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Understanding selenoprotein function and regulation through the use of rodent models

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Abstract

Selenium (Se) is an essential micronutrient. Its biological functions are associated with selenoproteins, which contain this trace element in the form of the 21st amino acid, selenocysteine. Genetic defects in selenocysteine insertion into proteins are associated with severe health issues. The consequences of selenoprotein deficiency are more variable, with several selenoproteins being essential, and several showing no clear phenotypes. Much of these functional studies benefited from the use of rodent models and diets employing variable levels of Se. This review summarizes the data obtained with these models, focusing on mouse models with targeted expression of individual selenoproteins and removal of individual, subsets or all selenoproteins in a systemic or organ-specific manner.

Keywords

selenium; selenocysteine; selenoproteins; mouse models

1. Selenoproteins: identification and oxidoreductase functions

Glutathione peroxidase 1 (GPx1) [1] was the first identified selenoprotein. Initially isolated from human erythrocytes, it was shown to protect hemoglobin from oxidative damage. Later, it was found to be dependent on selenium (Se), [2–5]. Se is incorporated into GPx1 in the form of the 21st amino acid, selenocysteine (Sec). In comparison to cysteine (Cys), Sec has a lower pKa and is a stronger nucleophile [6]. Almost all known selenoproteins are oxidoreductases with Sec in the active center. Sec insertion requires the presence of an in-frame UGA codon and the Sec insertion sequence (SECIS) element, a kink-turn RNA structure. In eukaryotes, the SECIS element is located in the 3’ UTRs of selenoprotein mRNAs. Biosynthesis of Sec occurs on its own tRNA, $tRNA^{[Ser]Sec}$, which is initially charged with Ser. SECIS-binding protein 2 (SBP2 or Secisbp2) binds the SECIS element and recruits $Sec\text{-}tRNA^{[Ser]Sec}$ along with other factors involved in Sec insertion [7, 8]. Characterization of the structure and conserved sequences of the SECIS element allowed

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development of computational programs for identification of selenoprotein genes in sequence databases [9–11]. The SECISsearch program was designed to recognize sequence, structural and thermodynamic parameters of SECIS elements [12]. By searching for SECIS elements, in-frame UGA codons in the ORFs and the presence of Cys-containing orthologs of selenoproteins, selenoprotein genes could be identified in genomic sequences. Accordingly, the human genome was found to contain 25 selenoprotein genes (Table 1). Most of these proteins participate in maintaining cellular redox homeostasis, including three thioredoxin reductases (TRs), five glutathione peroxidases (GPx1), methionine sulfoxide reductase (MsrB1), and three thyroid hormone deiodinases (DIs).

As shown in Table 1, the functions of several selenoproteins have been established, but the majority of selenoproteins have no known functions. Indeed, besides TRs, GPxs, MsrB1, DIs, and SPS2, the specific reactions catalyzed by selenoproteins are not known. However, conservation of selenoproteins among species and preservation of the complex biosynthetic pathway for their production indicate the importance of this class of proteins. So far, the common feature of all selenoproteins with the identified functions is their participation in oxidoreductase reactions. This type of reaction is important in intracellular redox homeostasis and antioxidant defense. GPxs (and possibly the N-terminal domain of SelP) are capable of reducing various peroxides [13, 14]. TRs and MsrB1 participate in the reduction of disulfides and methionine (Met) sulfoxide residues in proteins, respectively [15–18]. DIs catalyze reductive removal of iodine (I) from the outer ring of the prohormone thyroxine (T_4) yielding various forms of thyroid hormones [19, 20]. Sep15, SelM, SelH, SelS, SelK, SelN, SelT, SelW are less characterized, whereas almost no studies have been done on SelV, SelO, and SelI. Most likely, many these proteins are also oxidoreductases with Sec in the active site. More than half of mammalian selenoproteins are characterized by the thioredoxin-like fold. This fold is a two-layer $\alpha/\beta/\alpha$ sandwich structure that includes a conserved CxxC motif (i.e., two Cys separated by two other residues). In some cases, one of the Cys residues can be substituted with Ser or Thr. This fold is especially common for enzymes that catalyze formation or isomerization of disulfide bonds or perform other functions that change the redox state of cysteine residues. In addition, at least 6 out of 25 selenoproteins (Sep15, SelK, SelM, SelN, SelS, and SelT) reside in the ER lumen, an additional selenoprotein (D2 or Dio2) is associated with ER membranes (its catalytic site faces the cytosol), and several secreted selenoproteins pass through this compartment. The enrichment of the ER with selenoproteins suggests the roles of selenoproteins in ER-associated pathways, such as protein secretion/modification (Sep15, SelM [21]) and ER-associated protein degradation ERAD (SelS, SelK [22, 23, 24]).

2. Mouse models for studying selenoproteins

Knockout (KO) and transgenic models can be used for evaluating protein functions as well as for their impact on physiology and pathology. To examine selenoprotein functions, a number of mouse models have been developed and characterized. Generally, these models can be divided into two groups. The first group includes animals lacking (or overexpressing) one or two selenoproteins. The second group includes various mouse models characterized by the altered selenoprotein biosynthesis pathway. These animals develop systemic selenoprotein deficiency. The use of these animal groups is discussed in the following sections.

2.1 Targeted removal of individual selenoproteins

Several mouse models with targeted inactivation of one or two selenoproteins have been developed and characterized thus far [25, 26]. Their overview is given in Table 2. Three selenoproteins were found to be essential for embryogenesis: TR1, TR3 and GPx4. Knockout of cytosolic TR1 leads to embryonic death between days E8.5 and E10.5 [27, 28].

While the cardiomyocyte-specific TR1 KO mice developed normally, the neuronal system (NS)-specific TR1 KO caused severe neurological symptoms, such as ataxia and tremor [29]. These symptoms were the result of cerebellar hypoplasia, abnormal foliation, perturbed lamination and reduced proliferation of granule cell precursors in the cerebellum [29]. Mitochondrial TR3 KO induced embryonic lethality between days E13.5 and E15.5. Compared to controls, embryos were smaller, developed anemia and showed high levels of liver apoptosis. NS-specific TR3 KO mice developed normally without signs of neurodegeneration; however, the cardiomyocyte-specific TR3 KO mice died from the heart failure within a few hours of birth [30]. Disruption of mitochondrial TR3 in B and T cells did not affect viability and functions of immune cells [31]. Similar to mice, TR3 polymorphism was found to be associated with dilated cardiomyopathy in humans. Both nucleotide substitutions were in the open reading frame and were part of the FAD-binding domain [32].

GPx4 is another essential selenoenzyme: its homozygous genetic inactivation was found to be lethal by E7.5 [33–35]. GPx4 is represented by cytosolic (cGPx4), nuclear (nGPx4) and mitochondrial (mGPx4) isoforms. These isoforms are synthesized from the same gene by alternative initiation of transcription and differ by their N-terminal sequences. nGPx4 expression is driven by its own testes-specific promoter, which lies inside the first intron of the cytosolic GPx4 transcript [36]. The role of GPx4 in sperm maturation was supported by the finding that this protein was a structural component of the mitochondrial capsule of male germ cells [37]. In addition, spermatid-specific knockout of GPx4 led to infertility in mice [38]. Moreover, inducible inactivation of GPx4 in mice and primary cells led to an increased 12/15-lipoxygenase-derived lipid peroxidation followed by apoptosis triggered by activation of apoptosis-inducing factor. To further access the function of each isoform, several KO/ transgenic mouse models were prepared. nGPx4 KO mice developed normally; neither testicular structure nor fertility were affected in these mice; however, the delayed sperm chromatin condensation was observed [39]. The sequence between the two alternative translation initiation codons corresponding to mitochondrial and cytosolic forms encodes a mitochondrial signal peptide. Thus, introduction of the in-frame stop codon between these start codons resulted in the specific disruption of the mGPx4 form without affecting the expression of cGPx4. mGPx4 KO male mice were infertile [40]. These experiments revealed an essential role of mGPx4 in male reproduction.

KO of other GPxs did not affect viability and fertility. The major findings with GPx KO mice are summarized in Table 2. GPx1 KO mice did not show significant phenotypes; however, they were more susceptible to oxidative stress and viral myocarditis, as well as to reoxygenation damage due to ischemia-reperfusion injury [41]. GPx2 is mainly expressed in the epithelial tissues, and its disruption affects intestinal cells [42]. GPx1 and GPx2 double KO mice are characterized by severe colitis when maintained on an atherogenic diet [43]. Recently, GPx3 KO mice were developed [44]. Even though no significant phenotype was observed, this model revealed the specific binding of GPx3 to the basement membranes of renal cortical proximal and distal convoluted tubules.

Experiments designed to understand the function of selenoproteins in the thyroid gave ambiguous results. Both general and liver-specific knockout of DI1did not lead to significant changes in the thyroid hormone axis. [45, 46]. DI2 is expressed in the pituitary and is thought to be a T4 sensor, which is known to be a part of the negative feedback loop for thyroid hormone production. DI2 KO mice showed pituitary resistance to T4 [47]. In addition, DI2 was found to be important in the conversion of T4 to T3 in peripheral tissues (T3 stimulation is critical for the development of the auditory functions) [48, 49]. DI2 activity was increased in the WT mouse cochlea at postnatal day 7, and then declined by day 10. This DI2 activity correlated with the onset of hearing. This observation suggests that DI2 plays an important role in producing local T3 for the proper cochlear development [50]. At the same time, DI2 deficiency resulted in delayed cochlear differentiation that was the reason for irreversible deafness of DI2 KO mice [49]. DI1/DI2 double KO mice did not augment the phenotype of D1 or D2 KO mice; it was rather the sum of each single KO [51]. DI3 is responsible for inactivation of T3 and T4. DI3 KO mice showed signs of central hypothyroidism, suggesting the importance of T3 degradation for maintaining the thyroid hormone axis [52]. D3 KO mice, like DI2 KO mice, were characterized by impaired auditory function, but with different pathogenesis. Unlike DI2 KO, DI3 KO mice displayed accelerated cochlear differentiation, which also resulted in deafness. This might suggest a critical role of DI3 in local protection of the developing tissues from premature T3 exposure and differentiation [53].

Experiments with SelP KO mice revealed that the major function of SelP is the transport of Se from liver to peripheral tissues [54, 55]. SelP KO mice developed symptoms of general Se deficiency, such as ataxia, seizures and male infertility [56, 57]. All these symptoms (except male infertility) could be rescued by an increase in dietary Se. Some of these phenotypes could be rescued by an increase in dietary Se. Apparently, SelP KO mice are a particularly well suited model to study Se deficiency. Analysis of the liver-specific Sec tRNA$^{[Ser]Sec}$ KO (liver Trsp KO) mice (these mice lack expression of all selenoproteins in hepatocytes and will be discussed later in this review) showed decreased expression and activity of selenoproteins in peripheral tissues, which confirmed the transport function for the hepatic SelP [58]. The levels of Se in the brain remained unaffected in liver-specific Trsp KO mice; also, these mice did not show neurological phenotypes. These findings suggested another essential SelP function in the brain. Restoration of liver SelP expression in SelP KO mice restored Se transport and removed symptoms associated with Se deficiency [59]. Thus, hepatocyte-derived SelP provides the major Se supply for kidney, testis and brain. However, under Se deficiency, overexpression of SelP in the liver was unable to rescue the phenotypes of SelP KO mice, which indicates the importance of local SelP production to support selenoprotein biosynthesis under limiting Se conditions [59]. SelP was found to be recognized by two receptors: ApoER2 (mostly in the testes) and megalin. Mice, lacking these receptors demonstrated Se deficiency in testes and kidney proximal tubule epithelial cells, respectively [60, 61]. SelP consists of two parts. The N-terminal region contains a conserved UxxC motif, which is part of the domain characterized by the thioredoxin-like fold [62]. The C-terminal part of SelP contains multiple Sec residues and is involved in providing Se for the synthesis of other selenoproteins. Deletion of the C-terminal region of SelP resulted in a milder phenotype compared to the KO of the entire protein. Overall, the C-terminus plays a critical role in Se transport [63]. Infection of mice lacking the C-terminal domain of SelP with the African tripanosomiasis resulted in lower tissue injury in comparison with SelP KO mice. These mice also showed decreased production of reactive oxygen species and decreased apoptosis in the liver immune cells, increased parasite clearance capacity of myeloid cells, and increased survival. All these observations indicate that the N-terminal part of SelP plays an important role in these processes [64].

Recently, three additional KO models were described [65]. A KO of MsrB1 did not lead to strong phenotypes: the KO mice were viable and fertile. However, various tissues of MsrB1 KO mice were characterized by a decreased level of MsrA (methionine sulfoxide reductase specific for the *S*-diastereomer of Met sulfoxide) and increased levels of malondialdehyde, protein carbonyls, protein Met sulfoxide, as well as higher levels of oxidized glutathione and reduced levels of free and protein thiols; all this indicates the persistent oxidative stress in MsrB1 KO mice.

Systemic inactivation of SelK in mice also did not affect viability and reproduction [66]. However, as a result of the receptor mediated Ca^{2+} flux, SelK KO mice showed compromised functions of the immune cells, including T cell proliferation, T cell and neutrophil migration, and Fcγ receptor-mediated oxidative burst in macrophages; they also showed higher susceptibility to viral infection.

Unexpected results were obtained from the analysis of the mouse model characterized by targeted inactivation of the Sep15 gene. These Sep15 KO mice developed congenital nuclear cataracts. Sep15 mRNA was enriched during lens development, which suggested Sep15 function in lens formation. These cataracts did not appear to be due to severe oxidative stress or glucose dysregulation and presumably are associated with improper folding status of lens proteins caused by Sep15 deficiency [67].

Genetic defects in SEPN1 gene are associated with a human disorder called SEPN1 related myopathy, which includes early-onset muscle atrophy, myotendinous contractures and muscle weakness [68]. These symptoms lead to respiratory insufficiency, spine rigidity and severe scoliosis. Recently, a mouse model for SelN (Sepn1) deficiency was developed and characterized. Although SEPN1 KO mice showed normal embryogenesis and growth, they demonstrated limited motility and body rigidity during physical exercise [69]. By 4 months of age, these animals displayed a reduced pool of muscle satellite cells (SC), which are essential for adult muscle growth and repair. SelN expression was drastically increased during muscle regeneration followed by cardiotoxin-induced injury. Under these conditions, SelN KO mice showed poorer recovery, characterized by lower injured-to-colateral muscle mass ratio and excessive SC loss. The essential role of SelN in SC homeostasis is consistent with the observation that biopsies from patients with SEPN1 related myopathies showed a significant SC loss [70].

There are several selenoproteins, which are still poorly characterized, and which would benefit from the development and characterization of KO models. These proteins include SPS2, SelI, SelO, SelS, SelT, SelV, and SelW.

2.2 Overexpression of selenoproteins in mice

Besides selenoprotein gene KO mice, several studies described animals with overexpression of individual selenoproteins. One of the best such models is the GPx-overexpressing mice (GPx1oe). These animals were shown to develop hyperglycemia and hyperinsulinemia, and they also developed high levels of blood insulin and increased islet β-cell mass [71–73]. It should be noted that similar phenotypes were observed in Type 2 diabetes models. When maintained on a high fat diet, these mice developed obesity and insulin resistance, unlike GPx1 KO mice, which showed reduced insulin levels and decreased islet β-cell mass [72]. This phenotype of GPx1oe mice might be explained by insufficient ROS-mediated signaling in islet β-cells. In a different model of diabetes, expression of GPx1 had a beneficial effect. Here, overexpression of GPx1 in the islet β-cell of the db/db mice alleviated hyperglycemia at an early age and completely reversed it by 20 weeks of age [74]. Since redox signaling plays a critical role in β-cell signal transduction, both deficiency and excess of GPx1 are capable of deregulating signaling pathways. These results suggest the importance of controlled GPx1 expression for prevention of Type 2 diabetes.

Another research group developed mice with transgenic overexpression of mitochondrial GPx4 (mGPx4) [75]. Compared to littermate controls, these mice developed attenuated cardiac dysfunction in response to ischemia/reperfusion injury. Overexpression of mGPx4 reduced the levels of lipid peroxidation and slightly increased the activity of the electron transport chain (ETC) complexes I, III, and IV.

Another example of overexpression of a selenoprotein in an animal model is the overexpression of SelM in rats [76]. These animals showed a better response to oxidant treatment. When fed with high Se diet, transgenic rats showed altered ERK signal transduction in the brain, which was characterized by inhibition of the alpha/gamma-secretase activity and Tau protein phosphorylation. These observations suggest a possible protective role of SelM in the Alzheimer's disease [77].

3. Mouse models targeting the Sec biosynthesis pathway

Inactivating a single selenoprotein in the mouse can provide information about its function and reveal phenotypes associated with its deficiency. However, targeting the Sec incorporation machinery allows modulation of the expression of subsets or even all selenoproteins. Many such models have been developed.

3.1 Sec incorporating machinery

In eukaryotic cells, Sec biosynthesis and incorporation is a complex multi-stage process [7, 8, 78, 79]. The overall pathway of Sec incorporation is illustrated in Fig 1. Sec is synthesized on its own tRNA, tRNA$^{[Ser]Sec}$, which is the product of the Trsp gene. Initially, this tRNA is charged with Ser, forming Ser-tRNA$^{[Ser]Sec}$. This reaction is catalyzed by seryl-tRNA synthetase (SerRS). Ser-tRNA$^{[Ser]Sec}$ is further phosphorylated by phosphoseryl-tRNA$^{[Ser]Sec}$ kinase (PSTK). The Se donor compound for the Sec biosynthesis, selenophosphate, is synthesized by selenophosphate synthetase 2 (SPS2). Sec synthase (SecS or SepSecS) catalyzes the pyridoxal phosphate-dependent reaction which results in Sec-tRNA$^{[Ser]Sec}$ formation. Once formed, Sec-tRNA$^{[Ser]Sec}$ associates with EFSec and SBP2, and this supramolecular complex is translocated to the nucleus [80]. SBP2 recognizes the SECIS element, which is located in the 3’-UTR of selenoprotein mRNAs. This complex then supports the incorporation of Sec in response to the in-frame-UGA codon. There are several features which are critical for proper function of the pathway: 1) as shown in Fig 1, SBP2 and EFSec shuttle between the nucleus and cytosol. This allows binding selenoprotein mRNAs in the nucleus and inhibition of the nonsense mediated decay induced by the in-frame stop codon [81]; 2) SPS2 is itself a selenoprotein, forming a positive feedback loop [82]; 3) recently, it was found that SPS2 can also synthesize thiophosphate, promoting incorporation of Cys in place of Sec; in mice maintained on the Se-deficient diet, insertion of Cys at UGA codon of TR1 equaled that of Sec [83]; and 4) Sec tRNA$^{[Ser]Sec}$ is a unique tRNA, which undergoes multiple modifications, further regulating Sec incorporation. These modifications include isopentenyladenosine modification at position 37 and methylcarboxymethyl-5’-uridine (mcm^{5}U) at position 34. The last step in Sec-tRNA$^{[Ser]Sec}$ maturation is the methylation of mcm^{5}U, which may be assisted by Secp43 and results in the formation of methylcarboxymethyl-5’-uridine-2’-O-hydroxymethylribose (mcm^{5}Um) [84]. This process is highly sensitive to the primary, secondary and tertiary structure of the tRNA as well as to overall Se status. mcm^{5}U supports the synthesis of “housekeeping” selenoproteins, such as GPx4, TR1 and TR3, whereas the methylated tRNA is needed for expression of “stress-related” selenoproteins, such as GPx1, GPx3, and MsrB1. This change in selenoprotein expression pattern is commonly observed during Se deficiency, but the precise molecular mechanism is unknown.

There are several ways to regulate efficiency of Sec incorporation. In order to modulate expression of selenoproteins, the easiest way is to change the levels of dietary Se. To examine the effects of dietary Se on various health parameters, one can adjust Se concentration in rodent chow. For example, 0.1 ppm Se in the diet corresponds approximately to the human Recommended Dietary Allowance for adults, whereas 0.4 ppm Se may correspond to the diet supplemented with 200 μg Se/day, which is the dose most often used in clinical trials involving Se [85–87]. This approach was successfully applied to examine Se function in diabetes [88], cancer [89], the immune response [90, 91], etc. The disadvantage of this approach, however, is that with the change in dietary Se in order to regulate selenoprotein expression, the levels of low molecular weight Se compounds are also changed, which might itself influence certain pathways.

3.2 Trsp transgenic mouse models

Stable expression of mutant Trsp was shown to severely affect selenoprotein biosynthesis by interfering with the Sec incorporation pathway by a dominant-negative mechanism. According to this hypothesis, two mouse models were generated. In the first model, A37 was substituted with G37 [92], and in the second, T34 was replaced with A34 [93]. Both models lacked mcm^{5}Um34; thus, expression of stress-related selenoproteins was severely reduced, whereas expression of housekeeping selenoprotein genes was little affected. The effect of G37 transgene was tissue-specific: it was significant in the liver and kidney, but not in testes [92]. The G37 transgenic mice were studied for various health parameters. These mice were found to be more susceptible to viral infection [94], colon cancer [95] and X-ray damage [96]. Crossing the G37 and C3/Tag mice provided a good model for studying the function of selenoproteins in prostate cancer. Such mice were found to accelerate the development of prostatic epithelial neoplasia (PIN), suggesting a protecting role of selenoproteins during prostate cancer development [97]. The G37 mice demonstrated enhanced muscle growth in the setting that modeled exercise overload. These data correlated with the initial activation of the insulin signaling pathway, which included increased Akt and p70 phosphorylation [98]. Abnormal insulin signaling might be, in part, the reason for glucose intolerance and lead to a diabetes-like phenotype, that was recently observed in the G37 mice [88].

3.3 Trsp knockout mouse models

Another approach of inactivating selenoprotein function in mice is to target the Trsp gene. The complete KO of Trsp leads to embryonic lethality [99], but a conditional removal of Trsp is possible [100]. Development of the tissue-specific KO models helped examining important functions of selenoproteins in the heart and skeletal muscle, endothelial cells [101], skin [102], bone [103], neurons [104] and the immune cells (macrophages, T cells and hematopoietic tissues) [105–107], and also studying more dispensable selenoprotein functions in the liver [58, 108], mammary gland [100] and podocytes [109]. KO of Trsp in the endothelial cells led to embryonic death at day E14.5 due to necrosis of the central nervous system, erythrocyte immaturity and subcutaneous hemorrhage. Mice with the myocyte-specific Trsp KO died 12 days after birth from acute myocardial failure [101]. Deletion of Trsp in the skin resulted in runt phenotype, epidermal neoplasia, and abnormal development of the hair follicles. Altogether, these abnormalities induced weight loss and early death. Thus, selenoproteins have a role in maintaining skin integrity [110]. Osteo-chondroprogenitor-specific Trsp KO mice showed multiple skeletal abnormalities, including growth retardation, abnormal epiphyseal plates, delayed ossification, and chondronecrosis of cartilage [103]. The neuron-specific Trsp KO induced severe neurodegeneration in the hippocampus and led to the absence of certain interneurons [110] (similar to what was observed in the neuron-specific GPx4 KO model). Besides, these mice showed degeneration of the Purkinje and granule cells that led to cerebral hyperplasia. In several studies, Se modulated the immune response. To understand the function of selenoproteins in immune cells, T and B cell-specific Trsp KO mice were developed. The T-cell-specific Trsp KO decreased the pool of mature T-cells and impaired T-cell dependent antibody response. Lack of antioxidant enzymes caused extensive oxidative stress and weak proliferation in response to T-cell receptor stimulation [106]. Macrophage specific-Trsp KO mice showed impaired invasiveness, which might be explained by hyperproduction of ROS and altered expression of extracellular matrix proteins [105]. Ablation of the Trsp gene in hematopoietic tissues resulted in anemia, which led to an increased production of erythroid progenitors in the bone marrow as well as to thymus atrophy [107]. The liver-specific Trsp KO induced expression of phase II enzymes, including various GSTs [111]. By preventing SelP synthesis and secretion, the liver-specific Trsp KO dramatically decreased plasma SelP. Thus, these mice showed symptoms of Se deficiency, which could be rescued by increased Se intake [93]. The mammary gland-specific Trsp KO mice showed increased levels of p53 and decreased expression of BRCA1 tumor suppressor [100]. In addition, mice carrying a knockout of Trsp in the liver were found to have an increased apolipoprotein E (ApoE) level and elevated cholesterol levels in plasma that was accompanied by enhanced expressed of the genes involved in cholesterol biosynthesis, metabolism and transport [112]. Interestingly, transgenic mouse models that express housekeeping, but not stress-related selenoproteins restored the expression of these genes (made them close to the corresponding levels observed in wild type controls). These studies showed that housekeeping selenoproteins have a role in regulating lipoprotein biosynthesis and metabolism and were consistent with the earlier studies showing that selenium deficiency increased ApoE expression. Overall, mouse models with conditional Trsp KO turned out to be a powerful tool for understanding functions of selenoproteins in various tissues.

3.4 Knockout/transgenic mouse models

An additional strategy to investigate the effect of transgene overexpression is to develop KO/transgenic animal models. In the case of selenoproteins, liver Trsp KO mice were crossed with the G37 or A34 mice. In both cases, similar expression patterns of housekeeping selenoproteins were observed. As discussed above, restoration of housekeeping selenoprotein genes partially decreased elevated levels of ApoE and serum cholesterol that had been observed in the liver-specific Trsp KO. Another useful knockout/transgene mouse model was also described [113]. STAF (Sec tRNA gene transcription activating factor) is a transcription factor for several RNA PolII and RNA PolIII-dependent genes. In this study, the authors overexpressed Trsp lacking the STAF binding promoter region and afterwards removed the WT Trsp. Interestingly, removal of the STAF binding site did not affect Trsp levels in the heart and testis, but showed severe reduction of the transgene in the liver, kidney, lung, spleen, and brain. Moreover, methylation of Trsp at A34 was significantly decreased, and expression of stress-related selenoproteins was reduced. These mice demonstrated the neurological phenotype similar to that of SelP KO mice. These findings indicated the importance of the STAF binding region in regulation of Sec $tRNA^{[Ser]Sec}$ expression and its proper modification status.

4. Concluding remarks

Development of appropriate animal models is a critical step in the characterization of biological functions of genes. A great deal of research in the area of Se biology was devoted to the understanding of functions of this micronutrient and selenoproteins in health and disease. It is clear from the discussion above that the functions of several selenoproteins and their forms could not have been determined without the use of appropriate KO models. However, there are also several obstacles resulting from the analysis of human diseases associated with the genetic defects in selenoprotein biosynthesis. For example, the presence of hypomorphic alleles of SBP2 gene was associated with retarded growth due to thyroid axis imbalance in children. At the same time, these patients experienced myopathy, waddling gait and mental retardation [114, 115] and were characterized by bilateral hearing loss and infertility. Recent research demonstrated that mutations in SecS gene are associated with the development of autosomal-recessive progressive cerebellocerebral atrophy [116] and that the observed phenotypes could be partially reproduced in the corresponding KO animal models. Indeed, analysis of the SelP KO mice could explain all symptoms, except for abnormalities in the thyroid function. While hypothyroidism is one of the first complaints in patients with impaired Sec incorporation pathway, in mice this effect is less pronounced. There are also several open questions. For example, mice maintained on the Se-deficient diet survive for more than one year with no visible abnormalities. The lifespan of the G37 transgenic mice was not affected: these mice were fully fertile, and did not develop symptoms similar to those in the patients with defects in SBP2. Understanding the reasons for the differences between human and mouse phenotypes could provide important new insights into the role of Se, Sec and selenoproteins in human health, and also into the molecular mechanisms of Sec incorporation and selenoprotein function. This research may also reveal novel regulatory mechanisms.

Highlights

- ➢ Se regulates pathways through incorporation into selenoproteins in the form of Sec
- ➢ Recent findings in selenoprotein biosynthesis and functions are summarized
- ➢ Overview of available knockout mouse models relevant to Se biology is provided
- ➢ Mouse models with targeted expression of selenoproteins are described
- ➢ Limitations of using animal models and insights into human health are discussed

Abbreviations

Cys	cysteine
DI1	thyroid hormone deiodinase type 1
DI2	thyroid hormone deiodinase type 2
DI3	thyroid hormone deiodinase type 3
ER	endoplasmic reticulum
ERAD	ER associated degradation
ICP-MS	inductively coupled plasma mass spectrometry
GF	germ-free
GPx1	glutathione peroxidase 1
GPx2	glutathione peroxidase 2
GPx3	glutathione peroxidase 3
GPx4	glutathione peroxidase 4
GSH	glutathione
I	iodine
MsrA	methionine-*S*-sulfoxide reductase
MsrB	methionine-*R*-sulfoxide reductase
NS	neuronal system
SC	satellite cells

Se	selenium
Sec	selenocysteine
SECIS	selenocysteine insertion sequence
SelH	selenoprotein H
SelI	selenoprotein I
SelK	selenoprotein K
SelM	selenoprotein M
SelN	selenoprotein N
SelO	selenoprotein O
SelP	selenoprotein P
SelS	selenoprotein S
SelT	selenoprotein T
SelV	selenoprotein V
SelW	selenoprotein W
Sep15	the 15 kDa selenoprotein
SPS1	selenophosphate synthetase 1
SPS2	selenophosphate synthetase 2
TR1	thioredoxin reductase 1
TR3	thioredoxin reductase 3
Trsp	Sec tRNA$^{[Ser]Sec}$ gene
UGT	UDP-glucose:glycoprotein glucosyltransferase
UPR	unfolded protein response

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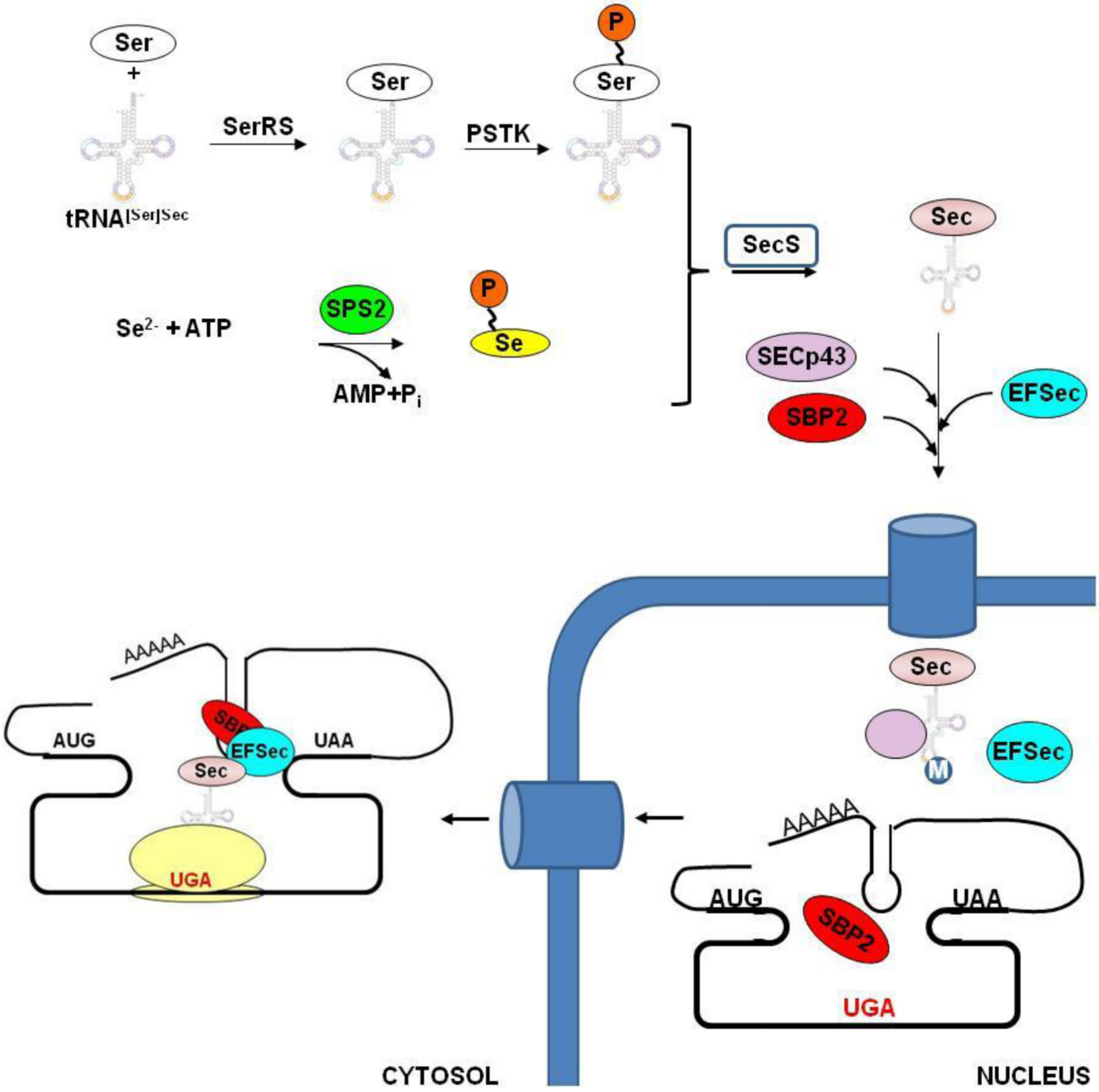

Figure 1. Mechanisms of eukaryotic Sec biosynthesis and incorporation

Sec tRNA$^{[Ser]Sec}$ is initially charged with Ser, which is further phosphorylated by PSTK. SPS2 facilitates the synthesis of selenophosphate, the selenium donor compound. SecS then catalyzes Sec formation. SECp43 may be involved in the methylation of Sec tRNA$^{[Ser]Sec}$ at the A34 position. Protein factors, including SBP2 and EFSec, bind the SECIS element, located in the 3’-UTRs of selenoprotein mRNAs. After translocation to the cytosol, protein factors support interaction with the ribosome and Sec incorporation.

Table 1

Mammalian selenoproteins: localization and functions

Selenoprotein	Localization	Function	References
15 kDa selenoprotein (Sep15)	ER	- Trx-like fold - regulated by ER stress - interacts with UDP-glucose: glycoprotein glucosyltransferase - potentially involved in glycoprotein folding	[21, 117–119]
Thyroid hormone deiodinase 1 (DI1, Dio1)	Plasma membrane	- removes iodine from the outer ring of T4 to produce plasma T3 - catalyzes deiodination and thus inactivation of T3	[120, 121]
Thyroid hormone deiodinase 2 (DI2, Dio2)	ER	- converts T4 to T3 locally in tissues	[48]
Thyroid hormone deiodinase 3 (DI3, Dio3)	Plasma membrane	- catalyzes deiodination of T4 to T3 in peripheral tissues	[121, 122]
Glutathione peroxidase 1 (GPx1)	Cytosol	- GSH-dependent detoxification of H_2O_2 (enriched in liver, kidney, erythrocytes)	[13]
Glutathione peroxidase 2 (GPx2)	Cytosol	- GSH-dependent detoxification of H_2O_2 (enriched in the epithelium, especially in the intestine and lung)	[123, 124]
Glutathione peroxidase 3 (GPx3)	Plasma	- GSH-dependent detoxification of H_2O_2 (synthesized predominantly by kidneys and secreted to plasma)	[125]
Glutathione peroxidase 4 (GPx4, PHGPx)	Cytosol Mitochondria nucleus (testis-specific)	- has cytosolic, nuclear and mitochondrial isoforms - protects lipids from H_2O_2-mediated oxidation	[36]
Glutathione peroxidase 6 (GPx6)	Cytosol	- GSH-dependent detoxification of H_2O_2(enriched in the olfactory epithelium)	[124]
Selenoprotein H (SelH)	Nucleus	- Trx-like fold - protects cells from H_2O_2, increases mitochondrial biogenesis and CytC production - AT-hook family protein. In response to redox changes facilitates synthesis of genes responsible for *de novo* GSH synthesis and phase II detoxification	[126–128]
Selenoprotein I (SelI)	Membrane	unknown function	[12]
Selenoprotein K (SelK)	ER membrane	- modulates Ca^{2+} influx that affects immune cell function - component of ERAD	[66]
Selenoprotein M (SelM)	ER	- Trx-like fold - protects neurons from oxidative stress	[129]
Selenoprotein N (SelN, SEPN1, SelN1)	ER membrane	- expressed in skeletal muscle, heart, lung, and placenta	[24, 130]

Selenoprotein	**Localization**	**Function**	**References**
		- controls redox state of the intracellular calcium-release channel (ryanodine receptor (RyR)), and therefore affects Ca^{2+} homeostasis - mutations in SelN gene cause congenital myopathy	
Selenoprotein O (SelO)	Mitochondria	- unknown function	[12]
Selenoprotein P (SelP)	Plasma	- Se transport to peripheral tissues and antioxidant function	[62, 131, 132]
Selenoprotein R (SelR, MsrB1, Selx1)	Cytosol	- reduces methionine-R-sulfoxide residues in proteins to methionine	[18]
Selenoprotein S (SelS, SEPS1, Tanis, VIMP, and SELENOS)	ER membrane	- upregulated upon treatment with pro-inflammatory cytokines and glucose deprivation - ERAD component	[23, 24]
SPS2	Cytosol	- synthesis of selenophosphate	[82, 133]
Selenoprotein T (SelT)	ER and Golgi	- Trx-like fold - redox regulation - plays a role in cell adhesion	[134]
Thioredoxin reductase 1 (TR1, Txnrd1)	Cytosol	- reduces the oxidized form of cytosolic thioredoxin - has at least 6 isoforms differing in N-terminal sequences	[15, 135]
Thioredoxin/glutathi one reductase (TGR, TR2, Txnrd3)	Cytosol	- has a glutaredoxin domain - catalyzes a variety of reactions, specific for thioredoxin and glutaredoxin systems - expressed in spermatids	[136]
Thioredoxin reductase 3 (Txnrd2, TR3)	Mitochondria	- reduces the oxidized form of mitochondrial thioredoxin and glutaredoxin 2	[137]
Selenoprotein V (SelV)	Cytosol	- Trx-like fold - unknown function - expressed in spermatids	[126]
Selenoprotein W (SelW)	Cytosol	- Trx-like fold - unknown function - expressed in skeletal muscle and other tissues	[138]

Table 2

Knockout of individual selenoprotein genes in mice

Gene	Approach	Phenotype	References
GPx1	Whole body	- no gross phenotypes - susceptibility to oxidative stress and viral miocardites - acceleration of cardiac hypertrophy and dysfunction - reduced blood insulin and reduced islet β-cell mass in the pancreas	[13, 139]
GPx2	Whole body	- no gross phenotypes - increased apoptosis in colon crypt cells during Se deficiency	[42]
GPx1+GPx2	Whole body	- microflora-dependent intestinal colitis - decreased levels of Paneth cells	[43]
GPx3	Whole body	- no gross phenotypes	[44]
GPx4	Whole body	- embryos die at E7.5	[33]
GPx4	Neuron specific	- severe neuodegeneration	[34, 36]
GPx4	Spermatid-specific	- male infertility	[38]
nGPx4	Whole body	- delayed sperm chromatin condensation	
mGPx4	Whole body	- male infertility	
TR1	Whole body	- embryos die at between E8.5 and E10.5	[27]
TR1	Cardiomyocyte-specific	- no gross phenotype	[27]
TR1	Neuron-specific	- neurological symptoms, including tremor and ataxia as a result of cerebral hypoplasia	[29]
TR3	Whole body	- embryos die at between E13.5 and E15.5	[30]
TR3	Cardiomyocyte-specific	- heart failure	[30]
TR3	Neuron-specific, T-and B cells specific	- no gross phenotype	[30, 31]
DI1	Whole body	- no gross phenotypes - increased iodine excretion	[46]
DI2	Whole body	- pituitary resistance to T4 - impaired thermogenic response to cold - at thermoneutral conditions, high fat diet induced glucose intolerance, and exacerbated hepatic steatosis - poor hearing, poorly differentiated sensory epithelium	[48, 49]
DI3	Whole body	- reduced levels of circulating T4 and T3	[52, 53]

Gene	Approach	Phenotype	References
		- retarded development - deafness with premature cochlear differentiation	
DI1+DI2	Whole body	- mild hypothyroidism - the sum of DI1 KO and DI2 KO phenotypes	[51]
SelP		- neuronal degeneration, leading to ataxia and seizures - reduced selenoprotein expression in peripheral tissues - male infertility	[54–56]
MsrB1	Whole body	- no gross phenotypes - increased markers of oxidative stress	[65]
Sep15	Whole body	- congenital cataract	[67]
SelK	Whole body	- no gross phenotypes - impaired function of immune cells	[66]
SEPN1	Whole body	- limited motility and body rigidity in response to physical exercise - poor muscle regeneration due to age and injury induced SC loss	[69, 70]