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Recognition of tRNAs with a long variable arm by aminoacyl-tRNA synthetases

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In prokaryotic cells three tRNA species, $tRNA^{Ser}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$, possess a long variable arm of 11–20 nucleotides (type 2 tRNA) rather than usual 4 or 5 nucleotides (type 1 tRNA). In this review we have summarized the results of our research on the structural basis for recognition and discrimination of type 2 tRNAs by Thermus thermophilus seryl-, tyrosyl- and leucyl-tRNA synthetases (SerRS, TyrRS and LeuRS) obtained by X-ray crystallography and chemical probing tRNA in solution. Crystal structures are now known of all three aminoacyl-tRNA synthetases complexed with type 2 tRNAs and the different modes of tRNA recognition represented by these structures will be discussed. In particular, emphasis will be given to the results on recognition of characteristic shape of type 2 tRNAs by cognate synthetases. In $tRNA^{Ser}$, $tRNA^{Tyr}$ and $tRNA^{Leu}$ the orientation of the long variable arm with respect to the body of the tRNA is different and is controlled by different packing of the core. In the case of SerRS the N-terminal domain and in the case of TyrRS, the C-terminal domain, bind to the characteristic long variable arm of the cognate RNA, thus recognizing the unique shape of the tRNA. The core of T. thermophilus $tRNA^{Leu}$ has several layers of unusual base-pairs, which are revealed by the crystal structure of $tRNA^{Leu}$ complexed with T. thermophilus LeuRS and by probing a ligand-free tRNA by specific chemical reagents in solution. In the crystal structure of the LeuRS-$tRNA^{Leu}$ complex the unique D-stem structure is recognized by the C-terminal domain of LeuRS and these data are in good agreement with those obtained in solution. LeuRS has canonical class I mode of tRNA recognition, approaching the tRNA acceptor stem from the D-stem and minor groove of the acceptor stem side. SerRS also has canonical class II mode of tRNA recognition and approaches $tRNA^{Ser}$ from opposite, variable stem and major groove of acceptor stem site. And finally, TyrRS in strong contrast to canonical class I system has class II mode of tRNA recognition.

Keywords: type 2 tRNA, long variable arm, aminoacyl-tRNA synthetase, tRNA recognition, aminoacyl-tRNA synthetase complexes.

Introduction. The fidelity of translation of the information stored in nucleic acids into proteins is essential for all living cells. The algorithm of translation of the genetic code is established in the process of aminoacyl-tRNA formation. Therefore, the fidelity of protein synthesis depends to a large extent on a high specificity with which aminoacyl-tRNA synthetases (aaRSs) charge their cognate tRNAs with a correct amino acid. aaRSs catalyse the aminoacylation reaction in two steps, firstly, the activation of amino acid using ATP to form the enzyme bound aminoacyl-adenylate, and secondly, the transfer of amino acid to the 2' or 3' hydroxyl of the 3' terminal tRNA ribose. The ester linkage of the aminoacyl-tRNA provides much of the energy required for peptide bond formation on the ribosome. The accuracy of the aminoacylation reaction is based on correct selection and recognition by aaRSs their cognate tRNAs. The selection of tRNA is a result of productive interaction between tRNA and its corresponding aaRS mediated by the recognition-defined elements (identity determinants) and non-productive interaction between this tRNA and 19 other aaRS species mediated by the rejection-defined elements (anti-identity determinants). Sum of identity and anti-identity determinants forms the identity set of a given tRNA system [1]. Given that tRNAs apparently have similar secondary and tertiary structures, the question ari-

ses about the structural basis for the specific recognition between aaRSs and tRNAs.

Evolution has resulted in two completely distinct structural solutions of the aminoacylation problem. The amino acid sequence analysis [2] and X-ray crystallography [3] have shown that the aaRSs are partitioned into two exclusive classes. The catalytic domain of class I enzymes contains the well-known Rossmann fold as a framework whereas that of class II enzymes is based around a different anti-parallel fold. The catalytic domain of each class includes short sequence motifs, HIGH and KMSK in class I and motifs 1, 2 and 3 in class II [2, 4]. A functional distinction between the two classes is that class I synthetases charge the 2' hydroxyl and class II synthetases (except for phenylalanyl-tRNA synthetase) charge the 3' hydroxyl of the ribose of A76 [2, 5, 6]. Class II aaRSs are almost all functional dimers whereas most class I enzymes are monomers except for tyrosyl-tRNA synthetase and tryptophanyl-tRNA synthetase. Now the crystal structures are available for all 20 canonical aaRSs [4] as well as for the non-canonical pyrrolysyl-tRNA synthetase and phosphoseryl-tRNA synthetase [7, 8]. In both classes, the tRNA binding ability of aaRS is augmented by RNA-binding modules which, because of their greater structural variability, have presumably been added to the catalytic domain at a later stage in evolution [9–11]. Classification of tRNAs according to the length of their extra (variable) arm, leads to dividing them into two classes: those with a short variable arm of 4–5 nucleotide (type 1) and those with a long variable arm, more than 10 nucleotides (type 2) [12]. Prokaryotic $tRNA^{Ser}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$, are classified as the type 2 tRNAs. The long variable arm of $tRNA^{Ser}$ and $tRNA^{Leu}$ shows variation in both length and sequence within the isoacceptor tRNAs. Thus, the long-standing question was how the discrimination between the type 2 tRNAs occurs and what is a role of the long variable arm in the process. In this article we would like to review what is known on the structural basis for recognition and discrimination of the type 2 tRNAs by *Thermus thermophilus* seryl-, leucyl- and tyrosyl-tRNA synthetases.

The serine system. The serine system has a number of interesting features. Firstly, the bacteria *Escherichia coli* and *T. thermophilus* possess five isoaccepting $tRNA^{Ser}$s in order to cope with the six codons for serine which correspond to distinct codon classes. In addition $tRNA^{Sec}$, the selenocysteine-specific tRNA species, which has an opal stop anticodon, is also specifically charged by seryl-tRNA synthetase (SerRS). As a result, there is no consistency in the anticodon bases of $tRNA^{Ser}$s, and their anticodons are apparently not involved in recognition by SerRS. Another special feature is that prokaryotic $tRNA^{Ser}$, as indicated above, has a long variable arm and refers to type 2 tRNAs. In fact, the serine aminoacylation system is unique in the sense that it is the only system where class II aaRS recognizes type 2 tRNA. Therefore the main question has arisen in the study of this system: what are the common features of $tRNA^{Ser}$s that are recognized by synthetase? Clearly only a crystal structure of the complex can give a comprehensive picture of the recognition of SerRS by its cognate tRNAs, but of course one expects it to be consistent with the biochemical and mutagenesis results. Therefore, we have studied the recognition of $tRNA^{Ser}$ by the cognate synthetase using two approaches: X-ray crystallography and chemical and enzymatic footprinting of tRNA in solution. The extreme thermophilic bacterium *T. thermophilus* has been chosen as a source for the isolation of tRNA and SerRS to investigate their structures and functions by biochemical and structural methods. The proteins and nucleic acids from this organism are very stable and crystallized easier than those from the methophilic organisms. At the beginning the scheme of simultaneous isolation of ribosome, tRNA, three elongation factors, several aaRSs was developed, and purification of SerRS from *T. thermophilus* HB27 was described [13, 14]. To study the mechanism of amino acid activation and specific recognition of cognate tRNA by *T. thermophilus* SerRS (SerRSTT) we tried to crystallize this enzyme alone and in a complex with substrates. The crystals of SerRSTT, obtained using mixed solutions of ammonium sulphate and 2-methyl-2,4-pentanediol, were very stable and diffracted to at least 2 Å [15]. The enzyme from two strains of *T. thermophilus* HB8 and HB27 has been cloned and sequenced (Tukalo *et al*. unpublished results). SerRSTTs from both strains have 421 residues per subunit, but differ in six positions, and have an overall sequence identity with *E. coli* enzyme of 37 %. The three-dimension structure of the HB27 SerRSTT has been determined and refined at 2.5 Å resolution [16]. The structures of *T. thermophilus* and

E. coli SerRSs are very similar [3, 16] and made up a central globular domain with a long coiled-coil extension (Fig. 1, see inset). The globular, catalytical domain consists of eight-stranded -sheet, of which seven strands are antiparallel, packed onto two -helices. One of these -helices interacts with the corresponding one in the other molecules of the dimer, forming the layer of four -helices. The antiparallel-fold is characteristic of class II aaRSs and has been found in all synthetases from this class [4]. N-terminal domain as coiled-coil extension of the protein, a remarkable feature of SerRS, stretches about 60 Å in the solution and its role in tRNA recognition will be discussed below.

The first step of the overall aminoacylation reaction catalyzed by SerRS is activation of serine by Mg^{2+}-ATP leading to a stable enzyme-bound intermediate, seryl-adenylate (Ser-AMP). The first information on ATP-binding mode in the active site of class II synthetase was obtained from the structure of SerRSTT in complex with ATP-analogue [17, 18]. In spite of the medium resolution of data, it was clear that ATP is bound in a part of the active site pocket formed by the class II conserved motifs 2 and 3. Later, a series of crystal structures at 2.3–3 Å resolution of complexes involving Ser RSTT, ATP, Mn^{2+} or Mg^{2+} and Ser-AMP (natural and analogues) have been determined, which provide the structural basis for explanation of the specificity and mechanism of serine activation [19, 20]. In the presence of a divalent cation (Mg^{2+} or Mn^{2+}) the ATP molecule is found in an unusual U-shaped conformation in which the - and -phosphates are bent back into an arginine-rich pocket (comprising Arg271, Arg344 and Arg386) towards the purine ring rather than extending away from it. A similar conformation of ATP has been observed in the yeast AspRS-$tRNA^{Asp}$-ATP ternary complex [21] and later in other class II synthetases [22]. This compact conformation of ATP is unique for class II synthetases as in the active sites of class I enzymes ATP adopts an extended conformation [23]. The superposition of the enzyme-bound ATP and Ser-AMP structures provides strong support for an in-line displacement mechanism of the serine activation [20]. The bent conformation of ATP and the position of serine are consistent with nucleophilic attack of the serine carboxyl group on the -phosphate leading to the release of inorganic pyrophosphate.

Structure of SerRSTT-$tRNA^{Ser}$ complex. Since the crystallization of the synthetase-tRNA complexes might require relatively large quantities of pure tRNA species we had to develop the method for preparative isolation of tRNA. Separation of biologically active pure and specific tRNAs is difficult due to the overall similarity in tertiary structure of different RNA molecules on the one hand and their heterogeneity on the other hand. This heterogeneity is conditioned by both degeneration of the genetic code (6 anticodons for serine) and the degree of maturation (post-transcriptional modification of nucleotide bases and their transformation into minor components). Therefore, we have developed a method for tRNA isolation from *T. thermophilus* cells which combines different techniques: chromatography on benzoyl-DEAE-cellulose and HPLC on anion-exchange and reverse phase columns [24]. This methodological approach allowed us to obtain two highly purified isoaccepting $tRNA^{Ser}$s, sufficient for the study of their primary structures and for the crystallization trials of $tRNA^{Ser}$ complexes with cognate SerRSTT. The nucleotide sequence of two serine isoacceptor tRNAs from *T. thermophilus*, containing different anticodons has been studied by the ultramicrospectrophotometrical method and rapid gel sequencing procedure [25]. Comparison of the sequences of $tRNA_1^{Ser}$ and $tRNA_2^{Ser}$ shows that the acceptor stem and T-stem are identical in both RNAs, and only three changes have been found in the structures of D-stems. The most significant differences were found in the anticodon stems and variable arms of two $tRNA^{Ser}$s. These data are in good agreement with the results of the study on the identity determinants in *E. coli* $tRNA^{Ser}$ [26, 27]. The authors have shown that the anticodon nucleotides are not involved in specific recognition of $tRNA^{Ser}$. However, the elements of tertiary structure play a critical role. For example, it was established that the length and orientation of the variable arm of $tRNA^{Ser}$ are more important for aminoacylation by SerRS than its sequence. In view of the fact that SerRS selectively recognizes $tRNA^{Ser}$ on the basis of its characteristic tertiary structure rather than the specific nucleotides, an important step in investigation of the recognition mechanism for serine system was to determine the characteristics of $tRNA^{Ser}$s spacial organization and topography of the complex with cognate synthetase. The most direct and informative method for

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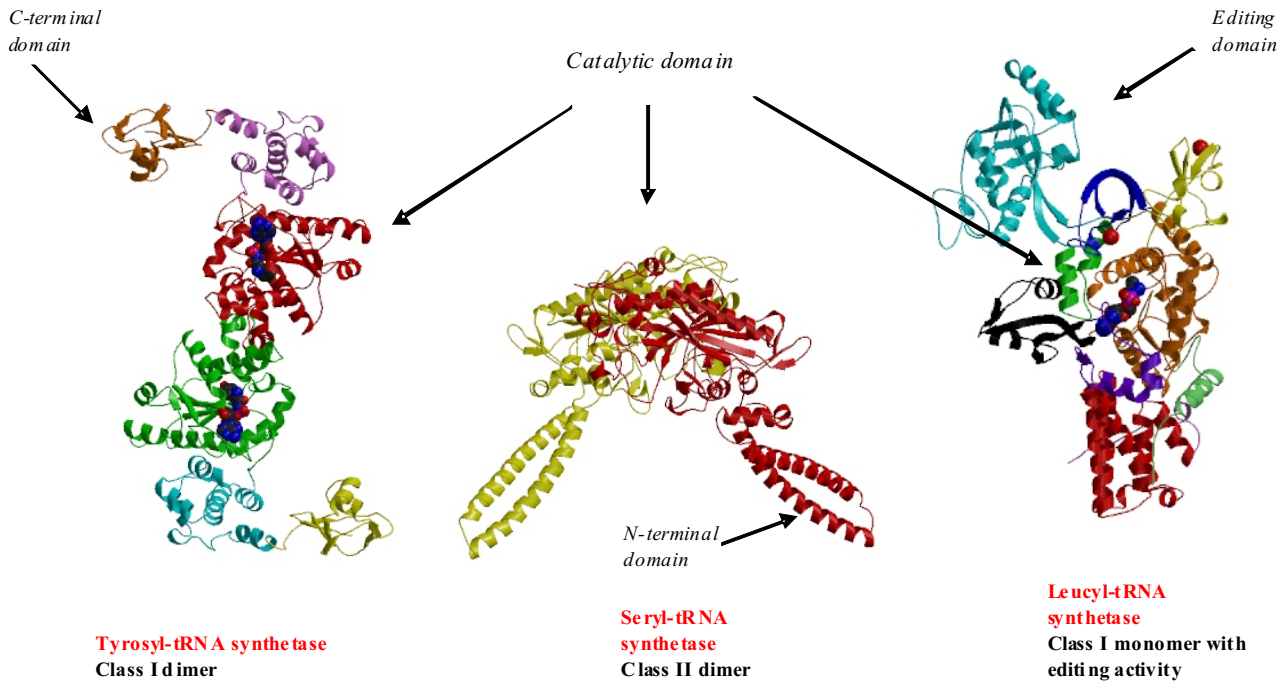

Fig. 1. Structures of *T. thermophilus* aminoacyl-tRNA synthetases (SerRSTT, TyrRSTT and LeuRSTT), which recognize tRNAs with a long variable arm

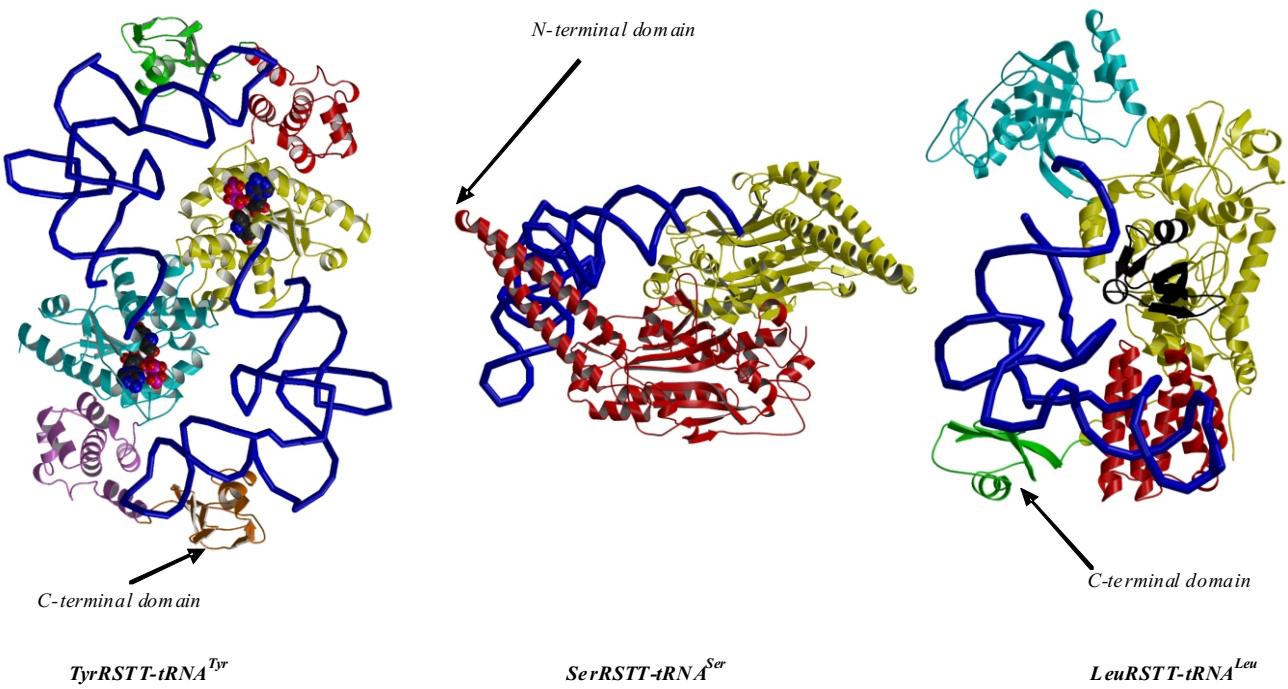

Fig. 2. Complexes of aminoacyl-tRNA synthetases recognizing long variable arm tRNAs

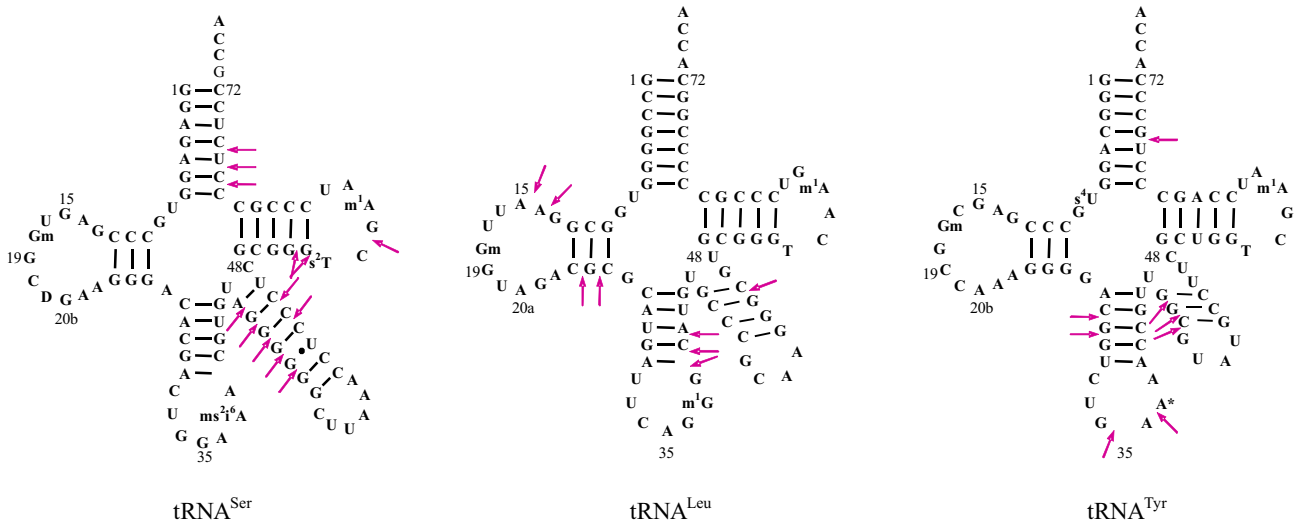

Fig. 3. Cleverleaf structures of *T. thermophilus* $tRNA^{Ser}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$ with position of phosphates protected by cognate synthetase from alkylation with ethylnitrosourea

achieving this goal is the X-ray analysis. However, it should be noted that the crystallization conditions, on the one hand, and the interaction with the enzyme, on the other, may lead to changes in the spatial organization of tRNA. Therefore, extremely important is the study of $tRNA^{Ser}$ in the free state and in the complex with protein in solution under conditions close to physiological.

In order to study the structure of $tRNA^{Ser}$ and the molecular basis of its recognition by the synthetase, we have obtained four crystal forms of the complex between SerRS and its cognate tRNA from the *T. thermophilus* [28, 29]. Of these, two (denoted by Form III and Form IV) have tetragonal crystal forms and both diffract to about 6 Å resolution [28]. Form III crystals diffract to about 3.5 Å and contain two synthetase dimmers with two tRNA molecules each. On the other hand, Form IV crystals, which diffract to 2.8 Å resolution, contain only one tRNA molecule bound to the synthetase dimer [28]. Using Form IV crystals the structure of *T. thermophilus* SerRS complexed with $tRNA^{Ser}$ molecule was solved at 2.9 Å resolution [18]. A ribbon diagram of the structure of SerRSTT-$tRNA^{Ser}$ complex is shown in Fig. 2 (see inset). The main conclusion can be summarized as follows: (1) the tRNA binds across the two subunits of the dimer; (2) the anticodon loop is not in contact with the synthetase; (3) upon tRNA binding the coiled-coil domain of the synthetase is stabilized in a particular orientation and curves between the T C loop and the long variable arm of the tRNA; (4) the synthetase makes several backbone contacts but few base-specific interactions; (5) the contacts with the tRNA long variable arm backbone extend out to the sixth base pair, explaining the need for a minimum length of the arm, but allowing longer arms (as, for instance, in $tRNA^{Sec}$) to be accommodated; and (6) the bases 20a and 20b inserted into the D loop in the $tRNA^{Ser}$ both play novel roles in the core formation of the tRNA. In particular, the base of G20b is stacked against the first base pair of the long variable arm and thus determines the orientation of the variable arm.

These crystallographic results show that both distinctive features of the serine system, the synthetase coiled-coil N-terminal domain and the $tRNA^{Ser}$ long variable arm, play the major role in the mutual recognition of these two macromolecules. Furthermore, this recognition is based on the shape rather than on the specific nucleotide sequence, which fits well with the biochemical data [26, 27]. These data are also in good agreement with those obtained by us in the solution, where SerRS TT protected from alkylation by ethylnitrosourea the phosphates residues located in three regions of $tRNA^{Ser}$: at the variable arm (phosphates 46–47c, 47o, 47p), the T stem-loop (P50, P53, P54) and the acceptor stem (P67-P69) [30] (Fig. 3).

The structure of complex provides also the first detailed description of the architecture of a type 2 tRNA.

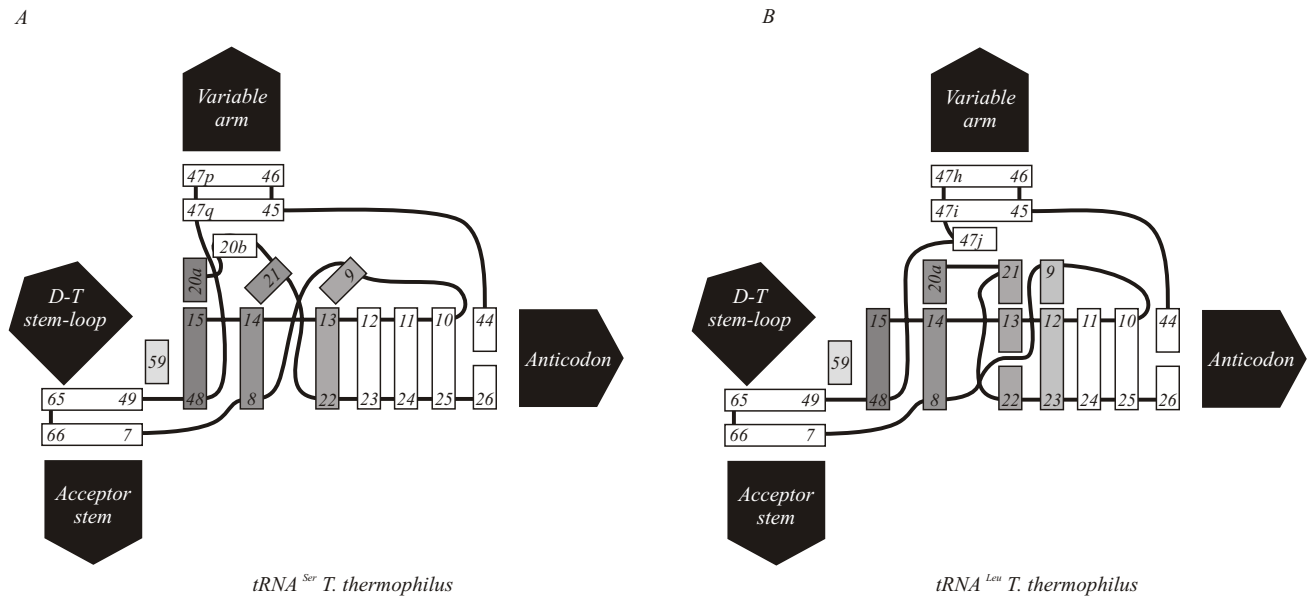

Fig. 4. Comparison of the core structure of *T. thermophilus* $tRNA^{Ser}$ (*A*) and $tRNA^{Leu}$ (*B*)

The tertiary interactions in the core of the $tRNA^{Ser}$ differ from those observed previously for a type 1 $tRNA^{Phe}$. The core of the latter includes four parallel stacked planes; three of them consist of a base triplet. The equivalent region in $tRNA^{Ser}$ (Fig. 4, *A*) is reorganized to accommodate the insertion of 20a-20b from the D loop and can be described by the notation D20a-[G15-C481, A21-[U8-A14], G9-[A22-G13], and [G23-C12]. In order to clarify the question, whether the resulting crystal structure of the $tRNA^{Ser}$ corresponds to that under physiological conditions, the elements of tertiary structure of $tRNA^{Ser}$ from *T. thermophilus* were studied by the methods of chemical modification in solution [31]. For this, ethylnitrosourea has been used to modify the phosphate residues; guanosines and cytidines were modified by dimethylsulphate, and adenosines by diethylpyrocarbonate [32, 33]. Summarizing the results (which will also be discussed below), we can conclude that the same interactions, that define an architecture of the solution structure of $tRNA^{Ser}$, exist in the crystal form. Thus, the crystal conformation of $tRNA^{Ser}$ in complex with the synthetase is very close to that for the free tRNA in solution.

In the original binary SerRSTT-$tRNA^{Ser}$ complex crystal structure [18], the end of the acceptor stem of tRNA was not ordered in the active site. However the ternary complex of SerRSTT-$tRNA^{Ser}$ with a non-hydrolysable seryl-adenylate analogue [34] shows a much better ordering of the active site, and the interactions inside the acceptor stem mainly made by the motif 2 loop of SerRS, are visible. Interestingly, in the absence of tRNA, but in the presence of ATP or Ser-AMP [20, 34] the motif 2 loop adapts a quite different conformation. Upon tRNA binding a number of motif 2 residues previously found interacting with ATP or adenylate now switch to participate in tRNA recognition. These results combined with those, obtained previously [18], provide strong evidence that the functional binding of $tRNA^{Ser}$ to SerRS occurs in at least two distinct steps: firstly the initial recognition and docking which depend largely on interaction of the N-terminal domain with a long variable arm and secondly, the correct positioning of the 3' end of the tRNA in the active site. The latter depends critically on a conformation switch of the motif 2 loop after the adenylate formation.

The leucine system. A subfamily of class 1a aaRSs, leucyl-, isoleucyl- and valyl-tRNA synthetases (LeuRS, IleRS and ValRS, respectively) are particularly closely related and probably evolved from a common ancestor. The three enzymes are large monomers (100 kDa) and contain an unusually large insertion often, called CP1 (connective polypeptide 1), [35] into the class 1 Rossmann-fold catalytic domain. Based on the structural data of IleRS and on the mutagenesis experiments, a putative hydrolytic editing active site was identified within the CP1 domain [36–38]. LeuRS was the least stu-

died of this triad of editing enzymes. We have found that *T. thermophilus* LeuRS is capable of editing homocysteine, norvaline and norleucine, and have studied the structure of editing active site of enzyme which binds the distinct pre- and post-transfer editing substrates [39]. The second interesting feature of LeuRS is that this synthetase recognizes tRNA with a long variable arm. However, unlike the other two such enzymes, SerRS and TyrRS, the bacterial LeuRS surprisingly does not generally use the long variable arm of $tRNA^{Leu}$ as an identity element [40]. Neither does LeuRS use the anticodon triplet [40]. Obviously, the complexity of LeuRS needs a deep on complexes of the enzyme with various substrates in combination with the mutagenesis and biochemical results for the understanding of the molecular mechanisms of tRNA recognition and catalysis. For this, the gene of *T. thermophilus* HB27 LeuRS has been cloned and sequenced [41]. The open reading frame encodes a polypeptide chain of 878 amino acid residues in length (molecular mass 101 kDa). Then the *T. thermophilus* LeuRS (LeuRSTT) was expressed in *E. coli* cells by cloning the corresponding gene into *pET29b* vector [41]. LeuRS from *T. thermophilus* was the first crystallized LeuRS, for which the crystal structure was described [42–44]. A high quality crystal form of the native enzyme and its complexes with leucine and leucyl-adenylate analogue diffracts to 1.9 Å resolution and contains one monomer in asymmetric unit [42]. The overall architecture of LeuRSTT is similar to that of IleRS, except for the fact that the editing domain is inserted at the different position in the primary structure [43] (Fig. 1, see inset). This feature is unique to prokaryotic LeuRS, as well as the presence of novel additional flexibly inserted domain (designated leucyl-specific domain). Comparison of the native enzyme and complexes with leucine and a leucyl-adenylate analogue shows that the binding of the adenosine moiety of leucyl-adenylate causes significant conformational changes in the active site required for amino acid activation and tight binding of the adenylate. These changes propagated to more distant regions of the enzyme, leading to a significantly more ordered structure ready for the subsequent aminoacylation and/or editing steps.

Structure of LeuRSTT-$tRNA^{Leu}$ complex. To obtain further insight into tRNA recognition by LeuRS and into mechanisms of aminoacylation and editing, we have determined the crystal structure of LeuRSTT complexed with the $tRNA^{Leu}$ transcript and leucine [45]. LeuRSTT-$tRNA^{Leu}$ co-crystals have been obtained only with the particular T7 transcript of *T. thermophilus* $tRNA^{Leu}$(CAG), which normally has 87 nucleotides, but in this work had a two base-pair deletion in the long variable arm stem. Such a truncation of the long variable arm does not affect the leucylation activity [40]. The crystal structure shows (Fig. 2, see inset) that the tRNA acceptor end enters the editing site in a fashion completely compatible with our previous structure of LeuRSTT complexed with a post-transfer editing substrate analogue, 2'-(L-norvalyl)amino-2'-deoxyadenosine (Nva2AA) [39]. Furthermore, the structure at the improved resolution of 2.9 Å, obtained by soaking the co-crystals with Nva2AA, shows that this compound, bound in the editing active site, displaces the 3' end of the tRNA. The anticodon stem of $tRNA^{Leu}$ is packed against the helical domain characteristic of class Ia. Neither the long variable arm, nor the anticodon loop, is in contact with the synthetase. The C-terminal domain of the synthetase, hitherto unseen in crystal structures of uncomplexed LeuRSTT, for the first time could be traced as a compact alpha-beta domain. To clarify the function of the C-terminal domain in LeuRSTT, a truncation mutant LeuRSTTdC with the C-terminal 60 residues deleted was expressed and purified. The C-terminal truncation mutant shows 152-fold reduction (the k_{cat} value) of the aminoacylation activity [46], indicating that the C-terminal domain is strictly required for the tRNA charging activity of Leu RSTT. This has also been demonstrated for the *E. coli* and *Pyroccocus horikoshii* LeuRSs (LeuRSHP) [47, 48]. Analysis of the $tRNA^{Leu}$ interaction with LeuRSTT shows, that there are non-specific backbone contacts in the regions of nucleotides 12–13, 22–26, and 42, involving residues in the regions 667–686 and 749–760. The C-terminal domain makes more extensive contacts to the base pair G19-C56 including some base specific interactions. All these contacts agree reasonably well with biochemical footprinting studies. The regions of the wild type $tRNA^{Leu}$ (the long variable arm has four base pairs) protected by LeuRSTT against alkylation with ethylnitrosourea are located at the 5' side of D-stem (phosphates P14 and P15), at the 3' side of the D-stem (phosphates P24 and P25), at the 3' side of the anticodon stem (P38-40) and at the variable arm (P47i). The anticodon loop

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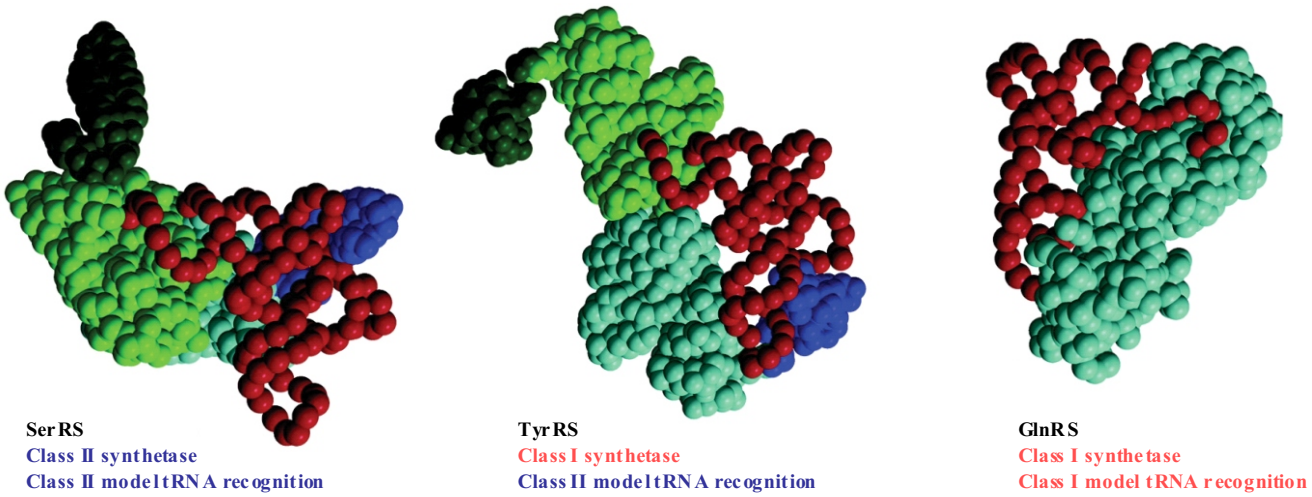

Fig. 5. A different modes of tRNA recognition by aminoacyl-tRNA synthetases. Adopted from [60]

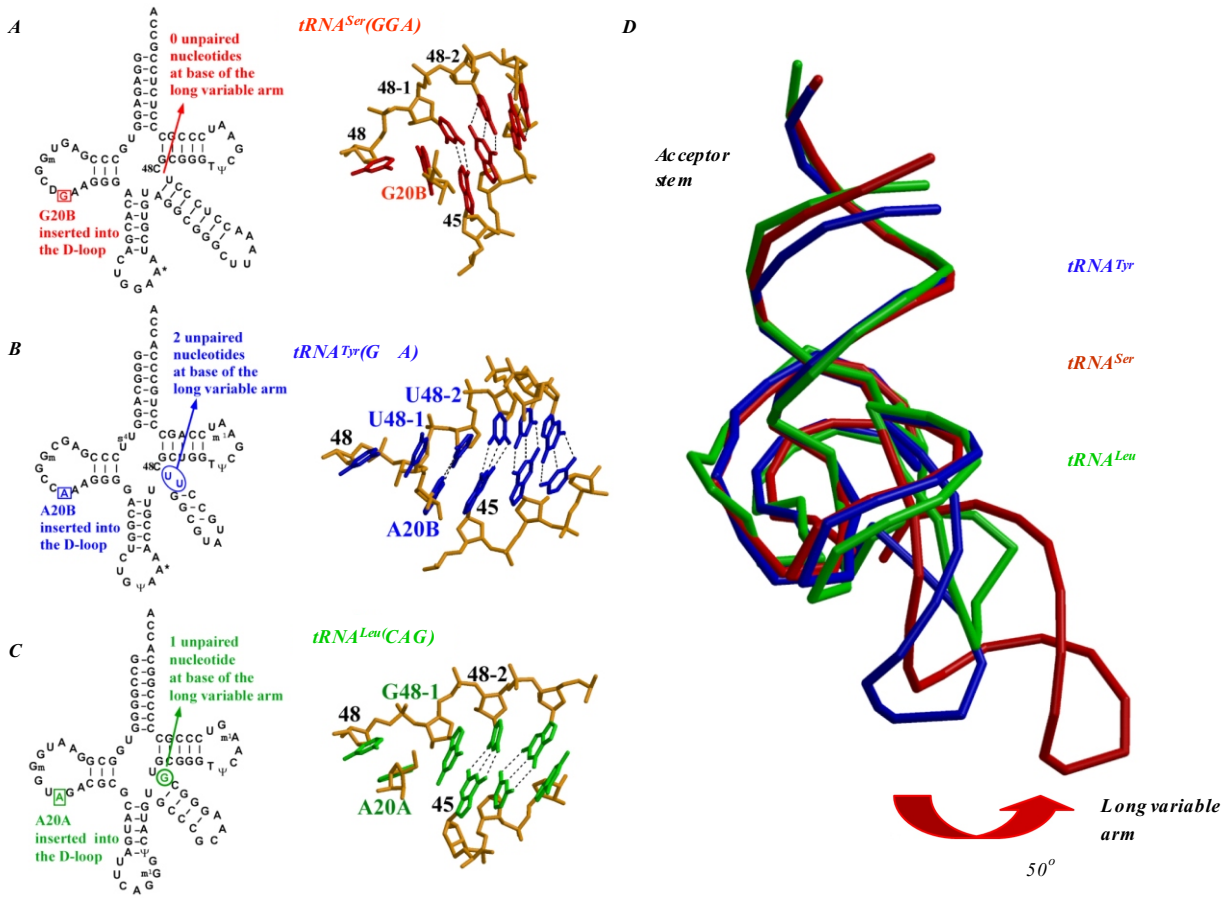

Fig. 6. Structural comparison of the long-variable arm tRNAs: *T. thermophilus* $tRNA^{Ser}$ (*A*), $tRNA^{Tyr}$ (*B*) and $tRNA^{Leu}$ (*C*). The structural alignment of $tRNA^{Tyr}$, $tRNA^{Ser}$ and $tRNA^{Leu}$ (*D*)

and practically entire variable arm of $tRNA^{Leu}$ are exposed for chemical modification [49], consistent with their not being in contact with the synthetase (Fig. 3). The fact of the protection of P47i in solution which is not in contact with the enzyme in the crystal may be explained by using truncated form of $tRNA^{Leu}$ for crystallization of the complex. In the recently reported crystal structures of *E. coli* LeuRS-$tRNA^{Leu}$ complexes in the aminoacylation or edititing conformations, the long variable arm is the same as in the wild type $tRNA^{Leu}$ and contacts the C-terminal domain via the variable arm phosphates P47i (in the aminoacylation conformation) or P47f-P47i (in the editing conformation) [50]. Regardless of the details of the interaction with the long variable arm, LeuRSTT has a canonical class I mode of tRNA recognition approaching the tRNA acceptor stem from the D-stem and minor groove of the acceptor stem side. Ser RSTT also has a canonical class II mode of tRNA recognition and approaches $tRNA^{Ser}$ from opposite, *i. e*. the variable stem and major groove of the acceptor stem side.

For the first time, the study on the structure of the LeuRS-$tRNA^{Leu}$ complex has shown a unique spatial structure of bacterial $tRNA^{Leu}$. There are two related aspects: firstly, the structure of the tRNA core and, secondly, the orientation of the long variable arm. The core structure of *T. thermophilus* $tRNA^{Leu}$ has several layers of unusual base-pairs, which are revealed by both the crystal structure and probing a ligand-free $tRNA^{Leu}$ with the specific chemical reagents in solution [45, 51] (Fig. 4, *B*). The orientation of the long variable arm in $tRNA^{Leu}$ is determined by the single unpaired base G48.1 which stacks against the first base-pair of the variable arm stem, G45:C48.2 (Fig. 6, *C*, see inset).

Unfortunately we failed to crystallize the LeuRSTT-$tRNA^{Leu}$ complex in aminoacylation conformation. But this complex was obtained for archaea LeuRSHP [52]. Due to the high homology of key amino acid residues in the catalytic domain of LeuRS, including LeuRSTT and LeuRSPH, we proposed a model aminoacylation conformation of the 3'-end of $tRNA^{Leu}$, which is located in the center of the catalytical domain of *T. thermopilus* LeuRS [45]. A key observation from this modelling is that the zinc-containing ZN-1 domain (residues 154–189 in LeuRSTT) must be mobile during the aminoacylation reaction. This model also shows a crucial role of the conserved motif **418-RLRDWLISRQRYW-431** in positioning the 3' end of the tRNA, in particular, by making specific main-chain hydrogen bonds to the discriminator base A73. Basic residues Arg418, Arg420 and Arg426 are also probably important for tRNA binding. Interestingly that recently solved crystal structure of *E. coli* LeuRS-$tRNA^{Leu}$ complex in the aminoacylation conformation has confirmed the basic assumptions for aminoacylation model of LeuRSTT [50]. Our study on the ternary complex LeuRSTT with $tRNA^{Leu}$ and boron derivative AN2690, which is a selective inhibitor to the editing active center of LeuRS, made it possible to understand the initial binding conformation of tRNA to the enzyme [53]. In the crystal it was obtained stable adduct of AN2690-$tRNA^{Leu}$ in the editing domain of LeuRSTT, by chemical cross-linking the boron atom of AN2690 with oxygen atoms of 2'- and 3'-OH end groups of adenosine tRNA. In addition, we showed such cross-linking in the solution. This indicates that the original, the most thermodynamically stable binding conformation of $tRNA^{Leu}$ to LeuRSTT is the conformation in which the 3'-end of tRNA interacts with the editing active center of the enzyme. After the formation of aminoacyl-adenylate in the synthetic active site, 3'-end of tRNA switches from the editing domain to the synthetic one. A superposition of the tRNA conformations from the various *Pyroccocus* and *Thermus* LeuRS-$tRNA^{Leu}$ complexes gives an impression of the trajectory of the 3' end of the tRNA from aminoacylation to editing and exit conformations. How does LeuRSTT recognize $tRNA^{Leu}$ and exclude noncognate tRNA? A biochemical insight into this problem has mainly come from the attempts to mutate $tRNA^{Leu}$ in the *E. coli* system [40, 54–56]. The generalisation of these results shows that A73, the configuration of the D loop (notably the position of the G18-G19 dinucleotide), the Levitt base pair A15-U48 and the single unpaired nucleotide at 3' end of the long variable arm are the crucial elements for effective leucylation. Examination of the crystal structures explained this finding to some extent but failed to provide complete understanding of the specificity of LeuRS for $tRNA^{Leu}$ in comparison to $tRNA^{Tyr}$, which has the same discriminator nucleotide A73. We noted that for discrimination between $tRNA^{Leu}$ and $tRNA^{Tyr}$ the LeuRS uses the differences in their tertiary structures, interacting simultaneously in several positions of a ribose-phosphate backbone of tRNA [45, 46].

The tyrosine system. Tyrosyl-tRNA synthetase (TyrRS) is a homodimeric class I aaRS, but is unusually a functional dimer, a feature only shared with tryptophanyl-tRNA synthetase [57]. This system is also unique because of having two types of $tRNA^{Tyr}$: with a long variable loop for prokaryotes and eukaryotic organelles and with a short variable loop for archaea and eukaryotes. Besides, the acceptor stems for $tRNA^{Tyr}$ of prokaryotes, mitochondria and chloroplasts have the G1-C72 base pair found in most tRNAs while the first base pair of $tRNA^{Tyr}$ of eukaryotic cytoplasm and archaea is C1-G72 [12]. Eukaryote cytoplasmic and prokaryote TyrRS cannot cross-aminoacylate their respective $tRNAs^{Tyr}$. Although the crystallographic structure of the *Bacillus stearothermophilus* TyrRS has been determined [58] this structure comprises only the N-terminal 320 amino acids of the molecule as the C-terminal 99 amino acids are disordered in the crystal. A long time there were no crystallographic data on the complex with ATP or with $tRNA^{Tyr}$. In order to fill up the gaps in structural information, we have begun the work on the tyrosine system from *T. thermophilus*. To obtain a sufficient amount of TyrRS for crystallization, the gene encoding TyrRS from the extreme thermophilic eubacterium *T. thermophilus* HB27 has been cloned and sequenced [59]. The open reading frame encodes a polypeptide chain of 432 amino acid residues in length (molecular mass 48717 Da). Comparison of the amino acid sequence of the *T. thermophilus* TyrRS (TyrRSTT) with that of TyrRS from various organisms shows that the *T. thermophilus* enzyme shares a branch in the phylogenetic tree of eubacterial TyrRSs with the enzymes from *Aquifex aeolicus*, *Deinococcus radiodurans*, *Haemophilus influenzae* and *Helicobacter pylori* (40–57 % amino acid identity), distinct from the branch containing *E. coli*, *Chlamydia trachomatis* and *Bacillus stearothermophilus*, for example (24–28 % amino acid identity). Non-bacterial TyrRSs, which recognize type 1 tRNAs without a long variable arm, are quite different and either lack (archaeal) or have an alternative (eukaryotic) C-terminal domain. We have determined a series of structures of TyrRSTT complexed with various combinations of ATP and tyrosine, which causes several questions relating to the mechanism of tyrosine activation. Crystallization of Tyr RSTT with tyrosinol (Fig. 1, see inset) allowed us to visualize for the first time the complete enzyme including the C-terminal domain at 2.0 Å resolution [60]. The fold of C-terminal domain of TyrRSTT is similar to part of the C-terminal domain of ribosomal protein S4 and its role in $tRNA^{Tyr}$ recognition will be discussed below.

Structure of TyrRSTT-$tRNA^{Tyr}$ complex. We have studied five different crystal forms of the complex between TyrRS and native or transcript $tRNA^{Tyr}$ and determined the structure of TyrRSTT-$tRNA^{Tyr}$ complex at 2.9 Å resolution [60]. The structure of complex was novel for several reasons since it (i) confirmed the cross-subunit binding of the tRNA to TyrRS dimer, (ii) showed that class I synthetase TyRS had a class II mode of tRNA recognition, (iii) revealed the detailed interactions of the TyrRS C-terminal domain with the tRNA long variable arm and anticodon stem-loop, and (iiii) demonstrated tertiary structural features in $tRNA^{Tyr}$ which determine the orientation of the long variable arm (Fig. 2, see inset).

The mode of binding tRNA to TyrRSTT is similar to the earlier model of the TyrRS-$tRNA^{Tyr}$ complex proposed by Bedouelle [61] on the basis of extensive mutational studies and very similar to one proposed by us on the basis of the study of phosphate protection upon $tRNA^{Tyr}$ binding to the synthetase [62]. Despite having an unambiguous class I catalytic domain, TyrRS in contrast to the canonical class I systems has a class II mode of tRNA recognition [60]. This means that it interacts with $tRNA^{Tyr}$ from the variable loop and acceptor stem major groove side as, for instance, in the case of class II AspRS [63] and SerRS [34] (Fig. 2, 5, see inset). This is in strong contrast to the canonical class I systems such as those of subclass Ib GlnRS [64], which approach cognate tRNA from the acceptor stem minor groove side. Despite the class II mode of tRNA recognition, TyrRS preferentially aminoacylates the 2' OH of A76 in accordance with other class I systems [65]. The evolutional scenario that led to those non-canonical features of TyrRS is not obvious. There is a suggestion that dimerization of synthetase and the class II mode of tRNA recognition may be evolutionary linked in TyrRS [60]. This hypothesis has been confirmed by the recent data on the structures of the complex *Methanococcus jannaschii* TyrRS with cognate tRNA [66] and the complex of human tryptophanyl-tRNA synthetase with $tRNA^{Trp}$ [67], where both homodimeric class I synthetases have the class II mode of tRNA recognition.

The C-terminal domain of TyrRSTT plays a critical role in the recognition of $tRNA^{Tyr}$, first by recognizing the tRNA's unique shape, and second by participating in specific interaction with one of the anticodon bases. These regions of contact agree very well with protection studies on the *T. thermophilus* system by chemical modification and nuclease hydrolysis methods [62] (Fig. 3). The experiments showed that the $tRNA^{Tyr}$ interacts with the cognate enzyme with the anticodon stem (on the 5' side), the anticodon, the variable stem and loop (on the 5' side) and the acceptor stem (on the 3' side). In the complex, the anticodon triplet of $tRNA^{Tyr}$ (G A) takes up a novel conformation, in which G34 and A36 are stacked on top of each other and 35 bulges out in the opposite direction. There is base-specific recognition of 35 by Asp423 and G34 by carboxyl group of Asp259. It was shown also by biochemical methods, that anticodon bases 34–35 are important recognition elements by TyrRS [26, 68]. The acceptor stem of the $tRNA^{Tyr}$ binds across the dimer interface onto the catalytic domain of the opposing subunit. G1 is not specifically recognized by TyrRSTT; instead, C72 is recognized by one hydrogen bond with Glu154. Specific recognition of the discriminator base A73 is made through a hydrogen bound between the N6 position and the main-chain carbonyl oxygen of Glu154 and a hydrogen bond between the N3 position to Arg198. Interestingly, the bacterial *T. thermophilus* TysRS and archaeal *M. jannaschii* TyrRS (TyrSMJ) recognize the acceptor stem of tRNA in a different manner [60, 66]. The acceptor stem of the *M. jannaschii* $tRNA^{Tyr}$ is the most important recognition element for TyrRSMJ, therefore archeael enzyme strictly recognizes the C1-G72 base pair, as it was shown by structural and biochemical methods [66, 70].

Conformation comparison of the long variable arm of the type 2 tRNAs. The structure of TyrRSTT-$tRNA^{Tyr}$ complex completes the trilogy of structures of long-variable-arm tRNAs with their cognate synthetases, allowing comparative studies of the unique conformations of these tRNAs. The structures of three *T. thermophilus* type 2 tRNAs, $tRNA^{Ser}$, $tRNA^{Tyr}$ and $tRNA^{Leu}$ are compared in Fig. 6 (see inset). The crystal structures of all three type 2 tRNAs show that the orientation of the variable arm differs with the respect to the globular main body of tRNA, depending on differences in the D and variable arm regions [45]. As has been proposed, a key determinant in the orientation of the long variable arm of type 2 tRNA is the number of unpaired nucleotides at the 3'-end of the long variable arm. In the case of $tRNA^{Tyr}$, this is of critical importance as a positive identity element for recognition by TyrRS and as a negative element which prevents the mischarging of $tRNA^{Tyr}$ by LeuRS and SerRS [36, 40]. In $tRNA^{Ser}$ and $tRNA^{Tyr}$ the D-loop has the same number of nucleotides and a similar conformation to nucleotide 20a, forming a planar base-triple with the Levitt pair (G15-C48) and with the base 20b inserted into the tRNA core. This makes the backbone conformation of the two tRNAs, apart from the variable loop, rather similar. However, the details of the core packing are significantly different, resulting in an 50^{o} change in orientation of the long variable arm helix (Fig. 6, *D*, see inset), which clearly permits the shape discrimination between these two type 2 tRNAs by their respective synthetases [60]. In $tRNA^{Ser}$, the significant tilt of bases A21 and G9 allows deep penetration of G20b into the core to stack against the first base pair of the long variable arm (A45-U48-1). In contrast, in $tRNA^{Tyr}$, the first base pair of the long variable arm is formed by reverse Hoogsteen base pair between A20b and U48-2, against which the unpaired U48-1 stacks. In bacterial $tRNA^{Leu}$ there are highly conserved features that distinguish it from the other two members of type 2 tRNAs ($tRNA^{Ser}$ and $tRNA^{Tyr}$) as already discussed above. Among them we should mention the less common Levitt pair A15-U48; the a4- 3 configuration of the D loop owing to the insertion of an additional base (nucleotide 17) before the G18-G19 dinucleotide and to the presence of base 20a but absence of 20b; and the occurrence of a single unpaired nucleotide (G48-1) at the base of the long variable arm. As the result of this unique configuration of $tRNA^{Leu}$ core, G48-1 stacks against the first base-pair of the variable arm and leads to its orientation, different than that of $tRNA^{Ser}$ and $tRNA^{Tyr}$ (Fig. 6, see inset). The different orientations of the variable arm among the tRNAs thus depend on the identity of the interacting D loop nucleotide, the number of other inserted D loop nucleotides, and the number and identities of the unpaired variable loop nucleotides that flank the 5' and 3' sides of the stem-loop motif.

Concluding remarks. Co-crystal structures of Ser RS, LeuRS, and TyrRS that aminoacylate the type 2

tRNAs together with the footprinting and biochemical data show that the enzymes recognize the unique core domain shape arising from the large stem-loop variable region. The structural description of three bacterial tRNAs with the long variable arms, $tRNA^{Ser}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$, has provided an explanation of how the systematic differences between them (correlate insertions in the D loop and the base of the long variable arm) lead to the unique core structure and long-variable-arm orientation in each case [18, 30, 45, 51, 60, 62]. The recognition by SerRS, LeuRS and TyRS of distinct globular shape in these type 2 tRNAs as a mechanism for selectivity is related to so-called «indirect readout», because usually most or all of the interactions are made with the sugar-phosphate backbone [45].

An example of indirect readout for the type 1 tRNA, is the recognition of the G15-G48 Levitt pair in *E. coli* $tRNA^{Cys}$ in its natural context by CysRS [71]. Similarly, in the type 2 $tRNA^{Leu}$ the substitution of A14, which is not in direct contact with the enzyme in the crystal structure of the complexes [45, 50], by any of the other three nucleo- sides decreased the activity by 100-fold or more [40]. Since the mechanism of indirect readout is important in the context of RNA-protein interactions in general, it requires further studies using different mutant forms of tRNAs and aaRSs by X-ray methods together with footprinting analysis and kinetic techniques.

Another important question, how the tRNA recognition mode for the type 2 tRNAs has evolved in association with the evolution of synthetases? Eukaryotic cells have only two of type 2 tRNAs ($tRNA^{Ser}$ and $tRNA^{Leu}$) as the length of $tRNA^{Tyr}$ dramatically changed during evolution. This has resulted in the fact that the recognition style of eukaryotic $tRNA^{Ser}$ and $tRNA^{Leu}$ varied in parralel with some changes in the structure of these tRNAs [72, 73]. There have also been changes in the structures of relevant synthetases [74, 75]. Though the footprinting studies have shown that in general the type of interaction of eukaryotic $tRNA^{Ser}$ and $tRNA^{Leu}$ with cognate enzymes is similar to that for prokaryotic tRNAs [76–78], the recognition elements of tRNAs are different [72, 73, 79]. X-ray structures of the complexes of eukaryotic SerRS and LeuRS with cognate tRNAs should shed light on the details of their recognition. The importance of information on tRNA selectivity by eukaryotic SerRS and LeuRS that would emerge from such studies is also related with a growing number of examples of their participation in a variety of cellular functions and pathological processes [80, 81].

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Упізнавання тРНК, які мають довгу варіабельну гілку, аміноацил-тРНК синтетазами

Резюме

У клітинах евкаріотів тРНК трьох специфічностей – $тРНК^{Ser}$, $тРНК^{Leu}$ і $тРНК^{Tyr}$ – мають довгу варіабельну гілку довжиною 11–20 нуклеотидів (2-га група тРНК) на відміну від чотирьох або п'яти нуклеотидів 1-ї групи тРНК. Підсумовано результати наших досліджень структурних основ упізнавання і дискримінації тРНК 2-ї групи серил-, тирозил- і лейцил-тРНК синтетазами з Thermus thermophilus (СерРС, ТирРС і ЛейРС), отриманих методами рентгенівської кристалографії і хімічної модифікації тРНК у розчині. На сьогодні кристалічна структура відома для всіх трьох комплексів аміноацил-тРНК синтетаз з відповідними тРНК 2-ї групи, різні типи впізнавання яких обговорюються в огляді. Зокрема, особливу увагу приділено результатам аналізу впізнавання гомологічними синтетазами характерних рис просторової структури тРНК 2-ї групи. У $тРНК^{Ser}$, $тРНК^{Leu}$ і $тРНК^{Tyr}$ орієнтація довгої варіабельної гілки відносно основного тіла тРНК відрізняється і контролюється різною упаковкою корової частини молекули. У разі СерРС N-кінцевий, а в разі ТирРС – С-кінцевий домени зв'язуються з певними структурами довгих варіабельних гілок гомологічних РНК, упізнаючи таким чином унікальну структурну форму тРНК. Корова частина $тРНК^{Leu}$ має кілька шарів незвичайних пар основ, виявлених при вивченні кристалографічної структури комплексу $тРНК^{Leu}$ з ЛейРС із T. thermophiles ma при дослідженні вільної тРНК у розчині методом хімічної модифікації з використанням специфічних реагентів. У кристалографічній структурі комплексу ЛейРС-$тРНК^{Leu}$ унікальна будова D-стебла впізнається С-кінцевим доменом ЛейРС і ці дані добре узгоджуються з результатами, отриманими в розчині. ЛейРС притаманний канонічний для синтетаз I структурного класу тип упізнавання тРНК – з боку D-стебла і малої борозенки акцепторного стебла. Для СерРС також характерний канонічний для синтетаз II структурного класу тип упізнавання тРНК – з протилежного боку, тобто з боку варіабельного стебла і великої борозенки акцепторного стебла. І, нарешті, ТирРС на відміну від канонічного для ферментів I класу типу має тип упізнавання тРНК, властивий синтетазам II класу.

Ключові слова: тРНК другого типу, довга варіабельна гілка, аміноацил-тРНК синтетаза, упізнавання тРНК, комплекси аміноацил-тРНК синтетази.

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Узнавание тРНК с длинной вариабельной веткой аминоацил-тРНК синтетазами

Резюме

В клетках эукариотов тРНК трех специфичностей – $тРНК^{Ser}$, $тРНК^{Leu}$ и $тРНК^{Tyr}$ – имеют длинную вариабельную ветку длиной

11–20 нуклеотидов (2-я группа мРНК) в отличие от четырех или пяти нуклеотидов 1-й группы мРНК. Суммированы результаты наших исследований структурных основ узнавания и дискриминации мРНК 2-й группы серил-, тирозил- и лейцил-мРНК синтетазами из Thermus thermophiles (СерРС, ТирРС и ЛейРС), полученные методами рентгеновской кристаллографии и химической модификации мРНК в растворе. На сегодня кристаллическая структура известна для всех трех комплексов аминоацил-мРНК синтетаз с соответствующими мРНК 2-й группы, разные типы узнавания которых обсуждаются в обзоре. В частности, особенное внимание уделено результатам анализа узнавания гомологичными синтетазами характерных черт пространственной структуры мРНК 2-й группы. У мРНК^{Ser}, мРНК^{Leu} и мРНК^{Tyr} ориентация длинной вариабельной ветви относительно основного тела мРНК отличается и контролируется разной упаковкой коровой части молекулы. В случае СерРС N-концевой, а в случае ТирРС – С-концевой домены связываются с определенными структурами длинных вариабельных веток гомологичных РНК, узнавая тем самым уникальную структурную форму мРНК. Коровая часть мРНК^{Leu} имеет несколько слоев необычных пар оснований, выявленных при изучении кристаллографической структуры комплекса мРНК^{Leu} с ЛейРС из T. thermophiles и при исследовании свободной мРНК в растворе методом химической модификации с использованием специфических реагентов. В кристаллографической структуре комплекса ЛейРС–мРНК^{Leu} уникальное строение D-стебля узнается С-концевым доменом ЛейРС и эти данные хорошо согласуются с результатами, полученными в растворе. ЛейРС свойствен канонический для синтетаз I структурного класса тип узнавания мРНК – со стороны D-стебля и малой бороздки акцепторного стебля. Для СерРС также характерный канонический для синтетаз II структурного класса тип узнавания мРНК – с противоположной стороны, т. е. со стороны вариабельного стебля и большой бороздки акцепторного стебля. И, наконец, ТирРС в отличие от канонического для ферментов I класса типа имеет тип узнавания мРНК, присущий синтетазам II класса.

Ключевые слова: мРНК второго типа, длинная вариабельная ветка, аминоацил-мРНК синтетаза, узнавание мРНК, комплексы аминоацил-мРНК синтетазы.

REFERENCES

1. *Giege R., Sissler M. Florentz C.* Universal rules and idiosyncratic features in tRNA identity // Nucleic Acids Res.–1998.–**26**, N 22.–P. 5017–5035.
2. *Eriani G., Delarue M., Poch O., Gangloff J., Moras D.* Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs // Nature.–1990.–**347**, N 6289.–P. 203–206.
3. *Cusack S., Berthet-Colominas C., Hartlein M., Nassar N., Leberman R.* A second class of synthetase structure revealed by X-ray analysis of *Escherichia coli* seryl-tRNA synthetase at 2.5 Å // Nature.–1990.–**347**, N 6290.–P. 249–255.
4. *Ibba M., Soll D.* Aminoacyl-tRNA synthesis // Annu. Rev. Biochem.–2000.–**69**.–P. 617–650.
5. *Fraser T. H., Rich A.* Amino acids are not all initially attached to the same position on transfer RNA molecules // Proc. Natl Acad. Sci. USA.–1975.–**72**, N 8.–P. 3044–3048.
6. *Sprinzl M., Cramer F.* Site of aminoacylation of tRNAs from *Escherichia coli* with respect to the 2'- or 3'-hydroxyl group of the terminal adenosine // Proc. Natl Acad. Sci. USA.–1975.–**72**, N 8.–P. 3049–3053.
7. *Kavran J. M., Gundllapalli S., O'Donoghue P., Englert M., Soll D., Steitz T. A.* Structure of pyrrolysyl-tRNA synthetase, an archaeal enzyme for genetic code innovation // Proc. Natl Acad. Sci. USA.–2007.–**104**, N 27.–P. 11268–11273.
8. *Kamtekar S., Hohn M. J., Park H. S. et al.* Toward understanding phosphoseryl-tRNA^{Cys} formation: the crystal structure of *Methanococcus maripaludis* phosphoseryl-tRNA synthetase // Proc. Natl Acad. Sci. USA.–2007.–**104**, N 8.–P. 2620–2625.
9. *Cusack S., Hartlein M., Leberman R.* Sequence, structural and evolutionary relationships between class 2 aminoacyl-tRNA synthetases // Nucleic Acids Res.–1991.–**19**, N 13.–P. 3489–3498.
10. *Delarue M., Moras D.* The aminoacyl-tRNA synthetase family: modules at work // Bioessays.–1993.–**15**, N 10.–P. 675–687.
11. *Cusack S., Yaremchuk A., Tukalo M.* tRNA recognition by aminoacyl-tRNA synthetases // The many faces of tRNA / Eds D. S. Eggleston, C. D. Prescott, N. D. Pearson.–London: Acad. Press, 1997.–P. 55–65.
12. *Sprinzl M., Vassilenko K. S.* Compilation of tRNA sequences and sequences of tRNA genes // Nucleic Acids Res.–2005.–**33** (Database issue).–D139–140.
13. *Yaremchuk A. D., Tukalo M. A., Konovalenko A. V., Egorova S. P., Matsuka G. Kh.* Isolation of seryl-tRNA synthetase from *Thermus thermophilus* HB-27 // Biopolym. Cell.–1989.–**5**, N 5.–P. 83–86.
14. *Garber M. B., Agalarov S. Ch., Eliseikina I. A. et al.* Purification and crystallization of components of the protein-synthesizing system from *Thermus thermophilus* // J. Crystal Growth.–1991.–**110**, N 1–2.–P. 228–236.
15. *Garber M. B., Yaremchuk A. D., Tukalo M. A., Egorova S. P., Berthet-Colominas C., Leberman R.* Crystals of seryl-tRNA synthetase from *Thermus thermophilus*. Preliminary crystallographic data // J. Mol. Biol.–1990.–**213**, N 4.–P. 631–632.
16. *Fujinaga M., Berthet-Colominas C., Yaremchuk A. D., Tukalo M. A., Cusack S.* Refined crystal structure of seryl-tRNA synthetase from *Thermus thermophilus* at 2.5 Å resolution // J. Mol. Biol.–1993.–**234**, N 1.–P. 222–233.
17. *Cusack S., Berthet-Colominas C., Biou V., Borel F., Fujinaga M., Hartlein M., Krikliviy I., Nassar N., Price S., Tukalo M. A., Yaremchuk A., Leberman R.* The crystal structure of seryl-tRNA synthetase and its complexes with ATP and tRNASer // The translation apparatus: structure, function, regulation, evolution.–New-York; London: Plenum Press, 1993.–P. 1–12.
18. *Biou V., Yaremchuk A., Tukalo M., Cusack S.* The 2.9 Å crystal structure of *T. thermophilus* seryl-tRNA synthetase complexed with tRNA(Ser) // Science.–1994.–**263**, N 5152.–P. 1404–1410.
19. *Belrhali H., Yaremchuk A., Tukalo M. et al.* Crystal structures at 2.5 angstrom resolution of seryl-tRNA synthetase complexed with two analogs of seryl adenylate // Science.–1994.–**263**, N 5152.–P. 1432–1436.
20. *Belrhali H., Yaremchuk A., Tukalo M., et al.* The structural basis for seryl-adenylate and Ap4A synthesis by seryl-tRNA synthetase // Structure.–1995.–**3**, N 4.–P. 341–352.
21. *Cavarelli J., Eriani G., Rees B. et al.* The active site of yeast aspartyl-tRNA synthetase: structural and functional aspects of the aminoacylation reaction // EMBO J.–1994.–**13**, N 2.–P. 327–337.
22. *Yaremchuk A., Tukalo M., Grotli M., Cusack S.* A succession of substrate induced conformational changes ensures the amino acid specificity of *Thermus thermophilus* prolyl-tRNA synthetase: comparison with histidyl-tRNA synthetase // J. Mol. Biol.–2001.–**309**, N 4.–P. 989–1002.

23. *Arnez J. G., Moras D*. Structural and functional consideration of the aminoacylation reaction // Trends Biochem. Sci.–1998.–**22**, N 6.– P. 211–216.
24. *Krikliviy I. A., Kovalenko O. P., Gudzera O. Y., Yaremchuk A. D., Tukalo M. A.* Isolation and purification isoaccepting $tRNA_1^{Ser}$ and $tRNA_2^{Ser}$ from *Thermus thermophilus* // Biopolym. Cell.–2006.–**22**, N 6.–P. 425–432.
25. *Petrushenko Z. M., Kovalenko O. P., Mal'chenko N. N., Krikliviy I. A., Yaremchuk A. D., Tukalo M. A.* The primary structure of $tRNA^{Ser}$ from *Thermus thermophilus* // Biopolym. cell.–1997.–**13**, N 3.–P. 202–208.
26. *Himeno H., Hasegawa T., Ueda T., Watanabe K., Shimizu M.* Conversion of aminoacylation specificity from $tRNA^{Tyr}$ to $tRNA^{Ser}$ *in vitro* // Nucleic Acids Res.–1990.–**18**, N 23.–P. 6815– 6819.
27. *Asahara H., Himeno H., Tamura K. et al. Escherichia coli* seryl-tRNA synthetase recognizes $tRNA^{Ser}$ by its characteristic tertiary structure // J. Mol. Biol.– 1994.–**236**, N 3.–P. 738–748.
28. *Yaremchuk A. D., Tukalo M. A., Krikliviy I. A. et al.* Crystallization of the seryl-tRNA synthetase-$tRNA^{Ser}$ complex from *Thermus thermophilus* // J. Mol. Biol.–1992.–**224**, N 2.–519–522.
29. *Yaremchuk A. D., Tukalo M. A., Krikliviy I. et al.* A new crystal form of the complex between seryl-tRNA synthetase and tRNA (Ser) from *Thermus thermophilus* that diffracts to 2.8 Å resolution // FEBS Lett.–1992.–**310**, N 2.–P. 157–161.
30. *Kovalenko O. P., Petrushenko Z. M., Mal'chenko N. N., Krikliviy I. A., Yaremchuk A. D., Tukalo M. A.* Studies of interaction sites between tRNA2Ser from *Thermus thermophilus* and seryl-tRNA synlhetase by chemical modification // Biopolym. Cell.–1997.–**13**, N 4.–P. 298–302.
31. *Kovalenko O. P., Kriklivyi I. A., Tukalo M. A.* Study of tertiary structure elements of $tRNA^{Ser}$ from *Thermus thermophilus* in solution // Biopolym. Cell.–2000.–**16**, N 2.–P. 115–123.
32. *Vlassov V. V., Giege R., Ebel J. P.* Tertiary structure of tRNA in solution monitored phosphodiester modification with ethylnitrosourea // Eur. J. Biochem.–1981.–**119**, N 1.–P. 51–59.
33. *Peattie D. A., Gilbert W.* Chemical probes for higher-order structure in RNA // Proc. Natl Acad. Sci. USA.–1980.–**77**, N 8.–P. 4679–4682.
34. *Cusack S., Yaremchuk A., Tukalo M.* The crystal structure of the ternary complex of *T. thermophilus* seryl-tRNA synthetase with $tRNA^{Ser}$ and seryl-adenylate analogue reveals a conformational switch in the active site // EMBO J.–1996.–**15**, N 11.–P. 2834–2842.
35. *Starzyk R. M., Webster T. A., Schimmel P.* Evidence for dispensable sequences inserted into a nucleotide fold // Science.–1987.–**237**, N 4822.–P. 1614–1618.
36. *Nureki O., Vassylyev D. G., Tateno M. et al.* Enzyme structure with two catalytic sites for double-sieve selection of substrate // Science.–1998.–**280**, N 5363.–P. 578–582.
37. *Silvian L., Wang J., Steitz T. A.* Insights into editing from an Ile-tRNA synthetase structure with $tRNA^{Ile}$ and mupirocin // Science.–1999.–**285**, N 5430.–P. 1074–1077.
38. *Schmidt E., Schimmel P.* Mutational isolation of a sieve for editing in a transfer RNA synthetase // Science.–1994.–**264**, N 5156.–P. 265–267.
39. *Lincecum T. L. Jr., Tukalo M., Yaremchuk A. et al.* Structural and mechanistic basis of pre- and posttransfer editing by leucyl-tRNA synthetase // Mol. Cell.–2003.–**11**, 4.–P. 951–963.
40. *Asahara H., Himeno H., Tamura K., Hasegawa T., Watanabe K., Shimizu M.* Recognition nucleotides of *Escherichia coli* $tRNA^{Leu}$ and its elements facilitating discrimination from $tRNA^{Ser}$ and $tRNA^{Tyr}$ // J. Mol. Biol.–1993.–**231**, N 2.– P. 219–229.
41. *Yaremchuk A. D., Kovalenko O. P., Gudzera O. I., Tukalo M. A.* Molecular cloning, sequencing and expression in *Escherichia coli* cells *Thermus thermophilus* leucyl-tRNA synthetase // Biopolym. Cell.–2011.–**27**, N 6.–P. 436–441.
42. *Yaremchuk A., Cusack S., Gudzera O., Grotli M., Tukalo M.* Crystallization and preliminary crystallographic analysis of *Thermus thermophilus* leucyl-tRNA synthetase and its complexes with leucine and a non-hydrolysable leucyl-adenylate analogue // Acta Crystallogr. D Biol. Crystallogr–2000.–**56**, Pt 5.–P. 667–669.
43. *Cusack S., Yaremchuk A., Tukalo M.* The 2 Å crystal structure of leucyl-tRNA synthetase and its complex with a leucyl-adenylate analogue // EMBO J.–2000.–**19**, N 10.–P. 2351–2361.
44. *Yaremchuk A. D., Gudzera O. I., Egorova S. P., Rozhko D. I., Krikliviy I. A., Tukalo M. A.* Leucyl-tRNA synthetase from *Thermus thermophilus*. Purification and some properties of the crystallizing enzyme // Biopolym. Cell.–2001.–**17**, N 3.–P. 216–220.
45. *Tukalo M., Yaremchuk A., Fukunaga R., Yokoyama S., Cusack S.* The crystal structure of leucyl-tRNA synthetase complexed with $tRNA^{Leu}$ in the post-transfer-editing conformation // Nat. Struct. Mol. Biol.–2005.–**12**, N 10.–P. 923–930.
46. *Gudzera O. I., Yaremchuk A. D., Tukalo M. A.* Functional role of C-terminal domain of *Thermus thermophilus* leucyl-tRNA synthetase // Biopolym. Cell.–2010.–**26**, N 6.–P. 478–485.
47. *Hsu J. L., Rho S. B., Vannella K. M., Martinis S. A.* Functional divergence of a unique C-terminal domain of leucyl-tRNA synthetase to accommodate its splicing and aminoacylation roles // J. Biol. Chem.–2006.–**281**, N 32.–P. 23075–23082.
48. *Fukunaga R., Yokoyama S.* The C-terminal domain of the archaeal leucyl-tRNA synthetase prevents misediting of isoleucyl-$tRNA^{Ile}$ // Biochemistry.–2007.–**46**, N 17.–P. 4985–4996.
49. *Kovalenko O., Kriklivyi I., Yaremchuk A., Tukalo M.* Comparative studies the tertiary structure of *T. thermophilus* $tRNA^{Ser}$ and $tRNA^{Leu}$ and the sites of interaction with cognate aminoacyl-tRNA synthetases by chemical modification methods // 18^{th} tRNA Workshop «tRNA 2000» (8^{th}–12^{th} April 2000, Cambridge).–Cambridge: BioDesign Publications, 2001.–P. 20.
50. *Palencia A., Crepin T., Vu M. T., Lincecum T. L. Jr., Martinis S. A., Cusack S.* Structural dynamics of the aminoacylation and proofeading functional cycle of bacterial leucyl-tRNA synthetase // Nat. Struct. Mol. Biol.–2012.–**19**, N 7.–P. 677–684.
51. *Kovalenko O. P., Kriklivyi I. A., Tukalo M. A.* Participation of nitrogen bases in the tertiary folding of $tRNA^{Leu}$ from *Thermus thermophilus* // Biopolym. Cell.–2003.–**19**, N 2.–P. 151–156.
52. *Fukunaga R., Ishitani R., Nureki O., Yokoyama S.* Crystallization of leucyl-tRNA synthetase complexed with $tRNA^{Leu}$ from the archaeon *Pyrococcus horikoshii* // Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun.–2005.–**61**, Pt 1.–P. 30–32.
53. *Rock F. L., Mao W., Yaremchuk A. et al.* An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site // Science.–2007.–**316**, N 5832.–P. 1759–1761.
54. *Asahara H., Nameki N., Hasegawa T. In vitro* selection of RNAs aminoacylated by *Escherichia coli* leucyl-tRNA synthetase // J. Mol. Biol.–1998.–**283**, N 3.–P. 605–618.
55. *Tocchini-Valentini G., Saks M. E., Abelson J.* tRNA leucine identity and recognition sets // J. Mol. Biol.–2000.–**298**, N 5.–P. 779–793.
56. *Larkin D. C., Williams A. M., Martinis S. A., Fox G. E.* Identification of essential domains for *Escherichia coli* $tRNA^{Leu}$ aminoacylation and amino acid editing using minimalist RNA molecules // Nucleic Acids Res.–2002.–**30**, N 10.–P. 2103–2113.
57. *Cusack S.* Eleven down and nine to go // Nat. Struct. Biol.–1995.–**2**, N 10.–P. 824–831.

58. *Brick P., Blow D. M.* Crystal structure of a deletion mutant of a tyrosyl-tRNA synthetase complexed with tyrosine // J. Mol. Biol.–1987.–**194**, N 2.–P. 287–294.
59. *Yaremchuk A. D., Kovalenko O. P., Gudzera O. I., Tukalo M. A.* Molecular cloning, sequencing and sequence analysis of *Thermus thermophilus* tyrosyl-tRNA synthetase // Biopolym. Cell.–2004.–**20**, N 1–2.–P. 144–149.
60. *Yaremchuk A., Kriklivyi I., Tukalo M., Cusack S.* Class I tyrosyl-tRNA synthetase has a class II mode of cognate tRNA recognition // EMBO J.–2002.–**21**, N 14.–P. 3829–3240.
61. *Bedouelle H., Guez-Ivanier V., Nageotte R.* Discrimination between transfer-RNAs by tyrosyl-tRNA synthetase // Biochimie.–1993.–**75**, N 12.–P. 1099–1108.
62. *Egorova S. P., Yaremchuk A. D., Krikliviy I. A., Tukalo M. A*. Comparative analysis of interaction sites of tRNA from *Thermus thermophilus* and *Escherichia coli* with cognate aminoacyl tRNA synthetases by the chemical modification and nuclease hydrolysis methods // // Bioor. Khimiya.–1998.–**24**, N 8.–P. 593–600.
63. *Ruff M., Krishnaswamy S., Boeglin M. et al.* Class II aminoacyl transfer RNA synthetases: crystal structure of yeast aspartyl-tRNA synthetase complexed with $tRNA^{Asp}$ // Science.–1991.–**252**, N 5013.–P. 1682–1689.
64. *Rould M. A., Perona J. J., Soll D., Steitz T. A.* Structure of *E. coli* glutaminyl-tRNA synthetase complexed with $tRNA^{Gln}$ and ATP t 2.8 Å resolution // Science.–1989.–**246**, N 4934.–P. 1135–1142.
65. *Cramer F., Faulhammer H., von der Haar F., Sprinzl M., Sternbach H.* Aminoacyl-tRNA synthetases from baker's yeast: reacting site of aminoacylation is not uniform for all tRNAs // FEBS Lett.– 1975.–**56**, N 2.–P. 212–214.
66. *Kobayashi T., Nureki O., Ishitani R. et al.* Structural basis for orthogonal tRNA specificities of tyrosyl-tRNA synthetases for genetic code expansion // Nat. Struct. Biol.–2003.–**10**, N 6.–P. 425–432.
67. *Yang X. L., Otero F. J., Ewalt K. L. et al.* Two conformations of a crystalline human tRNA synthetase-tRNA complex: implications for protein synthesis // EMBO J.–2006.–**25**, N 12.–P. 2919–2929.
68. *Hou Y. M., Schimmel P.* Modeling with *in vitro* kinetic parameters for the elaboration of transfer RNA identity *in vivo* // Biochemistry.–1989.–**28**, N 12.–P. 4942–4947.
69. *Himeno H., Hasegawa T., Ueda T., Watanabe K., Shimizu M.* Conversion of aminoacylation specificity from $tRNA^{Tyr}$ to $tRNA^{Ser}$ *in vitro* // Nucleic Acids Res.–1990.–**18**, N 23.–P. 6815–6819.
70. *Fechter P., Rudinger-Thirion J., Tukalo M., Giege R.* Major tyrosine identity determinants in *Methanococcus jannaschii* and *Saccharomyces cerevisiae* $tRNA^{Tyr}$ are conserved but expressed differently // Eur. J. Biochem.–2001.–**268**, N 3.–P. 761–767.
71. *Hauenstein S., Zhang C. M., Hou Y. M., Perona J. J.* Shape-selective RNA recognition by cysteinyl-tRNA synthetase // Nat. Struct. Mol. Biol.–2004.–**11**, N 11.–P. 1134–1141.
72. *Soma A., Himeno H.* Cross-species aminoacylation of tRNA with a long variable arm between *Escherichia coli* and *Saccharomyces cerevisiae* // Nucleic Acids Res.–1998.–**26**, N 19.–P. 4374–4381.
73. *Lenhard B., Orellana O., Ibba M., Weygand-Durasevic I.* tRNA recognition and evolution of determinants in seryl-tRNA synthesis // Nucleic Acids Res.–1999.–**27**, N 3.–P. 721–729.
74. *Sidorik L. L., Gudzera O. I., Dragovoz V. A., Tukalo M. A., Beresten S. F*. Immuno-chemical non-cross-reactivity between eukaryotic and prokaryotic seryl-tRNA synthetase // FEBS Let.–1991.–**292**, N 1, 2.–P. 76–78.
75. *Xu X., Shi Y., Zhang H. M. et al*. Unique domain appended to vertebrate tRNA synthetase is essential for vascular development // Nat.Commun.–2012.–**3**, N 2.–P. 1–9.
76. *Petrushenko Z. M., Tukalo M. A., Gudzera O. I et al.* Identification of interaction sites of $tRNA^{Leu}$ from cow mammary gland with the cognate aminoacyl-tRNA synthetase by the chemical modification method // Rus. J. Bioorg. Chem.–1990.–**16**, N 12.–P. 1647–1652.
77. *Dock-Bregeon A. C., Garsia A., Giege R., Moras D*. The contacts of yeast tRNASer with seryl-tRNA synthetase studied by footprinting experiments // Eur. J. Biochem.–1990.–**188**, N 2.–P. 283–290.
78. *Kalachnyuk L. G., Tukalo M. A., Matsuka G. Kh*. Identification of interaction sites of $tRNA_{GCU}^{Ser}$ from the bovine liver with cognate aminoacyl-tRNA synthetase by the chemical modification method // Ukr. Biochem. J.–1992.–**64**, N 6.–P. 38–43.
79. *Wu X. Q., Gross H. J.* The long extra arms of human tRNA (Ser(Sec)) and tRNASer function as major identity elements for serylation in an orientation-dependent, but not sequence-specific manner // Nucl. Acids Res.–1993.–**21**, N 24.–P.5589–5594.
80. *Bonfils G., Jaquenoud M., Bontron S. et al*. Leucyl-tRNA synthetase controls TORC1 via the EGO complex // Mol. Cell.–2012.–**46**, N 1.–P. 105–110.
81. *Han J. M., Jeong S. J., Park M. C.et al.* Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway // Cell.–2012.–**149**, N 2.–P. 410–424.

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