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Recognition of $tRNA^{Tyr}$ by tyrosyl-tRNA synthetase

H Bedouelle

Unité de Biochimie Cellulaire (CNRS URA D1129), Institut Pasteur, 28, rue du Docteur Roux, 75724 Paris Cedex 15, France

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Summary – In this review, I have brought together and compared the available data on the interaction between $tRNA^{Tyr}$ and tyrosyl-tRNA synthetases (TyrTS) of prokaryotic origins. The amino acid sequences of the heterologous TyrTS that can charge *Escherichia coli* $tRNA^{Tyr}$, show that the residues involved in the binding and recognition of tyrosine are strictly conserved whereas those involved in the interaction with $tRNA^{Tyr}$ are only weakly similar. The results of *in vivo* genetic complementation experiments indicate that the identity elements of tRNAs and the recognition mechanisms of such elements by the synthetases have been conserved during evolution. Heterologous or mutant $tRNA^{Tyr}$ are quantitatively charged by *E coli* TyrTS; the set of their common residues contains less than 10 elements if one excludes the invariant and semi-invariant residues of tRNAs. The residues of this set are candidates for a specific recognition by TyrTS. So far, adenosine-73 is the only residue for which a specific recognition of the base has been demonstrated. The residues that might serve as identity elements for *E coli* $tRNA^{Tyr}$ [McClain WH, Nicholas Jr HB (1987) *J Mol Biol* 194, 635–642] do not belong to the above set of conserved residues and therefore probably play negative roles, enabling $tRNA^{Tyr}$ to avoid non-cognate synthetases. Comparison of the charging and stability properties of mutant $tRNA^{Tyr}su^{+}3$ shows that bases 1 and 72 must pair (either by Watson-Crick or non-canonical hydrogen bonds) and adopt a geometry which is compatible with the helical structure of the acceptor stem in order for the mutant $tRNA^{Tyr}$ to be charged with tyrosine. If bases 1 and 72 or bases 2 and 71 cannot form such pairings, the suppressor phenotype of the mutant $tRNA^{Tyr}su^{+}3$ becomes thermosensitive. The weakening of base pair 1 / 72 by mutation or the change of adenosine-73 into guanosine results in the charging of $tRNA^{Tyr}su^{+}3$ with glutamine. Comparison of the structural model of the TyrTS / $tRNA^{Tyr}$ complex with the crystallographic structure of the GlnTS / $tRNA^{Gln}$ complex indicates that the mechanisms for the recognition of the acceptor arm are different in the 2 cases. Chemical attack and molecular modeling experiments have indicated that the acceptor end of $tRNA^{Tyr}$ ... CCA3′–OH, remains mobile after the initial binding of $tRNA^{Tyr}$ to TyrTS.

structural model / $tRNA^{Tyr}$ identity / glutaminyl-tRNA synthetase / *Bacillus stearothermophilus* / *Escherichia coli*

Introduction

The aminoacylation of transfer RNAs (tRNAs) by their cognate aminoacyl-tRNA synthetases (aaTS) is a key step in translation since it relates the amino acids to the anticodons. Each aaTS recognizes the tRNAs that correspond to the same amino acid, to the exclusion of other tRNAs. All the tRNAs interact with the same cellular components in the subsequent steps of translation and they have common structural elements. The synthetases must therefore discriminate between molecules whose general structures are identical. In some cases, discrete structure or sequence elements are sufficient to confer its identity to the tRNA. In other cases, the situation might be more complex [1–5].

The aaTS / tRNA interaction has been described in detail for 2 systems. The crystal structure of the *Escherichia coli* GlnTS / $tRNA^{Gln}$ complex has been solved at 2.8 Å resolution [6]. A structural model of the complex between the tyrosyl-tRNA synthetase (TyrTS) from *Bacillus stearothermophilus* and the acceptor arm of $tRNA^{Tyr}$ has been constructed on the basis of the crystal structures of free *B stearothermophilus* TyrTS and yeast $tRNA^{Phe}$ and of extensive genetic analyses of both partners of the complex, TyrTS and $tRNA^{Tyr}$ [7, 8]. Good crystals of the yeast AspTS / $tRNA^{Asp}$ complex have been obtained and the determination of its structure is in progress [9]. Finally a detailed genetic and biochemical analysis of the MetTS / $tRNA^{Met}$ interaction has been undertaken and the crystal structures of both partners are known [10, 11].

In this paper, we briefly describe our structural model of the TyrTS / $tRNA^{Tyr}$ complex. We then review the data on the recognition and interaction between these 2 macromolecules that are available. Finally, we compare the GlnTS / $tRNA^{Gln}$ and TyrTS / $tRNA^{Tyr}$ complexes.

The partners of the complex

The crystal structure of the tyrosyl-tRNA synthetase from *B stearothermophilus* has been determined, at 2.3 Å resolution, as well as those of its complexes with tyrosine, tyrosyl adenylate (Tyr-AMP) and the inhibitor tyrosinyl adenylate [12–14]. TyrTS is a dimer whose subunits are symmetrical by rotation through a 2-fold axis. Each monomer consists of 3 domains: an α/β domain (residues 1 to 220) containing a six-stranded β-sheet, an α-helical domain (248-318) containing

5 helices and a C-terminal domain (319-419) for which it has not been possible to trace the polypeptide chain. The electron density maps show that the C-terminal domain is adjacent to the α-helical domain (248-318). It extends for approximately 34 Å above this domain and has a diameter of 20 Å (fig 1).

The crystallographic structure of a tRNA specific for tyrosine has not yet been determined although *E coli* $tRNA^{Tyr}$ has been crystallized [15]. The solution structure of *E coli* $tRNA^{Tyr}$ has been probed by determining the chemical reactivity of its cytosines towards methoxyamine [16], and of its uridines and guanosines towards carbodiimide [17], by the binding of complementary oligonucleotides [18] and by mutagenesis [19]. The results of these studies are compatible with a structure which is similar to that of yeast $tRNA^{Phe}$ [20–22].

The prokaryotic and mitochondrial $tRNA^{Tyr}$ have a large extra-loop. The crystal structure of a tRNA with a large extra-loop has not yet been solved. However, the general structure of such a tRNA has been discussed [23] and the solution structure of yeast $tRNA^{Ser}$ has been characterized by chemical modification experiments and by model building [24, 25]. A model of $tRNA^{Tyr}$ from *B stearothermophilus* has been described [26]. These studies have indicated that the structures of the tRNAs with a large extra-loop do not differ greatly from those of the other tRNAs and that the extra-loop is positioned roughly in the plane of the L-shaped structure, on its outer face, in slight protuberance on the face of the tRNA that carries phosphates 65 to 67.

Other studies have indicated that the structures of the tRNAs in solution do not differ greatly from their crystal structures or modify upon association with their cognate aaTS. We have therefore used the crystal structure of yeast $tRNA^{Phe}$ to construct a model of interaction between TyrTS and $tRNA^{Tyr}$. The 3′-terminal residues ...CACCA3′–OH are identical in *E coli* or *B stearothermophilus* $tRNA^{Tyr}$ and in yeast $tRNA^{Phe}$ [27].

Surface scanning of TyrTS

To identify residues of the *B stearothermophilus* TyrTS that interact with $tRNA^{Tyr}$, we simplified the problem as follows. In a first step, we only considered those aminoacyl residues that are basic and conserved in the sequences of the TyrTS from *E coli* and *B stearothermophilus*. The 2 sequences have 56% identity [28]. We individually changed 40 basic residues of TyrTS by oligonucleotide site-directed mutagenesis [29] and found that 13 of them affect its activity in a genetic complementation assay. We performed kinetic analyses of the purified, mutant proteins and found that 4 residues, Lys82, Arg86, Lys230 and Lys233, are involved in the formation of Tyr-AMP [7]. These 4 residues belong to 2 loops in the TyrTS structure that move during the formation of Tyr-AMP [30] and they are also involved in the transfer of tyrosine from Tyr-AMP to $tRNA^{Tyr}$ (Fersht and Knill-Jones, personal communication).

Nine basic residues are only involved in the interaction between TyrTS and $tRNA^{Tyr}$ and not in the formation of Tyr-AMP. Six of these residues are located in the C-terminal domain of TyrTS (Arg368, 371, 407, 408 and Lys410, 411) and 3 others in the α/β domain (Arg207, Lys208 and Lys151). Among these 9 mutations, the change of Lys151 to Asn is peculiar because it only affects the k_{cat} parameter and not the K_M for $tRNA^{Tyr}$ in the aminoacylation reaction [7]. A truncated TyrTS (residues 1-317), lacking the C-terminal domain, has been constructed at the genetic level. It forms Tyr-AMP with kinetic parameters, k_{cat} and K_M, identical to those of the wild type enzyme but does not bind and does not charge $tRNA^{Tyr}$ [31]. The 6 point mutations and the deletion of the C-terminal domain have properties that demonstrate the essential role of this domain in the interaction between TyrTS and $tRNA^{Tyr}$.

$tRNA^{Tyr}$ straddles both subunits of TyrTS

We constructed heterodimers between the truncated TyrTS and the 13 mutant enzymes listed above and tested their activities in the aminoacylation reaction. We also constructed and characterized heterodimers between truncated and full length TyrTS, with mutation His45 to Asn, which prevents the formation of Tyr-AMP, in one of the 2 subunits. These experiments have shown that each tRNA binding site straddles both subunits of TyrTS and that, for each of the 13 mutations tested, only 1 of the 2 symmetrical copies of the wild type residue belongs to the binding site [7, 32]. They have also suggested an asymmetry of TyrTS in solution (table II in [7]), as was subsequently demonstrated [33, 34].

Structural model of the TyrTS / $tRNA^{Tyr}$ complex

We used the results of our surface scanning and heterodimer experiments and the crystal structures of *B stearothermophilus* TyrTS [12] and yeast $tRNA^{Phe}$ [20, 21], to construct a first structural model of the complex between the synthetase and $tRNA^{Tyr}$. In this model, the acceptor arm of $tRNA^{Tyr}$ interacts with Tyr-AMP, Lys151, Arg207 and Lys208 in the α/β domain of one TyrTS subunit and the anticodon arm interacts with the C-terminal domain of the other subunit (fig 1).

By examining this preliminary model, we identified potential contacts between the acceptor arm of $tRNA^{Tyr}$ and the α/β domain of TyrTS. We mutated the corresponding aminoacyl residues of TyrTS and found 2 strong additional contacts, involving Trp196 and

Asn146, and 2 weak ones, involving Gln15 and Thr17 [8]. We then used our genetic data [7, 8], the published data on $tRNA^{Tyr}$ (see below) and a refined structure of TyrTS [13, 14] to construct a detailed model of the complex between the acceptor arm of $tRNA^{Tyr}$ and TyrTS. We also performed multiple changes of selected amino acid residues, Lys151, Glu152 and Trp196, to determine the nature of their contacts with $tRNA^{Tyr}$ [8].

The detailed model is depicted in figures 2 and 3. Its main features are as follows [8]. There is a tight complementarity in shape between the acceptor arm of the tRNA and TyrTS. The phosphate groups of residues 67-70 of $tRNA^{Tyr}$ form salt bridges or H-bonds with the side chains of Arg207, Lys208 and Asn146. Guanine-1 is surrounded by the side chains of Thr17, Gln15 and Trp196 but does not appear to form H-bonds with them. Adenine-73 forms an H-bond between its N6-H2 group and the carbonyl oxygen of residue Ala150. Its C2 and N3 atoms are at Van-der-Waals distances of the Cδ1 atom of Trp196. These contacts imply a specific recognition of A73 by TyrTS. A correct interaction cannot be maintained when A73 is replaced by G. Phosphates 73 and 74 are close to and in electrostatic repulsion with Glu152. Residues 74 to 76 are in a stretched conformation with the bases oriented towards the solvent, as in the interaction of pancreatic ribonuclease with polynucleotides [35]. Adenine-76 inserts between residues Lys82 and Arg86 on one face, Thr40 on the other face. The 2′−OH and

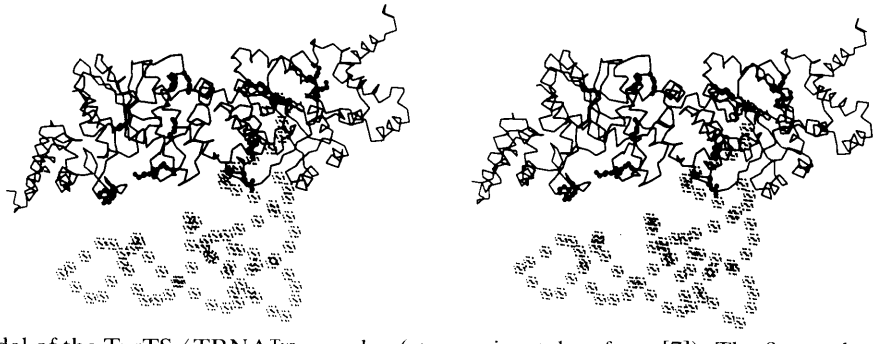

Fig 1. Preliminary model of the TyrTS / $TRNA^{Tyr}$ complex (stereo view taken from [7]). The figure shows the backbone of the TyrTS dimer with tyrosinyl-adenylate bound to the right subunit and the side chains of residues R207, K208, K151, K82, R86, K230 and K233. Only the N-terminal domain (residues 1-319) of each subunit is represented. The structure of $tRNA^{Phe}$ is indicated by the van der Waals' spheres of its phosphorous atoms.

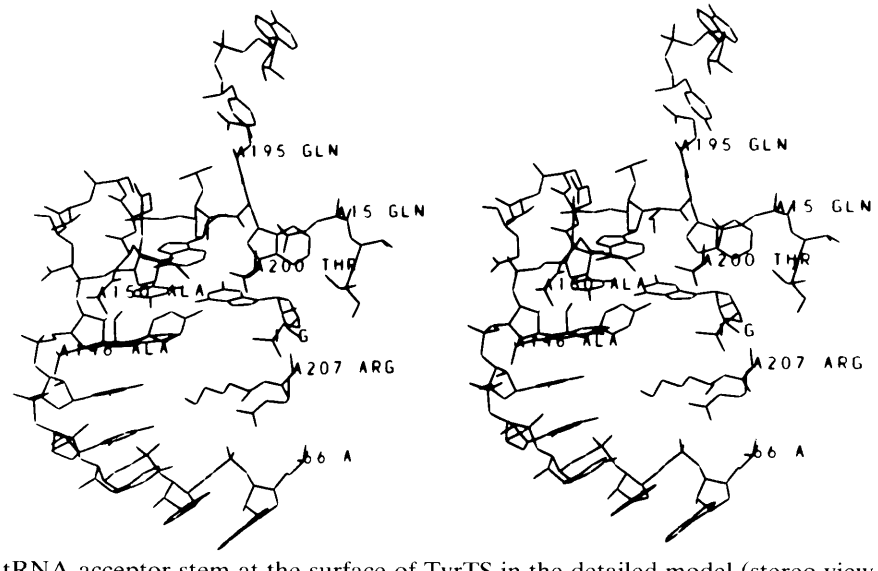

Fig 2. Docking of the tRNA acceptor stem at the surface of TyrTS in the detailed model (stereo view taken from [8]). Only the relevant fragments of $tRNA^{Phe}$ (residues 1 and 66 to 73) and TyrTS and the position of bound tyrosinyl-adenylate are represented. Residues 2 and following of $tRNA^{Phe}$ do not interact with TyrTS in the model (fig 1). The N or 5′-terminal residue of each TyrTS or $tRNA^{Phe}$ fragment is labelled. The latter A, at the beginning of each label, is drawn at the position of the Cα atom. Asn146 is represented and labelled as Ala because the side chain is not visible in the electron density map [14].

3′−OH of ribose-76 are close to the carbonyl of Tyr-AMP. C75 might be in stacking interaction with the adenine portion of Tyr-AMP. This model of the complex is compatible with the existence of a large extra loop in $tRNA^{Tyr}$. The anticodon stem and loop is close to the second subunit with the anticodon loop in a position suitable to interact with the C-terminal domain.

TyrTS binds only one $tRNA^{Tyr}$ molecule per dimer

Experiments on gel filtration at equilibrium [36], neutron diffraction [37], filtration through nitrocellulose [38] and protection of TyrTS by $tRNA^{Tyr}$ against an attack with acetic anhydride [39] have shown that only 1 molecule of $tRNA^{Tyr}$ binds each dimer of *B stearothermophilus* or *E coli* TyrTS. Our structural model does not forbid the binding of 2 tRNA molecules. The mechanism which prevents the binding of a second $tRNA^{Tyr}$ remains unknown.

The formation of only 1 molecule of Tyr-AMP per dimer of TyrTS is due to an asymmetry of the free enzyme in solution [33]. The kinetics of $tRNA^{Tyr}$ charging are biphasic towards the concentration of tyrosine because a second molecule of tyrosine binds to the same site as the first tyrosine, probably to help in displacing the charged tRNA [34].

The kinetics of $tRNA^{Tyr}$ binding to the TyrTS from *E coli* [40−43] and of $tRNA^{Tyr}$ aminoacylation by the TyrTS from *E coli* and *B stearothermophilus* [34, 36, 44, 45] have been investigated in detail.

Comparison of TyrTS from different organisms

The TyrTS from *E coli* and *B stearothermophilus* charge the $tRNA^{Tyr}$ from both organisms with similar efficiencies [26, 46]. We have therefore used commercial *E coli* $tRNA^{Tyr}$ in our *in vitro* characterization of mutant *B stearothermophilus* TyrTS [7, 8]. Other heterologous aminoacylations of $tRNA^{Tyr}$ from one origin by TyrTS from other origins have been reported ([47−55]; table I). This prompted us to compare the amino acid sequences of the corresponding TyrTS, when available, and to determine whether critical amino acid residues were conserved. The sequences of 5 TyrTS have been published together with their alignments: from *E coli* [56], *B stearothermophilus* [28], *Bacillus caldotenax* [53], *Bacillus subtilis* [54] and mitochondria of *Neurospora crassa* [57]. All of them can charge *E coli* $tRNA^{Tyr}$. In the following, we number the residues according to the *B stearothermophilus* sequence. The homologies are strong in the α/β and α domains of TyrTS but much weaker in the C-terminal domain.

Most of the residues involved in the binding and recognition of tyrosine and in the formation of Tyr-AMP are strictly conserved [14, 58]. The conservation is less strict for the residues of the $tRNA^{Tyr}$ binding site

Table I. Charging of $tRNA^{Tyr}$ by heterologous TyrTS. Eco: *Escherichia coli*; Bst: *Bacillus stearothermophilus*; Bca: *B caldotenax*; Bsu: *B subtilis*; Ncr(mit): mitochondria of *Neurospora crassa*; Sce(mit): mitochondria of *Saccharomyces cerevisiae*; Sce(cyt): cytoplasm of *S cerevisiae*. +: efficient charging. −: no charging. x: not reported.The references are indicated in parentheses.

TyrTS	*$tRNA^{Tyr}$*					
	Eco	*Bst*	*Bsu*	*Ncr (mit)*	*Sce (mit)*	*Sce (cyt)*
Eco	+	+ [26, 47]	+ [47, 48]	+ [49]	+ [50, 51]	− [51, 52]
Bst	+ [26, 46]	+	+ [47]	x	x	x
Bca	+ [53]	+ [53]	x	x	x	x
Bsu1	+ [54]	x	x	x	x	x
Bsu2	+ [48]	x	+	x	x	x
Sce (cyt)	+ [55]	x	+/− [47]	− [49]	x	+

that we have identified. To illustrate this last point, we can compare the TyrTS from *B stearothermophilus* and *N crassa* which only have 25% identities. Asn146 in *B stearothermophilus* might be replaced by an Arg in *N crassa*, in relative position −2. The dipeptide Lys151-Glu152 is replaced by Arg-Asp. Trp196 is replaced by Tyr. Remarkably, residues (189-199) are identical in 4 of the TyrTS, except Trp196. We have shown that the replacement of Trp196 by Phe does not alter the kinetics of aminoacylation of $tRNA^{Tyr}$ by TyrTS, contrary to its replacement by Gln or Ala [8]. The dipeptide Arg207-Lys208 is replaced by Lys-Ala-Ala-Arg. The Lys and Arg residues which are contiguous in the *B stearothermophilus* sequence, might be separated by 1 turn of α-helix and thus contiguous in space in *N crassa*. It is impossible to find residues, in *N crassa*, that are equivalent to Arg368, 371, 407 and 408 and Lys410 and 411 in *B stearothermophilus* because the sequence similarity between the 2 enzymes is too weak in the C-terminal domain. Nonetheless, the C-terminal ends of all the TyrTS are rich in positively charged residues. Thus, although the residues involved in $tRNA^{Tyr}$ binding are not conserved, one can find functional equivalents in nearby positions, at least in the N-terminal domain.

The strict identity of the residues that are involved in the recognition of tyrosine and the formation of Tyr-AMP, and the low similarity of those that are involved in the binding of $tRNA^{Tyr}$ suggest that the 2 sites have evolved independently and that the mechanisms of recognition of a small substrate, such as tyrosine, might differ from those for a large molecule, as $tRNA^{Tyr}$. The amino acyl residues involved in binding and specificity tend to be the same for small

substrates whereas they can be distinct for large substrates.

Genetic complementation experiments

The *tyrS* genes of *B stearothermophilus* and *B caldotenax* and the *tyrS1* gene of *B subtilis* can complement a mutation in the *tyrS* gene of *E coli, in vivo* [46, 53, 54]. Likewise, the *tyrS* gene of *E coli* can complement a mutation in the TyrTS gene of yeast mitochondria [50]. These experiments have profound consequences. They show that the foreign TyrTS can not only charge the heterologous $tRNA^{Tyr}$ but also discriminate against the heterologous non-cognate tRNAs. Indeed, aminoacylation of non-cognate tRNAs with tyrosine would be lethal to the cell. This implies that the tRNA identity elements and the mechanisms of recognition of such elements by the synthetases have been globally conserved during evolution. The results of the sequence comparisons and of the genetic complementation experiments therefore suggest that the residues of TyrTS and $tRNA^{Tyr}$ involved in binding and discrimination might be distinct. The discrimination could be due to negative determinants, preventing the binding of non-cognate molecules.

Cross-linking and protection experiments

The interaction between $tRNA^{Tyr}$ and TyrTS from *B stearothermophilus* has been studied by differential acetylation of the lysine residues in the free enzyme and in its complex with $tRNA^{Tyr}$. At least 2 lysines have shown a reduced reactivity towards acetic anhydride in the TyrTS / $tRNA^{Tyr}$ complex, at positions 225 or 230 and at position 233 [39]. We have found that mutations of residues Lys230 and Lys233 affect the formation of Tyr-AMP [7, 30]. The same mutations also affect the transfer of tyrosine from preformed Tyr-AMP to $tRNA^{Tyr}$ (Fersht and Knill-Jones, personal communication). In contrast, the mutation of Lys225 to Ala has no effect on the aminoacylation of $tRNA^{Tyr}$ *in vivo* [7].

Hountondji *et al* [59] have shown that Lys229, 234 and 237 of *E coli* TyrTS (homologous to Lys225, 230 and 233 of the *B stearothermophilus* enzyme) are specifically attacked by a 2′,3′-dialdehyde derivative of *E coli* $tRNA^{Tyr}$, in a mutually exclusive manner. These experiments have suggested that the ...CCA3′-OH end of $tRNA^{Tyr}$ remains mobile after the initial binding of $tRNA^{Tyr}$ to TyrTS when aminoacylation is blocked. We have checked this hypothesis as follows. After docking the acceptor stem of $tRNA^{Phe}$ at the surface of TyrTS, we performed rotations around the 2 bonds that bracket the phosphorus atoms of residues 74, 75 and 76 and found that it is possible, in this way, to bring the 2′-OH and 3′-OH of ribose-76 close to either the carbonyl of Tyr-AMP, as in the model of figure 3, or residue Lys230 or Lys233 (but not Lys225) [8]. The flexibility of the 3′-end of tRNAs is also illustrated by the hairpin loop structure of $tRNA^{Gln}$ when complexed to GlnTS [6].

Experiments of cross-linking between *E coli* $tRNA^{Tyr}$ and TyrTS by UV irradiation have indicated that the fragments 8-13 (D stem), 31-42 (anticodon stem and loop), 46-47d (extra loop) and residue U64 (Tψ stem and loop) are close to TyrTS in the complex [60, 61]. Our structural model does not show these regions of the complex.

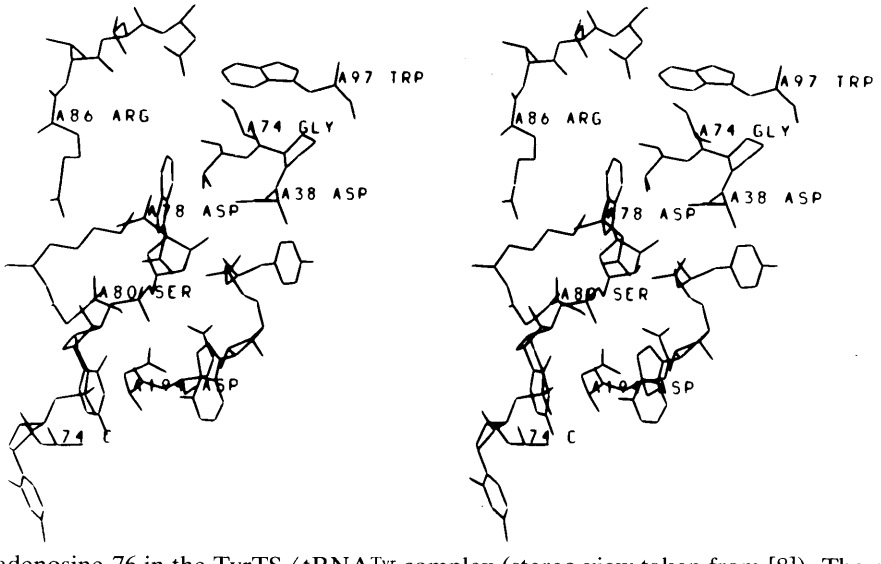

Fig 3. Environment of adenosine-76 in the TyrTS / $tRNA^{Tyr}$ complex (stereo view taken from [8]). The relevant fragments of TyrTS, residues 74 to 76 of $tRNA^{Phe}$ and tyrosinyl-adenylate are represented. The N or 5′-terminal residue of each TyrTS or $tRNA^{Phe}$ fragment is labelled.

Comparison of tRNA^{Tyr} sequences

E coli TyrTS can quantitatively charge the tRNA^{Tyr} from *E coli, B stearothermophilus* [26], *B subtilis* [48], *N crassa* mitochondria [49] and yeast mitochondria [51, 52] with tyrosine (table I). The nucleotide residues that are common to these 5 families of tRNA^{Tyr} are represented in figure 4 [27]. These common residues are

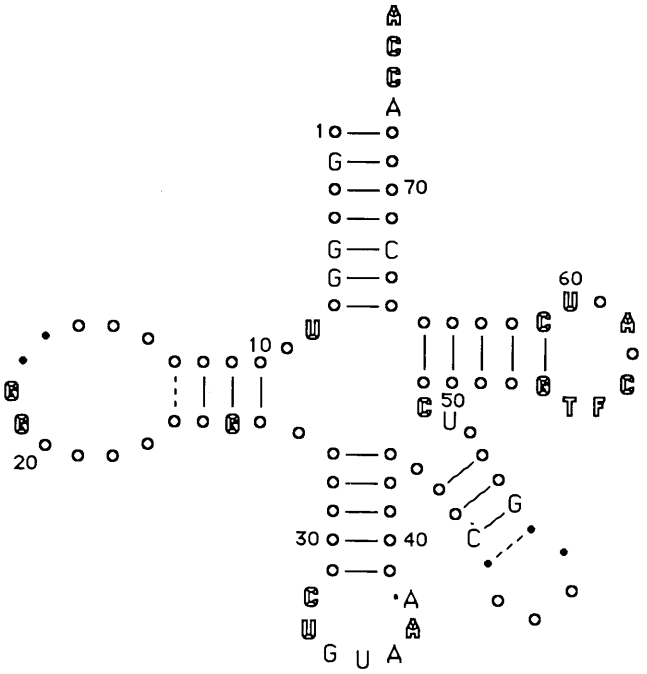

Fig 4. Comparison of the sequences of tRNA^{Tyr} that can be charged by *E coli* TyrTS. ●: Additional nucleotides in mitochondrial tRNA^{Tyr}; U: base residues that are not conserved; U: base residues that are conserved; **U**: invariant or semi-invariant bases.

candidates for a specific recognition by *E coli* TyrTS. Their set includes residues that are invariant or semi-invariant among all tRNAs.

Some of these common residues are differently modified according to the origin of tRNA^{Tyr}. The residue in position 8 can be uridine or 4-thiouridine; residue 18 can be 2′-*O*-methylguanosine or guanosine; residue 34, guanosine or queuosine; residue 37, 2-methyl-thio-*N*6-isopentyladenosine or *N*6-isopenthyladenosine. Moreover, the lengths of the D- and extra-stems and loops are not constant [27].

Mutants of *E coli* tRNA^{Tyr}

E coli possesses 2 different tRNA^{Tyr} which differ only by residues 47b and 47c and have identical charging properties [62, 63]. Amber and ochre suppressor tRNAs, charged with tyrosine, have been derived from these tRNA^{Tyr} [19, 64, 65].

Mutants of the amber suppressor tRNA^{Tyr}su^{+}3 have been obtained in 2 main ways.

1— A *lacZ*(am) strain and an indicator medium of β-galactosidase activity have been used to screen a derivative of phage ø80, encoding tRNA^{Tyr}su^{+}3, for altered suppressor properties.

2— A *supF, galK*(am), *galE* strain, in which suppression of the amber mutation in the galactokinase gene is lethal, has been used to select mutants defective for suppression.

The main problem with these screens and selections stems from the fact that the majority of the nucleotide residues are involved in the tertiary structure of the tRNA or of its precursor so that, in many cases, a sequence alteration results in a global structural change or it reduces the amount of processed tRNA [19, 66]. For example, mutant A18 is poorly processed so that the host strain has a Su^{-} phenotype, even though the kinetic properties of the purified, mutant tRNA are identical to those of the parental tRNA, mG18 [63].

Starting from mutants of tRNA^{Tyr}su^{+3} defective for suppression, double mutants have been selected as revertants having an increased suppressor activity. The double mutants allow us to conclude that the bases involved are not essential recognition elements for TyrTS. Such is the case for base pairs G1.C72 and G2.C71 in the acceptor stem; for the pair C11.G24 in the D-stem; for residues G15, C16, C20 and C20a in the D-loop; for pair G30.C40 in the anticodon stem; for residue C44 and pair G45.C47f in the extra-stem and loop ([67]; fig 5).

There are conflicting data about the role of residue Q34 in aminoacylation. Hou and Schimmel [68] have reported that mutation Q34 to C, as in tRNA^{Tyr}su^{+}3, does not change the K_M for tRNA^{Tyr} in the aminoacylation reaction but results in a 15-fold lower k_{cat}, thus showing that Q34 is a recognition element for TyrTS. In contrast, Abelson *et al* [69] have shown that tRNA^{Tyr}su^{+}3 has the same kinetic parameters as wild type tRNA^{Tyr}.

Chemical and enzymic modifications of *E coli* tRNA^{Tyr}

Several chemical modifications of tRNA^{Tyr}, listed in table II, do not alter its capacity for *in vitro* charging with tyrosine [16, 17, 70–72]. Similar results were reported for an enzymic cut, between residues 30 and 31 [73] and for a deletion of the D-loop [63]. A duplex between poly-G and a 3′-terminal fragment of tRNA^{Tyr}, obtained by digestion with RNAse T1 and containing 20 residues, is a competitive inhibitor of wild type tRNA^{Tyr} for aminoacylation by TyrTS [74]. This experiment has shown that the acceptor arm of tRNA^{Tyr} is recognized by TyrTS and that the recognition is not abolished by the simultaneous changes of U3 to G, A50 to G and A51 to G.

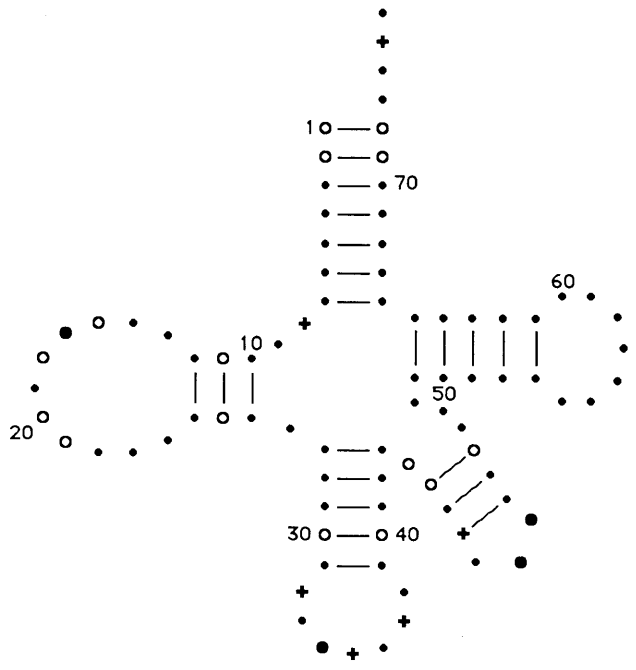

Fig 5. Modifications of *E coli* tRNA^{Tyr} that do not affect its charging capacity. o: Base or base-pair that can be mutated without altering the kinetics or capacity of charging; +: base for which chemical or enzymic modifications do not affect the capacity of charging; ●: combination of the 2 cases.

Conclusions on the variants of tRNA^{Tyr}

By combining our comparison of the tRNA^{Tyr} sequences from different origins with the properties of the mutant and chemically modified *E coli* tRNA^{Tyr}, we can conclude that the base residues that are recognized by TyrTS, in a specific and essential way, belong to the following list (figs 4, 5; table II): G5, G6, G19, U33, A36, A38, G47d, U47h, C48, G53, T54, F55, C56, A58, U60, C61, C68, A73, C74, A76. If one excludes the results of the chemical modifications, one must add: U8, C32, U35, A37, C47, C75.

In these lists, the invariant and semi-invariant residues are underlined. Several of these invariant residues are involved in tertiary interactions within the tRNA structures: U8, G19, C48, T54, F55, C56, A58 and, potentially, U47h [26] and therefore unlikely to participate in the TyrTS/tRNA^{Tyr} interaction [75]. So far, A73 is the only residue for which a specific recognition of the base by TyrTS has been demonstrated [67].

Except for A73, our structural model of the TyrTS/tRNA^{Tyr} complex does not show specific recognitions of bases in the acceptor stem of tRNA^{Tyr}. Thus, our model is consistent with the lack of recognition of residues 1 to 4 and 69 to 72.

tRNA^{Tyr} identity

By comparing the nucleotide sequences of the *E coli* and *Salmonella typhimurium* tRNAs, Mc Clain and Nicholas [76] have predicted the tRNA^{Tyr} residues that may serve as identity elements for this tRNA. These residues are: U9, C10, C20a, G25, C43 and A50.

Figure 4 shows that none of these residues is conserved among the heterologous tRNA^{Tyr} that are aminoacylated by *E coli* TyrTS. Thus, none of these residues is an essential recognition element for *E coli* TyrTS. This observation suggests that the potential role of these residues in the specificity of recognition is negative, *ie* they would prevent mischarging of tRNA-^{Tyr} by other aaTS of *E coli*.

Table II. Chemical modifications that do not inactive *E coli* tRNA^{Tyr} (wild type or su^{+}3). s^{4}U: 4-thiouridine; MS2I6A: 2-methylthio-*N*6-isopentyladenosine; I6A: *N*6-isopentyladenosine; *modified nucleoside; † the double modification of C74 and C75 inactivates tRNA^{Tyr}su^{+}3 [16].

wt Residue	*Modified residue*	*Chemicals*	*Ref*
s^{4}U8	U*	triiodide	[70]
s^{4}U8	U	–	
C16	C*	methoxyamine	[71]
C32	C*	methoxyamine	[16]
C34	C*	methoxyamine	[16]
U35	U*	cardoiimide	[17]
MS2I6A37	A*	triiodide	[70]
MS2I6A37	I6A	–	[72]
MS2I6A37	A	–	[72]
C47	C*	methoxyamine	[16]
U47b	U*	carbodiimide	[17]
C47c	C*	methoxyamine	[16]
C75†	C*	methoxyamine	[16]

Mischarging with glutamine

Mutants of *E coli* tRNA^{Tyr}su^{+}3 are mischarged with glutamine [67]. Some have lost their capacity of charging with tyrosine, others have retained it; some confer a thermosensitive suppressor phenotype to *E coli* whereas others are thermostable (table III). For example, mutants G73 and A1·C72 are not charged with tyrosine whereas G1·A72, G1·U72 and A1·U72 are. Mutant A2·C71 is thermosensitive whereas G2·U71 and A2·U71 are thermostable [77, 78]. Our structural model shows how adenine-73 is recognized by TyrTS

Table III. Mischarging mutants of $tRNA^{Tyr}su^{+}3$ and their revertants [67, 68, 77, 78, 80]. −: thermoresistant; +: weakly thermosensitive; +++: strongly thermosensitive; nd: not reported. The thermosensitivity of the phenotype is stronger when suppression is specific for tyrosine than when it is unspecific [77].

wt Residue or base pair	*Mutant residues*	*Charging properties*	*Thermo-sensitivity*
A73	G73	Gln	−
G1·C72	A1·C72	Gln	+++
G1·C72	G1·A72	Tyr + neutral	+
G1·C72	G1·U72	Tyr + Gln	−
G1·C72	A1·U72	Tyr + Gln	−
G2·C71	A2·C71	Tyr + Gln	++
G2·C71	G2·U71	Tyr + neutral	−
G2·C71	A2·U71	Tyr	−
U3·A70	G3·U70	Tyr + Ala	nd
G1·C72/A73	A1·C72/G73	Gln	nd

and why its binding site cannot accomodate a guanine (fig 2). We suggest the following explanation for the properties of the other mutants. Pairs G·A and G·U can form 2 hydrogen bonds and adopt a geometry which is close to that of a Watson-Crick base pair whereas pair A·C cannot [79]. The inability for a base pair to form hydrogen bonds and adopt a geometry that is compatible with the helical structure of the acceptor stem, would prevent the recognition of $tRNA^{Tyr}$ by TyrTS (pair A1·C72) or make it thermosensitive (pair A2·C71). Our structural model is compatible with non-specific interactions between TyrTS and base pairs 1/72 and 2/71.

The structure of the GlnTS/$tRNA^{Gln}$ complex shows a specific role of G73 in the interaction and implies the opening of base pair 1/72. It explains why the change of A73 into G or the weakening of base pair G1·C72 or G2·C71, by mutation, results in the charging of $tRNA^{Tyr}su^{+}3$ with glutamine. The combination of the structural and mutagenesis data shows that GlnTS discriminates between an A·U and a G·C base pair in position 1/72 because the former melts more easily than the latter. Note that neither a guanine in position 73 nor a weak base pair in positions 1/72 or 2/71 is an absolute requirement for charging of $tRNA^{Tyr}su^{+}3$ with glutamine (table III). Nevertheless, one would predict that the double mutant A1·C72/G73 of $tRNA^{Tyr}su^{+}3$ is more efficiently charged with glutamine than the 2 simple mutants A1·C72 or G73. This is indeed the case [80].

When GlnTS is overproduced by the cell, it mischarges $tRNA^{Tyr}su^{+}3$. This result has shown that an imbalance between the cellular concentrations of synthetase and tRNA can result in mischarging [81]. Mutants of GlnTS that mischarge $tRNA^{Tyr}su^{+}3$ have been selected *in vivo* or constructed *in vitro* at residue positions 129 and 235 [82−84]. These mutations are discussed in [84].

Comparison with the glutaminyl system

In the glutaminyl system [6], residues C74, C75 and A76 form a hairpin whereas in our model of the TyrTS/$tRNA^{Tyr}$ complex [7, 8], these residues are stretched in the general direction followed by the helix of the acceptor stem so that A76 can reach Tyr-AMP. It will be interesting to see, in the future, if the hairpin structure is characteristic of aaTS for which the tRNA binding site is included within 1 monomer or subunit and the extended structure, of aaTS for which the binding site straddles several subunits. There are extensive interactions between the ...CCA3′ of $tRNA^{Gln}$ and GlnTS, in the complex crystallized without glutamine, whereas experimental evidence indicates that the ...CCA3′ end remains mobile after the initial binding of $tRNA^{Tyr}$ to TyrTS.

In the glutaminyl system, the N2-H2 group of G73 makes a hydrogen bond with the phosphate oxygen of A72. Therefore, G73 specifically participates in the stabilization of the hairpin structure of the ...CCA3′. Residue G73 is an important recognition element by virtue of an RNA/RNA interaction rather than an RNA/protein interaction. In the tyrosyl system, A73 is recognized by TyrTS through a hydrogen bond between its N6-H2 group and the carbonyl oxygen of Ala150 and through Van-der-Waals contacts with Trp196. Thus, A73 is an essential recognition element by virtue of an RNA/protein interaction.

In the glutaminyl system, base pair 1/72 opens to let the Leu136 side chain insert between the 2 bases whereas in the tyrosyl system, the 2 bases must bond in a way that is compatible with the helical structure of the acceptor stem.

GlnTS makes sequence specific contacts, with G2, C71 and G3, in the minor groove of the acceptor stem of $tRNA^{Gln}$. Our structural model of the TyrTS/$tRNA^{Tyr}$ complex and the available sequence and genetic data on $tRNA^{Tyr}$ indicate that the bases of the pairs G1·C72, G2·C71, G3·C70 and U4·A69 are not essential recognition elements for TyrTS.

In the glutaminyl system, there are extensive interactions between the aaTS and the anticodon stem and loop. In particular, U35 is an important recognition element. In the tyrosyl system, the role of Q34 in the recognition of $tRNA^{Tyr}$ by TyrTS is controversial. Nevertheless, we have demonstrated the existence of essential contacts between the C-terminal domain of TyrTS and the anticodon stem and loop of $tRNA^{Tyr}$ [7].

Thus, the comparison of the TyrTS / $tRNA^{Tyr}$ and GlnTS / $tRNA^{Gln}$ complexes shows that the recognition mechanisms of the tRNAs are different in the 2 systems. The characterization of other complexes will complete our understanding of this crucial step of genetic translation.

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