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Specific Sequence Homology and Three-Dimensional Structure of an Aminoacyl Transfer RNA Synthetase

Abstract. *Few and limited amino acid sequence homologies have been found among eight bacterial aminoacyl transfer RNA (tRNA) synthetases whose primary structures are known. The entire 939–amino acid primary structure of* Escherichia coli *isoleucyl-tRNA synthetase is now reported. In a sequence of 11 consecutive amino acids matching a sequence in* E. coli *methionyl-tRNA synthetase, there are ten identical residues and one conservative change. This is the strongest homology recorded between any two aminoacyl tRNA synthetases. This part of the methionine enzyme's three-dimensional structure has been determined, and it occurs in a mononucleotide binding fold; a close three-dimensional structural homology of this part of the enzyme with* Bacillus stearothermophilus *tyrosyl-tRNA synthetase has also been reported. The three synthetases probably fold identically in this region.*

Aminoacyl transfer RNA (tRNA) synthetases presumably arose early in evolution and established the rules of the genetic code by means of the aminoacylation reaction, in which amino acids are matched with trinucleotide sequences within tRNA molecules. It has appeared that the evolutionary relations between these canonical enzymes are weak because (i) the enzymes vary in size, (ii) there are diverse quaternary structures (*1*), and (iii) sequence homologies between the bacterial enzymes sequenced so far are sparse or nonexistent (*2–5*). We now report the first strong homology between any two synthetases and further evidence that, in spite of the various sizes of these enzymes, the catalytic portions are toward the amino terminus.

A restriction map of an 8-kilobase (kb) segment of *Escherichia coli* K12 DNA cloned into plasmid pGM21 (*6*) (Fig. 1) shows three coding regions that have been defined: ribosomal protein S20 (*7*), Ile-tRNA synthetase, and an overlapping unidentified open reading frame that would encode a polypeptide approximately 17 kilodaltons (kD) in size. The organization of restriction sites in this region suggests that the open reading frame encodes the prolipoprotein signal peptidase (*8*); this suggestion has been confirmed (*9*). We sequenced the coding region for Ile-tRNA synthetase and extended the sequencing through the region encoding the 17-kD polypeptide.

The long open reading frame encoding Ile-tRNA synthetase is located 1.4 kb from the gene encoding S20 and is transcribed in the opposite direction from it. Extensive polypeptide sequence information was independently obtained from the enzyme isolated from *E. coli* MRE600. The amino and carboxyl terminal sequences determined for the polypeptide (*10*) match exactly those encoded by the long open reading frame of the DNA sequence, rigorously defining the location and span of the *ileS* coding region.

The translated amino acid sequence of *E. coli* K12 Ile-tRNA synthetase (Fig. 2) shows stretches of the translated polypeptide sequence that were established by amino acid sequencing of purified peptides isolated from digests of the *E. coli* MRE600 protein. Altogether about 70 percent of the sequence was independently confirmed in this way. There is a strong amino acid sequence homology between residues 57 to 67 of Ile-tRNA synthetase and residues 14 to 24 of *E. coli* Met-tRNA synthetase (*4*) (Fig. 3, a and b). The homology starts with a proline which, in the methionine enzyme's structure (*11*), is near the end of the first β segment of the alternating βαβ . . . structure that is characteristic of mononucleotide binding folds. The subsequent residues (15 to 24) complete the β piece and form a characteristic loop which joins that piece to the first α-helix segment.

The significance of this region is reinforced by its strong three-dimensional structural homology, if not perfect sequence homology, with an identical structural arrangement near the amino terminus of *Bacillus stearothermophilus* Tyr-tRNA synthetase (Fig. 3c) (*12, 13*). In that enzyme the corresponding proline occurs at position 39, and the local conformation of the chain is almost superimposable with that of the methionine enzyme. The α carbons of residues 14 to 28 of the methionine enzyme, for example, have a root-mean-square deviation of 1.8 Å when they are superimposed on residues 38 to 52 of the tyrosine enzyme (*12*).

It is with this part of the methionine enzyme that the strong amino acid sequence homology occurs with Ile-tRNA synthetase (Fig. 3b). This sequence homology and the close structural homology (in this region) between the Tyr- and Met-synthetases implies that Ile-tRNA synthetase is folded in the same way. The *E. coli* Gln-tRNA synthetase is also homologous in exactly the same region (Fig. 3d). We surmise that this is because of powerful selective pressures exerted on the structure in this region.

One constraint is possibly the contact points between adenosine triphosphate (ATP) (or the adenyl part of the adenylate intermediate) and the protein. In the tyrosine enzyme, His^{48} is probably hydrogen-bonded to a ribose oxygen and His^{45} is in close proximity (*12*). Although ATP binds in the analogous place in the methionine enzyme, structural details are still unresolved (*12*). The two analogous histidines are at positions 22 and 25 in the methionine enzyme and align with histidines 64 and 67 of Ile-tRNA synthetase.

The αβ mononucleotide fold in Tyr- and Met-tRNA synthetases extends over

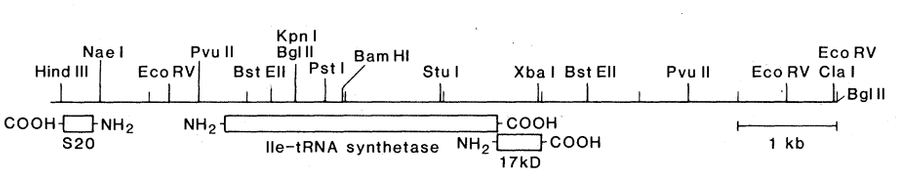

Fig. 1. Restriction map of an 8-kb insert of plasmid pGM21, showing the location of the coding regions for ribosomal protein S20, Ile-tRNA synthetase, and a 17-kD protein.

more than 200 amino acids, and the homology shown in Fig. 3 is at the beginning of this structure (*12*). Although the tyrosine and methionine enzymes show little sequence homology over this region, there is considerable topological equivalence (*12*). We suspect that the structure of Ile-tRNA synthetase is laid out in the same way and that the mononucleotide fold encompasses perhaps 150 to 200 residues beyond the sequence homology shown in Fig. 3.

N-Ethylmaleimide preferentially reacts with a specific cysteine in Ile-tRNA synthetase and inactivates the enzyme. This inactivation is prevented by bound isoleucine (*14*). L-Isoleucyl bromoethyl ketone reacts with the same cysteine and also inactivates the enzyme. The tryptic peptide that contains this cysteine has been sequenced (*15*). We aligned exactly this sequence (of 15 amino acids) with a section of the primary structure shown in Fig. 2 and found that Cys^{463} is the reactive residue. This places the isoleucine affinity–labeled site within the amino-terminal half and on the carboxyl-terminal side of the mononucleotide fold.

This arrangement would indicate that adenylate synthesis is done on the amino-terminal half of Ile-tRNA synthetase. The same arrangement is suggested for several other synthetases. An analysis of protein fragments generated by gene deletions shows that the carboxyl-terminal half of *E. coli* Ala-tRNA synthetase (875 amino acids) is dispensable for aminoacylation activity and that only the amino-terminal 385 residues are necessary for adenylate synthesis (*16*). At least 200 amino acids at the carboxyl terminus of Met-tRNA synthetase (approximately 750 amino acids in length) are dispensable for aminoacylation activity (*4*). About 100 residues can be removed from the carboxyl terminus of Tyr-tRNA synthetase (419 amino acids in length) with retention of adenylate synthesis activity (*17*).

Ultraviolet irradiation of an *E. coli* Ile-tRNA synthetase–ATP complex couples ATP to the enzyme with concomitant inactivation. A labeled pentapeptide has been isolated and sequenced (*18*), and four of five residues match with a sequence in the *E. coli* K12 enzyme. This sequence is just seven amino acids from the carboxyl terminus. Because the comparison is with proteins from different strains of *E. coli* and because the match is over only a short stretch of sequence, this particular assignment in the structure is not rigorous.

The sequence shown in Fig. 2 contains no significant repeats, in contrast with an earlier report of repeated sequences for

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M S D Y K S T L N L P E T G F P M R G D L A K R E P G M L A R W T D D D L Y G I   40
I R A A K K G K K T F I L H D G P P Y A N G S I H I G H S V N K I L K D I I V K   80
S K G L S G Y D S P Y V P G W D C H G L P I E L K V E Q E Y G K P G E K F T A A   120
E F R A K C R E Y A A T Q V D G Q R K D F I R L G V L G D W S H P Y L T M D F K   160
T E A N I I R A L G K I I G N G H L H K G A K P V H W C V D C R S A L A E A E V   200
E Y Y D K T S P S I D V A F Q A V D Q D A L K A K F A V S N V N G P I S L V I W   240
T T R R G L C L P T A Q S L L H Q I S T M R W W Q I D G Q A V I L A K D L V E S   280
V M Q R I G V T D Y T I L G T V K G A D V E L L R F T H P F M G F D V P A I L G   320
D H V T L D A G T G A V H T A P G H G P D D Y V I G Q K Y G L E T A N P V G P D   360
G T Y L P G T Y P T L D G V N V F K A N D I V V A L L Q E K G A L L H V E K M Q   400
H S Y P C C W R H K T P I I F R A T P Q W F V S M D Q K G L R A Q S L K E I K G   440
V Q W I P D W G Q A R I E S M V A N R P D W C I S R Q R T W G V P M S L F V H K   480
D T E E L H P R T L E L M E E V A K R V E V D G I Q A W W D L D A K E I L G D E   520
A D Q Y V K V P D T L D V W F D S G S T H S S V V D V R P E F A G H A A D M Y L   560
E G S D Q H R G W F M S S L M I S T A M K G K A P Y C Q V L T H G F T V D G Q G   600
R K M S K S I G N T V S P Q D V M N K L G A D I L R L W V A S T D Y T G Q M A V   640
S D E I L K R A A D S Y R R I R N T A R F L L A N L N G F D P A K D M V K P E E   680
M V V L D R W A V G C A K A A Q E D I L K A Y E A Y D F H E V V Q R L M R F C S   720
V E M V S F Y L D I I K D R Q Y T P K R T V W A R R S C Q T A L Y H I A E A L V   760
R W M A P I L S F T A D E V W G Y L P G E R E K Y V L T G E W Y E G L F G L A D   800
S E A M N D A F W D E L L K V R G E V N K V I E Q A R A D N K V G G S L E A A V   840
T L Y A E P E L S A K L T A L G D E L R F V L L T S D R R Y V A D Y N D A P A D   880
A Q Q S E V L K G L K V A L S K A E G E K C P R C W H Y T Q D V G K V A E H A E   920
I C G R C V S N V A G D G E K R K F A  939

...GCCTGATGAGT...

M S ...
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a  1.1 T C A L P Y A N G S I H L G H M L E H 29
b  53 H D G P P Y A N G S I H I G H S V N K 71
c  34 Y C G F D P T A D S L H I G H L A T I 52
d  30 T R F P P E P N G Y L H I G H A K S I 48
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Fig. 2 (left). Amino acid sequence of *E. coli* K12 Ile-tRNA synthetase, which is translated from the DNA sequence. Amino acids are numbered with +1 taken as the methionine initiator. The beginning of the DNA and amino acid sequence of an overlapping open reading frame is also shown. The A of the first of two Ile-tRNA synthetase TGA stop codons is the A of the ATG initiation codon of this open frame. Underlined are sequences obtained independently from Edman degradations of fragments of the *E. coli* MRE600 Ile-tRNA synthetase. Differences between the translated amino acid sequence for the *E. coli* K12 gene and the sequence of the *E. coli* MRE600 purified protein are indicated with a dashed line. DNA sequencing was carried out by the chain-termination method (*21*), and single-stranded DNA templates were generated by use of M13 mp8 phages as described (*22*). Overlapping 4- and 6-base recognition site restriction fragments from plasmid pGM21 were cloned into M13 and sequenced. The plasmid DNA isolations, restriction endonuclease digestions, isolation of DNA fragments, and materials were as described (*5*). The one-letter notation for amino acid residues is A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine. A partial nucleotide sequence is shown below the amino acid sequence at the location of the overlapping reading frame which starts with M and is followed by S.

Fig. 3 (right). Alignment of conserved amino acid sequences showing the strong homology between (a) *E. coli* Met-tRNA synthetase and (b) Ile-tRNA synthetase. The 11–amino acid homology is boxed and includes ten identical matches and one conservative substitution. An alignment with (c) *B. stearothermophilus* Tyr-tRNA synthetase (*23*) and with (d) *E. coli* Gln-tRNA synthetase (*3*) is also shown. Amino acids are numbered with respect to the first codon (ATG) of each structural gene. The one-letter notation for amino acid residues is as given in the legend to Fig. 2.

Ile-tRNA synthetase (*19*). This is the longest synthetase polypeptide sequenced and, together with the lack of repeats for the other sequenced synthetases, suggests that gene duplications did not play a role in the evolution of these enzymes.

The amino acid sequence homology between Ile- and Met-tRNA synthetases is remarkable in view of the lack of homology between other synthetases. Wetzel pointed out that, based on several correlations, Ile- and Met-tRNA synthetases are part of a small subfamily of synthetases that may have an evolutionary relation that is closer than average (*20*). Sequences of the valine and leucine enzymes are of special interest in this regard.

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Inability of Mouse Blastomere Nuclei Transferred to Enucleated Zygotes to Support Development in Vitro

Abstract. *More than 90 percent of enucleated one-cell mouse embryos receiving pronuclei from other one-cell embryos successfully develop to the blastocyst stage* in vitro. *In this investigation, nuclei from successive preimplantation cleavage stages were introduced into enucleated one-cell embryos and the embryos were tested for development* in vitro. *Although two-cell nuclei supported development to the morula or blastocyst stage, four-cell, eight-cell, and inner cell mass cell nuclei did not. The inability of cell nuclei from these stages to support development reflects rapid loss of totipotency of the transferred nucleus and is not the result of simultaneous transfer of membrane or cytoplasm.*

Ever since it was found that transplantation of blastula nuclei to enucleated *Rana pipiens* zygotes can result in normal embryogenesis (*1*), the ability of nuclei from various sources to support development has been a subject of investigation. Thus, when it was shown that endodermal nuclei support the development of fertile adults from ultraviolet-irradiated *Xenopus laevis* eggs (*2*), it was concluded that early embryonic nuclei remain unrestricted in their developmental potential in amphibians. The developmental potential of nuclei from later stage embryos and adults is less certain. Although several investigators have reported characteristic developmental abnormalities associated with transplantation of nuclei from differentiated tissues into *R. pipiens* zygotes (*3*), some nuclei from tadpole intestinal epithelium retain the ability to support complete development (*4*). Thus it remains unclear whether embryonic development and differentiation are accompanied by a restriction in nuclear potential to support normal development (*5*).

More recently, this restriction in mammals has become amenable to experimental analysis. It has been reported (*6*)

Table 1. Development of mouse embryos with transplanted nuclei. Embryos were obtained from spontaneous inter se matings of ICR (Swiss albino) or C57BL6/J mice. Embryo isolation and culture in vitro and the isolation of single ICM cells from immunosurgically obtained ICM's (*14*) were as previously described (*8*), as were enucleation and nuclear transfer. One-cell embryos were incubated in Whitten medium (*15*) containing cytochalasin B (5 μg/ml; Sigma) and demecolcine (0.1 μg/ml; Sigma) for 15 to 30 minutes before microsurgery. Enucleation of both pronuclei was performed and karyoplasts obtained from one-, two-, four-, or eight-cell embryos also exposed to enucleation medium were fused with enucleated one-cell embryos by using inactivated Sendai virus. Embryos having ICM cell nuclei were obtained by similarly fusing single ICM cells with enucleated one-cell embryos. All manipulations were performed at room temperature with a Leitz hanging-drop oil chamber. Embryos that underwent fusion were washed and cultured for 5 days in drops of Whitten medium containing 100 μ*M* disodium EDTA (*16*) under silicone oil in an atmosphere of 5 percent O_2, 5 percent CO_2, and 90 percent N_2. The proportion of embryos surviving the removal of both pronuclei was 99 percent (481 of 486).

Nuclear donor	Recipient	Number of embryos fused	Development			Percent*
			Sub-morula	Morula	Blastocyst	
Zygote	Enucleated zygote	21 of 21	1	0	20	95
Two-cell stage	Enucleated zygote	151 of 174	123	9	19	19
Four-cell stage	Enucleated zygote	81 of 84	77	4	0	5
Eight-cell stage	Enucleated zygote	111 of 116	111	0	0	0
ICM	Enucleated zygote	84 of 101	84	0	0	0
None	Nonenucleated zygote†		27	22	182	88
None	Nonenucleated zygote‡		1	0	11	92

*Percentage of embryos developing to morula or blastocyst stage. †Nonenucleated (control) zygotes were used in parallel with each series of experiments. ‡Nonenucleated (control) zygotes exposed to enucleation medium and Sendai virus.