ELSEVIER

Gene 215 (1998) 311–318

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

Cloning and characterization of mitochondrial methionyl-tRNA synthetase from a pathogenic fungi *Candida albicans*

Sang Won Lee, Yeong Joon Jo, Sunghoon Kim *

Department of Biology, Sung Kyun Kwan University, 300 Chunchundong, Jangangu, Suwon, Kyunggido 440-746, South Korea

Received 23 March 1998; accepted 18 May 1998; Received by J. Wild

Abstract

A genomic sequence encoding mitochondrial methionyl-tRNA synthetase (MetRS) was determined from a pathogenic fungi *Candida albicans*. The gene is distinct from that encoding the cytoplasmic MetRS. The encoded protein consists of 577 amino acids (aa) and contains the class I defining sequences in the N-terminal domain and the conserved anticodon-binding amino acid, Trp, in the C-terminal domain. This protein showed the highest similarity with the mitochondrial MetRSs of *Saccharomyces cerevisiae* and *Shizosaccharomyces pombe*. The mitochondrial MetRSs of these fungi were distinguished from their cytoplasmic forms. The protein lacks the zinc binding motif in the N-terminal domain and the C-terminal dimerization appendix that are present in MetRSs of several other species. *Escherichia coli* $tRNA^{Met}$ was a substrate for the encoded protein as determined by genetic complementation and in vitro aminoacylation reaction. This cross-species aminoacylation activity suggests the conservation of interaction mode between $tRNA^{Met}$ and MetRS.

Keywords: Class I tRNA synthetase; Gene location; Conserved motifs; Aminoacylation; Genetic complementation

1. Introduction

Aminoacyl-tRNA synthetases catalyze the transfer of specific aa to their cognate tRNAs, and thus are essential to maintain the fidelity of cellular protein synthesis. They are ancient enzymes that show a wide range of sequence and structural diversity (Martinis and Schimmel, 1996; Shiba et al., 1997). Eukaryotic cells contain cytoplasmic and mitochondrial forms of tRNA synthetases. The mitochondrial tRNA synthetases are encoded by the nuclear genome and transported into mitochondria (Myers and Tzagoloff, 1985; Pape et al., 1985; Koerner et al., 1987). Most of the studies on the eukaryotic tRNA synthetases have focused on the cytoplasmic forms and thus information on the mitochondrial tRNA synthetases is limited. Among the known MetRS sequences, only the mitochondrial enzyme of *Saccharomyces cerevisiae* has been studied for its genetic and biochemical characteristics (Tzagoloff et al., 1989). In the cases of yeast histidyl- and valyl-tRNA synthetases, the cytoplasmic and mitochondrial enzymes are encoded by a single gene but generated by differential transcription (Natsoulis et al., 1986; Chatton et al., 1988). Some of the mitochondrial tRNAs showed non-canonical structures and thus pose an interesting question as to how they are recognized by the mitochondrial tRNA synthetases (Dirheimer et al., 1995).

We have been interested in the mitochondrial tRNA synthetases of a pathogenic fungus *Candida albicans*, that is taxonomically related to the other well-known yeast species such as *S. cerevisiae* and *Shizosaccharomyces pombe*. *C. albicans* is a commensal fungus which causes opportunistic infection, especially to immunocompromised patients. In addition, repeated treatment with commonly used antifungal agents such as fluconazole (Powderly, 1994) has resulted in the emergence of resistant strains. Despite its increasing medical significance, insufficient genetic and molecular biological knowledge has been accumulated on *C. albicans*, and thus it is difficult to cope with the increasing threat of this species. Owing to their structural diversity and functional essentiality, aminoacyl-tRNA synthetases could be used as a molecular target to develop novel agents that can specifically control the growth of patho-

* Corresponding author. Tel: 001-82-331-290-7007; Fax: 001-82-331-290-7015; e-mail: shkim@yurim.skku.ac.kr

Abbreviations: aa, amino acid(s); IPTG, isopropyl-β-D-thiogalactopyranoside; MetRS, methionyl-tRNA synthetase; ORF, open reading frame; SDS, sodium dodecyl sulfate.

0378-1119/98/$19.00
PII: S0378-1119(98)00292-3

genic cells. As a part of our efforts to isolate and characterize the tRNA synthetases from pathogenic organisms, we report the isolation and characterization of the mitochondrial MetRS from *C. albicans*.

2. Materials and methods

2.1. Isolation of genomic fragments encoding mitochondrial and cytoplasmic MetRSs

High molecular weight genomic fragments (>50 kb) were isolated by grinding *C. albicans* (ATCC 36801) cells in liquid nitrogen. The crushed cells were subsequently suspended in 50 mM Tris–HCl buffer (pH 7.4), 10 mM $MgCl_2$, 50 mM NaCl and 1% (w/v) SDS. DNA fragments were cleaned up by phenol extraction and precipitated by ethanol. The degenerate PCR primers for the mitochondrial MetRS gene were designed and synthesized from the conserved regions identified from the multiple sequence alignment of MetRS sequences (Shiba et al., 1994). PCR was conducted using the isolated genomic DNA as a template with four different pairs of the degenerate primers. The PCR fragment was obtained (data not shown) and cloned into pBluescript SK+ (Stratagene, La Jolla, CA) using *Eco*RI.

To isolate the genomic fragment for the cytoplasmic MetRS, new primers were designed based on the sequences of the cytoplasmic MetRSs of *S. cerevisiae, Caenorhabditis elegans* and *Homo sapiens*. They were used in combination with the previous primers for PCR. Three PCR products were obtained and cloned into pGEM-T vector (ProMega, Madison, WI) to determine the sequences (data not shown). The sequences of the PCR products obtained were analyzed by homology search using the BLAST program (Altschul et al., 1990).

2.2. Southern blot analysis of mitochondrial and cytoplasmic MetRS genes

The DNA fragments that were thought to encode the mitochondrial and cytoplasmic MetRSs were used to probe the respective genes in the chromosome. Genomic DNA isolated from *C. albicans* was digested with *Cla*I and separated by agarose gel electrophoresis. The DNA fragments were subsequently denatured by alkaline treatment and transferred to Hybond nylon membrane (Amersham, UK). The DNA probes for the mitochondrial and cytoplasmic MetRSs were labeled with digoxigenin (DIG, Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions and used for Southern blot analysis.

2.3. Cloning and determination of ORF for mitochondrial MetRS

Two clones were found to contain parts of the mitochondrial MetRS of *C. albicans* by searching the genome database of *C. albicans*. They were 1776 and snc3 generated by Dr S. Scherer (Acacia Biosciences, Inc., Richmond, USA) and Dr P.T. Magee (University of Minnesota, St. Paul, USA), respectively, and kindly provided for further characterization. The sequences of the *Candida* genomic fragments in the two clones were determined and assembled with that of the original PCR product.

2.4. Expression and purification of recombinant MetRS protein

Analysis of the assembled sequence revealed an ORF putatively expressing the mitochondrial MetRS of *C. albicans*. To determine whether the gene expresses a functional MetRS protein, the gene was fused to six His tag (HIS) of pET-28b (Novagen, Madison, WI) in the N-terminal end using *Sac*I and *Hin*dIII. The resulting recombinant, pSW2, was introduced into the *E. coli* strain BL21(DE3) for expression and activity studies. The protein synthesis was induced by 0.1 mM IPTG for 3 h and subsequently the cells were harvested. The cells were then lysed by ultrasonication and the cell debris was removed by centrifugation. The HIS–MetRS fusion protein was purified using Ni^{2+} affinity chromatography (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

The active monomeric form of *E. coli* MetRS (N547mer) was purified from *E. coli* MJR strain expressing pJB104 (Burbaum and Schimmel, 1991). The harvested cells were resuspended in 50 mM potassium phosphate buffer (pH 7.4), 5% glycerol, 2 mM EDTA, 1 mM DTT, 0.24 M NaCl, 1.4 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonylfluoride (PMSF) and lysed by ultrasonication. The MetRS fraction in the lysate was separated by 30–50% ammonium sulfate precipitation. The pellet was dissolved into 50 mM potassium phosphate (pH 7.3), and dialyzed in the same buffer. The sample was loaded onto a DEAE–Sephadex column pre-equilibrated in 50 mM potassium phosphate buffer and eluted by a linear gradient of NaCl from 0 to 0.5 M.

2.5. In vitro aminoacylation assay

The aminoacylation reaction was carried out using *E. coli* $tRNA^{fMet}$ as a substrate. The protein was purified to test whether it shows aminoacylation activity. The reaction mixture contained 20 mM Hepes buffer (pH 7.5), 0.1 mM EDTA, 0.15 M NH_4Cl, 100 μg/ml bovine serum albumin, 2 mM ATP, 4 mM $MgCl_2$, 20 μM

[^{35}S]Met and 4 mg/ml *E. coli* tRNA^{fMet} (Sigma, St. Louis, MO). The reaction was initiated at 30°C by the addition of 5 nM of the *E. coli* and *Candida* mitochondrial MetRS proteins. Aliquots of the reactions were taken at time intervals and quenched by 10% trichloroacetic acid containing 1 mM Met on a filter paper (Whatman, 3MM, Maidston, UK). After washing the filter paper, the tRNA charged with the radioactive Met was measured with a liquid scintillation counter (LKB).

2.6. Genetic complementation

The aminoacylation activity of the protein was further confirmed by an in vivo genetic complementation test. The mitochondrial MetRS gene of *C. albicans* was transferred to the *E. coli* vector, pBluescript SK+ (Stratagene) using *Sac*I and *Pst*I. The resulting plasmid, pSW1, expressing the mitochondrial MetRS of *C. albicans*, was introduced into the *E. coli* strain MJR (*K12 A1E, metG, recA*) in which the chromosomally encoded MetRS gene was mutated (Starzyk and Schimmel, 1989). This strain encodes a defective MetRS that shows lower affinity to Met, and thus cannot grow on the minimal media without Met. If the mitochondrial MetRS of *C. albicans* is active to the *E. coli* tRNA^{Met} in this strain, it will rescue its auxotrophic phenotype on the minimal media. The *E. coli* MJR cells containing either the *E. coli* or *Candida* MetRS were inoculated on M9 minimal media containing 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.2% glucose and 0.1% thiamine.

3. Result and discussion

3.1. Generation of genomic fragments for mitochondrial and cytoplasmic MetRSs

To isolate a part of the gene encoding mitochondrial and cytoplasmic MetRSs from *C. albicans*, two sets (two forward and two backward) of the degenerate primers were synthesized from the known MetRS sequences. One pair of the primers generated the specific PCR product of 599 bp (data not shown). The determined sequence showed the highest homology to the mitochondrial MetRS gene of *S. cerevisiae*. This result suggested that the isolated PCR product could be a part of the gene encoding the mitochondrial MetRS of *C. albicans*. However, the cytoplasmic MetRS of *C. albicans* was not isolated using the combinations of these primers, implying that it should contain sequences different from those designed from the MetRS alignment. The result was not entirely unexpected, since the primers were designed from the sequence alignment that contained predominantly the bacterial MetRS sequences and thus would be preferential to the prokaryotic and mitochondrial MetRS sequences.

To isolate the genomic fragment of the cytoplasmic MetRS from *C. albicans*, new primers were designed from the sequence alignment of the cytoplasmic MetRSs of human, nematode and yeast. Combinations of the newly designed primers with the previously used primers generated three PCR products of difference sizes (data not shown). The sequences of these fragments were determined and assembled to generate 1154 bp encoding 384 aa. The sequence showed the highest homology to that of the cytoplasmic MetRS of *S. cerevisiae*.

3.2. Determination and Southern blotting of ORF for mitochondrial MetRS

The sequences of the three clones containing different parts of the mitochondrial MetRS were assembled to determine a complete gene structure. From the assembled sequence, an ORF of 1731 bp encoding 577 aa was established (Fig. 1). Since the mitochondrial histidyl- and valyl-tRNA synthetases of yeast are synthesized from the same gene encoding their cytoplasmic counterparts (Natsoulis et al., 1986; Chatton et al., 1988), we investigated whether the mitochondrial MetRS gene is different from that of the cytoplasmic MetRS, using Southern blot analysis. The PCR products that were thought to express the mitochondrial and cytoplasmic MetRSs of *C. albicans* were used to identify the genes in the chromosome fragments digested with *Cla*I. The two probes were hybridized to distinct DNA fragments, suggesting that the two MetRS genes are present in different genomic locations (Fig. 2).

3.3. Sequence analysis of mitochondrial MetRS

The deduced polypeptide sequence aligned well with the known MetRS sequences from other species (data not shown). The protein showed 43.2% and 35.9% identity with the mitochondrial MetRSs of *S. cerevisiae* and *Sh. pombe*. However, the enzyme showed 17.1% and 20.1% identity with the cytoplasmic MetRSs of these species. The N-terminal peptides of the mitochondrial MetRSs did not show a consensus sequence pattern, but positively charged and hydrophobic residues are relatively abundant (Fig. 1). This observation is consistent with the previous findings on the presequences for mitochondrial translocation (Roise and Schatz, 1988).

We generated the groups of the mitochondrial and cytoplasmic MetRSs of the three fungal species and compared the conserved sequence regions between them. The comparison of the sequences in the N-terminal catalytic domain revealed a few interesting differences between the two sequence groups. There are the two class-defining sequence elements, HIGH and KMSKS,

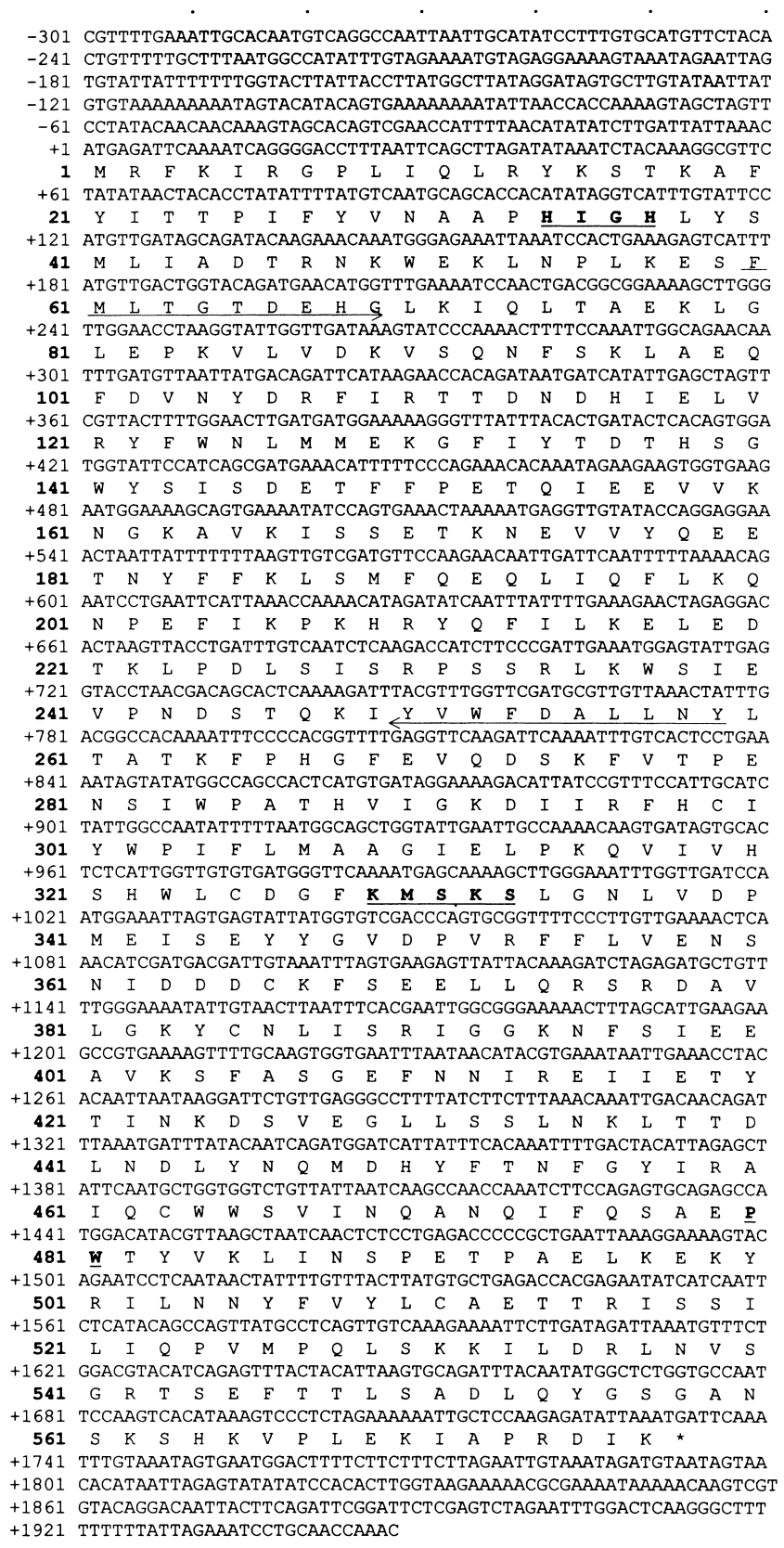

Fig. 1. DNA sequence and deduced aa of mitochondrial MetRS isolated from *C. albicans*. Class I defining sequence elements (HIGH and KMSKS) and the anticodon-binding Trp (preceded by Pro) are highlighted as bold characters with underline. The arrows indicate the sites from which the PCR primers were designed. The DDBJ accession number for the sequence is AB006140.

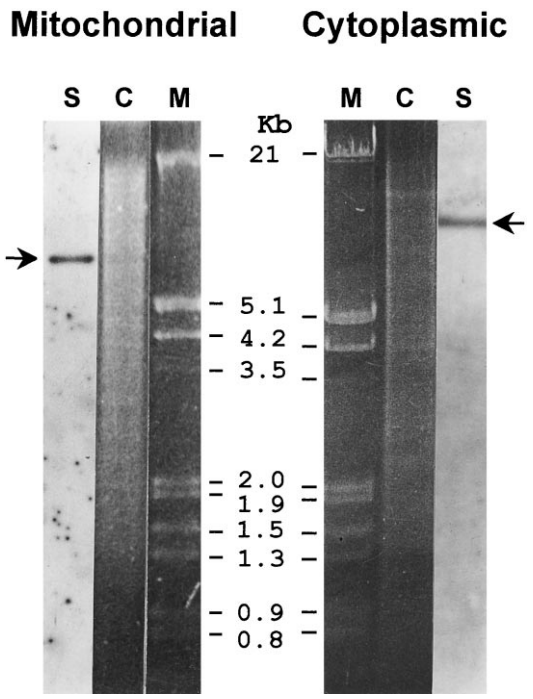

Fig. 2. Southern blotting of the genes for the mitochondrial and cytoplasmic MetRSs in *C. albicans*. Lanes, M, C and S indicate the molecular markers (λ *Hin*dIII and *Eco*RI cut), *Cla*I digested chromosome and its Southern blot, respectively. PCR products of 599 and 1154 bp encoding the mitochondrial and cytoplasmic methionyl-tRNA synthetases, respectively, were labeled by random priming with digoxigenin and used to detect the corresponding genes.

which are involved in the stabilization of the transition intermediate for the formation of aminoacyl-adenylate (Mechulam et al., 1991; First and Fersht, 1993; Schmitt et al., 1995). In the first conserved sequence motif, the mitochondrial and cytoplasmic MetRSs contain HLGH and HLGN, respectively (Fig. 3A). Earlier studies have suggested that the first His is more important than the second one for the enzyme activity (Lowe et al., 1985; Leatherbarrow and Fersht, 1987; Schmitt et al., 1995, 1997). Thus, substitutions at the second His must have been tolerated during the evolution.

In the second conserved sequences, the mitochondrial and cytoplasmic MetRSs of the three fungi contain KMSKSL and KFSKSR, respectively, showing the differences at the two aa (Fig. 3D). The three basic residues in this sequence element have been thought to be critical for the enzyme activity (Mechulam et al., 1991; Schmitt et al., 1994). Among them, the last basic residue was substituted for Leu in the mitochondrial MetRSs. Mutational analysis of these basic residues using *Bacillus* MetRS showed that the first two residues were more important than the last one (Schmitt et al., 1997), consistent with the sequence comparison.

Differences are also found in other important regions in the catalytic domain. The cytoplasmic MetRSs contain the Zn binding motif consisting of four Cys, whereas the mitochondrial MetRSs are devoid of it (Fig. 3B). The Zn binding motif is known to be involved in the maintenance of the active conformation of the catalytic site (Landro and Schimmel, 1993; Nureki et al., 1993; Schmitt et al., 1997). The absence of this motif in the mitochondrial MetRS suggests that it is dispensable, depending on the sequence context. Lastly, the two groups of MetRS showed difference in the motif that was shown to be involved in binding to aa (Fig. 3C) (Fourmy et al., 1991; Ghosh et al., 1991). Among the three important residues, V, H and W, only His is

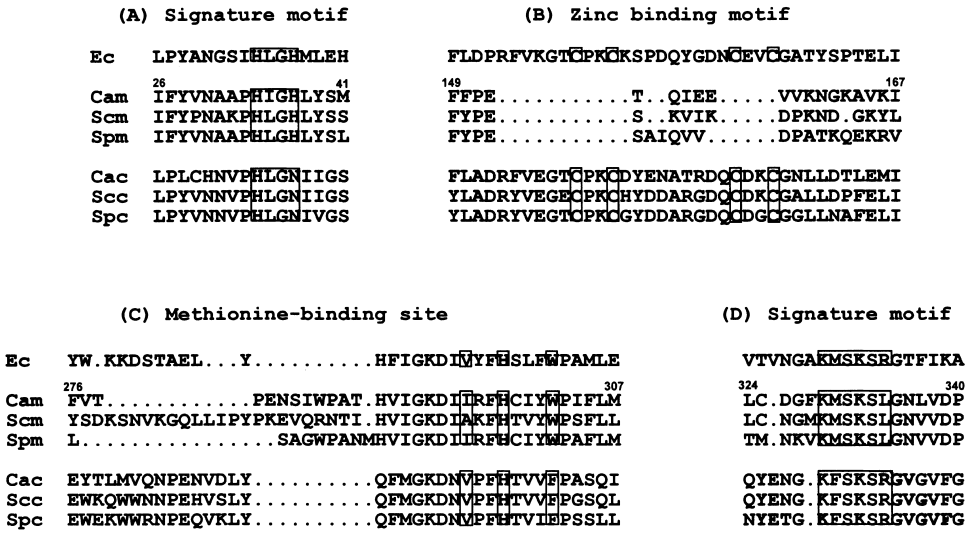

Fig. 3. Conserved sequence regions in the catalytic N-terminal domain of the mitochondrial and cytoplasmic MetRS of *E. coli*, *C. albicans*, *S. cerevisiae* and *Sh. pombe*. The conserved motifs are highlighted by boxes, and the numbers indicate the aa in the mitochondrial MetRS of *C. albicans*. Cam, mitochondrial MetRS of *C. albicans* (this work); Cac, cytoplasmic MetRS of *C. albicans*; Scm, mitochondrial MetRS *of S. cerevisiae* (Tzagoloff et al., 1989); Scc, cytoplasmic MetRS of *S. cerevisiae* (Walter et al., 1983); Spm, mitochondrial MetRS of *Sh. pombe* (Z98978); Scc, cytoplasmic MetRS of *Sh. pombe* (D1022268); Ec, *E. coli* MetRS.

completely conserved among all the known MetRSs, implying its functional significance. It was also confirmed by the mutational analyses of these residues in *Bacillus* MetRS (Schmitt et al., 1997).

3.4. In vitro aminoacylation activity

The mitochondrial MetRS protein of *C. albicans* was expressed as HIS tag fusion protein and purified using Ni^{2+}-affinity chromatography. The estimated molecular weight of the recombinant protein was consistent with the expected size (62.4 kDa) calculated from the deduced polypeptide (Fig. 4). The protein was found to react with the *E. coli* $tRNA^{fMet}$ for aminoacylation at much slower rate than the *E. coli* MetRS (Fig. 5). Although we believe that the lower activity of *Candida* MetRS results from its inefficient reaction with *E. coli* $tRNA^{fMet}$, we cannot rule out the possibility that the purified *Candida* MetRS contains a low proportion of the active enzyme. The *E. coli* MetRS contains the Zn binding motif that is critical for the enzyme activity and contains the C-terminal appendix that is involved in tRNA binding (Kim et al., 1993) as well as dimerization (Cassio and Waller, 1971). The mitochondrial MetRS of *C. albicans* lacks both of these motifs. Therefore, the aminoacylation activity of the mitochondrial MetRS of *C. albicans* to the *E. coli* $tRNA^{fMet}$ implies that the Zn binding and dimerization peptides are not essential for the enzyme activity. We recently found that the *Mycobacterium tuberculosis* MetRS also lacks the Zn binding motif and dimerization domain, but showed aminoacylation activity to the *E. coli* $tRNA^{Met}$ as well (Kim et al., 1998).

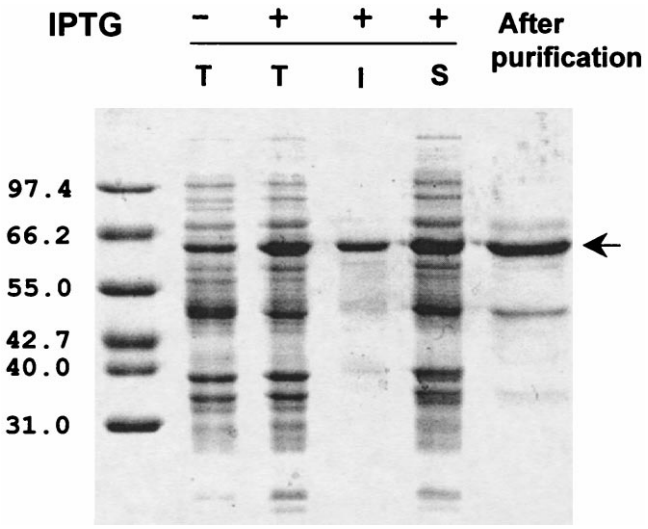

Fig. 4. Expression and purification of the recombinant mitochondrial MetRS of *C. albicans*. T, I and S indicate the total, insoluble and soluble fractions of the proteins extracted from the *E. coli* cells containing the recombinant mitochondrial MetRS of *C. albicans*.

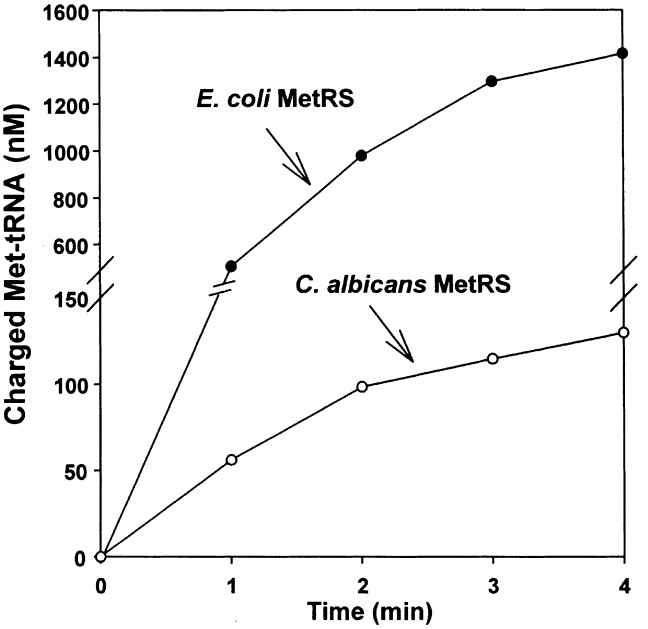

Fig. 5. In vitro aminoacylation of *E. coli* $tRNA^{fMet}$. The mitochondrial *E. coli* and *C. albicans* MetRSs were subjected to in vitro aminoacylation reaction.

3.5. Genetic complementation

The aminoacylation activity of the mitochondrial MetRS of *C. albicans* was further tested by genetic complementation. The tester *E. coli* host, MJR, shows Met auxotrophy because its intrinsic MetRS is defective in Met binding. The *Candida* MetRS was expressed in this strain to test whether it rescues the auxotrophic phenotype. The cells expressing the mitochondrial MetRS of *C. albicans* grew on the minimal media without Met at both 30°C and 37°C (Fig. 6). The growth of the cells containing the *Candida* MetRS was

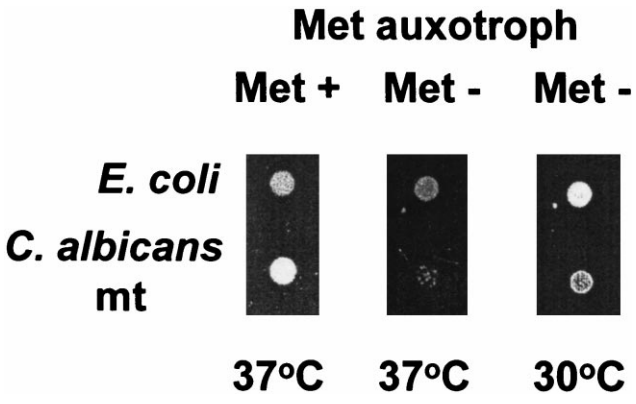

Fig. 6. In vivo genetic complementation. In vivo aminoacylation activity of *Candida* mitochondrial MetRS was determined by genetic complementation using the *E. coli* tester strain. This strain is a Met auxotroph due to the MetRS defective in Met binding. The growth of the cells containing the *E. coli* and *Candida* MetRSs was tested on M9 minimal media with (Met+) and without (Met−) the supplement of Met at 30°C and 37°C.

a little slower than those containing the *E. coli* MetRS. This result is consistent with the lower activity of the *Candida* enzyme in in vitro aminoacylation assay. *E. coli* contains initiator and elongator tRNAs that interact with the Met codon. The positive complementation result indicates that the *Candida* MetRS specifically recognized both types of the *E. coli* $tRNA^{Met}$.

3.6. Implications for evolution and tRNA recognition

In the present work, we have cloned the gene encoding the mitochondrial MetRS of a pathogenic fungi *C. albicans* and characterized its sequence and activity. Its sequence, together with those of the mitochondrial MetRSs of *S. cerevisiae* and *Sh. pombe*, was classified into the prokaryotic group (data not shown) and distinguished from the cytoplasmic tRNA synthetases of the same species (Fig. 3). Horizontal gene transfer of aminoacyl tRNA synthetases has been reported between the prokaryotes and eukaryotes (Shiba et al., 1997). A gene transfer from the eukaryotic type to prokaryotic type was observed in the case of a few bacterial MetRSs, including *E. coli*, *Haemophilus influenza* and *Neisseria gonorrhoeae* (Kim et al., 1998). However, the phylogenetic analysis suggested that a gene transfer did not appear to have occurred in the mitochondrial MetRS sequences (data not shown).

The putative sequence for the mitochondrial $tRNA^{Met}$ of *C. albicans* (obtained from the *Candida* Sequencing Project) showed 43.2% and 50.6% identity to that of the initiator and elongator tRNA of *E. coli*, differing at many locations except for the CAU anticodon (data not shown). The anticodon of $tRNA^{Met}$ is known to interact with the Trp residue in the C-terminal domain of *E. coli* MetRS (Ghosh et al., 1991; Schulman and Pelka, 1988). The mitochondrial MetRS of *C. albicans* contains Trp481 preceded by Pro (Fig. 2) that is expected to play a same role in the interaction with the cognate tRNA. Conservation of Trp in the C-terminal domain of MetRS protein implies that these proteins would interact with their cognate tRNAs in a similar molecular mechanism.

Acknowledgement

We wish to thank Drs S. Scherer and P.T. Magee for the kind gifts of the clones and Dr K. Manoharan for critical reading of the manuscript. This work was supported by grant 94-0403-20 from the Korea Science and Engineering Foundation.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Burbaum, J.J., Schimmel, P., 1991. Assembly of a class I tRNA synthetase from products of artificially split gene. Biochemistry 30, 319–324.

Cassio, D., Waller, J.-P., 1971. Modification of methionyl-tRNA synthetase by proteolytic cleavage and properties of the trypsin-modified enzyme. Eur. J. Biochem. 20, 283–300.

Chatton, B., Walter, P., Ebel, J.-P., Lacroute, F., Fasiolo, F., 1988. The yeast *VAS1* gene encodes both mitochondrial and cytoplasmic valyl-tRNA synthetases. J. Biol. Chem. 263, 52–57.

Dirheimer, G., Keith, G., Dumas, P., Westhof, E., 1995. Primary, secondary and tertiary structures of tRNAs. In: Söll, D., RajBhandary, U.L (Eds.), tRNA: Structure, Biosynthesis and Function, ASM Press, Washington, DC, pp. 93–126.

First, E.A., Fersht, A.R., 1993. Mutational and kinetic analysis of a mobile loop in tyrosyl-tRNA synthetase. Biochemistry 32, 13658–13663.

Fourmy, D., Mechulam, Y., Brunie, S., Blanquet, S., Fayat, G., 1991. Identification of residues involved in the binding of methionine by *Escherichia coli* methionyl-tRNA synthetase. FEBS Lett. 292, 259–263.

Ghosh, G., Pelka, H., Schulman, L.H., Brunie, S., 1991. Activation of methionine by *Escherichia coli* methionyl-tRNA synthetase. Biochemistry 30, 9569–9575.

Kim, S., Jo, Y.J., Lee, S.H., Motegi, H., Shiba, K., Sassanfar, M., Martinis, S.A., 1998. Biochemical and phylogenetic analyses of methionyl-tRNA synthetase isolated from a pathogenic microorganism, Mycobacterium tuberculosis. FEBS Lett 427, 259–262.

Kim, S., Landro, J.A., Gale, A.J., Schimmel, P., 1993. C-Terminal peptide appendix in a class I transfer RNA synthetase needed for acceptor–helix contacts and microhelix aminoacylation. Biochemistry 32, 13026–13031.

Koerner, T.J., Myers, A.M., Lee, S., Tzagoloff, A., 1987. Isolation and characterization of the yeast gene coding for the alpha subunit of mitochondrial phenylalanyl-tRNA synthetase. J. Biol. Chem. 262, 3690–3696.

Landro, J.A., Schimmel, P., 1993. Metal binding site in a class I tRNA synthetase localized to a cysteine cluster inserted into nucleotide binding fold. Proc. Natl. Acad. Sci. USA 90, 2261–2265.

Leatherbarrow, R.J., Fersht, A.R., 1987. Investigation of transition state stabilization by residues histidine-45 and threonine-40 in the tyrosyl-tRNA synthetase. Biochemistry 26, 8524–8528.

Lowe, D.M., Fersht, A.R., Wilkinson, A.J., 1985. Probing histidine–substrate interactions in tyrosyl-tRNA synthetase using asparagine and glutamine replacement. Biochemistry 24, 5106–5109.

Martinis, S.A., Schimmel, P., 1996. Aminoacyl tRNA synthetases. In: Neidhardt, F.C. (Ed), *Escherichia coli* and *Salmonella typhimurium* Cellular and Molecular Biology, 2nd ed., ASM Press, Washington, DC, pp. 887–901.

Mechulam, Y., Dardel, F., Le Corre, D., Blanquet, S., Fayat, G., 1991. Lysine 335, part of the KMSKS signature sequence, plays a crucial role in the amino acid activation reaction catalyzed by the *E. coli* methionyl-tRNA synthetase. J. Mol. Biol. 217, 465–475.

Myers, A.M., Tzagoloff, A., 1985. *MSW*, a yeast gene coding for mitochondrial tryptophanyl-tRNA synthetase. J. Biol. Chem. 260, 15371–15377.

Natsoulis, G., Hilger, F., Fink, G.R., 1986. The *HTS1* gene encodes both the cytoplasmic and mitochondrial histidine tRNA synthetases of *S. cerevisiae*. Cell 46, 235–243.

Nureki, O., Kohno, T., Sakamoto, K., Miyazawa, T., Yokoyama, S., 1993. Chemical modification and mutagenesis studies on zinc binding of aminoacyl-tRNA synthetases. J. Biol. Chem. 268, 15368–15373.

Pape, L.K., Koerner, T.J., Tzagoloff, A., 1985. Characterization of a yeast nuclear gene (*MST1*) coding for the mitochondrial threonyl-$tRNA_1$ synthetase. J. Biol. Chem. 260, 15362–15370.

Powderly, W.G., 1994. Resistant candidiasis. AIDS Res. Hum. Retroviruses 10, 925–929.

Roise, D., Schatz, G., 1988. Mitochondrial presequences. J. Biol. Chem. 263, 4509–4511.

Schmitt, E., Meinnel, T., Blanquet, S., Mechulam, Y., 1994. Methionyl-tRNA synthetase needs an intact and mobile $_{332}$KMSKS$_{336}$ motif in catalysis of methionyl adenylate formation. J. Mol. Biol. 242, 566–577.

Schmitt, E., Panvert, M., Blanquet, S., Mechulam, Y., 1995. Transition state stabilization by the 'high' motif of class I aminoacyl-tRNA synthetases: the case of *Escherichia coli* methionyl-tRNA synthetase. Nucleic Acids Res. 23, 4793–4798.

Schmitt, E., Panvert, M., Mechulam, Y., Blanquet, S., 1997. General structure/function properties of microbial methionyl-tRNA synthetase. Eur. J. Biochem. 246, 539–547.

Schulman, L.H., Pelka, H., 1988. Anticodon switching changes the identity of methionine and valine transfer RNAs. Science 242, 765–768.

Shiba, K., Motegi, H., Schimmel, P., 1997. Maintaining genetic code through adaptations of tRNA synthetases to taxonomic domains. Trends Biochem. Sci. 22, 453–457.

Shiba, K., Suzuki, N., Shigesada, K., Namba, Y., Schimmel, P., Noda, T., 1994. Human cytoplasmic isoleucyl-tRNA synthetase: selective divergence of a new structural unit. Proc. Natl. Acad. Sci. USA 91, 7435–7439.

Starzyk, R.M., Schimmel, P., 1989. Construction of intra-domain chimeras of aminoacyl-tRNA synthetases. J. Biomol. Struct. Dyn. 7, 225–234.

Tzagoloff, A., Vambutas, A., Akai, A., 1989. Characterization of *MSM1*, the structural gene for yeast mitochondrial methionyl-tRNA synthetase. Eur. J. Biochem. 179, 365–371.

Walter, P., Gangloff, J., Bonnet, J., Boulanger, Y., Ebel, J.-P., Fasiolo, F., 1983. Primary structure of the *Saccharomyces cerevisiae* gene for methionyl-tRNA synthetase. Proc. Natl. Acad. Sci. USA 80, 2437–2441.