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Cloning and Nucleotide Sequence of the Leucyl-tRNA Synthetase Gene of *Bacillus subtilis*

PETER B. VANDER HORN† AND STANLEY A. ZAHLER*

Section of Genetics and Development, Cornell University, Ithaca, New York 14853

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The leucyl-tRNA synthetase gene (*leuS*) of *Bacillus subtilis* was cloned and sequenced. A mutation in the gene, *leuS1*, increases the transcription and expression of the *ilv-leu* operon, permitting monitoring of *leuS* alleles. The *leuS1* mutation was mapped to 270° on the chromosome. Sequence analysis showed that the mutation is a single-base substitution, possibly in a monocistronic operon. The leader mRNA predicted by the sequence would contain a number of possible secondary structures and a T box, a sequence observed upstream of leader mRNA terminators of *Bacillus* tRNA synthetases and the *B. subtilis ilv-leu* operon. The DNA of the *B. subtilis leuS* open reading frame is 48% identical to the *leuS* gene of *Escherichia coli* and is predicted to encode a polypeptide with 46% identity to the leucyl-tRNA synthetase of *E. coli*.

The critical process of accurate charging of tRNAs by aminoacyl-tRNA synthetases presents a unique opportunity to study protein-nucleotide interaction. All of the tRNA synthetases of *Escherichia coli* have been cloned and sequenced (9). Several tRNA synthetases have been crystallized, some bound to their substrates (3, 4, 25). The enzymes have been divided into two types (4, 8, 27) based upon sequence and structural homologies. Type I enzymes include those for arginine, cysteine, glutamine, glutamic acid, isoleucine, leucine, methionine, tryptophan, tyrosine, and valine. These enzymes all contain several conserved amino acid sequences, including HIGH and KMSKS (part of the ATP-binding Rossman fold). Type II enzymes include those for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine. These enzymes lack the HIGH and KMSKS motifs but share (to a degree) three other motifs (8).

The *E. coli* leucyl-tRNA synthetase belongs to a subgroup of type I synthetases known as the XUX family (for the codons recognized by these enzymes). This group includes the leucine, isoleucine, valine, and methionine enzymes (27). Sequence and structural homologies suggest that these enzymes evolved from a common ancestral enzyme, an early divergence from the other type I aminoacyl-tRNA synthetases.

We cloned the *leuS* gene as part of a study of mutations that result in increased expression of the *ilv-leu* operon of *Bacillus subtilis*. The *ilv-leu* operon contains, in order, the genes *ilvB*, *ilvN*, *ilvC*, *leuA*, *leuC*, *leuB*, and *leuD*. The operon is transcriptionally regulated by the amino acid leucine (11, 31, 32). Strains carrying a transposon insertion in the *ilvN* gene (*ilvN*::Tn*917*) cannot grow in the presence of high concentrations of leucine. This sensitivity is overcome by the addition of isoleucine and valine. Presumably, the decreased transcription of the operon caused by the addition of leucine, combined with the decreased activity of the IlvN-inactivated IlvBN enzyme (acetohydroxyacid synthase), causes the cells to starve for valine and isoleucine. The *leuS1* mutation was isolated as a spontaneous mutation that resulted in an *ilvN*::Tn*917*-containing strain becoming leucine resistant (Leu^r). The measurements of Vandeyar et al. (31, 32) of β-galactosidase expression from an *ilvN*::*lacZ* fusion showed that *leuS1* increases transcription of the *ilv-leu* operon. (Note that the *leuS* gene was called *ilvX* in reference 31.) The β-galactosidase expression is high enough for *ilvN*::*lacZ leuS1* mutants to appear blue on complex media plus 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). *ilvN*::*lacZ leuS*$^+$ strains are colorless.

MATERIALS AND METHODS

Bacterial strains, plasmids, bacteriophages. The bacterial strains, plasmids, and bacteriophages used in this study are described in Table 1.

Media. Bacteria were grown on tryptose blood agar base (TBAB; Difco) solid medium or in Luria-Bertani (LB) broth (17). M13 was propagated in 2YT medium (2). Overlays contained LB and 0.75% agar. Additives for *E. coli* selections included 35 μg of ampicillin ml^{-1}. Additives for *B. subtilis* selections included 1 μg of erythromycin ml^{-1} and 25 μg of lincomycin ml^{-1} (Mls^r), 5 μg of chloramphenicol ml^{-1} (Cam^r), or 20 μg of 4-azaleucine ml^{-1} (Azl^r). *E. coli* transfections with M13mp19 and its clone-containing derivatives were plated with overlays onto defined minimal-glucose agar medium supplemented with 12 μg of thiamine ml^{-1}, 40 μg of X-Gal ml^{-1}, and 4 μM isopropyl-thiogalactoside.

DNA isolations. Plasmids were isolated on a small scale by the Holmes and Quigley boiling method (15). Isolation of plasmids on a large scale and double-stranded bacteriophage M13 DNAs was as described previously (2). DNAs from bacteriophage lambda and its derivatives were isolated by the method of Davis et al. (6). Single-stranded M13 clone DNA was isolated as described previously (17).

Bacterial transformations. Competent cells of *E. coli* were prepared by the method of Hanahan (12). *B. subtilis* was transformed by the procedure of Yasbin et al. (34).

Transposon tagging of *leuS*. As stated above, the *leuS1* allele can be monitored by plating an *ilvN*::*lacZ* transcriptional fusion-containing strain of *B. subtilis* on TBAB containing X-Gal. After incubation at 37°C for 24 h, *leuS*$^+$ strains are colorless and *leuS1* strains are blue.

Transposon Tn*917*[60] is the chr::Tn*917-lac*::pTV21Δ2 construct described by Youngman (35). The transposon

* Corresponding author.
† Present address: Department of Chemistry, Cornell University, Ithaca, NY 14853.

TABLE 1. Bacterial strains, plasmids, and bacteriophages

Strain, plasmid, or phage	Genotype	Source or reference
B. subtilis		
CU1065	*trpC2*	36
CU3985[a]	*leuS*$^+$ *ilvN*::Tn*917*[32] *trpC2*	This work
CU3986[a]	*leuS1 ilvN*::Tn*917*[32] *trpC2*	This work
CU3989[b]	*leuS*$^+$ *ilvN*::Tn*917*[60] *trpC2*	This work
CU3990[b]	*leuS1 ilvN*::Tn*917*[60] *trpC2*	This work
CU3992[b]	*ilvN*::*Tn917*[60] *ald-1 bioB141*	This work
CU4421[b,c]	*leuS*$^+$ *ilvN*::Tn*917*[60] *trpC2* Tn*917*[J]	This work
CU4422[b,c]	*leuS1 ilvN*::Tn*917*[60] *trpC2* Tn*917*[J]	This work
CU4435[c]	*leuS*$^+$ *ilvBΔ1 trpC2* Tn*917*[J]	This work
CU4436[c]	*leuS1 ilvBΔ1 trpC2* Tn*917*[J]	This work
CU4437[c,d]	*leuS*$^+$ *ilvBΔ1 trpC2* Tn*917*[J, 21Δ2]	This work
CU4438[c,d]	*leuS1 ilvBΔ1 trpC2* Tn*917*[J, 21Δ2]	This work
CU4444[b]	*ald-1 ilvN*::Tn*917*[60] *thrB5 trpC2*	This work
CU4643[a]	*leuS*$^+$ *ilvN*::Tn*917*[32]	This work
CU4631	*aroG1 thrB5 trpC2*	This laboratory
CU4644[a]	*leuS1 ilvN*::Tn*917*[32]	This work
E. coli		
JM101	*hsdR*$^-$*M*$^+$ *recA1* Δ(*lac-proAB*) *thi gyrA96 relA1* F′ (*proAB lacQZΔM15*)	J. Messing
JM2R$^-$	*mcrA*$^-$*B*$^-$ *hsdR*$^-$*M*$^+$ *recA1* Δ(*lac-proAB*) *thi gyrA96 relA1 srl*::Tn*10* F′ (*proAB lacQZΔM15*)	M. Weiner
Plasmids		
pTV1		35
pTV21Δ2		35
pVH401		This work
pVH402		This work
pVH403		This work
pVH404		This work
Bacteriophages		
λ EMBL4		10
λ-*leuS*$^+$		This work
λ-*leuS1*		This work
M13mp19-401.EH		This work
M13mp18-401.EH		This work
M13mp19-402.EH		This work
M13mp19-401.ES		This work
M13mp19-λ-*leuS*.A1		This work
M13mp19-λ-*leuS1*.G3		This work

[a] Tn*917*[32] is Mls^r and *lacZ*. This is the recombinant transposon present on plasmid pTV32Ts (35).
[b] Tn*917*[60] is Cam^r *lacZ* and contains a pBR322-derived replicon and *bla* gene that are active in *E. coli*. This transposon is the chr::Tn*917*-lac::pTV21Δ2 construct described previously (35).
[c] Tn*917*[J] is tightly linked to *leuS* on the downstream side.
[d] Tn*917*[21Δ2] is Cam^r and contains a pBR322-derived replicon and *bla* gene that are active in *E. coli*. This is the recombinant transposon present on plasmid pTV21Δ2 (35).

carries the *E. coli lacZ* gene, a *cat* gene, and portions of plasmid pBR322 that include an origin of replication for *E. coli* and the *bla* gene. We constructed the useful strain *B. subtilis* CU3990 (*leuS1 ilvN*::Tn*917*[60]). This strain is blue on TBAB containing X-Gal.

A PBS1 transducing lysate that contained random Tn*917* insertions in the *B. subtilis* chromosome was prepared as described previously (33). This lysate was used to transduce strain CU3990 to Mls^r on TBAB containing X-Gal, erythromycin, and lincomycin. Three events might lead to colorless colonies in this cross. (i) The *ilvN*::Tn*917*[60] could be replaced by the wild-type Tn*917* by intratransposon recombination. (ii) A transposon that is cotransduced with *ilvN* could replace the entire *ilv-leu* region of the recipient chromosome. This would replace the *ilvN*::*lacZ* fusion with wild-type *ilvN* linked to a transposon. Either of these events (i or ii) would result in cells that are Cam^s because of the loss of Tn*917*[60]. (iii) A transposon that is cotransduced with *leuS*$^+$ could replace the *leuS1* gene with *leuS*$^+$ linked to the transposon, resulting in cells that are still Cam^r.

Cloning of the DNA from the *leuS* region. The wild-type transposon was replaced with Tn*917*[21Δ2] (from plasmid pTV21Δ2) and junction fragments were cloned as described previously (35). Tn*917*[21Δ2] carries a *cat* gene and parts of plasmid pBR322 that encode ampicillin resistance and the ability to replicate in *E. coli*. *Eco*RI or *Hin*dIII restriction digests of *B. subtilis* DNA containing the recombinant transposon and either the *leuS*$^+$ or *leuS1* allele were ligated under dilute conditions (<200 ng of DNA ml^{-1}). The ligation mixture was transformed into *E. coli* strain JM2R$^-$ with selection for resistance to ampicillin. Four plasmids were recovered. Plasmid pVH401 was isolated from an *Eco*RI digest of *leuS*$^+$-containing DNA; pVH402 was isolated from an *Eco*RI digest of *leuS1*-containing DNA. Plasmid pVH403 was isolated from a *Hin*dIII digest of *leuS*$^+$-containing DNA; pVH404 was isolated from a *Hin*dIII digest of *leuS1*-containing DNA. λ-*leuS*$^+$ and λ-*leuS1* were isolated by probing bacteriophage lambda (EMBL4) libraries of *B. subtilis* chromosomal DNA with radiolabeled pVH401 as described previously (17).

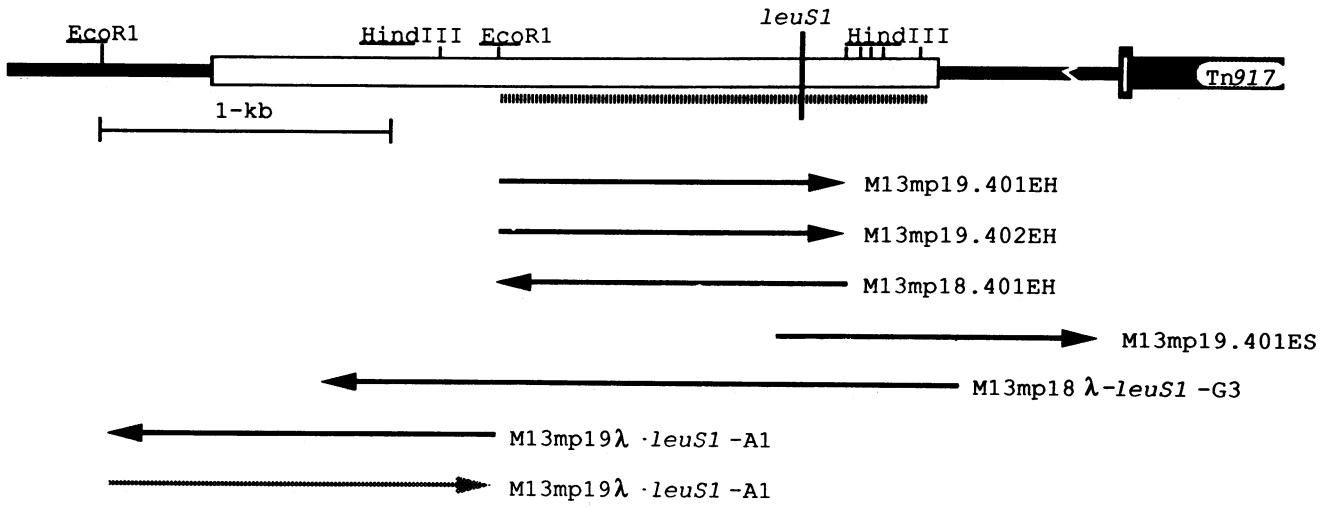

FIG. 1. Nucleotide sequencing strategy used for the *leuS* and *leuS1* genes. The top bar represents DNA of the *leuS* region of *B. subtilis*. The white, boxed portion is the putative open reading frame of the *leuS* gene, which is transcribed from left to right. The hatched bar indicates the *Eco*RI-*Hin*dIII restriction fragment (which contains more than one *Hin*dIII site) to which the *leuS1* mutation has been localized genetically. The arrows indicate the regions sequenced from the various indicated templates. The dotted arrow indicates the orientation that was sequenced by double-strand methodology.

Isolation and analysis of M13 subclones. Various fragments from pVH401, pVH402 or λ-*leuS* were ligated to replicative-form DNA of M13mp18 and M13mp19 (19) (Fig. 1). Ligation mixtures were transfected into *E. coli* JM101. Then 5 μl of M13 loading buffer (1% sodium dodecyl sulfate, 0.1% bromophenol blue, 50% glycerol, 250 mM EDTA) was added to 20 μl of a bacteriophage lysate prepared from colorless plaques. Dot blots of the mixtures were analyzed by hybridization to various radiolabeled DNA fragments. Hybridization was carried out as described previously (17), except that two types of probes were used as needed. First, double-stranded DNA probes were used to identify the presence of particular cloned fragments. These probes were radiolabeled by using the Prime-A-Gene (Promega) labeling system. Second, sequencing reactions were used as single-stranded DNA probes to identify the orientation of inserts.

The *Eco*RI fragment present in M13mp19-λ-*leuS1*.A1 resisted cloning in the opposite orientation. The missing strand was sequenced by double-strand sequencing of the replicative form of M13mp19-λ-*leuS1*.A1.

DNA sequencing. DNA sequencing of all templates was performed by the method of Sanger et al. (26). [α-^{32}P]dATP (specific activity, 3,000 Ci nmol^{-1}) was purchased from Amersham. All sequencing reactions were carried out with a Sequenase kit as specified by the manufacturer (U.S. Biochemical Corp.). Sequencing was carried out with custom-made primers synthesized by the Analytical and Synthetic Facility of Cornell University. Both strands of DNA were sequenced.

Enzymes and reagents. The T4 DNA ligase and all restriction endonucleases used in this study were purchased from and used under the conditions specified by Amersham.

Computer analysis. The DNA was initially analyzed with DNA Inspector II. The sequence was further analyzed with software from the Genetics Computer Group of the University of Wisconsin. The sequence was compared with se-

TABLE 2. PBS1 transducing data[a]

Donor	Recipient	Selection	Recombinant class	No.	Order of genes
CU3990 (*leuS1*)	CU3992 (*bioB141 ald-1*)	Bio$^+$	BioB$^+$ Ald$^+$ LeuS$^+$	(1)	*bioB, leuS, ald*
			BioB$^+$ Ald$^+$ LeuS1	(6)	
			BioB$^+$ Ald$^-$ LeuS$^+$	(5)	
			BioB$^+$ Ald$^-$ LeuS1	(25)	
CU4421 (Tn*917*[J])	CU4631 (*thrB5 aroG1*)	Mls^r	Mls^r Aro$^+$ Thr$^+$	(0)	*aroG*, Tn*917*[J], *thrB*
			Mls^r Aro$^+$ Thr$^-$	(36)	
			Mls^r Aro$^-$ Thr$^+$	(1)	
			Mls^r Aro$^-$ Thr$^-$	(15)	
CU4422 (*leuS1* Tn*917*[J])	CU4444 (*thrB5 leuS$^+$*)	Thr$^+$	ThrB$^+$ Mls^r LeuS1	(19)	*leuS*, Tn*917*[J], *thrB*
			ThrB$^+$ Mls^r LeuS$^+$	(3)	
			ThrB$^+$ Mls^s LeuS1	(0)	
			ThrB$^+$ Mls^s LeuS$^+$	(446)	

[a] All of the transductions involve three-point crosses. In the first and last crosses, the donors and recipients carried the indicator *ilvN*::Tn*917*[60]. In the second cross, Tn*917*[J] is located between *aroG* and *thrB*. The auxotrophic markers of this cross are unlinked to each other. The linkage data of the these experiments agree with previously published genetic maps (24).

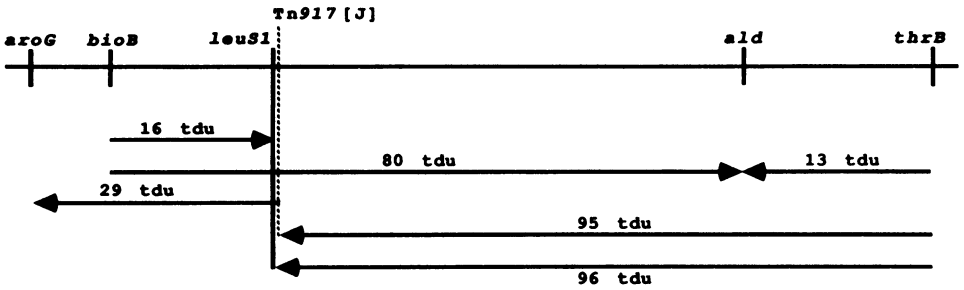

FIG. 2. Summary of the genetic mapping data of Table 2.

quences in the data bases of GenBank and EMBL by using FASTA, a program derived from Pearson and Lipman (23). The LeuS polypeptides of *B. subtilis* and *E. coli* were compared by using the program GAP (22) with a gap weight of 3.000 and a length weight of 0.100.

Nucleotide sequence accession number. The GenBank nucleotide sequence accession number for the *B. subtilis leuS* gene is M88581.

RESULTS

***leuS1* phenotype.** Cells containing *ilvN*::Tn*917* are sensitive to high concentrations of leucine (33). A mutant with a spontaneous suppressor mutation, *leuS1*, was isolated. When the mutation was introduced into an *ilvN*::Tn*917*::*lacZ* background, the mutant was blue on TBAB containing X-Gal, whereas *leuS*$^+$ colonies are colorless. Furthermore, *leuS1 ilv-leu*$^+$ cells are resistant to the leucine analog 4-azaleucine. We conclude that the *leuS1* mutation results in increased transcription of the *ilv-leu* operon. We found that *leuS1* mutants also grew more slowly than *leuS*$^+$ cells in broth and that the colony sizes of *leuS1* mutants were smaller on all agar media. Auxotrophies of the various *ilv-leu* genes did not suppress this slow-growing phenotype.

Transposon tagging of *leuS*. A PBS1 transducing lysate that contained random Tn*917* insertions in the *B. subtilis* chromosome was used to transduce CU3990 (*leuS1 ilvN*::Tn*917*[60]) with selection for Mls^r on TBAB containing X-Gal. DNA was isolated from colorless Mls^r Cam^r transductants and used to transform CU3990 to Mls^r on TBAB containing X-Gal. One of these DNA preparations cotransformed Tn*917* and *leuS*$^+$ with high frequency. The *leuS*$^+$ strain carrying this insertion was designated CU4421; a *leuS1* version was labeled CU4422. CU4422 DNA cotransformed Tn*917* and *leuS1* 67% of the time. The transposon insertion of strains CU4421 and CU4422, named Tn*917*[J], was chosen for further work.

Genetic mapping of *leuS* and Tn*917*[J]. The *leuS1* mutation and the transposon were mapped by PBS1 generalized transduction to the *bioB* region (270°) of the *B. subtilis* chromosome (Table 2). The order of the genes examined is *aroG*, *bioB*, *leuS*, Tn*917*[J], *ald*, and *thrB*. A summary of the genetic mapping data is shown in Fig. 2.

Cloning of *leuS*$^+$ and *leuS1*. Spontaneous Leu^r mutants occur at too high a frequency for the identification of a *leuS1* clone by direct selection in *B. subtilis*. For this reason, congression was used to test possible clones for the *leuS1* mutation. Congression is the cotransformation of unlinked markers. Nonselectable homologous DNA can be introduced into *B. subtilis* by mixing it with selectable DNA (5). The selectable marker allows selection for the competent cells. Some of the competent cells also take up the nonselected DNA and incorporate it into their chromosomes, resulting in allele exchange.

Cloning began with the standard Tn*917*[21Δ2] methodologies described in Materials and Methods and developed by

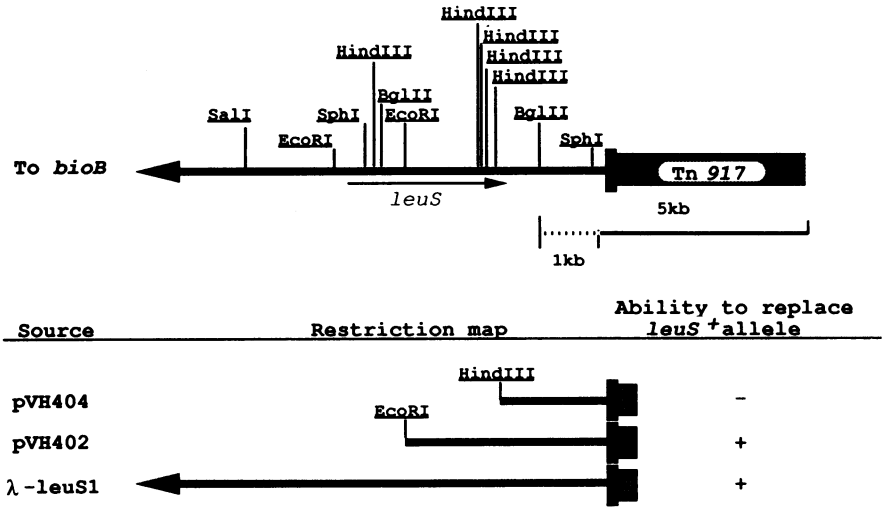

FIG. 3. Endpoint map of DNAs isolated from the *leuS1* region. Clones were tested for their ability to replace the *leuS*$^+$ allele in congression experiments.

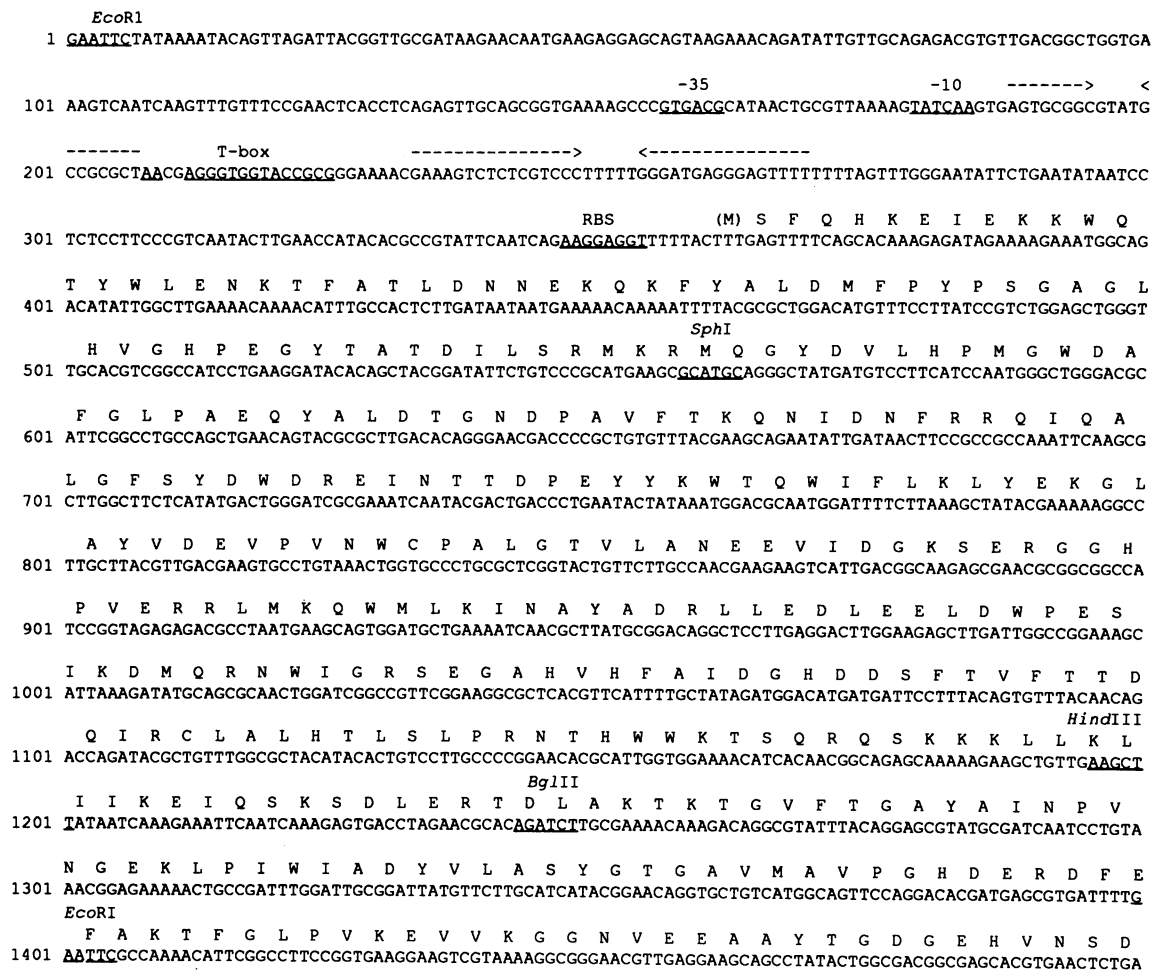

FIG. 4. Nucleotide sequence of the noncoding strand of DNA from the *leuS* region of *B. subtilis*. A possible promoter consensus sequence, the T-box, the ribosomal binding site (RBS), and various restriction endonuclease sites are underlined. Inverted repeats within the mRNA leader and the presumed transcriptional terminator have dotted-line arrows above them. Within the coding region the *leuS1* mutation, codon and amino acid changes are indicated in boldface type under the wild-type sequence.

Youngman (35). Plasmid pVH401, which later proved to contain part of the *leuS*$^+$ gene, was used as a probe to isolate bacteriophage lambda clones from DNA libraries constructed from *B. subtilis leuS*$^+$ or *leuS1*-containing DNA. The analysis below describes how the clones were tested.

Endpoint analysis. Clones containing DNA from the *leuS1* region were tested for their ability to replace the *leuS*$^+$ allele in transformed cells (Fig. 3). One microgram of DNA to be tested for the presence of *leuS1* was mixed with 0.1 μg of chromosomal DNA isolated from CU3989 (*leuS*$^+$ *ilvN*::Tn*917*[60]). The mixture was then used to transform CU1065 (*leuS*$^+$ *ilvN*$^+$) with selection for Cam^r on TBAB containing X-Gal. All transformants carry an *ilvN*::*lacZ* transcriptional fusion. Blue transformants (*leuS1* congressants) occurred at a rate higher than 10^{-2} when λ-*leuS1* or pVH402 DNA was cotransformed with the selected DNA. Blue transformants were never observed (rate, $<10^{-4}$) when plasmid pVH404 was added to the selectable DNA. When experimental DNA was omitted or *leuS*$^+$-derived DNA clones were added to the transformation, blue colonies occurred at a rate lower than 10^{-5}. The results of this experiment placed the *leuS1* mutation between the endpoints of the chromosomal DNA in plasmids pVH402 and pVH404.

The reverse experiment (replacing the *leuS1* allele with *leuS*$^+$) was conducted and confirmed that *leuS*$^+$ is located between the restriction sites of plasmids pVH401 and pVH403. A *leuS1*-to-*leuS*$^+$ revertant was never seen in these experiments, indicating that the *leuS1* mutation reverts at a frequency lower than 10^{-5}.

Analysis of the *B. subtilis leuS* nucleotide sequence. The DNA sequence was determined for the *leuS1*-containing junction fragments (Fig. 4). This region was found to contain an open reading frame that extends from the *Eco*RI site of pVH401 through the *Hin*dIII site and toward the transposon. Comparison of the DNA sequences cloned from plasmids pVH401 (*leuS*$^+$) and pVH402 (*leuS1*) identified a single-base-pair difference. An A in the open reading frame (nucleotide 2084) derived from pVH401 DNA is a G in pVH402-derived DNA. This substitution results in the amino acid glutamate being replaced by a lysine residue in the mutant polypeptide.

Inspection of DNA sequences flanking this region revealed a possible ribosomal binding site (21, 29) immediately upstream of the 5′ end of the open reading frame. Use of UUG as a start codon has been observed a number of times in *B. subtilis* (28). On the 3′-flanking region we identified the

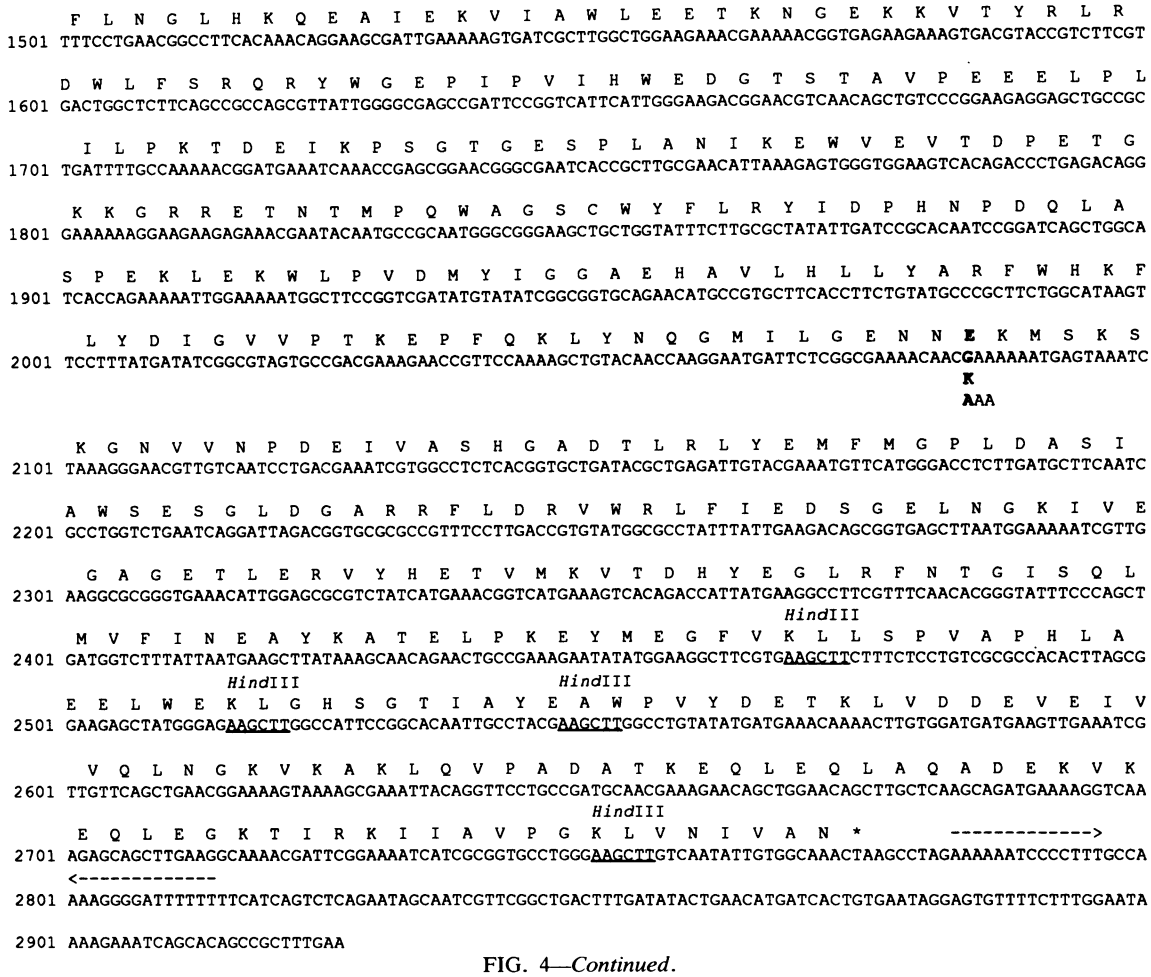

FIG. 4—*Continued*.

end of the open reading frame. A possible transcriptional terminator was identified shortly after the presumptive stop codon.

We identified a fairly good consensus sequence for a vegetative promoter 206 bases upstream of the start codon (21). The −35 region (beginning at base 156 in Fig. 4) is a four-of-six match with the consensus sequence (TTGACA). A 17-bp spacer is followed by a −10 region that matches four of six bases with the consensus sequence (TATAAT). Two inverted repeats were identified between the possible promoter and the predicted start of the open reading frame. The second repeat would be expected to function as a transcriptional terminator. Just upstream of this presumptive terminator we identified a T box. A T box is a sequence found upstream of leader-mRNA terminators of *Bacillus* tRNA synthetase genes and of the *B. subtilis ilv-leu* operon (14). The role of the T box has yet to be identified.

The DNA sequence of the open reading frame was compared with sequences in the GenBank and EMBL data bases by using the program FASTA (23). The *B. subtilis* DNA had the highest degree of identity with the *E. coli leuS* gene (initial [initn] score, 1,258; optimal score, 1,269). The nucleotide identity, 48%, suggests that the open reading frame encodes the leucyl-tRNA synthetase of *B. subtilis*. We named the gene *leuS*.

The second, third, and fourth most homologous DNAs also encoded leucyl-tRNA synthetases. They are from *Saccharomyces douglassi* (initn score, 785), *Neurospora crassa* (initn score, 685), and *Saccharomyces cerevisiae* (initn score, 659), respectively. The fifth most homologous sequence belonged to the valyl-tRNA synthetase (initn score, 202) gene of *Bacillus stearothermophilus*.

Predicted LeuS protein. The polypeptide predicted from the open reading frame is 804 amino acids long and has a molecular size of 92 kDa. The *B. subtilis* peptide was compared with the *E. coli* peptide by using the program GAP (22) (Fig. 5). The proteins show 46% amino acid identity.

The *leuS1* mutation results in glutamate residue 575 of the putative wild-type gene product being replaced with a lysine in the mutant gene product. The mutation is located immediately adjacent to the evolutionarily conserved peptide sequence KMSKS.

DISCUSSION

The entire *leuS* gene from λ-*leuS*$^+$ and λ-*leuS1* resisted several attempts at subcloning. The gene had to be cut into pieces to be sequenced. The upstream *Eco*RI fragment (nucleotides 1 to 1403) could be subcloned only in one orientation in M13mp19 (M13mp19-λ-*leuS*.A1). *Eco*RI digestion and religation of the replicative form of M13mp19-λ-*leuS*.A1 always gave rise to the same orientation. The lack of

FIG. 5. The *B. subtilis leuS* polypeptide sequence (top line) and the *E. coli leuS* polypeptide sequence (bottom line) were compared by the method of Needleman and Wunsh (22). Identical amino acids are joined by vertical lines. The amino acid sequences are 45.7% identical with 13 gaps. *B. subtilis* amino acid 575 (boldface type) is glutamate (E) in *leuS*$^+$ and lysine (K) in *leuS1*. The HIGH box starts with amino acid 48, and KMSKS starts with amino acid 576 (immediately after the gap after the *leuS1* mutation).

an intact gene made demonstration of leucyl-tRNA synthetase activity, either enzymically or by complementation, very difficult. Although we think that these barriers can be overcome, the evidence is strong that we have cloned the *leuS* gene of *B. subtilis*.

The best evidence is the striking homology between the *B. subtilis leuS* and the leucyl-tRNA synthetases of other species. There is also cross-regulation of the *ilv-leu* operon, the signature T box, and the fact that *leuS1* mutants grow slowly. Slow growth cannot be attributed to overexpression of the *ilv-leu* operon, since it was observed in several Ilv$^-$ and Leu$^-$ auxotrophs. Before the homology with the *E. coli* leucyl-tRNA synthetase was identified, we made several attempts to inactivate the gene by allele replacement with a Tn*5*::*cat*-mutagenized plasmid clone (data not shown). The failure of these experiments suggests that *leuS* is essential and is present in a single copy.

The *B. subtilis* leucyl-tRNA synthetase contains all of the consensus sequences relevant to known structural features and functional domains present in XUX and other type I tRNA synthetases (3, 18, 25). A large gap in the *B. subtilis* enzyme sequence (or an insertion in the *E. coli* enzyme sequence) has an endpoint immediately upstream of the KMSKS box, which is part of the nucleotide binding site. The *leuS1* mutation itself alters the residue just upstream of this conserved motif.

It is not surprising that a mutation in the *leuS* gene affects expression of the *ilv-leu* operon. The biosynthetic operon may be regulated by transcriptional attenuation (11). Analogous mutations affecting amino acid biosynthetic operons of *E. coli* were instrumental in learning the mechanism of attenuation (for a review, see reference 16).

Alternatively, the *leuS* gene may influence the *ilv-leu* operon by molecular mimicry. According to this model of regulation, first suggested by Ames et al. (1), synthetases and tRNA-modifying proteins bind to mRNA sequences that are homologous to their tRNA substrates. For example, the *thrS* operon of *E. coli* is autoregulated at the level of translation by the binding of threonyl-tRNA synthetase to its own mRNA (20) at two sites. At least one of these sites is believed to overlap the *thrS* ribosomal binding site and to prevent translational initiation. Scanning of the *leuS* and *ilv-leu* operon leaders for $tRNA^{Leu}$ sequences (data not shown) suggests that both contain regions homologous to the $tRNA^{Leu}$ D loop. Such homology was identified twice in the *ilv-leu* operon leader (30) and twice in the *leuS* mRNA.

In *leuS*, this method of regulation deserves special attention. No leader peptide sequence could be detected based upon the sequencing data. There are two regions of $tRNA^{Leu}$ homology. In the first, *leuS* nucleotides 223 through 241 (Fig. 4) share 13 of 19 identical bases with nucleotides 10 through 27 (the D loop) of leucyl-$tRNA^{CAG}$. This sequence appears in *leuS* at the upstream base and stem of the terminator in the leader mRNA, overlapping and between the T box and the terminator. The second site of homology is between nucleotides 390 and 402 of *leuS* and nucleotides 14 and 25 of leucyl-$tRNA^{CAG}$. Eleven of the 12 bases are identical between these sequences. Although the D loop of $tRNA^{Leu}$ is conserved in evolution and interacts tightly with leucyl-tRNA synthetase (7), more evidence is required before we can say that LeuS is a *trans*-acting regulatory element for the *leuS* and *ilv-leu* operons. That study should include an examination of the role of T boxes.

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