Chapter 22
Selenoprotein P and Selenium Distribution in Mammals

Ulrich Schweizer, Lutz Schomburg, and Josef Köhrle

Abstract Selenoprotein P (Sepp) is a remarkable selenoprotein. Among all mammalian selenoproteins, it is the only one containing more than one selenocysteine, and ten in humans. Sepp is a plasma protein mainly originating in the liver, but it is also expressed in other organs, notably the brain, placenta, and the lactating mamma. The main function of Sepp is transport of selenium (Se) to target tissues. Hepatocytes thus convert nutritional selenocompounds into Sepp for transport and distribution, and the mother's milk contains Sepp as an essential gift to the offspring. The fact that about 25 % of all selenoprotein mRNAs in hepatocytes code for Sepp alone highlights its central position in the body's Se homeostasis. Selenium status of an individual is thus reflected by the serum concentration of Sepp. Endocytic receptors of the lipoprotein receptor-related protein (Lrp) family participate in targeting cell-specific Sepp uptake and retention. A Sepp-cycle exists in brain, testes, and kidney and appears to preserve tissue Se during times of poor nutritional supply, explaining the long known hierarchical differences in tissue-specific Se retention in deficiency. Individual genotype differences may modulate these processes exerting an influence on the relative expression levels of selenoproteins, response to Se intake, and individual risk for Se-dependent diseases.

Keywords ApoER2 • Lrp2 • Lrp8 • Megalin • Plasma • SelP • SePP • Sepp1 • Transport

U. Schweizer (✉)
Institute für Biochemie und Molekularbiologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Nussallee 11, 53115 Bonn, Germany
e-mail: ulrich.schweizer@uni-bonn.de

L. Schomburg • J. Köhrle
Institut für Experimentelle Endokrinologie, Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
e-mail: lutz.schomburg@charite.de; josef.koehrle@charite.de

D.L. Hatfield et al. (eds.), *Selenium*, DOI 10.1007/978-3-319-41283-2_22

22.1 Introduction

Sepp was initially discovered as a plasma protein incorporating ^{75}Se in metabolically labeled rats [1]. In the same report, the authors demonstrated uptake of ^{75}Se-Sepp into extrahepatic organs and suggested a transport function for this protein. Later studies showed that Sepp contains up to 50 % of plasma Se in individuals with normal Se status [2]. When the cDNA for *Sepp* was cloned, it became apparent that it contains ten selenocysteine (Sec) codons within the open reading frame in both rodents and humans, and two separate **Sec**-**i**nsertion **s**equence (SECIS) elements in the 3′-untranslated regions of the transcripts [3]. This finding was important as it demonstrated that several UGA/Sec codons can share the same SECIS element in mammals, while in bacteria the SECIS elements are located immediately 3′ of the respective UGA codon [4]. Sepp carries one Sec within an N-terminal domain with a predicted similarity to thioredoxin and peroxidases, and a C-terminal Sec-rich domain with no known structure (Fig. 22.1). While peroxidase activity of the N-terminal part of Sepp has been demonstrated repeatedly, its physiological role remains unclear [5, 6].

22.2 Selenoprotein P (Sepp) Is a Plasma Selenium Transport Protein

Our current model of Sepp function is summarized as follows: Sepp is a plasma Se transport protein mainly secreted by liver and is taken up in target tissues by interaction with endocytic receptors. These key findings support our model: dietary Se intake determines plasma Sepp and plasma Se concentrations [7]. The unusual Sec-rich C-terminus of Sepp is partially conserved among species and its main property appears to be its content of many Sec residues [8]. Metabolic labeling studies indicated that dietary Se is rapidly taken up by the liver and incorporated into circulating Sepp which, slowly declining, gives rise to increasing kidney-derived plasma glutathione peroxidase (Gpx) concentrations [9]. Interestingly, Sepp constitutes the essential Se-containing component in serum-based cell culture media supporting growth and survival of primary neuronal cells [10, 11]. Transgenic mice with genetically disrupted Sepp biosynthesis (*Sepp*-knockout, *Sepp*$^{-/-}$) develop a number of Se-dependent phenotypes including growth defect, male infertility and neuronal abnormalities [12, 13] that can be rescued by liver-specific expression of human SEPP1 [14]. The identification of specific Sepp binding and uptake by members of the lipoprotein receptor-related protein (Lrp) family confirms transport by Sepp as a regulated and targeted process of Se supply to specific target tissues [15–17]. This concept has been integrated with the mechanisms of hierarchical Se retention and excretion [18].

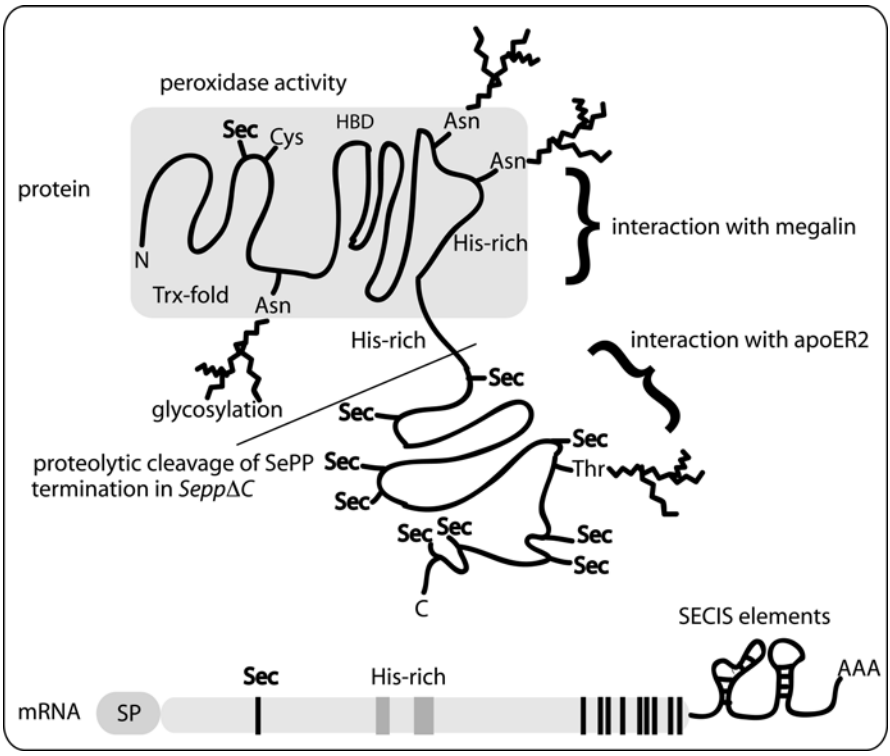

Fig. 22.1 Structural features of the two domains of selenoprotein P (Sepp). The N-terminal domain, predicted to adopt a thioredoxin (Trx)-fold, contains a Sec-X-X-Cys motif, showing weak peroxidase activity. The Sec-rich C-terminal domain is without homology to any known protein and likely serves a Se transport function. A classical N-terminal signal sequence (SP) directs Sepp biosynthesis into the ER lumen. The secreted protein carries three N-glycosylation sites (Asn) and one O-linked (Thr) glycosylation site. A heparin-binding-domain (HBD) has been identified along with two His-rich domains, which potentially mediate association to the extracellular matrix. Sepp isoforms may result from differential glycosylation, proteolytic cleavage or premature translational termination

22.3 Lipoprotein Receptor-Related Proteins as Endocytic Receptors Involved in Sepp Uptake

Lrps are endocytic receptors for lipoproteins, hormone- and vitamin-binding proteins, and morphogens [19–21]. These receptors usually are capable of binding many cargo proteins. Lrp2 (megalin or glycoprotein-330) is expressed along many epithelia, e.g., kidney proximal tubule cells, and binds vitamin D3/vitamin D binding globulin, progesterone/clara cell secretory protein, vitamin A/retinol binding protein, androgens and estrogens/sex-hormone binding globulin, thyroid hormones/transthyretin, vitamin B12/transcobalamin, and folate/soluble folate receptor [20, 22]. The physiological importance of Lrp2-mediated (re-)uptake and

internalization of hydrophobic ligands along with their high molecular weight carrier proteins is evident from their loss in a number of animal models and in patients with inherited deficiency in megalin/LRP2 [21, 23, 24]. The additional importance of Lrp2 for Se homeostasis by specific binding and re-uptake of Sepp came initially as a surprise to the field of Se biology [15, 25], but appears highly plausible as a logical addition to the list of functions for this internalizing system controlling the uptake of essential circulating serum factors such as nutrients, vitamins and hormones. Lrp-2 binds the N-terminus of Sepp and prevents the urinary loss of Se as found in *megalin*$^{-/-}$ mice [6, 25].

Lrp8 (Apolipoprotein E receptor 2, ApoER2) is highly homologous to the low density lipoprotein receptor (LDL-R) and the very low density lipoprotein-receptor (VLDL-R). Lipoprotein receptors can participate in signal transduction, e.g., Lrp8 is one of the cell-surface receptors involved in the reelin signaling pathway. Reelin is a large neuronal signaling molecule guiding neuronal cell migration during central nervous system development [26] and interacts with both Lrp8 and VLDL-R. Genetic inactivation of both receptors leads to neuronal migration deficits, tremor, and ataxia [27]. Mutations targeting the signaling function of Lrp8 do not affect Se metabolism [28]. However, inactivation of *Apoer2* leads to a similar neurological phenotype as observed in *Sepp*$^{-/-}$ mice when fed a low Se diet [17, 29]. This phenotypic similarity is explained by the interaction of Lrp8/Apoer2 with Sepp [16]. Accordingly, brain Se levels are strongly reduced in *Apoer2*$^{-/-}$ mice [17]. Lrp8 binds Sepp only if part of the Se-rich C-terminus is present [30]. Taken together, Sepp in combination with Lrp2 and Lrp8, respectively, constitute a Se-uptake and -delivery system essential for preferentially supplying Se to target organs and avoiding Sepp loss through the kidney.

22.4 Mouse Models of Modified Sepp, Lrp2 or Lrp8 Expression

22.4.1 Classical Gene Targeting

Genetic inactivation of *Sepp* profoundly disturbs Se metabolism in mice [12, 13]. As expected for the inactivation of the plasma Se transport protein, circulating Se concentrations were decreased in *Sepp*$^{-/-}$ mice. Moreover, Se content in brain and testes, organs normally preferentially supplied with Se, were strongly reduced. Male *Sepp*$^{-/-}$ mice were infertile and the appearance of sperm from *Sepp*$^{-/-}$ mice resembled sperm from *Apoer2*$^{-/-}$ mice [14, 31, 32]. A more detailed description of the role of Se in male fertility can be found in Chaps. 17 and 18.

Sepp$^{-/-}$ mice developed a neurological phenotype which was strictly dependent on Se content in their diet [29, 33]. The role of Se in brain is reviewed in Chap. 36. Sepp not only has a role in Se distribution, but can serve as a local Se storage device. It is not only expressed in the liver, but also in other organs, e.g., the brain. Provided

that Sepp is prevented from leaving the source organ (i.e., brain), it can be safely deposited outside of cells and taken up again, thus maintaining a stable Se level in the brain compartment during times of low nutritional Se supply. We called this mechanism a "Sepp-cycle" (Fig. 22.2) [18, 34].

Hepatic Sepp biosynthesis plays an important role in regulating whole body Se status [35]. *Sepp*$^{-/-}$ mice show increased loss of Se via the urine. In the absence of Sepp biosynthesis, increased hepatic Se becomes available for the production of small selenocompounds which are excreted via the urine, e.g., trimethylselenonium and selenosugars [18, 36]. Sepp also serves as the main source of Se in mouse and human milk [29, 37]. More recently it has been shown that glutathione peroxidase 3 (Gpx3) is also present in mouse milk. However, only Sepp contributed to Se transfer to offspring [38].

Lrp2$^{-/-}$ mice usually die perinatally [39], but *megalin*-deficient mice carrying a nonsense mutation on a different genetic background survive into adulthood [40]. We have used these mice as a model system for the analysis of the physiological functions of Lrp2 in adult mice, as discussed later, with respect to renal Sepp metabolism [25]. These results have recently been independently supported [6].

In contrast, *Lrp8*$^{-/-}$ mice are born at the expected Mendelian ratio and appear grossly normal. Reduced male fertility of these mice was associated with a reduced expression of the selenoenzyme Gpx4 in the initial segments of the epididymis [32]. Gpx4 is an important structural component of sperm [41]. Therefore, male infertility of *Lrp8*$^{-/-}$ mice appears secondary to decreased selenoprotein expression in sperm which in turn depends on Sepp-mediated Se supply [42]. Se deficiency also develops in the brains of *Lrp8*$^{-/-}$ mice and these could be rescued with increased dietary Se intake [17]. Selenite appears to be highly bioavailable to the brain, a finding which is of limited physiological importance as selenite is not a usual component of our diet, but needs to be considered in view of the supplementation of animal diets with sodium selenite or in rare diseases where normal Se transport is impaired.

22.4.2 Isoforms of Sepp

The structure of Sepp containing two clearly defined domains implies two separate functions for the protein, i.e., a peroxidase activity associated with the first Sec residue within the N-terminal thioredoxin-like domain and a Se supply function assigned to the C-terminus which harbors the remaining nine Sec residues [43]. Biochemical experiments have demonstrated that purified Sepp elicits phospholipid hydroperoxide peroxidase activity with glutathione [5], but thioredoxin was identified as a superior cofactor in vitro [44]. More recently, it was reported that the N-terminal domain of Sepp contains considerable peroxidase activity when coupled to thioredoxin reductase 1 [6]. In order to delineate the significance of the two domains in a physiological model, transgenic mice with a shortened Sepp isoform lacking the C-terminal Sec-rich domain were generated and compared to wild type

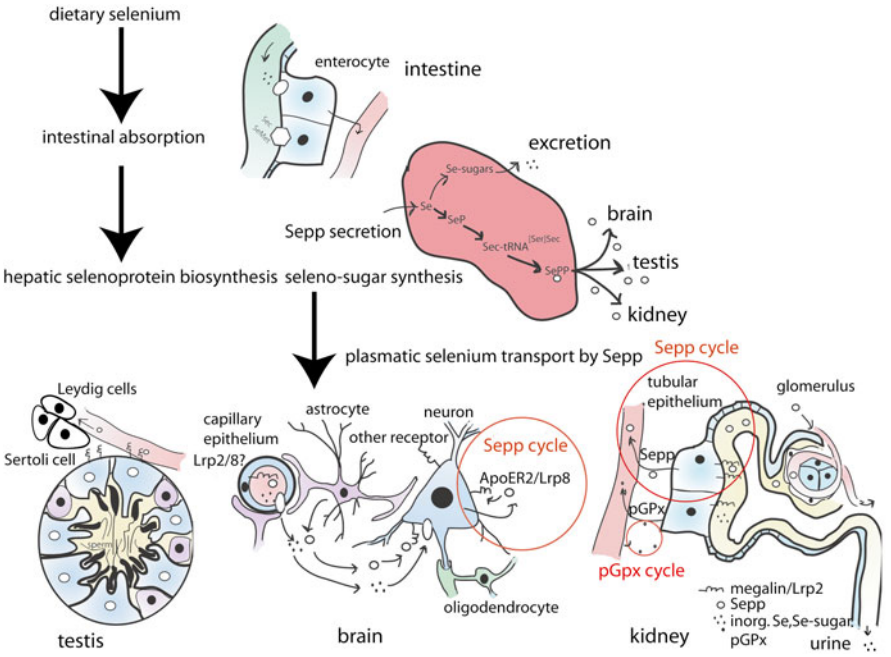

Fig. 22.2 Summary of Se transport processes described in vivo. Dietary Se is primarily taken up along the absorptive epithelium of the small bowel by transporters specific for inorganic divalent anions (e.g., the sodium-dependent sulfate transporter, NaSi-1) or transporters for amino acids and small peptides. Via portal circulation, different selenocompounds reach the liver where the selenium atom is converted into Sec on $tRNA^{[Ser]Sec}$ and subsequently translationally inserted into selenoproteins. Gpx1 has been proposed as a hepatic storage form for excess Se, which may also be converted into selenosugars or selenonium ions and excreted. The liver is central to Se metabolism as the major source of plasma Sepp, which transports Se to privileged target tissues, e.g., brain, testis, and kidney. Brain Se supply is complicated, since several cellular membranes must be crossed to finally reach the neurons. Megalin may be involved in Sepp uptake along the choroid plexus and ependymal epithelium, while ApoER2 is expressed by neurons. Astrocytes synthesize Sepp in vitro and may thus contribute to neuronal Se supply. Neurons express Sepp and may store excess Se outside the cell in the form of Sepp. Brain retains its privileged Se status during dietary restriction via reversible Sepp expression, extracellular deposition and re-uptake. We have termed neuronal Sepp synthesis and ApoER2-mediated Sepp re-uptake in brain as "Sepp-cycle". Testis function likewise depends on ApoER2-mediated Sepp uptake. Inactivation of either protein leads to decreased Gpx4 expression in maturing spermatozoa and infertility. Megalin expressed along the kidney tubular epithelium is involved in re-uptake of Sepp from the primary glomerular filtrate. Accordingly, inactivation of megalin leads to urinary loss of Sepp. Megalin-positive cells express the highest levels of Gpx1, Gpx3, and Sepp within the kidney, and inactivation of megalin decreases expression of all three proteins. Plasma Gpx3 originates from the kidney epithelium, but most Gpx3 is deposited locally within the kidney. Another "Sepp-cycle" can thus be proposed involving glomerular filtration, re-uptake, and renal re-synthesis of Sepp. Kidney insufficiency in patients is associated with low Se status. Tissues expressing selenoproteins, but not expressing ApoER2 and megalin likely operate by a still elusive Se uptake mechanism, which might rely on the poorly characterized selenocompounds from the gastrointestinal tract

and classical *Sepp*$^{-/-}$ mice [45]. Testis and brain Se concentrations were only slightly higher than in *Sepp*$^{-/-}$ mice. These findings corroborate that the C-terminus of Sepp is important for Se supply to the hierarchically preferred target tissues, which express ApoER2/Lrp8, the receptor for the Sepp C-terminus (Fig. 22.2). Apparently, evolution added a C-terminal extension to Sepp, whose only function is to make Se transport more efficient and targeted towards Lrp2 and Lrp8 expressing specific tissues. The peroxidase activity of the N-terminus of Sepp may be needed to limit tissue damage during infection with Trypanosomes [46].

22.4.3 Sepp in the Liver

Se organification, Se distribution, and Se metabolism/excretion are organized by the liver. Experimental studies have shown that liver is the organ converting dietary Se into circulating Sepp for supply of other tissues [9, 47]. Accordingly, liver disease leads to reduced serum Se and Sepp concentrations in patients [48]. Because Sepp is expressed in most tissues [49], cell type-specific gene targeting was used to delineate the role of hepatic Sepp expression for Se metabolism. Mice carrying a conditional allele of tRNA$^{[Ser]Sec}$ (*Trsp*$^{fl/fl}$) were crossed with a cell-specific Albumin-Cre strain abrogating selenoprotein biosynthesis specifically in hepatocytes [50]. The mice were viable and showed almost complete loss of hepatic *Trsp* at 3 weeks of age. As expected, circulating Se and Sepp concentrations were strongly decreased [50, 51]. In these mice, kidney Se and kidney-derived Gpx3 concentrations were also strongly reduced [51]. Testis and brain Se levels and brain selenoprotein expression were also lower in liver-specific *Trsp*-knockout mice indicating that liver-derived circulating Sepp is indeed the Se transfer protein bringing Se to Sepp-dependent tissues [51]. These studies were later independently supported by another group, who conditionally inactivated *Sepp* in hepatocytes [52]. Kidney and brain Se levels were decreased in liver-specific *Sepp*$^{-/-}$ mice and after feeding these mice for a prolonged time with a Se-deficient diet, the mice developed a neurological phenotype. The dependence of testis on circulating Sepp was further demonstrated by the morphological abnormalities of sperm from liver-specific *Sepp*$^{-/-}$ mice which were also described in classical *Sepp*$^{-/-}$ mice [52]. In addition, urinary Se excretion was increased, most likely because excess hepatic Se (not incorporated into Sepp and secreted into plasma) became available for hepatic selenosugar biosynthesis and excretion.

What role does hepatic Sepp play in the absence of *Sepp* expression in the rest of the body? This question was addressed in a complementary mouse model, in which hepatocyte-specific expression of a human *SEPP1* transgene was studied in a *Sepp*$^{-/-}$ background [14]. Compared to *Sepp*$^{-/-}$ mice, those with liver-specific expression of *SEPP1* had increased Se concentrations in most tissues, were less sensitive to Se restriction in terms of neurological dysfunction, and had restored male fertility [14]. Nevertheless, without locally expressed Sepp, the brain remained more sensitive to

dietary Se restriction despite hepatically-derived circulating SEPP1. These observations support the concept of a local "Sepp-cycle" in brain and possibly other organs (Fig. 22.2).

22.4.4 Sepp in the Brain

The brain depends on Se supply via circulating Sepp [13, 14, 33, 52]. SEPP is locally expressed in the human brain [53, 54]. Lrp8 is expressed on neurons and along the blood-brain-barrier and is of importance for Se uptake by neurons [17], although Lrp8 may not represent the only Sepp receptor in the brain (see Chap. 36 for a detailed discussion). Megalin contributes to Se uptake along the blood-brain-barrier [6, 25]. The model of a Sepp-cycle in brain has recently obtained additional support when it was shown that lower Se levels in the brain were tolerated as long as both Sepp and ApoER2 were expressed in the brain [55] (Fig. 22.2). The roles of various selenoproteins in brain development, function, and degeneration are treated in Chap. 36. Interestingly, a recent mouse study highlighted a potential competition between Sepp-mediated Se supply to testes versus to brain, where one tissue may profit from the other tissues in cases where uptake is tissue-specifically impaired [56].

22.4.5 Sepp in the Kidney

The importance of Sepp for kidney Se status and metabolism has not been addressed by tissue-specific knockout studies of *Lrp2*, *Trsp* or *Sepp*. Inactivation of selenoprotein expression (conditional targeting of *Trsp*) in kidney (using a *Pax8-Cre*, which is expressed in the developing kidney and thyroid) was lethal [57], possibly because loss of Gpx4 leads to ferroptosis in kidney tubules [58]. Instead, the physiological role of Sepp for kidney Se can be deduced from a number of findings in the aforementioned Sepp-specific mouse models. Kidney Se concentrations were strongly decreased in $Sepp^{-/-}$ mice. This finding implies that either hepatically-derived circulating Sepp transports Se to the kidneys or that renal Sepp biosynthesis itself is crucially important for controlling local tissue Se content [12, 13]. In order to solve this conundrum, hepatic or renal Sepp biosynthesis were specifically disrupted. Hepatocyte-specific inactivation of *Trsp* abrogated biosynthesis of all selenoproteins in hepatocytes and strongly reduced Sepp levels in plasma [51]. Kidney Se concentrations were decreased in this model and in hepatocyte-specific *Sepp*-deficient mice [52]. Reduced renal selenoprotein expression in $Sepp^{-/-}$ mice was rescued by hepatic expression of the human *SEPP1* transgene [14].

Lrp2 is abundantly expressed in the kidneys [59]. Lrp2/megalin has been implicated as a renal Sepp receptor [15], but $Lrp2^{-/-}$ mice analyzed in this pioneering

study did not survive birth, and thus a role of Lrp2 in renal Sepp uptake could not be directly demonstrated. We have taken advantage of a different *Lrp2* mutant mouse strain in which a significant fraction of *megalin*-deficient mice survive into adulthood. These mice carry a missense mutation in the extracellular domain of Lrp2 developing a less severe phenotype as compared to classical *Lrp2*$^{-/-}$ mice [40]. When Se metabolism was analyzed in these *Lrp2*-mutant mice, Se status was low, Gpx activities were decreased in kidney and serum, and Sepp concentrations were reduced in serum [25]. Movement coordination deteriorated in *Lrp2*-mutant mice when challenged by feeding a low Se diet. Interestingly, full-length Sepp was detected in the urine of these mice indicating, on the one hand, that Sepp is partially filtrated by the glomeruli into the primary urine and, on the other hand, that renal Lrp2/megalin recognizes, binds and removes Sepp from the primary urinary filtrate and prevents Sepp loss in wild type mice [25]. Based on this study, *megalin*$^{-/-}$ mice were extensively backcrossed on a genetic background which allows them to survive into adulthood and several of our initial observations have been supported by a more recent study [6]. Se metabolism in kidney is summarized in Fig. 22.2.

22.5 Regulation of Sepp Expression

Sepp gene expression has been studied in several cell types and experimental models. Proinflammatory cytokines such as interleukin-1α, TNFα, and interferon-γ suppress gene expression in cell lines involving activation of nitric oxide synthase-2 [60, 61], and TGFβ represses Sepp transcription by a SMAD-binding element in the proximal human promoter [62]. Similarly, interleukin-6 suppresses the Sepp promotor and down regulates Sepp biosynthesis in hepatocytes in culture [63]. Besides these cytokines, hypoxic conditions also negatively affect Sepp biosynthesis [64], which may collectively explain the particularly strong down-regulation of Sepp biosynthesis and Sepp serum concentrations in severe diseases like sepsis [65].

Recently, positive regulation of Sepp expression was also reported after activation of the forkhead box transcription factor, FoxO1a, and this effect was enhanced by overexpression of peroxisomal proliferator activated receptor-γ coactivator 1α (PGC-1α) [66]. These observations link Sepp expression to the glucose levels regulating hormones insulin and the adrenal glucocorticoids. In cultured hepatocytes, high glucose supported Sepp biosynthesis [67]. Accordingly, Sepp is proposed to serve as an insulin-antagonistic hepatokine, and higher Sepp concentrations were reported in diabetic subjects [68]. This study, unfortunately, has not addressed the established role of Sepp as a Se transport protein, and thus failed to consider Sepp-dependent stimulation of selenoprotein expression in insulin targeted tissues. The expression of selenoproteins in Sepp-target cells would have been important, since enhanced expression of Gpx1 is known to cause insulin resistance in mice [69]. Collectively, the role, if any, of Sepp in diabetes is a matter of current debate and research, and prospective clinical studies on its potential impact are missing.

22.6 Comparison of Experimental Concepts with Clinical Data

A systematic comparison of potential biomarkers of Se status in humans has been compiled and published [70]. In comparison to plasma or red blood cell Se concentrations or Gpx activities in serum, plasma or blood, SEPP concentration turned out as the more reliable and versatile biomarker of Se status [71–73]. However, due to its apparently saturated expression upon high Se intake, SEPP reliably reflects Se status in poorly or moderately supplied subjects only. In well supplied populations as found in the United States, SEPP concentrations do not correlate to Se intake or total serum Se concentrations, as maximal levels are already achieved in the majority of subjects [74].

Recent population-based studies on the associations between various cancer forms and SNPs of genes encoding for selenoproteins and enzymes involved in metabolism of ROS provided some evidence that *SEPP1* variants (Ala234Thr, rs3877899; G>A in 3′UTR, rs7579) affect Se availability to target tissues such as prostate or colon thereby modulating cancer risk in the context of other gene-gene (e.g., SOD2), gene-nutrient or lifestyle interactions [75–78]. Its direct contribution to colorectal cancer risk in moderately supplied subjects has just been determined in the European prospective investigation of cancer and nutrition cohort (EPIC), where higher SEPP concentrations were inversely associated with colorectal cancer risk, especially in women [79]. Decreased expression of *SEPP* mRNA and SEPP protein has been reported for several preneoplastic and cancerous lesions, e.g., of the respiratory and the GI tracts (gastric, colorectal and hepatic cancers) as well as for the prostate [80–83]. This down-regulation may limit Se loss from tumor cells causing an apparent Se accumulation in tumors. Accordingly, the first clinical study (SECAR) has tried to selectively poison tumors by high dose selenite treatment of cancer patients [84], and determined a maximally tolerated dose of around 10 mg Se/square meter body surface. It will be most interesting to monitor SEPP concentrations under such high dose supplementation attempts, and assess how far Se accumulation by tumor cells qualifies as a novel cancer treatment strategy.

Unfortunately, SEPP status has not been monitored in several other relevant and large cancer, cardiovascular or metabolic trials (e.g., SELECT, NPC, SU.VI.MAX, SETCAP, etc.) analyzing potential protective or therapeutic effects of supplementation with selenocompounds alone or in combination with other antioxidative compounds such as vitamin E [85–87]. The recent availability of several specific antibodies recognizing human SEPP helped to clarify the picture of systemic SEPP distribution vs. local production indicated by cellular expression of *SEPP* transcripts. These studies confirmed previous hints that SEPP reaches various tissues and cells via circulation, but also provided evidence for local SEPP production and secretion, e.g., within the cerebrospinal fluid (CSF) and in brain ependymal cells [53, 54]. SEPP expression and immunostaining showed specific spatial and temporal patterns during brain development and pathological alterations in brains from patients suffering from neurodegenerative diseases such as

Alzheimer's [88, 89]. Together, these observations suggest a strategic location of SEPP in brain potentially protecting cell types of high activity and functional relevance from Se deficiency, thereby ensuring regular development, differentiation and expression of Se-dependent antioxidative defense systems.

Impaired renal function and chronic hemodialysis markedly impact serum SEPP and Se homeostasis in patients with consequences on their thyroid hormone status [90]. Patients on chronic hemodialysis and apheresis develop Se deficiency and frequently receive Se supplements [91]. Currently, it is not clear, whether they lose SEPP or its (shorter) isoforms during the filtration process or whether their damaged renal tissue expresses insufficient Lrp2/megalin for adequate reabsorption of filtrated SEPP. Since a low Se status is a negative prognostic factor for long-term survival of chronically ill patients, it appears mandatory to control the trace element status of hemodialysis patients in order to avoid severe Se deficiency [92].

Besides cancer, chronic and degenerative diseases, the Se status and SEPP are implicated in male fertility, which is reviewed in detail in Chap. 18. An inherited defect causing impaired SEPP biosynthesis and low circulating SEPP concentrations has recently been described in humans. Individuals with certain mutations in *SECISBP2* display very low or undetectable levels of SEPP [93]. Interestingly, some of these subjects are reported with mental retardation and abnormal gait [94] or delayed neurological and motor skill milestones [95]. However, apparent SEPP-deficiency in patients carrying *SECISBP2* mutations does not lead to a phenotype as severe as in *Sepp*$^{-/-}$ mice, indicating that brain Se metabolism is not completely impaired in these individuals which is in line with some detectable, albeit considerably lower levels of circulating SEPP. In contrast, severe neurological symptoms involving brain atrophy and epilepsy were reported in patients carrying a mutation in another rate-limiting factor of selenoprotein biosynthesis, i.e., the Sec synthase gene, *SEPSECS* [96]. However, Se and SEPP status in the CSF have not been determined in *SEPSECS*-deficient patients, but are likely reduced.

22.7 Concluding Remarks

The hepatically-derived SEPP constitutes the Se transport form in blood circulation. Preferential supply to certain tissues like brain, testes, bone or muscles is achieved via SEPP recognition and specific uptake mediated by Sepp-receptors from the Lrp family, namely Lrp2 and Lrp8. This interaction not only ensures the hierarchical supply, but also an efficient retention of Se in prioritized tissues. Moreover, renal Lrp2 is essential for avoiding Se decline through Sepp loss via the urine. Pharmacological intervention of these endocytic receptor/Sepp interactions might become a therapeutic option in certain forms of male infertility, neurodegenerative disorders or in patients exposed to excess oxidative stress, e.g., during various forms of chemotherapy, systemic inflammation, bacterial or viral infections. However, no endogenous or synthetic modulators of Sepp/Sepp receptor interaction have been described so far.

One of the important experimental tasks in the future will thus be the molecular characterization of the different Sepp isoforms, their physiological functions, regulation and interaction with the different Sepp-receptors. Of particular interest is also the relation between Sepp-dependent delivery of Se to the kidneys in comparison to local recycling, biosynthesis and secretion of renal-derived Gpx3, which constitutes the second selenoprotein significantly contributing to circulating blood Se content. Our current knowledge is limited with regard to expression and regulation of Sepp during development, Se compartmentalization and supply to tissues and cells not depending on Sepp and devoid of the Lrp receptors. Nevertheless, with the identification of Sepp as the major transport, distribution and storage protein for Se, and the characterization of receptor-mediated tissue-specific uptake processes, a number of previous enigmatic findings are now explained by a plausible molecular pathway. SEPP's role as a functional biomarker reliably reflecting Se intake and Se status has become widely accepted. Now, both more experimental and clinical efforts are needed to apply this knowledge and the increasing number of available technologies for SEPP quantification to better understand its role in disease prevention and health support.

Acknowledgements Work on selenoproteins in the authors' labs is supported by a team of dedicated technicians and doctoral students, and by the German Research Foundation (DFG: Schw914/2, Scho 849/2-2) and German Cancer Aid (Deutsche Krebshilfe, 10-1792 Scho2, 108 426).

References

1. MA Motsenbocker, AL Tappel 1982 *Biochim Biophys Acta* 719:147
2. M Persson-Moschos et al 1998 *Eur J Clin Nutr* 52:363
3. KE Hill et al 1993 *Proc Natl Acad Sci U S A* 90:537
4. C Baron et al 1993 *Proc Natl Acad Sci U S A* 90:4181
5. Y Saito et al 1999 *J Biol Chem* 274:2866
6. S Kurokawa et al 2014 *Free Radic Biol Med* 69:67
7. JG Yang et al 1989 *J Nutr* 119:1010
8. AV Lobanov et al 2008 *Genome Biol* 9:R62
9. KT Suzuki et al 1999 *Analyst* 124:1749
10. Y Saito, K Takahashi 2002 *Eur J Biochem* 269:5746
11. J Yan, JN Barrett 1998 *J Neurosci* 18:8682
12. KE Hill et al 2003 *J Biol Chem* 278:13640
13. L Schomburg et al 2003 *Biochem J* 370:397
14. K Renko et al 2008 *Biochem J* 409:741
15. GE Olson et al 2008 *J Biol Chem* 283:6854
16. GE Olson et al 2007 *J Biol Chem* 282:12290
17. RF Burk et al 2007 *J Neurosci* 27:6207
18. RF Burk, KE Hill 2015 *Annu Rev Nutr* 35:109
19. J Herz, HH Bock 2002 *Annu Rev Biochem* 71:405
20. TE Willnow, A Nykjaer 2010 *Mol Cell Endocrinol* 316:93
21. A Christ et al 2015 *Dev Cell* 35:36
22. EI Christensen, H Birn 2002 *Nat Rev Mol Cell Biol* 3:256

23. TK Andreassen 2006 *Horm Metab Res* 38:279
24. AL Negri 2006 *Nephrology (Carlton)* 11:510
25. J Chiu-Ugalde et al 2010 *Biochem J* 431:103
26. S Hirotsune et al 1995 *Nat Genet* 10:77
27. M Trommsdorff et al 1999 *Cell* 97:689
28. I Masiulis et al 2009 *Biol Chem* 390:67
29. U Schweizer et al 2004 *Biochem J* 378:21
30. S Kurokawa et al 2014 *J Biol Chem* 289:9195
31. GE Olson et al 2005 *Biol Reprod* 73:201
32. OM Andersen et al 2003 *J Biol Chem* 278:23989
33. KE Hill et al 2004 *J Nutr* 134:157
34. L Schomburg et al 2004 *Cell Mol Life Sci* 61:1988
35. RF Burk et al 2006 *Biochim Biophys Acta* 1760:1789
36. KT Suzuki et al 2005 *Toxicol Appl Pharmacol* 206:1
37. CS Hoefig 2010 *Establishment, Validation and Application of Immunological and LC-MS/MS-Based Detection Methods to Study the Role of Human Aromatic L-Amino Acid Decarboxylase as an Enzyme Potentially Involved in Thyronamine Biosynthesis,* PhD Thesis, Humboldt University, Berlin
38. KE Hill et al 2014 *PLoS One* 9:e103486
39. TE Willnow et al 1996 *Proc Natl Acad Sci U S A* 93:8460
40. K Zarbalis et al 2004 *PLoS Biol* 2:E219
41. F Ursini et al 1999 *Science* 285:1393
42. S Kehr et al 2009 *J Mol Biol* 389:808
43. Y Saito et al 2004 *Biochem J* 381:841
44. G Takebe et al 2002 *J Biol Chem* 277:41254
45. KE Hill et al 2007 *J Biol Chem* 282:10972
46. T Bosschaerts et al 2008 *J Immunol* 180:6168
47. RF Burk et al 1991 *Am J Physiol* 261:E26
48. RF Burk et al 1998 *Hepatology* 27:794
49. I Dreher et al 1997 *J Trace Elem Med Biol* 11:83
50. BA Carlson et al 2004 *J Biol Chem* 279:8011
51. U Schweizer et al 2005 *Biochem J* 386:221
52. KE Hill et al 2012 *J Biol Chem* 287:40414
53. M Scharpf et al 2007 *J Neural Transm* 114:877
54. FP Bellinger et al 2008 *J Alzheimers Dis* 15:465
55. RF Burk et al 2014 *FASEB J* 28:3579
56. MW Pitts et al 2015 *J Neurosci* 35:15326
57. J Chiu-Ugalde et al 2012 *Antioxid Redox Signal* 17:902
58. JP Friedmann Angeli et al 2014 *Nat Cell Biol* 16:1180
59. TE Willnow et al 1999 *Nat Cell Biol* 1:E157
60. I Dreher et al 1997 *J Biol Chem* 272:29364
61. B Speckmann et al 2010 *Free Radic Biol Med* 49:777
62. V Mostert et al 2001 *Biofactors* 14:135
63. J Martitz et al 2015 *Metallomics* 7:1515
64. NP Becker et al 2014 *Metallomics* 6:1079
65. X Forceville et al 2009 *Eur Surg Res* 43:338
66. B Speckmann et al 2008 *Hepatology* 48:1998
67. B Speckmann et al 2009 *Biochem Biophys Res Commun* 387:158
68. H Misu et al 2010 *Cell Metab* 12:483
69. JP McClung et al 2004 *Proc Natl Acad Sci U S A* 101:8852
70. K Ashton et al 2009 *Am J Clin Nutr* 89:2025S
71. Y Xia et al 2005 *Am J Clin Nutr* 81:829
72. RF Burk et al 2006 *Cancer Epidemiol Biomarkers Prev* 15:804

73. C Meplan et al 2007 *FASEB J* 21:3063
74. GF Combs, Jr. 2015 *Nutrients* 7:2209
75. U Peters et al 2008 *Cancer Epidemiol Biomarkers Prev* 17:1144
76. ML Cooper et al 2008 *Cancer Res* 68:10171
77. C Meplan et al 2010 *Carcinogenesis* 31:1074
78. A Steinbrecher et al 2010 *Cancer Epidemiol Biomarkers Prev* 19:2958
79. DJ Hughes et al 2015 *Int J Cancer* 136:1149
80. H Mörk et al 2000 *Nutr Cancer* 37:108
81. A Calvo et al 2002 *Cancer Res* 62:5325
82. OH Al-Taie et al 2004 *Nutr Cancer* 48:6
83. CL Li et al 2007 *World J Gastroenterol* 13:2363
84. O Brodin et al 2015 *Nutrients* 7:4978
85. SM Lippman et al 2009 *JAMA* 301:39
86. S Hercberg et al 1998 *Control Clin Trials* 19:336
87. R Schnabel et al 2008 *Am Heart J* 156:1201.e1
88. RF Burk, KE Hill 2009 *Biochim Biophys Acta* 1790:1441
89. S Zhang et al 2010 *Mech Ageing Dev* 131:253
90. W Reinhardt et al 2015 *Thyroid* 25:1091
91. M Tonelli et al 2009 *BMC Med* 7:25
92. M Geoghegan et al 2006 *Curr Opin Crit Care* 12:136
93. AM Dumitrescu et al 2005 *Nat Genet* 37:1247
94. MF Azevedo et al 2010 *J Clin Endocrinol Metab* 95:4066
95. E Schoenmakers et al 2010 *J Clin Invest* 120:4220
96. O Agamy et al 2010 *Am J Hum Genet* 87:538