Molecular cloning and nucleotide sequence of the gene for *Escherichia coli* leucyl-tRNA synthetase

Michael Härtlein and Dominique Madern

European Molecular Biology Laboratory, 156X, 38042 Grenoble Cedex, France

Received September 29, 1987; Revised and Accepted November 24, 1987

Accession no. Y00869

ABSTRACT

The gene for *Escherichia coli* leucyl-tRNA synthetase leuS has been cloned by complementation of a leuS temperature sensitive mutant KL231 with an *E.coli* gene bank DNA. The resulting clones overexpress leucyl-tRNA synthetase (LeuRS) by a factor greater than 50. The DNA sequence of the complete coding regions was determined. The derived N-terminal protein sequence of LeuRS was confirmed by independent protein sequencing of the first 8 aminoacids. Sequence comparison of the LeuRS sequence with all aminoacyl-tRNA synthetase sequences available reveal a significant homology with the valyl-, isoleucyl- and methionyl-enzyme indicating that the genes of these enzymes could have derived from a common ancestor. Sequence comparison with the gene product of the yeast nuclear NAM2 -1 suppressor allele curing mitochondrial RNA maturation deficiency reveals about 30 % homology.

INTRODUCTION

The XUX family of aminoacyl-tRNA synthetases containing the leucine, isoleucine, valine, methionine and phenylalanine enzyme is a particularly interesting group of enzymes which share many common features like structural similarity in the aminoacids these enzymes bind and activate, 2' charging specificity (1), adenosine as fourth base from the 3' end of their cognate tRNA in the so-called discriminator position (2), misacylation pattern and codon group XUX. [For details see ref. 3] Furthermore the aminoacyl-tRNA synthetases for the aliphatic aminoacids leucine, isoleucine and valine enzymes are all monomeric proteins of about 100,000 molecular weight (3). These common features were taken as evidence for the generation of XUX family through divergent evolution by gene duplication and mutations from a common ancestral synthetase (4).

Leucyl-tRNA synthetases have been purified from different organisms as well as from cell organelles and the properties of the enzymes are well documented (5,6,7). However no sequence and tertiary structure have been reported so far. Recently a biochemical comparison of the cytoplasmic and mitochondrial LeuRS of <u>Neurospora crassa</u> wild type and a temperature sensitive leucine auxotroph mutant has been reported (7).

Temperature sensitive mutants of *E.coli* have been described in which conditional growth is the result of temperature sensitive leucyl-tRNA synthetase (8). The corresponding genetic locus <u>leuS</u> has been mapped at position 15 min. on the *E.coli* chromosome. The existence of a regulatory

gene unlinked to the <u>leuS</u> locus at position 79 min. on the *E.coli* chromosome has been reported which leads to elevated levels of LeuRS in <u>leuS</u> ts-mutants (9). The molecular mechanism of this specific regulation is yet unknown.

In this paper we report the cloning, overproduction and sequencing of <u>leuS</u> as well as the relationship of its gene product to other proteins.

MATERIALS and METHODS.

Bacterial Strains.

The <u>leuS</u> temperature sensitive strain KL231 (F^{-} , leuS31, thyA6, rpsL120) was obtained from the *E.coli* Genetic Stock Center, Yale University, New Haven through Dr. B.Bachmann. Strain P678-54 (F^{-} , minA, minB, thr, leu, thi, ara, lacY, gal, malA, xyl, mtl, tonA, rpsL, supE) for minicell preparation was donated by Dr.W.Goebel. Strain JM101, M13 tg130 and M13 tg131 DNA (10) were obtained from Amersham.

Gene library

The gene bank DNA of E.coli K12 strain 1100 was a generous gift from A. Wittinghofer.

Enzymes and Chemicals.

Calf intestine alkaline phosphatase, *E.coli* DNA polymerase I (Klenow fragment), restriction enzymes, T4 DNA ligase, egg white lysozyme, DNase I, nuclease Bal31 and unfractionated *E.coli* tRNA were all from Boehringer. Reverse transcriptase was obtained from Appligene. L- $[U-^{14}C]$ leucine, biotinylated protein A and streptavidine peroxidase were obtained from Amersham. All other chemicals were of analytical quality.

Cloning of the <u>leuS</u> gene.

KL231 was transformed with the gene library DNA (11) using the method described by Hanahan (12). The transformation mixture was incubated at 44° C on LB ampicillin plates (50µg/ml). A small fraction of the mixture was incubated at 28°C to determine the total number of transformants.

Aminoacylation assay for leucyl-tRNA synthetase

Bacterial cells were lysed by incubating for 30 min in a buffer containing 60mM Tris-HCl (pH7.4), 2mM EDTA, 1mg/ml lysozyme. After addition of 0.04% sodium deoxycholate, 10mM MgCl₂ and 10 mg of DNase I the crude extract was centrifuged for 5 min. and the supernatant assayed. The reaction mixture contained 60mM Tris-HCl (pH7.5), 10mM MgCl₂, 0.5mM DTE, 2mM ATP, 1mg/ml unfractionated tRNA, 20 mM L-[U-¹⁴C] leucine (330 mCi/mmol) and enzyme fraction. Incubation was for 10 min at 37°C. The reaction mixture was put on a Whatman GF-A filter. Filters were washed dried and counted as previously described (22).

Analysis of plasmid-specific proteins by minicells.

The minicell producing strain P678-54 was transformed with plasmids of <u>leuS</u> positive clones. Minicells were purified as described by Lewy (13) and labelled with 15mCi [35 S] methionine in methionine assay medium for 45 min. Cells were centrifuged, lysed in electrophoresis sample



Figure 1: Restriction map of plasmid pLeuS1 and sequencing strategy for the leuS gene. The gene for ampicillin resistance on pBR322 is marked by an arrow. Thick lines represent the inserted chromosomal DNA.

Abbreviations for restriction enzymes are: B=BamHI, Bg=BgIII, C=ClaI, E=EcoRI, EV=EcoRV, H=HindIII, K=KpnI, P=PstI, S=SaII, Sm=SmaI. Restriction site ClaI(Δ C) of pBR322 has been lost by the insertion of the chromosomal fragment.

Sequencing strategy for the leuS gene:

Arrows represent length and direction of sequences obtained by the method of Sanger et al. (19). The large arrow indicates the coding region for leucyl-tRNA synthetase.

o indicates M13 clones containing restriction fragments of pLeuS1, x M13 clones generated by Bal31 (see under METHODS) and p regions obtained by specific-primer-directed sequencing (20,21), using pLeuS1.

buffer (14). TCA precipitable counts were collected on Whatman GF-A filters. Samples were electrophoresed on 12% SDS-polyacrylamide gels (14). $[^{14}C]$ labelled marker proteins (Amersham) were run in a separate lane. Gels were dried and autoradiographed.



Figure 2: SDS-PAGE analysis of minicell proteins.

Minicells were prepared as described in METHODS, proteins were separated on a 12% SDSpolyacrylamide gel. Molecular weight markers were [U-¹⁴C] methylated proteins from Amersham; 30K carbonic anhydrase, 69K bovine serum albumin, 92K phosphorylase b and 200K myosin. Lane a; marker proteins; lane b, minicells pBR322; lane c, minicells pLeuS1;

DNA Purification.

Plasmid DNA was extracted by the alkaline lysis procedure (15), M13 single strand DNA was prepared by phenol extraction (16).

DNA manipulations.

DNA manipulations were as described by Manniatis et al. (17).

Nucleotide Sequencing.

Fragments cloned into M13 tg130 and M13 tg131 were sequenced by the chain terminator method (18). The strategy for sequencing was based on the generation of overlapping fragments by directional cloning and by the usage of Bal31 (19). Plasmid DNA (pLeuS1) was linearized by SmaI, digested with Bal31 for various periods of time, and treated with EcoRI to create a set of fragments with one blunt end and one cohesive end (EcoRI). For sequencing, these fragments were cloned into M13 tg131 cut with SmaI (blunt) and EcoRI. In a similar manner fragments were generated starting with a BgIII digest followed by a Bal31 kinetic and a HindIII digest. Additionally leuS containing restriction enzyme fragments of pLeuS1 (HindIII/SmaI, SmaI/ BgIII,



Figure 3: Expression of LeuRS in normal cells and identification of the enzyme by immunoblotting.

CSH26 (lane b) and CSH26 pLeuS1 (lane a) were grown to stationary phase, cells were lysed in Laemmli buffer (15) and proteins analysed on a 12% SDS-polyacrylamide gel. Additionally separated proteins of CSH26 (lane e) and CSH26 pLeuS1 (lane d) were electrophoretically transfered to a nitrocellulose filter and treated with a 1:200 dilution of rabbit antiserum raised against purified LeuRS. The binding of antibodies was detected by the biotine-streptavidineperoxidase system (24). MW standards (lane c): 21.5K, soybean trypsin inhibitor, 31K, carbonic anhydrase; 45K, ovalbumin; 66.2K, bovine serum albumin; 92.5K phosphorylase b.

PstI/HindIII, BgIII/PstI, KpnI/EcoRI, EcoRV) were cloned into M13 tg130 and M13 tg131. Finally several <u>leuS</u> regions were sequenced by specific-primer directed DNA sequencing (20,21) using pLeuS1 DNA.

Isolation of leucyl-tRNA synthetase.

The enzyme was purified as previously described for the E. coli seryl-tRNA synthetase (22).

Determination of polypeptide N-terminal sequences.

The N-terminal sequence of the expressed protein was determined by Beatrice Cortolezzis by manual Edman degradation and identification of the first 8 amino acids by HPLC.

RESULTS AND DISCUSSION

Cloning of leuS

A gene library from E.coli (strain 1100) was used, which contained chromosomal fragments of about 5kb generated by partial digestion with Sau3A and HpaII cloned into pBR322 ClaI and BamHI sites (11). The temperature sensitive mutant for <u>leuS</u> (strain KL231) was transformed with the library DNA and grown at 44°C on LB ampicillin plates. Clones which grew at KL231 non-

1 aagetttettaat

15 caccogrataaagaogtgtcagattettettetttcaggetetetteatageteatggggaactettecaggtcaogtetgacagetoggtcagetcategacetoggttcagettaget 13 caccogttettaatagogetggoggetgttcagetggtggaatatcagttegetettggtcagetggeggaaccagtteacggtaatattgggcaactgg 253 ttoatogtteactecogggtogtaactgaetaataatagagetettgtteettggttegtgaaaaccagtcaagetectgaaattagtgageacatg 272 caagtgagtgggggaaaggetgtttgaeenggaagttggtggtatggaagaetggaeacagetcaattgaeggaatggaacagggaecatggetagaacgggaecatgg 273 toatogttagetgggggaaaggetgtttgaeenggaagttggtggtatggaaaccagtcaattgaecaccagetaattggaecggtatgaaaagggaecatggetgaeacagg 274 caagtgagtggggaaaggetgtttgaeenggaagttggtggtatggaaaaccactcaaagetacatttgtageeggattgaaaacgggaecatggetggaecatggetgge

30 60 581 AGC AAA GAG AAG TAT TAC TOC CTG TCT ATG CTT CCC TAT CCT TCT GGT CGA CTA CAC ATG GGC CAC GTA CGT AAC TAC ACC ATC GGT GAC V T A R Y O H M L G K N V L O P I G W D A F G L P A E G A A 90 671 GTG ATC GCC COC TAC CAG CAT ATG CTG GGC AAA AAC GTC CTG CAG COC ATC GGC TGG GAC GOC TTT GGT CTG CCT GCG GAA GGC GOG GCG 120 D Y К Q G А Ρ т Y N I А м N L L G 761 GTG AAA AAC AAC ACC CCT CCG GCA CCG TCG ACG TAC GAC AAC ATC CCG TAT ATG AAA AAC CAG CTC AAA ATG CTG GGC TTT GGT TAT GAC 150 Р R TOG AGE CAE CHE GEA ACE TET AGE CEG GAA TAE TAE OFT TEE GAA CAE AAA TTE TTE ACE GAE CTE TAT AAA AAA GEE CTE GTA TAT 180 Α Ν C Ρ N D 0 Т v L А N E 0 D 941 AAG AAG AAC TICT GOD GTC AAC TIGE TOO COG AAC GAC CAG ACC GTA CTG GOG AAC GAA CAA GTT ATC GAC GOC TOO TIGE TOG COC TOO GAT 210 P E Ρ 0 F I к Ι Ν А Y А n N 1031 ÂCC ÀRA CIT GAR COT ARA GAG ÂTE COS CAG TOG TIT ÂTE ARA ÂTE ART CAT TAC COT GAC GAG ETG CTE ARE GAT ETG GAT ARA ETG GAT 240 n T K M 0 R N I G R E E N 1121 CAC TGG CCA GAC ACC GTT AAA ACC ATG CAG CGT AAC TGG ATC GGT CGT TCC GAA GGC GTG GAG ATC ACC TTC AAC GTT AAC GAC TAT GAC 270 м G R G 1211 AAC ÂGE ETE ÂCE ETT TAC ACT ÂCE CAC CCE GAC ACC TIT ATE GET TET ÂCE TAC ETE GOS ETA CET GOS GET CAT CCE ETE GOS CAG AAA 300 N Р E L A A F T D E. C R N т к v А E А Е м 1301 GOS GOS GAA AAT AAT CCT GAA CTG GOS GOC TTT ATT GAC GAA TGC CGT AAC ACC AAA GTT GOC GAA ACT GAA ATG GOG ACG ATG GAG AAA C ĸ А v u D τ G E F Т С v A N 330 1391 AAA GOC CTC GAT ACT GOC TIT AAA GOG GTT CAC CCA TTA ACG GOC GAA GAA ATT CCC GTT TGG GCA GCA AAC TTC GTA TTG ATG GAG TAC A D C D 0 R D v E A s 360 1481 GOC ACC GOC GCA GTT ATG GCG GTA COG GGG CAC GAC CAG COC GAC TAC GAG TTT GCC TCT AAA TAC GGC CTG AAC ATC AAA COG GTT ATC L A A D G S E P D L S Q Q A L T E K G V L F N S G E F N G L 1571 CTG GCA GCT GAC GCC TCT GAG CCA GAT CTT TCT CAG CAA GCC CTG ACT GAA AAA GGC GTG CTG TC AAC TCT GGC GAG TTC AAC GGT CTT 390 D м v 420 A к T. т A G G E R к Ν n 1661 GAC CAT GAA GOG SCC TTC AAC GOC ATC GOC GAT AAA CTG ACT GOC ATG GOC GTT GOC GAG CGT AAA GTG AAC TAC COC CTG COC GAC TOG D G v E м Р D D 450 480 D 1841 COS GTG ATC CTG COS GAA GAT GTG GTA ATG GAC GOC ATT ACC AGC COS ATT AAA GCA GAT COS GAG TGG GOS AAA ACT ACC GTT AAC GGT M P A L R E T D T F D T F M E S S W Y Y A R Y T C P Q Y K E 1931 ATG CCA GCA CTG CGT GAA ACC GAC ACT TTC GAC ACC TTT ATG GAG TCC TCG TAC TAT GCG CGC TAC ACT TCC CCG CAG TAC AAA GAA 510 L 540 2021 GET ATE CHE GAT TOC GAA GOG GOT AND TAC THE TEG CTE COE GTE GAT ATE TAC ATT GET GET ATT GAA CAC GOC ATT ATE CAC CTE CTE TAC 570 F R F F H K L M R D A G M V N S D E P A K Q L L C Q G M V L 2111 TTC COC TTC CAC AAA CTG ATG CGT GAT GCA GOC ATG GTG AAC TCT GAC GAA CCA GOC AAA CAG TTG CTG TGT CAG GGT ATG GTG CTG 600 2201 GCA GAT GOC TTC TAC TAT GTT GOC GAA AAC GOC GAA CGT AAC TGG GTT TCC CCG GTT GAT GCT ATC GTT GAA CGT GAC GAG AAA GOC CGT D G 630 A A Е L G 2291 ATC GTG AAA GOG AAA GAT GCG GCA GGC CAT GAA CTG GTT TAT ACC GGC ATG AGC AAA ATG TCC AAG TCG AAG AAC AAC GGT ATC GAC CCG Y G D v 660 E R A Т I. м м 2381 CAG GTG ATG GTT GAA COT TAC COC COG GAC ACC GTT COT CTG TTT ATG ATG TTT CCT TCT COG GCT GAT ATG ACT CTC GAA TGG CAG GAA 690 G Α N R R L н G D Y F. А 2471 TOC GET GTG GAA GOG GET AAC COC TTC CTG AAA CET GTC TOG AAA CTG GTT TAC GAG CAC ACA GCA AAA GET GAT GTT GCG GCA CTG AAC 720 0 D E 2561 GTT GAT GOG CTG ACT GAA AAT CAG AAA GCG CTG CGT CGC GAT GTG CAT AAA ACG ATC GCT AAA GTG ACC GAT GAT ATC GGC CGT CGT CAG 750 Α Α А Т м E τ. м N T. А к А P т D G E Q D 2651 ACC TTC AAC ACC GCA ATT GCG GCG ATT ATG GAG CTG ATG AAC AAA CTG GCG AAA GCA CCA ACC GAT GCC GAG GAT GCC GCT CTG ATG 780 А Τ. Α v R м N P т Ρ н С F Τ. Q E. Τ. 2741 CAS GAA GCA CTG CTG GCC GTT GTC CGT ATG CTT AAC CCG TTC ACC CCG CAC ATC TGC TTC ACG CTG TGG CAA GAC CTG AAA GOC GAA GGC 810 А D F. Α м D S Τ. 0 2831 GAT ATC GAC AAC GOS COS TOS COS GTT GCT GAC GAA AAA GCG ATG GTG GAA GAC TOC AOG CTG GTC GTG GTG CAG GTT AAC GGT AAA GTC 840 F D А E E R E R G E н A 2921 COT GOC ANA ATC ACC GTT COG GTG GAC GCA ACG GAA GAA CAG GTT COC GAA CAT CTG GCT ACC CAG GAA CAT CTG GTA GCA AAA TAT CTT GAT G V T V R K V I Y V P G K L L N L V V G 860 3011 GGC GTT ACT GTA CGT ANA GTG ATT TAC GTA CCA GGT ANA CTC CTC AAT CTG GTC GTT GGC taagoggggggggggggggggggggtatetetgggcaacattgt

29 adgcogtgoggfgócgfaaccattgacgtcfgaatggtggtgagttgoctgataaagaaaccacgggtaaggacgttccatócttgógttaggfaaagfgagcatogogaágatacc 18 gcatoggtattccgtaacggtcaaacagcagagtatcagatgatgacatgacggttaatgcgaccgtgttgatccccggccgtgatatctaccccgattagcgccaaagtcttccgttcg 3229 a 3586 ggatattcgttccgacgaagaacagacgtcgac

Figure 4: DNA sequence of leuS and derived aminoacid sequence for leucyl-tRNA synthetase. Only the sequence of the sense strand is shown. Numbering of the DNA sequence starts with AAGCTT, the HindIII site in pLeuS1. Possible regulatory sequences like -35 and -10 transcription control elements (25,26), ribosome binding site (27) and stop of transcription signal (25) are underlined.

permissive temperature were isolated (the total number of transformants at 28°C was 2x10⁶). The sizes of the plasmids in these clones were all in the range of 9-10 kb.

The leucyl-tRNA synthetase activity of one of the isolated clones (pLeuS1, see Fig.1) was measured by aminoacylation assay and compared with that of the wild type CSH26. A more than

50 fold overproduction of LeuRS activity at 37°C by the clone CSH26 pLeuS1 (18.8 nmol/min/mg) compared with CSH26 (0.3 nmol/min/mg) clearly indicates that pLeuS1 is responsible for this overproduction.

Expression of leuS in minicells

To analyse plasmid specific expression of proteins in pLeuS1 the minicell system was used. Minicell producing strain P678-54 was transformed with pLeuS1. Minicells were prepared as described in METHODS. An autoradiogram of the [35 S] methionine labelled proteins in minicells is shown in Fig.2. A predominant band at an apparent M_r of about 100,000 is consistent with a molecular weight of 105,000 reported for LeuRS (5).

Expression of leuS in normal cells

To estimate the quantity of LeuRS expressed in pLeuS1 a comparison was made of the total protein pattern of CSH26 with the clone CSH26 pLeuS1 by SDS-PAGE (Fig.3). The clone expresses strongly a 100K protein (Fig.3a), corresponding to the value found for the purified enzyme, whereas the strain CSH26 without plasmid does not (Fig.3b). The expression of this protein seems to be comparable with the expression of elongation factor Tu, which represents about 6% of the total cellular protein under standard conditions (23). The proteins, separated by SDS-PAGE, were transferred to nitrocellulose and were tested by immunoblotting using rabbit antiserum against highly purified LeuRS. Biotinylated protein A in combination with streptavidin-peroxidase was used (24). With CSH26 pLeuS1 cells, a specific strong reaction was observed (Fig.3d) with a 100K protein, in CSH26 pLeuS1 has the same molecular weight as that produced in wild type cells. This result together with the expression of LeuRS in minicells and the >50 fold enhancement of LeuRS activity clearly shows that pLeuS1 contains the whole structural gene for leucyl-tRNA synthetase.

Localization of <u>leuS</u> on pLeuS1

To localize <u>leuS</u> on the plasmid pLeuS1 restriction fragments were cloned into pUC19. KL231 clones containing the 4 Kb HindIII-EcoRI of pLeuS1 inserted in pUC19 were able to grow at KL231 non-permissive temperature and overexpressed LeuRS, whereas clones containing the PstI-EcoRI fragment were not able to grow at 44°C. The precise localization of the coding region was determined by DNA sequencing of the pLeuS1 HindIII-EcoRI fragment and comparison of the derived protein sequence with the N-terminal LeuRS sequence directly obtained by protein sequencing.

Sequencing of <u>leuS</u>

We sequenced the gene following the method of Sanger et al. (18). The sequencing strategy is shown in Fig.1, and the sequence obtained in Fig.4. The region sequenced starts with the HindIII site of pLeuS1, includes the 490bp of the 5' untranslated region, the 2580 bp of the coding region and 547 bp of the 3' untranslated region of <u>leuS</u>. The N-terminal sequence of the protein was confirmed by independent protein sequencing by sequential Edman degradation and amino acid

				1 au		S	econd		us gen	с.			
		U			C				A	G			
First		SEG	leuS		SEG	leuS		SEG	leuS		SEG	leuS	Third
U	Phe	0.26 0.74	0.41 0.59	Ser	0.36 0.34	0.38 0.42	Tyr	0.26 0.74	0.31 0.69	Cys	0.36 0.64	0.27 0.73	U C
	Leu	0.03 0.04	0.02 0.03		0.02 0.04	0.04		ochre amber		Trp	opal 1.00 1.00		A G
с	Leu	0.06	0.09	Pro	0.10	0.07	His Gin	0.20	0.41	Arg	0.67	0.61	U C A G
		0.01 0.79	0.02 0.75		0.12 0.77	0.17 0.71		0.18 0.82	0.13 0.87		0.01 0.00	0.02	
•		0.20 0.79	0.31		0.36 0.48	0.21 0.55	Asn	0.08	0.13	Ser	0.04 0.19	0.00 0.17	UC
-	Met	0.01 1.00	0.03	Inr	0.05 0.10	0.04 0.21	Lys	0.71	0.89 0.11	Arg	0.01 0.00	0.00 0.00	A G
G	Val	0.44	0.42	Ala	0.37	0.18	Asp Glu	0.36	0.43	Gly	0.54 0.41	0.35	U
		0.09 0.27 0.19	0.18		0.25	0.13		0.76 0.24	0.67 0.33		0.01	0.00	AG

Table 1. Codon usage in the leuS gene.

The codon usage of the leuS gene given as fraction of each codon within its redundancy family is compared with the average codon usage in many strongly expressed genes (SEG). Boxes indicate the same codon preferences in leuS as those characteristically found for strongly expressed genes (28).

identification by HPLC of the first 8 residues. In Fig.4 only the sense strand for <u>leuS</u> is shown. A possible promotor region (25,26) at position 398-404, 417-422 and a ribosome binding site (27) at position 476-479 are underlined. Between base 3143 and 3183 there is a region of two-fold symmetry, 69 bp after the TAA stop codon for the synthetase. This is probably the RNA polymerase termination site for <u>leuS</u> (25).

Aminoacid composition and codon usage.

The aminoacid composition of the protein sequence derived from the <u>leuS</u> DNA sequence was compared with the amino acid analysis of the enzyme isolated by Hayashi et al. (5). The agreement between the two sets of values is rather good, except the value for cysteine, with an average difference in the composition for each aminoacid of less than 9%. The codon usage of the <u>leuS</u> gene is shown in Tab.1. It resembles in general that of highly expressed genes (28) with exception of the codons for glycine. In <u>leuS</u> the preferred codon for glycine is GGC rather than GGT.

Homology with other E. coli aminoacyl-tRNA synthetases.

The structural relationships between the E. coli leucyl- and other aminoacyl-tRNA synthetases on the basis of sequence similarities shows as has been postulated before on the basis of biochemical and functional similarities (4), that the leucyl-, valyl-, isoleucyl- and methionyl enzymes are the

*** ·		ч к	040	****	*****	.		4 x · ·		****	****	
A. N.O ≼ U.N			40L ·			A			L B	- N - Z	00 % ¥ 2 ¥ H J	
	<u>و</u> ي ک		44.	****	1120	5 : : :				- 0 H >	0 0 1 1	
HU4 .	au j z	KOZ -		· 🖬 · ·	2 2 2 2	3	· • 🖬 •		· • # 0	Z · Z Z	K 1 K X	
ZNX	7) Y H	4 J 4 .	8 2 H N		0 F 10 >	¥ · · · ·			< > < >	N	8 > J 8 2 J 8 D	
XOX ·			× < X +		****	2		2	A N H A	2 · J F K · K J	0 K O N	
< > > · · · · · · · · · · · · · · · · ·	2 2 2 2	HOH	21242 28129		Z K <	K · · · ·			3 K F 4	< · 10	8 8 F F 8 3 3 3 2	
				· • • • •		<u>.</u>		***	ZUZH	1.14	HOUN	
4 H H	HAXX		EEE		0-63	3		~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	3366	44>0	O J Z Z	
<u></u>	****	0.18	40>>		OFOR		X>XX	****	N G J F	00>+	8 H Z H	
5 5 5 5 5 L I < I	>	8 6 8 F	• • • • • •	. .	004> 8×8×	2:::	>0011	T X F H H	4 > 3 O	111 2011	· · · · · · · · · · · · · · · · · · ·	
	1211		0 4 4 4	· • • •		.		****	4 N K A	KUKZ KUTH	<u> 탄구</u> 탄권	
1110	XZNN	7.44	2050		2624	<u>.</u>		4472	2223	0223		
H & > U	****		000-			ö · · ·	· · · ×	NONE	2		<	
0 × 3 F	NXXX	1 3 3 M				.	0 + > L	004>		0 - 1 4	> • • •	
> N H > 1 7 7 3	F 0 > ·	20	24FC		8688	.	222	****	8 8 8 7 0 1 2 1	•> ₽ ₽ 2 4 8	× N S N	
X D X D X D X X	~~ > 2	× • · · ·	A THX			> · · ·		8 4 8 J	# 4 0 0 0 X 0 0	44	> J K F	
0000	OHXN		222*		***		****	****			0 + 4 +	
	3 2 4 5		0000	1.			****		0 > 0 ¥	1.22	> 4 4 H	
pi×põ	~	F 2 · ·	0000	: 5 : :			WEDE'	FF00	><0+		1910 1910	
	化化化化 化化化化化		HOH ·	1211	2000			040H	4400			
3255		> * · ·	NAN -				+00>	<	****		000 ·	
>++>	UXX		>><	• E • •		<u>-</u>	>>* ·		X H Z		223	
UXI4	<u></u>	e	XON					F K K K	243		< 2 2 A	
- J - O		9 · · ·	400.	. h	· · # ·	× • • •		X M J M	KOL .	· · • • •	× 0 ¥ 0	
× × O ×		2 · · · ·	3 H 3 ·			9 M · · ·	4 H H N				4 4 4 J	
*>		ā · · ·	EEB :	-:5:::	· · •		0 > > 0		80×83		DOHA	
ÉŤÌÌ	2024	¥	MOR			Ha	40=>		****			
ZHZ-	F > Z =			****		***	XXFFF	: * : :	22		4 X 4 A	
<u> </u>	1 1 1 0 M	2	1 2 2 4	2018		4 8 · · ·	****		** • • •		2 2 7 4 2 7 4 4	
<u></u>		2:::	FF :	N C A C M				. .	1			
NEFE		E					MICK		: . : :			
<u> </u>			NR.					· • • • •		· · · ü	2 -	
144		X · 0 - 1	010	0×40		LLL ·	2120				н×	
50A		1 · 4 M	0 2 1 .	4 1 2 2 2	· · 20 00	6 2 G ·	41×1					
3 2 2			> 0 0 ·	****		F 3 4 -	761 ·	: : :::	:::::	:::4		
***	- 19 - 1		***			2+2:		· • • •				
***		N .00	M > 1 1	404	> 0		>===					
0		4 ·	HO	*		JXA ·	REFE					
9 (9 9) 9 (9 9)		4		227.		428.		> J J J K K K K M	· · · · ·			
0 × 0	:		11 H H + + + +	340.		101 ·		000	· • • • · ·	:::>		
HAR		***	M.C		::2::	240		ब्बम :	· ••• · · ·		· · • • •	10 0
			>>		E							5 20
HOF		000	1 1 1			2222			- E			3 10
×10		× ·	# 0 · ·	120			4400				: : : : :	<u>És</u>
80×	· · • • •	X 0 K ·		> h m ·		****	4=> -					X08 ·
303	××	***		**>			1 · M ·		· > · ·		:::::::::::::::::::::::::::::::::::::::	
- 2 -			• • • •			4144					· · F ·	
> < ->		****					a					>0×9
N X O X 1 F	180 .	0.000.	4 X · · ·	· · • • •		**>*	ц Ц	· · # ·			. C # C	X 4 > >
1 G 1	0 K H -		ын шн	H0H -		HHONE	a	· · • • • •	· • • • •	::: <u>:</u>	* 4 × 0	DOWN H4DE
	***	UX		444		000>	¥	· · × ·			335	
A × A		0									000	00××
x 4 z	4 6 0 ·		40 · ·	314.		<u>> = > = = =</u>	N · · · ·				물문원 :	2 2 0 2 0 2 0 0 4 4
00F	240 ·	> > · · ·	* - · ·			0 K 0 X N X 4 0	m · · · · · · · · · · · · · · · · · · ·	::8::	XO		F 4 F • •	X A A B A
	> F & F -	14 · · · · · · · · · · · · · · · · · · ·	48	811 ·	8 8 0 0 1 8 1 1 1	9 4 6 0 0 9 4 4 0	≥ :::	1121	34	· · 66 66		7×21 2210
7572	858 B		8823	1226		3882	835X	8886		****	2 3 5 S	3829
3338	2222					****	5 8 8 8		3455	4455		****
1131	7427	113X	2435	1471 1	1431	2432		2433	2233	2432	2433	2433

Figure 5. Alignment of Escherichia coli aminoacyl-tRNA synthetase sequences of the XUX tRNA family LeuRS : leucyl-tRNA synthetase, IleRS: isoleucyl-tRNA synthetase (30), VaIRS: valyl-tRNA synthetase (31), MetRS: methionyl-tRNA synthetase (33). Sequences were aligned using the program BESTFIT. For detail see text. Residues in LeuRS which are conserved in at least two aligned sequences are boxed.

10207

Nucleic Acids Research

LeuRS	10	IESKVQLHMDEKRITFEVTEDESKEKYYCLEMILPYPSCRLHACHVRNYTIGDVIARYQHMLCKNVLQPIGHDAFGLPAEGAAVKNNTAPAPMTYDNIAYMK 109
NAM2-1	24	IAIGEKWKOKTITGIPKODTINSGSKYILOOPPYPSGALHIGHLRVYVISDSINRFYKOKGINVIHPMGHDAFGIPAENRAIERSINPAIWIRDNIAWK 123
LeuRS	110	NOLKMILGFGYDMSRELATCTPEYYRMEOKFFTELYRKCLVYRKTSAVMICENDOTVLANEOV. IDGCCMPCDTKVERKEIPOMFIKINAYADELINDIDK 208
NAM2-1	124	QCMQSMLANFDMDREITTCDPEYYKFTOWIFIKIFENSLAYRKEAEINMDPVDMTVLANEONDAQGRSWRSCAIVEKKQLKONFIGTTKFAPKLKKHLNQ 223
LeuRS	209	LDH#PDTvKtMQRWIGRSEGVEITFMMD. YDNTLTVYTTRPDT#MGCTYLAVRAGPDAQKAAENNPELAAFIDECRNIKVAEAEMATMEXKGDTGF 307
NAM2-1	224	LKDMPSWKQMQKWWISESVGAELWFKVADPKFENLIVFTTRPETIFAVQYVALALDHPIVQKYCEEMPDLKEFIQKSDQLPNDTKEGPOLPNI 317
Leurs	308	KAVHPLITGEEIPWMAANFVLMEYGTGAWAAVPGHOGPOYEFASKYGLNIKPVILAADGSEPDLSQQALTEKGVLFNSGEFNSLDH 392
NAM2-1	318	KAWAPLIKEEVPIFAAPYVVSSYGSAPSAVMCCPGHDNPOFEFWJINCPGEHIKTCIAPFFEEASKVTECERORI IDTVPFTSTDGVLTKEOGEHSGVLT 417
LeuRS	393	EAAFNAIADKI.TAMSKGERKUNYRIRDKISKSRORVIKGAP IEMVTLEDGTVMPTPDDQLPVILPEDVMDGITSPIKADPEWAKTTAKSMPALREITDTF 490
NAM2-1	418	WARKSIMAMINSEGLSKSWRYKIROWILISRORVWSTPIPIIHCINOSPVPVPESDLPVKLPELEGLDTKONPLSTIDEFVWVACPSOSSPAKRETUTM 517
LeuRS	491	DIFMESSWYYARYTCPQYKECMIDSEANNYWLPVDIYIGGTEHAIMILLYFRFFHKIMFDACMNSDEPAKQLLQQMVLADAFYYVEENGERNWSPVD 590
NAM2-1	518	DIFIDSSWYFRELDEKNISKPFDREIASKMEVDIYIGSMEHAILHLLYSRFIAKFLGSINAKSDPAGIFEPFKKLVTQGMQGKTYVDPDSGKFLKPD 617
LeuRS	591	AIVERDEKGRIVKAKDAAGHELVYTGMSKMSKSKNIGIDPOMMERYGADIVRLEMMFASPADMILEMGESGNEGANRFLKRVMKLVYEHTAKGDVAALN 690
NAM2-1	618	ELTEVNOSPORITVIIKSNERVPVVSYERVSKSKYNGADENECILRHOPDATRAHILFOSPIADAIAMDESKIVGIERNLOKVLHLIRNILSLEKOLAIS 717
LeuRS	691	VDALTENOKAL REDVHKTLAKVIDDIG ROTENTA LAAIMELMIKLAKAPIDGEOORALMOEALLAWIMINPETPHICETTIAGELKGEGDIINA 785
NMM2-1	718	KOYKTPIDINDAEVKFINDFORFIKSITESFEWILSINTVISOYMLINIIESALKKGEVRNEMIVONICKUVIVIYPAVPSISEEAAEMINSOMEWOY 817
Leurs	786	PNPVADEKANVEDSTLVVVQVNCKVRAKTIVPVDATEEQVRERAGQEHL 834
NM2-1	818	RMPEVERTTESKEKKEPOIVANERAKEMYTADKAFIKI.GEDAVIETI 863

Figure 6. Homology with the gene product of the yeast nuclear NAM2 -1 gene

Sequences were aligned using the program BESTFIT. The serine at position 240 in the NAM2-1 gene product responsible for its suppressor activity (34) matches with glycine in the LeuRS sequence. In the *nam2* + wild type protein also a glycine is present at this position.

most closely related. The phenylalanine enzyme, which also belongs to the XUX tRNA family, has not been included in the alignment because conseved sequence motifs could not be found. Despite some common features this enzyme differs from the methionine, valine, leucine and isoleucine enzymes by its quaternary structure $(\alpha_2\beta_2)$ and that it aminoacylates an aromatic aminoacid. The alignment shown in Fig.5 was carried out using the program BESTFIT (29). The leucyl enzyme sequences was aligned with the isoleucyl enzyme sequence (30) and valyl enzyme sequence (31), using the standard gap weight of 5 and a gap length weight of 0.01 to allow relatively long gaps which are necessary for the alignment of sequence motifs in evolutionary distant sequences. The methionyl sequence (33) was aligned with the valyl- rather than with the leucyl- sequence because of its higher similarity to this enzyme using a gap weight of 2 and the gap length weight of 0.01. The alignment shown in Fig.5 is an assembly of these three pairwise alignments. Homologies in the leucyl sequence with two or three of the aligned sequences at a given position are boxed. There is a rather high level of homology in the N-terminal regions including the HIGH region as postulated ATP binding site (30) at position 42-52 in LeuRS. Additionally the KMSKS region involved in 3' end tRNA binding (32) at position 619-623 in LeuRS is also present in MetRS, ValRS and IleRS. There are further conserved regions such as

W X X S R X X X G whose importance for function and specificity remains unclear. The alignment of these four aminoacyl-tRNA synthetase sequences indicates that these enzymes could have derived from a common ancestor.

Homology with the gene product of the yeast nuclear NAM2-1 suppressor allele

Sequence comparison with the gene product of yeast nuclear NAM2 -1 suppressor allele curing mitochondrial RNA maturation deficiency (34) is shown in Fig.6. The sequences were aligned using the program BESTFIT with standard gap weight of 5 and standard gap length weight of 0.3. There is evidence that the wild type $nam2^+$ gene codes for yeast mitochondrial leucyl-tRNA synthetase (35). Both sequences show about 30 % homology. There is a high homology in the putative ATP binding and 3' end tRNA binding regions. The serine at position 240 in the NAM2-1 gene product responsible for the suppressor activity matches with glycine in the *E.coli* LeuRS sequence. The $nam2^+$ gene product contains also a glycine at this position.

ACKNOWLEDGEMENTS

We would like to thank Beatrice Cortolezzis for carrying out the N-terminal sequence analysis of the enzyme, Rita Neumann for excellent technical assistance, Prof. Piotr Slonimski and Dr. Reuben Leberman for helpful discussions.

REFERENCES

- 1. Sprinzl, M. and Cramer, F., (1975), Proc. Natl. Sci. USA, 72, 3049-3053.
- 2. Crothers, D. M., Seno, D. and Söll, D., (1972), Proc. Natl. Sci. (USA), 69, 3063-3067.
- 3. Schimmel, P. and Söll, D. (1979), Ann. Rev. Biochem. 48, 601-604.
- 4. Wetzel, R. (1978), Origins of Life, 9, 39-50.
- 5. Hayashi, H., Knowles, J.R., Katze, J.R., Lapointe, J. and Söll, D., (1970), J.Biol.Chem. 245, 1401-1406.
- 6. Lin, C.-S., Irwin, R. and Chirikjian, J.G., (1979), Nucleic Acids Res. 6, 3651-3660.
- 7. Kunugi, S., Uehara-Kunugi, Y., von der Haar, F., Schischkoff, J., Freist, W., Englisch, U. and Cramer, F., (1986), Eur. J. Biochem. 158, 43-49.
- 8. Low, B., Gates, F., Goldstein, T. and Söll, D. (1971), J. Bacteriol., 108, 742-750.
- 9. Theall, G. Low, K.B. and Söll, D., (1979), Molec. gen. Genet., 169, 205-211.
- 10. Kieny, M.P., Lathe, R. and Lecocq, J.P., (1983), Gene, 26, 91-99.
- 11. Brune, M., Schumann, R. and Wittinghofer, F., (1985), Nucleic Acids Res., 13, 7139-7151.
- 12. Hanahan, D. (1983) J. Mol. Biol., 166, 557-580.
- 13. Lewy, S.B., (1974), J. Bacteriol., 120, 1451-1463.
- 14. Laemmli, U.K , (1970), Nature (London) , 227, 680-685.
- 15. Birnboim, H.C. and Doly, J., (1979), Nucleic Acids Res., 7, 1513-1523.
- 16. Schreier, P.H. and Cortese, R., (1979), J. Mol. Biol., 129, 169-172.
- 17. Maniatis, T., Frisch, E.F. and Sambook, J., (1982), Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, New York
- 18. Sanger, F., Nicklen, S. and Coulson, A.R., (1972), Proc. Natl.Acad.Sci. (U.S.A.), 74, 5463-5467.
- Poncz, M., Solowiejczky, D., Ballantine, M., Schwartz, E. and Surrey, S., (1982), Proc. Natl. Acad. Sci. (U.S.A.), 79, 4298-4302.
- Strauss, E. C., Kobori, J. A., Siu, G. and Hood, L. E., (1986), Analytical Biochemistry, 154, 353-360.
- 21. Zagurski, R. J., Baumeister, K., Lomax, N. and Berman, M. L., (1985), Gene Anal. Techn., 2, 89-94.

- 22. Härtlein, M., Madern, D. and Leberman, R., (1987), Nucleic Acids Res., 15, 1005-1017.
- 23. Furano, A.V., (1975), Proc.Natl.Acad.Sci. (U.S.A.), 72, 4780-4784.
- 24. Graf, F. M. and Strauli, P., (1983), J. Histochem. Cytochem., 31, 803-810.
- 25. Rosenberg, M. and Court, D., (1979), Ann.Rev.Genet., 13, 319-353.
- 26. Siebenlist, U., Simpson, R.B. and Gilbert, W., (1980), Cell, 20, 269-281.
- 27. Shine, J. and Dalgarno, L., (1975), Nature (London), 254, 34-38.
- 28. Grosjean, H. and Fiers, W., (1982), Gene, 18, 199-208.
- 29. Smith, T.F. and Waterman, M. S., (1981), J. Mol. Biol., 147, 195-197.
- 30. Webster, T., Tsai, H., Kula, M., Mackie, G. and Schimmel, P., (1984), Science, 226, 1315-1317.
- 31. Härtlein, M., Frank, R. and Madern, D., (1987), Nucleic Acids Res. in press.
- 32. Hountondji, C., Dessen, P. and Blanquet, S., (1986), Biochimie, 68, 1071-1078.
- 33. Dardel, F., Fayat, G. and Blanquet, S., (1984), J. Bacteriol., 160, 1115-1122.
- 34. Labouesse, M., Herbert, C. J., Dujardin, G. and Slonimski P.P. (1987), EMBO J., 6, 713-721.
- 35. Herbert, C.J., Labouesse, M., Dujardin, G. and Slonimski P. P., EMBO J. submitted.