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A Recognition Model of $tRNA^{Ser}$ by Seryl-tRNA Synthetase in E. coli

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E. coli seryl-tRNA synthetase (SerRS) is a dimeric enzyme, having a subunit composed of two domains, i.e., a globular domain that includes a putative active site, and a long antiparallel α-helical coiled coil domain. The recognition sites on $tRNA^{Ser}$ by SerRS were searched using the T7 transcription system, and a new model of the recognition mechanism was proposed, consisting of a long coil shaped domain on one subunit which interacts with the long extra arm of $tRNA^{Ser}$, and a globular domain of the other subunit which interacts with the 3' end of $tRNA^{Ser}$.

$tRNA^{Ser}$ is classified along with $tRNA^{Leu}$ and prokaryotic $tRNA^{Tyr}$ as a class II tRNA, all of which possess a long extra arm composed of more than ten nucleotides, whereas in contrast the class I tRNAs have a shorter extra arm of only four or five nucleotides. A tertiary structure model of yeast $tRNA^{Ser}$ in solution was previously proposed using a chemical attack experiment as its basis,[1] where the long extra arm is in close contact with the D-loop. The interaction mode of yeast $tRNA^{Ser}$ with SerRS has also been studied by a footprinting experiment using a chemical probe, showing that the most extensively protected region from a chemical attack by addition of SerRS is aligned in a straight line, that is from a location between the 3' side of the D stem and the 5' side of the anticodon stem to the variable arm (P46, P47:2).[2] Genetic experiments have shown that an initial change of 12 nucleotides followed by a later change of 8 nucleotides (i.e., the discriminator base, being the 4th base from the 3' end of the tRNA, the five bases at positions 2, 3, 70, 71,and 72 in the acceptor stem, and the base pair 11-24 in the D stem) is sufficient to bring the serine identity to the suppressor $tRNA^{Leu}$.[3,4] The latter mutant showed a suppressor efficiency of 40%, yet in this experiment most of the $tRNA^{Ser}$ identity elements suggested were in common with the E. coli $tRNA^{Tyr}$ sequence.[4,5] This lead us to consider that the discrimination between the two tRNAs is very dependent on the long extra arm, and therefore an attempt was made at in vitro conversion of specificity of E. coli $tRNA^{Tyr}$ to $tRNA^{Ser}$ by using the T7 phage RNA polymerase transcription system.[5] The resulting expected change in the direction of the extra arm of $tRNA^{Tyr}$ by converting only five nucleotides, i.e., G_9, G_{20B}, and adding three

nucleotides to the extra arm, enabled almost complete conversion of aminoacylation specificity from $tRNA^{Tyr}$ to $tRNA^{Ser}$. Recently a crystal structure of E. coli SerRS (a dimer) was determined at 2.5 Å resolution, with the overall subunit having a "bean sprout" structure composed of two domains,[6] one domain being a globular protein possessing a specific nucleotide sequence similar to the prolyl- and threonyl-tRNA synthetases (classified as a subgroup of the class II synthetases[7]) and comprising mainly antiparallel β-sheets, and the other being a form of a straight rod (the bean's root) with an antiparallel α-helical coiled-coil structure having a length of 60 Å. It is interesting to speculate on the role of this domain, where it was argued that the coiled coil structure supports the anticodon stem of $tRNA^{Ser}$, as supposed from the role of an RNA binding protein which has a similar structural motif. The present study, however, leads us to a different hypothesis.

Three $tRNA^{Ser}_1$ variants were constructed using the T7 phage RNA polymerase system,[8] with a normal sequence (comprising unmodified bases), a deletion of one nucleotide pair at position 47B-47G ($tRNA^{Ser}$(1), Fig. 1), and a deletion of two nucleotide pairs at positions 47A-47H and 47B-47G ($tRNA^{Ser}$(2), Fig. 1). The time sequence of serine charging activity for each transcript is shown in Fig. 2. The normal $tRNA^{Ser}$ showed a serine acceptance of approximately 1000 pmol/A_{260}, whereas the initial velocities of mutants (1) and (2) respectively decreased by factors of 10 and 3000. This result indicates the involvement of the end of the long extra arm.

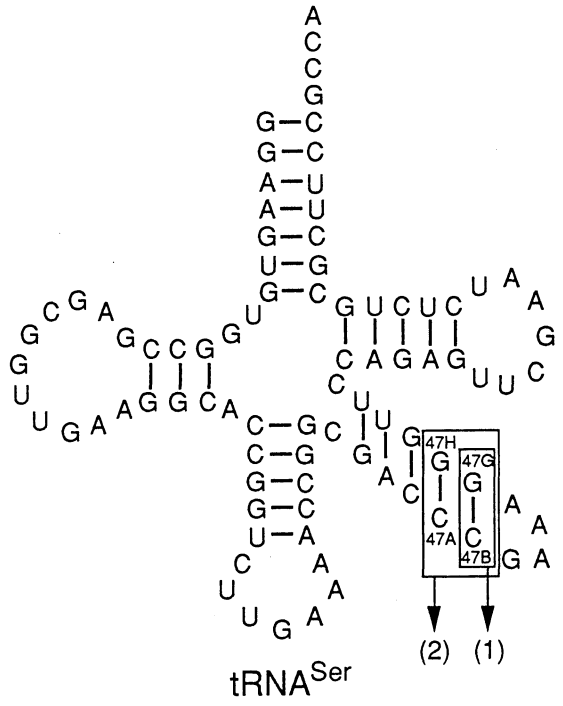

Fig. 1. The $tRNA^{Ser}$ transcript folded into a cloverleaf structure. Arrows indicate the deletions of the mutant transcripts (1) and (2). Transcript preparation was according to Refs. 5 and 8, with numbering according to Ref. 9.

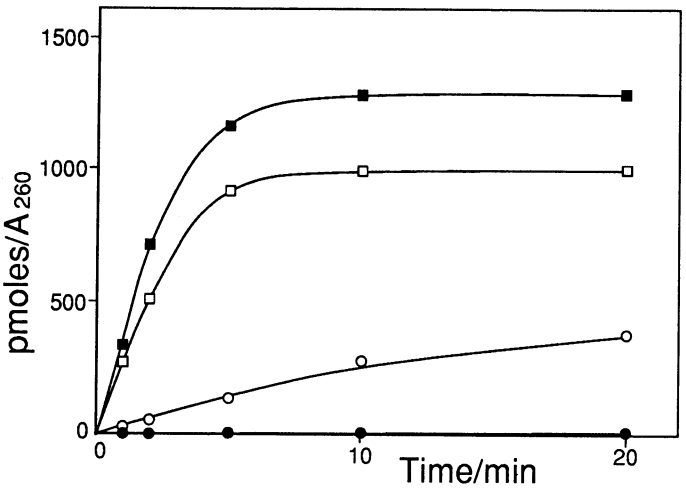

Fig. 2. Aminoacylation of the $tRNA^{Ser}$ transcripts. The reaction was carried out at 37 °C with partially purified SerRS from E. coli strain A19 in the reaction mixture containing 60 mM Tris-HCl (pH 7.5), 10 mM magnesium cloride, 2 mM dithiotheitol, 0.1 mg/ml bovine serum albumin, 2.5 mM ATP, 0.035 mM L-[U-^{14}C]serine (5.22 GBq/mmol)and 500 nM transcript RNA. Native $tRNA^{Ser}_1$ (1300 pmol/A_{260}) indicated by solid square was obtained from Subriden RNA. Transcripts with the normal sequence, (1) and (2) are respectively indicated by open square, open circle and solid circle.

These mutant deletions are believed to cause no significant damage to the tertiary structure of the $tRNA^{Ser}$ molecule because the region around the end of the long extra arm is considered to have no intramolecular interactions with any other region.[1,10] Thus, it is concluded that SerRS recognizes the vicinity of the end of the long extra arm. Based on previous studies in comparison to the present results, the envisioned complex structure of E. coli $tRNA^{Ser}$ with SerRS is shown in Fig. 3, where the extra arm can interact with the coiled coil, and the vicinity of the 3' CCA end can interact around the putative active site of the other subunit. It was previously thought that the long extra arm plays a crucial role in discrimination between $tRNA^{Tyr}$ and $tRNA^{Ser}$ for SerRS,[5] which is clearly supported by this model. The direction of the extra arm of $tRNA^{Ser}$ is possibly just suitable for interacting with SerRS's coiled coil, while the extra arm of $tRNA^{Tyr}$, which has been suggested by their cloverleaf structures to have a different direction from that of $tRNA^{Ser}$,[5] may prevent the coiled coil from successful interaction. This model is additionally consistent with a yeast system footprinting study.[2]

The previous model which indicates that the coiled coil supports the anticodon stem does not answer the new question as to how the long extra arm interacts with the enzyme. The assumption that only a single subunit interacts with $tRNA^{Ser}$ may be excluded, since the distance between the coiled coil and the putative active site on a single subunit does not cover the recognition sites indicated by the presented study.

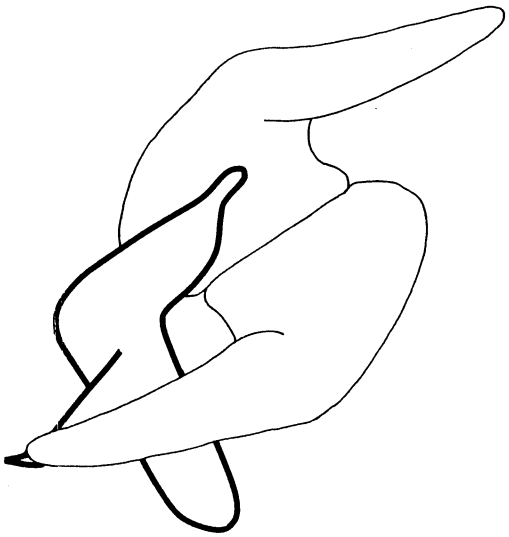

Fig. 3. Schematical view of the E. coli $tRNA^{Ser}$-SerRS complex. Bold and standard lines respectively indicate $tRNA^{Ser}$ and SerRS.

Gratitude is extended to the Radio Isotope Center at the University of Tokyo for the use of their facility.

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