Cell. Mol. Life Sci. 62 (2005) 2414–2437
1420-682X/05/212414-24
DOI 10.1007/s00018-005-5143-y

Cellular and Molecular Life Sciences

Review

Human selenoproteins at a glance

S. Gromer*, J. K. Eubel, B. L. Lee and J. Jacob

Biochemiezentrum der Universität Heidelberg (BZH), Im Neuenheimer Feld 504, 69120 Heidelberg (Germany),
Fax: +49 (6221) 54 5586, e-mail: stephan@gromer-online.de

Received 30 March 2005; received after revision 4 July 2005; accepted 13 July 2005
Online First 18 October 2005

Abstract. The public perception of selenium has changed significantly over the last decades. Originally mainly known for its high toxicity, it was later recognized as an essential trace element and is now (despite its narrow therapeutic window) almost being marketed as a lifestyle drug. Indeed, some clinical and preclinical studies suggest that selenium supplementation may be beneficial in a large number of clinical conditions. However, its mode of action is unresolved in most of these cases. Selenocysteine – identified as the 21st amino acid used in ribosome-mediated protein synthesis – is incorporated in at least 25 specific, genetically determined human selenoproteins, many of which have only recently been discovered. Restoration of normal selenoprotein levels may be – apart from direct supranutritional effects – one possible explanation for the effects of selenium supplements. In this review we provide a brief but up-to-date overview of what is currently known about these 25 acknowledged human selenoproteins and their synthesis.

Key words. Selenoprotein; selenium; selenoprotein biosynthesis; redox metabolism; antioxidant.

Historical landmarks

The essential trace element selenium was discovered in 1817 by the Swedish physician and chemist Jöns Jakob Berzelius when he was seeking the etiology of a mysterious disease amongst workers at a sulfuric acid plant in Gripsholm (Sweden).
With reference to the Greek moon goddess Selene, Berzelius named it selenium (Se), as it is closely related to the element tellurium (Te; tellus (Lat.) = earth) discovered afore. Most selenium derivatives are rather toxic, some even more than intravenously applied cyanide [1]. Moreover, selenium and most of its compounds exhibit a characteristic, very penetrative and acrid, garlicky smell, which is often already detectable at extremely low concentrations and persists on contaminated surfaces and skin. These features make selenium and selenocompounds a rather unattractive research problem. It is thus not surprising that biomedical studies remained scarce for over a century after selenium's discovery. Those published [2, 3] – such as the study by Gassmann [2] on selenium content of bones and teeth in healthy individuals (In this publication, Gassmann also speculated on the biological importance of selenium.) – were largely neglected, or – as pointed out by Behne and Kyriakopoulos [4] – rejected by the scientific authorities of the time.
Selenium's reputation went from bad to worse when field research showed that selenium poisoning was the leading cause of alkali and blind staggers disease [5], threatening livestock in large farming communities such as the Great Plains in the US and elsewhere. In addition, laboratory studies declared selenium a potential carcinogen [6, 7].
Today's favourable view of selenium, even referred to as selenophilia [8], is inseparably associated with the name of Klaus Schwarz. His publication (Schwarz and Foltz,

* Corresponding author.

1957), which provides strong evidence for a beneficial and essential role for selenium, is a milestone in biochemical and biomedical research [9] and changed the perception of the moon element. Schwarz, a German biochemist, had started his work on vitamins and selenium in Richard Kuhn's laboratory at the Kaiser-Wilhelm Institute for Medical Research (now Max-Planck Institute) in Heidelberg (Germany) in 1939. He emigrated to the US where he finished his studies on the protective effect of the selenium-containing (still not well defined) factor 3 against liver necrosis at the Bethesda National Institute of Health (NIH) [10,11]. During the same era, Patterson and coworkers independently published a study in 1957 showing that selenium supplements prevented exudative diathesis in poultry [12]. Selenium's essential role for certain physiological processes was confirmed later [13, 14].

In 1973, the same year Turner and Stadtman [15] established bacterial glycine reductase (EC 1.21.4.2) as a selenoprotein, glutathione peroxidase (GPx, EC 1.11.9.1) was the first specific (that is genetically coded) mammalian selenoprotein discovered. Following initial work in Hoekstra's group [16, 17], Leopold Flohé (granted the Klaus Schwarz Commemorative Medal in 1997 [8]) succeeded in showing that selenium is an integral part – covalently bound in stoichiometric quantities – of glutathione peroxidase [18]. Selenium was shown to be incorporated as selenocysteine (Sec; U in one-letter code) first in bacterial glycine reductase in 1976 [19] and in GPx in 1978 [20] where Sec is located and required in the enzyme's active site [21–23]. Still being in the pregenomic era, it took another 6 years until the amino acid sequence of glutathione peroxidase was solved by Günzler et al. [24]. This subsequently led to the establishment of selenocysteine as the 21st proteinogenic amino acid ('Proteinogenic' describes an amino acid used in ribosome-mediated protein synthesis.) [25]. In the next two decades following the discovery of selenium in GPx, only a handful of other proteins were detected in pro- and eukaryotic cells and shown to be specific selenoproteins [15, 26–28]. This difficulty in detecting selenoproteins is attributed to the fact that the codon used for selenocysteine incorporation is not as unambiguous as for the other proteinogenic amino acids. Indeed, the codon-defining selenocysteine, TGA, is normally interpreted as a stop signal by the cell's protein biosynthesis machinery (UGA also codes for tryptophan in mitochondria and some bacterial organisms [29].), (Recently, an additional amino acid, pyrrolysine, was identified as the 22nd proteinogenic amino acid. It is inserted in response to a UAG codon – serving normally also as a stop-codon – in some methanogenic archaea [30]. However, the incorporation mechanism of pyrrolysine seems to differ from the mechanism used for selenocysteine [31].). The dual use of the stop codon, another key observation in understanding selenoprotein biosynthesis, was first made by Chambers and co-workers for murine GPx [32] and later confirmed for all other (specific) selenoproteins [28, 33]. Therefore, selenocysteine insertion requires additional signals, allowing the reinterpretation of the stop signal as a selenocysteine incorporation command. This signaling is achieved via the interaction of several proteins with a special messenger RNA (mRNA) secondary structure, known as the SECIS element (*se*leno*c*ysteine *i*nsertion *s*equence). Our current understanding of this process is primarily built upon the work of August Böck and Thressa Stadtman [34–36]. The details of the steps involved apparently differ between species – particularly between pro- and eukaryotes [23, 37, 38]. Furthermore, no simple mRNA sequence exists that allows an easy and definite prediction of additional selenoproteins in a genome, even though radiolabeling studies can indicate their existence [39, 40].

With the rapid advancements of genome sequencing and its concomitant success in bioinformatics over the last decade, the number of newly identified selenoproteins has almost doubled within a short period of time [38, 41–43]. In fact, the number of identified prokaryotic selenoprotein genes has increased by more than 100 to a total now of approximately 310 in a recent publication by Zhang et al. using a computational approach [44]. Functional analysis of recently discovered selenoproteins has not kept pace with the rapid identification of new selenoproteins. Yet, their vital importance – at least for mammals – is underlined by selenocysteine transfer-specific RNA (tRNA)-knockout experiments, which are lethal in utero [45].

In addition to basic biochemical research, various studies, particularly in livestock farming [46], population-based surveys [47] and many clinical trials [48, 49] indicate the biological importance of selenium. One of the most influential, that is most cited, publications on clinical selenium research is the study by Clark et al. [48], indicating a tumor-preventive effect of selenium. However, as discussed later, in a subsequent follow-up reanalysis the protective effects were not reproducible for all carcinomas as initially reported [50, 51]. Furthermore, a closer look at the data reveals that patients with a very low baseline selenium status profited most from selenium supplementation, whereas those participants with higher levels might actually be at increased risk of cancer.

At present, most clinical and many animal studies are phenomenological in nature: the study participants or laboratory animals essentially serve as black boxes studied in the presence or absence of selenium (supplements). Since many of these studies do not consider a precise mode of action, the recorded parameters are difficult to interpret and often inadequate to reach valid conclusions. With uncontrolled confounding variables, the general applicability of the results is limited.

At this stage further progress in clinically applied selenology would be more substantial if a more rational approach was pursued. This requires a sounder understanding not only of the metabolic functions and effects of selenium and its derivatives, but also of selenoproteins and their roles in cellular and intercellular processes.

Since it is difficult to address all aspects of this highly complex subject, we will narrow our focus to human selenoproteins (Recently discovered selenoproteins, such as SelU, where only cysteine homologues are found in man [52] are not discussed here. Nor are prokaryotic selenoproteins dealt with. It is noteworthy, however, that unlike earlier assumptions [53], prokaryotic and eukaryotic selenoprotein sets exhibit significant overlap [44].) and review our current (still rather limited) knowledge about their roles and functions in physiological processes. Furthermore, many highly interesting topics – in particular metabolic aspects of low molecular weight selenium compounds – are not included in this review, and a few reviews are recommended: see e. g. [8, 54–57] and references therein.

Biochemistry in brief

Selenium is a rare element comprising only ~8×10^{-5}% of the earth crust's mass. Selenium is almost three orders of magnitude less abundant than its closely related neighbour sulfur (~ 0.05 %) [58]. Both elements belong to the chalcogen group and are similar in many of their physicochemical properties. However, the redox potential of selenium compounds is lower compared with their respective sulfur analogues. Furthermore, selenium compounds are generally more reactive [59, 60].

Three different possibilities for selenoprotein formation are currently known: (i) posttranslational binding as a cofactor, (ii) non-specific incorporation and (iii) specific incorporation during translation.

The posttranslational concept has so far only been found in a number of bacterial molybdenum-containing proteins [61–63], but not in higher eukaryotes.

Until now, only two selenium containing amino acids have been detected in proteins (However, further selenium-containing amino acids have been identified in nature [10].): selenomethionine (Sem) and selenocysteine (Sec). They can be incorporated in a non-specific (Sem), but also in a specific (Sec) way into proteins.

The cotranslational incorporation of selenoamino acids into proteins is affected by the close relationship between selenium and sulfur. The latter outnumbers selenium also in cells. In normal cellular metabolism methionine and selenomethionine are essentially treated equally (Apart from the classical methionine pathway, there is evidence – at least in rats – for an alternative pathway for the degradation of selenomethionine: γ-lyase activity seems to allow the direct conversion of selenomethionine to monomethylselenol [55].), leading to an unselective substitution of methionine for selenomethionine in $tRNA^{Met}$ and, therefore, in the total body protein pool [8]. The selenomethionine content in mammalian proteins thus correlates with the nutritional selenomethionine supply (Species capable of forming methionine using inorganic precursor provided by their environment may exhibit a higher content of selenomethionine in their proteins. This results from the fact that the metabolic routes for methionine and selenomethionine formation are identical. This is for example the case in some yeast [56].). The relative Sem content in tissues is proportional to its level in protein synthesis [56]. The non-specific incorporation of selenomethionine is also underlined by the fact that thus far, there is no evidence for a specific $tRNA^{Sem}$ in any organism. It should be noted that enzymes containing selenomethionine instead of their genome-encoded methionine counterparts may differ in their activities, even though no significant structural differences are expected [64–66]. Due to the unselective nature of selenomethionine incorporation, the physiological relevance of Sem-containing proteins remains unclear (yet it is a helpful tool for protein structure analysis [67, 68]).

Unlike selenomethionine, selenocysteine is (at least in mammals [The extent of non-specific incorporation is increased in organisms challenged with high selenium supply: e. g. plants grown on soil with high selenium content and appropriate pH conditions typically exhibit a higher degree of non-specific selenium incorporation.]) primarily incorporated specifically into proteins. However, it is not the free selenocysteine that is directly used for loading its respective tRNA for at least two reasons: the cellular abundance of cysteine compared with selenocysteine competitively inhibits this process [69]. Vice versa, the situation might be even worse: significant improper loading of Sec onto a $tRNA^{Cys}$ could result in a replacement of Cys by Sec, which would lead to significantly different properties of the protein, e. g. in iron-sulfur proteins [70]. In fact, when artificially reversing the selenium-to-sulfur ratio, selenocysteine is incorporated into proteins instead of cysteine via misloaded $tRNA^{Cys}$ [71, 72]. Thus, higher levels of free Sec, which would be required for an efficient direct $tRNA^{Sec}$ loading, are undesirable.

Nature has solved the problem by creating a multistep process. Most of our knowledge about the steps involved in selenoprotein biosynthesis is derived from studies of the bacterial system [25, 73, 74]. Even though overall concepts are comparable in prokaryotic and eukaryotic cells, differences certainly exist. Therefore, any model of this process in eukaryotes is still speculative, yet we outline a conceptual model that is mostly evidence based (figure 1). At first serine is loaded onto Sec-tRNA (SelC in prokaryotes) which is then converted to Sec while the amino acid remains bound to the tRNA [75, 76].

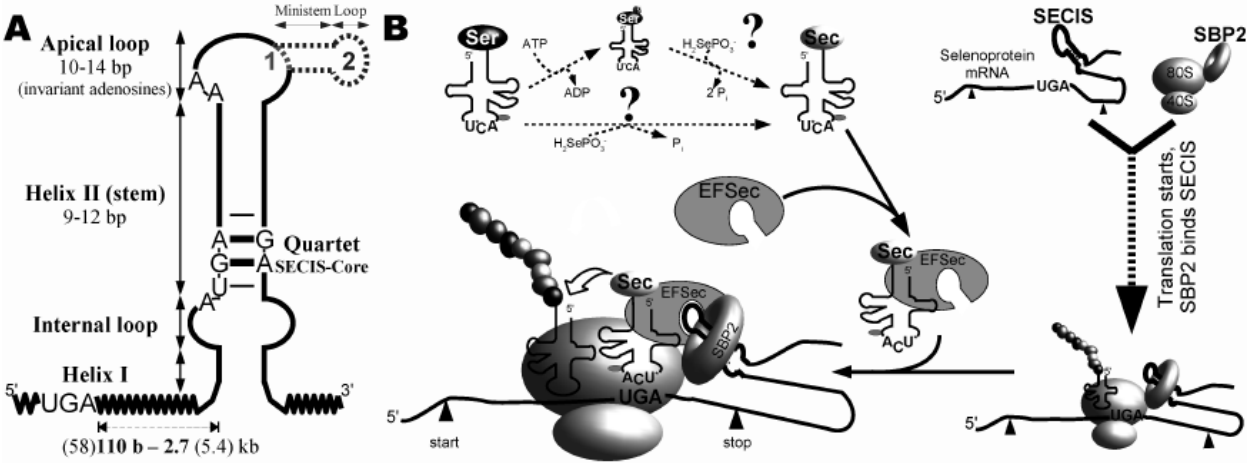


Figure 1. (A) Scheme of human SECIS elements. Two forms are known to exist, differing in the absence (form 1) or presence (form 2) of an additional (mini)stem-loop motif as part of the apical loop [107]. Overall, the total number of nucleotides in the apical loop is almost unchanged in form 2 since the main apical loop becomes smaller. The so-called invariant adenosines are occasionally replaced by cytosines. The quartet or SECIS core is invariant and formed by non-Watson-Crick base pairs (after [107–109] with modifications). (B) Simplified draft of selenoprotein biosynthesis in humans. Serine-loaded $tRNA^{[Ser]Sec}$ is either directly converted to selenocysteinyl-$tRNA^{[Ser]Sec}$ or via an O-phosphorylated intermediate involving a phosphoseryl-$tRNA^{[Ser]Sec}$ kinase (PSTK). Monoselenophosphate [formed by selenophosphate synthetases [SPS] (not shown)] is the assumed selenium donor. These steps are marked with dotted lines and question marks as their relative importance and existence is still not fully established. It should be noted that these two are not per se mutually exclusive. Sec-$tRNA^{[Ser]Sec}$ is now loaded on a specific elongation factor (EFSec). The mRNA attaches to the ribosome, to which SECIS-binding protein 2 is presumably already attached, and protein biosynthesis commences. The stem of the SECIS element is bound by SBP2, while translation progresses. Due to the distance between the coding UGA and the SECIS-element in the 3'-UTR (see A), any in-frame UGA can now be encoded as selenocysteine since EFSec-bound Sec-$tRNA^{[Ser]Sec}$ serves as a selenocysteine donor during translation. How the decision is made to cease translation is still not completely resolved [53, 84]. Not shown are standard factors required for protein biosynthesis.

Therefore, the selenocysteine tRNA is referred to as $tRNA^{[Ser]Sec}$ [77]. The converting step(s) are, however, still rather poorly defined: the presence of significant amounts of O-phosphoseryl-$tRNA^{[Ser]Sec}$ and a respective kinase (PSTK; 10q26.13, [78]) gave rise to the concept that phosphoseryl-$tRNA^{[Ser]Sec}$ may be the intermediate that is consecutively converted to tRNA-bound selenocysteine using monoselenophosphate as the selenium donor [78–81]. Others consider phosphoseryl-$tRNA^{[Ser]Sec}$ a storage form, and experimental data suggest that a direct conversion from seryl-$tRNA^{[Ser]Sec}$ to selenocysteinyl-$tRNA^{[Ser]Sec}$ is possible [76, 82]. The latter pathway would more closely resemble the established pathway found in prokaryotes [35, 83]. While both views have their strong supporters, it is noteworthy that they are not per se mutually exclusive.

It should be emphasized that the $tRNA^{[Ser]Sec}$ is not a standard serine tRNA used for selenocysteine incorporation, but in fact a unique tRNA that differs from other tRNAs in certain aspects [76] (for review see e. g. [84]). In the light of recent results, one structural feature of mammalian $tRNA^{[Ser]Sec}$ merits mention: adenosine 37 is N^6-isopentenylated (i^6A). This modification is not reserved solely to $tRNA^{[Ser]Sec}$, yet it is of importance because it is involved in the maturation process of $tRNA^{[Ser]Sec}$. The final maturation step requires the conversion of methylcarboxymethyl-5'-uridine (mcm^5U) of $tRNA^{[Ser]Sec}$ to methylcarboxymethyl-5'-uridine-2'-O-methylribose (mcm^5Um) at position 34 [85] – the site that forms the wobble position of $tRNA^{[Ser]Sec}$'s anticodon [84]. The mcm^5Um:mcm^5U ratio is dependent on the selenium status and increases with increasing selenium supply [84]. These two tRNA forms apparently provide different efficiencies for the formation of certain selenoproteins, which seem to be partly tissue dependent as well [86–88]. For example, mcm^5Um seems to favour GPx1 synthesis, whereas mcm^5U supports mitochondrial thioredoxin reductase (TrxR2) formation [87]. Indirect evidence also suggests that a lack of mcm^5Um $tRNA^{[Ser]Sec}$ affects the synthesis of mammalian selenoproteins to different degrees. Carlson et al.'s study suggests that thioredoxin reductase 1 and 2 expression is minimally affected by the absence of mcm^5Um, whereas the translation of other proteins – such as GPx2, GPx4, SelP and Sep15 – is influenced and in the case of GPx1, GPx3, SelR, SelT and SelW highly reduced [87, 89, 90]. However, the mRNA levels (as detected by Northern blot analysis) of some of the poorly transcribed proteins were lower as well, suggesting a potential bias in the interpretation of the relative proportions. This differential behaviour – if confirmed – is not fully understood, but implies a further possibility of explaining how the expression levels of individual selenoproteins are maintained in the organism under varying conditions of selenium supply [91].

As indicated above, the methylation step is dependent on cellular selenium, and the regulating effector(s) mediating selenium concentration to methylation activity apparently require a N^6-isopentenyladenosine at position 37 [85, 87]. This has potential clinical importance since statins [β-hydroxymethylglutaryl-coenzymeA-reductase inhibitors] inhibit the isopentenylation of adenosine 37 [92]. These drugs currently form the therapeutic backbone for the treatment of hypercholesterolemia and its associated cardiovascular diseases [93]. As recently suggested, this might be an important link between statins and their adverse side effects, which include myositis, fatal rhabdomyolysis and neuropathy [94–96]. A possible explanation for these adverse effects could involve selenoprotein N (SelN, see below), whose function is not elucidated. SelN involvement is suspected since several mutations in its gene cause rigid spine muscular dystrophy 1 (RSMD1, [97, 98]), which shares many symptoms with statin-induced myositis [94, 95]. However, it is necessary to add that mice overexpressing a mutant $tRNA^{[Ser]Sec}$ incapable of forming the i^6A did not show signs of muscular damage, but in fact an enhanced exercise-induced muscle growth [90]. The conclusions that might be drawn from this experiment are yet limited, as wild-type tRNA – and thus mcm^5Um $tRNA^{[Ser]Sec}$ – was still present in these mice. The selenoprotein levels determined are affected to different extents, but unfortunately SelN levels were not determined. Therefore, valuable information about the involvement of this selenoprotein is still missing. Nevertheless, if the statin hypothesis outlined above holds true and given convincing evidence for an involvement of selenoproteins in the anticancer effects of selenium, the question must be raised whether statin therapy – apart from its undoubtedly beneficial effects in cardiovascular diseases – increases one's cancer risk. So far, there is no compelling evidence for such a disastrous long-term effect of statins (nor for a proposed benefit [99]). However, considering the widespread use of statins, the point that statin therapy could affect selenoprotein synthesis should be further investigated. Current and future trials should closely monitor this possibility. A general recommendation for a nutritional selenium supplement to prevent possible adverse side effects associated with statin therapy cannot be made, as it is also unclear whether statins actually exert some of their therapeutic effects via inhibition of selenoprotein biosynthesis [95]. In fact, a clinical trial published by Brown et al. provides evidence that the beneficial cardiovascular effects of simvastatin + niacin are significantly attenuated by the addition of several antioxidants, including 100 μg of selenium per day to the therapeutic regime [100].

Once the serine residue in $tRNA^{[Ser]Sec}$ (provided as a single-copy gene at 19q13.2-q13.3) is converted to selenocysteine – a process presumably involving selenophosphate formed by selenophosphate synthetase (SPS, isoform 2 being a selenoenzyme by itself, see below) – incorporation into specific selenoproteins requires further factors. Apart from the encoding the UGA codon on the mRNA, a special structure – the cis-acting so-called SECIS-element (selenocysteine insertation sequence, fig. 1A) – is a conditio sine qua non for selenocysteine incorporation. The SECIS element is located in the 3'-untranslated region (UTR) of the mRNA. A minimum distance of 58–111 nucleotides (up to a maximum of ~2.7 kb or even 5.4 kb in some cases [101]) is required for the upstream Sec-encoding UGA [53,102]. This is in contrast to the prokaryotic system. where the SECIS element is typically found within the coding sequence (Recently it was shown that a SECIS element can also be functional in the 3'-UTR of prokaryotic selenoprotein mRNA [103]. However, this position is apparently less efficient and required an artificial base-pairing sequence to bring the SECIS element in sufficient proximity to the Sec-encoding UGA. This methodology is highly interesting for the recombinant production of eukaryotic selenoproteins and may provide further insight in the evolution of selenoproteins. Its relevance for prokaryotic selenoprotein expression in general has, however, not been shown yet.) in very close proximity to the UGA [44].

The SECIS element is recognized and bound by SECIS-binding protein 2 (SBP2; discovered by Copeland et al. [104, 105]), which is presumably already attached to ribosomes. This facilitates selenoprotein synthesis on such selected ribosomes [84].

A specific elongation factor (EFsec = mSelB), only if loaded with Sec-containing $tRNA^{[Ser]Sec}$, can now attach to the SBP2-SECIS-ribosome complex and provide selenocysteine for its incorporation as directed by the UGA (The functionality of the eukaryotic proteins SBP2 and EFSec (= mSelB) is combined within a single protein referred to as SelB in prokaryotes [106].) [106]. How this decision is made whether to terminate translation or to incorporate selenocysteine is not fully understood. Apparently, sequence information as well as trans-acting factors (including selenium and $tRNA^{[Ser]Sec}$ levels) are involved in this process [84].

Judged by their primary sequence the human selenoproteins show little homology. Since the three-dimensional structure is solved only in a few cases, structural comparison largely relies on in silico predictions. These predictions suggest that the overall structures are distinct, too. The selenocysteine is located either in the first half of the amino acid sequence or at the very end of the protein. It is assumed that in the first case, Sec is an essential part of an internally located active site, commonly in a loop between a (predicted) β-strand and an α-helix, whereas in the case of C-terminally located selenocysteines, Sec serves as some sort of (redox active) cofactor equivalent [84]. Many of the selenoproteins exhibit a CXXU motif, indicative of involvement in redox metabolism, transition

metal binding and/or antioxidative defense. The selective advantage of selenocysteine in the first group is thought to be the redox reactivity of the selenolate [84]. In the case of proteins with C-terminally located selenocysteines, the decreased sensitivity to pH changes (pK_a(Sec) = 5.24; pK_a(Cys) = 8–9 [60]) or increased reactivity with certain (seleno)substrates has been suggested to be a selective advantage [110]. Furthermore, several selenoproteins are localized in the endoplasmatic reticulum (ER). Severe selenium deficiency has been reported to be associated with ER disruption at least in chicks (whereas mitochondria remained structurally intact) [111]. This may suggest that ER-associated selenoproteins may be involved in disulfide formation, ER-stress response or calcium homeostasis.

Medical and nutritional implications at a glance

Now, with selenium having reached the tabloids, it should be recalled that selenocompounds are toxic and that their therapeutic index – the difference between beneficial and toxic effects – is rather small. Acute and chronic intoxication differ in their clinical symptoms: chronic selenosis is characterized by brittleness and loss of hair and nails, rashes, gastrointestinal disturbances, fatigue, depression, irritability and other neurological disturbances (Symptoms of chronic selenium poisoning are already documented by Marco Polo, who travelled the silk road in China and passed through areas whose soils are now known to be toxic [47].). Acute intoxications are characterized by severe gastrointestinal and neurological disturbances followed by an often lethal acute respiratory distress syndrome, myocardial infarction and renal failure [112]. Both intoxications share the garlicky smell.

True selenium deficiency-related diseases were first identified in livestock animals [11]. The most prominent examples in humans are Keshan disease, a dilatative cardiomyopathy primarily affecting children [113], and Kashin Beck disease, a disabling chondronecrosis [114–116]. As reviewed by Rayman in The Lancet [117], many diseases in different medical fields and clinical conditions seem to be ameliorated or even prevented by selenium supplementation. However, in many trials, study groups were small, and occasionally potential confounders were not fully ruled out. A critical review on the proposed mechanism of selenium and selenoproteins in diseases is found in [8].

The studies having gained the most attention provide evidence for a tumor preventive effect of selenium. The 1996 Clark study – mentioned above – indicates that selenium supplements may reduce the risk for some of the quantitatively and clinically most important types of cancer – namely prostate, lung and colon cancer – by about 50 % [48]. However, in a follow-up reanalysis the preventive effect against lung and colon carcinomas is almost lost and essentially limited to participants with low baseline selenium levels [50, 51]. Several subsequent studies also show a beneficial effect of selenium supplements; however, most report lower reduction rates than the prominent Clark study. About one-third even reported no beneficial effects at all. So far, the most consistently preventive effects are generally found in prostate cancer chemoprevention trials. Large-scale studies such as the selenium and vitamin E chemoprevention trial (SELECT), (http://www.crab.org/select) are still ongoing [118].

It is noteworthy that currently used selenium sources are not equally effective for all purposes, since absorption, tissue distribution and metabolic fate differ to some extent [10,119,120].

Despite the focus of this article on selenoproteins, it should be emphasized that low molecular weight selenium compounds [121] are at least as important for the beneficial effects of selenium – if not more important [122–124]. This might suggest a higher dosage for selenium supplementation than the currently recommended 55 μg per day, which is based on glutathione peroxidase activity in blood [112]. However, the narrow therapeutic window of selenium and potential negative effects must be carefully considered, and any recommendation to the general public should be made cautiously.

The known human selenoproteins

The selenoproteins identified so far in humans share little sequence homology and serve – at least to our knowledge – quite diverse functions. Many selenoproteins have no reliably designated function at all. With our rather fragmentary knowledge about (human) selenoproteins, it is impossible to review the proteins using a structural and functional classification. We have thus decided to list the proteins in alphabetic order, and provide information and references about each one individually. Although extensive reviews are available for some selenoproteins, we will attempt to consolidate this information and recommend referencing the included resources for a more in-depth look at certain topics.

15kDa selenoprotein

The 15kDa selenoprotein was first described in ^{75}Se-labeling studies in the prostate by Behne et al. [125]. It has an apparent size of 15 kDa and is referred to as Sep15 (Swissprot [http://www.expasy.org/sprot/]: o60613). After its discovery, Sep15 was later purified and sequenced by Gladyshev and co-workers from T cells [126]. Sep15 is mainly expressed in the prostatic gland, testes, brain, kidney and liver, yet low levels are also detected in skeletal muscle, mammary gland and trachea [127]. The

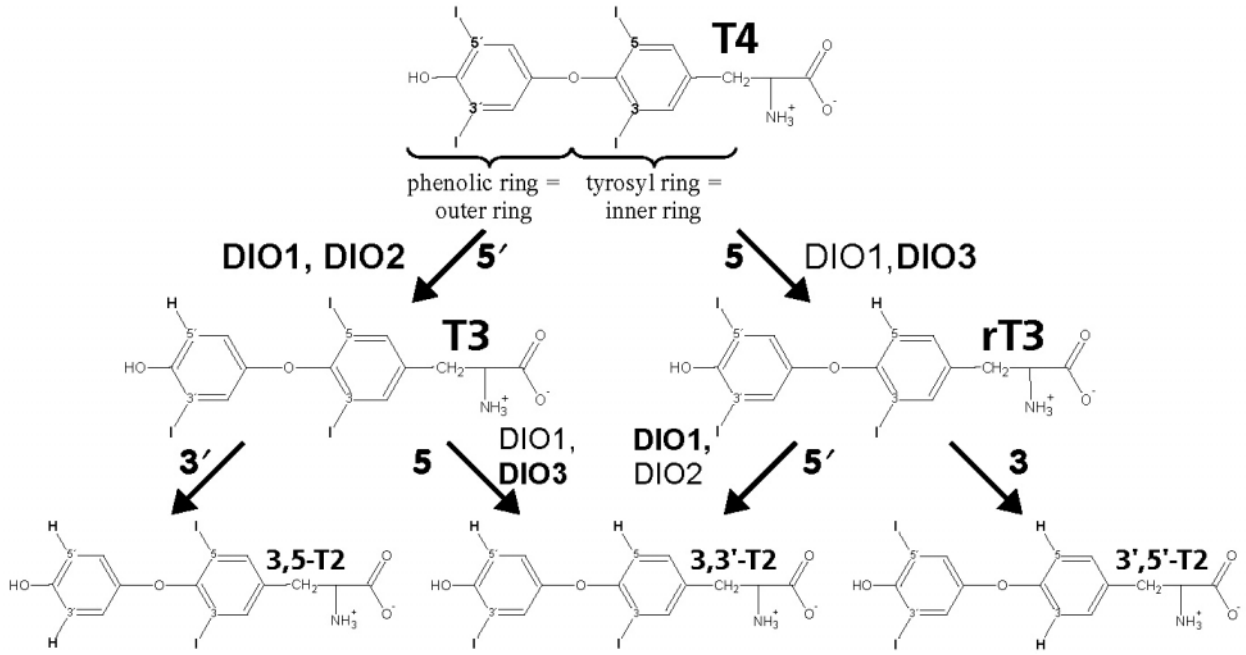


Figure 2. Metabolic pathways of L-thyroxine. Shown are the structures of L-thyroxine (T4), its prime metabolites T3 and reverse T3 and their metabolic fate. The respective deiodinases are indicated. Contrary to popular belief, the diiodo-metabolites 3,5-T2, 3,3'-T2 (but not 3',5'-T2) still exert hormonal function, at least in animals. Both diiodo-metabolites demonstrate a dose-dependent increase in the resting metabolic rate of muscle (greatest effect with 3,3'-T2), brown adipose tissue (greatest effect with 3,5-T2), liver and heart [143]. Under physiological conditions, 3,3'-T2-levels decline with age. Alternative pathways, such as glucuronidation or sulfation, are not shown [143].

Sep15 gene is mapped to 1p31 in humans [127], a site occasionally mutated or deleted in various human tumors [128–130]. Sep15 is localized in the ER, forming a complex with UDP-glucose:glycoprotein glucosyltransferase 1 (HUGT; Swissprot: q9nyu1) [131]. HUGTs are soluble ER enzymes, functioning as gatekeepers for quality control by glucosylating misfolded proteins, thereby preventing transport of improperly folded glycoproteins out of the ER until they are correctly folded or transferred to degradation pathways [132]. The interaction between Sep15 and HUGTs suggests that Sep15 is involved in quality control of protein transport; however, compelling evidence is still lacking. During fractionation on a molecular weight column, the ^{75}Se-15 kDa protein (presumably Sep15 [4]) migrates at an apparent molecular weight of ~300 kDa [125], which is attributed to oligomer formation or Sep15 binding to HUGT (150 kDa) [133]. Increased interest in Sep15 developed after supranutritional selenium supplementation was shown to reduce the incidence of prostatic cancer [49]. In support of these results, certain prostate cancer cell lines have reduced levels of Sep15 [127]. Two polymorphisms in the SECIS-element gene have also been identified that differ in their effectiveness to yield functional Sep15 at low selenium tissue levels [127]. However, at this stage relating Sep15 to cancer prevention/cancer development – even though tempting – is still speculative [130, 134].

Deiodinases

The first deiodinase (DIO) identified as a selenoenzyme was DIO1 in 1990 [27, 135]. Even though this finding occurred almost 20 years after Flohé's discovery, DIO1 was still amongst the first mammalian selenoproteins discovered.

Deiodinases cleave specific iodine carbon bonds in thyroid hormones (fig. 2), thereby regulating their hormonal activity. Thyroid hormones – particularly L-thyroxine (T4: 3,3´,5,5´-tetraiodo-L-thyronine; $t_{½}$ = 7 days), 3,3´,5-triiodo-L-thyronine (T3; $t_{½}$ = 1 day) and 3,3´,5´-triiodo-L-thyronine (reverse T3, rT3) – are of crucial importance to human health as they regulate most metabolic functions. Thyroid hormones act primarily via intracellular receptors as transcription factors and are required for normal growth and development, thermogenesis and regulation of the basal metabolic rate. Our understanding of thyroid hormones has been shaped primarily by clinical observations, where low and high levels of T4, T3 and rT3 cause different clinical disorders, and complete absence or excess of the thyroid hormones can cause life-threatening conditions [136]. The normal thyroid function depends on the two trace elements iodine and selenium, which are commonly low in most Western diets. In addition, the thyroid gland has the highest per gram selenium content [137] of all organs (present not only as deiodinases, but also in glutathione peroxidases, which are presumably

required for the peroxide-dependent formation of T4). Iodine seems to be solely used for thyroid hormone production [138], which makes the thyroid system particularly vulnerable to iodine deficiency. Diseases such as myxedematous cretinism and Kashin-Beck may be combined iodine-selenium deficiencies [116,139].

Today three types of deiodinases are known which not only differ in sequence and structure but also catalyze different reactions. However, most enzymatic deiodination reactions require an endogenous reductant that has not yet been identified for the deiodinases. In fact, it is suggested that deiodinases may act as single-use "enzymes" in vivo [140].

The thyroid hormone system is very complex, especially in the anterior pituitary of the brain, which releases the thyroid-stimulating hormone; but different tissues must also respond appropriately to circulating T3 and T4. The interactions of the thyroid system on a body at the tissue, cellular and subcellular levels are beyond the scope of this article. We recommend a number of excellent publications that further discuss thyroid system (see e. g. [136, 138, 141, 142]).

Deiodinase 1 (DIO1; Swissprot: p49895; EC 1.97.1.10, formerly 3.8.1.4) was identified in 1990 as a selenoenzyme by two groups independently [27,135]. The Sec-encoding UGA was soon discovered by Berry and co-workers [144,145]. DIO1's sequence is located at 1p32-p33 in the human genome [146]. At least two single nucleotide polymorphisms (SNPs) are known to alter plasma hormone levels and cause clinical phenotypes [147]. DIO1 is a homodimeric plasma membrane protein and primarily deiodinates the 5'-position of the phenolic ring (fig. 2), but it can also deiodinate the 5-position under certain circumstances. The first reaction is a reductive deiodination converting the -Se-H group of DIO1 (similarly in DIO2) into a -Se-I group. Reduction releases iodide and regenerates the enzyme's selenol group. Its 5'-deiodination activity converts L-thyroxine (T4) – the major form secreted by the thyroid – to T3, which is the major thyroid hormone in peripheral circulation. Furthermore, the almost inactive reverse T3 (rT3) can be converted to 3,3´-diiodo-L-thyronine. DIO1 expression is high in the liver, kidney, thyroid and pituitary gland, even though trace levels are found in most tissues except the brain, where deiodinase 2 predominates. The relative contribution of different tissues to plasma T3 levels via DIO1 activity is difficult to assess. Fast-exchanging tissues, such as liver and kidney, appear to be primary sources. More than 80 % of T4 is converted to T3 outside the thyroid, primarily in the kidneys and the liver (conversion to T2 and T1 is almost exclusively done outside the thyroid gland) [143]. Diminished DIO1 levels are frequently encountered in low-T3 syndrome (accompanied by elevated levels of rT3), a clinical condition occasionally seen in critically ill patients. The low-T3 syndrome indicates the pivotal role of DIO1 in the production of plasma T3 and rT3 degradation. DIO1 expression is induced by elevated T4 and T3 levels and responds to increased carbohydrate intake. The thyreostatic agent 6-propyl-2-thio-uracil (PTU) reportedly acts as a competitive inhibitor of the regenerating endogenous reductant (however, this contradicts the single-use theory) [148]. Gold(I) complexes, such as aurothioglucose and similar antirheumatics, are known to inhibit several selenoenzymes [149,150] and also DIO1 [144].

The denomination of **deiodinase 2** [151] (DIO2; Swissprot: q92813, also EC 1.97.1.10) as a selenoenzyme had been debated until Buettner et al. showed that the functional SECIS element was present at an unusual far distance (5.4 kb) from the UGA codon in the human enzyme [101]. DIO2 is an ER-membrane protein, whose gene is at 4q24.2-q24.3 [152]. It deiodinates the 5'-position with a preference for T4 over rT3. DIO2 is present in the central nervous system, pituitary and thyroid glands, skeletal and heart muscle, and in placental and brown adipose tissue, where its expression is under catecholamine control. Only low levels are detectable in the kidney and pancreas. It is the dominant form in the brain that it is responsible for more than 75 % of the local T3 production. T3 production within the brain is necessary, as there is minimal absorption of bloodstream T3 across the blood-brain barrier to enter the central nervous system [153]. Interestingly the T4:T3 ratio is approximately 1:1 in the brain compared with other tissues where T4 is more abundant. Located inside the cell, DIO2's primary function is the conversion of T4 into T3 in specific target tissues. However, total T3 produced in peripheral tissues provides ~50 % of total plasma T3. Unlike DIO1, DIO2 is downregulated with increasing T4 (as well as rT3) levels and rapidly degraded via ubiquitin-dependent pathways (half life: minutes to 1 h). DIO2 expression is responsive to cyclic AMP, which activates the CRE sequence using the DIO2 promotor [154]. All this allows for rapid fine tuning of local T3 production in response to changes in circulating T4 levels – presumably also in the TSH-feedback control mechanism in the pituitary. DIO2 knockout mice show little gross phenotype abnormalities, apart from mild growth retardation and hearing loss – again emphasizing the importance of thyroid hormones during development – and pituitary T4 resistance [155, 156]. DIO2 activity is only minimally affected by PTU and aurothioglucose.

The human **deiodinase 3** (DIO3; EC 1.97.1.11; Swissprot: p55073) discovered in 1995 by Salvatore et al. [157] is mapped to 14q32 [158]. Unlike DIO2, DIO3 deiodinates the 5-position of the tyrosyl ring (fig. 2). The resulting products cannot bind to the nuclear T3 receptor and have thus lost the classical thyromimetic effect. Therefore, the prime physiological function attributed to DIO3 is the inactivation of T3 and T4. The

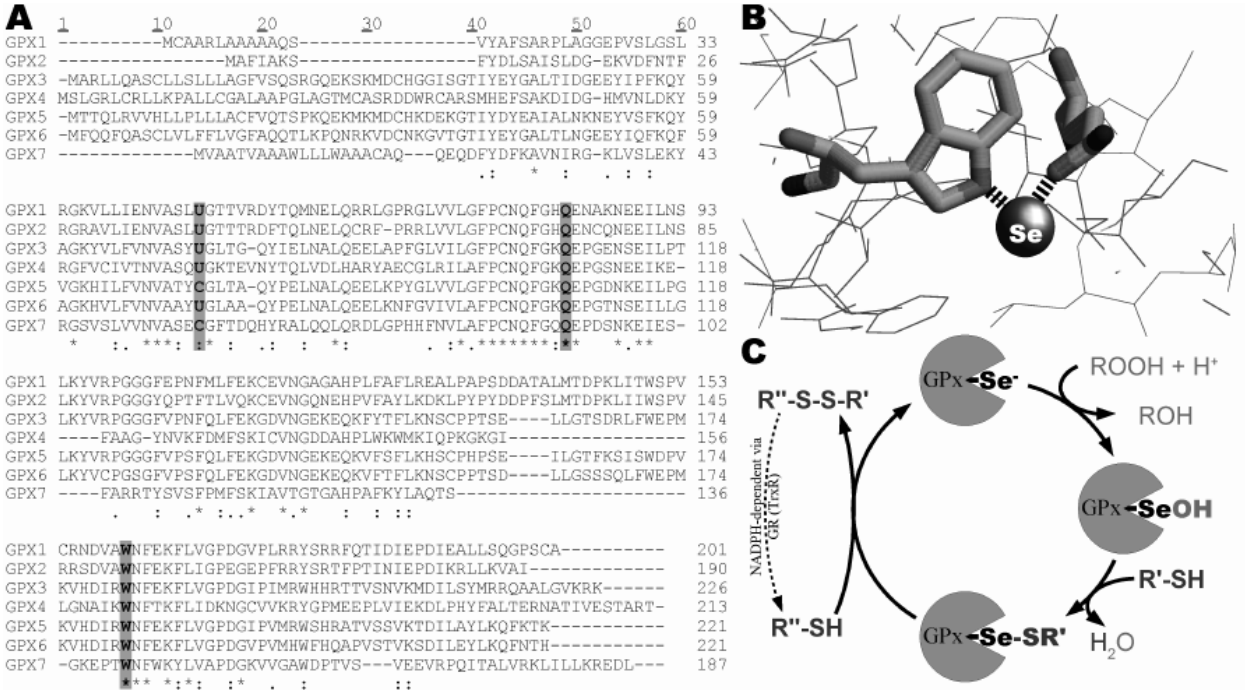


Figure 3. (A) Sequence alignment of the known six human glutathione peroxidase isoforms (using Clustal W V1.82 from http://www.ebi.ac.uk/clustalw). Highlighted are the catalytic triad residues. (B) The catalytic triad of GPx1. Hydrogen bonds are formed between the selenolate and tryptophan imino group as well as the glutamine amido group. The model is based upon structural data of bovine GPx 1 (PDB-code: 1GP1) [21] using H. Bernstein's RasMol V2.7.2.1.1. (C) Simplified sketch of glutathione peroxidase's catalytic mechanism. The enzyme's selenocysteine is oxidized by the peroxide to a selenenic acid, which is consecutively reduced back to the selenolate by thiols, typically glutathione. Mutational exchange of selenocysteine for cysteine reduces the enzyme's turnover by three orders of magnitude. Exchanges of the triad's glutamine or tryptophan for acidic residues affect the enzyme's activity to a similar degree [166, 167]. Modified after [8].

brain, placenta and pregnant uterus express considerably high amounts of DIO3. However, persistently high levels of DIO3 and low levels of T3 may have deleterious effects upon central nervous system development and brain function [157]. Thus, this expression pattern may reflect the organism's attempt to protect the fetal central nervous system from inappropriate levels of T4 and T3 [159]. DIO3 is induced with increasing T4 levels and, like DIO2, almost insensitive towards PTU and gold(I).

Glutathione peroxidases

Glutathione peroxidase [GPx, EC 1.11.1.9 (GPx4: 1.11.1.12)] was the first specific mammalian selenoprotein identified [16,18] and has since received increasing attention. Today the family of glutathione peroxidases includes seven isoenzymes in humans (fig. 3A). One of the last additions to the list, GPx6, was identified in 2003 [108] – 20 years after Flohé's discovery of GPx1 [18]. The black sheep of the family are GPx5 and GPx7, which are not selenoenzymes [160, 161]. Glutathione peroxidases reduce and thereby detoxify different types of peroxides to their respective alcohols at the expense of (typically) glutathione (R-OOH + 2 GSH → R-OH + H_2O + GSSG, fig. 3C). Apparently all of them share the same catalytic mechanism involving a strictly conserved triad formed by selenocysteine, tryptophan and glutamine (fig. 3A+B) [162, 163]. Glutathione peroxidases play an important role in the body's antioxdative armoury. In fact, GPx1 – at least on a quantitative basis – is probably the most important of all mammalian selenoproteins in this respect [164]. The number of research and review publications on these enzymes is countless [162, 163, 165].

Glutathione peroxidase 1 (GPx1; SwissProt: p07203), which was later found to have selenoprotein properties [18], was originally discovered in 1957 [168]. It is a ubiquitous homotetrameric cytosolic enzyme (therefore often referred to as cGPx). GPx1 is abundant in the liver and erythrocytes, with the concentration being dependent on the nutritional selenium status [112]. However, the vast majority of H_2O_2 formed in erythrocytes is not detoxified by GPx, but by catalase [169]. GPx1 knockout mice show no obvious phenotype under normal conditions [170], yet when challenged with oxidative stress, significant pathologies become evident [164]. One may interpret this as evidence that GPx1 plays a limited role under normal conditions. To the contrary, the following experiment demonstrated that we should not underestimate the importance of GPx1: an avirulent coxsackie virus was inoculated in GPx1 knockout mice, and the mice

subsequently developed a cardiomyopathy that closely resembles Keshan disease [113, 171, 172]. The human cardiomyopathy occurring in Keshan disease almost exclusively affects children and is endemic in certain parts of China, where dietary selenium is extremely low [47, 115]. Mice with a functional GPx1 did not develop cardiomyopathy under identical conditions. However, when coxsackie viruses isolated from cardiomyopathic GPx1 knockout mice are transferred to their normal littermates, an identical cardiomyopathy develops. This indicates that in the absence of GPx1 activity, e.g. during selenium deficiency, avirulent strains may mutate to virulent pathogens or at least these conditions select for virulent strains [172–174]. In this context it is also interesting to note that apparently the genomes of several viruses, including HIV-1, HIV-2, hepatitis C virus, coxsackievirus B3 and measles virus encode for GPx homologues [175]. In the case of HIV1, this GPx-like protein is reported to protect the cell from apoptosis [176] and to be involved in the regulation of virus production [177]. It is suggested that its synthesis may deprive the organism of selenium and other limiting resources, supporting the idea that selenium supplementation could be beneficial with viral infections [178], even though in vitro data are not convincing [179].

A number of GPx1-polymorphisms (particularly Pro^{198} Leu) are reported to be associated with an increased risk for cancer– mainly bladder cancer [180] – and vascular diseases [181].

Glutathione peroxidase 2 (GPx2; SwissProt: p18283) is found in the liver and within the gastrointestinal system (but absent in heart and kidney). Therefore, GPx2 is often referred to as GI-GPx. Its distribution varies in the intestine with a decline from the crypts to the luminal surface [182]. GPx2 is a homotetrameric, cytoplasmatic enzyme accepting organic hydroperoxides such as t-butylhydroperoxide, linolic acid hydroperoxides and cumene hydroperoxides (but not phosphatidylcholine hydroperoxide) as substrates. GPx2 is conserved under conditions of inadequate selenium supply [183, 184], and some authors assume that it is the first line of defense against ingested organic hydroperoxides [162, 184, 185]. However, regulatory functions are suggested as well, and GPx2 could be involved in apoptosis and proliferation [182].

GPx2 knockout mice do not have a unique phenotype; however, inflammatory bowl disease and bacteria-induced tumors are typically observed in the GPx1-GPx2 double knockout [186].

Glutathione peroxidase 3 (GPx3; SwissProt: p22352) [187] is located extracellularly in the plasma, hence the acronym pGPx, and in the intestine [188]. GPx3 has the second highest plasma concentration after selenoprotein P. The physiological function of this homotetrameric glycoprotein [189] is not convincingly resolved. So far, an efficient reductant [190] – present in sufficient concentrations in the plasma – has not been found. It is speculated that GPx3 may have regulatory functions. GPx3 expression is induced by hypoxia [191], and its deficiency seems to correlate with cardiovascular events and cancer [192]. Plasma GPx3 is primarily expressed in the renal proximal tubules and is used as a marker to monitor tubular integrity [193].

Glutathione peroxidase 4 (GPx4: SwissProt: p36969; EC 1.11.1.12) is a monomeric enzyme with a number of unusual features. By using alternative initiation sites (Met^{1} or Met^{28}), GPx4 synthesis can generate mitochondrial and cytoplasmatic isoforms. GPx4 also exhibits the broadest substrate specificity of all glutathione peroxidases and can even reduce phospholipid hydroperoxides (and therefore is often referred to as ph-GPx). It is even capable of reducing hydroperoxides still integrated in membranes and may thus play a role as a universal antioxidant in the protection of biomembranes [194, 195]. GPx4 is also involved in redox signaling and regulatory processes, such as inhibiting lipoxygenases and apoptosis [162, 196]. In the testes, where it accounts for almost the total selenium content [197], GPx4 transforms into a relevant structural component of the sperm's midpiece [198]. This requires alternative splicing to introduce a nuclear localization sequence [199]. As such, GPx4 is required for sperm fertilization and not for the cell's antioxidative defense [200]. Thus, it is not surprising that low selenium levels [201] and possibly GPx4 polymorphisms (although gene-specific mutations causing infertility have not yet been identified) [202] are associated with male infertility. GPx4 knockouts are lethal at an early embryonic stage, and the conceptus lacks normal structural compartmentalization. Heterozygous cells are also markedly more sensitive towards induced oxidative stress, which reinforces the notion that GPx4 has an important antioxidative function [203].

Glutathione peroxidase 5 (GPx5; SwissProt: o75715) is a non-selenocysteine containing isoform and found exclusively in the epididymis [161]. It is secreted or membrane bound, and some authors suggest it functions as a backup for the selenocysteine-containing isoforms in sperm [204]. However, it is argued that the human expression level of GPx5 is extremely low, which challenges its perceived importance as a potential radical scavenger [160]. Thus, further analyses are warranted to determine the relevance and function of GPx5.

Glutathione peroxidase 6 (GPx6; SwissProt: p59796) was discovered by Kryukov et al. using an in silico approach. So far, GPx6 expression (as judged by mRNA) is shown only in olfactory epithelium and embryonic tissues [108]. Its rat (Cys-) homologue was cloned by Dear et al. and is a putative odorant metabolizing enzyme [205]. GPx6 is expressed in or near the Bowman's glands, which is a site where several olfactory-specific biotransformation enzymes are localized. This finding solely suggests, yet does not prove, a function for GPx6 in olfaction.

Glutathione peroxidase 7 (GPx7; SwissProt: q96sL4) – like GPx5 – is also a non-selenocysteine-containing isoform. The sequence of this 22-kDa cytoplasmic protein is available in the databases (accession no: AF320068.1), where Gu et al. [unpublished] refer to it as GPx6. It has little detectable glutathione peroxidase activity in vitro [206]. GPx7 is, however, being reported to be involved in breast cancer cell defense against oxidative stress generated from polyunsaturated fatty acid metabolism [206].

The gene loci of these glutathione peroxidases are established: GPx1 is located at 3p21.3, GPx2 at 14q23.3, GPx3 at 5q23.1, GPx4 at 19p13.3, GPx5 at 6p21.32, GPx6 at 6p22.1 and GPx7 at 1p32 in the human genome (As published by Kryukov et al. [108] or online by the HUGO Nomenclature Committee http://www.gene.ucl.ac.uk/nomenclature). It should be noted that several pseudogenes are known. Like DIO1 and thioredoxin reductases, selenocysteine-containing GPxs are inhibited by gold(I) compounds [149,150].

Selenoprotein H

Selenoprotein H (SelH: Swissprot: q8izq5), a globular protein, comprises 122 residues, selenocysteine being the 44th. The genomic sequence of SelH is localized at 11q12.1 in humans [108]. SelH is expressed in numerous tissues, yet no experimental data about its function are available. The CXXU motif suggests a redox function with the selenocysteine possibly forming a selenenylsulfide bridge with Cys-40.

Selenoprotein I

Selenoprotein I (SelI; Swissprot: q9c0d9) contains 397 amino acids and its gene is mapped to 2p23.3 in humans. SelI mRNA is detected in many tissues [108]. In silico sequence analysis predicts up to 10 transmembrane helices, indicative of an integral membrane protein. Functional and structural data are unavailable for this protein.

Selenoprotein K

Selenoprotein K (SelK; Swissprot: q9y6d0) was originally cloned from CD34+ hematopoietic stem cells by Zhang et al. [207]. As in the case of thioredoxin reductase and other selenoproteins [208,209], the selenocysteine UGA was misinterpreted as a stop. Kryukov and co-workers then identified the correct sequence of SelK and located its gene at 3p21.31 [108]. Electron microscopy-assisted immunostaining and computational secondary structure analysis indicate that SelK is indeed a membrane protein [108], but its function remains to be elucidated.

Selenoprotein M

Selenoprotein M's (SelM, Swissprot: q8wwx9) sequence was identified at chromosomal position 22q12.2 [108,210]. Its SECIS element is unusual, as cytosines replace the invariant adenosines (fig. 1A) at the apical loop [210]. It is – as judged by mRNA levels – expressed in many tissues, with the highest levels in the brain and the lowest levels in liver and spleen. SelM is localized (and retained) in the endoplasmatic reticulum, since the first 23 residues contain an ER-signal sequence. The mature protein is presumably 122 residues in size. The retention of SelM in the ER is not accomplished by one of the known sequence motifs and suggests a novel retention mechanism. Similar to many other selenoproteins, the SelM sequence contains a CXXU motif indicative for a redox active protein.

Selenoprotein N

Selenoprotein N (SelN; Swissprot: q9nzv5) was identified by Alain Krol's group using a computational approach [41]. Its gene is located at 1p36.13 in the human genome [108, 211] and expresses two splice isoforms [212]. Isoform 1 corresponds to the full-length transcript, and when exon 3 is spliced out isoform 2 is produced. Both transcripts are detected in skeletal muscle, brain, lung and placenta, but isoform 2 is always more abundant. The exon 3 sequence corresponds to an *Alu* cassette and contains a second in-frame selenocysteine codon. SelN is retained within the ER [212] and seems to be a ubiquitously expressed glycoprotein, particularly during fetal development, but also at lower levels in adults. The fact that SelN is highly expressed in cultured myoblasts and downregulated in differentiating myotubes suggests an involvement in early development, as well as proliferation and regeneration in striated muscles [212]. However, no distinct function is definitely attributed to this selenoprotein so far. Probably a rare experimentum naturae may exist. As described in the biochemistry section, mutations in the SelN gene are associated with rare early onset myopathies: rigid spine muscular dystrophy 1 [211], severe classic multiminicore myopathy [97] and desmin-related myopathy with Mallory bodies. All of these conditions are now referred to as SEPN-related myopathy (SEPN-RM) [213] (see also NCBI-OMIm (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) entry #602771). The potential relationship of SelN to statin-induced myopathies has already been discussed above [94–96]. With a clinical and pharmacological connection, it is hoped that the physiological function of SelN will soon be elucidated – as this future step will probably make a significant contribution to the selenoprotein field.

Selenoprotein O

The selenoprotein O (SelO; Swissprot: q9bvL4) gene is mapped to 22q13.33 [108]. Similar to selenoprotein M, its SECIS element is also unusual because the invariant adenosines at the apical loop are replaced by cytosines [108]. With a total of 669 residues and a predicted molecular weight of 73.4 kDa, SelO is a fairly large protein. Its C-terminal Cys-XX-Sec motif may again be indicative for a redox-dependent activity. Despite its known size, no further information on its function and localization is reported.

Selenoprotein P

Selenoprotein P (SelP; Swissprot: p49908) not only represents the major selenoprotein in plasma, but also provides more than 50 % of the total plasma selenium [214].

It was originally discovered in 1982 in rats [215] and later confirmed in other species, including humans [216]. The gene of the SelP plasma glycoprotein [217, 218] was recently mapped to 5q31 in humans [108]. It is transcribed in many tissues, yet the majority of the plasma SelP (>80 % [219]) is secreted by the liver and presumably enters target cells via a receptor-mediated mechanism [220]. Unlike most selenoproteins that contain only one selenocysteine per polypeptide chain, human SelP contains up to 10 residues per chain [221–223]. Two selenocysteines apparently form a selenenylsulfide bridge with cysteine [224]. SelP is an established marker for the nutritional (liver) selenium status [219, 225]. Its extracellular localization and the repression of SelP expression during acute phase reaction [226], as well as its intrinsic high selenium content and plasma concentration, led researchers to advocate that the primary function of SelP is for storage and transport of selenium [215, 227, 228]. ^{75}Se-radiolabeling studies support this notion by showing selenium enrichment in the brain, kidney and testis [215, 229]. Selenium's tendency to bind heavy metal ions [59,230,231] and its redox properties also suggest a function as a plasma antioxidant and heavy metal antidote [232–236]. However, the belief that SelP functions as an effective antioxidant is challenged by the fact that no efficient reductant has yet been identified in sufficient concentrations in the plasma. In 2003, more than 20 years after its discovery, Schomburg et al. and Hill et al. published their results on SelP-gene disruption experiments in mice [237, 238]. Their results that SelP is a transport molecule carrying nutritional selenium from the liver to peripheral organs are convincing: in their experiment SelP-knockout decreased Se-plasma levels by 80–90 %. Selenium tissue concentrations and selenoenzyme activities dropped markedly in the brain, kidney and testis.

However, unlike in tRNA$^{[Ser]Sec}$ knockout experiments done by Bösl et al. [45], embryonal development showed no obvious phenotype in SelP knockout mice, and other symptoms did not become evident prior to the third postnatal week. This excludes that SelP by itself is of vital importance during early development and indicates that SelP is not the underlying cause of embryo lethality in selenoprotein (<tRNA$^{[Ser]Sec}$) knockout mice. However, starting with approximately the third week post partum, the mice showed reduced weight gain, sporadic fatalities as well as cerebral symptoms such as ataxia [239].

Interestingly, most symptoms, including the cerebral sign, that develop in SelP knockout mice are avoided if the supply of inorganic selenium is increased in the diet [238] or if selenium is indirectly transferred from the mother to the offspring by breast feeding [240]. The symptomatic recovery correlates with increasing selenoenzyme activities in the affected tissues. The only symptom not responding to supranutritional dietary selenium supplementation in SelP-knockout mice is reduced fertility in males. Testicular selenium levels and enzyme activities remain low [238, 241]. Whether this is a rare cause of male infertility in humans, e. g. via SelP-receptor defects, has not yet been addressed. A withdrawal of supranutritional selenium leads to a rapid loss of selenium in all organs, including the brain, and consecutive clinical symptoms (re)develop [219].

In a subsequent study, hepatic SelP release was selectively prevented via a liver-specific tRNA$^{[Ser]Sec}$ knockout [219]. As expected, SelP levels dropped markedly in the plasma. However, unlike in the complete SelP knockout experiments [239], neither a significant decrease in the brain's total selenium content or selenoprotein levels nor clinical cerebral symptoms were observed [219]. Only the kidneys' selenium content and ability to secrete GPx-3 were diminished. This indicates that SelP is required in the brain to retain selenium and that transport mechanisms other than hepatic SelP do exist to provide selenium to most organs at higher nutritional supply. Yet hepatic SelP – even though not essential for the individual – greatly facilitates the distribution of selenium in the organism.

Cumulatively, these results provide a solid basis for the current hypothesis of SelP function [219, 242]: nutritional selenium is delivered to the liver and used for SelP synthesis, which is toxicologically – in contrast to most low molecular weight selenium compounds [1] – rather inert. SelP is then secreted into the plasma and delivered to target tissues where SelP is transported intracellularly via receptor-mediated mechanisms. Within the cell, SelP and subsequently selenocysteine are degraded to liberate selenium that is recycled for the synthesis of novel selenoproteins.

Since the brain has a substantial need for selenium, a sufficient supply must be maintained. It seems that hepatic SelP is not essential as a selenium source for most tissues excluding the testes, which suggests that small molecules

such as selenite and selenomethionine could serve as the selenium supply. Intracellular selenium storage, however, is accompanied by the innate risk of selenium toxicity. Brain SelP may serve as a rapidly available local source of extracellular and thus less dangerous form of selenium. Since brain cells are separated from the bloodstream by a tight blood-brain barrier, the brain SelP pool should not be lost to general circulation. Furthermore, SelP may exert cell-protective functions, such as heavy metal trapping and protection from reactive oxygen species.

Decreased SelP mRNA levels occur often in prostate cancer and suggest that SelP expression is downregulated in this cancer [243]. Indeed, selenium levels are commonly reduced in the plasma of prostate cancer patients. However in contradiction, selenium concentrations are even lower in patients with benign prostate hyperplasia [244]. Furthermore, tissue selenium concentrations have also been reported to be increased in prostate cancer [245]. Thus, even though it is tempting to speculate, it is premature to correlate SelP fluctuations with the development and progression of prostate cancer.

Selenoprotein R

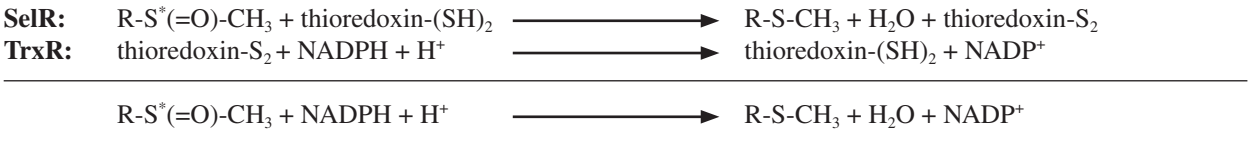
Selenoprotein R is a cytosolic and nucleic protein (SelR; Swissprot: q9nzv6) identified at position 16p13.3 in the human genome [109]. It is a selenoprotein in vertebrates, but its homologues in other eukaryotes and prokaryotes contain a cysteine instead of a selenocysteine. In addition to the selenocysteine residue, one Zn^{2+} ion is bound per 12 kDa molecules via four cysteine residues [246]. SelR is also referred to as selenoprotein X (SelX) [41] and more appropriately as methionine-R-sulfoxide reductase 1 (MsrB1) [246–248]. SelR exhibits the highest specific activity amongst the three principal types of methionine-R-sulfoxide reductases (MsrB) in humans. It should be noted that a second, distinct family of methionine-sulfoxide reductases, called MsrA, is present as well and the latter is required for methionine-S-sulfoxide reduction. MsrB1 (SelR) is the only selenoenzyme of the MsrA and B families known today. SelR and its Cys counterparts are detected in most genomes sequenced so far and are commonly clustered with MsrA. Their expression is stimulated by oxidative stress [246]. Both families, MsrA and MsrB (as judged by data obtained using the prokaryotic enzymes), essentially share the same catalytic mechanism, even though they are structurally unrelated [249].

Interestingly, the reductant used by methionine-sulfoxide reductases is thioredoxin, which in turn is reduced by the selenoenzyme thioredoxin reductase [249] linking the action of these two selenoenzymes:

The catalyzed reaction, the stereospecific and reversible conversion of methionine-R/S-sulfoxides to methionine, is involved in numerous important biological processes, including antioxidant functions, regulation of enzyme activity and cell signaling. Methionine sulfoxide reductases emerged very early during the evolution of life [250,251]. Disruption of this pathway is associated e. g. with Alzheimer's disease [252] and reduced life expectancy. In contrast, overexpression of MsrA increases life span [252]. As an aside, it is critical to mention that in periods of insufficient nutritional selenium supply, the brain's selenium content is preferentially spared [253]. Selenium depletion or abnormal distribution is shown to cause neurodegenerative symptoms and enhance pathological conditions [254,255]. In summary, although the relative importance of SelR is still debated, these findings support the belief that selenoenzymes are crucial players in the protective armory of the brain against oxidative challenge [251].

Selenoprotein S

Selenoprotein S (SelS, Swissprot: q9bqe4) was first predicted as a selenoprotein in silico. SelS's genomic localization is 15q26.3 [108]. Computational secondary structure analysis indicates a single transmembrane helix (as well as many putative phosphorylation and glycosylation sites) and electron microscopy-assisted immunostaining shows that SelS, like SelK, is indeed a plasma and ER-membrane protein [108,256]. Prior to its discovery as a selenoprotein in humans, a rodent homologue (as well as the human sequence) was found and cloned in 2002 by Walder et al., yet they missed Sec as the penultimate amino acid by interpreting the TGA as stop codon [257]. Using differential polymerase chain reaction. Walder and co-workers looked for differences in the expression pattern of hepatic proteins in a rat animal model of type II diabetes compared with their non-diabetic littermates. They called the protein Tanis (Hebrew for fasting), as its expression is increased in fasting diabetic mice. SelS (or Tanis) expression is inversely correlated to the plasma glucose concentration (as well as insulin and triacylglycerol). At least in rats, it is transcribed in almost all tissues, but glucose levels only affect hepatic expression of SelS in vivo [257]. Subsequent experiments using a – as we know now – truncated protein indicate an interaction with serum amyloid A 1β, SAA. This SelS-SAA interaction is



presumably dependent on SelS's C-terminus, where incidentally the Sec is located! SAA and other inflammatory markers are indeed elevated in type II diabetes and are believed to be involved in the long-term complications associated with diabetes (i.e. diabetic nephropathy, neuropathy, retinopathy and vascular disease). In review the in vivo and cell culture data are compelling and seem to show that a dysregulation of SelS expression occurs in diabetes and with impaired glucose tolerance, and SelS is important in hepatic glucose metabolism [258]. However, to completely understand the selenoprotein nature of SelS, more research is needed to examine the suggested SAA interaction.

More recent data suggest that SelS is involved in the retrotransport of misfolded luminal ER proteins to the cytosol for proteosome degradation in a ubiquitin-dependent manner. It is suggested that SelS acts as the link between Derlin-1 (DER1-like protein), an ER-membrane protein involved in the translocation of misfolded proteins to the cytosol, and VCP (including its cofactors Ufd1 and NpI4), an ATPase complex, which mediates translocation and ubiquitinylation of misfolded ER proteins. VCP acts by pulling the misfolded protein back into the cytosol via a protein channel and marks these proteins for degradation [259]. Since SelS recruits the cytosolic VCP to Derlin-1, SelS is also known by the acronym VIMP for VCP-interacting membrane protein. As shown by Ye et al., a large subset of misfolded proteins are retrotranslocated via this SelS-dependent pathway [259]. If and how these two different findings – SelS involvement in hepatic glucose metabolism and retrotranslocation of ER proteins – are related remains to be investigated.

Selenophosphate synthetase 2

Selenophosphate synthetase 2 (SPS2, EC 2.7.9.3, Swissprot: q99611) is a SelD homologue [260,261] and localized at 16p11.1 in the human genome. In contrast, selenophosphate synthetase 1 (SPS1) is not a selenoenzyme. Selenophosphate synthetases catalyze the formation of monoselenophosphate ($HSe^- + ATP + H_2O \rightarrow HSePO_3^{2-} + HPO_4^{2-} + AMP$) and are required not only for the formation of $tRNA^{[Ser]Sec}$-bound selenocysteine but also for 2-selenouridine synthesis in bacteria [35]. The in vivo selenium donor is, however, still not ultimately resolved as kinetic data obtained in vitro do not correlate very well with in vivo conditions [262]. However, local formation could well provide the required selenide concentrations in the vicinity of the SPS enzyme, leaving the concentration low in other regions of the cell. The SPS2 sequence contains Walker A- and B-like motifs, characteristic of α/β-nucleotide-binding folds. SPS2 is expressed in high levels particularly during the early development [261] and interestingly correlates with the lethal effects of $tRNA^{[Ser]Sec}$ knockouts [45]. Sec Cys-mutants of SPS2 are less active than the authentic enzyme, but still capable of performing selenoprotein synthesis in Escherichia coli SelD-knockout mutants [263]. Using SPS1 as the complementing enzyme, inorganic sources for selenide do not yield high amounts of selenoproteins, whereas L-selenocysteine as the selenium source does. The situation is reversed for SPS2: selenide derived from selenite serves as a good selenium source, whereas selenocysteine does not [263].

This led to the hypothesis that SPS1 and SPS2 serve different but complementary purposes: inorganic selenium sources provide selenide [8], which is incorporated via SPS2 forming monoselenophosphate. Free selenocysteine liberated from selenoproteins during proteolysis or accidentally formed in the trans-sulfuration pathway [8] is assumed to be guided to and recycled by SPS1, requiring further assistance by transport proteins and selenocysteine β-lyase activity [263–266]. It is also suggested that SPS2 – due to its selenocysteine residue – may be involved in autoregulation of its own biosynthesis [261]. However, even though this hypothesis is convincing, further experimental evidence is necessary.

Selenoprotein T

Selenoprotein T's (SelT, Swissprot: p62341) gene is localized at 3q24 [108,109]. Its sequence contains a Cys-X-X-Sec motif, similar to the active site in thioredoxins and glutaredoxins, which suggests that SelT has relevant redox properties. However, no further studies are available to validate this view.

Thioredoxin reductase family

The classical thioredoxin system is formed by thioredoxin reductase (TrxR; EC 1.8.1.9; $TrxS_2 + NADPH + H^+ \rightarrow Trx(SH)_2 + NADP^+$) and its associated substrate, the redox active protein thioredoxin (Trx). Trx is reduced at the expense of NADPH. Reduced thioredoxin is reoxidized to provide reducing equivalents to various target molecules such as ribonucleotide reductase [267]. Thioredoxin reductases belong to a family of homodimeric pyridine nucleotide-disulfide oxidoreductases, which includes lipoamide dehydrogenase, glutathione reductase and mercuric ion reductase [268]. Two very distinct classes of thioredoxin reductases have evolved: small thioredoxin reductases (subunit M_r ~35 kDa), present in prokaryotes and fungi, and large TrxRs (subunit M_r approx. 55 kDa), present in higher eukaryotes, including man. Historically, these two classes were considered to be mutually exclusive until Novoselov et al. published a report on the thioredoxin system in the green algae Chlamydomonas reinhardtii [43]. This organism is unusual since it harbours both classes of thioredoxin reductases. However, since small TrxRs are absent in the human genome, they

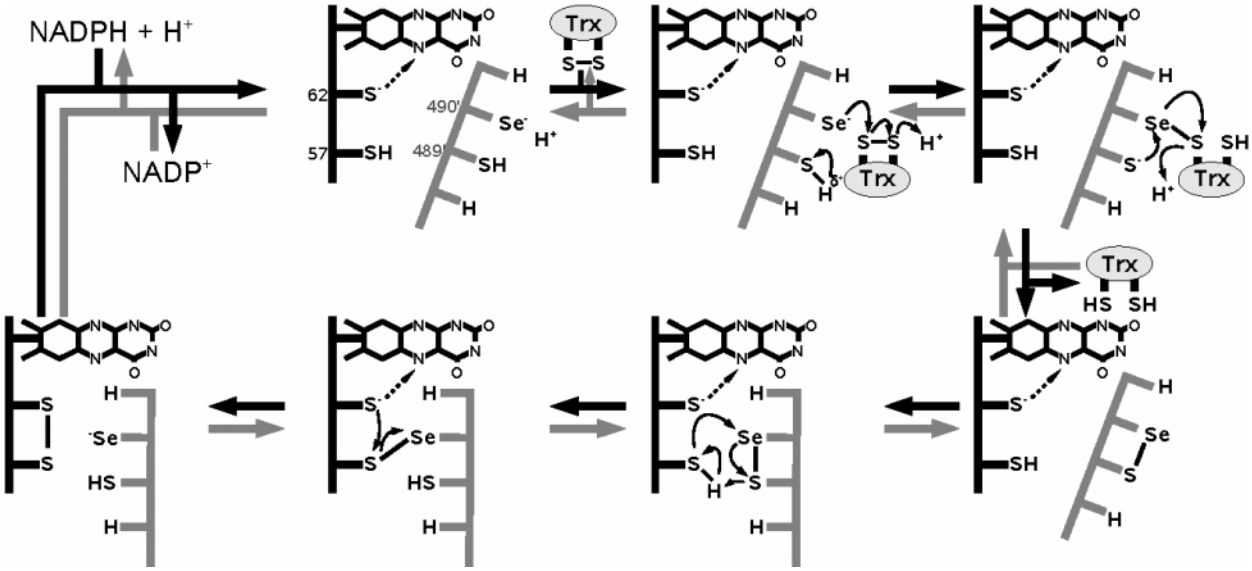


Figure 4. The catalytic mechanism of large thioredoxin reductases. Electrons from NADPH are transferred via the FAD flavin ring to the N-terminal buried Cys-Cys redox active site. The electrons are subsequently transferred to a second redox-active site containing the selenocysteine (GCUG), located on the enzyme's flexible C-terminus. The reduced C-terminal tail moves to a more solvent-exposed position and provides the electrons to the final (typically bulky) substrate, e. g. thioredoxin. In cysteine variants, polar serine residues activate the thiol [110]. However, recent results by Johannson et al. [280] as well as Brandt and Wessjohann [281] indicate that this is not the only requirement in eliminating the need for Sec.

will not be further discussed (even though small TrxRs are interesting targets for novel antibiotic drugs). A TrxR from human placenta was purified by Oblong et al. [269]. The enzyme was soon cloned, but turned out to be inactive. The reason remained unknown, until Stadtman's group – more or less accidentally ("Chance favours the prepared mind" [Louis Pasteur]) – found a selenium-containing thioredoxin reductase in human carcinoma cells [28], which was soon confirmed for all mammalian TrxRs [150, 270]. Its selenocysteine is identified as the penultimate amino acid [209].

Today, three distinct human thioredoxin reductases are known. However, a comparatively large number of splice variants exist. This may be relevant for regulating organelle and cell specific localization [271, 272]. Interestingly, knockout experiments that eliminated the two known isoforms of Trx are both lethal in utero [273, 274] as is a TrxR2 knockout (discussed below) [275]. The thioredoxin system is involved in a myriad of cellular and intercellular processes, and today it is difficult to distinguish the most important pathways. Numerous reviews have been published on the thioredoxin system and highlight different aspects of this system. We suggest referencing these reviews for more detailed discussions [267, 268, 276–279].

It should be noted that thioredoxin reductases exhibit an unusual broad substrate spectrum which includes low molecular weight compounds and large proteins. All large TrxRs essentially share the same catalytic mechanism, as depicted in figure 4 [110, 267].

A significant challenge in thioredoxin reductase research is the use of unstandardized nomenclature, which is frequently observed in the literature (Commonly used designations: TrxR1: = TR1 = TR α; TrxR2: = TR3 = TR β TGR: = TR2 = TrxR3). Here we will use the systematic nomenclature outlined in [267].

Thioredoxin reductase 1 (TrxR1, GENE-name: TXNRD1; Swissprot: q16881) is a ubiquitous cytoplasmatic housekeeping enzyme. Its gene has been mapped to 12q23-q24.1. It is involved in many aspects of cellular redox regulation [282]. It is capable of inducing apoptosis if the enzyme does not contain selenocysteine or if this residue is blocked e. g. by a chemotherapeutic agent [283]. These findings render it an even more interesting target for chemotherapy than its involvement in desoxyribonucleotide synthesis already does [284]. TrxR1 is also secreted in the plasma, yet the importance of this finding remains uncertain [285].

Thioredoxin reductase 2 (TrxR2, GENE-name: TXNRD2; Swissprot: q9nnw7) is located in mitochondria [286] with highest levels in the prostate, testis, liver, uterus and small intestine and intermediate levels in brain, skeletal muscle, heart and spleen. Two splice variants designated SelZf1 and SelZf2 are described at the mRNA level [41]. However, these isoforms lack the N-terminal redox active site CVNVGC and are thus catalytically inactive. Whether these isoforms are artefacts or serve a function remains to be established. TrxR2 knockout studies led to early embryonic death with signs of severe

anemia, apoptosis in the liver and heart abnormalities. A heart-specific knockout causes a dilatative cardiomyopathy and early death, similar to Keshan disease [113, 275]. TrxR2 has been mapped to 22q11.21, a gene locus associated with DiGeorge syndrome (NCBI-OMIM: #188400). DiGeorge syndrome is a congenital disease caused by a developmental defect of the embryonic pharyngeal system. However, no direct association between DiGeorge syndrome and TrxR2 has been made to date, and animal models as well as studies in humans suggest that DiGeorge syndrome is due to the loss of a transcription factor called T-box 1 (TBX1), which is apparently the most important cause for the clinically observed phenotype [287].

Thioredoxin glutathione reductase (TGR, GENEname: TXNRD3; Accession no: XP_051264.6) is a testis-specific enzyme mapped to 3p13-q13.33. Unlike TrxR1 and TrxR2, it can reduce glutathione disulfide since it contains a N-terminal 1-Cys glutaredoxin-like domain. It is located in the ER [288]. TGR's specific function is currently unknown.

Selenoprotein V

Selenoprotein V (SelV, Swissprot: p59797) was identified using an in silico approach and shows homology to SelW. Its sequence is localized at 19q13.13 [108]. SelV expression – as judged by mRNA in situ hybridization – seems to be limited to the seminiferous tubules of the testes [108]. Unfortunately, there is little information available about its physiological function. The CGLU motif in the SelV sequence may suggest a redox-related function.

Selenoprotein W

Selenoprotein W (SelW, Swissprot: p63302), first published in 1993, is a small protein (9.5 kDa) initially purified from rat muscle [289], but later also demonstrated in most other tissues [290]. Only trace amounts of SelW are found in liver, thyroid, pancreas, eye and pituitary gland [291–293]. Furthermore, it was shown in rats that different quantities were gender specific in certain tissues [294]. The "W" is derived from the fact that SelW is one of the missing selenoproteins in selenium-deficient lambs suffering from *w*hite muscle disease [293]. SelW levels in fetal heart and muscle correspond well to the selenium status in human fetuses [293]. SelW is mapped to 19q13.32 in the human genome [108,295]. It is present mainly in the cytosol, but small amounts are found associated with membranes as well [290]. Already in the first report on SelW, four forms had been isolated with slightly different masses [289,296]. These differences were later shown to be due to the binding of low molecular weight compounds, in particular glutathione, which is bound rather firmly under anaerobic conditions [297] to Cys-36. To remove glutathione, a 1000-fold excess of dithiothreitol at 50 °C is required. This finding suggests that SelW has a potential function in redox metabolism, a view supported by cell culture studies [296,298–300]. As such, SelW may act in the developing brain as it exhibits a different and non-overlapping distribution with another important antioxidant – thioredoxin – as indicated by mRNA studies [301].

The nature and function of an additional (42 Da) low molecular weight compound commonly bound to SelW is still unresolved [293].

SelW tissue levels respond to changes in the selenium supply, yet the pattern differs from that of glutathione peroxidase [290]. SelW is effectively retained in the brain during times of selenium deficiency.

However, despite all of this data, no definite function has yet been attributed to selenoprotein W.

Summary and outlook

Our understanding of selenium has progressed substantially since its discovery in 1817: from a hated toxin to an essential trace element. With the discovery of glutathione peroxidase as the first specific mammalian selenoprotein, scientists around the world have advanced our knowledge of selenium and selenoproteins, but many questions remain to be answered in the field. Today 25 distinct members of this elite protein family have been identified in humans – not counting the ever-increasing number of splice variants. New methodologies that allow the recombinant expression of these proteins [103, 302] will certainly be advantageous in our efforts to elucidate the specific function and potential clinical importance of each selenoprotein. Overall, most selenoproteins are, or at least seem, to be involved in antioxidative defense and redox metabolism – but in our next glance at human selenoproteins, we may discover new surprises…

Acknowledgement. We are indebted to the countless women and men whose research provided the basis for this review. This work would not have been possible without the aid of modern online research resources, open source software and the creators of these new technologies. We would like to thank R. Heiner Schirmer for stimulating discussions and insightful comments and Barbara Mattern for assistance with the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (GR 2028/1-1).

1 Falbe J. and Regitz M. (1999) Römpp kompakt Basislexikon Chemie, vol. 4 (Rf-Z), Thieme, Stuttgart
2 Gassmann T. (1916) Der Nachweis des Selens im Knochen- und Zahngewebe. Hoppe-Seyler's Z. Physiol. Chem. **97:** 307–310
3 Clayton C. C. and Baumann C. A. (1949) Diet and azo dye tumors: Effect of diet during periods when the dye is fed. Cancer Res. **9:** 575–582
4 Behne D. and Kyriakopoulos A. (2001) Mammalian selenium-containing proteins. Annu. Rev. Nutr. **21:** 453–473

5 Moxon A. L. (1937) Alkali disease, or selenium poisoning. So. Dak. Agric. Exp. Sta. Tech. Bull. **311:** 1–91
6 Nelson A. A., Fitzhugh O. G. and Calvery H. O. (1943) Liver tumors following cirrhosis caused by selenium in rats. Cancer Res. **3:** 230–236.
7 Tscherkes L. A., Volgarev M. N. and Aptekar S. G. (1963) Selenium-caused tumours. Acta Unio Int. Contra Cancrum **19:** 632–633
8 Birringer M., Pilawa S. and Flohé L. (2002) Trends in selenium biochemistry. Nat. Prod. Rep. **19:** 693–718
9 Schwarz K. and Foltz C. M. (1957) Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. **79:** 3292–3293
10 Ullrey D. E., Combs G. F. J., Conrad H. R., Hoekstra W. G., Jenkins K. J. W., Levander O. A. et al. (1983) Selenium in Nutrition, pp. 174, National Academy Press, Washington, DC
11 Oldfield J. E. (2002) A brief history of selenium research: From alkali disease to prostate cancer (from poison to prevention) J. Anim. Sci. Online supplement, 1–4
12 Patterson E. L., Milstrey R. and Stokstad E. L. (1957) Effect of selenium in preventing exudative diathesis in chicks. Proc. Soc. Exp. Biol. Med. **95:** 617–620
13 McCoy K. E. and Weswig P. H. (1969) Some selenium responses in the rat not related to vitamin E. J. Nutrr **98:** 383–389
14 Schroeder H. A., Frost D. V. and Balassa J. J. (1970) Essential trace metals in man: selenium. J. Chronic. Dis. **23:** 227–243
15 Turner D. C. and Stadtman T. C. (1973) Purification of protein components of the clostridial glycine reductase system and characterization of protein A as a selenoprotein. Arch. Biochem. Biophys. **154:** 366–381
16 Rotruck J. T., Pope A. L., Ganther H. E., Swanson A. B., Hafeman D. G. and Hoekstra W. G. (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science **179:** 588–590
17 Rotruck J. T., Hoekstra W. G., Pope A. L., Ganther H., Swanson A. B. and Hafeman D. G. (1972) Relationship of selenium to glutathione peroxidase. Fed. Proc. **31:** 691
18 Flohé L., Günzler W. A. and Schock H. H. (1973) Glutathione peroxidase: a selenoenzyme. FEBS Lett. **32:** 132–134
19 Cone J. E., Del Rio R. M., Davis J. N. and Stadtman T. C. (1976) Chemical characterization of the selenoprotein component of clostridial glycine reductase: identification of selenocysteine as the organoselenium moiety. Proc. Natl. Acad. Sci. USA **73:** 2659–2663
20 Forstrom J. W., Zakowski J. J. and Tappel A. L. (1978) Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry **17:** 2639–2644
21 Epp O., Ladenstein R. and Wendel A. (1983) The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution. Eur. J. Biochem. **133:** 51–69
22 Rocher C., Lalanne J. L. and Chaudière J. (1992) Purification and properties of a recombinant sulfur analog of murine selenium-glutathione peroxidase. Eur. J. Biochem. **205:** 955–960
23 Rocher C., Faucheu C., Herve F., Benicourt C. and Lalanne J. L. (1991) Cloning of murine SeGpx cDNA and synthesis of mutated GPx proteins in Escherichia coli. Gene **98:** 193–200
24 Günzler W. A., Steffens G. J., Grossmann A., Kim S. M., Otting F., Wendel A. et al. (1984) The amino-acid sequence of bovine glutathione peroxidase. Hoppe-Seylers Z. Physiol. Chem. **365:** 195–212
25 Böck A., Forchhammer K., Heider J., Leinfelder W., Sawers G., Veprek B. et al. (1991) Selenocysteine: the 21st amino acid. Mol. Microbiol. **5:** 515–520
26 Andreesen J. R. and Ljungdahl L. G. (1973) Formate dehydrogenase of Clostridium thermoaceticum: incorporation of selenium-75, and the effects of selenite, molybdate, and tungstate on the enzyme. J. Bacteriol. **116:** 867–873
27 Behne D., Kyriakopoulos A., Meinhold H. and Köhrle J. (1990) Identification of type I iodothyronine 5'-deiodinase as a selenoenzyme. Biochem Biophys Res Commun **173:** 1143–1149
28 Tamura T. and Stadtman T. C. (1996) A new selenoprotein from human lung adenocarcinoma cells: purification, properties and thioredoxin reductase activity. Proc. Natl. Acad. Sci. USA **93:** 1006–1011
29 Hatfield D. and Diamond A. (1993) UGA: a split personality in the universal genetic code. Trends Genet. **9:** 69–70
30 Hao B., Gong W., Ferguson T. K., James C. M., Krzycki J. A. and Chan M. K. (2002) A new UAG-encoded residue in the structure of a methanogen methyltransferase. Science **296:** 1462–1466
31 Zhang Y., Baranov P. V., Atkins J. F. and Gladyshev V. N. (2005) Pyrrolysine and selenocysteine use dissimilar decoding strategies. J. Biol. Chem. **280:** 20740–20751
32 Chambers I., Frampton J., Goldfarb P., Affara N., McBain W. and Harrison P. R. (1986) The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. EMBO J. **5:** 1221–1227
33 Zinoni F., Birkmann A., Stadtman T. C. and Böck A. (1986) Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from Escherichia coli. Proc. Natl. Acad. Sci. USA **83:** 4650–4654
34 Heider J. and Böck A. (1993) Selenium metabolism in microorganisms. Adv. Microb. Physiol. **35:** 71–109
35 Stadtman T. C. (1996) Selenocysteine. Annu. Rev. Biochem. **65:** 83–100
36 Böck A. and Stadtman T. C. (1988) Selenocysteine, a highly specific component of certain enzymes, is incorporated by a UGA-directed co-translational mechanism. Biofactors **1:** 245–250
37 Berry M. J., Martin G. W. 3rd and Low S. C. (1997) RNA and protein requirements for eukaryotic selenoprotein synthesis. Biomed. Environ. Sci. **10:** 182–189
38 Gladyshev V. N., Kryukov G. V., Fomenko D. E. and Hatfield D. L. (2004) Identification of trace element-containing proteins in genomic databases. Annu. Rev. Nutr. **24:** 579–596
39 Behne D., Weiss-Nowak C., Kalcklosch M., Westphal C., Gessner H. and Kyriakopoulos A. (1995) Studies on the distribution and characteristics of new mammalian selenium-containing proteins. Analyst **120:** 823–825
40 Behne D., Kyriakopoeulos A., Weiss-Nowak C., Kalckloesch M., Westphal C. and Gessner H. (1996) Newly found selenium-containing proteins in the tissues of the rat. Biol. Trace Elem. Res. **55:** 99–110
41 Lescure A., Gautheret D., Carbon P. and Krol A. (1999) Novel selenoproteins identified in silico and in vivo by using a conserved RNA structural motif. J. Biol. Chem. **274:** 38147–38154
42 Kryukov G. V. and Gladyshev V. N. (2004) The prokaryotic selenoproteome. EMBO Rep. **5:** 538–543
43 Novoselov S. V. and Gladyshev V. N. (2003) Non-animal origin of animal thioredoxin reductases: implications for selenocysteine evolution and evolution of protein function through carboxy-terminal extensions. Protein Sci. **12:** 372–378
44 Zhang Y., Fomenko D. E. and Gladyshev V. N. (2005) The microbial selenoproteome of the Sargasso Sea. Genome Biol. **6:** R37
45 Bösl M. R., Takaku K., Oshima M., Nishimura S. and Taketo M. M. (1997) Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp) Proc. Natl. Acad. Sci. USA **94:** 5531–5534
46 Oldfield J. E. (1999) The case for selenium fertilization: an update. Bulletin Selenium Tellurium Dev. Assoc. 1–3
47 Oldfield J. E. (2002) Se Atlas 2002, pp. 1–9 Selenium Tellurium Dev. Assoc. Grimbergen, Belgium
48 Clark L. C., Combs G. F. Jr, Turnbull B. W., Slate E. H., Chalker D. K., Chow J. et al. (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA **276:** 1957–1963

49 Clark L. C., Dalkin B., Krongrad A., Combs G. F. Jr, Turnbull B. W., Slate E. H. et al. (1998) Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. Br. J. Urol. **81:** 730–734

50 Duffield-Lillico A. J., Reid M. E., Turnbull B. W., Combs G. F. Jr, Slate E. H., Fischbach L. A. et al. (2002) Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. Cancer Epidemiol. Biomarkers Prev. **11:** 630–639

51 Reid M. E., Duffield-Lillico A. J., Garland L., Turnbull B. W., Clark L. C. and Marshall J. R. (2002) Selenium supplementation and lung cancer incidence: an update of the nutritional prevention of cancer trial. Cancer Epidemiol. Biomarkers Prev. **11:** 1285–1291

52 Castellano S., Novoselov S. V., Kryukov G. V., Lescure A., Blanco E., Krol A. et al. (2004) Reconsidering the evolution of eukaryotic selenoproteins: a novel nonmammalian family with scattered phylogenetic distribution. EMBO Rep. **5:** 71–77

53 Low S. C. and Berry M. J. (1996) Knowing when not to stop: selenocysteine incorporation in eukaryotes. Trends Biochem. Sci. **21:** 203–238

54 Mugesh G., du Mont W. W. and Sies H. (2001) Chemistry of biologically important synthetic organoselenium compounds. Chem. Rev. **101:** 2125–2179

55 Nakamuro K., Okuno T. and Hasegawa T. (2000) Metabolism of selenoamino acids and contribution of selenium methylation to toxicity. J. Health Sci. **46:** 418–421

56 Schrauzer G. N. (2000) Selenomethionine: a review of its nutritional significance, metabolism and toxicity. J. Nutr. **130:** 1653–1656

57 Gromer S. and Gross J. H. (2002) Methylseleninate is a substrate rather than an inhibitor of mammalian thioredoxin reductase. Implications for the antitumor effects of selenium. J. Biol. Chem. **277:** 9701–9706

58 Hollemann A. F. and Wiberg N. (1985) Lehrbuch der anorganischen Chemie, p. 1451, de Gruyter, Berlin

59 Kaim W. and Schwederski B. (1995) Bioanorganische Chemie: zur Funktion chemischer Elemente in Lebensprozessen, p. 460, Teubner, Stuttgart

60 Huber R. E. and Criddle R. S. (1967) Comparison of the chemical properties of selenocysteine and selenocystine with their sulfur analogs. Arch. Biochem. Biophys. **122:** 164–173

61 Dilworth G. L. (1982) Properties of the selenium-containing moiety of nicotinic acid hydroxylase from Clostridium barkeri. Arch. Biochem. Biophys. **219:** 30–38

62 Gladyshev V. N., Khangulov S. V., Axley M. J. and Stadtman T. C. (1994) Coordination of selenium to molybdenum in formate dehydrogenase H from Escherichia coli. Proc. Natl. Acad. Sci. USA **91:** 7708–7711

63 Gladyshev V. N., Khangulov S. V. and Stadtman T. C. (1994) Nicotinic acid hydroxylase from Clostridium barkeri: electron paramagnetic resonance studies show that selenium is coordinated with molybdenum in the catalytically active selenium-dependent enzyme. Proc. Natl. Acad. Sci. USA **91:** 232–236

64 Boles J. O., Cisneros R. J., Weir M. S., Odom J. D., Villafranca J. E. and Dunlap R. B. (1991) Purification and characterization of selenomethionyl thymidylate synthase from Escherichia coli: comparison with the wild-type enzyme. Biochemistry **30:** 11073–11080

65 Bernard A. R., Wells T. N., Cleasby A., Borlat F., Payton M. A. and Proudfoot A. E. (1995) Selenomethionine labelling of phosphomannose isomerase changes its kinetic properties. Eur. J. Biochem. **230:** 111–118

66 Huber R. E. and Criddle R. S. (1967) The isolation and properties of beta-galactosidase from Escherichia coli grown on sodium selenate. Biochim. Biophys. Acta **141:** 587–599

67 Hendrickson W. A. (1991) Determination of macromolecular structures from anomalous diffraction of synchrotron radiation. Science **254:** 51–58

68 Hendrickson W. A., Horton J. R. and LeMaster D. M. (1990) Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure. EMBO J. **9:** 1665–1672

69 Sliwkowski M. X. and Stadtman T. C. (1985) Incorporation and distribution of selenium into thiolase from Clostridium kluyveri. J. Biol. Chem. **260:** 3140–3144

70 Young P. A. and Kaiser, II (1975) Aminoacylation of Escherichia coli cysteine tRNA by selenocysteine. Arch. Biochem. Biophys. **171:** 483–489

71 Müller S., Senn H., Gsell B., Vetter W., Baron C. and Böck A. (1994) The formation of diselenide bridges in proteins by incorporation of selenocysteine residues: biosynthesis and characterization of $(Se)_2$-thioredoxin. Biochemistry **33:** 3404–3412

72 Kramer G. F. and Ames B. N. (1988) Isolation and characterization of a selenium metabolism mutant of Salmonella typhimurium. J. Bacteriol. **170:** 736–743

73 Böck A., Forchhammer K., Heider J. and Baron C. (1991) Selenoprotein synthesis: an expansion of the genetic code. Trends Biochem. Sci. **16:** 463–467

74 Böck A. (2000) Biosynthesis of selenoproteins – an overview. Biofactors **11:** 77–78

75 Leinfelder W., Zehelein E., Mandrand-Berthelot M. A. and Böck A. (1988) Gene for a novel tRNA species that accepts L-serine and cotranslationally inserts selenocysteine. Nature **331:** 723–725

76 Amberg R., Mizutani T., Wu X. Q. and Gross H. J. (1996) Selenocysteine synthesis in mammalia: an identity switch from $tRNA^{Ser}$ to $tRNA^{Sec}$. J. Mol. Biol. **263:** 8–19

77 Lee B. J., Worland P. J., Davis J. N., Stadtman T. C. and Hatfield D. L. (1989) Identification of a selenocysteyl-tRNA(Ser) in mammalian cells that recognizes the nonsense codon, UGA. J. Biol. Chem. **264:** 9724–9727

78 Carlson B. A., Xu X. M., Kryukov G. V., Rao M., Berry M. J., Gladyshev V. N. et al. (2004) Identification and characterization of phosphoseryl-$tRNA^{[Ser]Sec}$ kinase. Proc. Natl. Acad. Sci. USA **101:** 12848–12853

79 Mizutani T. (1989) Some evidence of the enzymatic conversion of bovine suppressor phosphoseryl-tRNA to selenocysteyl-tRNA. FEBS Lett. **250:** 142–146

80 Mizutani T., Kanaya K. and Tanabe K. (1999) Selenophosphate as a substrate for mammalian selenocysteine synthase, its stability and toxicity. Biofactors **9:** 27–36

81 Diamond A. M. (2004) On the road to selenocysteine. Proc. Natl. Acad. Sci. USA **101:** 13395–13396

82 Mizutani T., Kurata H., Yamada K. and Totsuka T. (1992) Some properties of murine selenocysteine synthase. Biochem. J. **284** (Pt 3): 827–834

83 Tormay P., Wilting R., Lottspeich F., Mehta P. K., Christen P. and Böck A. (1998) Bacterial selenocysteine synthase-structural and functional properties. Eur. J. Biochem. **254:** 655–661

84 Hatfield D. L. and Gladyshev V. N. (2002) How selenium has altered our understanding of the genetic code. Mol. Cell. Biol. **22:** 3565–3576

85 Kim L. K., Matsufuji T., Matsufuji S., Carlson B. A., Kim S. S., Hatfield D. L. et al. (2000) Methylation of the ribosyl moiety at position 34 of selenocysteine $tRNA^{[Ser]Sec}$ is governed by both primary and tertiary structure. RNA **6:** 1306–1315

86 Chittum H. S., Hill K. E., Carlson B. A., Lee B. J., Burk R. F. and Hatfield D. L. (1997) Replenishment of selenium deficient rats with selenium results in redistribution of the selenocysteine tRNA population in a tissue specific manner. Biochim. Biophys. Acta **1359:** 25–34

87 Moustafa M. E., Carlson B. A., El-Saadani M. A., Kryukov G. V., Sun Q. A., Harney J. W. et al. (2001) Selective inhibition of selenocysteine tRNA maturation and selenoprotein synthesis in transgenic mice expressing isopentenyladenosine-deficient selenocysteine tRNA. Mol. Cell. Biol. **21:** 3840–3852

88 Carlson B. A., Novoselov S. V., Kumaraswamy E., Lee B. J., Anver M. R., Gladyshev V. N. et al. (2004) Specific excision of the selenocysteine tRNA[Ser]Sec (Trsp) gene in mouse liver demonstrates an essential role of selenoproteins in liver function. J. Biol. Chem. **279:** 8011–8017
89 Carlson B. A., Xu X. M., Gladyshev V. N. and Hatfield D. L. (2005) Selective rescue of selenoprotein expression in mice lacking a highly specialized methyl group in selenocysteine tRNA. J. Biol. Chem. **280:** 5542–5548
90 Hornberger T. A., McLoughlin T. J., Leszczynski J. K., Armstrong D. D., Jameson R. R., Bowen P. E. et al. (2003) Selenoprotein-deficient transgenic mice exhibit enhanced exercise-induced muscle growth. J. Nutr. **133:** 3091–3097
91 Driscoll D. M. and Copeland P. R. (2003) Mechanism and regulation of selenoprotein synthesis. Annu. Rev. Nutr. **23:** 17–40
92 Warner G. J., Berry M. J., Moustafa M. E., Carlson B. A., Hatfield D. L. and Faust J. R. (2000) Inhibition of selenoprotein synthesis by selenocysteine tRNA[Ser]Sec lacking isopentenyladenosine. J. Biol. Chem. **275:** 28110–28119
93 Stein E. A. (2002) Management of dyslipidemia in the high-risk patient. Am. Heart J. **144:** S43–50
94 Moosmann B. and Behl C. (2004) Selenoproteins, cholesterol-lowering drugs, and the consequences: revisiting of the mevalonate pathway. Trends Cardiovasc. Med. **14:** 273–281
95 Moosmann B. and Behl C. (2004) Selenoprotein synthesis and side-effects of statins. Lancet **363:** 892–894
96 Baker S. K. (2005) Molecular clues into the pathogenesis of statin-mediated muscle toxicity. Muscle Nerve **31:** 572–580
97 Ferreiro A., Quijano-Roy S., Pichereau C., Moghadaszadeh B., Goemans N., Bonnemann C. et al. (2002) Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multiminicore disease: reassessing the nosology of early-onset myopathies. Am. J. Hum. Genet. **71:** 739–749
98 Venance S. L., Koopman W. J., Miskie B. A., Hegele R. A. and Hahn A. F. (2005) Rigid spine muscular dystrophy due to SEPN1 mutation presenting as cor pulmonale. Neurology **64:** 395–396
99 Friis S., Poulsen A. H., Johnsen S. P., McLaughlin J. K., Fryzek J. P., Dalton S. O. et al. (2005) Cancer risk among statin users: a population-based cohort study. Int. J. Cancer **114:** 643–647
100 Brown B. G., Zhao X. Q., Chait A., Fisher L. D., Cheung M. C., Morse J. S. et al. (2001) Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N. Engl. J. Med. **345:** 1583–1592
101 Buettner C., Harney J. W. and Larsen P. R. (1998) The 3'-untranslated region of human type 2 iodothyronine deiodinase mRNA contains a functional selenocysteine insertion sequence element. J. Biol. Chem. **273:** 33374–33378
102 Grundner-Culemann E., Martin G. W. 3rd, Harney J. W. and Berry M. J. (1999) Two distinct SECIS structures capable of directing selenocysteine incorporation in eukaryotes. RNA **5:** 625–635
103 Su D., Li Y. and Gladyshev V. N. (2005) Selenocysteine insertion directed by the 3'-UTR SECIS element in Escherichia coli. Nucleic Acids Res. **33:** 2486–2492
104 Copeland P. R. and Driscoll D. M. (1999) Purification, redox sensitivity and RNA binding properties of SECIS-binding protein 2, a protein involved in selenoprotein biosynthesis. J. Biol. Chem. **274:** 25447–25454
105 Copeland P. R., Fletcher J. E., Carlson B. A., Hatfield D. L. and Driscoll D. M. (2000) A novel RNA binding protein, SBP2, is required for the translation of mammalian selenoprotein mRNAs. EMBO J. **19:** 306–314
106 Lescure A., Fagegaltier D., Carbon P. and Krol A. (2002) Protein factors mediating selenoprotein synthesis. Curr. Protein. Pept. Sci. **3:** 143–151
107 Berry M. J., Banu L., Harney J. W. and Larsen P. R. (1993) Functional characterization of the eukaryotic SECIS elements which direct selenocysteine insertion at UGA codons. EMBO J. **12:** 3315–3322
108 Kryukov G. V., Castellano S., Novoselov S. V., Lobanov A. V., Zehtab O., Guigo R. et al. (2003) Characterization of mammalian selenoproteomes. Science **300:** 1439–1443
109 Kryukov G. V., Kryukov V. M. and Gladyshev V. N. (1999) New mammalian selenocysteine-containing proteins identified with an algorithm that searches for selenocysteine insertion sequence elements. J. Biol. Chem. **274:** 33888–33897
110 Gromer S., Johansson L., Bauer H., Arscott L. D., Rauch S., Ballou D. P. et al. (2003) Active sites of thioredoxin reductases: why selenoproteins? Proc. Natl. Acad. Sci. USA **100:** 12618–12623
111 Root E. J. and Combs G. F. Jr (1988) Disruption of endoplasmic reticulum is the primary ultrastructural lesion of the pancreas in the selenium-deficient chick. Proc. Soc. Exp. Biol. Med. **187:** 513–521
112 Food and Nutrition Board, Institute of Medicine (2000) Selenium. In: Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. A Report of the Panel on Dietary Antioxidants and Related Compounds, vol. 1, pp. 284–324, National Academy Press, Washington, DC
113 Ge K., Xue A., Bai J. and Wang S. (1983) Keshan disease-an endemic cardiomyopathy in China. Virchows Arch. A Pathol. Anat. Histopathol. **401:** 1–15
114 Peng X., Lingxia Z., Schrauzer G. N. and Xiong G. (2000) Selenium, boron, and germanium deficiency in the etiology of Kashin-Beck disease. Biol. Trace Elem. Res. **77:** 193–197
115 Ge K. and Yang G. (1993) The epidemiology of selenium deficiency in the etiological study of endemic diseases in China. Am. J. Clin. Nutr. **57:** 259S–263S
116 Moreno-Reyes R., Suetens C., Mathieu F., Begaux F., Zhu D., Rivera M. T. et al. (1998) Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and iodine status. N. Engl. J. Med. **339:** 1112–1120
117 Rayman M. P. (2000) The importance of selenium to human health. Lancet **356:** 233–241
118 Klein E. A., Thompson I. M., Lippman S. M., Goodman P. J., Albanes D., Taylor P. R. et al. (2001) SELECT: the next prostate cancer prevention trial. Selenum and Vitamin E Cancer Prevention Trial. J. Urol. **166:** 1311–1315
119 Underwood E. J. and Suttle N. F., eds (1999) Mineral nutrition in Livestock, p. 624, CAB International Publishing, Wallingford, UK
120 Finley J. W. and Davis C. D. (2001) Selenium (Se) from high-selenium broccoli is utilized differently than selenite, selenate and selenomethionine, but is more effective in inhibiting colon carcinogenesis. Biofactors **14:** 191–196
121 Ip C., Thompson H. J., Zhu Z. and Ganther H. E. (2000) In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. Cancer Res. **60:** 2882–2886
122 Combs G. F. Jr (1999) Chemopreventive mechanisms of selenium. Med. Klin. 94 Suppl. **3:** 18–24
123 Davis C. D. and Uthus E. O. (2003) Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one-carbon metabolism in rats. J. Nutr. **133:** 2907–2914
124 Davis C. D., Uthus E. O. and Finley J. W. (2000) Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon. J. Nutr. **130:** 2903–2909
125 Behne D., Kyriakopoulos A., Kalcklosch M., Weiss-Nowak C., Pfeifer H., Gessner H. et al. (1997) Two new selenoproteins found in the prostatic glandular epithelium and in the spermatid nuclei. Biomed. Environ. Sci. **10:** 340–345
126 Gladyshev V. N., Jeang K. T., Wootton J. C. and Hatfield D. L. (1998) A new human selenium-containing protein. Purification, characterization and cDNA sequence. J. Biol. Chem. **273:** 8910–8915

127 Kumaraswamy E., Malykh A., Korotkov K. V., Kozyavkin S., Hu Y., Kwon S. Y. et al. (2000) Structure-expression relationships of the 15-kDa selenoprotein gene. Possible role of the protein in cancer etiology. J. Biol. Chem. **275:** 35540–35547

128 Nagai H., Negrini M., Carter S. L., Gillum D. R., Rosenberg A. L., Schwartz G. F. et al. (1995) Detection and cloning of a common region of loss of heterozygosity at chromosome 1p in breast cancer. Cancer Res. **55:** 1752–1757

129 Cheung T. H., Chung T. K., Poon C. S., Hampton G. M., Wang V. W. and Wong Y. F. (1999) Allelic loss on chromosome 1 is associated with tumor progression of cervical carcinoma. Cancer **86:** 1294–1298

130 Apostolou S., Klein J. O., Mitsuuchi Y., Shetler J. N., Poulikakos P. I., Jhanwar S. C. et al. (2004) Growth inhibition and induction of apoptosis in mesothelioma cells by selenium and dependence on selenoprotein SEP15 genotype. Oncogene **23:** 5032–5040

131 Korotkov K. V., Kumaraswamy E., Zhou Y., Hatfield D. L. and Gladyshev V. N. (2001) Association between the 15-kDa selenoprotein and UDP-glucose:glycoprotein glucosyltransferase in the endoplasmic reticulum of mammalian cells. J. Biol. Chem. **276:** 15330–15336

132 Arnold S. M., Fessler L. I., Fessler J. H. and Kaufman R. J. (2000) Two homologues encoding human UDP-glucose: glycoprotein glucosyltransferase differ in mRNA expression and enzymatic activity. Biochemistry **39:** 2149–2163

133 Kumaraswamy E., Korotkov K. V., Diamond A. M., Gladyshev V. N. and Hatfield D. L. (2002) Genetic and functional analysis of mammalian Sep15 selenoprotein. Methods Enzymol. **347:** 187–197

134 Diwadkar-Navsariwala V. and Diamond A. M. (2004) The link between selenium and chemoprevention: a case for selenoproteins. J. Nutr. **134:** 2899–2902

135 Arthur J. R., Nicol F. and Beckett G. J. (1990) Hepatic iodothyronine 5'-deiodinase. The role of selenium. Biochem. J. **272:** 537–540

136 Bianco A. C., Salvatore D., Gereben B., Berry M. J. and Larsen P. R. (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr. Rev. **23:** 38–89

137 Dickson R. C. and Tomlinson R. H. (1967) Selenium in blood and human tissues. Clin. Chim. Acta **16:** 311–321

138 Köhrle J. (1999) The trace element selenium and the thyroid gland. Biochimie **81:** 527–533

139 Contempre B., de Escobar G. M., Denef J. F., Dumont J. E. and Many M. C. (2004) Thiocyanate induces cell necrosis and fibrosis in selenium- and iodine-deficient rat thyroids: a potential experimental model for myxedematous endemic cretinism in central Africa. Endocrinology **145:** 994–1002

140 Köhrle J. (1999) Local activation and inactivation of thyroid hormones: the deiodinase family. Mol. Cell. Endocrinol. **151:** 103–119

141 Köhrle J. (2000) The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. Cell. Mol. Life Sci. **57:** 1853–1863

142 St Germain D. L. and Galton V. A. (1997) The deiodinase family of selenoproteins. Thyroid **7:** 655–668

143 Kelly G. S. (2000) Peripheral metabolism of thyroid hormones: a review. Altern. Med. Rev. **5:** 306–333

144 Berry M. J., Banu L. and Larsen P. R. (1991) Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. Nature **349:** 438–440

145 Berry M. J., Banu L., Chen Y. Y., Mandel S. J., Kieffer J. D., Harney J. W. et al. (1991) Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3' untranslated region. Nature **353:** 273–276

146 Jakobs T. C., Koehler M. R., Schmutzler C., Glaser F., Schmid M. and Köhrle J. (1997) Structure of the human type I iodothyronine 5'-deiodinase gene and localization to chromosome 1p32-p33. Genomics **42:** 361–363

147 Peeters R. P., van Toor H., Klootwijk W., de Rijke Y. B., Kuiper G. G., Uitterlinden A. G. et al. (2003) Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. J. Clin. Endocrinol. Metab. **88:** 2880–2888

148 Leonard J. L. and Rosenberg I. N. (1978) Thyroxine 5'-deiodinase activity of rat kidney: observations on activation by thiols and inhibition by propylthiouracil. Endocrinology **103:** 2137–2144

149 Chaudière J. and Tappel A. L. (1984) Interaction of gold(I) with the active site of selenium-glutathione peroxidase. J. Inorg. Biochem. **20:** 313–325

150 Gromer S., Arscott L. D., Williams C. H. Jr, Schirmer R. H. and Becker K. (1998) Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. J. Biol. Chem. **273:** 20096–20101

151 Croteau W., Davey J. C., Galton V. A. and St Germain D. L. (1996) Cloning of the mammalian type II iodothyronine deiodinase. A selenoprotein differentially expressed and regulated in human and rat brain and other tissues. J. Clin. Invest. **98:** 405–417

152 Araki O., Murakami M., Morimura T., Kamiya Y., Hosoi Y., Kato Y. et al. (1999) Assignment of type II iodothyronine deiodinase gene (DIO2) to human chromosome band 14q24.2->q24.3 by in situ hybridization. Cytogenet. Cell Genet. **84:** 73–74

153 Escobar-Morreale H. F., Obregon M. J., Escobar del Rey F. and Morreale de Escobar G. (1999) Tissue-specific patterns of changes in 3,5,3'-triiodo-L-thyronine concentrations in thyroidectomized rats infused with increasing doses of the hormone. Which are the regulatory mechanisms? Biochimie **81:** 453–462

154 Bartha T., Kim S. W., Salvatore D., Gereben B., Tu H. M., Harney J. W. et al. (2000) Characterization of the 5'-flanking and 5'-untranslated regions of the cyclic adenosine 3',5'-monophosphate-responsive human type 2 iodothyronine deiodinase gene. Endocrinology **141:** 229–237

155 Schneider M. J., Fiering S. N., Pallud S. E., Parlow A. F., St Germain D. L. and Galton V. A. (2001) Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. Mol. Endocrinol. **15:** 2137–2148

156 Ng L., Goodyear R. J., Woods C. A., Schneider M. J., Diamond E., Richardson G. P. et al. (2004) Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. Proc. Natl. Acad. Sci. USA **101:** 3474–3479

157 Salvatore D., Low S. C., Berry M., Maia A. L., Harney J. W., Croteau W. et al. (1995) Type 3 lodothyronine deiodinase: cloning, in vitro expression, and functional analysis of the placental selenoenzyme. J. Clin. Invest. **96:** 2421–2430

158 Hernández A., Park J. P., Lyon G. J., Mohandas T. K. and St Germain D. L. (1998) Localization of the type 3 iodothyronine deiodinase (DIO3) gene to human chromosome 14q32 and mouse chromosome 12F1. Genomics **53:** 119–121

159 Mortimer R. H., Galligan J. P., Cannell G. R., Addison R. S. and Roberts M. S. (1996) Maternal to fetal thyroxine transmission in the human term placenta is limited by inner ring deiodination. J. Clin. Endocrinol. Metab. **81:** 2247–2249

160 Hall L., Williams K., Perry A. C., Frayne J. and Jury J. A. (1998) The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implications for the role of GPX5 in the male reproductive tract. Biochem. J. **333** (Pt 1): 5–9

161 Vernet P., Rigaudiere N., Ghyselinck N., Dufaure J. P. and Drevet J. R. (1996) In vitro expression of a mouse tissue specific glutathione-peroxidase-like protein lacking the selenocysteine can protect stably transfected mammalian cells against oxidative damage. Biochem. Cell Biol. **74:** 125–131

162 Brigelius-Flohé R. (1999) Tissue-specific functions of individual glutathione peroxidases. Free Radic. Biol. Med. **27:** 951–965

163 Aumann K. D., Bedorf N., Brigelius-Flohé R., Schomburg D. and Flohé L. (1997) Glutathione peroxidase revisited – simulation of the catalytic cycle by computer-assisted molecular modelling. Biomed. Environ. Sci. **10:** 136–155

164 Fu Y., Cheng W. H., Porres J. M., Ross D. A. and Lei X. G. (1999) Knockout of cellular glutathione peroxidase gene renders mice susceptible to diquat-induced oxidative stress. Free Radic. Biol. Med. **27:** 605–611

165 Arthur J. R. (2000) The glutathione peroxidases. Cell. Mol. Life Sci. **57:** 1825–1835

166 Maiorino M., Aumann K. D., Brigelius-Flohé R., Doria D., van den Heuvel J., McCarthy J. et al. (1995) Probing the presumed catalytic triad of selenium-containing peroxidases by mutational analysis of phospholipid hydroperoxide glutathione peroxidase (PHGPx) Biol. Chem. Hoppe Seyler **376:** 651–660

167 Maiorino M., Aumann K. D., Brigelius-Flohé R., Doria D., van den Heuvel J., McCarthy J. et al. (1998) Probing the presumed catalytic triad of a selenium-containing peroxidase by mutational analysis. Z. Ernährungswiss. 37 Suppl **1:** 118–121

168 Mills G. C. (1957) Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J. Biol. Chem. **229:** 189–197

169 Müller S., Riedel H. D. and Stremmel W. (1997) Direct evidence for catalase as the predominant H_2O_2 -removing enzyme in human erythrocytes. Blood **90:** 4973–4978

170 Ho Y. S., Magnenat J. L., Bronson R. T., Cao J., Gargano M., Sugawara M. et al. (1997) Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. J. Biol. Chem. **272:** 16644–16651

171 Yang G. Q., Chen J. S., Wen Z. M., Ge K. Y., Zhu L. Z., Chen X. C. et al. (1984) The role of selenium in Keshan disease. Adv. Nutr. Res. **6:** 203–231

172 Beck M. A., Esworthy R. S., Ho Y. S. and Chu F. F. (1998) Glutathione peroxidase protects mice from viral-induced myocarditis. FASEB J. **12:** 1143–1149

173 Beck M. A., Shi Q., Morris V. C. and Levander O. A. (1995) Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. Nat. Med. **1:** 433–436

174 Diamond A. M., Hu Y. J. and Mansur D. B. (2001) Glutathione peroxidase and viral replication: implications for viral evolution and chemoprevention. Biofactors **14: 2**05–210

175 Zhang W., Ramanathan C. S., Nadimpalli R. G., Bhat A. A., Cox A. G. and Taylor E. W. (1999) Selenium-dependent glutathione peroxidase modules encoded by RNA viruses. Biol. Trace Elem. Res. **70:** 97–116

176 Cohen I., Boya P., Zhao L., Metivier D., Andreau K., Perfettini J. L. et al. (2004) Anti-apoptotic activity of the glutathione peroxidase homologue encoded by HIV-1. Apoptosis **9:** 181–192

177 Taylor E. W., Cox A. G., Zhao L., Ruzicka J. A., Bhat A. A., Zhang W. et al. (2000) Nutrition, HIV, and drug abuse: the molecular basis of a unique role for selenium. J. Acquir. Immune Defic. Syndr. 25 Suppl. **1:** S53–61

178 Foster H. D. (2004) How HIV-1 causes AIDS: implications for prevention and treatment. Med. Hypotheses **62:** 549–553

179 Sandstrom P. A., Murray J., Folks T. M. and Diamond A. M. (1998) Antioxidant defenses influence HIV-1 replication and associated cytopathic effects. Free Radic. Biol. Med. **24:** 1485–1491

180 Ichimura Y., Habuchi T., Tsuchiya N., Wang L., Oyama C., Sato K. et al. (2004) Increased risk of bladder cancer associated with a glutathione peroxidase 1 codon 198 variant. J. Urol. **172:** 728–732

181 Hamanishi T., Furuta H., Kato H., Doi A., Tamai M., Shimomura H. et al. (2004) Functional variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in japanese type 2 diabetic patients. Diabetes **53:** 2455–2460

182 Florian S., Wingler K., Schmehl K., Jacobasch G., Kreuzer O. J., Meyerhof W. et al. (2001) Cellular and subcellular localization of gastrointestinal glutathione peroxidase in normal and malignant human intestinal tissue. Free Radic. Res. **35:** 655–663

183 Wingler K. and Brigelius-Flohé R. (1999) Gastrointestinal glutathione peroxidase. Biofactors **10:** 245–249

184 Wingler K., Bocher M., Flohé L., Kollmus H. and Brigelius-Flohé R. (1999) mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. Eur. J. Biochem. **259:** 149–157

185 Brigelius-Flohé R., Muller C., Menard J., Florian S., Schmehl K. and Wingler K. (2001) Functions of GI-GPx: lessons from selenium-dependent expression and intracellular localization. Biofactors **14:** 101–106

186 Chu F. F., Esworthy R. S., Chu P. G., Longmate J. A., Huycke M. M., Wilczynski S. et al. (2004) Bacteria-induced intestinal cancer in mice with disrupted Gpx1 and Gpx2 genes. Cancer Res. **64:** 962–968

187 Takahashi K., Akasaka M., Yamamoto Y., Kobayashi C., Mizoguchi J. and Koyama J. (1990) Primary structure of human plasma glutathione peroxidase deduced from cDNA sequences. J. Biochem. (Tokyo) **108:** 145–148

188 Tham D. M., Whitin J. C., Kim K. K., Zhu S. X. and Cohen H. J. (1998) Expression of extracellular glutathione peroxidase in human and mouse gastrointestinal tract. Am. J. Physiol. **275:** G1463–1471

189 Takahashi K., Avissar N., Whitin J. and Cohen H. (1987) Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch. Biochem. Biophys. **256:** 677–686

190 Björnstedt M., Xue J., Huang W., Akesson B. and Holmgren A. (1994) The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. J. Biol. Chem. **269:** 29382–29384

191 Bierl C., Voetsch B., Jin R. C., Handy D. E. and Loscalzo J. (2004) Determinants of human plasma glutathione peroxidase (GPx-3) expression. J. Biol. Chem. **279:** 26839–26845

192 Sarto C., Frutiger S., Cappellano F., Sanchez J. C., Doro G., Catanzaro F. et al. (1999) Modified expression of plasma glutathione peroxidase and manganese superoxide dismutase in human renal cell carcinoma. Electrophoresis **20:** 3458–3466

193 Whitin J. C., Tham D. M., Bhamre S., Ornt D. B., Scandling J. D., Tune B. M. et al. (1998) Plasma glutathione peroxidase and its relationship to renal proximal tubule function. Mol. Genet. Metab. **65:** 238–245

194 Ursini F., Maiorino M., Valente M., Ferri L. and Gregolin C. (1982) Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. Biochim. Biophys. Acta **710:** 197–211

195 Ursini F., Maiorino M. and Gregolin C. (1985) The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim. Biophys. Acta **839:** 62–70

196 Brigelius-Flohé R., Friedrichs B., Maurer S., Schultz M. and Streicher R. (1997) Interleukin-1-induced nuclear factor kappa B activation is inhibited by overexpression of phospholipid hydroperoxide glutathione peroxidase in a human endothelial cell line. Biochem. J. **328** (Pt 1): 199–203

197 Foresta C., Flohé L., Garolla A., Roveri A., Ursini F. and Maiorino M. (2002) Male fertility is linked to the selenoprotein phospholipid hydroperoxide glutathione peroxidase. Biol. Reprod. **67:** 967–971

198 Flohé L., Foresta C., Garolla A., Maiorino M., Roveri A. and Ursini F. (2002) Metamorphosis of the selenoprotein PHGPx during spermatogenesis. Ann. NY Acad. Sci. **973:** 287–288

199 Moreno S. G., Laux G., Brielmeier M., Bornkamm G. W. and Conrad M. (2003) Testis-specific expression of the nuclear

form of phospholipid hydroperoxide glutathione peroxidase (PHGPx) Biol. Chem. **384:** 635–643
200 Ursini F., Heim S., Kiess M., Maiorino M., Roveri A., Wissing J. et al. (1999) Dual function of the selenoprotein PHGPx during sperm maturation. Science **285:** 1393–1396
201 Maiorino M., Bosello V., Ursini F., Foresta C., Garolla A., Scapin M. et al. (2003) Genetic variations of gpx-4 and male infertility in humans. Biol. Reprod. **68:** 1134–1141
202 Vogt P. H. (2004) Molecular genetics of human male infertility: from genes to new therapeutic perspectives. Curr. Pharm. Des. **10:** 471–500
203 Yant L. J., Ran Q., Rao L., Van Remmen H., Shibatani T., Belter J. G. et al. (2003) The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. Free Radic. Biol. Med. **34:** 496–502
204 Okamura N., Iwaki Y., Hiramoto S., Tamba M., Bannai S., Sugita Y. et al. (1997) Molecular cloning and characterization of the epididymis-specific glutathione peroxidase-like protein secreted in the porcine epididymal fluid. Biochim. Biophys. Acta **1336:** 99–109
205 Dear T. N., Campbell K. and Rabbitts T. H. (1991) Molecular cloning of putative odorant-binding and odorant-metabolizing proteins. Biochemistry **30:** 10376–10382
206 Utomo A., Jiang X., Furuta S., Yun J., Levin D. S., Wang Y. C. et al. (2004) Identification of a novel putative non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase (NPGPx) essential for alleviating oxidative stress generated from polyunsaturated fatty acids in breast cancer cells. J. Biol. Chem. **279:** 43522–43529
207 Zhang Q. H., Ye M., Wu X. Y., Ren S. X., Zhao M., Zhao C. J. et al. (2000) Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in CD34+ hematopoietic stem/progenitor cells. Genome Res. **10:** 1546–1560
208 Gasdaska P. Y., Berggren M. M., Berry M. J. and Powis G. (1999) Cloning, sequencing and functional expression of a novel human thioredoxin reductase. FEBS Lett. **442:** 105–111
209 Gladyshev V. N., Jeang K. T. and Stadtman T. C. (1996) Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. Proc. Natl. Acad. Sci. USA **93:** 6146–6151
210 Korotkov K. V., Novoselov S. V., Hatfield D. L. and Gladyshev V. N. (2002) Mammalian selenoprotein in which selenocysteine (Sec) incorporation is supported by a new form of Sec insertion sequence element. Mol. Cell. Biol. **22:** 1402–1411
211 Moghadaszadeh B., Petit N., Jaillard C., Brockington M., Roy S. Q., Merlini L. et al. (2001) Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. Nat. Genet. **29:** 17–18
212 Petit N., Lescure A., Rederstorff M., Krol A., Moghadaszadeh B., Wewer U. M. et al. (2003) Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern. Hum. Mol. Genet. **12:** 1045–1053
213 Ferreiro A., Ceuterick-de Groote C., Marks J. J., Goemans N., Schreiber G., Hanefeld F. et al. (2004) Desmin-related myopathy with Mallory body-like inclusions is caused by mutations of the selenoprotein N gene. Ann. Neurol. **55:** 676–686
214 Mostert V. (2000) Selenoprotein P: properties, functions and regulation. Arch. Biochem. Biophys. **376:** 433–438
215 Motsenbocker M. A. and Tappel A. L. (1982) A selenocysteine-containing selenium-transport protein in rat plasma. Biochim. Biophys. Acta **719:** 147–153
216 Eberle B. and Haas H. J. (1993) Purification of selenoprotein Ph from human plasma. J. Trace Elem. Electrolytes Health Dis. **7:** 217–221
217 Read R., Bellew T., Yang J. G., Hill K. E., Palmer I. S. and Burk R. F. (1990) Selenium and amino acid composition of selenoprotein P, the major selenoprotein in rat serum. J. Biol. Chem. **265:** 17899–17905
218 Burk R. F. and Hill K. E. (1999) Orphan selenoproteins. Bioessays **21:** 231–237
219 Schweizer U., Streckfuss F., Pelt P., Carlson B. A., Hatfield D. L., Köhrle J. et al. (2005) Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply. Biochem. J. **386:** 221–226
220 Gomez B. Jr and Tappel A. L. (1989) Selenoprotein P receptor from rat. Biochim. Biophys. Acta **979:** 20–26
221 Hill K. E., Lloyd R. S., Yang J. G., Read R. and Burk R. F. (1991) The cDNA for rat selenoprotein P contains 10 TGA codons in the open reading frame. J. Biol. Chem. **266:** 10050–10053
222 Burk R. F. and Hill K. E. (1992) Some properties of selenoprotein P. Biol. Trace Elem. Res. **33:** 151–153
223 Motchnik P. A. and Tappel A. L. (1990) Multiple selenocysteine content of selenoprotein P in rats. J. Inorg. Biochem. **40:** 265–269
224 Ma S., Hill K. E., Burk R. F. and Caprioli R. M. (2005) Mass spectrometric determination of selenenylsulfide linkages in rat selenoprotein P. J. Mass Spectrom. **40:** 400–404
225 Persson-Moschos M., Huang W., Srikumar T. S., Akesson B. and Lindeberg S. (1995) Selenoprotein P in serum as a biochemical marker of selenium status. Analyst **120:** 833–836
226 Dreher I., Jakobs T. C. and Kohrle J. (1997) Cloning and characterization of the human selenoprotein P promoter. Response of selenoprotein P expression to cytokines in liver cells. J. Biol. Chem. **272:** 29364–29371
227 Saito Y. and Takahashi K. (2002) Characterization of selenoprotein P as a selenium supply protein. Eur. J. Biochem. **269:** 5746–51
228 Saito Y., Sato N., Hirashima M., Takebe G., Nagasawa S. and Takahashi K. (2004) Domain structure of bi-functional selenoprotein P. Biochem. J. **381:** 841–846
229 Burk R. F., Hill K. E., Read R. and Bellew T. (1991) Response of rat selenoprotein P to selenium administration and fate of its selenium. Am. J. Physiol. **261:** E26–30
230 Yoneda S. and Suzuki K. T. (1997) Equimolar Hg-Se complex binds to selenoprotein P. Biochem. Biophys. Res. Commun. **231:** 7–11
231 Suzuki K. T., Sasakura C. and Yoneda S. (1998) Binding sites for the (Hg-Se) complex on selenoprotein P. Biochim. Biophys. Acta **1429:** 102–112
232 Fujii M., Saijoh K. and Sumino K. (1997) Regulation of selenoprotein P mRNA expression in comparison with metallothionein and osteonectin mRNAs following cadmium and dexamethasone administration. Kobe J. Med. Sci. **43:** 13–23
233 Fujii M., Saijoh K., Kobayashi T., Fujii S., Lee M. J. and Sumino K. (1997) Analysis of bovine selenoprotein P-like protein gene and availability of metal responsive element (MRE) located in its promoter. Gene **199:** 211–217
234 Saito Y., Hayashi T., Tanaka A., Watanabe Y., Suzuki M., Saito E. et al. (1999) Selenoprotein P in human plasma as an extracellular phospholipid hydroperoxide glutathione peroxidase. Isolation and enzymatic characterization of human selenoprotein p. J. Biol. Chem. **274:** 2866–2871
235 Arteel G. E., Mostert V., Oubrahim H., Briviba K., Abel J. and Sies H. (1998) Protection by selenoprotein P in human plasma against peroxynitrite-mediated oxidation and nitration. Biol. Chem. **379:** 1201–1205
236 Hill K. E. and Burk R. F. (1997) Selenoprotein P: recent studies in rats and in humans. Biomed. Environ. Sci. **10:** 198–208
237 Schweizer U., Schomburg L. and Savaskan N. E. (2004) The neurobiology of selenium: lessons from transgenic mice. J. Nutr. **134:** 707–710
238 Hill K. E., Zhou J., McMahan W. J., Motley A. K., Atkins J. F., Gesteland R. F. et al. (2003) Deletion of selenoprotein P alters distribution of selenium in the mouse. J. Biol. Chem. **278:** 13640–13646
239 Schomburg L., Schweizer U., Holtmann B., Flohé L., Sendtner M. and Köhrle J. (2003) Gene disruption discloses role of se-

lenoprotein P in selenium delivery to target tissues. Biochem. J. **370:** 397–402
240 Schweizer U., Michaelis M., Kohrle J. and Schomburg L. (2004) Efficient selenium transfer from mother to offspring in selenoprotein-P-deficient mice enables dose-dependent rescue of phenotypes associated with selenium deficiency. Biochem. J. **378:** 21–26
241 Olson G. E., Winfrey V. P., Nagdas S. K., Hill K. E. and Burk R. F. (2005) Selenoprotein P is required for mouse sperm development. Biol. Reprod. **73:** 201–211
242 Richardson D. R. (2005) More roles for selenoprotein P: local selenium storage and recycling protein in the brain. Biochem. J. **386:** e5–7
243 Calvo A., Xiao N., Kang J., Best C. J., Leiva I., Emmert-Buck M. R. et al. (2002) Alterations in gene expression profiles during prostate cancer progression: functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors. Cancer Res. **62:** 5325–5335
244 Zachara B. A., Szewczyk-Golec K., Tyloch J., Wolski Z., Szylberg T., Stepien S. et al. (2005) Blood and tissue selenium concentrations and glutathione peroxidase activities in patients with prostate cancer and benign prostate hyperplasia. Neoplasma **52:** 248–254
245 Zachara B. A., Szewczyk-Golec K., Wolski Z., Tyloch J., Skok Z., Bloch-Boguslawska E. et al. (2005) Selenium level in benign and cancerous prostate. Biol. Trace Elem. Res. **103:** 199–206
246 Kryukov G. V., Kumar R. A., Koc A., Sun Z. and Gladyshev V. N. (2002) Selenoprotein R is a zinc-containing stereo-specific methionine sulfoxide reductase. Proc. Natl. Acad. Sci. USA **99:** 4245–4250
247 Kim H. Y. and Gladyshev V. N. (2004) Characterization of mouse endoplasmic reticulum methionine-R-sulfoxide reductase. Biochem. Biophys. Res. Commun. **320:** 1277–1283
248 Grimaud R., Ezraty B., Mitchell J. K., Lafitte D., Briand C., Derrick P. J. et al. (2001) Repair of oxidized proteins. Identification of a new methionine sulfoxide reductase. J. Biol. Chem. **276:** 48915–48920
249 Boschi-Muller S., Olry A., Antoine M. and Branlant G. (2005) The enzymology and biochemistry of methionine sulfoxide reductases. Biochim. Biophys. Acta **1703:** 231–238
250 Stadtman E. R., Moskovitz J. and Levine R. L. (2003) Oxidation of methionine residues of proteins: biological consequences. Antioxid. Redox Signal. **5:** 577–582
251 Hansel A., Heinemann S. H. and Hoshi T. (2005) Heterogeneity and function of mammalian MSRs: enzymes for repair, protection and regulation. Biochim. Biophys. Acta **1703:** 239–247
252 Gabbita S. P., Aksenov M. Y., Lovell M. A. and Markesbery W. R. (1999) Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. J. Neurochem. **73:** 1660–1666
253 Allan C. B., Lacourciere G. M. and Stadtman T. C. (1999) Responsiveness of selenoproteins to dietary selenium. Annu. Rev. Nutr. **19:** 1–16
254 Schweizer U., Brauer A. U., Kohrle J., Nitsch R. and Savaskan N. E. (2004) Selenium and brain function: a poorly recognized liaison. Brain Res. Brain Res. Rev. **45:** 164–178
255 Chen J. and Berry M. J. (2003) Selenium and selenoproteins in the brain and brain diseases. J. Neurochem. **86:** 1–12
256 Gao Y., Walder K., Sunderland T., Kantham L., Feng H. C., Quick M. et al. (2003) Elevation in Tanis expression alters glucose metabolism and insulin sensitivity in H4IIE cells. Diabetes **52:** 929–934
257 Walder K., Kantham L., McMillan J. S., Trevaskis J., Kerr L., De Silva A. et al. (2002) Tanis: a link between type 2 diabetes and inflammation? Diabetes **51:** 1859–1866
258 Gao Y., Feng H. C., Walder K., Bolton K., Sunderland T., Bishara N. et al. (2004) Regulation of the selenoprotein SelS by glucose deprivation and endoplasmic reticulum stress – SelS is a novel glucose-regulated protein. FEBS Lett. **563:** 185–190
259 Ye Y., Shibata Y., Yun C., Ron D. and Rapoport T. A. (2004) A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature **429:** 841–847
260 Low S. C., Harney J. W. and Berry M. J. (1995) Cloning and functional characterization of human selenophosphate synthetase, an essential component of selenoprotein synthesis. J. Biol. Chem. **270:** 21659–21664
261 Guimaraes M. J., Peterson D., Vicari A., Cocks B. G., Copeland N. G., Gilbert D. J. et al. (1996) Identification of a novel selD homolog from eukaryotes, bacteria, and archaea: is there an autoregulatory mechanism in selenocysteine metabolism? Proc. Natl. Acad. Sci. USA **93:** 15086–15091
262 Lacourciere G. M. and Stadtman T. C. (2001) Utilization of selenocysteine as a source of selenium for selenophosphate biosynthesis. Biofactors **14:** 69–74
263 Tamura T., Yamamoto S., Takahata M., Sakaguchi H., Tanaka H., Stadtman T. C. et al. (2004) Selenophosphate synthetase genes from lung adenocarcinoma cells: Sps1 for recycling L-selenocysteine and Sps2 for selenite assimilation. Proc. Natl. Acad. Sci. USA **101:** 16162–16167
264 Ogasawara Y., Lacourciere G. M., Ishii K. and Stadtman T. C. (2005) Characterization of potential selenium-binding proteins in the selenophosphate synthetase system. Proc. Natl. Acad. Sci. USA **102:** 1012–1016
265 Lacourciere G. M. (2002) Selenium is mobilized in vivo from free selenocysteine and is incorporated specifically into formate dehydrogenase H and tRNA nucleosides. J. Bacteriol. **184:** 1940–1946
266 Esaki N., Nakamura T., Tanaka H. and Soda K. (1982) Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. Mammalian distribution and purification and properties of pig liver enzyme. J. Biol. Chem. **257:** 4386–4391
267 Gromer S., Urig S. and Becker K. (2004) The thioredoxin system-from science to clinic. Med. Res. Rev. **24:** 40–89
268 Williams C. H. J. (1992) Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase, and mercuric ion reductase – a family of flavoenzyme transhydrogenases in: Chemistry and biochemistry of flavoenzymes, vol. 3, pp. 121–211, Müller F. (ed.), CRC Press, Boca Raton, FL
269 Oblong J. E., Gasdaska P. Y., Sherrill K. and Powis G. (1993) Purification of human thioredoxin reductase: properties and characterization by absorption and circular dichroism spectroscopy. Biochemistry **32:** 7271–7277
270 Gromer S., Schirmer R. H. and Becker K. (1997) The 58 kDa mouse selenoprotein is a BCNU-sensitive thioredoxin reductase. FEBS Lett. **412:** 318–320
271 Sun Q. A., Zappacosta F., Factor V. M., Wirth P. J., Hatfield D. L. and Gladyshev V. N. (2001) Heterogeneity within animal thioredoxin reductases. Evidence for alternative first exon splicing. J. Biol. Chem. **276:** 3106–3114
272 Rundlöf A. K., Janard M., Miranda-Vizuete A. and Arnér E. S. (2004) Evidence for intriguingly complex transcription of human thioredoxin reductase 1. Free Radic. Biol. Med. **36:** 641–656
273 Nonn L., Williams R. R., Erickson R. P. and Powis G. (2003) The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. Mol. Cell. Biol. **23:** 916–922
274 Matsui M., Oshima M., Oshima H., Takaku K., Maruyama T., Yodoi J. et al. (1996) Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. Dev. Biol. **178:** 179–185
275 Conrad M., Jakupoglu C., Moreno S. G., Lippl S., Banjac A., Schneider M. et al. (2004) Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. Mol. Cell. Biol. **24:** 9414–9423
276 Arnér E. S. J. and Holmgren A. (2000) Physiological functions of thioredoxin and thioredoxin reductase. Eur. J. Biochem. **267:** 6102–6109

277 Becker K., Gromer S., Schirmer R. H. and Müller S. (2000) Thioredoxin reductase as a pathophysiological factor and drug target. Eur. J. Biochem. **267:** 6118–6125
278 Gromer S., Schirmer R. H. and Becker K. (1999) News and views on thioredoxin reductases. Redox Rep. **4:** 221–228
279 Mustacich D. and Powis G. (2000) Thioredoxin reductase. Biochem. J. **346** (Pt 1): 1–8
280 Johansson L., Arscott L. D., Ballou D. P., Williams C. H. Jr and Arnér E. S. J. (2005) Characterization of a Drosophila-mimic active site mutant of mammalian thioredoxin reductase. Flavins and Flavoproteins 15, in press
281 Brandt W. and Wessjohann L. A. (2005) The functional role of selenocysteine (Sec) in the catalysis mechanism of large thioredoxin reductases: proposition of a swapping catalytic triad including a Sec-His-Glu state. Chembiochem **6:** 386–394
282 Sun Q. A. and Gladyshev V. N. (2002) Redox regulation of cell signaling by thioredoxin reductases. Methods Enzymol. **347:** 451–461
283 Anestal K. and Arnér E. S. J. (2003) Rapid induction of cell death by selenium compromised thioredoxin reductase 1 but not by the fully active enzyme containing selenocysteine. J. Biol. Chem. **6:** 6
284 Spyrou G. and Holmgren A. (1996) Deoxyribonucleoside triphosphate pools and growth of glutathione-depleted 3T6 mouse fibroblasts. Biochem. Biophys. Res. Commun. **220:** 42–46
285 Söderberg A., Sahaf B. and Rosén A. (2000) Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in human plasma. Cancer Res. **60:** 2281–2289
286 Miranda-Vizuete A., Damdimopoulos A. E. and Spyrou G. (2000) The mitochondrial thioredoxin system. Antioxid. Redox Signal. **2:** 801–810
287 Baldini A. (2005) Dissecting contiguous gene defects: TBX1. Curr. Opin. Genet. Dev. **15:** 279–284
288 Sun Q. A., Kirnarsky L., Sherman S. and Gladyshev V. N. (2001) Selenoprotein oxidoreductase with specificity for thioredoxin and glutathione systems. Proc. Natl. Acad. Sci. USA **98:** 3673–3678
289 Vendeland S. C., Beilstein M. A., Chen C. L., Jensen O. N., Barofsky E. and Whanger P. D. (1993) Purification and properties of selenoprotein W from rat muscle. J. Biol. Chem. **268:** 17103–17107
290 Yeh J. Y., Beilstein M. A., Andrews J. S. and Whanger P. D. (1995) Tissue distribution and influence of selenium status on levels of selenoprotein W. FASEB J. **9:** 392–396
291 Yeh J. Y., Gu Q. P., Beilstein M. A., Forsberg N. E. and Whanger P. D. (1997) Selenium influences tissue levels of selenoprotein W in sheep. J. Nutr. **127:** 394–402
292 Gu Q. P., Sun Y., Ream L. W. and Whanger P. D. (2000) Selenoprotein W accumulates primarily in primate skeletal muscle, heart, brain and tongue. Mol. Cell. Biochem. **204:** 49–56
293 Whanger P. D. (2002) Selenoprotein W. Methods Enzymol. **347:** 179–187
294 Yeh J. Y., Ou B. R., Gu Q. P. and Whanger P. D. (1998) Influence of gender on selenoprotein W, glutathione peroxidase and selenium in tissues of rats. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **119:** 151–155
295 Bellingham J., Gregory-Evans K., Fox M. F. and Gregory-Evans C. Y. (2003) Gene structure and tissue expression of human selenoprotein W, SEPW1, and identification of a retroprocessed pseudogene, SEPW1P. Biochim. Biophys. Acta **1627:** 140–146
296 Beilstein M. A., Vendeland S. C., Barofsky E., Jensen O. N. and Whanger P. D. (1996) Selenoprotein W of rat muscle binds glutathione and an unknown small molecular weight moiety. J. Inorg. Biochem. **61:** 117–124
297 Bauman A. T., Malencik D. A., Barofsky D. F., Barofsky E., Anderson S. R. and Whanger P. D. (2004) Selective production of rat mutant selenoprotein W with and without bound glutathione. Biochem. Biophys. Res. Commun. **313:** 308–313
298 Gu Q. P., Beilstein M. A., Barofsky E., Ream W. and Whanger P. D. (1999) Purification, characterization, and glutathione binding to selenoprotein W from monkey muscle. Arch. Biochem. Biophys. **361:** 25–33
299 Sun Y., Gu Q. P. and Whanger P. D. (2001) Selenoprotein W in overexpressed and underexpressed rat glial cells in culture. J. Inorg. Biochem. **84:** 151–156
300 Jeong D., Kim T. S., Chung Y. W., Lee B. J. and Kim I. Y. (2002) Selenoprotein W is a glutathione-dependent antioxidant in vivo. FEBS Lett. **517:** 225–228
301 Jeong D. W., Kim E. H., Kim T. S., Chung Y. W., Kim H. and Kim I. Y. (2004) Different distributions of selenoprotein W and thioredoxin during postnatal brain development and embryogenesis. Mol. Cells **17:** 156–159
302 Arnér E. S., Sarioglu H., Lottspeich F., Holmgren A. and Böck A. (1999) High-level expression in Escherichia coli of selenocysteine-containing rat thioredoxin reductase utilizing gene fusions with engineered bacterial-type SECIS elements and co-expression with the selA, selB and selC genes. J. Mol. Biol. **292:** 1003–1016