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Visions & Reflections

Selenium and selenoproteins in mammals: extraordinary, essential, enigmatic

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Abstract. Selenium (Se), once known only for its potential toxicity, is now well established as an essential trace element for mammals. Insufficient Se intake predisposes to and manifests in a variety of diseases. Recent studies have proven that it is the synthesis of selenocysteine (Sec)-containing proteins, designated selenoproteins, which represents an essential prerequisite for regular development and a long and healthy life. New transgenic mouse models analysing those selenoproteins with proven enzymatic functions displayed particular phenotypes and highlighted essential Se-dependent processes in development, growth or against specific challenges. While there is a growing molecular understanding of and general agreement on the importance of sufficiently high Se intake and undisturbed selenoprotein biosynthesis, many of the recently identified selenoproteins are still uncharacterised, and the effects and consequences of supraphysiological Se dosages are not biochemically understood. With the recent definition of the human and mouse selenoproteomes and a growing number of available tools, the Se field is now geared for a great leap forward. Se biology has already broadened our knowledge about the genetic code and about protein translation. It now holds great promises also for a better understanding of some key aspects of cancer, inflammation, fertility and prevention of age-associated diseases.

Key words. Selenium; selenoproteins; trace element; mouse model; translation; health; genetic code.

Selenium in mammalian proteins: finally at home

Traces of selenium (Se) are absolutely essential for mammals, and Se has been found in all tissues albeit at varying concentrations. Generally, Se is taken up with the diet mainly as selenoamino acids in which Se replaces sulfur; L-selenomethionine (SeMet), L-selenocysteine (Sec) and Se-methylselenocysteine from cereal grains, plants and animal proteins, in which the content of Se depends upon the area of growth, i.e. upon the soil and fodder quality [1]. In contrast, nutritional supplements consumed as over-the-counter drugs consist mainly of inorganic Se (selenite or selenate) or preparations from Se-enriched yeast (SeMet and a wide range of diverse Se compounds) [2]. During protein synthesis, SeMet is loaded directly onto methionine transfer RNA ($tRNA^{Met}$) and replaces the essential amino acid Met in the growing peptide chain. The SeMet content of newly synthesised proteins is directly related to the ratio of SeMet versus Met that is available for the methionyl-tRNA synthetase and is therefore very low and likely without functional relevance [3]. In contrast, cells actively synthesise Sec-loaded tRNA and specifically insert the aminoacyl moiety cotranslationally into selenoproteins at discrete positions within

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the peptide chain. This process has been elucidated in detail in prokaryotes [4] and is also becoming increasingly understood in mammals [5]. Sec insertion requires the recoding of a UGA stop codon as a codon for Sec which is achieved in conjunction with a specific RNA structure within the 3′-UTR, the so called Sec-insertion sequence (SECIS) element [6]. Thus, Sec expanded the genetic code and represents the 21st proteinogenic amino acid. The term 'selenoproteins' refers to Sec-containing proteins and is not used for Se-binding or SeMet-containing ones [7]. Despite different catabolic pathways for Se-containing compounds, all Se seems to finally end up in the Sec-loaded tRNA pool to be used for selenoprotein synthesis. Taking advantage of the completed genome projects and the characteristics of selenoprotein-encoding transcripts, computational strategies were developed and successfully used [8] to predict that the human and mouse selenoproteomes comprise 25 and 24 different members, respectively [9]. Currently, only about half of these selenoproteins have been characterised in detail and shown to display a defined biological function or known enzymatic activity (table 1).

Selenoenzymes: extraordinary catalysts of extraordinary reactions

Sec-containing proteins, i. e. selenoenzymes, are involved in very diverse metabolic processes and display unique qualities. Isozymes of the glutathione-peroxidase (GPx) family (GPx-1, -2, -3, -4 and -6) differ in tissue distribution and in their substrate specificity for peroxide degradation [10, 11]. GPx-4 is additionally involved in arachadonic acid metabolism and displays specific moonlightning activities during sperm maturation, where it is converted to a structural protein [12]. The recently identified GPx-6 seems to be expressed exclusively during development and in the olfactory epithelium [9]. While human GPx-6 contains Sec, its rodent orthologues have lost this feature and display a cysteine at the respective position, explaining the single difference between the human and mouse selenoproteomes. Three different mammalian thioredoxin reductases (TrxR) contain Sec at the penultimate position and accept a broad range of substrates [13]. They differ by tissue distribution and intracellular localisation; TrxR1 is a cytosolic enzyme, TrxR2 is active in mitochondria, while a third isozyme is mainly expressed in testis and carries an additional N-terminal

Table 1. Transgenic mouse models analysing selenoproteins and Se-dependent processes.

Class	OMIM	KO model	Health effects	
			In development	Upon challenge
Iodothyronine deiodinase (DIO)				
DIO 1	*147892	not rep.	(likely mild)	?
DIO 2	*601413	[31]	growth defect, hearing loss	defective cold adaptation
DIO 3	*601038	[30]	growth retardation	fatalities
Glutathione peroxidase (GPx)				
GPx-1	*138320	[38]	none reported	sensitive to oxidative stress
GPx-2	*138319	[39]	none reported	colitis/cancer in GPx1/GPx2 KO
GPx-3	*138321	not rep.	(likely mild)	?
GPx-4	*138322	[26]	embryonic lethal	reduced fitness of hemizygotes
GPx-6	*607913	impossible	Se dependent in human only	
Thioredoxin reductase (TrxR)				
TrxR1	*601112	[28]	embryonic lethal	–
TrxR2	*606235	[28]	embryonic lethal	–
TGR	*606448	not rep.	?	?
Methionine sulfoxide reductase (Msr)				
MsrB	*606216	not rep.	?	?
Selenophosphate synthetase (SPS)				
SPS2	*606218	not rep.	(likely lethal)	?
Selenoprotein P (SePP)				
SePP	*601484	[34, 35]	growth defect, ataxia, seizures	male infertility
All selenoproteins ($tRNA^{[Ser]Sec}$)				
in general	*165060	[25]	embryonic lethal	–
in mammary	*165060	[68]	none reported	altered tumor suppressor levels
in liver	*165060	[29]	none reported	liver and fat necrosis, fatalities

OMIM refers to entries in the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM). Since some selenoproteins have not yet been analysed by KO models, the authors gave a personal expectation for their importance for development (marked by brackets, pure assumptions only).

glutaredoxin domain (Trx-/GSH-reductase, TGR) [14]. A specific function of TGR can be expected through its direct connection to these two redox-regulatory systems. The iodothyronine deiodinase isozymes (DIOs) link Se biology to thyroid hormone metabolism. These selenoenzymes are responsible for the deiodination of thyroxine (T4) to the biologically active triiodothyronine (T3) (type 1 and 2) or the inactivation of T4 and T3 by 5-deiodination (mainly type 3) [15]. Thus, both activation and termination of thyroid hormone signalling is controlled by selenoenzymes, indicating coevolutionary processes between the two essential trace elements Se and iodine. This feature might also partly explain the severity of some forms of myxoedematous cretinism observed in combined iodine and Se deficiency [16]. Methionine-sulfoxide reductase B (MsrB) depends on Trx for the stereospecific reduction of R-methionine sulfoxide [17], indicating another redox-regulatory role of Se in mammals [18]. The biological importance of methionine oxidation and methionine-sulfoxide reduction for the ageing process and as reversible modifications for signalling proteins is slowly emerging. At least in *Drosophila*, transgenic overexpression of the non-Se-dependent Msr (MsrA) isozyme reduced oxidative damage and increased the life span [19]. Selenophosphate-synthetase 2 (SPS2) completes the list of functionally characterised selenoenzymes. It might be involved in an autoregulatory loop where it activates Se for Sec synthesis, thereby controlling the overall rate of selenoprotein expression [20]. Taken together, selenoenzymes take advantage of the chemical properties and enhanced reactivity of the trace element by placing it into the active site. Sulfur-containing analogues of selenoenzymes can become orders of magnitude less efficient [21, 22] or accept only a reduced range of substrates [13]. The consequences of insufficient or incomplete synthesis can be enormous for a particular cell: Se-compromised TrxR1 displays only little NADPH-disulfide oxidoreductase activity [21], but specifically and dominantly induces apoptosis when introduced into mammalian cells in culture [23].

Se-dependent processes and proteins: essential for life

A low Se diet induces various physiological and biochemical changes in mammals [24], but the contribution of individual selenoproteins has often remained elusive. Several recent knockout (KO) mouse models have provided additional insights and pointed directly to particular Se-dependent processes and Sec-containing proteins that are absolutely essential. Especially during development, crucial Se-dependent processes include Sec-dependent translation in general ($tRNA^{[Ser]Sec}$ KO) [25], the Se-dependent metabolism of phospholipid hydroperoxides and arachadonic acids (GPx-4 KO) [26] and the thioredoxin/TrxR system (Trx-KO) [27] (TrxR-KO) [28]. This fundamental importance is also proven through locally restricted ablation of selenoprotein synthesis, since severe hepatocellular degeneration and necrosis leading to early fatalities result from tissue-specific disruption of selenoprotein synthesis in liver [29]. Further adverse health effects were observed after prolonging thyroid hormone action by deleting iodothyronine deiodinase type 3 (DIO 3-KO) [30], i.e. the isozyme that terminates thyroid hormone action. In contrast, deletion of a major activating deiodinase (DIO 2-KO) pointed to specific thyroid hormone-dependent processes in brain development and led to retarded cochlear development and hearing loss [31]. A DIO 1-KO is not available, but a mouse strain with minimal DIO 1 activity (strain C3H/HeJ) is phenotypically normal in spite of altered serum hormone levels [32]. Similarly, dietary Se restriction was shown to impact tissue specifically on the expression of DIO 1, i.e. hepatic loss of DIO 1 was paralleled by an increase in thyroid DIO 1 expression [33]. Thus, transient changes in Se supply affect thyroid hormone metabolism, but there are no acute health defects – consistent with the phenotype of DIO 2-deficient mice and indicative of efficient compensatory mechanisms within the thyroid hormone feedback system. Without the major selenoprotein in plasma (SePP), a disturbance of Se tissue distribution was observed (SePP-KO) [34, 35] that also led to obvious neurological and growth defects [36, 37] and to infertility in male SePP-KO mice. In contrast to these obvious phenotypes, inactivation of the major ubiquitously expressed cellular GPx (GPx-1) encoding gene caused less severe and/or less overt phenotypes that became apparent only after specific challenges such as oxidative stress, viral infection, experimentally induced stroke, inflammation and so on (GPx-1 KO) [38]. The combined disruption of two GPx genes expressed in the gastrointestinal tract (GPx-1/GPx-2 KO) led to the development of tissue-specific pathologies such as colitis [39] and increased bacteria-induced cancer rates [40]. Thus, the transgenic mice display defined phenotypes, indicating that the function of these selenoproteins is not redundant, cannot be compensated for by non-Se-containing proteins and represents a clear evolutionary advantage. So far, the only inherited human disease linked to a selenoprotein has been described as a form of rigid spine muscular dystrophy caused by mutations in selenoprotein N [41], although the underlying molecular defects and functions of selenoprotein N are as yet not well understood.

Selenoprotein synthesis: Selene does it her way

The synthesis of selenoproteins is outstanding and fascinating. It depends entirely on the availability of the trace

element that has been named after the Greek goddess of the moon, Selene. After activation, a selenol group (-SeH) is used to replace the hydroxyl group of seryl-tRNA$^{[Ser]Sec}$, yielding Sec-tRNA$^{[Ser]Sec}$ [42]. Thus, there is no Sec-specific aminoacyl-tRNA transferase, but a single specific tRNA that has to be recognised by the modifying enzymes. This tRNA$^{[Ser]Sec}$ is exceptional with respect to synthesis, size and degree of posttranscriptional modification [5]. Moreover it comes in two specific forms that differ by the presence of an additional methyl group on the 2′-O-ribosyl moiety of the anticodon wobble position [43]. Generally, the amount of available tRNA$^{[Ser]Sec}$ seems not to be limiting [44], but shifts in the amount of methylated versus unmethylated tRNA are tissue- and Se-level dependent and likely influence the biosynthesis of selected selenoproteins [45]. Thus, a unique pathway for synthesis, loading and posttranscriptional modification of the tRNA$^{[Ser]Sec}$ has evolved. It is complemented by the specific translation factor EFsec [46] and the bridging protein SBP2, which recognises the SECIS element of selenoprotein-encoding transcripts [47, 48]. The assembled complex, the selenosome, competes with the eukaryotic release factors in the deciphering of the UGA codon for Sec insertion or translational termination. Obviously, readthrough with Sec integration is not a very efficient process and depends on additional parameters [49]. The recognition of the UGA codon as nonsense not only terminates translation but can also lead to nonsense-mediated decay of the messenger RNA (mRNA) and subsequent reduction in transcript levels [50]. Thus, the availability of Se and the levels of the two Sec-loaded tRNA$^{[Ser]Sec}$ species directly control the translational efficiency and the stability of certain selenoprotein-encoding transcripts [51]. These findings are surely relevant for the observed hierarchical Se retention in different tissues upon deprivation [52] and for the Se-dependent transcript-specific regulation of selenoprotein mRNA stabilities [33, 51, 53]. But a clear picture of the details controlling preferential tissue supply with Se and privileged translation of certain transcripts, i.e. the two enigmatic hierarchies within Se biology, is currently still missing (fig. 1).

Se distribution within the body: facilitated transport versus alternative routes

Se-containing compounds are taken up from the diet, but their immediate bioavailability depends on their bio-

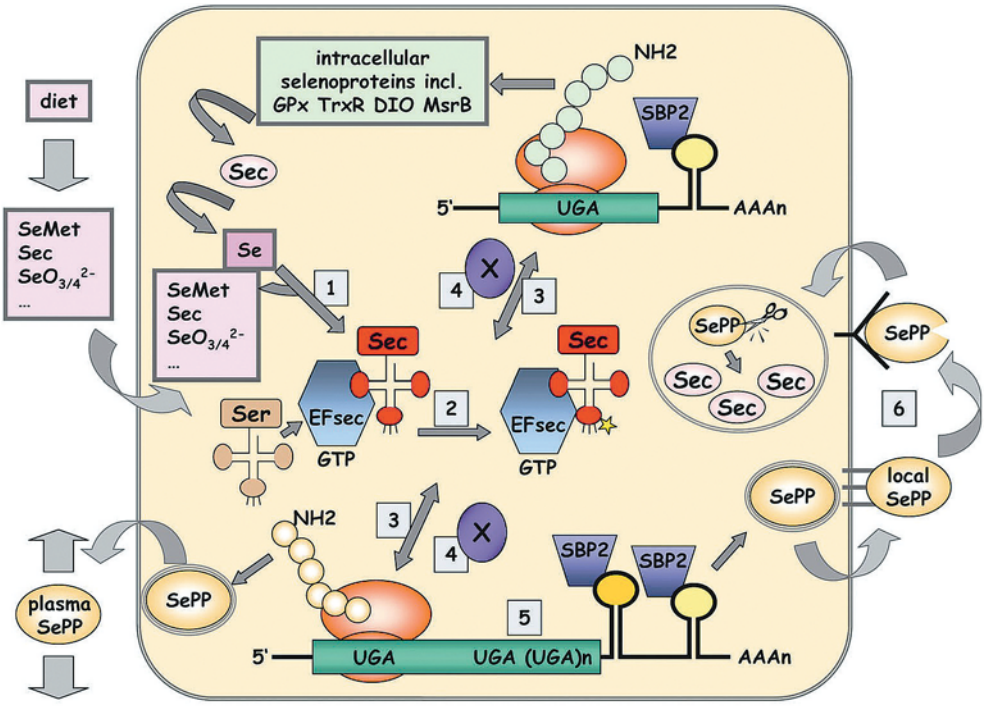

Figure 1. Illustration of central steps and unresolved issues in selenium (Se) metabolism. Step 1: Dietary-derived Se-containing compounds are taken up, and Se is liberated, activated by phosphorylation and charged onto its tRNA by incompletely defined pathways. Step 2: Specific posttranscriptional processes give rise to two different tRNA$^{[Ser]Sec}$ isoforms that are used with unequal efficiencies for the synthesis (step 3) of the different selenocysteine (Sec)-containing proteins. Step 4: Additional factors (X) might be required to assemble specific selenosomes for efficient translation of particular selenoproteins. Step 5: Two individual Sec-insertion elements are likely necessary to decipher multiple UGA-codons in selenoprotein P (SePP) mRNA for successful readthrough and consecutive insertion of up to 10 Sec residues per protein. Step 6: After synthesis, SePP is released into the extracellular space and found in plasma or locally bound to the cell surface from where it might be remobilised and taken up for Se retrieval (SePP cycle).

chemical nature, which determines the metabolism and toxicological potential [54]. With adequate intake and regular functioning of the organs, most of the trace element is taken up by the liver and used for synthesis of hepatic selenoenzymes, including SePP [55, 56]. SePP contains up to 10 Sec residues per protein and is a good marker of Se nutritional status [57]. Se levels in blood and tissues and the expression of selenoenzymes such as GPx or TrxR depend on SePP synthesis and secretion [34, 35], indicating that these selenoenzymes depend on hepatic or local SePP. Surprisingly, the neurological defects caused by disruption of SePP synthesis in SePP-KO mice could successfully be rescued by high selenite supplementation [36, 37]. This indicated additional, although less-effective SePP-independent Se uptake routes in brain, which turned out not to be ubiquitous. The liver-specific $tRNA^{[Ser]Sec}$-KO mice displayed reduced Se and SePP levels in plasma and also in testis but, interestingly, not in brain [29]. Of special interest is therefore also locally synthesised SePP and the recent data describing C-terminal Se-rich fragments of SePP as a most effective delivery form of the trace element into cells in culture [58]. It is presently not clear why supplementation of SePP KO mice with SeMet did not rescue the mice as efficiently as with selenite [37]. These findings, enigmatic as they are, underline our present ignorance of many aspects of Se uptake, transport and metabolism (fig. 1). Crucial differences in the metabolism of organic versus inorganic, i.e. Se-containing amino acids versus selenite or selenate, have been worked out with respect to resulting tissue levels and selenoprotein activities [59], and details on the metabolism of the different Se-containing amino acids have been elucidated [60]. It appears, though, that inorganic Se (which is usually not a major source of dietary Se) is more readily available to selenoprotein synthesis than e.g. SeMet, i.e. the major form of dietary Se from many plants. Se from animal sources is mostly present as Sec, a chemical form that is not a direct precursor for selenoprotein synthesis, but requires liberation of its Se via selenocysteine-beta-lyase [61]. A deeper biochemical understanding of the different metabolic pathways for the diverse Se-containing compounds and their relative contributions for the Se supply in vivo is currently evolving.

Enigmatic aspects: the bright side and the dark side of the element of the moon

Whatever aspect of Se biology is addressed, one is always confronted with riddles. Not only are the functions of the many recently identified selenoproteins (roughly half of all) still elusive, even some of the old acquaintances have not yet disclosed their biological role. Considering the long-known extracellular GPx-3 as an example, one wonders about its function given the low concentration of glutathione in plasma. It is hard to envision this protein working as an enzyme – even more so as there is probably no glutathione-regenerating system in the circulating system. The same is true for the second major selenoenzyme in plasma, i.e. SePP. Has nature really developed a transport or storage protein for Se that needs to be taken up and hydrolysed completely in order to deliver its cargo? At first glance it seems a waste of metabolic energy compared to the effective ways of iron or zinc binding, transport and distribution. But if it was not transport, the aforementioned lack of reducing systems argues against an enzymatic function in plasma. Detoxification of free selenium or other heavy metal ions or suicide-type reactions are further options [55]. A better understanding is expected via in-depth analyses of the uptake mechanism or from the molecular characterisation of potential SePP-specific reception devices that have not yet been identified (fig. 1).

Other unresolved issues lie within Se-protein biosynthesis. There is virtually no free Sec – and if it were available, it could not be charged onto $tRNA^{[Ser]Sec}$. Thus, Se must be liberated from selenoproteins [61], SeMet-containing proteins or other Se compounds to make it available for $tRNA^{[Ser]Sec}$ biosynthesis. The biochemistry is not yet understood, but many findings hint toward a ‘Se cycle’ in which Se from degraded proteins is directly funneled into Sec resynthesis. Since some organs (such as the brain and endocrine glands) retain their Se content even during prolonged severe Se restriction [52], this ‘Se stockpile’ might be Se proteins already destined for storage and degradation when they are synthesised. Such a function inside the cells has been suggested for the ubiquitously expressed major GPx, i.e. GPx-1 [62]. Since SePP is also expressed in almost all tissues, yet released into plasma mainly by the liver, we propose that it may serve such a local albeit extracellular storage and distribution function in an auto- or paracrine fashion. This scenario imposes additional relevance to a potential proteolytic fragmentation outside of target cells for effective uptake [58]. Moreover, synthesis itself is still elusive because current estimates from in vitro model systems predict termination to largely dominate over Sec insertion at a given UGA, yet with different methods mainly full-length SePP is detected in human and mouse plasma. This is even more disturbing when one additionally considers the apparent preferential supply of Se to different transcripts, i.e. the hierarchical synthesis of the different selenoproteins [63]. Is there really a competition between termination and Sec insertion at every single UGA codon, or rather is the transcript preloaded with one or more specific selenosomes, including $tRNA^{[Ser]Sec}$, SBP2, GTP, EFsec and further discriminating although not-yet defined components? This preloading could explain the necessity of two individual albeit different SECIS elements in the 3′-UTR of SePP mRNA for successful syn-

thesis of closely neighbored Sec codons. Here again, some additional cis- or trans-acting factors might discriminate between the two different $tRNA^{[Ser]Sec}$ forms and sense the overall concentration of available Se and Sec-loaded tRNA. How is the rate of the driving force, i.e. GTP hydrolysis regulated – also in a transcript-specific way? Most interestingly, tissue- and Se-specific regulation of methylated versus non-methylated $tRNA^{[Ser]Sec}$ levels in combination with differential integration rates into the UGA-displaying transcripts might also provide clues to the observed hierarchical expression rates of the different selenoenzymes in privileged tissues [64]. Thus, the importance of regular selenoprotein biosynthesis is obvious and undisputed even though many of these questions remain open.

Apart from these biochemical issues, many epidemiological studies have demonstrated the fundamental importance of sufficiently high Se levels also for human health, including aspects of immune function, viral infection, reproduction, mood, thyroid function, cardiovascular disease, cancer prevention and so on [65]. But the Se intake and Se plasma levels show considerable geographical variation among countries (they are, e.g. twice as high in the USA compared to most European countries) [66], although no clear correlation to health issues could be drawn. Nevertheless, the most important and certainly most Delphic mystery, especially for ageing societies, is given by the promising health-preserving and cancer-preventive potentials of Se compounds at pharmacological dosages. Comprehensive human trials are currently under way to examine the initial observations from smaller earlier studies. The plethora of effects of Se on almost all cellular functions and pathways has just been exemplified in an analysis of changes in the transcriptome of human prostate cancer cells treated with methylseleninic acid in vitro [67]. At the moment, therefore, due caution is needed when it comes to one-sided appraisals of the beneficial effects from pharmacological Se intake, at least until more and better data are available from the current clinical trials and model systems at hand. Appreciating the bright side should not necessarily mean that one must become blind toward Selene's other face, i.e. the toxicological potential of Se-containing compounds.

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