

Chapter 2

Selenocysteine Biosynthesis and the Replacement of Selenocysteine with Cysteine in the Pathway

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Abstract The biosynthetic pathway of selenocysteine (Sec), the 21st amino acid in the genetic code, has been established in eukaryotes and archaea using comparative genomic and experimental approaches. In addition, cysteine (Cys) was found to arise in place of selenocysteine in thioredoxin reductase (TR) in NIH 3T3 cells and in mice. An analysis of the selenocysteine biosynthetic pathway demonstrated that replacement of selenide with sulfide in generating the active cysteine donor, thio-phosphate, resulted in cysteine being donated to the acceptor molecule, which is likely dehydroalanyl-tRNA^{[Ser]Sec}, yielding Cys-tRNA^{[Ser]Sec}. The identification of the pathways for biosynthesis of selenocysteine and cysteine in mammals is discussed in this chapter.

2.1 Introduction

Selenocysteine (Sec) is a naturally occurring protein amino acid in the three domains of life, eukaryotes, archaea, and eubacteria, and is the only known protein amino acid in eukaryotes whose biosynthesis occurs on its tRNA, designated Sec tRNA^{[Ser]Sec} [1, 2]. Bock and collaborators established the biosynthesis of Sec in eubacteria in 1991 [3] but its biosynthesis in eukaryotes and archaea remained elusive until only in the last few years [1, 4]. In addition, cysteine (Cys) was reported to occur in place of Sec in naturally occurring selenoproteins, e.g., the thioredoxin reductases (TR) [5],

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but the mechanism of how Cys arose in place of Sec was not determined. We recently observed that Cys is also biosynthesized using the Sec biosynthetic machinery to generate Cys-tRNA^{[Ser]Sec} and the resulting Cys is inserted into some, but apparently not all, selenoproteins [6]. The biosynthesis of Sec and the de novo synthesis of Cys using the Sec biosynthetic machinery and the insertion of Cys into protein in place of Sec are discussed herein.

2.2 Sec Biosynthesis

The biosynthesis of Sec, as established in mammals, is shown in Fig. 2.1. The background on how the biosynthesis was deciphered in eukaryotes and archaea is discussed below.

2.2.1 Background

In 1970, two separate studies involving the same tRNA that would subsequently provide the foundation for the biosynthesis of Sec were reported. Maenpaa and Bernfield [7] found that a minor seryl-tRNA within the total seryl-tRNA population formed phosphoseryl-tRNA in rooster liver and these investigators speculated that this tRNA might have a role in the biosynthesis of the phosphoseryl moieties in phosvitin, a protein containing over 50% phosphoserine residues. At the same time, a minor seryl-tRNA was found in bovine and chicken livers that decoded specifically the nonsense (stop) codon, UGA [8]. Since a topic of considerable interest in the late 1960s and early 1970s was whether nonsense suppressor tRNAs occurred in higher vertebrates, it was suggested that the UGA decoding tRNA was an opal suppressor tRNA. Phosphoseryl-tRNA and the UGA decoding seryl-tRNA were subsequently shown to be the same tRNA [9]. Further characterization of this tRNA, including identifying it as the tRNA that inserts Sec into protein (designated as Sec tRNA^{[Ser]Sec}), and that it exists in organs and tissues of higher vertebrates in two isoforms, which are selectively used in synthesizing different subclasses of selenoproteins, can be found elsewhere ([10] and reviewed in [11] and Chap. 44).

It should also be noted that in 1989, Sec was shown to be biosynthesized on tRNA^{[Ser]Sec} in *Escherichia coli* [12] and mammalian cells [13] demonstrating unequivocally that Sec was the 21st amino acid in the genetic code. These observations ruled out the possibility that phosphoserine, which was known to be attached to tRNA^{[Ser]Sec}, was initially incorporated into selenoproteins and then modified posttranslationally to Sec that would of course had made phosphoserine the 21st amino acid.

In addition to Sec tRNA, the seryl-tRNA synthetase that attaches serine to Sec tRNA, and the kinase that phosphorylates seryl-tRNA in forming phosphoseryl-tRNA, there were other protein factors, reported by several investigators, whose role in selenium metabolism had not been identified. These included a 48 kD protein in

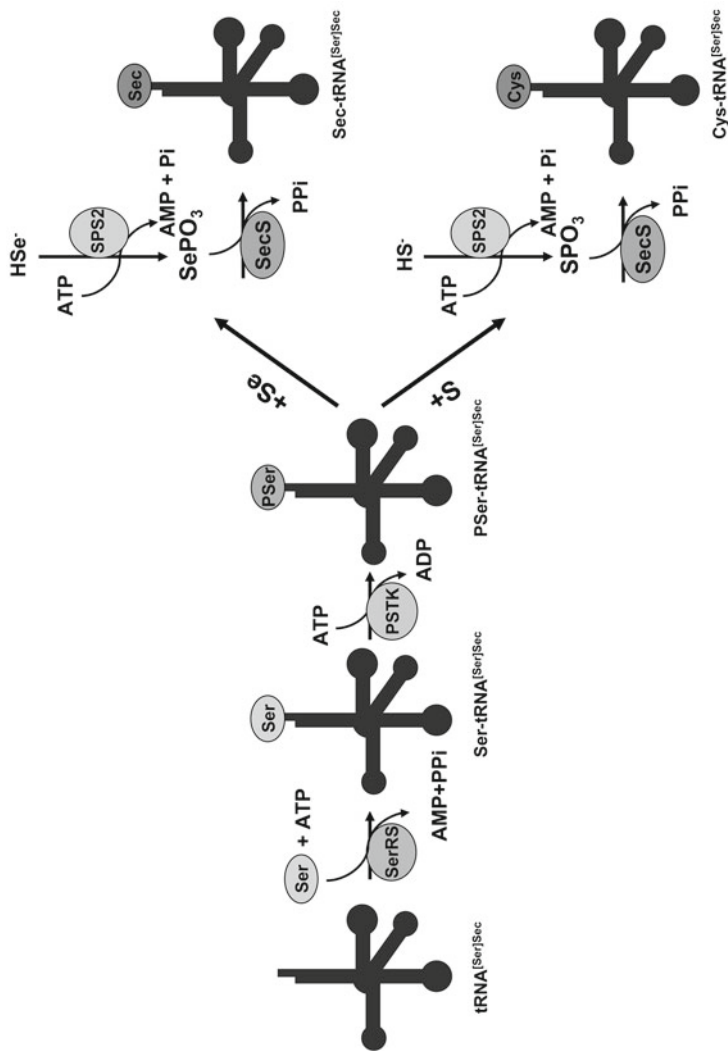


Fig. 2.1 Biosynthesis of Sec and de novo synthesis of Cys. The pathway of Sec biosynthesis in eukaryotes and archaea, wherein the synthesis is complete by the generation of selenophosphate from selenide and ATP in the presence of SecS and the donation of selenium to the active selenium acceptor to form Sec-tRNA^{Sec} is shown (see *upper portion* of the figure for the final steps in Sec biosynthesis). The de novo synthesis of Cys on Sec tRNA^{Sec}, wherein sulfide replaces selenide in generating thiophosphate and its donation to the active selenium acceptor to yield Cys-tRNA^{Sec} is also shown (see *lower portion* of the figure for the final steps in Cys biosynthesis)

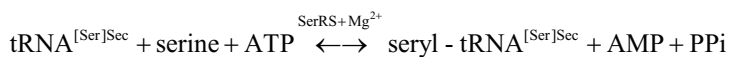
patients with an autoimmune chronic hepatitis that co-precipitated with Sec tRNA^{[Ser]_{Sec}} in cell extracts from such patients [13]. This protein was designated the soluble liver antigen (SLA) [14] and was found to form a complex with other proteins involved in the Sec insertion machinery [15]. SLA was subsequently reported to occur as a separate family within a large superfamily of diverse pyridoxal phosphate-dependent transferases [16] and was proposed to be selenocysteine synthase (SecS) in mammals [11, 16–18].

In addition, two genes were identified in mammals as having homology to the enzyme in *E. coli* that synthesized the active selenium donor, selenophosphate synthetase (SPS) [19]. SPS was initially designated as SelD in *E. coli* [20] and the mammalian genes coding for SPS as *sps1* [21, 22] and *sps2* [23]. The product of *sps2* is a selenoprotein (SPS2) suggesting that it might be an autoregulatory protein in selenoprotein biosynthesis [23, 24]. Initial experimental studies with SPS1 and SPS2 had shown that: (1) mutation of Sec to Cys in SPS2 had enzyme activity [23–25] and the Cys mutant form could complement *SelD*[−] cells following transfection of the *SelD*[−] cells with the Sec → Cys mutant *sps2* [25]; and (2) transformation of *E. coli* cells with *sps1* or *sps2* suggested that SPS2 had a role in the synthesis of selenophosphate and that SPS1 was involved in Sec recycling via a selenium salvage pathway [26].

The biosynthesis of Sec in eukaryotes and archaea was solved using the components in the above studies as described below.

2.2.2 Seryl-tRNA Synthetase (*SerRS*)

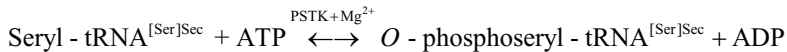
The biosynthesis of Sec begins with the aminoacylation of tRNA^{[Ser]_{Sec}} by seryl-tRNA synthetase (*SerRS*) in the presence of serine, ATP, and Mg⁺⁺ as follows:



2.2.3 Phosphoseryl-tRNA Kinase (*PSTK*)

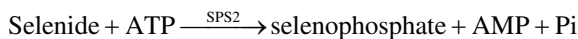
Identification of the kinase that is responsible for phosphorylating the serine moiety on the UGA decoding seryl-tRNA [7, 8] remained elusive for many years. This kinase, which was designated phosphoseryl-tRNA kinase (*PSTK*), was finally identified initially using a comparative genomic approach that searched completely sequenced genomes of archaea for a kinase-like protein that was present in those organisms that utilized the selenoprotein synthesizing machinery and was absent in those that did not [27]. Two candidates were detected, and their homology was next searched in eukaryotic genomes that did and did not use a functional Sec insertion machinery. Orthologs of one of these candidate kinases were present in eukaryotes

synthesizing selenoproteins but absent in eukaryotes lacking these proteins. The gene for this protein was cloned from mouse genomic DNA, the protein product expressed, isolated and its biochemical properties examined that unequivocally identified it as PSTK [27]. PSTK was shown to carry out the following reaction:



2.2.4 Selenophosphate Synthetases (SPS1 and SPS2)

The protein products of *sps1* and *sps2* were generated by cloning the corresponding mouse genes into expression vectors, expressing and isolating the proteins for further study [1, 28]. A mutation was initially introduced into *sps2* to change Sec to Cys in SPS2. Similarly, *Caenorhabditis elegans* *sps2* that normally contains Cys in place of Sec and *SelD* was cloned and the products expressed and isolated. Mouse SPS1 and SPS2 (mSPS1 and mSPS2(Cys)), *C. elegans* SPS (cSPS2) and *E. coli* SPS (*SelD*) were all examined for their ability to synthesize the active donor, monoselenophosphate [1]. Selenide and ATP were incubated individually with each protein to assess whether they could synthesize the active selenium donor. mSPS2(Cys), cSPS2, and *SelD* generated selenophosphate but SPS1 did not, demonstrating that eukaryotic SPS2 is responsible for making the active selenium donor, and that SPS1 likely has another metabolic role [1]. SPS2 acts on selenide and ATP in yielding selenophosphate by the following reaction:



2.2.5 Selenophosphate Synthase (SecS)

As no homologous sequences to bacterial SecS, previously designated by Böck and collaborators as Sela [29], could be found in eukaryotes that encode a functional Sec insertion machinery in their genomes, we applied a computational and comparative genomic strategy, similar to that used to identify *ptsk*, in searching for a *SecS* gene in eukaryotes [1]. The search was confined to eukaryotes and archaea whose genomes had been sequenced and the organisms synthesize selenoproteins, and the genomes of those organisms that did not synthesize selenoproteins were used as controls. In addition to identifying genes that are involved in the Sec insertion machinery, another gene was detected that might be SecS in mammals [1]. A homologous sequence was found in all eukaryotes and archaea encoding the functional selenoprotein insertion machinery but not in sequenced genomes of organisms not making selenoproteins.

The sequence of the purported mammalian SecS matched that of SLA [1], the 48 kD protein in patients with an autoimmune chronic hepatitis that co-precipitated

with tRNA^{[Ser]Sec} (see above). The mouse *SLA* gene was cloned, expressed, and the gene product characterized [1]. The purified protein bound tightly with phosphoseryl-tRNA^{[Ser]Sec}, less well with tRNA^{[Ser]Sec}, and poorly or not at all with seryl-tRNA^{[Ser]Sec}, seryl-tRNA^{Ser} or tRNA^{Ser}, strongly suggesting that the substrate for the *SLA* gene product was phosphoseryl-tRNA. Furthermore, this protein rapidly hydrolyzed the phosphate from the substrate leaving, most likely, dehydroalanyl-tRNA^{[Ser]Sec} bound to the enzyme [1]. This complex was then shown to accept the active selenium donor, selenophosphate, that resulted in selenocysteyl-tRNA^{[Ser]Sec} (see Fig. 2.1) demonstrating unequivocally that SLA is indeed mammalian SecS.

2.3 De novo Biosynthesis of Cys

Cys is considered to be an essential amino acid in mammals in that it was not thought to be synthesized de novo and must be obtained from the diet or synthesized from methionine. However, we recently reported that Cys can be biosynthesized de novo in mammals by using the Sec biosynthetic machinery [6]. Although this pathway synthesizes Cys de novo, the Cys synthesized does not substitute for the essential requirement of Cys insertion into protein in response to the UGU/UGC codons. The key steps in the replacement of Sec with Cys are: (1) sulfide can substitute for selenide in the SPS2 catalyzed reaction yielding thiophosphate; and (2) thiophosphate can react with the *O*-phosphoseryl-tRNA^{[Ser]Sec} intermediate which is most likely dehydroalanine that was generated by the interaction of SecS to yield Sec tRNA^{[Ser]Sec} (Fig. 2.1). The details of Cys replacement of Sec on tRNA^{[Ser]Sec} and the significance of Cys insertion into protein in mammalian cells in culture or in mouse liver as a consequence of selenium status are discussed below.

2.3.1 Cys/Sec Replacement *in vitro*

The precise means of how Cys replaces Sec in the biosynthetic pathway was determined by using the enzymes and other components used in establishing how the latter amino acid was synthesized [6]. The only differences were that (1) sulfide was used in place of selenide in the presence of SPS2 and ATP to generate thiophosphate, and (2) thiophosphate was used in place of selenophosphate as the active sulfur donor in the presence of SecS and phosphoseryl-tRNA^{[Ser]Sec}. Thiophosphate did indeed replace selenophosphate that served as the active sulfur donor to the active acceptor molecule generated by SecS that in turn yielded Cys-tRNA^{[Ser]Sec} (Fig. 2.1).

2.3.2 Cys/Sec Replacement in NIH 3T3 Cells

To elucidate the intracellular relevance of the replacement of Sec by Cys in the Sec biosynthetic pathway, we initially examined the effect of adding thiophosphate to

Table 2.1 C-Terminal peptide sequences of mouse TR1 and TR3 from NIH 3T3 cells and liver^a

Experiment	Source of TRs	Protein	Peptide sequences	Sec (%)	Cys (%)
I	NIH 3T3, control	TR1	R.SGGDILQSGCUG	90	10
		TR1	R.SGGDILQSGCCG		
	NIH 3T3, SPO ₃ treated	TR1	R.SGGDILQSGCUG	4	96
		TR1	R.SGGDILQSGCCG		
		TR1	KRSGGDILQSGCCG		
IIa	Liver, 0 ppm Se	TR1	R.SGGDILQSGCUG	49	51
		TR1	R.SGGDILQSGCCG		
		TR3	K.RSGLEPTVTGCCG		
IIb	Liver, 0.1 ppm Se	TR1	R.SGGDILQSGCUG	91	9
		TR1	R.SGGDILQSGCCG		
		TR1	K.RSGGDILQSGCUG		
		TR1	K.RSGGDILQSGCCG		
		TR3	R.SGLEPTVTGCUG		
		TR3	R.SGLEPTVTGCCG		
IIIc	Liver, 2 ppm se	TR1	R.SGGDILQSGCUG	100	ND ^b
		TR1	K.RSGGDILQSGCUG		
		TR3	R.SGLEPTVTGCUG		
		TR3	K.RSGLEPTVTGCUG		

^aTR1 and TR3 were affinity isolated from NIH 3T3 cells, either treated or untreated with thiophosphate (Experiment I), or from livers of mice fed selenium deficient, selenium sufficient or selenium supplemented diets (Experiments IIa–c). The percent of Sec/Cys in the resulting C-terminal selenoprotein peptides was analyzed by MS/MS as described previously [6]

^bND not detectable

NIH 3T3 cells in culture [6]. The resulting TR1 was isolated, purified, and the ratio of Sec to Cys encoded by UGA determined by mass-spectrometry analysis (Table 2.1, Experiment I). In untreated cells, wherein the cells were grown in the presence of adequate amounts of selenium, the Sec to Cys ratio was 9:1 demonstrating that Cys was incorporated into TR1 at the UGA-encoded site even in the presence of adequate amounts of selenium in the medium. On the other hand, more than 90% Cys was present at the UGA-Sec position in TR1 within cells treated with thiophosphate.

2.3.3 Cys/Sec Replacement in Mice

We next assessed the ratio of Cys/Sec in liver TR1 and TR3 of mice maintained on identical diets, except for varying amounts of selenium that included deficient, adequate, and supplemented levels (Table 2.1, Experiments IIa–c, respectively) [6]. The ratio of Sec to Cys in liver TR1 and TR3 was ~1:1 in mice fed a selenium-deficient diet and ~9:1 in mice fed a selenium-adequate diet. Mice maintained on a selenium-supplemented diet had undetectable levels of Cys in TR1 and TR3 at the UGA

encoded site. Interestingly, the degree of replacement of Cys with Sec intracellularly appeared to be a reflection of the level of selenium in the medium (Table 2.1, Experiment I) or diet (Table 2.1, Experiment II).

2.4 Conclusions

Sec is the 21st amino acid in the genetic code, the only known protein amino acid in eukaryotes whose biosynthesis occurs on its tRNA, and the last known protein amino acid in eukaryotes whose biosynthesis was finally resolved [1]. The enzymes and other factors involved in Sec biosynthesis were initially found and subsequently identified to be involved in Sec biosynthesis in a variety of ways. For example, Sec tRNA^{[Ser]Sec} was first reported to be a minor seryl-tRNA that decoded specifically the stop codon, UGA, in bovine and chicken livers [8] and subsequently was shown to be Sec tRNA^{[Ser]Sec} [13]. PSTK was described as a kinase that phosphorylated the serine moiety on a minor seryl-tRNA in rooster liver to form phosphoseryl-tRNA [7] and was also subsequently identified many years later by computational and comparative genomic and experimental approaches to be the kinase involved in synthesizing one of the intermediates in the Sec pathway in eukaryotes and archaea [27]. SecS was initially described as a protein factor that bound Sec tRNA^{[Ser]Sec} in human liver of patients with an autoimmune disease [14] and was then identified by computational and comparative genomic and experimental approaches as SecS [1]. SPS1 and SPS2 were originally found in the mammalian genome by their homology to the eubacterial SelD [21–23], but their experimental characterization demonstrated that only SPS2 was the enzyme responsible for making the active selenium donor [1]. Each of these components was cloned, expressed, isolated, and characterized to establish the biosynthesis of Sec [1].

In addition, Cys was found to be synthesized *de novo* by using SPS2 and replacing selenide with sulfide-yielding thiophosphate that donated sulfur to the active acceptor, most likely, dehydroalanine, that was attached to SecS yielding Cys-tRNA^{[Ser]Sec} [6]. This pathway was shown to be operative in mammalian cells and in mice ingesting normal amounts of selenium and to insert Cys in some, but likely not all, selenoproteins [6]. In mice on a selenium-deficient diet, the levels of Sec and Cys in TR1 and TR3 were approximately equal suggesting a possible physiological significance of such a pathway. It was suggested that the replacement of Sec by Cys in some selenoproteins “may provide possibilities for regulating the expression of (specific) selenoproteins and their functions as well as elucidating the biological roles of selenium” [6]. Clearly, the finding that Cys can replace Sec in certain selenoproteins opens the door to many additional and fascinating studies further elucidating the significance of this novel pathway involving Cys and selenoproteins.

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