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

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To test the hypothesis that tRNA 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'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 first two domains of the human tyrosyl-tRNA synthetase are 52, 36, and 16% identical to tyrosyl-tRNA synthetases from S. cerevisiae, Methanococcus 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 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 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).

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E + AA + ATP \rightleftharpoons E \cdot AA-AMP + PP, \quad (Eq. 1)
\]

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

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 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 Tyr substrates suggesting that tRNA 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%

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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) through 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); cDNA clones 53277 and 124918 (I.M.A.G.E. clone accession numbers) were obtained from the ATCC. 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 primers:

- M13 reverse primer (−190), HYTS Exp Fwd (21), HYTS-S1 (181), HYTS-S3 (460), HYTS-S5 (794), HPRT (1083), HP9 (1338), HYTS-S2 (193), HYTS-S4 (494), HYTS-S6 (818), HPBR (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 Institute of FASTA (26), the Stanford University implementation of BLAST (27), and the Baylor College of Medicine implementation of BEAUTY (28). Multiple sequence alignments were performed using the Baylor College of Medicine implementation of ClustalW (29, 30). All data base searches and multiple sequence alignments were used to analyze each cDNA sequence. The results of these analyses and possible implications of the findings are presented here.

**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:

- 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 nucleotide 2069.

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 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 synthetase\(^5\) (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\(^{\text{tyr}}\) 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\(^{\text{tyr}}\) and the amino acid sequences of the tyrosyl-tRNA synthetases. Comparison of tRNA\(^{\text{tyr}}\) sequences indicates that in bacteria, tRNA\(^{\text{tyr}}\) is a type II tRNA, whereas in eukaryotes tRNA\(^{\text{tyr}}\) is a type I tRNA (16, 40, 41), 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\(^{\text{tyr}}\) 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 \(E\)\(^{\text{Tyr}}\)-AMP intermediate, tRNA\(^{\text{tyr}}\) 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\(^{\text{tyr}}\) anticodon recognition domain (amino acids 330–419 in the \(B.\) stearothermophilus enzyme).

\(\text{Human Tyrosyl-tRNA Synthetase Contains an EMAP II-like Domain—}\)

FASTA and BLAST searches of the GenBank\textsuperscript{TM} nucleotide sequence data base identified proteins with significant homology to the carboxyl-terminal domain of human tyrosyl-tRNA synthetase\(^5\). Assignment of secondary structure is based on the structure of the \(B.\) stearothermophilus enzyme as determined by x-ray crystallography (10). Conerved amino acids are boxed, shaded regions indicate conserved amino acids that participate in tyrosyl-adenylate formation, and amino acids in boldface type indicate those amino acids in the \(B.\) stearothermophilus enzyme that are highly conserved in other bacterial homologues.

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\(^5\) C. L. Quinn, GenBank\textsuperscript{TM} accession number U40714.
ported (50). 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 domains 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 ESTs have been incorrectly assigned due to the similarity of their sequences to the C. elegans methionyl-tRNA synthetase.

The recent finding that, in contrast to the human methionyl-tRNA synthetase does not have an EMAP II-like domain at its carboxyl terminus is consistent with this 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 recombinant human tyrosyl-tRNA synthetase expressed in E. coli, lane C, shows the recombinant enzyme 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 synthetase isoforms (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^{Tyr} substrates corresponding to tRNA^{Tyr} from B. steaothermophilus (35, 53) and human placenta (54) were transcribed in vitro, gel purified, and annealed. These tRNAs were used as substrates for aminoacylation by human and B. steaothermophilus tyrosyl-tRNA synthetase. As shown in Fig. 4, recombinant human tyrosyl-tRNA synthetase fully aminoacylates the human tRNA^{Tyr} but fails to aminoacylate more than 10% of the B. steaothermophilus tRNA^{Tyr} during the time course of the assay. This is in contrast to the recombinant B. steaothermophilus tyrosyl-tRNA synthetase, which fully aminoacylates B. steaothermophilus tRNA^{Tyr} but fails to aminoacylate the human tRNA^{Tyr} above background levels.

FIG. 2. Alignment of human tyrosyl-tRNA synthetase carboxyl-terminal domain homologues. Although only the human tyrosyl-tRNA synthetase, human EMAP II, S. cerevisiae Arc1p, and C. elegans, E. coli, and H. influenzae methionyl-tRNA synthetase sequences are shown, the actual alignment of these sequences is based on a ClustalW alignment of the carboxyl-terminal domain of human tyrosyl-tRNA synthetase (TyrRS), H. sapiens and M. musculus endothelial monocyte-activating protein II (EMAP II), S. cerevisiae Arc1p (Arc1p), and the carboxyl-terminal domains of methionyl-tRNA synthetase (MetRS) from C. elegans, S. cerevisiae, Bacillus subtilis, B. steaothermophilus, T. ferrooxidans, E. coli, H. influenzae, and M. genitalium.

FIG. 3. SDS-polyacrylamide gel electrophoresis of recombinant human tyrosyl-tRNA synthetase expressed in E. coli. Lane A, molecular mass standards (shown in kDa on figure) myosin (200,000 Da), B-galactosidase (116,250 Da), phosphorylase B (97,400 Da), bovine serum albumin (66,200 Da), ovalbumin (45,000 Da), and carbonic anhydrase (31,000 Da); lane B, uninduced BL21 DE3 pLysS E. coli cells harboring the phytos2 plasmid; lane C, isopropyl-1-thiogalactopyranoside-induced BL21DE3 pLysS E. coli cells harboring the phytos2 plasmid; lane D, recombinant human tyrosyl-tRNA synthetase purified on Ni-NTA resin.

**DISCUSSION**

If the hypothesis that tRNA^{Tyr} 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^{Tyr} substrates should differ significantly between bacteria and eukaryotes. In this regard, tRNA^{Tyr} is unique among tRNAs in that the nucleotide sequence of bacterial tRNA^{Tyr} is a type II tRNA sequence, whereas eukaryotic tRNA^{Tyr} sub-

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* M. Mirande, personal communication.
tyrosyl-tRNA synthetase is the result of a relatively recent evolutionary event. The presence of a distantly related homologue of this domain in other organisms, including bacteria and eukaryotes, suggests that the carboxyl-terminal domain is responsible for dimerization of the enzyme.

A third possible function for the carboxyl-terminal domain of human tyrosyl-tRNA synthetase is suggested by the observation that Arc1p participate in directing tRNA to the active sites of aminoacyl-tRNA synthetases. This function is further supported by the observation that Arc1p binds to EMAP II and binds tRNA nonspecifically (22). 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 as a component of the tRNA nuclear export machinery (22). 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 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 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.

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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 carboxy-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 carboxy-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 aminoacylation of RNA-Tyr. The validity of this hypothesis is currently being determined.

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