— **Minireview** —

Mammalian Selenocysteine tRNA, Its Enzymes and Selenophosphate

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Selenocysteine (Sec) is the 21st amino acid, because Sec has a specific tRNA and codon UGA, and shares a major stop codon UGA. The present article surveys the structure of mammalian $tRNA^{Sec}$, the identity elements, the enzymes to synthesize Sec, and selenophosphate as a Se donor. Sec–tRNA is converted from Ser–tRNA by Sec synthase. $tRNA^{Sec}$ has a long 9 bp AA-stem, 6 bp D-stem and 4 bp T-stem, differing from Ser tRNAs. The 9 bp AA-stem and 6 bp D-stem were identity elements necessary for selenylation, however, the 4 bp T-stem is not essential. This finding was supported by the active mutants derived from major $tRNA^{Ser}$ by changing the AA-stem to 9 bp and the D-stem to 6 bp. There are many enzymes and factors that produce and bring Sec-tRNA to ribosomes. The reaction proceeds as follows. The first step is Ser–tRNA synthetase which recognizes the discrimination base G73 and the long extra arm. Bovine Sec synthase, composed of two protein species, recognizes Ser–$tRNA^{Sec}$ among three Ser–tRNAs and changes Ser–$tRNA^{Sec}$ to Sec–$tRNA^{Sec}$, by the addition of Se from selenophosphate (SeP). The stability of SeP and some properties of the synthetase are discussed. Sec–tRNA is recognized by an elongation factor specific to Sec–tRNA, and is brought to Se-protein mRNA. The discrimination mechanism of the Sec UGA codon from a major stop codon UGA remains to be resolved.

Key words —— selenocysteine, tRNA, Selenium, selenophosphate

History of Mammalian Selenocysteine tRNA

Selenocysteine (Sec) is recognized as the 21st amino acid from the finding that Sec has the codon UGA and tRNA specific to Sec. Three decades ago, Hatfield found that one species of $tRNA^{Ser}$ in liver and brain from chickens, cows, and rabbits bound to the UGA codon.[1] This tRNA was sequenced[2] and the sequence was later corrected with evidence of no-editing.[3] The position of the natural suppressor $tRNA^{Ser}$ gene on human chromosome 19 was also clarified.[4] Meanwhile, cDNA of murine glutathione peroxidase containing Sec was sequenced and the Sec codon was reported to be UGA.[5] From these findings, it was suggested that the $tRNA^{Ser}$ with an affinity to UGA must be the Sec tRNA. In addition, the carbon backbone of Sec in glutathione peroxidase comes from ^{14}C-Ser.[6] Subsequently, [^{75}Se]Sec was prepared *in vitro* using this natural suppressor tRNA and the T7 transcript tRNA by bovine liver cytosol as an enzyme source and [^{75}Se]H_2Se as the Se donor.[7] [^{75}Se]Sec was also found on this tRNA prepared from rat mammary tumor cells cultured in the presence of [^{75}Se]selenite.[8] Our working hypothesis of Sec synthesis is shown in Fig. 1. Two $tRNA^{Ser}$ and one $tRNA^{Sec}$ species are recognized by one kind of seryl–tRNA synthetase (SerRS) dimer and are aminoacylated to Ser–tRNAs.[9] However, only Ser–$tRNA^{Sec}$ is recognized by Sec synthase (SecS) and converted to Sec–$tRNA^{Sec}$. In this step, Se was supplied by selenophosphate (SeP), produced from HSe^- and ATP by selenophosphate synthetase, as described at the last chapter. Then, Sec–$tRNA^{Sec}$ is brought by its specific elongation factor (SePF, a mammalian candidate of SELB homolog) to ribosomes.[10–12] The mechanism of the discrimination between the in-frame Sec UGA codon and the major stop codon UGA is unclear, but SECIS (Sec insertion sequence) on the 3′untranslated region of mRNA of Se-proteins may play a key role to incorporate Sec.[13,14] SECIS is recognized by its binding protein (SBP).[15–17] However, it is not clear how the SECIS element, in the 3′UTR at distances greater than sev-

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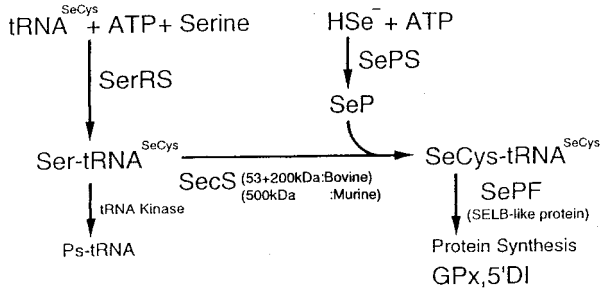

Fig. 1. Schematic Model of Mammalian Sec Synthesis

eral thousands of nucleotides, can mediate Sec insertion at the in-frame UGA Sec codon. In order to show that SECIS is essential for expression of Se-proteins, most studies have been done by combination of Se-protein mRNAs and these SECIS elements. It is rare to find the experimental result of conversion from non-Se-protein to Se-protein, with general protein mRNA. Meanwhile, it was suggested that there must be a signal near the UGA Sec codon for discrimination of one codon sharing two means, stop or Sec. We identified and reported the general conserved region upstream of the in-frame UGA Sec codon of most Se-protein mRNAs.[18] This model also explains how the UGA Sec codon escapes from attack by eRF of the peptide releasing factor. We showed that mutations upstream of the in-frame UGA Sec codon decreased the expression level of GPx.[19] Recently, it has also been shown that changes in the codons at the positions –2 and –1 of the Sec UGA in PHGPx can markedly affect Sec incorporation efficiency.[20]

Secondary Structure of tRNA^{Sec}

In the first model of the secondary structure of tRNA^{Sec}, the AA-stem was 7 bp and the T-stem was 5 bp with a bulged C (7/5 model).[2] In the second model, tRNA^{Sec} had a 9 bp AA-stem and 4 bp T-stem (9/4 model),[21] which was derived from the 8/5 model of the secondary structure of *E. coli* tRNA^{Sec}, as well as that in *Aquifex aeolicus* bacteria. The 9/4 model was strongly supported using enzymatic and chemical probes.[22] The secondary structure of tRNA^{Sec} in Archae *Methanococcus jannaschii* also shows the 9/4 structure.[23] Thus, all tRNA^{Sec} possess the 13 bp domain II made by stacking the colinear AA and T-stems, where they present the 9/4 structure in Eukarya and Archaea or the 8/5 structure in bacteria,[24] as shown in Fig. 2A. Thus, tRNA^{Sec} was found in Archaea, Bacteria, Eukarya (animal) and their secondary structures were clarified. However, the yeast genome, *Saccharomyces cerevisiae*, has no

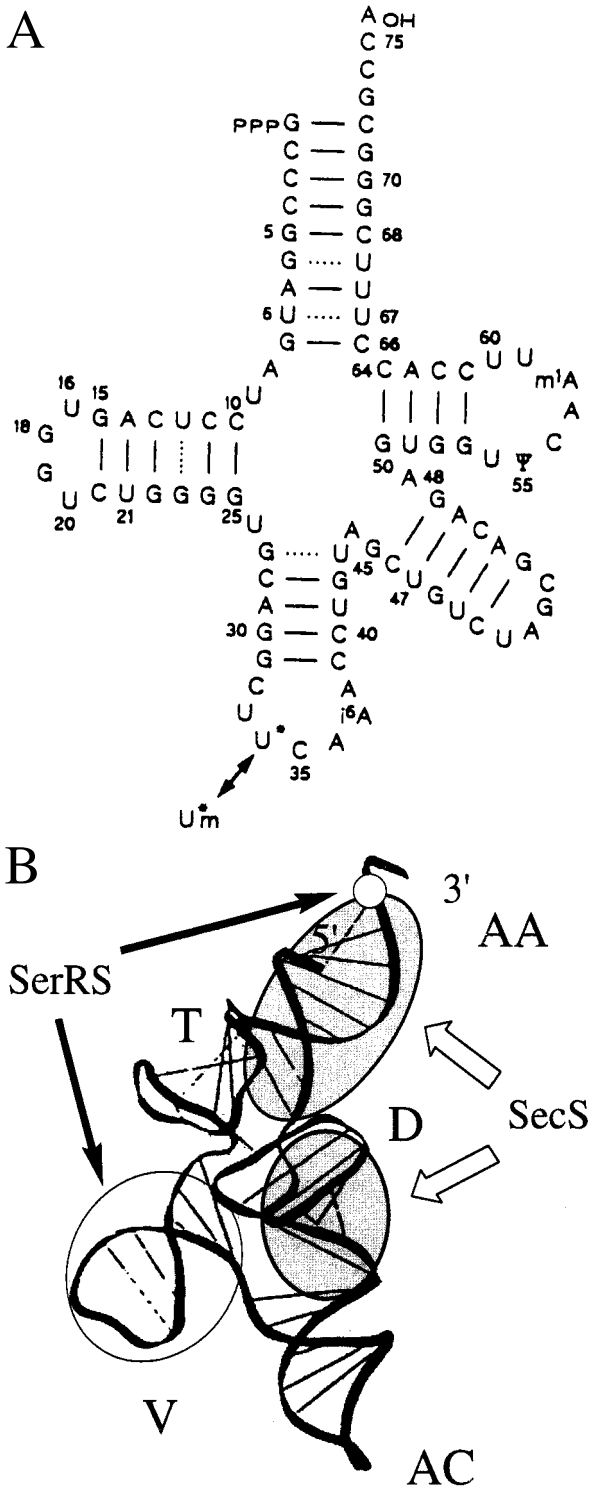

Fig. 2. A; Secondary Structure of tRNA^{Sec}, B; Tertiary Structure Model of tRNA^{Sec}, White Arrows Indicate the Identity Elements (Shaded Area) for SecS and Black Arrows Indicate the Identity Elements (White Aarea) for SerRS

tRNA^{Sec} gene and there is no evidence of the presence of tRNA^{Sec} in higher plants. It is possible that the gene for tRNA^{Sec} in yeast and higher plants was deleted during evolution. Mammalian tRNA^{Sec} has

Table 1. The Influence of Length of AA- + T-Stems on Serylation and Selenylation

tRNA	Length	Relative V_{max}/Km value	
		Serylation	Selenylation
Native tRNA^{Sec}	(9 bp AA + 4 bp T)	1.00	1.00
X33[a)]	(9 bp AA + 5 bp T)	0.015	0.20
X34	(9 bp AA + 3 bp T)	0.96	0.35
Native tRNA^{Ser}	(7 bp AA + 5 bp T)	1.00	0
Y8K	(8 bp AA + 5 bp T)	0.48	0.003
Y11	(7 bp AA + 4 bp T)	0	0

a) X33 showed low serylation but significant selenylation. These measurements were obtained from the radiospecific activity of [^{14}]serine (0.025 Ci/mmol) and ^{75}Selenide (2 Ci/mmol). Selenylation of X33 was measured in a 50 μl reaction mixture containing 200 ng (5 pmol) tRNA in which 75 fmol were serylated and 10 fmol were selenylated. The Sec level with native tRNA^{Sec} in the same reaction mixture was 50 fmol. We measured the amount of 10 fmol using the high radiospecific activity of ^{75}Se and a sensitive device, BioImage BAS 2000.

the generally rigid 6 bp D-stem and we can find a similar rigid 6 bp D-stem structure in *E. coli* tRNA^{Sec}. This suggests that this 6 bp D-stem is essential for selenylation activity,[25)] as described later.

Identity Elements on tRNA^{Sec} for Serylation and Selenylation

SerRS recognizes the G73 and the long extra arm on three Ser tRNAs (two tRNAs^{Ser} and one tRNA^{Sec}).[26)] In addition, two key structures for selenylation in tRNA^{Sec} were manifested using many mutants of tRNA^{Sec}, as shown by white arrows in Fig. 2B (inside the L-shape form of tRNA). This first identity element is the long 9 bp AA-stem.[27)] The deletion mutants with the 8 bp AA-stem were very low or showed no selenylation activity and this recognition of 9 bp AA-stem was not base-specific but structure-specific, because none of the point mutations on the AA-stem significantly modified the selenylation level.[25)] tRNA^{Sec} has the 6 bp D-stem, the 4 bp T-stem and the 9 bp AA-stem. The second key structure is the identity element for selenylation, which is 6 bp D-stem.[25)] Mutants with a disrupted 6 bp D-stem or 5 bp D-stem were inactive for selenylation. This indicates that the role of the 6 bp D-stem is structure-specific, not base-specific.[25)] The 9 bp AA-stem and 6 bp D-stem on tRNA^{Sec} are identity sites for selenylation. This finding was derived from experimental results using some active mutants from major Ser tRNA by changing the AA-stem to 9 bp and the D-stem to 6 bp.[25,27,28)] The length of 4 bp T-stem is not essential, because the serylation and selenylation activities of mutants with various chain lengths are shown in Table 1.[28)] tRNAs having 3 bp T-stem (X34), 4 bp T-stem (WT) and 5 bp T-stem (X33) are good selenylation substrates. Meanwhile, the length of domain II (AA-stem + T-stem) should be 12 or 13 bp for serylation, because tRNA mutants with 11 bp (Y11) and 14 bp (X33) were inactive. This suggests that the length of domain II does not directly depend upon the recognition site (identity elements) for SerRS, but is affected by the distance between the G73 and the long extra arm, as shown by black arrow in Fig. 2B, whose steric distances on tRNA were measured by SerRS. This finding clarified that the distance between two recognition sites is another key factor for recognition by SerRS.

Enzymes to Produce Sec–tRNA

Sec on tRNA^{Sec} is changed from Ser on tRNA^{Sec}. There are three tRNAs accepting Ser. The first tRNA^{Ser} has anticodon GCU corresponding to codon AGU and AGC and the second tRNA^{Ser} has anticodon IGA corresponding to codon UCX. The last tRNA^{Sec} has anticodon UCA (U is modified, mcmU) corresponding to codon UGA. Bovine SerRS made of two subunits, recognizes these three tRNAs with the same Km and Vmax values.[9,29)] The recognition sites to SerRS are the G73 and the long extra arm,[26)] as shown by black arrows in Fig. 2B. The sequence of human SerRS has been reported.[30)] As shown in Fig. 1, SecS recognized only Ser–tRNA^{Sec} among three Ser–tRNAs. SecS in *E. coli* is one kind of protein[31)] and presents oligomer as an active state. Meanwhile, bovine SecS is composed of two main protein species of different molecular sizes and the selenylation reaction proceeds sequentially (Mizutani, unpublished data). Gel filtration patterns of S100 from bovine liver and COS-7 cells showed that the size of one protein species of SecS activity was relatively small, approximately 53 kD. The SecS activity pattern of S100 from mouse and guinea pigs shows relatively large size, about 500 kD. The activity in mice may come from a complex of several proteins. It is possible that the intermediate from Ser–

Table 2. Comparison of $tRNA^{Sec}$ and Its Enzymes in Mammals and *E. coli*

	Mammals	*E. coli*
tRNA		
AA-stem	9 bp	8 bp
T-stem	4 bp	5 bp
D-stem	6 bp	6 bp
SerRS (dimer)	66 kD	48 kD
Identity elements for SerRS	G73, long V-arm	G73, long V-arm, C11-G24
SecS	53 kD+200 kD	600 kD
Identity elements for SecS	AA-stem, D-stem	AA-stem (D-stem?)
Reaction	2 steps by 2 enzymes	One enzyme
Se-donor	Selenophosphate	Selenophosphate
SeP synthetase	57 kD	37 kD (SELD)
Elongation factor	SePF (60 kD) + SBP2 (120 kD)	68 kD (SELB)
Products	DI, GPx, TRR	FDH

$tRNA^{Sec}$ to Sec–$tRNA^{Sec}$ is aminoacrylyl–$tRNA^{Sec}$, like the intermediate in *E. coli*,[31] because we found a weak spot of [^{14}C]Ala on TLC after the reduction of the intermediate with $NaBH_4$ (Mizutani, unpublished data). We found the EF specific to Sec–tRNA, which protected the tRNA from alkaline hydrolysis.[10–12] This factor, called SePF, is different from eEF-1α which protected [^{14}C]Phe–tRNA.[11] Meanwhile, this factor was separated from the SECIS–binding protein (SBP) on a column of Sephacryl S-300.[32] The SBP activity was eluted almost at the void volume of the column and SePF activity was eluted at the point of molecular size 60 kD.[32] There are three other studies on the proteins which reported an affinity to $tRNA^{Sec}$.[33–36] These proteins may possible be SerRS, SecS or SePF. Table 2 shows the comparison of some factors in mammals and *E. coli*. Some mammalian factors, acting in the initiation and elongation processes of protein translation on ribosomes, are more complex than *E. coli* factors. This suggests that some factors in Sec translation might be more complex than *E. coli* factors. Sec is converted from Ser on tRNA and there are two other known examples of amino acid conversion on tRNA, such as the case of Met–tRNA to formyl-Met–tRNA and Glu–tRNA to Gln–tRNA.[37] The transcriptional mechanism of tRNASec by RNA polymerase III has been clarified.[38]

Selenophosphate as an Activated Se Donor

Selenium is an essential trace element for humans; its deficiency causes Keshan disease, a condition endemic in China.[39] Keshan disease is healed by administration of selenite solution. This phenomenon has ascertained that $tRNA^{Sec}$ gene knock-out mice cannot grow and cannot be born.[40] We normally consume Se as inorganic Se, such as selenite and hydrogen selenide, and/or organic Se, such as seleno-methionine in rice and flour. In the human body, these compounds are converted to hydrogen selenide in blood (red cells) and in cells,[41] and Se presents hydrogen selenide in cytosol in reduced environments. We previously showed that the Se donor for synthesis of Sec was HSe^- in mammals[42] and found a factor which had an affinity to HSe^- in the presence of ATP.[8] This factor was shown to be essential for the synthesis of Sec.

In *E. coli*, the Se donor for Sec synthesis was clarified as selenophosphate (SeP).[43,44] There is a SeP synthetase in *E. coli*, called SELD, which makes SeP from hydrogen selenide and ATP.[45] The product of this enzyme, as confirmed by NMR, was SeP,[46–48] and this study also revealed that SeP was stable during measurement at room temperature. The phosphate moiety of SeP is derived from the γ-phosphate of ATP.[49] A cation-dependency of SELD was also clarified.[50] From the site-directed mutagenesis of Cys17, it was shown to be essential in the catalytic process;[47] the activity was also abolished by mutation of Lys20 to Gln.[51]

Recently, the genes of a protein resembling SELD (SeP synthetase) were cloned from mammals,[52–55] but the products (SeP) of a mammalian SeP synthetase have not yet been confirmed by NMR measurement.[54] The most plausible SELD–like protein is Se-protein and has a Sec-residue in the protein frame.[54] This SELD–like protein and SeP is hen-and-egg (SeP is necessary to produce SeP synthetase containing Sec and SeP synthetase is necessary to produce SeP). Meanwhile, SeP is the Se-donor that

produces a minor base containing Se, mnm^{5}Se^{2}U, in mammalian tRNA.[56]

It was suggested that our protein factor with an affinity to Se, as described previously,[8] might be the SeP synthetase of mammals. We provided evidence that [^{75}Se]Sec is produced by bovine Sec synthase from Ser–tRNA^{Sec} and [^{75}Se]SeP, synthesized from elemental ^{75}Se and Tris(trimethylsilyl)-phosphite according to the previously described procedure.[57,58] This finding indicates that SeP is the Se donor of Sec synthesis and strongly suggests that the product of Se-binding protein and ATP is SeP, as described in our paper.[58] We also studied the stability of SeP by NMR measurement.[58] SeP was stable during storage under nitrogen at –80˚C for 3 months in 0.2 M Hepes buffer at pH 6.8. However, SeP decomposed at 0˚C in air (half-life 32 h) or at 22˚C under nitrogen (half-life 30 h) at pH 6.8. Thus, SeP was shown to be stable at low temperatures, under acidic and anaerobic conditions, but labile under neutral and alkaline conditions. The LD_{50} of SeP administered i.p. to mice was 37 mg/kg body weight,[58] this value indicates that SeP is non-toxic in humans, because the Se level in humans is 0.2 ppm (0.2 mg/kg).

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