# Um34 in selenocysteine tRNA is required for the expression of stress-related selenoproteins in mammals

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## Abstract

Selenium is an essential micronutrient in the diet of mammals and has many health benefits. Selenium-containing proteins are responsible for most, if not all, of these benefits. This element is incorporated into protein as selenocysteine (Sec), the 21st amino acid in the genetic code. There are two species of Sec tRNA in mammalian cells that differ by a single 2'-O-hydroxymethyl group on the ribosyl moiety at position 34 (Um34). The relationship between this modification and selenoprotein synthesis was examined in mice in which the wild type Sec tRNA gene was replaced with a mutant Sec tRNA transgene incapable of forming Um34. This mouse line did not express several stress-related selenoproteins, whereas the levels of several selenoproteins thought to serve housekeeping functions were normal. This novel form of protein regulation occurred at the translational level. The Um34 modification in Sec tRNA, therefore, plays a crucial role in regulating the expression of a subset of mammalian selenoproteins and is a requisite for the synthesis of several stress-related selenoproteins.

## 1 Introduction

Selenium is a vital component in the diet of humans and other mammals and has numerous health benefits. It was reported that this element decreases the incidence of certain forms of cancer, and alleviates heart disease and other cardiovascular and muscle anomalies (Hatfield 2001). Furthermore, selenium has been observed to inhibit viral expression, delay the aging process, slow the progression of AIDS in HIV-positive patients and it has roles in mammalian development, male reproduction and immune function (Hatfield 2001). The underlying mechanisms of how selenium promotes these benefits are just beginning to be understood. The available evidence strongly indicates that selenoproteins are the responsible agents (Diwadkar-Navsariwala and Diamond 2004). There are 25 selenoprotein genes in the human genome and 24 in the genomes of rodents (Kryukov et al. 2003). The functions of less than half of the selenoprotein gene products have been characterized

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Selenium is incorporated into protein as the 21st amino acid, selenocysteine (Sec), in the genetic code (Hatfield and Gladyshev 2002). The codeword for Sec is UGA and Sec is biosynthesized on its tRNA (designated $tRNA^{[Ser]Sec}$) following aminoacylation of the tRNA with serine by seryl-tRNA synthetase. The reason UGA can be used as a Sec codon instead of its usual role as a termination codon is the presence of a stem-loop structure in the 3'-untranslated region of eukaryotic selenoprotein mRNAs designated as the Sec insertion sequence or SECIS element (Low and Berry 1996). The machinery involved in the insertion of Sec into protein includes several additional specific factors such as an elongation factor and a SECIS binding protein (reviewed in Driscoll and Copeland 2003). Interestingly, none of the known factors involved in the insertion of Sec into protein appear to have a regulatory role in translation of selenoprotein mRNAs. However, as described in the present review, base modification in Sec $tRNA^{[Ser]Sec}$ (for review, see Hatfield and Gladyshev 2002) plays a key role in determining which selenoprotein mRNAs are translated.

## 2 Sec $tRNA^{[Ser]Sec}$

Sec $tRNA^{[Ser]Sec}$ has many unique characteristics as it is the longest eukaryotic tRNA sequenced to date and it is highly undermodified compared to other tRNAs (Hatfield and Gladyshev 2002). It has only four modified bases and biosynthesis of the base modifications have been characterized in *Xenopus* oocytes (Choi et al. 1994; Sturchler et al. 1994). The Sec $tRNA^{[Ser]Sec}$ population in mammals consists of two isoforms that differ from each other by a single methyl modification on the ribosyl moiety at position 34 (Um34; Fig. 1). Addition of the methyl group is a highly specialized last step in maturation and determines both the structure and function of $tRNA^{[Ser]Sec}$ (reviewed in Hatfield and Gladyshev 2002). This methylation is dependent on the prior synthesis of four other modified bases and on an intact tertiary structure, whereas synthesis of the other modified bases is less stringently connected to primary and tertiary structure (Kim et al. 2000). Furthermore, methylation of U34 is enhanced by enriched selenium levels (reviewed in Hatfield and Gladyshev 2002) and its presence dramatically affects secondary and tertiary structure (Diamond et al. 1993). In addition, as shown in Figure 1, the occurrence of the Um34 isoform correlates with the expression of several selenoproteins (see also Chittum et al. 1997; Moustafa et al. 2001; and below). Recently, the selenium-induced, Sec $tRNA^{[Ser]Sec}_{mcmUm}$ isoform has been reported to have a specialized role in selenoprotein biosynthesis in that it is likely the major isoacceptor used in expressing this protein class (Jameson and Diamond 2004). One of our major goals, therefore, has been to better understand the role of this methyl group in selenoprotein expression.

Changing A to G at position 37 results in a $tRNA^{[Ser]Sec}$ that lacks both isopentenyladenosine ($i^6A$) at this position ($i^6A37$) and Um 34 (Kim et al. 2000). This observation provided an opportunity to generate a mutant $tRNA^{[Ser]Sec}$ without this highly specialized methyl group and examine its role in selenoprotein synthesis.