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REVIEW

Interaction of Aminoacyl-tRNA Synthetases with tRNA: General Principles and Distinguishing Characteristics of the High-Molecular-Weight Substrate Recognition

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Abstract—This review summarizes results of numerous (mainly functional) studies that have been accumulated over recent years on the problem of tRNA recognition by aminoacyl-tRNA synthetases. Development and employment of approaches that use synthetic mutant and chimeric tRNAs have demonstrated general principles underlying highly specific interaction in different systems. The specificity of interaction is determined by a certain number of nucleotides and structural elements of tRNA (constituting the set of recognition elements or specificity determinants), which are characteristic of each pair. Crystallographic structures available for many systems provide the details of the molecular basis of selective interaction. Diversity and identity of biochemical functions of the recognition elements make substantial contribution to the specificity of such interactions.

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Aminoacyl-tRNA synthetases (aaRSs) are key enzymes of protein biosynthesis, which determine accuracy of reproduction of genetic information. They catalyze the esterification of a particular tRNA with its corresponding amino acid. The first reaction step includes activation of the appropriate amino acid (aa): it is recognized by the enzyme (E) and reacts with ATP to form an enzyme-bound aminoacyl adenylate (aa-AMP) and release of pyrophosphate (PP_i):

$$E + aa + ATP \rightarrow E{\cdot}aa\text{-}AMP + PP_i.$$

During the second step the activated amino acid is transferred onto the 3′-end ribose of the corresponding tRNA:

$$E{\cdot}aa\text{-}AMP + tRNA \rightarrow aa\text{-}tRNA + AMP + E.$$

As a rule, each amino acid has its "own" tRNA (or a set of isoacceptor tRNAs [1]) and aaRS and there are 20 enzymes of various specificity, although some exceptions are known in some organisms. For example, archaebacteria, Gram-positive eubacteria, and eukaryotic organelles lack functionally active glutaminyl-tRNA synthetase (GlnRS) [2, 3]. AsnRS and CysRS have not been found in some archaebacteria [4]. In these cases, synthesis of Gln-$tRNA^{Gln}$ and Asn-$tRNA^{Asn}$ includes an alternative pathway that employs non-discriminating GluRS and AspRS (which synthesize Glu-$tRNA^{Gln}$ and Asp-$tRNA^{Asn}$, respectively) and amidotransferases [4, 5].

TWO CLASSES OF AMINOACYL-tRNA SYNTHETASES

In spite of their common functional role in protein biosynthesis, the aaRSs significantly differ in size, amino acid sequence, and three-dimensional (3D) structure. Discovery of characteristic structural motifs reflecting differences in topology of active sites [6, 7] resulted in

Abbreviations: aaRS(s)) aminoacyl-tRNA synthetase(s); GlnRS) glutaminyl-tRNA synthetase (specificity of other aaRSs is defined in the same style using three letter abbreviation of amino acids); XSA) X-ray structure analysis; $tRNA^{Gln}$) glutamine specific tRNA (specificity of other tRNAs is defined in the same style using three letter abbreviation of amino acids); 3D structure) three-dimensional structure.

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Table 1. Structural and functional differences of two classes of aminoacyl-tRNA synthetases (summarized from [3, 8] with additions)

Characteristics	Class I	Class II
	Subclass Ia	*Subclass IIa*
Members (subunit composition)	ArgRS (α), ValRS (α), IleRS (α), MetRS (α, α_2), CysRS (α), LeuRS (α, αβ [9])	SerRS (α_2), ThrRS (α_2), ProRS (α_2), GlyRS (α_2), HisRS (α_2), AlaRS (α)
	Subclass Ib	*Subclass IIb*
	GlnRS (α), GluRS (α), LysRS1 (α)	AspRS (α_2), AsnRS (α_2), LysRS2 (α_2)
	Subclass Ic	*Subclass IIc*
	TrpRS (α_2), TyrRS (α_2)	PheRS [$(\alpha\beta)_2$], AlaRS (α_4), GlyRS [$(\alpha\beta)_2$]
Conservative motifs*	φHφ**G**h KmS**K**s	(1) gφxxφxxPφφ (2) f**R**xex$_{n=4-12}$(h/r)xxxFxxx(d/e) (3) gφgφgφ(d/e)**R**φφφφφ
Active site topology	parallel β-sheet (Rossmann fold)	seven-stranded antiparallel β-sheet
Position specificity	2′-OH group	3′-OH group (PheRS is exception)

* Conservative residues are indicated with capital letters, strictly conservative residues are indicated in bold, φ is any hydrophobic residue, x shows any residues, the chain of various length ($x_{n=4-12}$) forms a loop in motif 2.

subdivision of these enzymes into two classes (Table 1). Each class consists of 10 enzymes of various specificities. The only exception is the existence of two types of LysRS with structural topology of classes I and II [4, 10]. Subsequent subdivision of classes into subclasses is based on structural resemblance of catalytic and non-catalytic domains and their compositions [11, 12].

Enzymes of class I (members of subclasses Ia and Ib are monomers, members of subclass Ic are homodimers) contain two conservative sequences, φHφ**G**h (His-Ile-Gly-His) and KmS**K**s (Lys-Met-Ser-Lys-Ser), which form the nucleotide-binding Rossmann fold of the active site. Class II aaRSs (usually dimers or tetramers) are characterized by three motifs. Motif 1 containing a conserved proline residue is involved in formation of the intersubunit contacts. Motifs 2 and 3 (each of which contains a conservative Arg residue) form a part of the active site. Two classes of aaRSs are characterized by structural difference in the conformations of the bound nucleotide substrate: in the active sites of class I and class II aaRSs, ATP triphosphate group adopts extended or bent conformation, respectively [8, 13]. The second principal difference between enzymes of these two classes consists in the mode of binding of the acceptor arm of tRNA. Class I aaRSs (except TyrRS and TrpRS) are characterized by an interaction with the acceptor stem from the minor groove side and hairpin structure of 3′-terminal tetranucleotide, whereas binding of the acceptor stem from the major groove and extended conformation of the 3′-end are typical for class II enzymes. Subdivision of aaRSs into two classes also correlates with different catalytic behavior of these enzymes: class I enzymes attach the corresponding amino acid to the 2′-OH group, whereas class II enzymes (except PheRS) attach the amino acid to the 3′-OH group of 3′-terminal adenosine of tRNA. Structural principles underlying interactions with low-molecular-weight substrates (ATP and amino acids) have been studied for most aaRSs; within each class, enzymes share common catalytic mechanisms of amino acid activation (see for review [3, 8, 13]). However, the mode of tRNA binding is very individual due to lack of common tRNA-binding motifs (see for review [8, 14]).

METHODS FOR DETERMINATION OF SPECIFICITY ELEMENTS IN tRNA

Significant progress in characterization of structural elements responsible for recognition of specific tRNA by corresponding aaRS has been achieved due to numerous studies employing genetic methods *in vivo* and kinetic experiments *in vitro* using mutant tRNAs [14-17]. Nucleotides whose mutations are accompanied by loss of tRNA specificity are considered as specificity determinants (elements); these identified *in vivo* or *in vitro* are designated as identity or recognition elements, respectively [15, 17], although no general rules exist in terminology used. The most frequently used experimental approach *in*

vivo is based on the expression of a reporter protein encoded by DNA containing a stop-codon at a certain position. Thus, this approach implies determination of specificity of suppressor tRNA obtained on the basis of the tested tRNA and its various mutant forms. Such study is carried out under physiological conditions when all aaRSs specific to 20 amino acids compete for the substrate tRNA. Table 2 shows several examples. Specificity of suppressor tRNA aminoacylation was determined by analysis of amino acids incorporated into the translated protein. Frequency of amino acid incorporation varies from 0 to 100%. Significant effects (>90% incorporation of the wrong amino acids) determined for examples by mutation A20U in the suppressor *E. coli* $tRNA^{Arg}$ result in total loss of specificity and thus A20 is the major identity determinant in the Arg-specific system. Wrong incorporation of Lys observed for the wild-type suppressor $tRNA^{Arg}$ was explained by substitution of $tRNA^{Arg}$ anticodon C35 for U35 corresponding to the identity element of $tRNA^{Lys}$. Multiple specificity is a common feature of mutant suppressor tRNAs; it may be attributed to removal of identity elements of a particular system and/or addition of identity elements of other systems. *In vivo* studies of the role of anticodon employ an alternative method utilizing mutant initiator tRNAs [16, 17].

A completely different strategy is used for determination of the recognition elements *in vitro*: instead of evaluation of the final effect of tRNA mutations in protein synthesis, this strategy implies evaluation of the physicochemical effects of such mutations on the aminoacylation reaction by measuring K_m and k_{cat} parameters for tRNA transcripts and their mutant forms. The decrease in k_{cat}/K_m value (specificity constant or catalytic efficiency of reaction [18]) caused by a mutation is a measure of the loss of specificity. tRNAs are synthesized using transcription of a synthetic DNA template by T7 RNA polymerase. For most systems [15, 16] lack of modified bases in the transcripts insignificantly influenced efficiency of their aminoacylation (compared with natural tRNAs). The presence of 5′-guanosine as ultimate precondition for effective transcription limits use of *in vitro* synthesized tRNAs [19, 20]. However, the use of NpG dinucleotide for initiation of transcription allows incorporation of any base at the tRNA 5′-end [21]. Chemical synthesis of tRNA molecules containing minor bases [22] can be employed to elucidate the role of post-translational modification in the tRNA specificity.

Kinetic studies traditionally using trichloroacetic acid precipitation of aminoacyl-tRNAs on filters [23] now employ a more effective and sensitive method based on separation of free and aminoacylated tRNA in gel [24]. Although *in vitro* experiments are carried out under non-physiological conditions because of use of individual tRNA and enzymes (in the absence of other aaRSs), they can nevertheless quantitatively evaluate the contribution of each element into the effectiveness of tRNA recognition by corresponding aaRS. Completeness of the set of positive recognition elements (for particular tRNA-specific aaRS) is verified by experiments on the specificity switch by "transplantation" of this set of elements into other tRNAs. Identification of the specificity determinants among tertiary structure elements requires additional analysis of the effect of mutations on the general fold of tRNA [14]. In cases when the loss of specificity is associated with destabilization of tertiary structure of tRNA, structural studies of the tRNA–aaRS complex are needed.

Elements of specificity determined by different approaches may be non-identical, because effects pro-

Table 2. Some examples of *in vivo* studies of aminoacylation specificity ([15] with modifications)

E. coli suppressor tRNA*	Mutations	Amino acid incorporation**, %	
		natural specificity	acquired specificity
$tRNA^{Ala}$ (amber)	G3–U70→A3–U70	n.d.	n.d.
$tRNA^{Arg}$ (amber)	(amber anticodon)	Arg (37%)	Lys (55%)
$tRNA^{Arg}$ (amber)	A20→U20	n.d.	Lys (91%)
$tRNA^{Arg}$ (amber)	A59→U59	Arg (38%)	Lys (50%)
$tRNA^{Cys}$ (amber)	U73→A73	Cys (0%)	Lys (63%), Tyr (20%), Gln (8%)
$tRNA^{Cys}$ (amber)	A31–U39→C31–G39	Cys (90%)	Gln (10%)
$tRNA^{Cys}$ (amber)	G29–C41→U29–A41	Cys (93%)	Gln (5%)
$tRNA^{Cys}$ (amber)	G27–U43→C27–G43	Cys (89%)	Gln (11%)

Note: n.d., not determined (incorporation did not exceed the background level).

* Amber anticodon is 5′-CUA-3′.

** Loss of specificity was evaluated by relative content of the amino acid incorporated at the 10th position of dehydrofolate reductase expressed in *E. coli* cells (its gene carried amber-mutation at the 10th codon).

duced in *in vivo* experiments reflect changes in the interactions of mutant tRNAs with all competing aaRSs and also with other components of the translation machinery.

Use of integrated approaches *in vivo* and *in vitro* allows a complete set of specificity determinants to be detected for a particular system: positive recognition elements and anti-determinants (negative elements) preventing tRNA interaction with nonspecific aaRSs.

DISTRIBUTION OF SPECIFICITY ELEMENTS IN tRNA

Using the above-considered methods, the tRNA specificity elements have been determined for all 20 systems from *E. coli*, some systems from *T. thermophilus* and archaebacteria, 15 systems from yeasts, and 11 systems from higher eukaryotes including humans (Table 3). Analysis of these data shows that a relatively small number of tRNA nucleotides are critical for specificity of aminoacylation, and a set of these nucleotides is highly specific for each pair (aaRS-tRNA). Figure 1 shows distribution of the recognition elements over *E. coli* tRNA for all pairs aaRS-tRNA grouped according to enzyme classification into classes I and II. In both cases, they are preferentially positioned in peripheral regions of tRNA: anticodon and acceptor arm. A hypothesis on the decisive role of anticodon in tRNA recognition proposed by Russian scientists at early stages of consideration of this problem [94, 95] was perfectly supported by results of subsequent studies: indeed, the anticodon nucleotides make the major contribution to specificity of most of the investigated systems (Table 3). The middle position 35 is always important for recognition. Nucleotides at positions 34 (varying in isoacceptor tRNAs) and 36 are used less frequently as the specificity determinants. For some tRNAs anticodon importance was confirmed by independent biochemical experiments. It has been demonstrated that interaction of yeast AspRS, ValRS, MetRS, *E. coli* HisRS, *E. coli* and *T. thermophilus* TyrRS, and *B. burgdorferi* LysRS with corresponding tRNAs protect the anticodon region against chemical modification and nuclease hydrolysis [15, 29, 57, 96]. Interestingly, in some cases substitutions of anticodon nucleotides resulting in changes of amino acid specificity of tRNA (corresponding to the genetic code) are not accompanied by the loss of tRNA ability to be recognized by initial aaRS. Besides, three nucleotides of anticodon additional recognition elements positioned in the anticodon loop have been found for several class I enzymes of various specificity and for one class II aaRS (AspRS from various organisms); specificity of yeast $tRNA^{Met}$ is determined by the sequence of the whole anticodon loop (Table 3).

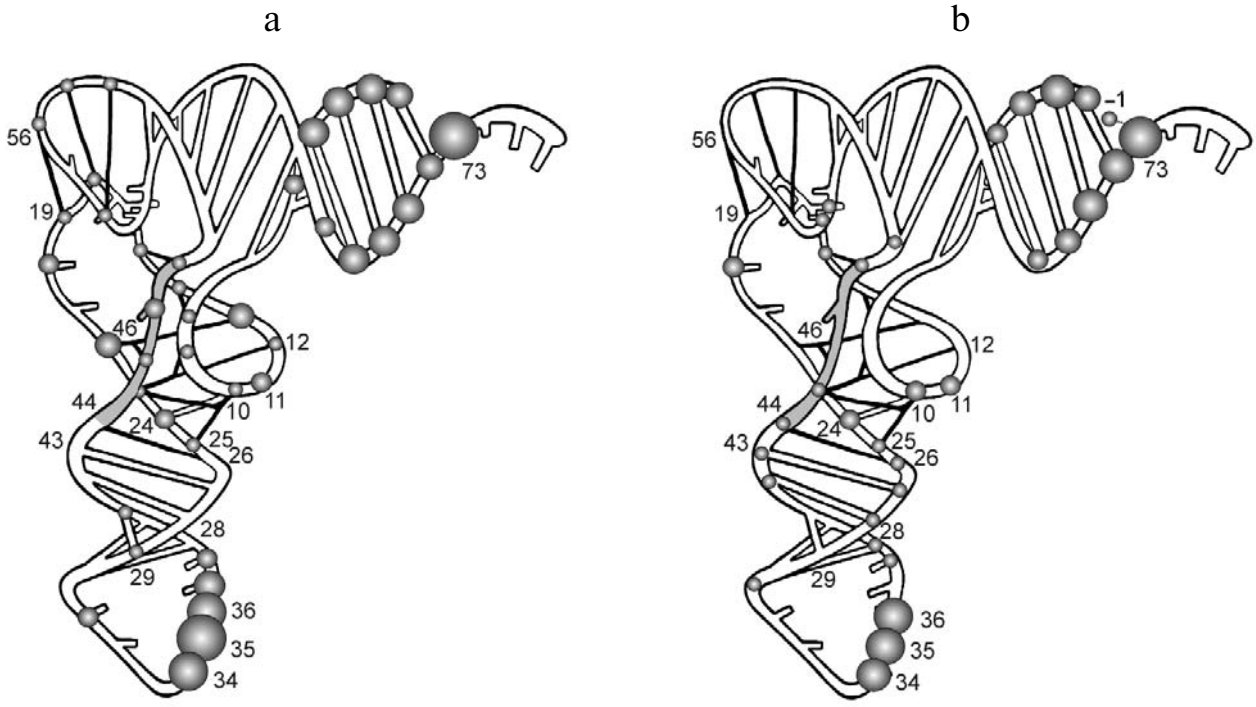

Fig. 1. Distribution of the recognition elements of *E. coli* tRNA by aminoacyl-tRNA synthetases class I (a) and class II (b) in three-dimensional model ($tRNA^{Phe}$). Sphere sizes are proportional to the frequency of recognition elements at a given position in systems of various specificity within the same class. The variable arm marked with gray color is recognized as structural feature of $tRNA^{Tyr}$ and $tRNA^{Ser}$ (arm sizes increase up to 13 and 16-21 nucleotides, respectively).

Anticodon is not essential for recognition of at least three *E. coli* tRNAs specific for Leu, Ser, and Ala. This is quite understandable for $tRNA^{Ser}$ because none of the anticodon nucleotides is common for all six isoacceptors [92]; the recognition elements are positioned in the acceptor arm, D-stem, and long variable arm [15, 60]. The Leu-specific systems are characterized by significant species differences: only yeast $tRNA^{Leu}$ contains the recognition elements in the anticodon loop [15]. Size and sequence of the long V-arm of $tRNA^{Leu}$ are crucial for recognition by human and *H. volcanii* archaebacterium LeuRS, but these elements do not determine specificity in *E. coli* and yeast [15, 31-35]. A unique wobble pair G3-U70 represents the evolutionarily conservative specificity determinant of $tRNA^{Ala}$; its transplantation into other tRNAs provides their aminoacylation with alanine [15, 91, 97].

The acceptor arm contains necessary recognition elements even in the systems where the anticodon is the major specificity determinant. The earlier studies suggested that the acceptor arm might play the decisive role in aminoacylation due to its "neighborhood" to the catalytic site of aaRS, and nucleotide 73 of the single-stranded region serves as the primary discrimination site ("the discriminator base") [98]. Subsequent studies confirmed correctness of this hypothesis: nucleotide nature at this position is really important for 18 systems of *E. coli*, for 10 yeast systems, and most archaebacterial systems studied (Table 3). The discriminator bases of Trp-, Pro-, Gly-, and His-specific tRNAs differ for prokaryotes, eukaryotes, and archaebacteria. Many tRNAs (most *E. coli* tRNAs, $tRNA^{Thr}$ and $tRNA^{Gly}$ from various organisms, and other tRNAs) are characterized by the presence of the identity elements in the first four base pairs of the acceptor stem. At the 5′-end, $tRNA^{His}$ contains a unique additional nucleotide $G^{-}1$, its base (forming a pair with the discriminator base) and a phosphate group being essential for recognition by HisRS of *E. coli* and yeast [15, 74-76]. *E. coli* AlaRS is sensitive to substitution of atoms or groups of the first pair G1-C72, whereas the pair G2-C71 may be replaced by other combinations of purine and pyrimidine without significant loss of aminoacylation efficiency [89, 90]. It is suggested that the second pair determines local structure and electrostatic potential of the acceptor stem near the main recognition element (G3-U70). Using the method of selection of functionally active tRNAs (from a large number of mutants) by their ability to support growth of knockout cells (lacking the gene encoding $tRNA^{Ala}$), other researchers have proposed the acceptor stem of $tRNA^{Ala}$ being recognized as a whole fragment; individual nucleotides make different contribution to the specificity [88].

In most systems of aaRS classes I and II, the recognition elements are commonly located in the central regions of the tRNA molecule (positions 8-31 and 39-65) (Table 3). Their number and localization vary. (In the case of *E. coli* tRNA the small size spheres are in positions 8-15, 18-20, 22-25, 29, 41, 46, 47, 48, 54-56, and 58 (Fig. 1a) and in positions 10, 11, 15, 20, 24-28, 42-45, 48, 49, 59, and 60 (Fig. 1b).) Nucleotides of the anticodon stem are effectively recognized only by several enzymes: *E. coli* IleRS and LysRS, *M. barkeri* SerRS, and human PheRS [15, 64, 81]; identity of the anticodon stem of *E. coli* $tRNA^{Phe}$ was found only in *in vivo* experiments [99]. Many specificity elements of variable, D- and T-arms (Table 3) are involved in tertiary interactions of tRNA that are responsible for the optimal folding of the substrate. An unusual tertiary base pair G15-G48 of eubacterial $tRNA^{Cys}$ and long V-arm of all $tRNA^{Ser}$, eubacterial $tRNA^{Tyr}$, and some $tRNA^{Leu}$ are recognized as structural features of corresponding tRNAs.

In rare cases, minor bases of natural tRNAs act as the specificity elements. For example, lysidine (modified U) and inosine at position 34 are positive recognition elements of *E. coli* and yeast $tRNA^{Ile}$ [15]. Base modifications may prevent tRNA recognition by nonspecific aaRS. In *E. coli* $tRNA^{Ile}$ lysidine blocks its acylation by MetRS; 1-methylguanosine at position 37 of yeast $tRNA^{Asp}$ prevents incorrect aminoacylation by ArgRS [15]. Table 4 shows other examples of anti-determinants of homologous (from the same organism) and heterologous systems. They show that tRNA specific for class I aaRSs contain anti-determinants against class II enzymes and vice versa. Although such elements are known to date only for certain systems, it is suggested that each tRNA contains anti-determinants against some noncognate synthetases; this provides necessary specificity *in vivo*.

RELATIVE CONTRIBUTION OF RECOGNITION ELEMENTS TO KINETIC PARAMETERS OF AMINOACYLATION

Studies *in vitro* evaluate the effect of mutations by the loss of kinetic specificity (L) as the ratio k_{cat}/K_m for the wild-type tRNA to corresponding value for the mutant. The contribution of individual elements to recognition differs for tRNAs of various specificity: L values may vary from <10 to >1000. Differences in recognition determinant strength are observed for the same tRNA from various organisms. For example, the loss of specificity brought by mutations of anticodon and the discriminator base in $tRNA^{Asp}$ does not exceed 10^3 in eukaryotes and does exceed 10^3 in prokaryotes (see for review [15]). On the contrary, the contribution of the discriminator base and nucleotides at the 20th position to specificity of $tRNA^{Phe}$ is higher in eukaryotes than in prokaryotes (see for review [99]). When the number of recognition elements is small, their contribution to specificity is maximal. The main recognition elements of *E. coli* $tRNA^{Ala}$ (pair G3-U70) and *E. coli* $tRNA^{His}$ (C73 and $G^{-}1$) represent a bright example; their substitutions produced $L > 10^3$ [75, 85]. However, some systems with a limited network of recognition elements are characterized by relatively low individ-

Table 3. tRNA recognition elements for aminoacyl-tRNA synthetases class I (A) and II (B)

A	Organism[a]	Acceptor arm[b]	Anticodon arm[b]	Other regions of tRNA[b]
Arg	*E.c.* [15, 25]	**A/G73**[c]	**C35**, G/U36[c]	**A20**
	T.t. [26]		C35	A20
	S.c. [15, 27]		**C35, G/U36**[c]	
	mammalian [28]		C35	A20
Val	*E.c.* [15, 29]	A73, G3-C70[d], U4-A69[d]	**A35**, *C36*	*G20*[d], *G45*[d]
	T.t. [30]		A35[e], C36[e]	G18, G19-C56
	S.c. [15]	A73	A35	
Ile	*E.c.* [15]	A73, C4-G69	L/**G34**[c], **A35**, **U36**, t^6A37, A38, C29-G41	U12-A23
	S.c. [15]		I/Ψ34[c], A35, U36	
Leu	*E.c.* [15, 31-34]	**A73**		A20a, U8-A14, G47j, **A15-U48**, G18-U55, G19-C56, U54-A58
	S.c. [15, 34]	A73	A35, G37	
	H.s. [15, 34]	A73, C3-G70, A4-U69, G5-C68		G18, G19, C20a, V-arm[f,g,h]
	H.v. [35]	A73		A20b, A47c, G47d, U47h, V-arm[f]
Met (fMet)	*E.c.* [15]	A73, (G2-C71, C3-G70)[i], *U4-A69*, *A5-U68*	C34, A35, U36 (C32, U33, A37)[i]	
	S.c. [15]	A73	C32, U33, C34, A35, U36, A37, A38	D-arm[j]
	H.v. [36]	n.i.[k]	*C34, A35, U36*	n.i.[k]
Cys	*E.c.* [15, 37-40]	**U73**, *G2-C71*, *C3-G70*	**G34,** *C35, A36*	G15-G48, A13-A22-A46, U21, Δ47[l]
	S.c. [15, 37]	U73	G34, C35, A36	
	H.i. [41]	U73	G34, C35, A36	G15-G48, A13-A22, A9, A46
	H.s. [37]	U73	G34, C35, A36	A13-A22
	H. NRC-1 [42]	U73	G34, C35, A36	
Glu	*E.c.* [15, 43]	G1-C72, U2-A71	**mnm^{5}s^{2}U34, U35**, A37	U11-A24, U13-G22-A46, Δ47[l]
Gln	*E.c.* [15, 44-46]	**G73, U1-A72, G2-C71, G3-C70,** *G4-C69, G5-C68*	U/C34[c], **U35**, G36, A37, *U32*-**U38**	**C9, G10**-C25, *C11-G24*, **A13-A22**, **G15-C48**
Tyr	*E.c.* [15, 47, 48]	**A73**, G1-C72[d]	**G34[d], U35**	V-arm[f,g]
	S.c. [15, 48-50]	**A73, C1-G72**	**G34, Ψ35**, A36	
	H.s. [51]	C1-G72	galQ34	n.i.[k]
	M.j. [48, 50, 52]	A73, C1-G72	G34, U35[d], A36[d]	
	A.p. [47]	A73, C1-G72	G34, U35	
Trp	*E.c.* [15]	**G73, A1-U72,** *G2-C71, C3-G70*	**C34, C35, A36**	
	B.s. [15, 53, 54]	**G73, A1-U72**[d], G2-C71, C3-G70, G4-C69, **G5-C68**[d]	**C34, C35, A36**	**A9**[d]
	S.c. [15]	A73	**C34, C35**	
	A.t. [55]	**A73**	**C34, C35, A36**	
	H.s., mammalian [54]	A73, G1-C72	n.i.[k]	n.i.[k]
	A.f. [54]	A73	n.i.[k]	n.i.[k]
Lys I	*B.b.* [56-59]	A73, G2-U71, G3-C70	**U35, U36**	
	M.m. [56, 58, 59]	A73	U36	

[a] The following abbreviations of organisms have been used: bacteria *B. b. – Borrelia burgdorferi*, *B. s. – Bacillus subtilis*, *E. c. – Escherichia coli*, *H. i. – Haemophilus influenzae*, *T. t. – Thermus thermophilus*; archaebacteria *A. f. – Archaeoglobus fulgidus*, *A. p. – Aeropyrum pernix*, *H. NRC-1 – Halobacterium NRC-1*, *H. v. – Haloferax volcanii*, *M. b. – Methanosarcina barkeri*, *M. j. – Methanococcus jannaschii*, *M. m. – Methanococcus maripaludis*; eukaryotes *A. t. – Arabidopsis thaliana*, *B. m. – Bombyx mori*, *S. c. – Saccharomyces cerevisiae*, *H. s. – Homo sapiens.*

Table 3 (Contd.)

B	Organism[a]	Acceptor arm[b]	Anticodon arm[b]	Other regions of tRNA[b]
Ser	*E.c.* [15, 60, 61]	*G73*, **G1-C72, G2-C71, A3-U70**		V-arm[f,g], *C11-G24*, D-loop[m]
	S.c. [15, 60, 62]	acceptor stem[j]		V-arm[f,h]
	H.s., mammalian [15, 63]	G73		V-arm[f,h]
	M.b. 1[n] [64]	G73	G30-C40	V-arm[f]
	M.b. 2[n] [64]	G73, G1-C72	G30-C40	V-arm[f,g], G46
Thr	*E.c.* [15]	G1-C72, C2-G71	G35, U36	
	T.t. [15]	U73, G1-C72, U3-A70	G35, U36	
	S.c. [15]	G1-C72	G35, U36	
	A.p. [65]	G1-C72, C2-G71, C3-G70	G35, U36	
	H.v. [66]	U73, G1-C72, C2-G71	G35, U36	
Pro	*E.c.* [15, 67, 68]	A73, G72	G35, G36	G17a, G15-C48, G49, D-stem[j]
	H.s. [15, 67]	C73[d]	G35, G36	
	M.j. [69]	A73, C72	G35, G36	
	A.p. [70]	A73, G1-C72	G35, G36	
Gly	*E.c.* [15, 71]	**U73, G1-C72, C2-G71, G3-C70**	**C35**, C36	
	T.t. [71, 72]	G1-C72, C2-G71, G3-C70	C35, C36	G10, U16, C50-G64
	S.c. [71]	A73, C2-G71, G3-C70	C35, C36	
	mammalian [15]	A73, C2-G71		
His	*E.c.* [15, 73-75]	**C73**, (5'p)G^{-1}	G34[d], U35[d], G36[d]	
	S.c. [15, 76]	A73, (5'p)G^{-1}	G34, U35	
Asp	*E.c.* [15, 77-79]	G73, G1-C72, G2-C71	G34, U35, C36, C38	G10
	T.t. [15, 78]	G73	G34, U35, C36, C38	G10
	S.c. [15, 78, 80]	G73	G34, U35, C36, C38	G10-U25
Lys	*E.c.* [15, 81]	**A73**	mnm^{5}s^{2}U34, **U35**, U36, **A31-U39**	
	H.s. [81-83]		U35, U36	U8-A14, G15-C48
Asn	*E.c.* [15]	*G73*	*G34, U35, U36*	n.i.[k]
Phe	*E.c.* [15]	**A73**	G34, A35, A36, *G27-C43*, *G28-C42*	**U20**, A26-**G44, U45, U59**, *U60*
	T.t. [15, 84]	A73[d]	G34, A35, A36	U20, G19-C56, A26-G44, U45-G10-C25
	S.c. [15]	A73	G34, A35, A36	G20
	H.s. [15]	A73	G34, A35, A36, G30-C40, A31-U39	G20
Ala	*E.c.* [15, 85-91]	A73, G1-C72, **G2-C71, G3-U70, G4-C69**		G20
	S.c., B.m., *H.s.*, mammalian, *A.t.* [15]	G3-U70		

[b] Recognition elements (borrowed from review [15] and supplemented by additional data) are listed by their position in the acceptor arm, anticodon hairpin, and in other regions of tRNA. Elements identified *in vitro*, *in vivo*, and both *in vivo* and *in vitro* are written in plain type, italic, and bold, respectively. Modified nucleotides representing specificity elements are underlined. Generally accepted numeration of nucleotides and designation of modified nucleotides are used [92, 93]; purine and pyrimidine are designated as R and Y, respectively.

[c] At this position two bases may be the specificity elements.

[d] Weak determinant.

[e] Data obtained by XSA.

[f] The length of V-arm is crucial for specificity.

[g] Number of unpaired nucleotides at 5′- or/and 3′-end of the V-arm is critical for recognition.

[h] Nucleotide sequence of the V-arm is critical for specificity.

[i] Additional elements required for recognition of initiator tRNA^{fMet} are shown in brackets. Pairs U4-A69, A5-U68 are the recognition elements of tRNA^{Met}.

[j] Positions of determinants have not been identified.

[k] n.i., region has not been investigated.

[l] Lack of nucleotide 47 (Δ47), influencing stability of the tertiary triplet U13-G22-A46 is considered as the specificity determinant.

[m] Number of additional nucleotides at the 20th position is critical for discrimination of tRNA^{Leu} and tRNA^{Tyr}.

[n] Genome of *M. barkeri* contains two genes encoding SerRS: bacterial type (1) and methane producing archaebacteria type (2).

Table 4. Examples of the recognition anti-determinants in homologous and heterologous systems

Anti-determinant	tRNA (organism)/class	AaRS[a]/class
G3-U70 [15]	$tRNA^{Ala}$ (yeast)/II	ThrRS/II
C6-G67 [100]	$tRNA_2^{Arg}$ (yeast)/I	AspRS/II[b]
U30-G40 [15]	$tRNA^{Ile}$ (yeast)/I	GlnRS/I[c], LysRS/II[c]
U34 [15]	$tRNA^{Ile}$ (yeast)/I	MetRS/I
L34 [15]	$tRNA^{Ile}$ (*E. coli*)/I	MetRS/I
A36 [15]	$tRNA^{Arg}$ (*E. coli*)/I	TrpRS/I
G37 [15]	$tRNA^{Ser}$ (yeast)/II	LeuRS/I[b]
m^1G37 [15]	$tRNA^{Asp}$ (yeast)/II	ArgRS/I[b]
U28-A42 and A37 [101]	$tRNA^{Trp}$ (yeast)/I[d]	TrpRS/I (mammalian)
A73 [15]	$tRNA^{Leu}$ (human)/I	SerRS/II[b]
G2-U71 [56]	$tRNA^{Lys}$ (*B. burgdorferi*)/I	LysRS/II (*E. coli*)
G37 [37]	$tRNA^{Cys}$ (human)/I	CysRS/I (*E. coli*)

[a] Enzyme involved into wrong aminoacylation is blocked by anti-determinant; the name of the organism for heterologous system is given in parenthesis.
[b] Anti-determinants against synthetases of the other class.
[c] Data obtained for amber suppressor yeast $tRNA^{Ile}$ expressed in *E. coli*.
[d] Unmodified $tRNA^{Trp}$.

ual contribution to total specificity. For example, in the yeast Asp- and Phe-specific systems the effects of individual substitutions do not exceed 10^3 [15, 99]. Results of some studies [102-106] have demonstrated that effects of mutations depend on experimental conditions of aminoacylation (concentrations of magnesium ions or low-molecular-weight substrates). This fact should be taken into consideration during comparison of results obtained for various systems, because many publications do not contain information about optimal conditions used.

Substitution of recognition elements may influence catalytic efficiency in three ways: due to preferential changes in k_{cat}, K_m or a mixed effect of k_{cat} and K_m. Relative contribution of k_{cat} and K_m to kinetic specificity differs for various elements. The most pronounced effects (mainly due to changes in k_{cat} and influences on the catalysis of reaction) are typical for the direct recognition elements, forming base-specific contacts with the enzyme. Mutations of the elements responsible for formation of correctly folded structure of tRNA often cause changes in K_m values. For example, substitution of the pair G10-U25 in $tRNA^{Asp}$ for A10-U25 was accompanied by K_m-dependent decrease in the efficiency of aminoacylation [15]. This element of tertiary structure of tRNA did not form contacts with AspRS [107]; its indirect involvement in recognition may be attributed to formation of local conformation and mutual orientation of D-stem and the anticodon loop (due to interaction of U25 with G37 typical for the complex) required for interactions of these regions with the enzyme.

tRNA recognition is not a process of separate interaction of certain nucleotide residues with the enzyme. Since the recognition elements are most frequently localized in three domains of tRNA (anticodon arm, acceptor arm, and central part), a reasonable question arises, how do they contribute to specificity of tRNA–aaRS interaction? Study of aminoacylation of double mutants of $tRNA^{Phe}$ by yeast PheRS has demonstrated independent functioning of three specificity elements (G34, A73, and G20) [108]: 2-3-fold differences between experimental and calculated values of k_{cat}/K_m for double mutants (G20A, A73U or G34A, A73U) were significantly lower than the effects of individual mutations. Functional interdependence of specificity determinants of yeast $tRNA^{Asp}$ (G73, G34, U35, C36, and G10-U25) was investigated using transcripts carrying two and more substitutions [109]; multiple mutations influenced efficiency of aminoacylation mainly at the k_{cat} level. Significant differences in experimental and calculated k_{cat}/K_m values determined for double mutants (in anticodon and acceptor arm, in D- and acceptor arms, and in D- and anticodon arms) revealed that two nucleotides located far apart in the three-dimensional structure of $tRNA^{Asp}$ act in cooperative manner, whereas nucleotides of the anticodon triplet exhibit additive or anti-cooperative effect. Similar properties have also been found for the recognition elements of *T. thermophilus* $tRNA^{Asp}$ [110]. The existence of cooperative effects suggests an important role of conformational changes of macromolecules in formation of functionally active $tRNA^{Asp}$·AspRS complex.

NATURE OF CONTACTS DETERMINING SPECIFICITY OF INTERACTION

Crystal structures of 18 complexes of aaRSs with corresponding homologous tRNAs and five complexes with heterologous tRNAs are known to date (Table 5). For many complexes (yeast AspRS, ArgRS, PheRS, ValRS, TyrRS, and SerRS [15, 146-148], *E. coli* IleRS, SerRS, and ThrRS [15, 149], *E. coli* and human CysRS [150], and also *T. thermophilus* PheRS [151]) data on nuclease and chemical mapping (foot-printing) have been obtained. Using s^4U-induced affinity labeling, the close contacts between $tRNA^{Phe}$ and *T. thermophilus* PheRS have been localized [152]. Results of these studies are ultimately important for understanding the structural basis for tRNA recognition by synthetases, because they describe the detailed nature of contacts and conformational changes

Table 5. Existence of structural data for complexes of aminoacyl-tRNA synthetases with tRNAs

Enzyme[a]	Organism[b]	Complexes with tRNA[c,d]
Subclass Ia		
ArgRS, α	*S.c.*	$tRNA^{Arg}_{ICG}$ (–/+Arg) [111]
ValRS, α	*T.t.*	$tRNA^{Val}_{CAC}$ (+Val-AMS) [30, 112]
IleRS, α	*S.a.*	$tRNA^{Ile}_{CAU}$[e] *E.c.* (+mupirocin) [113]
LeuRS, α	*T.t.*	$tRNA^{Leu}_{CAG}$[e] [114]
	P.h.	$tRNA^{Leu}_{CAA}$[e] [115]
MetRS, α_2	*A.a.*	$тPHK^{Met}_{CAU}$[e] [116]
CysRS, α	*E.c.*	$tRNA^{Cys}_{GCA}$ [117]
Subclass Ib		
GlnRS, α	*E.c.*	$tRNA^{Gln}_{CUG}$ (+ATP, Gln-AMS, AMPcPP+Glu) [118-123]
GluRS, α	*T.t.*	$tRNA^{Glu}_{CUC}$[e] (–/+GluOH-AMP) [124, 125]
Subclass Ic		
TyrRS, α_2	*T.t.*	$tRNA^{Tyr}_{G\Psi A}$ (+TyrOH+ATP) [126]
	M.j.	$tRNA^{Tyr}_{G\Psi A}$ (+Tyr) [52]
TrpRS, α_2	*H.s.*	$tRNA^{Trp}_{CCA}$[e] mammalian (–/+Trp) [127, 128]
Subclass IIa		
ProRS, α_2	*T.t.*	$tRNA^{Pro}_{CGG}$ (–/+Pro-AMS, Pro-AMP, ProOH+ATP) [129-131]
ThrRS, α_2	*E.c.*	$tRNA^{Thr}_{CGU}$ (+AMP) [132]
SerRS, α_2	*T.t.*	$tRNA^{Ser}_{GGA}$ (–/+Ser-AMS) [133, 134]
Subclass IIb		
AspRS, α_2	*S.c.*	$tRNA^{Asp}_{GUC}$ (–/+ATP, AMPPcP) [135-138]
	T.t.	$tRNA^{Asp}_{GUC}$ [68]; $tRNA^{Asp}_{QUC}$ *E.c.* [139]
	E.c.	$tRNA^{Asp}_{QUC}$ (+Asp-AMP) [140, 141]; $tRNA^{Asp}_{GUC}$ *S.c.* (+Asp-AMP) [142]
LysRS2, α_2	*T.t.*	$tRNA^{Lys}_{CUU}$[e], $tRNA^{Lys}_{U*UU}$ *E.c.* (–/+Lys-AMS) [143]
Subclass IIc		
PheRS, $(\alpha\beta)_2$	*T.t.*	$tRNA^{Phe}_{GAA}$ (–/+PheOH-AMP) [144, 145]

[a] Subunit composition of enzymes exhibiting given specificity is shown.

[b] List of abbreviations: *A. a.*, *Aquifex aeolicus*; *E. c.*, *Escherichia coli*; *H. s.*, *Homo sapiens*; *M. j.*, *Methanococcus jannaschii*; *P. h.*, *Pyrococcus horikoshii*; *S. a.*, *Staphylococcus aureus*; *S. c.*, *Saccharomyces cerevisiae*; *T. t.*, *Thermus thermophilus*.

[c] Structure of complex with tRNA in the absence (–) or in the presence (+) of various sets of ligands. Source of tRNA is indicated for heterologous complexes only; in all other cases, homologous complexes of tRNA and the enzyme are indicated. Anticodon sequences are shown: I, inosine; Ψ, pseudouridine; U*, 5-methylaminomethyluridine; Q, queuosine.

[d] Substrate analogs: TyrOH, ProOH) tyrosinol, prolinol; AMPPcP, AMPcPP) ATP analogs—β-γ- or α-β-methylene ATP; Val-AMS, Gln-AMS, Pro-AMS, Ser-AMS, and Lys-AMS) aminoacyl adenylate analogs containing sulfamoyl group instead of phosphate group; GluOH-AMP, PheOH-AMP) aminoalkyl adenylates obtained by condensation of amino alcohols with AMP.

[e] tRNA synthesized by transcription *in vitro*.

of the interacting macromolecules. Figure 2 shows a comparison of 3D structures of aaRSs from both classes, GlnRS and AspRS. Class I enzymes (with the exception of subclass Ic) form contacts with the minor groove of the acceptor stem, whereas class II aaRSs, TyrRS and TrpRS, form contacts with the major groove. This results in exposure of the variable loop into solution (class I) or its orientation towards protein (class II). Different regions of tRNA interact with certain structural domains of the enzyme. The total number of domains, modes of their folds, and involvement in binding and recognition of tRNA are rather specific and vary in each system. Most nucleotide residues of the anticodon loop (especially in the Gln system) involved in interaction with the enzyme are recognition elements. The acceptor arm of $tRNA^{Gln}$ forms a more intensive network of contacts with GlnRS compared with corresponding region of $tRNA^{Asp}$ in its complex with AspRS; this correlates with the existence of a larger number of the recognition elements in it.

Interaction between tRNA and aaRS is accompanied by conformational changes in both macromolecules. Changes in tertiary structure of the enzyme varies in different systems and includes ordering of some domains that contact with tRNA (the anticodon binding domain of TyrRS [126] and CysRS [117], the N-terminal domain of SerRS [133], and the N-terminal domain of small subunit of PheRS [144]), their conformational shifts with respect to each other and the catalytic domain. The most pronounced structural changes in tRNA are observed in the anticodon loop: in most complexes, it undergoes deformation followed by disruption of stacking of some bases. Only in $tRNA^{Cys}$, $tRNA^{Glu}$, and $tRNA^{Phe}$ the anticodon loop maintains almost the same U-shaped conformation as in free $tRNA^{Phe}$. The loop conformation is stabilized by its contacts with the protein and new intramolecular RNA interactions. The general structure of $tRNA^{Asp}$ in the complex with enzyme becomes more compact (than in free tRNA molecule) due to a decrease in the hinge angle between the two arms of the L-shaped tRNA [137]. The CCA-end of $tRNA^{Gln}$ forms a U-shaped conformation and the first base pair of the acceptor stem is disrupted; in $tRNA^{Asp}$, the 3′-end sequence is in helical continuity with the acceptor stem (Fig. 2). Bend in the acceptor arm at N73 typical for many complexes of class I aaRSs is required for correct orientation of the acceptor end at the active site. Disruption of the first base pair is not universal for this class of enzymes: according to XSA [112] and ^{19}F-NMR [153], the base-pair G1-C72 is preserved in the complex of ValRS with $tRNA^{Val}$.

Crystallographic studies have demonstrated that direct recognition of the major specificity determinants in anticodon is a common property of aaRSs [30, 52, 111-113, 116, 117, 119, 124, 126, 129, 132, 135, 139, 140, 143, 144]. Hydrogen bonds are often used for such interactions. It has been revealed that in the Asp-specific system the decrease in aminoacylation efficiency caused by mutations was proportional to the loss of the number of hydrogen bonds [109]. Mechanisms responsible for recognition of the discriminator base are dissimilar in different systems. For example, base G73 in $tRNA^{Asp}$ (yeast and *E. coli*) directly contacts homologous AspRS [135, 140]. However, in *E. coli* $tRNA^{Gln}$ G73 stabilizes the functional conformation of the acceptor end by forming nonspecific intermolecular (with GlnRS) and intramolecular (with A72 and C75) interactions [118]. General architecture of tRNAs and structural features of their central regions are also recognized by enzymes in different manners. In crystal complexes some specificity determinants of the central regions of bacterial tRNAs specific for Arg, Ile, and Val form contacts that involve bases [30, 111, 113]; in similar regions of $tRNA^{Cys}$ and $tRNA^{Phe}$ only nonspecific interactions of ribose-phosphate backbone with protein have been found [117, 144]. A nucleotide located at position 20 of $tRNA^{Val}$ varies in isoacceptor tRNAs and in different organisms; although it is a weak specificity determinant [29] in the crystal complex, it is involved in specific interactions with ValRS [30]. A long V-loop of bacterial $tRNA^{Tyr}$ forms a few contacts that involve bases, whereas in $tRNA^{Ser}$ it forms a wide network of specific and nonspecific interactions in corresponding complexes [126, 133]. In the anticodon recognizing systems, the number of specific contacts with anticodon nucleotides is significantly higher than with tertiary structure elements; this is consistent with their relative contribution to aminoacylation efficiency. In these systems the functional role of structural elements consists of formation of general configuration of tRNAs (including configuration formed during conformational adaptation of the substrate) required for optimal binding of tRNA and structural interrelationship between direct recognition elements with each other and the acceptor end. The identity element, G10, crucial for $tRNA^{Asp}$ aminoacylation is free of contacts with yeast AspRS [135] or it interacts with a single amino acid residue of *E. coli* AspRS [140]. This suggests its primary importance for formation of optimal structure of the tRNA–substrate due to intramolecular interactions.

Preferential use of indirect mechanisms for recognition of elements responsible for formation of 3D structure and configuration of its regions is typical for some systems in which the anticodon does not determine their specificity. For example, base pairs A15-U48, A20a, G18-U55, and G19-C56 play the key role in the recognition of $tRNA^{Leu}$ by LeuRS [31-34], but only for the universal tertiary pair G19-C56 extensive interactions with LeuRS have been found [114]. Contradictory results have been obtained for the *E. coli* Ala-specific system: some results indicate direct recognition of the major specificity determinant, base pair G3-U70, whereas others suggest its structural role in the formation of a unique conformation of the acceptor stem [15, 85-91]. Final conclusions on the mechanisms of discrimination of $tRNA^{Ala}$ require crystallographic study of $tRNA^{Ala}$–AlaRS complex(s).

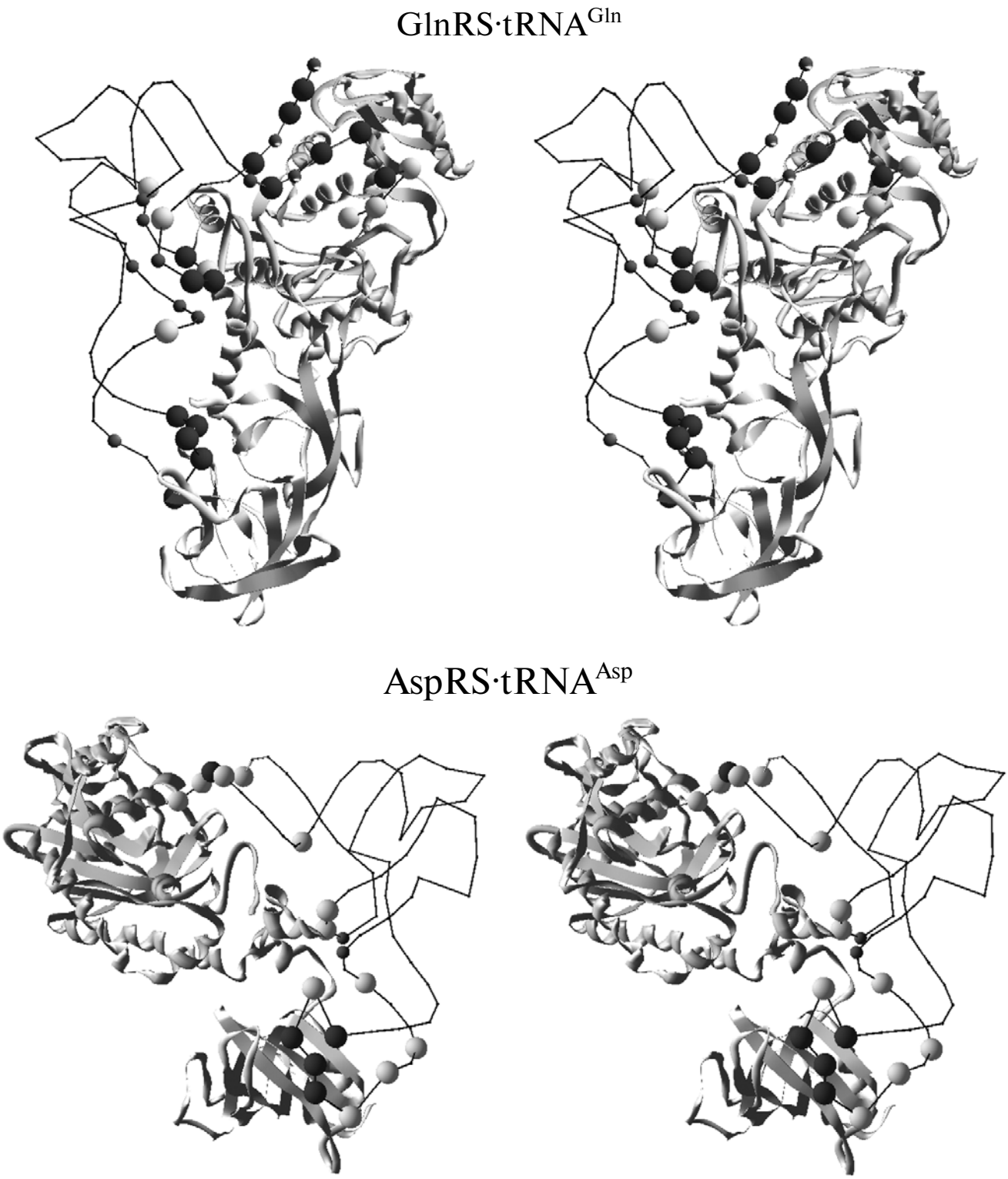

Fig. 2. Comparison of tRNA binding by class I (GlnRS *E. coli*) and class II (yeast AspRS) aminoacyl-tRNA synthetases. Nucleotides forming contacts with aaRS (involving bases 2, 3, 10, 16, 34-38, 71, 74, and 76 of $tRNA^{Gln}$ or 1, 32-36, 38, and 72-76 of $tRNA^{Asp}$ or ribose-phosphate backbone at positions 5, 8, 11-13, 27, 69, 72, 73, and 75 of $tRNA^{Gln}$ or 11, 12, 27, 30, 37, and 67 of $tRNA^{Asp}$) are shown as large spheres; the recognition elements are shown in dark color; small spheres show specificity elements which do not form contacts with the protein. Structures have been borrowed from Protein Data Bank (codes 1gts and 1asz for complexes of GlnRS and AspRS, respectively). The stereoscopic image has been generated using the program DeepView.

FUNCTIONAL ROLE OF RECOGNITION ELEMENTS IN SPECIFICITY AT VARIOUS STAGES OF INTERACTION

Aminoacylation of tRNA is the multistage process that includes initial binding of substrates, conformational rearrangement of the enzyme–substrate complex, chemical reaction at the active site, and release of products. Results of numerous studies employing genetic methods *in vivo* and kinetic experiments *in vitro* with mutant tRNAs elucidate the molecular basis underlying highly specific interactions of tRNAs with synthetases. However, problems related to the functional role of recognition elements in specific tRNA–aaRS interaction still require additional investigation using independent methods. Effects of mutations of suppressor tRNAs on protein biosynthesis *in vivo* may be attributed to changes in their interaction not only with aaRSs, but also with translation factors. Experimental evidence shows that the factor EF-Tu binds with equal efficiency correct aminoacyl-tRNAs, whereas its affinity for wrong aminoacyl-tRNAs significantly varies depending on the nature of the amino acid and tRNA [154]. Complex nature of the kinetic parameters of aminoacylation cannot discriminate contribution of recognition elements into specificity at various stages of tRNA–aaRS interaction. Results of *in vitro* studies suggest that specificity of interactions is mainly determined by kinetic factors (due to higher values of k_{cat} for specific tRNA) rather than thermodynamic parameters (because of differences in K_m values) [155, 156]. This simplifies the real picture of the multistage control of specificity in various systems.

In early studies on tRNA recognition by aaRS using the method of fast kinetics, Krauss et al. proposed a dynamic model of the two-step process of complex formation [157]. The first bimolecular step (controlled by diffusion) is considered as recombination due to long-range electrostatic interactions between protein molecule and sugar-phosphate backbone of tRNA. The second monomolecular stage consists of conformational changes of the tRNA–aaRS complex providing induced fit of enzyme and substrate. Usually the increase in ionic strength destabilizes specific and nonspecific complexes in solution; this suggests involvement of ionic interactions in the complex formation [158]. XSA data obtained for various tRNA–aaRS pairs confirm previously obtained results on the importance of electrostatic contacts for the complex formation. Each aaRS generates a unique configuration of the overall positive potential that determines the mode of binding of corresponding negatively charged tRNA [159, 160]. In most cases, complexes of aaRS with nonspecific homologous or specific heterologous tRNA are less stable than specific complexes (Table 6). Effectiveness of tRNA recognition at the binding step significantly varies in the systems studied and depends (to different extent) on pH, ionic strength, and the presence of Mg^{2+} ions. It is apparently determined by the stability of aaRS complex with cognate tRNA as well as structural differences between specific and nonspecific tRNAs. Hydrogen bonds and van der Waals and hydrophobic interactions play crucial roles in the specificity of this interaction. Positively charged clusters located at terminal regions of eukaryotic aaRSs (extended compared with their prokaryotic analogs) are involved in tRNA binding; this significantly increases complex stability and catalytic efficiency of aminoacylation [169-172]. The existence of such domains decreases effectiveness of discrimination of noncognate tRNAs as it has been demonstrated for yeast AspRS [172]. However, some systems employ electrostatic interactions for tRNA recognition upon binding: negatively charged amino acid residues (or positively charged residues in the case of TrpRS) of aaRS act as anti-determinants with respect to nonspecific or heterologous tRNAs [173-177]. A cluster of arginine residues is involved in recognition of a unique pair $G^{-}1$-C73 in $tRNA^{His}$ [74].

The structural changes of aaRS complex with tRNA registered in studies of PheRS [157] are typical for effec-

Table 6. K_d values for complexes of aminoacyl-tRNA synthetases with specific and nonspecific tRNAs

Enzyme (source)	K_d (specif. homolog.), μM	K_d (heterolog.)/ K_d (homolog.)[a]	K_d (non-specif.)/ K_d (specif.)[a]
ValRS (yeast) [158, 161]	0.01[b]/ 0.001-0.12[c]	3	15-10000
IleRS (*E.c.*) [161]	0.01[b]		5-10000
PheRS (yeast) [157, 162-165]	0.03[b]/1.2[b]/ 1.25[d]/0.04-1.0[b]		30-300
PheRS (*E.c.*) [157, 166]	0.25[e]	4-36	25
PheRS (*T.t.*) [84, 167]	0.0025[b]; 0.005[f]	1-16	100-3000
TyrRS (*E.c.*) [162]	0.05[b]/<1; 4.0[d, g]		n.b.
SerRS (yeast) [162, 168]	0.05[b]/0.67[b]/<1; 7.7[d, g]	93	n.b.

Note: The range of K_d values determined at various pH, ionic strength, and magnesium ions concentrations is shown; n.b., no binding.

[a] Ratio of K_d values for specific heterologous (from another organism) or nonspecific tRNA to corresponding value for specific homologous tRNA. The interval of values for various heterologous or nonspecific tRNAs is shown.

[b-f] K_d value was determined by fluorescent titration, nitrocellulose filter binding assay, ultracentrifugation, laser light scattering, and gel retardation, respectively.

[g] Constants for nonequivalent centers of tRNA binding were measured.

tively interacting pairs: in a homologous system (yeast PheRS and $tRNA^{Phe}$) it occurs 3-4-fold faster than in a heterologous pair (*E. coli* PheRS and yeast $tRNA^{Phe}$); binding of $tRNA^{Tyr}$ which is not a PheRS substrate occurs in one step. Binding of tRNA 3′-terminal nucleotide induces conformational fit [164, 178]. The complex formation in the presence of a stable phenylalanyl adenylate analog also followed a two-step mechanism, but both steps were much slower [178, 179]. Based on these observations, it was concluded that conformational changes of the complex PheRS with tRNA in the absence or in the presence of the adenylate analog reflect distinct processes; however, the relationship between tRNA and low-molecular-weight substrates was not elucidated due to lack of structural data at that time.

Several studies on the role of the recognition elements of tRNA in distinct steps of tRNA–aaRS interaction have shown involvement of various mechanisms used by different enzymes. Interactions of yeast AspRS with $tRNA^{Asp}$ anticodon make a major contribution to stability of the enzyme–substrate complex and provide effective discrimination of tRNA at the binding step: single mutations of amino acid residues interacting with the important determinants at $tRNA^{Asp}$ anticodon (G34 and U35) resulted in the most significant increase in K_d value (up to 35-fold) [180]. Perturbations of contacts responsible for recognition of G73 were induced by substitutions at the conservative motif 2; these substitutions caused 3-7 times less dramatic destabilization effect. Lack of additivity of various mutations of amino acid residues contacting with various bases of anticodon or with anticodon and G73 is mainly reflected by altered rates of aminoacylation (positive cooperativity of the effects of double mutations) rather than stability of the complex (weak positive or negative cooperativity). Results of these studies suggest a functional interrelationship between the major recognition elements and the trigger role of anticodon in structural rearrangement of the complex, which optimize productive binding of the acceptor end at the active site of aaRS. The major role of anticodon in specific selection of tRNA during its binding has been demonstrated when investigating the stability of complexes of *E. coli* HisRS (and its mutant forms carrying substitutions in the anticodon-binding domain) with various tRNAs and $tRNA^{His}$ mutants [96]. Effectiveness of discrimination of nonspecific $tRNA^{Phe}$ increased in the presence of a stable histidyl adenylate analog. The unique pair $G^{-}1$-C73 in the acceptor stem of $tRNA^{His}$ is essential only for catalysis, and it is not recognized at the step of binding.

Interactions of *E. coli* GlnRS with specificity elements of $tRNA^{Glu}$ determine efficacy of glutamine recognition: substitutions of G73, tertiary base pair G10-C25 and three anticodon bases result in 5-20-fold increase in K_m for glutamine and decrease in catalytic efficiency of aminoacylation (up to 640 times) [104]. Involvement of terminal adenosine of $tRNA^{Gln}$ in formation of the binding site for Gln substrate demonstrated in structural studies [122] explains the requirement of the tRNA presence for the amino acid activation. Mutations of amino acid residues forming specific contacts with A76 of $tRNA^{Gln}$ and glutamine caused preferential influence on productive interaction with Gln substrate [181]. Reverse influence of glutamine on $tRNA^{Gln}$ binding by GlnRS was found by fast kinetics methods [182]. In the case of *E. coli* GluRS, a close structural homolog of GlnRS, the mutation in the conservative motif involved in recognition of the acceptor stem caused more than 10-fold decrease in affinity to glutamate and 2-fold decrease in affinity to $tRNA^{Glu}$ [183]. Thus, the functional role of the recognition elements of $tRNA^{Gln}$ and $tRNA^{Glu}$ in correct orientation of the 3′-end is closely related to its direct involvement in modulation of the active site structure required for effective interaction with corresponding amino acids. Conformational changes induced by coordinated interactions between enzyme and its amino acid and tRNA substrates make substantial contribution to specificity.

Optimization of aaRS interaction with amino acid induced by tRNA has been demonstrated not only for tRNA-dependent systems. Structural changes in $tRNA^{Trp}$ influenced the affinity (K_m) of *E. coli* TrpRS for Trp [184]. The catalytic efficiency of serine activation by yeast SerRS increased, whereas activation of the wrong substrate, threonine, decreased in the presence of non-aminoacylated $tRNA^{Ser}$ (with truncated 3′-end) [185]. The effect induced by $tRNA^{Ser}$ depends on mutations of amino acid residues of aaRS motif 2 responsible for recognition of the acceptor arm of the substrate. The nature of tRNA-dependent control in amino acid recognition by SerRS remains unclear: according to structural data obtained for bacterial SerRS, $tRNA^{Ser}$ modulates interaction of the motif 2 loop with ATP [134].

Studies directly demonstrating the importance of tRNA identity for complementary interaction between enzyme and its substrate at the transition state have been carried out using Trp- and Gln-specific systems of *E. coli* [184, 186]. Mutations of minor recognition elements of $tRNA^{Trp}$ (G73, A36, removal of A1) caused a 3-20-fold reduction in the rate constant of Trp transfer onto the tRNA substrate without any influence on the efficiency of $tRNA^{Trp}$ binding to the TrpRS·Trp-AMP complex. The complex of TrpRS with chimeric $tRNA^{Gln}$ (CCA) containing anticodon of $tRNA^{Trp}$ is characterized by 30 times less effective rate of the catalytic reaction and only 4 times less stability than the specific complex. Thus the most effective discrimination of noncognate tRNAs by TrpRS occurs at the catalytic stage. Mutation of the major recognition element of *E. coli* $tRNA^{Gln}$, U35A, was accompanied by 30-fold decrease in the rate constant for glutamine transfer onto the tRNA substrate and 20-fold increase in the K_d value for glutamine.

Inorganic pyrophosphatase selectively influenced aminoacylation of yeast $tRNA^{Phe}$ mutants by homologous PheRS: it caused 12-30-fold increase in catalytic efficien-

cy for structural mutants carrying substitutions G19C/C56G or G20U (which, however, remained significantly lower than the corresponding value obtained for the wild-type $tRNA^{Phe}$ transcript), and just 1.4-2.2 times lower for A73U- and A35U-mutants [187]. The authors suggested that perturbations of tertiary structure of $tRNA^{Phe}$ change the reaction mechanism: dissociation of pyrophosphate becomes the rate-limiting step. These effects reflect events that occur at the active site; they may be attributed to different degree of disorientation of the acceptor end of $tRNA^{Phe}$ induced by mutations.

The function of universal specificity determinant of $tRNA^{Cys}$ (in various organisms), U73, consists of formation of a unique local conformation of the acceptor arm: disruption of staking interactions in the helical structure provides increased conformational mobility of the terminal nucleotide [39]. Lack of strict orientation of terminal ribose with respect to the acceptor stem explains the ability of *E. coli* CysRS to use 2′- and 3′-OH groups for primary addition of cysteine. The particular role of a single nucleotide in breaking the stereo barrier of the aminoacylation reaction has been demonstrated in experiments with *E. coli* ValRS; this enzyme loses positional specificity after A73 substitution for U in $tRNA^{Val}$ [39]. The formation of the acceptor end conformation required for productive interaction is a common function of the discriminator base in different tRNAs; this function is realized by various molecular mechanisms.

The decisive role of anticodon in specific and effective recognition of tRNA by *T. thermophilus* PheRS at the binding step has been demonstrated by independent measurements of kinetic parameters of aminoacylation and K_d values for complexes with various tRNAs [84]. Nucleotides of the tertiary base pair G19-C56 and triplet U45-G10-C25 make major contributions to stabilization of the correctly folded structure of $tRNA^{Phe}$ required for optimal binding; U20 and the tertiary base pair A26-G44 are important for conformational adaptation of the complex. Affinity labeling of PheRS with 3′-s^4U-substituted analogs of $tRNA^{Phe}$ and its mutants has demonstrated the importance of fine adjustment of PheRS complex with substrates at the step preceding the catalytic stage. This involved all recognition elements of $tRNA^{Phe}$, and any mutations caused more pronounced effects on orientation of the acceptor end of $tRNA^{Phe}$ in the presence of Phe and ATP rather than in their absence [188]. Involvement of indirect recognition elements, U20 and the tertiary base pair A26-G44 in conformational changes of the complex induced by PheRS interaction with low-molecular-weight substrates has been confirmed by XSA data [145]. The functional role of Phe and ATP in productive binding of the acceptor end of $tRNA^{Phe}$ is evolutionarily conservative in spite of differences in composition of domains of prokaryotic and eukaryotic PheRSs [189].

In conclusion, we should emphasize that in spite of significant progress in understanding of specificity of tRNA–aaRS interaction, this problem still requires further investigations. Most systems of higher eukaryotes have not been investigated at the functional level; structural data have been obtained for only one system, the complex of human TrpRS with heterologous $tRNA^{Trp}$. Biochemical functions of the recognition elements have been studied for a limited number of systems; they are characterized by diversity and identity. Such studies of systems of various specificity and origin would determine previously unknown mechanisms.

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