JOURNAL OF BACTERIOLOGY, Aug. 1992, p. 4871–4877
0021-9193/92/154871-07$02.00/0

Vol. 174, No. 15

Multiple Transcripts Encoded by the *ilvGMEDA* Gene Cluster of *Escherichia coli* K-12

FEI HUANG, GEORGE COPPOLA, AND DAVID H. CALHOUN*

Biochemistry Doctoral Program, Department of Chemistry, City College of New York, Convent Avenue and 138th Street, New York, New York 10031

Received 14 January 1992/Accepted 18 May 1992

We report here that, using Northern (RNA) blots, we identified two relatively stable transcripts of 4.6 and 1.1 kb that correspond to the products of the *ilvEDA* and *ilvE* genes and two relatively unstable transcripts of 6.7 and 3.6 kb that correspond to the products of the *ilvGMEDA* and *ilvDA* genes. The transcripts were identified by the use of eight probes derived from segments of the *ilvGMEDA* cluster. In addition, we used two strains with deletions of *ilvG* or *ilvDA* and observed the expected decrease in transcript size in Northern blots. Primer extension with reverse transcriptase generated a 169-nucleotide product corresponding to a 5′ end within the *ilvED* intercistronic region, 37 nucleotides from the AUG codon of the *ilvD* gene. This primer extension product presumably indicates the 5′ end of the *ilvDA* transcript that we detected in Northern blots. The stability of the transcripts was monitored, and RNase E was found to play a major role in *ilv* transcript degradation. Transcript levels varied in response to growth in the presence of the end product amino acids and in response to the presence of the polar frameshift site in *ilvG*. Although there have been speculations about the identities and numbers of transcripts derived from the *ilvGMEDA* cluster on the basis of the identification of some of the sites of transcription initiation and termination, this is the first report of the use of Northern blots to determine the actual sizes and distribution of mRNAs present in vivo.

The *ilvGMEDA* cluster (Fig. 1) encodes five gene products needed for the biosynthesis of leucine, isoleucine, and valine in *Escherichia coli* K-12 (10, 16, 27). Three promoters, $ilvGp_2$, *ilvEp*, and *ilvAp*, which have been well characterized, initiate transcription just upstream of the *ilvG*, *ilvE*, and *ilvA* genes, respectively (1, 15, 17, 23, 28). The presence of two internal promoters, *ilvEp* and *ilvDp*, was first inferred by use of strains with polar insertions or mutations located in the *ilvGMEDA* cluster (4–6), λ*ilv* phage carrying only *ilvEDA*, *ilvDA*, or *ilvA* (13), and fusions to reporter genes (7, 17, 28). Internal promoter *ilvAp*, which was first reported by Lopes and Lawther (17), is preferentially expressed in cells grown under anaerobic conditions. A site of transcription termination has been characterized downstream of the *ilvA* gene (25).

Identification of some of the potential sites of transcription initiation and termination is not sufficient to predict the actual populations of *ilvGMEDA* transcripts present in a cell, particularly during the response to regulatory signals. Specifically, several questions remain about factors affecting the expression of these genes, including possible sites of internal transcription termination, differential mRNA stability of specific segments, the presence of specific mRNA segments generated during the early stages of mRNA turnover (8), and changes in transcription patterns during repression or derepression in response to changes in the end product amino acids, leucine, isoleucine, and valine. The downstream amplification model (26) suggests that, under repressing conditions, premature transcription termination occurs at a site(s) within the cluster, whereas under derepressing conditions, the majority of transcripts are full length.

In the present experiments, our goal was to identify the major transcripts produced by using Northern (RNA) blots. We found the transcripts expected if initiation occurred at the major $ilvGp_2$ promoter and at the internal *ilvEp* promoter and extended to the termination site at the end of the cluster. We did not detect the transcript expected if initiation occurred at the weak internal *ilvAp* promoter. We detected *ilvDA* and *ilvE* mRNAs that could be either primary transcription products or the products of posttranscriptional events.

MATERIALS AND METHODS

Strains and growth conditions. Strains C600 (F^- *thi-1 thr-1 leuB6 lacY1 tonA21 supE44* λ^-) (2), N3431 [*lacZ43 relA1 spoT1 thi-1 rne-3071*(Ts)] (21), N3433 (*lacZ43 relA1 spoT1 thi-1*) (21), and FD1054 (*rbs-301*::Tn*5* Δ*ilvGMEDA723*::Tn*5*-131) (12), with or without plasmids, were grown on a rotary shaker at 200 rpm and 37°C in M9 medium (20) supplemented as required with 0.3 mM threonine, isoleucine, valine, leucine and 0.05 mM thiamine and used for RNA isolation (see Fig. 2 to 4). For the amino acid limitation experiments (see Fig. 3), cells were grown on a rotary shaker at 200 rpm and 37°C to the early exponential phase (A_{550} = 0.3) in the presence of excess leucine, isoleucine, and valine, pelleted by centrifugation, resuspended in medium containing a limiting concentration of one amino acid (0.015 mM for isoleucine and leucine and 0.034 mM for valine) as described previously (11), and incubated on a rotary shaker at 200 rpm and 37°C for 3 h prior to RNA isolation.

Recombinant DNA methodology and DNA sequencing. Restriction enzymes, T4 polynucleotide kinase, T4 DNA polymerase, and T4 DNA ligase were purchased from New England BioLabs, Inc. (Beverly, Mass.), the Klenow fragment of DNA polymerase was purchased from International Biotechnologies Inc. (New Haven, Conn.), *Taq* DNA polymerase was purchased from Perkin-Elmer Corp. (Norwalk, Conn.), avian myeloblastosis virus reverse transcriptase was purchased from GIBCO-BRL (Gaithersburg, Md.), RNA molecular weight markers were purchased from Boehringer

* Corresponding author.

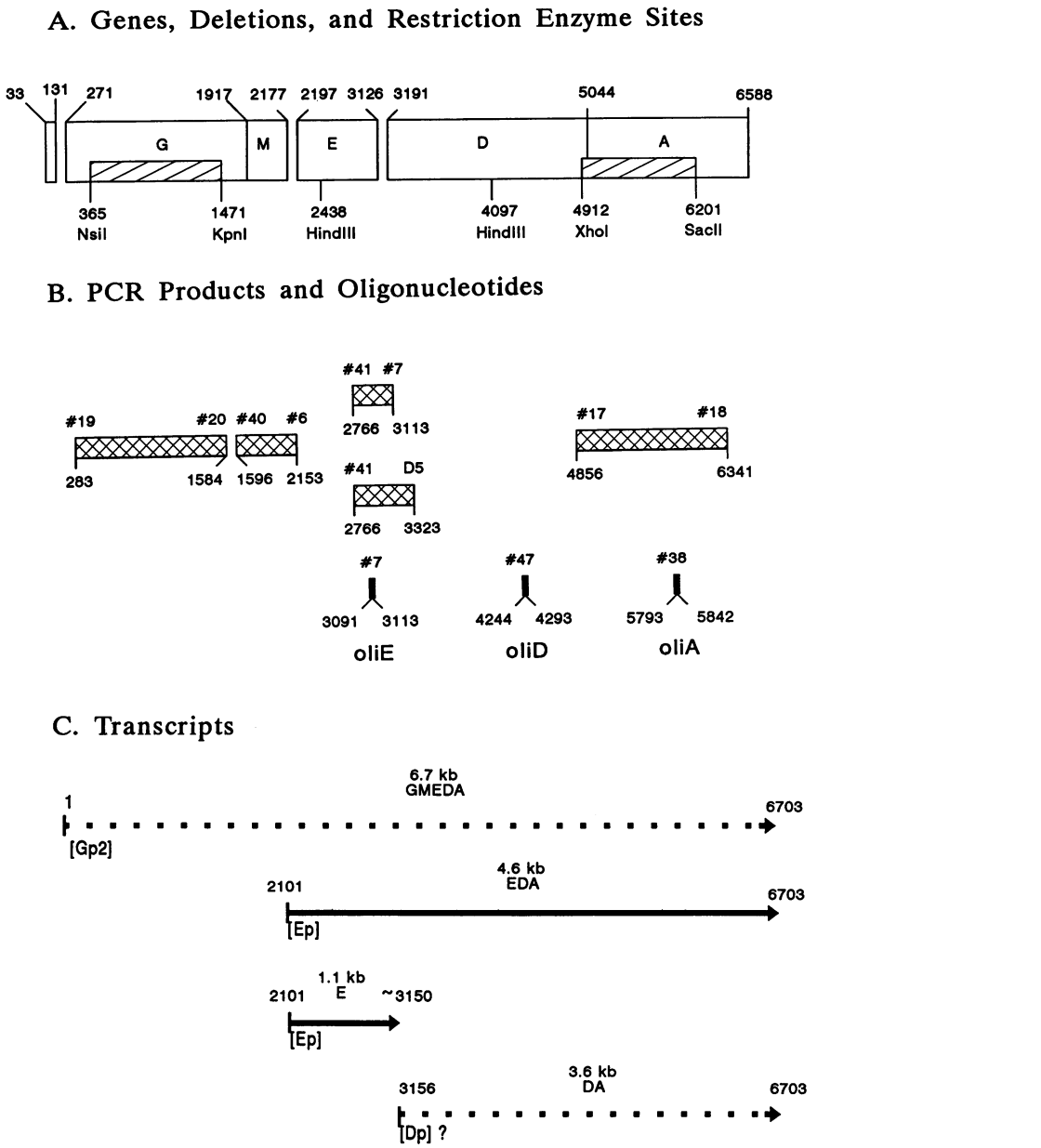

FIG. 1. *ilvGMEDA* cluster, probes used in Northern blots, and major transcripts. (A) Genes, locations of the coding regions for each polypeptide, locations of selected restriction enzyme sites, and deletions (hatched bars) introduced in *ilvG* to construct plasmid pCC17 and in *ilvDA* to construct plasmid pCC20. (B) PCR products and oligonucleotides used as probes in Northern blots. The PCR products (cross-hatched bars) were generated with oligonucleotides 19 and 20 (pcrG), 40 and 6 (pcrGM), 41 and 7 (pcrE), 41 and D5 (pcrED), and 17 and 18 (pcrDA). Synthetic oligonucleotides used as probes were 7 (oliE), 47 (oliD), and 38 (oliA). Oligonucleotide D5 was also used as a primer for reverse transcriptase (see Fig. 5). (C) Relatively unstable (broken lines) 6.7- and 3.6-kb transcripts and relatively stable (solid lines) 4.6- and 1.1-kb transcripts.

Mannheim (Indianapolis, Ind.), and RNasin was purchased from Promega Corp. (Madison, Wis.). The presence of the expected deletions was confirmed by direct DNA sequence analysis with asymmetric polymerase chain reaction (PCR) products as templates for the dideoxy sequencing protocol.

Construction of plasmids pCC17, pCC20, and pCC36. Plasmids pCC17 and pCC20 were generated by standard methodologies (3). In brief, plasmid pJG51 (13), which has unique sites for *Nsi*I and *Kpn*I separated by 1,110 bp in the *ilvG* gene, was treated with both enzymes and then with T4 DNA polymerase to remove the protruding 3′ ends and T4 DNA ligase to join the blunt ends. The deletion in the resulting plasmid, pCC17, generated a protein fusion within the *ilvG* gene product, so that translation would proceed without the formation of a polar frameshift site. To generate plasmid pCC20, we treated plasmid pJG51 with *Xho*I, which cleaves in the *ilvD* gene, and *Sac*II, which cleaves in the *ilvA* gene. Phage T4 DNA polymerase was used to remove two overhanging bases at the 3′ end of the *Sac*II site, the Klenow fragment was used to fill in 4 bases at the *Xho*I site, and T4

DNA ligase was used to join the blunt ends. This deletion of 1,290 nucleotides (nt) generates a protein fusion with the amino-terminal region encoded by *ilvD* and the carboxyl-terminal region encoded by *ilvA*.

To construct plasmid pCC36, we used phage P1 grown on strain CU2501 (*rbs-200 ilvG468* [Val^r]) (24) to transduce strain CC84 (strain FD1054 containing plasmid pJG51) to Val^r. A plasmid (pCC36) obtained from a Val^r transductant was used to transform strain FD1054 to Val^r, generating strain CC221. Strains CC84 (Val^s) and CC221 (Val^r) are Ilv prototrophs, since the plasmid contains the *ilvGMEDA* genes and the chromosome contains the *ilvYC* genes.

Hybridizations and probes. RNA was isolated from cells harvested in the log phase as previously described (9). The probes (Fig. 1) used for the Northern blots (see Fig. 2 to 4) were either synthetic oligonucleotides derived from sequences encoding the *ilvE* gene product (oligonucleotide 7: nt 3113 to 3091; oliE), the *ilvD* gene product (oligonucleotide 47: nt 4293 to 4244; oliD), or the *ilvA* gene product (oligonucleotide 38: nt 5842 to 5793; oliA) or PCR products derived from primers in *ilvG* (oligonucleotide 19: nt 283 to 302; and oligonucleotide 20: nt 1584 to 1565; pcrG), in *ilvE* (oligonucleotide 41: nt 2766 to 2785; and oligonucleotide 7: nt 3113 to 3091; pcrE), from the *ilvGM* junction (oligonucleotide 40: nt 1596 to 1615; and oligonucleotide 6: nt 2153-2131; pcrGM), from the *ilvED* junction (oligonucleotide 41: nt 2766 to 2785; and oligonucleotide D5: nt 3323 to 3301; pcrED), and from the *ilvDA* junction (oligonucleotide 17: nt 4856 to 4876; and oligonucleotide 18: nt 6363 to 6341; pcrDA). Oligonucleotides 19, 40, 41, and 17 anneal to the coding DNA strand and prime from left to right in Fig. 1, and oligonucleotides 20, 6, 7, 18, D5, 47, and 38 anneal to the opposite strand. The asymmetric PCR protocol of McCabe (19) was used to generate preferentially a product complementary to the mRNA, and the antisense primer was labeled with ^{32}P in the presence of T4 polynucleotide kinase prior to amplification. The synthetic oligonucleotides were prepared by phosphoramidite chemistry on a Cyclone Plus DNA synthesizer (Milligen/Biosearch, Burlington, Mass.) and purified on polyacrylamide gels or Oligo-Pak columns (Milligen/Biosearch). The effect of RNase E on transcript stability was determined as described by Mudd et al. (21). For the Northern blots (see Fig. 2 to 4), ethidium bromide-stained gels confirmed that comparable quantities of RNA were loaded in each lane, and we detected no differences in plasmid copy number.

Primer extension. Primer D5 (3 ng), labeled at the 5′ end with [γ-^{32}P]ATP by use of T4 polynucleotide kinase, and 50 μg of RNA were hybridized in a reaction volume of 20 μl (250 mM KCl, 5 mM Tris-HCl, 0.5 mM EDTA) at 80°C for 10 min and then at 42°C for 2 h. RNasin (0.5 μl containing 20 U), reverse transcriptase (1 μl containing 400 U), and 130 μl of reaction buffer (250 mM Tris-HCl [pH 8.3], 375 mM KCl, 50 mM dithiothreitol, 15 mM $MgCl_2$) provided by the supplier were added, and the mixture was incubated at 37°C for 60 min. The reaction was terminated by the addition of 4 μl of EDTA (0.5 M), and RNA was ethanol precipitated and loaded onto an 8% polyacrylamide sequencing gel.

RESULTS

Characterization of *ilvGMEDA* cellular transcripts. We used Northern blot hybridization to determine the identities of the mRNA species encoded in vivo by the *ilvGMEDA* cluster. As probes we used synthetic oligonucleotides and asymmetric PCR products (Fig. 1). We noted, as has been previously reported by others (22), that the rRNAs (1,541 and 2,904 bases) in the samples saturated the binding sites on the membrane and created "shadows" in the *ilv* mRNA pattern (Fig. 2 to 4). The *ilv* mRNAs accumulated just ahead of the rRNAs, thus providing internal controls for the size estimation, which was based on RNA markers in parallel lanes.

Transcripts of 6.7, 4.6, and 1.1 kb were detected with probe oliE (Fig. 2 to 4), whereas transcripts of 6.7, 4.6, and 3.6 kb were detected with probes pcrDA and oliD (Fig. 2 and 3). The 6.7- and 3.6-kb transcripts, which were much less stable than the 4.6- and 1.1-kb transcripts, were not always detectable on short exposures of the blots to film or at low levels of expression (Fig. 2 to 4).

The 6.7-kb transcript was the size predicted for the full-length *ilvGMEDA* cluster, and it also hybridized to the other five probes (Fig. 1) specific for the *ilvGMEDA* genes. The 6.7-kb transcript was reduced to 5.6 kb by the 1,110-bp *ilvG* deletion present in plasmid pCC17 and to 5.4 kb by the 1,290-bp deletion in the *ilvDA* junction present in plasmid pCC20 (Fig. 2, lanes G′ and A′, respectively).

The 4.6-kb transcript hybridized to all probes except pcrG and was the size predicted if initiation occurred at the *ilvEp* promoter at nt 2101 and continued to the terminator at nt 6703. It should be noted that probe pcrGM extends from nt 1596 to 2131, so that it overlaps the site of transcription initiation of *ilvEp* at nt 2101 by 30 nt, a length that is sufficient to account for the observed hybridization to the 4.6-kb transcript. The 4.6-kb transcript was not affected by the *ilvG* deletion (Fig. 2, lane G′), but the *ilvDA* deletion resulted in the loss of the 4.6-kb transcript and the appearance of a 3.3-kb transcript (Fig. 2, lane A′).

The 3.6-kb transcript (Fig. 2 and 3) hybridized to probes pcrED, oliD, pcrDA, and oliA but not pcrG, pcrGM, pcrE, or oliE and was the size predicted for a transcript encoding the *ilvD* and *ilvA* genes. The 3.6-kb transcript was not affected by the *ilvG* deletion (Fig. 2, lane G′, probe pcrDA), but the *ilvDA* deletion resulted in the loss of the 3.6-kb transcript and the appearance of a 2.3-kb transcript that migrated just ahead of the 23S RNA (Fig. 2, lane A′, probe pcrDA).

The 1.1-kb transcript (Fig. 2 to 4) hybridized to probes pcrGM, pcrE, oliE, and pcrED but not pcrG, pcrDA, oliD, or oliA. Thus, this transcript contained internal coding regions but lacked the 5′ and 3′ ends of the *ilvGMEDA* cluster. The 1.1-kb transcript was not affected by the deletions in plasmids pCC17 and pCC20 (Fig. 2, compare lanes wt, G′, and A′, probe oliE), confirming that this mRNA was derived from sequences outside the regions deleted. This transcript could have been derived from a longer precursor, such as the 6.7- or 4.6-kb transcript, or it could have been a primary transcription product. In the latter case, it could have been derived from initiation at *ilvEp*, which is predicted to yield a transcript extending from nt 2101 to approximately nt 3201 and encoding the *ilvE* gene but little or none of the *ilvD* gene.

Thus, four major transcripts were detected, and the 4.6- and 1.1-kb messages formed stronger, more distinct bands than the 6.7- and 3.6-kb messages. Similar variations in intensity and sharpness were also seen with transcripts derived from the *lac* operon (see Discussion).

It should be noted that the *ilvG* and *ilvDA* deletions generated in-frame protein fusions, so that polar effects due to the termination of translation would not be expected. The deletion in plasmid pCC17 also removed the polar frameshift site present in the wild-type *ilvG* (Val^s) allele present in

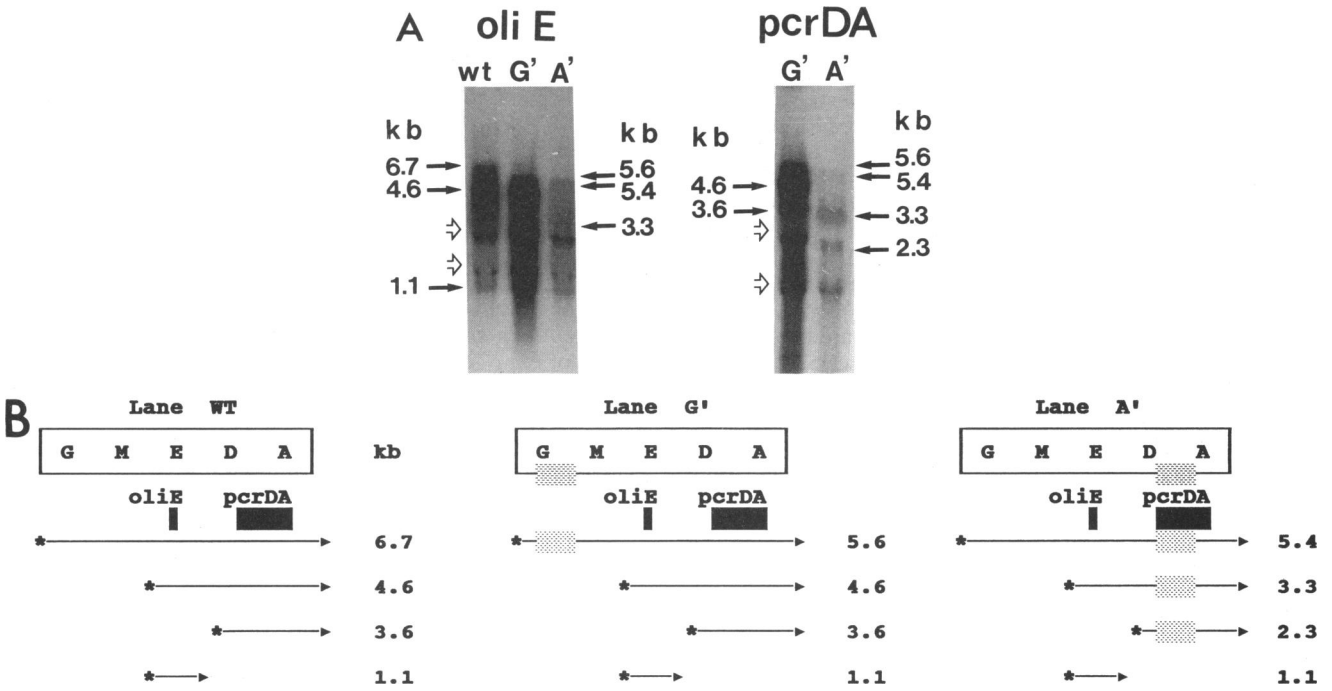

FIG. 2. (A) Detection of *ilv* transcripts in Northern blots. RNA was extracted from strain C600 derivatives grown with threonine, leucine, isoleucine, and valine and containing plasmid pJG51, which contains the full-length *ilvGMEDA* cluster (lane wt), plasmid pCC17, which contains a deletion in the *ilvG* gene (lane G'), or plasmid pCC20, which contains a deletion in the *ilvDA* region (lane A') and hybridized to probe oliE or pcrDA. Solid arrows indicate the sizes of the full-length transcripts (left) or shortened transcripts (right) generated as a result of the deletions in plasmids pCC17 and pCC20. Open arrows (left) indicate the positions of the large (2,904-bp) and small (1,541-bp) rRNA bands. (B) Approximate positions of the deletions (▦), PCR (■) and synthetic (▮) hybridization probes, and transcripts (*——►), including approximate sizes in kilobases, detected by each probe. Since the 6.7- and 3.6-kb transcripts were much less stable than the 4.6- and 1.1-kb transcripts, they were not always detectable (see also Fig. 3 and 4) on some of the shorter exposures or at lower levels of expression (see Fig. 3).

plasmid pJG51, and this fact presumably accounts for the increased transcript levels derived from plasmid pCC17 (Fig. 2, compare lane G' with lanes wt and A'). As predicted, probe oliA, which is based on sequences from nt 5842 to 5793, failed to hybridize to any transcripts derived from plasmid pCC20 (data not shown), which has a deletion extending from the *Xho*I site at nt 4912 to the *Sac*II site at nt 6201.

Primer extension detects a 5' end between *ilvE* and *ilvD*. The 5' ends of the 6.7-kb *ilvGMEDA* and 4.6-kb *ilvEDA* transcripts can be attributed to the well-characterized $ilvGp_2$ and *ilvEp* promoters, respectively. The existence of an *ilvDp* promoter has been inferred (see above), but no direct in vivo or in vitro experiments characterizing the 5' end of such a transcript have been reported. Accordingly, oligonucleotide D5, with a sequence derived from nt 3323 to 3301 (Fig. 1),

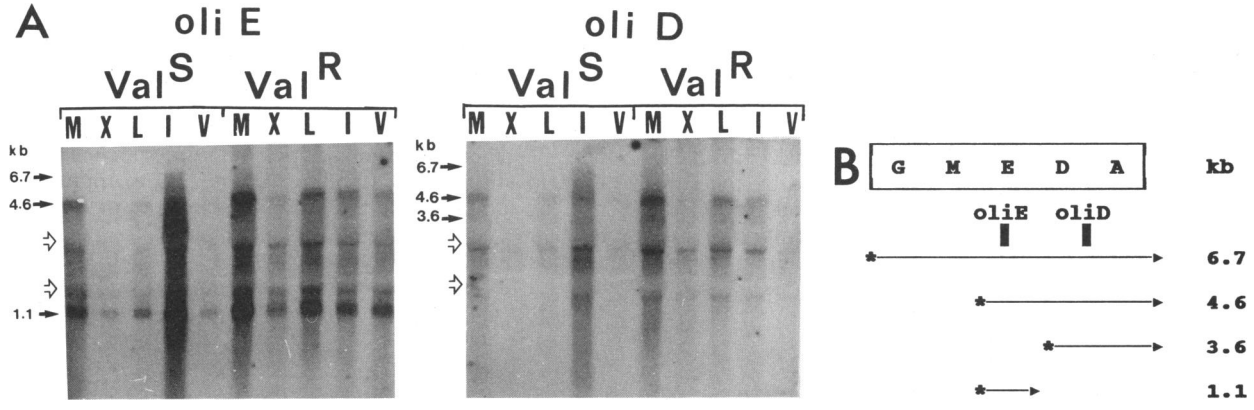

FIG. 3. (A) Effect of end product amino acids on *ilv* transcripts. RNA was isolated from derivatives of strain FD1054 containing plasmid pJG51 (Val^s) or pCC36 (Val^r) and hybridized to probe oliE or oliD. Cells were grown in unsupplemented minimal medium (lane M), in the presence of excess (lane X) leucine, isoleucine, and valine, or with a limiting concentration of leucine, isoleucine, or valine (lane L, I, or V, respectively). Solid and open arrows are as described in the legend to Fig. 2. (B) Probes used and transcripts detected by each probe.

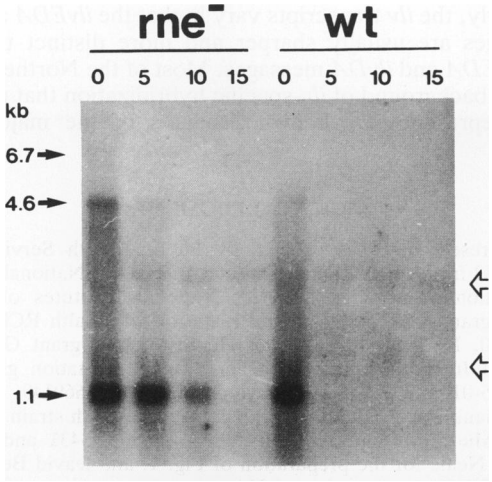

FIG. 4. Effects of RNase E on *ilv* transcript stability. Strains N3431 (*rne*) (rne⁻) and N3433 (*rne*⁺) (wt) were transformed with plasmid pJG51, grown in the absence of leucine, isoleucine, and valine, and used to examine transcript stability. Rifampin was added, and RNA samples were prepared 0, 5, 10, and 15 min later and analyzed with probe oliE. Solid and open arrows are as described in the legend to Fig. 2. Figure 3B indicates the transcripts detected with probe oliE. The 6.7-kb transcript is detectable in the original film at time zero, particularly at longer exposure times, but it is not seen in this photograph of the film.

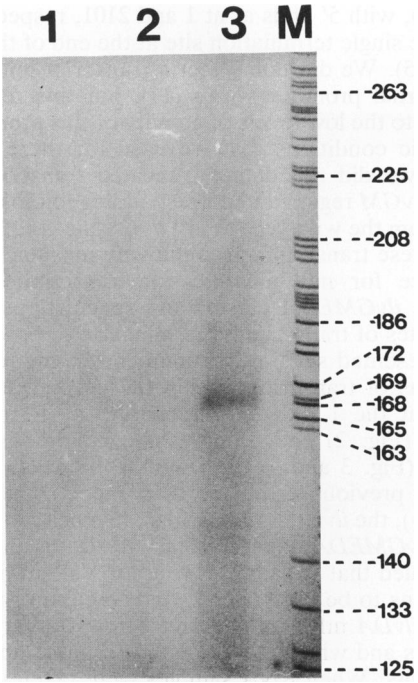

FIG. 5. Primer extension with total *E. coli* mRNA as a template for reverse transcriptase and synthetic oligonucleotide D5, derived from sequences near the 5′ end of the *ilvD* gene (Fig. 1), as a primer. The RNA was from strain FD1054, which contains an *ilvGMEDA* deletion (lane 1), or from a strain C600 derivative containing plasmid pJG51 (lanes 2 and 3). Lane 2 is a control in which reverse transcriptase was omitted. Lane M contains molecular size markers (in nucleotides).

was used as a primer for reverse transcriptase with mRNA as a template. An extension product of approximately 169 nt was detected (Fig. 5), corresponding to a 5′ end at nt 3154, located approximately 37 nt upstream of the start codon of *ilvD*, within the intercistronic region between *ilvE* and *ilvD*. This result, in conjunction with those of previous reports cited above, is consistent with the existence of an *ilvDp* promoter, but at present we cannot exclude the possibility of a processing event that generated this 5′ end.

Effect of supplementation with branched-chain amino acids. The expression of the *ilvGMEDA* cluster is regulated by the levels of branched-chain amino acids and is affected by various regulatory loci, both linked and unlinked (27). We isolated RNA from strain FD1054 derivatives (Fig. 3) containing plasmid pJG51 (Val^s) or pCC36 (Val^r) and grown in medium containing no branched-chain amino acids (lane M), repressing concentrations of all three end products (lane X), or a limiting concentration of one of the three end products (lanes L, I, and V). On the basis of a direct enzyme assay (27), these prototrophic strains would be predicted to have the lowest mRNA levels in the presence of an excess of all three end product amino acids, intermediate levels in the absence of the three end products, and the highest levels in the presence of a limiting concentration of isoleucine, leucine, or valine. Isoleucine limitation (the presence of excess valine and leucine) inhibits the growth of the Val^s but not the Val^r strain and therefore causes a more severe limitation in the Val^s strain. Furthermore, the Val^r strain does not have the polar frameshift site in *ilvG*, so that higher levels of the downstream *ilvEDA* transcripts would be predicted.

As expected for the Val^s strain (Fig. 3), the lowest, intermediate, and highest mRNA levels resulted from growth with an excess of all three end products, no end products added, and isoleucine starvation, respectively. Leucine or valine limitation resulted in mRNA levels intermediate between those detected with an excess of all three end products and unsupplemented minimal medium.

As expected for the Val^r strain (Fig. 3), levels of transcripts higher than those in the Val^s strain were found in the presence of all three end products, with no end products added, and with leucine or valine limitation. The Val^r strain had the lowest mRNA levels in the presence of all three end products, intermediate levels with leucine, isoleucine, or valine limitation, and the highest levels in unsupplemented minimal medium.

The levels of the 6.7-, 4.6-, 3.6-, and 1.1-kb transcripts appeared to change in parallel in response to amino acid levels. The Northern blots represent steady-state mRNA levels, and we have not yet measured the separate rates of synthesis and degradation of each transcript under growth conditions used.

Stability of transcripts and effect of RNase E. RNase E, encoded by the *rne* (also designated *ams*) gene, has been postulated to have a general role in *E. coli* mRNA turnover (21). We examined the decay of labeled RNA in cells grown in the absence of branched-chain amino acids following rifampin addition to an isogenic *rne* and *rne*⁺ pair containing plasmid pJG51 (Fig. 4). All three transcripts decayed in parallel, and the rate of decay was significantly reduced in the *rne* strain relative to its *rne*⁺ parent.

DISCUSSION

We detected four transcripts derived from the *ilvGMEDA* cluster, and these encode the products of the *ilvGMEDA*, *ilvEDA*, *ilvDA*, and *ilvE* genes. The *ilvGMEDA* and *ilvEDA* transcripts can be interpreted in terms of the previously characterized transcription start sites, $ilvGp_2$ and *ilvEp* (1,

17, 23, 28), with 5′ ends at nt 1 and 2101, respectively (Fig. 1), and the single termination site at the end of the cluster at nt 6703 (25). We did not detect a transcript initiated at the weak internal promoter *ilvAp* (17), but this result can be attributed to the low levels of activity of this promoter under the aerobic conditions that we used for these studies. In addition, we did not detect truncated transcripts derived from the *ilvGM* region and corresponding to those generated in vitro from the wild-type (Val^s) *ilvG* gene (18, 29), suggesting that these transcripts are relatively unstable in vivo.

Evidence for multiple sites of transcription initiation within the *ilvGMEDA* cluster and speculations relating to internal sites of transcription termination or processing (see above) presented several possibilities relating to the actual populations of transcripts within the cells. Our results revealed that the 1.1-kb *ilvE* transcript is one of the most abundant (Fig. 2) and, under some conditions, the most abundant (Fig. 3 and 4) form within the cells. Our results confirmed previous estimates that under some conditions (see below), the *ilvEDA* transcripts are much more abundant than the *ilvGMEDA* transcripts in Val^s strains and unexpectedly revealed that the same is true for Val^r strains (Fig. 3).

It remains to be established what fraction of the *ilvEDA*, *ilvE*, and *ilvDA* mRNAs is derived from primary transcription events and what fraction is the result of posttranscriptional events. When RNA samples were taken immediately after rifampin addition (Fig. 4), we did not detect obvious differences in stability among the *ilv* transcripts, and there was no evidence for precursor-product relationships in either the *rne* or the *rne*$^+$ host. Certainly some of the *ilvEDA* mRNAs are primary transcripts, since it was previously estimated that, in wild-type (Val^s) cells growing under repressing conditions, *ilvEp* accounts for 41% (29), 61% (7), and 90% (18) of downstream gene expression. Under derepressing conditions or in the presence of the Val^r allele, the relative contribution of *ilvEp* was reported to be reduced (7, 18, 29). Therefore, the *ilvGMEDA* and *ilvEDA* transcripts are both potential precursors for the *ilvE* and *ilvDA* transcripts.

We previously noted (10) for the relatively large intercistronic region between *ilvE* and *ilvD* an inverted repeat sequence at nt 3134 to 3146 that could form a particularly stable stem-loop structure of the type predicted to stabilize the 3′ ends of transcripts. Our primer extension analysis identified the 5′ end of the *ilvDA* transcript at nt 3153 in the *ilvED* intercistronic region, consistent with the origin of the *ilvE* and/or *ilvDA* transcript by the processing of an *ilvEDA* precursor. Although an *ilvDA* transcript derived from a potential *ilvDp* promoter was predicted by the genetic data (4–6, 13) we have been unable to detect such an *ilvDp* promoter on the 1,659-nt *Hin*dIII fragment extending from nt 2438 to 4079 (Fig. 1) with either fusions to β-galactosidase or in vitro transcription (unpublished data).

It is apparent that the relative intensities of the *ilv* transcripts were not identical in derivatives of strain C600 (Fig. 2), strain FD1054 (Fig. 3), and strains N3431 and N3433 (Fig. 4). This result could reflect differences in genetic backgrounds or amino acid supplements present during growth. It is also apparent that whereas the *ilvEDA* and *ilvE* transcripts often were present as discrete and relatively intense transcripts (Fig. 3 and 4), the *ilvGMEDA* transcripts were accompanied by what apparently was a broad distribution of degradation forms (Fig. 2 and 3).

Multiple transcripts were reported for the *lacZYA* operon of *E. coli* (22), including distinct *lacZYA*, *lacZY*, *lacZ*, and *lacA* messages and more diffuse *lacY* and *lacYA* messages. Similarly, the *ilv* transcripts vary in that the *ilvEDA* and *ilvE* messages are usually sharper and more distinct than the *ilvGMEDA* and *ilvDA* messages. Most of the Northern blots have a background of *ilv*-specific hybridization that presumably represents breakdown products of the major transcripts.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM23182 from the National Institutes of Health, National Science Foundation award DMB8703610, National Institutes of Health MBRS grant RR08168, National Institutes of Health RCMI grant RR03060, National Institutes of Health MARC grant GM07639, National Institutes of Health Shared Instrumentation grant S10 RR04715-01, and PSC/CUNY awards 668279 and 669149.

We thank Robert Lawther, who provided us with strain FD1054, E. A. Mudd, who provided us with strains N3431 and N3433, Chance Neale for the preparation of Fig. 1, and David Bechhoffer for helpful discussions about RNA extractions.

REFERENCES

1. **Adams, C. W., M. Rosenberg, and G. W. Hatfield.** 1985. Analysis of the in vivo RNA transcription products of the *ilvGEDA* attenuator region of *Escherichia coli* K-12. J. Biol. Chem. **260:**8538–8544.
2. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39:**440–449.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1991. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
4. **Berg, C. M., and K. J. Shaw.** 1981. Organization and regulation of the *ilvGEDA* operon in *Salmonella typhimurium* LT2. J. Bacteriol. **145:**984–989.
5. **Berg, C. M., K. J. Shaw, J. Vender, and M. Borucka-Mankeiwicz.** 1979. Physiological characterization of polar Tn-5 induced isoleucine-valine auxotrophs in *Escherichia coli* K-12: evidence for an internal promoter in the *ilvOGEDA* operon. Genetics **93:**309–319.
6. **Blazey, D. L., and R. O. Burns.** 1979. Transcriptional activity of the transposable element Tn10 in the *Salmonella typhimurium ilv* gene cluster. Mol. Gen. Genet. **177:**1–11.
7. **Calhoun, D. H., J. W. Wallen, L. Traub, J. E. Gray, and H.-F. Kung.** 1985. Internal promoter in the *ilvGEDA* transcription unit of *Escherichia coli* K-12. J. Bacteriol. **161:**128–132.
8. **Cannistraro, V. J., M. N. Subbarao, and D. Kennell.** 1986. Specific endonucleolytic cleavage sites for decay of *Escherichia coli* mRNA. J. Mol. Biol. **192:**257–274.
9. **Coppola, G., F. Huang, J. Riley, J. L. Cox, P. Hantzopoulos, L.-B. Zhou, and D. H. Calhoun.** 1991. Sequence and transcriptional activity of the *Escherichia coli* chromosome region between *rrnC* and *ilvGMEDA*. Gene **97:**21–27.
10. **Cox, J. L., B. J. Cox, V. Fidanza, and D. H. Calhoun.** 1987. The complete nucleotide sequence of the *ilvGMEDA* cluster of *Escherichia coli* K-12. Gene **56:**185–198.
11. **Driver, R. P., and R. P. Lawther.** 1985. Physical analysis of deletion mutations in the *ilvGMEDA* operon of *Escherichia coli* K-12. J. Bacteriol. **162:**598–606.
12. **Gayda, D. J., T. D. Leathers, J. D. Noti, F. J. Smith, J. M. Smith, C. S. Subrahmanyam, and H. E. Umbarger.** 1980. Location of the multivalent control site for the *ilvEDA* operon of *Escherichia coli*. J. Bacteriol. **142:**556–567.
13. **Gray, J. E., D. C. Bennett, H. E. Umbarger, and D. H. Calhoun.** 1982. Physical and genetic localization of *ilv* regulatory sites in *ilv* bacteriophages. J. Bacteriol. **149:**1071–1081.
14. **Gray, J. E., J. W. Wallen, and D. H. Calhoun.** 1982. Identification of a protein of 15,000 daltons related to isoleucine-valine biosynthesis in *Escherichia coli* K-12. J. Bacteriol. **151:**127–134.
15. **Harms, E. H., and H. E. Umbarger.** 1991. The absence of branched-chain amino acid and growth rate control at the internal *ilvEp* promoter of the *ilvGMEDA* operon. J. Bacteriol.

173:6446–6452.

16. **Lawther, R. P., R. C. Wek, J. M. Lopes, R. Pereira, B. E. Taillon, and G. W. Hatfield.** 1987. The complete nucleotide sequence of the *ilvGMEDA* operon of *Escherichia coli* K-12. Nucleic Acids Res. **15:**2137–2155.
17. **Lopes, J. M., and R. P. Lawther.** 1989. Physical identification of an internal promoter, *ilvAp*, in the distal portion of the *ilvGMEDA* operon. Gene **76:**255–269.
18. **Lopes, J. M., N. Soliman, P. K. Smith, and R. P. Lawther.** 1989. Transcriptional polarity enhances the contribution of the internal promoter, *ilvEp*, in the expression of the *ilvGMEDA* operon in wild-type *Escherichia coli* K-12. Mol. Microbiol. **3:**1039–1051.
19. **McCabe, P. C.** 1990. Production of single-stranded DNA by asymmetric PCR, p. 76–83. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols. A guide to methods and applications. Academic Press, Inc., New York.
20. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 431–432. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Mudd, E. A., H. M. Krisch, and C. F. Higgins.** 1990. RNase E, an endoribonuclease, has a general role in the chemical decay of *Escherichia coli* mRNA: evidence that *rne* and *ams* are the same genetic locus. Mol. Microbiol. **4:**2127–2135.
22. **Murakawa, G. J., C. Kwan, J. Yamashita, and D. P. Nierlich.** 1991. Transcription and decay of the *lac* messenger: role of an intergenic terminator. J. Bacteriol. **173:**28–36.
23. **Pereira, R. F., M. J. Ortuno, and R. P. Lawther.** 1988. Binding of integration host factor (IHF) to the *ilvGp* promoter of the *ilvGMEDA* cluster of *Escherichia coli* K-12. Nucleic Acids Res. **16:**5973–5989.
24. **Pledger, W. J., and H. E. Umbarger.** 1973. Isoleucine and valine metabolism in *Escherichia coli*. XXII. A pleiotropic mutation affecting induction of isomeroreductase activity. J. Bacteriol. **114:**195–207.
25. **Sameshima, J. H., R. C. Wek, and G. W. Hatfield.** 1989. Overlapping transcription and termination of the convergent *ilvA* and *ilvY* genes of *Escherichia coli*. J. Biol. Chem. **264:**1224–1231.
26. **Smith, J. M., F. J. Smith, and H. E. Umbarger.** 1976. Polarity and the regulation of the *ilv* gene cluster of *Escherichia coli* K-12. Mol. Gen. Genet. **148:**111–124.
27. **Umbarger, H. E.** 1987. Biosynthesis of the branched-chain amino acids, p. 352–367. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
28. **Wek, R. C., and G. W. Hatfield.** 1986. Examination of the internal promoter, P_E, in the *ilvGMEDA* operon of *E. coli* K-12. Nucleic Acids Res. **14:**2763–2777.
29. **Wek, R. C., J. H. Sameshima, and G. W. Hatfield.** 1987. Rho-dependent transcriptional polarity in the *ilvGMEDA* operon of wild-type *Escherichia coli* K12. J. Biol. Chem. **262:**15256–15261.