RNA (1998), *4*:241–245. Cambridge University Press. Printed in the USA.

LETTER TO THE EDITOR

The secondary structure of eukaryotic selenocysteine tRNA: 7/5 versus 9/4

SERGEY V. STEINBERG,[1] ANATOLI IOUDOVITCH, and ROBERT CEDERGREN
Département de Biochimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada

Keywords: selenocysteine tRNA; tRNA; tRNA identity; tRNA structure

INTRODUCTION

Insertion of selenocysteine into a growing peptide requires the unusual tRNA^{Sec} (Zinoni et al., 1987; Stadtman, 1990; Böck et al., 1991). This tRNA has an extended D-stem containing six base pairs, which, in the case of eukaryotic tRNA^{Sec} (euk-tRNA^{Sec}), is the key identity element for selenylation and phosphorylation (Wu & Gross, 1994; Amberg et al., 1996). Two secondary structures have been proposed for the euk-tRNA^{Sec}, which differ in the base pairing of the acceptor/T helical domain (Diamond et al., 1981; Böck et al., 1991; Sturchler et al., 1993). One structure has the normal seven base pairs in the acceptor stem and five base pairs in the T-stem (7/5 structure, Fig. 1, left), and is characterized by an unusually long four-nucleotide unpaired region between the acceptor and D-stems (Connector 1) and an unpaired nucleotide, C64a, in the T-stem. The alternate structure features the normal two nucleotides in Connector 1 and a 13-base pair acceptor/T domain comprised of nine base pairs in the acceptor stem and four in the T-stem (9/4 structure, Fig. 1, right). This 9/4 structure was initially proposed by analogy with the prokaryotic tRNA^{Sec} (prok-tRNA^{Sec}), which also contains 13 base pairs in the acceptor/T helical domain. However, in this case, there are eight and five base pairs in the acceptor and T-stems, respectively. The acceptor/T helical domain having 13 base pairs is thought to be a key structural element determining the functionalities pattern of tRNA^{Sec} in both prokaryotes and eukaryotes (Böck et al., 1991).

Using enzymatic and chemical probing, Sturchler et al. (1993) favored the 9/4 structure, for which a three-dimensional model was proposed. Since then, new experimental data have been collected on serylation, selenylation, and phosphorylation of the euk-tRNA^{Sec} and mutants thereof (Wu & Gross, 1993, 1994; Ohama et al., 1994; Sturchler-Pierrat et al., 1995; Amberg et al., 1996). The point by point analysis presented here shows that the activities of the euk-tRNA^{Sec} and its mutants in serylation, selenylation, and phosphorylation are better explained by the 7/5 structure.

Reprint requests to: Sergey V. Steinberg, Département de Biochimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada; e-mail: serguei.chteinberg@umontreal.ca.
[1]On the leave from Engelhardt Institute of Molecular Biology, Vavilova 32, Moscow, Russia.

GENERAL CRITERIA

Recently, criteria for the juxtaposition of the acceptor/T and anticodon/D helical domains have been proposed based on the lengths of paired and unpaired regions in the tRNA secondary structure (Steinberg et al., 1997). One criterion requires a minimum of two nucleotides in Connector 1 to facilitate the connection between the acceptor and D-stems. Another states that the T-stem should consist of five or six layers of stacked nucleotides to allow for the normal D/T loop interaction. Violation of either criterion, if not compensated (Steinberg et al., 1997), leads to deformations in the arrangement of the helical domains, which may render the tRNA nonfunctional. Compensations include extension of the anticodon stem to more than the normal six base pairs for a shorter Connector 1 (Steinberg & Cedergren, 1994) and extension of the anticodon/D helical domain to more than the normal 12 layers for a shorter T-stem (Steinberg et al., 1997). In the following analysis, we have assumed that tRNA in serylation, selenylation, and phosphorylation must have the normal juxtaposition of the acceptor/T and anticodon/D helical domains and thus must fulfil the above criteria.

Analysis of the wt euk-tRNA^{Sec}

1. The "7/5" structure could have either five or six nucleotide layers in the T-stem, depending on whether the unpaired nt C64a is bulged or stacked into the helical domain. However, either way, the criteria for a

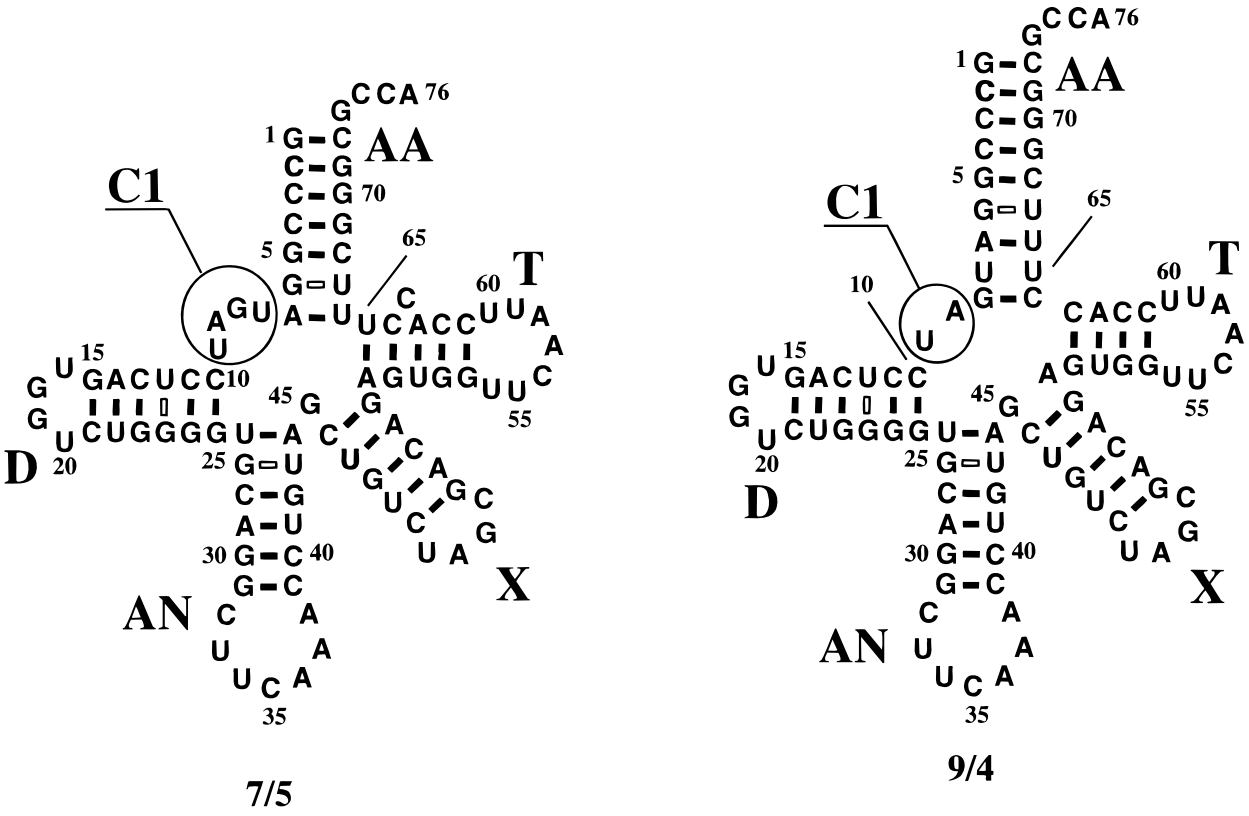

FIGURE 1. Nucleotide sequence of the human tRNA^{Sec} folded into alternate secondary structures: the 7/5 structure to the left and the 9/4 structure to the right. Numbering of nucleotides is taken from Sprinzl et al. (1996) and is different from that used in Sturchler et al. (1993). Nucleotides G9, U20, and C64 are followed by A9a and U9b, by C20b, and by C64a, respectively. AA, D, AN, T, X, and C1 represent the acceptor, D-, anticodon, and T-stems, the extra arm, and Connector 1, respectively. Structure 7/5 has a longer Connector 1 and an unpaired nucleotide in the T-stem.

normal D/T-loop interaction is satisfied (Fig. 2). The 9/4 structure, due to a T-stem of only four base pairs (Steinberg et al., 1997), does not provide for a normal D/T-loop interaction.

2. The 9/4 structure predicts two base pairing combinations, 8–65 and 9–64a. Nucleotide variations at these positions, however, do not support these pairs. Pair 8–65 is U-U in all euk-tRNAs^{Sec} and its conversion into a Watson–Crick or G-U combination has no major effect on either serylation or selenylation (Ohama et al., 1994; Sturchler-Pierrat et al., 1995). The nature of pair 9–64a does not have a Watson–Crick requirement either, because the mutant harboring the G9 → A replacement was effectively serylated and phosphorylated (Wu & Gross, 1994). In contrast, nt 8–65 and 9–64a in the 7/5 model belong to different domains and therefore would not be expected to have Watson–Crick relationships.

The bulged nucleotide in the T-stem

3. A deletion of nt C64a accompanied by replacement G9 → A does not affect either serylation or phosphorylation (mutant X12, Wu & Gross, 1994). The inability of the 9/4 structure to accommodate this mutant was recognized by Wu and Gross (1994, Fig. 1), because no more than seven base pairs could be formed in the acceptor stem. To the contrary, the 7/5 structure is not affected by this deletion (Fig. 2).

4. The replacement of the acceptor/T domain in the euk-tRNA^{Sec} by the corresponding region from the tRNA^{Ser} preserves both serylation and phosphorylation (mutant X9, Wu & Gross, 1993, 1994). This mutant folds exclusively in the 7/5 structure (Fig. 2).

5. The deletion of U65, together with the replacement G9 → A, does not seriously affect either selenylation or phosphorylation (mutant X12H, Amberg et al., 1996). The A49–C64a pair in this mutant can be accommodated in the 7/5 structure (Fig. 2), and, as described in #2 above, the G9 → A replacement does not affect selenylation. The 9/4 structure (see Fig. 5 in Amberg et al., 1996) is an unlikely form for this mutant because, in addition to the formation of pair A9–C64a, the intercalation of the unpaired U8 into the acceptor stem is required. The combination of both irregularities would damage the stability of the acceptor stem.

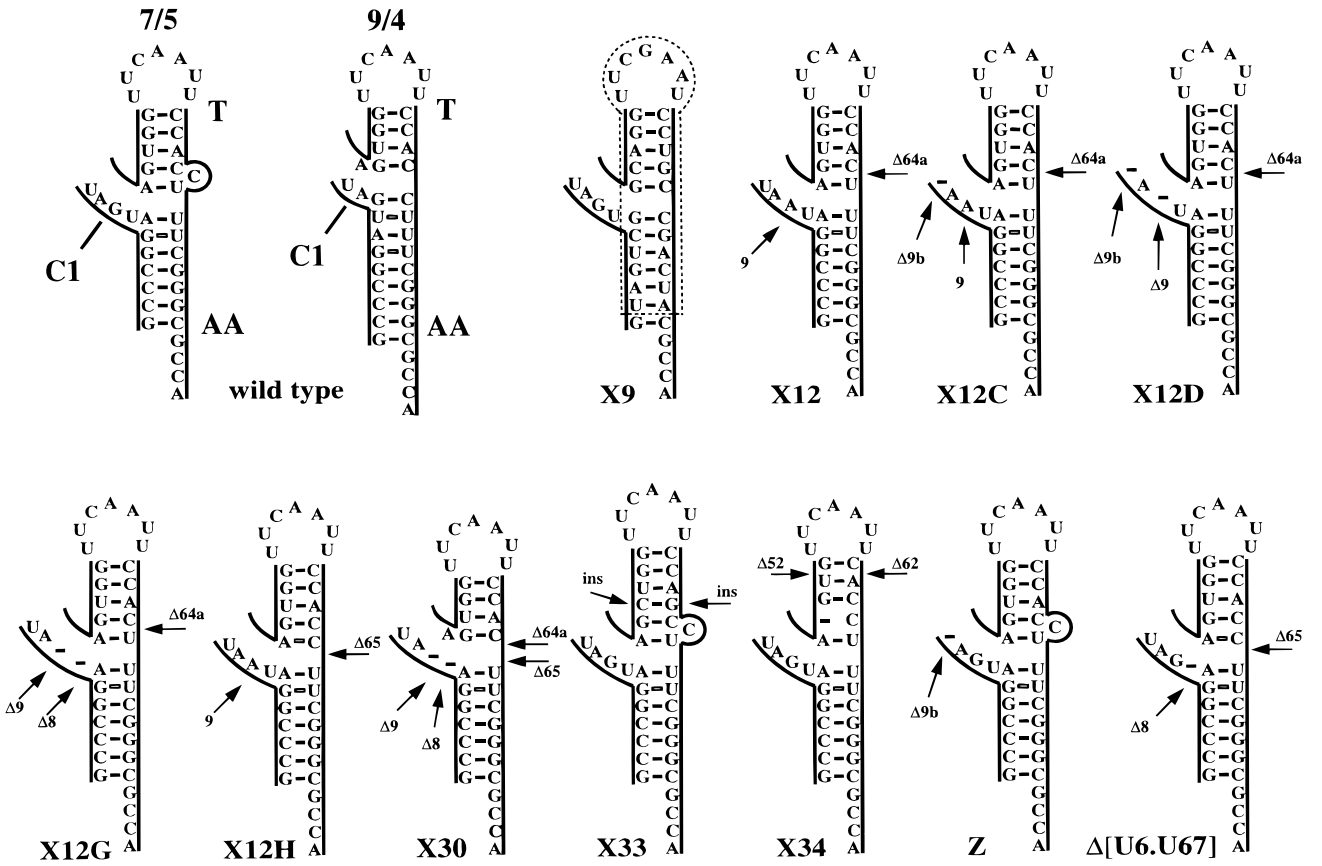

FIGURE 2. Structure of the acceptor/T helical domain in human $tRNA^{Sec}$ and mutants thereof discussed in this paper. For the wt tRNA, both the 7/5 and the 9/4 structures are presented, whereas, for the mutant tRNAs, only the 7/5 structures are shown. AA, T, and C1 in the wt tRNA structures stand for the acceptor stem, the T-stem, and Connector 1, respectively. Arrows indicate the nucleotides in mutant tRNAs that differ from those in the wt euk-$tRNA^{Sec}$. Numbers correspond to the nucleotide positions in Figure 1. Δ and "ins" stand for deletions and insertions, respectively. The region in mutant X9 surrounded by a dashed line, including the D-stem and loop and a part of the acceptor stem, was taken from the $tRNA^{Ser}$ (Wu & Gross, 1994). In mutant Z, nucleotide U9b is not deleted, but rather a part of the D-stem (see #7 in the text and Fig. 3).

The length of Connector 1

6. Deletion of U9b and C64a accompanied by the replacement G9 → A does not seriously affect either selenylation or phosphorylation (mutant X12C, Amberg et al., 1996). However, deletion of C64 deprives mutant X12C of the ability to be folded into the 9/4 structure. Moreover, the intercalation of A9 needed to form a nine-base pair acceptor stem (see Fig. 5, Amberg et al., 1996) leaves only one nucleotide in Connector 1, rendering the normal connection between the acceptor and D-stems sterically impossible. On the other hand, in the 7/5 structure, three nucleotides in Connector 1 would be retained (Fig. 2).

7. Shortening of Connector 1 by one nucleotide does not affect serylation. Ohama et al. (1994) reported that the mutant having two replacements C11 → G and G23 → C in the D-stem (Fig. 3, left) fully preserved the serylation capacity, even though these mutations result in two mismatches, G11–G24 and U12–C23, in the D-stem. A more probable structure of this region involves bulging U12 and forming three new pairs, G11–C23, C10–G24, and U9b-G25 (Fig. 3, right; Fig. 2Z). Because U9b comes from Connector 1 in this structure, Connector 1 must have more than two nucleotides, as in the 7/5 but not in the 9/4 structure.

8. Deletion of two nucleotides from Connector 1 and nt C64a in mutants X12D and X12G does not abolish either selenylation or phosphorylation (Amberg et al., 1996). Only the 7/5 structure is possible for these mutants (Fig. 2): a deletion of two nucleotides from Connector 1 would not affect this secondary structure, because two connector nucleotides remain. However, the attempt to restore the nine-base pair acceptor stem leaves no nucleotides for Connector 1 in the 9/4 structure (see Fig. 5 in Amberg et al., 1996).

The lengths of the acceptor and T-stems

9. Deletion of nt U8–U65 (mutant [U6.U67], Sturchler-Pierrat et al., 1995) is less detrimental for selenylation

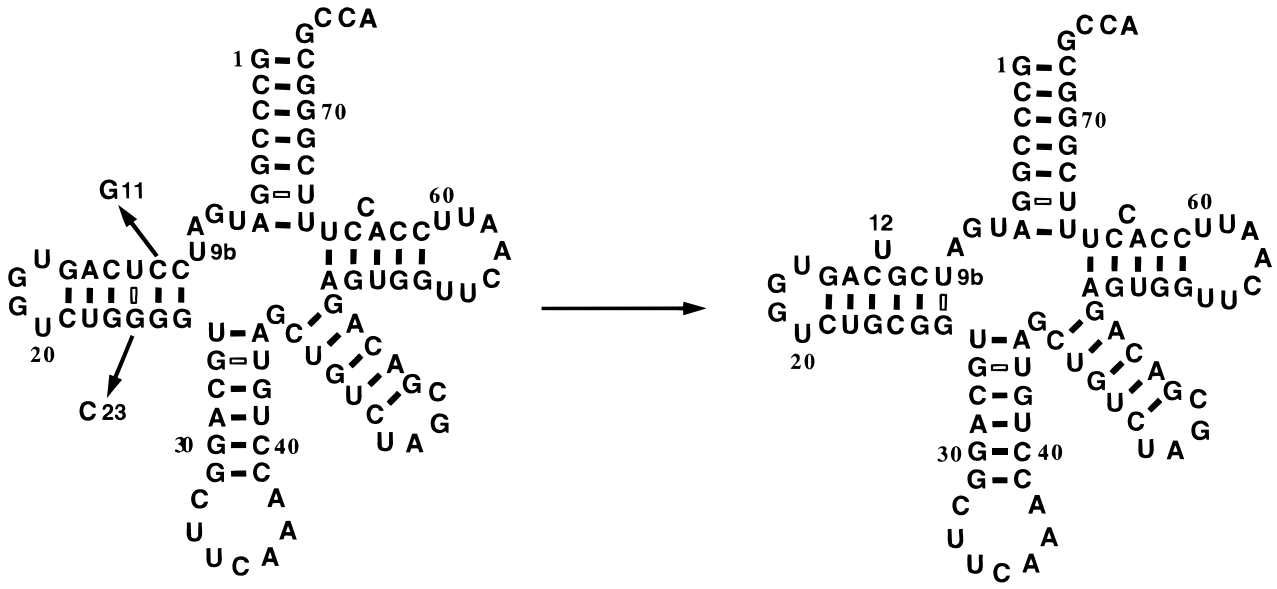

FIGURE 3. Nucleotide sequence of the wt and mutant tRNA^{Sec} from *Homo sapiens* (Ohama et al., 1994, see the acceptor/T domain representation in Fig. 2Z). Replacement of the C11 and G23 by G and C, respectively, results in two mismatches in the D-stem. The normal base pairing can, however, be restored, if U12 is bulged out and U9b is involved in the base pairing. Only 7/5 structure can accommodate this rearrangement. The 9/4 structure leaves only one nucleotide in Connector 1.

than deletion of base pairs C3-G70, G6–U67, or A7–U66 (respectively, [C3-G70], [G5a-U67b], and [A5b-U67a]). None of these deletions can be accommodated in the 9/4 structure, because they result in no more than eight base pairs in the acceptor stem. In the 7/5 structure, however, the U8–U65 combination, unlike the three other combinations, does not form a base pair (Fig. 2), whereas deletion of U65 or a nucleotide from Connector 1, as mutants X12H and X12C have shown, has only a minor effect on selenylation.

10. A deletion of base pair G52–C62 from the T-stem improves serylation and only slightly diminishes selenylation and phosphorylation (mutant X34, Amberg et al., 1996). The 9/4 model cannot explain this fact because a deletion of a base pair from an already shortened T-stem would make it even more difficult to create the proper D/T-loop interaction. Although the 7/5 model is also affected by this deletion, intercalation of nt C64a could compensate for the deletion and restore the normal D/T-loop interaction (Fig. 2).

11. Deletion of nt U8, G9, C64a, and U65 abolishes both serylation and selenylation (mutant X30, Amberg et al., 1996). This mutant differs from X12G by the additional deletion of U65. In the 7/5 model, this deletion deprives A49 of its Watson–Crick partner in the T-stem, which would leave the latter with only four base pairs, thus preventing the normal D/T-loop interaction (Fig. 2).

12. Insertion of a base pair in the T-stem abolishes serylation (mutant X33, Amberg et al., 1996). Both the 9/4 and 7/5 structures are able to accommodate this mutation: in the 9/4 structure, the addition of a base pair in the T-stem provides the optimal five base pairs, whereas, in the 7/5 structure, it increases the length of the T-stem to the maximally allowable six base pairs (Fig. 2). The situation with the 7/5 structure is different, however, because the unpaired nt C64a (or C64), would have to be bulged, unlike in the wt sequence, to avoid extending of the T-stem to more than six layers. If this nucleotide was bulged, it could prevent the normal interaction with the seryl-tRNA synthetase and abolish the serylation.

This suggestion is compatible with the experimental data indicating that the eukaryotic seryl-tRNA synthetase probably interacts directly with the T-stem. It was recently shown by Acshel and Gross (1993) and by Ohama et al. (1994), that even minor modifications, such as changing of Watson–Crick pairs in this region of the T-stem, decreased the efficiency of serylation. We note that, of all mutants presented here, only those able to fold into a 7/5-type structure without requiring a bulged nucleotide in the T-stem are active in serylation. A bulge in the T-stem abolishing serylation is used in a further analysis (Ioudovitch & Steinberg, 1998) to explain the behavior of euk-tRNA^{Ser} mutants.

CONCLUSION

The above analysis strongly supports the 7/5 structure for the euk-tRNA^{Sec}. It also predicts that the acceptor/T helical domain does not contain any major identity elements for the enzymes involved in selenylation and phosphorylation. The existence of the unpaired nucle-

otide in the T-stem of the wt euk-tRNA^{Sec} (nt C64 or C64a) is neither necessary nor harmful for the serylation, selenylation, or phosphorylation. Whether either C64 or C64a is bulged in the solution euk-tRNA^{Sec} structure is not known, although the fact that the backbone between C64a and U65 is sensitive to ribonuclease V1 (specific for stacked and helical regions) while insensitive to ribonuclease T2 (cleaving single-stranded regions) points to the possible insertion of C64a into the double helix (Sturchler et al., 1993). Whether C64 bulges or not is less clear, because the linkage between C64 and C64a was not cleaved by either of V1 or T2. The interpretation of these results may be compromised, however, by the inconsistent behavior of enzymes V1 and T2: ribonuclease V1 cleaved between two unstacked nt U60 and C61, whereas ribonuclease T2 cleaved efficiently in the middle of the D-stem (Sturchler et al., 1993).

Chemical protection experiments (Sturchler et al., 1993) show a higher reactivity of N^3-U8 than N^3-U65, which is consistent with the fact that U8 belongs to the connector region in the 7/5 structure, whereas U65 pairs to A49. On the other hand, the complete accessibility observed for nt U12, G50, G52, G53, and A63, known to form base pairs in the D- and T-stems, raises questions about the applicability of this approach. It seems that the probing experiments do not distinguish well between the two alternate secondary structures, whereas the activity data strongly support the 7/5 model.

ACKNOWLEDGMENTS

This work was supported by a grant from the Natural Science and Engineering Council of Canada. R.C. is Richard Ivey Fellow of the Canadian Institute of Advanced Research.

REFERENCES

Acshel T, Gross HJ. 1993. Identity determinants of human tRNA^{Ser}: Sequence elements necessary for serylation and maturation of a tRNA with a long extra arm. *EMBO J 12*:3333–3338.

Amberg R, Takaharu M, Wu XQ, Gross HJ. 1996. Selenocysteine synthesis in mammalia: An identity of switch from tRNA^{Ser} to tRNA^{Sec}. *J Mol Biol 263*:8–19.

Böck A, Forchhammer K, Heider J, Baron C. 1991. Selenoprotein synthesis: An expansion of the genetic code. *Trends Biochem Sci 16*:463–467.

Diamond A, Dudock B, Hatfield D. 1981. Structure and properties of a bovine liver UGA suppressor serine tRNA with a triptophane anticodon. *Cell 25*:497–506.

Ioudovitch A, Steinberg SV. 1998. Modeling the tertiary interactions in the eukaryotic selenocysteine tRNA. *RNA 4*:in press.

Ohama T, Yang DCH, Hatfield DL. 1994. Selenocysteine tRNA and serine tRNA are aminoacylated by the same synthetase, but may manifest different identities with respect to the long extra arm. *Archives Biochem Biophys 315*:293–301.

Sprinzl M, Steegborn C, Hübel F, Steinberg S. 1996. Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res 24*:68–72.

Stadtman TC. 1990. Selenium biochemistry. *Annu Rev Biochem 59*:111–127.

Steinberg S, Cedergren R. 1994. Structural compensation in atypical mitochondrial tRNAs. *Nature Struct Biol 1*:507–510.

Steinberg S, Leclerc F, Cedergren R. 1997. Structural rules and conformational compensations in the tRNA L-form. *J Mol Biol 266*:269–282.

Sturchler C, Westhof E, Carbon P, Krol A. 1993. Unique secondary and tertiary features of the eukaryotic selenocysteine tRNA^{Sec}. *Nucleic Acids Res 21*:1073–1079.

Sturchler-Pierrat C, Hubert N, Totsuka T, Mizutani T, Carbon P, Krol A. 1995. Selenocysteylation in eukaryotes necessitates the uniquely long aminoacyl acceptor stem of selenocysteine tRNA^{Sec}. *J Biol Chem 270*:18570–18574.

Wu XQ, Gross HJ. 1993. The long extra arms of human tRNA$^{(Ser)Sec}$ and tRNA^{Ser} function as major identity elements for serylation in an orientation-dependent, but not sequence specific manner. *Nucleic Acids Res 21*:5589–5594.

Wu XQ, Gross HJ. 1994. The length and the secondary structure of the D-stem of human selenocysteine tRNA are the major identity determinants for serine phosphorylation. *EMBO J 13*:241–248.

Zinoni F, Birkmann A, Leinfelder W, Böck A. 1987. Cotranslational insertion of selenocysteine into formate dehydrogenase from *Escherichia coli* directed by a UGA codon. *Proc Natl Acad Sci USA 84*:3156–3160.