

Eukaryotic selenocysteine tRNA has the 9/4 secondary structure

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Abstract There are two secondary structure models for the eukaryotic selenocysteine (Sec) tRNA^{Sec}. One model, the 9/4 structure, was experimentally tested and possesses acceptor and T-stems with 9 and 4 bp, respectively [Sturchler et al., 1993; Hubert et al., 1998]. The other one, the 7/5 secondary structure with a bulge in the T-stem, was derived from theoretical calculation [Ioudovitch and Steinberg, 1999]. In this report, we show more experimental results supporting the 9/4 secondary structure. Several tRNA^{Sec} mutants, whose secondary structure can adopt only the 9/4 structure, were active for serylation and selenylation. Some mutants that cannot base-pair between positions 26 and 44 to provide the 6 bp anticodon stem were still active, inconsistent with the model by Steinberg. We also show that the orientation of the V-arm directly or indirectly influences the selenylation activity, and that the rigid 6 bp D-stem is important. Finally, we conclude that all tRNAs^{Sec} possess the 13 bp domain II made by the stacking of the colinear AA and T-stems, whether they present the 9/4 structure in Eukarya and Archaea or the 8/5 structure in bacteria.

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Key words: tRNA; Selenocysteine; Selenium; tRNA structure

1. Introduction

Selenocysteine (Sec) plays a key role in the redox function of glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases. Sec is formed on its tRNA^{Sec} from seryl-tRNA^{Sec} by Sec synthase. The selenocysteine tRNA^{Sec} has two characteristic features. It possesses the anticodon UCA complementary to the selenocysteine codon UGA. The other feature is the peculiar 9/4 secondary structure, differing from the general standard 7/5 tRNA structure; 9 and 7 stand for the lengths of the AA stem, 4 and 5 for those of the T-stem. The 9/4 secondary structure of the eukaryotic tRNA^{Sec} was suggested based on the secondary structure of the *Escherichia coli* tRNA^{Sec} having the 8/5 structure [1]. Experimental evidence arguing in favor of the 9/4 secondary structure of the eukaryotic tRNA^{Sec} has been shown [2,3]. This secondary structure was different from the 7/5 model which has an unexpected bulge in the T-stem [4]. Recently, it has been shown that the archaeal tRNA^{Sec} can adopt the 9/4 secondary structure as well [3,5]. Thus, it is considered that tRNA^{Sec} can fold into the 9/4 or 8/5 secondary structures. However, Ioudovitch

and Steinberg [5] have maintained firmly their 7/5-bulge secondary structure model for the eukaryotic tRNA^{Sec}, based on theoretical considerations [6–8]. They however agreed for the existence of the 8/5 and 9/4 structures of eubacterial and archaeal tRNAs^{Sec}, respectively [5]. Their theory can mislead the readers that the eukaryotic tRNA^{Sec} adopts the 7/5-bulge secondary structure only. The purpose of this report is to clarify the situation by bringing new arguments in favor of the 9/4 secondary structure of the eukaryotic tRNA^{Sec}.

The selenocysteine synthesis and its incorporation into selenoproteins is well established in bacteria. The tRNA^{Sec} is first charged with Ser by the conventional Ser-tRNA synthetase (SerRS). The product Ser-tRNA^{Sec} is subsequently bound by selenocysteine synthase (SecS), an enzyme which converts the Ser-residue to Sec, using an activated phosphoselenoate compound as the Se-donor. Sec-tRNA^{Sec} is then brought to an in frame Sec-specifying UGA codon by a specific elongation factor different from EF-Tu.

The current state of research on the eukaryotic tRNA^{Sec} can be summarized as follows. The functional sites on tRNAs for the aminoacyl-tRNA synthetases have been termed the identity sites on tRNA [9]. We determined the recognition sites on for the selenocysteine synthase on the tRNA^{Sec} [10,11]. Eukaryotic tRNA^{Sec} exhibits an aminoacyl acceptor-stem with a unique length of 9 bp. None of the point mutations on the 9 bp AA-stem significantly modified the selenylation level. In contrast, reduction of the AA-stem length to 8 bp led the tRNA^{Sec} to lose or reduce its ability to efficiently support selenylation [12]. This result provided strong evidence that the length of the acceptor stem is of prime importance for the serine to selenocysteine conversion step.

The tRNA^{Sec} has a short 4 bp T-stem and an elongated 6 bp D-stem, as well as the long 9 bp AA-stem described above. We showed that the elongated 6 bp D-stem was another essential element on this tRNA, because tRNA^{Sec} mutants having a D-stem length decreased to 4 bp were inactive in selenylation. Therefore, the long 9 bp AA-stem and the elongated 6 bp D-stem are two essential recognition sites for selenylation. This was confirmed by an identity switch from tRNA^{Ser} to tRNA^{Sec}. In this experiment, the 7 bp AA-stem and 4 bp D-stem of tRNA^{Ser} were converted to the active mutants having the 9 bp AA-stem and 6 bp D-stem [13].

We succeeded in the conversion of tRNA^{Ser} to tRNA^{Sec}, but the selenylation activity was weak and about 1/20 of that of the native tRNA^{Sec} [14]. This suggests an influence of base specificity on the tRNA^{Sec} and/or the involvement of the orientation of the V-arm. We also showed that the 4 bp T-stem of the tRNA^{Sec} is not important for selenylation, because tRNA^{Sec} mutants having 3, 4 or 5 bp T-stems still possessed selenylation activity [14]. The length of the coaxial helix (domain II) formed by the stacking of the AA and T-stems is essential for serylation, because tRNA^{Sec} having 12 or 13 bp

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Abbreviations: Sec, selenocysteine; SecS, selenocysteine synthase; SerRS, Seryl-tRNA synthetase; AA, aminoacyl-acceptor; T, TψC; D, dihydrouridine; AC, anticodon; V, variable arm

of domain II were still active, but not the mutants carrying 11 or 14 bp of domain II. This suggests that the SerRS measures the length between the discriminating base G73 and the V-arm [14,15].

The secondary structure model for the tRNA^{Sec} was proposed based on enzymatic and chemical probing [2]. RNase T2 did not digest the bulge position in the 7/5-bulge model and modification with dimethyl sulfate showed the absence of this bulge in the 7/5-bulge structure of Steinberg et al. Despite this experimental evidence, Ioudovitch and Steinberg [8] con-

cluded that our data fit the 7/5-bulge model better than the 9/4 secondary structure model. Their analysis revealed the ability to comply with their L-form compensatory rules within the 7/5 structure. After the publication by Ioudovitch and Steinberg [8], Krol and coworkers presented more experimental data supporting the eukaryotic 9/4 model, as well as a secondary structure model for the tRNA^{Sec} of the Archaea *Methanococcus jannaschii* which can also fold into the 9/4 structure model [3]. This secondary structure model for the archaeal tRNA^{Sec} has been also confirmed by Ioudovitch and Steinberg [5]. They

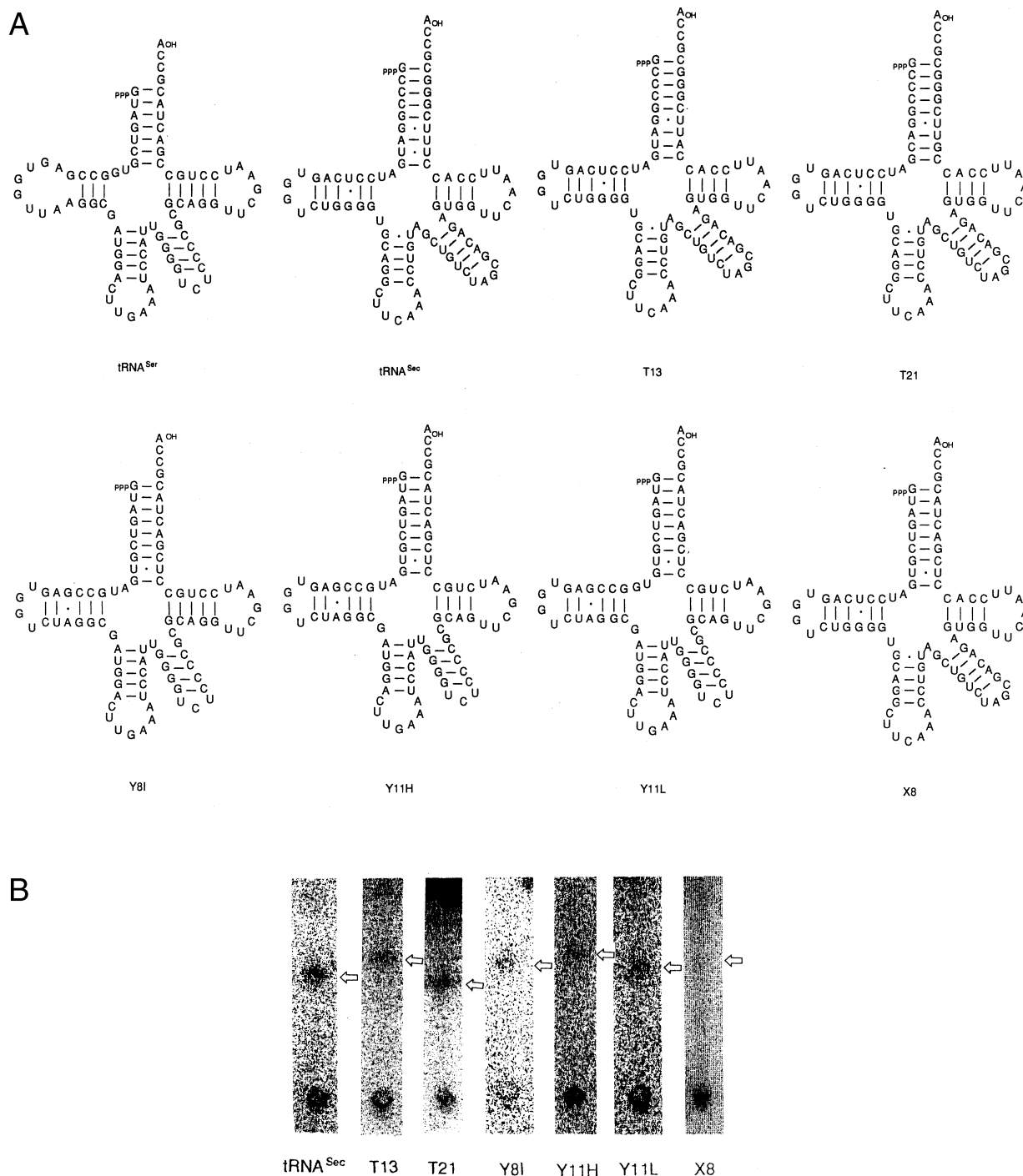


Fig. 1. Eukaryotic active selenocysteine tRNAs. A: Secondary structure models of various tRNAs. B: The TLC patterns of [⁷⁵Se]Sec produced on and liberated from those tRNAs.

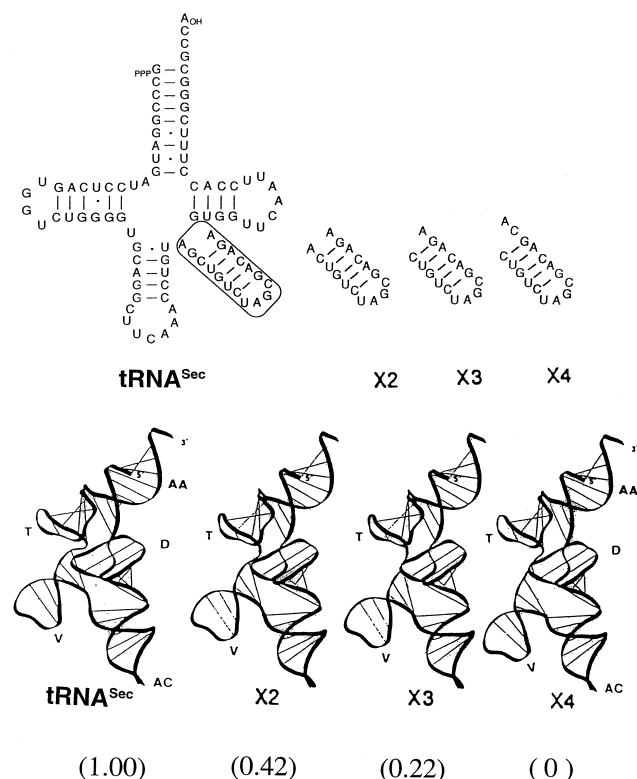


Fig. 3. Relationships between the orientation of V-arm and the selenylation activity. Upper panel: Secondary structure of the V-arm in the wt $tRNA^{Sec}$, mutants X2, X3 and X4. Lower panel: Proposed tertiary structure models for these mutants; parentheses indicate the relative selenylation activity (V_{max}/K_m of wt = 1).

the 6 bp D-stem is essential. T16 with A14*A21 and U2 with A14*C21 were inactive. Thus, the mutants having unpaired portions, such as X15, T16, and U2 were very weak or inactive. This shows that a rigid 6 bp D-stem is better for recognition by SecS. Base pair U12-G23 in the 6 bp D-stem may act in a base-specific manner. The 6 bp D-stem is completely different from the 3 bp D-stem in classical tRNAs. This rigid 6 bp D-stem lifts up domain II of $tRNA^{Sec}$ and the junction between domains I and II should be influenced by this 6 bp D-stem.

The model by Ioudovitch and Steinberg [5] proposed the existence of a 6 bp AC-stem. However, a 6 bp AC-stem is not essential, because the three active mutants Y20 Y23 and Y24 [13] have G26 and A44, which cannot base-pair. The active X3 mutant described in the next section has also an unpaired U26 and C44. They only have a 5 bp AC-stem, showing that a 6 bp AC-stem is not essential for serylation and selenylation.

3.3. The orientation of the V-arm influences the selenylation activity, not serylation

Fig. 3 (upper panel) shows the structure of the V-arms of a few $tRNA^{Sec}$ mutants. These mutants differ at positions 44-45 and 48, at the junction of the V-arm. Therefore, the orientation of the V-arm is different and proposed tertiary structure models for these mutants are shown in Fig. 3 (lower panel), derived from [2]. According to the number at position 48, the decrease of the position 44-45, the V-arm bends to the AC-arm and leaves from the T-loop. The selenylation activity, as shown in the parentheses of Fig. 3, decreased according to the bend of the V-arm. Mutants X2 and X3 were active, while

mutant X4 was inactive. As a matter of fact, the native $tRNA^{Sec}$ mutants X2, X3 and X4 all displayed full serylation activity (data not shown). These results suggest that SecS directly or indirectly recognizes the orientation of the V-arm as well as the other two key elements, the 9 bp AA-stem and the 6 bp D-stem. SecS may bind inside the L-shape tRNA and may cover the V-arm. However, it is possible that the orientation of the V-arm indirectly influences the active tRNA structure for recognition by SecS and there may be some base-specificity for recognition of the V-arm by SerRS and SecS.

We suppose that Steinberg and coworkers insisted on the 7/5-bulge model in order to deduce a general 7/5 secondary structure for all tRNAs. However, the 5 bp T-stem+one bulge is not identical to the 5 bp-only in a tRNA structure. And their model of 7/5 secondary structure contains the four bases between AA-stem and D-stem, inconsistent with two bases of almost all tRNAs. Steinberg stands that all tRNAs must fit the L-type secondary structure model. Surprisingly, even mitochondrial tRNAs missing the D or T-arms fit the L-type [6]. However, one should take into account that biological systems can contain exceptions, such as the $tRNA^{Sec}$ with the 9/4 secondary structure. The 9/4 structure of the $tRNA^{Sec}$ is a true fact among Archaea and Eukarya. Mutant X33 having the 9/5 structure is well selenylated but scarcely serylated [14]. This means that SecS did not recognize the length of the T-stem but that SerRS measures the length of domain II. The tRNA mutants having 12 or 13 bp of domain II were acceptable by SerRS but mutants having 11 or 14 bp of domain II were not recognized by SerRS. Thus, SecS accepts mutants having 12, 13, and 14 bp of domain II.

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