JOURNAL OF BACTERIOLOGY, Oct. 1991, p. 6446–6452
0021-9193/91/206446-07$02.00/0

Vol. 173, No. 20

The Absence of Branched-Chain Amino Acid and Growth Rate Control at the Internal *ilvEp* Promoter of the *ilvGMEDA* Operon†

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Received 20 May 1991/Accepted 12 August 1991

The question of whether the promoter *ilvEp*, located in the coding region of *ilvM*, the second structural gene in the *ilvGMEDA* operon, is subject to either amino acid- or growth rate-mediated regulation is examined. The experiments described here were performed with *ilvEp-cat* and *ilvEp-lac* fusions carried as single copies on the chromosome. The activity of the *ilvEp* promoter was found to respond neither to the availability of branched-chain amino acids nor to a wide range of growth rates between 35 to 390 min. In the absence of any known role for the products of the *ilvGMEDA* operon when repressing levels of branched-chain amino acids are present, there appears to be only a gratuitous role for the transcription at *ilvEp*.

The regulation of the *ilvGMEDA* operon is very complex. The operon is transcribed in vivo from the proximal of two tandem in vitro-detected promoters, $ilvGp_1$ and $ilvGp_2$ (1, 7, 20, 27). Transcription from the upstream promoter, $ilvGp_1$, is prevented in vivo by binding to integration host factor (IHF), and that from $ilvGp_2$ is enhanced (30, 39). For this reason, the upstream region will be referred to as an IHF binding site, and the promoter that functions in vivo will be referred to as *ilvGp*. However, transcription into the *ilvG* gene has been reported to be reduced owing to an additional IHF binding site in the leader region which is thought to enhance attenuation (28). The expression of the entire operon is specifically regulated by the presence of all or the absence of any one of the branched-chain amino acids via translational control of transcription termination at an attenuator site preceding *ilvG*, the first structural gene of the operon (10, 19, 26, 34). Two internal promoters, potential sites for modulation of expression, have been described (3, 4, 21, 22). Promoter *ilvEp* is located upstream of *ilvE* within the coding region for *ilvM*, and promoter *ilvAp* is located upstream of *ilvA* within the coding region of *ilvD* (22, 37). The transcription initiated at the *ilvEp* promoter has been shown to be enhanced in wild-type *Escherichia coli* K-12 because of the frameshift mutation in the *ilvG* gene which exposes three Rho-dependent transcriptional polarity sites located in the distal part of *ilvG* and the proximal part of *ilvM* (23, 38). Therefore, in *E. coli* K-12 grown under repressing conditions, the amount of transcription into the structural genes, initiated at *ilvGp* but not terminated at the attenuator site, will be further reduced at these polarity sites, upstream of *ilvE*. Indeed, transcription of the structural genes in wild-type *E. coli* K-12 has been attributed mainly to transcription initiation at the internal promoter *ilvEp* under repressing conditions (4) and in minimal medium (21). *ilvEp* was shown to be a weaker promoter than the major operon promoter *ilvGp* (4, 37). Owing to the truncated nature of the product of *ilvG*, the first structural gene in the *ilvGMEDA* operon, many of the experiments assessing its regulation by the availability of branched-chain amino acids actually measured only the effect on expression of the distal genes *ilvE*, *ilvD*, and *ilvA*, downstream of promoter *ilvEp*, or of *lacZ* fusions within that part of the operon. The question arises as to whether *ilvEp* itself is subject to either amino acid-mediated or any other kind of regulation and whether any such kind of regulation contributes significantly to downstream gene expression under various growth conditions. Previous studies have been contradictory, some indicating that *ilvEp* might be regulated (21, 33) and others proposing that it is constitutively expressed (3, 37). The experiments described below address this question.

MATERIALS AND METHODS

Strains and plasmids. The bacterial strains and plasmids used are described in Tables 1 and 2.

Growth of cells. The media and methods used to grow cells under repressing and derepressing conditions were those described by Gayda et al. (11). Repressing conditions were obtained by growing cells in minimal medium supplemented with 50 μg each of L-isoleucine and L-leucine per ml and 100 μg of L-valine per ml. Derepressing conditions were obtained by transferring the cells from a repressed culture at 0.3 A_{660} to the same volume of fresh medium supplemented with two branched-chain amino acids of the repressing concentration and one at 1/20 of the repressing concentration. To obtain different growth rates, cells were grown in either LB or LB supplemented with 0.5% glucose or in minimal medium with either 0.5% glucose, 0.5% sodium succinate, or 0.2% ammonium acetate added as a carbon source. Cells were harvested at mid-log phase by centrifugation.

Enzyme assays. Preparation of cell extracts and acetohydroxy acid synthase II (AHASII) assays were described earlier (32). Valine-resistant AHASII was measured in the presence of 1 mM valine. Chloramphenicol acetyltransferase activity was determined by the method of Gorman et al. (13) and modified as described previously (17). β-Galactosidase assays were performed as described by Miller (25). Restriction enzymes, Klenow fragment of DNA polymerase, T4 DNA ligase, and T4 DNA polymerase were used as suggested by their suppliers.

Plasmid constructions. A 1.14-kb *Pvu*II-*Hin*dIII *ilv'GME'* fragment from pPU119 (2) containing the *ilvEp* promoter was inserted into the *Sma*I site of the polylinker of pRS415 (31), upstream of the *lacZ* gene, to yield pPU377 (Fig. 1). The

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† Dedicated to Professor Gunter B. Kohlhaw on the occasion of his 60th birthday.

TABLE 1. Strains used

Strain	Genotype	Reference
CU1257	Δ(*pro-lac*) *ara thi* Δ*ilv*(*G-C*) *2049 arg*::Tn*10*	6
CU1766	Δ(*pro-lac*) *ara thi* Δ*ilv*(*G-C*) *2243*::*aadA*$^+$ *arg*::Tn*10*	6
CU1648	Δ(*ilvG-Y*) *2249*::Δ*aadA*$^+$::[pPU325 φ(*lacZpZ'-ilv 'DA'Y'*)3 φ(*cat-ilvEp*)1 3.1 kb] *leuB6 proA2 trpD9579 his-4 argE3 thi-1 hsdR18 ara-14 lacY1 galK2 xyl-5 mtl-3 rpsL31 tsx-33 tonB56 supE44 recB21 recC22 sbcB15* λ$^-$	16

*Hin*dIII site was filled in with the Klenow fragment of DNA polymerase prior to ligation. pRS415 contains transcriptional terminators upstream of the multiple cloning site and retains the translational start signals for *lacZ* but not the promoter. Strain CU1257 was transformed with pPU377 to yield Amp^r Lac$^+$ colonies that contained the plasmid with the desired insert.

A series of plasmids that contain *ilv* DNA fragments with either the *ilvGp* promoter region or *ilvEp* promoter region or both upstream of *lacZ* was constructed. pPU378 was obtained by insertion of the 4.5-kb *Sma*I fragment from pPU336 (5) into the *Sma*I site of pRS415 upstream of *lacZ*. The *Sma*I fragment carries the *ilvGMED'* genes, including *ilvGp*, the IHF binding site, and the *ilvG2200* mutation, which restores *ilvG* function. The *Sal*I restriction site within the vector sequence of pPU378 was removed by a partial *Sal*I digestion and subsequent filling-in reaction with Klenow fragment to obtain pPU379. To suppress the promoter function of *ilvEp*, an *Sst*I linker was inserted into pPU379 at the *Sal*I site, which lies between the −35 and −10 regions. The resulting plasmid is called pPU380. To assess expression from *ilvEp* or the modified *ilvEp* only, the IHF binding site and *ilvGp* were removed from plasmids pPU378 and pPU380 by deleting the 1.45-kb *Eco*RI-*Kpn*I fragments to yield pPU381 and pPU382, respectively. Several attempts to cross the inserts of the plasmids onto phage λRZ5 (31) failed. Therefore, the inserted fragments of pPU378, pPU380, and pPU381 were shortened by deleting the 1.13-kb *Hin*dIII-*Bam*HI *ilv'ED'* fragments to yield pPU383, pPU384, and pPU385. The construction of all plasmids was verified by restriction analysis.

Strain constructions. Single copies of the *ilv-lac* fusions were obtained by using the pRS415/λRZ5 *lac*-fusion system developed by Simons et al. (31), which allows transfer from plasmid to phage by homologous recombination. Strain CU1257 was transformed with pPU377, and strain CU1766 was transformed with plasmids pPU381, pPU383, and pPU384, respectively. The transformed strains were subsequently infected with λRZ5, and phage lysates were made as described by Miller (25). Strains CU1257 or CU1766 (carrying no plasmid) were infected with the resulting phage lysates, and recombinant lysogens were selected on L agar in presence of ampicillin (25 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; 40 μg/ml). Several Lac$^+$ colonies were chosen in each case and examined for β-galactosidase activity. Those showing lowest activity were inferred to be single lysogens. The strains obtained, CU1819(pPU377), CU1820(pPU383), CU1821(pPU384), and CU1822(pPU385), are described in Table 3.

Chemicals and molecular biological reagents. T4 ligase, restriction endonucleases, T4 DNA polymerase, and Klenow fragment of *E. coli* DNA polymerase were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. The *Sst*I linker was purchased from Pharmacia Inc., Piscataway, N.J.

RESULTS

Specific control. For characterization of the *ilvEp* promoter, a protein product more readily assayed than transaminase B was desired. The *cat* gene from pKK232-8 had previously been placed under the *ilvEp* promoter and used for a different purpose (16). The strain carrying the *ilvEp-cat* fusion, CU1648, offered a convenient system to examine whether *ilvEp* is regulated specifically by the availability of valine, isoleucine, and leucine, the end products of the pathway, or is affected by the growth rate of the cells as proposed earlier (21, 33). Strain CU1648 carries the *ilvEp* promoter on a 100-bp *Hae*II-*Aat*II fragment upstream of the promoterless *cat* gene, with about 50 bp upstream of the −35 region. CU1648 was grown under conditions of excess branched-chain amino acids (repressing conditions), under conditions in which one of the branched-chain amino acids was in limited supply (derepressing conditions), and, as a control, under conditions of another amino acid limiting

TABLE 2. Plasmids used

Plasmid	Genotype	Reference or description
pRS415	*bla*$^+$ *trrnB* Δ(*lacZpZo*) *lacZYA*	30
pPU119	pPU24 Δ[λh80d*ilv* −2.0/−0.45 kb; pBR322 *tet* 0.29–2.065 kb]1	5
pPU336	pPU333 Ω[0.029 kb:pPU334 *ilv'ED'* 1.659 kb (−)]2	6
pPU377	pRS415 Ω[0.009 kb:pPU119 *ilv'GE'* 1.14 kb (+)]1	Insertion of 1.14-kb *Pvu*II-*Hin*dIII *ilv'GE'* fragment from pPU119 into *Sma*I site of pRS415
pPU378	pRS415 Ω[0.009 kb:pPU336 *ilvGpeaG2200ED'* 4.5 kb (+)]2	Insertion of 4.5-kb *Sma*I *ilvGpeaG2200ED'* fragment from pPU336 into *Sma*I site of pRS415
pPU379	pPU378 Ω[6.301 kb:0.004 bp]1	Removal of pRS415 *Sal*I site in pPU378 by filling in with Klenow fragment (to remove the *Sal*I site in the vector)
pPU380	pPU379 Ω[3.05 kb:*Sst*I site 0.006 kb (+)]1	Insertion of *Sst*I linker into *Sal*I site of *ilvM* to yield the *ilvM2304* allele
pPU381	pPU378 Δ[*ilvGpeaG'* 0.001/2.431 kb]2	Removal of 2.4-kb *Eco*RI-*Kpn*I *ilvG'* fragment from pPU378
pPU382	pPU380 Δ[*ilvGpeaG'* 0.001/2.431 kb]1	Removal of 2.4-kb *Eco*RI-*Kpn*I *ilvGpeaG'* fragment from pPU380
pPU383	pPU378 Δ[*ilv'ED'* 3.498/4.561 kb]3	Deletion of 1.063-kb *Hin*dIII-*Bam*HI *ilv'ED'* fragment from pPU378
pPU384	pPU380 Δ[*ilv'ED'* 3.504/4.567 kb]2	Deletion of 1.063-kb *Hin*dIII-*Bam*HI *ilv'ED'* fragment from pPU380
pPU385	pPU381 Δ[*ilv'ED'* 1.057/2.12 kb]1	Deletion of 1.063-kb *Hin*dIII-*Bam*HI *ilv'ED'* fragment from pPU381

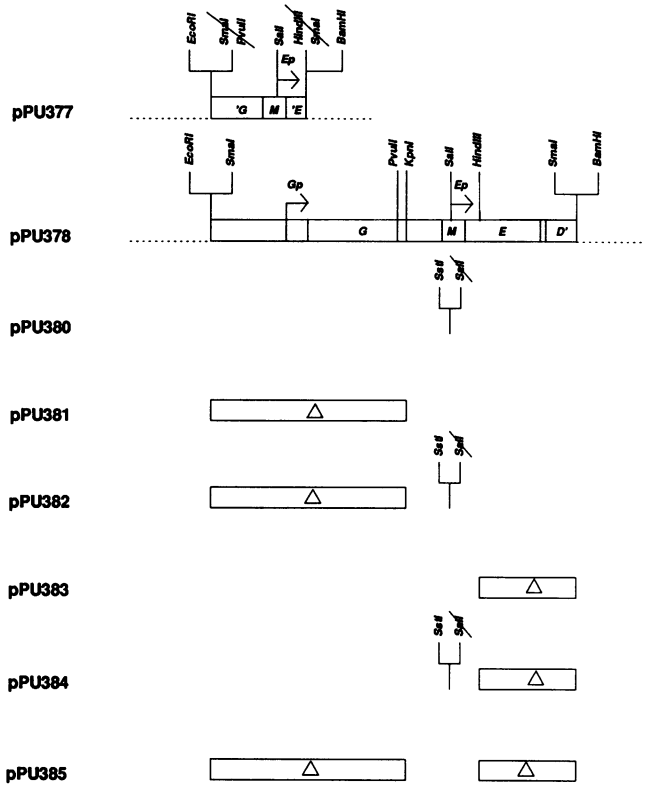

FIG. 1. Plasmid constructs. Broken lines represent pRS415 vector sequences. Open boxes depict *ilvGMED'* DNA. *Gp* and *Ep* are the *ilvGp* promoter at the beginning of the *ilv* leader region and the *ilvEp* internal promoter, respectively. Restriction sites with diagonal bars are those that were destroyed during plasmid construction. The adjacent destroyed *Sal*I and *Sst*I sites demonstrate the insertion of the *Sst*I linker into the *Sal*I site. Open boxes with triangles represent sequences that were deleted from the plasmid.

growth. Table 4 shows the expression of the *cat* gene under the control of the *ilvEp* promoter. When either valine, isoleucine, or leucine was limiting, chloramphenicol acetyltransferase activity increased by about 20%. This small increase was consistently seen also in other similarly constructed strains described by Harms et al. (16; data not shown). This effect is not due to the slower growth rate occurring under derepressing conditions, since a decreased growth rate caused by the limited availability of either proline or arginine was actually accompanied by a small decrease in *cat* expression.

The *ilvEp-cat* fusion in CU1648 was inserted into the *ilvY* region of the chromosome in such a way that transcription of *ilvY* from the *ilvY* promoter and *cat* from the *ilvEp* promoter proceeded into the same direction. The possibility of transcription beginning at the *ilvY* promoter and continuing through the *ilvEp* promoter region into *cat* could not be eliminated. Therefore, we constructed a plasmid, pPU377, which did eliminate this possibility. In this recombinant plasmid, the *ilvEp* promoter is located 800 bp into a 1.2-kb *Pvu*II-*Hin*dIII *ilv'GME'* fragment. This plasmid also reduces the possibility that DNA sequences in *ilvG* necessary for modulation of expression from *ilvEp* had been deleted in strain CU1648, since it contains all the DNA that would be left untranscribed following the Rho-dependent termination studied by Wek et al. (38) and Lopes et al. (23). This

TABLE 3. Plasmids, promoter fusions, and λRZ5 derivatives used for strain constructions

Plasmid	Fusion	Promoter	λRZ5 derivative	Strain
pPU325	*ilv'ME'-lacZ*	*ilvEp*		CU1648
pPU377	*ilv'GME'-lacZ*	*ilvEp*	λRZ5-*ilv4*	CU1819
pPU383	*ilvGpeaG2200 ME'-lacZ*	*ilvGpEp*	λRZ5-*ilv5*	CU1820
pPU384	*ilvGpeaG2200 M2304 E'-lacZ*	*ilvGp*	λRZ5-*ilv6*	CU1821
pPU385	*ilv 'G2200 ME'-lacZ*	*ilvEp*	λRZ5-*ilv7*	CU1822

fragment was inserted into the multiple cloning site of pRS415 (31) upstream of the *lacZ* gene to yield pPU377. The *ilvEp-lacZ* fusion from the plasmid was subsequently crossed into λRZ5, followed by lysogeny of strain CU1257 to obtain strain CU1819. CU1822 was obtained in a similar way. In this strain, *lacZ* is transcribed from the *ilvEp* promoter located on a 0.96-kb *Kpn*I-*Hin*dIII *ilv'GME'* fragment ligated to the *lacZ* gene of pRS415. CU1819 and CU1822 were grown under repressing conditions and under limiting conditions as described above. β-Galactosidase activities, reflecting the expression of *lacZ* transcribed from the *ilvEp* promoter, are also shown in Table 4. No branched-chain amino acid-specific control could be detected. The β-galactosidase activities in cells grown with an amino acid limitation were generally lower than those in cells with amino acid excess, possibly because of errors in translation of this large protein caused by amino acid limitation (29).

Growth rate control. It has been repeatedly observed that when cells are grown in rich medium (L broth), the genes of the *ilv* operon are more strongly repressed than they are in cells growing in the presence of all three branched-chain amino acids (9, 21, 33). Furthermore, this effect is independent of the operon promoter *ilvGp* (11, 21). This repression might reflect a more general type of regulation, i.e., a growth rate-dependent control. To test whether expression from the *ilvEp* promoter responded to different growth rates, strains CU1648, CU1819, and CU1822 were grown in media allowing a variety of doubling times ranging from 35 to 390 min (Table 5). No significant change in *cat* gene expression could be detected in strain CU1648. When β-galactosidase activity was measured in strain CU1819 and CU1822, somewhat lower activity was observed in cells grown in rich medium than in cells grown in the other media. However, a similar effect was observed when β-galactosidase was expressed from the *ilv* operon promoter *ilvGp* (see below) and can therefore not be attributed to the *ilvE* promoter activity.

TABLE 4. Expression of *ilvEp-cat* and *ilvEp-lac* fusions under conditions of limited amino acid supply

Growth condition	CU1648 chloramphenicol acetyltransferase activity[a]	CU1819 β-galactosidase activity[b]	CU1822 β-galactosidase activity
Repressing	72	71	52
Limiting valine	92	42	43
Limiting isoleucine	98	42	38
Limiting leucine	94	54	30
Limiting proline	75	34	34
Limiting arginine	63	35	35

[a] Percent chloramphenicol converted to acetylchloramphenicol per 15 min per 10 μg of protein.
[b] Miller units (25).

TABLE 5. Expression of *ilvEp-cat* and *ilvEp-lac* fusions under different growth conditions

Growth condition	CU1648		CU1819		CU1822	
	Chloramphenicol acetyltransferase activity[a]	Doubling time (min)	β-Galactosidase activity[b]	Doubling time (min)	β-Galactosidase activity	Doubling time (min)
L broth	67	40	23	35	22	42
L broth + glucose	68	38	23	35	22	35
Minimal + glucose	71	50	51	55	50	65
Minimal + succinate	63	255	46	340	28	300
Minimal + acetate	80	335	51	360	30	390

[a] Percent chloramphenicol converted to acetylchloramphenicol per 15 min per 10 μg protein.
[b] Miller units (25).

From these data, it appears that *ilvEp* promoter activity is not controlled by the growth rate of the cell.

Contribution of *ilvEp* to downstream *ilv* gene expression. The control of gene expression in the *ilvGMEDA* operon has often been assessed by measuring expression of genes downstream of the *ilvEp* promoter. For that reason, it was important to determine the contribution of the internal promoter *ilvEp* to expression of the downstream genes. We constructed a derivative of pRS415, pPU383, which carried in its multiple cloning site upstream of *lacZ* the 3.4-kb *Sma*I-*Hin*dIII ilvGME′ fragment containing *ilvGp*, the IHF binding site, and about 500 bp upstream, as well as an *ilvG* (*ilvO*) mutation that results in the synthesis of AHASII. Transcription into the inserted fragment from a vector initiation site is prevented by an *rrnB* terminator located immediately upstream of the multiple cloning site (31). Plasmid pPU384 is a derivative of pPU383 in which the *ilvEp* promoter was inactivated by inserting an *Sst*I linker into the *Sal*I site located between the −35 and −10 regions of the *ilvEp* promoter. A third derivative of pPU383, pPU385, was obtained by deleting the 2.4-kb *Eco*RI-*Kpn*I fragment that contains the upstream IHF binding site as well as *ilvGp*. To determine the extent of inactivation of *ilvEp* by the *Sst*I insert, which extended the spacing between the −35 and −10 regions by six bases, the operon promoter *ilvGp* and the upstream IHF site were deleted from pPU380 by removing the 2.4-kb *Eco*RI-*Kpn*I fragment to obtain pPU382. Comparison of β-galactosidase activities expressed from pPU381 and pPU382 revealed a 75% decrease in activity from a plasmid containing the modified *ilvEp* promoter (data not shown). Whether the residual expression arose from some still weaker promoter in the vector or insert or from residual *ilvEp* activity is not known. *ilvEp* is located within the coding region of *ilvM*. Therefore, the insertion of the *Sst*I linker introduced two serine residues at amino acid position 57 into the small subunit of AHASII, generating *ilvM2304* and rendering the enzyme inactive (data not shown).

The *ilv* promoter-*lacZ* fusions were introduced as single copies into the chromosome of strain CU1766 to yield CU1820(pPU383), CU1821(pPU384), and CU1822(pPU385) as described in Materials and Methods. The β-galactosidase activities in these strains were determined under various growth conditions (Table 6). As had been observed with strain CU1819 (Table 4), strain CU1822, with only *ilvEp* directing *lacZ* expression, exhibited no specific regulation by branched-chain amino acids. With strains CU1820 and CU1821, *ilv*-specific attenuation control was observed, a four- to eightfold derepression when valine and isoleucine were limiting. Leucine-mediated derepression was low, a phenomenon discussed earlier and attributed to the high frequency of leucine codons in the *lacZ* message (6). That the effect is not due to the strains not responding to leucine limitation was shown by the fact that the AHASII activity measured in strain CU1820 under those conditions was derepressed. Limitation for proline and arginine led to a slight decrease in β-galactosidase activity, similar to the decrease observed when *ilvEp* directed *lacZ* expression under conditions of limited amino acid supply. Under repressing conditions (excess branched-chain amino acids and either limiting proline or limiting arginine), expression from *ilvEp* (CU1822) and *ilvGp* (CU1821) is similar, assuming 25% expression in CU1821 surviving insertional inactivation of *ilvEp*. When both promoters are present, *lacZ* expression is not the sum of *ilvGp* and *ilvEp* expression but rather is some intermediate level. This might imply an interference of *ilvEp* promoter activity by *ilvG* transcription-translation. Either promoter *ilvGp* or *ilvEp* alone allows a similarly low level of downstream gene expression in an *ilvG*$^+$ strain. Strains CU1820, CU1821, and CU1822 also show a slightly lower β-galactosidase activity when grown in rich media than in the other media and are consistently highest when grown in minimal medium with glucose added as a carbon source. This effect is independent of the promoter from which the *lacZ* gene is expressed, is observed only when *lacZ* is the reporter gene, and does not follow the growth rate of the cells. Therefore, it seems to reflect not the promoter activity under the conditions tested but rather a β-galactosidase-specific effect. Valine-resistant AHASII activity expressed

TABLE 6. Expression from *ilvGp* and *ilvEp* promoters

Growth condition	CU1820 *ilvGp + Ep*		CU1821 *ilvGP*	CU1822 *ilvEp*
	β-Galactosidase activity[a]	Valine-resistant AHASII activity (nmol/min/mg of protein)	β-galactosidase activity	β-galactosidase activity
Repressing	73	2.2	60	52
Limiting valine	329	28.1	385	43
Limiting isoleucine	355	54.5	457	38
Limiting leucine	103	39.1	102	30
Limiting proline	62	3.0	49	34
Limiting arginine	66	3.2	59	35
L broth	33	1.3	28	22
L broth + glucose	34	1.0	27	22
Minimal medium + glucose	111	1.8	86	50
Minimal medium + succinate	69	2.5	35	28
Minimal medium + acetate	58	1.5	32	30

[a] Miller units (25).

from the operon promoter *ilvGp* remains similar under these conditions. Taken together, the data show that *ilvEp* is a constitutive internal promoter regulated neither by growth rate nor specifically by the availability of branched-chain amino acids. Therefore, the presence of *ilvEp* within a fully translated *ilvGM* gene might moderate slightly a regulatory response of downstream genes. Furthermore, the *ilv* operon promoter *ilvGp* is also not subject to growth rate control.

DISCUSSION

Internal promoters have been found in several operons of *E. coli*. For some, their activity is required as modulating expression of distal genes within an operon in response to the physiological environment of the cell (24, 35, 40). For other internal promoters, their physiological roles, or indeed whether they have any significant role in the cell, are not immediately obvious. When their activities are constitutive, internal promoters are thought to serve to maintain a basal level of expression of distal genes (12, 18). An example of the first type of internal promoter is P_{hs}, one of a set of promoters located in the *dnaG* gene within the *rpsU-dnaG-rpoD* operon (24). The promoter is inducible by heat and thus allows an increased expression of the *rpoD* gene product, σ^{70}, at elevated temperatures. Likewise, transcription of the last two genes of the *deoCABD* operon is induced by inosine or guanine at the internal P3 promoter (35). The internal promoters within the amino acid biosynthetic operons *argCBH*, *his*, and *trp*, however, are in contrast to the main operon promoters not subject to regulation by the end products of the pathways (12, 15, 18). They therefore serve to maintain a low level of transcription of the distal genes even under repressing conditions, but convincing argument for any selective advantage appears to be missing. The existence of an internal promoter in the *ilv* operon was first suggested by Berg et al. (3) based on analysis of strains with polar insertions and subsequently supported by enzyme assays with lambda d*ilv* phage lysogens carrying parts of the *ilv* operon (14), RNA polymerase binding studies (32), and DNA sequence analysis of the region upstream of *ilvE* and by determination of the 5′ end of the message in vivo and in vitro (21, 37). A number of transcriptional fusion plasmids were used to compare the strength of the internal promoter *pE* with that of the operon promoter *ilvGp* as well as with that of the in vitro-detected promoter, $ilvGp_1$, lying within the IHF binding site (37), and to assess the contribution of *ilvEp* to downstream gene expression (4, 23, 37). In this study, the question of whether the internal promoter *ilvEp* is regulated either by the end products of the pathway or by the growth rate has been examined. Lopes and Lawther (21) reported reduction in expression from *ilvEp* carried on a multicopy plasmid upon adding the three branched-chain amino acids to a minimal medium and a marked decrease when rich medium was used, but copy number variations of the plasmids under the various conditions may have been a factor. The rich-medium effect had also been observed earlier by Subrahmanyam et al. (33), again with a multicopy plasmid, but no effect was seen under derepressing conditions in plasmid constructions (pPU36) in which transcription of the downstream genes ought to have been initiated at *ilvEp* (33). The data presented in this report were obtained with transcriptional fusions that had been transferred to the chromosome in order to eliminate any ambiguity that might arise from plasmid copy number effects. The results (Table 4) demonstrate that *ilvEp* does not seem to be regulated by the products of the pathway, isoleucine, leucine, and valine. The generally lower activity of β-galactosidase when any of the amino acids are in limited supply may be caused by increased mistranslation that occurs under these conditions (29). The slightly higher *cat* gene expression when branched-chain amino acids are limiting growth than when proline or arginine is limiting or when all of the required amino acids are present was not observed when *lacZ* was used as a reporter gene or when the *ilvEp-cat* fusion was introduced into the *lacZ* gene of pRS415 (data not shown). One difference between the *ilvEp-cat* fusion is in the nucleotide sequence upstream of *ilvEp*. In the former there are only about 50 bp of contiguous *ilvG* sequence (the rest being derived from *ilvY* and its promoter and *ilvC*), whereas in the latter the contiguous *ilvG* DNA upstream of *ilvEp* included all of the DNA that is left untranscribed beyond the Rho-dependent termination in the wild-type K-12 *ilv* operon. The *ilvEp* promoter activity did also not seem to be modulated by widely different growth rates. Doubling times of 40 to 335 min did not affect significantly chloramphenicol acetyltransferase activity in strain CU1648 (Table 5). When β-galactosidase activity was measured in strains CU1819 and CU1822, the activity seemed to be slightly lower when cells are grown in rich medium. Such an effect was also observed with strain CU1821 (Table 6), in which *lacZ* transcription is initiated at the *ilv* operon promoter *ilvGp*, and in strain CU1820, in which *ilvGp* and *ilvEp* are both present. This variation in *lacZ* expression might be a medium-specific effect as described by Wanner et al. (36), which could influence the transcriptional as well as the translational process (8). Thus, it was important to compare the activity not only of different reporter genes but also of different promoters in order to eliminate subtle variations in gene expression not arising from differences in *ilvEp* promoter activity.

The participation of *ilvEp* in downstream gene expression is accentuated in wild-type *E. coli* K-12 strains which carry a frameshift mutation in *ilvG*. In these strains, transcripts from the operon promoter *ilvGp* that are not terminated at the attenuator of the regulatory region are exposed to the three Rho-dependent terminator sites near the junction of the *ilvGM* genes (23, 38). Under repressing conditions, the *ilvEp* promoter is variously reported to account for 41 to 96% of downstream gene expression (4, 23, 37). When the Rho-dependent polarity effect is eliminated as it is in *ilvG* mutants that synthesize AHASII, the *ilvEp* promoter activity is reported to contribute 15 to 18% (4, 23, 37). The experiments described here with CU1821 (Table 3) have the advantage of allowing direct measurement of expression from the *ilvGp* operon promoter of genes located downstream of the *ilvEp* promoter without the interference of the *ilvEp* promoter which was inactivated. In addition the Rho-dependent polarity effects were eliminated by using a fully translated *ilvG* gene. Under repressing conditions as well as in rich medium, expression of *lacZ* resulting from read-through from *ilvGp* alone was slightly higher than when initiated at *ilvEp* alone. When both promoters were present, β-galactosidase activity was higher still but not the sum of the activities of the two promoters individually. These results indicate interference of transcription initiation at *ilvEp* with transcription elongation and translation of *ilvG*. The role of the *ilvEp* promoter has been suggested to ensure a low level of downstream gene expression. This might be important under repressing conditions for wild-type *E. coli* strains, in which *ilvG* transcripts might be terminated at the polarity sites upstream of *ilvE*.

In *Salmonella typhimurium*, an *ilvEp* promoter has been found by sequence homology and was found to be active (21). *S. typhimurium* contains an *ilvG* gene that is fully

translated and in that respect is comparable to an *E. coli ilvG* mutant that expresses *ilvG* (e.g., CU1820; Table 6). This observation eliminated the possibility that *ilvEp* arose as a compensatory mutation in response to the frameshift in the K-12 *ilvG* gene. A comparison of CU1820 (*ilvGp*$^+$ *ilvEp*) and CU1821 (*ilvGp* only) reveals that under all conditions examined, *ilvEp* contributes very little to downstream gene expression. The role of the *ilvEp* promoter has been suggested to ensure at least a low level of downstream gene expression. How this might be important under repressing conditions even for wild-type *E. coli* K-12 in which those *ilvG* transcripts escaping attenuation are mostly terminated at the polarity sites upstream of *ilvE* is difficult to explain. Even in a minimal medium, any restriction on *ilv* gene expression owing to the polarity sites are at least partially overcome by deattenuation at the end of the leader. Therefore, there seems to be no need for the *ilvE* promoter to be conserved unless the *ilv* gene products also serve some unknown role. However, strains in which all functions of the *ilv* gene cluster have been abolished by deletion grow as well as prototrophic strains as long as isoleucine and valine are available. It is tempting to suggest that the *ilvEp* promoter is a fortuitous element that offers no selective advantage to the cell. It did permit one means of compensating for the *ilvG* polar mutations in the emergence of the K-12 strain, albeit at the expense of a less effective control of operon expression by attenuation-deattenuation.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant GM12522 from the National Institute of General Medical Sciences.

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