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Growth Rate-Related Regulation of the *ilvGMEDA* Operon of *Escherichia coli* K-12 Is a Consequence of the Polar Frameshift Mutation in the *ilvG* Gene of This Strain

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In *Escherichia coli* K-12 the intracellular levels of threonine deaminase and transaminase B, products of *ilvA* and *ilvE*, respectively, in the *ilvGMEDA* operon, increase with increasing growth rates (S. Pedersen, P. L. Bloch, S. Reeh, and F. C. Neidhardt, Cell 14:179–190, 1978). However, the transcriptional activities of the upstream $ilvp_G$ and the internal $ilvp_E$ promoters do not increase. Therefore, the growth rate-related expression of this operon is not regulated at the level of transcription initiation. Unlike other wild-type *E. coli* strains, *E. coli* K-12 contains a polar frameshift mutation in the *ilvG* gene (R. P. Lawther, D. H. Calhoun, C. W. Adams, C. A. Hauser, J. Gray, and G. W. Hatfield, Proc. Natl. Acad. Sci. USA 78:922–925, 1981). In an *E. coli* K-12 (IlvG$^+$) derivative strain, where the reading frame of the *ilvG* gene is restored, no growth rate-related expression of the *ilvGMEDA* operon is observed. Thus, the growth rate-related expression of the *ilvGMEDA* operon in *E. coli* K-12 is the fortuitous consequence of the polar frameshift mutation in the *ilvG* gene of this strain.

The *ilvGMEDA* operon of *Escherichia coli* K-12 is an amino acid-biosynthetic operon required for the synthesis of the branched-chain amino acids isoleucine, leucine, and valine (21). Transcription through the leader region of this operon into the structural genes is regulated by the leucine-responsive regulatory protein, Lrp (18), and a multivalent attenuation mechanism responsive to the intracellular levels of isoleucine, valine, and leucine (8, 11). The operon is transcribed from a strong σ^{70} promoter, $ilvp_G$. Transcription from $ilvp_G$ is activated by two upstream activating sequences, UAS1 and UAS2 (13, 14). UAS1, centered 92 bp upstream from the transcriptional start site, contains a binding site for integration host factor (IHF) (17, 20, 25). IHF binding at this site forms a nucleoprotein complex that alters the structure of a supercoiled DNA template in a way that unwinds the DNA helix in the −10 region of the downstream promoter and facilitates open complex formation (14). This IHF-mediated, DNA structural transmission mechanism amplifies the sensitivity of the downstream promoter to increases in superhelical density of the bacterial chromosome and activates transcription up to fivefold (15). UAS2 contains an UP element (19) that activates transcription from the $ilvp_G$ promoter another 15-fold. A basal level of transcription through the downstream *EDA* genes is maintained by an internal promoter, $ilvp_E$ (22).

Pedersen et al. (16) cataloged the relative abundance of a large number of proteins in *E. coli* grown in minimal glucose medium (doubling time, 0.83 h) and minimal acetate medium (doubling time, 2.5 h) by separating the proteins of *E. coli* whole-cell extracts on two-dimensional O'Farrell protein gels (12). They observed that the amounts of the products of the *ilvE* and *ilvA* genes, transaminase B and threonine deaminase, respectively, increased relative to total cell mass and growth rate (16). To study the mechanism of this growth rate-related regulation of the *ilvGMEDA* operon, we performed threonine deaminase assays (1) with whole-cell extracts prepared from wild-type *E. coli* K-12 cells grown in minimal media containing either glucose, glycerol, or acetate and measured growth rate doubling times of 0.83, 1.68, and 2.67 h, respectively. As previously noted (16), threonine deaminase activity increased with increasing growth rate (Fig. 1A), suggesting that like the well-studied operons of the protein synthesis system (5), the expression of the *ilvGMEDA* operon was subject to growth rate-dependent control.

To determine if this growth rate-related expression of the *ilvGMEDA* operon was the consequence of a transcriptional control mechanism, the $ilvp_G$ promoter region (base pair positions −248 to +6 relative to the transcriptional start site of $ilvp_G$ [9]) was transcriptionally fused to a *lacZ* reporter gene

TABLE 1. $ilvp_G$::*lacZ* transcriptional fusion expression at different growth rates

Strain	Relevant genotype	Carbon source[a]	β-Galactosidase sp act (nmol of ONP/min/mg of protein)[b]
IHRS551	Δ$ilvp_G$::*lacZ*	Glucose	ND
IH-100	$ilvp_G$::*lacZ*	Glucose	10,520 ± 1,250
IH-105	$ilvp_G$::*lacZ himA*	Glucose	2,504 ± 175
IH-100	$ilvp_G$::*lacZ*	Glycerol	10,024 ± 1,345
IH-105	$ilvp_G$::*lacZ himA*	Glycerol	2,350 ± 215
IH-100	$ilvp_G$::*lacZ*	Acetate	11,035 ± 1,450
IH-105	$ilvp_G$::*lacZ himA*	Acetate	No growth[c]

[a] Each strain was grown in M63 minimal salts medium supplemented with glucose (doubling time = 0.83 h), glycerol (doubling time = 1.68 h), or acetate (doubling time = 2.67 h).

[b] β-Galactosidase activities were measured by assaying for *o*-nitrophenol-β-galactopyranoside hydrolysis in sonicated cells obtained from log-phase cultures (10). The β-galactosidase activities were measured at four time points and two extract concentrations under conditions where the assay is linear with respect to time and extract concentration. Rates of *o*-nitrophenol (ONP) formation were determined by a linear regression analysis of an ONP concentration-versus-time plot, and specific activities were calculated according to the method of Miller (10). ND, not detectable.

[c] IHF$^-$ (*himA*) *E. coli* K-12 strains are unable to grow in media containing acetate as the carbon source (2).

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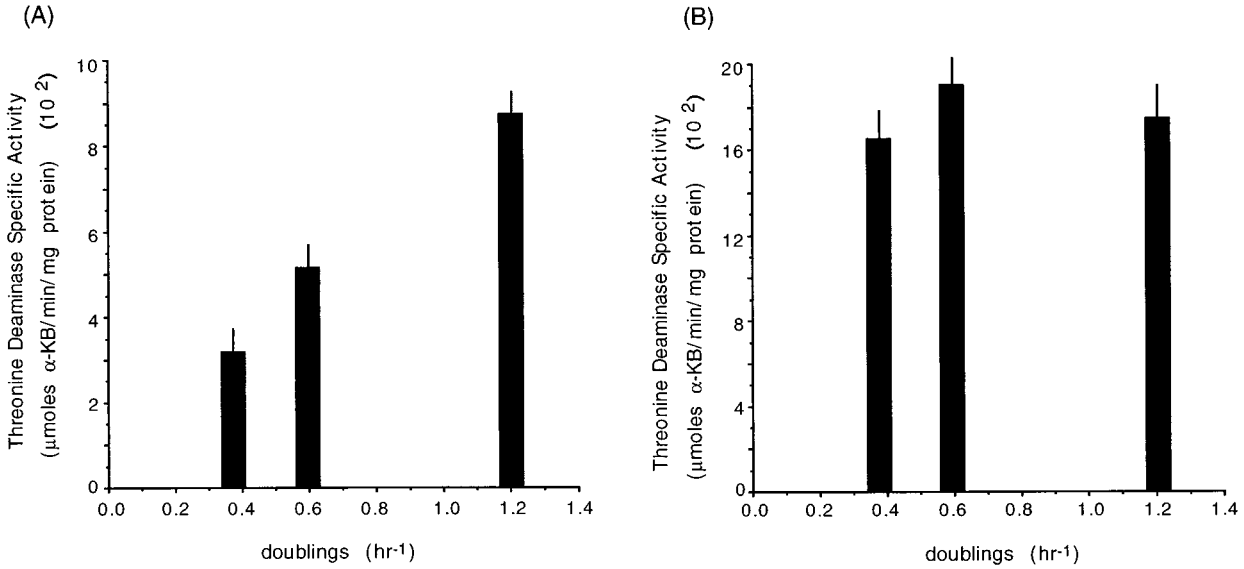

FIG. 1. Threonine deaminase levels in *E. coli* K-12 and *E. coli* K-12 IlvG$^+$ strains. Threonine deaminase was assayed as described by Calhoun et al. (1) in *E. coli* K-12 strains T31-4-4 [*thi-1 trpE9829*(Am) *trpA9761* (Am)] (A) and T31-4-590 [*ilvG468* (IlvG$^+$) *trpE9829*(Am) *trpA9761*(Am)] (B) grown in M63 minimal medium (10) supplemented with acetate, glycerol, or glucose. Cells grew with doubling times of 2.67 h in acetate, 1.68 h in glycerol, and 0.83 h in glucose. Threonine deaminase specific activity is about threefold higher in the IlvG$^+$ strain due to relief of transcriptional polarity caused by the frameshift mutation in the promoter-proximal *ilvG* gene. α-KB, α-ketobutyrate.

and integrated, in a single copy, into the genome of wild-type and *himA* (IHF$^-$) *E. coli* K-12 strains (3, 23). These integrant strains, IH-100 and IH-105, respectively (14), were grown in minimal media containing either glucose, glycerol, or acetate, and the growth rates were measured. The results of this experiment (Table 1) showed that the growth rate-related expression of the *ilvGMEDA* operon is not mediated by either IHF or $ilvp_G$ promoter activity.

In *E. coli* K-12 strains, approximately 41% of the transcription into the operon distal *ilvEDA* genes originates from the internal promoter, $ilvP_E$, when cell growth is not limited by isoleucine, valine, or leucine (22). Although it has been demonstrated that this internal promoter is not regulated by the intracellular levels of endproduct amino acids (4, 22), it remained possible that it might be growth rate regulated. To examine the in vivo transcriptional activity of $ilvp_E$ at different cellular growth rates, a DNA fragment containing this promoter (base pair positions −223 to +109 relative to the transcription start site of $ilvp_E$ [9]) was transcriptionally fused to the *lacZ* gene and integrated into the genome in single copy (3, 23). The data in Table 2 show that the expression of $ilvP_E$ also does not change as a function of growth rate of the cell. These data suggest, therefore, that the growth rate-related expression of the *ilvGMEDA* operon of *E. coli* K-12 is effected by a posttranscriptional mechanism.

A unique feature of the *E. coli* K-12 strain is that, unlike other wild-type *E. coli* strains, it contains a polar frameshift mutation in the middle of the *ilvG* gene (6, 7). The polar frameshift mutation in the *ilvG* gene causes premature polypeptide chain termination and unmasks three latent rho-dependent termination sites (24). To determine if this mutation affects the growth rate-related expression of the *ilvGMEDA* operon, the activity of threonine deaminase was examined in an *E. coli* K-12 IlvG$^+$ strain growing in the presence of different carbon sources and at different rates. In this strain, the specific activity of threonine deaminase did not change as a function of the growth rate of the cell (Fig. 1B). These results demonstrate, therefore, that the growth rate-related expression of the *ilvGMEDA* operon in *E. coli* K-12 is the fortuitous consequence of the polar frameshift mutation in the *ilvG* gene in this strain. Thus, the expression of this operon is not regulated by the growth rate control mechanisms described for the operons of the protein synthesis system (5).

TABLE 2. $ilvp_E$::*lacZ* transcriptional fusion expression at different growth rates[a]

Strain	Relevant genotype	Carbon source	β-Galactosidase sp act (nmol of ONP/min/mg of protein)
IHRS551	Δ$ilvp_E$::*lacZ*	Glucose	ND
IH-RSP$_E$	$ilvp_E$::*lacZ*	Glucose	340 ± 40
IH-RSP$_E$	$ilvp_E$::*lacZ*	Glycerol	290 ± 35
IH-RSP$_E$	$ilvp_E$::*lacZ*	Acetate	375 ± 43

[a] Cell growth conditions, β-galactosidase assay, and generation times are as described in Table 1. ND, not detectable.

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