Biosynthesis of Selenocysteine, the 21st Amino Acid in the Genetic Code, and a Novel Pathway for Cysteine Biosynthesis[1,2]

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ABSTRACT

The biosynthetic pathway for selenocysteine (Sec), the 21st amino acid in the genetic code whose codeword is UGA, was recently determined in eukaryotes and archaea. Sec tRNA, designated $tRNA^{[Ser]Sec}$, is initially aminoacylated with serine by seryl-tRNA synthetase and the resulting seryl moiety is converted to phosphoserine by *O*-phosphoseryl-tRNA kinase to form *O*-phosphoseryl-$tRNA^{[Ser]Sec}$. Sec synthase (SecS) then uses *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ and the active donor of selenium, selenophosphate, to form Sec-$tRNA^{[Ser]Sec}$. Selenophosphate is synthesized from selenide and ATP by selenophosphate synthetase 2 (SPS2). Sec was the last protein amino acid in eukaryotes whose biosynthesis had not been established and the only known amino acid in eukaryotes whose biosynthesis occurs on its tRNA. Interestingly, sulfide can replace selenide to form thiophosphate in the SPS2-catalyzed reaction that can then react with *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ in the presence of SecS to form cysteine-(Cys-)$tRNA^{[Ser]Sec}$. This novel pathway of Cys biosynthesis results in Cys being decoded by UGA and replacing Sec in normally selenium-containing proteins (selenoproteins). The selenoprotein, thioredoxin reductase 1 (TR1), was isolated from cells in culture and from mouse liver for analysis of Cys/Sec replacement by MS. The level of Cys/Sec replacement in TR1 was proportional to the level of selenium in the diet of the mice. Elucidation of the biosynthesis of Sec and Sec/Cys replacement provides novel ways of regulating selenoprotein functions and ultimately better understanding of the biological roles of dietary selenium. *Adv. Nutr. 2: 122–128, 2011.*

Introduction

Selenocysteine (Sec) is the 21st amino acid in the genetic code and this selenium containing amino acid is cotranslationally incorporated into selenium-containing proteins, designated selenoproteins, in response to the codon, UGA (1–3). Although UGA is normally a termination codon that dictates the cessation of protein synthesis, it is also used as a Sec codon by numerous organisms in each of the 3 domains of life: eubacteria, archaea, and eukaryotes. Of the >500 genomes sequenced in eubacteria, only ~20% encode the machinery for inserting Sec into protein, and in archaea, ~10% have this machinery (4,5). In eukaryotes, the Sec insertion machinery has been found in a number of lower organisms such as green algae, kinetoplastida, and slime molds and it is widespread in animals but absent in fungi and higher plants (4,5).

The mechanisms responsible for designating UGA as a Sec codon instead of termination involve a stem-loop structure in the 3′-untranslated region of selenoprotein mRNA in eukaryotes known as the Sec insertion sequence (SECIS)[6] element (6) and a SECIS binding protein, designated SBP2 (7). SECIS-binding protein 2 (SBP2) binds to the SECIS element and forms a complex with the specific elongation factor for Sec $tRNA^{[Ser]Sec}$, EF_{Sec}, for incorporation of Sec into protein in response to the UGA Sec codon. Because the synthesis of some selenoproteins is terminated by a UGA stop signal, the Sec insertion machinery has the capability of distinguishing between the UGA for amino acid incorporation and the UGA for termination. The distance between the Sec

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[6] Abbreviations used: SECIS, Sec insertion sequence; Um34, methyl group located on the 2′-hydroxylribose at wobble position of selenocysteine tRNA.

codon and the SECIS element plays an essential role in this distinction (8, and refs. therein).

Even though the biosynthesis of Sec was established in eubacteria in the early 1990s (9–11), only in the last several years was the complete biosynthetic pathway of this selenium-containing amino acid determined in eukaryotes and archaea (12). Very recently, it was also shown that cysteine (Cys) can be synthesized de novo by replacing sulfide with selenide in the Sec biosynthetic pathway, forming Cys on tRNA$^{[Ser]Sec}$ (13). The biosynthesis of Sec and the novel pathway of Cys biosynthesis are the subjects of this review.

Biosynthesis of Sec

The biosynthesis of Sec in *Escherichia coli* proceeds as follows. The enzyme that synthesizes Sec, Sec synthase (designated SelA in eubacteria and SecS in eukaryotes and archaea), is a pyridoxal phosphate dependent protein (9–11). This enzyme interacts with seryl-tRNA$^{[Ser]Sec}$ and removes the hydroxyl group from the seryl moiety to yield aminoacrylyl- (dehydroalanyl-) tRNA$^{[Ser]Sec}$ as an intermediate. Dehydroalanyl-tRNA$^{[Sec]Sec}$ then accepts the active selenium donor, which was identified in eubacteria as monoselenophosphate (9,11), to form selenocysteyl-tRNA$^{[Ser]Sec}$. Selenophosphate is synthesized from selenide and ATP by *E. coli* selenophosphate synthetase (SelD) (14).

The biosynthesis of Sec in eukaryotes and archaea (**Fig. 1**) proceeds by a similar pathway as in eubacteria except that an immediate, *O*-phosphoseryl-tRNA$^{[Ser]Sec}$, is involved and it arises by action of the kinase, *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ kinase (PSTK), on seryl-tRNA$^{[Ser]Sec}$ (15). SecS then acts on phosphoseryl-tRNA$^{[Ser]Sec}$, converting it to the proposed intermediate, dehydroalanyl-tRNA$^{[Ser]Sec}$ (12), accepts the active selenium donor, also identified as monoselenophosphate in eukaryotes, and generates selenocysteyl-tRNA$^{[Ser]Sec}$ from the dehydroalanyl-tRNA$^{[Ser]Sec}$ intermediate (12). In our studies, we relied on bioinformatics for identification of genes involved in Sec biosynthesis. One distinct benefit aiding us in identifying these genes was that several factors were previously found in higher vertebrates that were associated with selenium metabolism in some manner. For example, in 1970, a kinase that phosphorylated a minor rooster liver seryl-tRNA forming *O*-phosphoseryl-tRNA (16) and a minor mammalian and chicken liver seryl-tRNA recognizing specifically the stop codon, UGA (17), were reported. The minor seryl-tRNA$_{UGA}$ was subsequently shown to form phosphoseryl-tRNA (18) and identified as Sec tRNA$^{[Ser]Sec}$ (19), and the kinase originally found by Maaenpa and Bernfield (15) was shown to be PSTK. In addition, a 48-kDa protein that bound Sec tRNA$^{[Ser]Sec}$ in human liver and designated the soluble liver antigen (SLA) was found to be targeted by antibodies in patients with an autoimmune chronic hepatitis (20). SLA was found to occur in a separate family within a large superfamily of diverse pyridoxal phosphate dependent transferases (21), had been proposed to be the SecS in mammals (21–24), and was subsequently identified as SecS (12).

To identify the enzyme responsible for making the active selenium donor in mammals, we examined the protein products of 2 previously identified genes, *sps1* and *sps2* (25–28), in their ability to generate selenophosphate (12,29). Both genes were previously found in mammals as having homology to SelD and their products were proposed to be SPS (25–28). The product of one of these genes, SPS2, could serve as an autoregulator of selenoprotein synthesis because it was a selenoprotein (25,27). Several other studies involving the roles of SPS1 and SPS2 in Sec biosynthesis should also be noted. For example, the Sec residue in SPS2 was changed to Cys and the resulting mutant was found to have enzyme activity (25,27,29) but could complement *SelD* deficient *E. coli* cells following their transfection with the Cys mutant

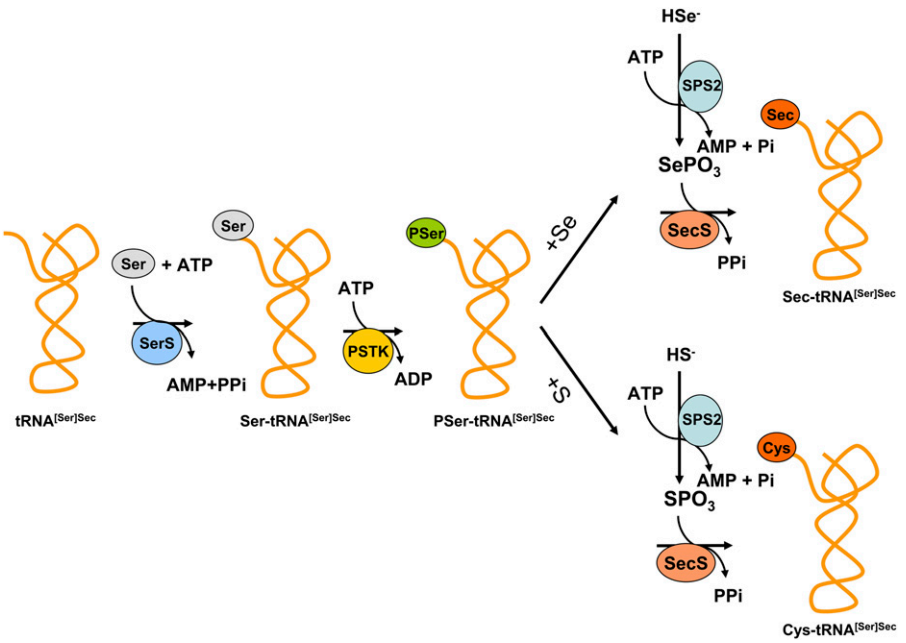

Figure 1 Biosynthesis of Sec and de novo synthesis of Cys. The complete synthesis of Sec on its tRNA in eukaryotes and archaea wherein selenite and ATP are substrates for SPS2 yielding selenophosphate that interacts with SecS, and the intermediate, likely dehydroalanine, to generate Sec-tRNA$^{[Ser]Sec}$ is shown on the right in the upper pathway. In the lower pathway, the de novo synthesis of Cys is shown in mammals wherein sulfide and ATP are substrates for SPS2 yielding thiophosphate that interacts with SecS, and the intermediate, likely dehydroalanine, to yield Cys-tRNA$^{[Ser]Sec}$. See text for further details.

form (29). In addition, transformation of *SelD* deficient *E. coli* cells with *sps1* or *sps2* suggested that SPS1 was involved in Sec recycling via a selenium salvage pathway, whereas SPS2 played a role in the synthesis of selenophosphate (30).

Further elucidation of the roles of SPS1 and SPS2 in the biosynthesis of Sec and characterization of seryl-tRNA$^{[Ser]Sec}$ synthetase, tRNA$^{[Ser]Sec}$, seryl-tRNA$^{[Ser]Sec}$, O-phosphoseryl-tRNA$^{[Ser]Sec}$, PSTK, SLA, and selenophosphate in the pathway of Sec biosynthesis are discussed below.

Sec tRNA$^{[Ser]Sec}$ and its aminoacylation

As noted above, Sec tRNA$^{[Ser]Sec}$ was discovered in 1970 and characterized as a minor seryl-tRNA that decoded specifically the stop codon, UGA, in bovine and chicken liver (17). Because there was considerable interest at that time in assessing whether nonsense suppressor tRNA occurred in mammalian cells, this minor "seryl-tRNA" was thought to be a nonsense suppressor tRNA. Subsequent studies demonstrated numerous unique features of this tRNA, including that it: *1*) formed *O*-phosphoseryl-tRNA (17); *2*) has a primary sequence unlike that of other tRNA in that it is 90 nucleotides long (and the longest eukaryotic sequenced) with relatively few base modifications (reviewed in 24); *3*) suppresses the UGA termination codon in rabbit β-globin mRNA (31); *5*) is transcribed unlike any other known tRNA in that transcription begins at the first nucleotide within the coding sequence (32); *6*) is transcribed through the regulation of several novel transcription regulatory elements encoded both 5′-proximally and 5′-distally of its gene, *Trsp* (reviewed in 33); *7*) has a unique cloverleaf model in that there are 9 bp in the acceptor stem and 4 in the TψC stem compared with a 7/5 cloverleaf model in other tRNA (24, and refs. therein); and *8*) exists in 2 isoforms that differ from each other by a single methyl group, Um34 (34). In addition, the first tRNA transgenic mice involving tRNA were generated with wild-type or mutant *Trsp* transgenes (35). Overexpression of the wild-type Sec tRNA$^{[Ser]Sec}$ population did not appear to result in any detectable alteration in selenoprotein expression in the cells, tissues, and organs examined, suggesting that the Sec tRNA population was not limiting in selenoprotein expression. Furthermore, rescue of the knockout of wild-type *Trsp*, which is embryonic lethal (36,37), with a mutant Sec tRNA$^{[Ser]Sec}$ transgene lacking Um34 demonstrated that the noncontaining Um34 isoform synthesized housekeeping (essential) selenoproteins, whereas the Um34 isoform was responsible for synthesizing stress related selenoproteins that are nonessential to survival (38).

In addition to the many unique features of Sec tRNA discussed above, transgenic mouse models encoding a mutant Sec tRNA transgene were developed as well as numerous conditional *Trsp* knockout mouse models targeting specific tissues and organs that demonstrated roles for selenoproteins in development and in a variety of health issues (reviewed in 39). What was initially thought to be a nonsense suppressor seryl-tRNA (17) was subsequently found to be Sec tRNA$^{[Ser]Sec}$ that expanded the genetic code by including Sec as the 21st amino acid (19,40) and the only known protein amino acid in eukaryotes that is biosynthesized on its tRNA. Sec tRNA$^{[Ser]Sec}$ is aminoacylated with serine in the presence of seryl-tRNA synthetase (SerS), ATP, and Mg^{2+} to begin the biosynthesis of Sec:

$$\text{tRNA}^{[\text{Ser}]\text{Sec}} + \text{ATP} + \text{serine} \overset{\text{SerS, Mg}^{2+}}{\rightleftharpoons} \text{seryl-tRNA}^{[\text{Ser}]\text{Sec}} + \text{AMP} + \text{PP}.$$

Identifying the gene for PTSK and characterizing the gene product

Our rationale in identifying the PSTK gene (*pstk*) involved an initial proposal that Sec biosynthesis proceeded by identical pathways in organisms making selenoproteins within eukaryotes and archaea and that *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ is an intermediate in the pathway (15). The strategy involved a computational and genomic comparison approach by initially searching for kinase-like genes that were present in *Methanococcus jnnaschii* and *Methanopyrus kandleri*, archaea that encode the Sec insertion machinery, and comparing all such genes to the other archaea whose genomes had been sequenced but did not make selenoproteins (15, and refs. therein). Two kinase-like genes were detected in the 2 archaea synthesizing selenoproteins that were not found in the other 12 archaea not synthesizing selenoproteins. The sequenced eukaryotic genomes that did (nematodes, *Drosophila*, and mice) and did not (yeasts) encode the Sec insertion machinery were scanned for homologous sequences of the 2 candidate genes found in archaea. A single candidate kinase (gi: 20095115) was detected and this putative *pstk* from mice was cloned into an expression vector, expressed, and the product isolated and characterized (15). The coding sequence specifically used seryl-tRNA$^{[Ser]Sec}$ as a substrate to form phosphoseryl-tRNA$^{[Ser]Sec}$, unequivocally demonstrating that the putative kinase indeed coded for PSTK. The phosphorylation of seryl-tRNA$^{[Ser]Sec}$ proceeded as follows:

$$\text{Seryl-tRNA}^{[\text{Ser}]\text{Sec}} + \text{ATP} \overset{\text{PSTK, Mg}^{2+}}{\rightleftharpoons} \textit{O}\text{-Phosphoseryl-tRNA}^{[\text{Ser}]\text{Sec}} + \text{ADP}.$$

The function of PSTK was not resolved by the above studies, because the biosynthetic pathway in *E. coli* (i.e., in eubacteria encoding the Sec insertion machinery) did not involve *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ as an intermediate (9–11) and this form of tRNA$^{[Ser]Sec}$ had been proposed as a storage form (41). The hypothesis that *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ was as an intermediate in Sec biosynthesis (15) was shown to be correct when the biosynthesis in eukaryotes and archaea was resolved (see below).

Characterization of SecS

We also used a computational and genomic comparison strategy to identify a SecS gene (*SecS*), because no sequences with homology to SelA could be detected in eukaryotes that synthesize selenoproteins. The genomes of archaea and eukaryotes that had been sequenced were examined for the presence of selenoproteins and genes that co-occur with the

identified selenoprotein genes (12). Genes corresponding to the Sec insertion machinery were identified in addition to another gene that might be SecS in mammals. Homologous sequences of this gene were found in all archaea and eukaryotes encoding selenoproteins and not in the examined organisms in these kingdoms that did not make selenoproteins. The sequence of this putative SecS gene corresponded to the sequence of a protein in patients with autoimmune chronic hepatitis that coprecipitated with Sec $tRNA^{[Ser]Sec}$ in cell extracts from such individuals, designated SLA (20; and see above). SLA was found to bind other proteins involved in Sec metabolism, further suggesting a role of this protein in Sec biosynthesis (42).

The putative mouse *SecS* (*mSecS*) was then cloned and characterized for binding to a number of potential $tRNA^{[Ser]Sec}$ substrates, including $tRNA^{[Ser]Sec}$, seryl-$tRNA^{[Ser]Sec}$, and *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ by using $tRNA^{Ser}$ and seryl-$tRNA^{Ser}$ as controls (12). *O*-Phosphoseryl-$tRNA^{[Ser]Sec}$ bound tightly to the expressed product. $tRNA^{[Ser]Sec}$ also bound, but less well, and seryl-$tRNA^{[Ser]Sec}$ bound poorly, whereas $tRNA^{Ser}$ and seryl-$tRNA^{Ser}$ did not bind at all, suggesting that *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ is a substrate for mSecS. In addition, mSecS rapidly removed the phosphate group from *O*-phosphoserine, suggesting that the intermediate product was not seryl-$tRNA^{[Ser]Sec}$ because seryl-$tRNA^{[Ser]Sec}$ bound poorly to this protein. These results provided strong evidence that mSecS was indeed the missing SecS in eukaryotic Sec biosynthesis (12).

Characterization of mammalian SPS

Mouse *sps2* (*msps2*), wherein the Sec codeword was changed to a Cys codeword, and mouse *sps1* (*msps1*) were cloned into an expression vector, along with *Caenorhabditis elegans sps2* (*csps2*), because this gene normally contains Cys in place of Sec in *msps2*. *E. coli selD* was cloned as a control to elucidate whether 1 or both of the mammalian SPS proteins were responsible for making the active selenium donor (12). The resulting proteins from each clone were expressed, purified, and their ability to generate monoselenophosphate from ATP and selenite examined. mSPS2(Cys), cSPS2, and SelD were all capable of synthesizing selenophosphate, but mSPS1 was not, providing strong evidence that eukaryotic SPS2 is responsible for making the active selenium donor and SPS1 must have another role (12,43).

Sec biosynthesis

In vitro studies. One important issue that needed to be resolved was whether selenophosphate could itself serve as the active selenium donor, because previous studies had not shown that this compound per se could be used to directly donate active selenium to reactions synthesizing Sec (10, 11,14). We chemically synthesized monoselenophosphate and incubated it with *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ and mSecS, which produced Sec attached to its tRNA (12). This study unequivocally demonstrated that selenophosphate is the active selenium donor in Sec biosynthesis and that SLA is the missing SecS and *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ is an intermediate (12).

We also reconstituted the entire Sec biosynthetic pathway in vivo that included cloning and expressing mouse seryl-tRNA synthetase (*mSerS*) as well as each of the other components and substrates involved in the biosynthesis of the 21st amino acid (12). The only component not fully characterized was the intermediate attached to SecS that accepted activated selenium to form Sec attached to its tRNA. Dehydroalanine had been identified as the intermediate in *E. coli* wherein SelA acted on seryl-$tRNA^{[Ser]Sec}$ to yield dehydroalanyl-$tRNA^{[Ser]Sec}$ (9,11); however, the available evidence suggested that the intermediate formed by the action of SecS on *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ was indeed the same as in *E. coli* (12). The Sec biosynthetic pathway, based on these in vitro studies and on additional in vivo studies, is presented in Figure 1.

In vivo studies. Although earlier, conflicting reports suggested that SPS1 was either the mammalian enzyme responsible for making the active selenium donor (25–28) or was involved in recycling selenium in Sec biosynthesis (30), our in vitro studies demonstrated that SPS2 synthesizes selenophosphate and SPS1 has another role (12). To resolve the discrepancies between the earlier studies (25,28) and ours (12), and to elucidate the roles of SPS1 and SPS2 intracellularly, these proteins were individually knocked down using RNAi technology and the consequences of their resulting loss examined (43). Selenoprotein biosynthesis was abolished in NIH 3T3 cells by loss of SPS2 expression, but loss of SPS1 had no effect. Complementation experiments were also carried out wherein selenoprotein synthesis was restored in SPS2 deficient NIH 3T3 cells with either mSPS2 (Cys) or SelD but not with mSPS1. These studies unequivocally established that SPS2, which synthesizes selenophosphate in vivo, is essential to selenoprotein synthesis and that SPS1 most likely has another role in cells other than Sec or selenoprotein synthesis (12,43). It should also be noted that *sps1* is an essential gene in *Drosophila* development (44) and has been shown to have a role in preventing megamitochondrial formation (45). Interestingly, SPS1 has been retained in insects such as the red flour beetle and silkworm that have lost all selenoprotein synthetic machinery, providing further evidence that *sps1* must have a role independent of Sec and selenoprotein biosynthesis (46).

The complete Sec biosynthetic pathway

The complete biosynthesis of Sec on its tRNA is shown in Figure 1. Initially, $tRNA^{[Ser]Sec}$ is aminoacylated with serine by SerS (17) and the seryl moiety is then converted to *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ by PSTK (15). SecS acts on *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ to hydrolyze the phosphate group forming the acceptor molecule, likely dehydroalanyl-$tRNA^{[Ser]Sec}$, which in turn accepts the active selenium donor, selenophosphate, synthesized by SPS2 (12, 14,43). SecS then converts the active acceptor to Sec (12), completing the biosynthesis of Sec, the 21st amino acid

in the genetic code, the last known protein amino acid whose biosynthesis had not been established in eukaryotes, and the only known amino acid in eukaryotes whose biosynthesis occurs on its tRNA.

De novo synthesis of Cys and Cys/Sec replacement in protein synthesis

Cys is not synthesized by mammals and therefore is 1 of several of their essential amino acids. It is 1 of the 20 commonly used amino acids in protein synthesis and its genetic codewords are UGU/UGC. Although Cys and Sec use a different genetic language and have different biosynthetic mechanisms, they are structurally similar and the replacement of sulfur with selenium in methionine (Met) synthesis has been reported wherein selenomethionine can be inserted into protein in place of methionine (46,47). Cys in place of Sec in normally selenium containing proteins has also been reported in selenium deficient rodents (48), but the specific mechanism of how this occurred was not addressed. We examined whether SPS2 would engage sulfide in place of selenide in generating thiophosphate that would be used as an active sulfur donor in making Cys attached to $tRNA^{[Ser]Sec}$ (reaction catalyzed by SecS), whereby replacing Sec with Cys in normally selenium containing proteins. The replacement of Cys for Sec on $tRNA^{[Ser]Sec}$ and the incorporation of Cys into protein by this pathway is discussed below.

In vitro studies. The incorporation of Cys in place of Sec was established in cell free reactions by adding all the appropriate enzymes and other components. For example, incubation of thiophosphate with *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ and mSecS resulted in Cys-$tRNA^{[Ser]Sec}$ (13). Similarly, incubation of sodium sulfide, ATP, mSPS2(Cys), *O*-phosphoseryl-$tRNA^{[Ser]Sec}$, and mSecS also yielded Cys-$tRNA^{[Ser]Sec}$. Replacing mSecS with SelD in either of these reactions did not result in Cys-$tRNA^{[Ser]Sec}$, demonstrating that the insertion of Cys in place of Sec on $tRNA^{[Ser]Sec}$ likely does not occur in *E. coli*.

In vivo studies. Thiophosphate was added to NIH 3T3 cells in culture and the treated cells were examined for the insertion of Cys in place of Sec in thioredoxin reductase (TR) 1 and TR3 (13). Initially, NIH 3T3 cells were labeled with ^{75}Se either with or without thiophosphate and then a sample of protein extract was electrophoresed to separate the various labeled selenoproteins on polyacrylamide gels. The level of ^{75}Se labeling in TR1 from thiophosphate-treated cells appeared to be slightly less than from control cells. On the other hand, analysis of TR1 by Western blotting of the same extracts showed a dramatic enrichment in TR1 levels treated with thiophosphate, suggesting that indeed TR1 must either be truncated and/or another amino acid was inserted in place of Sec. It should also be noted that the enrichment of TR1 shown by Western blotting in cells exposed to thiophosphate was dependent on the SECIS element in the 3′-untranslated region of TR1 mRNA (13). This experiment demonstrated that the insertion of Cys into selenoprotein, which was confirmed by MS analysis of purified TR1 (see below), was, like that of Sec incorporation, contingent on the presence of the SECIS element.

A major question was whether Cys occurred naturally in place of Sec in selenoproteins of animals. To resolve this issue, TR1 and TR3 were purified from livers of mice that had been fed selenium deficient (0 ppm selenium), selenium adequate (0.1 ppm selenium), or selenium enriched (2.0 ppm selenium) diets (13). MS analysis of TR1 and TR3 showed that the ratio of Sec:Cys was ~1:1 in mice maintained on a selenium deficient diet and this ratio changed to ~9:1 in mice receiving a selenium adequate diet, whereas Cys was not detected in these selenoproteins on a selenium enhanced diet. In NIH 3T3 cells, the ratio of Sec:Cys was 9:1 in control cells and 1:24 in cells treated with thiophosphate (17). Thus, Cys is inserted into normally selenium containing proteins in vivo that occurs by sulfide competing with selenide in generating the active donor catalyzed by SPS2. The active donor, thiophosphate, interacts with the acceptor molecule generated from *O*-phosphoseryl-$tRNA^{[Ser]Sec}$ by SecS, wherein SecS in turn converts to Cys-$tRNA^{[Ser]Sec}$. In mammals, de novo synthesis of Cys on $tRNA^{[Ser]Sec}$ is shown in Figure 1.

Conclusions

A computational, comparative genomics approach was used to identify a single kinase that had close homologs only in those archaea and eukaryotes that encode the Sec protein insertion machinery. This protein was confirmed to be PSTK by biochemical and genetic studies (15). Furthermore, a similar comparative genomics approach was used to predict SLA as the likely SecS that was indeed confirmed to serve this function (12). With few exceptions, orthologs of both SLA and PSTK were found in eukaryotes and archaea that utilize the Sec protein insertion machinery, but not in organisms that do not, suggesting that this pathway is used by all eukaryotes and archaea that synthesize selenoproteins (12,15). Establishing the mechanism by which selenium makes its way into proteins demonstrates how this essential element becomes a component of Sec that is incorporated into selenoproteins as the 21st amino acid in the genetic code in eukaryotes and archaea. Many health benefits of selenium have been reported that include roles in decreasing incidence of cancer and heart disease, development and immune function, and delaying the aging process and the onset of AIDS in HIV positive patients. Selenoproteins are likely the components responsible for most of these health benefits.

Cys, an essential amino acid in mammals, was also found to be synthesized de novo by replacing Sec in the synthesis of selenium containing proteins. The precise means of how this synthesis occurs was determined in vitro and the presence of Cys in place of Sec was shown to occur in vivo in both cells in culture and in mice. In addition to establishing the pathway of Sec biosynthesis, the replacement of Sec with Cys provides unique possibilities for regulating the expression

of selenoproteins and their functions as well as elucidating the biological roles of dietary selenium.

Acknowledgments

All authors analyzed data, wrote the paper, and had primary responsibility for final content. All authors read and approved the final manuscript.

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