MOLECULAR RECOGNITION AND EVOLUTION OF *Escherichia coli* TYROSINE tRNA BY TYROSYL-tRNA SYNTHETASE

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(Abstract)

Identity determinants of *Escherichia coli* $tRNA^{Tyr}$ were examined using *in vitro* transcripts. The first and second anticodon nucleotides were base-specifically involved in tRNA recognition of tyrosyl-tRNA synthetase, whereas the third anticodon nucleotide was not. None of the identity determinants were found in the acceptor stem, except the discriminator base A73. With respect to the long variable arm, a stem of three base pairs in length was required for tyrosylation, while the sequence of the arm was not essential. From the results of identity conversion experiments, the discrimination modes of $tRNA^{Tyr}$ from other amino acid specific tRNAs sharing some of the identity determinants of $tRNA^{Tyr}$ were discussed.

(Keywords)

$tRNA^{Tyr}$; tRNA identity; tyrosyl-tRNA synthetase; *Escherichia coli*

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1. Introduction

The correct attachment of amino acid to tRNAs by its cognate aminoacyl-tRNA synthetase (aaRS) is important for the precise translation of genetic information in protein synthesis. An aaRS must discriminate its cognate tRNAs from the pool of tRNAs having a similar overall tertiary structure. The sequence and structural features of a tRNA that define this discrimination are named "identity determinants". A number of studies using several genetic, biochemical and biophysical approaches have shown that many aaRSs recognize a small set of nucleotides in the corresponding tRNA, which are often located in the anticodon, the fourth base from the 3' terminus subsequent to the universal CCA3' sequence (the discriminator base) and the acceptor stem [1-6]. In addition to these positive identity determinants important for recognition by cognate aaRS, negative identity determinants were found to function in the prevention of misacylation by non-cognate aaRSs [6-11].

tRNAs are divided into two groups according to the length of the variable region. This classification is conserved throughout kingdoms in all tRNAs except $tRNA^{Tyr}$. In eubacteria, $tRNA^{Tyr}$, along with $tRNA^{Ser}$ and $tRNA^{Leu}$, possesses a long variable arm composed of more than ten nucleotides, and these three tRNAs are classified as class II tRNAs. In eukaryotes and archaebacteria, however, $tRNA^{Tyr}$ belongs to class I tRNAs, which have four or five nucleotides in the variable region [12]. This structural difference seems to be responsible for the difference in the way that $tRNA^{Tyr}$ is recognized by tyrosyl-tRNA synthetase (TyrRS) between organisms. *Saccharomyces cerevisiae* TyrRS does not aminoacylate *Escherichia coli* $tRNA^{Tyr}$ *in vitro* [13]. In contrast, *E. coli* TyrRS does not aminoacylate *S. cerevisiae* amber suppressor $tRNA^{Tyr}$ *in vivo* [13]. Although tRNA identity in the tyrosine system is interesting from an evolutionary point of view, data of the identity determinants is still incomplete. In *E. coli*, *in vivo* and *in vitro* mutational studies demonstrated that the first and second anticodon nucleotides and the discriminator base A73 are involved in aminoacylation with tyrosine [14-16]. However, other nucleotides, including the third anticodon nucleotide A36, have not yet been closely examined. As for the long variable arm, the mutational study has suggested the importance of its direction for tyrosylation [16]. However, the role of the length and the sequence of the long variable arm in tyrosylation remain unclear.

In this study, we constructed various tRNA mutant transcripts, and measured their aminoacylation kinetics with *E. coli* TyrRS *in vitro* in order to systematically examine the identity determinants of $tRNA^{Tyr}$. Moreover, identity conversion experiments from both class I (Lys and

Asp) and class II (Ser and Leu) tRNAs were performed in order to clarify how TyrRS discriminates $tRNA^{Tyr}$ from the other tRNAs. On the basis of the results obtained, a set of $tRNA^{Tyr}$ identity determinants that enables $tRNA^{Tyr}$ discrimination from other tRNAs was determined.

2. Materials and Methods

2.1. Preparation of template DNAs and in vitro transcripts

Synthetic DNA oligomers carrying the T7 promoter and tRNA genes were ligated into pUC19 and transformed into *E. coli* strain JM109. The template DNA sequences were confirmed by dideoxy sequencing. Transcripts of the tRNA genes were prepared in a reaction mixture containing 40 mM Tris-HCl (pH 8.1), 5 mM dithiothreitol, 2 mM spermidine, 10 mM magnesium chloride, 50 μg/ml bovine serum albumin, 2 mM each NTP, 20 mM 5'GMP, *Bst*NI-digested template DNA (0.2 mg/ml), 2 U of inorganic pyrophosphatese (Sigma), and T7 RNA polymerase (50 μg/ml) that had been purified from the overproducer *E. coli* BL21/pAR1219 [17]. The transcripts were purified by 15 % polyacrylamide gel electrophoresis.

2.2. Aminoacylation assay

TyrRS was partially purified from *E. coli* strain Q13 by anion exchange column chromatography (DEAE-Toyopearl 650, Tosoh, Tokyo). The TyrRS fraction had a specific activity of 56 U/mg (One unit of aminoacyl-tRNA synthetase activity was defined as the amount of the enzyme that catalyzes the incorporation of 1 nmol of amino acid into tRNA in 10 min under the reaction conditions described below.). The aminoacylation reaction proceeded at 37 ^{o}C in 30 μl of reaction mixture containing 60 mM Tris-HCl (pH 7.5), 10 mM magnesium chloride, 2 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 2.5 mM ATP, 11 μM L-[U-^{14}C] tyrosine (17.6 GBq/mmol), and various concentrations of tRNA transcripts and *E. coli* TyrRS. The initial rates of aminoacylation were determined by using six concentrations of tRNA transcripts ranging from 0.1 to 10 μM at a fixed concentration of the synthetase, depending on the mutant tRNA transcripts. The Km and Vmax values were determined from Lineweaver-Burk plots with the use of linear least-squares analysis. The Vmax/Km values for two or three independent determinations were within ±15 %.

3. Results

To investigate the effects of the base modification on tyrosylation, the kinetic parameter of the native $tRNA^{Tyr}$ was compared with that of the wild-type sequence of the $tRNA^{Tyr}$ transcript. *E. coli* $tRNA^{Tyr}$ has seven modified nucleotides including a hypermodified G, queuosine (Q), at the position of

the first anticodon nucleotide. As shown in Table 1, the native $tRNA^{Tyr}$ possessed a Vmax/Km value only 1.5-fold larger than that of the transcript, indicating that this bulky modification, together with other base modifications, is not essential for recognition by *E. coli* TyrRS.

According to cross-species aminoacylation experiments, *E. coli* TyrRS can aminoacylate $tRNA^{Tyr}$s from *Bacillus stearothermophilus*, *Bacillus subtilis*, *Neurospora crassa* mitochondria, and yeast mitochondria as efficiently as those from homogeneous $tRNA^{Tyr}$ [18]. As shown in Figure 1, except for invariant nucleotides only limited numbers of nucleotides were conserved among these five $tRNA^{Tyr}$s. Since the nucleotides that are not preserved among these five $tRNA^{Tyr}$s do not base-specifically contribute to aminoacylation by *E. coli* TyrRS, a base or a base-pair substitution was introduced into the positions of the conserved nucleotides among these tRNAs.

3.1. Acceptor stem

In the acceptor stem, G2, G5-C68 and G6 are conserved among heterogeneous $tRNA^{Tyr}$s aminoacylated by *E. coli* TyrRS (Fig. 1). Substitutions of G2-C71 by C2-G71, G5-C68 by C5-G68 and G6-C67 by C6-G67 resulted in decreases in the Vmax/Km value by 1.7-, 3.1-, and 4-fold,

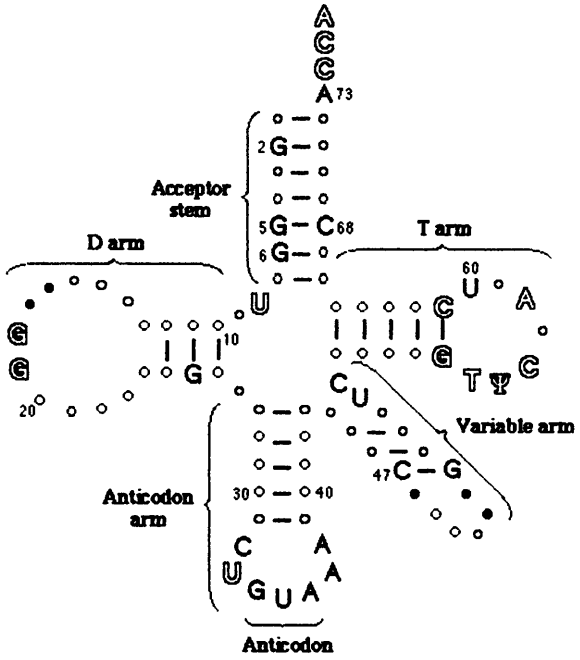

Fig. 1. Consensus sequences of $tRNA^{Tyr}$ from *E. coli*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Neurospora crassa* mitochondria and yeast mitochondria. These $tRNA^{Tyr}$s can be aminoacylated by *E. coli* TyrRS. Additional nucleotides in mitochondrial $tRNA^{Tyr}$ are indicated by closed circles. Invariant or semi-invariant bases are designated by outlined letters. This figure is cited from [18].

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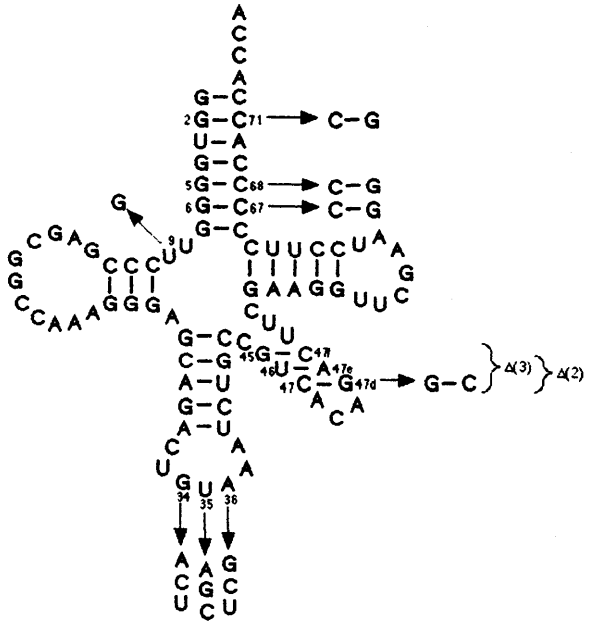

Fig. 2. The cloverleaf structure of *E. coli* $tRNA^{Tyr}$ with the base modifications omitted. Numbering is according to [12]. Arrows indicate the substitutions made in this study.

respectively (Fig. 2, Table 1). These findings indicate that these three base pairs in the acceptor stem are not involved in tyrosylation. U9, located between the acceptor stem and the D arm, is not only conserved among heterogeneous $tRNA^{Tyr}$s aminoacylated by *E. coli* TyrRS, but is also characteristic of $tRNA^{Tyr}$ because the other class II tRNAs possess G9. However, the substitution of G9 for U9 had only a slight effect on tyrosylation activity (Fig. 2, Table 1). Thus, we concluded that the identity determinants of $tRNA^{Tyr}$, except the discriminator base A73, do not reside in the acceptor stem.

3.2. Anticodon

To study the involvement of the anticodon nucleotides in tyrosylation in detail, each nucleotide was substituted by the other three nucleotides (Fig. 2). The substitution of the first anticodon nucleotide G34 by the other three nucleotides decreased Vmax/Km values by 25- to 56-fold (Table 1). The kinetic data of the C34 mutant were approximately in agreement with those reported by Hou & Schimmel [15]. The effects of mutation on tyrosylation appeared most drastically when the mutation occurred at the second anticodon nucleotide. The substitution of U35 by A35 reduced the Vmax/Km value 3100-fold (Table 1). The other substitutions, U35 by G35 and U35 by C35, decreased the Vmax/Km value 240- and 670-fold, respectively (Table 1). In contrast, the substitution of the third anticodon nucleotide A36 by

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Table 1

Kinetic parameters with *E. coli* tyrosyl-tRNA synthetase for $tRNA^{Tyr}$ transcripts

	Km (μM)	Vmax (nmol/min per mg)	Vmax/Km (relative)	loss of specificity (relative)
Native $tRNA^{Tyr}$	0.34	5.6	1.5	
transcript $tRNA^{Tyr}$	0.53	5.9	1.0	1
$tRNA^{Tyr}$ (C2-G71)	1.4	8.9	0.58	1.7
$tRNA^{Tyr}$ (C5-G68)	1.0	3.7	0.32	3.1
$tRNA^{Tyr}$ (C6-G67)	3.3	9.2	0.25	4
$tRNA^{Tyr}$ (G9)	0.58	4.4	0.67	1.5
$tRNA^{Tyr}$ (G47-C47d)	1.1	9.5	0.81	1.2
$tRNA^{Tyr}$ Δ(2)	1.7	0.91	0.048	21
$tRNA^{Tyr}$ Δ(3)	-	-	<0.0001	>10000
$tRNA^{Tyr}$ (A34)	2.3	1.0	0.040	25
$tRNA^{Tyr}$ (C34)	3.6	0.79	0.020	50
$tRNA^{Tyr}$ (U34)	1.9	0.38	0.018	56
$tRNA^{Tyr}$ (A35)	67	0.24	0.00032	3100
$tRNA^{Tyr}$ (G35)	5.9	0.28	0.0042	240
$tRNA^{Tyr}$ (C35)	22	0.38	0.0015	670
$tRNA^{Tyr}$ (G36)	0.52	2.4	0.42	2.4
$tRNA^{Tyr}$ (C36)	0.91	5.3	0.52	1.9
$tRNA^{Tyr}$ (U36)	2.8	7.8	0.25	4

Numbered nucleotides and base pairs in parentheses refer to the substitutions of tRNAs (Fig. 2).
Δ(2) means the deletion of two base pairs; 46-47e, 47-47d, and Δ (3) means the deletion of three base pairs; 45-47f, 46-47e, 47-47d, in variable arm of $tRNA^{Tyr}$ transcripts.

any other nucleotide decreased the Vmax/Km values only a few-fold (Table 1). These results indicate that the first and second anticodon nucleotides are base-specifically required for aminoacylation by TyrRS, whereas the third anticodon nucleotide is not.

3.3. Variable arm

In the variable arm, only C47-G47d is conserved among heterogeneous $tRNA^{Tyr}$s aminoacylated by *E. coli* TyrRS (Fig. 1). Substitution by G47-C47d did not affect the tyrosylation activity (Fig. 2, Table 1).

The variable arm of $tRNA^{Tyr}$ contains three Watson-Crick base pairs, which form the variable stem. The deletion of one base pair reduced the Vmax/Km value 21-fold (Fig. 2, Table 1). Additional one-base-pair deletion more severely decreased tyrosylation activity (Fig. 2, Table 1). These results indicate that a variable arm with a stem of three base pairs in length is required for tyrosylation, but that a specific sequence is not.

3.4. Identity conversion

The above and previous results indicated that the anticodon nucleotides and the discriminator base are crucial identity determinants of $tRNA^{Tyr}$. Here, the $tRNA^{Tyr}$-type anticodon nucleotides and the discriminator base were introduced into $tRNA^{Ser}$ and $tRNA^{Leu}$ to clarify to what extent these elements are involved in $tRNA^{Tyr}$ discrimination from the other class II tRNAs (Fig. 3(a), 3(b)). These transcripts with a wild-type sequence were not charged with tyrosine (Table 2). For $tRNA^{Ser}$, the change of both the anticodon nucleotides from U34G35A36 to G34U35A36, and the discriminator base from G73 to A73, increased the Vmax/Km value of the tyrosylation activity up to 1/45 compared with that of wild-type $tRNA^{Tyr}$ (Table 2). In case of $tRNA^{Leu}$, which possesses the same discriminator base as $tRNA^{Tyr}$, the conversion of the anticodon nucleotides from C34A35G36 to G34U35A36 alone dramatically increased the tyrosylation activity up to 1/13 (Table 2). Thesé results indicate that the $tRNA^{Tyr}$-type anticodon nucleotides and discriminator base are sufficient to allow the discrimination of $tRNA^{Tyr}$ from the other class II tRNAs.

Identity conversion experiments were also applied to class I tRNAs, $tRNA^{Lys}$ and $tRNA^{Asp}$ (Fig. 3(c), 3(d)). Wild-type $tRNA^{Lys}$ transcript contains U34U35U36 and A73, and thus among the major identity determinants of $tRNA^{Tyr}$ (excepting the long variable arm) only the first anticodon nucleotide is different from that of $tRNA^{Tyr}$. Although the wild-type $tRNA^{Lys}$ transcript had no tyrosylation activity, the substitution of U34 by G34 increased the Vmax/Km value up to a detectable level, 1/2100 compared with that of $tRNA^{Tyr}$ (Table 2). Likewise, for $tRNA^{Asp}$ containing G34U35C36 and G73, only the discriminator base is different from that of $tRNA^{Tyr}$. The wild-type $tRNA^{Asp}$ transcript was not charged with tyrosine (Table 2). The substitution of G73 by A73 slightly decreased the Vmax/Km value up to 1/3600 (Table 2). To investigate whether the third anticodon nucleotide is important in this discrimination mode, C36 of this $tRNA^{Asp}$ mutant was changed to A36. This additional mutation improved the Vmax/Km value by only about 5-fold (Table 2). These results show that the substitution of the anticodon nucleotides and the discriminator base alone does not confer efficient tyrosylation activity

on class I tRNAs. It seems most likely that the long variable arm plays an important role in the discrimination by TyrRS in addition to the first and second anticodon nucleotides and the discriminator base.

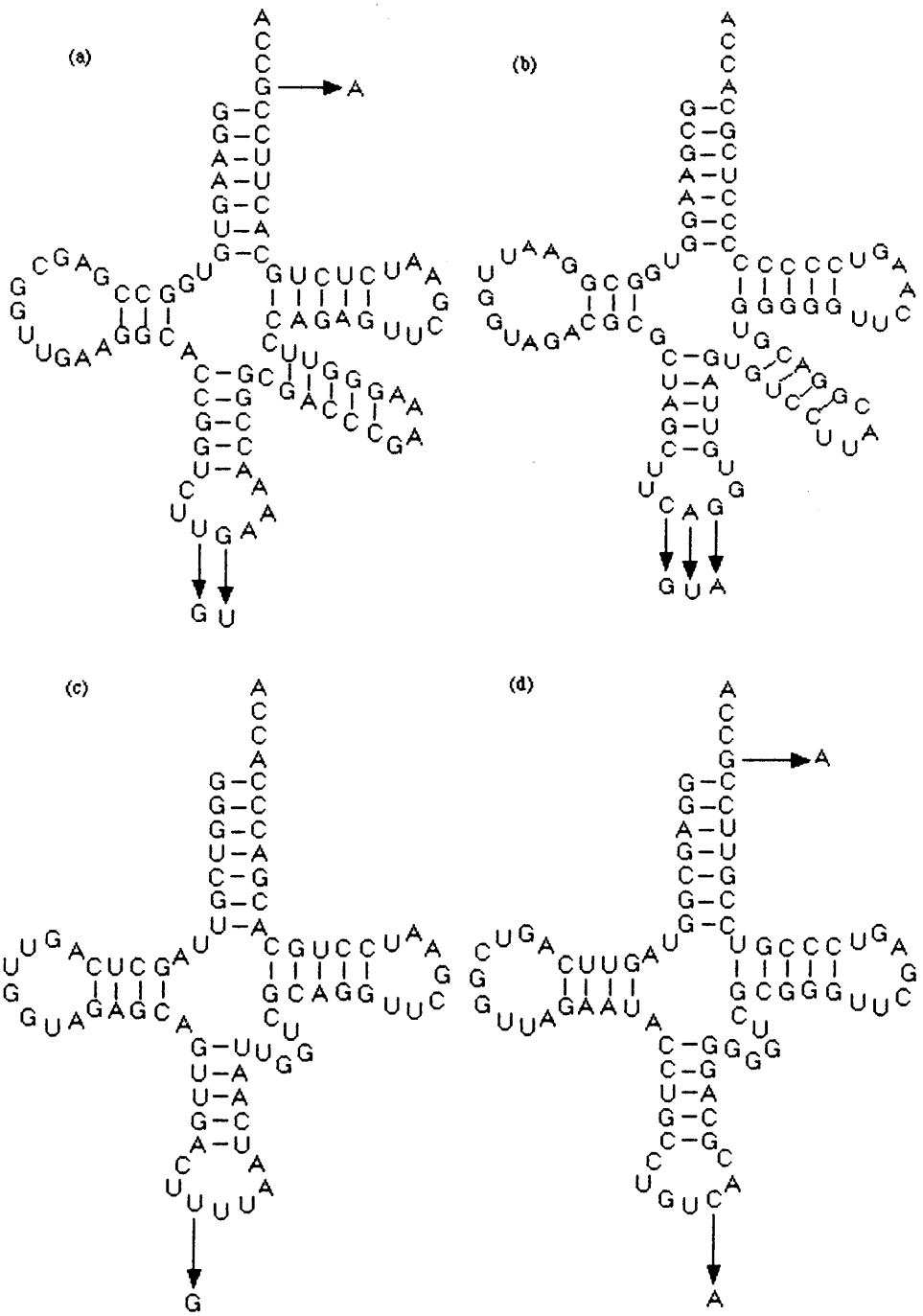

Fig. 3. The transcripts for the identity conversion from $tRNA^{Ser}$ (a), $tRNA^{Leu}$ (b), $tRNA^{Lys}$ (c), and $tRNA^{Asp}$ (d). Arrows indicate the substitutions made in this study.

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Table 2

Kinetic parameters with *E. coli* tyrosyl-tRNA synthetase for tRNA transcripts

	Km (μM)	Vmax (nmol/min per mg)	Vmax/Km (relative)	loss of specificity (relative)
$tRNA^{Tyr}$	0.53	5.9	1.0	1
$tRNA^{Ser}$	-	-	<0.0001	>10000
$tRNA^{Ser}$ (GUA, A73)	1.3	0.31	0.022	45
$tRNA^{Leu}$	-	-	<0.0001	>10000
$tRNA^{Leu}$ (GUA)	2.6	0.22	0.079	13
$tRNA^{Lys}$	-	-	<0.0001	>10000
$tRNA^{Lys}$ (G34)	6.3	0.034	0.00048	2100
$tRNA^{Asp}$	-	-	<0.0001	>10000
$tRNA^{Asp}$ (A73)	9.7	0.029	0.00028	3600
$tRNA^{Asp}$ (A36, A73)	2.4	0.036	0.0014	710

Numbered nucleotides in parentheses correspond to mutations of tRNAs at numbered positions. The triplets in parentheses purport the substitutions of ainticodon (Fig. 3).

4. Discussion

Our present findings together with those of other studies demonstrated that none of the nucleotides in the acceptor stem, except the discriminator base A73, are involved in tyrosylation and that the first and second anticodon nucleotides are required for tyrosylation, while the third is much less important.

As for the long variable arm, a stem of three base pairs in length was shown to be important for tyrosylation, while the sequence of the arm was not. Various studies on TyrRS based on the crystal structure of *Bacillus stearothermophilus* TyrRS suggest that TyrRS approaches $tRNA^{Tyr}$ on the side of the variable arm, straddling both subunits of the TyrRS [18]. Experiments on cross-linking between *E. coli* $tRNA^{Tyr}$ and TyrRS by UV irradiation indicated that the fragment constituting the variable arm, U46-G47d, is close to TyrRS in the complex [19]. These findings together with our mutation studies suggest that *E. coli* TyrRS recognizes the long variable arm, directly binding to the sugar-phosphate backbone in the vicinity of U46-G47d.

The recognition mode of the variable arm in aminoacylation is divergent among class II tRNAs. For $tRNA^{Ser}$, both the length and the direction of the long variable arm are crucial for serylation in *E. coli*

[9, 20]. The cocrystal structure of *Thermus thermophilus* seryl-tRNA synthetase (SerRS) and $tRNA^{Ser}$ demonstrated that SerRS binds to the sugar-phosphate backbone of the long variable arm [21]. In contrast, the long variable arm of *E. coli* $tRNA^{Leu}$ does not serve as a positive identity determinant because the primary and secondary structures of the variable arm are not correlated to leucylation activities [22-23]. These findings imply that leucyl-tRNA synthetase (LeuRS) does not recognize the long variable arm. For $tRNA^{Tyr}$, the present mutational study showed that the length of the stem in the variable arm, but not its sequence, is required for tyrosylation. The identity conversion experiments showed that $tRNA^{Ser}$ and $tRNA^{Leu}$ acquired good tyrosylation activities through changes in the anticodon and the discriminator base alone. This indicates that differences in the direction of the variable arm within class II tRNAs do not definitely affect the recognition of $tRNA^{Tyr}$ by TyrRS. This is in contrast to the serine system in which the change in the direction of the variable arm was requisite for the identity conversion from $tRNA^{Tyr}$ and $tRNA^{Leu}$ to $tRNA^{Ser}$ [9, 16]. A previous mutational study showed that the deletion of U47g and U47h at the base of the variable arm or the insertion of two adenosines between C44 and G45 to pair with U47g and U47h severely impaired the tyrosylation activity [16]. These mutations are thought to either cause the destruction of the overall tertiary structure or change the direction of the long variable arm far beyond the differences within class II tRNAs.

The uninvolvement of the third anticodon nucleotide in tyrosylation is intriguing from the view point of $tRNA^{Tyr}$ discrimination. Four amino acid-specific tRNAs, $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$, possess the same first and second anticodon nucleotides Q34 and U35, while the third nucleotides differ between them; i.e., A36, G36, U36, and C36, respectively. The differences between $tRNA^{Tyr}$ and the remaining three tRNAs lie in the long variable arm and the discriminator base: C73 for $tRNA^{His}$, and G73 for $tRNA^{Asp}$ and $tRNA^{Asn}$. Moreover, both elements are used as the identity determinants of $tRNA^{Tyr}$. This indicates that $tRNA^{Tyr}$ is discriminated from other tRNAs containing Q34 and U35 by the existence of the long variable arm and A73, not by the third anticodon nucleotide.

Among tRNAs containing Q34 and U35, neither $tRNA^{Tyr}$ nor $tRNA^{His}$ uses the third anticodon nucleotide as a major positive identity determinant [24]. In contrast, C36 of $tRNA^{Asp}$ and U36 of $tRNA^{Asn}$ are important for aminoacylation by their cognate aaRS [25, 26]. It is worthy of note that $tRNA^{Tyr}$ and $tRNA^{His}$ possess the characteristic tertiary structure, the long variable arm of $tRNA^{Tyr}$ and the extra G-1 of $tRNA^{His}$, both of which are positive identity determinants of the respective

tRNAs [27]. Because these tRNAs are easily discriminated from the other tRNAs by the existence of the characteristic tertiary structure, they might not need to use the third anticodon nucleotide as an identity determinant.

Neither eukaryotic nor archaebacterial $tRNA^{Tyr}$ possesses the long variable arm [12]. It has been reported that C1-G72, G34, Ψ35, and A73 are identity determinants of yeast $tRNA^{Tyr}$, whereas the involvement of A36 in tyrosylation has not been examined yet [28-30]. Taking into account that of all tRNAs from eukaryotes and archaebacteria only $tRNA^{Tyr}$ has C1-G72, thus this unique base pair is thought to play a role similar to that of the long variable arm of eubacterial $tRNA^{Tyr}$ in discrimination among tRNAs with G34Ψ35. We infer that, in eukaryotes and archaebacteria, A36 is also not involved in recognition by TyrRS.

TyrRS and tryptophanyl-tRNA synthetase (TrpRS) are structural isomers that diverged more recently than most aaRSs [31]. A proposed evolutionary scenario is that $tRNA^{Tyr}$ acquired the long variable arm for the discrimination from $tRNA^{Trp}$ when TyrRS and TrpRS were separated and UAY and UGG codons were assigned to tyrosine and tryptophan, respectively [32]. However, $tRNA^{Trp}$ possesses C34C35A36 and G73, which are not the identity determinants of $tRNA^{Tyr}$, and vice versa [33]. Thus, $tRNA^{Tyr}$ is thought not to need the long variable arm for the discrimination from $tRNA^{Trp}$. It is more likely that the long variable arm was inserted to $tRNA^{Tyr}$ for the discrimination from tRNAs with the same first and second anticodon nucleotides, which already existed in cells. Eukaryotes and archaebacteria would have chosen to have C1-G72 instead of the long variable arm.

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References

1. Shimizu, M., Asahara, H., Tamura, K., Hasegawa, T. and Himeno, H. The role of anticodon bases and the discriminator nucleotide in the recognition of some *E. coli* tRNAs by their aminoacyl-tRNA synthetases, J. Mol. Evol. 35, 436-443 (1992).
2. Giegé, R., Puglisi, J.D. and Florentz, C.

tRNA structure and aminoacylation efficiency, Prog. Nucleic Acid Res. Mol. Biol. 45, 129-206 (1993).

3. McClain, W.H. Rules that govern tRNA identity in protein synthesis, J. Mol. Biol. 234, 257-280 (1993).
4. Saks, M.E., Sampson, J.R. and Abelson, J.N. The transfer RNA identity problem: a search for rules, Science 263, 191-197 (1994).
5. Hou, Y.-M. Discriminating among the discriminator bases of tRNAs, Chem. Biol. 4, 93-96 (1997).
6. Giegé, R., Sissler, M. and Florentz, C. Universal rules and idiosyncratic features in tRNA identity, Nucleic Acids Res. 26, 5017-5035 (1998).
7. Muramatsu, T., Nishikawa, K., Nemoto, F., Kuchino, Y., Nishimura, S., Miyazawa, T. and Yokoyama, S. Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification, Nature 336, 179-181 (1988).
8. Perret, V., Garcia, A., Grosjean, H., Ebel, J.P., Florentz, C. and Giegé, R. Relaxation of a transfer RNA specificity by removal of modified nucleotides, Nature 344, 787-789 (1990).
9. Asahara, H., Himeno, H., Tamura, K., Nameki, N., Hasegawa, T. and Shimizu, M. *Escherichia coli* seryl-tRNA synthetase recognizes $tRNA^{Ser}$ by its characteristic tertiary structure, J. Mol. Biol. 236, 738-748 (1994).
10. Pütz, J., Florentz, C., Benseler, F. and Giegé, R. A single methyl group prevents the mischarging of a tRNA, Nat. Struc. Biol. 1, 580-582 (1994).
11. Nameki, N., Asahara, H. and Hasegawa, T. Identity elements of *Thermus thermophilus* $tRNA^{Thr}$, FEBS Lett. 396, 201-207 (1996).
12. Sprinzl, M., Horn, C., Brown, M., Ioudovitch, A. and Steinberg, S. Compilation of tRNA sequences and sequences of tRNA genes, Nucleic Acids Res. 26, 148-153 (1998).
13. Ohno, S., Yokogawa, T., Fujii, I., Asahara, H., Inokuchi, H. and Nishikawa, K. Co-expression of yeast amber suppressor $tRNA^{Tyr}$ and tyrosyl-tRNA synthetase in *Escherichia coli*: possibility to expand the genetic code, J. Biochem. 124, 1065-1068 (1998).
14. Shimura, Y., Aono, H., Ozeki, H., Sarabhai, A., Lamfrom, H. and Abelson, J. Mutant tyrosine tRNA of altered amino acid specificity, FEBS Lett. 22, 144-148 (1972).
15. Hou, Y.-M. and Schimmel, P. Modeling with *in vitro* kinetic parameters for the

elaboration of transfer RNA identity *in vivo*, Biochemistry 28, 4942-4947 (1989).

16. Himeno, H., Hasegawa, T., Ueda, T., Watanabe, K. and Shimizu, M. Conversion of aminoacylation specificity from $tRNA^{Tyr}$ to $tRNA^{Ser}$ *in vitro*, Nucleic Acids Res. 18, 6815-6819 (1990).
17. Grodberg, J. and Dunn, J.J. ompT encodes the *Escherichia coli* outer membrane protease that cleaves T7 RNA polymerase during purification, J. Bacteriol. 170, 1245-1253 (1988).
18. Bedouelle, H. Recognition of $tRNA^{Tyr}$ by tyrosyl-tRNA synthetase, Biochimie 72, 589-598 (1990).
19. Schoemaker, H.J. and Schimmel, P.R. Photo-induced joining of a transfer RNA with its cognate aminoacyl-transfer RNA synthetase, J. Mol. Biol. 84, 503-513 (1974).
20. Asahara, H., Himeno, H. and Shimizu, M. A recognition model of $tRNA^{Ser}$ by seryl-tRNA synthetase in *E. coli*, Chem. Lett. 363-366 (1991).
21. Biou, V., Yaremchuk, A., Tukalo, M. and Cusack, S. The 2.9 Å crystal structure of *T. thermophilus* seryl-tRNA synthetase complexed with $tRNA^{Ser}$, Science 263, 1404-1410 (1994).
22. Asahara, H., Himeno, H., Tamura, K., Hasegawa, T., Watanabe, K. and Shimizu, M. Recognition nucleotides of *Escherichia coli* $tRNA^{Leu}$ and its elements facilitating discrimination from $tRNA^{Ser}$ and $tRNA^{Tyr}$, J. Mol. Biol. 231, 219-229 (1993).
23. Asahara, H., Nameki, N. and Hasegawa, T. *In vitro* selection of RNAs aminoacylated by *Escherichia coli* leucyl-tRNA synthetase, J. Mol. Biol. 283, 605-618 (1998).
24. Nameki, N., Asahara, H., Shimizu, M., Okada, N. and Himeno, H. Identity elements of *Saccharomyces cerevisiae* $tRNA^{His}$, Nucleic Acids Res. 23, 389-394 (1995).
25. Nameki, N., Tamura, K., Himeno, H., Asahara, H., Hasegawa, T. and Shimizu, M. *Escherichia coli* $tRNA^{Asp}$ recognition mechanism differing from that of the yeast system, Biochem. Biophys. Res. Commun. 189, 856-862 (1992).
26. Li, S., Pelka, H. and Schulman, L.H. The anticodon and discriminator base are important for aminoacylation of *Escherichia coli* $tRNA^{Asn}$, J. Biol. Chem. 268, 18335-18339 (1993).
27. Himeno, H., Hasegawa, T., Ueda, T., Watanabe, K., Miura, K. and Shimizu, M. Role of the extra G-C pair at the end of the acceptor stem of $tRNA^{His}$ in aminoacylation, Nucleic Acids Res. 17, 7855-7863 (1989).

28. Bare, L.A. and Uhlenbeck, O.C. Aminoacylation of anticodon loop substituted yeast tyrosine transfer RNA, Biochemistry 24, 2354-2360 (1985).

29. Bare, L.A. and Uhlenbeck, O.C. Specific substitution into the anticodon loop of yeast tyrosine transfer RNA, Biochemistry 25, 5825-5830 (1986).

30. Lee, C.P. and RajBhandary, U.L. Mutants of *Escherichia coli* initiator tRNA that suppress amber codons in *Saccharomyces cerevisiae* and are aminoacylated with tyrosine by yeast extracts, Proc. Natl. Acad. Sci. USA 88, 11378-11382 (1991).

31. Doublié, S., Bricogne, G., Gilmore, C. and Carter, J., C. W. Tryptophanyl-tRNA synthetase crystal structure reveals an unexpected homology to tyrosyl-tRNA synthetase, Structure 3, 17-31 (1995).

32. Lenhard, B., Orellana, O., Ibba, M. and Weygand-Durasevic, I. tRNA recognition and evolution of determinants in seryl-tRNA synthesis, Nucleic Acids Res. 27, 721-729 (1999).

33. Himeno, H., Hasegawa, T., Asahara, H., Tamura, K. and Shimizu, M. Identity determinants of *E. coli* tryptophan tRNA, Nucleic Acids Res. 19, 6379-6382 (1991).