Cross-species aminoacylation of tRNA with a long variable arm between Escherichia coli and Saccharomyces cerevisiae

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INTRODUCTION

Each aminoacyl-tRNA synthetase must distinguish its cognate isoacceptors from the pool of tRNA molecules with apparently undistinguishable structures to ensure that the proper amino acid is inserted in response to a given codon. It has been shown that many aminoacyl-tRNA synthetases recognize a few sets of nucleotides in the L-shaped tertiary structure, which are concentrated on the anticodon arm and the acceptor stem containing the discriminator base, although the relative importance of these two widely spaced tRNA domains varies among tRNA species (1–3). In addition to these positive identity determinants, specific aminoacylation would require the absence of incorrect interactions with 19 noncognate synthetases, and some nucleotides have been exemplified to function as a negative identity determinant against noncognate aminoacyl-tRNA synthetase to avoid misaminoacylation. These studies have shown that identity elements of a tRNA often vary during evolution (9–16), although some are maintained (17–20). An artificial aminoacylation system comprised of a tRNA and an aminoacyl-tRNA synthetase from different sources often causes a unilateral aminoacylation specificity and sometimes causes a lack of amino acid specificity (21–23). These studies raise an interesting question as to how the mode of the tRNA identity determination has been changed in association with the evolution of tRNA.

Although most tRNAs have a similar cloverleaf-like secondary and L-shaped tertiary structure due to many constraints in the translational processes, they can be divided into two classes according to the length of the variable arm. Class I tRNAs have a short variable arm with 4–5 nucleotides, while class II tRNAs have a long variable arm with >10 nucleotides which can contribute to discrimination from many class I tRNAs. Prokaryotes, chloroplasts and mitochondria from lower eukaryotes have three amino acid specific class II tRNAs, tRNA^Ser, tRNA^Leu and tRNA^Tyr. On the other hand, eukaryotes and archaeabacteria have only two amino acid specific class II tRNAs, tRNA^Ser and tRNA^Leu. Animal mitochondria have no tRNA with a long variable arm. Concomitant with this different composition of tRNA in the class II tRNA system, there is an apparent difference in the recognition manner between prokaryotes and eukaryotes, as described below.

Aminoacyl-tRNA synthetases specific for Escherichia coli class II tRNAs have a novel style of tRNA recognition (Fig. 1a) (24). Escherichia coli leucyl-tRNA synthetase (LeuRS) recognizes the discriminator base (A73) but not the anticodon nucleotides, despite the fact that the second base (A35) is completely conserved within the tRNA^Leu isoacceptors (2,25). Escherichia coli seryl-tRNA synthetase (SerRS) recognizes the long variable

ABSTRACT

Prokaryotes have three amino acid specific class II tRNAs that possess a characteristic long variable arm, tRNA^Ser, tRNA^Leu and tRNA^Tyr, while eukaryotes have only two, tRNA^Ser and tRNA^Leu. Because eukaryotes have a long variable arm between Escherichia coli and Saccharomyces cerevisiae, both SerRS and LeuRS from E.coli were unable to aminoacylate yeast class II tRNAs; in contrast, the yeast counterparts were able to aminoacylate E.coli class II tRNAs. Yeast seryl-tRNA synthetase was able to aminoacylate not only E.coli tRNA^Ser but also tRNA^Leu and tRNA^Tyr, and yeast LeuRS was able to aminoacylate not only E.coli tRNA^Leu but also tRNA^Tyr. These results indicate that the recognition manner of class II tRNA, especially the discrimination strategy of each aminoacyl-tRNA synthetase against noncognate class II tRNAs, is significantly divergent between E.coli and yeast. This difference is thought to be due mainly to the different composition of class II tRNAs in E.coli and yeast.

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Figure 1. Cloverleaf secondary structures of class II tRNAs from *E. coli* (a) and *S. cerevisiae* (b). The sequences of *E. coli* and *S. cerevisiae* class II tRNAs, RL1660, RL1661, RL1662, RS1660, RS1661, RS1662, RS1663, RS1664, RL6280, RL6281, RL6282, RS6280, RS6281, and RS6282 (51), were used in the compilation, with nucleotide modifications ignored. The indicated nucleotides are those that are absolutely conserved within the isoacceptor tRNA species specific to each amino acid. Filled circles indicate the positions of sequence variation. Recognition elements for each cognate aminoacyl-tRNA synthetase are highlighted by white with a black background. A35 is indicated by open square in this figure, since one of the base substitutions at 35 causes a significant reduction in activity while others do not (28). Arrowheads indicate nucleotides required for conversion of aminoacylation specificity from the other class II tRNAs (see text). The sequences of *E. coli* tRNA Ser show variation in the number of nucleotides comprising the variable arm, indicated by a continuous line in (a). One of the three *S. cerevisiae* tRNA Leu isoacceptors (RL6282) has one extra base-pair at the base of the variable arm, designated by parentheses in (b). The G-U or U-G wobble base pair is shown by an open circle.

The recognition style of class II tRNAs in yeast is significantly different from that in *E. coli*. We found that yeast LeuRS recognizes the discriminator base (A73) and a few bases in the anticodon loop (A35 and G37) (28). Introduction of these three bases into tRNA Ser confers it with an efficient leucine acceptor ability. Note that the requirement of the anticodon is not conserved between *E. coli* and yeast (25) and even within eukaryotes (29). Yeast SerRS recognizes the long variable arm but not the discriminator base or the anticodon. Only one nucleotide insertion into the long variable arm confers an ability as an efficient serine acceptor upon yeast tRNA Leu (30).
elements involved in the identity change of class II tRNAs are concentrated in the hinge region of the molecule in *E. coli*, whereas they are dispersed in yeast. The specific aminoacylations of class II tRNAs in *E. coli* considerably depend on global structure-specific recognitions by SerRS and LeuRS, while those in yeast are mainly dependent on base-specific or local conformation-specific recognitions.

What has caused such a substantial difference in the recognition manner of class II tRNAs among species? It seems plausible that the recognition manner is significantly influenced by the structure and the number of cognate and noncognate tRNAs during evolution. In this study, we focused on the evolution of the recognition style of class II tRNAs and attempted a cross-aminoacylation study between *E. coli* and a yeast, *Saccharomyces cerevisiae*. It was revealed that unilateral aminoacylation specificity occurs between *E. coli* and yeast. From the results of the present study, as well as some earlier cross-species aminoacylation studies, we propose a rationale for the evolution of the tRNA identity.

**MATERIALS AND METHODS**

**Preparation of template DNAs and in vitro transcripts**

Synthetic DNA oligomers carrying the tRNA gene under the T7 promoter sequence were ligated into pUC19 and transformed into *E. coli* strain JM109 (31,32). The template DNA sequences were confirmed by dideoxy sequencing (33). 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, bovine serum albumin (50 µg/ml), 2.0 mM each NTP, 20 mM 5′-A260 unit) and tRNA Leu (UAG) (1500 pmol/A260 unit) were obtained from Subriden, A260 unit), tRNA Leu (CAG) (1450 pmol/A260 unit) and tRNA-Ser (VGA) (1300 pmol/A260 unit) were purified from *S. cerevisiae* tRNA. The transcripts were purified by 15% polyacrylamide gel electrophoresis.

**Aminoacylation assay**

LeuRS (35) and SerRS (36) were purified from *E. coli* strain Q13 by anion exchange column chromatography (DEAE-Toyopearl 650, Tosoh, Tokyo) and subsequent hydroxyapatite column chromatography (Gigapite, Seikagaku Corporation, Tokyo). The final SerRS fraction had a specific activity of 2734 U/mg (1 U of aminoacyl-tRNA synthetase activity was defined as the amount of the enzyme that catalyzes the incorporation of 1 nmol of amino acid into aminoacyl-tRNA in 10 min), which contains no detectable LeuRS. The final LeuRS fraction had a specific activity of 293 U/mg, which contains no detectable SerRS activity.

Yeast LeuRS (37,38) and SerRS (39) were purified from *S. cerevisiae* strain BJ926 (provided by Dr Y. Ohsumi, University of Tokyo) by anion exchange column chromatography and subsequent hydroxyapatite column chromatography. The final SerRS fraction had a specific activity of 53 U/mg, which contains no detectable LeuRS. The final LeuRS fraction had a specific activity of 500 U/mg, which contains no detectable SerRS.

*Escherichia coli* native tRNASer(VGA) (1300 pmol/A260 unit), tRNALeu(CAG) (1450 pmol/A260 unit) and tRNA-Ser(IGA) (1500 pmol/A260 unit) were obtained from Subriden. *Saccharomyces cerevisiae* tRNASer(JGA) (1500 pmol/A260 unit) and tRNALeu(UAG) (1500 pmol/A260 unit) were purified from *S. cerevisiae* crude tRNA fraction according to the method of Tsurui et al. (40), described earlier (28,30).

The aminoacylation reaction proceeded at 37 and 30°C for *E. coli* and *S. cerevisiae* aminoacyl-tRNA synthetases, respectively. The reaction mixture contained 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, 21 µM L-[U-14C]leucine (11 GBq/mmol) or 35 µM L-[U-14C]serine (5.22 GBq/mmol), 0.5–1.25 µM transcript RNA and various concentrations of SerRS and LeuRS.

Each kinetic parameter was determined from a plot of [S] against [S]/v ([S], tRNA concentration; v, observed initial velocity of serylation or leucylation).

**RESULTS**

Neither SerRS nor LeuRS from *E. coli* aminoacylates class II tRNAs from *S. cerevisiae*

In general, the *in vitro* transcript of tRNA with no modified nucleotides is a good substrate for aminoacylation. This is also applicable to tRNASer, tRNALeu and tRNA-Tyr from *E. coli*, and to tRNASer and tRNALeu from *S. cerevisiae* (25–28,30,41). In this study, we prepared class II tRNA transcripts from *E. coli* and *S. cerevisiae* (Fig. 2), and we examined their aminoacylation specificities towards *E. coli* SerRS and LeuRS. As shown in Figure 3a and b and Tables 1 and 2, neither *S. cerevisiae* tRNA tRNA Ser transcript nor *tRNA Leu* transcript was aminoacylated by *E. coli* SerRS and LeuRS. This is also the case for native *S. cerevisiae* tRNA Ser and tRNA Leu (Fig. 3a and b).

<table>
<thead>
<tr>
<th>tRNA</th>
<th>Km (µM)</th>
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<th>Vmax/Km (relative)</th>
<th>Loss of activity</th>
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<tr>
<td>LeuRS</td>
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<td>–</td>
<td>&lt;0.001</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>tRNA</th>
<th>Km (µM)</th>
<th>Vmax (relative)</th>
<th>Vmax/Km (relative)</th>
<th>Loss of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LeuRS</td>
<td>–</td>
<td>–</td>
<td>&lt;0.001</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

Table 1. Kinetic parameters of *E. coli* seryl-tRNA synthetase for class II tRNA transcripts

Table 2. Kinetic parameters of *E. coli* leucyl-tRNA synthetase for class II tRNA transcripts
Figure 2. The transcripts of class II tRNAs from *E.coli* (a) and *S.cerevisiae* (b) used in this study. The sequences were from RS1664, RL1661 and RY1661 for *E.coli* tRNAs and from RS6281 and RL6281 for *S.cerevisiae* tRNAs. The primary sequences of native tRNAs used in this study correspond to those of their transcripts (Materials and Methods; 51).

Considering that *S.cerevisiae* tRNA<sup>Ser</sup> and tRNA<sup>Leu</sup> have some positive recognition elements for *E.coli* synthetases, such as the variable arm for SerRS and the discriminator base (A73) for LeuRS, the above results can be attributed to the lack of some structural requirements for *E.coli* synthetases (Fig. 1a). As mentioned above, both *E.coli* SerRS and LeuRS recruit a discrimination style that is mainly based on the difference in the tertiary structure, which is created by several tertiary nucleotides, i.e. the location of G18G19 in the D-loop, the tertiary Pu15–Py48 base pair and the number of the unpaired nucleotides at the base of the long variable arm. In addition, SerRS requires a certain length of the long variable arm, although in a base-non-specific manner. G15–C48 and the base number comprising the D-loop as well as G73 in *S.cerevisiae* tRNA<sup>Ser</sup>, and G15–C48 and the location of the G18G19 sequence in *S.cerevisiae* tRNA<sup>Leu</sup> are not appropriate for *E.coli* LeuRS. For *E.coli* SerRS, both *S.cerevisiae* tRNA<sup>Ser</sup> and tRNA<sup>Leu</sup> do not have the proper number of unpaired nucleotides at the base of the long variable arm.

The present results are consistent with an *in vivo* study showing that tRNA<sup>Ser</sup> from eukaryotes (*Schizosaccharomyces pombe* and human) are not functional in *E.coli* (42).

*Saccharomyces cerevisiae* SerRS can recognize all the three class II tRNAs from *E.coli*.

Since serine is coded by two distinct codon boxes, no base in the anticodon is conserved among the tRNA<sup>Ser</sup> isoacceptors. As a consequence, SerRS adopts a quite unique recognition system of tRNA<sup>Ser</sup>. In *E.coli*, *Thermus thermophilus* and *S.cerevisiae*, the anticodon is not involved in recognition by SerRS (27,30,43). The discriminator base G73, which is absolutely conserved among prokaryotic and eukaryotic serine tRNAs, is not important for recognition by SerRS in *E.coli* and *S.cerevisiae*, although this nucleotide serves as a negative identity determinant against *S.cerevisiae* LeuRS (28). Instead of these typical recognition elements for class I tRNAs, the long projecting variable arm plays a dominant role in recognition by SerRS in *E.coli* (26,27,30,44), in *T.thermophilus* (43), and in *S.cerevisiae* and humans (45,46). The importance of the long variable arm of yeast tRNA<sup>Ser</sup> has also been suggested by a footprinting study (47). In *S.cerevisiae*, only one nucleotide insertion into the long variable arm confers an efficient serine acceptor activity upon tRNA<sup>Leu</sup> (30).
Escherichia coli tRNA$^{\text{Ser}}$, tRNA$^{\text{Leu}}$ and tRNA$^{\text{Tyr}}$ possess variable arms with 15–20, 14 and 12 nucleotides, respectively (Fig. 1a). All of these variable arms seem long enough for aminoacylation by S.cerevisiae SerRS. We examined the serylation ability of three E.coli class II tRNA transcripts toward S.cerevisiae SerRS. As shown in Figure 3c, yeast SerRS efficiently aminoacylated the E.coli tRNA$^{\text{Ser}}$ transcript. The $K_m$ and $V_{\text{max}}$ values were $\sim$2-fold higher and 2-fold lower, respectively, than those of the S.cerevisiae tRNA$^{\text{Ser}}$ transcript (Table 3). This result is consistent with an in vivo study showing that E.coli tRNA$^{\text{Ser}}$ can be recognized by yeast SerRS expressed in E.coli (19). The E.coli tRNA$^{\text{Leu}}$ and tRNA$^{\text{Tyr}}$ transcripts were also serylated by S.cerevisiae SerRS, although with a $V_{\text{max}}/K_m$ that is 200-fold lower than that of the S.cerevisiae tRNA$^{\text{Ser}}$ transcript (Table 3).
This type of broad specificity has sometimes been observed for unmodified tRNA, probably because the modifications serve as a negative identity determinant by themselves or because they contribute to make a less relaxed tertiary structure (48). Indeed, S.cerevisiae tRNALeu transcript, but not native tRNALeu, is a weak substrate for S.cerevisiae SerRS (30). We therefore examined the seryl transfer ability of native E.coli class II tRNAs towards S.cerevisiae SerRS. A similar broad specificity was also observed for these native E.coli class II tRNAs (Fig. 3c). Among 10 tRNAs examined in the present study, native S.cerevisiae tRNALeu is the only tRNA that has no detectable serine acceptor activity towards S.cerevisiae SerRS (Fig. 3c).

These results emphasize the importance of the length of the variable arm for recognition by S.cerevisiae SerRS. Besides, the observed broad specificity against E.coli class II tRNAs indicates that S.cerevisiae SerRS can accommodate various types of long variable arms as well as various types of tertiary structures. Taken together, the mode of discrimination against noncognate class II tRNAs by SerRS appears less stringent than that in E.coli.

**S.cerevisiae LeuRS can recognize E.coli tRNALeu and tRNA^{Tyr}**

A previous study showed that a few bases in the anticodon arm (A35 and G37) and the discriminator base (A73) are important for leucylation in S.cerevisiae and that introduction of these identity elements converted tRNA^{Ser} into an efficient leucine acceptor with a $V_{\text{max}}/K_m$ that is 2-fold lower than that of the wild type tRNA^{Leu} (28). These identity elements of yeast tRNA^{Leu} are located at both ends of the L-shaped tertiary structure. This sort of base-specific recognition manner is quite popular among the recognitions for class I tRNAs. However, it is significantly different from that of E.coli tRNA^{Leu}, in which the anticodon is not involved (25). In other eukaryotes, beans and humans, the anticodon is also not important for recognition by LeuRS (29,49,50). The tRNA^{Leu} recognition system is the only example that shows variability in the requirement of the anticodon for aminoacylation among species.

The E.coli tRNA^{Leu} transcript as well as native tRNA^{Leu} was efficiently aminoacylated by S.cerevisiae LeuRS (Fig. 3d). The $K_m$ value was almost the same as that of S.cerevisiae tRNA^{Leu}, and the $V_{\text{max}}$ value was ∼4-fold lower (Table 4). It appears quite reasonable that this cross-species aminoacylation proceeded efficiently, since E.coli tRNA^{Leu} possesses all of the above three identity elements for S.cerevisiae LeuRS, A73, A35 and G37 (Fig. 1a).

### Table 3. Kinetic parameters of S.cerevisiae seryl-tRNA synthetase for class II tRNA transcripts

<table>
<thead>
<tr>
<th>S.cerevisiae transcripts</th>
<th>$K_m$ (µM)</th>
<th>$V_{\text{max}}$ (relative)</th>
<th>$V_{\text{max}}/K_m$ (relative)</th>
<th>Loss of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA^{Ser}</td>
<td>0.218</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>tRNA^{Leu}</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>tRNA^{Tyr}</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>E.coli transcripts</td>
<td></td>
<td></td>
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<tr>
<td>tRNA^{Ser}</td>
<td>0.486</td>
<td>0.534</td>
<td>0.240</td>
<td>4</td>
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<tr>
<td>tRNA^{Leu}</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>tRNA^{Tyr}</td>
<td></td>
<td></td>
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</table>

### Table 4. Kinetic parameters of S.cerevisiae leucyl-tRNA synthetase for class II tRNA transcripts

<table>
<thead>
<tr>
<th>S.cerevisiae transcripts</th>
<th>$K_m$ (µM)</th>
<th>$V_{\text{max}}$ (relative)</th>
<th>$V_{\text{max}}/K_m$ (relative)</th>
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<td>tRNA^{Ser}</td>
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<tr>
<td>tRNA^{Tyr}</td>
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<tr>
<td>E.coli transcripts</td>
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<td>tRNA^{Leu}</td>
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<td>0.278</td>
<td>0.257</td>
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<td>tRNA^{Ser}</td>
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</tr>
<tr>
<td>tRNA^{Tyr}</td>
<td></td>
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The E.coli tRNA^{Tyr} transcript was also aminoacylated by S.cerevisiae LeuRS with a $V_{\text{max}}/K_m$ that is 18-fold lower than that for S.cerevisiae tRNA^{Leu} (Table 4). Native E.coli tRNA^{Tyr} was also aminoacylated by S.cerevisiae LeuRS to a similar extent (Fig. 3d). Escherichia coli tRNA^{Tyr} has U35, A37 and A73, only the last of which fulfills the requirement for recognition by S.cerevisiae LeuRS (Fig. 1a). Our previous study has shown that a G37→A37 mutation in S.cerevisiae tRNA^{Leu} causes an ∼20-fold decrease in LeuRS activity, while a A35→U35 mutation exerts a much less significant effect (28). Therefore, the observed low efficiency of leucylation for E.coli tRNA^{Tyr} in the present study is attributed mainly to A37. This result is consistent with a previous in vivo study showing that E.coli tRNA^{Tyr} can behave as a leucine tRNA in yeast (23).

Native E.coli tRNA^{Ser} or its transcript, which has none of these three identity elements for S.cerevisiae LeuRS (Fig. 1a), had no detectable leucine acceptor activity (Fig. 3d).

**DISCUSSION**

Specific aminoacylation for every tRNA is maintained not only by the specific interaction of the identity elements with its cognate aminoacyl-tRNA synthetase, but also by the absence of incorrect interactions with 19 noncognate synthetases. Has such an elaborate system evolved and undergone evolution while maintaining a high degree of accuracy? Because of several phylogenetic differences, class II tRNAs are expected to provide an evolutionarily conserved system appeared and undergone evolution while maintaining a high degree of accuracy (51). Mitochondria of lower eukaryotes or plants and chloroplasts are categorized to the prokaryotic type, and archaebacteria to the eukaryotic type.

The second difference is in the composition of class II tRNAs. A clear line can be drawn between prokaryotes and eukaryotes (51). Chloroplasts and mitochondria of lower eukaryotes or plants, while a structural similarity is widely conserved among eukaryotes (52).

The third difference is in the sequence and structure of SerRS and LeuRS. Bacterial SerRS is a homodimer, each subunit having two domains, the N-terminal α-helical coiled coil domain, which
interacts productively with the long variable arm of tRNA^Ser, and the C-terminal catalytic domain typical to class 2 aminoacyl-tRNA synthetases (43). The C-terminal half has high amino acid sequence similarity between E.coli and S.cerevisiae, but the N-terminal half does not (24,36,39,52,53). On the other hand, only a faint sequence similarity between E.coli and S.cerevisiae can be observed over the entire region of LeuRS, which is categorized as class 1 aminoacyl-tRNA synthetase (35,38).

The fourth difference is in the recognition manner of class II tRNAs (25–28,30). Both E.coli SerRS and LeuRS recognize their cognate tRNAs mainly in a global structure-specific manner, based on the characteristic spatial arrangement of the recognition groups in the L-shaped structure. In contrast, yeast SerRS and LeuRS recognize mainly in a base-specific or local conformation-specific manner, based on the characteristic chemical groups on a common global tertiary structure.

The results of the present study revealed a fifth difference: the substrate stringency upon recognition by synthetase. Both SerRS and LeuRS in E.coli are exclusive, while those in yeast can accommodate various L-frameworks possessing a long variable arm.

These five differences are thought to be closely interrelated. The substantial difference in the recognition of tRNA^Leu between E.coli and S.cerevisiae might reflect the lack of sequence conservation of LeuRS. The presence of the structural characteristic in each tRNA is a prerequisite for structure-dependent recognition. Therefore, it is reasonable that E.coli has acquired a global structure-dependent recognition style, while yeast has not. The structural characteristic can act as an obstacle for some noncognate synthetases, and it also modulates the spatial arrangement of positive recognition elements. Such a structural obstacle would allow more stringent discrimination, as in the E.coli class II tRNA system. It is also conceivable that the composition of tRNAs in each system causes the difference of substrate stringency of aminoacyl-tRNA synthetase, as discussed below.

A similar unilateral aminoacylation has also been observed between bovine mitochondria and eubacteria (21,22). Animal mitochondria have only a minimal set of tRNAs, 22 species. Although they often have non-canonical secondary structures, none of them has a long variable arm. Escherichia coli or T.thermophilus phenylalanyl-tRNA synthetase, threonyl-tRNA synthetase, arginyl-tRNA synthetase, lysyl-tRNA synthetase and SerRS cannot aminoacylate bovine mitochondrial tRNAs. In contrast, bovine mitochondrial aminoacyl-tRNA synthetase are capable of aminoacylating E.coli tRNAs. Among these five synthetases in mitochondria, only SerRS can aminoacylate not only its amino acid-specific tRNA, tRNA^Ser, but also many serine-non-specific tRNAs of eubacteria, while the aminoacylations by the other synthetases maintain the amino acid-specificity for tRNA. This is probably due to the absence of anticondon recognition only for SerRS (54).

Judging from the two heterologous aminoacylation systems between E.coli and yeast class II tRNAs and between animal mitochondrial and eubacterial tRNAs, the number of tRNAs comprising one system seems to significantly influence the evolution of the mode of tRNA discrimination. Either E.coli SerRS or LeuRS is obliged to exclude two amino acid-specific noncognate class II tRNAs, whereas excluding only one is sufficient for yeast SerRS or LeuRS. Each eubacterial aminoacyl-tRNA synthetase is required to exclude >50 tRNAs, whereas excluding only 20 or 21 tRNAs is sufficient for each animal mitochondrial synthetase. Thus, we can draw the rationale that the higher the number of similar-shaped tRNAs in a system are, the more exclusive the recognition manner aminoacyl-tRNA synthetase becomes during evolution.

The length of the variable arm of tRNA^Tyr is dramatically changed during evolution, probably reflecting the divergence of TyrRS recognition. Its recognition manner would have exerted some influence on the structures of tRNA^Ser and tRNA^Leu in order to avoid misrecognition. Escherichia coli TyrRS may reject tRNA^Ser and tRNA^Leu by discriminating the anticodon sequence (and also the discriminator base for tRNA^Ser) (26). Saccharomyces cerevisiae TyrRS may reject tRNA^Ser and tRNA^Leu by discriminating the anticodon sequence (55) and the first base pair of the acceptor stem (56,57). In E.coli, the tertiary structural variation of class II tRNAs may also contribute, as has been suggested by the involvement of the unpaired nucleotides at the base of the long variable arm in recognition (58). This would reasonably explain an earlier finding that E.coli TyrRS cannot recognize S.cerevisiae tRNA^Tyr, while S.cerevisiae TyrRS can recognize E.coli tRNA^Tyr (58). It is tempting to speculate which type of tRNA^Tyr structure is the prototype and how it changed the recognition system of class II tRNAs.

In some groups of Candida species classified as an asporogenic yeast, the CUG leucine codon is translated as serine (59). This change of codon assignment is suggested to be caused by a change in the acceptor identity of tRNA possessing a CAG anticodon from a leucine acceptor to a serine acceptor (60). In S.cerevisiae, such identity conversion requires at least three mutations on tRNA^Leu, one nucleotide insertion into the long variable arm for SerRS, and base substitutions at 37 and 73 to avoid misrecognition by LeuRS. In fact, Candida zeylanoides tRNA^Ser(CAG) possessing m^G37 and G73 functions as a weak leucine acceptor as well as a full serine acceptor in vitro and also in vivo. The combination of LeuRS and SerRS, both possessing a less exclusive discrimination manner as in the case of S.cerevisiae shown in this study, would have allowed the appearance of tRNA having multiple specificity during the evolution of Candida species.

The recognition elements of tRNA^Ser and tRNA^Leu are significantly diverged between yeast and higher eukaryotes (48,49,61). It has yet to be clarified whether the class II tRNA recognition manner potentially involving a broad specificity is limited to yeast or it occurs widely in other eukaryotes. It is conceivable that such a recognition manner could accelerate the evolution of identity elements, as well as that of the sequence and structure of aminoacyl-tRNA synthetase.

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