The Molecular Basis for Unilateral Aminoacylation Specificity between Mitochondria and Bacteria: A Case for Seryl-tRNA Synthetase

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1 Introduction

Mitochondrial (mt) aminoacyl-tRNA synthetases (aaRSs) are able to charge both mt and bacterial cognate tRNAs. Conversely, most bacterial synthetases, including those for phenylalanine (Phe), threonine (Thr), arginine (Arg), lysine (Lys), serine (Ser) and aspartic acid (Asp), are only able to charge bacterial cognate tRNAs. This phenomenon is termed unilateral aminoacylation specificity between mitochondria and bacteria. Comparison of the amino acid sequences of bacterial synthetases from *Escherichia coli* (*E. coli*) and *Thermus thermophilus* (*T. thermophilus*) with those from bovine (or human for AspRS) mitochondria, revealed that, in most cases, amino acid sequences are very similar, except for the existence of some extra insertions that are present only in mt synthetases.

In general, the identity elements (or identity determinants, which are the defined nucleotides or regions in tRNA recognized by the cognate aaRS) of *E. coli* tRNAs are indistinguishable or very similar to those of mt tRNAs for five species of amino acids (Phe, Thr, Arg, Lys and Asp); however, the identity elements of *E. coli* serine tRNAs (tRNAs\(^{\text{Ser}}\)) are quite different from those of mt tRNAs\(^{\text{Ser}}\).

Since none of the identity elements of *E. coli* tRNAs\(^{\text{Ser}}\) are found in mt tRNAs\(^{\text{Ser}}\), and mt tRNAs\(^{\text{Ser}}\) consist of two species with quite different secondary structures, namely tRNA\(^{\text{Ser}}\)\(_{\text{GCU}}\) and tRNA\(^{\text{Ser}}\)\(_{\text{UGA}}\), we predicted that structural analysis of mt SerRS recognizing both *E. coli* tRNAs\(^{\text{Ser}}\) and mt tRNAs\(^{\text{Ser}}\) would be useful for elucidating the molecular mechanism underlying unilateral aminoacylation specificity. The tertiary structure of bovine mt SerRS analyzed by X-ray crystallography at 1.65 Å was almost identical to that of *T. thermophilus* SerRS with the exception of the existence of three extra insertions in bovine mt SerRS, including the distal helix at the N-terminus, a tip loop between the two α-helical domains, and the C-tail at the C-terminus. The distal helix and C-tail were demonstrated to be required for recognition of mt tRNAs\(^{\text{Ser}}\) by mt SerRS. Thus, the RNA-sandwich model was proposed, in which the TΨC loop of mt tRNA\(^{\text{Ser}}\) is clamped by the distal helix and the C-tail at one side, and by the helical arm of SerRS at the opposite side. It is presumed that the unilateral aminoacylation specificity is conferred by these two extra domains, which exist only in mt SerRS.

This review will discuss whether the recognition mechanism of mt SerRS is common to other aaRS systems and how such a mechanism would have emerged in mt synthetases during the course of evolution.

2 Unilateral Aminoacylation Specificity of Aminoacyl-tRNA Synthetases between Mitochondria and Bacteria

It is widely accepted that mitochondria arose from a bacterial origin; hence their translational apparatus are more similar to bacterial than cytoplasmic counterparts (Margulis, 1970). We have studied animal mt genetic systems extensively, including the genetic code, tRNAs, enzymes, ribosomes, and factors involved in translation (Watanabe, 2010). This review deals with the aminoacylation specificity of aaRSs from bovine mitochondria and bacteria (*E. coli* and *T. thermophilus*) towards their respective tRNAs.
### Table 1: Cross-species aminoacylation among bovine mitochondrial (mt), *E. coli*, and *T. thermophilus* aminoacyl-tRNA synthetases (aaRSs) in Phe-, Thr-, Arg-, Lys- and Ser-specific reactions. Data represent pmol of acid-insoluble \( ^{14} \)C radioactivity for 30 min at 37°C (bovine mt and *E. coli* synthetases) or 65°C (*T. thermophilus* synthetases), and are cited from Kumazawa et al. (1991) for Thr-, Arg-, Lys- and Ser-specific reactions, and from Kumazawa et al. (1989) for Phe-specific reactions. The numbers in parentheses are percent aminoacylation activities when the values obtained by combination of bovine mt tRNA and bovine mt aaRSs are estimated as 100%. All the tRNAs used in this table are native unfractionated tRNAs isolated from bovine mitochondria, *E. coli*, and *T. thermophilus*.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>aaRS</th>
<th>tRNA</th>
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<tbody>
<tr>
<td></td>
<td>Bovine mt</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Phe</td>
<td>Bovine mt</td>
<td>2.4 (100)</td>
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<tr>
<td></td>
<td><em>E. coli</em></td>
<td>&lt;0.1 (&lt;4.2)</td>
</tr>
<tr>
<td></td>
<td><em>T. thermophilus</em></td>
<td>&lt;0.1 (&lt;4.2)</td>
</tr>
<tr>
<td>Thr</td>
<td>Bovine mt</td>
<td>3.1 (100)</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>0.1 (3.2)</td>
</tr>
<tr>
<td></td>
<td><em>T. thermophilus</em></td>
<td>0.3 (9.7)</td>
</tr>
<tr>
<td>Arg</td>
<td>Bovine mt</td>
<td>2.5 (100)</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
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<tr>
<td></td>
<td><em>T. thermophilus</em></td>
<td>0.2 (8)</td>
</tr>
<tr>
<td>Lys</td>
<td>Bovine mt</td>
<td>2.5 (100)</td>
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<tr>
<td></td>
<td><em>E. coli</em></td>
<td>&lt;0.1 (&lt;4)</td>
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<tr>
<td></td>
<td><em>T. thermophilus</em></td>
<td>&lt;0.1 (&lt;4)</td>
</tr>
<tr>
<td>Ser</td>
<td>Bovine mt</td>
<td>2.6 (100)</td>
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<tr>
<td></td>
<td><em>T. thermophilus</em></td>
<td>0.1 (3.8)</td>
</tr>
</tbody>
</table>

Table 1 displays the unilateral cross-species aminoacylation among bovine mitochondria, *E. coli* and *T. thermophilus* in Phe-, Thr-, Arg-, Lys- and Ser-specific reactions (Kumazawa et al., 1989; Kumazawa et al., 1991). These amino acid-specific reactions were selected due to the relatively high activities of the corresponding aaRSs from bovine mitochondria. Although there may exist some other aminoacylation systems with lower activities in the *in vitro* assay system, they have not been examined. As shown in Table 1, bacterial synthetases are unable to aminoacylate mt tRNAs, whereas mt synthetases are able to efficiently aminoacylate both their homologous mt tRNAs and heterologous bacterial tRNAs. The Arg-specific reaction is an exception, since mt tRNA appears to be charged to some extent by *E. coli* aaRS (Kumazawa et al., 1991; see also Table 1 and Fig. 1). Recently, an aspartic acid (Asp)-specific reaction with mt enzyme, in which the human mt system was used, also displayed efficient aminoacylation of both homologous mt tRNAs and heterologous bacterial tRNAs (Fender et al., 2006; Fender et al., 2012).
Figure 1: Cross-species aminoacylation between bovine mitochondrial (mt) and bacterial aaRSs. Cross-species aminoacylation was examined in five amino acid-specific reactions between bovine mitochondria and *E. coli* (a), and between bovine mitochondria and *T. thermophilus* (b). Histograms display the percentage aminoacylation of tRNA by heterologous synthetase as compared with that by homologous synthetase. The data are taken from Table 1. Percent cross-species aminoacylation is represented by white (when mt aaRS was used) or black bars (when *E. coli* aaRS or *T. thermophilus* aaRS was used).

Figure 1 displays the data from Table 1 as a histogram representing the extent of heterologous aminoacylation as a percentage. Under the standardized conditions, mt synthetases charged greater than 40% of *E. coli* and *T. thermophilus* tRNAs (white bars in Fig. 1), whereas bacterial synthetases generally charged less than 10% of mt tRNAs (black bars in Fig. 1), except for the case of Arg. It is, therefore, evident that unilateral aminoacylation specificity exists between mitochondrial and bacterial aaRSs for Phe-, Thr-, Arg-, Lys-, and Ser-specific reactions.

Similar results were obtained when a set of 23 purified *E. coli* tRNA species corresponding to 18 amino acids (excluding tRNA.Pro and tRNA.Asn), as well as two bovine mt tRNA.Ser isoacceptors for AGR (where R=A or G) codons and UCN (where N=U, C, A or G) codons, were used (Kumazawa, *et al*., 1991). The tRNA.Phe, tRNA.Thr, tRNA.Arg, tRNA.Lys, tRNA.Ser and tRNA.Asp species from *E. coli* were all aminoacylated by their corresponding mt synthetases, albeit with varying degrees of efficiency (Fender *et al*., 2006; Fender *et al*., 2012; Kumazawa *et al*., 1989; Kumazawa *et al*., 1991). Interestingly, mt ArgRS was able to aminoacylate both *E. coli* tRNA.Arg for CGN codons and *E. coli* tRNA.Arg for AGR codons; mt tRNA.Arg for AGR codons is missing in the mt system because AGR are termination codons in mammalian mitochondria (Anderson *et al*., 1981).
3 Comparison of Amino Acid Sequences for Six Aminoacyl-tRNA Synthetase Genes from Mitochondria, *E. coli* and *T. thermophilus*

To elucidate the molecular basis for unilateral aminoacylation specificity between mitochondria and bacteria, it is necessary to reveal the recognition mechanism of aaRSs toward their cognate tRNAs. For this purpose, the genome sequences of aaRSs from bovine (or human for AspRS) mitochondria, *E. coli* and *T. thermophilus* were compared (Fig. 2) (Gouet et al., 1999; Thompson et al., 1994).

3.1 PheRS

The subunit structures of *E. coli* and *T. thermophilus* PheRSs are $\alpha_2\beta_2$, whilst that of mt PheRS is $\alpha$ (Bullard et al., 1999), in which approximately 350 N-terminal amino acids are homologous to the $\alpha$ sub-unit and approximately 100 C-terminal amino acids are homologous to the $\beta$-subunit from the C terminus of bacterial PheRSs (Fig. 2(a)). The amino acid sequences of *E. coli* and *T. thermophilus* PheRSs are very similar, with the exception of a 15 amino acid insertion sequence in the *T. thermophilus* enzyme between $\eta_4$ and $\beta_5$. The sequence of bovine mt PheRS is very similar to those of the *E. coli* and *T. thermophilus* PheRSs, with the exception of two extra sequences, that are non-existent in the bacterial synthetases; an additional region of 35 amino acids is located between $\beta_7$ and $\alpha_6$, and an additional region of 8-11 amino acids is located at the junction between the $\alpha$ and $\beta$ subunits of the bacterial synthetases (Fig. 2(a)).

3.2 ThrRS

The subunit structures of *E. coli*, *T. thermophilus*, and bovine mt ThrRSs are all $\alpha_2$. The amino acid sequences of ThrRSs from bovine mitochondria, *E. coli*, and *T. thermophilus* display many conserved amino acids. At the N-terminus, a protruding sequence of approximately 10 amino acids is present in bovine mt ThrRS, but not in *E. coli* or *T. thermophilus* ThrRS. In addition, 17 amino acid residues in *E. coli* and 12 residues in *T. thermophilus* at the central part (the region between amino acids 330 and 350) of mt ThrRS, are missing from the two bacterial enzymes (Fig. 2(b)).

3.3 ArgRS

The subunit structures of *E. coli*, *T. thermophilus*, and bovine mt ArgRSs are all $\alpha$. Bovine mt ArgRS lacks the 12-13 amino acid N-terminal region found in the bacterial synthetases; aside from this difference, bovine mt and *E. coli* enzymes are similar, but a 28 amino acid insertion sequence is present in the central region of the *T. thermophilus* enzyme. Overall, the amino acid sequences are similar between all three synthetases and the whole chain lengths of the enzymes are almost the same at 562, 574 and 580 amino acids for bovine mt, *E. coli* and *T. thermophilus* synthetases, respectively (Fig. 2(c)). These characteristic features may be related to the less defined unilateral specificity between mitochondrial and bacterial ArgRSs, as mentioned earlier in the review.
(a) Aminoacyl-tRNA synthetases for Phenylalanine (PheRS). Mitochondrial enzymes are aligned with the bacterial α subunit, followed by the C-terminal domain of the β subunit. Dashed lines indicate continuous sequence in the mitochondrial enzyme.

Continued on next page...
(b) Aminoacyl-tRNA synthetases for Threonine (ThrRS).

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(c) Aminoacyl-tRNA synthetases for Arginine (ArgRS).

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(d) Aminoacyl-tRNA synthetases for Lysine (LysRS).
(e) Aminoacyl-tRNA synthetases for Serine (SerRS).
Aminoacyl-tRNA synthetases for Aspartate (AspRS). Alignments were performed using the CLUSTAL W (Thompson et al., 1994) and ESPript programs (Gouet et al., 1999). Numbering is adopted from the sequences of mt maturation peptides. Conserved residues are indicated by bold-faced type and highly conserved residues are indicated by a black-background. NCBI-GI numbers for each sequence are as follows: Bovine (Bos taurus) mt PheRS (116003835), ThrRS (156120539), ArgRS (115495861), LysRS (77735669), SerRS (27806133); Human (Homo sapiens) mt AspRS (40789249); E. coli PheRS (16129670), ThrRS (16129675), ArgRS (16129828), LysRS (16130792), SerRS (16128860), AspRS (16129819); T. thermophilus PheRS (55981927), ThrRS (55981844), ArgRS (55980067), LysRS (55981010), SerRS (55980844), and AspRS (55980680).

**Figure 2:** Comparison of amino acid sequences for six aaRSs from bovine (mtBovine) or human mitochondria (mtHuman), *E. coli* (Ecoli), and *T. thermophilus* (Thermus).
3.4 LysRS
The subunit structures of *E. coli*, *T. thermophilus*, and bovine mt LysRSs are all \( \alpha_2 \) (Sissler *et al.*, 2005). There is extremely high sequence similarity in the central part of the three enzymes; however, a long N-terminal protruding sequence of approximately 80 amino acids, a seven amino acid-sequence between \( \beta_{12} \) and \( \beta_{13} \), and an extra 11-18 amino acid C-terminal sequence exist only in bovine mt LysRS (Fig. 2(d)).

3.5 SerRS
The subunit structures of *E. coli*, *T. thermophilus*, and bovine mt (Chimnaronk *et al.*, 2005; Yokogawa *et al.*, 2000) SerRSs are all \( \alpha_2 \). Three additional sequences present in bovine mt SerRS are absent from the bacterial SerRSs. These additional sequences include an N-terminal helical region of approximately 20 amino acids, a short loop of 8-10 amino acids between the \( \alpha_4 \) and \( \alpha_5 \) helices, and a C-terminal region of 16 amino acids (Fig. 2(e)). These insertions, particularly the N- and C-terminal insertions, in mt SerRS are commonly found in vertebrates (for example, human, mouse and zebra fish). The detailed recognition mechanism of these enzymes towards mt and *T. thermophilus* tRNAs will be described later in this review.

3.6 AspRS
The mt synthetase described (Fender *et al.*, 2006; Fender *et al.*, 2012) is of human origin. The subunit structures of *E. coli*, *T. thermophilus*, and mt AspRSs are all \( \alpha_2 \). The chain lengths of the three enzymes are almost identical; no extra insertions are present in mt AspRS. Although there is overall high sequence similarity between the three enzymes, the region between amino acids 291 and 405 is the most variable between species (Fig. 2(f)), which may represent an exception to the main hypothesis of this review, which states that only mt synthetases possess extra sequences that may be responsible for the unilateral amino acid specificity.

In summary, the six synthetases described above display some common characteristics; there are many sequence similarities between the individual enzymes from mt, *E. coli*, and *T. thermophilus*. With the exception of ArgRS and AspRS, the mt synthetases possess additional sequences at N-terminal, C-terminal, or internal regions. These additional regions are assumed to be related to unilateral amino acid specificity; the additional regions in the mt enzymes may serve to recognize not only cognate mt tRNAs, but also non-cognate *E. coli* and *T. thermophilus* tRNAs. The cases for the exceptions ArgRS and AspRS will be discussed later in this review.

4 Identity Elements of *E. coli* tRNAs Recognized by *E. coli* Aminoacyl-tRNA Synthetases
Another factor which influences unilateral amino acid specificity is the nature of the identity elements in the relevant tRNAs, which should be almost identical when mt synthetases aminoacylate both cognate mt tRNAs and non-cognate bacterial tRNAs.
4.1 tRNA\textsuperscript{Phe}

The anticodon nucleotides, G\textsubscript{34}, A\textsubscript{35}, and A\textsubscript{36} are the major determinants of tRNA\textsuperscript{Phe} specificity in all systems studied, including both \textit{E. coli} and \textit{T. thermophilus} (Safro \textit{et al.}, 2005). Among these identity elements, G\textsubscript{34} provides the maximum contribution to recognition, with A\textsubscript{35} and A\textsubscript{36} playing smaller roles. Candidate identity elements with much smaller contributions to tRNA\textsuperscript{Phe} recognition are distributed over the entire \textit{E. coli} and \textit{T. thermophilus} tRNAs\textsuperscript{Phe} sequence and include U\textsubscript{20}, U\textsubscript{59}, and A\textsubscript{73}, and tertiary base pairs A\textsubscript{9}–A\textsubscript{23}–U\textsubscript{12}, U\textsubscript{45}–G\textsubscript{10}–G\textsubscript{25}, and C\textsubscript{13}–G\textsubscript{22}–G\textsubscript{46} (Fig. 3(a)). Mitochondrial tRNA\textsuperscript{Phe} contains the same three major identity elements (G\textsubscript{34}, A\textsubscript{35}, and A\textsubscript{36}) as bacterial tRNA\textsuperscript{Phe}. With respect to the other minor identity elements, A\textsubscript{73} and A\textsubscript{9}–A\textsubscript{23}–U\textsubscript{12} are the only nucleotides retained in mt tRNA\textsuperscript{Phe} (Wakita \textit{et al.}, 1994) (Fig. 3(a)).

4.2 tRNA\textsuperscript{Thr}

The tRNA\textsuperscript{Thr} identity elements are located within the two distal regions of the tRNA, namely the anticodon loop and the acceptor stem (Dock-Bregeon \textit{et al.}, 2005). The wobble base at the first position of the anticodon of tRNA\textsuperscript{Thr} is not essential for aminoacylation, because tRNA isoacceptors can have different bases at that position (i.e., C, G, and U in \textit{E. coli}). However, the second (G\textsubscript{35}) and third (U\textsubscript{36}) positions of the anticodons have been conserved as common identity elements between ThrRS from \textit{E. coli}, \textit{T. thermophilus}, and bovine mitochondria (Fig. 3(b)). Similarly, the first base pair (G\textsubscript{1}–C\textsubscript{72}) in the acceptor stem of tRNA\textsuperscript{Thr} is another common identity element in \textit{E. coli}, \textit{T. thermophilus}, and bovine mitochondria. The C\textsubscript{2}–G\textsubscript{71} base pair is required for efficient aminoacylation in \textit{E. coli}, whereas mutations in the discriminator base (A\textsubscript{73}) have no effect. Conversely, replacement of the wild-type U\textsubscript{73} by a purine residue in \textit{T. thermophilus} tRNA\textsuperscript{Thr} results in loss of aminoacylation, which explains both the failure of \textit{E. coli} tRNA\textsuperscript{Thr} to be aminoacylated by \textit{T. thermophilus} ThrRS, and capacity of the \textit{E. coli} ThrRS to aminoacylate \textit{T. thermophilus} tRNA\textsuperscript{Thr} as well as its own tRNA (see Table 1). The structure of the \textit{E. coli} tRNA\textsuperscript{Thr}:ThrRS complex revealed that the identity elements interact directly with the enzyme (Sankaranarayanan \textit{et al.}, 1999), which is also supported by footprinting data, that showed clear protections in the anticodon and the acceptor arms of \textit{E. coli} tRNA\textsuperscript{Thr} (Theobald \textit{et al.}, 1988).

4.3 tRNA\textsuperscript{Arg}

A quasi invariant adenosine nucleoside at canonical position 20 (A\textsubscript{20}) in the variable pocket of the D loop is crucial for tRNA-mediated arginylation in \textit{E. coli}. (Eriani & Cavarelli, 2005). In addition, the invariant C at canonical position 35 (C\textsubscript{35}) of tRNA\textsuperscript{Arg} (the middle anticodon nucleotide) is the other identity element required for arginylation in \textit{E. coli}. Nucleotides A\textsubscript{20} and C\textsubscript{35} are both well conserved in \textit{T. thermophilus} and bovine mt tRNAs\textsuperscript{Arg} (Fig. 3(c)). Two positive determinants (A\textsubscript{20} and C\textsubscript{35}) for \textit{E. coli} ArgRS works in the L-shaped tRNA structure. Therefore, if mt ArgRS recognizes mt tRNA\textsuperscript{Arg} in the same manner, the tertiary structure of mt tRNA\textsuperscript{Arg} seems to be similar to the L-shaped tRNA structure.
(a) tRNA^Phe (GAA).

(b) tRNA^Thr (UGU).

(c) tRNA^Arg (UCG/ACG).

(d) tRNA^Lys (UUU).

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Figure 3: Comparison of the cloverleaf structures of tRNAs for six amino acids species from bovine (Bos taurus) or human (Homo sapiens) mitochondria, *E. coli*, and *T. thermophilus*. Identity elements determined on *E. coli* tRNAs are indicated by grey shading and lines. Modifications are sometimes partial, but it is not especially mentioned. Some tRNAs without modifications are depicted in the figure, because in these cases, tRNAs with modifications are not published in the database.

(e) Upper: tRNA$_{\text{Ser}}$ (UGA); Lower: tRNA$_{\text{Ser}}$ (GCU).

(f) tRNA$_{\text{Asp}}$ (GUC).
4.4 tRNA\textsubscript{Lys}

In \textit{E. coli}, the major identity elements of tRNA\textsubscript{Lys} recognized by the synthetase include the anticodon bases U\textsubscript{34}, U\textsubscript{35}, and U\textsubscript{36}, as well as the discriminator base A\textsubscript{73} (Blanquet \textit{et al.}, 2005). These identity elements are conserved in bovine mt tRNA\textsubscript{Lys} as well as \textit{T. thermophilus} tRNA\textsubscript{Lys} (Fig. 3(d)). The \textit{E. coli} tRNA\textsubscript{Lys} contains two hypermodified nucleotides in the anticodon loop, namely mnm\textsuperscript{5}s\textsuperscript{2}U\textsubscript{34} (or mnm\textsuperscript{5}se\textsuperscript{2}U\textsubscript{34}) and \textsuperscript{t}6A\textsubscript{37}. These modifications, which are likely to be important for the base pairing between codon and anticodon (Grosjean \textit{et al.}, 1976), have only a minor role in the recognition by LysRS.

4.5 tRNA\textsubscript{Ser}

Like tRNA\textsubscript{Leu} and tRNA\textsubscript{Ala}, it is characteristic to tRNA\textsubscript{Ser} that the anticodon does not contain the identity elements and is not recognized by SerRS (Normanly \textit{et al.}, 1986; Weygand-Durasevic\& Cusack, 2005). \textit{In vivo} identity switch experiments, in which tRNA\textsubscript{Leu} was switched to tRNA\textsubscript{Ser}, highlighted the importance to serine identity of the discriminator base, bases from the first three pairs of the acceptor stem, and the D stem base pair C\textsubscript{11}–G\textsubscript{24} (Breitschopf \textit{et al.}, 1995). Serine isoaccepting tRNAs possess a helical long variable arm of 14-20 nucleotides, rather than the typical four to five nucleotides (Fig. 3(e)); with the exception of tRNA\textsubscript{Tyr}, this characteristic feature is shared by bacterial tRNA\textsubscript{Tyr} and tRNA\textsubscript{Leu} isoacceptors. Base-addition or base-deletion experiments have highlighted the importance of the long variable arm as an essential recognition element (Himeno \textit{et al.}, 1990). In contrast to tRNA\textsubscript{Tyr} and tRNA\textsubscript{Leu} isoacceptors, which have one and two unpaired nucleotides at the proximal stem in the variable arm, respectively, a conserved feature of bacterial tRNA\textsubscript{Ser} isoacceptors is the absence of unpaired nucleotides at this region. These differences, which are correlated with the number of insertions in the D loop at position 20, influence the orientation of the variable arm and are crucial for the discrimination between long variable arm tRNAs, as demonstrated for tRNA\textsubscript{Tyr}, tRNA\textsubscript{Ser}, and tRNA\textsubscript{Leu} (Breitschopf \textit{et al.}, 1995; Normanly \textit{et al.}, 1986; Normanly \textit{et al.}, 1992).

The long variable arm is the domain that makes the largest contribution to \(k_{cat}/K_m\) of aminoacylation. The acceptor stem is the second most important domain for recognition by SerRS, whereas the anticodon stem/loop does not make a significant contribution. The roles of the acceptor stem and variable arm in tRNA recognition by the synthetase \textit{in vitro} have been studied extensively and it was concluded that SerRS selectively recognizes tRNA\textsubscript{Ser} on the basis of its characteristic tertiary structure rather than on sequence specificity (Asahara \textit{et al.}, 1994).

Interestingly, bovine mt tRNAs\textsubscript{Ser} have secondary structures that are quite different from those of \textit{E. coli} (Fig. 2(e)). Firstly, mt tRNAs\textsubscript{Ser} do not have the long variable arm that is the major identity element of \textit{E. coli} tRNAs\textsubscript{Ser}. Secondly, mt tRNAs\textsubscript{Ser} have the discriminator base adenosine (A), which is different to that of \textit{E. coli} (G). Thirdly, the first three base-pairs in the acceptor stem are distinct between bovine mt and \textit{E. coli} tRNAs\textsubscript{Ser}. The lack of \textit{E. coli} tRNA\textsubscript{Ser} identity elements in bovine mt tRNAs\textsubscript{Ser} strongly suggests that mt tRNAs\textsubscript{Ser} would not be serylated by \textit{E. coli} SerRS (Table 1).

It is notable that the secondary structures of bovine mt tRNAs\textsubscript{Ser} are quite different from each other (Ueda \textit{et al.}, 1983; Yokogawa \textit{et al.}, 1991) (Fig. 3(e)). The mechanism by which these tRNAs\textsubscript{Ser} are recognized by a single species of mt SerRS is discussed later in this review.
4.6 tRNA\textsuperscript{Asp}

The *E. coli* aspartate identity elements are G\textsubscript{34} and U\textsubscript{35} in the anticodon, the discriminator base G\textsubscript{73} for major elements, and the G\textsubscript{2}–C\textsubscript{71} base pair for minor elements. Human mt tRNA\textsuperscript{Asp} has the same anticodon identity elements, but the discriminator base for the major element and the N\textsubscript{2}–N\textsubscript{71} base pair for the minor elements are different (A\textsubscript{73} and A\textsubscript{2}–U\textsubscript{71}, respectively) (Fig. 3(f)). When these identity elements were converted to those found in *E. coli* tRNA\textsuperscript{Asp}, aspartate accepting activity of the altered mt tRNA\textsuperscript{Asp} by *E. coli* AspRS was enhanced (Fender et al., 2012).

5 Dual-Mode Recognition of Two Mammalian Mitochondrial Isoacceptor tRNAs\textsuperscript{Ser} by the Cognate Seryl-tRNA Synthetase

As described earlier in this review, the sequences of most aaRSs from mitochondria, *E. coli*, and *T. thermophilus* are very similar, but in the majority of cases extra insertions are present in mt synthetases. These extra insertions might be the cause of unilateral aminoacylation specificities between mt and bacterial synthetases toward their cognate tRNAs. With the exception of ArgRS and AspRS, it is likely that most of the aaRSs described in the present review fit with this assumption.

To confirm this notion more clearly, our laboratory focused on mt SerRS, because it possesses three extra insertions as compared with *E. coli* and *T. thermophilus* SerRSs (Fig. 2(e)). It is also interesting to investigate this recognition mechanism of mt SerRS toward mt tRNAs, because mt tRNA\textsuperscript{Ser} consists of two species that display quite different secondary structures (Fig. 3(e)), namely tRNA\textsuperscript{Ser\_GCU} (Ueda et al., 1983) and tRNA\textsuperscript{Ser\_UGA} (Yokogawa et al., 1991). The tRNA\textsuperscript{Ser\_GCU} (for AGY codons, where Y=U or C) structure is highly truncated and lacks the entire D arm, whereas the tRNA\textsuperscript{Ser\_UGA} (for UCN codons, where N=U, C, A, or G) structure has an unusual secondary configuration with an extended anticodon stem. We previously demonstrated that mt SerRS recognizes these topologically distinct isoacceptors with the same affinity (Yokogawa et al., 2000). Recombinant mt SerRS clearly footprints at the TΨC loop of each isoacceptor and at the bottom of the acceptor stem; kinetic studies revealed that mt SerRS specifically recognizes the TΨC loop sequence in each isoacceptor (Fig. 4). However, in the case of tRNA\textsuperscript{Ser\_UGA}, TΨC loop–D loop interaction is also required for recognition, suggesting that mt SerRS recognizes the two substrates by distinct mechanisms. These different modes of recognition for the two substrates are accentuated by the fact that the TΨC loop-containing mini-helix variant of mt tRNA\textsuperscript{Ser\_GCU} is a good substrate for mt SerRS, whereas that of mt tRNA\textsuperscript{Ser\_UGA} is not (Shimada et al., 2001); therefore, it is clear that mt SerRS operates on two distinct mt tRNAs\textsuperscript{Ser} by dual-mode recognition.
6 Structural Basis for Dual-Mode Recognition of Mitochondrial Seryl-
tRNA Synthetase

The tertiary structure of bovine mt SerRS (in complex with seryl adenylate) was analyzed by X-ray crystallography, which gave a diffraction pattern at 1.65Å resolution (Chimnaronk et al., 2005). Fig. 5 shows a superposition of the Ca chain of mt SerRS onto the already determined structure of T. thermophilus SerRS (Biou et al., 1994; Cusack et al., 1996). Notably, the tertiary structures of the two SerRSs are almost identical, except for extra three insertions in mt SerRS that are absent from bacterial SerRS, including the distal helix (39 amino acids) in the N-terminal region, the tip loop (10 amino acids) at the top of the two long α-helical arms (α4 and α5 in Fig. 2(e)), and the C tail (24 amino acids) in the C-terminal region.

Mutation of SerRS revealed that, among these three extra insertions, the distal helix and the C-tail are critical to the recognition of two species of mt tRNAs\textsuperscript{Ser} (Chimnaronk et al., 2005). Single Ala-substitution of various Arg residues in the distal helix significantly reduced aminoacylation activity against both mt tRNAs\textsuperscript{Ser} to a similar extent; removal of the entire distal helix completely eliminated enzymatic activity for both mt tRNAs\textsuperscript{Ser} (Fig. 6). Likewise, deletion of the 24 amino acid C-tail from mt SerRS resulted in complete loss of aminoacylation activity. While the C-tail partly contributes to dimer interface interactions, its deletion scarcely affects dimerization (Chimnaronk et al., 2005), suggesting that the C-tail plays an essential role in recognition of mt tRNAs\textsuperscript{Ser}. Interestingly, the last eight residues could be deleted from the C-tail (ΔC-8) without altering enzymatic activity, whereas the neighboring eight residues in the middle of the C-tail (ΔC-16), which is mostly comprised of charged residues, were critical to enzymatic activity. However, the ΔC-16 mutant displayed a significant difference in its enzymatic activi-
ty towards the two isoacceptor tRNAs\textsuperscript{Ser}; compared with the wild-type enzyme, ΔC-16 mutation reduced the aminoacylation rate for tRNA\textsuperscript{Ser\_GCU} to approximately 30%, whereas the aminoacylation rate dropped to 10% for tRNA\textsuperscript{Ser\_UGA}. This difference was derived from distinct kinetics of interaction with SerRS (Chimnaronk et al., 2005). Deletion of the C-tail caused augmentation of the $K_{\text{m}}$ value with tRNA\textsuperscript{Ser\_GCU} but not tRNA\textsuperscript{Ser\_UGA}, whereas the $k_{\text{cat}}$ value was greatly affected for tRNA\textsuperscript{Ser\_UGA} only. These results suggest that the C-tail of mt SerRS interacts with each of two isoacceptor tRNAs\textsuperscript{Ser} in a different manner, and thus plays a specific role in dual-mode recognition.

\textbf{Figure 5:} Superposition of the Ca chain of bovine mt SerRS (pink) onto the known structure of \textit{T. thermophilus} SerRS (blue) (Chimnaronk et al., 2005). The N-terminal helical region (Distal helix), the top region of the two long α-helical arms (tip loop), and the C-terminal region (C-tail) are indicated; these regions are not present in \textit{T. thermophilus} SerRS.
Mitochondrial tRNAs\textsubscript{Ser} do not possess the canonical identity element of the long variable arm present in bacterial tRNAs\textsubscript{Ser} (Biou \textit{et al.}, 1994), and mt SerRS has evolved alternatively to recognize the TΨC loop of both mt isoacceptors (12) (see Fig. 4). It is not obvious whether mt SerRS recognizes the sequence or the structure of the TΨC loop, or both. The crystal structure showed that mt SerRS forms an active dimer where the distal helix makes contact with the C-tail of the other subunit (Fig. 7), which likely coincides with the corner of bound tRNA substrate. Our laboratory found that mt SerRS switches its tRNA-binding mode between two isoacceptors by utilizing a distinct combination of tRNA-recognition sites comprised of the distal helix, the C-tail, and a limited positive patch in the α-helical arm (Chimnaronk \textit{et al.}, 2005). For mt tRNA\textsubscript{Ser\textsubscript{GCU}}, Arg\textsuperscript{129} in the helical arm is essential for serylation, whereas Lys\textsuperscript{93} and Arg\textsuperscript{122} are exclusively indispensable for serylation of tRNA\textsubscript{Ser\textsubscript{UGA}}. The C-tail likely plays a specific role in dual-mode recognition since deletion of this region caused augmentation of the kinetic $K_m$ value exclusively with tRNA\textsubscript{Ser\textsubscript{GCU}}. While detailed molecular mechanisms for recognition of mt tRNAs\textsubscript{Ser} await determination of the crystal structure of the mt SerRS-tRNA\textsubscript{Ser} complex, current structural and biochemical studies have revealed that mt SerRS utilizes a smart strategy to discriminate two conformationally distinct mt tRNAs\textsubscript{Ser} by recruiting extra domains to form the new tRNA-binding plat-
form, and by altering the canonical tRNA-binding site in the helical domain to enable accommodation of each mt tRNA\textsuperscript{Ser}.

**Figure 7**: Docking model of SerRS and the substrate tRNA in bacterial (left panel) and mt (right panel) systems. In the bacterial system (upper left panel), the long-helical arms of one subunit of bacterial SerRS recognizes the long variable arm of tRNA\textsuperscript{Ser}; another subunit recognizes the weak identity elements, the discriminator base, the top three base-pairs, and the C–G pair in the D arm of tRNA\textsuperscript{Ser}. In the mitochondrial system (upper right panel), the distal helix and the C-tail of mt SerRS recognizes the T arm of mt tRNA\textsuperscript{Ser}\textsubscript{GCU} and tRNA\textsuperscript{Ser}\textsubscript{UGA}. The protein-sandwich model is applicable to the bacterial system (lower left panel), in which the long-helical arms of one subunit of bacterial SerRS are positioned between the T arm and variable arm of bacterial tRNA\textsuperscript{Ser}. The RNA-sandwich model is applicable to the mitochondrial system (lower right panel), in which the T arm of mt tRNA\textsuperscript{Ser} is positioned between the helical arm and the extra domains (the distal helix and C-tail) of mt SerRS.

**7 The Origin of Unilateral Aminoacylation Specificity in the Case of Mitochondrial Seryl-tRNA Synthetase**

As mentioned earlier in the present review, mt SerRS is capable of serylating bacterial tRNAs\textsuperscript{Ser}, during evolution, the distal helix and the C-tail would have been added to SerRS to enable both proper interaction with mt tRNAs\textsuperscript{Ser} and rejection of other tRNA species. Simple addition of mitochondria-specific extensions to both the N- and C-termini of *E. coli* SerRS did not tolerate the recognition of truncated mt tRNAs\textsuperscript{Ser} (Chimnaronk *et al.*, 2005), which indicates that the correct combination with the helical arm is
required. All residues in the helical domain (α4 and α5 in Fig. 2(e)) that are crucial for recognition of mt tRNAs\textsuperscript{Ser} such as Arg\textsuperscript{129}, Lys\textsuperscript{93}, and Arg\textsuperscript{122}, are conserved exclusively in mammalian mt SerRSs but not in their bacterial counterparts. There is little doubt regarding that a strong evolutionary force would have driven shortening of mt tRNAs, which would thereby require mt aaRSs to increase in sizes in order to compensate for the loss in RNA.

RNA abbreviation may have caused drastic alteration of identities and recognition mechanisms of mt tRNAs\textsuperscript{Ser}. We have proposed the RNA-sandwich model as a possible mechanism for discrimination of mt SerRS:tRNA\textsuperscript{Ser} complexes, in which the TΨC loop is clamped by the distal helix and the C-tail at one side, and by the helical arm at the opposite side (Chimnaront \textit{et al.}, 2005; Fig. 7). Because the TΨC loop of mt tRNA\textsuperscript{Ser}_\text{UGA} closely resembles that of bacterial tRNAs\textsuperscript{Ser}, the RNA-sandwich recognition model of mt SerRS should be capable of accommodating bacterial tRNAs\textsuperscript{Ser}. However, since the sequence and structure of the TΨC loop are highly conserved among bacterial tRNA species, it is also possible that mt SerRS loosely discriminates tRNA identities, and may mischarge serine to other bacterial non-isoacceptor tRNAs. In fact, it has been elucidated that bovine mt SerRS can mischarge \textit{E. coli} tRNA\textsuperscript{T}ur, tRNA\textsuperscript{Leu}, tRNA\textsuperscript{Val}, tRNA\textsuperscript{Glu}, tRNA\textsuperscript{Gly} and tRNA\textsuperscript{Gly} to a great extent (Kumazawa \textit{et al.}, 1991). Hence, the unilateral aminoacylation specificity may have arisen from escalating ambiguity of tRNA discrimination.

8 Other Mitochondrial Aminoacyl-tRNA Synthetases

Compared with bacterial synthetase genes, the mt synthetase genes for four of the six amino acid systems described in this review possess extra insertions at N-terminal, C-terminal, or internal sites. The additional insertion(s) likely function in recognition of the substrate tRNAs of bacteria as well as mitochondria. The discrimination mechanism of these synthetases possessing extra insertions may be similar to that of the RNA-sandwich model, as suggested by the discrimination mechanism of mt SerRS.

However, the remaining two amino-acid systems, namely Arg and Asp, whose synthetase genes have almost the same chain lengths for \textit{E. coli}, \textit{T. thermophilus}, and bovine mitochondria, may have different mechanisms for tRNA recognition. Since bovine mt ArgRS has a very similar sequence and chain length to ArgRSs from \textit{E. coli} and \textit{T. thermophilus} (Fig. 2(c)), and the identity elements of \textit{E. coli} and \textit{T. thermophilus} tRNAs\textsuperscript{Arg} (A\textsubscript{20} and C\textsubscript{35}) are also well conserved in bovine mt tRNAs\textsuperscript{Arg} (Fig. 3(c)), the strict unilateral amino acid specificity may not be formed. In fact, bovine mt ArgRS charges \textit{E. coli} and \textit{T. thermophilus} tRNAs with 72 % and 80 % efficiencies, respectively, whereas \textit{E. coli} ArgRS charges bovine mt tRNA with 28 % efficiency (Kumazawa \textit{et al.}, 1991) (Table 1, Fig. 1).

In the case of AspRS, the chain lengths of \textit{E. coli}, \textit{T. thermophilus}, and human mt enzymes are almost the same, with no extra insertions present in mt AspRS. There are high sequence similarities in the former half and the latter third of the mt enzyme, but a dissimilar region exists between amino acid 291 and 405 (Fig. 2(f)). In this case, complex factors, such as dissimilar regions of the AspRS between \textit{E. coli} and mitochondria, may be involved in the unilateral amino acid specificity. In addition, human mt tRNA\textsuperscript{Asp} has the same anticodon bases (G\textsubscript{34} and U\textsubscript{35}) for the major identity elements as \textit{E. coli} tRNA\textsuperscript{Asp}, but the discriminator base for the major element G\textsubscript{73} and the N\textsubscript{2}–N\textsubscript{71} base pair for the minor element G\textsubscript{2}– C\textsubscript{71} are different (A\textsubscript{73} and A\textsubscript{2}–U\textsubscript{71}, respectively) (Fig. 3(f)); therefore, partially different elements may also underlie the unilateral amino acid specificity. Because neither quantitative nor structural analyses
have been performed, further discussion requires more detailed studies (Fender et al., 2006; Fender et al., 2012).

9 Summary and Perspective

This review describes unilateral aminoacylation specificity between mitochondria and bacteria for the six amino acid systems of mt synthetases. The six amino acid systems can be divided into two groups; the first group possesses extra insertions in the mt aaRSs, which are not present in the E. coli and T. thermophilus synthetases; the second group does not possess extra insertions in the mt synthetases, and amino acid sequences of the synthetases from mitochondria and bacteria are similar to each other. The former group, containing PheRS, ThrRS, LysRS and SerRS, can be further divided into two subgroups; one subgroup possesses the same identity elements in the cognate tRNAs (PheRS, ThrRS, and LysRS), whereas the other subgroup possesses identity elements in mt tRNA that are different to those of E. coli and T. thermophilus (SerRS). The extra insertions of these two subgroups of synthetases may serve to recognize bacterial non-cognate tRNAs as well as mt cognate tRNAs. Such extra insertions in mt synthetases may have been generated to compensate for the lack of certain regions in mt tRNAs, that resulted from shortening of mt genomes during the course of evolution.

In the case of mt SerRS, the enzyme probably recognizes tRNA by positioning the TΨC loop between the distal helix and the C-tail (the extra insertions of mt SerRS) at one side and the helical arm (the body of the enzyme) at the opposite side; this mechanism was proposed by our laboratory as the RNA-sandwich recognition model (Chimnaronk et al., 2005).

The latter group of aaRSs that lack extra insertions in the mt synthetases consists of ArgRS and AspRS. This group may also be further divided into two subgroups; one subgroup (ArgRS) contains the same identity elements in the cognate tRNAs of mitochondria, E. coli, and T. thermophilus, whereas the second subgroup (AspRS) does not necessarily contain the same identity elements in the cognate tRNAs of mitochondria, E. coli, and T. thermophilus. As mentioned earlier, synthetases such as ArgRS show no strict unilateral amino acid specificity; even bacterial ArgRS is able to charge the mt tRNAs to some extent. With regard to AspRS, quantitative analyses have not been performed to date; therefore, further detailed study is required (Fender et al., 2006; Fender et al., 2012).

Additional structural studies are required to analyze the detailed mechanism of unilateral amino acid specificity. We plan to perform X-ray diffraction studies of mt SerRS, mt tRNA$^{Ser}_{GCU}$, and/or tRNA$^{Ser}_{UGA}$ crystals. We expect that these studies will provide additional insight into the mechanism of unilateral amino acid specificity.

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Appendix A

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References


Cusack, S., Yaremchuk, A. & Tukalo, M. (1996). The crystal structure of the ternary complex of T. thermophilus seryl-tRNA synthetase with tRNA\textsuperscript{Ser} and a seryl-adenylate analogue reveals a conformational switch in the active site. EMBO Journal, 15, 2834-2842.


