Human Tyrosyl-tRNA Synthetase Shares Amino Acid Sequence Homology with a Putative Cytokine*

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Theresa A. Kleeman‡, Dongbing Wei‡, Keith L. Simpson, and Eric A. First§

From the Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, Louisiana 71130-3932

To test the hypothesis that tRNA\(^{\text{Tyr}}\) recognition differs between bacterial and human tyrosyl-tRNA synthetases, we sequenced several clones identified as human tyrosyl-tRNA synthetase cDNAs by the Human Genome Project. We found that human tyrosyl-tRNA synthetase is composed of three domains: 1) an amino-terminal Rossmann fold domain that is responsible for formation of the activated E\(^{\text{Tyr}}\)-AMP intermediate and is conserved among bacteria, archaea, and eukaryotes; 2) a tRNA anticodon recognition domain that has not been conserved between bacteria and eukaryotes; and 3) a carboxyl-terminal domain that is unique to the human tyrosyl-tRNA synthetase and whose primary structure is 49% identical to the putative human cytokine endothelial monocyte-activating protein II, 50% identical to the carboxyl-terminal domain of methionyl-tRNA synthetase from Caenorhabditis elegans, and 45% identical to the carboxyl-terminal domain of Arc1p from Saccharomyces cerevisiae. The two first domains of the human tyrosyl-tRNA synthetase are 52, 36, and 16% identical to tyrosyl-tRNA synthetases from S. cerevisiae, Methanothermobius jannaschii, and Bacillus stearothermophilus, respectively. Nine of fifteen amino acids known to be involved in the formation of the tyrosyl-adenylate complex in B. stearothermophilus are conserved across all of the organisms, whereas amino acids involved in the recognition of tRNA\(^{\text{Tyr}}\) are not conserved. Kinetic analyses of recombinant human and B. stearothermophilus tyrosyl-tRNA synthetases expressed in Escherichia coli indicate that human tyrosyl-tRNA synthetase aminoacylates human but not B. stearothermophilus tRNA\(^{\text{Tyr}}\), and vice versa, supporting the original hypothesis. It is proposed that like endothelial monocyte-activating protein II and the carboxyl-terminal domain of Arc1p, the carboxyl-terminal domain of human tyrosyl-tRNA synthetase evolved from gene duplication of the carboxyl-terminal domain of methionyl-tRNA synthetase and may direct tRNA to the active site of the enzyme.

Aminoacyl-tRNA synthetases catalyze the aminoacylation of tRNA by their cognate amino acid. For most aminoacyl-tRNA synthetases (E), tRNA aminoacylation can be separated into two steps: formation of a stable enzyme-bound aminoacyl-adenylate intermediate (E:AA-AMP, Equation 1), followed by transfer of the amino acid (AA) from the aminoacyl-adenylate intermediate to the 3’ end of the tRNA substrate (Equation 2).

\[
E + AA + ATP \rightleftharpoons E \cdot AA-AMP + PP_i \quad \text{(Eq. 1)}
\]

\[
E \cdot AA-AMP + tRNA \rightleftharpoons E + AA-tRNA + AMP \quad \text{(Eq. 2)}
\]

The 20 different aminoacyl-tRNA synthetases fall into two distinct structural classes (1, 2). Class I aminoacyl-tRNA synthetases (of which tyrosyl-tRNA synthetase is a member) are characterized by a structurally well conserved amino-terminal Rossmann fold domain which contains the signature sequences “HIGH” and “KMSKS” (3, 4). In contrast, the carboxyl-terminal domains of class I aminoacyl-tRNA synthetases are structurally diverse suggesting that the primordial aminoacyl-tRNA synthetase consisted solely of the amino-terminal Rossmann fold (5–8). Both x-ray crystallography and site-directed mutagenesis of class I aminoacyl-tRNA synthetases indicate that the amino-terminal domains are responsible for catalyzing the first step of the aminoacylation reaction and recognizing the 3’ end of the tRNA substrate, whereas the carboxyl-terminal domain is responsible for recognition of the anticodon loop of the tRNA substrate (6, 7, 9–15).

Transfer RNA molecules also occur as one of two distinct types, although there does not appear to be a correlation between the two types of tRNA molecules and the two classes of aminoacyl-tRNA synthetase. Tyrosyl-tRNA (tRNA\(^{\text{Tyr}}\)) is unique among tRNAs in that in bacteria it is type II (which contains an extended variable loop), whereas in eukaryotes it is type I (16, 17). Furthermore, previous studies indicate that bacterial and eukaryotic tyrosyl-tRNA synthetases do not catalyze the aminoacylation of other tRNA\(^{\text{Tyr}}\) substrates suggesting that tRNA\(^{\text{Tyr}}\) recognition differs between bacteria and eukaryotes (17–21). If this hypothesis is correct, it should be possible to exploit this species specificity to design novel antibiotics that selectively inhibit bacterial tyrosyl-tRNA synthetases. To test this hypothesis, we have sequenced the cDNA for human tyrosyl-tRNA synthetase and expressed the recombinant protein in Escherichia coli cells. During the course of these investigations, it was discovered that the human tyrosyl-tRNA synthetase consists of three distinct domains, an amino-terminal Rossmann fold domain, an anticodon recognition domain, and an idiosyncratic carboxyl-terminal domain whose amino acid sequence is 49% identical to the putative human cytokine endothelial monocyte-activating protein II (EMAP II), \(^1\) 50% identical to the carboxyl-terminal domain of methionyl-tRNA synthetase from Caenorhabditis elegans, and 43% by guest on September 24, 2017 http://www.jbc.org/ Downloaded from

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\textsuperscript{TM}/EBI Data Bank with accession number(s) U89436.

‡ These authors contributed equally to this research.

§ To whom correspondence should be addressed. Tel.: 318-675-7779; Fax: 318-675-5180; E-mail: efirst@lsumc.edu.

\(^1\) The abbreviations used are: EMAP II, endothelial monocyte-activating protein II; Ni-NTA, nickel-nitrilotriacetic acid.
identical to the Arc1p protein (also known as G4p1), which has been postulated to direct tRNA to active sites of the methionyl- and glutamyl-tRNA synthetases in *Saccharomyces cerevisiae* (22). This is in contrast to all other known tyrosyl-tRNA synthetase sequences, which possess only the first two domains. The results of these analyses and possible implications of the findings are presented here.

**EXPERIMENTAL PROCEDURES**

**Materials**—cDNA clones 53277 and 124918 (I.M.A.G.E. clone accession numbers) were obtained from the I.M.A.G.E. Consortium (23). All other cDNA clones were obtained from The Institute for Genome Research (TIGR) or the American Type Culture Collection (ATCC) and are designated by their TIGR clone numbers. Reagents and vectors were purchased from the following sources: pET-30a vector and S-Tag Western blot kit (Novagen), Ni-NTA resin and QIAquick gel extraction kit (Qiagen), Wizard PCR prep system and recombinant enterokinase (Promega), NuSieve low melting point agarose (FMC), Taq polymerase (Amersham), λ [γ-32P]dATP (Life Technologies, Inc.), and oligonucleotides (Life Technologies, Inc.). Automated DNA sequencing was performed by the DNA sequencing facility at Iowa State University using dye-labeled dideoxy terminators. All other chemicals and reagents were purchased from Fisher.

**Plasmid Purification and DNA Sequencing**—All plasmids were purified on CsCl gradients (24, 25). For clone 186313 and the pHYTS1 expression plasmid, both DNA strands were sequenced using the following primers2: M13 reverse primer (−190), HYTS Exp Fwd (21), HYTS-S1 (181), HYTS-S3 (460), HYTS-S5 (794), HP97 (1083), HP99 (1338), HYTS-S2 (193), HYTS-S4 (494), HYTS-S6 (818), HP87 (1104), HP10 (1407), HYTS Exp Rvs (1626). For all other clones, the sense strand was sequenced in entirety, and ambiguities in the sequences were resolved by sequencing the appropriate regions of the complementary strand. SeqEd (v1.0.3, Applied Biosystems) was used to align overlapping sequences, assess the quality of the sequencing results, and determine the consensus sequence for each cDNA.

Data base searching was performed using the European Bioinformatics Institute implementation of FASTA (26), the Stanford University implementation of BLAST (27), and the Baylor College of Medicine implementation of *BEAUTY* (28). Multiple alignment of amino acid sequences was performed using the Baylor College of Medicine implementation of ClustalW (29, 30). All data base searches and multiple sequence alignment methods used the parameters optimized for each program implementation.

**Construction of the pHYTS2 Expression Plasmid**—Subcloning of the human tyrosyl-tRNA synthetase cDNA from clone 186313 into the pET-30a vector for expression of recombinant tyrosyl-tRNA synthetase protein in *E. coli* was performed in the following manner. First, the full-length human tyrosyl-tRNA cDNA from clone 186313 was amplified using the polymerase chain reaction with the HYTS Exp Fwd and HYTS Exp Rvs primers described above. These primers contain *Bgl*II and *HinIII* sites near their 5′ termini, respectively. Following amplification, the human tyrosyl-tRNA synthetase cDNA was gel-purified, digested with *Bgl*II and *HinIII*, and ligated into a pET-30a (+) vector that had been previously digested with *BamHI* and *HindIII*. The resulting plasmid, designated pHYTS1, contained an in-frame gene fusion between the 44-amino acid amino-terminal His-tag/S-tag leader sequence present in the pET-30a (+) vector and the human tyrosyl-tRNA synthetase cDNA. Both strands of pHYTS1 were sequenced, and a single *Bgl*II cleavage site was found at nucleotide 1000 (converting a lysyl codon into an isoleucyl codon in the mRNA sequence). To correct this mutation clone 186313 was digested with *BamHI* and *NheI*, and the fragment corresponding to nucleotides 963–1582 was purified on 1.5% agarose. This 620-base pair fragment was ligated into pHYTS that had been digested with *BamHI* and *NheI*. The resulting construct, designated pHYTS2, was sequenced to ensure that its nucleotide sequence is identical to the human tyrosyl-tRNA synthetase sequence found in clone 186313.

**Expression of Recombinant Human and *B. stearothermophilus* Tyrosyl-tRNA Synthetases in *E. coli**—pB21DE3 pLYS8 *E. coli* cells harboring the pHYTS2 plasmid were grown to saturation at 37 °C in 2 × TY medium containing 50 μg/liter kanamycin and 0.5 mM isopropyl-β-D-thiogalactopyranoside. Recombinant human tyrosyl-tRNA synthetase was harvested as described by Qiagen (protocol 5 in the QIAPrepation-ist). Briefly, this involved lysing the cells and binding the His-tagged recombinant human tyrosyl-tRNA synthetase to Ni-NTA resin. After washing the Ni-NTA resin with 50 mM sodium phosphate, 300 mM NaCl, 10% glycerol, pH 6.0 buffer, recombinant human tyrosyl-tRNA synthetase was eluted from the column using a step gradient from 0 to 0.5 M imidazole. Recombinant human tyrosyl-tRNA synthetase was then dialyzed against buffer A (20 mM Tris, pH 7.8, 1 mM EDTA, 5 mM β-mercaptoethanol) containing 1 mM sodium pyrophosphate (to remove any enzyme-bound tyrosyl adenylate) and 0.1 mM phenylmethylsulfonyl fluoride, followed by three changes of buffer A without sodium pyrophosphate. The final dialysis buffer contained 10% glycerol. *B. stearothermophilus* tyrosyl-tRNA synthetase was purified from TG2 cells harboring the pYTS5 plasmid as described previously (31). The concentrations of human and *B. stearothermophilus* tyrosyl-tRNA synthetases were determined by active site titration using λ-[γ-32P]tyrosine to monitor tyrosyl-adenylate formation (32). SDS-polyacrylamide gel electrophoresis (33) and modified Western blot analysis using the S-Tag Western blot kit (34) were used to assess the purity of the tyrosyl-tRNA synthetases and to estimate the molecular weight of the recombinant human tyrosyl-tRNA synthetase.

**In Vitro Transcription and Purification of Human and *B. stearothermophilus* tRNA<sup>tyr</sup>**—*B. stearothermophilus* tRNA<sup>tyr</sup> was transcribed *in vitro* from pGFX-WT, a derivative of the plasmid pGAG2 (35) in which a FoK1 restriction site replaces the BstN1 cleavage site in pGAG2 such that digestion with FoK1, and subsequent in *vitro* transcription of the digested pGFX-WT plasmid by T7 RNA polymerase, produces tRNA<sup>tyr</sup> containing an intact -CCA 3′ terminus. Details of the construction of the pGFX-WT plasmid will be published elsewhere.

Three consecutive rounds of polymerase chain reaction mutagenesis (36) were used to replace the *B. stearothermophilus* tRNA<sup>tyr</sup> in pGFX-WT with the cDNA sequence for human placental tRNA<sup>tyr</sup> (GenBank<sup>3</sup> accession number HsTrny1), generating the pHYR-WT plasmid. Digestion of pHYR-WT by FoK1, followed by *in vitro* transcription using T7 RNA polymerase, yields the human tRNA<sup>tyr</sup> product. *In vitro* transcription of the *B. stearothermophilus* and human tRNA<sup>tyr</sup> substrates from the pGFX-WT and pHYR-WT plasmids was performed as described by Avis *et al.* (35) with the modification that the plasmids were digested with FoK1 rather than BstN1. *In vitro* transcribed tRNA<sup>tyr</sup> products were purified on 12% polyacrylamide gels (37). Purified tRNA<sup>tyr</sup> products were resuspended in diethylylcarbonate treated water containing 10 mM MgCl₂, heated to 75 °C, and slowly cooled to room temperature over a period of 30–60 min to allow annealing of the tRNA<sup>tyr</sup> substrates to occur.

**RESULTS**

**Comparison of Human Tyrosyl-tRNA Synthetases from Different cDNA Libraries**—To ensure that the tyrosyl-tRNA synthetase cDNA used in subsequent experiments corresponds to the predominant form found in humans, cDNAs from several different libraries were sequenced. Specifically, the following cDNAs were sequenced:4 186313 (TIGR, adult white blood cells), 151265 (TIGR, female infant brain), 160622 (TIGR, adult white blood cells), 109082 (TIGR, adult colon), 132369 (TIGR, six week old infant), 53277 (I.M.A.G.E. Consortium, infant brain), and 124918 (I.M.A.G.E. Consortium, fetal liver, spleen). Clones 186313, 151265, and 160622 contain full-length cDNA sequences, clone 109082 is missing the first 181 nucleotides of the coding sequence, clone 132369 is missing the first 792 nucleotides of the coding sequence, clone 53277 is missing the first 426 nucleotides of the coding sequence, and the nucleotide sequence of clone 124918 corresponds to that of the human tyrosyl-tRNA synthetase cDNA coding sequence through nu-

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2 The first seven primers are complementary to the antisense strand of the cDNA, and the remaining six primers are complementary to the sense strand; positions of the 3′ ends of each primer relative to the ATG start codon are indicated in parentheses following the primer; all primers except HYTS Exp Fwd and HYTS Exp Rvs are 18 to 21 nucleotides in length.

3 Clone numbers refer to either the TIGR or I.M.A.G.E. Consortium accession numbers; clone source and tissues used to construct the cDNA libraries are shown in parentheses following the clone number.

4 The sequences of the initial clones we received bearing these accession numbers did not correspond to their expressed sequence tag sequences. We subsequently obtained the correct clones bearing these accession numbers from ATCC. TIGR and ATCC have since withdrawn these clones from their inventory of publicly available clones.
cleotide 685, and then diverges into an unrelated sequence. In all of the cDNA clones examined (with the exception of the truncated clone 124918), the nucleotide sequence differs from the previously reported cDNA sequence for human tyrosyl-tRNA synthetase5 (39) by the insertion of a cytosine at position 1061. This additional nucleotide base alters the reading frame of the cDNA sequence, which extends the open reading frame an additional 525 nucleotides.

Within the coding sequence, we observed the following heterogeneity among the cDNA clones. In clone 151265, nucleotides A484 through C 489 are absent, resulting in deletion of serine 161 and glycine 162 in the translated protein. Clone 160622 has a guanosine deleted at position 7 in the coding sequence and a thymine inserted at position 479, resulting in an open reading frame from nucleotide 631 to nucleotide 1584.

Comparison of the Human Tyrosyl-tRNA Synthetase Amino Acid Sequence with Tyrosyl-tRNA Synthetase from Other Species—If the hypothesis that tRNA Tyr recognition differs between eukaryotic and bacterial tyrosyl-tRNA synthetases is correct, one would expect these differences to be reflected in both the nucleotide sequences of tRNA Tyr and the amino acid sequences of the tyrosyl-tRNA synthetases. Comparison of tRNA Tyr sequences indicates that in bacteria, tRNA Tyr is a type II tRNA, whereas in eukaryotes tRNA Tyr is a type I tRNA (16, 17), supporting the above hypothesis. Comparison of the amino acid sequences of tyrosyl-tRNA synthetases further supports the above hypothesis. Human tyrosyl-tRNA synthetase is 53% identical at the amino acid level to the S. cerevisiae homologue (excluding the carboxyl-terminal domain of human tyrosyl-tRNA synthetase), 36% identical to the homologue from the archaeon Methanococcus jannaschii, and 16% identical to the B. stearothermophilus homologue (Fig. 1). Notably, nine of the fifteen amino acids involved in stabilizing the transition state for the first step of the reaction in the B. stearothermophilus enzyme (11, 40, 41) are conserved in the human, M. jannaschii, and S. cerevisiae enzymes. In contrast, none of the eleven amino acids known to be involved in tRNA Tyr recognition (42–47) are conserved between the human and B. stearothermophilus tyrosyl-tRNA synthetases, suggesting that in contrast to the mechanism for formation of the E Tyr-AMP intermediate, tRNA Tyr recognition differs between eukaryotes, archaea, and bacteria. This is most apparent in the M. jannaschii amino acid sequence, which surprisingly is missing a substantial portion of the tRNA Tyr anticodon recognition domain (amino acids 330–419 in the B. stearothermophilus enzyme).

Human Tyrosyl-tRNA Synthetase Contains an EMAP II-like Domain—FASTA and BLAST searches of the GenBank nucleotide sequence data base identified proteins with significant homology to the carboxyl-terminal domain of human tyrosyl-tRNA synthetase. In particular, the EMAP II initially isolated from murine fibrosarcoma cells (48), methionyl-tRNA synthetase from C. elegans (49), and the Arc1p protein from S. cerevisiae (22) were found to be 49, 50, and 43% identical to the carboxyl-terminal domain of human tyrosyl-tRNA synthetase (Fig. 2). The sequence similarities observed between Arc1p, EMAP II, and the methionyl-tRNA synthetases have been previously re-

\[5 \text{C. L. Quinn, GenBank}^\text{TM} \text{ accession number U40714.}\]
port(M. Mirande, personal communication).

Furthermore, EMAP II has recently been found to be identical to the p18 protein component of the 24 S aminoacyl-tRNA synthetase complex found in mammalian cells. To quantify the similarity between the amino acid sequences for the carboxyl-terminal domains of the human tyrosyl- and methionyl-tRNA synthetases, we analyzed expressed sequence tags that have been putatively identified as the carboxyl-terminal domain of human methionyl-tRNA synthetase (I.M.A.G.E. clone accession numbers 33689, L1914, and 46655).

The nucleotide sequence of the carboxyl-terminal domain of tyrosyl-tRNA synthetase was 100% identical to a consensus sequence of these expressed sequence tags, suggesting that the human tyrosyl-tRNA synthetase was 100% identical to a consensus sequence of these expressed sequence tags, suggesting that the human methionyl-tRNA synthetase does not have an enzyme, the human methionyl-tRNA synthetase does not have an enzyme, the human methionyl-tRNA synthetase does not have an enzyme, the human methionyl-tRNA synthetase does not have an enzyme, the human methionyl-tRNA synthetase does not have an enzyme, the human methionyl-tRNA synthetase does not have an enzyme, the human methionyl-tRNA synthetase does not have an enzyme. This is consistent with our hypothesis.

Expression of the Recombinant Human Tyrosyl-tRNA Synthetase in E. coli—To further characterize the human tyrosyl-tRNA synthetase, the cDNA sequence from clone 186313 was subcloned into the pET-30a vector, and the resulting plasmid was designated pHYTS2. Recombinant human tyrosyl-tRNA synthetase purified from BL21DE3 pLysS cells harboring pHYTS2 contains both a His-tag and an S-tag on its amino terminus (34, 51). SDS-polyacrylamide gel electrophoresis of the recombinant human tyrosyl-tRNA synthetase at various stages of purification is shown in Fig. 3. Based on its mobility on SDS-polyacrylamide gel electrophoresis, the recombinant protein has an apparent molecular mass of approximately 65,500 Da. This is in good agreement with the predicted molecular mass of 69,773 Da and previously published molecular mass estimates for other mammalian tyrosyl-tRNA synthetases (18, 52) and indicates that the carboxyl-terminal domain of the recombinant enzyme is translated in E. coli. Active site titration confirms that the purified recombinant enzyme is active and able to form the E-Tyr-AMP intermediate (data not shown).

To determine whether there are mechanistic differences in the way human and bacterial tyrosyl-tRNA synthetases recognize and aminoacylate their cognate tRNA substrates, tRNA<sup>Tyr</sup> substrates corresponding to tRNA<sup>Tyr</sup> from B. stearothermophilus and human placenta (54) were transcribed in vitro, gel purified, and annealed. These tRNAs were used as substrates for aminoacylation by human and B. stearothermophilus tyrosyl-tRNA synthetase. As shown in Fig. 4, recombinant human tyrosyl-tRNA synthetase fully aminoacylates the human tRNA<sup>Tyr</sup> but fails to aminoacylate more than 10% of the B. stearothermophilus tRNA<sup>Tyr</sup> during the time course of the assay. This is in contrast to the recombinant B. stearothermophilus tyrosyl-tRNA synthetase, which fully aminoacylates B. stearothermophilus tRNA<sup>Tyr</sup> but fails to aminoacylate the human tRNA<sup>Tyr</sup> above background levels.

**DISCUSSION**

If the hypothesis that tRNA<sup>Tyr</sup> recognition differs between bacterial and eukaryotic tyrosyl-tRNA synthetases is correct, one would predict that these differences should show up in three different ways. First, the nucleotide sequences of the tRNA<sup>Tyr</sup> substrates should differ significantly between bacteria and eukaryotes. In this regard, tRNA<sup>Tyr</sup> is unique among tRNAs in that the nucleotide sequence of bacterial tRNA<sup>Tyr</sup> is a type II tRNA sequence, whereas eukaryotic tRNA<sup>Tyr</sup> substrate...
tRNATyr by the human ( ) and B. stearothermophilus ( ) tyrosyl-tRNA synthetases. Solid lines represent tyrosylation of B. stearothermophilus tRNA^Tyr by human ( ) and B. stearothermophilus ( ) tyrosyl-tRNA synthetases.

Fig. 4. Aminoacylation kinetics of human and B. stearothermophilus tRNA^Tyr. Dashed lines represent tyrosylation of human tRNA^Tyr by the human ( ) and B. stearothermophilus ( ) tyrosyl-tRNA synthetases. Solid lines represent tyrosylation of B. stearothermophilus tRNA^Tyr by human ( ) and B. stearothermophilus ( ) tyrosyl-tRNA synthetases.

The absence of an EMAP II-like domain in human tyrosyl-tRNA synthetase is as a mediator of protein-protein interactions. In E. coli methionyl-tRNA synthetase, the carboxyl-terminal domain is responsible for dimerization of the enzyme (65). Although these carboxyl-terminal domains are distantly related to the carboxyl-terminal domain of human tyrosyl-tRNA synthetase, the human tyrosyl-tRNA synthetase presumably dimerizes through the Rossmann fold domain, as is the case for the B. stearothermophilus enzyme (10, 38). In addition, purification of tyrosyl-tRNA synthetase from porcine and rabbit tissue (18, 52) indicates that in mammalian cells perform a common function in tRNA aminoacylation. The observation that Arc1p binds tRNA nonspecifically (22) is consistent with this hypothesis. In this regard it is interesting that for S. cerevisiae and humans the carboxyl-terminal EMAP II-like domain in methionyl-tRNA synthetase has been lost, and its function appears to have been replaced by Arc1p in S. cerevisiae and EMAP II in humans (EMAP II is identical to the p18 component of the 24 S aminoacyl-tRNA synthetase complex), both of which interact with more than one aminoacyl-tRNA synthetase. The selective advantage of allowing multiple aminoacyl-tRNA synthetases to have access to the EMAP II-like domain is reflected in the observation that replacement of the carboxyl-terminal domain by a separate protein occurred at least twice during the evolution of eukaryotic methionyl-tRNA synthetases since this domain is absent in the methionyl-tRNA synthetases from both S. cerevisiae and humans but is present in methionyl-tRNA synthetase from C. elegans (49, 60, 61).

As discussed above, the homologous domains in human tyrosyl-tRNA synthetase, Arc1p, EMAP II, and C. elegans methionyl-tRNA synthetase may perform similar functions. The most thoroughly characterized of these proteins is Arc1p from S. cerevisiae. Arc1p was initially isolated using a synthetic lethal genetic screen to identify proteins that interact with components of the tRNA nuclear export machinery (22). Arc1p consists of three domains: an amino-terminal domain that binds the methionyl- and glutamyl-tRNA synthetases, a lysine- and alanine-rich central domain that is 34% identical to histone H1, and a carboxyl-terminal domain that is 54% identical to EMAP II and binds tRNA nonspecifically (22). Steady state kinetic analyses indicate that Arc1p increases the rate of methionyl-tRNA synthetase-catalyzed tRNA^Met aminoacylation (22). Based on these observations, Simos et al. (22) postulated that Arc1p participates in directing tRNA to the active sites of the methionyl- and glutamyl-tRNA synthetases. It is likely that the carboxyl-terminal domain in human tyrosyl-tRNA synthetase has a function similar to that of Arc1p namely directing tRNA to the active site of tyrosyl-tRNA synthetase. This hypothesis is consistent with the observation that in mammalian cells tRNAs are not free to diffuse in the cytoplasm but appear to be “channeled” by the translation machinery (62, 63).

While it is likely that both the carboxyl-terminal domain of human tyrosyl-tRNA synthetase and the EMAP II protein participate in directing tRNA to the active sites of aminoacyl-tRNA synthetases, the ability of the EMAP II protein to induce cell migration, tissue factor activity, and cell surface expression of P- and E-selectins in endothelial cells, as well as its ability to induce mRNA synthesis of tumor necrosis factor α, tissue factor, and interleukin 8 in monocytes, suggests that EMAP II may have an alternate role as a cytokine (48, 64). As the amino acid sequence of the carboxyl-terminal domain of human tyrosyl-tRNA synthetase is 49% identical to the EMAP II sequence, it is possible that it may also exhibit cytokine-like activities. At the present time, however, there is no evidence supporting this hypothesis.

A third possible function for the carboxyl-terminal domain of human tyrosyl-tRNA synthetase is as a mediator of protein-protein interactions. In E. coli methionyl-tRNA synthetase, the carboxyl-terminal domain is responsible for dimerization of the enzyme (65). Although these carboxyl-terminal domains are distantly related to the carboxyl-terminal domain of human tyrosyl-tRNA synthetase, the human tyrosyl-tRNA synthetase presumably dimerizes through the Rossmann fold domain, as is the case for the B. stearothermophilus enzyme (10, 38). In addition, purification of tyrosyl-tRNA synthetase from porcine and rabbit tissue (18, 52) indicates that in mammalian cells...
this enzyme is not part of a larger multiprotein complex, suggesting that it does not form strong protein-protein interactions in vivo.

Sequence analysis of the human tyrosyl-tRNA synthetase coding sequence has revealed the existence of a novel carboxyl-terminal domain that is not present in previously sequenced tyrosyl-tRNA synthetases from other organisms. The similarity of this domain to the amino acid sequences of the carboxyl-terminal domains of *C. elegans* methionyl-tRNA synthetase, the Arc1p protein, and EMAP II suggests that in the human tyrosyl-tRNA synthetase this domain may be involved in directing tRNA to the active site of the enzyme, thereby facilitating the aminocacylation of tRNA\textsubscript{Tyrr}.

The validity of this hypothesis is currently being determined.

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Theresa A. Kleeman, Dongbing Wei, Keith L. Simpson and Eric A. First

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