Identification and Characterization of Human Mitochondrial Tryptophanyl-tRNA Synthetase*

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A full-length cDNA clone encoding the human mitochondrial tryptophanyl-tRNA synthetase (hmtTrpRS) has been identified. The deduced amino acid sequence shows high homology to both the mitochondrial tryptophanyl-tRNA synthetase (mtTrpRS) from Saccharomyces cerevisiae and to different eu-bacterial forms of tryptophanyl-tRNA synthetase (TrpRS). Using the baculovirus expression system, we have expressed and purified the aminocyl-tRNA synthetase (TrpRS). Using the baculovirus and to different eubacterial forms of trypto-

The only components of the mitochondria encoded by the mitochondrial genome include 12 S and 16 S rRNAs, 22 tRNAs, and 13 proteins: 3 cytochrome c oxidase subunits, 2 ATPase subunits, cytochrome b, and 6 components of respiratory-chain NADH dehydrogenase (1–3). Mitochondrial protein-synthesizing machinery is required for synthesis of these 13 proteins. Therefore, proteins like the mitochondrial translational release factors, mtRF1 and mtRRF (4), and the aminocyl-tRNA synthetases (aaRS)1 are encoded by the nuclear genome, trans-

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Biotechnology Information using the sequence encoding the h\textsubscript{mt}TrpRS from \textit{S. cerevisiae} (accession no. M12081). The IMAGE clone was fully sequenced by the primer walking approach (16). Sequencing was performed on an Applied Biosystems 373A sequencer using a sequencing kit (ABI Prism Dye Terminator Cycle, Perkin Elmer). Each strand was sequenced at least twice.

**RACE**—RACE was performed as described from Frohman (17) using total RNA from HeLa cells. The primers for the second nested polymerase chain reaction (PCR) contained XhoI and XhoI endonuclease restriction sites, which allow the resulting PCR product to be cloned into the corresponding sites of the Bluescript SK\textsuperscript{+} vector (Stratagene). The clone was sequenced using the M13 MID ABI sequencing primer (DNA technology A/S, Aarhus, Denmark). Primer sequences were as follows: Gene-specific primer 1 (GSP-1), 5'-GGATGTGAGAATGGACTTACGG-3'; GSP-2 (including an XhoI site), 5'-GGGGCTAGATGACCGAAGAAGACG-3'; linker primer 1, 5'-CAATCAGAAGATCCGTCTAGAGTGAA-3'; linker primer 2 (including an XhoI site), 5'-GGGCACTTATGCAACCATCCTATC-3'; and M13-21 ABI sequencing primer, 5'-CTGTGTTAAGACGACGCCAGT-3'.

**Alignment**—The alignments were performed using the following TrpRS amino acid sequences:

\textit{S. cerevisiae} (Swissprot P04803), \textit{Caenorhabditis elegans} (Swissprot P46579), \textit{Escherichia coli} (Swissprot P00954), \textit{Bacillus stearothermophilus} (Swissprot P00955), \textit{Bacillus subtilis} (Swissprot P21656), \textit{human} (sequence from \textit{Homo sapiens} (Swissprot P23381), and the translated human h\textsubscript{mt}TrpRS cDNA (GenBank\textsuperscript{TM} accession no. A0247239). The alignments were carried out using the ClustalW and the Boxshade programs from the Molecular Bioinformatics of Gene Regulation.

**Cell Lines**—Human amniotic cells (7), 293 cells (ATCC CRL-1573), and HeLa cells (ATCC CCL-2) were cultured as monolayers in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10\% newborn calf serum and 1\% penicillin/streptomycin (Life Technologies, Inc.). AMA cells were either untreated or treated with 500 units/ml IFN-\alpha or 100 units/ml IFN-\gamma for 24 h. The insect cell lines SF9 (Spodoptera frugiperda, ATCC CRL1711) and HighFive\textsuperscript{TM} (BTI-NT-5B1-4) were grown as suspension cultures in BaculoGold\textsuperscript{TM} (Pharmacia Biotech). AMA cells were either untreated or treated with AJ242739. The alignments were carried out using the ClustalW and the Boxshade programs from the Molecular Bioinformatics of Gene Regulation.

**Cloning the Full-length h\textsubscript{mt}TrpRS cDNA and Expression of Histidine-tagged h\textsubscript{mt}TrpRS in the Baculovirus Expression System—**Using the sequencing results from RACE, we were able to design primers and to obtain a full-length cDNA clone encoding h\textsubscript{mt}TrpRS. The forward primer containing a XhoI site and the Zozori site indicated by RACE was 5'-CGGGATCCATGAGGCTGCTACACTGACGGAGAACCGTGATGAGGCGGACG-3', and the reverse primer containing a 6\% His tag, a stop codon, and an XhoI site was 5'-GGGCACTTAAGGAACTTATCTTTCTTC-3'. These primers were used to amplify the IMAGE clone using a Pfu proofreading polymerase (Stratagene) thus obtaining a full-length h\textsubscript{mt}TrpRS cDNA, which was cloned into the pFastBac\textsubscript{TM} vector (Life Technologies, Inc.). Preparation of recombinant bacmid DNA was performed following the instruction manual for the Bacto-to-Bac\textsuperscript{TM} Baculovirus Expression system (Life Technologies, Inc.). This bacmid DNA was then used to transfet SF9 insect cells, and the h\textsubscript{mt}TrpRS recombinant baculovirus was isolated (18). The h\textsubscript{mt}TrpRS cDNA (8) was cloned into the EcoRI/SmaI sites of the baculovirus transfer vector pXCL1932, and the recombinant h\textsubscript{mt}TrpRS baculovirus was isolated using the BaculoGold\textsuperscript{TM} transfection method (PharMingen) following the manufacturer’s instructions.

**Expression of Recombinant Human Histidine-tagged h\textsubscript{mt}TrpRS in Human 293 Cells and Fluorescence Microscopy—**One day prior to transfection, coverslips were incubated for 2 h with 10 \mu g/ml poly-l-lysine in PBS (Sigma) and dried. Human 293 cells were seeded on coverslips in 32-mm dishes (Nunc, Denmark) in 2 ml of medium, and at 50\% confluence they were transfected with 0.3 \mu g of pEGFP-h\textsubscript{mt}TrpRS plasmid and Superfect (Qiagen), according to the manufacturer’s instructions, and incubated at 37 \degree C for 24 h. In vivo fluorescent red staining of mitochondria was achieved by incubating cells for 5 min with 25 \mu M MitoTracker\textsuperscript{TM} Red CMXRos (Molecular Probes) in growth medium. Cells were fixed with PBS, washed with PBS, and coverslips were mounted on the coverslips. Fluorescence microscopy was done using a Leica DMRB fluorescence microscope equipped with a cooled charge-coupled device camera (Sensys, Photometrics) and an automatic filter wheel (Ludl). Images were captured and analyzed using SmartCaptureVP (Digital Scientific) and IPLab software (Scanalytics).
with a modification of a Trp-dependent PP\_ATP exchange assay using \([\gamma-\text{32P}]\text{ATP}\) instead of \([\gamma-\text{32P}]\text{pyrophosphate}\); Trp + ATP + Enzyme \(\rightarrow\) Trp-adenylate enzyne + PP, (22). The reaction contained 4 mM ATP; 0.02 \(\mu\text{Ci}\) of \([\gamma-\text{32P}]\text{ATP}\); 50 mM Tris-HCl, pH 9.0; 1 mM KF; 0.02% gelatin; 10 mM MgCl\(_2\); 0.1 mM \(\gamma\)-Trp; 4 mM PP\(_1\); and 10 \(\mu\)M of purified enzyme in a total volume of 20 \(\mu\)l. The last components to be added were PP\(_1\), and then the purified enzyme (final concentration, 0.14 \(\mu\)M). The samples were incubated for 30 min at room temperature and stopped by rapid cooling to 0 \(^\circ\)C. 3 \(\mu\)l was spotted on a polyethyleneimine-cellulose chromatogram (Polygram\(_\text{R}\), Macherey-Nagel), which was developed with 1 \(\text{m}\) KH\(_2\)PO\(_4\), dried, and visualized using a PhosphorImager\(_\text{R}\) (Molecular Dynamics). Hs-tagged hTrpRS purified from E. coli, kindly provided by Bente Vestergaard, Aarhus University, Denmark, was marked as a positive control. Two negative controls were included. One with \text{in vitro} translated luciferase from the control DNA in the TNT\(_\text{R}\)777/773 Coupled Reticulocyte Lysate System (Promega), and one in which 0.1 \(\mu\)M Leu was added instead of Trp. In reactions with varying ATP concentrations, Trp was held constant at 2 mM. When the concentration of Trp was varied, the ATP was kept at 4 mM. Exchange reactions were measured at 1, 5, 10, 15, and 20 min, and initial velocities were calculated. Each assay was repeated four times. These data were used to make plots of the reaction velocity (\(V\)) as a function of the substrate concentration ([S]), and to calculate the \(K_v\) and \(V_{max}\) values for ATP and Trp. Nonlinear regression was made by fitting the data to the Michaelis-Menten equation.

**RESULTS**

The Nucleotide Sequence of \(h_{mt}\text{TrpRS}—\)** We searched the human EST data base for clones with similarity to the gene encoding the \(h_{mt}\text{TrpRS}\) from *S. cerevisiae* as described by Myers and Tzagoloff (24) and to TrpRS in different eubacteria. Several human EST clones belonging to the same tentative human consensus group (THC) appeared. One of these clones (IMAGE 667474) had been partly sequenced from the 3' end and included the poly(A) signal and tail. The entire clone was sequenced, and to obtain a full-length cDNA sequence we performed RACE using total RNA from HeLa cells. The \(h_{mt}\text{TrpRS}\) primers used in the PCR reactions were \(5'-\text{CATTCAACCTA}-\text{CAGGAATC}-3'\) and \(5'-\text{CTTCTTCTAATGTCCTTGTCCA}-3'\). The PCR setting was 25 cycles with denaturation at 95 \(^\circ\)C for 40 s, annealing at 61 \(^\circ\)C for 40 s, and extension at 72 \(^\circ\)C for 1 min, resulting in a 277-bp PCR product.

As a control, a PCR was performed using the same cDNA with primers in glyceraldehyde-3-phosphate dehydrogenase, 5'\(-\text{CCAGCATCGCCCCACTTGA}-3'\) and 5'\(-\text{TCAGGAGGTGGCAGGGGATA}-3'\). The PCR was carried out with 25 cycles of denaturation at 95 \(^\circ\)C for 40 s, annealing at 56 \(^\circ\)C for 40 s, and extension at 72 \(^\circ\)C for 2 min, resulting in an 880-base pair (bp) PCR product. To compare it with IFN-\(\gamma\)-induced hTrpRS, the PCR was carried out with the same cDNA using the hTrpRS primers 5'\(-\text{GCTCAAGAGGCACTCATAGGGTTGC}-3'\) and 5'\(-\text{TATTGATACATACAGCGCTCAGAGG}-3'\). This PCR was carried out with 25 cycles of denaturation at 95 \(^\circ\)C for 40 s, annealing at 61 \(^\circ\)C for 40 s, and extension at 72 \(^\circ\)C for 1 min, resulting in a 277-bp PCR product.

As a control, a PCR was performed using the same cDNA with primers in glyceraldehyde-3-phosphate dehydrogenase, 5'\(-\text{GTGCCAGTCAACAGGAGGATT}-3'\) and 5'\(-\text{CCAGCATCGCCCCACTTGA}-3'\). The condition for this PCR was as for \(h_{mt}\text{TrpRS}\) except annealing was at 55 \(^\circ\)C.

Alignment of \(h_{mt}\text{TrpRS} with Different Forms TrpRS}—Us-\) ing the ClustalW program, an alignment was made with \(h_{mt}\text{TrpRS}\) from *S. cerevisiae*, the putative \(h_{mt}\text{TrpRS}\) from *C. elegans*, and TrpRSs from the eubacteria *E. coli*, *B. stearothermophila*, *B. subtilis*, and *H. influenzae*. The sequence identity of \(h_{mt}\text{TrpRS}\) to \(h_{mt}\text{TrpRS}\) in *S. cerevisiae* and *C. elegans* is 37% and 38%, respectively. As for \(h_{mt}\text{TrpRS}\) from *S. cerevisiae* and *C. elegans*, the \(h_{mt}\text{TrpRS}\) also has a very high homology to the bacterial forms of TrpRS (Fig. 1). The \(h_{mt}\text{TrpRS}\) has between 37% and 40% identical residues compared with the four eubacterial TrpRSs. Some of the well-conserved domains are the consensus sequences HXGH (HLGN for mitochondria and TIGN for eubacteria) and KMSKS responsible for the Rossmann fold.

Both the human and *S. cerevisiae* \(h_{mt}\text{TrpRSs}\) have amino-terminal extensions of approximately 32 amino acid residues, which are not represented in the eubacterial variants. These extensions are rich in Lys and Arg residues and thus most likely constitute the mitochondrial localization signal.

An alignment was made with \(h_{mt}\text{TrpRSs}\) and hTrpRS, which showed that \(h_{mt}\text{TrpRSs}\) aligns to the carboxyl-terminal part of hTrpRS and that the sequence identity is only 11%. The computer program MitoProt II predicts a probability of 73.5% for the human \(h_{mt}\text{TrpRSs}\) to be localized to the mitochondria. The predictions are done according to the theory of Claros et al. (25), taking into account the net charge, hydrophobicity, and the ability to form \(\alpha\)-helical amphiphilic structures. The MitoProt II program was run with \(h_{mt}\text{TrpRSs}\) from human, *S. cerevisiae*, and *C. elegans* and hTrpRS. In comparison to \(h_{mt}\text{TrpRSs}\), *S. cerevisiae* \(h_{mt}\text{TrpRSs}\) is given a probability of 98.8% of being mitochondrial, whereas the \(h_{mt}\text{TrpRSs}\) from *C. elegans* is only given a probability of 22.5%. As expected, the hTrpRS is only given a probability of 8.4%. The cleavage sites in human, *S. cerevisiae*, and *C. elegans* \(h_{mt}\text{TrpRSs}\) are predicted to be between the amino acids 46 and 47, 24 and 25, and 22 and 23, respectively, whereas the cleavage site for hTrpRS is not predictable.

**Localization of \(h_{mt}\text{TrpRSs}—To investigate the localization of** \(h_{mt}\text{TrpRSs}\), we fractionated lysates of insect cells expressing His-tagged \(h_{mt}\text{TrpRSs}\) into three different crude fractions. One fraction contained the nuclei, one contained the cytoplasm, and one the mitochondria. Immunoblots with anti-His-tag antibodies revealed that \(h_{mt}\text{TrpRSs}\) is highly expressed in the mitochondrial fraction and to a much weaker extent in the nuclear fraction (Fig. 2A), whereas this band was completely absent from the cytoplasmatic fraction. The His-tagged \(h_{mt}\text{TrpRSs}\) migrates with a molecular mass of 35 kDa, which is less than the predicted 40.9 kDa of the noncleaved precursor of \(h_{mt}\text{TrpRSs}\) with the \(6 \times\) His tag. However, this correlates with the loss of the signal peptide during mitochondrial localization. The faint band of \(-60\) kDa in the cytoplasmatic fraction of both \(h_{mt}\text{TrpRSs}\) and hTrpRS cells most likely corresponds to unspecific binding of the antibody.

To compare the localization of \(h_{mt}\text{TrpRSs}\) with hTrpRS, a similar immunoblot was carried out using polyclonal rabbit hTrpRS antibody. The hTrpRS antibody recognized a distinct band of \(-53\) kDa corresponding to hTrpRS in both the cytoplasmic and nuclear fractions, whereas only a weak band appeared in the mitochondrial fraction of the hTrpRS-expressing cells (Fig. 2B). No bands were detected in any of the fractions from the \(h_{mt}\text{TrpRSs}\)-expressing insect cells (Fig. 2B), which indicates that there is no cross-reactivity between the hTrpRS antibody and \(h_{mt}\text{TrpRSs}\). Furthermore, no endogenous insect cell TrpRS could be detected by the hTrpRS antibody. To confirm the quality of the mitochondrial fractionation, we performed an assay for the activity of the succinate dehydrogenase. This enzyme catalyzes the oxidation of succinate to fumarate in the citric acid cycle in mitochondria. We found the highest values for succinate dehydrogenase activity in the mitochondrial fraction, although the fraction containing the nuclei also showed some activity (Table 1). These data correlate with the weak \(h_{mt}\text{TrpRSs}\) band in the nuclear fraction of the \(h_{mt}\text{TrpRSs}\)-expressing insect cells.
fected with the pEGFP-hmtTrpRS vector. As can be seen on Fig. 3A, the expressed GFP-tagged hmtTrpRS is localized to subcellular compartments surrounding the nucleus. Fig. 3B shows the same cells stained red by MitoTracker dye, and it can be seen that the mitochondria co-localize with the GFP-tagged hmtTrpRS. It is noteworthy that the GFP staining is confined to the periphery of the mitochondria.

The Mitochondrial Import Signal Peptide—Recombinant carboxyl-terminally His-tagged hmtTrpRS from baculovirus was purified on a Ni-NTA column. The gel shows a single band of the expected size of ~35 kDa in the eluate (Fig. 4A).

The cleavage site for the signal peptide of His-tagged hmtTrpRS was determined by amino-terminal sequencing of the recombinant protein purified from insect cells. The first seven amino-terminal amino acids were LHKGSAA. This suggests that 18 amino acids were cleaved off during the mitochondrial localization.

Activity Assay of hmtTrpRS—To test the activity of the purified His-tagged hmtTrpRS, we used a PPi-ATP exchange assay with [γ-32P]ATP, which has the advantage that mitochondrial

FIG. 1. Multiple sequence alignment of TrpRSs. Mitochondrial forms from human, S. cerevisiae, C. elegans, and the eubacterial forms from B. stearothermophilus, B. subtilis, H. influenzae, and E. coli were used. The HXGH and KMSKS motifs responsible for the Rossmann fold are enclosed in the box.
tRNA-Trp is not needed in the reaction. The activity is demonstrated by autoradiography of a polyethyleneimine-cellulose chromatograph (Fig. 4B) of an exchange assay containing 0.14 mM of the purified enzyme. Recombinant hTrpRS was used as a positive control, whereas Leu and luciferase was used as negative controls. Formation of radioactive PPi is Trp-dependent both for hmtTrpRS and hTrpRS.

The kinetic parameters \( V_{\text{max}} \), \( K_m \), \( k_{\text{cat}} \), and \( k_{\text{cat}}/K_m \) for the interaction of hmtTrpRS with Trp and ATP were determined by measuring the initial rate of the PPi-ATP exchange followed by nonlinear regression fitting to the Michaelis-Menten equation. With a constant saturating concentration of 4 mM ATP, the concentration of Trp was varied between 10 and 800 mM (Fig. 5A). The \( K_m \) value of hmtTrpRS is comparable to the \( K_m \) value for E. coli TrpRS (26), whereas the value for B. subtilis is much higher (27) and the value for hTrpRS is much lower (28) (Table II). In addition, the \( k_{\text{cat}} \) value is almost 100-fold lower than the \( k_{\text{cat}} \) for E. coli. A similar pattern is seen with the \( k_{\text{cat}} \) values for E. coli phenylalanyl-tRNA synthetase (PheRS) and hmtPheRS (29, 30).

At a constant Trp concentration of 2 mM, the ATP concentration was varied between 0.04 and 4 mM (Fig. 5B). This \( K_m \) value for ATP is more than twice as high as for the \( K_m \) values of E. coli and B. subtilis TrpRS, as well as the value for hTrpRS (Table II). Again, the \( k_{\text{cat}} \) value for hmtTrpRS is almost 100-fold lower than the \( k_{\text{cat}} \) for E. coli, and this pattern is also seen with \( k_{\text{cat}} \) values for hmtPheRS and E. coli PheRS. There seems to be a tendency for higher \( K_m \) values for ATP in mitochondrial aaRS than for eubacteria and cytoplasmic aaRS, which might be explained by the higher concentration of ATP in mitochondria.

The Effect of Interferon on Expression of hmtTrpRS—To get an indication of whether the hmtTrpRS, like the cytoplasmic hTrpRS, is induced by interferons, RT-PCR with primers in the hmtTrpRS gene was carried out using cDNA from AMA cells treated with IFN-\( \alpha \) or IFN-\( \gamma \) compared with nontreated cells. For comparison, PCR with primers in the hTrpRS gene was performed on the same cDNA, and as a control for RNA integrity and comparable RT reactions, PCR with primers in the

![Table I](https://example.com/table1.png)

**Table I** Subcellular fractions of insect cells expressing hmtTrpRS

| Succinate dehydrogenase | Nuclei | Cytoplasm | Mitochondria |
|------------------------|--------|-----------|--------------|
| (A490/15 min)          | 0.039  | 0         | 0.239        |
| Units*/ml              | 0.026  | 0         | 0.159        |
| % units of total       | 25     | 0*        | 75           |

*Units = A490/min.  
*<5%.

![Figure 2](https://example.com/f2.png)

**Fig. 2.** Cell fractionation of HighFive™ cells expressing hmt-TrpRS or hTrpRS. HighFive™ cells infected with either His-tagged hmtTrpRS or hTrpRS baculovirus, as indicated, were harvested 3 days postinfection. Subcellular fractionation was carried out and applied to 10% SDS-PAGE. 1, nucleic fractions; 2, cytoplasmic fractions; and 3, mitochondrial fractions. Immuno-blotting using anti-His-tag (A) and anti-hTrpRS (B). The positions of hmtTrpRS and hTrpRS are indicated by arrows.

![Figure 3](https://example.com/f3.png)

**Fig. 3.** Expression of GFP-tagged hmtTrpRS in human 293 cells. Human 293 cells were grown on coverslips, transfected with pEGFP-hmtTrpRS, cultured for 24 h, and stained with MitoTracker™ Red CMXRos. A, green fluorescence from GFP; B, red fluorescence from CMXRos mitochondrial staining.

![Figure 4](https://example.com/f4.png)

**Fig. 4.** Recombinant His-tagged hmt-TrpRS. A, purification of His-tagged hmt-TrpRS expressed in HighFive™ cells on an Ni-NTA column. Fractions were applied to 10% SDS-PAGE, and the gel was stained with Coomassie Blue. The position of His-tagged hmt-TrpRS is indicated by an arrow. Lanes 1 and 2, flow through; lanes 3–6, column washes; lane 7, eluate. B, PPi-ATP exchange assay with purified His-tagged hmt-TrpRS. Autoradiography of a polyethyleneimine-cellulose chromatogram with samples incubated for 30 min at 25 °C and additions as indicated. Positions of PPi and ATP are marked with arrows.
glyceraldehyde-3-phosphate dehydrogenase gene was also performed on the same cDNA. It can be seen in Fig. 6 that neither IFN-α nor IFN-γ induced the transcription of the h$_{mt}$TrpRS gene. This is in contrast to the hTrpRS gene, which is highly induced by IFN-γ as expected.

**DISCUSSION**

We have identified a human cDNA encoding the h$_{mt}$TrpRS, which is different from the human gene encoding hTrpRS. A BLAST search with mtTrpRS from *S. cerevisiae* against the human EST data base identified several different EST clones with a very high homology to the gene encoding mtTrpRS from *S. cerevisiae*. The high homology of several yet unidentified human EST clones to the mtTrpRS gene from *S. cerevisiae* gave us reason to believe that they could encode the h$_{mt}$TrpRS. Complete sequencing of one of these clones (IMAGE 667474) and performing 5’ RACE resulted in an open reading frame of 1080 bp. The entire cDNA was cloned and expressed with a carboxyl-terminal His-tag in the baculovirus expression system. Activity assays, together with kinetic studies and localization experiments using both GFP-tagged h$_{mt}$TrpRS-expressing insect cells, identified with certainty that our cDNA clone was human mitochondrial TrpRS.

In addition, we have recently used radiation hybrids to locate the gene encoding the h$_{mt}$TrpRS (designated *WARS2*) to chromosome 1 (1p13.3-p13.1) (31), thus confirming the human origin of the IMAGE 667474 EST clone.

The existence of h$_{mt}$TrpRS makes it unlikely that a hTrpRS splice variant should be localized to the mitochondria to provide tryptophanyl-tRNA synthetase activity to the mitochondria. Using a monoclonal antibody labeled with gold particles against a conserved epitope of TrpRS, Popenko et al. (14) showed that rodent TrpRS localized not only to the cytoplasm but also to the mitochondria and to the chromatin region in the nucleus. In addition to immunostaining of ultrathin sections of rat pancreas tissue and rat fibroblasts, *E. coli* and Methanococcus halophilus cells were also stained. The distribution of TrpRS in *E. coli* and *M. halophilus* was mainly concentrated in the cytoplasm, but some was also detected adjacent to the nucleoid border. This detection was feasible because the antibody could cross-react with TrpRS from prokaryotic, archaeobacterial, and eukaryotic cells (32). This particular antibody most likely also recognizes mtTrpRS in eukaryotes, taking into account its high homology to prokaryotic TrpRS. However, this does not explain why TrpRS also was found localized to the

![Fig. 5](http://www.jbc.org/)

**Fig. 5.** Plots of reaction velocity (v) as a function of substrate concentration [S] in PP-ATP exchange assays. A, Trp as substrate and ATP at 4 mM. B, ATP as substrate and Trp at 2 mM. The concentration of h$_{mt}$TrpRS was held constant at 0.14 μM in all reactions.

![Fig. 6](http://www.jbc.org/)

**Fig. 6.** RT-PCR of nontreated (+) AMA cells and AMA cells treated with IFN-α or IFN-γ. Primers specific for h$_{mt}$TrpRS, hTrpRS, and glyceraldehyde-3-phosphate dehydrogenase were used as indicated.

**Table II**

| Kinetic parameters | K$_M$ (μM) | h$_cat$ (μM) | h$_cat$/K$_M$ (s$^{-1}$) | K$_M$ (μM) | h$_cat$ (μM) | h$_cat$/K$_M$ (s$^{-1}$) | Ref. |
|--------------------|------------|-------------|------------------------|------------|-------------|------------------------|-----|
| h$_{mt}$TrpRS      | 27 ± 7     | 1.2 ± 0.1   | 0.044                  | 400 ± 75   | 1.4 ± 0.1   | 0.00035                |     |
| hTrpRS             | 2.8        | nd$^a$      | nd                     | 160        | nd          | nd                     | (28) |
| *E. coli* TrpRS    | 18 ± 3     | 111 ± 7     | 6.1                    | 132 ± 9    | 110 ± 8     | 0.8                    | (26) |
| *B. subtilis* TrpRS| 160 ± 12   | nd          | nd                     | 150 ± 10$^b$| nd          | nd                     | (27) |
| Phe                | 33         | 1.1         | 0.033                  | 2500       | 2.9         | 0.0012                 | (29) |
| *E. coli* PheRS    | 40         | 140         | 3.5                    | 800        | 180         | 0.23                   | (30) |

$^a$ nd, not determined.
$^b$ Calculation of K$_M$ for ATP in *B. subtilis* was done using a tRNA charging assay.
nucleus. In addition, our immunoblot data, using antibodies against hTrpRS not cross-reacting with hmtTrpRS, showed hTrpRS mainly in nucleic and cytoplasmic fractions of hTrpRS-expressing insect cells.

The GFP-tagged hmtTrpRS seems to localize to the mitochondrial periphery. Similar localization is observed when the GFP derivative yellow fluorescence protein is anchored in the outer mitochondrial membrane using the signal peptide from the Tom70 protein from yeast (33, 34). However, GFP fusion proteins can also be targeted to the mitochondrial matrix, as has been demonstrated by the presence from citrate synthase (35). We can thus conclude that the hmtTrpRS-GFP protein is located in the mitochondrial periphery and not in the matrix.

Suggestions have been made that the amino-terminal part of the cytoplasmic TrpRS could possess a secondary unknown function (36). Such a secondary function could be related to the mitochondrial and nuclear distribution, together with the IFN induction and the alternatively spliced 5′-end. Although several views have been put forward about the involvement of TrpRS in the antiviral effect of IFN (37–40), it is at present not certain why the human TrpRS is among the most strongly induced proteins.

The signal peptide of 18 amino acids deviates from the predicted 46 amino acids using the MitoProtII program, which most likely is due to a wrong prediction by the program. However, another explanation for this discrepancy might be that the hmtTrpRS was expressed in insect cells and not in human cells. It is possible that there is a difference in the mitochondrial localization process between the two cell types, although earlier results from expression of mitochondrial proteins in the baculovirus expression system have indicated a tendency for correct processing (41–43). Therefore, it is likely that the mitochondrial import mechanisms in human and insect cells are closely related and that the signal peptide of hmtTrpRS expressed in human cells is identical to the signal peptide observed in insect cells.

Like the human cytoplasmic and the bacterial TrpRs, the hmtTrpRS also belong to class I aaRSs harboring sequences similar to HXGH and KMSKS. Some variation of these motifs has been observed between species. The bacterial and mitochondrial HXGH sequence in TrpRS deviates by having an Asn instead of the last His (HLGN). In addition, bacterial forms have a Thr instead of the first His (TI/LGN). Instead of KMSKS, the hTrpRS has the sequence KMSAS, which is a change from a basic hydrophilic amino acid (Lys) to an aliphatic hydrophobic one (Ala). These changes are interesting because the imidazole side chain of both His residues in HXGH and the second Lys in KMSKS have been shown to be closely involved in binding of ATP and formation of the aminoacl-adenylate in tyrosyl- and glutamyl-tRNA synthetases from B. steatorrhophilus and E. coli, respectively (44, 45). The homology of hmtTrpRS to B. subtilis TrpRS, which has been shown to be an α2 dimer (27), makes it likely that the hmtTrpRS also functions as an α2 dimer. This correlates well with cytoplasmic hTrpRS, which also has a homodimeric structure (22). However, as illustrated by the recent description of hPhRS (29), a correlation between cytoplasmic and mitochondrial aaRS structures does not always apply. Cytoplasmic and prokaryotic PhRS belong to class II of aaRS with an α2β2 tetramer structure, whereas the hPhRS is shown to be a monomer containing the three sequence motifs normally present in the α subunit as well as a well-conserved sequence from the β subunit.

The present data describe hTrpRS as a mitochondrial enzyme from the translational system, which will need much further investigation to clarify the increasing number of functions attributed to mitochondrial proteins in health and disease.

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