JOURNAL OF BACTERIOLOGY, Oct. 1992, p. 6580–6589
0021-9193/92/206580-10$02.00/0

Vol. 174, No. 20

# Branched-Chain Amino Acid Biosynthesis Genes in *Lactococcus lactis* subsp. *lactis*

JEAN-JACQUES GODON,* MARIE-CHRISTINE CHOPIN, AND S. DUSKO EHRLICH

*Laboratoire de Génétique Microbienne, Institut National de la Recherche Agronomique, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France*

Received 21 April 1992/Accepted 12 August 1992

**The genes for biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine in *Lactococcus lactis* subsp. *lactis* NCDO2118 were characterized by cloning, complementation in *Escherichia coli* and *Bacillus subtilis*, and nucleotide sequence analysis. Nine structural genes are clustered on a 12-kb DNA fragment in the order *leuABCD ilvDBNCA*. Upstream of these genes, the nucleotide sequence suggests the existence of regulation by transcriptional attenuation. Between the *leuD* and *ilvD* genes is an unexpected gene, encoding a protein which belongs to the ATP-binding cassette protein superfamily.**

The branched-chain amino acid (BCAA) pathway, by which leucine, isoleucine, and valine are synthesized, has been widely studied in bacteria, fungi, and plants (8, 31, 59, 60). Regulation of the expression of BCAA genes is complex because of the common steps for synthesis of the three amino acids, and the pathway is often presented as a model for organization and regulation studies. However, the sequences of all of the genes from the same organism have never been reported. Organization of the genes of the BCAA pathway has been characterized for *Escherichia coli* (59), *Salmonella typhimurium* (59), *Bacillus subtilis* (37, 62, 63), *Corynebacterium glutamicum* (9), and *Staphylococcus aureus* (44). On the *E. coli* chromosome, the genes are located in three clusters (3). The largest, at 85 min, is organized into one large and two small transcription units, comprising the *ilvGMEDA*, *ilvY*, and *ilvC* genes, respectively (36, 64). Another cluster, at 2 min, is composed of two transcription units, comprising the *ilvIH* and *leuACBD* genes (17, 56), and the last cluster, at 82 min, groups the *ilvBN* genes in a single transcription unit (65). A similar organization is found in other enterobacteriaceae (12). In *B. subtilis*, *ilvBNC* and *leuACBD* genes are found in one chromosomal region (37) and *ilvAD* genes are found in another (46). Three noncontiguous chromosomal fragments cloned from *C. glutamicum* carry five BCAA genes, *ilvCBN*, *ilvA*, and *ilvE* (9). In *S. aureus*, eight structural genes are clustered in the order *ilvABCD leuABCD*, as found by genetic mapping (44). Here we report the cloning, characterization, and sequences of *L. lactis* subsp. *lactis* BCAA genes. Similar analyses of the genes of the tryptophan and histidine biosynthesis pathways are described in the accompanying reports (5, 10).

## MATERIALS AND METHODS

The bacterial strains and plasmids used are described in Table 1. Media and growth conditions and DNA cloning and manipulation procedures are described in an accompanying report (5). The reported sequence was determined for both strands. Restrictionless *B. subtilis* strains mutated in the *ilvA* gene were constructed by transforming MT119-competent cells with pHV438 (41) to $Cm^r$. Integration of this plasmid into the chromosome by double crossing over replaces the *ilvA* gene by the $Cm^r$ gene (42). A representative *ilvA leuB6* $r^- m^- Cm^r$ clone was designated IL2685. A restrictionless *B. subtilis ilvD4 leuB6* double mutant was constructed by congression, using GSY276 DNA to transform 1012 competent cells to methionine independence. Transformants were further tested for isoleucine and leucine requirements and for the absence of restriction by titration of phage rho. One *ilvD4 leuB6* $r^- m^+$ clone was designated IL3151.

**Nucleotide sequence accession number.** The sequence shown in Fig. 2 has been assigned GenBank accession number M90761.

## RESULTS

**Cloning of BCAA biosynthetic genes.** Total DNA from *L. lactis* subsp. *lactis* NCDO2118 was partially digested with endonuclease *Sau*3AI. Twenty micrograms of >10-kb DNA segments was ligated to 10 μg of *Bam*HI-cleaved plasmid vector pIL253 DNA at a final concentration of 500 μg/ml. The ligation mixture was used to transform competent cells of the *B. subtilis leuB6 ilvD4* mutant strain IL3151 to isoleucine independence. Four transformants were also $Em^r$ and contained plasmids of 18, 14.2, 13.5, and 9.5 kb, designated pIL384, pIL371, pIL374, and pIL373, respectively. In a similar experiment, *Xba*I-cleaved *L. lactis* subsp. *lactis* DNA was cloned into pIL253, using the *B. subtilis ilvA* mutant strain IL2685 as the recipient. Transformants were selected on minimal medium supplemented with leucine and tryptophan but lacking isoleucine. Two $Em^r$ $Ile^+$ clones, containing an apparently identical 23.5-kb plasmid designated pIL500, were obtained.

**Complementation experiments.** The cloned DNA segments were used for complementation of *leu* and *ilv* mutants in *B. subtilis* and, after subcloning in pBluescript, in *E. coli*. The results are summarized in Fig. 1. The *E. coli* nomenclature is used; *leuB* and *leuC* genes from *E. coli* correspond to *leuC* and *leuB* genes from *B. subtilis*, respectively, and the three isoenzymes for acetolactate synthase and acetohydroxyacid synthase activity, encoded by *ilvBN*, *ilvIH*, and *ilvGM* in *E. coli*, correspond to a single enzyme, encoded by *ilvBN*, in *B. subtilis*. Three genes (*leuABC*) were complemented in both hosts, and three (*leuD* and *ilvC* in *E. coli* and *ilvA* in *B. subtilis*) were complemented in only one. One of the two genes tested in one host only (*ilvD*) was complemented, and the other (*ilvB*) was not. We have no explanation for the complementation pattern, which probably is due to the

* Corresponding author.

TABLE 1. Strains and plasmids

| Strain or plasmid | Characteristics | Source or reference |
|---|---|---|
| Strains | | |
| *L. lactis* subsp. *lactis* NCDO2118 | Natural isolate | National Collection of Dairy Organisms |
| *B. subtilis* | | |
| CU740 | *leuA5 trpC2* (SPβ) | 63 |
| CU741 | *leuC7 trpC2* | 63 |
| CU315 | *leuD117 trpC2* (SPβ) | 38 |
| IL2685 | *leuB6 trpC2 ilvA* $r^-$ $m^-$ $Cm^r$ | This work |
| MT119 | *leuA8*[a] *trpC2* $r^-$ $m^-$ | 57 |
| GSY184 | *ilvC1 trpC2* | 4 |
| IL3151 | *ilvD4 leuB6* $r^-$ $m^+$ | This work |
| 1012 | *leuA8*[a] *metB5* $r^-$ $m^+$ | 26 |
| GSY276 | *ilvD4 trpC2* | 4 |
| *E. coli* | | |
| CU518 | *leuA371* | 54 |
| CU514 | *leuB401* | 54 |
| CU520 | *leuC171* | 54 |
| CU526 | *leuD101* | 54 |
| AB1255 | *tonA2 lacY1 tsx-5 supE44 gal-6* $\lambda^-$ *hisG1 rpsL8 malA1 xyl-7 mtl-2 ilvA201 metB1 argH1 thi-1* | 39 |
| FD1062 | *ara-14 ilvI614 ilvH612* $\lambda^-$ *glyA18 relA1 spoT1 ilvB619 bglR20 rbs-5*::Tn*5 ilvG468*(*ilvG*$^+$) *thi-1* | 34 |
| JP58 | *galK2* $\lambda^-$ *rpsL704 xyl-5 mtl-1 ilvC7 argE3 thi-1* | 7 |
| TG1 | *supE thi* Δ(*lac-proAB*) *hsdD5* (*F'*$^+$ *traD36 proAB lacI*$^q$*ZΔM15*) | 18a |
| Plasmids | | |
| pIL253 | $Em^r$, 4.9 kb | 52 |
| pHV438 | Hybrid between pBR322, $Cm^r$ gene of pC194, and *thyB* and X segments of *B. subtilis* DNA | 42 |
| pIL371 | 9.2-kb *Sau*3A fragment of *L. lactis* chromosome in pIL253 | This work |
| pIL373 | 4.5-kb *Sau*3A fragment of *L. lactis* chromosome in pIL253 | This work |
| pIL374 | 8.5-kb *Sau*3A fragment of *L. lactis* chromosome in pIL253 | This work |
| pIL384 | 13-kb *Sau*3A fragment of *L. lactis* chromosome in pIL253 | This work |
| pIL389 | 7.5-kb *Sau*3A-*Sma*I left fragment from pIL384 in pBluescript | This work |
| pIL500 | 18.5-kb *Xba*I fragment of *L. lactis* chromosome in pIL253 | This work |
| pIL505 | 6.5-kb *Sma*I-*Sau*3A right fragment from pIL384 in pBluescript | This work |
| pIL533 | 2.5-kb *Sau*3A-exonuclease III left fragment from pIL384 | This work |
| pBluescript | $Ap^r$, M13 *ori*, pBR322 *ori* | Stratagene |

[a] This mutation is in fact *leuB6* (68).

presence of active promoters upstream of the tested genes and the interaction of the gene products with the host proteins. The *L. lactis* subsp. *lactis* genes for histidine biosynthesis also gave inconsistent complementation patterns in *B. subtilis* and *E. coli* (10). Nevertheless, we were able to tentatively identify seven genes required for BCAA biosynthesis.

**Nucleotide sequence of the genes.** The nucleotide sequence of a 12,720-bp region has been determined (Fig. 2). A computer analysis performed according to Gribskov et al. (22) revealed 10 open reading frames (ORFs) larger than 200 bp. Each ORF is preceded by a putative ribosome binding site, complementary to the 3′ end of the *L. lactis* subsp. *lactis* 16S rRNA (11) (Fig. 2). All ORFs begin with ATG except for the second, which starts with a TTG codon. In addition, four 72-bp direct repeats are present between the second and third ORFs.

**Assignment of the ORFs.** The proteins deduced from the 10 ORFs were compared with those in the GENPRO and NBRF protein data bases. Significant homologies were found for 10 ORFs, 9 of which correspond to the BCAA biosynthetic genes (Table 2). In addition to genes detected by complementation (see above), *ilvB* (homologous to *E. coli ilvB*, *ilvI*, and *ilvG*) and *ilvN* (homologous to *E. coli ilvH*) genes, for which complementation data were not obtained, were identified. Most of the *L. lactis* proteins are similar in size to their homologs. However, three exceptions were observed. IlvA and IlvC lactococcal proteins lack 73 and 147 carboxy-terminal amino acids, respectively, and IlvD had a 36-amino-acid gap compared with the *E. coli* proteins. One of the ORFs, designated ORF2, encodes a protein which has no homology with BCAA biosynthetic enzymes but carries two boxes conserved within a superfamily of closely related ATP-binding cassette (ABC) proteins (Fig. 3) (23).

Organization of the sequenced fragment is shown in Fig. 1. All of the genes except the first, upstream of *leuA* (ORF1), are transcribed in the same direction. *leu* and *ilv* genes are clustered, and the two clusters are separated by 121 bp. The *leu* genes are spaced by less than 19 bp except for *leuB* and *leuC*, which are separated by four direct repeats of 72 bp. The distance between *ilv* genes ranges from 10 to 42 bp except for *ilvB* and *ilvN*, which have a 9-bp overlap.

**Transcription signals.** Sequences which conform to the consensus for lactococcus promoters (11) were found upstream of the *leu* and *ilv* gene clusters (p1 and p2; Fig. 1). The region between p1 and *leuA* strongly resembles regulatory regions of amino acid biosynthetic operons controlled by attenuation (32). The transcript initiated at p1 can fold in two ways. One leads to the formation of a rho-independent transcription terminator (Fig. 4A), whereas the other does

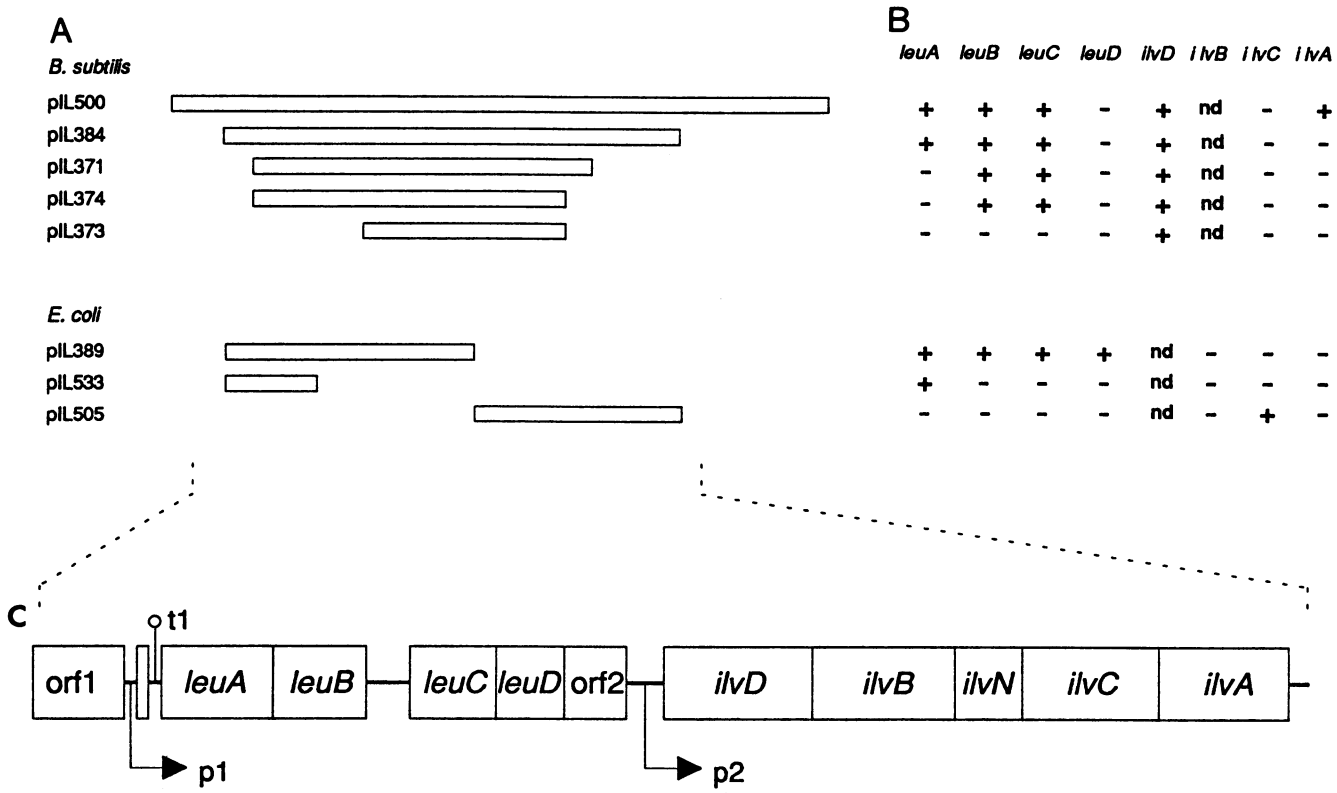


FIG. 1. Structure of the DNA region carrying the *L. lactis* BCAA genes. (A) Segments used for complementation experiments in *B. subtilis* and *E. coli*. These segments are carried on the indicated plasmids, which were constructed as described in Table 1 and the text. (B) Results of complementation experiments with mutants listed in Table 1, indicating growth (+) or no growth (−) on media lacking the corresponding amino acids. (C) Organization of the BCAA region as deduced from sequence results. p1 and p2, putative transcription promoters; t1, putative transcription terminator. The open box between p1 and t1 represents the leader peptide.

TABLE 2. Conservation of proteins involved in BCAA biosynthesis between *L. lactis* subsp. *lactis* and various organisms

| Organism compared | % Identical amino acids[a] | | | | | | | | | Reference(s) |
|---|---|---|---|---|---|---|---|---|---|---|
| | LeuA | LeuB | LeuC | LeuD | IlvD | IlvB | IlvN | IlvC | IlvA | |
| *Escherichia coli* | —[b] | — | — | 46 | 42 | 42[c]<br>43[e]<br>40[g] | <20[d]<br>37[f]<br><20[h] | 34 | 36 | 17, 36, 64, 65<br>55<br>36 |
| *Salmonella typhimurium* | 41 | — | 49 | — | — | — | — | — | — | 47, 49 |
| *Bacillus subtilis* | — | 53 | — | — | — | — | — | — | 50 | 2, 27 |
| *B. coagulans* | — | 53 | — | — | — | — | — | — | — | 50 |
| *Thermus aquaticus* | — | 42 | — | — | — | — | — | — | — | 29 |
| *Saccharomyces cerevisiae* | 25 | 46 | 50[i] | 52[j] | — | 41 | — | 33 | 38 | 1, 6, 14, 30, 45, 53 |
| *Phycomyces blakesleeanus* | — | — | 50[i] | 49[j] | — | — | — | — | — | 28 |
| *Mucor circinelloides* | — | — | 49 | — | — | — | — | — | — | 48 |

[a] Calculated by Kanehisa software (67) as the ratio of perfect matches to the shorter protein length.
[b] —, Sequence not available.
[c] Comparison with IlvB.
[d] Comparison with IlvN.
[e] Comparison with IlvI.
[f] Comparison with IlvH.
[g] Comparison with IlvG.
[h] Comparison with IlvM.
[i] $NH_2$ end of Leu1.
[j] C end of Leu1.

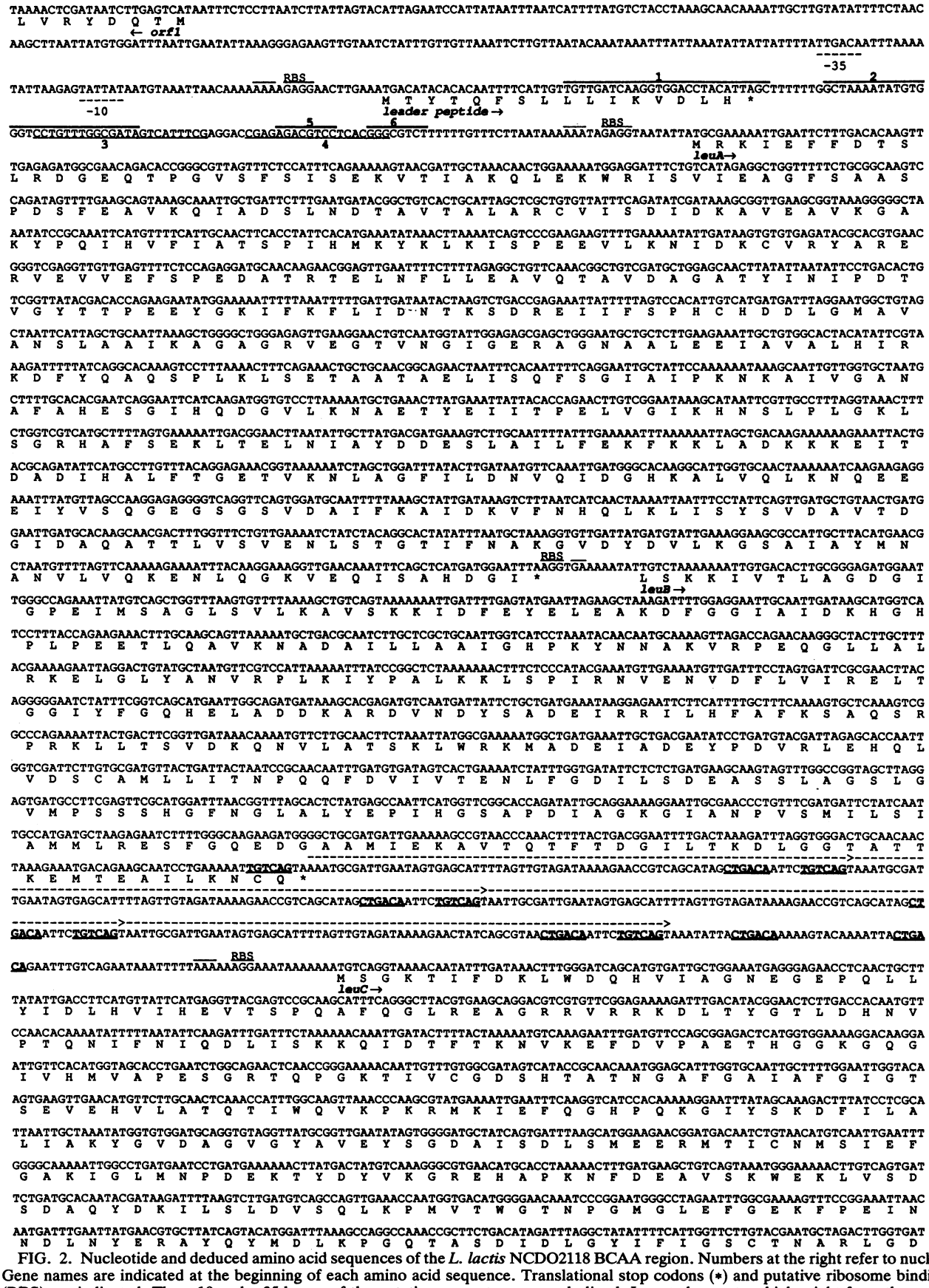

FIG. 2. Nucleotide and deduced amino acid sequences of the *L. lactis* NCDO2118 BCAA region. Numbers at the right refer to nucleotides. Gene names are indicated at the beginning of each amino acid sequence. Translational stop codons (*) and putative ribosome binding sites (RBS) are indicated. The −10 and −35 boxes of the putative promoters are underlined. Inverted repeats which might form the preemptor (numbered 1 and 2) and terminator (numbered 5 and 6) and those which might form the antiterminator (numbered 3 and 4) are indicated (see Fig. 4). Long dashed arrows indicate the 72-bp direct repeats; bold characters show the 6-bp inverted repeats contained within them.

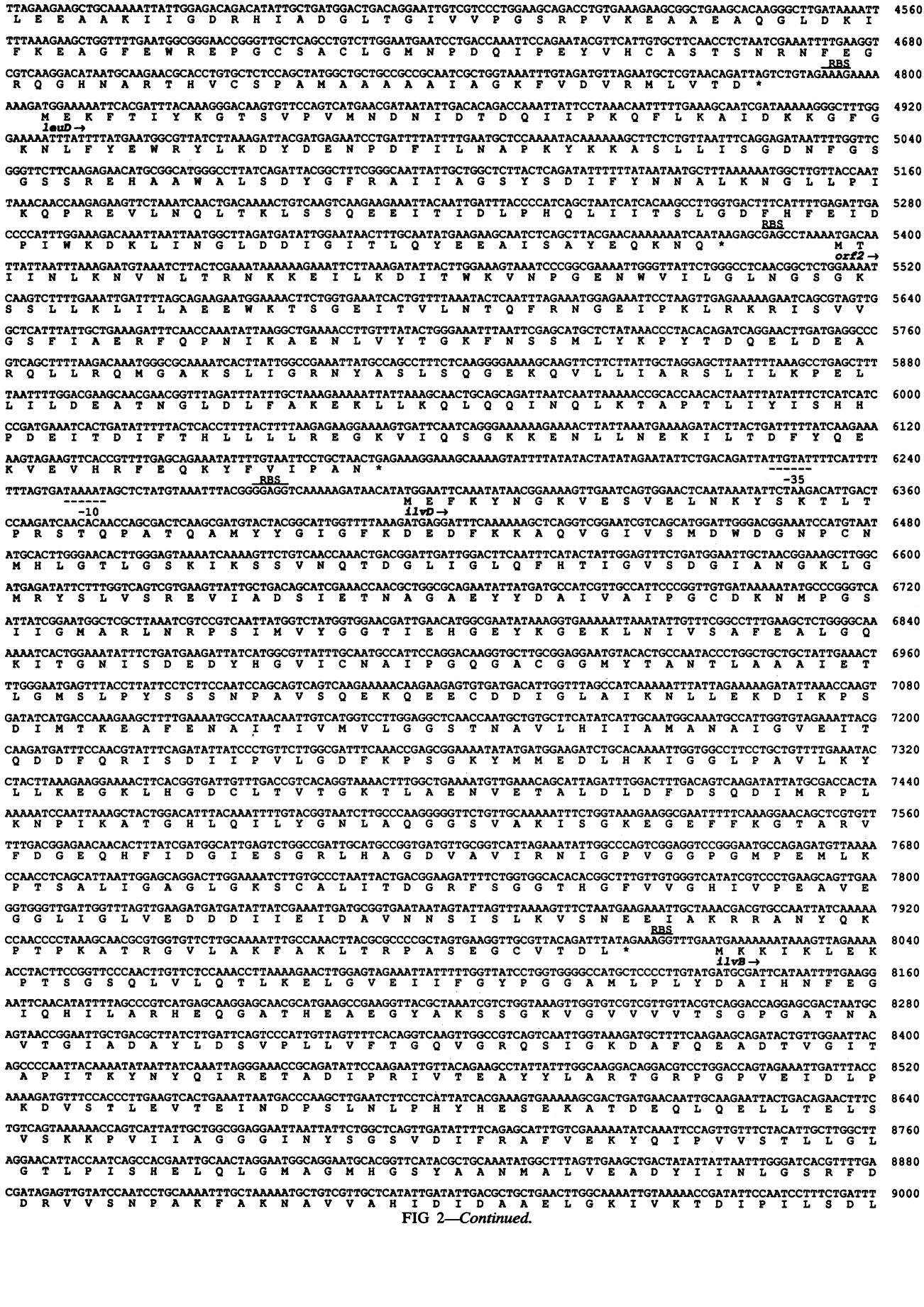


FIG 2—*Continued.*

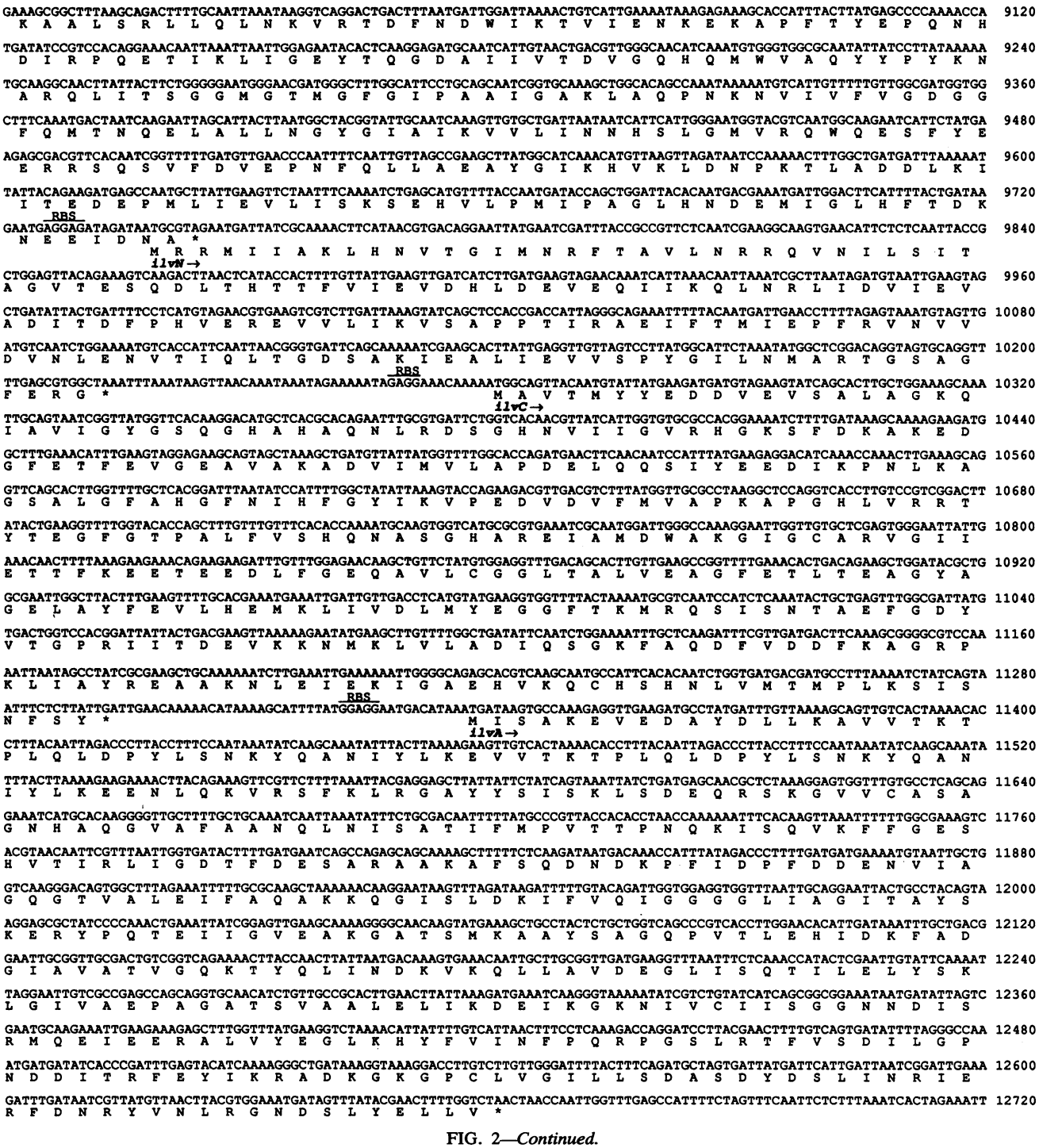

FIG. 2—*Continued.*


FIG. 3. Alignment of six ABC proteins with *L. lactis* ORF2. Software of Needleman and Wunsch was used (40). Nucleotide binding domains (23) are indicated by NB1 and NB2; a short sequence shared by all members of the ATP-dependent transport family is highlighted (#). Other notation: *, same amino acid in all proteins; ., conservative substitutions; bold characters, same amino acid in at least five proteins. Numbers at the left correspond to amino acid positions; numbers in parentheses indicate protein size in amino acids. The functions of the various proteins are as follows: [1], nodulation in *Rhizobium leguminosarum* (13); [2], maltose transport in *E. coli* (19); [3], glutamine transport in *E. coli* (43); [4], glycine, betaine, and proline transport in *E. coli* (21); [5], hemolysin secretion in *E. coli* (15); [6], cyclolysin secretion in *Bordetella pertussis* (20).

not (Fig. 4B). In addition, the transcript carries a 51-bp message, starting with an ATG and ending with a TAG codon, specifying a leader peptide of 16 amino acids, 4 of which are consecutive leucines and isoleucines. Ribosome stalling at Leu and Ile codons is expected to prevent formation of the termination signal and lead to transcription of the downstream *leu* genes. Rho-independent transcription terminators were not found between the two gene clusters or downstream of the *ilvA* gene.

## DISCUSSION

**Organization of the *ilv* operon.** The BCAA genes in *L. lactis* subsp. *lactis* are organized in a large cluster which is divided into two units, grouping *leu* and *ilv* genes. Both units are necessary for leucine biosynthesis, while only the second is required for the synthesis of isoleucine and valine. A transaminase, which carries out the last step of BCAA biosynthesis (the *ilvE* gene product in *E. coli*), is not encoded within the cluster, which suggests that this reaction is performed by a nonspecific transaminase or that the corresponding *L. lactis* subsp. *lactis* gene maps elsewhere on the chromosome. Both the *leu* and *ilv* gene clusters are preceded by a putative promoter. However, they are not separated by a rho-independent transcription terminator, which suggests that they might form a single operon. The putative operon extends past the last biosynthetic gene, *ilvA*.

**Regulation of the *ilv* operon.** Sequence analysis strongly suggests that the operon is regulated by an attenuation mechanism, mediated by a leucine-rich leader peptide (Fig. 4). This peptide is very similar to the leader peptides of the *E. coli* and *S. typhimurium leu* operons (18, 66) but differs from those of the *E. coli ilvBN* (16) and *ilvGMEDA* (35) operons.

The current model proposes that the strength of attenuation depends on the availability of charged $tRNA^{Leu}$ during translation of the leader peptide. The presence of rare codons increases the response to leucine deprivation by increasing the duration of ribosome stalling. In *E. coli*, the codon specifying the four leucine residues present in the leader peptide is CUA, which corresponds to only 2% of leucine codons used for the proteins of this organism (51). In contrast, the leucine codon UUG, found three times in the *L. lactis* BCAA leader transcript, corresponds to 24% of the leucine codons in proteins of *L. lactis* (61). The isoleucine codon is also present within the *L. lactis* leader peptide, closely following the Leu codons, which is not the case in *E. coli* and might affect the response of the operon to BCAA starvation. Further studies are needed to determine whether the model proposed for regulation in *E. coli* can be directly applied to *L. lactis*.

**ORF2.** ORF2 is not a BCAA biosynthetic gene, since it specifies a product which is a member of the ABC protein superfamily. Proteins from this family are found in prokaryotes and eukaryotes and are similar in basic organization (24, 25). In prokaryotes, most members of this superfamily are components of transport systems which involve periplasmic binding proteins. These genes are generally cotranscribed with other carrier protein genes (25) and have never been found within biosynthetic operons. Further work is required to establish the function and role of ORF2, but it is tempting to speculate that it might be involved in the transport of BCAA or in the regulation of BCAA genes, by analogy with another ABC protein, MalK, which regulates negatively the expression of the *mal* regulon in *E. coli* (33).

Isoleucine, leucine, and valine residues constitute at least 20% of the amino acid content of *L. lactis* (58), while tryptophan and histidine, the two other amino acids for which biosynthetic pathway genes were studied in *L. lactis* (5, 10), correspond to less than 2%. This finding suggests that

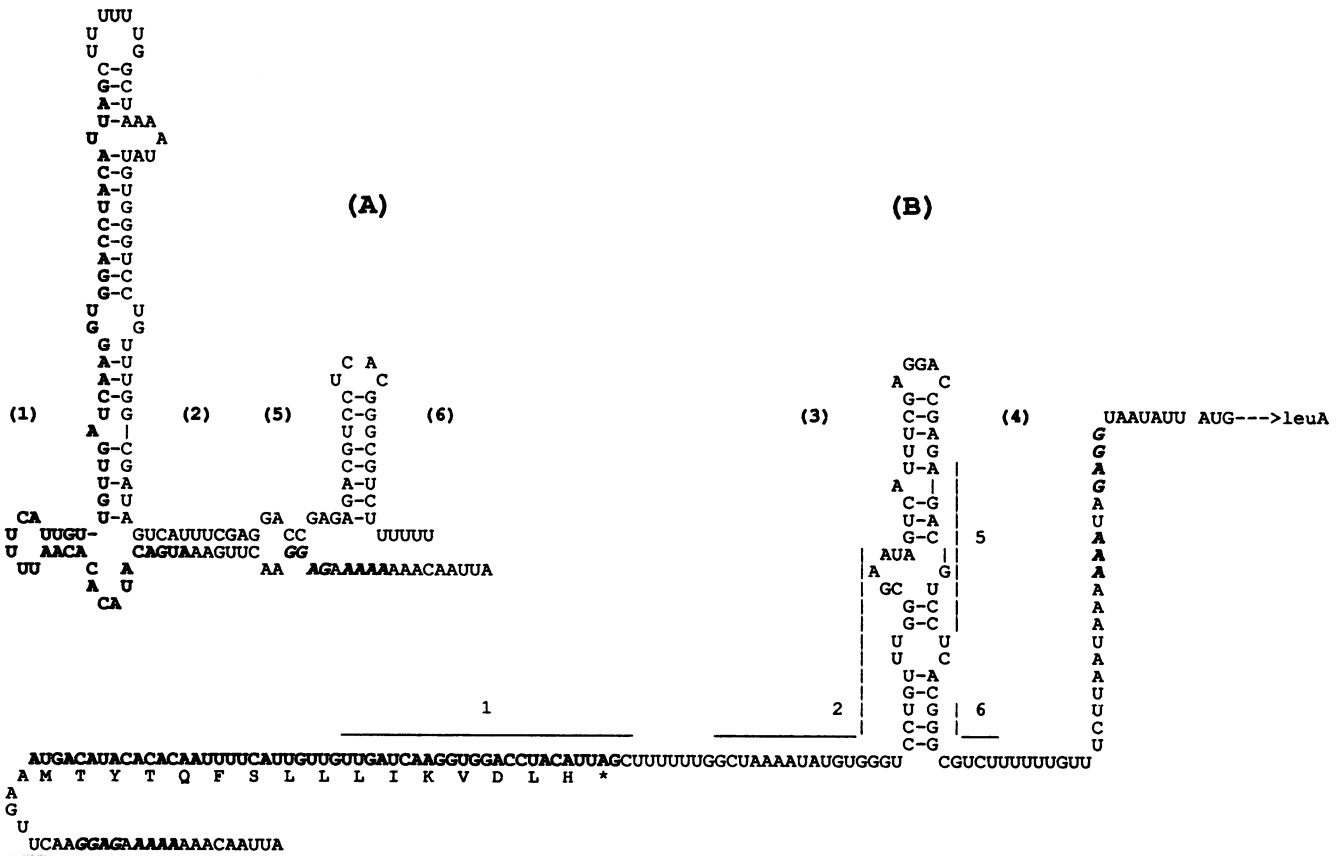


FIG. 4. Secondary structures of the leader transcript which might mediate transcriptional attenuation. (A) Termination configuration. Repeats 1 and 2 are annealed, and repeats 5 and 6 form a transcription terminator. (B) Antitermination configuration. Repeats 3 and 4 are annealed, which sequesters repeats 5 and 6. Bold characters indicate the sequence encoding the leader peptide; bold italics indicate the ribosome binding sites of the leader peptide and the *leuA* gene. Ribosome stalling at the successive Leu and Ile residues might sequester repeat 1 and favor this configuration.

a fine regulation of BCAA gene expression might be required. Furthermore, the three BCAA genes use a common pathway, which also requires a special regulation. In *E. coli*, in which the BCAA genes are scattered, the regulation is complex and not as yet fully understood. In *L. lactis*, in which the BCAA genes are clustered, the regulation is probably different. The coordination of expression of these genes is presently being studied.

## ACKNOWLEDGMENTS

We are grateful to P. Renault for helpful comments and to C. Anagnostopoulos for critical reading of the manuscript.

This work was supported in part by BRIDGE contract Biot-CT91-0263 of the Commission of the European Communities.

## REFERENCES

1. **Andreadis, A., Y.-P. Hsu, G. B. Kohlhaw, and P. Schimmel.** 1982. Nucleotide sequence of yeast *leu2* shows 5'-noncoding region has sequences cognate to leucine. Cell **31**:319–325.
2. **Armpriester, J. M., Jr., and P. S. Fink.** 1990. Unpublished data.
3. **Bachmann, B. J.** 1987. Linkage map of *Escherichia coli* K-12, p. 807–877. *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. 2. American Society for Microbiology, Washington, D.C.
4. **Barat, M., C. Anagnostopoulos, and A. M. Schneider.** 1965. Linkage relationships of genes controlling isoleucine, valine, and leucine biosynthesis in *Bacillus subtilis*. J. Bacteriol. **90**: 357–369.
5. **Bardowski, J., S. D. Ehrlich, and A. Chopin.** 1992. Tryptophan biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **174**:6563–6570.
6. **Beltzer, J. P., L.-F. L. Chang, A. E. Hinkkanen, and G. B. Kohlhaw.** 1986. Structure of yeast *leu4*: the 5' flanking region contains features that predict two modes of control and two productive translation starts. J. Biol. Chem. **261**:5160–5167.
7. **Butlin, J. D., G. B. Cox, and F. Gibson.** 1971. Oxydative phosphorylation in *Escherichia coli* K12. Biochem. J. **124**:75–81.
8. **Calvo, J. M.** 1983. Leucine biosynthesis in prokaryotes, p. 267–284. *In* K. M. Herrman and R. L. Somerville (ed.), Amino acids, biosynthesis and genetic regulation. Addison-Wesley, Reading, Mass.
9. **Cordes, C., L. Eggeling, and H. Sahm.** 1990. Production of L-isoleucine by recombinant *Corynebacterium glutamicum* strains after cloning, analysis and amplification of genes involved in isoleucine biosynthesis, p. 339–351. *In* H. Heslot, J. Davies, J. Florent, L. Bobichon, G. Durand, and L. Penasse (ed.), 6th International Symposium on Genetics of Industrial Microorganisms. Société Française de Microbiologie, Paris.
10. **Delorme, C., S. D. Ehrlich, and P. Renault.** 1992. Histidine biosynthesis genes in *Lactococcus lactis* subsp. *lactis*. J. Bacteriol. **174**:6571–6579.
11. **de Vos, W. M.** 1987. Gene cloning and expression in lactic streptococci. FEMS Microbiol. Rev. **46**:281–295.
12. **Driver, R. P., and R. P. Lawther.** 1985. Restriction endonuclease analysis of the *ilvGEDA* operon of members of the family

*Enterobacteriaceae*. J. Bacteriol. **162:**1317–1319.
13. **Evans, I. J., and J. A. Downie.** 1986. The nod1 gene product of *Rhizobium leguminosarum* is closely related to ATP-binding bacterial transport proteins; nucleotide sequence analysis of the *nodI* and *nodJ* genes. Gene **43:**95–101.
14. **Falco, S. C., K. S. Dumas, and K. J. Livak.** 1985. Nucleotide sequence of the yeast *ilv2* gene which encodes acetolactate synthase. Nucleic Acids Res. **13:**11.
15. **Felmlee, T., S. Pellett, and R. A. Welch.** 1985. Nucleotide sequence of an *Escherichia coli* chromosomal hemolysin. J. Bacteriol. **163:**94–105.
16. **Friden, P., T. Newman, and M. Freundlich.** 1982. Nucleotide sequence of the *ilvB* promoter-regulatory region: a biosynthetic operon controlled by attenuation and cyclic AMP. Proc. Natl. Acad. Sci. USA **79:**6156–6160.
17. **Friedberg, D., E. R. Rosenthal, J. W. Jones, and J. M. Calvo.** 1985. Characterization of the 3′ end of the leucine operon of *Salmonella typhimurium*. Mol. Gen. Genet. **199:**486–494.
18. **Gemmill, R. M., S. R. Wessler, E. B. Keller, and J. M. Calvo.** 1979. *leu* operon of *Salmonella typhimurium* is controlled by an attenuation mechanism. Proc. Natl. Acad. Sci. USA **76:**4941–4945.
18a. **Gibson, T. J.** 1984. Ph.D. thesis. University of Cambridge, Cambridge, England.
19. **Gilson, E., H. Nikaido, and M. Hofnung.** 1982. Sequence of the *malK* gene in *E. coli* K12. Nucleic Acids Res. **10:**7449–7458.
20. **Glaser, P., H. Sakamoto, J. Bellalou, A. Ullmann, and A. Danchin.** 1988. Secretion of cyclolysin, the calmodulin-sensitive adenylate cyclase-haemolysin bifunctional protein of *Bordetella pertussis*. EMBO J. **7:**3997–4004.
21. **Gowrishankar, J.** 1989. Nucleotide sequence of the osmoregulatory *proU* operon of *Escherichia coli*. J. Bacteriol. **171:**1923–1931.
22. **Gribskov, G., J. Devereux, and R. R. Burgess.** 1984. The codon preference plot: protein coding sequences and prediction of gene expression. Nucleic Acids Res. **12:**539–549.
23. **Higgins, C. F., M. L. Gallagher, M. L. Mimmack, and S. R. Pearce.** 1988. A family of closely related ATP-binding subunits from prokaryotic and eukaryotic cells. BioEssays **8:**111–116.
24. **Higgins, C. F., I. D. Hiles, G. P. C. Salmond, D. R. Gill, J. A. Downie, I. J. Evans, I. B. Holland, L. Gray, S. D. Bucked, A. W. Bell, and M. A. Hermodson.** 1986. A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria. Nature (London) **323:**448–450.
25. **Higgins, C. F., I. D. Hiles, K. Whalley, and D. J. Jamieson.** 1985. Nucleotide binding by membrane components of bacterial periplasmic binding protein-dependent transport systems. EMBO J. **4:**1133–1040.
26. **Ikawa, S., T. Shibata, T. Ando, and H. Saito.** 1980. Genetic studies on site-specific endodeoxyribonucleases in *Bacillus subtilis*: multiple modification and restriction systems in transformants of *Bacillus subtilis* 168. Mol. Gen. Genet. **177:**359–368.
27. **Imai, R., T. Sekiguchi, Y. Nosoh, and K. Tsuda.** 1987. The nucleotide sequence of 3-isopropylmalate dehydrogenase gene from *Bacillus subtilis*. Nucleic Acids Res. **15:**4988.
28. **Iturriaga, E. A., J. M. Diaz-Minguez, E. P. Benito, M. I. Alvarez, and A. T. Eslava.** 1990. Nucleotide sequence of *Phycomyces blakesleeanus leu1* gene. Nucleic Acids Res. **18:**4612.
29. **Kagawa, Y., H. Nojima, N. Nukiwa, M. Ishizuka, T. Nakajima, T. Yasuhara, T. Tanaka, and T. Oshima.** 1984. High guanine plus cytosine content in the third letter of codons of an extreme thermophile. J. Biol. Chem. **259:**2956–2960.
30. **Kielland-Brandt, M. C., S. Holmberg, J. G. L. Petersen, and T. Nilsson-Tillgren.** 1984. Nucleotide sequence of the gene for threonine dehydratase *ilv-1* of *Saccharomyces cerevisiae*. Carlsberg Res. Commun. **49:**567–575.
31. **Kohlhaw, G. B.** 1983. Regulation of leucine biosynthesis in lower eukaryotes, p. 285–299. *In* K. M. Herrman and R. L. Somerville (ed.) Amino acids, biosynthesis and genetic regulation. Addison-Wesley, Reading, Mass.
32. **Kolter, R., and C. Yanofsky.** 1982. Attenuation in amino acid biosynthetic operons. Annu. Rev. Genet. **16:**113–134.
33. **Kuhnau, S., M. Reyes, A. Sievertsen, H. A. Shuman, and W. Boos.** 1991. The activities of the *Escherichia coli* MalK protein in maltose transport, regulation, and inducer exclusion can be separated by mutations. J. Bacteriol. **173:**2180–2186.
34. **Lawther, R. P.** Unpublished data.
35. **Lawther, R. P., and G. W. Hatfield.** 1980. Multivalent translational control of transcription termination at attenuator of *ilvGEDA* operon of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **77:**1862–1866.
36. **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.
37. **Mackey, C. J., R. J. Warburg, H. O. Halvorson, and S. A. Zahler.** 1984. Genetic and physical analysis of the *ilvBC-leu* region in *Bacillus subtilis*. Gene **32:**49–56.
38. **Mackey, C. J., and S. A. Zahler.** 1982. Insertion of bacteriophage SPβ into the *citF* gene of *Bacillus subtilis* and specialized transduction of the *ilvBC-leu* genes. J. Bacteriol. **151:**1222–1229.
39. **Marsch, N. J., and D. E. Duggan.** 1972. Ordering of mutant sites in the isoleucine-valine genes of *Escherichia coli* by use of merogenotes derived from $F^{14}$: a new procedure for fine-structure mapping. J. Bacteriol. **109:**730–740.
40. **Needleman, S. B., and C. D. Wunsch.** 1970. A general method applicable to the search for similarities in the amino acid sequence of two proteins. J. Mol. Biol. **48:**443–453.
41. **Niaudet, B., and S. D. Ehrlich.** 1982. Insertional mutagenesis: use in cloning of *Bacillus subtilis* genes, p. 201–209. *In* M. Polsinelli and G. Mazza (ed.), Transformation-80. Cotswold Press, Oxford, England.
42. **Niaudet, B., L. Jannière, and S. D. Ehrlich.** 1985. Integration of linear, heterologous DNA molecules into the *Bacillus subtilis* chromosome: mechanism and use in induction of predictable rearrangements. J. Bacteriol. **163:**111–120.
43. **Nohno, T., T. Saito, and J.-S. Hong.** 1986. Cloning and complete nucleotide sequence of the *Escherichia coli* glutamine permease operon (*glnHPQ*). Mol. Gen. Genet. **205:**260–269.
44. **Pattee, P. A.** 1976. Genetic linkage of chromosomal tetracycline resistance and pigmentation to a purine auxotrophic marker and the isoleucine-valine-leucine structural genes in *Staphylococcus aureus*. J. Bacteriol. **127:**1167–1172.
45. **Petersen, J. G. L., and S. Holmberg.** 1986. The *ilv5* gene of *Saccharomyces cerevisiae* is highly expressed. Nucleic Acids Res. **14:**9631–9651.
46. **Piggot, P. G., and J. A. Hoch.** 1985. Revised genetic linkage map of *Bacillus subtilis*. Microbiol. Rev. **49:**158–179.
47. **Ricca, E., and J. M. Calvo.** 1990. The nucleotide sequence of *leuA* from *Salmonella typhimurium*. Nucleic Acids Res. **18:**1290.
48. **Roncero, M. I. G., L. P. Jepsen, P. Stroman, and R. van Heeswijck.** 1989. Characterization of a *leuA* gene and an ARS element from *Mucor circinelloides*. Gene **84:**335–343.
49. **Rosenthal, E. R., and J. M. Calvo.** 1990. The nucleotide sequence of *leuC* from *Salmonella typhimurium*. Nucleic Acids Res. **18:**3072.
50. **Sekiguchi, T., J. Ortega-Cesena, Y. Nosoh, S. Ohashi, K. Tsuda, and S. Kanaya.** 1986. DNA and amino-acid sequences of 3-isopropylmalate dehydrogenase of *Bacillus coagulans*. Comparison with the enzymes of *Saccharomyces cerevisiae* and *Thermus thermophilus*. Biochim. Biophys. Acta **867:**36–44.
51. **Sharp, P. M., E. Cowe, D. G. Higgins, D. C. Shieds, K. H. Wolfe, and F. Wright.** 1988. Codon usage patterns in *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila melanogaster*, and *Homo sapiens*; a review of the considerable within-species diversity. Nucleic Acids Res. **16:**8207–8211.
52. **Simon, D., and A. Chopin.** 1988. Construction of a vector plasmid family for molecular cloning in *Streptococcus lactis*. Biochimie **70:**559–566.
53. **Skala, J., E. Capieaux, E. Balzi, W. Chen, and A. Goffeau.** 1991. Complete sequence of the *Saccharomyces cerevisiae leu1* gene encoding isopropylmalate isomerase. Yeast **7:**281–285.

54. **Somers, J. M., A. Amzallag, and R. B. Middleton.** 1973. Genetic fine structure of the leucine operon of *Escherichia coli* K-12. J. Bacteriol. **113**:1268–1272.
55. **Squires, C. H., M. DeFelice, J. Devereux, and J. M. Calvo.** 1983. Molecular structure of *ilvIH* and its evolutionary relationship to *ilvG* in *Escherichia coli*. Nucleic Acids Res. **11**:5299–5313.
56. **Squires, C. H., M. DeFelice, S. R. Wessler, and J. M. Calvo.** 1981. Physical characterization of the *ilvHI* operon of *Escherichia coli* K-12. J. Bacteriol. **147**:797–804.
57. **Tanaka, T.** 1979. Restriction of plasmid-mediated transformation in *Bacillus subtilis* 168. Mol. Gen. Genet. **175**:235–237.
58. **Thomas, T. D., and G. G. Prichard.** 1987. Proteolytic enzymes of dairy starter cultures. FEMS Microbiol. Rev. **46**:245–268.
59. **Umbarger, H. E.** 1983. The biosynthesis of isoleucine and valine and its regulation, p. 245–266. *In* K. M. Herrman and R. L. Somerville (ed.), Amino acids, biosynthesis and genetic regulation. Addison-Wesley, Reading, Mass.
60. **Umbarger, H. E.** 1987. Biosynthesis of the branched-chain amino acids, p. 353–367. *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. 2. American Society for Microbiology, Washington, D.C.
61. **Van de Guchte, M., J. Kok, and G. Venema.** 1992. Gene expression in *Lactococcus lactis*. FEMS Microbiol. Rev. **88**:73–92.
62. **Vandeyar, M. A., C. J. Mackey, R. H. Lipsky, and S. A. Zahler.** 1986. The *ilvBC-leu* operon of *Bacillus subtilis*, p. 295–305. *In* A. T. Ganesan and J. A. Hoch (ed.), Bacillus molecular genetics and biotechnology applications. Academic Press, Inc., New York.
63. **Ward, J. B., and S. A. Zahler.** 1973. Genetic studies of leucine biosynthesis in *Bacillus subtilis*. J. Bacteriol. **116**:719–726.
64. **Wek, R. C., and G. W. Hatfield.** 1986. Nucleotide sequence and in vivo expression of *ilvY* and *ilvC* genes in *Escherichia coli* K12. J. Biochem. Chem. **261**:2441–2450.
65. **Wek, R. C., C. A. Hauser, and G. W. Hatfield.** 1985. The nucleotide sequence of the *ilvBN* operon of *Escherichia coli*: sequence homologies of the acetohydroxy acid synthase isozymes. Nucleic Acids Res. **13**:3995–4010.
66. **Wessler, S. R., and J. M. Calvo.** 1981. Control of *leu* operon expression in *Escherichia coli* by transcription attenuation mechanism. J. Mol. Biol. **149**:579–597.
67. **Wilbur, W. J., and D. Lipman.** 1983. Rapid similarity searches of nucleic acid and protein data banks. Proc. Natl. Acad. Sci. USA **80**:726–730.
68. **Zahler, S. A.** Personal communication.