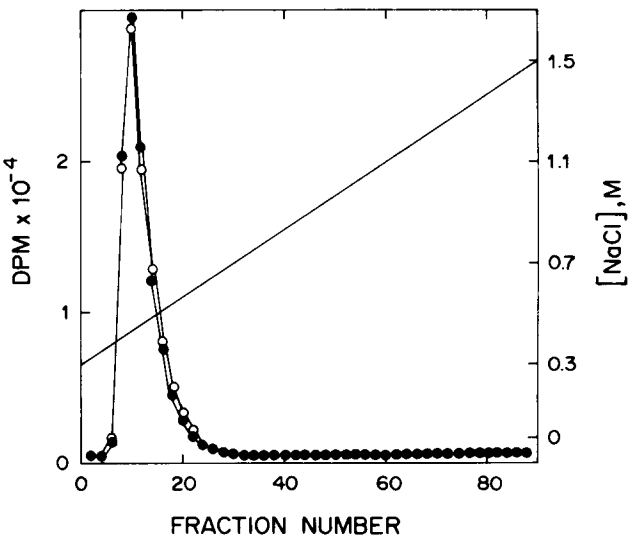

FIG. 5. **Analysis of tRNA^{Val} in strain CU152.** tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labeled with [^{14}C]valine. tRNA from cells grown under limitation for isoleucine (○) was labeled with [^{3}H]valine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. ^{14}C dpm are per 1.11 ml. ^{3}H dpm are per 1.16 ml.

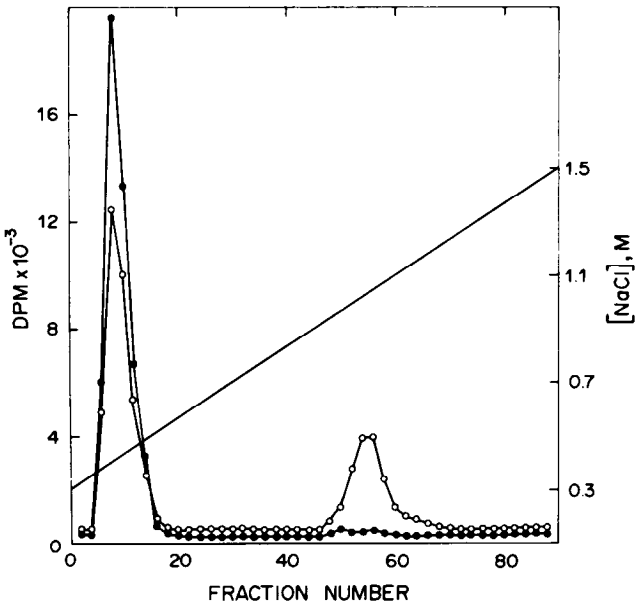

FIG. 6. **Comparison of tRNA^{Ile} in strains CU176 and CU879.** Strain CU176 (●) was labeled with [^{3}H]isoleucine. Strain CU879 (○) was labeled with [^{14}C]isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. ^{14}C dpm are per 1.00 ml. ^{3}H dpm are per 1.00 ml. Cells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

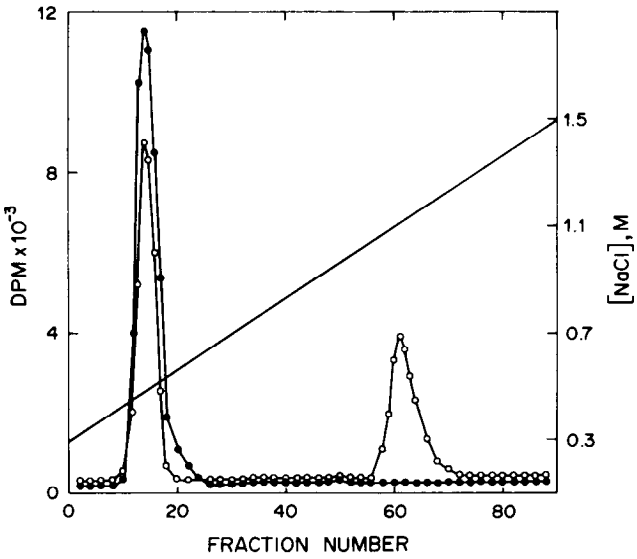

FIG. 7. **Comparison of tRNA^{Val} in strains CU176 and CU879.** Strain CU176 (●) was labeled with [^{14}C]valine. Strain CU879 (○) was labeled with [^{3}H]valine. Samples were normalized to indicate equivalent amounts of tRNA per dpm. ^{14}C dpm are per 1.00 ml. ^{3}H dpm are per 0.97 ml. Cells were grown in minimal medium.

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 *ilvU*$^+$ or an *ilvU*$^-$ strain was treated with chloramphenicol, species 2 of both tRNA^{Ile} and tRNA^{Val} 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 tRNA^{Ile} and tRNA^{Val} 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 [^{3}H]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 tRNA^{Ile} and tRNA^{Val} 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 ^{14}C-labeled amino acid. As shown in Fig. 8 (tRNA^{Ile}), and Fig. 9 (tRNA^{Val}), 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 tRNA^{Ile}, 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 tRNA^{Ile} 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 tRNA^{Ile} 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 tRNA^{Val} (Fig. 9), except that, during recovery from chloramphenicol treatment, the overall RPC-5 profile steadily approached the normal pattern, and at no time was a greater than normal amount of tRNA$_1^{Val}$ nor a less than normal amount of tRNA$_2^{Val}$ observed.

The simplest model to account for the change in profiles during recovery from chloramphenicol treatment is that tRNA$_2^{Val}$ and tRNA$_2^{Ile}$ are converted to tRNA$_1^{Val}$ and tRNA$_1^{Ile}$. 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 ilvU Mutants—To provide additional evidence that the effects on tRNA profiles observed in *ilvU459* strains were indeed due to the *ilvU* lesion, additional *ilvU* mutants were examined. These mutants included strain CU418, in which the *ilvU459* 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 *ilvU* lesions. The new lesions were obtained spontaneously by selecting for thiaisoleucine 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 thiaisoleucine resistance was characteristic of the *ilvU* strains but in which the lesion was not linked to *proA* or *proB*. Presumably one or more of the lesions conferring thiaisoleucine resistance in strain CU1018 is present in strain CU899. Examination of tRNA^{Ile} and tRNA^{Val} profiles from strain CU418 grown in minimal medium revealed profiles analogous to those observed in the other *ilvU459* strain, CU426. Examination of new *ilvU*$^-$ strains, CU900 and CU901, grown in minimal medium supplemented with excess leucine, isoleucine, and valine revealed profiles for tRNA^{Ile} and tRNA^{Val} similar to those observed in the *ilvU459* mutants, strains CU426 and CU418. Strain CU901 revealed a tRNA^{Ile} profile in which the increase in Species 2 was slightly greater than that in strain CU900. The tRNA^{Val} profile from strain CU901

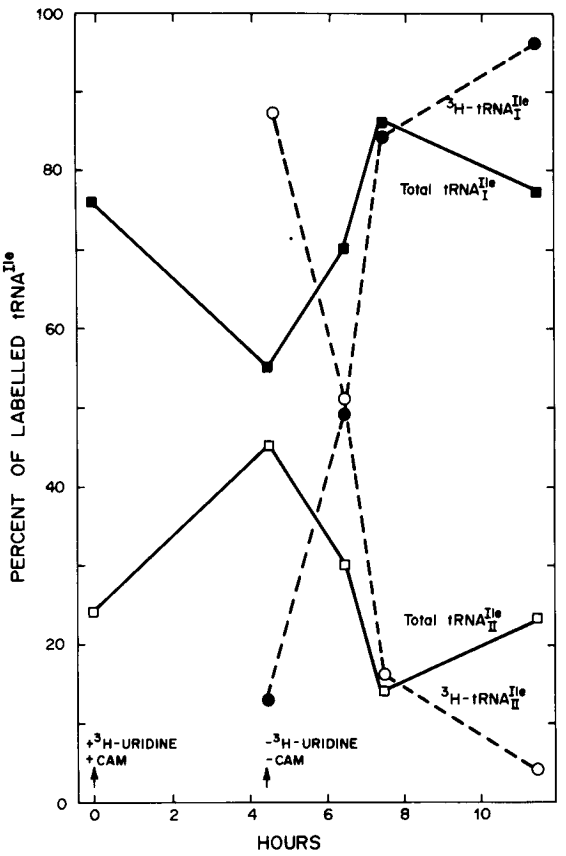

FIG. 8 (*left*). **Recovery from the effect of chloramphenicol on the distribution of tRNA^{Ile} between Species 1 and 2.** Strain CU426 growing in minimal medium was treated with chloramphenicol in the presence of [^{3}H]uridine. Recovery was in the presence of cold uridine. In all cases, the total tRNA isolated was charged with [^{14}C]isoleucine. ^{14}C radioactivity indicated total Species 1 (■) or 2 (□) as indicated. ^{3}H radioactivity indicated the tRNA^{Ile} 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.)

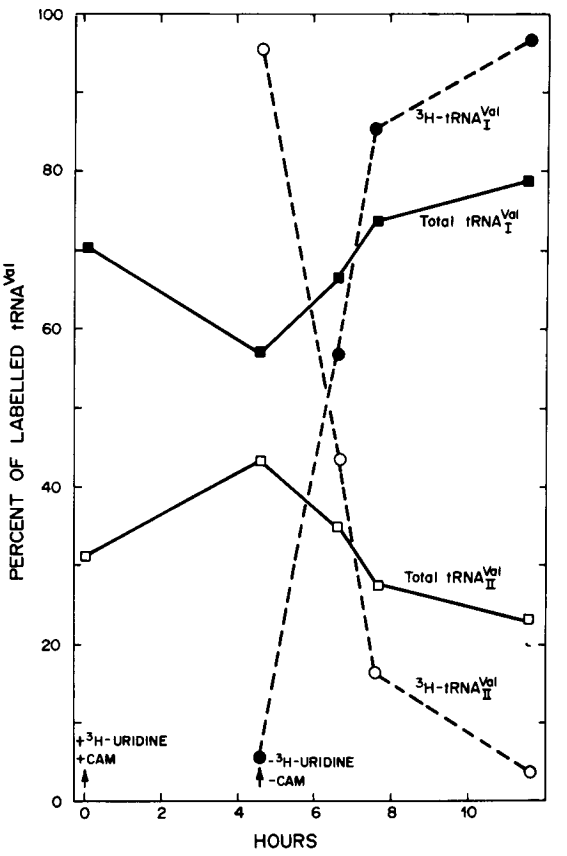

FIG. 9 (*right*). **Recovery from the effect of chloramphenicol on the distribution of tRNA^{Val} between Species 1 and 2.** Cells growing in minimal medium were treated with chloramphenicol in the presence of [^{3}H]uridine. Recovery was in the presence of cold uridine. In all cases, the total tRNA isolated was charged with [^{14}C]valine. ^{14}C radioactivity indicated total Species 1 (■) or 2 (□) as indicated. ^{3}H radioactivity indicated the tRNA^{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^{Ile} and tRNA^{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 *ilvEDA* operon (7).

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 CU882) 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. It thus 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 aminoacyl-tRNA synthetases

Strain (Pertinent Genotype)	Growth Medium[a]	Threonine Deaminase (*ilvA*)	Dihydroxy Acid Dehydrase (*ilvD*)	Trans-aminase B (*ilvE*)	Acetohydroxy Acid Isomero-reductase (*ilvC*)	Acetohydroxy Acid Synthase (*ilvB* and *ilvHI*)		Isoleucyl tRNA Synthetase	Valyl tRNA Synthetase	Leucyl tRNA Synthetase
		nmol/min/mg protein						*pmol/min/mg protein*		
						-Valine	+Valine[b]			
CU210 (*ilvU*$^+$)	LPT	32.6	24.8	21.0	24.3	39.8	7.8	6.1	10.0	10.6
	Repressing	15.1	7.5	7.8	0.4	16.5	2.5	5.2	8.4	12.2
	Limiting Leucine	99.5	67.8	65.5	1.5	89.5	14.3	8.5	8.7	18.6
	Limiting Isoleucine	114.2	68.7	68.6	0.1	1.46	0.4	12.9	20.6	12.9
	Limiting Proline	6.3	10.2	7.1	1.4	10.6	3.1	23.5	25.4	20.4
	L-Broth	8.5	5.5	5.0	--	--	--	--	--	--
CU426 (*ilvU459*)	LPT	34.2	22.8	24.6	26.7	32.9	7.5	8.1	10.2	9.7
	Repressing	31.5	15.7	21.4	<0.1	19.2	3.1	7.2	8.9	13.6
	Limiting Leucine	101.0	61.0	70.3	1.0	36.2	10.3	11.0	10.3	25.4
	Limiting Isoleucine	120.0	74.3	81.4	1.6	9.6	1.4	7.8	13.9	16.1
	L-Broth	12.5	7.6	2.7	--	--	--	--	--	--
CU418 (*ilvU459*)	Repressing	32.5	15.4	25.3	0.9	10.5	2.6	6.9	8.7	--
	Limiting Isoleucine	116.9	67.5	97.4	0.5	8.5	1.6	7.4	17.5	--
CU900 (*ilvU2126*)	Repressing	15.3	7.6	8.7	0.4	14.7	3.0	5.9	8.6	--
	Limiting Isoleucine	122.1	61.9	64.6	0.4	8.2	1.9	6.3	18.0	--
CU901 (*ilvU2127*)	Repressing	20.0	8.1	8.2	0.7	15.9	2.7	6.9	9.0	--
	Limiting Isoleucine	118.8	58.6	63.7	0.6	6.3	2.1	7.1	17.0	--
CU899 (*ilvU*$^+$ plus thiaisoleucine resistance marker)	Repressing	16.2	7.4	8.0	0.2	12.9	2.8	5.7	8.8	--
	Limiting Isoleucine	109.3	62.8	61.8	0.4	7.5	2.0	11.9	19.1	--

[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

Strain (Pertinent Genotype)	Growth Conditions[a]	Threonine Deaminase		Dihydroxy Acid Dehydrase	Acetohydroxy Acid Isomero reductase	Trans-aminase B	Acetohydroxy Acid Synthase		Isoleucyl tRNA Synthetase	Valyl tRNA Synthetase
		nmol/min/mg protein							*pmol/min/mg protein*	
		-Iso-leucine	+Iso-leucine[b]				-Valine	+Valine[c]		
CU18 (*ilvU2141*)	Repressing	16.89	15.51	10.91	0	8.92	6.63	1.14	6.71	9.60
	Limiting	183.30	122.92	69.50	0	65.89	5.77	0.67	9.55	22.00
CU882 (*ilvU2141 lac*)	Repressing	19.30	12.98	10.50	0.12	7.73	6.35	1.23	6.05	8.37
	Limiting	232.99	171.78	70.50	0.22	73.33	4.73	0.89	9.55	15.89
CU896 (F *ilvU459 lac*$^+$/*ilvU2141 lac*)	Repressing	16.30	11.09	11.50	0.07	20.21	8.28	1.69	6.88	9.25
	Limiting	204.62	160.26	68.34	0.14	62.31	6.69	0.44	6.83	17.73
CU897 (F *ilvU*$^+$ *lac*$^+$/*ilvU2141 lac*)	Repressing	20.48	16.62	9.69	0.64	19.38	8.74	2.99	5.90	11.10
	Limiting	148.89	94.81	65.76	0.14	63.26	4.12	0.52	10.30	20.10
CU176 [Δ(*pro-lac*)]	Repressing	19.66	1.22	5.75	1.99	18.43	13.42	1.60	6.19	9.10
	Limiting	96.25	8.33	45.49	0.63	59.61	3.52	1.75	9.66	21.60
CU152 [Δ(*pro-lac*)]	Repressing	15.15	1.77	4.01	1.45	9.84	10.42	2.55	6.07	8.89
	Limiting	99.28	11.71	54.89	1.24	52.87	4.48	0.59	9.43	18.63
CU852 [F *ilvU*$^+$*lac*$^+$/Δ(*pro-lac*)]	Repressing	18.69	2.37	5.28	1.23	15.71	11.67	3.01	6.62	9.40
	Limiting	99.89	9.21	58.93	1.01	64.78	5.98	1.03	12.81	19.20
CU879 [F *ilvU459 lac*$^+$/Δ(*pro-lac*)]	Repressing	14.08	1.64	6.59	0.76	15.08	13.76	3.60	6.48	12.80
	Limiting	101.44	11.46	60.98	0.23	63.64	11.90	1.75	7.23	21.60

[a] Repressing conditions: cells grown in excess branched-chain amino acids; derepressing conditions: cells grown in excess valine and leucine, limiting isoleucine. Note: The acetohydroxy acid synthase and isomeroreductase 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] Acetohydroxy 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 $tRNA^{Ile}$, $tRNA^{Val}$, and $tRNA^{Leu}$ profiles that were altered relative to those of the *E. coli* wild type, strain CU1. Two additional *ilvU* lesions (*ilvU2126* and *ilvU2127*) 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 $ilvU^-$ over $ilvU^+$. Furthermore, when an episome carrying either an $ilvU^+$ or $ilvU^-$ marker was transferred to *ilvU* deletion strains, the deletion phenotype (a missing Species 2 of $tRNA^{Ile}$ and $tRNA^{Val}$) was suppressed.

It is likely that, for both $tRNA^{Ile}$ and $tRNA^{Val}$, 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 $ilvU^+$ locus impedes the modification of Species 2 into Species 1. This restriction of modification might be due to inhibition or to repression of one or more modification enzymes that might require continued resynthesis. The $ilvU^-$ allele presumably permits even less modification of Species 2 into Species 1 than does the $ilvU^+$ 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 $tRNA_2^{Ile}$ and $tRNA_2^{Val}$ 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 $tRNA^{Ile}$ (21, 22) and *E. coli* B and K-12 $tRNA^{Val}$ (23–27) revealed that the differences between two forms of $tRNA^{Ile}$ separated by MAK column chromatography are not the same as those between two forms of $tRNA^{Val}$. For $tRNA^{Ile}$, one form contained one less dihydrouridine residue than did the other. For $tRNA^{Val}$, there were a number of modification differences between the two species but not the same differences as those between the two forms of $tRNA^{Ile}$. Furthermore, there was some difference between the primary sequences of the two forms of $tRNA^{Val}$.[3] 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 $tRNA^{Ile}$ 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 $tRNA^{Ile}$ 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 $tRNA_1^{Ile}$, $tRNA_1^{Val}$, and $tRNA_3^{Leu}$, it is all three modifications that the *ilvU* product retards.

[3] Sequencing had been done on species referred to as 1, 2A, and 2B. Species 2A and 2B were often coeluted from MAK columns, and, although their modifications were exactly the same, they differed somewhat in primary sequence. The relation of these forms to those separated by RPC-5 chromatography is not known.

It seems unlikely that the *ilvU459* mutation is an "up-promoter" mutation, for the $ilvU^-$ phenotype was neither mimicked in $ilvU^+/ilvU^+$ merodiploids, nor further enhanced in $ilvU^-/ilvU^-$ merodiploids. The *ilvU* product may be under autogenous control and the difference between the $ilvU^+$ and $ilvU^-$ 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'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 $tRNA^{Leu}$ profiles like those we describe (Fig. S-3).

The property of the *ilvU459* allele of enhancing the physiological effect exerted by the $ilvU^+$ allele probably accounts for the dominance of the mutant allele over the wild type. An analogous situation may be found in *lacI* 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 $ilvU^+$ 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 $tRNA^{Ile}$ or $tRNA^{Val}$. 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 $ilvU^+$ strain, whereas the $ilvU^-$ 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 $tRNA^{Ile}$ and $tRNA^{Val}$ 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 synthase 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.

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SUPPLEMENT TO

ilvU, A Locus in *Escherichia coli* Affecting the Derepression of Isoleucyl tRNA Synthetase and the RPC-5 Chromatographic Profiles of $tRNA^{Ile}$ and $tRNA^{Val}$

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EXPERIMENTAL PROCEDURES

Aminoacyl tRNA Synthetase Assays—Aminoacyl tRNA synthetase activities were assayed at 37° in 0.6 ml systems that contained 33 μmoles Tris buffer, pH 7.3, 3.3 μmoles magnesium chloride, 3.3 μmoles potassium chloride, 10 μmoles reduced glutathione, pH 7.0, 2 μmoles ATP, pH 6.8, 1.0 mg tRNA (from *E. coli* K-12, obtained from Calbiochem), 0.02 μmole ^{3}H- or ^{14}C-amino acid with a specific activity of 20 μCi/μmole to 100 μCi/μmole, and 0.1 ml of crude extract with a protein concentration of 2 mg/ml. Control samples contained no extract. The reaction was conducted for five or ten minutes and was terminated with three ml of 5% (w/v) trichloroacetic acid. An equal volume of 100% ethanol was added, and, after a two-hour precipitation period at 4°, the precipitate was collected by filtration on Millipore filters. The filters were washed and dried completely prior to determination of radioactivity. Enzyme activity was expressed as picomoles of amino acid attached per minute per milligram of extract protein.

tRNA Preparation—To extract RNA, cells from liter cultures in minimal medium were harvested by centrifugation for 8 min at 10,400 x g and resuspended in 10 ml 0.05 M acetate buffer, pH 5.5, containing 0.01 M magnesium chloride and 0.06 M potassium chloride. Water-saturated phenol was added to 50% (v/v) and the RNA extraction was performed by shaking at 37° for 40 minutes. Following centrifugation for 15 min at 12,000 x g, the aqueous phase was precipitated in three volumes of 100% ethanol. The precipitate was resuspended in 2.5 ml acetate buffer and passed over a DEAE-cellulose column (35 ml bed volume) that had been prepared by washing successively in 1 M sodium hydroxide followed by 1 M hydrochloric acid, and again in 1 M sodium hydroxide prior to decanting of particulate matter and equilibration in acetate buffer. A batch elution with 0.1 M lithium chloride until there was no further decrease in A_{260} was followed by a batch elution with 40 ml of 1.0 M lithium chloride. The entire 1.0 M lithium chloride eluate was precipitated in 2.5 volumes of 100% ethanol. The precipitate was then resuspended in 2 ml distilled water, and the solution was examined for purity and nucleic acid content by measuring absorbency on a model 250 Gilford spectrophotometer at 260 nm and 280 nm. Twenty absorbence units at 260 nm was considered to be equivalent to a concentration of 1.0 mg/ml of RNA. A ratio of A_{260}/A_{280} between 1.90 and 2.10 was considered indicative of a pure preparation. Ratios greater than 2.10 indicated the presence of phenol, while ratios less than 1.90 indicated protein contamination. RNA preparations to be stored for longer than 24 hours were kept as precipitated pellets at -20°. RNA to be stored for periods under 24 hours was kept in solution at 4° until used.

tRNA was deacylated by resuspension in 0.1 M Tris buffer, pH 8.7 and incubation for 30 min at 37°. The RNA was precipitated by mixing with three volumes of 100% ethanol and storing two hours at -20°. The precipitate was removed from the ethanol by centrifugation in a tabletop centrifuge. Isoleucyl $tRNA^{Ile}$ was deacylated at pH 8.0 instead of pH 8.7. The higher pH rendered $tRNA^{Ile}$ less stable in subsequent charging and chromatographic applications. Aminoacylation of tRNA was performed in the presence of 55 mM Tris-HCl, pH 7.3, 5.5 mM potassium chloride, 5.5 mM magnesium chloride, 16.7 mM reduced glutathione, 3.3 mM ATP previously adjusted to pH 6.8, tRNA at a concentration yielding between 50-200 A_{260} units per ml, 3.3×10^{-5} M ^{3}H- or ^{14}C-amino acid (20-100 μCi per μmole), and 0.33 mg of crude extract protein per ml or, when a partially purified synthetase preparation was used, 0.1 mg protein per ml. Incubation was at 37° for 30 min. The reaction was stopped by precipitation with three volumes of 100% ethanol at -20°.

The tRNA was prepared for reversed phase chromatography by dissolving the precipitate from the aminoacylation reaction in the previously mentioned pH 5.5 acetate buffer and extracted with a 50% final concentration (v/v) of phenol. The aqueous phase was dialyzed overnight at 4° against distilled water and precipitated in three volumes of 100% ethanol at -20°. The precipitate was removed from the ethanol by centrifugation and stored at -20° until required. The stored precipitate was resuspended in the pH 5.5 acetate buffer just prior to loading on the reversed phase column.

Reversed Phase Chromatography—The RPC-5 matrix was obtained commercially from Miles Laboratories or Astro Chemicals, Inc. Teflon tubing was used throughout. Whitey valves with Swagelok fittings were used in conjunction with a column manufactured by Glenco and a Pall syringe pump. The bed volume in the columns was 75 ml. Gradient and equilibration buffers contained sodium chloride as indicated, 0.01 M magnesium chloride and 1 mM β-mercaptoethanol in a 0.01 M acetate buffer, pH 4.5. The total volume of the gradients was 100 ml, except in the experiments with $tRNA^{Leu}$, when a 200 ml gradient was employed. Radioactivity of each sample was determined in a Nuclear Chicago Isocap 300 scintillation counter.

RESULTS

Reversed Phase Chromatographic Separation of the Isoacceptor tRNA's for the Branched-Chain Amino Acids from Escherichia coli and from a Thiaisoleucine-Resistant Derivative—*Escherichia coli* strain CU1, a K-12 wild type strain, and a derivative of it that had been selected for growth in the presence of thiaisoleucine, strain CU1018, were grown in a minimal glucose-salts medium. The tRNA of each strain was prepared and charged with labelled isoleucine as described in the Experimental Procedures of the text, ([^{14}C]isoleucine for strain CU1 and [^{3}H]isoleucine for strain CU1018). The isoaccepting species of isoleucyl tRNA were separated by RPC-5 chromatography. Figure S-1 shows that both strains contained two readily separated isoacceptors which appeared in the same column fractions for both strains. A striking difference between the two strains was that in strain CU1 the major isoacceptor ($tRNA_1^{Ile}$) comprised a larger proportion of the total than did that in strain CU1018.

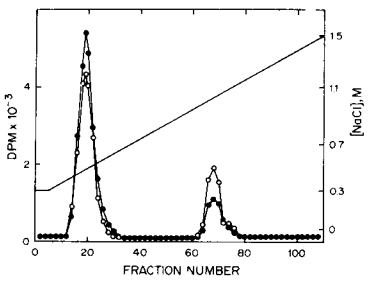

Fig. S-1. Comparison of $tRNA^{Ile}$ in Strains CU1 and CU1018. Strain CU1 (●) was labelled with ^{14}C-isoleucine. Strain CU1018 (o) was labelled with ^{3}H-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 0.97 ml. Cells were grown in minimal medium.

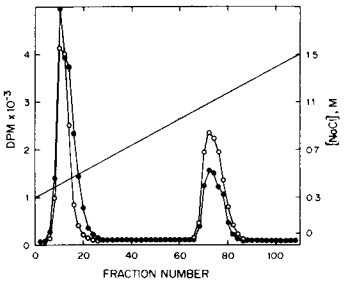

Fig. S-2. Comparison of $tRNA^{Val}$ in Strains CU1 and CU1018. Strain CU1 (●) was labelled with ^{14}C-valine. Strain CU1018 (o) was labelled with ^{3}H-valine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 0.96 ml. Cells were grown in minimal medium.

Figure S-2 shows the RPC-5 chromatographic profile for the valyl tRNA for the two strains. Although the positions of the major charged isoacceptor (valyl $tRNA_1^{Val}$) and the minor charged isoacceptor (valyl $tRNA_2^{Val}$) were the same for the two strains, the difference between the profiles of the sensitive and resistant strains was similar to that shown in Figure 1 for isoleucyl tRNA. It appeared that thiaisoleucine resistance had been accompanied by an increase in $tRNA_2^{Val}$ and a corresponding decrease in $tRNA_1^{Val}$.

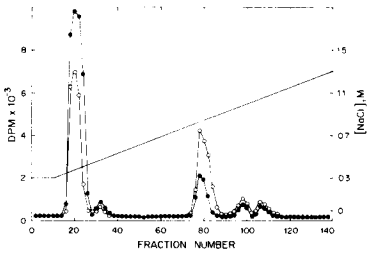

Fig. S-3. Comparison of $tRNA^{Leu}$ in Strains CU1 and CU1018. Strain CU1 (●) was labelled with ^{14}C-leucine. Strain CU1018 (o) was labelled with ^{3}H-leucine. Actual ^{3}H-DPM's were adjusted to indicate approximately equivalent amounts of tRNA per ^{3}H-DPM and ^{14}C-DPM plotted. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 0.63 ml. Cells were grown in minimal medium.

The same tRNA preparations for the two strains were also charged with labelled leucine and the isoacceptors were separated by RPC-5 chromatography. The elution profile of the leucine isoacceptors is shown in Figure S-3. Both strains yielded five well separated isoacceptors, all of which were eluted in the same way for both strains. The striking difference between the sensitive (CU1) and the resistant (CU1018) strains was that the amount of the earliest eluted isoacceptor ($tRNA_1^{Leu}$) appeared to be elevated in the mutant and the amount of $tRNA_3^{Leu}$ appeared to be correspondingly decreased. No signficant differences between the two strains could be seen with isoacceptors 2, 4, and 5, but their amounts were so low that real differences might not be apparent.

The Effect of Growth under Repressing or Derepressing Conditions on the Relative Amounts of the Two Isoacceptors for Isoleucine and Valine—Strain CU1018 differed from its thiaisoleucine parent by mutations at three loci, *ilvS*, *ilvY* and *ilvU*. As Figures 2 and 3 of the text show, the altered tRNA profile exhibited by CU1018 could be accounted for by the *ilvU459* lesion. Subsequent studies were therefore confined to strains in which the only thiaisoleucine resistance markers were in the *ilvU* locus. Since the changes in the isoleucine and valine isoacceptor profiles were less complex than those in the leucine isoacceptor profiles, subsequent studies were also confined to the isoleucine and valine isoacceptors.

Since the differences between the isoacceptor profiles of the parent and mutants shown in Figures 2, 3, S-1, S-2, and S-3 were exhibited by cells grown in the absence of isoleucine and valine, it was possible that the mutant profiles were the result of an isoleucine restriction. (Strain CU1018 is highly derepressed and strain CU426 is slightly derepressed for the *ilvEDA* operon, implying an "isoleucine-limiting" signal.) This question was examined by comparing the profiles exhibited by cells grown in the presence of an excess of the branched-chain amino acids and by cells grown with an amino acid restriction.

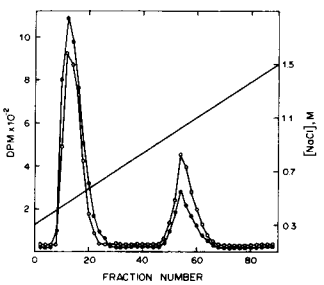

Fig. S-4. Effect of Isoleucine Limitation on $tRNA^{Ile}$ in Strain CU210. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-isoleucine. tRNA from cells grown under limitation for isoleucine (o) was labelled with ^{3}H-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 0.97 ml.

Figure S-4 shows that the ilvU$^+$ strain CU210 exhibited a profile of isoleucine acceptors when grown with excess branched-chain amino acids, much like that exhibited by cells of strain CU1 (Figure 2, text) grown in 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 $tRNA_2^{Ile}$ appeared to have been increased at the expense of isoleucyl $tRNA_1^{Ile}$. In this respect, isoleucine limitation has appeared to mimic the ilvU$^-$ lesion.

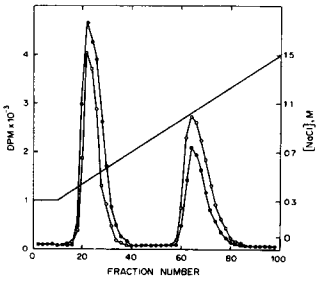

Fig. S-5. Effect of Isoleucine Limitation on $tRNA^{Ile}$ in Strain CU426. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-isoleucine. tRNA from cells grown under limitation for isoleucine (o) was labelled with ^{3}H-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 0.98 ml.

Figure S-5 shows that the ilvU$^-$ strain, CU426, showed the elevated isoleucyl $tRNA_2$ even when repressing conditions were employed. Similarly, it also exhibited a further increase in the second isoacceptor when isoleucine was made limiting. Thus, the effect of the ilvU$^-$ lesion was probably not due simply to an isoleucine limitation.

Figures S-6 and S-7 show that for both strains $tRNA_1^{Val}$ and $tRNA_2^{Val}$ underwent similar shifts in relative amounts when the isoleucine limitation signal was imposed. The shift was again superimposed upon the difference due to the ilvU$^-$ lesion.

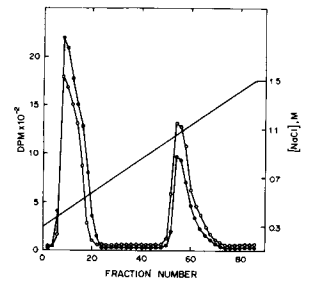

Fig. S-6. Effect of Isoleucine Limitation on $tRNA^{Val}$ in Strain CU210. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-valine. tRNA from cells grown under limitation for isoleucine (o) was labelled with ^{3}H-valine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 1.02 ml.

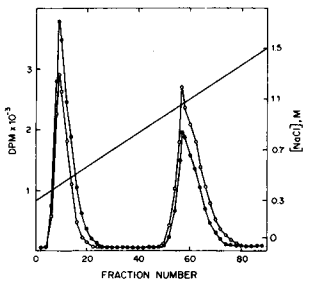

Fig. S-7. Effect of Isoleucine Limitation on $tRNA^{Val}$ in Strain CU426. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-valine. tRNA from cells grown under limitation for isoleucine (o) was labelled with ^{3}H-valine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 0.97 ml.

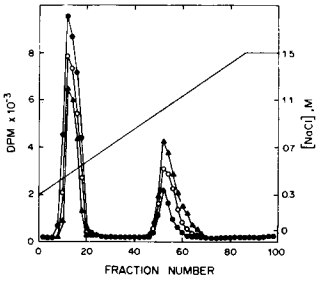

Fig. S-8. Effects of Isoleucine and Valine Limitation on $tRNA^{Ile}$ in Strain CU1014. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-isoleucine. tRNA from cells grown under limitation for isoleucine (o) was labelled with ^{3}H-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 0.98 ml. tRNA from cells grown under limitation for valine (▲) was labelled with ^{14}C-isoleucine and analyzed separately with the data superimposed on the dual labelled chromatogram illustrated in this figure. The ^{14}C-DPM's in this separate analysis are per 1.00 ml.

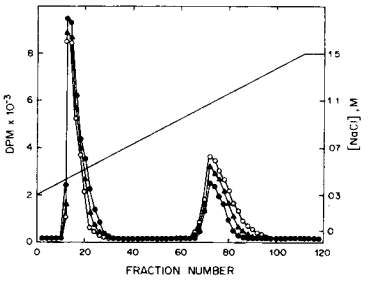

Fig. S-9. Effects of Isoleucine and Valine Limitation on $tRNA^{Val}$ in Strain CU1014. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-valine. tRNA from cells grown under limitation for isoleucine (o) was labelled with ^{3}H-valine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 0.87 ml. ^{3}H-DPM's are per 0.85 ml. tRNA from cells grown under limitation for valine (▲) was labelled with ^{14}C-valine and analyzed separately with the data superimposed on the dual labelled chromatogram illustrated in this figure. The ^{14}C-DPM's in this separate analysis are per 0.98 ml.

Since a valine limitation cannot be readily induced in a strain prototrophic for isoleucine and valine, an isoleucine and valine auxotroph, CU1014, was used. As Figures S-8 and S-9 show, limitation of either amino acid led to increases in the amounts of the second isoacceptors 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 isoacceptor was greater when the non-cognate amino acid was limiting.

The Effect of Growth in a Rich Medium on the RPC-5 Profile—Although there was no difference between the isoacceptor profiles of cells grown in the absence of the branched-chain amino acids and those of cells grown in excess branched-chain amino acids for either the thiaisoleucine-sensitive strain or the thiaisoleucine-resistant strain, there was a striking effect of growth in a rich medium (L-broth). As Figures S-10 and S-11 show, species 2 for both valine and isoleucine were greatly reduced in amount and there were no differences noted between strain CU210 (ilvU$^+$ and CU426 (ilvU$^-$). In both strains, the reduction in amount of the second isoacceptor was essentially compensated by increases in the first isoacceptor.

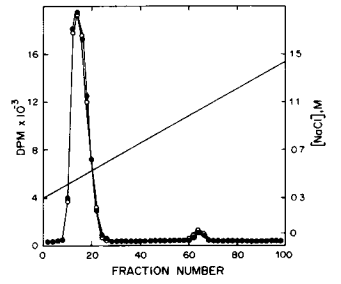

Fig. S-10. Comparison of $tRNA^{Ile}$ in Strains CU210 and CU426 Grown in L-broth. Strain CU210 (●) was labelled with ^{3}H-isoleucine. Strain CU426 (o) was labelled with ^{14}C-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 1.03 ml.

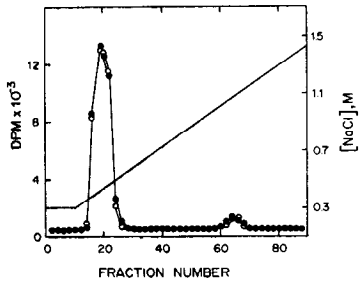

Fig. S-11. Comparison of $tRNA^{Val}$ in Strains CU210 and CU426 Grown in L-broth. Strain CU210 (●) was labelled with ^{3}H-valine. Strain CU426 (○) was labelled with ^{14}C-valine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 1.00 ml.

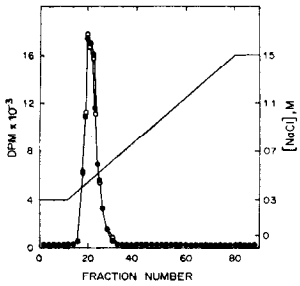

Fig. S-12. Analysis of $tRNA^{Ile}$ in Strain CU18. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-isoleucine. tRNA from cells grown under limitation for isoleucine (○) was labelled with ^{3}H-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.48 ml. ^{3}H-DPM's are per 1.44 ml.

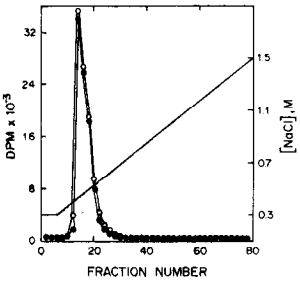

Fig. S-13. Analysis of $tRNA^{Val}$ in Strain CU18. tRNA from cells grown in minimal medium with excess leucine, isoleucine, and valine (●) was labelled with ^{14}C-valine. tRNA from cells grown under limitation for isoleucine (○) was labelled with ^{3}H-valine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.39 ml. ^{3}H-DPM's are per 1.39 ml.

The Isoacceptor Profile in Strain CU18—During the course of screening many strains altered in control of the isoleucine and valine biosynthetic enzymes for correlation with an *ilvU*⁻ or *ilvU*⁺ type of isoacceptor profile, one strain was found in which the second species for both $tRNA^{Ile}$ and $tRNA^{Val}$ was completely absent. The profiles for this strain, CU18, are shown in Figures S-12 and S-13. Even when isoleucine was limiting, there was no detectable species 2 of either tRNA. In this respect, strain CU18 resembled the *pro-lac* deletion strain, CU152, described in the text (see Figures 4 and 5). It is assumed that it bears an *ilvU*⁻ lesion that has inactivated the gene, whereas the *ilvU459* lesion in strain CU426 caused the formation of an altered *ilvU* product.

So that the F episome bearing the *ilvU* locus could be transferred to strain CU18, a *lac*⁻ derivative was prepared. This strain exhibited the same profiles as did the original strain CU18 (data not shown). It was converted to Lac⁺ by transfer of either an episome containing the *ilvU459* lesion (strain CU896) or an episome containing the *ilvU*⁺ allele (strain CU897). Figures S-14 and S-15 show the profiles of $tRNA^{Ile}$ and $tRNA^{Val}$, respectively, in these two episome-bearing derivatives of strain CU18. Not only did the second isoacceptor appear, but when the *ilvU459* allele had been received, the second isoacceptor was higher than when the *ilvU*⁺ allele had been received.

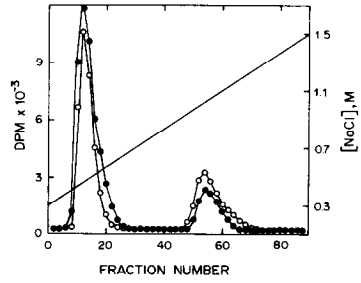

Fig. S-14. Effect of *ilvU459* and *ilvU*⁺ Episomes on the $tRNA^{Ile}$ Profiles of Derivatives of Strain CU18. A *lac* derivative of strain CU18 (CU882) was infected with an F *pro lac* episome bearing either the *ilvU459* marker (to yield strain CU896) or the *ilvU*⁺ marker (to yield strain CU897). Strain CU896 (○) was labelled with ^{14}C-isoleucine. Strain CU897 (●) was labelled with ^{3}H-isoleucine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.10 ml. ^{3}H-DPM's are per 1.15 ml. Cells were grown in minimal medium.

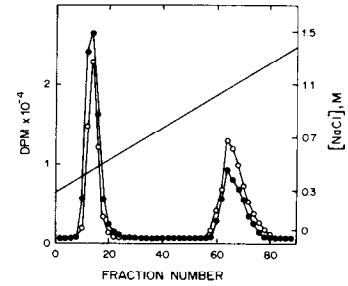

Fig. S-15. Effect of *ilvU459* and *ilvU*⁺ on the $tRNA^{Val}$ Profiles of Derivatives of Strain CU18. Strains prepared as in legend of Fig. S-14. Strain CU896 (○) was labelled with ^{14}C-valine. Strain CU897 (●) was labelled with ^{3}H-valine. Samples were normalized to indicate equivalent amounts of tRNA per DPM. ^{14}C-DPM's are per 1.00 ml. ^{3}H-DPM's are per 1.02 ml. Cells were grown in minimal medium.

The Effect of Chloramphenicol on the Formation of Valine and Isoleucine Isoacceptor Profile in Strain CU426; Recovery from Chloramphenicol Treatment—Figures 8 and 9 in the text showed that, during chloramphenicol treatment, nearly all of the $tRNA^{Ile}$ and $tRNA^{Val}$ appeared as isoacceptors 2. Furthermore, the amount formed was such that the distribution of the total acceptor activity between species 1 and 2 was shifted significantly. The data in those figures were obtained by plotting the areas under the curves in Figures S-16 and S-17 against time of incubation. The zero time control was obtained from the data for CU426 in Figures 2 and 3. The graphs labelled A in both Figures S-16 and S-17 show typical profiles of strains exposed to chloramphenicol. The closed circles indicate a ratio of species 1 to species 2 much lower than obtains under other conditions. The open circles (representing ^{3}H-containing tRNA) indicate that the perturbation of the ratio can be accounted for by the newly formed tRNA. Of particular interest is the profile of isoleucyl tRNA shown in Figure S-16C (closed circles). This profile resembles a profile of isoleucine isoacceptors of cells grown in a rich medium and unlike that of minimal grown cells. It would thus appear that the maturation process was transiently more active than it normally is in minimal medium grown cells. No such evidence for an "overshoot" was observed in the normalization of the valine isoacceptor profile (Figure S-17C, D).

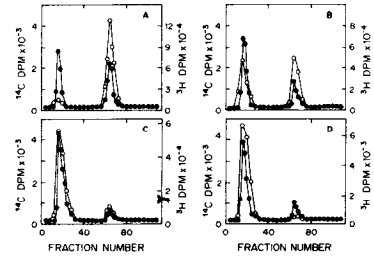

Fig. S-16. RPC-5 Profiles of $tRNA^{Ile}$ in Strain CU426 Following Treatment with Chloramphenicol. Strain CU426 growing in minimal medium was treated with chloramphenicol in the presence of ^{3}H-uridine. Recovery was in the presence of unlabelled uridine. Total $tRNA^{Ile}$ was separated from other tRNA's and charged with ^{14}C-isoleucine. The ^{3}H $tRNA^{Ile}$ (tRNA formed during chloramphenicol treatment) and ^{14}C isoleucyl $tRNA^{Ile}$ (total chargeable $tRNA^{Ile}$) profiles immediately following 4.5 hours of chloramphenicol treatment are shown in Graph A. The ^{3}H and ^{14}C profiles at two, three, and seven hours after cessation of chloramphenicol treatment are shown in Graphs B, C, and D, respectively. The ^{3}H profiles are indicated by open circles (○) and the ^{14}C profiles by closed circles (●).

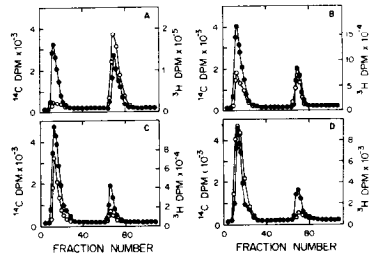

Fig. S-17. RPC-5 profiles of $tRNA^{Val}$ in Strain CU426 following Treatment with Chloramphenicol. Strain CU426 growing in minimal medium was treated with chloramphenicol in the presence of ^{3}H-uridine. Recovery was in the presence of unlabelled uridine. Total $tRNA^{Val}$ was separated from the other tRNA's and charged with ^{14}C-valine. The ^{3}H $tRNA^{Val}$ (tRNA formed during chloramphenicol treatment) and ^{14}C-valyl $tRNA^{Val}$ (total chargeable $tRNA^{Val}$) profiles immediately following 4.5 hours of chloramphenicol treatment are shown in Graph A. The ^{3}H and ^{14}C profiles at two, three, and seven hours after cessation of chloramphenicol treatment are shown in Graphs B, C, and D, respectively. The ^{3}H profiles are indicated by open circles (○) and the ^{14}C profiles by closed circles (●).

ilvU, a locus in Escherichia coli affecting the derepression of isoleucyl-tRNA synthetase and the RPC-5 chromatographic profiles of tRNAIle and tRNAVal.

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