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Translational Coupling between the *ilvD* and *ilvA* Genes of *Escherichia coli*

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The hypothesis that translation of the *ilvD* and *ilvA* genes of *Escherichia coli* may be linked has been examined in strains in which *lacZ-ilvD* protein fusions are translated in all three reading frames with respect to *ilvD*. In these strains, the nucleotide sequence was altered to obtain premature termination of *ilvD* translation, and in one strain translation termination of *ilvD* DNA occurred two bases downstream of the *ilvA* initiation codon. In the wild-type strain, the *ilvD* translation termination site was located two bases upstream of the *ilvA* start codon. In each of the mutant strains, expression of *ilvA*, as determined by the level of threonine deaminase activity, was strikingly lower than in the wild-type strain. The data suggest that expression of *ilvD* and *ilvA* is translationally coupled. By inserting a promoterless *cat* gene downstream of *ilvA*, it was shown that the differences in enzyme activity were not the result of differences in the amount of *ilvA* mRNA produced.

In members of the family *Enterobacteriaceae*, the biosynthesis of isoleucine and valine occurs by parallel pathways with enzymes specified by a cluster of genes, *ilvGMEDAYC* (39). The first five genes in the cluster form the *ilvGMEDA* operon; *ilvY* and *ilvC* are separate transcriptional units. Blazey and Burns (4) first implicated regulation at the translational level in the *ilv* operon and raised the possibility of translational coupling. Their experiments showed that Tn*10* insertions in both orientations in the *ilvD* gene of *Salmonella typhimurium* exerted an absolute polarity on *ilvA* expression. In *Escherichia coli*, a similar strong polar effect has been demonstrated in strains containing bacteriophage Mu-1 insertions (32) or Tn*5* insertions (3). Even earlier, it had been noted that the *ilvD188* ochre mutation resulted in a 60% reduction in threonine deaminase activity, although at the time, the effect was thought to be "antipolar" (42). Nucleotide sequence analysis has revealed that the *ilvD* translation stop codon and *ilvA* start codon are separated by 2 bases, TAA-TA-ATG (8, 18). (In this paper, we use the phrase "*n* bases upstream [or downstream]" to indicate that *n* bases separate the stop and start codons.) Proximity of translational termination and initiation signals, including overlapping signals, has been described as a feature in coupling translation of adjacent genes (11, 24, 33, 35). Translational coupling has been reported for a number of gene pairs, including *galK-galT* (29), *trpD-trpE* (26), and *trpB-trpA* (1, 9). In this paper we present evidence that efficient translation of the downstream *ilvA* gene is dependent on translation of the upstream *ilvD* gene.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study and their sources are listed in Table 1. The preparation of some of the strains requires special mention. Strain CU1644 was prepared by replacing the wild-type *ilv* region of CU1318 with that in pPU316, which carries Δ(*ilvGY*)*2243*::*aad*$^+$. The *aad*$^+$ gene, inserted at the site of the *ilv* deletion, was derived from Omega, the Spc^r Str^r interposon described by Prentki and Krisch (27) (see below). Plasmid pPU316 was cleaved at the *Eco*RI and *Bam*HI sites and used to transform CU1318, a *recBC* strain. Transformants were selected on L agar plates containing 25 μg of spectinomycin per ml and scored for sensitivity to ampicillin and a requirement for branched-chain amino acids for growth. One such colony was designated CU1644. This strain contains the Omega fragment, carrying the *aadA* gene, in place of the *ilvGMEDA* operon.

Strains CU1646 through CU1651 were obtained by integration of DNA from plasmids pPU323 through pPU328, respectively, into the chromosome of strain CU1644. Each of the above plasmids was cut at two unique restriction sites within the vector and used to transform CU1644. Recombinants were selected on L agar plates containing chloramphenicol (4 μg/ml). Colonies were scored for sensitivity to ampicillin and spectinomycin. Two Amp^s Spc^s colonies from each plasmid transfer were maintained without selection pressure and used for threonine deaminase assays. Threonine deaminase activities in extracts prepared from both members of each transfer were well within experimental error of the activity expected. Therefore, one of each type of colony was assigned a strain number. They are designated CU1646 through CU1651 in the strain table.

Growth of cells and enzyme assays. Cells were grown in L broth at 37°C to an OD_{660} of 0.2 before the inducer isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM. Portions of the culture were harvested by centrifugation at four times up to 4 h after induction; pellets were suspended in 0.1 M potassium phosphate (pH 7.3)–1 mM isoleucine–0.5 mM dithiothreitol, and cells were disrupted by sonic oscillation. Cell debris was removed by centrifugation, and the supernatant fluid was assayed for enzyme activity. Threonine deaminase activity was determined as described by Szentirmai and Umbarger (38). Chloramphenicol acetyltransferase levels were measured by the method of Gorman et al. (12) modified as described previously (15).

General procedures. Plasmid DNA was purified either by equilibrium density centrifugation in cesium chloride or by the alkaline lysis procedure described by Maniatis et al. (20).

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TABLE 1. Strains used

E. coli strain	Genotype	Source or reference
CU815	*ΔilvDAC galT12*	Smith et al. (31)
CU825	*ilvA454 Δ(pro-lac) ara thi rbs-221*	Watson et al. (41)
CU1230	*ΔilvGME2130 ara Δ(pro-lac) thi recA56 hsdR*	Harms et al. (14)
CU1318	*leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Strain W5449 (CGSC 5913) from B. Bachmann
CU1644	*Δ(ilvG-Y)2243::aadA*$^+$ (Omega fragment, Str^r Spc^r) *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Transformation of CU1318 with pPU316, digested with *Eco*RI and *Bam*HI
CU1646	*Δ(ilvG-Y)2247::ΔaadA*$^+$ (Omega fragment, Str^r Spc^r)::[pPU323 *Φ(lacZpZ'ilv'DAY')1 Φ(cat-ilvpE)1*, 3.1 kb] *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Transformation of CU1644 with pPU323, digested with *Kpn*I and *Pst*I; selected for Cm^r and scored for Spc^s
CU1647	*Δ(ilvG-Y)2248::ΔaadA*$^+$ (Omega fragment, Str^r Spc^r)::[pPU324 *Φ(lacZpZ'-ilv'DAY')2 Φ(cat-ilvEp)1*, 3.1 kb] *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Transformation of CU1644 with pPU324, digested with *Pst*I and *Nar*I; selected for Cm^r and scored for Spc^s
CU1648	*Δ(ilvG-Y)2249::ΔaadA*$^+$ (Omega fragment, Str^r Spc^r)::[pPU325 *Φ(lacZpZ'-ilv'DAY')3 Φ(cat-ilvEp)1*, 3.1 kb] *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Transformation of CU1644 with pPU325, digested with *Kpn*I and *Pst*I; selected for Cm^r and scored for Spc^s
CU1649	*Δ(ilvG-Y)2250::ΔaadA*$^+$ (Omega fragment, Str^r Spc^r)::[pPU326 *Φ(lacZpZ'-ilv'DAY')4 Φ(cat-ilvEp)1*, 3.7 kb] *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Transformation of CU1644 with pPU326, digested with *Nar*I and partially with *Pst*I; selected for Cm^r and scored for Spc^s
CU1650	*Δ(ilvG-Y)2251::ΔaadA*$^+$ (Omega fragment, Str^r Spc^r)::[pPU327 *Φ(lacZpZ'-ilv'DAY')5 Φ(cat-ilvEp)1*, 3.7 kb] *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Transformation of CU1644 with pPU327, digested with *Nar*I and partially with *Pst*I; selected for Cm^r and scored for Spc^s
CU1651	*Δ(ilvG-Y)2252::ΔaadA*$^+$ (Omega fragment, Str^r Spc^r)::[pPU328 *Φ(lacZpZ'-ilv'DAY')6 Φ(cat-ilvEp)1*, 3.7 kb] *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ^-	Transformation of CU1644 with pPU328, digested with *Pst*I and *Nar*I; selected for Cm^r and scored for Spc^s
JM103	F' *traD36 proAB*$^+$ *lacI*^{q}*ZΔM15/Δ (pro-lac) supE44 thi rpsL sbcB15 endA hsdR4*	Yanisch-Perron et al. (44)

Restriction enzymes, Klenow fragment of DNA polymerase, T4 DNA polymerase, T4 DNA ligase, and S1 nuclease were used as suggested by the suppliers. DNA fragments were isolated by using either an electroeluter, IBI model UEA, or dialysis bags as described by Maniatis et al. (20).

Plasmid constructions. Plasmids pPU307 through pPU309 (Fig. 1, Table 2) carry a 2.9-kilobase (kb) *Xho*I-*Hin*dIII DNA fragment containing the promoter-distal 129 base pairs (bp) of *ilvD*, the *ilvA* gene, and part of the *ilvY* gene from pPU118 (D. C. Bennett, Ph.D. thesis, Purdue University, West Lafayette, Ind., 1984). This fragment was first inserted into the *Sal*I-*Hin*dIII sites of the vectors pUC8 and pUC18 to yield pPU307 and pPU308, respectively. In pPU307 the *ilvD* gene is fused to *lacZ* so that *ilvD* is translated in the +1 frame with respect to the natural reading frame of *ilvD*. In pPU308 the fusion resulted in translation of *ilvD* in the −1 frame. To obtain a fusion in which *ilvD* was translated in its original reading frame, pPU307 was cut with *Bam*HI in the multiple cloning region, and the protruding 5′ ends were filled in by using the large fragment of *E. coli* DNA polymerase and ligated to yield pPU309.

A second, similar set of plasmid constructions, pPU311 through pPU313, contained an additional 473 bp of *ilvD* DNA, to which the *lac* promoter and *lacZ* translational start were fused (Fig. 2). A 3.5-kb *Pst*I-*Hin*dIII fragment of pPU118 (*ilv'DAY'*) was inserted at the *Pst*I-*Hin*dIII sites into pUC8 and pUC18 to obtain pPU311 and pPU312, respectively. Translation of *ilvD* sequences terminates prematurely 546 bases upstream of the *ilvA* initiation codon in pPU311 (−1 frame) and 572 bases upstream in pPU312 (+1 frame). Translation in the natural *ilvD* reading frame was obtained by digestion of pPU312 with *Pst*I, removal of the 3′ overhanging ends with T4 DNA polymerase, and subsequent ligation to yield pPU313.

The nucleotide sequence in the vicinity of the multiple cloning sites of all plasmids constructed in this work was determined by the method of Sanger et al. (28) to verify that the manipulations yielded the expected sequences.

pPU329 and pPU330 were constructed from pPU311 by replacing the 1.15-kb *Stu*I-*Hin*dIII fragment downstream of *ilvA* with the 1.65-kb *Sma*I-*Hin*dIII fragment from pPU320 containing the *cat* gene with its own ribosome-binding site but no promoter. pPU320 is a derivative of pKK232-8 (5) in which the 2-kb Omega fragment of pHP45Ω (27) is inserted in the *Pvu*II site downstream of the *cat* gene and the *rrn* terminators.

pPU316 is a pUC18 derivative that contains the Omega fragment of pHP45Ω flanked by a 1.3-kb DNA fragment from the region upstream of the *ilv* attenuator on one side and by a 1-kb *ilv'Y-C'* fragment on the other. The inserted fragment is thus a deletion of the *ilvGMEDA* operon that can be selected after transduction by the Spc^r Str^r phenotype. pPU316 was formed from two pUC18 derivatives, pPU314 and pPU315. pPU314 was constructed by an insertion of the 1.3-kb *Hin*dIII-*Sma*I fragment from pPU24 into the same sites of pUC18, followed by insertion of the 2.0-kb Omega

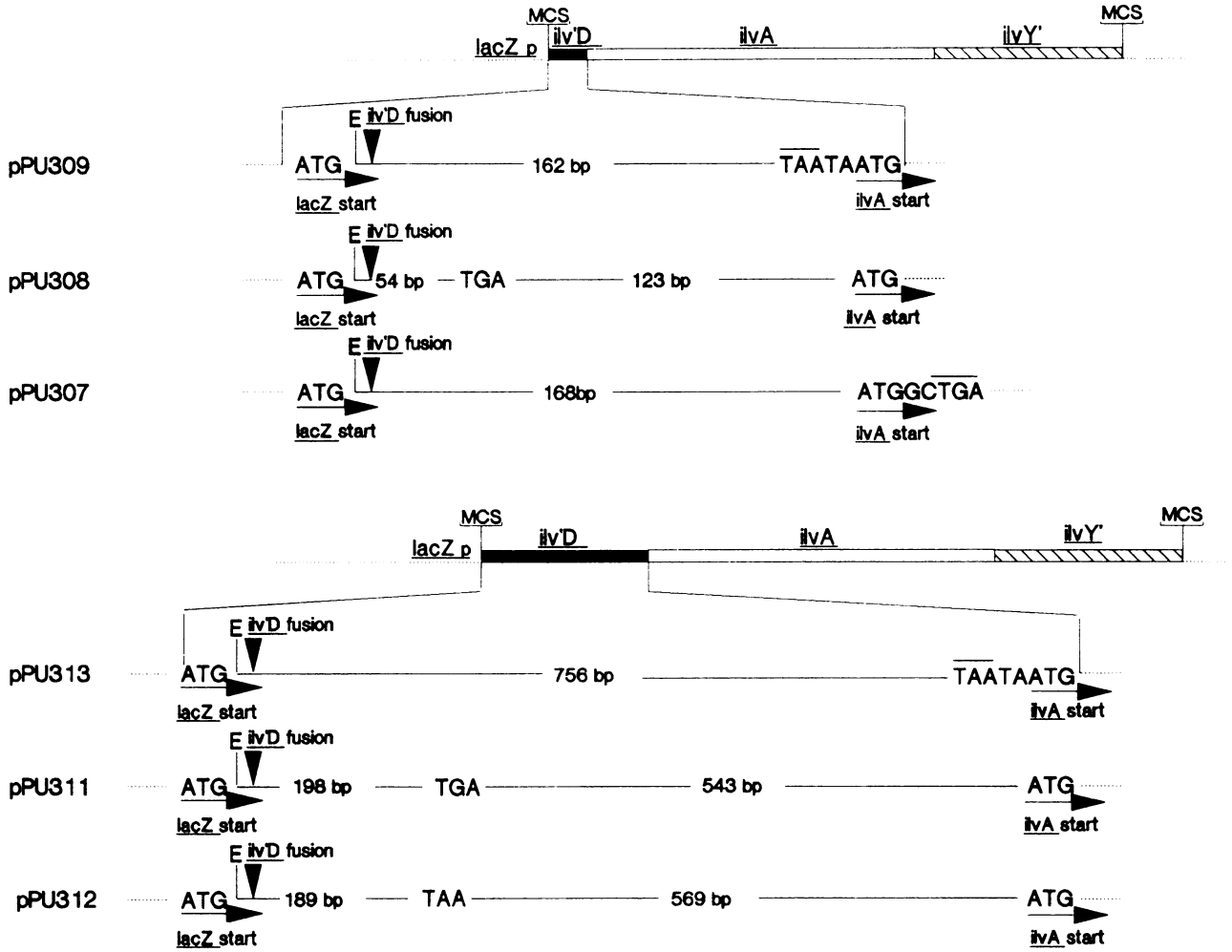

FIG. 1. Configuration of the altered translational reading frames in plasmids pPU307 through pPU309 and pPU311 through pPU313. The *lacZ* and *ilvA* start codons are indicated by arrows. The stop codons of the *lacZ'-ilv'D* fusion peptides are overlined. Vector DNA (·····) was pUC8 (as indicated in Table 2) or pUC18. E, *Eco*RI; MCS, multiple cloning site; P, promoter.

*Sma*I fragment into the *Sma*I site. The 1-kb *Bgl*II-*Kpn*I fragment of pPU5 containing part of *ilvY* and part of *ilvC* was ligated into the *Bam*HI and *Kpn*I sites of pPU18 to give pPU315. Finally, pPU316 was derived by ligation of the 3.66-kb *Nar*I-*Xba*I fragment of pPU315 to the 2.76-kb *Nar*I-*Kpn*I fragment of pPU314. The 3′ recessed ends created by *Xba*I digestion were filled in by use of the large fragment of *E. coli* DNA polymerase, and the 3′ protruding ends of the *Kpn*I site were digested with T4 DNA polymerase before ligation.

pPU318 is a plasmid containing the *cat* gene under the control of the *ilvEp* promoter, and pPU319 is the same except that it has the Omega fragment downstream of *cat*. For these constructions, a 480-bp *Hae*III-*Hin*dIII fragment from pPU119 containing the internal promoter located upstream of the *ilvE* gene, *Ep*, was inserted into pUC18 digested with *Pst*I and *Hin*dIII (19, 43). The 3′ protruding ends created by *Pst*I digestion of pUC18 were removed with T4 DNA polymerase before ligation. Strain JM103 was transformed with the resultant plasmid, pPU317, and white colonies were selected on L agar plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) and IPTG. Plasmid DNA was isolated, and insertion of the 480-bp fragment was verified by restriction enzyme analysis. The *ilvEp* promoter was transferred from this plasmid to pKK232-8, a promoter selection vector allowing assay of promoter activity by *cat* gene expression, to yield pPU318 by the following manipulations. pPU317 was cut with *Aat*II, and the 3′ protruding ends were digested with T4 DNA polymerase; the linear plasmid was then cut with *Bam*HI to obtain a 100-bp fragment containing the *ilvEp* promoter; this fragment was inserted into pKK232-8 at the *Bam*HI-*Hin*dIII sites after the *Hin*dIII site had been filled in by the Klenow fragment of *E. coli* DNA polymerase. This construction ensured the proper orientation of the promoter. Strain CU1230 was transformed with pPU318, and plasmid DNA was isolated from colonies that were selected for the ability to grow in the presence of chloramphenicol (30 μg/ml). This plasmid DNA was characterized by restriction site analysis. Additional restriction sites, carried on the *Sma*I-*Sma*I Omega fragment of pHP45Ω, were added to pPU318 to form pPU319. They were inserted into the *Pvu*II site downstream of the *cat* gene and the *rrn* terminators in pPU318.

pPU323 through pPU328 (Fig. 2) are plasmids that allow integration of desired sequences into the chromosome by recombination followed by selection for chloramphenicol resistance. They contain the several *lacZpZ'-ilv'DAY'* fusions inserted into pPU322, a plasmid in which the Omega

TABLE 2. Plasmids used

Plasmid	Genotype[a]	Description	Source or reference
pUC8	*bla*$^+$ *lacZpZoZ'*	Primary cloning vector with multiple cloning sites	Vieira and Messing (40)
pUC18	*bla*$^+$ *lacZpZoZ'*	Primary cloning vector with multiple cloning sites	Yanisch-Perron et al. (44)
pKK232-8	*bla*$^+$ *cat*$^+$	Promoter selection vector	Brosius (5)
pHP45Ω	*bla*$^+$ *aadA'*	pBR322 derivative containing Omega (*aadA*$^+$ gene of the R100.1 plasmid)	Prentki and Krisch (27)
pPU24	pBR322 Ω[0.029 kb; λh*80*d*ilv ilvG2200 ilvE2201 ilvGME'* −2.1/+2.24 kb (−)]*13*	*Hin*dIII fragment carrying *ilvGM'* inserted into pBR322	Subrahmanyam et al. (36); formerly called pCS7
pPU5	pBR313 Ω[0.375 kb; λh*80*d*ilv* −1.4/11.6 kb (−)]*5*	*ilvGMEDAYC* inserted into pBR313	Subrahmanyam et al. (37); formerly called pGMM101
pPU118	pBR328 Ω[0.375 kb; λd*ilv73* λ' *ilv'EDAY'* −0.42/0.81 λ3.0/7.41 (*ilv*) kb(+)]*1*	*ilv'EDAY'* and lambda DNA inserted into pBR328	3.8-kb *Bgl*II fragment of λ*ilv73* inserted into the *Bam*HI site of pBR328; C. Bennett
pPU119	pPU24 Δ[λh*80*d*ilv* 9.4/11.1 kb; pBR322 *tet* 0.29/2.065 kb]*1*	Removal of *Pvu*II-*Hin*dIII fragments of pBR322 and of sequences upstream of the *ilv* operon from pPU24	Bennett and Umbarger (2)
pPU307	pUC8 Δ[0.397/0.415 kb]*8* Ω[0.400 kb: pPU118 *ilv'DAY'* 2.9 kb (−)]*9*	*ilv'DAY'* DNA inserted into multiple cloning site of pUC8	2.9-kb *Xho*I-*Hin*dIII fragment of pPU118 carrying *ilv'DAY'* sequences inserted into *Sal*I-*Hin*dIII site of pUC8 to generate Φ(*lacZpZ'-ilv'DAY'*)*1* (*ilvD* transcript read in +1 frame)
pPU308	pUC18 Δ[0.399/0.421 kb]*1* Ω[0.422 kb: pPU118 *ilv'DAY'* 2.9 kb (−)]*1*	*ilv'DAY'* DNA inserted into multiple cloning site of pUC18	2.9-kb *Xho*I-*Hin*dIII fragment of pPU118 inserted into *Sal*I-*Hin*dIII sites of pUC18 to generate Φ(*lacZpZ'-ilv'DAY'*)*2* (*ilvD* transcript read in −1 frame)
pPU309	pPU307 Ω[3.29 kb: 4 bp]*1*	pPU307 derivative, change in reading frame of *ilvD* sequence	pPU307 digested with *Bam*HI, filled in with Klenow fragment, and religated to generate Φ(*lacZpZ'-ilv'DAY'*)*3* (*ilvD* transcript read in wt^c frame)
pPU311	pUC8 Δ[0.399/0.411 kb]*9* Ω[0.412 kb: pPU118 *ilv'DAY'* 3.5 kb (−)]*10*	*ilv'DAY'* DNA inserted into multiple cloning site of pUC8	3.5-kb *Pst*I-*Hin*dIII fragment from pPU118 inserted into the same sites of pUC8 to generate Φ(*lacZpZ'-ilv'DAY'*)*4* (*ilvD* transcript read in −1 frame)
pPU312	pUC18 Δ[0.399/0.415 kb]*2* Ω[0.416 kb: pPU118 *ilv'DAY'* 3.5 kb (−)]*2*	*ilv'DAY'* DNA inserted into multiple cloning site of pUC18	3.5-kb *Pst*I-*Hin*dIII fragment of pPU118 inserted into same sites of pUC18 to generate Φ(*lacZpZ'-ilv'DAY'*)*5* (*ilvD* transcript read in +1 frame)
pPU313	pPU312 Δ[3.89 kb: 4 bp]*1*	pPU312 derivative, change in reading frame of *ilvD* sequences	pPU312 digested with *Pst*I, 3' protruding ends digested with T4 polymerase, religation to generate Φ(*lacZpZ'-ilv'DAY'*)*6* (*ilvD* transcript read in wt frame)
pPU314	pUC18 Δ[0.399/0.436 kb]*3* Ω[0.437 kb: pPU24 *ilv* −0.8/2.1 kb (−)]*3* Ω[1.937: pHP45Ω *aadA*$^+$ (Omega, Spc^r Str^r) 2.0 kb]*4*	Omega fragment of pHP45Ω and DNA from upstream of the *ilv* regulatory region inserted into the multiple cloning site of pUC18	1.3-kb *Hin*dIII-*Sma*I fragment of pPU24 inserted into same sites of pUC18, followed by insertion of 2.0-kb Omega fragment of pHP45Ω into retained *Sma*I site
pPU315	pUC18 Δ[0.433/0.442 kb]*4* Ω[0.443 kb: pPU5 *ilv'YC'* 1.0 kb (−)]*5*	Insertion of *ilv'YC'* junction fragment into multiple cloning site of pUC18	1.0-kb *Bgl*II-*Kpn*I fragment of pPU5 inserted into *Bam*HI-*Kpn*I sites of pUC18
pPU316	pPU315 Δ[0.235/0.443 kb]*1* Ω[0.234 kb: pPU314 *ilv* −0.8/−2.1 kb) *aadA*$^+$ (Omega; Spc^r Str^r) 2.76 kb]*1*	Omega frament of pHP45Ω flanked by DNA from upstream of the *ilv* regulatory region and from the *ilv'YC'* region inserted into the multiple cloning site of pUC18	Insertion of 3.5-kb *Nar*I-*Kpn*I fragment of pPU314 into pPU315 cut with *Nar*I and *Xba*I; the *Xba*I site was filled in and the *Kpn*I site was digested with T4 DNA polymerase before ligation; this generated *ilv* allele *2243*

Continued on following page

TABLE 2—*Continued*

Plasmid	Genotype[a]	Description	Source or reference
pPU317	pUC18 Δ[0.399/0.415 kb]*5* Ω[0.416 kb: pPU119 *ilvEp* 0.48 kb (−)]*6*	Internal *ilvE* promoter *Ep* inserted into multiple cloning site of pUC18	0.42-kb *Hae*III-*Hin*dIII fragment from pPU119 inserted into *Pst*I-*Hin*dIII sites of pUC18; *Pst*I site was digested with T4 DNA polymerase prior to ligation
pPU318	pKK232-8 Δ[0.162/0.182 kb]*11* Ω[0.163 kb: pPU317 *ilvEp* 0.1 kb (+)]*11*[b]	*ilvEp* promoter inserted into cloning sites of pKK232-8	0.1-kb *Aat*II-*Bam*HI fragment of pPU317 inserted into *Bam*HI-*Hin*dIII sites of pKK232-8 to generate Φ(*ilvEp-cat*)*1*; the *Hin*dIII site was filled in, the *Aat*II site was digested with T4 polymerase prior to ligation
pPU319	pPU318 Ω[1.8 kb: pHP45Ω *aadA*$^+$ (Omega; Spc^r Str^r) 2.0 kb]*1*	*aadA*$^+$ gene from pHP45Ω inserted downstream of the *cat* gene in pPU318	pPU318 was partially digested with *Pvu*II and ligated with the 2.0-kb *Sma*I Omega fragment from pHP45Ω
pPU320	pKK232-8 Ω[1.7 kb: pHP45Ω *aadA*$^+$ (Omega; Spc^r Str^r) 2.0 kb]*12*	*aadA*$^+$ gene from pHP45Ω inserted into pKK232-8 downstream of the $rrnt_1t_2$ terminators	2.0-kb *Sma*I Omega fragment was ligated into pKK232-8 partially digested with *Pvu*II
pPU321	pPU316 Ω[2.99 kb: pPU319 Φ(*ilvEp-cat*)*1* 1.8 kb]*1*	*cat* gene under control of the *ilvEp* promoter was inserted into pPU316 downstream of Omega	1.8-kb *Bam*HI fragment of pPU319, containing the *cat* gene and the *ilvEp* promoter, was ligated into pPU316, partially digested with *Bam*HI
pPU322	pPU321 Δ[1.9/3.9 kb *aadA*$^+$]*1*	Omega fragment removed from pPU321	pPU321 partially digested with *Hin*dIII and religated
pPU323	pPU322 Δ[1.9/1.91 kb]*1* Ω[1.9 kb: pPU307 Φ(*lacZpZ'-ilv'DAY'*)*1* 3.1 kb (−)]*1*	Insertion of Φ(*lacZpZ'-ilv'DAY'*)*1* into pPU322 (*ilvD* read in +1 frame)	3.1-kb *Pvu*II-*Hin*dIII fragment from pPU307 was ligated into pPU322, partially digested with *Sma*I and *Hin*dIII
pPU324	pPU322 Δ[1.9/1.91 kb]*2* Ω[1.9 kb: pPU308 Φ(*lacZpZ'-ilv'DAY'*)*2* 3.1 kb (−)]*2*	Insertion of Φ(*lacZpZ'-ilv'DAY'*)*2* into pPU322 (*ilvD* read in −1 frame)	Same as pPU323, except fragment derived from pPU308
pPU325	pPU322 Δ[1.9/1.91 kb]*3* Ω[1.9 kb: pPU309 Φ(*lacZpZ'-ilv'DAY'*)*3* 3.1 kb (−)]*3*	Insertion of Φ(*lacZpZ'-ilv'DAY'*)*3* into pPU322 (*ilvD* read in wt frame)	Same as pPU323, except fragment derived from pPU309
pPU326	pPU322 Δ(1.9/1.91 kb)*4* Ω[1.9 kb Φ(*lacZpZ'-ilv'DAY'*)*4* 3.7 kb (−)]*4*	Insertion of Φ(*lacZpZ'-ilv'DAY'*)*4* into pPU322 (*ilvD* read in −1 frame)	Same as pPU323, except 3.7-kb *Pvu*II-*Hin*dIII fragment from pPU311 was inserted into pPU322, partially digested with *Sma*I and *Hin*dIII
pPU327	pPU322 Δ(1.9/1.91 kb)*5* Ω[1.9 kb Φ(*lacZpZ'-ilv'DAY'*)*5* 3.7 kb (−)]*5*	Insertion of Φ(*lacZpZ'-ilv'DAY'*)*5* into pPU322 (*ilvD* read in +1 frame)	Same as pPU326, except fragment from pPU312 was used
pPU328	pPU322 Δ(1.9/1.91)*6* Ω[1.9 kb Φ(*lacZpZ'-ilv'DAY'*)*6* 3.7 kb (−)]*6*	Insertion of Φ(*lacZpZ'-ilv'DAY'*)*6* into pPU322 (*ilvD* read in wt frame)	Same as pPU326, except fragment from pPU313 was used
pPU329	pPU311 Δ[2.65/3.90 kb]*1* Ω[2.65 kb: pPU320 *cat* 1.8 kb]*1*	cat gene inserted between end of *ilvA* and *ilvAt* in pPU311	1.25-kb *Stu*I-*Hin*dIII fragment was excised from pPU311 and replaced with the 1.8-kb *Sma*I-*Hin*dIII fragment from pPU320
pPU330	pPU313 Δ[2.65/3.90 kb]*1* Ω[2.65 kb: pPU320 *cat* 1.8 kb (+)]*1*	*cat* gene inserted between end of *ilvA* and *ilvAt* in pPU312	1.25-kb *Stu*I-*Hin*dIII fragment in pPU313 was replaced with the 1.8-kb *Sma*I-*Hin*dIII fragment from pPU320

[a] The genotype designations follow the conventions recommended by Novick et al. (25) and those in the Instructions to Authors. They are similar to those followed in earlier publications. The convention for the designation of cleavage sites in plasmids for which the entire nucleotide sequence is known is that the first T in the *Eco*RI recognition site is designated base 1 in derivatives of pBR322 and the first T in the sequence TCGCGCGTTC is designated base 1 in derivatives of pUC plasmids. The position shown for restriction endonuclease cleavage sites is that of the 5′ base at the site of cleavage. Thus, the *Eco*RI site is at position 4362 in pBR322 and at position 430 in pUC8. The sites of insertion or deletion in some previously described plasmids have been changed in accordance with these conventions. A (+) indicates that the orientation of the insert is the same with respect to the vector as the chromosome and vector maps are usually drawn; a (−) indicates that the orientation of the insert with respect to the vector is different from the usual map representation. With the six *lacZpZ'-ilv'DAY'* fusions, the map orientations of the *lac* and *ilv* sequences are reversed from one another, and (+) and (−) refers to the orientation of the *ilv* sequence. Thus, the orientation of the *ilv* inserts in all the pPU322 derivatives (Fig. 2) is designated (−).

[b] Designations in kilobases of the multiple cloning site in pKK232-8 are deduced from Brosius (5).

[c] wt, Wild type.

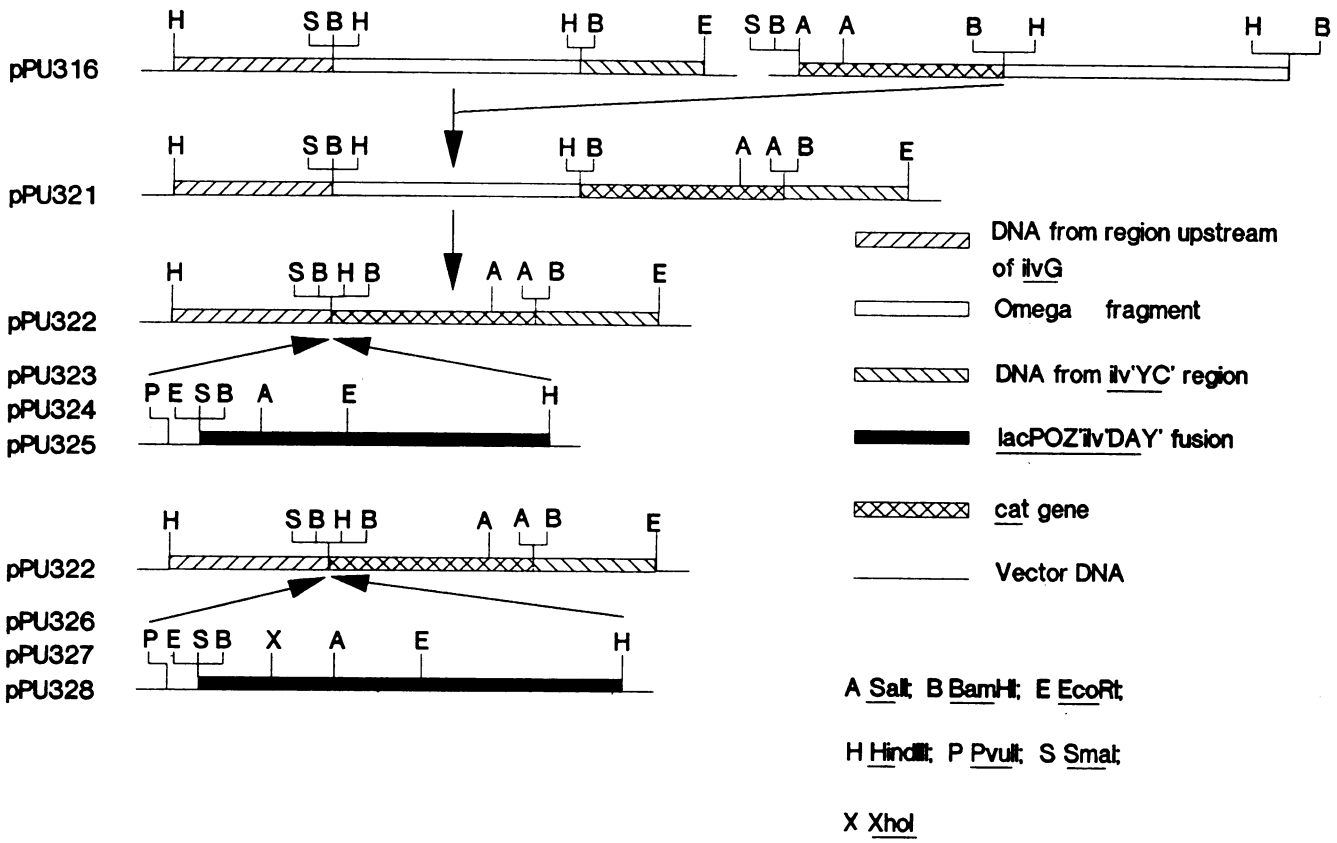

FIG. 2. Construction of plasmids that were transferred to the chromosome. pPU323, pPU324, and pPU325 carry a 3.1-kb insert containing the *lacPOZ'-ilv'DAY'* fusion and differ in the *ilv'D* reading frame. pPU326, pPU327, and pPU328 contain a 3.7-kb *lacPOZ'-ilv'DAY'* insert. They differ from each other in the *ilvD* reading frame.

fragment of pPU316 is replaced by the *cat* gene. pPU316 was used as the starting vector for these constructions. The pPU322 construction was done in two steps in order to preserve a *Hin*dIII site that would facilitate later steps. First, a 1.8-kb *Bam*HI fragment of pPU319 containing the *cat* gene under the control of the internal *ilv* promoter *Ep* was inserted into the *Bam*HI site immediately downstream of the Omega fragment of pPU316 to yield pPU321. This plasmid was introduced into strain CU1230, and transformants resistant to chloramphenicol, spectinomycin, and ampicillin were selected. Plasmid DNA from one such colony was isolated. The construction was verified by restriction enzyme analysis. The *cat* gene under control of the *ilvEp* promoter was inserted into pPU321 downstream of the Omega fragment and transcribed toward Omega. The Omega fragment was removed by partially digesting pPU321 with *Hin*dIII, religating, and introducing it into CU1230. Plasmid DNA from a resulting colony that was ampicillin and chloramphenicol resistant but spectinomycin sensitive was isolated, characterized by restriction enzyme analysis, and designated pPU322.

Fragments from pPU307, pPU308, pPU309, pPU311, pPU312, and pPU313 containing the various *lacZpZ'-ilv'DAY'* fusions were inserted into pPU322 and subsequently transferred to the chromosome. The 3.1-kb *Pvu*II-*Hin*dIII fragments from pPU307, pPU308, and pPU309 and the 3.7-kb *Pvu*II-*Hin*dIII fragments from pPU311, pPU312, and pPU313 containing the *lac* promoter operator region and the *ilvD-ilvA* fusions were ligated into pPU322, cut partially with *Sma*I and *Hin*dIII, and introduced into CU825 (*ilvA454*). Ampicillin-resistant transformants were selected and scored for growth on minimal agar containing ampicillin (25 μg/ml). Plasmid DNA from such colonies was examined by restriction enzyme analysis to verify the constructions. The plasmids were designated pPU323, pPU324, pPU325, pPU326, pPU327, and pPU328.

Chemicals and molecular biological reagents. T4 ligase, restriction endonucleases, and the Klenow fragment of *E. coli* DNA polymerase were obtained from Bethesda Research Laboratories, Gaithersburg, Md., Boehringer Mannheim Biochemicals, Indianapolis, Ind., New England Bio-Labs, Beverly, Mass., and Pharmacia Inc., Piscataway, N.J. X-gal, IPTG, and S1 nuclease were purchased from Boehringer Mannheim. The M13 nucleotide sequence analysis kit and [^{32}P]dATP were obtained from Amersham Corp., Arlington Heights, Ill. [^{14}C]Chloramphenicol was purchased from New England Nuclear Corp., Boston, Mass.

RESULTS

Effect of frame shift in *ilvD* on *ilvA* expression. The effect of incomplete or altered translation of *ilvD* mRNA on the expression of *ilvA* was examined by changing the *ilvD* reading frame and thereby causing premature termination of translation at different positions in *ilvD*. Figure 1 illustrates the plasmid constructions and DNA modifications made for this purpose as described in Materials and Methods. pPU309 and pPU313 contain *ilvD* sequences translated in the natural *ilvD* reading frame. Transcription in these plasmids is initiated at the *lac* promoter of the parent pUC vector and

TABLE 3. Dependence of *ilvA* expression on translation of *ilvD*

Strain	*ilv'D* reading frame	Sequence surrounding the *ilvA* start codon[a]	Differential rate of threonine diaminase synthesis [Δ(nmol/min)/Δmg of protein]	Relative threonine diaminase activity (% of wt[b])
CU1648	wt	. . . UAAUAAUGGCUGAC . . .	1,128	100
CU1647	−1	. . . UAG . . . 123n . . . . . AUGGCUGAC . . .	51	4.5
CU1646	+1	. . . AUGGCUGAC . . .	685	61
CU1651	wt	. . . UAAUAAUGGCUGAC . . .	446	100
CU1649	−1	. . . UGA . . . 546n . . . . . AUGGCUGAC . . .	79	18
CU1650	+1	. . . UAA . . . 572n . . . . . AUGGCUGAC . . .	61	14

[a] The *ilvA* translation start codon is underlined. The stop codon in the *ilvD* reading frames are overlined. n, Nucleotides.
[b] wt, Wild type.

proceeds through the multiple cloning site into the inserted fragment, presumably to the normal *ilv* operon termination signal. Translation is initiated at the *lacZ* initiation codon, proceeds through the *ilvD* sequences, and stops at the normal *ilvD* stop signal 2 bases upstream of the *ilvA* translation initiation codon. pPU309 contains 129 bp of the promoter-distal portion of *ilvD*; pPU313 carries the distal 702 bp of *ilvD*.

In pPU308, the translation of *ilvD* is in the −1 reading frame with respect to the natural reading frame, but termination occurs in *ilvD* immediately beyond the pUC-*ilvD* (*Sal*I-*Xho*I) fusion site, leaving 123 bases upstream of *ilvA* untranslated. In pPU307, translation of the *ilvD* sequences occurs in the +1 reading frame and is terminated at a stop codon 2 bases downstream of the *ilvA* initiation codon. pPU311 (−1 frame) and pPU312 (+1 frame) contain 546 and 572 untranslated bases upstream of *ilvA*, respectively.

Integration into the *E. coli* chromosome. Differences in gene expression caused by different numbers of plasmid copies per cell were avoided by integrating the six different *lac-ilv* fusions into the *E. coli* chromosome. CU1644 [*recBC sbcB* Δ(*ilvGMEDAY'*)*2246*] was prepared to be the host strain for fragment integration, and plasmid derivatives of pPU307 through pPU309 and pPU311 through pPU313 that carried the *cat* gene were constructed (Fig. 2). These derivatives, pPU323 through pPU328, carry the *cat* gene and DNA that provides homology between the plasmids and the chromosome of strain CU1644 for integration by crossover events. The *cat* gene facilitated selection for cells containing the desired integrated *lacZpZ'-ilv'DAY'* fusions. Because *cat* gene expression is under control of the constitutive *ilvEp* promoter, the measurement of *cat* activity provided a good method for ensuring that multiple copies of the inserted region had not been selected. Each of these plasmids, shown in Fig. 2, was opened by restriction enzyme digestion and used to transform strain CU1644. The desired recombinants were obtained. They were chloramphenicol resistant, sensitive to ampicillin, and had lost the Str^r Spc^r carried by strain CU1644. One colony containing DNA derived from each of the plasmids was maintained. These strains were designated CU1646 through CU1651 and were used to examine the effect of altered translation of *ilvD* on *ilvA* expression.

Dependence of expression of *ilvA* on translation of *ilvD*. The strains containing the *lacZpZ'-ilv'DA* fusions were grown in L broth and induced by addition of IPTG, and the increase in threonine deaminase activity relative to the increase in protein content was determined at several times after induction. The results are shown in Table 3. In absence of IPTG induction, no threonine deaminase activity could be detected in any of the strains, confirming that *ilvA* transcription is under the control of the *lac* promoter. Furthermore, the differential rate of increase in threonine deaminase activity was constant for at least 195 min after induction in all strains. Since no nucleotide changes had been introduced within the 130 bases upstream and none were introduced downstream of the *ilvA* translation initiation site, the values for increases in threonine deaminase activity are assumed to reflect accurately the effect of altered translation termination on downstream *ilvA* expression.

Strain CU1648, containing the wild-type configuration of *ilvD* stop and *ilvA* start codons, exhibited the highest level of threonine deaminase activity. In strain CU1647, in which translation of the upstream sequences was terminated 126 bases upstream of the *ilvA* start codon, *ilvA* expression decreased to 4.5% of the CU1648 (wild-type) level. This decrease occurred even though there is a sequence at the very end of the *ilvD* gene that can be predicted to base-pair with between 6 and 8 of the last 9 bases at the 3' end of 16S RNA (30). If, however, this potential ribosome-binding site were masked in some secondary structure (see Discussion), it might be readily accessible only if that portion of the *ilvD* transcript were translated. In the +1 reading frame of *ilvD* DNA, which is found in strain CU1646, this region of the transcript was translated, but translation stopped 2 bases beyond the *ilvA* start codon. Expression of *ilvA* thus was higher than in the strain (CU1647) in which translation of *ilvD* DNA stopped early but was still about one-third less than that in the strain in which translation was in the proper frame.

The other three strains allowed examination of the effect of terminating translation even earlier in *ilvD*. In the −1 reading frame, *ilvD* translation was terminated 549 bases upstream of *ilvA* (strain CU1649), and in the +1 reading frame, it was terminated 575 bases upstream (strain CU1650). In both strains, the differential rate of threonine deaminase formation was about like that observed when translation stopped only 126 bases upstream. As expected, *ilvA* expression in these two strains was considerably lower than that in the control strain, CU1651, in which the *lacZpZ'-ilvD* fusion was the same length but was translated in the wild-type *ilvD* reading frame. As Table 3 shows, however, strain CU1651 produced threonine deaminase activity after induction only one-third as effectively as did the corresponding control strain (CU1648), in which the *lacZ-ilvD* fusion was short.

That the difference in *ilvA* expression between the two strains with different lengths of *lacZ-ilvD* fusion was due, at least in part, to the *ilvD* DNA itself was demonstrated by deleting the extra DNA to recreate a fusion very similar to that in strain CU1648. This manipulation was done with pPU328, the plasmid from which Φ(*lacZpZ'-ilv'D*)*6* was transferred to the chromosome in strain CU1651. The DNA between the *Sma*I site in the multiple cloning site and the *Xho*I site in *ilvD* was removed to form a plasmid virtually

TABLE 4. Dependence of *ilvA* and *cat* expression on translation of *ilvD*

Plasmid	*ilv'D* reading frame	Sequence surrounding the *ilvA* start codon[a]	Threonine deaminase activity (μmol/min per mg of protein)	Chloramphenicol acetyltransferase activity[b] (%)
pPU329	wt[c]	. . . UAAUAAUG-*ilvA-cat* . . .	4.91	22.3
pPU330	−1	. . . UGA . . . 546n . . . AUG-*ilvA-cat* . . .	1.21	17.8

[a] The *ilvA* translation start codon is underlined. The stop codon in the *ilvD* reading frames are overlined. n, Nucleotides. Both enzyme activities were determined in the same cell extract. pPU329 carries an *ilv-cat* operon fusion in addition to the *lacZ'-ilv'D* protein fusion carried in strain CU1651. pPU330 carries the same operon fusion in addition to the *lacZ'-ilv'D* protein fusion carried in strain CU1649. CU815 is the host strain for both plasmids.

[b] Percent chloramphenicol converted to acetylchloramphenicol per minute per microgram of protein.

[c] wt, Wild type.

identical to pPU325 except for minor differences in the multiple cloning site. Upon transfer of this shortened fusion to the *recBC* recipient CU1644, a strain was obtained that produced twice as much threonine deaminase as did strain CU1651 (data not shown). Although the rate of formation was still less than that in strain CU1648, both sets of strains can be used to demonstrate the importance of translation of *ilvD* to a point very close to the *ilvA* translational start site for significant *ilvA* expression to occur.

Transcription termination after frameshift in *ilvD*. The question arose whether reduction in *ilvA* expression when *ilvD* is not completely translated is due to premature transcription termination. For example, termination might occur at possible rho-dependent sites in the promoter-distal portion of the *ilvD* gene, since the 123 bases of untranslated mRNA in pPU308 (and CU1647) would be sufficient for rho-dependent termination to occur (22). Therefore, an operon fusion was constructed that placed the promoterless *cat* gene from pKK232-8 (5) between of the *ilvA* translational stop codon and the operon transcriptional termination site. Because *ilvA* is the last gene of the operon, it is not possible to test for a polar effect on a downstream gene. The *cat* gene, including its own ribosome-binding site, was inserted into pPU311, which contains 543 bases of untranslated *ilvD* mRNA, and, as a control, into pPU313, in which the correct *ilvD* reading frame had been restored. The *cat* gene is thus transcribed from the *lac* promoter (after the *ilvA* gene is transcribed). Threonine deaminase activity and chloramphenicol acetyltransferase activity were measured in the same extract obtained from cells containing this fusion plasmid. Cells for these assays were grown in L broth. Table 4 shows that early termination of *ilvD* translation led to a 75% reduction in *ilvA* expression but only a 20% reduction in *cat* expression. Thus, transcription proceeded almost unimpaired through the *ilvA* and *cat* genes even though about 543 bases of *ilvD* mRNA remained untranslated. The data presented in Table 4 show clearly that reduction in the expression of *ilvA* when translation of *ilvD* was terminated early cannot be attributed to a decreased amount of transcription into or through the *ilvA* gene.

DISCUSSION

The data in this paper demonstrate the dependence of expression of *ilvA* on translation of the upstream *ilvD* gene. Efficient expression of *ilvA* occurred when translation of the upstream sequences was terminated at the natural *ilvD* termination site 2 bases upstream of the *ilvA* start codon. When termination occurred at the same distance downstream of the *ilvA* initiator ATG, threonine deaminase activity decreased by 39%. This observation might be explained as follows. A ribosome upstream of and close to a start codon can perhaps "slide" into initiation position and start translation, whereas a ribosome downstream and out of frame may hinder translation initiation at a proximal start site. On the other hand, the decrease might have been due, at least in part, to a reduced efficiency in translation of the *lacZ'-ilv'D* peptide in an altered reading frame. Termination of translation upstream of the normal *ilvD* stop, i.e., far from the ribosome-binding area for *ilvA*, reduced threonine deaminase activity drastically, to 4.5%. Similar studies performed with the adjacent *galT-galK* genes show looser dependence of expression of the *galK* gene on translation of the upstream *galT* gene (29). Reduction of 12% in galactokinase activity was observed when a stop codon was located 2 nucleotides downstream of a start codon, and translation termination occurring 176 bases upstream of *galK* decreased *galK* expression by 62%. In contrast, the dependence of *ilvA* expression on correct and complete translation of the upstream sequences is much stronger.

By introducing a gene with an independent ribosome-binding site between *ilvA* and the *ilvGMEDA* transcription terminator, it was shown that the reduction in *ilvA* expression when *ilvD* translation was terminated prematurely was a direct effect of translation efficiency and was not due to a decrease in transcript availability arising from early transcription termination. With the *ilv'DA-cat* transcriptional unit of pPU311, the expression of the downstream *cat* gene was reduced by 20%, in contrast to 75% reduction for the upstream *ilvA* gene. In this particular construction, 543 bases of mRNA upstream of *ilvA* remain untranslated. Schümperli et al. (29) found 5% fewer transcripts of the downstream *galK* gene in a construction that left the 126 promoter-distal bases of the upstream *galT* gene untranslated. The difference between the two results might directly reflect the difference in the length of untranslated mRNA.

The mechanism underlying translational coupling is not well understood. Two main views have been put forward (11, 24, 33, 35). One suggests that there is a weak ribosome-binding site for the downstream gene that is aided in translation initiation by ribosomes terminating nearby. The second view postulates the masking of a good ribosome-binding site by RNA secondary or tertiary structure that may form in the absence of a translating ribosome.

Weak stem and loop structures have been found at the *trpE-trpD* and the *trpB-trpA* boundaries (9). Experiments described by Sor et al. (33) on translational coupling in the L11 ribosomal protein operon did not provide any evidence that mRNA secondary structure was responsible for masking the initiation site for L1 translation. Schümperli et al. (29) postulated that the coupling of the *galT-galK* gene pair is effected by ribosomes terminating *galT* translation and reinitiating at *galK* or by the nearby termination event leading to an increase in the local concentration of ribosomal sub-

units facilitating initiation at *galK*. However, mRNA secondary structures involving a ribosome-binding site or the translation initiation codon have been shown to be responsible for reduction in initiation efficiency for a number of different systems, including bacteriophage T4 lysozyme early mRNA (21), *lamB* (13), *lacZ* (23), *lacI* (6, 7, 34), and the Tn*10* transposase gene (10).

Analysis of the nucleotide sequence around the *ilvA* translation initiation site reveals the possibility for formation of a relatively strong stem and loop structure ($\Delta G = -16.6$ kcal). Formation of this structure would sequester the proposed *ilvA* ribosome-binding site and might impair independent translation initiation of *ilvA*. Whether this structure is formed and, if so, is indeed involved in the mechanism of translational coupling has not been examined.

Strains CU1646, CU1647, and CU1648 contain approximately 130 bases of unaltered *ilvD* DNA in addition to the wild-type *ilvA* gene. There are only minor differences between these strains, and they are restricted to the multiple cloning sites of the plasmid-derived fragments that have been integrated into their chromosomes. The same is true for strains CU1649 through CU1651, except that they contain about 700 bp of unaltered *ilvD* DNA. It seems highly unlikely that the small differences in primary structure of DNA more than 130 bases upstream of translation start signals should influence translation initiation efficiency by means other than RNA folding. Sor et al. (33) demonstrated that changes in nucleotide sequence immediately upstream of the Shine-Dalgarno sequence of the L1 ribosomal gene contribute to the efficiency of translation initiation by means other than base pairing. The analogous sequences in strains CU1649 through CU1651, however, do not differ from each other. The reduction of *ilvA* expression in strains CU1646 through CU1651 should record precisely the effect of altered termination of translation of the upstream sequences.

Interestingly, there was a threefold difference in *ilvA* expression between strains CU1648 and CU1651, although in both strains the natural *ilvD* stop codon is the translation termination site. Strain CU1651, however, contains 440 more bases of *ilvD* DNA than CU1648 does, and the strains have some minor differences around the *lacZ-ilvD* fusion point. It was confirmed that the lower *ilvA* expression in strain CU1651 than in strain CU1648 was due at least in part to the additional *ilvD* DNA and not to any fortuitously acquired *lac* promoter-operator mutation. Thus, the additional *ilvD* sequences in CU1651 seem to exert an inhibitory effect on *ilvA* translation. Several independent transfers of the pPU325- and pPU328-derived fragments to strain CU1644 gave strains which, when analyzed as in Table 3, gave results similar to those reported for CU1648 and CU1651. Furthermore, chloramphenicol acetyltransferase activity (driven by the constitutive *ilvEp* promoter) was the same in strains CU1646 through CU1651 (data not shown). Therefore, differences between the strains other than those due to the newly integrated fragment seem highly unlikely. Although the reason for this reduction is not known, a contributory factor might be the number of less commonly used codons in the vicinity of the *lacZ'-ilv'D* fusion point within the integrated fragment (8 of 17 in strain CU1651, 3 of 17 in CU1648).

Translational coupling is thought to be an additional means of ensuring coordinate synthesis of physiologically related gene products, beyond the coordination achieved by simultaneous transcription of a polycistronic mRNA from a single promoter. Coupling of translation can be a device to obtain equimolar amounts of polypeptides that constitute an enzyme complex, as suggested for the gene pairs *trpB* and *trpA* and for *trpE* and *trpD*. On the other hand, the ribosomal protein L7/L12, although its formation is dependent on translation of the upstream *rplJ* gene, is made in fourfold excess over the *rplJ* product (16, 45). Therefore, the translation initiation complex must be able to form and initiate independently at the L7/L12 translational start site. The question remains, however, whether the terminating ribosome or its 30S subunit takes part in any of the initiation events occurring at the beginning of the downstream gene. The enzymes specified by *ilvD*, dihydroxy acid dehydrase, and *ilvA*, threonine deaminase, are required at nonconsecutive steps in the pathway to isoleucine biosynthesis. Moreover, threonine deaminase is required only for isoleucine biosynthesis, whereas dihydroxy acid dehydrase is needed for synthesis of valine and leucine as well. Thus, depending on the physiological state of the cell, these enzymes are at times needed in different amounts. It may be, therefore, that the translational coupling that occurs between *ilvD* and *ilvA* is exploited in some as yet unknown way to achieve this variable balance between *ilvD* and *ilvA* expression.

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