A G·U base pair in the eukaryotic selenocysteine tRNA is important for interaction with SePF, the putative selenocysteine-specific elongation factor

Takaharu Mizutani*, Kazutaka Tanabe, Kenichiro Yamada

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

Received 11 April 1998

Abstract In *Escherichia coli*, selenocysteine biosynthesis and incorporation into selenoproteins requires the action of four gene products, including the specialized selenocysteine tRNA^{Sec} and elongation factor SELB, different from the universal EF-Tu. In this regard, the situation is less clear in eukaryotes, but we previously reported the existence of SePF, a putative SELB homologue. The secondary structure of the tRNA^{Sec} differs slightly in eukaryotes, due to a change in the lengths of several stems. Two non-Watson-Crick base pairs, G5a·U67b and U6·U67, reside in the acceptor stem and are conserved in the course of evolution. Since it has already been reported that changing them to Watson-Crick base pairs did not affect the serylation or selenylation levels of tRNA^{Sec}, we asked whether these non-Watson-Crick base pairs are required for the interaction with SePF. To this end, tRNA^{Sec} variants carrying Watson-Crick changes at these positions were tested for their ability to maintain the interaction with SePF. In these assays, the tRNA^{Sec}-SePF interaction was determined by the protective action it confers against hydrolysis of the amino acid ester bond, under basic conditions. All the changes introduced at U6·U67 did not significantly affect the interaction. Interestingly, however, the G5a·U67b to G5a-C67b substitution was sufficient, by itself, to lead to unprotection of the ester bond. Therefore, our finding strongly suggests that SePF is unable to interact with a tRNA^{Sec} mutant version carrying a Watson-Crick G5a-C67b instead of the wild-type G5a·U67b base pair, establishing that G5a·U67b constitutes a structural determinant for SePF interaction.

Key words: Selenium; Selenocysteine; tRNA; SELB

1. Introduction

Selenium is an essential trace element. This was proved by its administration to patients suffering from Keshan disease, endemic in the Keshan area in China [1]. Selenium is mainly found as selenocysteine (Sec), its major biological form. This amino acid resides in the catalytic centers of selenoenzymes involved in oxidation-reduction reactions, such as glutathione peroxidase [2] and type I iodothyronine 5′ deiodinase [3]. Biosynthesis and co-translational incorporation of this amino acid into selenoproteins requires four gene products in bacteria [4]. Briefly, a specialized tRNA, tRNA^{Sec}, is charged with serine by the conventional seryl-tRNA synthetase (SerRS). The seryl residue is converted to a selenocysteyl residue on the tRNA, by the enzyme selenocysteine synthase which utilizes a phosphoselenoate moiety, provided by the selenophosphate synthetase. Since selenocysteine is encoded by UGA [5], otherwise used as a stop codon, a mechanism allows discrimination between stop and selenocysteine codons. Two actors participate in this mechanism: a stem-loop structure in selenoprotein mRNAs, lying immediately 3′ to the UGA codon, and SELB, a specific translation factor acting in place of the classical EF-Tu. SELB binds both the tRNA^{Sec} and the mRNA stem-loop, thereby bringing the Sec-tRNA^{Sec} to the A site of the ribosome.

Much less is known about the eukaryotic machinery and not all the participants have been identified yet. cDNAs for selenophosphate synthetases were isolated, and it was intriguing to discover that one form of this enzyme also contains selenocysteine [6]. We characterized biochemical fractions of selenocysteine synthase in the cytosol of bovine and murine liver [7,8]. It looks as if this enzyme is constituted by different types of subunits, in contrast to the bacterial counterpart [4]. This mammalian Sec synthase reaction proceeds like the concerted mechanisms of subunits of Glu-tRNAGln amidotransferase in *Bacillus subtilis* [9]. In an earlier work we found, in bovine liver microsomes, a 50 kDa protein that carries a protective activity against hydrolysis of the aminoacyl-tRNA ester bond [10,11]. We called this factor SePF (for selenocysteyl-tRNA protecting factor) and showed that it also has the property of bringing the selenocysteyl-tRNA^{Sec} to a UGA codon on microsomes. SePF therefore represents the best candidate for being the eukaryotic-SELB homologue.

Structure-function studies in tRNA^{Sec} established that the long acceptor and D-stems of this tRNA constitute structural determinants for selenocysteine synthase recognition [12–14]. In the course of this work, it was found that two non-Watson-Crick base pairs, G5a·U67b and U6·U67, which are rather well conserved in the course of evolution, are not essential for the serylation step, nor for the seryl to selenocysteyl conversion step. In this work, we asked whether they could be implicated in the interaction with SePF and found that the universal wobble G5a·U67b base pair, by itself, is important for the SePF protecting activity.

2. Materials and methods

2.1. tRNA constructs and in vitro transcription by T7 polymerase

Synthetic bovine wild-type and mutant Sec tRNAs were constructed by hybridizing six couples of 14–24mer oligonucleotides containing the desired sequences as described in [12]. In these constructs, the promoter of the T7 RNA polymerase is included immediately 5′ of the coding sequence. The sequences of the oligo couples, which were used to synthesize the wild-type tRNA^{Sec}, are given in Fig. 1.

*Corresponding author. Fax: (81) (52) 834-9309.
E-mail: mizutani@phar.nagoya-cu.ac.jp

Abbreviations: AA-stem, aminoacyl stem; Sec, selenocysteine; SePF, Sec-tRNA protecting factor; SerRS, seryl-tRNA synthetase; - between two bases, a Watson-Crick base pair; · between two bases, a non-Watson-Crick base pair

PII S0014-5793(98)00589-4

Conditions for transcription in vitro with T7 RNA polymerase were as described in [12]. The RNA products were purified by gel electrophoresis and electroeluted.

2.2. Selenocysteylation of tRNA^{Sec}

Prior to use, tRNA transcripts were renatured by heating to 65°C for 3 min and then at 25°C for 5 min. tRNA^{Sec} (0.02 nmol) in 20 μl of 0.2 M HEPES-Na (pH 7.4), 20 mM $MgCl_2$, 20 mM KCl, 20 mM mercaptoethanol, 0.4 mM serine, 10 mM ATP, and 5 μg SerRS were mixed with 10 μl of [^{75}Se]HSe^- (2 Ci/mmol), 10 μl of selenocysteine synthase and 10 μl of a selenide-activating protein and incubated at 30°C for 2 h [7]. [^{75}Se]Sec-tRNA was collected by ethanol precipitation, after the addition of 1 μl glacial acetic acid to protect the Sec-tRNA^{Sec} ester bond. This precipitate contained [^{75}Se]HSe^- and enzymes. In order to remove these contaminants, some treatments were performed as follows [10,11]. In the treatment with buffered phenol, the crude [^{75}Se]Sec-tRNA precipitate was dissolved in 40 μl of distilled water and the equivalent volume of buffered phenol was added. After mixing vigorously for 5 min, the tube centrifuged for 5 min at 2000×*g*. The upper layer was recovered carefully. Then the [^{75}Se]Sec-tRNA was reprecipitated with ethanol just as in the other treatments. In order to measure the amount of [^{75}Se]Sec-tRNA^{Sec}, the recovered [^{75}Se]Sec-tRNA was hydrolyzed with a small volume of 2.5% NH_4OH for 30 min at 37°C and the tRNA reprecipitated with ethanol. The supernatant which contained the hydrolyzed [^{75}Se]Sec arising from Sec-tRNA^{Sec} was immediately analyzed by TLC on a silica gel G plate developed with *n*-butanol/acetic acid/water (4:1:1). After development, the [^{75}Se]Sec was detected with a BioImage Analyzer BAS2500. Thus, contaminants, such as [^{75}Se]HSe^-, were removed from the above crude [^{75}Se]Sec-tRNA precipitate with buffered phenol. This purified Sec-tRNA was used for assaying for the SePF protecting activity against alkaline hydrolysis of the Sec-tRNA^{Sec} ester bond.

2.3. Assay of [^{75}Se]Sec-tRNA protecting activity against alkaline hydrolysis (SePF assay)

The standard assay mixture (final volume of 50 μl) contained 50 mM Tris-HCl (pH 7.5), 75 mM KCl, 0.2 mM DTT, 0.1 mM unhydrolyzable GTP analogue (GMP-P(CH_2)P), 5 μg BSA, [^{75}Se]Sec-tRNA and an appropriate amount of SePF [10]. Incubation was carried out for 10 min at 30°C. Generally, aminoacyl-tRNAs were labile and easily hydrolyzed under these conditions (pH 7.5 for 30 min). Alkaline hydrolysis was terminated by ethanol precipitation, and the free [^{75}Se]Sec liberated from tRNA was removed from by centrifugation. [^{75}Se]Sec-tRNA protected in the precipitate was hydrolyzed in 2 N ammonia for 60 min at 30°C. The solution was mixed with 2 volumes of ethanol and the mixture was re-centrifuged. The supernatant was immediately analyzed by TLC on silica-gel G. After development, [^{75}Se]Sec on the silica plate was detected and measured with a BioImage Analyzer. Authentic selenocysteine, used as a marker, was co-chromatographed and colored by the ninhydrin reaction.

2.4. Chromatography condition

The post-mitochondrial supernatants of bovine liver extracts were centrifuged at 150 000×*g*. The precipitates and the supernatants at 150 000×*g* were used as microsomes and cytosol (S-100), respectively. Microsomal proteins were extracted with 1 M NH_4Cl from bovine liver microsomes, collected at 150 000×*g*. CM-Sephadex C-25 column (3×30 cm) chromatography was performed with a standard buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 10% (v/v) glycerol [10]. The elution was performed with a 0–0.8 M KCl. The volume of each fraction was 10 ml. All the steps were carried out in a cold room at 4°C.

3. Results

The secondary structure of eukaryotic tRNA^{Sec} shows some variation with respect to classical tRNAs [15]. In particular, the AA- and D-stems are longer, containing 9 and 6 base pairs, respectively. In addition to this length, the acceptor stem contains the two non-Watson-Crick base pairs G5a·U67b and U6·U67 (Fig. 1). The latter is conserved in vertebrates, but also in *Drosophila melanogaster* and *Caenorhabditis elegans* [16]. The wobble G5a·U67b base pair is conserved in vertebrates, *C. elegans* and the archaeon *Methanococcus jannaschii* [17]. Surprisingly, an A65a·G67b base pair is found at the same location in *Drosophila*. This strongly suggests that, even though a wobble base pair per se is not strictly required, the structure and/or function of the tRNA^{Sec} necessitates the occurrence of a non-Watson-Crick base pair at this position. In an earlier work, it was shown that neither the G5a·U67b nor the U6·U67 base pairs are crucial to the serylation and selenylation steps [12]. This finding raised the possibility that one or the other base pair might be required for further steps in the decoding of the selenocysteine codon. In this regard we found, after biochemical fractionation of microsomal extracts, a protein activity that has the ability to protect the ester bond of the Sec-tRNA^{Sec}, under basic conditions [10]. This activity, which we called SePF, is different from the elongation factor eEF-1α and may well be the eukaryotic homologue of the prokaryotic selenocysteine-specific elongation factor SELB. Therefore, we set out to determine whether the non-Watson-Crick base pairs are necessary for interaction with the SePF.

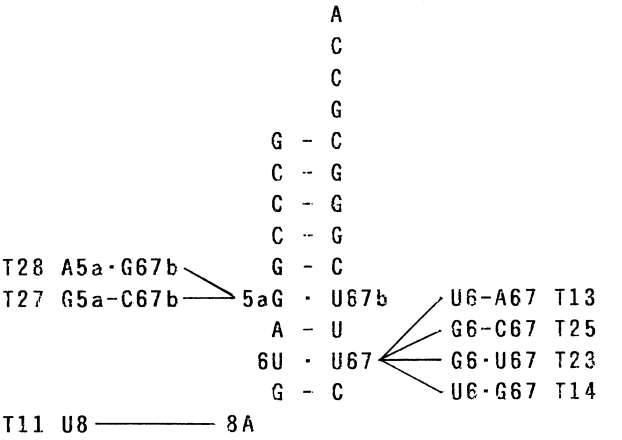

Fig. 1. The acceptor stem of the vertebrate selenocysteine tRNA with the mutant constructs used in this study. G5a·U67b was replaced by either of the two base pairs mentioned on the left, and U6·U67 by one of the four base pairs on the right.

In preliminary experiments, we wished to know whether SePF could also be detected in cytosol from bovine liver. When extracted from bovine liver microsomes at 1 M NH_4Cl, SePF activity elutes at 0.25 M KCl on CM-Sephadex chromatography as shown in the lower panel of Fig. 2. A similar elution profile could be obtained, starting from the cytosol fraction, as shown in the upper part of Fig. 2, in which the SePF activity was eluted at 0.25 M KCl. Thus, the SePF activity was found in both S-100 and microsomal fractions. As the next step, we measured the activity content in the fraction of SePF activity (fraction 26) in Fig. 2 by the dose-dependent manner as shown in Fig. 3A. Fig. 3B shows the results of dot-blot analyses of Sec. The activity of SePF in cytosol was weak at 2 μl and 5 μl, and was clearly found at 10 μl. Meanwhile, the SepF activity of the microsomal fraction was found at 2 μl and saturated at 2 μl. The fractions applied on the column of CM-Sephadex were obtained from the same bovine liver preparation. From Fig. 3, it can be concluded that microsomal fractions contained about 10 times as much SePF activity as those from cytosol. This result is interesting,

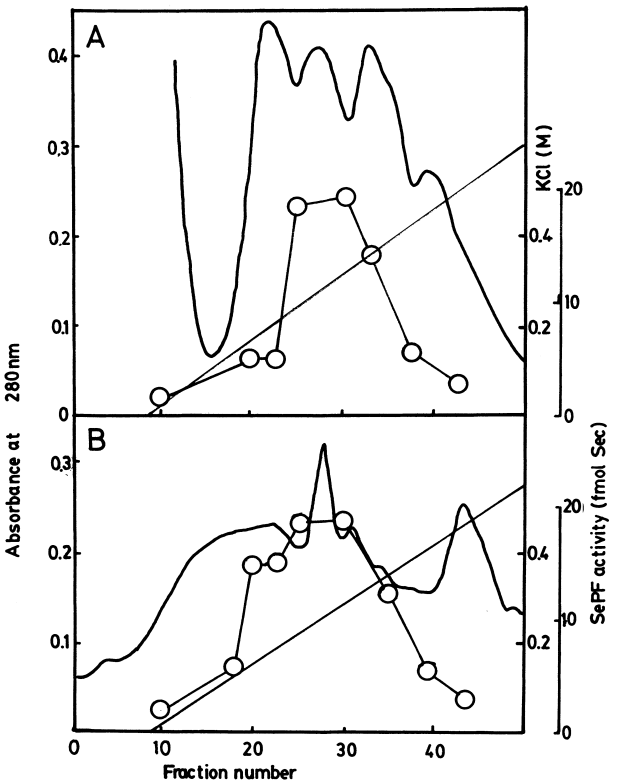

Fig. 2. Elution patterns of cytosol (A, upper panel) and microsomal extracts (B, lower panel) on CM-Sephadex chromatography. The thick lines indicate the absorbance at 280 nm and the circles the SePF activity in 10 ml fractions. The KCl gradient is indicated.

because this distribution of SePF is different from that of eEF-1α, much of which is contained in the cytosol. Almost all translational components, such as tRNAs and aminoacyl-tRNA synthetase, are contained as a compartment around ribosomes [18] and it is plausible that SePF is also associated with ribosomes.

The mutants tRNAs^{Sec} carrying the base pair substitutions in the acceptor stem, shown in Fig. 1, were prepared by in vitro transcription with T7 RNA polymerase. Four mutants were designed to change U6·U67 to U-A and G-C Watson-Crick base pairs on the one hand, and to U·G and G·U wobble base pairs, on the other. The wobble G5a·U67b was substituted to an Watson-Crick G-C and a non-Watson-Crick A·G base pair. These tRNA^{Sec} mutants were subsequently selenylated in vitro and the protection capabilities of SePF toward the corresponding [^{75}Se]Sec-tRNA^{Sec} ester bonds were assayed. The typical patterns after protection reaction

Table 1
Summary of the activity of tRNA mutants recognized by SePF

tRNA	Activity[a]
Wild-type	++
T11 A8→U	+
T13 U6·U67→U-A	++
T14 U6·U67→U·G	++
T23 U6·U67→G·U	++
T25 U6·U67→G-C	++
T27 G5a·U67b→G-C	−
T28 G5a·U67b→A·G	++

[a]The symbol ++ indicates a value of 70% or more of wild-type tRNA^{Sec} and − is a value of 30% or less. + is the middle value.

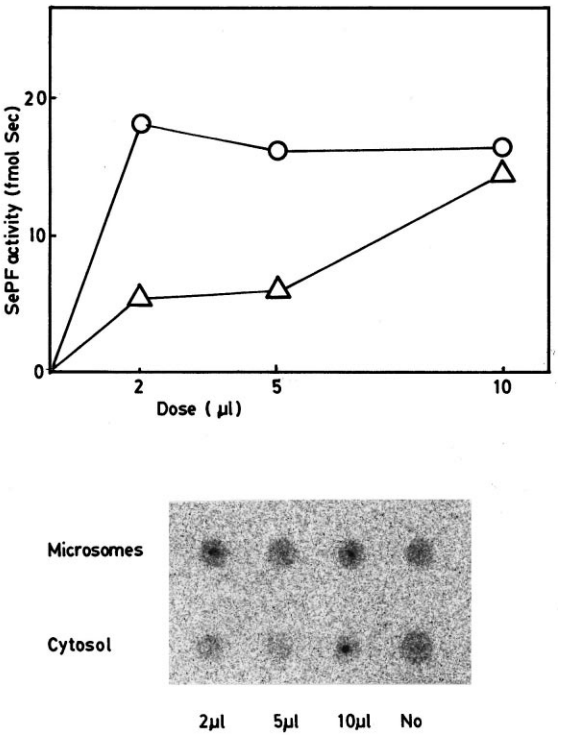

Fig. 3. Dose-response relationships of SePF activity in both fractions 26 in Fig. 2. The upper part shows the relationship between dose and SePF activity. Circles indicate fraction 26 from chromatography of microsomal extracts and triangles indicate fraction 26 from cytosol in Fig. 2. The abscissa gives the dose indicated by volume of both fractions 26 in Fig. 2. The bottom part represents the results of a radio-image pattern. No is non-treated wild-type [^{75}Se]Sec-tRNA and original substrate for SePF assay.

are shown in Fig. 4. In Fig. 4, we found that the spot density of [^{75}Se]Sec of T27 was weak, compared to the spots of the wild-type. This indicates that the mutation from G5a·U67b to G-C (T27) was not protected by SePF. The density of T25 (the mutation from U6·U67 to G-C) and T28 (from G5a·U67b to A·G) was clear and this indicated that these two mutants were protected and recognized by SePF. Table 1 shows that

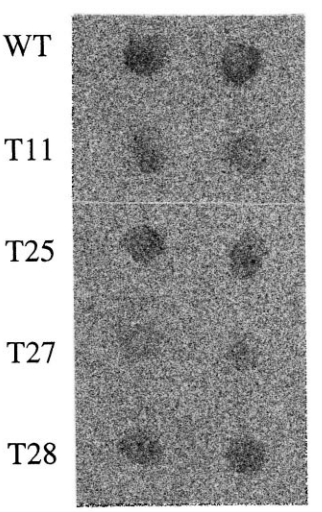

Fig. 4. Dot-blotted patterns (in duplicate) of [^{75}Se]Sec-tRNA wild-type and mutants after the assay of Sec-tRNA protecting activity.

none of the mutations engineered at U6·U67 affected the SePF activity in a significant manner.

This peculiar U6·U67 pair did not function to SePF, as well as serylation and selenocysteylation [12]. The substitution at G5a·U67b was more interesting since conversion to a G-C Watson-Crick base pair was sufficient to completely inhibit the SePF activity. This finding strongly suggests that the SePF is unable to interaction with a $tRNA^{Sec}$ mutant version carrying a G-C base pair, which may constitute one determinant for SePF recognition. In contrast, the substitution to A·G (T28), another type of non-Watson-Crick base pair, did not lead to abolition of this activity. From these experiments, we conclude that a $tRNA^{Sec}$ mutant carrying G5a-C67b instead of G5a·U67b is deleterious to the SePF activity, suggesting a functional role of G5a·U67b, but not U6·U67, for the interaction with this protein.

4. Discussion

We have examined in this work the putative function of two non-Watson-Crick base pairs, G5a·U67b and U6·U67, residing in the long AA-stem of mammalian $tRNA^{Sec}$ [15]. The interest that prompted us to focus our attention on these base pairs arose from phylogenetic considerations: U6·U67 is invariant in vertebrates, *Drosophila* and *C. elegans* [16]; G5a·U67b is conserved in vertebrates and *C. elegans*, but in *Drosophila* this wobble base pair is replaced by A5a·G67b [16], another non-Watson-Crick base pair, suggesting that a non-Watson-Crick status is required for some reason. In fact, an earlier work showed that neither the serylation nor selenylation steps were particularly affected by Watson-Crick replacements at these positions [12]. Instead, we have shown here that the protective activity provided by SePF, the putative selenocysteine elongation factor, against hydrolysis of the Sec-$tRNA^{Sec}$ ester bond is dramatically affected by a G5a-C67b conversion. This finding strongly suggests that this G·U base pair is a structural determinant for recognition or binding of SePF to the $tRNA^{Sec}$. This is not unprecedented and the G3·U70 base pair in $tRNA^{Ala}$ is one important determinant for recognition by the cognate aminoacyl-tRNA synthetase [19]. Several other examples have been described in the literature to involve G·U pairs as unique determinants for RNA-RNA or RNA-protein recognition [20]. There are three G·U base pairs in $tRNA^{Sec}$. The first is G5a·U67b, which has a role in the interaction with SePF, and the second is U12·G23, which should have a role in the interaction with selenocysteine synthase in a base-specific manner [14], although this interaction is not indispensable but increases the affinity with selenocysteine synthase. This second U12·G23 base pair is well conserved between $tRNA^{Sec}$ of *E. coli* and mammalia. The third is G27·U43, whose function is not clear but it may have a role in the maintenance of the active structure of $tRNA^{Sec}$.

The other result of this study shows that a G·U to A·G substitution does not affect protection by SePF. This is perfectly consistent with the fact that the *Drosophila* $tRNA^{Sec}$ possesses an A·G pair at this position. However, this poses the question as to why a G-C pair is deleterious while an A·G is not. In a G·U pair, the exocyclic amino group of the guanine projects into the minor groove of the RNA helix, thus providing a functional group available for ligand recognition. This group is not longer available in the Watson-Crick G-C pair, since it is hydrogen bonded to the O2 of the cytosine. An A·G pair, whatever the geometry, is not isosteric to the G·U pair and will not provide a free NH2 group. One possibility is that the sole presence of a non-Watson-Crick base pair, G·U or A·G, provokes a structural modification of the phosphodiester backbone of the RNA helix, sufficient to provide a recognition signal for SePF. In any event, it is worth noting that the $tRNA^{Sec}$ of the archaeon *M. jannaschii*, despite a great overall sequence divergence with the eukaryotic homologue, also possesses a G·U base pair at this position [17]. This base pair is therefore universal and, taking into account the fact that archaea appear to possess a selenocysteine machinery resembling that in eukaryotes [21], must play a key role in this process.

Meanwhile, recently, the translation machinery on ribosomes has been clarified. EF-Tu in *E. coli* recognized the helical AA-stem and CCA terminal having aminoacyl residues and 5′-terminal and T-stem by all three domains in its EF-Tu [22,23]. The recognition of the AA strand and T-stem by EF-Tu was also manifested by the study with minihelix RNA [24]. The recognition site on EF-Tu was analyzed by X-ray [25] and A, P, and E sites on ribosomes have also been shown by electron cryomicroscopy [26]. The pattern of a complex of EF-Tu-aa-tRNA binding on ribosomes has also been shown [27]. Meanwhile, it has been reported that EF-G takes a structure mimicking that of tRNA [22]. This molecular mimicry becomes the general rule, because RF recognizing stop codon also takes a tRNA-like structure [28].

In *E. coli*, there is an elongation factor (SELB) specific to Sec-tRNA, except EF-Tu which does not recognize Sec-tRNA. The N-terminal of SELB in *E. coli* recognizes Sec-$tRNA^{Sec}$. The C-terminal half of SELB recognizes a stem-loop structure downstream of the Sec UGA codon. This stem-loop structure has a role of discrimination between the Sec UGA codon and the stop UGA codon [4]. Sec UGA codon embedding in this stem-loop structure may be protected from attack of RF. Similar stem-loop structures were found on 5′ UTR and 3′ UTR separating from Sec UGA codon in some bacterial systems [21]. The molecular mass of SELB is 80 kDa, larger than that of EF-Tu, which is 45 kDa. This different region has the function to interact with this stem-loop structure. EF-Tu could not recognize 13 bp of AA-stem and T-stem of Sec-$tRNA^{Sec}$ [29]. But, there is a discrepant result of EF-Tu recognizing the minihelix having 13 bp [24]. It has been reported that the hinge region between AA- and T-stems on $tRNA^{Sec}$ of *E. coli* is anti-determinant to EF-Tu [30]. EF-Tu also did not recognize the AA-stem of initiator tRNA which has a non-base pair at position 1–72 [31].

A Sec-tRNA-protein-antibody complex was found in sera from patients with autoimmune chronic active hepatitis [32]. It is possible that the protein of 50 kDa in the complex is our SePF because of its consistency of molecular mass and not SerRS of molecular mass 65 kDa. We suppose that this phenomenon depends on the increase of Sec-tRNA by some pathogenic condition such as inflammation. We showed that the level of Sec-$tRNA^{Sec}$ was increased three times in dystrophic muscle of inflammation as well as an increase in Se-GPx [33]. It was considered that this increase was dependent upon the oxidative stress in the muscle [34], because Sec-tRNA is essential for the production of Se-GPx, which decomposes hydroxyl peroxide in cytosol.

Acknowledgements: We thank Alain Krol for giving us the $tRNA^{Sec}$ and its mutant transcripts and for improving the manuscript, and Toshinobu Fujiwara for preparation of the manuscript.

References

[1] Keshan Disease Research Group (1979) Chin. Med. J. 92, 477–482.
[2] Sukenaga, Y., Ishida, K., Takeda, T. and Takagi, K. (1987) Nucleic Acids Res. 15, 7178.
[3] Berry, M.J., Banu, L. and Larson, P.R. (1991) Nature 349, 438–440.
[4] Baron, C. and Bock, A. (1995) in tRNA: Structure, Biosynthesis, and Function (Soll, D. and RajBhandary, U.L., Eds.), pp. 524–544, ASM Press, Washington, DC.
[5] Hatfield, D. and Portugal, F.H. (1970) Proc. Natl. Acad. Sci. USA 67, 1200–1206.
[6] Kim, I.Y., Guimaraes, M.J., Zlotnik, A., Bazan, J.F. and Stadtman, T.C. (1997) Proc. Natl. Acad. Sci. USA 94, 418–421.
[7] Mizutani, T., Kurata, H., Yamada, K. and Totsuka, T. (1992) Biochem. J. 284, 827–834.
[8] Mizutani, T., Kurata, H. and Yamada, K. (1991) FEBS Lett. 289, 59–63.
[9] Curnow, A.W., Hong, K., Yuan, R., Kim, S., Martius, O., Winkler, W., Henken, T.M. and Soll, D. (1997) Proc. Natl. Acad. Sci. USA 94, 11819–11826.
[10] Yamada, K., Mizutani, T., Ejiri, S. and Totsuka, T. (1994) FEBS Lett. 347, 137–142.
[11] Yamada, K. (1995) FEBS Lett. 377, 313–317.
[12] Sturchler-Pierrat, C., Hubert, N., Totsuka, T., Mizutani, T., Carbon, P. and Krol, A. (1995) J. Biol. Chem. 270, 18570–18574.
[13] Amberg, R., Mizutani, T., Wu, X.-Q. and Gross, H.J. (1996) J. Mol. Biol. 263, 8–19.
[14] Mizutani, T., Kanaya, K., Ikeda, S., Fujiwara, T., Yamada, K. and Totsuka, T. (1998) Mol. Biol. Rep. (in press).
[15] Sturchler, C., Westhof, E., Carbob, P. and Krol, A. (1993) Nucleic Acids Res. 21, 1073–1079.
[16] Lee, B.J., Rajagopalan, M., Kim, Y.S., You, K.H., Jacobson, K.B. and Hatfield, D. (1990) Mol. Cell. Biol. 10, 1940–1949.
[17] Bult, C.J., White, O., Olsen, G.J., Zhou, L., Fleischmann, R.D., Sutton, G.G., Blake, J.A., FitzGerald, L.M., Clayton, R.A., Gocayne, J.D., Kerlavage, A.R., Dougherly, B.A., Tomb, J.F., Adams, M.D., Reich, C.I., Overbeek, R., Kirkness, E.F., Weinstock, K.G., Merrick, J.M., Glodek, A., Scott, J.L., Geoghagen, N.S.M., Weidman, J.F., Fuhrmann, J.L., Nguyen, D., Utterback, T.R., Kelley, J.M., Peterson, J.D., Sadow, P.W., Hanna, M.C., Cotton, M.D., Roberts, K.M., Hurst, M.A., Kaine, B.P., Borodovsky, M., Klenk, H.P., Fraser, C.M., Smith, H.O., Woese, C.R. and Venter, J.C. (1996) Science 273, 1058–1073.
[18] Stapulionis, R. and Deutsher, M.P. (1995) Proc. Natl. Acad. Sci. USA 92, 7158–7161.
[19] Gabriel, K., Schneider, J. and McClain, W.H. (1996) Science 271, 195–197.
[20] Benard, L., Mathy, N., Grunberg-Manago, M., Ehresmann, B., Ehresmann, C. and Portier, C. (1998) Proc. Natl. Acad. Sci. USA 95, 2564–2567.
[21] Wilting, R., Schorling, S., Persson, B.C. and Bock, A. (1997) J. Mol. Biol. 266, 637–641.
[22] Nissen, P., Kjeldgaad, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B.F.C. and Nyborg, J. (1995) Science 270, 1464–1472.
[23] Sprang, S.R. (1997) Annu. Rev. Biochem. 66, 639–678.
[24] Rudinger, J., Blechschmidt, B., Ribeiro, S. and Sprinzl, M. (1994) Biochemistry 33, 5682–5688.
[25] Berchtold, H., Reshetnikova, L., Reiser, C.O., Schirmer, N.K., Sprinzl, M. and Hilgenfeld, R. (1993) Nature 365, 126–132.
[26] Agrawal, R.K., Penczek, P., Grassucci, R.A., Li, Y., Leith, A., Nierhaus, K.H. and Frank, J. (1996) Science 271, 1000–1002.
[27] Stark, H., Rodnina, M.V., Rinke-Appel, J., Brimacombe, R., Wintermeyer, W. and van-Heel, M. (1997) Nature 389, 403–406.
[28] Nakamura, Y., Ito, K. and Isaksson, L.A. (1996) Cell 87, 147–150.
[29] Baron, C. and Bock, A. (1991) J. Biol. Chem. 266, 20375–20379.
[30] Rudinger, J., Hillenbrandt, R., Sprinzl, M. and Giege, R. (1996) EMBO J. 15, 650–657.
[31] Seong, B.L. and RajBhandary, L.L. (1987) Proc. Natl. Acad. Sci. USA 84, 8859–8863.
[32] Gelpi, C., Sontheimer, E.J. and Rodriguez-Sanchez, J.L. (1992) Proc. Natl. Acad. Sci. USA 89, 9739–9743.
[33] Hitaka, T., Mizutani, T., Watanabe, K. and Totsuka, T. (1990) Biochem. J. 266, 201–206.
[34] Watanabe, K., Yamada, K., Mizutani, T. and Totsuka, T. (1993) Free Radical Res. Commun. 19, 93–100.