PHYSIOLOGICAL CHARACTERIZATION OF POLAR Tn*5*-INDUCED ISOLEUCINE-VALINE AUXOTROPHS IN *ESCHERICHIA COLI* K-12: EVIDENCE FOR AN INTERNAL PROMOTER IN THE *ilvOGEDA OPERON*[1]

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ABSTRACT

The properties of 22 isoleucine-valine auxotrophs induced in *Escherichia coli* K-12 by the transposable element, Tn*5*, were characterized on the basis of growth requirements, cross-feeding behavior, and enzyme activity. Mutants defective in *ilvA*, *ilvC*, *ilvD* and *ilvE* were found. Mutations in *ilvE* were not completely polar on *ilvD* and *ilvA* enzyme activities (that is, *ilvE* mutants possessed a low constitutive level of expression of the enzymes coded by *ilvD* and *ilvA*), while mutations in *ilvD* were completely polar on *ilvA* enzyme activity. The data suggest that there is an internal promoter between the sites of Tn*5* insertion in *ilvE* and *ilvD*.

THE isoleucine-valine (*ilv*) biosynthetic genes comprise four transcriptional units: *ilvB*, *ilvC*, and *ilv*OGEDA, which were mapped at 83 min, and *ilvHI*, which was mapped at 2 min (BACHMANN, LOW and TAYLOR 1976; COHEN and JONES 1976; BERG and SHAW 1979). *ilvA* codes for the first enzyme in isoleucine biosynthesis, threonine deaminase (Figure 1); *ilvB*, *ilvHI* and *ilvG* code for three acetohydroxyacid synthase isozymes, which mediate the first common step in isoleucine-valine biosynthesis. *ilvC*, *ilvD* and *ilvE* code for the enzymes catalyzing the last three common steps in isoleucine-valine biosynthesis: hydroxyacid isomeroreductase, dihydroxyacid dehydrase and transaminase B, respectively.

Transposable elements are mutagenic when they insert into genes, and they are polar because they interrupt transcription. Twenty-two *ilv* mutants induced by insertion of the kanamycin-neomycin resistance-determining transposable element, Tn*5* (D. BERG 1977), have been characterized by growth requirement and cross-feeding data. Enzyme assays of the mutant strains confirmed the gene assignments to *ilvA*, *ilvC*, *ilvD* and *ilvE*, and further showed that *ilvD*::Tn*5* mutants completely lack *ilvA* enzyme activity, while *ilvE*::Tn*5* mutants have a

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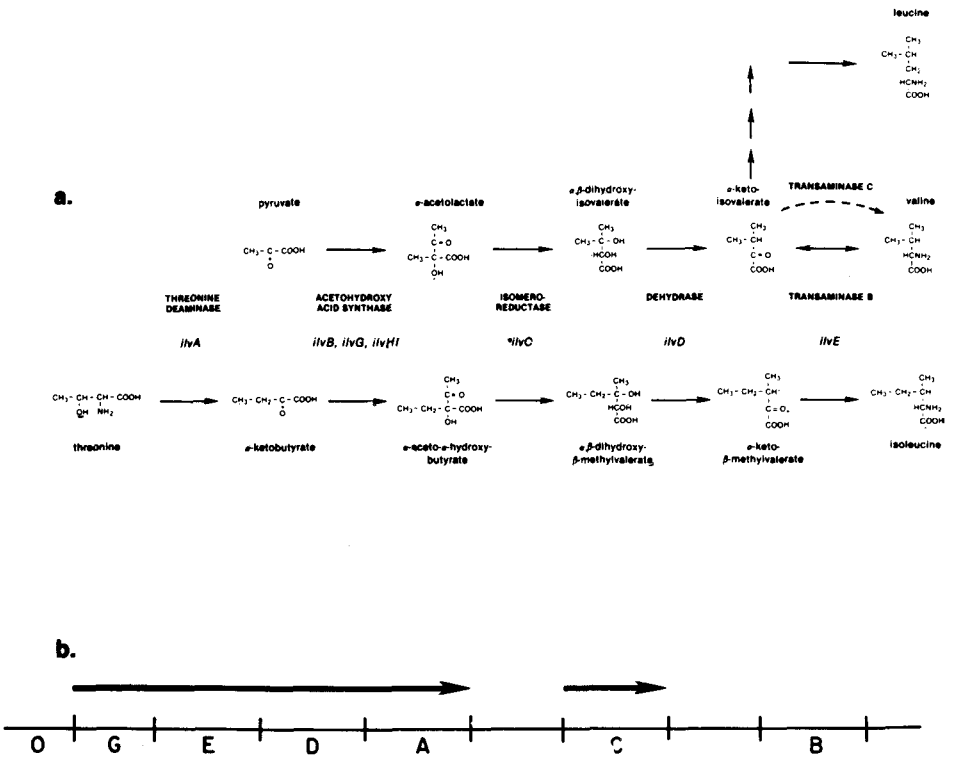

FIGURE 1.—(a). The biosynthetic pathway for isoleucine, valine and leucine (UMBARGER and DAVIS 1962). The genes of the *ilv* cluster specifying these enzymes are given beneath the respective enzyme. *In vivo*, the transaminase B-mediated interconversion of α-ketoisovalerate and valine is reversible, but the reversibility of the conversion of α-keto-β-methylvalverate to isoleucine has not been demonstrated. Transaminase C is also able to mediate the conversion of α-ketoisovalerate to valine, but not of α-keto-β-methylvalerate to isoleucine (UMBARGER and DAVIS 1962; MCGILVRAY and UMBARGER 1974).

(b.) Genetic organization of the isoleucine-valine cluster. The gene order determined by COHEN and JONES (1976) is given, except that *ilvG* is also shown (see text) (BERG and SHAW 1979). *ilvJ* (GUARDIOLA 1977) is not shown since "*ilvJ*" mutants are probably defective in a single bifunctional isoleucine-valine transaminase (TrB) (see text), *ilvHI*, which codes for an acetohydroxyacid synthase isozyme, is not in this cluster.

low constitutive level of both *ilvD* and *ilvA* enzyme activities. These data are consistent with the order OEDA for that operon (COHEN and JONES 1976) and suggest that there is an internal promoter between *ilvE* and *ilvD*.

MATERIALS AND METHODS

Strains and growth conditions: The strains of *E. coli* K-12 used were W3110(*thy*) and 22 isoleucine or isoleucine-valine requiring derivatives that had been induced by insertion of the transposable element Tn5 (D. BERG *et al.* 1975; D. BERG 1977). The isolation and preliminary characterization of these mutants are described elsewhere (SHAW and BERG 1979).

Strains CU1008 (*ilvA454*), CU1010 (*ilvC462*) and CU2 (*ilvE12*) were used in preliminary cross-feeding tests. CU592 (*ilvE2066*::Mu) and CU625 (*ilvE2070*::Mu) (SMITH, SMOLIN and

UMBARGER 1976) were used in one set of enzyme assays. These strains, from the collection of E. UMBARGER, were obtained from E. KLINE and E. UMBARGER.

The broth (LENNOX 1955) was supplemented with 10 μg per ml thymine, and the minimal medium E (VOGEL and BONNER 1956) was supplemented with thymine (10 μg per ml), and thiamine (2 μg per ml). The carbon source was glucose at 0.018% in glucose-limited minimal medium and at 0.5% in all other minimal media. Glucose-limited medium contained, in addition, L-isoleucine, L-valine, and L-leucine at 0.15 mM. Repressing medium contained excess L-isoleucine (0.4 mM), L-valine (1 mM) and L-leucine (0.4 mM). Derepressing (isoleucine-limited) medium contained excess L-valine and L-leucine, but a growth-limiting level of L-isoleucine (0.02 mM) (KLINE *et al* 1974). Valine-limited medium contained excess L-isoleucine and L-leucine, but a growth-limiting level of L-valine (0.08 mM). Cells were grown with aeration at 37°.

Determination of of growth requirements: About 0.05 ml of solutions of isoleucine (76 mM), valine (85 mM), leucine (76 mM) and α-ketobutyrate (100 mM) were spotted 4 cm apart on minimal medium plates that had been spread with approximately 10^7 cells. The presence of growth radiating from or between individual spots was scored after 24 to 48 hr of incubation.

Cross-feeding tests: Cells were grown in 2 ml of broth, without aeration, to log or late-log phase. Approximately 0.05 ml of the tester strain was applied in one or two vertical lines to the surface of a minimal medium plate. After drying, loopfuls of cultures of up to 20 other strains were streaked horizontally across the tester strain (Figure 2). CU1008, CU1010 and CU2 were used as testers in preliminary classifications. Subsequently, strains isolated in this study were used

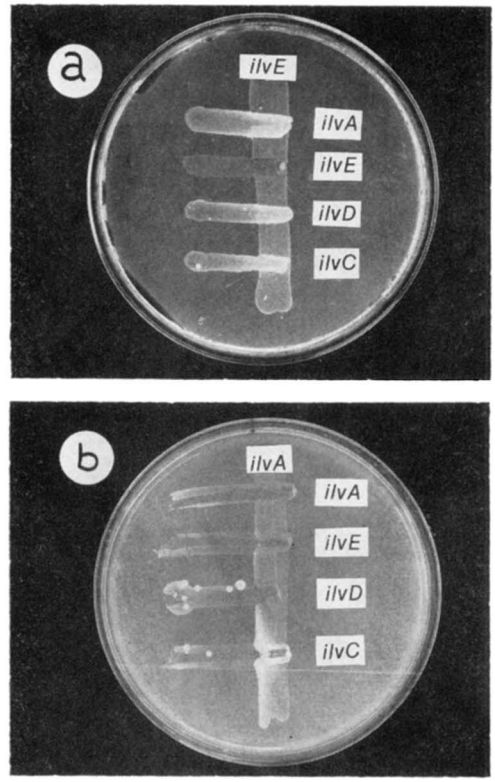

FIGURE 2.—Cross-feeding interactions of *ilv*::Tn5 mutants. The tester strain was streaked vertically and a representative of each class was streaked horizontally. *ilvA*, Class I (CBK007); *ilvE*, Class II (CBK086), *ilvD*, Class IIIa (CBK084); *ilvC*, Class IIIb (CBK252). Tester strains: a, CBK086 (*ilvE*); b, CBK007 (*ilvA*).

as tester strains: CBK007 (*ilvA700*::Tn5), CBK252 (*ilvC711*::Tn5), CBK084 (*ilvD714*::Tn5), CBK086 (*ilvE720*::Tn5). After 24 to 48 hr of incubation, the plates were scored. Heavy growth where streaks intersected indicated that cross-feeding had occurred. Growth extended vertically from the intersection indicated that the tester had been fed, while growth extending horizontally indicated that the tester had fed the other strain (Figure 2). Isogenic F- strains were used, so that mixing of the two cell types in the cross-streak did not lead to growth due to complementation or recombination.

Chemicals and substrates: α-acetolactate and α,β-dihydroxy-β-methylvalerate were generously provided by E. L. KLINE, Clemson University. For protein determinations, the Protein Assay Kit was purchased from Bio-Rad Laboratories. All other substrates and cofactors were purchased from Sigma Chemical Company.

Preparation of extracts: Cells were grown for 18 hr in glucose-limited medium. For most assays, derepressing conditions were achieved by harvesting the cells by centrifugation at room temperature, washing them once in isoleucine-limited medium, and resuspending them at an A_{660} of about 0.2 in isoleucine-limited medium. The same protocol was followed for the isomeroreductase (*ilvC*) assay except that valine-limited medium was employed. Repressing conditions were achieved by harvesting the cells by centrifugation, and resuspending them at an A_{660} of about 0.15 in repressing medium. After resuspension, cells were incubated for 2.5 hr with aeration (final A_{660} of 0.45 to 0.6), harvested by centrifugation, washed in chilled minimal salts, and resuspended at a 50 to 200-fold increased concentration in chilled 0.05 M potassium phosphate buffer, pH 7.2, containing 2×10^{-4} M dithiothreitol. Extracts were prepared by sonic disruption of the cells, and clarified by centrifugation at 15,000 × g for 10 min.

Enzyme assays: Protein was assayed by the protein dye-binding method of BRADFORD (1976), using bovine gamma globulin as the protein standard. All specific activities are expressed in nmol min^{-1} mg^{-1} protein, and are based on three or four time points for each assay, in addition to the control.

Transaminase B (*ilvE*) (EC 2.6.1.6) was assayed by the method of DUGGAN and WECHSLER (1973), using either valine or isoleucine (final concentration 25 mM) as the substrate. This assay is specific for transminase B (KLINE, MANROSS and WARWICK 1977).

Dehydrase (*ilvD*) (dihydroxyacid dehydrase, EC 4.2.1.9) and threonine deaminase (*ilvA*) (EC 4.2.1.16) were assayed by the method of UMBARGER (KLINE, personal communication). The reaction mixture for the dehydrase assay contained 0.1 M potassium phosphate buffer (pH 7.5), 0.02 M α,β-dihydroxy-β-methylvalerate, 0.01 M $MgCl_2$ and crude extract to a total volume of 4 ml. The reaction mixture for the threonine deaminase assay contained 0.1 M potassium phosphate buffer (pH 7.2), 0.1 M KCl, 0.1 mM pyridoxal-5-phosphate, 0.04 M threonine, and crude extract in a total volume of 4 ml. For both assays, the extract was preincubated at 37° in the reaction mixture without substrate. The reactions were started by the addition of substrate and incubated at 37°. One ml aliquots were removed after 10, 20 and 30 min, and the reactions were stopped by the addition of 0.1 ml of 50% trichloroacetic acid (TCA). The control tube contained 0.1 ml TCA, to which the reaction mixture and extract were added. Three ml of 0.025%, 2,4-dinitrophenylhydrazine in 0.5 M HCl were added to each tube and vortexed briefly. After 15 min at room temperature, 1 ml of 40% KOH was added, and five min later the absorbance was read at 540nm.

Acetohydroxyacid synthase (EC 4.1.3.12) was assayed by the method of ARFIN and UMBARGER (1969), as modified by KLINE *et al.* (1974).

Isomeroreductase (*ilvC*) (hydroxyacid isomeroreductase, EC 1.1.1.78) was assayed at room temperature by the modified method of ARFIN and UMBARGER (1969; KLINE *et al.* 1974; KLINE, personal communication). The reaction mixture contained 83 mM potassium phosphate buffer (pH 7.5), 0.12 mM NADPH, 8.3 mM α-acetolactate (pH 7.0), and crude extract.

RESULTS

We have used growth requirement and cross-feeding data to classify 22 Tn5-induced isoleucine-valine mutants (Table 1) obtained by direct selection of

TABLE 1

Physiological behavior of Tn5-induced ilv *mutants*

Class	Growth requirements*	Crossfeeding† I	II	IIIa	IIIb	No. of mutants	Representative strain
I	ile or α-KB	—	—	—	—	4	CBK007
II	ile, stimulated by val	↑	—	↑	↑	2	CBK086
IIIa	ile + val	—	—	—	—	7	CBK084
IIIb	ile + val	↑	—	—	—	9	CBK252

* ile: isoleucine. α-KB: α-ketobutyrate. val: valine.
† The arrowhead points to the class that is crossfed. A dash indicates no crossfeeding.

auxotrophs from kanamycin-resistant survivors of W3110(*thy*) infected with λb221 carrying Tn5 (SHAW and BERG 1979).

Fidelity of Tn5 insertion

Tn5 generally inserts into a chromosome without altering chromosomal DNA, since kanamycin-sensitive true revertants of Tn5-induced mutations can be obtained (D. BERG 1977). Representative strains of each mutant class gave rise to kanamycin-sensitive true revertants (at frequencies of 10^{-10} to 5×10^{-8}). Therefore, the mutant phenotypes are not due to chromosomal deletions or other aberrations that might affect transcription of adjacent cistrons.

Growth requirements

Class I: Four mutants required either isoleucine or α-ketobutyrate for growth, suggesting that they are defective in *ilvA*.

Class II: Two mutants required isoleucine but did not grow on α-ketobutyrate. They were slightly stimulated by valine in the presence of isoleucine, suggesting that they are defective in *ilvE*, but express *ilvD* and *ilvA*. Completely polar *ilvE*::Tn5 mutants, like *ilvE-ilvD* double mutants (KIRITANI and INUZUKA 1970; KLINE, MANROSS and WARWICK 1977), were expected to lack both *ilvE* and *ilvD* enzyme activities and, therefore, require all three branched-chain amino acids because α-ketoisovalerate is the precursor of leucine as well as valine (Figure 1). No such mutants were found among the 305 Tn5-induced autotrophs isolated (SHAW and BERG 1979) [although mutants requiring all three amino acids are found in this genetic background among *ilvEDA* deletion strains (D. BERG and C. BERG 1978)]. The two *ilvE*::Tn5 mutants do not show a strict requirement for valine, because an unrelated valine transaminase (transaminase C) can partially substitute for the missing *ilvE* product (transaminase B) (see MCGILVRAY and UMBARGER 1974). The alternative explanation, that there is a second transaminase B in *E. coli*, specific for the valine pathway (*ilvJ*) (GUARDIOLA 1977) is probably incorrect (see below).

Class III: Sixteen mutants required both isoleucine and valine suggesting that they are defective in *ilvD* or *ilvC*.

Cross-feeding

The class II (*ilvE*) mutants cross-fed all other mutants (Figure 2a). Nine of the 16 class III mutants cross-fed class I (*ilvA*) mutants (Figure 2b). The two *ilvE* mutants cross-fed members of all other classes, but more weakly than a nonpolar *ilvE* mutant, CU2 (data not shown), suggesting that in these *ilvE*::Tn*5* mutants the expression of *ilvD* and of *ilvA* is reduced. These observations indicate that the *ilvE* (class II) mutants are not completely polar, and that class III comprises two subclasses: the seven mutants (class IIIa) that did not feed *ilvA* mutants are completely polar *ilvD* mutants, while the nine mutants (class IIIb) that did feed *ilvA* mutants are *ilvC* mutants (Figure 2b, Table 1).

Regulation of ilv *operon expression*

The cistron assignments were confirmed by assaying the relevant enzymes in each mutant. Data on a representative strain of each class are presented in Table 2.

The lack of *ilvA* enzyme activity in the *ilvD*::Tn*5* mutants indicates that these insertion mutations are completely polar on operator-distal gene expression, while the presence of *ilvD* and *ilvA* enzyme activities in the *ilvE*::Tn*5* mutants indicates that these insertion mutations are not completely polar on operator-distal gene expression.

The *ilvE* mutants lack transaminase B activity when either valine or isoleucine is used as the substrate (Table 2). GUARDIOLA (1977) suggested that the *ilvE* gene product might have only isoleucine transaminase activity, while the product of a hypothetical gene, *ilvJ*, might be the valine transaminase. He was, however, unable to exclude the alternative explanation that both enzyme activities are present in a single bifunctional enzyme. We favor this latter interpretation since our insertion mutants, which are deficient in transaminase B activity on both substrates (Table 2), have the growth requirement attributed by GUARDIOLA (1977) to mutants lacking the isoleucine transaminase alone. Furthermore, in *S.*

TABLE 2

Expression of the ilv *genes in representative Tn5-induced mutants**

Class	Strain	Specific activity† TrB *ilvE*		DH *ilvD*	TD *ilvA*	IR *ilvC*	AHAS *ilvB,G,HI*
ilv+	W3110(*thy*)	13.8‡	7.9§	21.1	59.0	Nd‖	6.1
I	CBK007 (*ilvA700*::Tn*5*)	20.0	9.1	11.3	<1.5	Nd	6.5
II	CBK086 (*ilvE720*::Tn*5*)	<1.8	<1.8	1.2	3.3	20.6	9.1
IIIa	CBK084 (*ilvD714*::Tn*5*)	20.8	9.1	<0.6	<1.5	28.0	7.4
IIIb	CBK252 (*ilvC711*::Tn*5*)	13.4	7.4	21.3	57.6	<1.4	7.6

* For the *ilvC* enzyme assay, cells were grown in valine-limited medium. For all other assays, cells were grown in isoleucine-limited medium.

† Enzyme abbreviations: TrB, transaminase B; DH, dihydroxyacid dehydrase; TD, threonine deaminase; IR, hydroxyacid isomeroreductase; AHAS, acetohydroxyacid synthase.

‡ Isoleucine was used as the substrate.

§ Valine was used as the substrate.

‖ Not done.

TABLE 3

Expression of ilv *operon genes in Tn5-induced mutants*

Strain	Specific activity* TrB(*ilvE*)† D‡	R§	DH(*ilvD*) D	R	TD(*ilvA*) D	R	Ratio (D/R) TrB	DH	TD
W3110 (*thy*)	13.7 ± 2.6	5.5 ± 0.4	13.6 ± 5.3	1.9 ± 0.6	51.8 ± 7.6	5.2 ± 2.4	2.5	7.2	10.0
CBK086 (*ilvE720*::Tn5)	<1.8	<1.8	1.9 ± 1.1	1.9 ± 1.7	3.5 ± 0.4	3.6 ± 1.0	—	1.0	1.0
CBK089 (*ilvE721*::Tn5)	<1.8	<1.8	1.0 ± 0.1	1.2 ± 0.2	3.7 ± 1.5	3.3 ± 1.2	—	0.8	1.1
CBK084 (*ilvD714*::Tn5)	18.4 ± 2.6	7.4 ± 2.1	<0.65	<0.65	<1.5	<1.5	2.5	—	—
CBK007 (*ilvA700*::Tn5)	17.9 ± 5.9	7.7 ± 2.4	4.1 ± 1.4	1.2 ± 0.3	<1.5	<1.5	2.3	3.4	—

* Enzyme abbreviation: TrB, transaminase B; DH, dihydroxyacid dehydrase; TD, threonine deaminase. The means and standard deviations were based upon four to eight determinations for the TrB and TD assays and upon two to six determinations for the DH assay.

† Isoleucine was used as the substrate.

‡ Cells were grown in derepressing (isoleucine-limited) medium.

§ Cells were grown in repressing medium containing excess isoleucine, valine and leucine.

typhimurium, we find that the growth requirements of Tn*10*-induced mutants lacking transaminase B activity on both substrates are correlated with the level of expression of *ilvD*: mutants with relatively high levels of *ilvD* enzyme activity require isoleucine, while mutants with negligible levels of *ilvD* enzyme activity require isoleucine plus either valine or leucine (unpublished).

To test whether the low level expression of *ilvD* and *ilvA* enzyme activities in the *ilvE* mutant is under normal regulation, enzyme assays were performed on strains grown under derepressing and repressing conditions (Table 3). In both *ilvE* mutants, *ilvD* and *ilvA* were expressed at a low constitutive level. *ilvD* enzyme activity in the *ilvA* mutant, and *ilvE* enzyme activity in both the *ilvD* and *ilvA* mutants was subject to repression, although *ilvD* enzyme activity in the *ilvA* strain was lower than that in the *ilv*$^+$ strain. We have examined *ilvD* enzyme activity in the three other *ilvA*::Tn*5* mutants and find that one, like CBK007, has a reduced level of *ilvD* enzyme activity, while two have wild-type levels (data not shown). The basis of the reduced expression of *ilvD* enzyme activity in two of our four *ilvA*::Tn*5* mutants and in one *ilvA*::Mu mutant, previously described (KLINE and BAYLISS 1975), is not known.

The data in Table 3 indicate that in each insertion mutant, cistrons proximal to the site of Tn*5* insertion were subject to repression, and cistrons distal to the site of insertion were either not expressed (in *ilvD* mutants) or had a low constitutive expression (in *ilvE* mutants).

The data obtained in this study support the interpretation that in *E. coli ilvE*, *ilvD* and *ilvA* comprise one operon, which is transcribed in the order OEDA (COHEN and JONES 1976). These data do not test the finding that, as in *S. typhimurium*, the first structural gene in this operon is *ilvG* (BERG and SHAW 1979) because no *ilvG* insertion mutants were obtained.

The observation that both *ilvE* mutants were not completely polar and that all seven *ilvD* mutants were completely polar suggests that there is an internal promoter between *ilvE* and *ilvD*. If so, mutations induced in *ilvE* by any insertion element should be partially polar.

Properties of ilvE::*Mu strains.*

The only two other *ilvE* insertion mutants that have been described in *E. coli* were reported to lack *ilvD* and *ilvA* enzyme activity completely (SMITH, SMOLIN and UMBARGER 1976). We have obtained these mutants from E. UMBARGER and find that, like the *ilvE*::Tn*5* mutants, they cross-feed all other classes of mutants, and require only isoleucine (or isoleucine plus valine in some single colony isolates). They do not require all three branched-chain amino acids. Furthermore, they lack detectable *ilvE* enzyme activity and retain some *ilvD* and *ilvA* enzyme activity. Hence, these *ilvE* mutants are not completely polar (confirmed by E. UMBARGER, personal communication).

DISCUSSION

We have analyzed 22 *ilv* mutants induced by insertion of the transposable element Tn*5*. Growth requirement and cross-feeding data permitted us to dis-

tinguish four mutant classes: *ilvA*; *ilvE*, not completely polar on *ilvD* and *ilvA*; *ilvD*, completely polar on *ilvA*; and *ilvC* (Table 1, Figure 2). Enzyme assays confirmed the gene assignments and further showed that the *ilvE* mutants had low constitutive levels of *ilvD* and *ilvA* enzyme activities (Table 2, Table 3). Because Tn*5* has a polar effect on distal gene expression, we were able to detect an internal promoter between *ilvE* and *ilvD*, which is responsible for the constitutive expression of *ilvD* and *ilvA*.

To extend these findings, we have examined a larger collection of *Salmonella typhimurium ilv*::Tn*10* mutants (kindly provided by J. ROTH). As in *E. coli*, all 18 *ilvE*::Tn*10* mutants expressed low levels of *ilvD* and *ilvA* enzyme activities, while none of 13 *ilvD*::Tn*10* mutants had any detectable *ilvA* expression (unpublished). Every one of the *ilvE* mutants examined in both genera, using Tn*5* and Tn*10*, as well as Mu (see RESULTS), is not completely polar. This strongly suggests that the promoter activity detected is operon—rather than transposable element—encoded.

Our analysis of the Salmonella *ilv*::Tn*10* mutants indicates that the *ilv* operon is more complex than previously thought. An additional class of 33 isoleucine-requiring mutants, which are neither *ilvA* nor *ilvE*, was found. These mutants are defective in AHAS II (*ilvG*), are located operator-proximal to *ilvE* and have a low constitutive level of *ilvE* and *ilvA* enzyme activities (BERG and SHAW 1979). Therefore, we conclude that *ilvG* is the first gene in the *ilvOGEDA* operon and that there are two internal promoters in the operon, one between *ilvE* and *ilvD* and the other (which was not detected in the present study) between *ilvG* and *ilvE*.

Although internal promoters have been detected in several operons, too few studies have been performed to determine if they are common, perhaps important, features of bacterial operons (CAMPBELL 1979). Until recently, it was necessary to have operator-deletion mutants in order to detect an internal promoter (BAUERLE and MARGOLIN 1967; CUNIN *et al.* 1969; ATKINS and LOPER 1970). More recently, insertion mutants have been used to identify both the internal promoters previously described in the *his* operon of *S. typhimurium* (KLECKNER *et al.* 1975) and a new internal promoter in the *gua* operon of *E. coli* (FUKUMAKI, SHIMADA and TAKAGI 1977). As we have shown in the *ilv* operon, screening for internal promoters can be further facilitated if it is possible to detect the expression of distal genes in the operon on the basis of growth requirements and/or cross-feeding behavior, since these biological assays are easier and more sensitive than enzyme assays performed on crude extracts.

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Note added in proof: Our conclusion that *ilvE* codes for a single bifunctional transaminase was confirmed by recent reports of the purification of transaminase B: F.-C. LEE-PENG, M. A. HERMODSON and G. B. KOHLHAW, 1979 Transaminase B from *Escherichia coli*: quarternary structure, amino-terminal sequence, substrate specificity, and absence of a separate valine-α-ketoglutarate activity. J. Bacteriology **139:** 339–345; C. W. ADAMS, R. P. LAWTHER and G. W. HATFIELD, 1979 The *ilvEDA* operon of *Escherichia coli* K12 encodes only one valine-α-ketoglutarate transaminase activity. Biochem. Biophys. Res. Commun. **89:** 650–658.

LITERATURE CITED

ARFIN, S. M. and H. E. UMBARGER, 1969 Purification and properties of the acetohydroxy acid isomeroreductase of *Salmonella typhimurium*. J. Biol. Chem. **244:** 1118–1127.

ATKINS, J. F. and J. C. LOPER, 1970 Transcription initiation in the histidine operon of *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A. **65:** 925–932.

BACHMANN, B. J., K. B. LOW and A. L. TAYLOR, 1976 Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40:** 116–167.

BAUERLE, R. H. and P. MARGOLIN, 1967 Evidence for two sites for initiation of gene expression in the tryptophan operon of *Salmonella typhimurium*. J. Mol. Biol. **26:** 423–436.

BERG, C. M., and K. J. SHAW, 1979 Organization of the *ilvOGEDA* operon in *Salmonella typhimurium*. Abstr. Ann. Mtg. Amer. Soc. Microbiol. p. 156.

BERG, C. M., K. J. SHAW, J. VENDER and M. BORUCKA-MANKIEWICZ, 1978 Transposable element-induced isoleucine-valine auxotrophs in *Escherichia coli* K-12. Abstr. Ann. Mtg. Amer. Soc. Microbiol. p. 160.

BERG, D. E., 1977 Insertion and excision of the transposable kanamycin resistance determinant Tn*5*, pp. 205–212. *DNA Insertion Elements, Plasmids and Episomes*. Edited by A. I. BUKHARI, J. A. SHAPIRO and S. L. ADYHA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

BERG, D. E. and C. M. BERG, 1978 Deletions in the isoleucine-valine cluster in *E. coli*. Abstr. Ann. Mtg. Amer. Soc. Microbiol. p. 112.

BERG, D. E., J. DAVIES, B. ALLET and J-D. ROCHAIX, 1975 Transposition of R factor genes to bacteriophage λ. Proc. Natl. Acad. Sci. U.S. **72:** 3628–3632.

BRADFORD, M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

CAMPBELL, A., 1979 Structure of complex operons, pp. 19–55. In: *Biological Regulation and Devolpment*. Edited by R. F. GOLDBERGER, Plenum Press, New York.

COHEN, B. M. and E. W. JONES, 1976 New map location of *ilvO* in *Escherichia coli*. Genetics **83:** 201–225.

CUNIN, R., D. ELSEVIERS, G. SAND, G. FREUNDLICH and N. GLANSDORFF, 1969 On the functional organization of the *argECBH* cluster of genes in *Escherichia coli* K-12. Mol. Gen. Genet. **106:** 32–47.

DUGGAN, D. E. and J. A. WECHSLER, 1973 An assay for transaminase B enzyme activity in *Escherichia coli* K-12. Anal. Biochem. **51:** 67–79.

FUKUMAKI, Y., K. SHIMADA, and Y. TAKAGI, 1977 Secondary promoter of the guanine operon of *Escherichia coli* K-12. J. Bacteriol. **131:** 685–688.

GUARDIOLA, J., 1977 Requirement of the newly recognized *ilvJ* gene for the expression of a valine transaminase activity in *E. coli* K-12. Mol. Gen. Genet. **158:** 157–164.

KIRITANI, K. and N. INUZUKA, 1970 Mutations affecting the branched-chain-amino-acid aminotransferase in *Salmonella typhimurium*. Japan. J. Genet. **45:** 293–304.

KLECKNER, N., R. K. CHAN, B.-K. TYE and D. BOTSTEIN, 1975 Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. **97:** 561–575.

KLINE, E. L. and F. T. BAYLISS, 1975 The effect of an *ilvA* Mu phage insertion on *ilv* gene expression in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **63:** 1048–1055.

KLINE, E. L., C. S. BROWN, W. G. COLEMAN and H. E. UMBARGER, 1974 Regulation of isoleucine-valine biosynthesis in an *ilvDAC* deletion strain of *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **57:** 1144–1151.

KLINE, E. L., D. N. MANROSS, JR. and M. L. WARWICK, 1977 Multivalent regulation of isoleucine-valine transaminase in an *Escherichia coli* K-12 *ilvA* deletion strain. J. Bacteriol. **130:** 951–953.

LENNOX, E. S., 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology. **1:** 190–206.

MCGILVRAY, D. and H. E. UMBARGER, 1974 Regulation of transaminase C synthesis in *Escherichia coli*: conditional leucine auxotrophy. J. Bacteriol. **120:** 715–723.

SHAW, K. J. and C. M. BERG, 1979 *Echerichia coli* K-12 auxotrophs induced by insertion of the transposable element Tn5. Genetics **92:** 741–747.

SMITH, J. M., D. E. SMOLIN and H. E. UMBARGER, 1976 Polarity and the regulation of the *ilv* gene cluster in *Escherichia coli* strain K-12. Mol. Gen. Genet. **148:** 111–124.

UMBARGER, E. and B. D. DAVIS, 1962 Pathways of amino acid biosynthesis p. 167–251. In: *The Bacteria, vol. 3*, edited by I. C. GUNSALUS and R. Y. STANIER, Academic Press, New York..

VOGEL, H. J. and D. M. BONNER, 1956 Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol Chem. **218:** 97–106.

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