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Synthesis and Activities of Branched-Chain Aminoacyl-tRNA Synthetases in Threonine Deaminase Mutants of *Escherichia coli*

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Valyl-, isoleucyl-, and leucyl-tRNA synthetase activities were examined in an *Escherichia coli* K-12 strain that possessed a deletion of three genes of the *ilv* gene cluster, *ilvD, A,* and *C,* and in a strain with the same deletion that also carried the λd*ilvCB* bacteriophage. It was observed that the branched-chain tRNA synthetase activities of both strains were considerably less than those of the normal strain during growth in unrestricted medium. Furthermore, during an isoleucine limitation, there was a further reduction in isoleucyl-tRNA synthetase activity and an absence of the isoleucine-mediated derepression of valyl-tRNA synthetase formation in both of these mutants, as compared with the normal strain. In addition, it was observed that these branched-chain synthetase activities were reduced in steady-state cultures of several *ilvA* point mutants. However, upon the introduction of the *ilv* operon to these *ilvA* mutants by use of lambda bacteriophage, there was a specific increase in the branched-chain synthetase activities to levels comparable to those of the normal strain. These results support our previous findings that the stability and repression control of synthesis of these synthetases require some product(s) missing in the *ilvDAC* deletion strain and strongly suggest this component is some form of the *ilvA* gene product, threonine deaminase.

In both *Escherichia coli* and *Salmonella typhimurium*, the synthesis of valyl-, isoleucyl-, and leucyl-tRNA synthetases (VRS, IRS, and LRS, respectively) is subject to a repression control process similar to that for repression control of the *ilv* and *leu* gene products (2, 6, 16, 17, 19, 26). In addition, Parker et al. (20, 21) and Reeh et al. (23) have shown that the formation of these tRNA synthetases is subject to metabolic regulation and responsive to nutritional enrichment. Furthermore, modes of metabolic regulation have been reported for lysyl-tRNA synthetase of *E. coli* (8) and for most of the aminoacyl-tRNA synthetases of bakers' yeast (10).

For the branched-chain amino acid-mediated repression control process, VRS formation is multivalently controlled by the supply of both valine and isoleucine, whereas IRS and LRS formation is derepressed in specific response to a growth rate-limiting supply of the respective branched-chain amino acid (16, 17). That the branched-chain tRNA synthetases and biosynthetic enzymes may have a common control element(s) is based on several lines of evidence. (i) Jackson et al. (9) have reported that some regulatory mutants, with mutations not linked to the structural genes for the *ilv* and *leu* clusters or to those for the three branched-chain tRNA synthetases, exhibit altered repression control of both classes of branched-chain enzymes. (ii) Levinthal et al. (13, 14) have shown that the *ilvA* gene product, threonine deaminase, is essential for generation of the derepression signal(s) for VRS, IRS, and LRS formation as well as that for the *ilv* and *leu* biosynthetic enzymes. However, it was not clear whether threonine deaminase had a direct role in regulating synthesis of these enzymes. (iii) More recently, Coleman et al. (3) reported that these tRNA synthetases exhibited altered stability of activity and control of synthesis in an *E. coli* strain deleted for the *ilvD*, *-A*, and *-C* genes.

Since this *ilvDAC* deletion strain would have lacked all three of these gene products, it was not possible to attribute the alterations in stability and synthesis of these tRNA synthetases to the absence of a specific *ilv* gene product. We therefore undertook the present study using the same deletion strain and compared it to a strain that has the same deletion but that also carries the λd*ilvCB* bacteriophage. In this paper, we

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report evidence that the reduction in stability of activity and alteration in repression control of synthesis of the VRS, IRS, and LRS is independent of the *ilvD*, *-C* and *-B* gene products. Thus, the results of the present report can more reasonably be explained by the absence of the *ilvA* gene product, threonine deaminase.

A preliminary report of these results has appeared (A. L. Williams, S. M. Whitfield, and L. S. Williams, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, K166, p. 213).

MATERIALS AND METHODS

Organisms. The *E. coli* strains used in this study are listed in Table 1. Strain CU367 is isogenic to the deletion strain CU356 except for the *ilv* region and served as the normal cell in this study. Strain CU464 was prepared by selecting for *cya*$^+$ transductants grown on minimal maltose agar plates supplemented with isoleucine and valine. Several transductants were purified, tested for the presence of the λ bacteriophage by resistance to reinfection, and scored for temperature sensitivity and the Ilv$^-$ phenotype. Strains CU514 and CU473 were also tested periodically for temperature sensitivity and the presence of the bacteriophage.

Media and methods of cultivation. The minimal medium used was the basal salts solution described by Fraenkel and Neidhardt (5) supplemented with 0.4% glucose and 0.2% ammonium sulfate as carbon and nitrogen sources, respectively. The cells carrying the λ bacteriophage were incubated at 30°C, while all other strains were grown at 37°C. Unrestricted growth was achieved by incubation in minimal medium supplemented with L-valine (100 μg/ml), L-isoleucine (50 μg/ml), and L-leucine (100 μg/ml). Specific amino acid limitations were achieved by use of glycyl-DL-amino acid (branched-chain) as described earlier (16, 17). The culture conditions, measurement of growth, and cell extract preparation followed previously described methods (16, 17). The determination of protein content of extracts (15), measurement of aminoacyl-tRNA synthetase activity (16), and the growth procedures for deuterium oxide-to-water media transfers (27) were as previously described.

Centrifugation in CsCl and measurement of the rate of enzyme synthesis. Centrifugation and measurement of the rate of enzyme synthesis were as described by Williams and Neidhardt (27). The amount of protein per gradient tube was essentially the same and was standardized for each experiment. The equilibrium centrifugation was performed in a Spinco model SW50.1 rotor in a Spinco-Beckman L5-65 ultracentrifuge.

Chemicals. *E. coli* K-12 tRNA was purchased from General Biochemical Corp., Chagrin Falls, Ohio and/or Grand Island Biological Co., Grand Island, N.Y. *U*-^{14}C-labeled amino acids were obtained from Amersham/Searle Corp., Arlington Heights, Ill. Cesium chloride was obtained from Pierce Chemical Co., Rockford, Ill. Glycyl-DL-amino acids were from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Activities of VRS, IRS, and LRS of the *ilvDAC* deletion strain as compared with a strain with the same deletion that also carries λd*ilvCB*. We initially examined the activities of the tRNA synthetases of the normal (CU367) and *ilvDAC* deletion/λd*ilvCB* (CU464) strain grown in minimal medium supplemented with excess amounts of isoleucine, valine, and leucine. The *ilvDAC* deletion λd*ilvCB* strain maintained VRS, IRS, and LRS activities at 59, 54, and 63%, respectively, of the levels of the corresponding activities in the normal strain (Table 2). This result is identical to that ob-

TABLE 1. *Organisms used*

Strain	Genotype	Source
CU367	*gal ilvC462 leu-455*	H. E. Umbarger
CU356	*gal ilvDAC115 leu-455*	H. E. Umbarger
CU464	*gal ilvDAC115 cya*(λc*St*d*ilvCBcya*$^+$)	A. L. Williams
CU406	*gal ilvA454*	H. E. Umbarger
CU514	*gal ilvA454*(λcI857*St*68h80)(λcI857*St*68h80d*ilv*)	H. E. Umbarger
CU473	*gal ilvA454*(λcI857*St*68h80)	H. E. Umbarger
CU370	*ilvA454*	H. E. Umbarger
CU1008	*ilvA454*	H. E. Umbarger

TABLE 2. *VRS, IRS, and LRS activities of strains CU367, CU356, and CU464*[a]

Strain	Genotype	Sp act[b]				
		VRS	IRS	LRS	HRS	ARS
CU367	*gal ilvC462 leu-455*	0.29	0.11	0.16	0.13	0.24
CU464	*gal ilvDAC115cya*(λd*ilvCB*)	0.17	0.06	0.10	0.11	0.26
CU356	*gal ilvDAC115 leu-455*	0.16	0.07	0.08	0.14	0.27

[a] Cells were grown exponentially in minimal medium supplemented with excess amounts of isoleucine, valine, and leucine. VRS, IRS, LRS, HRS, and ARS activities were determined by the amino acid attachment assay system, with a standard error of 9 to 12%.

[b] Expressed as micromoles of product formed per hour per milligram of protein.

tained with strain CU356, which possesses the *ilvDAC* deletion alone (Table 2). The rather small differences observed for histidyl- and arginyl-tRNA synthetase (HRS and ARS, respectively) activities of these two deletion strains clearly indicate that this reduction in activity is specific to the branched-chain tRNA synthetases.

Measurement of the de novo rate of synthesis and stability of VRS, IRS, and LRS. In view of our previous findings (3) that growth of strain CU356 with limiting amounts of isoleucine, valine, or leucine caused a further reduction in these synthetase activities and an altered control of synthesis of these enzymes, we examined the effect of an isoleucine limitation on the activity and rate of synthesis of these synthetases in strain CU464, which contains a normal *ilvC* genes and displays a gene dosage effect (bacterial and phage) for the *ilvB* gene. The rationale for the imposition of an isoleucine limitation on this mutant strain is as follows: (i) an isoleucine limitation has been shown to specifically affect the stability of the corresponding synthetase activity, and (ii) an isoleucine-limiting signal causes derepression of synthesis of both IRS and VRS in a normal strain (16, 17). Upon an isoleucine limitation of this strain, there was a loss of IRS activity (Fig. 1). In contrast, the rates of formation of LRS, ARS, and HRS were essentially unchanged. More importantly, the rate of VRS formation was not derepressed in response to the limiting-isoleucine signal. Both of these results (i.e., loss of IRS activity and lack of derepression of VRS formation) are in contrast to the response of these activities during isoleucine-limited growth of a normal strain (16, 17). However, the fact that these results are identical to those obtained with strain CU356 (*ilvDAC* deletion alone) indicates that the *ilvC* and *ilvB* gene products have no role in the regulatory responses of this mutant strain. Furthermore, since the response of the repression control process to branched-chain amino acid limitations and the apparent stability of the branched-chain tRNA synthetase activities in an *ilvD* mutant are the same as those observed for a normal strain and *ilvC* mutants (results not shown), these data suggest that the *ilvD* gene product also has no role in generating the altered regulatory responses of these deletion mutants.

The essentially unchanged differential rate of VRS formation shown in Fig. 1 could be explained by a true refractory response of the control process to isoleucine limitation. Alternatively, this observation could be the result of balanced rates of synthesis and degradation of this synthetase during the isoleucine-limited growth of this mutant. To distinguish between

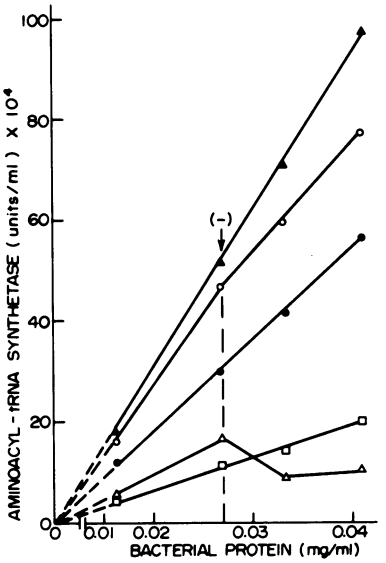

FIG. 1. *Effect of isoleucine limitation on IRS, VRS, LRS, ARS, and HRS formation in the ilvDAC deletion/λdilvCB strain. Experimental conditions were as described in Table 2. Isoleucine-limited growth was achieved by use of 10 μg of glycyl-DL-isoleucine per ml. The cultures were transferred from unrestricted to isoleucine-limited media at the time indicated by the vertical line and arrow. Symbols: △, IRS; □, LRS; ●, HRS; ○, VRS; and ▲, ARS. Results are plotted as enzyme units per milliliter of culture as a function of total protein per milliliter of culture.*

these two possibilities, we performed density labeling studies. Strains CU367 and CU464 were adapted to growth in 80% deuterium oxide (D_2O) medium, and the D_2O-to-water transfers were conducted as described earlier (27). Figure 2A shows the band of IRS activity found in CsCl gradients of samples collected during growth in D_2O-unrestricted medium for strain CU464 (*ilvDAC* deletion/λd*ilvCB*). Figures 2B and C show this activity in samples collected after 25 and 50% increases in mass, respectively, following the transfer from D_2O-unrestricted to isoleucine-limited water media. It is quite obvious that there was considerable inactivation of IRS activity (i.e., loss of IRS activity at a rate in excess of that predicted by the rate of dilution due to cell growth) during one-half generation of isoleucine-limited growth of strain CU464. As expected from previous experiments, the normal strain (CU367) exhibited a severalfold increase (derepression) of IRS synthesis, and there was not evidence of inactivation of this activity even during two generations of isoleucine-limited growth (Fig. 2).

The question of whether VRS formation is refractory to the limiting-isoleucine derepression

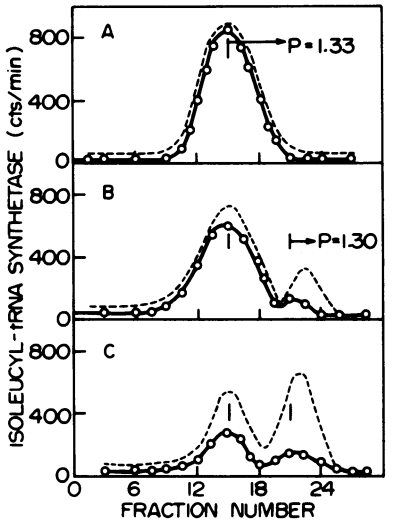

FIG. 2. *Banding in CsCl gradients of IRS from the normal and the ilvDAC deletion/λdilvCB strains grown with limiting isoleucine. (A) Banding of IRS in CsCl gradients of the sample taken during growth in D_2O minimal medium supplemented with isoleucine, valine, and leucine; (B and C) the same activity in samples collected after 25 and 50% mass doublings, respectively, following the transfer to water-isoleucine-limited medium. The IRS activity of the normal strain is indicated by the broken line. The bottom of the gradient is to the left of each panel. The activity is expressed as counts per minute of* L-[^{14}C]*isoleucine attached to tRNA under standard conditions. The protein added to the gradient tubes was 0.126 mg.*

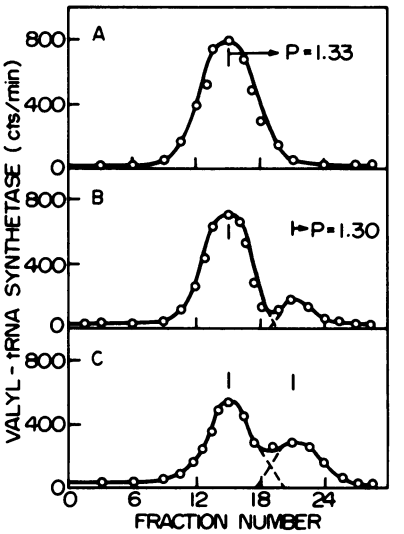

FIG. 3. *Banding in CsCl gradients of VRS from the ilvDAC deletion/λdilvCB strain grown with limiting isoleucine. Samples were taken during D_2O-unrestricted growth (A) and 25% (B) and 50% (C) mass increases following the transfer to water-isoleucine-limited medium. All other procedures were as described for Fig. 2. The activity is expressed as counts per minute of* L-[^{14}C]*valine attached to tRNA under standard conditions. The protein added to each gradient tube was the same as indicated for Fig. 2.*

signal is provided in Fig. 3. The CsCl gradient profiles of VRS in samples taken during growth in D_2O-unrestricted (A) and isoleucine-restricted water (B and C) media for strain CU464 clearly indicate that there was little (if any) inactivation of this activity during a 50% mass increase in isoleucine-restricted medium. More significantly, as judged from the rate of appearance of light (newly synthesized) enzyme in these gradients, there was no derepression of VRS formation in this mutant grown with limiting amounts of L-isoleucine (Fig. 3B and C). This lack or response of VRS formation to an isoleucine limitation in this mutant carrying normal *ilvC* and *ilvB* genes is identical to that observed for the *ilvDAC* deletion strain, but in striking contrast to the regulatory patterns of a normal strain (3, 16, 17). In addition, the results shown in Fig. 4 indicate that the rate of LRS formation was unaffected by isoleucine-limited growth of this *ilvDAC* deletion/λd*ilvCB* strain (CU464). This observation is exactly the same as that observed for the strain with this *ilvDAC* deletion alone. That there was no significant inactivation of LRS activity in this mutant (Fig.

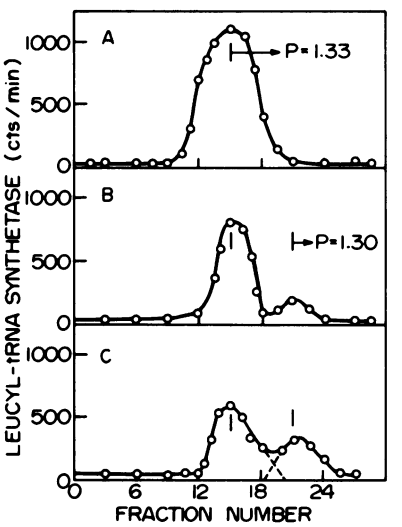

FIG. 4. *Effect of isoleucine limitation of the ilvDAC deletion/λdilvCB strain on LRS formation as revealed by density labeling. The panels show the banding in CsCl gradients of LRS of samples taken during growth in D_2O-unrestricted medium (A) and after 25% (B) and 50% (C) mass increases following the transfer to water-isoleucine-limited medium. All procedures and symbols are as described for Fig. 2. The protein added to each gradient tube was 0.140 mg.*

4B and C) is also in agreement with the results reported for the *ilvDAC* deletion strain, CU356 (3).

Activities of aminoacyl-tRNA synthetases in *ilvA* mutants. To further examine the possible role of threonine deaminase in the synthesis and stability of the branched-chain tRNA synthetases, we compared these activities in strains possessing point mutations in *ilvA* with the same strains also carrying a λ bacteriophage that contained the *ilv* operon. The results (Table 3) indicate that the *ilvA* mutants (i.e., CU406, CU1008, CU370) exhibited reduced VRS and IRS activities. However, upon the return of the *ilv* operon, restoring functional threonine deaminase activity via λ bacteriophages, these two activities were increased to levels comparable to those of the normal strain (Table 3, compare strains CU406 and CU514). Conversely, the LRS, ARS, and HRS activities were unaffected by the functional state or existence of the *ilvA* gene product in these cells (Table 3). Given the reduced VRS and IRS activities in these *ilvA* mutants, it was of interest to ascertain whether their synthesis was responsive to a limiting isoleucine signal. The results (Table 4) indicate that, upon an isoleucine limitation, VRS synthesis was derepressed in each mutant, while IRS formation was generally refractory to the limiting isoleucine signal. As expected, synthesis of LRS, ARS, and HRS was unaltered during isoleucine-limited growth (Table 4).

The reduced VRS and IRS activities of these *ilvA* mutants could be explained by (i) a decreased in vivo stability and/or (ii) a reduced rate of synthesis. There is a direct correlation between the functional state of threonine deaminase, the *ilvA* gene product, and the rate of synthesis of VRS and IRS (e.g., strains CU367, CU406, and CU514) during unrestricted growth (Table 5). Thus, these results (Tables 4 and 5) clearly show that some mutations in *ilvA*, conferring an altered form and/or reduced amounts of threonine deaminase, have no affect on the leucine-specific repression control of LRS synthesis and confer no change in the stability of

TABLE 5. *Relative differential rates of aminoacyl-tRNA synthetase formation in threonine deaminase mutants*[a]

Strain	Relevant genotype	Rate[b]			
		VRS	IRS	LRS	ARS
CU367	*ilvC462 leu-455*	1.00	1.00	1.00	1.00
CU406	*ilvA454*	0.42	0.61	0.88	0.97
CU514	*ilvA454*(λdil*v*)	0.81	1.07	0.85	0.95
CU1008	*ilvA454*	0.51	0.69	0.97	1.06

[a] Growth of the cells, composition of the unrestricted growth medium, and measurement of synthetase activities were as described in Table 2.

[b] Relative differential rates of synthesis were calculated from differential plots of the data and expressed as synthetase units per milliliter of culture as a function of total protein per milliliter of culture. The values for the mutant strains are expressed relative to those of the normal strain, CU367, which are set to equal 1.00.

TABLE 3. *Activities of aminoacyl-tRNA synthetases in ilvA mutants*[a]

Strain	Relevant genotype	Sp act[b]				
		VRS	IRS	LRS	ARS	HRS
CU367	*ilvC462 leu-455*	0.31	0.14	0.18	0.26	0.14
CU406	*ilvA454*	0.19	0.08	0.15	0.24	0.15
CU514	*ilvA454*(λdil*v*)	0.27	0.12	0.19	0.27	0.13
CU1008	*ilvA454*	0.20	0.09	0.16	0.26	0.14
CU370	*ilvA454*	0.19	0.09	0.15	0.28	0.13
CU473	*ilvA545*(λh80)	0.21	0.10	0.16	0.27	0.14

[a] Growth of the cells and all other procedures were as indicated in Table 2.

[b] Expressed as micromoles of product formed per hour per milligram of protein.

TABLE 4. *Effects of isoleucine limitation on synthesis of aminoacyl tRNA synthetases of ilvA mutants*[a]

Strain	Relevant genotype	Sp act[b]				
		VRS	IRS	LRS	ARS	HRS
CU367	*ilvC462 leu-455*	0.49	0.28	0.18	0.26	0.15
CU406	*ilvA454*	0.33	0.09	0.19	0.25	0.14
CU370	*ilvA454*	0.37	0.15	0.18	0.27	0.16
CU1008	*ilvA454*	0.38	0.11	0.17	0.25	0.17

[a] Cells were initially grown exponentially in minimal medium supplemented with excess isoleucine, valine, and leucine. Then each culture was collected by centrifugation, washed twice with minimal medium, and suspended in minimal medium with excess valine and leucine and 10 μg of glycyl-DL-isoleucine per ml.

[b] Expressed as micromoles of product formed per hour per milligram of protein.

this synthetase in such mutants. However, such mutations lead to a reduced rate of formation of VRS, even though its synthesis remains responsive to the supply of valine and isoleucine (i.e., multivalent control by these two amino acids is unaltered). In contrast, IRS formation is refractory to the isoleucine-mediated derepression signal(s), and its rate of synthesis is reduced relative to that of the normal strain (i.e., wild-type *ilvA* gene).

DISCUSSION

We have previously shown that synthesis of VRS, IRS, and LRS is controlled by a repression process in a parallel but noncoordinate fashion with controlled synthesis of the isoleucine-, valine-, and leucine-forming enzymes (2, 6, 16). In addition to these noncoordinate patterns of control, the regulation of these tRNA synthetases differs from multivalent repression control of the *ilv* and *leu* biosynthetic enzymes in the following respects. Among these three tRNA synthetases, only the control of VRS formation is multivalent in nature, requiring both valine and isoleucine for complete repression (16, 17). Repression control of IRS and LRS formation is mediated by the supply of the respective branched-chain amino acid (16, 17). However, it has been observed that several mutations, unlinked to the *ilv* and *leu* operons as well as the *valS*, *leuS*, and *ileS* genes, confer altered regulatory patterns for both classes (i.e., biosynthetic and synthetase) of these branched-chain enzymes (9; L. S. Williams, E. Kline, and H. E. Umbarger, manuscript in preparation). Consistent with models that postulate the involvement of aminoacylated $tRNA^{Val}$, $tRNA^{Leu}$, and $tRNA^{Ile}$ in repression control of the *ilv* and *leu* gene products (2, 4, 7, 27), one could explain the parallel alterations in regulation of both classes of these enzymes in some of these mutants by "cause and effect relations." Specifically, some of these mutations may be in genes encoding elements specific for synthetase regulation. Thus, changes in the repression/derepression mode for the *ilv* and *leu* gene products may be the result of deficient or excess aminoacylation of specific isoaccepting species of $tRNA^{Val}$, $tRNA^{Leu}$, and $tRNA^{Ile}$, owing to alterations in the levels of the branched-chain tRNA synthetases.

Other observations reported for these two classes of enzymes are not easily explained by the above model. Levinthal et al. (13, 14) have shown that a mutation in *ilvA*, the structural gene for threonine deaminase, altered the derepression responses *valS*, *ileS*, and *leuS* and the *ilv* and *leu* operons. These results lead to the suggestion that threonine deaminase has a positive role in repression control of both classes of these enzymes. Further evidence for the involvement of threonine deaminase (in some form) in control of synthesis of these enzymes was provided by pyridoxine restriction of cultures otherwise supplemented with excess amounts of isoleucine, valine, and leucine. It was observed that a normal strain and this same *ilvA* mutant strain described by Levinthal et al. (14) exhibited differential regulatory responses to pyridoxine limitations (L. S. Williams, unpublished data). In addition, Coleman et al. (3) have demonstrated that an *E. coli* K-12 strain possessing a deletion of three genes of the *ilv* cluster, *ilvD*, *-A*, and *-C*, exhibited alterations in stability and synthesis of VRS, IRS, and LRS. It was suggested that the specific inactivation of these synthetase activities, and even alterations in control of synthesis, were due to absence of these three *ilv*-specific gene products. In view of other results, we suggested that these observations could be reasonably explained by the absence of threonine deaminase in this deletion mutant (9, 13, 14).

In the present paper, we report that introduction of the *ilvC* gene (via λd*ilvCB* bacteriophage) to this *ilvDAC* deletion strain was not sufficient to restore the normal steady-state unrestricted levels of these synthetases. In fact, upon an isoleucine limitation of this strain, IRS activity was further inactivated, and the cells were unable to elicit derepression of VRS formation. Both of these results are identical to those found for the deletion strain missing the *ilvD*, *-A*, and *-C* gene products. Furthermore, since there were no detectable alterations in activity or synthesis of these synthetases in *ilvD* mutants, we further suggest that the product of this gene has no role in the regulatory responses of this *ilvDAC* deletion mutant. It would, therefore, appear reasonable to ascribe these alterations in stability of activity and control of synthesis of IRS, VRS, and LRS to the absence of threonine deaminase in these mutants. Furthermore, in each mutant strain examined, a single point mutation in *ilvA* caused a reduction in VRS and IRS activities. That these activity changes were due to the mutation in *ilvA* is supported by the observation that the introduction of the *ilv* operon in such mutants restored these synthetase activities to the normal levels. It is then suggested that the in vivo activity of VRS as well as IRS is mediated by a functional threonine deaminase, which serves as a regulatory effector for these synthetases.

Based on the results reported by Levinthal et al. (13, 14) and Coleman et al. (3) and those of the present report, it is suggested that all these observations could be explained by the following

model. Repression of synthesis of the branched-chain tRNA synthetases results from the formation of complexes (regulatory) between threonine deaminase: val-tRNA^{Val} and tRNA^{Ile} for VRS; threonine deaminase: ile-tRNA^{Ile} for IRS; and threonine deaminase: leu-tRNA^{Leu} for LRS. On the other hand, branched-chain amino acid-specific derepression of synthesis would be mediated by a complex of threonine deaminase and the respective branched-chain tRNA synthetases as free molecules (Eo), owing to the reduction in the supply of these amino acids (i.e., "low pools" of aminoacyl-adenylates and aminoacyl-tRNA's). In this respect, these synthetases would serve as positive control elements for their own synthesis (autoregulatory). This model is supported in part by recent indications of the existence of complexes of threonine deaminase and specific branched-chain tRNA synthetases (L. Dickson and L. S. Williams, unpublished data).

Furthermore, with the exception of studies employing *hisT*(tRNA) mutants (4, 24) and certain azaleucine-resistant isolates (L. S. Williams and H. E. Umbarger, unpublished data), most reports on the role of aminoacylated tRNA^{Val}, tRNA^{Ile}, and tRNA^{Leu} in repression control of the *ilv* and *leu* operons do not exclude the direct involvement of IRS, VRS, and LRS in these control processes. It is possible that these enzymes have a direct role in the generation of the repression control signal(s) for these biosynthetic operons. This suggestion should be viewed in the context of recent reports that the isoleucine-valine (25) as well as the histidine (11) and tryptophan (1, 12, 18, 22) operons contain a positive control element in addition to a "classical-repressor recognition site" leading to dual control mechanisms for expression of these operons.

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LITERATURE CITED

1. **Bertrand, K., L. Korn, F. Lee, C. Squires, C. Squires, and C. Yanofsky.** 1975. New features of the regulation of the tryptophan operon. Science **189**:22-26.
2. **Calvo, J. M., M. Freundlich, and H. E. Umbarger.** 1969. Regulation of branched-chain amino acid biosynthesis in *Salmonella typhimurium*: isolation of regulatory mutants. J. Bacteriol. **97**:1272-1282.
3. **Coleman, W., Jr., E. L. Kline, C. S. Brown, and L. S. Williams.** 1975. Regulation of branched-chain aminoacyl-transfer ribonucleic acid synthetases in an *ilvDAC* deletion strain of *Escherichia coli* K-12. J. Bacteriol. **121**:785-793.
4. **Cortese, R., R. Landsberg, R. A. Vonder Haar, H. E. Umbarger, and B. N. Ames.** 1974. Pleiotropy of *hisT* mutants blocked in pseudouridine synthesis in tRNA: leucine and isoleucine-valine operons. Proc. Natl. Acad. Sci. U.S.A. **71**:1857-1861.
5. **Fraenkel, D. G., and F. C. Neidhardt.** 1961. Use of chloramphenicol to study control of RNA synthesis in bacteria. Biochim. Biophys. Acta **53**:96-110.
6. **Freundlich, M., R. O. Burns, and H. E. Umbarger.** 1962. Control of isoleucine, valine and leucine biosynthesis. I. Multivalent repression. Proc. Natl. Acad. Sci. U.S.A. **48**:1804-1808.
7. **Hatfield, G. W., and R. O. Burns.** 1970. Specific binding of leucyl-transfer RNA to an immature form of L-threonine deaminase: its implication in repression. Proc. Natl. Acad. Sci. U.S.A. **66**:1027-1035.
8. **Hirshfield, I. N., C. Liu, and F. M. Yeh.** 1977. Two modes of metabolic regulation of lysyl-transfer ribonucleic acid synthetase in *Escherichia coli* K-12. J. Bacteriol. **131**:589-591.
9. **Jackson, J., L. S. Williams, and H. E. Umbarger.** 1974. Regulation of synthesis of the branched-chain amino acids and cognate aminoacyl-transfer ribonucleic acid synthetases of *Escherichia coli*: a common regulatory element. J. Bacteriol. **120**:1380-1386.
10. **Johnson, R. C., P. R. Vanatta, and J. R. Fresco.** 1977. Metabolic regulation of aminoacyl-tRNA synthetase biosynthesis in baker's yeast. J. Biol. Chem. **252**:878-882.
11. **Kasai, T.** 1974. Regulation of the expression of the histidine operon in *Salmonella typhimurium*. Nature (London) **249**:523-527.
12. **Lee, F., C. L. Squires, C. Squires, and C. Yanofsky.** 1976. Termination of transcription in vitro in the *Escherichia coli* tryptophan operon leader region. J. Mol. Biol. **103**:383-393.
13. **Levinthal, M., M. Levinthal, and L. S. Williams.** 1976. The regulation of the *ilvADGE* operon: evidence for postive control by threonine deaminase. J. Mol. Biol. **102**:433-465.
14. **Levinthal, M., L. S. Williams, M. Levinthal, and H. E. Umbarger.** 1973. Role of threonine deaminase in the regulation of isoleucine and valine biosynthesis. Nature (London) New Biol. **246**:65-68.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
16. **McGinnis, E., and L. S. Williams.** 1971. Regulation of synthesis of the aminoacyl-transfer ribonucleic acid synthetases for the branched-chain amino acids of *Escherichia coli*. J. Bacteriol. **108**:254-262.
17. **McGinnis, E., A. C. Williams, and L. S. Williams.** 1974. Derepression of synthesis of the aminoacyl-transfer ribonucleic acid synthetases for the branched-chain amino acids of *Escherichia coli*. J. Bacteriol. **119**:554-559.
18. **Morse, D., and A. Morse.** 1976. Dual control of the tryptophan operon by the repressor and tryptophanyl-tRNA synthetase. J. Mol. Biol. **103**:209-226.
19. **Neidhardt, F. C., J. Parker, and W. G. McKeever.** 1975. Function and regulation of aminoacyl-tRNA synthetases in prokaryotic and eukaryotic cells. Annu. Rev. Microbiol. **29**:215-250.
20. **Parker, J., M. Flashner, W. G. McKeever, and F. C. Neidhardt.** 1974. Metabolic regulation of the arginyl-

and valyl-transfer ribonucleic acid synthetases in bacteria. J. Biol. Chem. **249**:1044–1053.

21. **Parker, J., and F. C. Neidhardt.** 1972. Metabolic regulation of aminoacyl-tRNA synthetase formation in bacteria. Biochem. Biophys. Res. Commun. **49**:495–501.
22. **Powels, P. H., and H. Pannekoek.** 1976. A transcriptional barrier in the regulatory region of the tryptophan operon of *Escherichia coli*: its role in the regulation of repressor-independent RNA synthesis. Mol. Gen. Genet. **149**:255–265.
23. **Reeh, S., S. Pederson, and F. C. Neidhardt.** 1977. Transient rates of synthesis of five aminoacyl-transfer ribonucleic acid synthetases during a shift-up of *Escherichia coli*. J. Bacteriol. **129**:702–706.
24. **Rizzino, A. A., R. S. Bresalier, and M. Freundlich.** 1974. Derepressed levels of the isoleucine-valine and leucine enzymes in *hisT1504*, a strain of *Salmonella typhimurium* with altered leucine transfer ribonucleic acid. J. Bacteriol. **117**:449–455.
25. **Smith, J. M., D. E. Smolin, and H. E. Umbarger.** 1976. Polarity and the regulation of the *ilv* gene cluster in *Escherichia coli* strain K-12. Mol. Gen. Genet. **148**:111–124.
26. **Umbarger, H. E.** 1971. The regulation of enzyme levels in the pathways to the branched-chain amino acids, p. 44–462. *In* H. J. Vogel (ed.), Metabolic regulation, vol. 5. Academic Press Inc., New York.
27. **Williams, L. S., and F. C. Neidhardt.** 1969. Synthesis and inactivation of aminoacyl-tRNA synthetases during growth of *Escherichia coli*. J. Mol. Biol. **43**:529–550.