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CONTROL OF DOWNSTREAM AMPLIFICATION IN THE ilvEDA OPERON IN ISOLEUCYL-, VALYL-, AND LEUCYL-tRNA SYNTHETASE MUTANTS OF ESCHERICHIA COLI K-12

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SUMMARY: The role of isoleucyl-, valyl-, and leucyl-tRNA synthetases in attenuation of the ilvEDA operon was examined. The results indicate that the activities of isoleucyl- and valyl-tRNA synthetases are necessary to maintain attenuation of the ilvEDA operon. Leucyl-tRNA synthetase activity is nonessential for attenuation. These studies imply that uncharged $tRNA^{Ile}$ and $tRNA^{Val}$ each may cause deattenuation.

The importance of attenuation in the control of gene expression for biosynthetic enzymes has been extensively studied for the tryptophan and histidine pathways (1,2). Recent evidence suggests that isoleucine-valine biosynthesis is subject to attenuation control (3). When the ilvEDA operon is attenuated, the number of transcripts of ilvE, coding for transaminase B, may exceed the number of transcripts of ilvA, which codes for threonine deaminase. Isoleucine or valine restriction of growth in isoleucine-valine auxotrophs amplifies the promoter-distal ilvA expression relative to ilvE (3). This phenomenon is termed deattenuation or downstream amplification. Specific activities of threonine deaminase {L-threonine hydrolyase (deaminating), EC 4.2.1.16} and transaminase B (aliphatic-L-amino acid 2-oxoacid amino transferase, EC 2.6.1.6) probably reflect the number of ilvA and ilvE transcripts respectively.

Mutants of Escherichia coli K-12 with temperature-sensitive leucyl-tRNA synthetase[1] (EC 6.1.1.4, leucine: sRNA ligase, AMP) or valyl-tRNA

[1]The following abbreviations will be used throughout the text: $LeuRS^{ts}$ for temperature-sensitive leucyl-tRNA synthetase; $ValRS^{ts}$, for temperature-sensitive valyl-tRNA synthetase; and $IleRS^{km}$, for isoleucyl-tRNA synthetase with a high Km for L-isoleucine.

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synthetase[1] (EC 6.1.1.9, valine: sRNA ligase, AMP), and a mutant containing isoleucyl-tRNA synthetase[1] (EC 6.1.1.5, isoleucine: sRNA ligase, AMP) with a high K_m for isoleucine were used. We present evidence that IleRS and ValRS activities are required to control downstream amplification of the ilvEDA operon. A preliminary account of this work has appeared previously (4).

MATERIALS AND METHODS

Escherichia coli K-12 strains KL231 (gift from D. Söll, 5) and NP29 (gift from L.S. Williams) were grown in a glucose-salts minimal medium (6) at a growth permissive temperature (30°C) and shifted to a growth restrictive temperature (41°C or 37°C) as indicated. Strains MI1 and PB154 (gifts from M. Iaccarino, 7) were grown at 37° in minimal medium supplemented with 0.4 mM L-isoleucine for permissive conditions, and shifted to minimal medium to restrict growth of strain MI1. Strain MJ1 is a prototrophic K-12 strain (formerly CU1 from H.E. Umbarger) and PB154 is the parent of MI1. Culture samples were removed at approximate mass increments of 20% of the initial cell mass. Threonine deaminase and transaminase B were assayed in crude extracts (8) and by a detergent-permeabilized cell technique described elsewhere (9, and manuscript in preparation). Protein was measured by the biuret method (10) and cell culture density was determined in a Klett-Summerson colorimeter with a no. 42 (blue) filter. Amino acid supplementation of growth media was at 0.4 mM for L-isoleucine and L-leucine, and 1.2 mM for L-valine. All chemicals were of reagent grade or of the highest purity commercially available.

RESULTS

The differential rates of synthesis of threonine deaminase and transaminase B were measured in E. coli strains MJ1 and KL231 under permissive (30°) and non-permissive (41°) growth conditions in repressing medium (minimal medium supplemented with L-isoleucine, L-valine and L-leucine) and in minimal medium. The results are expressed as specific activity versus the change in culture density (Figs. 1A and 1B). At 41°C the $LeuRS^{ts}$ activity in KL231 was sufficiently low to restrict the cell growth rate. The differential rates of synthesis of threonine deaminase and transaminase B increased coordinately. Thus, downstream amplification did not occur, since the ratio of threonine deaminase to transaminase B did not change under restrictive conditions (Table 1).

Strain NP29 ($ValRS^{ts}$) was examined by a similar procedure and compared to strain MJ1, a strain identical in phenotype to the original parent strain

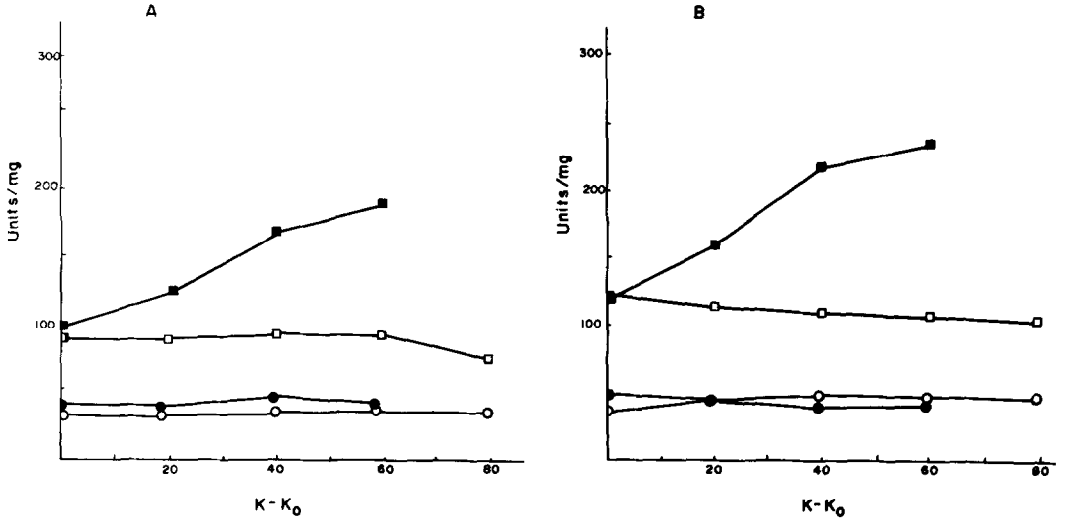

Fig. 1. Effect of leucyl-tRNA synthetase activity on expression of ilvA and ilvE. (A) Specific activity of threonine deaminase: Strain MJ1 (w.t.) grown at 30°C (○), and after a shift to 41°C (●); Strain KL231 (LeuRSts) grown at 30°C (□) and after a shift to 41°C (■). Culture growth is plotted as the change in turbidity (K-Ko) during growth in repressing medium measured by a Klett-Summerson colorimeter with a no. 42 filter. One unit of activity is defined as 1.0 n mole of α-oxobutyrate formed per min. (B) Same as 1A except transaminase B was measured, where one unit of activity is defined as 1.0 n mole of α-oxoisovalerate formed per min.

NP2 (11). After a shift to restrictive growth conditions (37°C) in repressing medium, the specific activity of threonine deaminase increased whereas that of transaminase B did not, relative to growth at 30°C (Figs. 2A and 2B). Thus, a slight downstream amplification was evident from the increased ratio of threonine deaminase to transaminase B specific activities (Table 1). Growth of NP29 in minimal medium at 30°C resulted in an increased specific activity for both enzymes when compared to growth in repressing medium (Figs. 2A and 2B). However, no further increases were observed upon shifting to restrictive growth conditions (37°C). The ratio of threonine deaminase to transaminase B specific activity was approximately two-fold higher in minimal than in repressing medium at a permissive growth temperature (Table 1). We take this result to indicate downstream amplification of ilvA expression. Thus, strain NP29, grown in minimal medium, appeared to be both fully de-

TABLE 1. Ratios of maximum specific activities[1]: threonine deaminase/transaminase B

Strain and Relevant Phenotype	Growth[2] Medium	Permissive[3] Growth Conditions	Restrictive[3] Growth Conditions
MJ1 (K-12 w.t.)	Minimal	0.957(±.060)	0.796(±.069)
	Minimal + IVL	0.767(±.062)	0.664(±.050)
KL231 (LeuRS^{ts})	Minimal	0.755(±.183)	0.831(±.123)
	Minimal + IVL	0.795(±.057)	0.793(±.022)
NP29 (ValRS^{ts})	Minimal	2.252(±.041)	2.134
	Minimal + IVL	1.237(±.169)	1.969
PB154 (Parent of MI1)	Minimal	———	0.680(±.034)
	Minimal + I	0.625(±.062)	———
MI1 (IleRS-High K_m for isoleucine)	Minimal	———	3.058
	Minimal + I	1.189(±.048)	———

[1]Maximum specific activity is the highest single value obtained under growth conditions in which the value would be expected to change. Under growth conditions in which the specific activity remains constant, the value reported is the average from four samples taken during the course of cell growth and corresponds to the differential rate of synthesis of the enzyme. Ratios are reported as the average value ± one mean deviation, except where only one sample displayed the maximum specific activity ratio.

[2]Minimal medium was supplemented with L-isoleucine (I), L-valine (V), or L-leucine (L) where indicated.

[3]Permissive and restrictive growth conditions are defined under materials and methods. Strains PB154 and MI1 are not temperature-sensitive, therefore a restrictive condition for these strains is defined as growth in minimal medium, whereas growth in minimal + I is permissive.

repressed and maximally deattenuated.

Strain PB154 (parent of MI1) and MI1 (IleRS^{km}) were examined to determine the requirement for isoleucyl-tRNA synthetase activity in attenuation of *ilvEDA*. The strains were grown in minimal medium supplemented with L-isoleucine for permissive conditions. Cultures were divided after reaching a turbidity of 100 Klett Units, and one-half was centrifuged at room temperature and resuspended for growth in minimal medium, which constituted restrictive conditions for strain MI1. Since the IleRS^{km} mutant required a

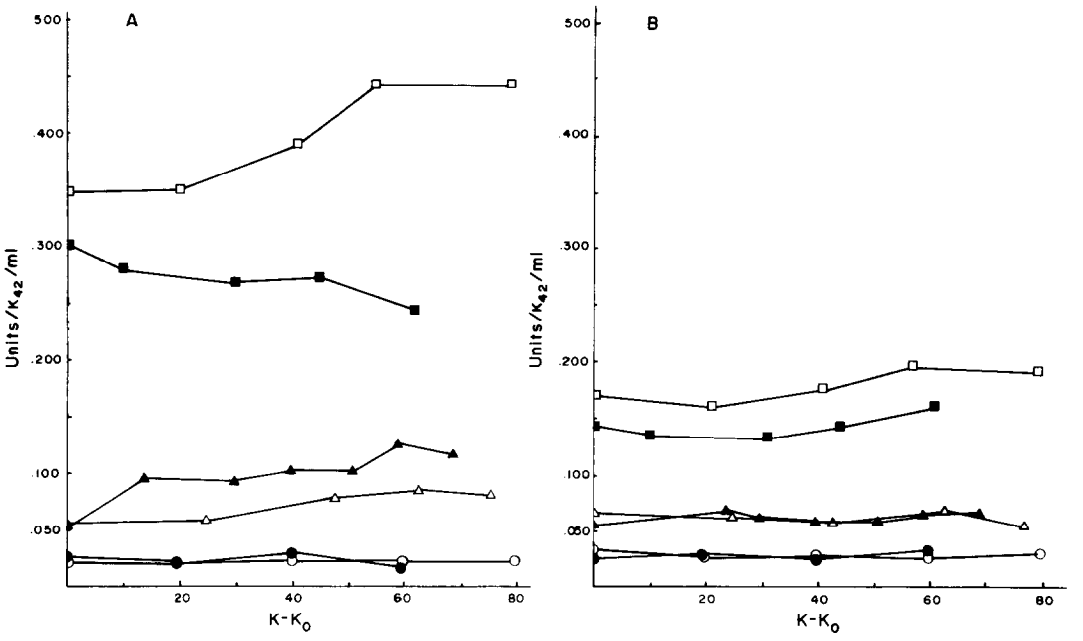

Fig. 2. Effect of valyl-tRNA synthetase activity on expression of ilvA and ilvE. (A) Specific activity of threonine deaminase: Strain MJ1 (w.t.) grown in minimal medium at 30°C (O), and after a shift to 37°C (●); Strain NP29 (ValRSts) grown in minimal medium at 30°C (□), and after a shift to 37°C (■); Strain NP29 grown in repressing medium at 30°C (△), and after a shift to 37°C (▲). Culture growth is plotted as the change in turbidity (K-Ko) measured by a Klett-Summerson colorimeter with a no. 42 filter. One unit of activity is defined as 1.0 n mole of α-oxobutyrate formed per min. One unit of cell mass is defined as 1.0 ml of cell culture with a turbidity of one Klett unit (K_{42}/ml). (B) Same as 2A except transaminase B was measured, where one unit of activity is defined as 1.0 n mole of α-oxoisovalerate formed per min.

high concentration of L-isoleucine to grow normally, the absence of supplementation with L-isolecuine caused a growth restriction, presumably caused by insufficient Ile-tRNA^{Ile} to support a normal rate of protein synthesis. Consequently, the rate of synthesis of threonine deaminase rapidly increased following the shift to restrictive growth conditions, whereas the rate of transaminase B synthesis did not change (Figs. 3A and 3B). Thus the ratio of threonine deaminase to transaminase B specific activity increased approximately two and one-half times as a consequence of the shift to restrictive conditions of growth (Table 1). Thus, downstream amplification of ilvA expression occurred as a consequence of isoleucyl-tRNA synthetase modification

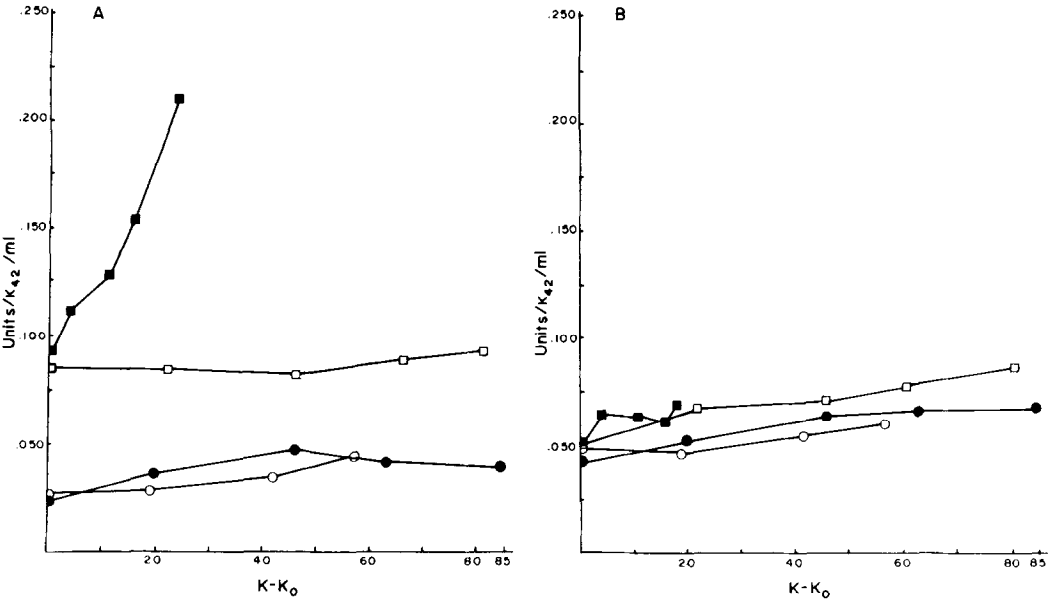

Fig. 3. Effect of IleRS^{Km} activity on expression of ilvA and ilvE. (A) Specific activity of threonine deaminase: Strain PB154 (parent of MI1) grown at 37°C in minimal medium supplemented with 0.4 mM L-isoleucine (○) and after a shift to minimal medium (●); Strain MI1 (IleRS^{Km}) grown at 37°C in minimal medium supplemented with 0.4 mM L-isoleucine (□) and after a shift to minimal medium (■). Culture growth and enzyme activity were measured as described in Fig. 2. (B) Same as 3A except transaminase B activity was measured.

and/or restriction of Ile-tRNA^{Ile} supply.

DISCUSSION

Since the specific activity and the differential rate of synthesis of transaminase B and threonine deaminase probably reflect the number of mRNA transcripts of ilvE and ilvA, then the ratio of specific activities would be expected to be proportional to the ratio of the number of transcripts. This ratio does not change when LeuRS^{ts} activity is decreased at restrictive temperatures, because the rates of synthesis of both threonine deaminase and transaminase B increase by the same factor. Therefore, heat inactivation of LeuRS^{ts} *in vivo* causes a coordinate derepression of ilvEDA but does not cause deattenuation. In contrast, heat inactivation of ValRS^{ts} *in vivo* not only causes the rates of synthesis of both threonine deaminase and transaminase

B to increase, it also increases the ratio of threonine deaminase to transaminase B. Therefore, loss of $ValRS^{ts}$ activity causes both derepression and deattenuation of *ilvEDA*. $ValRS^{ts}$ activity is low even under repressing conditions at a permissive temperature, which causes strain NP29 to have a higher generation time (T = 3.5 h) than the control strain (T = 2.7 h), presumably caused by a depletion of valyl-$tRNA^{Val}$. The gradual decrease in specific activity of threonine deaminase at the restrictive temperature indicates that no further deattenuation occurs, and that this enzyme loses activity, probably because of increased protease activity. The apparent derepression that we observe is consistent with published reports for leucly- and valyl-tRNA synthetase effects (5,12), although their effects on coordinate expression have not been effectively measured previously. Modification of isoleucyl-tRNA synthetase activity results in an increase in the ratio of threonine deaminase to transaminase B upon isoleucine restriction, with no significant increase in the rate of transaminase B synthesis. We interpret this to mean that deattenuation, but not derepression of *ilvEDA* occurs in response to restriction of Ile-$tRNA^{Ile}$ supply or modification of isoleucyl-tRNA synthetase activity.

These results immediately suggest two possible interpretations: 1) Uncharged $tRNA^{Val}$ and $tRNA^{Ile}$ release *ilvEDA* from attenuation control; or 2) Valyl- and isoleucyl-tRNA synthetases and/or valyl-$tRNA^{Val}$ and isoleucyl-$tRNA^{Ile}$ function to establish attenuation. In view of the proposed role for rho-factor in causing attenuation (1, 13) we suggest that uncharged $tRNA^{Val}$ and $tRNA^{Ile}$ may inactivate an *ilv*-specific rho-factor attenuation complex. Alternatively, the functional tRNA synthetases or their aminoacylated tRNA products may function as part of an attenuation complex.

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