THE EFFECT OF AN *ilvA* Mu PHAGE INSERTION ON *ilv* GENE EXPRESSION IN *Escherichia coli* K-12.*

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Summary: Physiological analysis of an *E. coli* K-12 strain carrying a Mu phage integrated into the *ilvA* structural gene shows that there is a polar affect on *ilvD* gene expression, whereas, the *ilvE* gene maintains a normal multivalent regulation response. It was also demonstrated that the *ilvC* and *ilvB* genes can be derepressed and repressed in response to *ilv* multivalent control. These experiments demonstrate that the *ilvE* structural gene can be regulated independently from the *ilvA* and *ilvD* structural genes and that the *ilvC* structural gene does not require the complete *ilvA* gene product (threonine deaminase) for its induction.

Genetic and physiological evidence presented by Ramakrishnan and Adelberg show that the gene expression of the products encoded by the *ilvADE* structural genes (Figure 1) are highly elevated when there is a

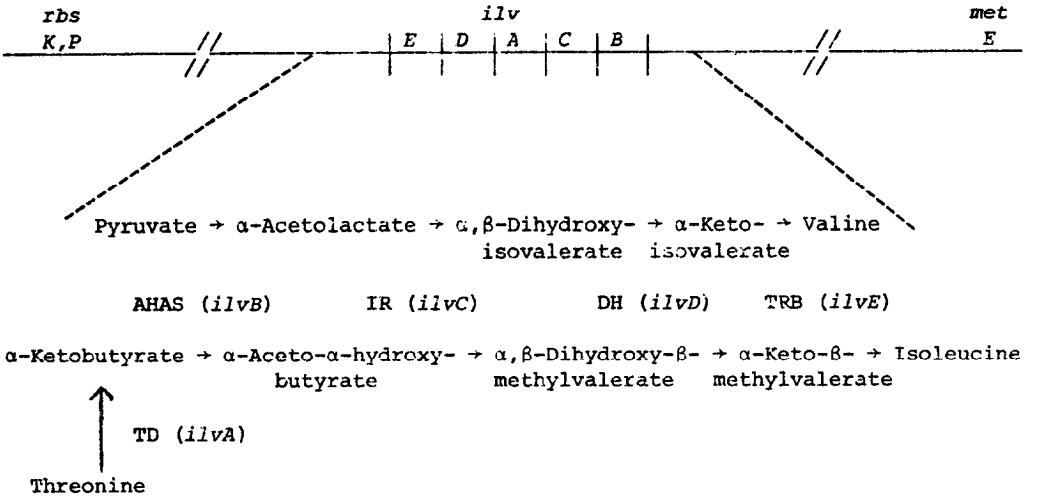

Figure 1. The intermediates formed and enzymes encoded by the *ilv* gene cluster. The genetic order of *ilv* genes is that reported previously (14). Abbreviations of *ilv* enzymes are as follows: TD, threonine deaminase; AHAS, acetohydroxy acid synthase; IR, acetohydroxy acid isomeroreductase; DH, dihydroxy acid dehydrase; TRB, transaminase B.

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lesion in a region they defined as *ilvO* (13). Recent physiological analysis of isogenic strains carrying these different *ilvO* lesions demonstrate that the apparent *ilvO* region also markedly affects the expression of the *ilvB* and *ilvC* structural genes and therefore may be involved in more than a repression recognition site for the *ilvADE* structural genes (7). It was suggested by Kline *et al.* (7), after their investigation of a strain carrying an *ilvDAC* deletion, that the *ilvE* gene may in fact be multivalently regulated independently of the *ilvAD* structural genes.

This analysis of an *E. coli* K-12 strain carrying the Mu phage integrated into the *ilvA* structural gene allows us to more clearly define whether the *ilvE* and *ilvAD* structural genes are regulated via the

TABLE 1. Bacterial Strains

Strains	Genotype	Source or Reference
CU358	*gal*$^-$	P1kc CU12 → CU357
CU370	*gal*$^-$ *ilvA* Mu phage[a] insertion (requires isoleucine and valine)	H. E. Umbarger and M. Levinthal[c]
ES81	*gal*$^-$ *ilvA21*[b]	UV induced isoleucine requiring revertant from CU370
ES82	*gal*$^-$ *ilvD535*	P1kc CU97 → CU12[d]
ES83	*gal*$^-$ *ilvE12*	P1kc CU2 → CU12
ES54	*gal*$^-$ *ilvC462*	P1kc CU1010 → CU12

[a]Strain CU370 was shown in this laboratory to give Ilv$^+$ or Val$^+$ derivatives after UV mutagenesis.

[b]ES81 was shown to give Ile$^+$ derivatives in the presence of diethylsulfonate (DES) or ethyl methanesulfonate (EMS).

[c]The authors appreciate the provision of CU370 by Dr. H. E. Umbarger and Dr. M. Levinthal for this investigation.

[d]The genotype and the derivation of strains not given can be found in references 7, 12, and 17.

previously defined *ilvO* region (14). It also affords us with an opportunity to asses whether the holo-form of threonine deaminase is necessary for isomeroreductase induction as has been previously postulated (2).

EXPERIMENTAL

Organisms and basal medium. The genotypes and derivatives of *Escherichia coli* K-12 used in this study are summarized in Table 1. All bacterial cultures were grown in a modified minimal medium (without citrate) defined by Davis and Mingioli (4).

Utilization of *ilv* biosynthetic intermediate and genetic analysis of CU370. Growth of CU370 on the different *ilv* biosynthetic intermediates demonstrates that the *ilvA* Mu phage insertion strain can only grow on substrates for the *ilvE* structural gene, α-ketoisovalerate and α-keto-β-methylvalerate (Table 2). An isoleucine requiring revertant ES81 (induced by UV mutagenesis) on the other hand is capable of using any

TABLE 2. Utilization of *ilv* biosynthetic intermediates

Strain	THR[a]	KB	VAL KB	AB	AL AB[c]	DMV	DIV DMV[c]	KMV	KIV KMV	ILE	VAL ILE
CU370 (*ilvA* Mu)	-[b]	-	-	-	-	-	-[d]	-	+	-	+
ES81 (*ilvA21*)	-	+	+	+	+	+	+	+	+	+	+
ES82 (*ilvD535*)	-	-	-	-	-	-	-	-	+	-	+
ES83 (*ilvE12*)	-	-	-	-	-	-	-	-	-	-	+
ES54 (*ilvC462*)	-	-	-	-	-	-	+	-	+	-	+

[a] Abbreviations for the *ilv* intermediates and amino acids are given as follows: Threonine (THR), α-ketobutyrate (KB), valine (VAL), α-aceto-α-hydroxybutyrate (AB), α-acetolactate (AL), α,β-dihydroxy-β-methylvalerate (DMV), α,β-dihydroxy-isovalerate (DIV), α-keto-β-methylvalerate (KMV), α-ketoisovalerate (KIV), and isoleucine (ILE).

[b] + or - designates the presence or absence of growth after 24 hours at 37° on glucose minimal medium that was supplimented with the above supplements.

[c] AB, AL, DMV and DIV were synthesized at Edinboro State College and can be obtained through the Edinboro Foundation.

[d] Growth on the dihydroxy acids was determined on glucose minimal medium at a pH of 6.4.

TABLE 3. Effect of an *ilvA* polar lesion on *ilv* gene expression

Strain	Genotype	Growth Conditions[a]	mμMoles/min/mg protein[b] TD	AHAS -val	AHAS +val[c]	IR	DH	TRB
CU358	*gal*⁻ (wild type)[d]	Excess	28	11	3	2	18	20
		Minimal	63	31	4	46	45	48
ES81	*gal*⁻ *ilvA21*	Excess	0	13	2	2	17	21
		Lim ile	0	9	1	5	84	67
CU370	*gal*⁻ *ilvA* Mu	Excess	0	12	3	2	6	19
		Lim ile	0	9	1	5	8	61
		Lim val	0	64	5	112	10	68

[a] Excess grown cultures were grown at 37° in glucose minimal medium that contained 1×10^{-3} M valine and 4×10^{-4} M isoleucine and leucine. The cultures were grown to an optical density (OD) of 0.55 at 660nm. Cells were grown in the same manner for the wild type minimal control without the presence of valine, isoleucine and leucine. Experiments for limitation of a branched-chain amino acid were grown in the same way as the excess grown cultures to an OD of 0.25 at 660nm, washed twice in glucose minimal medium supplemented with an excess of two branched-chain amino acids and a limiting concentration of the third. The cultures were limited for two hours after which each limiting culture had obtained virtually the same optical density. The limiting concentration of each amino acid was as previously described (7). All excess, minimal and limitation experiments were analysed at the same time.

[b] Enzyme assays of crude extracts for threonine deaminase (TD), acetohydroxy acid synthase (AHAS), isomeroreductase (IR), dihydroxy acid dehydrase (DH), and transaminase B (TRB) were as previously described (5, 7, 15).

[c] 1×10^{-3} M valine present in enzyme assay.

[d] Strain CU358 and CU4 were virtually isogenic.

one of the *ilv* biosynthetic intermediates for isoleucine biosynthesis with the exception of threonine (Table 2).

It was also demonstrated through P1 transduction (6) that CU370 (*ilvA* Mu phage insertion strain) would allow isoleucine-valine prototrophic recombinants with any of the *ilvA*⁻, *ilvC*⁻ and *ilvD*⁻ *Escherichia coli* K-12 mutant derivative strains in this laboratory (*ilvA454, ilvA467, ilvA451, ilvA483, ilvA2001, ilvA2002, ilvA2003, ilvC1, ilvC462, ilvC485, ilvC486, ilvC487,* and *ilvD535*).

The effect of the *ilvA* Mu phage insertion on *ilv* gene regulation. The pattern of *ilv* gene expression of strains used in this study are summarized in Tables 3 and 4. Growth of the *ilvA* mu phage insertion

TABLE 4. Effect of an *ilvA* Mu phage insertion on isomeroreductase catalytic expression

Strain	Genotype	mμMoles/min/mg protein[a]		
		Non-induced[b]	Internal induction[b]	External induction[b]
CU358	*gal*⁻ (wild type)	1.6	-----	86.3
ES81	*gal*⁻ *ilvA21*	2.1	-----	73.6
ES370	*gal*⁻ *ilvA* Mu	1.7	109.0	94.0
ES54	*gal*⁻ *ilvC462*	0.8	1.0	0.7

[a]Enzyme assays of crude extracts for acetohydroxy acid isomeroreductase were according to the modification previously described by Kline *et al.* (7).

[b]Freshly grown cultures of isogenic derivative strains (0.05 ml) and their wild type strain were used to inoculate separate one liter flasks containing 350 ml of glucose minimal medium which was supplemented with excess isoleucine (4×10^{-4} M), leucine (4×10^{-4} M) and valine (1×10^{-3} M). The cultures were grown at 37° with shaking until an OD of 0.3 at 660nm was obtained. Each culture was divided into three equal portions and washed twice with prewarmed minimal medium (37°). One third (150 ml) of the culture was resuspended in glucose (0.5%) minimal medium with excess branched-chain amino acid (non-induced) and allowed to grow to an OD of 0.55 at 660nm. The externally induced culture was resuspended and treated in the same manner except that the medium contained a 0.6 mM (pH 7) concentration of α-acetohydroxybutyrate. The internally induced cultures (Lim val) were also resuspended in the same manner except that the medium contained 8×10^{-5} M valine and was allowed to grow for three hours. The method of Lowry *et al.* was used to determine protein concentrations in crude extracts (9).

strain (CU370) in the presence of excess branched-chain amino acids resulted in a normal repression response of transaminase B (*ilvE*), acetohydroxy acid synthase (*ilvB*), and isomeroreductase (*ilvC*) when compared to the closely related wild type strain (CU358). The level of dehydrase (*ilvD*), however, was one fourth that of the wild type activity and there was no detectable activity for the *ilvA* gene product (threonine deaminase).

The expression of the gene products encoded by the *ilvE*, *ilvC*, and *ilvB* structural genes were increased when grown on limiting valine (Tables 3 and 4). Derepression of the isomeroreductase (IR) did not occur on limiting isoleucine whereas there was an elevated expression of the *ilvE* and *ilvB* structural genes. These results are indicative of a normal multivalent regulation pattern previously described (1, 7, 11, 12). The derepression of the *ilvC* structural gene under substrate induction

with 6 mM acetohydroxybutyrate (Table 4) and valine limitation in a strain devoid of the complete *ilvA* gene product, demonstrates that the intact *ilvA* gene product is not required for isomeroreductase production. No catalytic expression of the *ilvA* gene product occurred in CU370 under either isoleucine or valine limitation. Furthermore, it was shown that CU370 was incapable of allowing significant derepression of the *ilvD* structural gene and gave a normal multivalent derepression response for the *ilvE* and *ilvB* structural genes (Table 3).

DISCUSSION

The suggestion that the *ilvADE* structural genes constitute a single operon with the operator (*ilvO*) located at the *ilvA* proximal end was proposed by Ramakrishnan and Adelberg (13, 14). They observed that the products encoded by these structural genes (*ilvADE*) were elevated with several independently isolated *ilv* linked valine resistant *E. coli* K-12 strains. It was also demonstrated that these lesions still allowed high *ilvADE* gene expression in the presence of the wild type allele (14). Subsequent investigations of these same *ilvO* lesions, however, show that while the *ilvADE* gene products are amplified in their catalytic expression, the level of repressed and nonrepressed expression of the *ilvA*, *ilvD* and *ilvE* structural genes remains unaltered from that of the isogenic wild type (7). In the same investigation it was evidenced that the catalytic expression of the *ilvB* structural gene as well as the *ilvC* structural gene were distinctly altered and that the *ilvO* region defined previously as being between the *ilvA* and the *ilvC* genes (14) was outside of a deletion (*ilvDAC115*) which deleted all of the *ilvA* structural gene and extended into the *ilvD* and *ilvC* structural genes (5, 7). Therefore suggesting that this region (*ilvO*) is affecting more than an operator site for the *ilvADE* structural genes. Indeed, it is possible that the *ilvO* region may be such as to alter the components that are involved in the *ilv* repressor itself.

The presence of an apparent normal multivalent control for the expression of the *ilvE* structural gene in a strain carrying the *ilvDAC115* deletion raised the question as to whether the *ilvE* structural gene was a constituent of the same operon as the *ilvAD* structural genes (7). If the polar affect on the *ilvD* gene expression in the *ilvA* Mu phage insertion strain (Table 3) is due to the lack of mRNA transcription, the apparent normal multivalent control of *ilvE* gene expression would lead to the conclusion that the region controlling the repression recognition site for the *ilvD* and *ilvA* structural genes is not necessary for the control of transaminase B (*ilvE*). Therefore, the *ilvE* structural gene would be separate in its control from the *ilvA* and *ilvD* structural genes. Similar analysis by Thèze and Saint-Girons (16) was used to invoke the presence of an operon in the contiguous structural genes for threonine biosynthesis.

Another question with respect to the necessity for a complete *ilvA* gene product for isomeroreductase induction could be examined in the *ilvA* Mu phage insertion genetic background. It was postulated by previous investigators (2, 3) from their examination of an *Escherichia coli* K-12 strain and a *Salmonella typhimurium* strain carrying a lesion in threonine deaminase (TD) that some form of the complete *ilvA* gene product (threonine deaminase) was necessary for isomeroreductase induction. The ability of CU370 (*ilvA* Mu) to allow full expression of isomeroreductase (the *ilvC* gene product) by internal induction and external induction (Table 4) demonstrates that while lesions in the *ilvA* gene can cause alterations in the catalytic expression of isomeroreductase (2, 3, 12) the intact *ilvA* gene product is not obligatory for induction of the *ilvC* gene.

The results presented in this communication establish that the *ilvE* structural gene can be multivalently controlled independently from the *ilvD* and *ilvA* structural genes. It further reveals that the expression of the *ilvC* structural gene does not require the complete *ilvA* gene product for its induction.

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