

*Review*

## Methionyl-tRNA synthetase<sup>★</sup>

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**Methionyl-tRNA synthetase (MetRS) belongs to the family of 20 enzymes essential for protein biosynthesis. It links covalently methionine with its cognate tRNA. Crystal structures solved for bacterial MetRSs have given a number of interesting insights into enzyme architecture and methionylation catalysis. A comparison of sequences of MetRSs belonging to all kingdoms of life, as well as numerous biochemical and genetic studies have revealed the presence of various additional domains appended to the catalytic core of synthetase. They are responsible for interactions with tRNA and proteins. Tertiary structure of C-terminal tRNA-binding appendices can be deduced from those determined for their homologues: tRNA binding protein 111 and endothelial monocyte-activating polypeptide II. Contacts between MetRS and other proteins could be mediated not only by noncatalytic peptides but also by structural elements present in the catalytic core, e.g. Arg-Gly-Asp (RGD) motifs. Additional activities involve MetRS in the maintenance of translational fidelity and in coordination of ribosome biogenesis with protein synthesis.**

Protein synthesis is based on genetic information transcribed from DNA to mRNA, which is then decoded through recognition of each codon by a specific tRNA anticodon. Translational fidelity depends on specificity of codon-anticodon interactions and on tRNA

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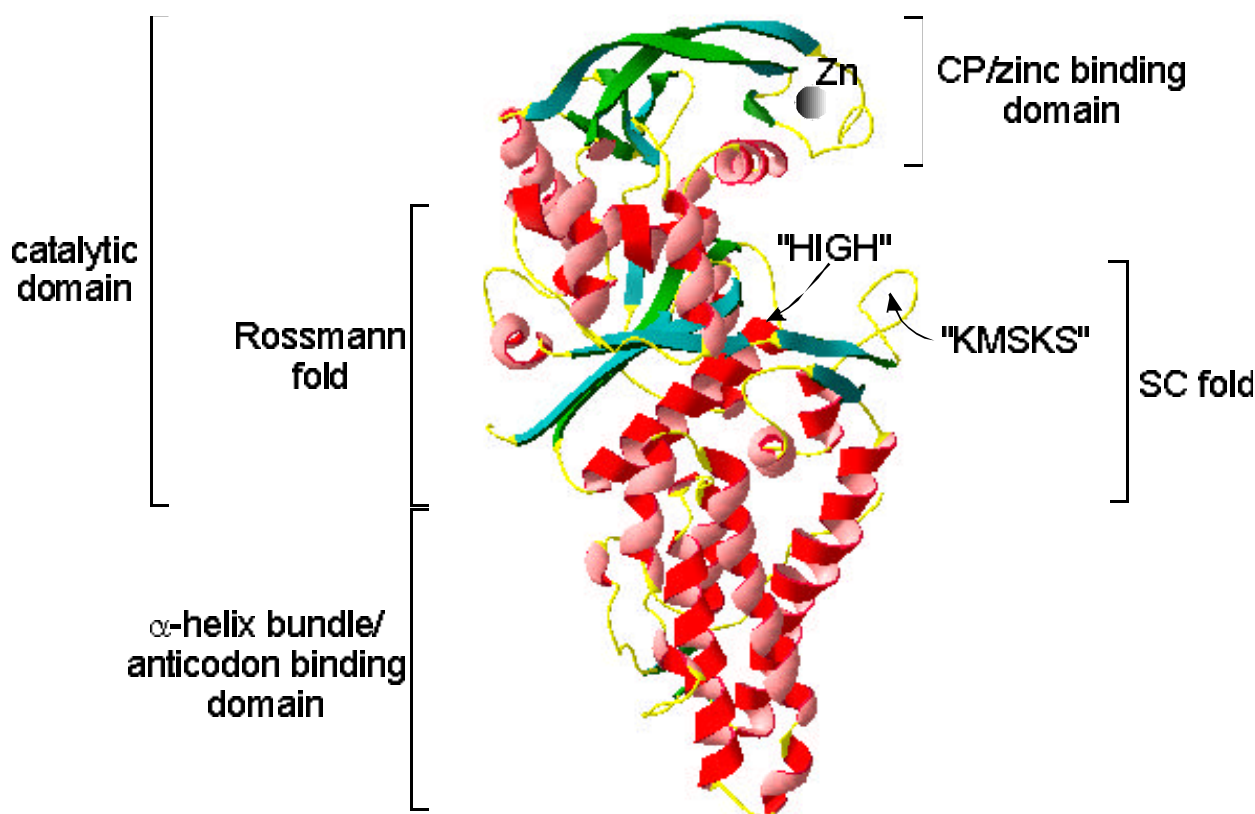
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**Abbreviations:** AARS, aminoacyl-tRNA synthetase (e.g. MetRS, methionyl-tRNA synthetase); CP, connective peptide; EMAP II, endothelial monocyte-activating polypeptide II; Hcy, homocysteine; OB-fold, oligonucleotide-oligosaccharide-binding fold; SC fold, stem contact fold; Trbp 111, tRNA-binding protein 111.

aminoacylation. The latter process is catalyzed by a family of 20 aminoacyl-tRNA synthetases (AARSs). They perform a two step reaction, in which an activated amino acid is transferred to the 3'-terminal adenosine of the tRNA. All aminoacyl-tRNA synthetases have been divided into two classes, each consisting of 10 enzymes [1–4]. Their classification is based on structural and biochemical data: sequence similarity (although very lim-

LeuRS, CysRS, and ArgRS, respectively). The other members of class I are: glutamyl- and glutamyl-tRNA synthetases (GlnRS, GluRS) belonging to subclass Ib, and tyrosyl-tRNA synthetase (TyrRS) forming, with tryptophanyl-tRNA synthetase (TrpRS), class Ic.

Amongst the enzymes of its class, MetRS is especially interesting. It recognizes an initiator tRNA as well as the tRNA delivering methionine for elongation of protein chain.



**Figure 1.** Structural features of MetRSs as observed in a crystal of *T. thermophilus* methionyl-tRNA synthetase (PDB ID 1A8H) [6].

Catalytic domain is formed by Rossmann fold and SC fold domains where "HIGH" and "KMSKS" signature motifs, respectively, are present. Correct structure of active site is maintained by zinc finger domain present in connective peptide (CP) in ser-tion. Anticodon binding domain forms an  $\alpha$ -helix bundle.

ited to short motifs), fold of catalytic domain, the way of interaction with and charging of tRNA. Each class has been additionally divided into subclasses the members of which show higher sequence and structural homology. Methionyl-tRNA synthetase (MetRS) belongs to subclass Ia, together with isoleucyl-, valyl-, leucyl-, cysteinyl- and arginyl-tRNA synthetases (IleRS, ValRS,

Moreover, unlike most of other AARSs, MetRS isolated from a variety of sources (species) shows structural diversity, connected with the ability to interact with other proteins to form functional complexes.

All class I AARSs, despite differences in amino-acid sequence, share a common structural motif being their catalytic center (Fig. 1). It is a nucleotide binding fold (called

Rossmann fold), consisting of five parallel  $\beta$  strands connected by  $\alpha$  helices. Until recently it has been assumed that Rossmann fold comprises both of the so-called class I signature motifs: "HIGH" and "KMSKS", located in close proximity and forming structural elements critical for aminoacyl-adenylate formation. Now crystal structures of MetRSs from *Escherichia coli* [5] and *Thermus thermophilus* [6] provided new informations concerning structural organization of their catalytic domains and regions responsible for substrates recognition.

### STRUCTURAL FEATURES OF BACTERIAL MetRSs

The Rossmann fold of *T. thermophilus* MetRS is formed by two polypeptide segments separated by an insertion of 117 amino acids, called the "connective polypeptide" domain (CP domain) [6]. The "HIGH" motif (HLGH in the case of *T. thermophilus* MetRS) is located in a loop between  $\beta 1$  and  $\alpha 1$  elements, with the two histidine residues stacked upon each other. The "KMSKS" peptide (KMSKT) – previously recognized to occur within the Rossmann fold was finally localized in a separate  $\beta$ - $\alpha$ - $\alpha$ - $\beta$ - $\alpha$  structure, named SC fold (stem contact fold) [6], between the Rossmann fold and C-terminal  $\alpha$ -helix-bundle. The SC fold is responsible for interactions of MetRS with tRNA<sup>Met</sup> anticodon (Fig. 1). It is conserved very well in other synthetases of class Ia and Ib: IleRS, ArgRS, GlnRS and GluRS. Study of the GlnRS–tRNA<sup>Gln</sup> complex lead to the conclusion that it interacts with the acceptor, dihydrouridine and anticodon stems of tRNA [7]. The KMSKS forms a loop, the conformation of which is conserved in free form of the mentioned enzymes, but changes dramatically upon substrate binding. This change probably affects the structure of HIGH region [6]. The flexibility of the KMSKS loop could be a consequence of aminoacylation complexity, requiring MetRS

substrates to be bound to the active site at subsequent steps of the reaction.

The connective polypeptide domain of MetRS from *T. thermophilus* is built mainly of antiparallely arranged eight  $\beta$  strands (Fig. 1) [6]. The core of CP domain is a four-stranded  $\beta$  sheet, conserved in all class Ia and Ib AARSs. An other four stranded  $\beta$  sheet is inserted between  $\beta$  strands of the core. This insertion coordinates one Zn<sup>2+</sup> ion. The zinc finger structure is crucial for enzyme activity. It plays a key role in methionine activation as well as in correct positioning of the 3' end of tRNA [8]. Results of studies carried out on MetRSs from different bacterial sources suggest that the zinc ion contributes to the correct orientation of the enzyme active site.

There is a difference in topology of CP domain observed between *T. thermophilus* and *E. coli* MetRSs. Namely, the zinc-binding domain of *E. coli* MetRS seems to be a result of duplication of the structure found in *T. thermophilus* protein [5]. Moreover, a comparison of sequences of known MetRSs enabled to distinguish four groups (families) differing in the number of knuckles and of zinc fingers in the CP region [5]. Connective peptides of *Eukaryota*, *Archaea* and *Spirochaetales* probably bind two zinc ions. Two other families possessing two knuckles and one zinc ion or one knuckle coordinating zinc are represented by *E. coli* and *T. thermophilus* MetRSs, respectively. The fourth group contains enzymes structurally similar to *T. thermophilus* MetRS, although they do not bind any zinc ion. MetRS from *Mycobacterium tuberculosis* belongs to this group [9].

Superimposition of the Rossmann folds of *E. coli* and *T. thermophilus* MetRSs has revealed distinct orientations of the CP domains with respect to the catalytic center. This can argue for the connective peptide being mobile with respect to the enzyme active site [5]. The tryptophan fluorescence of *E. coli* MetRS pointed to changes in the enzyme conformation accompanying methionylation step and MetRS–tRNA<sup>Met</sup> complex formation [10–12].

The SC fold of *T. thermophilus* MetRS is followed by an  $\alpha$ -helix rich cylindrical domain, unique for class Ia synthetases (Fig. 1). It has been shown that amino-acid residues involved in tRNA<sup>Met</sup> anticodon recognition are clustered on one face of this  $\alpha$ -helix bundle structure. The recognition surface of *T. thermophilus* MetRS contains invariant Asn355, Arg359 and Trp424 [6]. Mutagenesis performed for MetRS of *E. coli* has shown that corresponding Asn391, Arg395 and Trp461 also interact with the anticodon [13–16]. The C-terminal helix of *E. coli* MetRS folds back towards the KMSKS containing loop, probably playing a role in productive binding of the tRNA acceptor stem inside the KMSKS and CP regions.

Two amino-acid regions surrounding residues 253 and 300 of *E. coli* MetRS are well conserved in all known sequences of its orthologues (Fig. 2). These residues are possibly involved in methionine binding [17–19]. This prediction is reinforced by results of studies on mutations of His301 and Trp305 of *E. coli* enzyme [17, 20]. It has been also pointed out that the two regions mentioned above face one another forming a hydrophobic cleft, similar to that of TyrRS, where L-tyrosine binding occurs [21].

Two main domains present in the majority of AARSs structures – the catalytic center and the anticodon binding domain – reflect the L-shaped structure of the tRNA molecule. It has been shown in the case of *E. coli* MetRS that mutations of particular residues located in the anticodon binding domain and in a helix joining it to the catalytic core of the enzyme break the domain–domain functional communication in a synthetase complexed with tRNA<sup>Met</sup> [22]. These residues have no contact with tRNA<sup>Met</sup> but they contribute to the stability of the MetRS–tRNA<sup>Met</sup> complex and to the catalytic efficiency of aminoacylation. It has been proposed that they can be important for induced-fit-directed binding of methionine specific tRNA [22].

## MetRSs STRUCTURAL DIVERSITY

Methionyl-tRNA synthetases belong to those of AARSs which show structural diversity depending on the source the enzyme was isolated from. On that base MetRSs of different origin have been divided into five structural groups (Fig. 3) [23]. A minimal core enzyme is found in organisms belonging to all kingdoms of life and in eukaryotic organelles. A large group of eubacterial and some archaeal MetRSs possess additional C-terminal domains. An early study carried out on *E. coli* MetRS proved that this domain was dispensable for enzyme activity but was responsible for its dimerization [24, 25]. Interestingly, it is highly homologous to eubacterial peptide Trbp 111, found in *E. coli* and *A. aeolicus* genomes [26, 27]. Trbp 111 binds nonspecifically tRNAs [27]. A similar C-terminal domain was identified in MetRSs from worm *C. elegans*, plants *O. sativa* and *A. thaliana* and some eubacteria (*Borrelia burgdorferi*, *Treponema pallidum*) [23]. A functional study of that domain in monomeric *O. sativa* MetRS has led to the conclusion that it provides the enzyme with better catalytic efficiency in binding the amino acid acceptor arm of tRNA<sup>Met</sup> and facilitates its aminoacylation [23]. This kind of C-terminal MetRS peptide is similar to a monomeric cytokine EMAP II (endothelial monocyte-activating polypeptide II) [2–31]. EMAP II was originally identified as a product of murine methylcholanthrene A (Met A)-induced fibrosarcoma cells [28] but it is expressed also in normal cells upon apoptosis – a physiological process by which multicellular organisms get rid of injured, infected or developmentally unnecessary cells. EMAP II is generated after proteolytic cleavage of p43, one of the components of multisynthetase complex, where it probably facilitates tRNA-substrate binding [26]. Interestingly, an EMAP II-like cytokine was also generated after proteolytic cleavage of human TyrRS [32]. Its effect encompasses induction

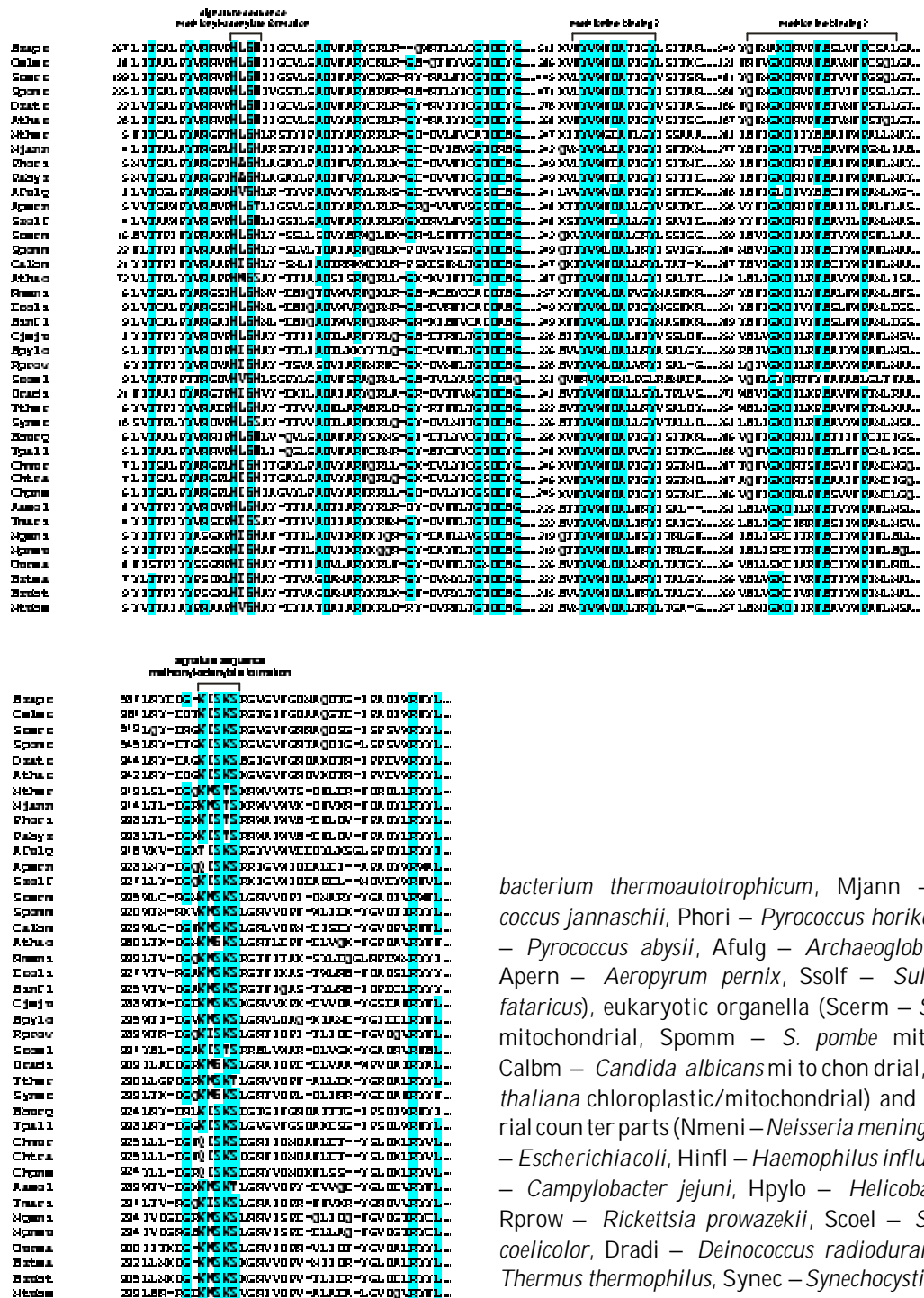
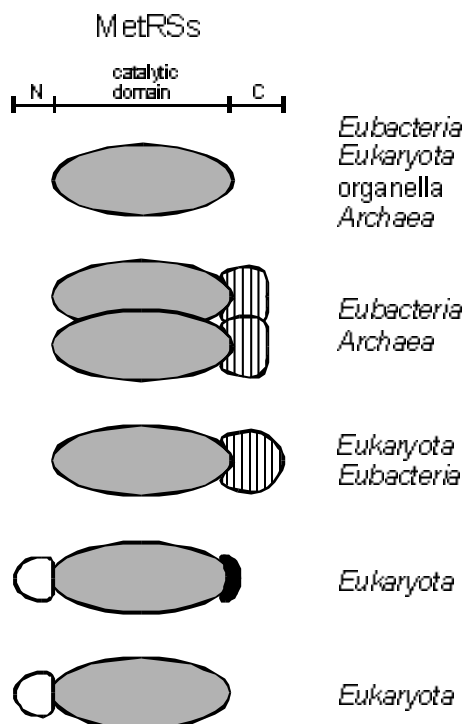


Figure 2. Alignment of MetRSs sequences.

Only critical regions of the active site (signature sequences) and possible methionine binding sites are shown. Among the presented primary structures are MetRSs of eukaryotic or i gin (Hsapi – *Homo sapiens* cytoplasmic, Celec – *Caenorhabditis elegans* cytoplasmic, Scerm – *Saccharomyces cerevisiae* cytoplasmic, Spom – *Schizosaccharomyces pombe* cytoplasmic, Osat – *Oryza sativa* cytoplasmic, Atha – *Arabidopsis thaliana* cytoplasmic), archaeobacterial enzymes (Mther – *Methano-*

*bacterium thermoautotrophicum*, Mjann – *Methanococcus jannaschii*, Phori – *Pyrococcus horikoshii*, Paby – *Pyrococcus abyssi*, Afulg – *Archaeoglobus fulgidus*, Aperr – *Aeropyrum pernix*, Ssol – *Sulfolobus solfataricus*), eukaryotic organella (Scerm – *S. cerevisiae* mitochondrial, Spom – *S. pombe* mitochondrial, Calbm – *Candida albicans* mitochondrial, Atha – *A. thaliana* chloroplastic/mitochondrial) and their bacterial counterparts (Nmen – *Neisseria meningitidis*, Ecol – *Escherichia coli*, Hinf – *Haemophilus influenzae*, Cjeju – *Campylobacter jejuni*, Hpylo – *Helicobacter pylori*, Rpro – *Rickettsia prowazekii*, Ssol – *Streptomyces coelicolor*, Dradi – *Deinococcus radiodurans*, Tther – *Thermus thermophilus*, Synec – *Synechocystis* sp., Bburg – *Borrelia burgdorferi*, Tpal – *Treponema pallidum*, Chmur – *Chlamydia muridarum*, Chtr – *Chlamydia trachomatis*, Chpn – *Chlamydophila pneumoniae*, Aaeol – *Aquifex aeolicus*, Tmar – *Thermotoga maritima*, Mgen – *Mycoplasma genitalium*, Mneu – *Mycoplasma pneumoniae*, Urea – *Ureaplasma urealyticum*, Bstea – *Bacillus stearothermophilus*, Bsub – *Bacillus subtilis*, Mtube – *Mycobacterium tuberculosis*). Amino-acid residues identical for at least 80% of analyzed sequences are shaded. So-called signature motifs are marked in bold.

of migration of mononuclear phagocytes (MPs) and polymorphonuclear leukocytes



**Figure 3. Structural diversity of MetRSs.**

A minimal core enzyme has been found in organisms belonging to all kingdoms as well as in organelles. Most of *Eubacteria* possess MetRSs bearing an additional C-terminal domain responsible for dimerization, homologous to *A. aeolicus* Trbp 111 peptide, which is also a dimer and is able to bind tRNAs. A similar C-terminal domain, more related to a cytokine EMAP II, is adjoined to eukaryotic and some bacterial MetRSs (plant MetRS belonging to this group is a monomer). EMAP II and Trbp 111 are close sequence and structure homologues. Two last structural variants of enzyme have been found only in eukaryotic cells. MetRS possessing an N-terminal extension and short C-terminal appendix forming another kind of tRNA-binding domain is a member of multienzymatic complex found in higher eukaryotes (N-terminal domain mediates association in the complex). Enzymes possessing only an N-terminal extension exist in yeast cytoplasm, where they form a complex with GluRS and Arc1p protein, which is an EMAP II homologue, responsible for tRNA recognition and delivery.

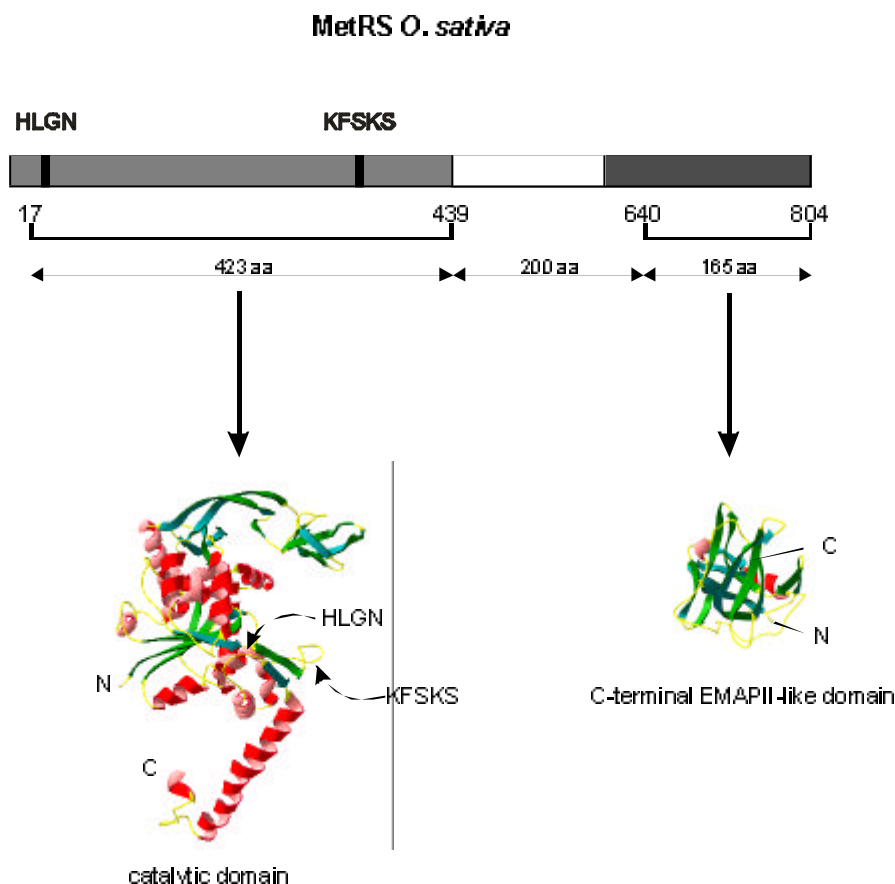
(PMNs), stimulation of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and tissue factor production, as well as activation of cell surface expression of P- and E-selectins. It is surprising, that this same kind of structure appended to MetRS or

p43 directs tRNA to the active site of synthetase.

Trbp 111 and EMAP II have highly similar sequences and tertiary structure, called the OB-fold (oligonucleotide-oligosaccharide-binding fold) [33, 34]. This kind of  $\beta$ -barrel has been also found at N-termini of AspRS [35], LysRS [36] and AsnRS [37], where it is responsible for specific binding of tRNA anticodon. The function of OB-fold localized in another AARS – *T. thermophilus* PheRS – is still unknown [38].

Two last structural variants of MetRSs are found only in eukaryotic cells. The enzyme being a component of multisynthetase complex found in higher eukaryotes cytoplasm, possesses a large N-terminal extension, mediating association with other proteins forming that particle [39, 40], and a shorter C-terminal appendix. Its sequence is related to that of linker domain of GluProRS, which forms another kind of RNA-binding module [41]. MetRSs isolated from yeast cytoplasm possess only the N-terminal extension. This additional domain enables association with Arc1p peptide (aminoacyl-tRNA synthetase cofactor 1 (ARC1) gene product), which is also a homologue of EMAP II/Trbp 111 [42, 43]. MetRS together with GluRS and Arc1p form a functional complex, where the latter peptide plays an important role in tRNA binding and delivery to the active sites of the two synthetases [43].

The crystal structures of bacterial MetRSs were determined using proteins bearing catalytic and anticodon binding domains, with the C-terminal extension removed by genetic engineering [5, 6]. Sequence similarity suggests that the missing MetRS domain is of tertiary structure similar to that recently determined for human EMAP II [34]. This set of information enabled us to propose a possible structure for both catalytic and additional C-terminal domains of plant MetRS (Fig. 4). Matrixes for modeling the *O. sativa* enzyme were *E. coli* MetRS and human EMAP II, respectively. The last  $\alpha$ -helix of the bacterial en-



**Figure 4. Eukaryotic MetRS modeling.**

3D models were generated using SWISS-MODEL program available on EXPASY server (<http://www.expasy.ch/swissmod/SWISS-MODEL.html>). An *E. coli* MetRS (PDB ID 1QQT) [5] and human EMAP II cytokine (1EUJ) [34] were taken as matrices for structural alignment. Sequence regions corresponding to obtained models are marked in gray on the top scheme.

zyme anticodon-binding domain folds back towards the active site. On the other hand, a study of EMAP II-like domain of plant enzyme showed that it could interact with tRNA<sup>Met</sup> acceptor stem [23]. Both facts lead to the conclusion that EMAP II-like C-terminal extension of plant enzyme is located near its active site, so as to be able to take a part in tRNA delivery and correct positioning for aminoacylation catalysis [23].

The OB-fold present in EMAP II and Trbp 111 seems to be characteristic for other peptides of similar sequence, e.g. C-termini of *H. sapiens* TyrRS and *E. coli* MetRS. They are represented by model structures shown in Fig. 5, all generated using EMAP II as a reference. Our Trbp 111 model is consistent with the published structure (not shown) [33].

## MetRS AND PROTEIN-PROTEIN INTERACTIONS

As it was mentioned above, interactions between MetRSs and other proteins are mediated by additional domains appended to the catalytic core of enzyme. They take a part in the assembly of multisynthetase complex [44–46] or interactions with tRNA binding proteins, enhancing MetRS catalytic efficiency [43]. Complexes isolated from cytoplasm of *Metazoa* contain eight synthetases—GluProRS, IleRS, LeuRS, MetRS, GlnRS, ArgRS, LysRS, AspRS, and three nonenzymatic peptides of molecular mass of 43 kDa, 38 kDa and 18 kDa. Interactions between all the components of the multienzymatic complex were studied extensively using genetic

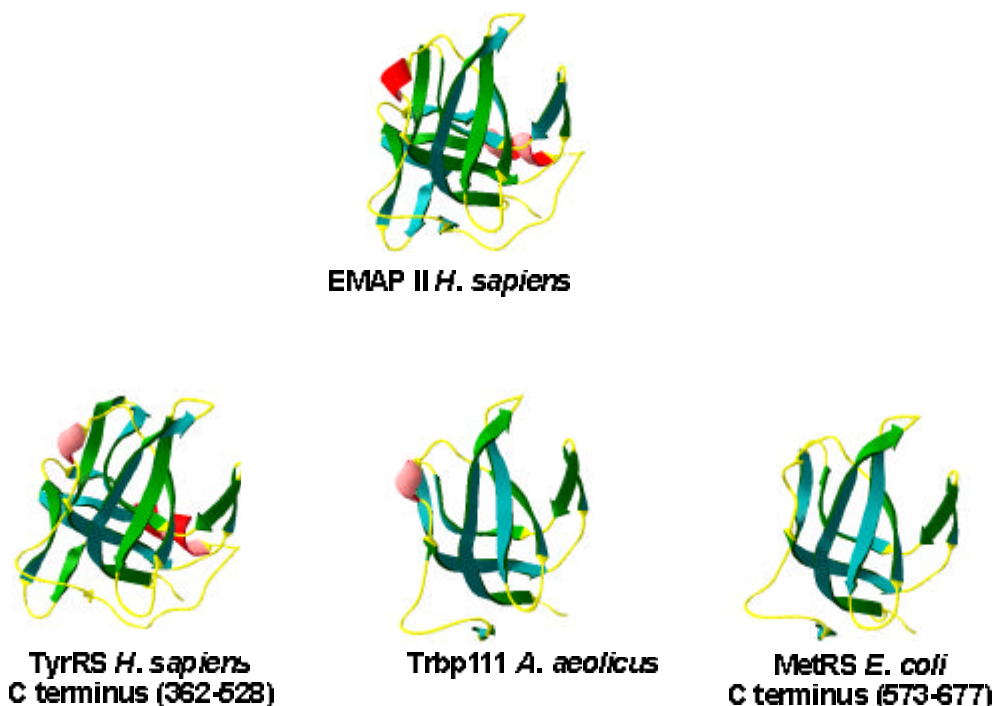


Figure 5. Tertiary structure of human EMAP II [34] and models made on its base: C-terminal domain of human TyrRS, Trbp 111 from *A. aeolicus* and C terminus of *E. coli* MetRS. Modelling was done by SWISS-MODEL.

[40, 47] and chemical crosslinking methods [48]. The genetic approach has resulted in determination of several contacts occurring between AARSs N- or C-terminal appendices [40], namely, the N-terminal noncatalytic domain of MetRS could make homologous (MetRS–MetRS) and heterologous (MetRS–ArgRS, MetRS–GlnRS) interactions. On the other hand, studies conducted using chemical crosslinking have shown that some syntheses are in close proximity in the complex (e.g. MetRS and AspRS) but they do not interact through N or C termini [48]. This observation suggests that additional contacts involving core domains of enzymes are also important. Looking for possible elements involved in such interactions we have analyzed MetRSs amino-acid sequences deposited in EMBL/GenBank. This search resulted in identification of a tripeptide – Arg-Gly-Asp (RGD) – present in 17 of 40 inspected primary structures (Table 1). This motif is a characteristic feature of the proteins involved in cell adhesion events, e.g. fibronectin [49], vitronectin [50], and fibrinogen [51]. In the structure of

those proteins RGD forms a loop which is directly engaged in interactions with specific integrins [52]. We wonder whether the presence of RGD peptide in MetRSs mostly of eukaryotic and archaeobacterial origin (Fig. 6) can be related to possible protein–protein interactions, which, for example, could locate the enzyme in a particular cell compartment (through binding to membrane proteins) or linking it with another peptide assisting MetRS function or being a member of some multienzyme complex. Such questions need, of course, further experimental explorations, starting from looking for possible MetRSs ligands.

#### ADDITIONAL ACTIVITIES OF MetRSs

Aminoacyl-tRNA synthetases are multifunctional proteins. Apart from tRNAs aminoacylation they play an important role in maintaining the translational fidelity, RNA processing and cellular transport, apoptosis, synthesis of signal molecules and trans-

**Table 1. The presence of RGD sequence in known MetRSs.**

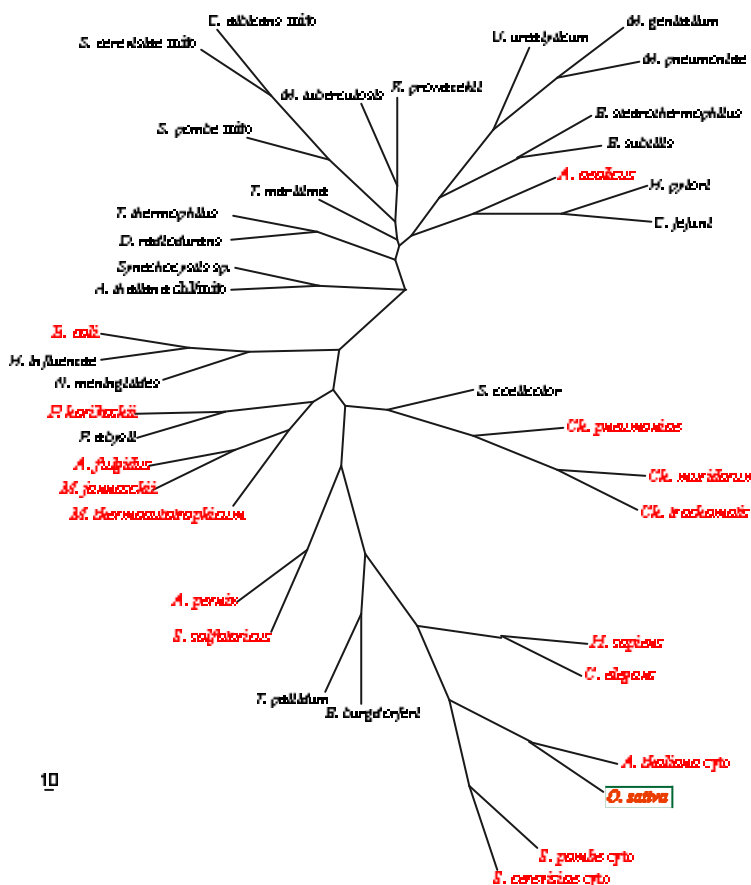
From 40 analyzed primary structures 17 contained at least one RGD peptide.

RGD present in MetRSs sequences	
Organism	Sequence
<i>A. pernix</i> (2)	147 GYEKARGDQCDEC 267 LFIMRRGDPEEWK
<i>Ch. muridarum</i>	149 GFDRARGDECCQC
<i>A. aeolicus</i>	111 EECYKRGDIYLGE
<i>A. thaliana</i>	164 NYDSARGDQCEKC
<i>A. fulgidus</i>	143 CGALARGDECDQG
<i>C. elegans</i>	180 AYDDARGDQCDCG
<i>Ch. trachomatis</i>	149 GFDRARGDECCQC
<i>Ch. pneumoniae</i>	148 GFDHARGDECCSC
<i>E. coli</i>	266 NLCDKRGDSVSFD
<i>H. sapiens</i>	409 GYEEARGDQCCKC
<i>M. thermoautotrophicum</i> (3)	34 RYRRLRGDDVLFV 74 YHEMIRGDLERCD 147 GSEGARGDHCEGC
<i>M. jannaschii</i>	145 CGGEARGDHCEVC
<i>O. sativa</i>	166 NYEAARGDQCENC
<i>P. horikoshii</i>	147 GAENQRGDQCEVC
<i>S. cerevisiae</i>	341 HYDDARGDQCCKC
<i>S. pombe</i>	367 GYDDARGDQCDCG
<i>S. solfataricus</i>	147 GFEDARGDQCDCN

lational or transcriptional regulation [53]. MetRS participates in the maintenance of translational fidelity through editing of homocysteine (Hcy), a non-protein amino acid which is an obligatory precursor of methionine [54, 55]. Due to its structural similarity to methionine Hcy can enter the first step of protein biosynthesis and be activated forming MetRS-bound homocysteinyl-adenylate, which is not transferred to tRNA but rejected in an intramolecular conversion reaction, leading to production of homocysteine thio-lactone. This same Hcy editing mechanism was also confirmed for IleRS, LeuRS, ValRS and LysRS [56, 57]. However, it has been shown that in cultured human cells Hcy can be incorporated into proteins in two ways:

- ◆ posttranslationally, through acetylation of side chain amino groups of lysine residues by thio-lactone,
- ◆ translationally, through incorporation of S-nitroso-Hcy into protein [58].

Both situations can occur in cells from endothelial cell cultures maintained on Hcy in fo-



**Figure 6. Phylogenetic tree of MetRS sequences.**

Enzymes containing RGD tripeptide are marked in red. The tree was obtained using NEIGHBOR and CONSENSE programs from PHYLIP package.

late-limited media. Translational incorporation of Hcy into proteins abolishes their biological activity and has significant medical consequences, contributing to induction of pathogenesis in atherosclerosis [54, 58].

Multienzyme complexes similar to those localized in the cytoplasm of higher eukaryotes have been recently found also in the nucleus [59]. Moreover, an investigation of cellular distribution of different human AARSs has led to the discovery, that MetRS was uniquely localized in nucleolus of proliferative cells [60]. The MetRS was translocated to nucleolus upon a mitogenic signal induced by different growth factors (insulin, platelet-derived growth factor, epidermal growth factor). The nucleolus is that part of nucleus where rRNA biogenesis takes place. It has been observed that the presence of MetRS in nucleolus was dependent on the integrity of rRNA and the activity of RNA polymerase I. Immunological tests have confirmed that MetRS is required for nucleolar synthesis of rRNA [60]. This enzyme can be a part of the cellular system coordinating biogenesis of ribosome and protein synthesis.

## CONCLUSIONS

From the systematically growing body of data concerning methionyl-tRNA synthetase an interesting picture emerges, showing that the enzyme might be of diverse structure, related not only to its function but also to cellular organization. This latter point still needs further exploration, especially in the case of plant enzymes. Genomics and proteomics, developing dynamically during the last few years, try to answer general questions concerning the organization of cellular apparatus as a whole. Taking into account the involvement of many AARSs in different metabolic pathways, going beyond protein biosynthesis itself, one can expect new findings showing a complicated network of relations of synthetases to other components of the cell.

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