Volume 15 Number 24 1987 Nucleic Acids Research

# 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 Neurospora crassa 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 leuS has been mapped at position 15 min. on the *E.coli* chromosome. The existence of a regulatory

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

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

## MATERIALS and METHODS.

### Bacterial Strains.

The leuS 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 leuS 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 $MgCl_2$ 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 $MgCl_2$ , 0.5mM DTE, 2mM ATP, 1mg/ml unfractionated tRNA, 20 mM L-[U-$^{14}$C] 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 leuS 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

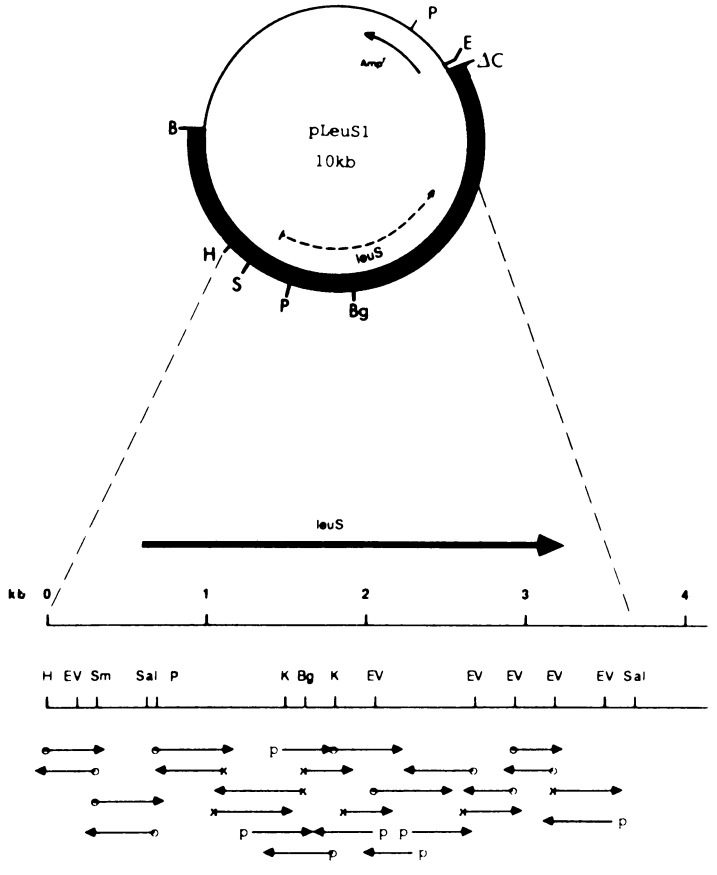


Figure 1: **Restriction map of plasmid pLeuS1 and sequencing strategy for the leuS gene.**
The gene for ampicillin resistance on pBR322 is marked by an arrow. Thick lines represent the inserted chromosomal DNA.
Abbreviations for restriction enzymes are: B=BamHI, Bg=BglII, C=ClaI, E=EcoRI, EV=EcoRV, H=HindIII, K=KpnI, P=PstI, S=SalI, Sm=SmaI. Restriction site ClaI(ΔC) 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.

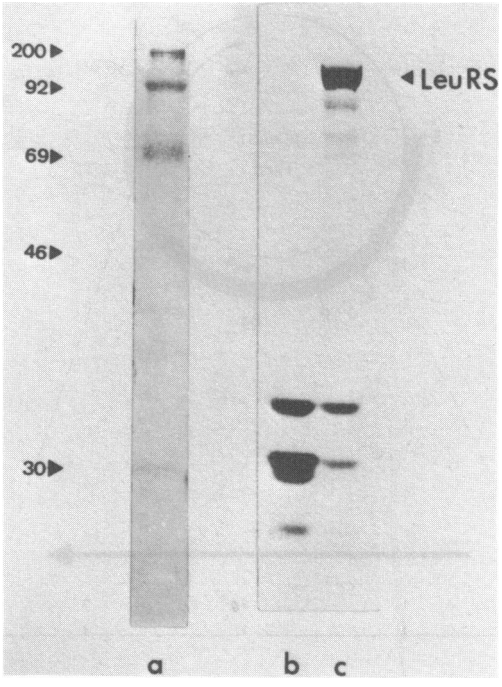


Figure 2: **SDS-PAGE analysis of minicell proteins.**
Minicells were prepared as described in METHODS, proteins were separated on a 12% SDS-polyacrylamide gel. Molecular weight markers were [U-$^{14}$C] methylated proteins from Amersham; 30K carbonic anhydrase, 69K bovine serum albumin, 92K phosphorylase 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 BglII digest followed by a Bal31 kinetic and a HindIII digest. Additionally leuS containing restriction enzyme fragments of pLeuS1 (HindIII/SmaI, SmaI/ BglII,

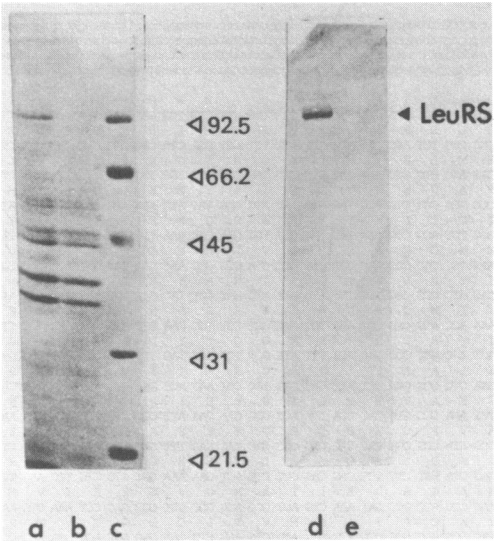


Figure 3: **Expression of LeuRS in normal cells and identification of the enzyme by immunoblotting.**
CSH26 (lane b) and CSH26 pLeuS1 (lane a) were grown to stationary phase, cells were lysed in Laemmli buffer (15) and proteins analysed on a 12% SDS-polyacrylamide gel. Additionally separated proteins 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-streptavidine-peroxidase 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, BglII/PstI, KpnI/EcoRI, EcoRV) were cloned into M13 tg130 and M13 tg131. Finally several leuS 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 leuS (strain KL231) was transformed with the library DNA and grown at 44°C on LB ampicillin plates. Clones which grew at KL231 non-

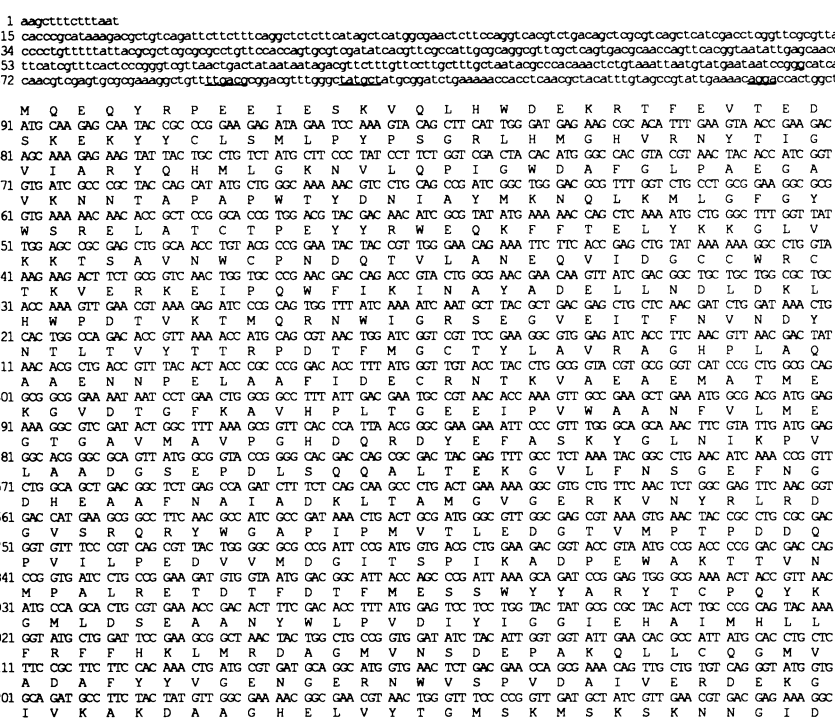

Figure 4: **DNA sequence of leuS and derived aminoacid sequence for leucyl-tRNA synthetase.** Only the sequence of the sense strand is shown. Numbering of the DNA sequence starts with AAGCTT, the HindIII site in pLeuS1. Possible regulatory sequences like -35 and -10 transcription control elements (25,26), 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 $2x10^6$). 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 cells the reaction was very weak (Fig.3e). This indicates that the protein overproduced 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 leuS on pLeuS1**

To localize leuS 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 leuS**

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 leuS. The N-terminal sequence of the protein was confirmed by independent protein sequencing by sequential Edman degradation and amino acid

Table 1. **Codon usage in the leuS gene.**

| First | Second U | SEG | leuS | Second C | SEG | leuS | Second A | SEG | leuS | Second G | SEG | leuS | Third |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| U | Phe | 0.26 | 0.41 | Ser | 0.36 | 0.38 | Tyr | 0.26 | 0.31 | Cys | 0.36 | 0.27 | U |
| | | 0.74 | 0.59 | | 0.34 | 0.42 | | 0.74 | 0.69 | | 0.64 | 0.73 | C |
| | Leu | 0.03 | 0.02 | | 0.02 | - | | ochre | | | opal | | A |
| | | 0.04 | 0.03 | | 0.04 | 0.04 | | amber | | Trp | 1.00 | 1.00 | G |
| C | Leu | 0.06 | 0.09 | Pro | 0.10 | 0.07 | His | 0.20 | 0.41 | Arg | 0.67 | 0.61 | U |
| | | 0.08 | 0.09 | | 0.01 | 0.05 | | 0.80 | 0.59 | | 0.30 | 0.37 | C |
| | | 0.01 | 0.02 | | 0.12 | 0.17 | Gln | 0.18 | 0.13 | | 0.01 | 0.02 | A |
| | | 0.79 | 0.75 | | 0.77 | 0.71 | | 0.82 | 0.87 | | 0.00 | 0.00 | G |
| A | Ile | 0.20 | 0.31 | Thr | 0.36 | 0.21 | Asn | 0.08 | 0.13 | Ser | 0.04 | 0.00 | U |
| | | 0.79 | 0.66 | | 0.48 | 0.55 | | 0.92 | 0.88 | | 0.19 | 0.17 | C |
| | | 0.01 | 0.03 | | 0.05 | 0.04 | Lys | 0.71 | 0.89 | Arg | 0.01 | 0.00 | A |
| | Met | 1.00 | 1.00 | | 0.10 | 0.21 | | 0.29 | 0.11 | | 0.00 | 0.00 | G |
| G | Val | 0.44 | 0.42 | Ala | 0.37 | 0.18 | Asp | 0.36 | 0.43 | Gly | 0.54 | 0.35 | U |
| | | 0.09 | 0.11 | | 0.10 | 0.15 | | 0.64 | 0.57 | | 0.41 | 0.61 | C |
| | | 0.27 | 0.18 | | 0.25 | 0.23 | Glu | 0.76 | 0.67 | | 0.01 | 0.00 | A |
| | | 0.19 | 0.29 | | 0.28 | 0.44 | | 0.24 | 0.33 | | 0.03 | 0.04 | G |

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 leuS 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 leuS (25).

**Aminoacid composition and codon usage.**

The aminoacid composition of the protein sequence derived from the leuS 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 leuS gene is shown in Tab.1. It resembles in general that of highly expressed genes (28) with exception of the codons for glycine. In leuS 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

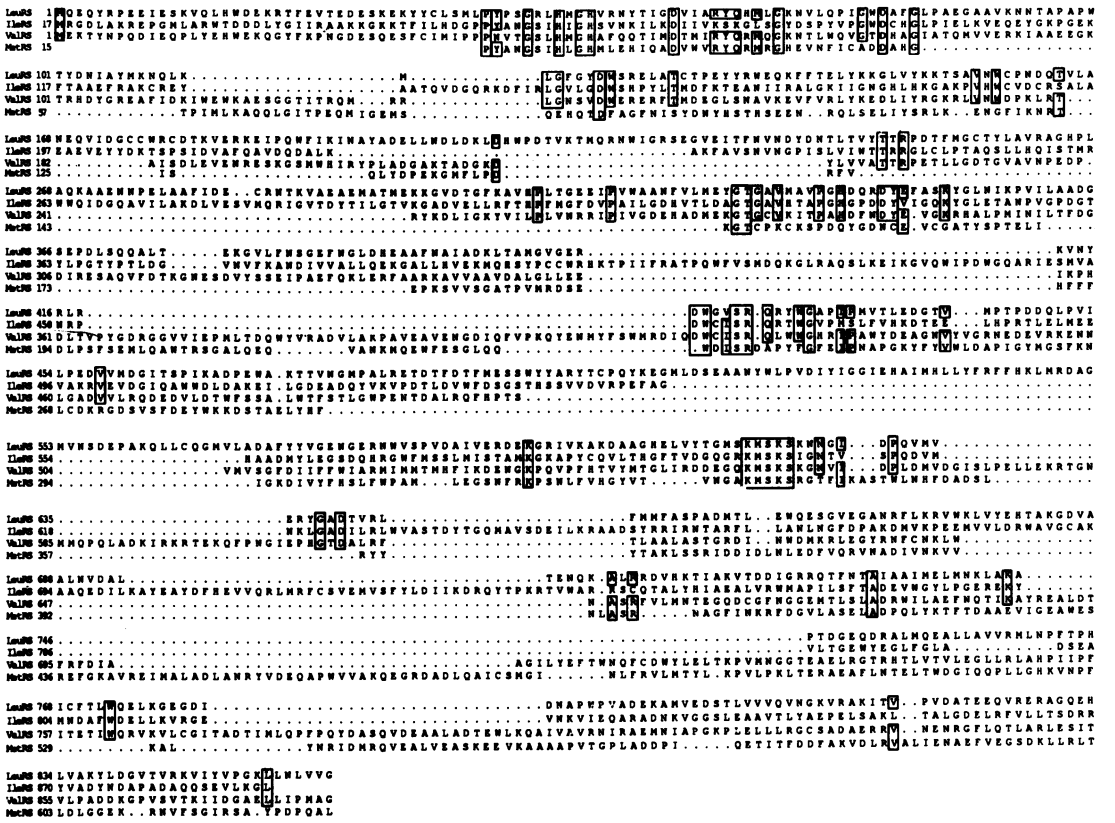

Figure 5. **Alignment of Escherichia coli aminoacyl-tRNA synthetase sequences of the XUX tRNA family**
LeuRS : leucyl-tRNA synthetase, IleRS: isoleucyl-tRNA synthetase (30), ValRS: valyl-tRNA synthetase (31), MetRS: methionyl-tRNA synthetase (33). Sequences were aligned using the program BESTFIT. For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

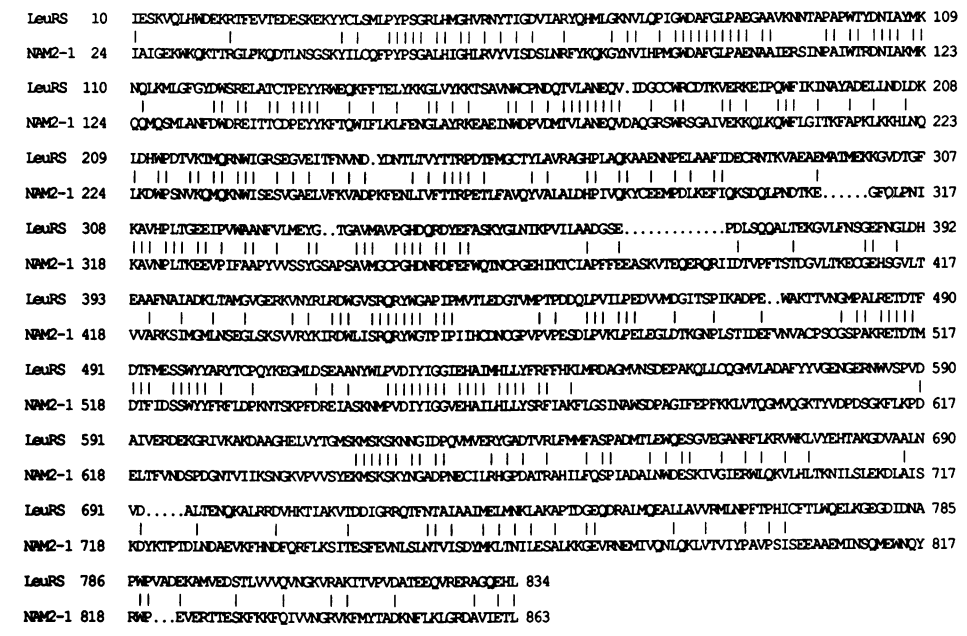

**Figure 6. Homology with the gene product of the yeast nuclear *NAM2 -1* gene**
Sequences were aligned using the program BESTFIT. The serine at position 240 in the *NAM2-1* gene product responsible for its suppressor activity (34) matches with glycine in the LeuRS sequence. In the *nam2* + wild type 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 H I G H region as postulated ATP binding site (30) at position 42-52 in LeuRS. Additionally the K M S K S 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 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.
19. Poncz, M., Solowiejczky, D., Ballantine, M., Schwartz, E. and Surrey, S., (1982), Proc. Natl. Acad. Sci. (U.S.A.), **79**, 4298-4302.
20. 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.