Synthesis and decoding of selenocysteine and human health

Selenocysteine, the 21st amino acid, has been found in 25 human selenoproteins and selenoenzymes important for fundamental cellular processes ranging from selenium homeostasis maintenance to the regulation of the overall metabolic rate. In all organisms that contain selenocysteine, both the synthesis of selenocysteine and its incorporation into a selenoprotein requires an elaborate synthetic and translational apparatus, which does not resemble the canonical enzymatic system employed for the 20 standard amino acids. In humans, three synthetic enzymes, a specialized elongation factor, an accessory protein factor, two catabolic enzymes, a tRNA, and a stem-loop structure in the selenoprotein mRNA are critical for ensuring that only selenocysteine is attached to selenocysteine tRNA and that only selenocysteine is inserted into the nascent polypeptide in response to a context-dependent UGA codon. The abnormal selenium homeostasis and mutations in selenoprotein genes have been causatively linked to a variety of human diseases, which, in turn, sparked a renewed interest in utilizing selenium as the dietary supplement to either prevent or remedy pathologic conditions. In contrast, the importance of the components of the selenocysteine-synthetic machinery for human health is less clear. Emerging evidence suggests that enzymes responsible for selenocysteine formation and decoding the selenocysteine UGA codon, which by extension are critical for synthesis of the entire selenoproteome, are essential for the development and health of the human organism.
A Swedish chemist, Jön J. Berzelius, isolated selenium from the lead chambers in a sulfuric acid factory in 1817. Because of its similarities to tellurium, which was previously named after the Roman god of earth Tellus (1), Berzelius decided to name the newly discovered element after Selene, the Hellenic moon goddess (2). The unique properties of selenium prompted its wide use in engineering, chemical industry, and glass manufacturing. It is this use that revealed the poisonous properties of selenium, which caused frequent poisoning and selenium-induced deaths in industrial workers (3,4). Reports that selenium is toxic to farm animals (5-7), that it has teratogenic effects in birds (8), non-human primates and man (9-11), and that its high doses might also be carcinogenic (11) further strengthened the notion that selenium is toxic to any living organism. This view held firm until the mid-1950s when it was first reported that trace amounts of selenium (and molybdate) are required for optimal enzymatic properties of intestinal Escherichia coli (12) and that selenium is essential for rodent survival (13). This was followed by a series of observations that selenium deficiency is a cause of a variety of livestock diseases such as white muscle disease in cattle and sheep (14,15), exudative diathesis in chicken (16,17), male infertility in mammals (18-20), and mulberry heart disease in pigs (21). In spite of these reports, it was not until the early 1980s that selenium was considered beneficial for humans. The anonymous report that the cardiovascular disease, known as Keshan disease, is caused by selenium deficiency (22) and epidemiological studies on the effect of selenium on cancer and cardiovascular diseases (23), Kashin-Beck disease (24), and myxoedematous cretinism (25) marked the turn of the tide for the field of selenium biology.

Concurrently with the reports on its beneficial role, observations that selenium is a constitutive component of mammalian glutathione peroxidase (GPx) (26-32) and other microbial enzymes such as protein A component of the clostridial glycine dehydrogenase (33), formate dehydrogenase (34,35), nicotinic acid hydroxylase, and xanthine dehydrogenase (36) have been published. It took several years of tedious work to show that selenium is present in those and other selenoproteins as the newly identified amino acid – selenocysteine (37-40). Initially, it was thought that selenium is incorporated post-translationally into certain enzymes and proteins, though the mechanism by which this could have taken place was not proposed. Almost a decade later, by a combination of macromolecular x-ray crystallography (41), and protein and gene sequencing (42,43) it was convincingly shown that the selenocysteine residue in the active site of the murine GPx is encoded by a UGA codon (43-45). It is now known that the human selenoproteome comprises 25 selenoproteins that are expressed in various tissues and organs (Table 1) (46). Some selenoproteins are ubiquitous, whereas the others have more restricted tissue and organ distribution. Also, while the biological role of certain selenoproteins is well established, there are still those for which the physiological and cellular role is less clear. With the notable exception of selenoprotein P (SelP), selenoproteins typically contain a single selenocysteine residue that is critical for their structure and function. That selenoproteins are essential for life has been convincingly shown by an embryonically lethal phenotype of the mouse tRNA^sec knockout mutant (47). Furthermore, emerging studies have just began to unravel all the ways selenoproteins affect cell signaling cascades and cell cycle, protein and RNA expression, and disease development in humans. This picture is growing into a complex mosaic, which stands in contrast to an earlier assumption that selenoenzymes and selenoproteins simply protect the cell from oxidative stress and thus play a preventive role against disease development.

The observation that selenocysteine is a constitutive component of enzymes immediately raised a series of questions that intrigued the scientific community to the present day: 1) How is selenocysteine synthesized? 2) Does it require a special tRNA to be incorporated into proteins? and 3) Which mechanism distinguishes the selenocysteine UGA codon from the typical translational stop UGA codon? Here, major discoveries in the field of selenocysteine and selenoprotein synthesis, which provided some of the answers to these fundamental questions, will be summarized. Additionally, the most recent clinical observations suggesting that the integrity of the selenocysteine-synthetic and translational machinery is of great importance for human health will be discussed.

THE MECHANISM OF SELENOCYSTEINE SYNTHESIS

Selenocysteine, also known as the 21st amino acid, is unique among the proteinogenic amino acids. It is the only amino acid containing an essential dietary micronutrient (selenium) as a constitutive component, the only amino acid encoded by a UGA codon and the only one synthesized on its tRNA in all domains of life. Moreover, selenocysteine is the only amino acid among over 140 amino acids found in proteins thus far (this count includes 20 standard amino acids, pyrrollysine and all post-translationally modified amino acids), which requires a complex tRNA-dependent
| Name          | Protein family            | Physiological role                                                                 | Role in human health                                                                 | Special notes                                                                 |
|--------------|--------------------------|-----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Gpx1-4, Gpx6 | Glutathione peroxidase   | Catalyzes the reduction of hydrogen peroxide and/or lipid peroxides. First line of defense against oxidative stress. | Plays a role in defense against cancer, cardiovascular and neurodegenerative disease. | Gpx1 – first identified selenoprotein.                                          |
| T6nrd1-3     | Thioredoxin reductases    | Catalyzes the reduction of oxidized thioredoxin (Trx). Regulate various signaling cascades. | Important for cancer progression and viral suppression.                             | T6nrd1 and 2 – Housekeeping proteins T6nrd3 – Expressed in testes               |
| DIO 1-3      | Iodothyronine deiodinases | Membrane-anchored selenoenzymes that activate/inactivate thyroid hormone.          | Important for development and regulating overall metabolic rate.                     | Stable mRNA under conditions of low selenium – suggests a high place in selenoprotein expression hierarchy. |
| SeH          | Thioredoxin fold-like protein | Regulates expression of enzymes involved in glutathione synthesis.            | Not known                                                                            | Widely distributed in various tissues.                                         |
| SeIM, Sep15  | Thioredoxin fold-like protein | Thiol-disulfide oxidoreductases that play a role in protein folding quality control. | Not known                                                                            | Localized to the endoplasmic reticulum (ER).                                  |
| SeT          | Thioredoxin fold-like protein | Not known                                                                        | Not known                                                                            | Localized to the ER membrane.                                                  |
| SeIV         | Thioredoxin fold-like protein | Not known                                                                        | Not known                                                                            | Expressed in testes.                                                          |
| SeW          | Thioredoxin fold-like protein | Interacts with glutathione and protein 14-3-3.                                    | Potential antioxidant role.                                                         | Expressed in all tissues.                                                      |
| SeI          | Seven transmembrane domains and a CDP-alcohol synthesis phosphatidylytransferase motif | Involved in phospholipid synthesis.                                              | Not known                                                                            | Perhaps localized to the ER.                                                   |
| SeK          | Integral membrane protein | Not known                                                                        | Not known                                                                            | Localized to the ER. High expression in the heart.                             |
| SeIS         | Integral membrane protein | Responsible for removal of misfolded proteins, protection from oxidative damage and ER stress induced apoptosis. | Mutations linked to cancer, cardiovascular disease, preeclampsia and rheumatoid arthritis. | Localized to the ER and plasma membranes.                                      |
| SeIN (SepN)  | Integral membrane protein | Function in calcium mobilization by direct modulation of the ryanodine receptor.  | Mutations linked to multiple muscle system disorders including muscular dystrophy and multiminicore disease. | Localized to the ER. Mutations in the 3’UTR of SeIN led to identification of the Sec redefinition element (SRE). |
| SeIP         | Methionine sulfoxide reductase | Reduction of R-form of methionine sulfoxides, oxidized methionines.             | Plays a role in protection from neurodegeneration, maintaining lens cell viability, and reducing oxidative damage during aging. | Only selenoprotein containing multiple selenocysteine residues. Accounts for 40%-50% of the total selenium in plasma. |
| SeIR         | Methionine sulfoxide reductase | Reduction of R-form of methionine sulfoxides, oxidized methionines.             | Plays a role in protection from neurodegeneration, maintaining lens cell viability, and reducing oxidative damage during aging. | Only selenoenzyme involved in selenoprotein synthesis.                          |
| SPS2         | Selenophosphate synthetase | Converts selenide into selenophosphate for Sec synthesis.                        | Not known                                                                            | Only selenoenzyme involved in selenoprotein synthesis.                          |
| SeIO         | Contains Cys-X-X-Sec motif | Not known                                                                        | Not known                                                                            |                                                                                 |
selenocysteine. However, it defies the canonical pathway; its synthesis is reminiscent of but more complex than the indirect aminoacylation pathway of asparagine and glutamine found in some archaea. First, a cellular pool of free selenocysteine does not exist, and second, because putative selenocysteinyl-tRNA synthetase never evolved, free selenocysteine, even if it were available, could not be attached to the cognate tRNA. So, how is selenocysteine synthesized and how is it paired with its tRNA? In all selenocysteine-containing organisms, the synthetic cycle of selenocysteine begins with an essential “error:” seryl-tRNA synthetase (SerRS) erroneously charges selenocysteine tRNA (tRNA^{Sec}) with serine thus yielding seryl-tRNA^{Sec} (Figure 1). The mischarged Ser-tRNA^{Sec} is not edited and is released into solution to serve as an intermediate for the subsequent enzymatic reactions. The ability of SerRS to aminoacylate two tRNAs with completely different anticodon sequences is quite peculiar. It is even more puzzling that SerRS is capable of acting on both tRNA^{Ser} and tRNA^{Sec} with significant efficiency considering that the two tRNAs adopt completely distinct folds. While tRNA^{Ser} is a canonical elongator tRNA that adopts a 7/5 fold (where 7 and 5 indicate a number of base pairs in the acceptor and TΨC arms, respectively), tRNA^{Sec} is an unusual tRNA that adopts either a 9/4 fold in eukaryotes (49,50) or an 8/5 fold in prokaryotes (51). In either case, the acceptor-TΨC helix of tRNA^{Sec} contains 13 base pairs as opposed to a standard length of 12 base pairs observed in all other tRNAs. This difference in the acceptor arm length should impose significant structural and spatial constraints on the interactions between tRNA^{Sec} and selenocysteine-synthetic enzymes. For instance, all tRNA-binding enzymes contain a 5'-phosphate binding groove, among other recognition motifs, which accommodates the 5'-end of the substrate tRNA. Because of the additional base pair in the acceptor arm, the 5'-phosphate in tRNA^{Sec} is translated by ~ 3.4 Å and rotated by ~ 33° clockwise around the helix-axis (when viewed down the helix and toward the 3' and 5' ends) relative to the corresponding group in canonical tRNAs. However, SerRS is capable of binding tRNAs in which the 5' phosphate and perhaps other structural elements are positioned quite differently. From the structure of the T. thermophilus SerRS-tRNA^{Sec} complex it is evident that the enzyme binds the elbow of the acceptor-TΨC 'helix' of tRNA^{Sec} with its N-terminal domain (52). However, large parts of the acceptor and variable arms were disordered in the crystal and it remains obscure how SerRS interacts with tRNA^{Sec} (52). Further mechanistic and structural studies on the mechanism of substrate promiscuity of SerRS will perhaps explain why this enzyme is capable of “charging” two structurally different tRNAs. Also, it is not clear how frequently human

![FIGURE 1. Synthesis and co-translational incorporation of selenocysteine in humans. The cycle, which is conserved in archaea and eukaryotes, begins with a mischarging reaction in which seryl-tRNA synthetase attaches L-serine (L-Ser) to a non-cognate tRNA. A specific kinase, O-phosphoseryl-tRNA synthase (PSTK), phosphorylates the seryl group yielding a phosphoseryl (Sep)-tRNA intermediately. In the terminal synthetic reaction, O-phosphoseryl-tRNA:seleocysteinyl-tRNA synthase (SepSecS), catalyzes conversion of Sep-tRNA into seleocysteinyl (Sec)-tRNA by a mechanism that requires selenophosphate and a co-factor pyridoxal phosphate (PLP). Selenophosphate, the main selenium donor in man, is a product of the catalytic activity of selenophosphate synthetase (SPS2). Human SPS2 is a selenoenzymes that utilizes as a reaction substrate the final product of selenoprotein/selenoenzyme degradation, selenide, and adenine triphosphate (ATP). Finally, Sec-tRNA^{Sec} is targeted and delivered to the ribosome by a specialized elongation factor – EFsec. An auxiliary protein factor, SECIS-binding protein 2 (SBP2), is required for decoding of the selenocysteine UGA codon in all vertebrates, whereas a shorter ortholog is functional in invertebrates. Selenocysteine (green sphere) is inserted into the nascent selenoprotein (orange spheres) in response to a specific UGA codon. SECIS, an in-cis element in the selenoprotein mRNA located in the 3'-UTR, forms a stem loop structure and is required for decoding of the selenocysteine UGA codon. In bacteria, a single enzyme, SelA, converts Ser-tRNA^{Sec} to Sec-tRNA^{Sec}, elongation factor SelB binds directly to SECIS, which is, in turn, a part of the coding sequence.](image-url)
SerRS misacylates tRNA^{Sec} under physiological conditions, whether this process is regulated and how putative regulatory processes might be affecting synthesis of selenocysteine in particular, and selenoproteins in general. Interestingly, the serylation of tRNA^{Sec} is the only reaction in the cycle of selenocysteine that is conserved in all domains of life; beyond this point the bacterial mechanism significantly diverges from the archaeal and eukaryotic processes.

While a single bacterial enzyme, the homodecameric SelA, catalyzes the conversion of Ser-tRNA^{Sec} into Sec-tRNA^{Sec} (53), two enzymatic steps are needed to complete the conversion in archaea and eukaryotes. In the first step, O-phosphoseryl-tRNA^{Sec} kinase (PSTK), phosphorylates Ser-tRNA^{Sec} into phosphoseryl-tRNA^{Sec} (Sep-tRNA^{Sec}) (54,55), and in the second, O-phosphoseryl-tRNA^{Sec}:selenocysteinyl-tRNA^{Sec} synthase (SepSecS) substitutes phosphate with selenium thus yielding Sec-tRNA^{Sec} (Figure 1) (56-58). In striking contrast to a promiscuous SerRS, PSTK and SepSecS have stringent substrate specificities: PSTK acts on Ser-tRNA^{Sec} and not on Ser-tRNA^{Ser}, whereas SepSecS acts only on Sep-tRNA^{Sec} and not on Ser-tRNA^{Sec} or Ser-tRNA^{Ser}. Biochemical, biophysical and x-ray crystallographic studies have provided a structural basis for the substrate specificity of these enzymes (Figure 2).

Identification of the bacterial SelA as the enzyme that directly converts serine to selenocysteine in a tRNA-dependent manner stimulated a search for archaeal and eukaryotic homologs. However, all efforts to identify a SelA-like enzyme in these organisms failed. Ultimately, it was studies published in the early 1980s by Hatfield et al that paved the way toward the solution for the conundrum (59,60). The authors have characterized a distinct opal (UGA) suppressor tRNA isolated from bovine liver that could suppress the termination of protein synthesis at the UGA codon, that carried an unusual phosphoseryl group attached to its 3’ end and that it participated in the phosphorylation reaction. Two decades later, a tRNA-dependent kinase responsible for formation of Sep-tRNA^{Sec} was identified as PSTK (54,55). An intriguing question is what would be the purpose of the phosphorylation event. The simplest explanation is that Sep-tRNA^{Sec} intermediate contains phosphate in its aminoacyl moiety, which is a better leaving group than water. In other words, phosphorylation activates the β-OH group of serine for a subsequent substitution with selenium. Also, insertion of the tRNA-dependent kinase adds yet another checkpoint that ensures proper decoding of an in-frame selenocysteine UGA codon. The crystal structure of the archaeal PSTK-tRNA^{Sec} binary complex revealed that the dimeric PSTK recognizes the distinct 8/5 fold of the archaeal tRNA^{Sec} by acting as a molecular ruler that “measures” the distance between the enlarged D arm and the tip of the long acceptor arm of tRNA^{Sec} (Figure 2) (51). The kinase interacts with the upper body of tRNA and it does not interact with the anticodon arm or anticodon sequence, which is a trademark of all enzymes involved in selenocysteine synthesis and insertion (ie, SerRS, SepSecS, and EFsec). Biochemical assays have confirmed the mode of tRNA^{Sec} recognition utilized by the archaeal PSTK (51,61) and by extension it was proposed that the human ortholog might employ the same mechanism for tRNA recognition. However, no structural and functional data are available on the human PSTK and the exact molecular detail on how this enzyme might be binding its substrate tRNA is not understood.

An observation that patients suffering from severe autoimmune hepatitis develop autoantibodies against a soluble liver antigen/liver-pancreas (SLA/LP) protein factor (62), which is in turn, often found in complex with Sep-tRNA^{Sec}, led to the identification of the terminal synthetic enzyme in the cycle of selenocysteine (63,64). It turned out that SLA/LP is an enzyme termed SepSecS (see above), which...

![Figure 2. PSTK and SepSecS recognize the distinct fold of tRNA^{Sec} by binding to different structural elements in tRNA^{Sec} (Left). Surface diagram of the human SepSecS tetramer (olive) complexed with human tRNA^{Sec} (red) shows that SepSecS binds the major groove of the extended acceptor-TΨC “helix” (based on PDB ID: 3HL2) (49) and it “measures” the distance between the variable arm and the CCA end. (Right) Archaeal PSTK (gray) binds the opposite side of tRNA^{Sec} (red) and ‘measures’ the distance between a longer D arm and the CCA end (based on PDB ID: 3ADD) (51). Acronyms explained in Figure 1 legend.](www.cmj.hr)
promotes the phosphoserine-to-selenocysteine conversion in archaea and eukaryotes. The crystal structure of the human SepSecS-tRNA<sup>sec</sup> binary complex, which is the first structure derived from the components of the human selenocysteine synthetic machinery, revealed that SepSecS, just like PSTK, primarily binds the unique 13bp-long acceptor-TΨC arm of tRNA<sup>sec</sup> (Figure 2) (49). In contrast to PSTK, however, SepSecS binds the major groove of the acceptor arm and the long variable arm, thus effectively “measuring” the distance between the variable arm and the tip of tRNA<sup>sec</sup> (Figure 2). Further, SepSecS and PSTK approach tRNA<sup>sec</sup> from opposite sides suggesting that larger multienzyme complexes may be responsible for formation of selenocysteine. In addition, the structure revealed that SepSecS binds tRNA<sup>sec</sup> in a cross-dimer fashion: one homodimer serves as a platform that binds tRNA<sup>sec</sup> and orients its CCA end to the catalytic site located in the other SepSecS homodimer. Finally, the results of the structural, mutational, and biochemical studies suggest that SepSecS requires phosphoserine covalently attached to tRNA<sup>sec</sup>, selenophosphate, and a cofactor, pyridoxal phosphate (PLP), for catalysis (49). Selenophosphate is the major selenium donor in human cells and it is a product of another selenoenzyme, selenophosphate synthetase 2 (SPS2) (65). Selenophosphate is synthesized from ATP and selenide, the end product of selenocysteine degradation promoted by selenocysteine lyase (66). Thus, selenium must first be extracted from the degraded selenoproteins, ingested or endogenous, and turned into selenophosphate before its incorporation into selenocysteine and nascent selenoproteins. SepSecS and SPS2 are positioned at the crossroad between selenium recycling and release, and thus could represent the control points for the synthesis of the entire selenoproteome.

The catalytic functions of SerRS, PSTK, and SepSecS substitute the missing function of the putative SecRS and they serve as distinct checkpoints that ensure that only selenocysteine is paired with tRNA<sup>sec</sup>. In addition to the elaborate mechanism of selenocysteine synthesis, a separate mechanism evolved to facilitate delivery of Sec-tRNA<sup>sec</sup> to the ribosome, the co-translational incorporation of selenocysteine into the nascent protein, and the decoding of the in-frame UGA codon.

**DECODING OF THE SELENOCYSTEINE UGA CODON**

A general elongation factor, EF-Tu in prokaryotes and eEF1A in eukaryotes, delivers to the ribosome all but one aminoacylated tRNA. That sole exception is Sec-tRNA<sup>sec</sup>. In all domains of life, the delivery of Sec-tRNA<sup>sec</sup> to the site of translation requires a specialized elongation factor, SelB in prokaryotes (67,68) and EFsec in eukaryotes (69,70), and a structural element in the selenoprotein mRNA located either within the coding sequence or in the 3′ untranslated region (UTR) (Figure 1). While this general mechanism holds true in all organisms that contain selenocysteine, some very important differences have been noted between bacteria, archaea, and eukaryotes.

The decoding mechanism for the selenocysteine UGA codon was initially studied using bacterial model systems. It was shown that the process depends critically on a 40 nucleotide-long sequence immediately downstream of the UGA codon, which is termed SElenoCysteine Insertion Sequence (SECIS) (71). The SECIS element forms a hairpin structure, which interacts with a ternary complex composed of SelB, GTP, and Sec-tRNA<sup>sec</sup>. Each selenocysteine codon in the bacterial selenoprotein mRNA is followed by the SECIS element and thus the bacterial SECIS is often a part of the coding sequence. The crystal structure of the archaeal SelB revealed that the protein contains an N-terminal EF-Tu-like domain and a unique C-terminal domain that contains motifs for binding to SECIS (72). However, the mode of tRNA<sup>sec</sup> recognition, the mechanism by which SelB facilitates decoding of the selenocysteine UGA codon and the nature of its interactions with the ribosome are not well understood. In archaea, the SECIS loop is typically in the 3′-UTR of the selenoprotein mRNA and a single SECIS element is capable of coordinating insertion of multiple selenocysteine residues (73,74).

The eukaryotic selenocysteine decoding machinery has another layer of complexity when compared to the bacterial system. For simplicity, we shall discuss primarily the human system. First, human selenoprotein genes typically encode a single selenocysteine residue and they contain a single SECIS element located in the 3′-UTR instead of being a part of the coding sequence (75,76). Exceptions are human selenoprotein P (SelP), which contains 10 selenocysteine residues and two functional SECIS elements, and selenoprotein L (SelL) that contains two selenocysteine residues and one SECIS element (77). Eukaryotic SECIS belongs to the kink-turn family of RNAs comprised of two helical stems separated by an internal loop of 4-18 nucleotides and the distance between the SECIS loop and an in-frame UGA varies significantly among selenoprotein genes (78). One may ask what the purpose of the SECIS element is and why selenocysteine requires this RNA element for its insertion into protein. An explanation would be that binding to...
the SECIS element localizes both Sec-tRNA\textsuperscript{Sec} and EFsec/SeIB near the site of translation, thus effectively increasing their local concentrations. This is important because selenoprotein synthesis is encoded by a UGA codon, which otherwise signals the end of translation. The ribosome always stalls at the UGA triplet since under normal circumstances there is no cognate tRNA that reads the UGA codon. This allows for a release factor protein to eventually associate and promote the termination of protein synthesis and the subsequent peptide release from the ribosome. Therefore, if the local concentrations of tRNA\textsuperscript{Sec} and EFsec/SeIB are low then the release factors might prematurely abort selenoprotein synthesis. Additionally, the presence of SECIS allows for a more complex regulation of selenoprotein synthesis and selenoprotein synthesis as it was already shown in the case of GPx and phospholipid hydroperoxide GPx where the interplay of auxiliary protein factors (eg, SBP2 and eIF4a3) regulates expression levels of these selenoenzymes in response to varying levels of selenium in the liver (79). Further biochemical studies, however, are needed to better understand how different SECIS elements coordinate and regulate selenoprotein synthesis.

Second, in contrast to the prokaryotic SeIB, EFsec cannot directly bind a SECIS loop without an auxiliary protein factor, SECIS-binding protein 2 (SBP2) (69,80,81). Mammalian SBP2 is a large protein composed of 854 amino-acid residues. It has been shown that ~70% of SBP2 is unstructured at the UGA triplet since under normal circumstances there is no cognate tRNA that reads the UGA codon. This allows for a release factor protein to eventually associate and promote the termination of protein synthesis and the subsequent peptide release from the ribosome. Therefore, if the local concentrations of tRNA\textsuperscript{Sec} and EFsec/SeIB are low then the release factors might prematurely abort selenoprotein synthesis. Additionally, the presence of SECIS allows for a more complex regulation of selenoprotein synthesis and selenoprotein synthesis as it was already shown in the case of GPx and phospholipid hydroperoxide GPx where the interplay of auxiliary protein factors (eg, SBP2 and eIF4a3) regulates expression levels of these selenoenzymes in response to varying levels of selenium in the liver (79). Further biochemical studies, however, are needed to better understand how different SECIS elements coordinate and regulate selenoprotein synthesis.

In spite of the numerous studies, the exact mechanism by which human EFsec and SBP2 bind SECIS and tRNA\textsuperscript{Sec}, and how they facilitate decoding of the selenocysteine UGA on the ribosome is not well understood. Further structural, biochemical, and mechanistic studies on the mechanism of decoding of the selenocysteine UGA in humans are thus warranted.

**DISRUPTION OF THE SYNTHESIS AND CO-TRANSLATIONAL INSERTION OF SELENOCYSTEINE CAUSES VARIOUS DISEASES IN HUMANS**

Selenium deficiency, and numerous mutations in selenoproteins and selenoenzymes have been linked to various disorders of the endocrine, central nervous, muscular, cardiovascular, and immune systems in man (93) (Table 1). In contrast, very few mutations in the enzymes responsible for synthesis and co-translational insertion of selenocysteine have been shown to cause any phenotype in humans. This is not entirely surprising considering the essentiality of the selenocysteine cycle for the integrity of the human selenoproteome and for the development of a healthy organism. In other words, given the mouse tRNA\textsuperscript{Sec} knockout mutant displayed embryonically lethal phenotype (47), it is expected that any mutation that has detrimental effect on the structure and function of SerRS, PSTK, SepSecS, SPS2, SBP2, EFsec, or tRNA\textsuperscript{Sec} would most likely completely inhibit selenoprotein synthesis. This, in turn, would yield an embryo incapable of developing into a healthy organism that can reach adulthood and thus such
mutations would be selected against in the early stages of embryogenesis. Only in instances in which mutations do not completely impair selenoprotein synthesis, would the phenotype be displayed in some form of disease or disorder. Indeed, recent studies have identified mutations in human SepSecS, SBP2, and the mRNA regulatory elements that cause disorders in humans of different severity and complexity (see below). While mutations affecting selenocysteine synthesis have generally been found to be associated with severe neurological disorders, mutations in the auxiliary components of the decoding apparatus (ie, SBP2, SECI, and SRE) have been shown, with a few notable exceptions, to cause somewhat milder systemic disorders.

Mutations in SepSecS cause progressive cerebello cerebral atrophy

Progressive cerebello cerebral atrophy (PCCA) was first identified in nonconsanguineous Jewish Sephardic families of Moroccan and Iraqi ancestry (94). Clinically, PCCA is associated with severe spasticity, profound mental retardation, and progressive microcephaly. Radiological examination revealed that patients exhibit progressive cerebellar atrophy and cerebral atrophy involving both white and gray matter. Most individuals never advanced past the first neurological milestone (smiling) and they rarely lived past 12-13 years of age. Their phenotype most closely resembled pontocerebellar hypoplasia type 2 (PCH2). However, because PCH2 is not associated with progressive cerebellar changes, the search for the cause of this new autosomal recessive syndrome ensued. Sequencing of four inflicted individuals revealed that PCCA is linked to two mutations in the gene encoding SepSecS. It was found that unrelated inflicted individuals of Iraqi Jewish ancestry were homozygous for a mutation in which a nucleotide A1001 is replaced with G. At the protein level, this mutation leads to a substitution of a highly conserved tyrosine residue in position 334 with cysteine (Y334C). On the other hand, inflicted individuals of mixed Iraqi-Moroccan were compound heterozygous for two mutations in the SepSecS gene. Besides the A1001G mutation in one allele, they also carried a G715A mutation in the second allele (termed “Moroccan”). This second mutation leads to a substitution of the conserved alanine 239 with threonine (A239T) in the SepSecS protein. Individuals carrying only one of these mutations exhibit no phenotype, suggesting that one wild-type allele is enough to compensate completely for a single mutation. It was thus hypothesized that the catalytic activity of these mutants is decreased and, indeed, these mutants were not able to complement a strain of *Escherichia coli* lacking the endogenous selenocysteine synthase, SelA (57). Thus, it is likely that the severe neurological phenotype in the inflicted individuals is a consequence of the reduced levels of selenoproteins. Although the selenium content of the brain is not particularly high, selenoprotein P (SelP) delivers selenium preferentially to the brain at the expense of other organs under selenium deficiency, which suggests that the constant selenium content in the brain is essential for normal development. This is in agreement with the observations that mice in which either SelP (95) or its receptor, ApoER2, (96) were knocked out, displayed ataxia and seizures (97,98) and that those in which tRNA^Sec^ was deleted from neurons had neurodevelopmental and degenerative phenotype in the cerebellum, hippocampus, and cerebral cortex (99). However, it is not clear how these mutations affect the structure and function of SepSecS. It is likely that the insertion of Thr instead of Ala239 causes steric clashes between two α helices that form a binding pocket for the variable arm of tRNA^Sec^ (Figure 3A). Thus, the

![FIGURE 3](image-url). Point mutations in SepSecS give rise to progressive cerebello cerebral atrophy by affecting the tRNA^Sec^ binding pocket and the active-site groove. (A) In the first mutant, alanine in position 239 (highlighted in red) in helix α8 is mutated into threonine. This substitution is likely to cause a change in positioning of helices α8 and α9. Because residues in helix α9 (Arg271 and Gln268) interact with the variable arm of tRNA^Sec^ (raspberry), any structural change in this part of the enzyme might reduce the binding affinity of the A239T SepSecS mutant for tRNA^Sec^. (B) In the second mutant, a highly conserved tyrosine in position 334 is replaced with cysteine. The Y334C mutation would almost certainly remove an important hydrogen bond between the hydroxyl group in Tyr334 and the backbone carbonyl oxygen in a turn preceding Lys284 to which an obligatory co-factor PLP is covalently attached. Thus, it is likely that the orientation and position of PLP in the Y334C mutant be different than that in the wild-type enzyme. It is plausible that this mutation impairs the catalytic prowess of SepSecS. The enzyme is beige, tRNA^Sec^ is raspberry, and the important amino-acid residues are green stick-and-balls. The ribbon diagrams are based on PDBID 3HL2 (49). Acronyms explained in Figure 1 legend.
would bind tRNA

be repositioned. Therefore, it is likely that this mutant the hydrogen bond would not be formed and PLP would attached (Figure 3B). Because Cys is much shorter than Tyr, the hydrogen bond would not be formed and PLP would be repositioned. Therefore, it is likely that this mutant would bind tRNA

of the turn that carries Lys284 to which PLP is covalently Tyr334 forms a hydrogen bond with the backbone atoms

ativity compared to the wild type protein, whereas its cata-

the active site and particularly the positioning of the co-

of iodothyronin deiodinase 2 (DIO2), which is responsible

thyroid function tests: elevated blood thyrotropin (TSH),

vided and dysfunctional protein factor. However, the pheno-

Selenium selenoprotein synthesis in general, and that of the enzymes of thyroid hormone metabolism in particular. A similar phenotype observed in an unrelated family of Irish origin was caused by a compound heterozygous defect in SBP2 (100). The patient’s inherited paternal allele carried a nonsense mutation K438X in exon 10, which resulted in a truncated protein devoid of any function, and a maternal allele with a mutation IVS8ds +29GA, which created an alternative splice site in the SBP2 transcript that caused a frameshift and a complete change in protein sequence. The abnormally spliced transcripts represented ~52% of the transcripts generated from the maternal allele. Consequently, the patient had ~25% of normal SBP2 transcripts and presumably that same level of functional protein compared to a normal and healthy individual. In both instances, it was shown that the activity of glutathione peroxidase in serum (GPx-3) was reduced and that serum levels of SeP and selenium were significantly reduced compared to healthy individuals.

Subsequently, Refetoff et al identified yet another mutation in the SBP2 gene in a family of African origin (104). In this peculiar case, the patient was homozygous to a R128X mutation, which was supposed to yield a severely truncated and dysfunctional protein factor. However, the phenotype was mild and reminiscent of the previously described partial SBP2 deficiency (100) albeit with normal TSH levels. Further examination of the expression profile generated from the SBP2 minigene revealed that at least three ATG codons downstream of the regular start codon support synthesis of SBP2. The N-terminal domain of SBP2 is dispensable and the truncated SBP2 constructs supported selenoprotein expression at a somewhat attenuated level, but which was sufficient to prevent expression of a more severe phenotype. Similar phenotype to the R128X was also described in Japan, but the cause was a distinct compound heterozygous defect in SBP2 (105). In this case, the maternal copy carried a Q79X nonsense mutation and the paternal allele had a mutation that caused a frame shift and insertion of the stop codon after 48 amino acids. The Q79X allele, just like R128X, supposedly yields a truncated and functional SBP2 by utilizing downstream

Two completely different mutations in SepSecS than those described in the PCCA study have been identified in three unrelated families in the Finnish population (personal communication with Dr Henna Tyynismaa). Compound heterozygous individuals exhibit a similar neurological phenotype as those with PCCA; the inflicted individuals display severe mental retardation, progressive cerebellar and cerebral atrophy, microcephaly, extreme spasticity, and other features characteristic of that disorder (personal communication with Dr Henna Tyynismaa). Additional symptoms have been observed, which suggest that this may be a distinct disorder related to SepSecS mutations.

**Human diseases caused by mutations in the selenocysteine UGA decoding apparatus, SBP2 and SECIS/SRE**

Numerous mutations have been found in the human SBP2 gene leading to multisystemic disorders. Also, links between human disorders and two mutations in in-cis regulatory elements (ie, SECIS and SRE) that coordinate decoding of the selenocysteine UGA codon have been characterized.

Refetoff et al were first to report on three mutations in SBP2 linked to abnormal thyroid hormone metabolism (100). In case of the Bedouin Saudi family, the affected individuals carried a homozygous missense mutation in exon 12 which resulted in the R540Q amino acid substitution in the SBP2 protein. The inflicted individuals had abnormal thyroid function tests: elevated blood thyrotropin (TSH), elevated T3 levels, and reduced T4 levels. Also, the levels of iodothyronin deiodinase 2 (DIO2), which is responsible for producing the most active form of thyroid hormone (101), were reduced and the patients displayed delayed bone growth resulting in a short stature. In a subsequent study it has been shown that the R540Q mutant of SBP2 had reduced affinity for a subset of SECIS RNAs including those for DIO1, DIO2, and GPx1 (102). Given that SBP2 preferentially binds certain SECIS elements (103), it has been suggested that the R540Q mutation affected the hierarchy of selenoprotein synthesis in general, and that of the enzymes of thyroid hormone metabolism in particular. A similar phenotype observed in an unrelated family of Irish origin was caused by a compound heterozygous defect in SBP2 (100). The patient’s inherited paternal allele carried a nonsense mutation K438X in exon 10, which resulted in a truncated protein devoid of any function, and a maternal allele with a mutation IVS8ds +29GA, which created an alternative splice site in the SBP2 transcript that caused a frameshift and a complete change in protein sequence. The abnormally spliced transcripts represented ~52% of the transcripts generated from the maternal allele. Consequently, the patient had ~25% of normal SBP2 transcripts and presumably that same level of functional protein compared to a normal and healthy individual. In both instances, it was shown that the activity of glutathione peroxidase in serum (GPx-3) was reduced and that serum levels of SeP and selenium were significantly reduced compared to healthy individuals.

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expectation would be that, if properly folded, the A239T mutant of SepSecS would bind tRNA

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ATG codons, whereas the mRNA derived from the second copy is targeted to the nonsense-mediated mRNA decay.

More recently, researchers from Brazil reported on a 12-year old girl with a syndrome of abnormal thyroid metabolism, delayed bone maturation, congenital myopathy, and impaired mental and motor coordination development (106), which was a result of yet another compound heterozygous defect in SBP2. The patient inherited a paternal allele with a R120X nonsense mutation and a maternal allele with another nonsense mutation, R770X. While the allele carrying the R120X mutation could still support expression of the truncated but functional protein (like R128X and Q79X), the R770X substitution yields dysfunctional SBP2 because the truncation occurs in the C-terminal domain critical for binding to SECIS and EFsec. Because only one allele could support synthesis of the somewhat functional SBP2, the phenotype was more severe than in the case of the R128X homozygous mutation. The patient had reduced levels of DIOs and undetectable levels of SelP, which are perhaps responsible for growth defects and neurological abnormalities. Finally, the patient exhibited myopathy similar to that observed in patients harboring mutations in SEPN1 gene suggesting that R770X mutation perhaps affected the expression of selenoprotein N (SeIN).

Perhaps the most complex multisystem disorder caused by mutations in the SBP2 gene was described by Chatterjee et al (107). Two patients had a very complex clinical picture: abnormal thyroid metabolism, low levels of selenium, reduced synthesis of selenoproteins including SelP and GPx3, azoospermia and spermatogenesis failure (infertility), axial muscular dystrophy, myopathy, skin photosensitivity, and abnormal immune cell function. In addition, increased reactive oxygen species production, accelerated telomere shortening, membrane lipid peroxidation and DNA damage were observed at the cellular level. Both patients were compound heterozygous for particular yet different mutations in SBP2. The first patient inherited a paternal allele in which a premature stop codon was introduced in exon 5 as a result of a frameshift mutation. The resulting protein is likely a truncated but functional isoform of SBP2. The maternal allele contained a splicing defect between exons 6 and 7, which presumably yields SBP2 devoid of the functionally important C-terminal domain. The second patient carried a maternally inherited C691R mutation and a paternally inherited splicing defect in SBP2. While the functional protein could not be expressed from the paternal allele, the maternal allele could give rise to a full-length C691R mutant of SBP2. The C691R mutation not only affects the RNA binding of SBP2, but also targeting of this mutant to the proteasome is enhanced thus reducing the overall level of SBP2.

Besides mutations in SBP2, two mutations in the regulatory elements of the selenoprotein mRNAs have been identified to cause disease in humans. It has been shown that the apical helix of the SECIS element of the Sep15 mRNA contains a single nucleotide polymorphism 1125G’A (108). Diamond et al have shown that frequency of this SNP varies with ethnicity and that might contribute to cancer risk (109). However, it is still not clear how this change in the region of SECIS not critical for SBP2 binding might affect sele-
nokprotein synthesis. Further, Guicheney et al have shown for the first time that a single homozygous mutation in the SECIS element causes a disease in humans. In particular, a 17195T>C substitution in the 3′-UTR of the gene encoding the SelN protein causes SEPN1-related myopathy characterized with marked muscle weakness and a significant restrictive respiratory insufficiency (110). The mutation, which affects the 5′ U in the non-Watson-Crick quartet in the core of the SECIS stem, impairs SelN expression in vivo and SBP2 binding to SECIS in vitro. More recently, Howard et al identified a series of mutations (G463V, heterozygous; R469W, homozygous; R469Q, homozygous; R466Q, heterozygous) within the SRE element that cause SEPN1-related myopathies of various severities (92). All patients exhibited congenital muscle weakness, spinal rigidity, scoliosis, and respiratory insufficiency. While most patients underwent spinal fusion and required nasal ventilation, they remained ambulant. Two patients developed severe phenotypes and died at the age of 5.5 and 7 years. The SRE element is immediately adjacent to the UGA codon and it regulates the expression of a subset of selenoproteins including SelN. It has been shown that the mutations affect the secondary structure of SRE, dampen the efficiency of co-translational insertion of selenocysteine, and reduce levels of the SelN mRNA and the SelN protein in muscle.

CONCLUDING REMARKS

Selenocysteine, the 21st amino acid, is absolutely essential for human health and survival. The most prominent members of the human selenoproteome regulate thyroid metabolism, remove reactive oxygen species and protect the cell from oxidative damage, maintain the cellular redox balance and participate in redox reactions, regulate signaling cascades, promote protein folding, and maintain selenium homeostasis. Mutations in selenoproteins and selenium deficiency have long been linked to a variety of human disorders, some of which have been treated by dietary selenium supplements. Synthesis of selenoproteins and the integrity of the selenoproteome, however, depend critically on formation and accurate co-translational incorporation of selenocysteine. In contrast to the 20 standard amino acids, selenocysteine is synthesized from a serine precursor through a series of reactions taking place on its unique tRNA. Moreover, selenocysteine is targeted to the site of translation by a specialized elongation factor, an in-trans protein factor, and an in-cis elements in the selenoprotein mRNA, which coordinate decoding of the selenocysteine UGA codon. Emerging evidence accumulated over the last two decades convincingly argues that the integrity of the selenocysteine-synthetic and decoding apparatus is essential to the development of a normal and healthy human organism (Figure 4). Mutations in the terminal synthetic enzyme, SepSecS, cause extremely detrimental neurological disorders in which the afflicted individuals do not develop past puberty. In addition, mutations in the components of the decoding apparatus (i.e., SBP2, SECIS, SRE) cause disorders of endocrine, muscular, skeletal, and cardiovascular systems. With the exception of two cases, phenotypes of the decoding system are generally milder than the ones linked to the malfunctioning synthetic cycle. However, it remains to be seen how these mutations affect the structure and function of the mutated proteins, enzymes, and regulatory mRNA elements, and if such information could be rationally used in remediating at least some of the disorders. It is reasonable to expect that more disorders linked to selenocysteine cycle will be uncovered, in particular, providing possible insight into neurological disorders of unknown etiology. Also, because of its essentiality for survival of all human cells, in particular, life-threatening cancer cells, the selenocysteine machinery should at least be considered as a potential therapeutic target. Finally, it is important to mention that several protozoan pathogens such as Trypanosoma, Plasmodium, and Leishmania depend on selenium, suggesting that structural and functional studies on the selenocysteine cycle in these organisms could reveal differences that could, in turn, be employed for design of novel therapies. It has become abundantly clear that research centered on selenium and its role in development and maintenance of the human organism will continue to grow in importance as more selenocysteine related pathologies are uncovered. We are just beginning to understand the wide-spread dependence on selenium for a majority of living organisms, in particular the entire animal kingdom.

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References

1 Klaproth MH. Chemical examination of the Transylvanian gold ores, collection of German papers, which were read aloud at the Royal Academy of Sciences in Berlin in the years 1789-1800 [in German].
The importance of selenocysteine for human health

43 Chambers I, Frampton J, Goldfarb P, Affara N, McBain W, Harrison PR. The structure of the mouse glutathione peroxidase: the catalytic site of rat liver glutathione peroxidase as selenocysteine. Hoppe Seylers Z Physiol Chem. 1984;365:195-212. Medline:6714945 doi:10.1515/bchm2.1984.365.1.195

44 Mullenhach GT, Fabrizi A, Irving BD, Bell GI, Tainer JA, Hallewell RA. Selenocysteine’s mechanism of incorporation and evolution revealed in cDNAs of three glutathione peroxidases. Protein Eng. 1988;2:239-46. Medline:2976939 doi:10.1093/protein/2.3.239

45 Zinoni F, Birkmann A, Stadtman TC, Böck A. Selenocysteine synthase from Escherichia coli. Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from Escherichia coli. Proc Natl Acad Sci U S A. 1986;83:4650-4. Medline:2941757 doi:10.1073/pnas.83.13.4650

46 Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehntal O, Guigo R, et al. Characterization of mammalian selenoproteomes. Science. 2003;300:1439-43. Medline:12775843 doi:10.1126/science.1083516

47 Böös MR, Takaku K, Oshima M, Nishimura S, Taketo MM. Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Tsp). Proc Natl Acad Sci U S A. 1997;94:5331-6. Medline:9159106 doi:10.1073/pnas.94.11.5331

48 Leinfelder W, Forchhammer K, Zinoni F, Sawers G, Mandrand-Berthollet MA, Böck A. Escherichia coli genes whose products are involved in selenium metabolism. J Bacteriol. 1988;170:540-6. Medline:2962989

49 Palouš A, Sherr er RL, Steitz TA, Döll D, Simonović M. The human SepSec-tRNAse complex reveals the mechanism of selenocysteine formation. Science. 2003;325:321-5. Medline:19608919 doi:10.1126/science.1173755

50 Itoh Y, Chiba S, Sekine Y, Yokoyama S. Crystal structure of human selenocysteine tRNA. Nucleic Acids Res. 2009;37:6259-68. Medline:19692584 doi:10.1093/nar/gkp648

51 Chiba S, Itoh Y, Sekine Y, Yokoyama S. Structural basis for the major role of O-phosphoseryl-tRNA kinase in the UGA-specific encoding of selenocysteine. Mol Cell. 2010;39:410-20. Medline:20705242 doi:10.1016/j.molcel.2010.07.018

52 Biou V, Yaremchuk A, Tukalo M, Cusack S. The 2.9 Å crystal structure of T. thermophilus seryl-tRNA synthetase complexed with tRNA(Ser). Science. 1994;263:1404-10. Medline:8128220 doi:10.1126/science.8128220

53 Forchhammer K, Leinfelder W, Boesmiller K, Veprek B, Böck A. Selenocysteine synthase from Escherichia coli. Nucleotide sequence of the gene (sela) and purification of the protein. J Biol Chem. 1991;266:6318-23. Medline:2007584

54 Carlson BA, Xu XM, Kryukov GV, Rao M, Berry MJ, Gladyshev VN, et al. Identification and characterization of phosphoseryl-tRNA[Ser]Sec kinase. Proc Natl Acad Sci U S A. 2004;101:12848-53. Medline:15317934 doi:10.1073/pnas.0402636101

55 Sherr er RL, Ho JM, Döll D. Divergence of selenocysteine tRNA recognition by archaeal and eukaryotic O-phosphoseryl-tRNAse kinase. Nucleic Acids Res. 2008;36:1871-80. Medline:18267971 doi:10.1093/nar/gkn036

56 Lee BJ, Worland PJ, Davis JN, Stadtman TC, Hatfield DL. Identification of a selenocysteinyll-tRNA[Ser]Sec in mammalian cells that recognizes the nonsense codon. UGA. J Biol Chem. 1989;264:9724-7. Medline:2498338

57 Yuan J, Palouš A, Salazar JC, Su D, O’Donoghue P, Hohn MJ, et al. RNA-dependent conversion of phosphoserine forms selenocysteine in eukaryotes and archaea. Proc Natl Acad Sci U S A. 2006;103:18923-7. Medline:17142313 doi:10.1073/pnas.0609703104

www.cmj.hr
58 Xu XM, Carlson BA, Mix H, Zhang Y, Saira K, Glass RS, et al. Biosynthesis of selenocysteine on its tRNA in eukaryotes. PLoS Biol. 2007;5:e4. Medline:17194211 doi:10.1371/journal.pbio.0050004
59 Diamond A, Dudock B, Hatfield D. Structure and properties of a bovine liver UGA suppressor serine tRNA with a tryptophan anticodon. Cell. 1981;25:497-506. Medline:6912798 doi:10.1016/0092-8674(81)90068-4
60 Hatfield D, Diamond A, Dudock B. Opal suppressor serine tRNAs from bovine liver form phosphoseryl-tRNA. Proc Natl Acad Sci U S A. 1982;79:6215-9. Medline:6815648 doi:10.1073/pnas.79.20.6215
61 Araso Y, Sherrer RL, Ishitani R, Ho JM, Soll D, Nureki O. Structure of a tRNA-dependent kinase essential for selenocysteine decoding. Proc Natl Acad Sci U S A. 2009;106:16215-20. Medline:19805283 doi:10.1073/pnas.0908861106
62 Wies I, Brunner S, Henninger J, Herkel J, Kanzler S., Meyer zum Buschenfelde KH, et al. Identification of target antigen for SLA/SLP autoantibodies in autoimmune hepatitis. Lancet. 2000;355:1510-5. Medline:10801173 doi:10.1016/S0140-6736(00)02166-8
63 Su D, Hohn MJ, Paliourea S, Sherrer RL, Yuan J, Soll D, et al. How an obscure archaeal gene inspired the discovery of selenocysteine biosynthesis in humans. IUBMB Life. 2009;61:35-9. Medline:18798524 doi:10.1002/iub.136
64 Paliourea S, Herkel J, Simonovic M, Lohse AW, Soll D. Human SsSec or SLa/LP: selenocysteine formation and autoimmune hepatitis. Biol Chem. 2010;391:771-6. Medline:20623998 doi:10.1515/bc.2010.078
65 Xu XM, Carlson BA, Iorns R, Mix H, Zhong N, Gladyshev VN, et al. Selenophosphatase synthetase 2 is essential for selenoprotein biosynthesis. Biochem J. 2007;404:115-20. Medline:17346238 doi:10.1042/bj20070165
66 Esaki N, Nakamura T, Tanaka H, Soda K. Selenocysteine yase, a novel enzyme that specifically acts on selenocysteine. Mammalian distribution and purification and properties of pig liver enzyme. J Biol Chem. 1982;257:4386-91. Medline:6461656
67 Forchhammer K, Leinfelder W, Bock A. Identification of a novel translation factor necessary for the incorporation of selenocysteine into protein. Nature. 1989;342:453-6. Medline:2531290 doi:10.1038/342453a0
68 Rother M, Wilting R, Commans S, Böck A. Identification and characterisation of the selenocysteine-specific translation factor SelB from the archaeon Methanococcus jannaschii. J Mol Biol. 2000;299:351-8. Medline:10860743 doi:10.1006/jmbi.2000.3756
69 Tutejaeva RM, Copeland PR, Xu XM, Carlson BA, Harney JW, Driscoll D, et al. Decoding apparatus for eukaryotic selenocysteine insertion. EMBO Rep. 2000;1:158-63. Medline:11265756 doi:10.1093/embo/re0/2000/033
70 Fagegaltier D, Hubert N, Yamada K, Mizutani T, Carbon P, Krol A. Characterization of mSelB, a novel mammalian elongation factor for selenoprotein translation. EMBO J. 2000;19:4796-805. Medline:10970870 doi:10.1093/emboj/19.17.4796
71 Zimoni F, Heider J, Böck A. Features of the formate dehydrogenase mRNA necessary for decoding of the UGA codon as selenocysteine. Proc Natl Acad Sci U S A. 1990;87:4660-4. Medline:2141170 doi:10.1073/pnas.87.12.4660
72 Leibundgut M, Frick C, Thanbichler M, Böck A, Ban N. Selenocysteine tRNA-specific elongation factor SelB is a structural chimera of elongation and initiation factors. EMBO J. 2005;24:11-22. Medline:15616587 doi:10.1038/sj.emboj.7600505
73 Rother M, Resch A, Wilting R, Böck A. Selenoprotein synthesis in archaea. Biofactors. 2001;14:75-83. Medline:11568443 doi:10.1002/biof.5520014011
74 Wilting R, Schorling S, Persson BC, Böck A. Selenoprotein synthesis in archaea: identification of an mRNA element of Methanococcus jannaschii probably directing selenocysteine insertion. J Mol Biol. 1997;266:637-41. Medline:9102456 doi:10.1006/jmbi.1996.0812
75 Berry MJ, Banu L, Chen YY, Mendel SJ, Kiessner JD, Harney JW, et al. Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3′ untranslated region. Nature. 1991;353:237-6. Medline:1832744 doi:10.1016/S0034-575X(00)00166-2
76 Shen Q, Chu FF, Newburger PE. Sequences in the 3′-untranslated region of the human cellular glutathione peroxidase gene are necessary and sufficient for selenoprotein incorporation at the UGA codon. J Biol Chem. 1993;268:11463-9. Medline:7684384
77 Schchedrina VA, Novoselov SV, Malinowski MY, Gladyshev VN. Identification and characterization of a selenoprotein family containing a diselenide bond in a redox motif. Proc Natl Acad Sci U S A. 2007;104:13919-24. Medline:17715293 doi:10.1073/pnas.0703448104
78 Krol A. Evolutionarily different RNA motifs and RNA-protein complexes to achieve selenoprotein synthesis. Biochimie. 2002;84:765-74. Medline:12457564 doi:10.1016/S0300-9126(02)01405-0
79 Budilman ME, Bubenik JL, Miniard AC, Middleton LM, Gerber CA, Cash A, et al. Eukaryotic initiation factor 4a3 is a selenium-regulated RNA-binding protein that selectively inhibits selenoprotein incorporation. Mol Cell. 2009;35:479-89. Medline:19716792 doi:10.1016/j.molcel.2009.06.026
80 Copeland PR, Driscoll DM. Purification, redox sensitivity, and RNA binding properties of SECIS-binding protein 2, a protein involved in selenoprotein biosynthesis. J Biol Chem. 1999;274:25447-54. Medline:10464275 doi:10.1074/jbc.274.36.25447
81 Copeland PR, Fletcher JE, Carlson BA, Hatfield DL, Driscoll DM. A novel RNA binding protein, SBP2, is required for the translation of mammalian selenoprotein mRNAs. EMBO J. 2000;19:306-14. Medline:10637234 doi:10.1093/emboj/19.2.306
82 Olieric V, Wolff P, Takeuchi A, Bec G, Birk C, Vitorino M, et al. SECIS-binding protein 2, a key player in selenoprotein synthesis, is an intrinsically disordered protein. Biochimie. 2009;91:1003-9. Medline:19467292 doi:10.1016/j.bioch.2009.05.004
83 Donovan J, Copeland PR. Evolutionary history of selenocysteine
The importance of selenocysteine for human health

84 Donovan J, Copeland PR. Selenocysteine insertion sequence binding protein 2L is implicated as a novel post-transcriptional regulator of selenoprotein expression. PLoS ONE. 2012;7:e35581. Medline:22530054 doi:10.1371/journal.pone.0035581

85 Caban K, Kinzy SA, Copeland PR. The L7ae RNA binding motif is a multifunctional domain required for the ribosome-dependent Sec incorporation activity of Sec insertion sequence binding protein 2. Mol Cell Biol. 2007;27:6350-60. Medline:17636016 doi:10.1128/MCB.00632-07

86 Copeland PR, Stepanik VA, Driscoll DM. Insight into mammalian selenocysteine insertion: domain structure and ribosome binding properties of Sec insertion sequence binding protein 2. Mol Cell Biol. 2001;21:1491-8. Medline:11238886 doi:10.1128/MCB.21.5.1491-1498.2001

87 Caban K, Copeland PR. Selenocysteine insertion sequence (SECIS)-binding protein 2 alters conformational dynamics of residues involved in RNA accommodation in 80S ribosomes. J Biol Chem. 2012;287:10664-73. Medline:22992746 doi:10.1074/jbc.M112.415463

88 Donovan J, Caban K, Ranaweera R, Gonzalez-Flores JN, Copeland PR. A novel domain introduces high affinity selenocysteine insertion sequence binding and elongation factor recruitment. J Biol Chem. 2008;283:35129-39. Medline:18948268 doi:10.1074/jbc.M806008200

89 Gonzales-Flores JN, Gupta N, DeMong LW, Copeland PR. The selenocysteine-specific elongation factor contains a novel and multi-functional domain. J Biol Chem. 2012;287:38936-45. Medline:22992746 doi:10.1074/jbc.M112.415463

90 Howard MT, Aggarwal G, Anderson CB, Khatri S, Flanagan KM, Atkins JF. Recoding elements located adjacent to a subset of eukaryal selenocysteine-specifying UGA codons. EMBO J. 2005;24:1596-607. Medline:15791204 doi:10.1038/sj.emboj.7600642

91 Howard MT, Moyle MW, Aggarwal G, Carlson BA, Anderson CB. A recoding element that stimulates decoding of UGA codons by Sec tRNA[Sec]Sec. RNA. 2007;13:912-20. Medline:17456565 doi:10.1261/rna.473907

92 Maiti B, Arbogast S, Allamand V, Moyle MW, Anderson CB, Richard P, et al. A mutation in the SEPN1 selenocysteine redefinition element (SRE) reduces selenocysteine incorporation and leads to SEPN1-related myopathy. Hum Mutat. 2009;30:411-6. Medline:19607361 doi:10.1002/humu.20879

93 Rayman MP. Selenium and human health. Lancet. 2012;379:1256-68. Medline:22381456 doi:10.1016/S0140-6736(11)61452-9

94 Agamy O, Ben Zeew B, Lev D, Marcus B, Fine D, Su D, et al. Mutations disrupting selenoprotein formation cause progressive cerebello-cerebral atrophy. Am J Hum Genet. 2010;87:538-44. Medline:20920667 doi:10.1016/j.ajhg.2010.09.007

95 Hill KE, Zhou J, McMahan WJ, Motley AK, Atkins JF, Gesteland RF, et al. Deletion of selenoprotein P alters distribution of selenium in the mouse. J Biol Chem. 2003;278:13640-6. Medline:12574155 doi:10.1074/jbc.M300075200

96 Burk RF, Hill KE, Olson GE, Weeber EJ, Motley AK, Winfrey VP, et al. Deletion of apolipoprotein E receptor-2 in mice lowers brain selenium and causes severe neurological dysfunction and death when a low-selenium diet is fed. J Neurosci. 2007;27:6207-11. Medline:17553992 doi:10.1523/JNEUROSCI.1153-07.2007

97 Hill KE, Zhou J, McMahan WJ, Motley AK, Burk RF. Neurological dysfunction occurs in mice with targeted deletion of the selenoprotein P gene. J Nutr. 2004;134:157-61. Medline:14704310

98 Valentine WM, Abel TW, Hill KE, Austin LM, Burk RF. Neurodegeneration in mice resulting from loss of functional selenoprotein P or its receptor apolipoprotein E receptor 2. J Neuropathol Exp Neurol. 2008;67:68-77. Medline:18172410 doi:10.1097/NEN.0b013e31816f0347

99 Wirth EK, Conrad M, Winterer J, Wozny C, Carlson BA, Roth S, et al. Neuronal selenoprotein expression is required for interneuron development and prevents seizures and neurodegeneration. FASEB J. 2010;24:844-52. Medline:19890015 doi:10.1096/fj.09-143974

100 Dumitrescu AM, Liao XY, Abdullah MS, Lado-Abel J, Majed FA, Moeller LC, et al. Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. Nat Genet. 2005;37:1247-52. Medline:16228000 doi:10.1038/ng1654

101 Bianco AC, Larsen PR. Cellular and structural biology of the deiodinases. Thyroid. 2005;15:777-86. Medline:16131321 doi:10.1089/thy.2005.15.777

102 Bubenik JL, Driscoll DM. Altered RNA binding activity underlies abnormal thyroid hormone metabolism linked to a mutation in selenocysteine insertion sequence-binding protein 2. J Biol Chem. 2007;282:34653-62. Medline:17901054 doi:10.1074/jbc.M806008200

103 Squires JE, Stoytchev I, Forry EP, Berry MJ. SBP2 binding affinity is a major determinant in differential selenoprotein mRNA translation and sensitivity to nonsense-mediated decay. Mol Cell Biol. 2007;27:7848-55. Medline:17846120 doi:10.1128/MCB.00793-07

104 Di Cosmo C, McLellan N, Liao XH, Khanna KK, Weiss RE, Papp L, et al. Clinical and molecular characterization of a novel selenocysteine insertion sequence-binding protein 2 (SBP2) gene mutation (R128X). J Clin Endocrinol Metab. 2009;94:4003-9. Medline:19602558 doi:10.1210/jc.2009-0686

105 Hamajima T, Mushimoto Y, Kobayashi H, Saito Y, Onigata K. Novel compound heterozygous mutations in the SBP2 gene: characteristic clinical manifestations and the implications of GH and triiodothyronine in longitudinal bone growth and maturation. Eur J Endocrinol. 2012;166:757-64. Medline:2247018 doi:10.1530/EJE-11-0812

106 Azevedo MF, Barra GB, Naves LA, Ribeiro Velasco LF, Godoy Garcia
Castro P, de Castro LC, et al. Selenoprotein-related disease in a young girl caused by nonsense mutations in the SBP2 gene. J Clin Endocrinol Metab. 2010;95:4066-71. Medline:20501692 doi:10.1210/jc.2009-2611

107 Schoenmakers E, Agostini M, Mitchell C, Schoenmakers N, Papp L, Rajanayagam O, et al. Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. J Clin Invest. 2010;120:4220-35. Medline:21084748 doi:10.1172/JCI43653

108 Gladyshev VN, Jeang KT, Wootton JC, Hatfield DL. A new human selenium-containing protein. Purification, characterization, and cDNA sequence. J Biol Chem. 1998;273:8910-5. Medline:9535873 doi:10.1074/jbc.273.15.8910

109 Hu YJ, Korotkov KV, Mehta R, Hatfield DL, Rotimi CN, Luke A, et al. Distribution and functional consequences of nucleotide polymorphisms in the 3'-untranslated region of the human Sep15 gene. Cancer Res. 2001;61:2307-10. Medline:11280803

110 Allamand V, Richard P, Lescure A, Ledeuil C, Desjardin D, Petit N, et al. A single homozygous point mutation in a 3'-untranslated region motif of selenoprotein N mRNA causes SEP15-related myopathy. EMBO Rep. 2006;7:450-4. Medline:16498447