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Mutations Affecting the Formation of Acetohydroxy Acid Synthase II in *Escherichia coli* K-12

John M. Smith*, Frank J. Smith, and H.E. Umbarger**
Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

Summary. Genetic mapping experiments have established that two recently isolated valine-resistant mutants of the K-12 strain of *Escherichia coli* have lesions lying between *ilvE* and *rbs*. These lesions allowed expression of the *ilvG* gene, specifying the valine-insensitive acetohydroxy acid synthase (synthase II) and an increased expression of the *ilvEDA* operon. In this respect, they resembled an earlier described *ilvO* lesion that was reported to lie between *ilvA* and *ilvC*. All three lesions were *cis*-dominant in *cis-trans* tests. Reexamination of the earlier studied *ilvO* lesion revealed that it, too, lies between *ilvE* and *rbs*. Valine-sensitive derivatives with lesions presumed to be in *ilvG* were selected from each of the valine-resistant strains. In two of the valine-resistant strains, the *ilvG* mutations were on the *rbs* side of *ilvO*, indicating a gene order *rbs-ilvG-ilvO-ilvE-ilvD-ilvA-ilvC*. In one of the recently isolated valine-resistant stocks, however, the apparent *ilvG* mutation was found to be between *ilvE* and the aline resistance marker. This finding suggests that either *ilvO* and *ilvG* mutations are interspersed or there is another locus, *ilvR*, that behaves phenotypically like *ilvO* and which lies between *ilvG* and *rbs*.

Introduction

In the K-12 strain of *Escherichia coli*, the biosynthesis of isoleucine and valine occurs in five enzymic steps catalyzed by enzymes specified in part by a cluster of genes that lie in a region designated 84 minutes on the chromosome (Fig. 1). In that cluster, only three genes, *ilvE*, *ilvD*, and *ilvA*, constitute a multicistronic operon; the *ilvC* gene is a separate transcriptional unit expressed upon substrate induction. Expression of the *ilvG* gene is dependent upon mutation of the *ilvO* locus. The *ilvB* gene has traditionally been thought to lie adjacent to the *ilvC* gene. However, recent attempts to demonstrate *ilvB* gene activity with fragments of DNA adjacent to *ilvC* have been unsuccessful (McCorkle and Leathers, unpublished observations).

In a previous report from this laboratory, it was shown that Mu-1 phage insertions into *ilvE* exerted polar effects on *ilvD* and *ilvA* (Smith et al., 1976). It was thus concluded that transcription of that operon proceeded from *ilvE* to *ilvA*. These findings were in agreement with the earlier findings of Cohen and Jones (1976) that *ilvO* lesions, long thought to lie between *ilvC* and *ilvA* and therefore to define the operator of an *ilvADE* operon, were actually between *ilvE* and *rbs*. The findings were also in agreement with the demonstration by Kline et al. (1974) that a group of *ilvO* lesions were outside of an *ilvDAC* deletion. However, genetic mapping data (Smith et al., 1976) for one of the presumed *ilvO* lesions (*ilvO468*) used by Kline et al. indicated that its position was in the traditional location between *ilvC* and *ilvA*. A role other than that of operator was therefore postulated for the *ilvO* locus, to account for the increased expression of the *ilvEDA* operon and the expression of the *ilvG* gene that were due to *ilvO468* mutations.

During the course of those studies other mutations were found that led to a phenotype identical to that of the *ilvO468* mutation, but which were clearly shown to lie between *ilvE* and *rbs*. It therefore seemed important to determine whether the *ilvO468* lesion was unique, or whether the earlier mapping data were in error. (For example, it was possible that the *ilvDAC* deletion strain used by Kline et al. (1974) also contained an *ilvG*$^-$ mutation. Such a lesion would have

* Present address: Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19174
** To whom reprint requests should be addressed

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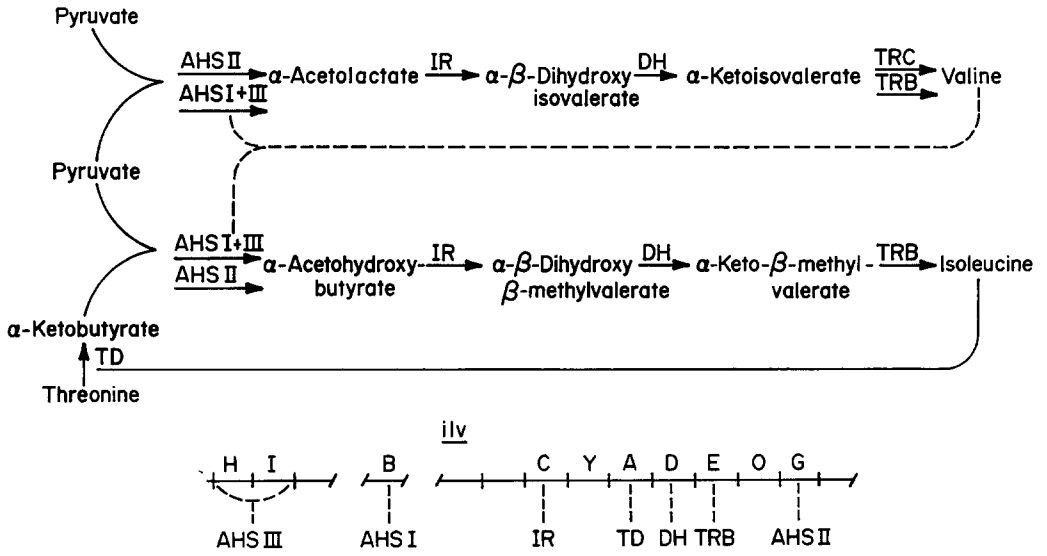

Fig. 1. Biosynthesis of isoleucine and valine. The enzymes catalyzing the indicated steps are abbreviated and the corresponding structural genes (where known) are indicated in parentheses as follows: TD (*ilvA*), threonine deaminase; AHS I (*ilvB*) and AHS III (*ilvHI*), endproduct-inhibited acetohydroxy acid synthases; AHS II (*ilvG*), endproduct-noninhibited acetohydroxy acid synthase; IR (*ilvC*), acetohydroxy acid isomeroreductase; DH (*ilvD*), dihydroxy acid dehydrase; TRB (*ilvE*), transaminase B; TRC, transaminase C. *ilvG* exhibits no activity in *E. coli* strain K-12. In this strain, *ilvO* mutations stimulate transcription initiated at the EDA promotor and are absolutely essential for transcription initiated at the *ilvG* promoter. *ilvY* specific a control element for isomeroreductase induction by substrate. Whether it precedes or follows *C* is uncertain.

accounted for the discrepancy between the two studies.) This paper reports the results of an examination of several valine resistance markers that give rise to *ilvG* expression as well as to elevated *ilvEDA* expression. All, including *ilvO468*, are shown to lie between *ilvE* and *rbs* as found by Cohen and Jones (1976) for *ilvO268* and *ilvO269*. The location of the lesions with respect to *ilvG* is also reported.

Materials and Methods

Bacterial Strains. All bacterial strains used in this study were derivatives of the K-12 strain of *E. coli* and are listed in Table 1. Diethyl sulfate, ethyl methyl sulfonate and Mu-1 induced mutagenesis, and ampicillin enrichment of auxotrophs was performed as described earlier (Smith et al., 1976).

Media. The minimal medium used was that of Davis and Mingioli (1950) modified by the omission of citrate and by increasing carbon source (glucose unless specified) concentration to 0.5%. L-Valine was used at a final concentration of 100 μg per ml. All other amino acid supplements were also L-form and were used at 50 μg per ml. Thymine, cytidine, guanine, adenine, uridine and xanthine were used at a final concentration of 50 μg per ml. Supplements of vitamins were added at final concentrations of 1 μg per ml. L-Broth was used as the rich medium (Bertani, 1951). Agar (Difco) at a final concentration of 1.5% was added to the media described above when solid media were employed.

Transduction. Generalized transductions were performed with the Plcm *clr100* (Plcm) transducing phage following the procedures of Rosner (1972).

Preparation of Cell Extracts. Cells to be used for assay of enzyme activity were grown at 37° C with shaking on a New Brunswick Gyrotory shaker. Growth of cultures was monitored by measuring the absorbency of the culture at 660 nm in a Spectronic 88 spectrophotometer. Repressed cells were obtained by growing cells in the minimal medium supplemented initially with excess branched chain amino acids (50 μg each of L-isoleucine and L-leucine per ml and 100 μg of L-valine per ml). Repressed cells were harvested from such a medium at an A_{660} of approximately 0.6. For derepression of the isoleucine and valine biosynthetic enzymes, cells were grown in the repressing medium until they reached an A_{660} of approximately 0.3. At that time, they were harvested at room temperature by centrifugation under aseptic conditions and resuspended in a fresh medium in which one of the three branched chain amino acids was limiting. The limiting concentrations were 2.5 μg per ml for L-isoleucine and L-leucine and 5.0 μg per ml for L-valine. Whenever one amino acid was limiting, the other two were present in excess. The resuspended cultures were then incubated under these limiting conditions for an additional three hours, during which time the cultures usually doubled once.

Crude extracts were usually prepared from 200 ml cultures. The cells were harvested by centrifugation at 8,000 rpm for 8 min in a Sorvall GS rotor at an A_{660} of 0.6 (7.3×10^8 cells/ml). The cell pellet was resuspended in 30 ml of 0.05 M potassium phosphate buffer, pH 7.0, containing 0.1 mM L-isoleucine. After a second centrifugation at 8,000 rpm in a Sorvall SS-34 rotor, the pellet was resuspended in 4 ml of disruption buffer containing (unless otherwise indicated) 0.05 M potassium phosphate, pH 7.0, 0.1 mM L-isoleucine, 0.1 mM magnesium sulfate and 0.5 mM dithiothreitol. The cell suspension was disrupted by sonic oscillation in a Branson Sonifier, model W185 at a setting of 7 for two 15 sec bursts. The cell suspension was cooled for 30 sec between each burst. After disruption, the extracts were centrifuged at 15,000 rpm for 20 min in a Sorvall SS-34 rotor. The supernatant fluids, which contained 6 to 8 mg protein per ml, were used for assay of the

Table 1. Strains used in this study

Strain	Genotype	Source or Reference
AB1160	F⁻ *ilvC7 proA2 his-4 argE3 thi-1 lacY1 galK2 xyl-5 mtl-1 supE44* λ⁻	Coli Stock Culture Collection
CSH26	*ara thi proAB-lac*Δ	Cold Spring Harbor Laboratory
CU1	F⁺ Wild type K-12 λ⁺	Laboratory collection
CU2	*ilvE12 ilv-2025*	McGilvray and Umbarger (1974)
CU4	F⁻ *galT12* λ⁻	Pledger and Umbarger (1973a)
CU8	*ilvA451*	Pledger and Umbarger (1973a)
CU17	F⁻ *ilvA467 metE200 rbs-215 galT12* λ⁻	Pledger and Umbarger (1973b)
CU29	F⁻ *ilvE488 galT12* λ⁻	Nitrous acid mutagenesis of CU4 by W. J. Pledger
CU41	F⁻ *ilvE499 metE200 rbs-215 galT12* λ⁻	UV mutagenesis of CU4 by A. L. Williams
CU339	F⁺ *ilvO468 rbs-215* λ⁺	Kline et al. (1974)
CU344	F⁻ *ilvDAC115 galT12* λ⁻	Kline et al. (1974)
CU356	F⁻ *ilvDAC115 leu-455 galT12* λ⁻	Kline et al. (1974)
CU420	*ilvA2022*::Mu-1 *thy leu*	R. E. Bird (Louarn et al. 1974)
CU447	*rbs-221 thi arg trp rho-221 lac*Δ	Smith et al. (1976)
CU452	*ilvDAC115 thi arg trp lac*Δ	Smith et al. (1976)
CU457	*ilvA2022*::Mu-1 *ilv-2095 thy leu*	Spontaneous Val^R derivative of CU420
CU458	*ilvA2022*::Mu-1 *ilv-2096 thy leu*	Spontaneous Val^R derivative of CU420
CU467	*ilvDAC115 metE thi arg trp lac*Δ	DES mutagenesis of CU452 by D. E. Smolin
CU504	F⁻ *rbs-221 leu-455 galT12* λ⁻	P1 transduction of CU356 with CU447 as donor
CU519	F⁻ *ilvDAC115 metE201 leu-455 galT12* λ⁻	P1 transduction of CU504 with CU467 as donor
CU520	F⁻ *metE201 rbs-221 leu-455 galT12* λ⁻	Smith et al. (1976)
CU532	F⁻ *ilvE2050 leu-455 galT12* λ⁻	Smith et al. (1976)
CU533	F⁻ *ilvE2050 rbs-221 leu-455 galT12* λ⁻	Smith et al. (1976)
CU545	F⁻ *ilvC44 leu-455 galT12* λ⁻	Smith et al. (1976)
CU555	F⁻ *ilvD513 rbs-221 leu-455 galT12* λ⁻	Smith et al. (1976)
CU588	F⁻ *ilv-2095 rbs-221 leu-455 galT12* λ⁻	P1 transduction of CU533 with CU457 as donor
CU589	F⁻ *ilv-2096 rbs-221 leu-455 galT12* λ⁻	P1 transduction of CU533 with CU458 as donor
CU590	*ilvO468 ilvC2065*::Mu-1 *rbs-215*	Smith et al. (1976)
CU592	*ilvO468 ilvE2066*::Mu-1 *rbs-215*	Smith et al. (1976)
CU595	*ilvO468 ilvA2058 rbs-215*	Spontaneous Ilv⁻ derivative of CU339
CU609	*ilvO468 ilvE2061 rbs-215*	Spontaneous Ilv⁻ derivative of CU339
CU613	F⁻ *ilvO468 ilvE2061 leu-455 rbs-221 galT12* λ⁻	P1 transduction of CU520 with CU609 as donor
CU625	*ilvE2070*::Mu-1	Smith et al. (1976)
CU626	*ilvO468 ilvA2071*::Mu-1 *rbs-215*	Smith et al. (1976)
CU627	*ilvO468 ilvD2072*::Mu-1 *rbs-215*	Smith et al. (1976)
CU631	F⁻ *ilvE12 leu-455 galT12* λ⁻	P1 transduction of CU520 with CU2 as donor
CU633	F⁻ *ilvE2050 leu-455 galT12* λ⁻ (Mu-1 lysogen)	Mu-1 lysogenization of CU532
CU634	F⁻ *ilvO468 ilvD2072*::Mu-1 *leu-455 galT12* λ⁻	P1 transduction of CU633 with CU627 as donor
CU639	*ilvO468 ilvG2075*::Mu-1 *rbs-215*	Mu-1 mutagenesis of CU339
CU653	*ilvDA2076 ara thi proAB-lac*	Spontaneous Ilv⁻ derivative of CSH26
CU654	F⁻ *ilvDA2076 leu-455 galT12* λ⁻	P1 transduction of CU520 with CU653 as donor
CU655	F⁻ *ilvDA2076 rbs-221 leu-455 galT12* λ⁻	P1 transduction of CU520 with CU653 as donor
CU665	*ilvO468 ilvE2061*	P1 transduction of CU609 with CU1 as donor
CU666	*ilvO468 ilvG2075*::Mu-1	P1 transduction of CU639 with CU532 as donor
CU690	*ilvO468 ilvE2099 ilvG2075*::Mu-1 *rbs-215*	Spontaneous Ilv⁻ derivative of CU639
CU691	*ilvO468 ilvE2099 ilvG2075*::Mu-1	P1 transduction of CU690 with CU666 as donor
CU692	F⁻ *ilv-2095 ilvE2104 leu-455 rbs-221 galT12* λ⁻	Spontaneous Ilv⁻ derivative of CU588
CU693	F⁻ *ilv-2096 ilvE2105 leu-455 rbs-221 galT12* λ⁻	Spontaneous Ilv⁻ derivative of CU589
CU694	F⁻ *ilv-2095 ilvE2104 leu-455 galT12* λ⁻	P1 transduction of CU692 with CU457 as donor
CU695	F⁻ *ilv-2096 ilvE2105 leu-455 galT12* λ⁻	P1 transduction of CU693 with CU458 as donor
CU696	*rbs-221 ara thi proAB-lac*Δ	P1 transduction of CU653 with CU520 as donor
CU697	*metE201 rbs-221 ara thi proAB-lac*Δ	P1 transduction of CU653 with CU520 as donor
CU698	F⁻ *metE201 leu-455 galT12* λ⁻	P1 transduction of CU519 with CU520 as donor
CU705	F⁻ *ilvE499 rbs-215 galT12* λ⁻	P1 transduction of CU41 with CU1 as donor
CU713	*ilvC2083*::λp1(209) *ara thi proAB-lac*Δ	Smith and Umbarger (1977)
CU735	*ilvC7 ara thi proAB-lac*Δ	P1 transduction of CU697 with AB1160 as donor
CU814	F⁻ *ilvE499 leu-455 galT12* λ⁻	P1 transduction of CU698 with CU705 as donor
CU815	F⁻ *ilvA467 galT12* λ⁻	P1 transduction of CU344 with CU17 as donor
CU816	F⁻ *ilv-2096 ilvG2106*::Mu-1 *leu-455 rbs-221 galT12* λ⁻	Mu-1 mutagenesis of CU589
CU821	F⁻ *ilv-2096 ilvG2106*::Mu-1 *leu-455 galT12* λ⁻	P1 transduction of CU816 with CU458 as donor
CU823	*ilvD493 ara thi proAB-lac*Δ	P1 transduction of CU697 with CU5123 as donor
CU853	F⁻ *ilv-2096 ilvG2113 leu-455 rbs-221 galT12* λ⁻	DES mutagenesis of CU589
CU856	F⁻ *ilv-2095 ilvG2111 leu-455 rbs-221 galT12* λ⁻	EMS mutagenesis of CU588
CU857	F⁻ *ilv-2096 ilvG2112 leu-455 rbs-221 galT12* λ⁻	EMS mutagenesis of CU589
CU858	F⁻ *ilv-2096 ilvG2106*::Mu-1 *ilvE2108 leu-455 rbs-221 galT12* λ⁻	Spontaneous Ilv⁻ derivative of CU816
CU859	F⁻ *ilv-2095 ilvG2111 ilvE2109 leu-455 rbs-221 galT12* λ⁻	Spontaneous Ilv⁻ derivative of CU856
CU860	F⁻ *ilv-2096 ilvG2112 ilvE2110 leu-455 rbs-221 galT12* λ⁻	Spontaneous Ilv⁻ derivative of CU857
CU1104	F⁻ *ilvO468 ilvE2061 leu-455 rbs-221 galT12* λh80d*ilvA2094*	Lysogenization of CU619
CU1105	F⁻ *ilv-2095 ilvE2104 leu-455 rbs-221 galT12* λh80d*ilvA2094*	Lysogenization of CU692
CU1106	F⁻ *ilv-2096 ilvE2105 leu-455 rbs-221 galT12* λh80d*ilvA2094*	Lysogenization of CU693

isoleucine and valine biosynthetic enzymes. The extracts employed in the experiments reported in Table 5 were made with a disruption buffer containing 5% glycerol and 0.1 mM $MgCl_2$. Isoleucine was omitted and dithiothreitol was reduced to 0.5 mM. The inclusion of glycerol stabilized the acetohydroxy acid synthase activity specified by the *ilvB* gene (H. Grimminger, personal communication). Glycerol did not interfere with any of the enzyme assays employed. $MgCl_2$ appears to stabilize the dehydrase. Harvesting of the cells and all subsequent operations were performed as close to 0° C as possible.

Assay of Enzyme Activities. Threonine deaminase, acetohydroxy acid synthase, and dihydroxy acid dehydrase activities were assayed immediately following centrifugation of the crude extracts. Protein was determined by the method of Lowry et al. (1951) with crystallized bovine serum albumin as standard. Specific activities are expressed as μmol product formed per g protein per min.

Threonine deaminase activity was assayed in a one ml system containing 100 μmol tris (hydroxymethyl) aminomethane-hydrochloride (Tris) buffer, pH 8.0, 100 μmol ammonium chloride, 0.1 μmol pyridoxal 5′-phosphate, 40 μmol L-threonine and 0.1 ml of crude extract. The reaction mixtures were prepared in triplicate and incubated at 37° C for 5, 10 and 15 min, or at 10, 20, and 30 min, depending upon the activity expected. The reactions were terminated by adding 0.1 ml 50% (w/v) trichloroacetic acid. The α-ketobutyrate formed was determined by a modification of the method of Friedemann and Haugen (1943). A measured sample of the reaction mixture was made up to one ml with water. Three ml of 0.025% 2,4-dinitrophenyl-hydrazine in 0.5 M HCl were added and held at room temperature for 15 min. One ml of 40% KOH was added and the color read immediately at 540 nm. A reaction mixture containing no threonine served as a blank.

Acetohydroxy acid synthase activity was assayed in a one ml system containing 100 μmol potassium phosphate, pH 8.0, 0.1 μmol thiamine pyrophosphate, 20 μg flavin-adenine dinucleotide (FAD), 40 μmol sodium pyruvate, 10 μmol magnesium chloride, and 0.1 ml crude extract. The reaction mixtures were prepared in triplicate and incubated for 5, 10, and 15 min, or 10, 20, and 30 min at 37° C. The reactions were stopped by adding 0.1 ml of 50% (v/v) H_2SO_4. A second set of reaction mixtures containing 1 μM L-valine was prepared and was used to minotor the valine-resistant acetohydroxy acid synthase activity which was assumed to be due to the expression of the *ilvG* gene. The acetolactate formed was converted to acetoin by incubating the acidified reaction mixture for an additional 30 min at 37° C. Acetoin was determined by the method of Westerfeld (1945).

Acetohydroxy acid isomeroreductase activity was determined spectrophotometrically by following the disappearance of reduced nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm in a Gilford model 240 spectrophotometer. The complete system contained in 0.5 ml: 50 μmol potassium phosphate, pH 7.5, 0.1 μmol NADPH, 1.5 μmol magnesium chloride, 5 μmol acetohydroxybutyrate and 0.02 to 0.10 ml of crude extract, depending on the activity of the extract. The blank cuvette contained neither acetohydroxybutyrate nor NADPH. Two control cuvettes were employed, one without acetohydroxybutyrate (to correct for NADPH oxidase activity) and one in which 0.15 μmol of ethylenediamine-tetraacetate replaced magnesium chloride (to correct for a magnesium-independent reductase present in some extracts and which is unrelated to the *ilvC* gene product).

Dihydroxy acid dehydrase activity was assayed in a one ml system containing: 100 μmol potassium phosphate, 10 μmol magnesium chlorate, and 20 μmol sodium dihydroxyisovalerate and 0.2 ml of crude extract. The reaction mixtures were prepared in triplicate and incubated for 5, 10, and 15 min or 10, 20, and 30 min at 37° C. The reactions were stopped with 0.1 ml 50% trichloroacetic acid. The α-ketoisovalerate formed was determined by the modification of the Friedemann and Haugen (1943) procedure described above. A reaction mixture from which the substrate was omitted served as the blank.

Transaminase B activity was assayed in the reverse of the biosynthetic direction with L-isoleucine and α-ketoglutarate as substrates. The assay procedure was that of Duggan and Wechsler (1973). The reaction mixtures were prepared in triplicate and incubated for three different times. A tube from which L-isoleucine was omitted served as the blank.

Isolation of Mutants. Diethylsulfate (DES) or ethylmethylsulfonate (EMS) mutagenesis was performed on stationary phase cells grown in L-broth overnight at 37° C. The cells were centrifuged in a tabletop centrifuge and were resuspended in $^1/_2$ their growth volume of 1 M tris, pH 7.5. Twenty-five μl of DES or EMS was then added to 0.2 ml of the resuspended cells. The cells were shaken at 37° C in the presence of DES or EMS for 5–10 minutes. The mutagenized cells were washed twice with minimal medium without a carbon source and then resuspended in appropriately supplemented minimal medium to allow segregation of mutants.

Mu-1 mutagenesis was performed by the method of Howe and Bade (1975).

Ampicillin counterselection was performed for the enrichment of mutants that could not be selected directly. The medium used for the ampicillin counterselections was minimal medium supplemented with purine and pyrimidine bases (thymine, cytidine, guanine, adenine, uridine and xanthine), vitamins, 0.5% glucose and all amino acids with the exception of isoleucine, valine, leucine and alanine. When isoleucine and valine auxotrophs were desired, leucine was added to the medium. Isoleucine and valine were added for the isolation of leucine auxotrophs. The omission of alanine allowed the use of cycloserine as an alternative counterselective agent (Curtiss et al., 1965).

After overnight growth, the mutagenized cells were washed and resuspended in unsupplemented minimal medium without a carbon source. A portion of the washed cells was then resuspended in the counterselection medium and incubated at 37° C. After sufficient growth had occurred to allow one to two cell doublings, ampicillin was added at a final concentration of 50 μg/ml. The cultures were then reincubated and growth was monitored until lysis had occurred and the density of the culture had reached a constant A_{660}. (Short term ampicillin treatment was particularly important for valine resistant strains, since killing of the auxotrophs occurred rapidly, perhaps owing to overproduction of isoleucine and valine by the prototrophs.) The surviving cells were then harvested by centrifugation, washed with minimal medium and resuspended in a medium supplemented for the growth of the desired mutants if additional cycles of enrichment were desired. After the final ampicillin counterselection, the surviving cells were harvested by centrifugation, washed with minimal medium and then diluted before plating on solid medium for incubation.

For the isolation of isoleucine and valine auxotrophs, the surviving cells were plated on a solid medium containing suboptimal amounts of isoleucine and valine (2 μg isoleucine/ml and 4 μg valine/ml). After incubation, the resulting small colonies formed by the isoleucine and valine auxotrophs were picked and scored for their Ilv phenotypes.

ilv mutants isolated after counterselection were initially characterized by their growth responses on solid media containing various intermediates of the isoleucine and valine biosynthetic pathway. From the growth responses of each auxotroph on the isoleucine and valine biosynthetic intermediates, a tentative site could be assigned for the mutational block. This tentative designation was usually confirmed by enzyme assays and genetic mapping.

Strains diploid for the *ilv* gene cluster were prepared from $ilvE^-$ stocks, selecting Ilv^+ lysogens carrying an $ilvA^-$ derivative of λh80*dilv* (Avitabile et al., 1972).

Table 2. Isoleucine and valine biosynthetic enzyme activity in several Val^R strains

Strain	Growth conditions	Specific activities[a]					
		Acetohydroxy acid synthase		Acetohydroxy acid isomero reductase	Threonine deaminase	Dihydroxy acid dehydrase	Transaminase B
		−Val	+1 mM Val				
CU420	Repressing	2.8	0.4	2.8	−[b]	12.2	37.5
ilvA2122::Mu-1	Limiting Ile	7.6	1.7	2.5	–	74.0	80.2
	Limiting Leu	30.7	2.4	3.2	–	82.2	100.5
CU457	Repressing	3.0	0.4	5.9	–	58.7	98.2
ilvA2122::Mu-1	Limiting Ile	13.0	8.5	14.4	–	210	449
ilv-2095	Limiting Leu	24.2	2.5	9.2	–	426	515
CU458	Repressing	1.9	0.3	9.4	–	61.7	95.0
ilvA2122::Mu-1	Limiting Ile	23.6	13.7	21.0	–	201	435
ilv-2096	Limiting Leu	24.1	3.0	14.7	–	494	545
CU613	Repressing	5.5	0.3	3.3	76.8	58.9	−[b]
ilvO468	Limiting Val	242.	33.5	130	1180	974	–
ilvE2061	Limiting Ile	26.0	15.8	11.5	790	379	–
	Limited Leu	43.0	5.3	23.1	270	296	–

[a] μmoles/min/g protein
[b] not detectable

Chemicals. The ethyl ester of α-aceto-α-acetoxybutyrate and sodium α,β-dihydroxyisovalerate were obtained from the Edinboro Foundation (Edinboro, Pennsylvania). NADPH, FAD, thiamine pyrophosphate and sodium pyruvate were obtained from Sigma. The L-amino acids were obtained from Calbiochem. All other chemicals were of reagent grade.

Results

Selection of Valine Resistance in a Strain Containing Mu-l in the ilvA Gene. The strain initially employed for the selection of valine-resistant derivatives was strain CU420, which, with the designation MX-213, was shown by Louarn et al. (1974) to contain Mu-l inserted in the *ilv* region and presumably in the *ilvA* gene, since it would grow on either α-amino-butyrate or isoleucine alone. As Table 2 shows, only threonine deaminase activity was missing in this strain. The absence of any polarity due to the Mu-l insertion is in accord with the earlier studies of Smith et al. (1976) indicating that the direction of transcription in the *ilvEDA* operon is from *E* to *A*.

Since growth on α-aminobutyrate requires acetohydroxy acid synthase function, *ilvA* mutants of *E. coli* K-12 will not grow on this precursor in the presence of valine. Such was true for strain CU420, which, as Table 2 shows, has only the valine-sensitive acetohydroxy acid synthase typical of K-12 derivatives. It was therefore possible to select valine-resistant (Val^R) strains by plating cultures grown from small inocula on medium containing 50 μg of each L-α-aminobutyrate and L-valine per ml. Twelve independent valine-resistant clones were selected and purified by single colony isolation.

The initial characterization of these Val^R mutants was a screen for linkage of the valine resistance lesions to the *ilv* gene cluster (potential *ilvO* lesions) and to the *ara* operon (potential *ilvH* mutations). Pl_{cm} lysates prepared from each Val^R strain were used to transduce appropriate Ilv$^-$ and Ara$^-$ strains to Ilv$^+$ and Ara$^+$, respectively. The transductants from each genetic cross were then scored for valine sensitivity. Of the twelve mutants, two were found to have *ilv*-linked lesions and two *ara*-linked lesions. Since the *ilv*-linked lesions were of primary interest, subsequent analysis was performed with those strains. The other non-*ilv*-linked valine resistance mutations were not characterized further. The strains that contained the *ilv*-linked mutations were designated CU457 and CU458 and assigned the allele numbers *ilv-2095* and *ilv-2096*, respectively.

Enzymic and Genetic Analysis of the ilv-2095 and ilv-2096 Lesions. Table 2 shows the isoleucine and valine biosynthetic enzyme activities of strains CU457, CU458, and, for comparison, strain CU613, an *ilvE* mutant bearing the *ilv0468* lesion studied earlier. Under repressing conditions, transaminase B and dihydroxy acid dehydrase activities were elevated several fold over the respective levels of the parent strain activities. With limiting isoleucine, there was a derepression of a valine-resistant acetohydroxy acid syn-

Table 3. Genetic analysis of strains CU457 and CU458

Cross	Donor	Recipient	Markers selected	Donor markers scored	Major crossover class						Probable order
					Order I			Order II			
1	CU457 *ilvA2122*::Mu-1 *ilv-2095*	CU1010 *ilvC462*	Ilv$^+$	7/52 Val^R	+ C *462*	*2095* Val^R +	*2122* *A* +	+ *C* *462*	*2122* *A* +	*2095* Val^R +	II
2	CU458 *ilvA2122*::Mu-1 *ilv-2096*	CU1010 *ilvC462*	Ilv$^+$	2/52 Val^R	+ C *462*	*2096* Val^R +	*2122* *A* +	+ *C* *462*	*2122* *A* +	*2096* Val^R +	II
3	CU457 *ilvA2122*::Mu-1 *ilv-2095*	CU2 *ilvE12*	Ilv$^+$	51/52 Val^R	*2095* Val^R +	*2122* *A* +	+ *E* *12*	*2122* *A* +	+ *E* *12*	*2095* Val^R +	II
4	CU458 *ilvA2122*::Mu-1 *ilv-2096*	CU2 *ilvE12*	Ilv$^+$	46/51 Val^R	*2096* Val^R +	*2122* *A* +	+ *E* *12*	*2122* *A* +	+ *E* *12*	*2096* Val^R +	II

thase activity which is characteristic of *ilvO* mutations and is presumably due to expression of the *ilvG* gene (Favre et al., 1976). The effect of the mutation is also revealed in the transaminase and dehydrase activities, which show derepressed levels several fold higher than the wild type derepressed levels.

Since all the strains represented in Table 2 carried *leu* markers, the effect of a leucine limitation could be examined. The derepression of transaminase and dehydrase activities in the parent and Val^R derivatives is as expected. Also, the derepression of acetohydroxy acid synthase activity in the parent strain is expected, and since this activity is valine-sensitive, little internal induction of the isomeroreductase was possible. It appears, however, that the *ilvG*-dependent (valine-resistant) acetohydroxy acid synthase activity, which can be derepressed in *ilvO* strains by limiting valine and by limiting isoleucine (Smith et al., 1976), was not derepressed by limiting leucine. Thus, the controls affecting the *ilvEDA* and the *ilvG* genes are different even in *ilvO* strains. This observation is in accord with the earlier proposal from this laboratory that *ilvG* and *ilvEDA* are separate transcriptional units. On the other hand, the isomeroreductase activities, which are presumably due to internal induction, are elevated upon leucine limitation in all three Val^R strains even though the valine-resistant activity is apparently not expressed. The question to be raised is whether in vivo there was considerably more formation of acetolactate or acetohydroxybutyrate than the enzyme measurements reveal. If so, the increase in isomeroreductase activity upon mutation to Val^R could be explained.

Since the response of strains CU457 and CU458 appeared to be phenotypically similar to the previously described *ilvO* mutants, a more detailed genetic analysis of these valine resistance lesions was undertaken to see if they were at the same locus. The initial crosses performed were for the purpose of mapping the lesions with respect to *ilvA*. Pl_{cm} lysates prepared from strains CU457 and CU458 were used to transduce strain CU1010 (*ilvC462*) to prototrophy. The resulting transductants were then scored for their valine sensitivity. The results, summarized in Table 3, are consistent with either of the two orders shown, but, if the Val^R marker were between *ilvC* and *ilvA*, more Val^S recombinants would be expected. Thus, order II is more likely, since a quadruple crossover would be required to account for the infrequent occurrence of Val^R recombinants. The same lysates were used to transduce strain CU2 (*ilvE12*) to prototrophy. The resulting transductants were scored for their valine sensitivity. The results are summarized in Table 3. The results confirm the initial gene order and furthermore are consistent with a gene order in which the *ilv-2095* and *ilv-2096* lesions lie very close to or outside of *ilvE*. Thus, neither lesion is between *ilvA* and *ilvC*. More specific mapping was carried out after the necessary strain preparation was performed.

Pl_{cm} lysates from strains CU457 and CU458 were used to transduce strain CU533 (*ilvE2050 rbs-221*) to prototrophy (Ilv$^+$). The valine sensitivity and Rbs character of each transductant was then analyzed. The results of these crosses as well as those in which donor and recipient were reversed, summarized in Table 4, confirm the results of the initial crosses and

Table 4. Genetic analysis of the *ilv-2095* and *ilv-2096* mutations

Cross	Donor	Recipient	Markers selected	Donor markers scored	Major crossover class								Probable order
					Order I				Order II				
1	CU457	CU533	Ilv$^+$	46/50 Val^R	*2095*	*2122*	+	+	*2122*	+	*2095*	+	
	ilvA2122::Mu-1	*ilvE2050*		44/50 Rbs$^+$	Val^R	*A*	*E*	*rbs*	*A*	*E*	Val^R	*rbs*	II
	ilv-2095	*rbs-221*			+	+	*2050*	*221*	+	*2050*	+	*221*	
2	CU458	CU533	Ilv$^+$	48/50 Val^R	*2096*	*2122*	+	+	*2122*	+	*2096*	+	
	ilvA2122::Mu-1	*ilvE2050*		44/50 *Rbs*$^+$	Val^R	*A*	*E*	*rbs*	*A*	*E*	Val^R	*rbs*	II
	ilv-2096	*rbs-221*			+	+	*2050*	*221*	+	*2050*	+	*221*	
3	CU533	CU457	Ilv$^+$	0/50 Val^S	+	+	*2050*	*221*	+	*2050*	+	*221*	
	ilvE2050	*ilvA2122*::Mu-1		11/50 Rbs$^-$	Val^R	*A*	*E*	*rbs*	*A*	*E*	Val^R	*rbs*	II
	rbs-221	*ilv-2095*			*2095*	*2122*	+	+	*2122*	+	*2095*	+	
4	CU533	CU548	Ilv$^+$	0/50 Val^S	+	+	*2050*	*221*	+	*2050*	+	*221*	
	ilvE2050	*ilvA2122*::Mu-1		11/50 Rbs$^-$	Val^R	*A*	*E*	*rbs*	*A*	*E*	Val^R	*rbs*	II
	rbs-221	*ilv-2096*			*2096*	*2122*	+	+	*2122*	+	*2096*	+	
5	CU588	CU532	Ilv$^+$	102/160 Val^R	*2095*	+	*221*		+	*2095*	*221*		
	ilv-2095	*ilvE2050*	Rbs$^+$		Val^R	*E*	*rbs*		*E*	Val^R	*rbs*		II
	rbs-221				+	*2050*	+		*2050*	+	+		
6	CU589	CU532	Ilv$^+$	48/50 Val^R	*2096*	+	*221*		+	*2096*	*221*		
	ilv-2096	*ilvE2050*	Rbs$^+$		Val^R	*E*	*rbs*		*E*	Val^R	*rbs*		II
	rbs-221				+	*2050*	+		*2050*	+	+		
7	CU694	CU696	Ilv$^+$	49/110 Val^R	*2095*	*2104*	+		*2104*	*2095*	+		
	ilv-2095	*rbs-221*	Rbs$^+$		Val^R	*E*	*rbs*		*E*	Val^R	*rbs*		II
	ilvE2104				+	+	*221*		+	+	*221*		
8	CU694	CU696	Ilv$^+$	84/110 Rbs$^+$	*2095*	*2104*	+		*2104*	*2095*	+		
	ilv-2095	*rbs-221*	Val^R		Val^R	*E*	*rbs*		*E*	Val^R	*rbs*		II
	ilvE2104				+	+	*221*		+	+	*221*		

show that both Val^R lesions are to the *rbs* side of *ilvE2050* and probably between *ilvE* and *rbs*. A valine-resistant, Rbs$^-$ transductant was retained from each of the first two crosses, and these were designated CU588 (*ilv-2095*) and CU589 (*ilv-2096*).

The position of *ilv-2095* with respect to *ilvE* and *rbs* was further defined in a cross with strains CU588 as the donor and strain CU532 as the recipient in which Ilv$^+$ and Rbs$^+$ were the selected markers. The frequency of 64% occurrence of the unselected Val^R marker is too high for the quadruple crossover event required if the order were *ilvE-rbs*-Val^R. The results are also compatible with order I, but this order is eliminated on the basis of the earlier crosses. The analogous cross with strain CU589 (*ilv-2096*) as the donor revealed an even closer linkage of the Val^R marker to *rbs-221*. The order *ilvE-ilv-2095-rbs* is further supported by the crosses between strains CU694 and CU696 in which selection for Val^R and Rbs$^+$ phenotypes was compared. This gene order is different from that previously reported for the *ilvO468* lesion (Smith et al., 1976) but is consistent with that reported by Cohen and Jones (1976) for the lesions designated *ilvO268* and *ilvO269*.

Cis-trans Analysis of the ilv-2095, ilv-2096 and ilvO468 Lesions. An additional characteristic of the *ilvO* lesions described in the past has been the *cis*-dominance that they exhibit. Tests for *cis*-dominance were performed on strains bearing the *ilv-2095* and *ilv-2096* lesions and an *ilvA*$^-$ derivative of the λh80d*ilv* phage (Avitabile et al., 1972). The strains were prepared by placing a drop of lysate containing λh80d*ilvA2094* on lawns of strains CU692 and CU693, *ilvE*$^-$ derivatives of strains CU588 and CU589, respectively. Ilv$^+$ transductants of each that were both temperature sensitive and resistant to phage λ were assumed to be the desired strains diploid for the *ilv* region and were used in the *cis-trans* tests. The specific activities of the isoleucine and valine biosynthetic enzymes of

Table 5. *Cis-trans* analysis of the *ilv-2095, ilv-2096* and *ilvO468* mutation in cells grown under repressing conditions

Strain	Genotype		Specific Activities					
	Bacterial genote	λh80d*ilv* genote	Acetohydroxy acid synthase		Isomero-reductase	Threonine deaminase	Dihydroxy acid dehydrase	Trans-aminase B
			−Valine	+Valine				
CU4	*ilv*$^+$	–	11.9	1.2	0.7	25.7	22.6	23.8
CU588	*ilv-2095*	–	19.9	4.7	2.0	99.0	44.1	58.0
CU692	*ilv-2095 ilvE2104*	–	14.2	6.2	3.5	170	0.9[b]	–[a]
CU1105	*ilv-2095 ilvE2104*	*ilvA2094*	19.2	3.5	3.4	156	23.8	28.2
CU589	*ilv-2096*	–	23.8	9.4	4.6	96.2	50.1	53.5
CU693	*ilv-2096 ilvE2105*	–	12.3	4.3	3.5	107	31.6	0
CU1106	*ilv 2096 ilvE2105*	*ilvA2094*	20.8	8.7	5.4	125	96.7	33.9
CU1	*ilv*$^+$	–	12.7	1.8	2.4	26.3	21.5	27.6
CU339	*ilvO468*	–	23.7	4.0	2.7	91.0	34.2	66.3
CU613	*ilvO468 ilvE2061*	–	13.4	3.1	2.0	55.7	22.2	–
CU1104	*ilvO468 ilvE2061*	*ilvA2094*	23.7	5.7	2.1	99.1	47.3	30.66

[a] Not detectable
[b] The *ilvE2104* mutation may extend into the *ilvD* gene, since strain CU692 exhibits so little dehydrase activity

these merodiploid strains grown under repressing conditions are given in Table 5. In the two merodiploids, transaminase B activity is derived exclusively from the phage genome. Even though the valine resistance mutation has not had as strong an effect on the transaminase as it did on threonine deaminase, it is clear that the expression of the *ilvE* gene on the phage was not enhanced by the mutation. Threonine deaminase activity which arises only from the chromosomal *ilvA* gene remains elevated in a valine resistant strain even in the presence of a valine-sensitive marker on the phage genome. It should be noted that the *ilvE2104* mutation in strains CU692 and CU1105 has virtually abolished the dehydrase activity arising from the chromosomal *ilvD* gene. Thus this mutation may extend into the *ilvD* gene.

A *cis-trans* analysis of the earlier studied *ilvO468* lesion is also included in Table 5. The merodiploid was also prepared by lysogenizing an *ilvE* derivative with the λh80d*ilvA2094* phage. Thus, transaminase B was the product of the phage *ilvE* gene, and threonine deaminase was the product of the chromosomal *ilvA* gene. As occurred with the *ilv-2095* and *ilv-2096* lesions, transaminase B activity in the merodiploid was about like that in the valine-sensitive strain (CU1), whereas threonine deaminase activity remained at the level of that in the valine-resistant strain (CU339).

Because the physiological and genetic dominance effects of the *ilv-2095* and *ilv-2096* lesions were so similar to those of the *ilvO468* but were apparently in a different location, it seemed imperative to reexamine the location of the *ilvO468* lesion.

Genetic Analysis of the ilvO468 Lesion. The initial experiments to reexamine the location of the *ilvO468* lesion were designed to determine whether *ilvO468* lies between *ilvC44* and *ilvA2058* as reported earlier. *ilvC44* is the most *ilvA*-proximal lesion examined in the experiments of Marsh and Duggan (1972). *ilvA2058* is a stable, spontaneous mutation selected in this laboratory. The earlier study had involved Mu-1 induced mutations. The results of a series of genetic crosses using Plcm as the transducing phage are given in Table 6. The first cross with strain CU595 as the donor and strain CU545 as the recipient yielded only a minority of transductants containing the *ilvO468* marker of the donor, but of those that did, most also received the *rbs* marker of the donor. Thus the Val^R transductants were probably the result of a quadruple crossover. Similarly, when the Val^R marker was selected in the second cross, all the Val^R transductants were Rbs$^-$. These results show that *ilvO468* cannot be between *ilvC* and *ilvA* but is rather between *ilvA2058* and *rbs-215*. The remaining crosses in Table 6 in which donor and recipient were reversed either support or are compatible with that conclusion. Crosses 3 and 5 deserve special comment, since neither order would require quadruple crossovers to account for the results. However, as was observed in the crosses represented in Table 3, if order I were correct, a greater linkage between *ilvC*$^+$ and the *ilvO468* allele would have been expected.

The results of crosses to establish whether the *ilvO468* lesion lies between *ilvE* and *rbs* are given in Table 7. The first cross between strains CU654, containing a deletion extending from *ilvD* into *ilvA*,

Table 6. Mapping of the *ilvO468* mutation with respect to *ilvC* and *ilvA* mutations

Cross	Donor	Recipient	Markers selected	Donor markers scored	Major crossover class								Probable order
					Order I				Order II				
1	CU595 *ilvA2058* *ilvO468* *rbs-215*	CU545 *ilvC44*	Ilv$^+$	15/50 Val^R 21/50 Rbs$^-$ 12/50 Val^R,Rbs$^-$ 3/50 Val^R,Rbs$^-$ 9/50 Val^S,Rbs$^-$	+	*468*	*2058*	*215*	+	*2058*	*468*	*215*	II
					C	*O*	*A*	*rbs*	*C*	*A*	*O*	*rbs*	
					44	+	+	+	*44*	+	+	+	
2	CU595 *ilvA2058* *ilvO468* *rbs-215*	CU545 *ilvC44*	Ilv$^+$ Val^R	50/50 Rbs$^-$	+	*468*	*2058*	*215*	+	*2058*	*468*	*215*	II
					C	*O*	*A*	*rbs*	*C*	*A*	*O*	*rbs*	
					44	+	+	+	*44*	+	+	+	
3	CU595 *ilvA2058* *ilvO468* *rbs-215*	CU545 *ilvC44*	Ilv$^+$ Rbs$^+$	10/52 Val^R	+	*468*	*2058*	*215*	+	*2058*	*468*	*215*	II
					C	*O*	*A*	*rbs*	*C*	*A*	*O*	*rbs*	
					44	+	+	+	*44*	+	+	+	
4	CU545 *ilvC44*	CU595 *ilvA2058* *ilvO468* *rbs-215*	Ilv$^+$ Val^R	12/50 Rbs$^+$	*44*	+	+	+	*44*	+	+	+	II
					C	*O*	*A*	*rbs*	*C*	*A*	*O*	*rbs*	
					+	*468*	*2058*	*215*	+	*2058*	*468*		
5	CU545 *ilvC44*	CU595 *ilvA2058* *ilvO468* *rbs-215*	Ilv$^+$ Rbs$^+$	38/50 Val^S	*44*	+	+	+	*44*	+	+	+	II
					C	*O*	*A*	*rbs*	*C*	*A*	*O*	*rbs*	
					+	*468*	*2058*	*215*	+	*468*	*2058*	*215*	

Table 7. Genetic analysis of the *ilvO468* mutation

Cross	Donor	Recipient	Markers selected	Donor markers scored	Major crossover class								Probable order
					Order I				Order II				
1	CU654 *ΔilvDA2076*	CU609 *ilvE2061* *ilvO468* *rbs-215*	Ilv$^+$	30/50 Val^S,Rbs$^+$ 5/50 Val^S,Rbs$^-$ 1/50 Val^R,Rbs$^+$	*2076*	+	+	+	*2076*	+	+	+	I
					AD	*E*	*O*	*rbs*	*AD*	*O*	*E*	*rbs*	
					+	*2061*	*468*	*215*	+	*468*	*2061*	*215*	
2	CU654 *ΔilvDA2076*	CU609 *ilvE2061* *ilvO468* *rbs-215*	Ilv$^+$ Rbs$^+$	40/50 Val^S	*2076*	+	+	+	*2076*	+	+	+	I
					AD	*E*	*O*	*rbs*	*AD*	*O*	*E*	*rbs*	
					+	*2061*	*468*	*215*	+	*468*	*2061*	*215*	
3	CU609 *ilvE2061* *ilvO468* *rbs-215*	CU654 *ΔilvDA2076*	Ilv$^+$ Rbs$^+$	7/32 Val^R	+	*2061*	*468*	*215*	+	*468*	*2061*	*215*	I
					AD	*E*	*O*	*rbs*	*AD*	*O*	*E*	*rbs*	
					2076	+	+	+	*2076*	+	+	+	
4	CU609 *ilvE2061* *ilvO468* *rbs-215*	CU654 *ΔilvDA2076*	Ilv$^+$ Val^R	42/50 Rbs$^-$	+	*2061*	*468*	*215*	+	*468*	*2061*	*215*	I
					AD	*E*	*O*	*rbs*	*AD*	*O*	*E*	*rbs*	
					2076	+	+	+	*2076*	+	+	+	
5	CU609 *ilvE2061* *ilvO468* *rbs-215*	CU625 *ilvE2070*::Mu-1	Ilv$^+$	15/50 Rbs$^-$ 6/50 Val^R	+	*2061*	*468*	*215*	*2061*	+	*468*	*215*	I
					E	*E*	*O*	*rbs*	*E*	*E*	*O*	*rbs*	
					2070	+	+	+	+	*2070*	+	+	
6	CU665 *ilvE2061* *ilvO468*	CU655 *ΔilvDA2076* *rbs-221*	Ilv$^+$ Val^R	37/50 Rbs$^+$	+	*2061*	*468*	+	+	*468*	*2061*	+	I
					AD	*E*	*O*	*rbs*	*AD*	*O*	*E*	*rbs*	
					2076	+	+	*221*	*2076*	+	+	*221*	

and CU609 clearly established that *ilvO468* is outside the deleted region and is between *ilvE2061* and *rbs-215*. The majority of the transductants received the donor Val^S and Rbs^+ markers, since the selection for Ilv^+ forced a crossover before the *ilvE2061* marker of the recipient. Of those that retained the Val^R of the recipient, the majority also retained the Rbs^- marker of the recipient. If *ilvO* were between *ilvD* and *ilvE*, the Val^R recombinants would have been predominantly Rbs^+. Direct selection for Ilv^+ and Rbs^+ in an identical cross resulted in the predominant occurrence of the double crossover type, Val^S. The fewer Val^R recombinants would have required a quadruple crossover. If *ilvO* were between *ilvD* and *ilvE*, the Val^R and Val^S recombinants would have occurred with nearly equal frequencies. In the reverse cross, in which selection was also for Ilv^+ and Rbs^+, the double crossover type, Val^S, was again predominant. The fourth cross is similar except that, when a quadruple crossover was selected (Ilv^+, Val^R), the Rbs^- marker of the donor appeared predominantly among the transductants. The fifth cross, between strain CU609 and a Mu-1-induced *ilvE* mutant, gave results analogous to those with the deletion strain and indicated that the insertion was later in the *ilvE* gene than the *ilvE2061* mutation. The final cross was similar to the fourth cross, except that a Rbs^- marker was carried in the deletion strain rather than in the Val^R strain. The results support completely the order deduced from the other crosses.

The Location of Several Mu-1 Insertions with Respect to ilvO468. It is of interest that no inconsistency was obtained in the above crosses when a Mu-1-induced mutant was employed. In the earlier study in which it was concluded that *ilvO468* was between *ilvA* and *ilvC*, all of the crosses involved Mu-1-induced *ilv* derivatives of the *ilvO468* strain, CU339. For these reasons, a series of crosses were performed with the same Mu-1 lysogens previously used and, in addition, several additional Mu-1-induced *ilv* derivatives of strain CU339. The crosses also served to locate some of the Mu-1 insertion sites in the laboratory collection.

The first cross represented in Table 8 was between strain CU823 containing a nitrous acid-induced mutation in *ilvD* and strain CU627, one of the Mu-1 derivatives of the *ilvO* mutant used in the earlier study. The majority class of transductants had the recipient phenotype, Val^R and Rbs^-, and indicated the order: *ilvD2072::Mu-1-ilvD493-ilvO468-rbs*. The second cross, with a nitrosoguanidine-induced *ilvD* mutant and the same Mu-1 lysogen, gave similar results. Several crosses were performed between the *ilvE* Mu-1 lysogen used in the earlier study and two different *ilvE* mutants, one, strain CU814, containing a UV-induced mutation and the other, strain CU631, containing a small deletion. In all the crosses involving the *ilvE* lysogen as recipient or as donor, the order was again *ilvE-ilvO-rbs*. The Mu-1 insertion site was observed to be earlier in the *ilvE* gene than either of the other two *ilvE* markers. The close linkage of the *rbs-215* and *ilvO468* lesions in all the crosses with the two Mu-1 lysogens was opposite to that recorded earlier. It therefore appears that, in the earlier study, the scoring of Rbs^- and Rbs^+ was reversed. With respect to those Mu-1 lysogens, the problem was one of record keeping and not with the strains themselves.

The location in *ilvC* of Mu-1 in strain CU590 used in the previous study was determined first with respect to *ilvC7*, the most *ilvA* distal *ilvC* lesion studied by Marsh and Duggan (1972) in cross number 7. The insertion site was clearly to the *ilvA* or *rbs* side of *ilvC7*. The virtually identical results in cross number 8 with strain CU545 containing the most *ilvA* proximal *ilvC* marker studied by Marsh and Duggan (1972), *ilvC44*, indicated that the insertion was very early in the *ilvC* gene. This result was verified when donor and recipient were reversed in cross number 9. Only one other marker had been encountered that was more promoter-proximal in the *ilvC* gene than *ilvC44* and that was the *ilvC-lac* fusion (Smith and Umbarger, 1977). The strain containing this fusion, which had been derived from an earlier Mu-1 insertion, strain CU713, was also crossed with strain CU590. That the majority of the Ilv^+ transductants were of the donor phenotype, Val^S and Rbs^+, indicated that the *ilvC2065*::Mu-1 lesion was even closer to *ilvA* and the *ilvC* control region than the *ilvC-lac* fusion. These crosses also supported the position of *ilvO* to the *rbs* side of the *ilv* gene cluster.

The other Mu-1 lysogen used in the earlier study was strain CU626, which contained Mu-1 in *ilvA*. In this reexamination, it was transduced to prototrophy with two *ilvA* mutants and with the wild type. In the two crosses involving *ilvA* mutants, the low linkage between *ilvO468* and *rbs-215* that had been observed in the earlier study was again encountered. In this case, scoring errors were eliminated as a possibility. Thus, the anomalous behavior was attributable to the strain itself. Indeed, even the cross with a wild type strain (CU1), which should have given primarily wild type transductants with close linkage of the Val^S and Rbs^+ markers, gave instead an unexpected result. It is quite possible that in the generation of the Mu-1 lysogen, CU626, some chromosomal rearrangement has occurred.

The Location of ilvG. In the study of the direction of transcription of the *ilv* gene cluster, it was shown that a Mu-1 insertion into *ilvE* had a polar effect

Table 8. Mapping of several Mu-1 induced mutations

Cross	Donor	Recipient	Markers selected	Donor markers scored	Major crossover class								Probable order
					Order I				Order II				
1	CU823 *ilvD493*	CU627 *ilvD2072*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	13/50 Val^S,Rbs$^-$ 4/50 Val^S,Rbs$^+$ 8/50 Val^R,Rbs$^+$	*493* *D* +	+ *D* *2072*	+ *O* *468*	+ *rbs* *215*	+ *D* *2072*	*493* *D* +	+ *O* *468*	+ *rbs* *215*	II
2	CU555 *ilvD513*	CU634 *ilvD2072*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	9/50 Val^S 12/50 Rbs$^+$	*513* *D* +	+ *D* *2072*	+ *O* *468*	+ *rbs* *215*	+ *D* *2072*	*513* *D* +	+ *O* *468*	+ *rbs* *215*	II
3	CU592 *ilvE2066*::Mu-1 *ilvO468* *rbs-215*	CU814 *ilvE499*	Ilv$^+$	0/50 Val^R 0/50 Rbs$^-$	*2066* *E* +	+ *E* *499*	*468* *O* +	*215* *rbs* +	+ *E* *499*	*2066* *E* +	*468* *O* +	*215* *rbs* +	II
4	CU631 *ilvE12*	CU592 *ilvE2066*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	27/33 Val^S 20/33 Rbs$^+$	*12* *E* +	+ *E* *2066*	+ *O* *468*	+ *rbs* *215*	+ *E* *2066*	*12* *E* +	+ *O* *468*	+ *rbs* *215*	I
5	CU592 *ilvD2066*::Mu-1 *ilvO468* *rbs-215*	CU29 *ilvE488*	Ilv$^+$	3/50 Val^R 2/50 Rbs$^-$	*2066* *E* +	+ *E* *488*	*468* *O* +	*215* *rbs* +	+ *E* *488*	*2066* *E* +	*468* *O* +	*215* *rbs* +	II
6	CU29 *ilvE488*	CU592 *ilvE2066*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	44/50 Val^S 46/50 Rbs$^+$	+ *E* *2066*	*488* *E* +	+ *O* *468*	+ *rbs* *215*	*488* *E* +	+ *E* *2066*	+ *O* *468*	+ *rbs* *215*	II
7	CU735 *ilvC7*	CU590 *ilvC2065*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	46/50 Val^S 44/50 Rbs$^+$	*7* *C* +	+ *C* *2065*	+ *O* *468*	+ *rbs* *215*	+ *C* *2065*	*7* *C* +	+ *O* *468*	+ *rbs* *215*	I
8	CU545 *ilvC44*	CU590 *ilvC2065*::Mu-1 ilvO468 rbs-215	Ilv$^+$	46/50 Val^S 45/50 Rbs$^+$	*44* *C* +	+ *C* *2065*	+ *O* *468*	+ *rbs* *215*	+ *C* *2065*	*44* *C* +	+ *O* *468*	+ *rbs* *215*	I
9	CU590 *ilvC2065*::Mu-1 *ilvO468* *rbs-215*	CU545 *ilvC44*	Ilv$^+$	0/50 Val^R 0/50 Rbs$^-$	*2065* *C* +	+ *C* *44*	*468* *O* +	*215* *rbs* +	+ *C* *44*	*2065* *C* +	*468* *O* +	*215* *rbs* +	II
10	CU713 *ilvC2083*:: *lacZYλ*-Mu-1	CU590 *ilvC2065*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	58/78 Val^S 60/78 Rbs$^+$	*2083* *C* +	+ *C* *2065*	+ *O* *468*	+ *rbs* *215*	+ *C* *2065*	*2083* *C* +	+ *O* *468*	+ *rbs* *215*	I
11	CU815 *ilvA467*	CU626 *ilvA2071*::Mu-1 *ilvO468*, *rbs-215*	Ilv$^+$	50/50 Val^S 1/50 Rbs$^+$	*467* *A* +	+ *A* *2071*	+ *O* *468*	+ *rbs* *215*	+ *O* *468*	+ *A* *2071*	*467* *A* +	+ *rbs* *215*	II
12	CU8 *ilvA451*	CU626 *ilvA2071*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	49/50 Val^S 0/50 Rbs$^+$	*451* *A* +	+ *A* *2071*	+ *O* *468*	+ *rbs* *215*	+ *O* *468*	+ *A* *2071*	*451* *A* +	+ *rbs* *215*	II
13	CU1 wild type	CU626 *ilvA2071*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$	1/50 Val^S 13/50 Rbs$^+$	+ *A* *2071*	+ *O* *468*	+ *rbs* *215*		+ *O* *468*	+ *A* *2071*	+ *rbs* *215*		II

Table 9. Genetic location of *ilvG*

Cross	Donor	Recipient	Markers selected	Donor markers scored	Major crossover class						Probable order
					Order I			Order II			
1	CU665 *ilvE2061* *ilvO468*	CU639 *ilvG2075*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$ Val^R	77/110 Rbs$^+$	*2061* *E* +	+ *G* *2075*	+ *rbs* *215*	*2061* *E* +	+ *rbs* *215*	+ *G* *2075*	I or II
2	CU666 *ilvG2075*::Mu-1 *ilvO468*	CU609 *ilvE2061* *ilvO468* *rbs-215*	Ilv$^+$ Val^R	2/110 Rbs$^+$	+ *E* *2061*	*2075* *G* +	+ *rbs* *215*	+ *E* *2061*	+ *rbs* *215*	*2075* *G* +	I
3	CU339 *ilvO468* *rbs-215*	CU691 *ilvE2099* *ilvO468* *ilvG2075*::Mu-1	Ilv$^+$ Rbs$^+$	44/50 Val^R	+ *E* *2099*	+ *G* *2075*	*215* *rbs* +	+ *E* *2099*	*215* *rbs* +	+ *G* *2075*	I
4	CU665 *ilvE2061* *ilvO468*	CU639 *ilvG2075*::Mu-1 *ilvO468* *rbs-215*	Ilv$^+$ Rbs$^+$	25/110 Val^R	*2061* *E* +	+ *G* *2075*	+ *rbs* *215*	*2061* *E* +	+ *rbs* *215*	+ *G* *2075*	I
5	CU695 *ilvE2105* *ilv-2096*	CU853 *ilv-2096* *ilvG2113* *rbs-221*	Ilv$^+$ Rbs$^+$	20/111 Val^R	*2105* *E* +	+ *G* *2113*	+ *rbs* *221*	*2105* *E* +	+ *rbs* *2113*	+ *G* *221*	I

on *ilvD* and *ilvA* but none on *ilvG*. It was therefore inferred but was not proven that *ilvG* was outside the *ilvEDA* operon and probably between *ilvE* and *rbs*$^+$. Since the position of *ilvG* might be helpful in distinguishing between the three Val^R markers described here, an effort was made to define the position of *ilvG*. For this purpose, Val^S derivatives with lesions in *ilvG* were obtained from the Val^R strains by Mu-1, EMS or DES mutagenesis.

The Val^S derivatives so obtained from all three Val^R mutants were found to be of two types when examined by a lysed cell assay for threonine deaminase activity. One type retained the elevated enzyme levels of the original Val^R mutants and the second had normal threonine deaminase levels. The latter were presumed to have mutations that abolished the effect of the original Val^R mutation, while the former were presumed to have lost only *ilvG* function by mutation.

In the mapping experiments involving *ilvG*, care was taken to insure that both donor and recipient contained the same Val^R lesion. This precaution was necessary, since the *ilvG* gene in *E. coli* K-12 is only expressed in the presence of an *ilvO* (Favre et al., 1976; Guardiola et al., 1977; Smith et al., 1976) or the *ilv-2095* and *ilv-2096* lesions.

Table 9 shows the results from a series of genetic crosses used to locate *ilvG* with respect to *ilvE* and *rbs*. In each cross, a double selection was employed, either Ilv$^+$, Rbs$^+$ or Ilv$^+$, Val^R, and the recipient always contained the negative allele of the marker that was to be selected. Since both donor and recipient bore the *ilvO468* lesion, its location did not enter into the selection. In the first cross, the majority of the transductants were Rbs$^+$, as was the donor. This observation eliminates the possibility that *ilvG* could be on the *ilvA* side of *ilvE* but does not strongly favor a position one side or the other of *rbs*. The second, reciprocal cross, however, indicated a very low linkage between *ilvE* and *rbs* on the transducing fragment derived from strain CU666 such as might arise if Mu-1 were inserted between *ilvE* and *rbs*. The result therefore strongly favors a position between *ilvE* and *rbs* for *ilvG*. The third cross, which yielded 88% Val^R phenotype of the donor among the transductants, could only be explained by the proposed order. The fourth cross, which is the same as the first except that Rbs$^+$ transductants were

Table 10. Mapping of *ilvG* with respect to several Val^R mutations

Cross	Donor	Recipient	Markers selected	Donor markers scored	Major crossover class								Probable order
					Order I				Order II				
1	CU1 (wild type)	CU690 *ilvO468* *ilvG2075*::Mu-1 *ilvE2099* *rbs-215*	Ilv^+ Val^R	27/38 Rbs^+	+ *E* *2099*	+ *O* *468*	+ *G* *2075*	+ *rbs* *215*	+ *E* *2099*	+ *G* *2075*	+ *O* *468*	+ *rbs* *215*	I
2	CU1 (wild type)	CU690 *ilvO468* *ilvG2075*::Mu-1 *ilvE2099* *rbs-215*	Ilv^+	86/110 Val^S,Rbs^+ 19/110 Val^R,Rbs^+ 4/110 Val^S,Rbs^-	+ *E* *2099*	+ *O* *468*	+ *G* *2075*	+ *rbs* *215*	+ *E* *2099*	+ *G* *2075*	+ *O* *468*	+ *rbs* *215*	I
3	CU1 (wild type)	CU859 *ilv-2095* *ilvG2111* *ilvE2109* *rbs-221*	Ilv^+ Val^R	71/110 Rbs^+	+ *E* *2109*	+ Val^R *2095*	+ *G* *2111*	+ *rbs* *221*	+ *E* *2109*	+ *G* *2111*	+ Val^R *2095*	+ *rbs* *221*	I
4	CU1 (wild type)	CU858 *ilv-2096* *ilvG2106*::Mu-1 *ilvE2108* *rbs-221*	Ilv^+ Val^R	50/110 Rbs^+	+ *E* *2108*	+ Val^R *2096*	+ *G* *2106*	+ *rbs* *221*	+ *E* *2108*	+ *G* *2106*	+ Val^R *2096*	+ *rbs* *221*	II
5	CU1 (wild type)	CU860 *ilv-2096* *ilvG2112* *ilvE2110* *rbs-221*	Ilv^+ Val^R	49/110 Rbs^+	+ *E* *2110*	+ Val^R *2096*	+ *G* *2112*	+ *rbs* *221*	+ *E* *2110*	+ *G* *2112*	+ Val^R *2096*	+ *rbs* *221*	II
6	CU639 *ilvO468* *ilvG2075*::Mu-1 *rbs-215*	CU532 *ilvE2050*	Ilv^+ Val^R	25/88 Rbs^-	+ *E* *2050*	*468* *O* +	*2075* *G* +	*215* *rbs* +	+ *E* *2050*	*2075* *G* +	*468* *O* +	*215* *rbs* +	I
7	CU856 *ilv-2095* *ilvG2111* *rbs-221*	CU532 *ilvE2050*	Ilv^+ Val^R	1/50 Rbs^-	+ *E* *2050*	*2095* Val^R +	*2111* *G* +	*221* *rbs* +	+ *E* *2050*	*2111* *G* +	*2095* Val^R +	*221* *rbs* +	I
8	CU666 *ilvO468* *ilvG2075*::Mu-1	CU533 *ilvE2050* *rbs-221*	Ilv^+ Val^R	0/110 Rbs^+	+ *E* *2050*	*468* *O* +	*2075* *G* +	+ *rbs* *221*	+ *E* *2050*	*2075* *G* +	*468* Val^R +	+ *rbs* *221*	I
9	CU666 *ilvO468* *ilvG2097*	CU533 *ilvE2050* *rbs-221*	Ilv^+ Val^R	1/110 Rbs^+	+ *E* *2050*	*468* *O* +	*2097* *G* +	+ *rbs* *221*	+ *E* *2050*	*2097* *G* +	*468* *O* +	+ *rbs* *221*	I
10	CU821 *ilv-2096* *ilvG2106*::Mu-1	CU533 *ilvE2050* *rbs-221*	Ilv^+ Val^R	48/50 Rbs^+	+ *E* *2050*	*2096* Val^R +	*2106* *G* +	+ *rbs* *221*	+ *E* *2050*	*2106* *G* +	*2096* Val^R +	+ *rbs* *221*	II

selected, also favors the proposed order. The fifth cross involves the *ilv-2096* Val^R marker in both donor and recipient and a DES-induced *ilvG* mutation in the recipient. The results are virtually the same as those of the fourth cross.

These results clearly indicate that *ilvG*, like *ilvO468*, *ilv-2095* and *ilv-2096*, lies between *ilvE* and *rbs*. Such a location would account for the failure to observe a polar effect on *ilvG* when Mu-1 was inserted into the *ilvE* gene of an *ilvO* mutant (Smith et al., 1976). It was of further interest to determine the location of *ilvG* with respect to the Val^R lesions, *ilvO468*, *ilv-2095*, and *ilv-2096*. Such experiments should also aid in deciding whether *ilv-2095* and *ilv-2096* were also in the *ilvO* locus. Val^S derivatives were selected from strains CU457 and CU458. They were found to be of two types, just as had been found for the Val^S derivatives of strain CU339. Only the presumed $ilvG^-$ strains were employed in the mapping experiments.

The results of the series of crosses are given in Table 10. In the first cross in which the wild type strain was the donor and a presumed *ilvG*, *ilvE* derivative of strain CU339 was the recipient, the Ilv^+ Val^R phenotype was selected so that both the $ilvE^+$ and $ilvG^+$ markers from the donor were required. The finding that the majority of the transductants selected received the donor's rbs^+ marker, makes gene order I the more likely. When Val^R was not selected in the same cross, the double crossover type, Val^S, was the majority recombinant type. Gene order I is still favored, since those Val^R recombinants that did occur were mostly Rbs^+, which with order I did not require quadruple crossovers. The third cross is identical to the first, except that the Val^R marker was *ilv-2095*. The results were also essentially the same as those in cross 1, so that again gene order I is favored. Cross 4 is another cross of the same type in which the Val^R marker was *ilv-2096*. The results are different from those with the other Val^R markers, and the majority phenotype would require a quadruple crossover event if gene order I existed, whereas only a double crossover would be required if gene order II existed. *ilv-2095* and *ilv-2096* are clearly differentiated in this type of cross.

Virtually identical results were obtained with strain CU860 in which the *ilvG* lesion had been induced by EMS mutagenesis rather than by Mu-1.

The additional crosses reported in Table 10 support the gene orders indicated by the first five crosses. Crosses 6 and 7 provided a comparison between an *ilvO468* strain and an *ilv-2095* strain as donors in a transduction with an *ilvE* recipient. Although in cross 6 there were more of the minority (quadruple crossover) class of recombinants among the transductants than in cross 7, the fact that the majority class can be explained by a double crossover event favors gene order I for both. Crosses 8, 9, and 10 are similar to crosses 6 and 7 and compare two different Val^S derivatives of the *ilvO468* strain as donors with the Val^S derivative of the *ilv-2096* strains as a donor. The clearly contrasting results with the two kinds of donors support the different gene orders for the two donors.

The results obtained in the mapping experiments reported in Table 10 suggest that the *ilv-2095* marker is allelic with *ilvO468*, but that *ilv-2096* is not. Whether the *ilv-2096* marker represents a second class of regulatory lesions or is the result of some chromosomal abnormality that developed in strain CU458 cannot be decided until more mutants of this class are identified, or until an abnormality can be genetically or physically demonstrated.

Discussion

The *ilvO* mutations in *E. coli* strain K-12 are known to have two phenotypic effects. One is an increase in expression of the *ilvEDA* operon without apparently qualitatively affecting either repressibility or derepressibility. The second is the appearance when either valine or isoleucine is limiting of a valine-resistant acetohydroxy acid synthase activity. As Favre et al. (1976) have shown, the appearance of this activity is due to the expression of the *ilvG* gene which the *ilvO* mutations make possible. Until very recently, the *ilvO* mutations, first described by Ramakrishnan and Adelberg (1965), were assumed to lie between *ilvA* and *ilvC*. For two mutations of this type, Cohen and Jones (1976) have shown them to lie between *ilvE* and *rbs*, an observation that led them to conclude correctly that transcription was from *ilvE* to *ilvA*.

With the finding that two mutations, *ilv-2095* and *ilv-2096*, phenotypically similar to *ilvO* mutations, were located between *ilvE* and *rbs*, it was decided to reexamine the location of *ilvO468*, which had been reported to lie between *ilvA* and *ilvC* (Smith et al., 1976). It is now evident that the *ilvO468* mutation is also between *ilvE* and *rbs*.

The earlier study from this laboratory had shown that the *ilvG* gene could not be between *ilvE* and *ilvD*, since a Mu-1 insertion into *ilvE* of the strain carrying the *ilvO468* mutation did not abolish *ilvG* function (Smith et al., 1976). Indeed, it now appears that *ilvG* and the *ilvEDA* operon are separate transcriptional units. Thus *ilvG* function can be destroyed by Mu-1-induced mutagenesis without affecting the

elevated expression of the *ilvEDA* operon (with a resultant β-chloroalanine resistance). Furthermore, the *ilvEDA* operon in both *ilvO*$^+$ and *ilvO*$^-$ strains undergoes derepression with limiting valine, limiting isoleucine or limiting leucine. *ilvG*, on the other hand, responds only to limiting valine or limiting isoleucine.

The Mu-1-induced *ilvG* mutations, which render *ilvO* strains valine sensitive, along with some other *ilvG* mutations permitted the mapping of *ilvG*. It, too, was found to lie between *ilvE* and *rbs*.

By locating the position of *ilvG* also between *ilvE* and *rbs*, it became possible to map *ilvO468, ilv-2095,* and *ilv-2096* with greater precision. It was found that *ilvO468* and *ilv-2095* were between *ilvE* and *ilvG*. It is therefore reasonable to assume that *ilv-2095* is also an *ilvO* lesion. The *ilv-2096* lesion thus far seems to lie between *ilvG* and *rbs*. This finding may indicate either that *ilvO* and *ilvG* lesions are interspersed and are in fact lesions in but a single locus or that there is a second regulatory locus, *ilvR*, that affects expression of *ilvG* and the *ilvEDA* operon. However, until additional lesions can be found that appear allelic to *ilv-2096*, any claim that a second regulatory locus lies between *ilvG* and *rbs* must be considered tentative. That the designation, *ilvO*, should be retained for the locus between *ilvE* and *ilvG* is strengthened by the accompanying report by Baez et al. (1978) of an independently isolated valine-resistant strain with a lesion between *ilvE* and *ilvG*.

The demonstration that *ilvO* is between *ilvE* and *ilvG* renders unwarranted for the present the role for *ilvO* postulated in the earlier report when it was assumed to lie between *ilvA* and *ilvC*. However, it is still unclear what its role is. The finding of a regulatory region between two transcriptional units that affects both units invites the suggestion that in *ilvoO*$^-$ strains there may be divergent transcription. However, preliminary evidence is against divergent transcription. For example, experiments by C.S. Subrahmanyam in this laboratory have indicated that strain CU592 (*ilvO468, ilvE2066: :* Mu-1) upon limitation for isoleucine yields message that hybridizes only with the same strand of λh80d*ilv* with which message from a wild type strain limited by isoleucine hybridizes (C.S. Subrahmanyam and H.E. Umbarger, Abstr. Ann. Mtg., Amer. Soc. Microbiol., 1978). Under these conditions, strain CU592 should form only *ilvG* and *ilvC* message (see Table 3, Smith et al., 1976), and the wild type should form only *ilvEDA* message. [It should be pointed out that the Mu-1 insertion in strain CU592 has not resulted in an absolute polarity. Since this insertion is early in *ilvE*, the low level of expression of the *ilvD* and *ilvA* genes could be due to the internal promoter preceding *ilvD* described by Berg et al. (C.M. Berg, K.J. Shaw, J. Vender and M. Borucha-Mankiewicz, Abstr. Ann. Mtg., Amer. Soc. Microbiol., 1978, K202, p. 160). Under one condition, this expression was less than ten percent that in a wild type strain, so there could have been a small amount of *ilvDA* transcript in the RNA of strain CU592 that hybridized to the phage DNA.]

Whatever model is considered, it must account for the finding of two kinds of Val^S derivatives of *ilvO* strains: those that have lost both *ilvG* expression and elevated *ilvEDA* expression and those that have lost *ilvG* expression only. These classes are found among Mu-1-induced mutations as well as among spontaneous and by DES or EMS-induced mutation. Thus, although elevated *ilvEDA* expression and the appearance of *ilvG* expression are dependent upon an *ilvO*$^-$ mutation, they appear to be independent transcriptional events.

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Note added in proof

The genotype for strain CU634 given in Table 1 omitted the *rbs-215* marker, which the strain also carries. Table 1 did not include strain CU1010 (a spontaneous derivative of strain CU1 isolated by D. McGilvray in this laboratory), which carries the *ilvC422* marker.

Recent experiments in this laboratory indicate that the *ilvG* gene is derepressed in *ilvO* strains when leucine is limiting. The results in Table 2 indicating relatively less of the *ilvG* product can be explained by the fact that acetohydroxy acid synthase III was repressed in all extracts except those prepared from cells grown with limiting leucine.