4374–4381 *Nucleic Acids Research, 1998, Vol. 26, No. 19*

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

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Received June 29, 1998; Revised and Accepted August 18, 1998

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 of such a phylogenetic divergence in the composition of tRNA, the class II tRNA system is a good candidate for studying how the tRNA recognition manner has evolved in association with the evolution of tRNA. We report here a cross-species aminoacylation study of the class II tRNAs, showing the unilateral aminoacylation specificity between *Escherichia coli* and a yeast, *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.

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 synthetases to avoid misaminoacylation. The coordination of these two types of identity determinants keep misaminoacylation by noncognate aminoacyl-tRNA synthetase free, although it can occur under special conditions such as in an organic solvent (4–8).

Recent 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 archaebacteria 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

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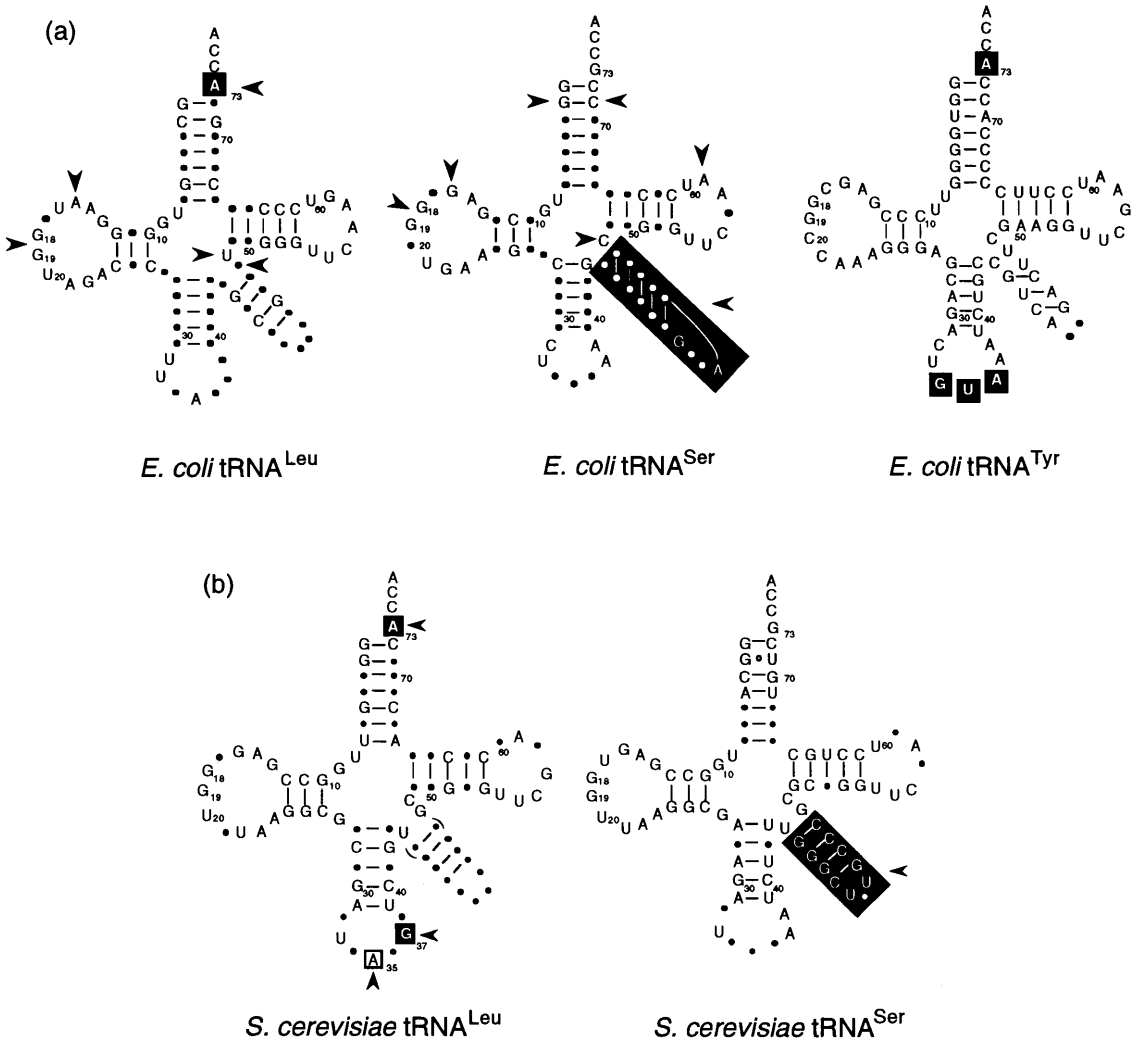

Figure 1. Cloverleaf secondary structures of class II tRNAs from *E.coli* (**a**) and *S.cerevisiae* (**b**). The sequences of *E.coli* and *S.cerevisiae* class II tRNAs, RL1660, RL1661, RL1662, RS1660, RS1661, RS1662, RS1663, RS1664, RY1660, RY1661, RL6280, RL6281, RL6282, RS6280, RS6281 and RS6282 (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.

arm of $tRNA^{Ser}$ but not the discriminator base (G73), which is completely conserved among the isoacceptors, or the anticodon (26,27). Among the three aminoacyl-tRNA synthetases for class II tRNAs, tyrosyl-tRNA synthetase (TyrRS) is the only one that recognizes both the discriminator base and the anticodon like many aminoacyl-tRNA synthetases for class I tRNAs. In addition to these positive recognition elements, a high degree of accurate discrimination among the three class II tRNAs by SerRS and LeuRS is accomplished in a unique style, based mainly on their tertiary structural characteristics created by the locations of the invariant G18G19 sequence in the D-arm, the semi-invariant Pu15–Py48 tertiary base pair, the base at 59 in the TψC loop, and also the unpaired nucleotides at the base of the variable arm (25–27). Exchanging a set of these structural elements (indicated by arrowheads in Fig. 1a) is required to transform either $tRNA^{Ser}$ or $tRNA^{Tyr}$ into an efficient leucine acceptor, and it is also required to transform either $tRNA^{Leu}$ or $tRNA^{Tyr}$ into a serine acceptor.

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). These

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′ GMP, *Bst*NI-digested template DNA (0.2 mg/ml), 2 U of inorganic pyrophosphatase (Sigma, St Louis, MO), and pure T7 RNA polymerase (50 µg/ml) (31,34). 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 hydroxy apatite 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 hydroxy apatite 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 $tRNA^{Ser}_1$(VGA) (1300 pmol/A_{260} unit), $tRNA^{Leu}_1$(CAG) (1450 pmol/A_{260} unit) and $tRNA^{Tyr}_1$(QUA) (1500 pmol/A_{260} unit) were obtained from Subriden RNA. *Saccharomyces cerevisiae* $tRNA^{Ser}$(IGA) (1500 pmol/A_{260} unit) and $tRNA^{Leu}$(UAG) (1500 pmol/A_{260} 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-^{14}C]leucine (11 GBq/mmol) or 35 µM L-[U-^{14}C]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 $tRNA^{Ser}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$ from *E.coli*, and to $tRNA^{Ser}$ and $tRNA^{Leu}$ 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* $tRNA^{Ser}$ 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 1. Kinetic parameters of *E.coli* seryl-tRNA synthetase for class II tRNA transcripts

	K_m (µM)	V_{max} (relative)	V_{max}/K_m (relative)	Loss of activity
E.coli transcripts				
$tRNA^{Ser}$	0.54	1	1	1
$tRNA^{Leu}$	–	–	<0.001	>1000
$tRNA^{Tyr}$	–	–	<0.001	>1000
S.cerevisiae transcripts				
$tRNA^{Leu}$	–	–	<0.001	>1000
$tRNA^{Ser}$	–	–	<0.001	>1000

Table 2. Kinetic parameters of *E.coli* leucyl-tRNA synthetase for class II tRNA transcripts

	K_m (µM)	V_{max} (relative)	V_{max}/K_m (relative)	Loss of activity
E.coli transcripts				
$tRNA^{Leu}$	0.54	1	1	1
$tRNA^{Ser}$	–	–	<0.001	>1000
$tRNA^{Tyr}$	–	–	<0.001	>1000
S.cerevisiae transcripts				
$tRNA^{Leu}$	–	–	<0.001	>1000
$tRNA^{Ser}$	–	–	<0.001	>1000

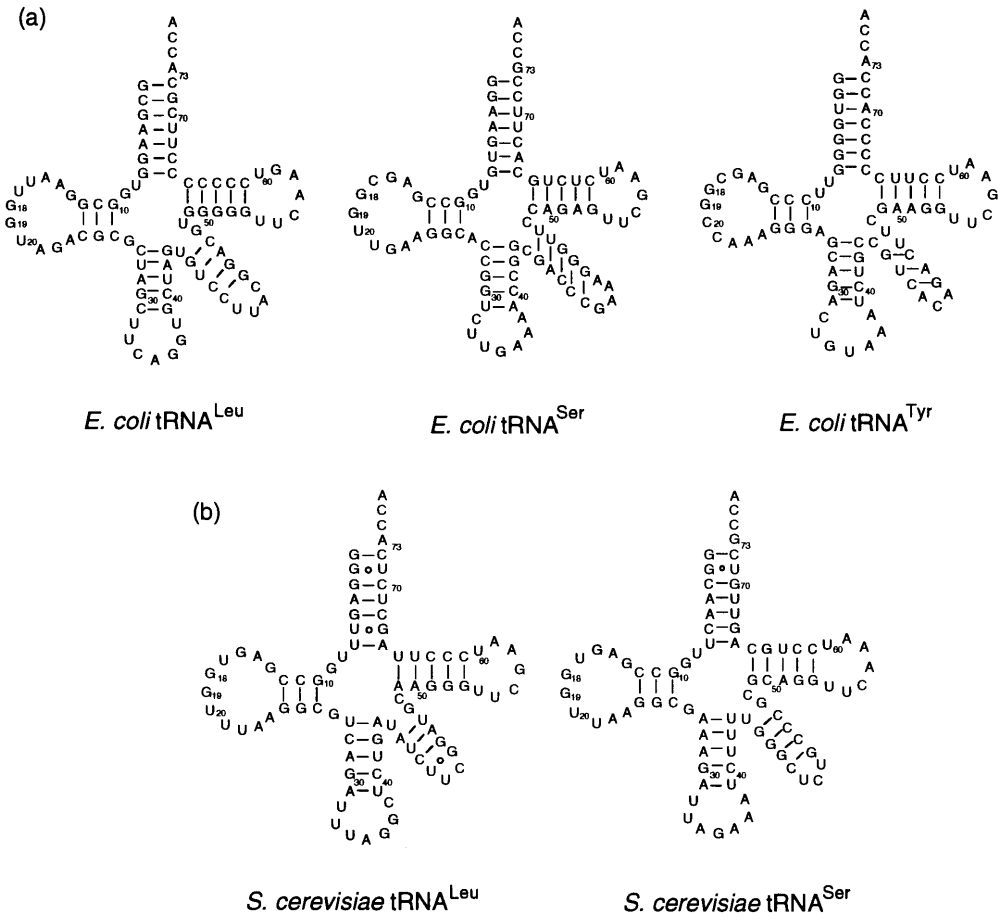

Figure 2. The transcripts of class II tRNAs from *E.coli* (**a**) and *S.cerevisiae* (**b**) used in this study. The sequences were from RS1664, RL1661 and RY1661 for *E.coli* tRNAs and from RS6281 and RL6281 for *S.cerevisiae* tRNAs. The primary sequences of native tRNAs used in this study correspond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

Considering that *S.cerevisiae* $tRNA^{Ser}$ and $tRNA^{Leu}$ 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* $tRNA^{Ser}$, and G15–C48 and the location of the G18G19 sequence in *S.cerevisiae* $tRNA^{Leu}$ are not appropriate for *E.coli* LeuRS. For *E.coli* SerRS, both *S.cerevisiae* $tRNA^{Ser}$ and $tRNA^{Leu}$ 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 $tRNA^{Ser}$ 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 $tRNA^{Ser}$ isoacceptors. As a consequence, SerRS adopts a quite unique recognition system of $tRNA^{Ser}$. 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 $tRNA^{Ser}$ 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 $tRNA^{Leu}$ (30).

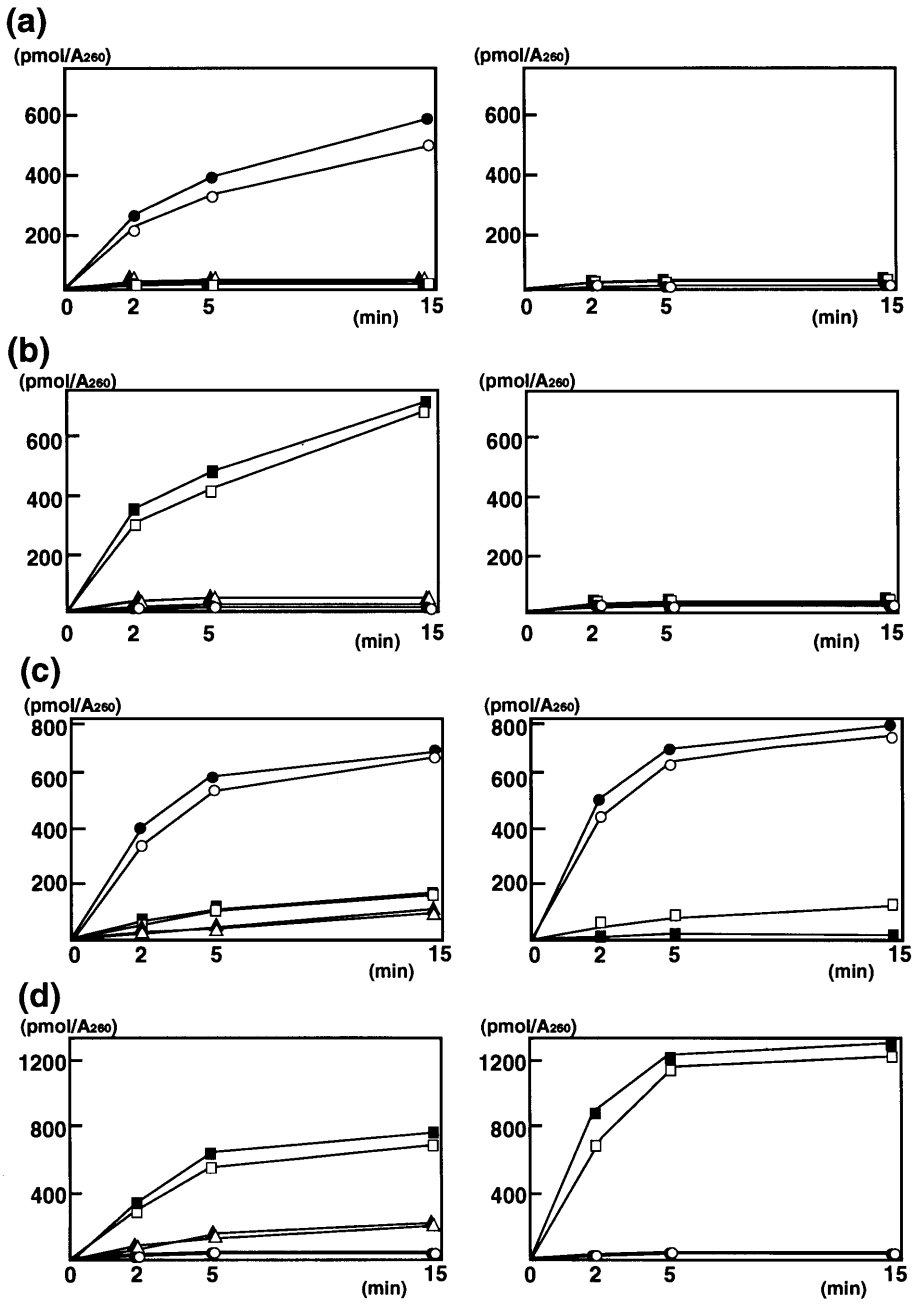

Figure 3. Aminoacylation of class II tRNAs from *E.coli* (left) and *S.cerevisiae* (right) with *E.coli* SerRS (**a**), *E.coli* LeuRS (**b**), *S.cerevisiae* SerRS (**c**) and *S.cerevisiae* LeuRS (**d**). $tRNA^{Ser}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$ are designated by a circle, square and triangle, respectively. Native tRNA and tRNA transcript are designated by filled and open symbols, respectively. Forty microlitres of the reaction mixture contained 15 pmol of tRNA substrate, and 3.9×10^{-3} U *E.coli* SerRS (a), 1.8×10^{-3} U *E.coli* LeuRS (b), 5.56×10^{-3} U *S.cerevisiae* SerRS (c) and 6.36×10^{-3} U *S.cerevisiae* LeuRS (d). At each time point, a 10 µl aliquot was withdrawn and spotted on Whatman 3MM filter paper, and the cold 5% trichloroacetic acid insoluble fraction was counted in a liquid scintillation counter.

Escherichia coli $tRNA^{Ser}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$ 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* $tRNA^{Ser}$ transcript. The K_m and V_{max} values were ~2-fold higher and 2-fold lower, respectively, than those of the *S.cerevisiae* $tRNA^{Ser}$ transcript (Table 3). This result is consistent with an *in vivo* study showing that *E.coli* $tRNA^{Ser}$ can be recognized by yeast SerRS expressed in *E.coli* (19). The *E.coli* $tRNA^{Leu}$ and $tRNA^{Tyr}$ transcripts were also serylated by *S.cerevisiae* SerRS, although with a V_{max}/K_m that is 200-fold lower than that of the *S.cerevisiae* $tRNA^{Ser}$ transcript (Table 3).

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

	K_m (μM)	V_{max} (relative)	V_{max}/K_m (relative)	Loss of activity
S.cerevisiae transcripts				
$tRNA^{Ser}$	0.218	1	1	1
$tRNA^{Leu}$	–	–	0.0008	1250
E.coli transcripts				
$tRNA^{Ser}$	0.486	0.534	0.240	4
$tRNA^{Leu}$	–	–	0.005	200
$tRNA^{Tyr}$	–	–	0.005	200

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

	K_m (μM)	V_{max} (relative)	V_{max}/K_m (relative)	Loss of activity
S.cerevisiae transcripts				
$tRNA^{Leu}$	0.455	1	1	1
$tRNA^{Ser}$	–	–	<0.0005	>2000
E.coli transcripts				
$tRNA^{Leu}$	0.493	0.278	0.257	4
$tRNA^{Ser}$	—	—	<0.0005	>2000
$tRNA^{Tyr}$	—	—	0.055	18

This type of broad specificity has sometimes been observed for unmodified tRNA transcript, 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* $tRNA^{Leu}$ transcript, but not native $tRNA^{Leu}$, is a weak substrate for *S.cerevisiae* SerRS (30). We therefore examined the serylation 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* $tRNA^{Leu}$ 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 accomodate 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* $tRNA^{Leu}$ 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_{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_{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).

The *E.coli* $tRNA^{Tyr}$ transcript was also aminoacylated by *S.cerevisiae* LeuRS with a V_{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 an A35→U35 mutation exerts a much less significant effect (28). Therefore, the observed low effciency 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. How has such an elaborate system appeared and undergone evolution while maintaining a high degree of accuracy? Because of several phylogenetic differences, class II tRNAs are expected to provide a good model system to study how the tRNA recognition has evolved in association with the evolution of tRNA, both in shape and in number. The differences between the *E.coli* and yeast class II tRNA systems can be summarized to the following points.

The first difference is in the composition of class II tRNAs. A clear line can be drawn between prokaryotes and eukaryotes (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 secondary or tertiary structure of the tRNA molecule (Fig. 1). Class II tRNAs in *E.coli* have an apparent structural variation, while yeast class II tRNAs share a similar secondary structure. A structural variation within class II tRNAs is observed in many other prokaryotes, 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 accomodate 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 synthetases 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 anticodon 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 an 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^1G37 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.

ACKNOWLEDGEMENTS

We would like to thank Drs K. Nishikawa, Y. Kumazawa, T. Yokogawa, T. Suzuki, N. Nameki, H. Asahara, T. Hasegawa, S. Nakamura, C. Ushida, A. Muto and M. Shimizu for their kind advice concerning this work and for their many useful discussions. We also thank the Gene Research Center of Hirosaki University for the use of the facility. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan.

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