FEBS Letters 584 (2010) 342–349

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Distinct genetic code expansion strategies for selenocysteine and pyrrolysine are reflected in different aminoacyl-tRNA formation systems

Jing Yuan [a,1,*], Patrick O'Donoghue [a,1,*], Alex Ambrogelly [c], Sarath Gundllapalli [c], R. Lynn Sherrer [a], Sotiria Palioura [a], Miljan Simonović [d], Dieter Söll [a,b]

[a] Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8114, USA
[b] Department of Chemistry, Yale University, New Haven, CT 06520-8114, USA
[c] Schering-Plough Corporation, Kenilworth, NJ 07033-0530, USA
[d] Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL 60607, USA

ARTICLE INFO

Article history:
Received 21 October 2009
Revised 3 November 2009
Accepted 4 November 2009
Available online 11 November 2009

Edited by Michael Ibba

Keywords:
o-phosphoseryl-tRNA^{Sec} kinase
Sep-tRNA:Sec-tRNA synthase
Pyrrolysyl-tRNA synthetase
Stop codon re-coding
Natural suppression

ABSTRACT

Selenocysteine and pyrrolysine, known as the 21st and 22nd amino acids, are directly inserted into growing polypeptides during translation. Selenocysteine is synthesized via a tRNA-dependent pathway and decodes UGA (opal) codons. The incorporation of selenocysteine requires the concerted action of specific RNA and protein elements. In contrast, pyrrolysine is ligated directly to tRNA^{Pyl} and inserted into proteins in response to UAG (amber) codons without the need for complex re-coding machinery. Here we review the latest updates on the structure and mechanisms of molecules involved in Sec-tRNA^{Sec} and Pyl-tRNA^{Pyl} formation as well as the distribution of the Pyl-decoding trait.

1. Selenocysteine biogenesis

Selenocysteine (Sec) is the major biological form of the element selenium, which in trace amounts is essential for human health. Sec is incorporated into polypeptides to form selenoproteins during translation. The 21st amino acid is typically found in catalytic centers of selenoproteins where it plays a functionally essential role. Unlike most amino acids, Sec is universally synthesized on its cognate tRNA [1–4]. During translation, selenocysteinyl-tRNA^{Sec} (Sec-tRNA^{Sec}) is delivered to the ribosome by a specific translation factor that requires a characteristic stem-loop structure in the mRNA to actively recode an in-frame UGA from stop codon to Sec sense codon. The human genome encodes only 25 selenoproteins [5], yet variations in these Sec-containing proteins or their synthetic machinery is linked to a range of human disorders including cancer and numerous diseases affecting the nervous, immune, and endocrine systems [6].

The machineries to synthesize Sec and incorporate it into selenoproteins are divergent in bacteria compared to archaea and eukaryotes. In bacteria, serine (Ser) as the precursor of Sec is initially attached to tRNA^{Sec} by seryl-tRNA synthetase (SerRS). The resulting Ser-tRNA^{Sec} is then converted to Sec-tRNA^{Sec} by selenocysteine synthase (SelA) in the presence of the selenium donor selenophosphate. This pathway has been well characterized in *Escherichia coli* [7] and extensively reviewed before [8,9].

The pathway for Sec biosynthesis in archaea and eukaryotes (Fig. 1) was revealed only in the last few years. The missing component was an archaeal/eukaryotic analog of SelA, since no clear sequence-based homolog could be found. An additional enzymatic step is involved in Sec biosynthesis in archaea and eukaryotes. *O*-phosphoseryl-tRNA kinase (PSTK) [10] catalyzes the phosphorylation of Ser-tRNA^{Sec} to form *O*-phosphoseryl-tRNA^{Sec} (Sep-tRNA^{Sec}). The Sep-tRNA:Sec-tRNA synthase (SepSecS), an independently evolved protein that is distantly related to SelA [11], then forms the final product Sec-tRNA^{Sec} from selenophosphate and Sep-tRNA^{Sec} [2–4]. Phylogenetic analysis indicated that PSTK and SepSecS co-evolved and are restricted to the archaeal and eukaryotic domains [2,12]. An interesting similarity, as reviewed previously [13], exists between the archaeal and eukaryotic Sec biosynthetic pathway and archaeal tRNA-dependent cysteine biosynthesis, which also proceeds via a Sep-tRNA intermediate [14]. In the first section of this paper, we focus on recent biochemical and structural work that further elucidated the mechanism and specificity

* Corresponding authors.
E-mail addresses: jing.yuan@yale.edu (J. Yuan), patrick.odonoghue@yale.edu (P. O'Donoghue).
[1] Both authors contributed equally.

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.11.005

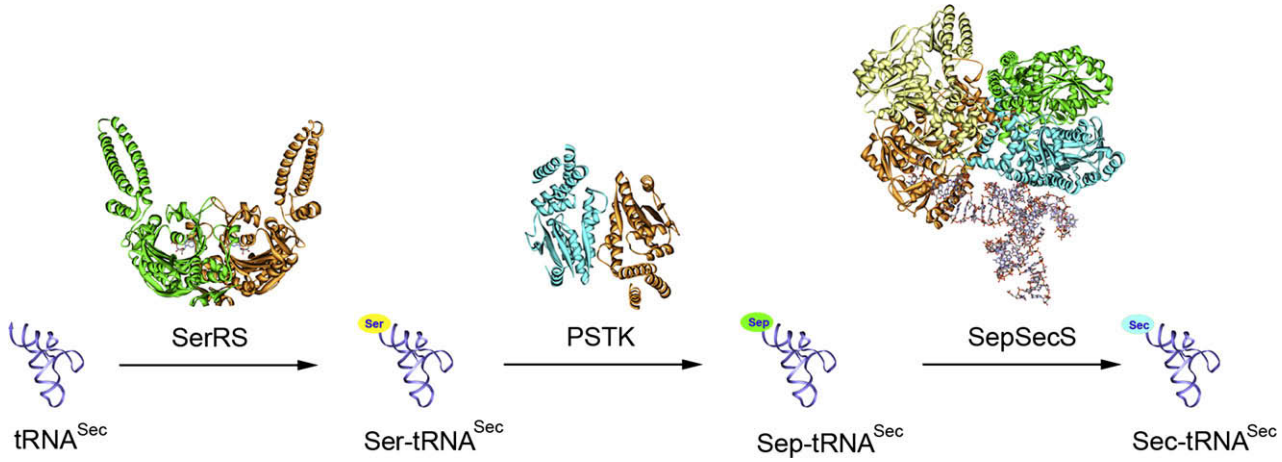

Fig. 1. The Sec biosynthesis pathway in archaea and eukaryotes. Sec is synthesized on tRNA^{Sec} in three steps. (1) The unacylated tRNA^{Sec} is serylated by SerRS; (2) the resulting Ser-tRNA^{Sec} is phosphorylated by PSTK forming Sep-tRNA^{Sec}; (3) the phosphorylated intermediate is converted to the final product Sec-tRNA^{Sec} by SepSecS. The crystal structures of tRNA and enzymes in this pathway are presented: tRNA^{Sec} (ribbon) from *Homo sapiens* [19], SerRS (ribbon) with AMP (stick) from *Pyrococcus horikoshii* [64], PSTK (ribbon) from *M. jannaschii* [26], and SepSecS (ribbon) with tRNA^{Sec} (stick, only one tRNA is shown) from *Homo sapiens* [18].

of the tRNA and enzymes involved in Sec biosynthesis in archaea and eukaryotes.

2. tRNA^{Sec} has a distinct structure

tRNA^{Sec} was identified more than two decades ago [1]. It is the longest tRNA with an extended acceptor stem resulting from an abnormal RNase P cleavage specificity [15]. tRNA^{Sec} has an 8-bp acceptor stem and 5-bp T-stem (a 8/5 secondary structure) in bacteria and a 9/4 arrangement in archaea and eukaryotes, and both can fold into a 13-bp long acceptor-TΨC helix (Fig. 2A). In contrast, canonical tRNAs typically have a 7-bp acceptor stem and a 5-bp T-stem forming a 12-bp acceptor-TΨC helix. Several other features of tRNA^{Sec}, including an elongated D-stem (6-bp instead of 4-bp), a smaller D-loop (4-bp instead of 8-bp), a long variable arm, and the absence of the highly conserved U8 residue, make tRNA^{Sec} distinct from canonical tRNAs.

The tertiary structures of *E. coli* and eukaryotic tRNA^{Sec} were investigated by chemical and enzymatic probing during the 1990s [16,17]. Very recently, the first tRNA^{Sec} crystal structure was solved. The unacylated human tRNA^{Sec} transcript was crystallized in complex with human SepSecS [18] and in an unbound state

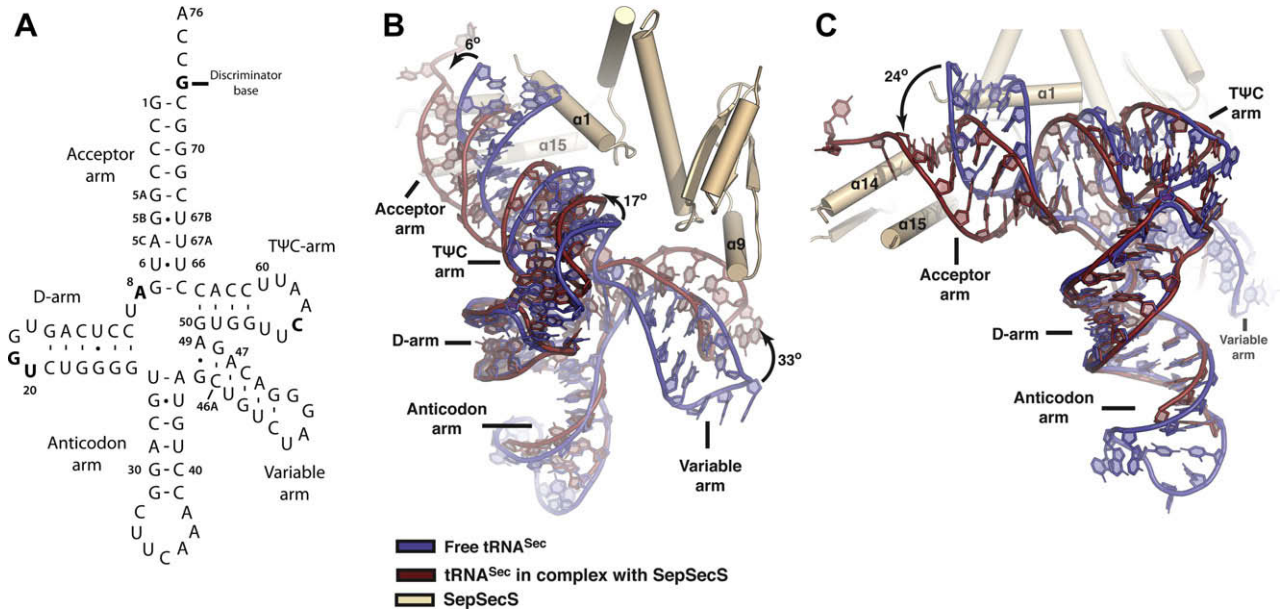

Fig. 2. Binding to SepSecS promotes a conformational change in tRNA^{Sec}. (A) The secondary structure of human tRNA^{Sec} with bases mentioned in the text highlighted in bold. The correct tRNA^{Sec} sequence is shown here (Fig. 2C in Ref. [18] omitted a base from the D-arm). (B) Superposition of the sugar–phosphate backbone of both the D- and anticodon arms of free tRNA^{Sec} (blue) on the corresponding atoms of tRNA^{Sec} complexed with SepSecS (red) reveals a conformational change in the tRNA molecule on binding to the enzyme. The variable arm of tRNA^{Sec} rotates by 33°, the T arm swings 17° and the acceptor arm rotates by 6° around the axis that runs parallel to both the D- and anticodon arms. The free tRNA^{Sec} conformer cannot bind to SepSecS because the tip of its acceptor arm would clash with the helix α1 and the variable arm would be positioned away from the helix α9. (C) The acceptor arm also slides down towards the anticodon arm on binding to SepSecS through a 24° rotation around the axis that is parallel to the variable arm. This movement positions G73 to interact with Arg398 in the helix α14 and orients the CCA end toward the active site. Free tRNA^{Sec} is blue, tRNA^{Sec} complexed with SepSecS is red and SepSecS is beige. Only the secondary structure elements of SepSecS that interact with tRNA are shown. The view is first rotated ~30° clockwise around the horizontal axis, and then another 30° anticlockwise around the vertical axis relative to panel B.