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# Cysteinyl-tRNA synthetase is a direct descendant of the first aminoacyl-tRNA synthetase

Javier Avalos*, Luis M. Corrochano and Sydney Brenner

*MRC Molecular Genetics Unit, Hills Road, Cambridge, CB2 2QH, UK*

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The gene encoding the cysteinyl-tRNA synthetase of *E. coli* was cloned from an *E. coli* genomic library made in λ2761, a lambda vector which can integrate and which carries a chloramphenicol resistance gene. A thermosensitive *cysS* mutant of *E. coli* was lysogenised and chloramphenicol-resistant colonies able to grow at 42°C were selected to isolate phages containing the wild-type *cysS* gene. The sequence of the gene was determined. It codes for a 461 amino-acid protein and includes the sequences HIGH and KMSK known to be involved in the ATP and tRNA binding respectively of class I synthetases. The cysteinyl enzyme has segments in common with the cytoplasmic leucyl-tRNA synthetase of *Neurospora crassa*, the tryptophanyl-tRNA synthetase of *Bacillus stearothermophilus*, and the phenylalanyl-tRNA synthetase of *Saccharomyces cerevisiae*. Sequence comparisons show that the amino end of the cysteinyl-tRNA synthetase has similarities with prokaryotic elongation factors Tu; this region is close to the equivalent acceptor binding domain of the glutaminyl-tRNa synthetase of *E. coli*. There is a further similarity with the seryl enzyme (a class II enzyme) which has led us to propose that both classes had a common origin and that this was the ancestor of the cysteinyl-tRNA synthetase.

Cysteinyl-tRNA synthetase; *cysS* gene; Elongation factor Tu; Genetic code; *Escherichia coli*

## 1. INTRODUCTION

Aminoacyl-tRNA synthetases are the enzymes that join an amino acid with its corresponding tRNA (reviewed in [1,2]). Since the genetic code depends on the specificity of this association, there is an interest in understanding the origin and evolution of these enzymes to look for clues to the early stages of the evolution of protein synthesis. Surprisingly, sequence studies have revealed that many of the enzymes are related to each other; there is close correspondence between the overall sequences of the leucine, isoleucine and valine enzymes which suggests that they had a common source [3,4]; the enzymes for glutamine and glutamic acid are also related [5]. These enzymes also share at least two important regions with the enzymes that activate methionine, arginine, tyrosine and tryptophan: the HIGH domain [6], which is the ATP binding site, and the KMSK domain which is involved in the attachment of the tRNA [7,8]. The determination of the tertiary structures of the methionyl [9,10], tyrosyl [11,12], and the glutaminyl [13] enzymes have shown that these domains have similar nucleotide binding folds.

The enzymes that activate aspartic acid, asparagine, and lysine are related to each other [14–17], and the aspartic enzyme has a short motif in common with the lysine, the phenylalanine, the alanine, and the histidine enzymes [16,18]. The serine, proline and threonine enzymes are also related [19,20]. In fact, a closer inspection of the sequences of all of these enzymes and that of phenylalanine and glycine led Eriani et al. [20] to suggest that they shared three motifs except for the glycine and alanine enzymes which only had one. None of these motifs are present in any of the enzymes with the HIGH and KMSK sequences. Thus, two exclusive classes of aminoacyl-tRNA synthetases can be defined depending on which motif they have: those with the HIGH and KMSK sequences are in class I and the rest are in class II. The serine enzyme (a class II enzyme) has a structure which is different from that of the class I enzymes [19] and the differences in primary and tertiary structure between the two classes have led to the proposal that they had different evolutionary origins [19,20].

Cysteinyl-tRNA synthetase could not be classified because there was no sequence information available. We therefore undertook to clone and sequence the cysteinyl-tRNA synthetase, using a thermosensitive mutant of the enzyme in *E. coli* [21]. We show here that while the cysteinyl-tRNA synthetase is a class I enzyme, it also has similarities with elongation factors and with a serine-tRNA synthetase.

After the completion of this work, a similar sequence was reported by Eriani et al. [22], our sequence differs from theirs at position 337, where an arginine should be replaced by an alanine. A second report by Hou et al. [23] contains the same sequence as ours.

*Correspondence address:* S. Brenner, MRC Molecular Genetics Unit, Hills Road, Cambridge, CB2 2QH, UK. Fax: (44) (223)-210136

*Present address:* Departamento de Genética y Biotecnia, Universidad de Sevilla, Apartado 1095, E-41080 Sevilla, Spain



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## 2. MATERIALS AND METHODS

### 2.1. *Strains*

UT181 is a strain of *Escherichia coli* K12 with *cysS818*, a thermosensitive mutation in the cysteinyl-tRNA synthetase gene [21]. DH5$\alpha$ has a wild-type *cysS* gene and its DNA was used to construct the *E. coli* gene library in λ2761. This bacteriophage lambda vector carries a chloramphenicol resistance gene and a phage attachment site. It is $C_I$ and requires a helper phage for integration in the *E. coli* genome (Koh and Brenner, unpublished). Wild-type lambda was obtained by UV induction from *E. coli* WX9. The purification and handling of bacterial and phage DNA and the transformation and infection procedures followed standard methods [24,25].

### 2.2. *Enzymes and chemicals*

Restriction enzymes and T4 ligase were from New England Biolabs, [$^{35}$S]$\alpha$-dATP from Amersham and Qiagen columns from Diagen GmbH. Methods followed the recommendations of the suppliers.

### 2.3. *Construction of an* E. coli *gene library*

DNA from DH5$\alpha$ was partially digested with *Sau*3A and separated by electrophoresis in low melting point agarose. DNA fragments ranging from 12–20 kb were excised from the gel, purified through Qiagen columns and ligated into λ2761 digested with *Bam*HI. The ligation mixture also contained a 10% of λ2761 digested with *Bam*HI and *Eco*RI. This will create central parts with *Eco*RI ends which cannot religate, therefore increasing the relative amount of right and left

```
    TTCAACCCAGTTCGGGTCATATATAGGGTGGTGTTATAGCATAACCGCACGATCGGATCATCACGCAATGTA
    TGCTGATTCGCGCGGGAAATATGGGTATTATACGCAACTCAATTACCCACACATGTCTAAACGGAATCTTCG

    ATGCTAAAAATCTTCAATACTCTGACACGCCAAAAAGAGGAATTTAAGCCTATTCACGCCGGGGAAGTCGGC
  1 M  L  K  I  F  N  T  L  T  R  Q  K  E  E  F  K  P  I  H  A  G  E  V  G

    ATGTACGTGTGTGGAATCACCGTTTACGATCTCTGTCATATCGGTCACGGGCGTACCTTTGTTGCTTTTGAC
 25 M  Y  V  C  G  I  T  V  Y  D  L  C  H  I  G  H  G  R  T  F  V  A  F  D

    GTGGTTGCGCGCTATCTGCGTTTCCTCGGCTATAAACTGAAGTATGTGCGCAACATTACCGATATCGACGAC
 49 V  V  A  R  Y  L  R  F  L  G  Y  K  L  K  Y  V  R  N  I  T  D  I  D  D

    AAAATCATCAAACGCGCCAATGAAAATGGCGAAAGCTTTGTGGCGATGGTGGATCGCATGATCGCCGAAATG
 73 K  I  I  K  R  A  N  E  N  G  E  S  F  V  A  M  V  D  R  M  I  A  E  M

    CACAAAGATTTTGATGCTTTGAACATTCTGCGCCCGGATATGGAGCCGCGCGCGACGCACCATATCGCAGAA
 97 H  K  D  F  D  A  L  N  I  L  R  P  D  M  E  P  R  A  T  H  H  I  A  E

    ATTATTGAACTCACTGAACAACTGATCGCCAAAGGTCACGCTTATGTGGCGGACAACGGCGACGTGATGTTC
121 I  I  E  L  T  E  Q  L  I  A  K  G  H  A  Y  V  A  D  N  G  D  V  M  F

    GACGTCCCGACCGATCCAACTTATGGCGTGCTGTCGCGTCAGGATCTCGACCAGCTGCAGGCAGGCGCGCGC
145 D  V  P  T  D  P  T  Y  G  V  L  S  R  Q  D  L  D  Q  L  Q  A  G  A  R

    GTTGACGTGGTCGACGACAAACGCAACCCAATGGACTTCGTTCTGTGGAAGATGTCGAAAGAGGGCGAACCG
169 V  D  V  V  D  D  K  R  N  P  M  D  F  V  L  W  K  M  S  K  E  G  E  P

    AGCTGGCCGTCTCCGTGGGGCGCGGGTCGTCCTGGCTGGCACATTGAATGTTCGGCAATGAACTGCAAGCAG
193 S  W  P  S  P  W  G  A  G  R  P  G  W  H  I  E  C  S  A  M  N  C  K  Q

    CTGGGTAACCACTTTGATATCCACGGCGGCGGTTCAGACCTGATGTTCCCGCACCACGAAAACGAAATCGCG
217 L  G  N  H  F  D  I  H  G  G  G  S  D  L  M  F  P  H  H  E  N  E  I  A

    CAGTCCACCTGTGCCCATGATGGTCAGTATGTGAACTACTGGATGCACTCGGGGATGGTGATGGTTGACCGC
241 Q  S  T  C  A  H  D  G  Q  Y  V  N  Y  W  M  H  S  G  M  V  M  V  D  R

    GAGAAGATGTCCAAATCGCTGGGTAACTTCTTTACCGTCGCGATGTGCTGAAATACTACGACGCGGAAACC
265 E  K  M  S  K  S  L  G  N  F  F  T  V  R  D  V  L  K  Y  Y  D  A  E  T

    GTGCGTTACTTCCTGATGTCGGGCCACTATCGCAGCCAGTTGAACTACAGCGAAGAGAACCTGAAGCAGGCG
289 V  R  Y  F  L  M  S  G  H  Y  R  S  Q  L  N  Y  S  E  E  N  L  K  Q  A

    CGTGCGGCGCTGGAGCGTCTCTACACTGCGCTGCGCGGCACAGATAAAACCGTTGCGCCTGCCGGTGGCGAA
313 R  A  A  L  E  R  L  Y  T  A  L  R  G  T  D  K  T  V  A  P  A  G  G  E

    GCGTTTGAAGCGCGCTTTATTGAAGCGATGGACGACGATTTCAACACCCCGGAAGCCTATTCCGTACTGTTT
337 A  F  E  A  R  F  I  E  A  M  D  D  D  F  N  T  P  E  A  Y  S  V  L  F

    GATATGGCGCGTGAAGTAAACCGTCTGAAAGCAGAAGATATGGCAGCGGCGAATGCAATGGCATCTCACCTG
361 D  M  A  R  E  V  N  R  L  K  A  E  D  M  A  A  A  N  A  M  A  S  H  L

    CGTAAACTTTCCGCTGTATTGGGCCTGCTGGAGCAAGAACCGGAAGCGTTCCTGCAAAGCGGCGCGCAGGCA
385 R  K  L  S  A  V  L  G  L  L  E  Q  E  P  E  A  F  L  Q  S  G  A  Q  A

    GACGACAGCGAAGTGGCTGAGATTGAAGCGTTAATTCAACAGCGTCTGGATGCCCGTAAAGCGAAAGACTGG
409 D  D  S  E  V  A  E  I  E  A  L  I  Q  Q  R  L  D  A  R  K  A  K  D  W

    GCGGCGGCGGATGCGGCGCGTGATCGTCTTAACGAGATGGGGATCGTGCTGGAAGATGGCCCGCAAGGGACC
433 A  A  A  D  A  A  R  D  R  L  N  E  M  G  I  V  L  E  D  G  P  Q  G  T

    ACCTGGCGTCGTAAGTAATTGCGCTATTGCCGGATGCGAGTTTTCGCATCCGGTTATCGTCTGCGCCACCAC
457 T  W  R  R  K
```

Fig. 1. Nucleotide sequence of the cysteinyl-tRNA synthetase gene of *E. coli*. The −10 and −35 sequences and a possible transcription termination signal sequence are underlined. This nucleotide sequence is in the EMBL Data Library under the accession number X59293.

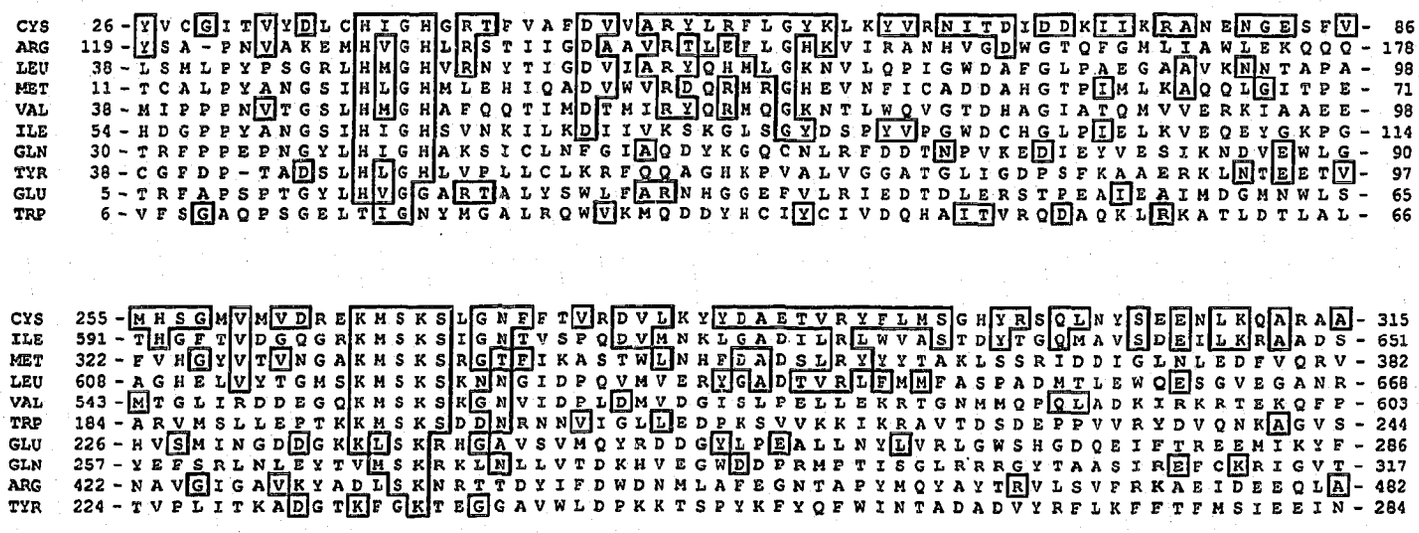

Fig. 2. Comparison of the amino-acid sequences around the HIGH and KMSK regions of the ten class I synthetases of *Escherichia coli*. The DNA sequences from all of them except for the isoleucyl enzyme [6] were obtained from the EMBL and GenBank databases where the corresponding references can be found. Only the amino acids shared with the cysteinyl-tRNA synthetase are boxed.

arms to that of the central part. It was expected that this particular mix will increase the frequency of ligation of *E. coli* DNA into the vector. The library contained $3 \times 10^5$ recombinant clones with inserts ranging from 12 to 20 kb.

2.4. *Cloning and sequencing the* cysS *gene*

2 ml of permisive cells of the thermosensitive mutant UT181 were coinfected with $5 \times 10^{14}$ phage particles from the library and $8 \times 10^8$ helper phages. The mixtures was incubated for 2 h at 30°C to allow the expression of the chloramphenicol resistance and cysteinyl-tRNA synthetase genes and then grown at 42°C on TYE plates with chloramphenicol (10 μg/ml). Twelve colonies appeared after 24 h of incubation. Phages were UV-induced from 25 ml cultures from these colonies and their DNA purified with Qiagen columns.

The DNA from 4 phages was partially digested with *Sau*3A and ligated into the *Bam*HI site of pBluescript KS$^+$ (Stratagene). UT181 was transformed with the ligation mixture and $2.5 \times 10^4$ transformants were isolated as resistant to ampicillin (100 μg/ml) at 30°C. Replica plating and incubation at 42°C led to the isolation of 70 colonies that grew vigorously at 42°C.

Plasmid DNA was extracted from 14 colonies and one of them, pJLE1, gave transformants of UT181 that grew at 42°C. This plasmid contains a 2.4 kb insert which we have sequenced using the chain termination method and a Sequenase II kit (United States Biochemicals). The sequencing was performed on both strands using synthetic oligonucleotides as primers.

2.5. *Database searchers*

Searchers from the Protein Identification Resource (PIR) database were carried out using the Prosrch5 program run on an AMT (Active Memory Technology) DAP (Distributed Array Processor) accessed from the Human Genome Mapping Program Resource Centre (Harrow, UK).

## 3. RESULTS

The sequence of the 2.4 kb insert in the plasmid pJLE1 showed a long open reading frame of 461 amino acids which contained the HIGH and KMSK sequences (Fig. 1). The cysteinyl-tRNA synthetase of *E. coli* is therefore a class I enzyme.

A comparison of the amino acids around the HIGH and KMSK regions in 10 aminoacyl-tRNA synthetases from *E. coli* shows that the HIGH region of the cysteine enzyme is most similar to that of the enzymes for arginine [26] (14 residues in common out of 61) and leucine [27] (13 out of 61). By contrast, its KMSK region is most similar to that of the isoleucine enzyme [6] (21 out of 61) (Fig. 2). All the KMSK domains presumably had a single origin among these enzymes, as shown by the use of the same codon for the serine (TCC) (Table I).

A more detailed analysis of the sequence of the cysteinyl-tRNA synthetase reveals that the enzyme can be divided in segments with similarities to synthetases and other proteins. The sequence at the carboxyl end of the HIGH domain of the cysteinyl-tRNA synthetase (amino acids 56–96) resembles one in the elongation factor Tu, the protein which promotes the GTP-dependent binding of aminoacyl tRNA to the A-site of ribosomes in protein biosynthesis (Fig. 3).

Between the HIGH and the KMSK domains there is a segment (amino acids 103–138) shared with the human glutaminyl-tRNA synthetase [28] and a further segment shared with the cytoplasmic leucyl-tRNA syn-

Table I

The DNA sequence for the KMSK region of Class I aminoacyl-tRNA synthetases of *E. coli*

| | Lys | Met | Ser | Lys |
|---|---|---|---|---|
| Cys | AAG | ATG | **TCC** | AAA |
| Leu | AAA | ATG | **TCC** | AAG |
| Val | AAG | ATG | **TCC** | AAA |
| Met | AAG | ATG | **TCC** | AAG |
| Trp | AAG | ATG | **TCC** | AAG |
| Gln | GTG | ATG | **TCC** | AAG |
| Glu | AAA | CTG | **TCC** | AAA |
| Arg | GAT | CTC | **TCC** | AAA |

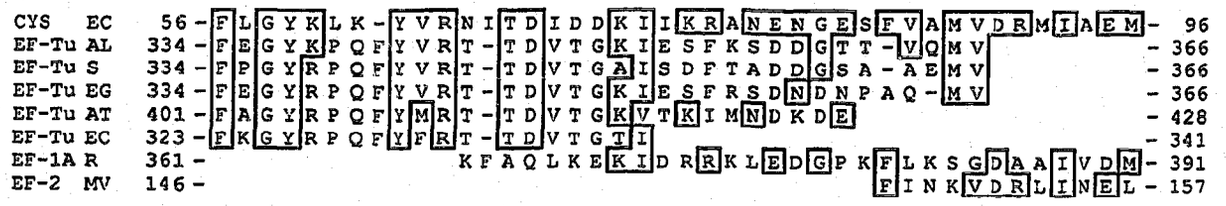


Fig. 3. Sequence comparison between the cysteinyl-tRNA synthetase and elongation factors. The protein sequences were obtained from the PIR database, where the corresponding references can be found. The abbreviations are: EC, *Escherichia coli*; AL, *Astasia longa* chloroplast; S, *Synechococcus* sp; EG, *Euglena gracilis* chloroplast; AT, *Arabidopsis thaliana* chloroplast; R, *Oryctolagus cuniculus* (domestic rabbit); MV, *Methanococcus vannielii*. Only the amino-acids shared with the cysteinyl-tRNA synthetase are boxed.

thetase of *Neurospora crassa* [29] (amino acids 158–193) (Fig. 4). The glutaminyl domain is also present in the *E. coli* and yeast enzymes and is included in a larger segment of similarities with the glutamic enzymes [5]. This segment is in the acceptor binding domain of the *E. coli* glutaminyl-tRNA synthetase [19].

Interestingly, a segment of 34 amino acids (from 360 to 393) appears in a synthetase of the class II: the seryl-tRNA synthetase of *E. coli* [30] (Fig. 4). The corresponding segment in the serine enzyme is located at the end of motif 2 of class II enzymes and is included in the region with similarities to the prolyl and threonyl enzymes around the putative active site of the seryl-tRNA synthetase [19]. Another part of this cysteinyl domain resembles a segment of the tryptophanyl-tRNA synthetase of *Bacillus stearothermophilus* [31]. Finally, a lesser degree of similarity can be found between amino acids 309 and 324 and a segment of the $\alpha$ subunit of the mitochondrial phenylalanyl-tRNA synthetase of *Saccharomyces cerevisiae* [32] (Fig. 4).

The distribution of these domains along the cysteinyl-tRNA synthetase is shown in Fig. 5.

## 4. DISCUSSION

The cyteinyl-tRNA synthetase is clearly a member of the class I synthetases since it has the HIGH and the KMSK domains, as was already found by others [22,23]. Since aminoacyl-tRNA synthetases and translation elongation factors interact with aminoacyl-tRNAs, it is not surprising to find a protein segment shared by the two groups of proteins. Fett and Knippers [28] found the human glutaminyl-tRNA synthetase to have some sequence similarities with eukaryotic elongation factors, and the peptide chain release factor in the rabbit resembles the bacterial and yeast tryptophanyl-tRNA synthetases [33]. It is also known that some aminoacyl-tRNA synthetases of fungal mitochondria are involved in intron splicing [34]. The similarity of the amino-terminal end of the cysteinyl-tRNA synthetase to the acceptor binding domain of the glutaminyl enzyme further supports the idea that the amino end of the cysteinyl-tRNA synthetase is an important domain involved in tRNA recognition. This result also suggests that the proteins involved in the protein translation machinery share common ancestors and have a close evolutionary relationship. A deeper study of the RNA binding domains of the cysteinyl-tRNA synthetase and other synthetases is in progress.

The cysteinyl-tRNA synthetase also has other segments in common with the glutaminyl, the tryptophanyl, and the leucyl enzymes. This further supports a common evolutionary origin for this class of synthetases of which the cysteine enzyme seems to have

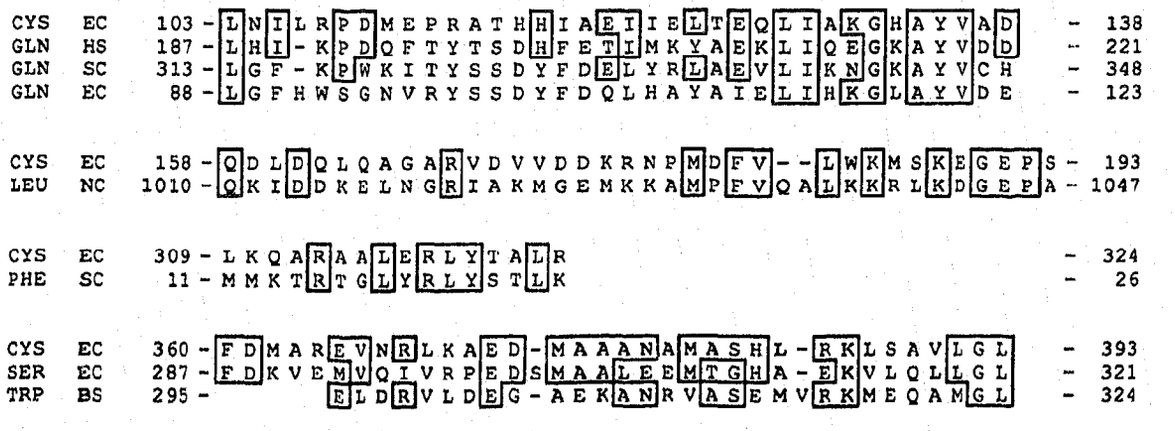


Fig. 4. Sequence comparisons between the cysteinyl-tRNA synthetase and other aminoacyl-tRNA synthetases. The abbreviations are: EC, *Escherichia coli*; HS, *Homo sapiens*; SC, *Saccharomyces cerevisiae*; NC, *Neurospora crassa*; BS, *Bacillus stearothermophilus*. Only the amino-acids shared with the cysteinyl-tRNA synthetase are boxed.

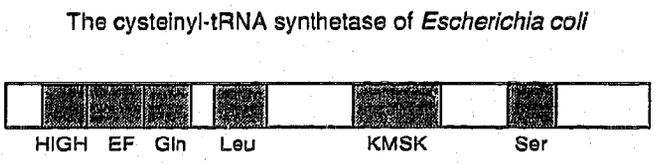


Fig. 5. The most significant domains in the cysteinyl-tRNA synthetase of *Escherichia coli*.

conserved the most ancestral segments. Moreover, the presence in the cysteinyl-tRNA synthetase of a structural and, possibly functional, domain of the seryl-tRNA synthetase (a class II enzyme) suggests that both classes once had a common ancestor.

It is tempting to speculate that the first aminoacyl-tRNA synthetase was like the modern cysteinyl enzyme and perhaps another version of this charged homocysteine. The latter would be the precursor of the modern methionyl enzyme, and the precursor of the enzymes for leucine, valine and isoleucine. The cysteinyl enzyme would then be the direct precursor of the arginine, tryptophan and tyrosine enzymes whose codons differ by one base change from that of cysteine; and arginine to glutamine and then to glutamic acid is another pathway with sequential one base differences. Initially, the cysteinyl enzyme could have been the precursor of the serine-tRNA synthetase, and we note again that both codon representations for serine are one base different from the cysteine codons. Serine would then be the parent of the class II enzymes and it would sequentially give rise to the proline, threonine, asparagine, aspartic acid and lysine enzymes, all of which can be connected by one base change in their respective codons.

We are undertaking studies of the surviving intron structures of these enzymes and this may throw further light on the pathway of evolution.

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