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Selenium. Role of the Essential Metalloid in Health

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Abstract

Selenium is an essential micronutrient in mammals, but is also recognized as toxic in excess. It is a non-metal with properties that are intermediate between the chalcogen elements sulfur and tellurium. Selenium exerts its biological functions through selenoproteins. Selenoproteins contain selenium in the form of the 21st amino acid, selenocysteine (Sec), which is an analog of cysteine with the sulfur-containing side chain replaced by a Se-containing side chain. Sec is encoded by the codon UGA, which is one of three termination codons for mRNA translation in non-selenoprotein genes. Recognition of the UGA codon as a Sec insertion site instead of stop requires a Sec insertion sequence (SECIS) element in selenoprotein mRNAs and a unique selenocysteyl-tRNA, both of which are recognized by specialized protein factors. Unlike the 20 standard amino acids, Sec is biosynthesized from serine on its tRNA. Twenty-five selenoproteins are encoded in the human genome. Most of the selenoprotein genes were discovered by bioinformatics approaches, searching for SECIS elements downstream of in-frame UGA codons. Sec has been described as having stronger nucleophilic and electrophilic properties than cysteine, and Sec is present in the catalytic site of all selenoenzymes. Most selenoproteins, whose functions are known, are involved in redox systems and signaling pathways. However, several selenoproteins are not well characterized in terms of their function. The selenium field has grown dramatically in the last few decades, and research on selenium biology is providing extensive new information regarding its importance for human health.

Keywords

essential nutrient; selenium; selenocysteine; selenoprotein

1 Introduction

1.1 History of Selenium

Selenium is a non-metal element, but sometimes considered as a metalloid, with the symbol Se and atomic number 34. It was first described in 1818 by the Swedish chemist Jöns Jacob Berzelius (1779–1848). He named this element selenium (Greek σελήνη selene meaning Moon) after the Greek moon goddess Selene (reviewed in [1]). Berzelius was investigating the cause of illness among workers at a sulfuric acid manufacturing plant when he found the

element in the bottom sludge of a sulfuric acid preparation. He reported that selenium had similarities with the previously known element tellurium (named for the Earth). He also reported close similarities between selenium and sulfur. At that time, selenium was thought to be a toxic element.

1.2 Identification of Selenium as an Essential Micronutrient

Most of the early research on selenium was done with the goal of addressing selenium toxicity. In the 1930s, selenium was found to cause poisoning of livestock in areas with high selenium content in the soil. In the mid 20th century, selenium was recognized as a micronutrient and its biological function was studied with regard to its importance in human nutrition. In 1957, Klaus Schwarz, a German scientist working at the National Institutes of Health in Bethesda first reported on the health benefits of selenium. Schwarz had studied yeast as a protein source in Germany during World War II and continued the study in the United States, eventually discovering that feeding torula yeast instead of brewer's yeast as a protein source to vitamin E-deficient rats led to necrotic liver formation. Schwarz and Foltz announced that the selenium contained in the fractionated brewer's yeast was responsible for preventing liver necrosis [2]. Selenium deficiency was also recognized in studies in Oregon [3], which showed a myopathy known as 'white muscle disease' in calves and lambs to be associated with selenium-depleted soil. Selenium supplementation to livestock has subsequently had great economic impacts in several countries, including New Zealand and Finland.

In 1979 in China, a congestive cardiac myopathy termed Keshan disease was the first reported human disease associated with selenium deficiency [4]. Keshan County in northeastern China, for which the disease was named, is a predominantly rural region where the diet consisted almost entirely of food produced locally on selenium-deficient land. The disease was also reported in New Zealand and Finland where the level of selenium in the soil is low [5]. Further details are discussed in Section 4.1.

In the 1970s selenium was found to be present in glutathione peroxidase as the amino acid selenocysteine (Sec) [6], and the focus on selenium studies shifted to the field of molecular biology. As a micronutrient, a recommended dietary allowance (RDA) for selenium was established in 1989 (70 µg/d for men and 55 µg/d for women) [7] and revised in 2000 (55 µg/d) [8]. In 1996, dietary recommendations from the World Health Organization (WHO) were issued (34 µg/d for men and 26 µg/d for women) [9].

2 Selenium in Biomolecules

2.1 Selenocysteine, the 21st Amino Acid in Proteins

Selenoproteins are defined as proteins containing the 21st amino acid, Sec [10]. The discovery of selenoproteins occurred in 1973, when Hoekstra and coworkers at the University of Wisconsin identified the presence of selenium in glutathione peroxidase as the first animal selenoprotein [6]. Research then focused on the catalytic role of the amino acid in the active site of selenoproteins. In 1976, Thressa Stadtman et al. identified glycine reductase as a selenoprotein [11] and in the 1980s, Böck and colleagues identified additional selenoproteins in bacteria [12]. The application of bioinformatics and SECIS specific

algorithms allowed for identification of selenoprotein genes from the expressed sequence tags (ESTs) database [13,14]. All of the 25 selenoprotein genes in humans were thus identified in 2003 [15].

Sec (Figure 1, left) contains a selenol group which is highly reactive at physiological pH. The reactivity of the thiol group (pK_a 8.3) of cysteine is modulated by microenvironmental conditions, and when deprotonated, is nucleophilic and easily oxidized. Since the selenol group of Sec has a lower pK_a (5.47), it is fully deprotonated. Due to the higher reduction potential of Sec, it is more efficient in participating in redox reactions, and is specifically used as a catalytic amino acid in most selenoproteins.

The other selenium-containing amino acid is selenomethionine (Figure 1, right). Like methionine, selenomethionine is not synthesized *de novo* in humans, but is supplied from plants. Selenium is misincorporated at random in place of sulfur in methionine biosynthesis, followed by the ribosome failing to distinguish between selenomethionine- and methionine-loaded tRNA during translation [16]. Since its selenium is covalently bound between two carbon atoms, selenomethionine is considerably less reactive than Sec.

2.2 Selenocysteine tRNA and Biosynthesis of Selenocysteine

Sec is a naturally occurring amino acid in eukaryotes, archaea, and eubacteria. Sec is cotranslationally incorporated into selenoproteins by Sec tRNA decoding of UGA, which is normally a termination codon [10,17,18]. In 1970, a seryl-tRNA was identified that specifically decoded the stop codon, UGA [19]. In assessing whether nonsense suppressor tRNAs occurred in mammalian cells, the minor seryl-tRNA was identified as a possible candidate. Extensive characterization of this tRNA subsequently revealed it to be Sec tRNA^[Ser] (reviewed in [20]). Unlike the other 20 amino acids, the biosynthesis of Sec occurs on its transfer RNA (tRNA^{[Ser]Sec}) [21]. The biosynthesis of Sec was first characterized in bacteria [22] by Böck and coworkers in the late 1980s to early 1990s [23,24], and subsequently in mammalian cells [21]. In the first step, tRNA^{[Ser]Sec} is aminoacylated with serine, providing the carbon backbone for Sec, thus the tRNA has been designated as tRNA^{[Ser]Sec} [21,25]. The pathway for mammalian Sec biosynthesis has been elucidated more recently, and is discussed below.

Sec tRNA^{[Ser]Sec} has been isolated and sequenced from bovine liver [19,26,27], rat liver [28], mouse liver, and HeLa cells [29]. tRNA^{[Ser]Sec} is the longest tRNA in mammals with a length of 90 nucleotides [26,27,30], and contains several modified bases. It was the first mammalian tRNA shown to contain mcm⁵U34 and mcm⁵Um34 [28] (Figure 2). Full expression of selenoproteins requires modification of tRNA^{[Ser]Sec} [31]. Interestingly, two major isoforms of tRNA^{[Ser]Sec} differ by a single methyl group at the wobble position (Um34) and synthesize different subclasses of selenoproteins [20]. The non-Um34 isoform supports the synthesis of a subclass of selenoproteins, designated housekeeping, while the Um34 isoform supports the expression of another subclass, designated stress-related selenoproteins [32]. The modification of i⁶A37 is also required for stress-related selenoproteins [33]. Um34 methylation of tRNA^{[Ser]Sec} requires aminoacylated tRNA^{[Ser]Sec}, most likely with Sec [34]. Recently, it was shown that N6-isopentenylolation of base A37 is catalyzed by Trit1, a dimethylallyl:tRNA^{[Ser]Sec}-transferase [35].

In the first step of Sec biosynthesis, seryl-tRNA synthetase (SerRS) attaches serine to Sec tRNA in the presence of ATP and Mg^{2+} as follows [36–38]:

In 1970, a kinase that phosphorylated what was presumed to be a minor species of seryl-tRNA to form O-phosphoseryl-tRNA was reported. Because it recognized the stop codon UGA, it was initially thought to be a suppressor tRNA [19,39]. This seryl-tRNA^{UGA} was eventually identified as Sec tRNA^{[Ser]Sec}, and the kinase originally found by Mäenpää and Bernfield was shown to be O-phosphoseryl-tRNA^{[Ser]Sec} kinase (PSTK) [40]. PSTK was identified using a comparative genomics approach that searched completely sequenced genomes of archaea for a kinase-like protein that was present in those organisms (*Methanococcus jannaschii* and *Methanopyrus kandleri*) that utilized the selenoprotein synthesizing machinery and was absent in those that did not. Two kinase-like genes were identified in the two archaea that synthesize selenoproteins. However, they were not found in the other twelve archaea which lack that ability. Further comparison was carried out in eukaryotic genomes that synthesize selenoproteins (nematodes, *Drosophila*, and mice) and those that do not (yeasts) for homologous sequences to the two candidate genes. A single candidate was detected and the putative *pstk* was cloned from mouse genomic DNA [40]. The protein's biochemical properties showed it to be phosphoseryl-tRNA kinase (PSTK). The reaction is as follows:

The selenium donor is monoselenophosphate, which is formed from selenite and ATP by selenophosphate synthetase (SPS). SPS has two homologs, SPS1 and SPS2 [41,42]. The gene product of SPS2 is a selenoprotein [42]. Biochemical analysis demonstrated that mouse SPS2 generates selenophosphate in the presence of selenide and ATP [43]. The reaction is as follows:

Sec synthetase (SecS) then mediates the generation of selenocysteyl-tRNA^{[Ser]Sec} and inorganic phosphate. Hatfield's group identified SecS using a computational and comparative genomic strategy, similar to that used to identify *pstk*, in searching for a *SecS* gene in eukaryotes [44]. The sequence of the purported mammalian SecS matched a 48 kDa soluble liver antigen (SLA) [45], which was previously reported as an antigen in patients with autoimmune chronic hepatitis and co-precipitated with Sec-tRNA^{[Ser]Sec} in extracts from such patients. SecS binds tightly with phosphoseryl-tRNA^{[Ser]Sec} [46]. The reaction is as follows (Figure 3):

Recently, it was shown that cysteine can be synthesized on the tRNA^{[Ser]Sec} by replacing selenide with sulfide in the Sec biosynthetic pathway [47] (*de novo* cysteine biosynthesis is discussed in Section 3.1.6).

2.3 Selenoprotein mRNAs

The specific, cotranslational incorporation of Sec into eukaryotic proteins requires the presence of a SECIS element within the selenoprotein mRNA. The SECIS element was identified through bioinformatic and functional studies of the selenoprotein genes encoding cytosolic glutathione peroxidase (Gpx1) and type 1 deiodinase (DII) [48,49]. Deletion analyses of the respective 3'UTRs resulted in production of truncated selenoproteins where the Sec UGA codon was recognized as a stop codon. Analysis of the DII and Gpx1 cDNAs from multiple species revealed the consensus SECIS element to be a conserved stem-loop structure containing three short, highly conserved nucleotide sequences [48].

Typical SECIS elements are depicted in Figure 4 and are based on an experimental secondary structure model [50]. Alignment of selenoprotein 3'-UTRs revealed the consensus sequences of AUGA, AAA, and UGAU as the SECIS core [48]. The core (quartet non-Watson-Crick base pairs; two shared tandem G•A, A•G motif) is a conserved feature of SECIS elements which introduces a kink-turn like structure [50]. This structure is recognized by SECIS binding protein 2 (SBP2) and plays a key role for SECIS function [51]. For example, mutation of the SECIS core of selenoprotein N (AUGA to ACGA) causes myopathy [52]. The secondary structure of the SECIS element shows conserved AA residues in an apical loop or internal bulge [53]. This motif is essential and is recognized by the RNA binding protein nucleolin [54] (SECIS binding proteins are further discussed in Section 2.4).

The sequences flanking the SECIS element need to be open and non-base paired. The stem length of the SECIS is between 9 to 15 Watson-Crick base pairs, a distance which approximates a single turn of an A-form double helix. Interestingly, human selenoprotein P (Sepp1), which has 10 UGA Sec codons, has two distinct SECIS elements, both necessary for synthesis of the full-length protein. The 5' element has an extended apical loop (SECIS1, form 2) and the 3' element has the basic stem-loop structure (SECIS2, form 1) [55]. These Sepp1-SECISs have distinct functions, SECIS1 being more efficient than SECIS2 [56]. SECIS elements have been utilized to develop software-based search algorithms in an attempt to identify potential selenoprotein cDNAs in the GenBank [13]. The algorithms search databases for the primary sequence and secondary structure features of SECIS elements, calculating the free energy of the secondary structures. This strategy was initially used to identify several selenoproteins (SelT and SelR [13]) (SelX, SelN, and SelZ [14]) and the SECIS elements activity was validated by incorporation of Sec into Gpx1 protein [57]. A more sophisticated approach was subsequently developed, searching for SECIS elements, conservation of open reading frames downstream of UGA codons between species (which identified SelH, SelI, SelK, SelO, SelS, and SelV), and conservation between Sec and Cys-containing orthologs (Gpx6) [15]. These novel proteins indeed contain selenium, which was verified by subsequent radioactive ⁷⁵Se labeling analysis.

2.4 Selenocysteine Incorporation into Proteins

SECIS element and RNA binding protein interaction is required for decoding of UGA codons as Sec. SECIS binding protein-2 was identified by Copeland et al. as a 94 kDa protein that specifically recognizes the AUGA core of SECIS elements [51,58]. SBP2 works as a factor for the recoding of an in-frame UGA as Sec, activity uncovered by an *in vitro* translation system in rabbit reticulocyte lysate. SBP2 contains an RNA binding domain found in several ribosomal proteins and eukaryotic translation termination release factor 1.

Sec incorporation requires the eukaryotic selenocysteyl-tRNA-specific elongation factor (eEFSec). eEFSec was identified using searches of the murine and human EST databases for homology to a putative archaeal alternative translational elongation factor SELB sequence [59,60]. eEFSec reveals a high degree of conservation in the amino-terminal elongation factor domain and purified recombinant eEFSec protein specifically binds to selenocysteyl-tRNA but not seryl-tRNA [59]. This discrimination between correctly charged selenocysteyl-tRNA and seryl-tRNA reveals the mechanism for preventing misincorporation of serine at UGA codons instead of Sec.

eEFSec has been shown to interact with SBP2 in an RNA-dependent manner [59]. Electrophoretic mobility shift assays (EMSA) with *in vitro* transcribed ³²P-labeled SECIS elements and recombinant purified proteins showed SBP2 and eEFSec bind the SECIS element independently. In contrast, co-immunoprecipitation studies of the mutant SECIS elements with eEFSec and SBP2 revealed a lack of ability to bind the SECIS element. This result suggests eEFSec has the ability to bind SECIS elements and SBP2 enhances its binding specificity.

L30 is a component of the large ribosomal subunit that binds to a specific sequence in 28S rRNA. It is a ubiquitously expressed abundant protein in mammalian tissues, and it has been demonstrated to overlap with SBP2 in binding to SECIS elements [61]. *In vitro* binding studies demonstrated that L30 binds to the SECIS element and forms a kinked conformer to facilitate SBP2 binding since SBP2 only binds to the kinked SECIS form.

Eukaryotic initiation factor 4A-III (eIF4a3) has been identified as a selenium status sensitive RNA-binding protein [62]. eIF4a3 is ubiquitously expressed and belongs to the DEAD box family of RNA-dependent ATPases [63].

Nucleolin, a protein that facilitates ribosome biogenesis in the nucleolus, has been demonstrated to exhibit SECIS element binding [64]. This protein alters mRNA stability or translation in the cytoplasm of specific selenoprotein mRNAs. It binds the upper part of the basal stem of SECIS elements but the consensus sequence for nucleolin binding in SECIS elements has not been identified [54]. siRNA knockdown of nucleolin demonstrated synthesis of essential selenoproteins was reduced but without effect on non-essential selenoprotein synthesis [54]. It is postulated that nucleolin may bind to the essential selenoproteins' SECIS element and facilitate SECIS element-protein interactions with SBP2 or other factors in the Sec incorporation pathway (Figure 5).

Supramolecular protein-protein and protein-RNA interactions are implicated in both the biosynthesis of selenocysteyl-tRNA and the incorporation of Sec [65]. Co-immunoprecipitation studies described the following interactions that have been characterized to date.

Protein:RNA interactions—SecS-selenocysteyl-tRNA

Secp43- selenocysteyl-tRNA

eEFsec- selenocysteyl-tRNA

SBP2-SECIS mRNA and rRNA

L30- SECIS mRNA and rRNA

Protein:protein interactions—SBP2-eEFSec

SecS-SPS1

SecS-Secp43

As several selenoprotein biosynthesis factors have both nuclear localization signals and nuclear export signals, this suggests that these factors may shuttle between the cytoplasm and the nucleus. SPS1, Secp43, and eEFSec were observed in both the cytoplasmic and nuclear fractions [65]. In summary, the co-immunoprecipitation studies suggest that SPS1, SecS, Secp43, and selenocysteyl-tRNA may form a complex in the cytoplasm that subsequently enters the nucleus. SecS may then leave the complex, replaced by eEFSec and SBP2 and subsequent shuttling to the cytoplasm.

3 Function of Selenoproteins

Twenty-five selenoproteins have been identified in human genome databases [15]. Comparative genomic analyses of selenoproteins provide insights into the biological functions of selenium. Figure 6 summarizes the human selenoprotein families.

3.1 Human Selenoproteins

3.1.1 Glutathione Peroxidases (Gpx1, Gpx2, Gpx3, and Gpx6)—In the early 1970s, glutathione peroxidase was identified as the first true selenoprotein [6]. The Gpx family has 8 known homologous proteins (Gpx1–Gpx8) and in humans, Gpx1, Gpx2, Gpx3, Gpx4, and Gpx6 are Sec-containing. Gpxs break down hydroperoxides in a reduced glutathione (GSH)-dependent reaction. The selenolate (R-Se-) in Gpx is oxidized by hydroperoxide and forms seleninic acid (R-SeOH). The seleninic acid reacts with a GSH to form the GS-Se-R. A second GSH reduces the GS-Se-R intermediate back to R-SeH and releases GSSG and water as the by-product. The reaction is a ping-pong mechanism:

Gpx kinetics are identical for Gpx1, Gpx3, and Gpx4. The oxidizing substrates for Gpx2 have not been identified. Gpx1 is expressed in all cells and is more abundant in liver and kidney. It is a homotetramer and localizes to cytosol and mitochondria, reacting with hydrogen peroxide and soluble low molecular mass hydroperoxides such as *t*-butyl hydroperoxide, cumene hydroperoxide, hydroperoxy fatty acids and hydroperoxy lysophosphatides. In tissues, which do not express GSH synthetase, Gpx1 can use γ -glutamylcysteine as a reductant for H_2O_2 . Gpx1 knockout mice show increased susceptibility of liver and lung to ROS [66,67].

Gpx2 is expressed in the gastrointestinal epithelium [68]. It is a homotetramer and localizes to the cytosol. Gpx2 is upregulated in epithelium-derived tumors which include colon adenocarcinoma [69–72], Barrett's esophagus [73], squamous cell carcinoma [74], and lung adenocarcinomas of smokers [75]. It is suggested to act as a barrier against absorption of food-born hydroperoxides. Gpx2-knockout mice display an increased rate of spontaneous apoptosis at crypt bases, an effect that is stimulated under restricted selenium supply [68,76]. Since selenium supplementation partially prevented the spontaneous apoptosis in crypt bases, Gpx1 might be compensating the Gpx2 depletion. Gpx1/Gpx2 double-knockout mice spontaneously develop colitis and intestinal cancer, a model now used to study spontaneous inflammatory bowel disease and predisposition to intestinal cancer [77].

Gpx3 is a tetramer, primarily synthesized in kidney proximal tubule cells and secreted into plasma [78,79]. Gpx3 produced by the kidney binds to renal proximal tubules, basement membranes of epithelial cells throughout the intestine, the epididymis, bronchi, and lung type II pneumocytes [80,81]. The epididymis synthesizes Gpx3 and releases it into its lumen [81]. Gpx3 knockout mice did not show an obvious phenotype. Since the apparent K_m of Gpx3 for GSH (5.3 mM) [82] is significantly higher than plasma GSH concentrations (<0.5 μM) [83], Gpx3's enzymatic function is unknown. Down-regulation of Gpx3 is observed in many types of cancer and hypermethylation of the Gpx3 promoter is detected in patients with Barrett's esophagus cancer [84] and prostate cancer [85]. Gpx3 has been suggested to be a novel tumor suppressor.

Gpx4 has 3 isoforms (cytosolic, sperm nuclear, and mitochondrial forms), which catalyze reduction of lipid peroxides and cholesterol ester hydroperoxides inside cellular membranes. Gpx4 is a monomer protein [86] that reacts with GSH and can use protein thiols instead of GSH when GSH is limiting. This has been shown for Gpx4 reactivity with sperm mitochondria-associated cysteine rich protein and also for Gpx4 against itself [87]. The mitochondrial form of Gpx4 has vital function in the sperm midpiece serving a structural role during spermatogenesis [87]. While total Gpx4 knockout is lethal [88,89], mitochondrial Gpx4-knockout mice developed normally [90]. Male mitochondrial Gpx4 mice are infertile since Gpx4 is necessary for sperm as an inactive structural protein. Mice in

which the nuclear form of Gpx4 was knocked out were not only viable but also fully fertile [91].

Gpx6 is closely related to Gpx3, and is a homodimer selenoprotein only in humans [15].

3.1.2 Thyroid Hormone Deiodinases (DI1, DI2, and DI3)—The iodothyronine deiodinase family has three homologues (DI1, DI2, and DI3) in mammals. DIs catalyze reductive deiodination of thyroid hormones, regulating their activity. T3 enters the nucleus and binds to thyroid hormone receptors which bind T3-responsive genes and regulate their transcription [92]. Thyroxine (T4) is predominantly produced in the thyroid gland but its affinity for the thyroid hormone receptors is about tenfold lower than T3 [93]. DI1 and DI2 are involved in activation of the thyroid hormone by outer ring deiodination of T4 to produce T3 [94]. DIs are homodimeric thioredoxin-like fold membrane-spanning proteins. DI1 and DI3 are found in the plasma membrane, while DI2 is present in endoplasmic reticulum (ER) [95–97]. DI1 is expressed at highest levels in liver and kidney, and produces most of the circulating T3 [98] while DI2 is most abundant in thyroid, heart, skeletal muscle, brown adipose tissue, and the central nervous system. DI2 on the ER may preferentially supply T3 to the nucleus.

Interestingly, human DI2 has an active site Sec and a second Sec 7 amino acids from the C-terminus. The second Sec and the remaining seven C-terminal amino acids are not critical for DI2 enzyme activity and the function of the C-terminal region is unknown [99]. DI2 is expressed in the brown adipose tissue (BAT) and its activity increases in BAT during cold stress, resulting in increased intracellular T3 levels [100–102]. DI2-knockout mice exhibit a mild phenotype; they are unable to control normal body temperature following cold exposure and also show bone development defects [103,104]. Further analysis of DI2-knockout mice on a high fat diet showed a tendency of weight gain and development of insulin resistance [105]. On the other hand, DI3 inactivates T3 and T4 to biologically inactive T2 or reverse T3 (rT3) by preferentially removing the iodine from the inner ring of the molecule. DI3 is found in fetal tissue and placenta and is thought to function in protecting tissues from premature exposure to T3 [106].

3.1.3 Thioredoxin Reductases (TR1, TR2, and TR3)—The thioredoxin reductase (TR) family has three selenoprotein homologues (TR1, TR2, and TR3). TRs reduce oxidized thioredoxin (thioredoxin-S₂) with NADPH as a cofactor. A C-terminal conserved motif (-GCUG) contains Sec, which is crucial for the enzyme activity. Reduced thioredoxin is reoxidized by disulfides in proteins generating thiols.

TRs also have broad substrate specificity, being able to use hydrogen peroxide, selenite, lipoic acid, ascorbate, and ubiquinone [107] as substrate. TR1 is localized in the cytosol. TR2, known as thioredoxin/glutathione reductase (TGR) [108], has the function of formation/isomerization of disulfide bonds during sperm maturation [109]. TR3 is a mitochondrial protein. Knockout of either TR1 or TR3 in mice is fatal [110,111]. Knockout

of the TR1 gene results in early embryonic death at day 6.5 (E6.5). TR3-knockout mice develop exencephaly and die during midgestation (E10.5). Thus both genes are indispensable for mouse embryo development.

3.1.4 Methionine-R-Sulfoxide Reductase (MsrB1)—Methionine, a sulfur containing amino acid, is highly susceptible to accumulated reactive oxygen species. Since sulfur of methionine is a prochiral atom, oxidized methionine generates two diastereomers, methionine-S-sulfoxide and methionine-R-sulfoxide. Methionine sulfoxide reductases (Msrs) reduce oxidized methionine residues in proteins and free methionine sulfoxides. Msrs have two stereospecific families, MsrA (reduces S-form of methionine sulfoxide) and MsrB (reduces R-form of methionine sulfoxide) [112]. Oxidized methionine may lead to conformational changes of proteins, e.g., ribosomal protein L12 and calmodulin are impaired by oxidation of methionines and restored by MsrA. On the other hand, methionine oxidation of calcium/calmodulin-dependent protein kinase II in the absence of calcium activates the protein. MsrB1 was identified through bioinformatics and initially designated as selenoprotein X but was subsequently renamed selenoprotein R [13,14]. MsrB1 efficiently acts on methionine sulfoxide in protein but it has very low activity on the free form of methionine sulfoxide. MsrB1 is a zinc-containing protein, primarily synthesized in liver and kidney, and localizes in the cytosol and nucleus. Its activity is the highest of the Msrs because of the presence of Sec in its active site. MsrB1 expression and activity is highly dependent on selenium status, and selenium deficiency decreases MsrB1 activity. Although MsrB1 affects redox regulation in liver and kidney, knockout of the gene in mice showed that MsrB1 is not an essential selenoprotein for development.

3.1.5 15kDa Selenoprotein (Sep15)—Sep15 has an ER signal peptide and localizes in the ER [113]. The C-terminal domain has a thioredoxin-like fold [114,115]. Sep15 has been suggested to take part in the process of rearrangement of disulfide bonds or reduction of incorrectly formed disulfide bonds in misfolded glycoproteins bound to UDP-glucose:glycoprotein glucosyltransferase [114,116,117]. There are several studies suggesting an association of Sep15 with cancer, but reports are contradictory as to whether it promotes or restricts cancer growth. Earlier studies had shown that Sep15 expression was reduced substantially in a malignant prostate cell line and in hepatocarcinoma [115]. An increase of Sep15 expression in colon cancer has been found [118], and targeted down-regulation of Sep15 inhibited growth of colon cancer cells [119]. Additionally, Sep15 knockout mice form significantly fewer carcinogen-induced aberrant crypt foci in colonic epithelia *in vivo* compared to controls [120]. Thus, the tumor-suppressor activity or oncogenic activity of Sep15 may be tissue-dependent.

3.1.6 Selenophosphate Synthetase 2 (SPS2)—SPS2 is a factor for selenoprotein biosynthesis, with an enzymatic activity to synthesize selenophosphate from ATP and selenite [44]. SPS2 could serve as an autoregulator of selenoprotein synthesis because it is a selenoprotein [42]. Sodium sulfide is also a substrate for SPS2 and generates thiophosphate that would be used as an active sulfur donor in making Cys attached to tRNA^{[Ser]Sec}. Mass spectrometry (MS) analysis of purified TR1 and TR3 from livers of mice that had been fed selenium-deficient (0 ppm selenium), or selenium-adequate (0.1 ppm selenium) diets

showed Cys in place of Sec [47]. Cys was not detected in these selenoproteins on a selenium-enriched (2.0 ppm selenium) diet. Cysteine insertion instead of Sec occurs by sulfide competing with selenide in generating the active donor catalyzed by SPS2.

3.1.7 Selenoprotein P (Sepp1)—Sepp1 is synthesized primarily in the liver and secreted into the plasma. Sepp1 is the only selenoprotein with multiple Sec residues. Human Sepp1 has 10 Sec residues [55]. The N-terminal domain contains a Sec residue in a thioredoxin-like motif and the C-terminal domain contains nine Sec residues in human Sepp1. Four isoforms of Sepp1 have been identified; the shortest isoform terminates at the second UGA, and has been verified as encoding Sec by mass spectrometry of this isoform purified from rat plasma [121]. Sepp1 is a secreted, heparin-binding glycoprotein. Two histidine-rich domains separate the N-terminal and C-terminal regions. Sepp1 delivers selenium to organs where apolipoprotein E receptor 2 or megalin are expressed [122–124]. More details on selenium transport via Sepp1 receptor mechanisms are provided in Section 5.2.

3.1.8 Selenoprotein W (SelW)—SelW was identified in 1993 as a 6 kDa protein, the smallest selenoprotein in mammals. SelW is primarily expressed in muscle, where its absence was notable in muscle of Se-deficient sheep. The expression level of SelW in vertebrates is highly sensitive to dietary Se intake. SelW has a thioredoxin-like fold structure and a Sec-containing redox center located in an exposed loop [125]. SelW was first identified in Se-deficient sheep [126]. SelW contains a CXXU redox motif (where C is Cys, X is any amino acid, and U is Sec) that is conserved among various mammalian species. SelW may be involved in oxidative metabolic pathways and functions as an antioxidant protein [127,128]. SelW was the first selenoprotein to be linked to muscular disorders [125].

3.1.9 Selenoprotein V (SelV)—SelV is a homologue of SelW with additional N-terminal sequence and unknown function. SelV is primarily expressed in testis [15].

3.1.10 Selenoprotein T (SelT)—SelT is highly expressed in kidney, brain, heart, thymus, and testis [129]. SelT has a thioredoxin-like fold and belongs to a new redox protein family. SelT is likely an ER resident protein and the thioredoxin fold domain is exposed to the ER or cytosol. Recently, the role of SelT in regulation of calcium homeostasis and neuroendocrine secretion in response to a pituitary adenylate cyclase-activating polypeptide was demonstrated [130].

3.1.11 Selenoprotein M (SelM)—SelM is a homologue of Sep15 and has an ER signal [15]. SelM contains a thioredoxin fold motif and is abundantly expressed in the brain [116]. SelM knockdown experiments in cell culture revealed a role for SelM in calcium regulation [131]. Overexpression of SelM reduced peroxide-induced calcium influx in a neuronal cell line [131]. Knockdown of SelM increased cytosolic calcium concentrations and resulted in apoptotic cell death [131].

3.1.12 Selenoprotein H (SelH)—SelH has a thioredoxin-like fold motif with a small DNA-binding domain (AT-hook motif) and is localized in the nucleus [132]. SelH is highly responsive to selenium status and is upregulated under conditions of elevated copper in mouse liver [133]. SelH overexpression was shown to upregulate expression levels of genes

involved in *de novo* GSH synthesis and phase II detoxification [134]. Chromatin immunoprecipitation experiments demonstrated SelH binds to sequences containing heat shock and/or stress response elements, suggesting SelH may function in a regulatory role in response to redox status. Overexpression of SelH demonstrates neuroprotection against UVB-induced cell death in neurons in culture and increases the levels of mitochondrial biogenesis regulators, mitochondrial cytochrome c content, mitochondria mass and enhanced respiration [135]. SelH may transduce oxidant signals by modulating gene expression.

3.1.13 Selenoprotein O and I (SelO and SelI)—The functions of SelO and SelI are unknown. SelI localizes in the ER.

3.1.14 Selenoprotein S (SelS)—SelS has an ER signal and is induced by ER stress [15,136,137]. SelS is a component of the ER-associated protein degradation (ERAD) system [138,139]. ERAD protects cells from accumulation of misfolded proteins, transferring these proteins from the ER to the proteasome [140,141]. SelS interacts with Derlin-1, a ER membrane integral protein [141]. The specific function of SelS in the ERAD system is unknown. An association of SelS expression and type 2 diabetes has been reported [142]. Further details are discussed in Section 4.3.

3.1.15 Selenoprotein K (SelK)—SelK has been postulated to function in regulation of endoplasmic reticulum stress [143] and facilitation of calcium flux in immune cells [144]. SelK mRNA is expressed in immune cells and lymphoid tissues, spleen, intestine, and testis. SelK predominantly localizes to the ER but has no ER localization signal [144]. Thus, the protein may be bound by a chaperone or other ER-localized protein for insertion into the ER. Studies with SelK-knockout mice suggest that this protein is not required for growth or development of mice [144]. SelK is cleaved by calpain and the cleaved form is highly abundant in unactivated macrophages [145]. SelK cleavage is inhibited by upregulation of the Toll-like receptor-induced calpain inhibitor, calpastatin [145].

3.1.16 Selenoprotein N (SelN)—SelN is ubiquitously expressed with highest expression in muscle [146] and is localized in the ER membrane [146]. It has a predicted redox active CUGS motif. Loss of SelN function is associated with congenital muscular dystrophy [147]. SelN has been reported to interact with ryanodine receptors, and may affect calcium flux [148]. Another study demonstrated SelN deficiency was associated with oxidative stress [149,150].

4 Selenium and Disease

4.1 Overview of Selenium-Related Diseases

Recently, selenium supplementation trials found that moderately higher selenium intake may influence redox status through selenoprotein synthesis to cause type 2 diabetes. Thus selenium homeostasis needs to be tightly regulated for healthy life. The range of selenium intake for optimal health in humans and animals is narrow, such that low selenium intake is associated with developmental defects and disease states and high selenium results in

toxicity. We discuss below the conditions associated with both cases, specifically myopathies, selenosis, brain degeneration, type 2 diabetes, and male infertility.

4.1.1 Selenium Deficiency—Three diseases have been reported to be associated with severe selenium deficiency, due to their occurrence in areas with selenium poor soils and their reversal upon selenium supplementation. It should be noted that selenium may be a cofactor in these diseases, with other factors contributing to their incidence or severity.

(1) *Keshan disease*, was first described as a juvenile cardiomyopathy in the early 1930s in the Chinese medical literature [151]. Women and children were susceptible to the development of Keshan disease, which had a high mortality rate. Supplementation of individuals with sodium selenite tablets could prevent the development of Keshan disease [4]. Since the incidence of Keshan disease fluctuated seasonally and annually, viral infection was also considered as a possible cofactor [152]. Heart tissues from Keshan disease victims were subsequently shown to contain coxsackie viruses. Further, studies in selenium-deficient mice demonstrated that coxsackie virus B4 infection induced severe heart pathology [153]. Selenium-adequate mice showed mild heart pathology when infected with the virus, which suggests that selenium deficiency in combination with coxsackie virus infection was required for the development of Keshan disease.

(2) *Kashin-Beck disease* is a chronic, endemic osteochondropathy, accompanied by joint necrosis [154]. This syndrome affects individuals in specific regions of Tibet, northeastern to southwestern China, Siberia, and North Korea. While individuals with this disease show skeletal pathology, they are not reported to develop dysfunction of other organs or tissues. Kashin-Beck disease may require other factors since the disease is clustered within specific regions and/or families. A polymorphism in the Gpx1 gene was reported as a potential genetic risk factor for Kashin-Beck disease [155].

(3) *Myxedematous endemic cretinism*, which is induced by thyroid atrophy, results in mental retardation [156,157]. Myxedematous endemic cretinism is highly prevalent in central Africa, where iodine and selenium-poor areas overlap with thiocyanate-rich areas.

4.1.2 Selenium Toxicity (Selenosis)—Blood selenium levels greater than 100 µg/dL can lead to selenosis. Symptoms of selenosis include hair loss, white blotchy nails, a garlic breath, gastrointestinal disorders, fatigue, irritability, and neurological damage [158]. Selenosis in humans is a rare event except in very high selenium areas. Extreme cases of selenosis can be fatal, due to cirrhosis of the liver [159].

Elemental selenium and metallic selenides have relatively low toxicities because of their low bioavailability. Selenates and selenites are very toxic. Organic selenium compounds which occur in metabolic processes, such as Sec, selenomethionine, and methylated selenium compounds are toxic in large doses. In the 10th edition of RDAs in 1989, it was pointed out that sensitive biochemical indices of selenium overexposure were not available and no attempt was made to establish an upper limit of selenium intake [7]. In 2000, The Institute of Medicine of the National Academy of Science provided a DRI, which set the tolerable upper

intake levels of selenium to 400 µg/d [8]. The Lowest Observed Adverse Effect Level is 910 µg/d [160], and the No Observed Adverse Effect Level is 200 µg/d.

4.2 Selenium in Brain Function

The supply of selenium to the brain is prioritized for normal development and brain function. There is a “hierarchy” of tissues with respect to selenium supply under low selenium status, whereby brain tends to maintain its selenium compared to other tissues [161,162]. Brain expresses almost all selenoproteins, especially within neurons [163]. Sepp1-knockout mice produce severe brain selenium deficiency when maintained on a selenium-deficient diet, with neurological impairments that lead to death within weeks [164,165]. Sepp1-knockout mice fed a 0.10 ppm selenium diet developed spasticity and abnormal movements in addition to poor performance on the rotarod test and pole climb. Sepp1-knockout mice fed 0.25 ppm selenium diet produced no neurological dysfunction.

Recently, syndromes of congenital selenoprotein biosynthetic deficiency have been discovered. Progressive cerebellar-cerebral atrophy (PCCA) was identified in several non-consanguineous Jewish Sephardic families of Moroccan or Iraqi ancestry [166]. The syndrome was mapped to homozygous or compound heterozygous missense mutations of the SecS gene, with no enzymatic activity. PCCA involves mental retardation, progressive microcephaly, and spasticity [166]. PCCA represents the first clinical syndrome related to selenocysteine biosynthesis in humans. Clinically, cerebral and cerebellar atrophy involves gray and white matter [166].

4.3 Selenium in Diabetics

Body glucose homeostasis is regulated by functional insulin circulation and signaling. Type 2 diabetes is characterized by high blood glucose in the context of insulin resistance. The Nutrition Prevention Cancer (NPC) trial which is a double-blind, randomized, placebo-controlled clinical trial to test micronutrients for cancer prevention revealed a more than two-fold increase in type 2 diabetes incidence in the selenium-supplemented compared to the placebo group [167–169]. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) revealed a similar trend [170], however non-significant in a 10-year follow-up, but still concerning enough to lead to the trial’s termination.

A recent study reported that Sepp1 is associated with development of type 2 diabetes [171]. Sepp1 mRNA levels were increased in people with insulin resistance. Hepatic Sepp1 mRNA is upregulated by glucose and hepatic Sepp1 mRNA levels correlated with insulin resistance [171]. Furthermore, insulin suppressed Sepp1 protein expression in hepatocytes. It is postulated that Sepp1 induces insulin resistance in liver and muscle, resulting in hyperglycemia [171].

Targeted removal of the tRNA^{[Ser]Sec} gene in hepatocytes revealed increases in plasma apolipoprotein E and cholesterol levels, up-regulation of cholesterol biosynthesis genes and down-regulation of cholesterol metabolism or transport genes in the hepatocytes [172]. These results suggest hepatic selenoproteins are responsible for ApoE and cholesterol metabolism.

Recently, overproduction of the antioxidant selenoprotein, Gpx1 in mice resulted in a type 2 diabetes-like phenotype [173]. Lei's group developed Gpx1-overproducing mice, which became obese at 6 months of age. Gpx1 catalyzes the reduction of hydrogen peroxide and organic hydroperoxides using GSH as the cofactor. Insulin production is regulated by pancreatic duodenal homeobox 1, forkhead box A2, and mitochondrial uncoupling protein 2, expression and function of which are affected by intracellular ROS [174,175]. Pancreatic β cells express relatively low amounts of Gpx1, and may be susceptible to oxidative stress [176].

ER stress caused by a disruption in ER homeostasis is associated with type 2 diabetes [177]. Up-regulation of SelS mRNA in liver, adipose tissue and skeletal muscle is associated with type 2 diabetes in *Psammomys obesus* [142]. Hepatic SelS mRNA expression and protein content are increased by deprivation of glucose in *P. obesus* [137] but not in non-diabetic *P. obesus* [142]. In addition, a high concentration of glucose reduces SelS mRNA expression in cultured hepatocytes [142]. A recent study demonstrated that SelS mRNA expression was increased by insulin stimulation in human subcutaneous adipocytes from type 2 diabetic patients but not in nondiabetic subjects [178]. Thus, SelS may be involved in development of type 2 diabetes.

ER stress arrests translation in DI2 synthesis, which leads to a reduction in T3 production [179]. Chemical chaperones (tauroursodeoxycholic acid or 4-phenylbutyric acid) can resolve ER stress and restore glucose tolerance in a DI2-dependent manner [180]. DI2 is necessary for T3-induced adaptive thermogenesis [103] and DI2-knockout mice showed obesity and glucose intolerance when placed on thermoneutrality and a high-fat diet [180]. The SNP of DI2 (A/G) at codon 92 has been identified [181] and this SNP leads to the Thr92Ala variant, which strongly associates with insulin resistance [182] and subsequently type 2 diabetes [181]. DI3-knockout mice were found to be glucose-intolerant and exhibited a reduction in pancreatic β -cell mass and insulin content [183]. The absence of DI3 in the β -cells exposes them to T3 and leads to impaired β -cell function [183] and insulin secretion.

4.4 Selenium in Reproduction

For at least five decades, selenium was recognized as an important factor for male fertility in rats, mice, and livestock. Selenium deficiency in human reproduction data is contradictory because of the limited number of patients analyzed. Thus, the role of selenium in human reproduction was largely inferred from studies in laboratory animals. Testis uptake and retain selenium even under conditions of substantial selenium deficiency. Feeding a selenium-deficient diet for two generations generated abnormal spermatozoa in rats [184,185]. In severe selenium deficiency, male rats and mice become sterile as spermatogenesis is arrested, the seminiferous epithelium is degenerated and abnormal sperm morphology is observed. Interestingly, when ^{75}Se was injected into rats, most of the selenium accumulated in the mid-piece of the spermatozoon that harbors the helix of mitochondria embedded in a keratinlike matrix [186]. Recently, TR2 was shown to be abundant in elongating spermatids at the site of the mitochondrial sheath formation [109].

In 1999 the Ursini and Flohé laboratories identified Gpx4 as the major component of the mitochondrial capsule [87]. Gpx4 activity is not detected with the specific substrate

phosphatidylcholine hydroperoxide in spermatozoa but immunohistochemical staining of Gpx4 was observed. Gpx4 in the mitochondrial mid-piece of mature spermatozoa is a chemically inactive form, likely due to disulfide and selenyl sulfide bridges that form high molecular weight complexes with other capsular proteins [87]. The Gpx4 protein can be solubilized out of this complex by strong reduction and chaotropic agents and detected by MALDI-TOF mass spectrometry or Western blotting [87]. Prolonged preincubation with 0.1M DTT or mercaptoethanol leads to recovery of enzymatic activity. Unlike Gpx1-3, Gpx4 can accept electrons from protein thiols instead of GSH when GSH is limiting. As the spermatids mature, GSH and protein thiols decrease, resulting in Gpx4 polymerization. The inactive Gpx4 complex contains sperm mitochondrion-associated cysteine-rich protein fragments (SMCP), voltage-dependent anion channel and three types of keratins (type II keratin kb1, keratin k5, and acidic keratin complex I) [187]. SMCP with its 30% cysteine residues is the most likely candidate to react with Gpx4. SMCP-knockout mice showed infertility and asthenozoospermia in some mice, but marginal disturbance of spermatogenesis in others [188].

Reduced Gpx4 production in spermatozoa resulted in bent tails with slight angulations to hairpin structures and abnormal kinking at the mid-pieces [189]. Knockout of the mitochondrial Gpx4 isoform resulted in viability but male infertility, and the spermatozoa presented severe morphological abnormalities [90]. Knockout of the entire Gpx4 gene was embryonic lethal, whereas nuclear Gpx4-knockout mice are viable and fertile but exhibit transient nuclear instability and delay in chromatin condensation of spermatozoon [91]. Since cytosolic Gpx4 exists in the nucleus, lack of nuclear Gpx4 function may be compensated by cytosolic Gpx4 [91,190].

5 Health Benefits of Selenium in Humans

5.1 Molecular Forms of Selenium in Diet

Selenium exists in the environment in several inorganic and organic forms. Elemental selenium exists stably as selenite and selenate. Organic forms of selenium are found in biological matter, and include the methylated selenium compounds, selenoamino acids, and selenoproteins. However selenium is ubiquitous, and the amounts can vary widely [191,192].

Selenium is primarily supplied in diets from grains and animal products [193]. Plants have no true selenoproteins. Selenomethionine is produced in plants due to indiscriminate substitution of selenium for sulfur in methionine biosynthesis. Drinking water contains very low amounts of water-soluble inorganic forms of selenium (0.12 to 0.44 µg/L) [194,195] and this contribution to selenium as a dietary source is very minor. In the United States, the amount of selenium in water is regulated by the Environmental Protection Agency under the Safe Drinking Water Act. The federal standards allow up to 50 mg/L in drinking water [196].

Grains, wheat, and corn used for breads and other food products contain selenomethionine (~55%) as a bioavailable selenium source [197]. Sec and selenite/selenate are also detectable in substantial amounts in wheat (~20% respectively). The content of selenium in plants can

vary widely, as much as 500-fold, depending on the soil selenium. In regions where soil selenium is low, such as Southwestern Oregon in the United States, Northeastern China, Finland, and New Zealand, sodium selenite is added to fertilizers as well as animal feed [198,199]. High-selenium regions, where soils exceed 600 mg/kg selenium, are found in parts of North and South America, China, and Russia [200,201]. In regions with high selenium in the soil, plants may accumulate up to 3,000 µg selenium per gram and may potentially be toxic [202]. Selenium absorption by plants depends on the pH of the soil, i.e., selenite in acidic soils and selenate in alkaline soils. Because selenate is a more water-soluble form of selenium, it is thought to be more available for plant uptake [203,204].

Fruit and vegetables contain only small amounts of selenium. Some vegetables can grow under selenium-rich soil and accumulate selenium (onions, leeks, garlic, and broccoli) [205]. These vegetables accumulate selenium up to 50-fold or higher. Vegetables grown in high-selenium soil contain mostly selenomethionine, methylselenocysteine, and γ-glutamyl-methylselenocysteine [206,207]. Fungi, such as mushrooms and yeast, can accumulate selenium and may contain more than 20 selenium-containing compounds (Sec, selenomethionine, methylselenocysteine, and Se-adenosylselenohomocysteine) [208].

Meats are good sources of selenium, but the selenium content in livestock is dependent on diet and the region in which the animals feed. Selenium supplementation of cattle, hogs, and chicken is a common practice [209]. Animal meat contains mostly selenomethionine (up to 60%) and Sec (up to 50%). The remaining selenium species are small selenium-containing molecules. These ratios can vary depending on what form of selenium is consumed. Selenite or selenate in food will be converted into Sec. Animals fed selenomethionine-containing food increase the content of selenomethionine and Sec in the meat [210].

5.2 Selenium Transport in Mammals

5.2.1 Tissue-Oriented Selenium Transport—Selenium is differentially distributed into different organs. It has been proposed there is a hierarchy of selenium requirements for selenium in tissues. Brain and testis tend to preserve selenium for their essential functions under selenium deficiency [161,211]. Dietary selenium is absorbed in intestine and transferred into liver. The body selenium content is regulated by hepatic production of methylated selenium compounds and its urinary excretion, not by intestinal absorption of selenium. Selenium is primarily transported in the plasma to the organs via Sepp1 [164,165]. Kidney expresses Sepp1 at 38% of the liver level. Skeletal muscle, heart, and testis followed with 10, 6, and 6%, respectively, in mouse [212]. Brain expresses Sepp1 at less than 2% of the liver level. Sepp1 is also expressed in many tissues at very low levels. The human plasma concentration of Sepp1 is approximately 5.6 mg/L. Since the liver exports selenium in the form of Sepp1, dietary selenium deficiency dramatically decreases liver selenium concentrations. As discussed above, Sepp1-knockout mice exhibited a severe phenotype, including neurological dysfunction and male infertility. Testis requires selenium for sperm maturation, and testis of Sepp1-knockout mice becomes severely selenium-deficient unless a high-selenium diet is fed. Testis selenium concentrations in Sepp1-knockout mice decrease to 8% of the wild-type value, whereas brain retains 56% of the wild-type value when mice were fed 0.25 ppm selenite diet for 4 weeks after weaning, a diet considered as selenium-

adequate. Deletion of Sepp1 causes increased excretion of selenium in the urine [213]. Selective deletion of Sepp1 in hepatocytes showed liver selenium is maintained but whole-body selenium concentration decreased to 58% of the control value in mice fed 0.25 ppm selenium diet for 4 weeks beginning at weaning [212]. However, in other tissues selenium concentrations also decreased to varying degrees, but brain and testis retained selenium better than other tissues. Under conditions of selenium deficiency, selective deletion of Sepp1 in hepatocytes resulted in elevation of liver selenium concentration to 500% of the control value, accounting for 53% of whole-body selenium. These results demonstrate the central role of Sepp1 production by hepatocytes and the critical role of secretion of Sepp1 from liver in maintaining selenium homeostasis [212].

Sepp1 is observed in testis Sertoli cells in endocytosed vesicles. A lipoprotein receptor family member, apolipoprotein E receptor-2 (apoER2) was the first identified Sepp1 receptor in testis Sertoli cells [122]. ApoER2-knockout mice exhibit very low testis selenium concentrations. Thus, most of the selenium in testis is taken up in the form of Sepp1 by apoER2. ApoER2 is also highly expressed in the brain [214], where it was shown to play a crucial role in uptake of Sepp1 and preservation of selenium when dietary selenium is limiting [123].

Megalin, also a lipoprotein receptor family member, was identified as a Sepp1 receptor in kidney brush border of proximal convoluted tubule (PCT) cells [124]. Several isoforms of Sepp 1 have been identified in rat [121,215]. Shortened isoforms may result from termination of Sepp1 translation at UGAs in the open reading frame of Sepp1 mRNA. One of the shortest isoforms of mouse Sepp1^{240–361} is small enough to pass through the renal glomerulus. Since megalin-knockout mice lose Sepp1 in urine, megalin prevents loss of selenoproteins in urine [216]. However, another plasma selenoprotein, Gpx3, which accounts for 21% of selenium in plasma [217], is not a selenium source of selenoproteins [218].

When selenium intake is high, non-Sepp1 selenium forms including low molecular weight selenium compounds are taken up by kidney. Under selenium deficiency, low molecular weight selenium compounds are not sufficient to support tissue selenium requirements [212].

In summary, selenium is transported to tissues primarily via Sepp1 and small molecules. Plasma Sepp1 is an efficient form of selenium transporter. Gpx3 is another plasma selenoprotein but does not appear to transport selenium for specific uptake by cells. Small selenium compounds can transfer selenium but this requires much higher selenium intake and the pathway appears to be nonspecific. The functions of small molecule selenium compounds need further characterization.

5.2.2 Selenium Excretion—Selenium is excreted in urine, in feces, and by other routes, which include exhalation in breath and loss through hair and skin cells.

Urine: Once selenium is absorbed by the body, it is excreted mostly into the urine. Urinary selenium excretion increased with increases in dietary selenium intake.

Trimethylselenonium ion was identified as a prominent form of selenium in rat urine [219], and is the major excreted form when selenium is in excess [220]. Recently, Suzuki's group identified the major selenium metabolite in urine as 1 β -methylseleno-N-acetyl-D-galactosamine (selenosugar) within the required to low-toxic range [221]. This selenosugar is synthesized in liver [221] (Figure 7).

Breath: Volatilization of selenium into breath is observed only at high selenium intakes [222]. The volatile compound dimethylselenide was identified as one of the methylated forms of selenium that account for most selenium excretion in urine and breath [223].

Feces: Fecal selenium excretion was regulated by dietary selenium intake at deficient to moderately high selenium intakes. Fecal selenium excretion plateaued at moderately high selenium intake [224]. Characterization of fecal selenium excretion has been relatively minimal.

5.3 Human Dietary Standards for Selenium

In 1980, the National Research Council (NRC) established an estimated safe and adequate daily dietary intake for selenium in humans [225]. The recommendation for adults was set from 50 to 200 $\mu\text{g}/\text{d}$ based on extrapolations from animal studies. In 1989, the Dietary Reference Intake (DRI) was established for selenium, with a RDA of 70 $\mu\text{g}/\text{d}$ for men and 55 $\mu\text{g}/\text{d}$ for women in accordance with the World Health Organization [7].

Selenoproteins are the major form of functional selenium, thus selenium nutritional requirements have been assessed through selenoprotein optimization [226]. Plasma contains two selenoproteins, Gpx3 and Sepp1. Plasma Gpx activity and Sepp1 concentration decrease to less than 5% of selenium-replete values in animals with severe selenium deficiency. Thus, plasma levels of these selenoproteins are used primarily as nutritional biomarkers of selenium.

In 2001, Burk's group carried out a study in a low-selenium area of China [227]. They concluded that full expression of glutathione peroxidase was achieved with 37 $\mu\text{g Se}/\text{d}$ as selenomethionine and with 66 $\mu\text{g}/\text{d}$ as selenite. However, full expression of selenoprotein P was not achieved at the highest doses of either form. There are several forms of selenium that exist in dietary food and supplements (e.g., high-selenium yeast and selenomethionine). Yeast contains selenium mostly as selenomethionine but has a significant amount of selenium in other forms. Burk's group carried out a study supplementing moderate (approximately 200 $\mu\text{g}/\text{d}$) to high levels (approximately 600 $\mu\text{g}/\text{d}$) of selenium supplements in three forms (selenized yeast, selenomethionine, selenite) to selenium-replete individuals in the US [228]. Since selenomethionine is nonspecifically incorporated to proteins, high-yeast selenium supplement and selenomethionine raised the plasma selenium concentration in a dose-dependent manner but plasma selenoproteins did not respond to selenium supplementation in selenium-replete individuals. Selenite intake did not increase plasma selenium concentration and was excreted into urinary selenium compounds. In the study, total intakes of over 800 $\mu\text{g}/\text{d}$ selenium for 16 weeks showed no signs of selenium toxicity. The authors concluded that the 800 $\mu\text{g}/\text{d}$ can be used safely in studies of limited duration if the subjects are monitored closely for signs of selenium toxicity.

In 2010, the Burk group further studied the effect of selenium supplementation in a selenium-deficient human population in China [229]. They studied healthy Chinese individuals who had a daily dietary selenium intake of 14 µg/d and showed that supplementation with 35 µg selenium/d for 40 weeks optimized the Sepp1 in the healthy selenium-deficient Chinese individuals. Gpx activities were optimized by a total intake of 35 µg selenium/d. The investigators concluded that adjustment for the difference in weight between the Chinese subjects (58 kg) and US residents (76 kg) and for variation among individuals would yield a selenium requirement for US adults of ≈ 75 µg/d. These studies indicate that once the selenium requirement has been met, selenoproteins are not increased and plasma Sepp1 concentration is the best marker of human selenium nutritional status.

6 General Conclusions

It has been two centuries since the identification of selenium by Berzelius. Selenium is essential for life processes. Although it is a rare element, many organisms have evolved to maximize selenium's properties. It is integrated into the biology of many life forms, to the extent of being critical for life. The selenium field has been dramatically expanding over the last few decades. However, functions of most of the selenoproteins and selenium containing molecules still remain unclear. Continued research of the biochemical properties of selenium will hopefully lead to new discoveries to improve human health.

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Abbreviations and Definitions

Ψ55	tRNA pseudouridine position at 55
28S	28S ribosomal RNA
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
apoER2	apolipoprotein E receptor-2
ATP	adenosine 5'-triphosphate
BAT	brown adipose tissue
cDNA	complementary DNA
Cys	cysteine
DI	iodothyronine deiodinase
DRI	dietary reference intake
DTT	dithiothreitol
EF	elongation factor
eEFSec	eukaryotic selenocysteyl-tRNA-specific elongation factor

eIF4a3	eukaryotic initiation factor 4A-III
EMSA	electrophoretic mobility shift assays
ER	endoplasmic reticulum
ERAD	ER-associated protein degradation
EST	expressed sequence tag
γ-GCS	γ -glutamylcysteine synthetase
Gpx	glutathione peroxidase
GS	glutathione synthetase
GSH	reduced form of glutathione or γ -glutamylcysteinylglycine
GSSG	oxidized form of glutathione, glutathione disulfide
i⁶A	isopentenyladenosine
kDa	kilo dalton
L12	large ribosomal subunit protein L12
L30	large ribosomal subunit protein L30
m¹A	N1-methyladenosine
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
mcm⁵U	methylcarboxymethyl-5'-uridine
mcm⁵Um	methylcarboxymethyl-5'-uridine-2'-O-methylribose
mRNA	messenger ribonucleic acid
MS	mass spectrometry
Msr	methionine sulfoxide reductase
mV	millivolts
NADP⁺	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NPC	Nutrition Prevention Cancer
NRC	National Research Council
Nrf2	leucine zipper transcription factor NF-E2 factor 2
PCCA	progressive cerebellar-cerebral atrophy
PCT	proximal convoluted tubule
pK_a	acid dissociation constant
PPi	pyrophosphate (diphosphate)
PSTK	O-phosphoseryl-tRNA ^[Ser] Sec kinase

RDA	recommended dietary allowance
ROS	reactive oxygen species
rRNA	ribosomal ribonucleic acid
rT3	reverse triiodothyronine
SBP2	SECIS binding protein-2
Sec	selenocysteine
SECIS	Sec insertion sequence
Secp43	Sec tRNA ^{[Ser]Sec} associated 43 kDa protein
SecS	Sec synthetase
Sel(X)	selenoprotein X (X is any selenoprotein)
SELB	Sec-specific translation elongation factor
SELECT	Selenium and Vitamin E Cancer Prevention Trial
selenosugar1	β -methylseleno-N-acetyl-D-galactosamine
Sep15	15 kDa selenoprotein
Sepp1	human selenoprotein P
siRNA	small interfering RNA
SLA	soluble liver antigen
SMCP	sperm mitochondrion-associated cysteine-rich protein
SPS	selenophosphate synthetase
T3	3,3',5-triiodo-L-thyronine or triiodothyronine
T4	thyroxine
TGR	thioredoxin/glutathione reductase
TR	thioredoxin reductase
Trit1	tRNA isopentenyltransferase, mitochondrial
tRNA	transfer ribonucleic acid
UDP-glucose	uridine diphosphate glucose
Um34	single methyl group on the ribosyl moiety at position 34
UTR	untranslated region
UVB	ultraviolet, 315-280 nm wave length
WHO	World Health Organization

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Figure 1.

Selenium-containing amino acids in mammals. Selenocysteine is found in selenoproteins. At the physiologic pH, its selenol is mostly deprotonated. Sec can be synthesized by mammals. Selenomethionine is classified as non-polar amino acid. Like the essential amino acid methionine, selenomethionine is also not synthesized *de novo* in mammals. Selenomethionine appears to be distributed nonspecifically in the proteins in place of methionine residues.

Figure 2.

Cloverleaf model of bovine liver Sec tRNA^{[Ser]Sec} and its modifications. Sec tRNA^{[Ser]Sec} sequences in mammals are 90 nucleotides long. The tRNA contains base modifications at position 34 (methyl carboxymethyl-5'-uridine; mcm⁵U), 37 (isopentenyladenosine; i⁶A), 55 (pseudouridine; Ψ) and 58 (N1-methyladenosine; m¹A). The two isoforms differ from each other by a single methyl group on the position 34 (mcm⁵U or mcm⁵Um).

Figure 3.

Biosynthesis of Sec and *de novo* synthesis of cysteine. Sec is synthesized on tRNA^{[Ser]Sec} by generation of selenophosphate from selenide and ATP (upper portion of the figure for the final steps in Sec biosynthesis). The *de novo* synthesis of cysteine on Sec tRNA^{[Ser]Sec} occurs when sulfide replaces selenide (lower portion of the figure for the final steps in cysteine biosynthesis).

Figure 4.

Diagram of two classes of eukaryotic SECIS elements. Secondary structure models of Form 1 and 2 SECIS. The critical structural features are labeled. The eukaryotic SECIS element is located in 3' UTR. N, any nucleotide; A/G and A/C indicate that A is the prevalent base.

Figure 5.

A model of Sec biosynthesis and incorporation. Aminoacylation of tRNA^{[Ser]Sec} and its conversion to Sec-tRNA^{[Ser]Sec} is depicted along the top. Recruitment of transcription factors (SBP2, eEFSec) are depicted top right. Shuttling of the complex of Sec-tRNA^{[Ser]Sec} into the nucleus and the association with eEFSec, SBP2, and SECIS elements are depicted along the right bottom. Cytoplasmic export and translation are shown along the left bottom.

Figure 6. Selenoproteins found in humans. SECIS type and Sec residues belong to the thioredoxin motif as shown (C; cysteine, x: any amino acid, U; Sec residue). On the right, relative size of selenoproteins are shown (relative to a 100 amino acid scale). The location of Sec within the protein sequence is shown by a black line.

Figure 7.

Selenium containing compounds in mammals. Methylselenocysteine is supplied by plants.

Selenosugar is a major urinary selenium compound which is synthesized in liver.

Dimethylselenide is found in breath.