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Review

## Selenium in Biology: Facts and Medical Perspectives

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**Several decades after the discovery of selenium as an essential trace element in vertebrates approximately 20 eukaryotic and more than 15 prokaryotic selenoproteins containing the 21<sup>st</sup> proteinogenic amino acid, selenocysteine, have been identified, partially characterized or cloned from several species. Many of these proteins are involved in redox reactions with selenocysteine acting as an essential component of the catalytic cycle. Enzyme activities have been assigned to the glutathione peroxidase family, to the thioredoxin reductases, which were recently identified as selenoproteins, to the iodothyronine deiodinases, which metabolize thyroid hormones, and to the selenophosphate synthetase 2, which is involved in selenoprotein biosynthesis. Prokaryotic selenoproteins catalyze redox reactions and formation of selenoethers in (stress-induced) metabolism and energy production of *E. coli*, of the clostridial cluster XI and of other prokaryotes. Apart from the specific and complex biosynthesis of selenocysteine, selenium also reversibly binds to proteins, is incorporated into selenomethionine in bacteria, yeast and higher plants, or posttranslationally modifies a catalytically essential cysteine residue of CO dehydrogenase. Expression of individual eukaryotic selenoproteins exhibits high tissue specificity, depends on selenium availability, in some cases is regulated by hormones, and if impaired**

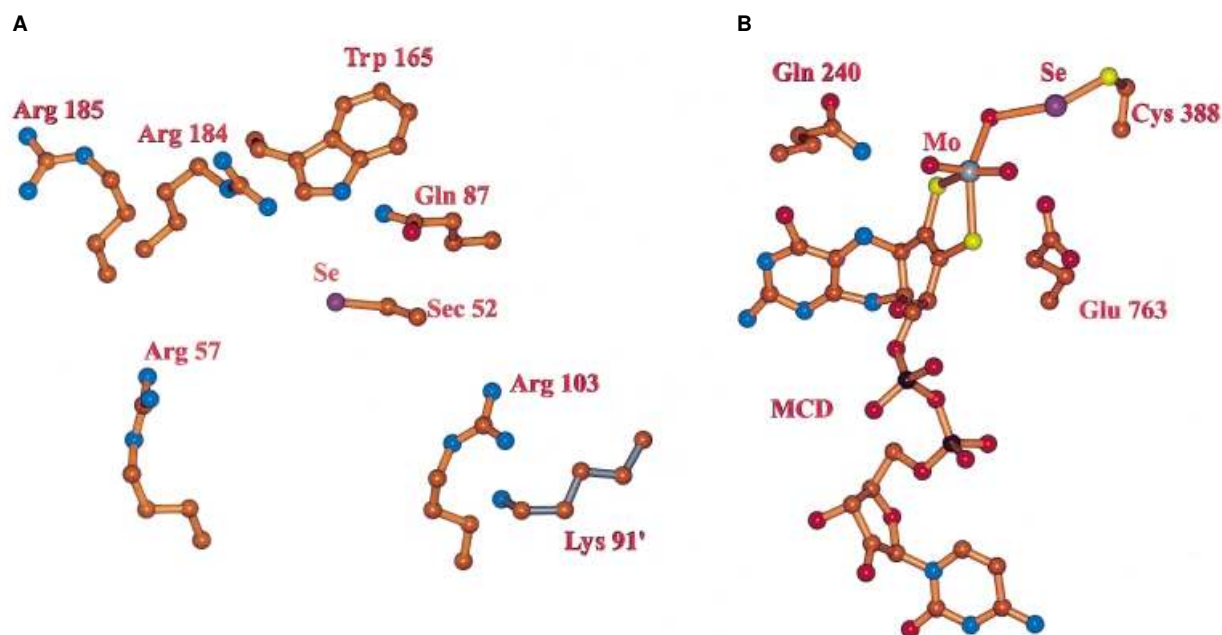
**contributes to several pathological conditions. Disturbance of selenoprotein expression or function is associated with deficiency syndromes (Keshan and Kashin-Beck disease), might contribute to tumorigenesis and atherosclerosis, is altered in several bacterial and viral infections, and leads to infertility in male rodents.**

*Key words:* Biosynthesis / Medical implications / Redox regulation / Selenoproteins / Sperm maturation / Thyroid function.

### Introduction: Some Historical Landmarks

The element selenium, discovered in 1817 and named after the Greek goddess of the moon by Berzelius, gained biomedical interest after Schwarz and Foltz (1957) reported that it is an essential trace element for mammals. The link between nutritional science and enzymology was, however, established appreciably later when Flohé *et al.* (1973), intrigued by a preliminary report from Hoekstra's group (Rotruck *et al.*, 1972), identified selenium as a stoichiometric, covalently bound component of glutathione peroxidase (GPx), an enzyme previously demonstrated to dominate mammalian hydroperoxide metabolism (Sies *et al.*, 1972). Selenium proved to be present in this enzyme as a selenocysteine residue (Forstrom *et al.*, 1978, Wendel *et al.*, 1978) that is integrated into the amino acid chain (Günzler *et al.*, 1984). The selenocysteine residue in GPx is responsible for the catalytic efficiency, as demonstrated by site-directed mutagenesis (Rocher *et al.*, 1992), and the X-ray analysis performed by Epp *et al.* (1983) enabled a detailed understanding of the catalytic mechanism (Aumann *et al.*, 1997; see also Figure 1A).

Starting in the mid-eighties, further selenoproteins were discovered that broadened the scope of selenium biochemistry from antioxidant defense to multiple aspects of mammalian metabolism. The metabolic activation (Behne *et al.*, 1990; Arthur *et al.*, 1990; Davey *et al.*, 1995), as well as degradation of thyroid hormones (Croteau *et al.*, 1995), were shown to depend on selenium-containing deiodinases. Most surprisingly, selenium was also discovered in a well-documented enzyme of basic metabolic relevance, in thioredoxin reductase (Tamura and Stadtman, 1996). Finally, a homolog of GPx, the phospholipid hydroperoxide glutathione peroxidase (PHGPx) originally described by Ursini *et al.* (1982), proved to be involved in sperm maturation (Ursini *et al.*, 1999). Further surprises may be anticipated from the growing number of mammalian selenoproteins.



**Fig. 1** Diversity of Reaction Centers of Selenoproteins.

(A) Active site of the classical mammalian glutathione peroxidase in its ground state, as modeled by Aumann *et al.* (1997) based on the X-ray crystallographic analysis of Epp *et al.* (1983). The dissociated selenol function of selenocysteine (Sec 52) forms a catalytic triad with Trp<sup>165</sup> and Glu<sup>87</sup>, as demonstrated for PHGPx by Maiorino *et al.* (1985). The basic residues Arg<sup>57</sup>, 103, 184, 185 and Lys<sup>91</sup> from the second subunit contribute to the optimum orientation of the reducing substrate GSH (Aumann *et al.*, 1997).

(B) Reaction center of CO dehydrogenase from *Oligotropha carboxidovorans* according to Dobbek *et al.* (1999). A molybdenum cofactor is buried in the center of the large subunit of CO dehydrogenase. The geometry of the first coordination sphere around the Mo ion forms a distorted square pyramid containing the dithiolene group of molybdopterin cytosine dinucleotide (MCD), two oxo- and one sulfido-group. The CO dehydrogenase preparations obtained from the bacteria contain active (sulfido-group present) and inactive enzyme (sulfido-group replaced by a hydroxy-group). The apical oxo-group is in hydrogen-bonding distance to the N2 of the conserved Gln<sup>240</sup>. The other oxo-group is in hydrogen-bonding distance to the O1 of the conserved Glu<sup>763</sup>. The sequence Val-Ala-Tyr-Arg-Cys<sup>388</sup>-Ser-Phe-Arg at the active site contains the residue Cys<sup>388</sup>, which has been post-translationally modified to *S*-selenylcysteine. The *S*-selenylcysteine is catalytically essential and in a distance of 2.2 Å to the sulfido-group. For further details see Dobbek *et al.* (1999).

Color codes: C, brown; N, blue; O, red; S, yellow; Se, magenta; P, dark red; grey backbone indicate second subunit of GPx. The figure was kindly prepared by H.-J. Hecht, GBF, Braunschweig, Germany.

The selenium biochemistry of mammalian systems was paralleled, in fact often preceded, by related discoveries in prokarya. Already in 1954 selenium was rated as a growth factor of certain bacteria (Pinsent, 1954). The first bacterial selenoproteins were described in 1973 (Andreesen and Ljungdahl, 1973; Turner and Stadtman 1973), and the growing diversity of enzymatic functions of such prokaryotic enzymes remains a valuable guide to unraveling the roles of less accessible mammalian enzymes. The present understanding of selenoprotein biosynthesis could not have been achieved without the aid of microbial genetics. The key observation, though, was made with a mouse gene: it obviously encoded a mouse GPx and displayed the stop codon TGA precisely at the position that should encode selenocysteine in the homologous bovine enzyme (Chambers *et al.*, 1986) that had been previously completely sequenced by protein chemistry (Günzler *et al.*, 1984). Within the same year Zinoni *et al.* (1986) established that TGA also encoded the selenocysteine discovered in bacterial formate dehydrogenase by Jones *et al.* (1979). Starting from these observations, the complex biosynthesis of selenoproteins was unraveled for bacteria in a

transatlantic cooperation that remains inseparably associated with the names of Stadtman and Böck (Zinoni *et al.*, 1986; Böck and Stadtman, 1988; Böck *et al.*, 1991a,b). The attempts to understand eukaryotic selenoprotein biosynthesis has revealed homologies but also marked differences (Berry *et al.*, 1991, 1993), and has not yet yielded a satisfying comprehensive view.

### Identified Selenoproteins

Selenium usually exerts its influence on physiology as an integral component of proteins, into which it is incorporated in the form of selenocysteine (Cone *et al.*, 1976; see below). Selenoproteins can be selectively labeled by <sup>75</sup>Se in selenium-deficient animals and autoradiographically visualized after electrophoretic separation (Behne *et al.*, 1996). According to such experiments, the number of selenoproteins in mammals has been estimated to reach 30–50. Less than 20 of such bands have been characterized by sequence analysis up to now and an enzymatic function was assigned to more than ten of them. Among

**Table 1** Mammalian Selenoproteins.

Selenoprotein (common abbreviations)	Key references
Glutathione peroxidases (GPx)	
Cytosolic or classical GPx (cGPx, GPx-1)	Mills 1957; Flohé <i>et al.</i> , 1973
Phospholipid hydroperoxide GPx (PHGPx, GPx-4)	Ursini <i>et al.</i> , 1982; Brigelius-Flohé <i>et al.</i> , 1994
Plasma GPx (pGPx, GPx-3)	Takahashi <i>et al.</i> , 1987
Gastrointestinal GPx (GI-GPx, GPx-GI, GPx-2)	Chu <i>et al.</i> , 1993
Iodothyronine deiodinases	
5'-deiodinase, type I (5'DI)	Behne <i>et al.</i> , 1990; Arthur <i>et al.</i> , 1990
5'-deiodinase, type II (5'DII)	Davey <i>et al.</i> , 1995
5-deiodinase, type III (5-DIII)	Croteau <i>et al.</i> , 1995
Thioredoxin reductases	
Thioredoxin reductase (TrxR)	Tamura and Stadtman, 1996
Mitochondrial thioredoxin reductase (TrxR-2)	Lee <i>et al.</i> , 1999; Watabe <i>et al.</i> , 1999;
	Miranda-Vizuete <i>et al.</i> , 1999; Gasdaska <i>et al.</i> , 1999
Thioredoxin reductase homologs (SelZf1; SelZf2)	Lescure <i>et al.</i> , 1999
Selenophosphate synthetase-2	Guimaraes <i>et al.</i> , 1996
Functionally undefined	
15 kDa selenoprotein of T cells	Gladyshev <i>et al.</i> , 1998
Selenoprotein P 10 (SelP)	Motsenbocker and Tappel, 1984
Selenoprotein P 12	Sajjoh <i>et al.</i> , 1995
Selenoprotein W (SelW)	Vendeland <i>et al.</i> , 1995; Whanger <i>et al.</i> , 1997
Selenoprotein R (SelR)	Kryukov <i>et al.</i> , 1999
Selenoprotein T (SelT)	Kryukov <i>et al.</i> , 1999
Selenoprotein X (SelX)	Lescure <i>et al.</i> , 1999
Selenoprotein N (SelN)	Lescure <i>et al.</i> , 1999

the identified and relatively well-characterized selenoproteins are four glutathione peroxidases (GPx), the cytosolic GPx (cGPx), the gastrointestinal GPx (GI-GPx), plasma GPx (pGPx) and phospholipid hydroperoxide GPx (PHGPx), at least three thioredoxin reductases (TrxR), three deiodinases (D), the selenophosphate synthetase-2, the selenoprotein P (SelP) present in plasma and a related variant in bovine brain (SelP12), the selenoprotein W in muscle, and some others of unknown function (Table 1).

In the majority of known mammalian selenoproteins, selenium occurs in the form of selenocysteine, which has proved to be essential for efficient catalysis (Maiorino *et al.*, 1995; Gromer *et al.*, 1998; Köhrle, 2000b; Lee *et al.*, 2000). If selenocysteine is substituted by cysteine, the activity of the selenoenzymes falls by 2 to 3 orders of magnitude. Accordingly, the natural cysteine-containing PHGPx homolog from *Plasmodium falciparum* is approximately 1000-fold less active than 'true' Se-PHGPx (Sztajer *et al.*, 2000).

In prokarya, enzymes that were post-translationally modified with selenium were characterized. The carbon monoxide dehydrogenase, for instance, is an extremely complex molybdopterine-containing iron-sulphur-flavo-protein (Dobbek *et al.*, 1999), in which a selanyl group is bound to a cysteine residue (Figure 1B). Such seleno-

proteins have not yet been described in vertebrates. However, in prokarya the overwhelming number of selenoproteins contain selenocysteyl residues (Table 2). They comprise selenoproteins that are present in eukarya as sulfur homologs like the peroxiredoxins (Rhee *et al.*, 1999; Flohé *et al.*, 1999). Most prokaryotic selenoproteins, however, are unique and catalyze highly-varied processes that have not been discovered in eukarya. In the clostridial cluster XI (Kreimer and Andreesen, 1995; Wagner *et al.*, 1999; Kabisch *et al.*, 1999), selenoproteins are vital for energy production, particularly under stress, and appear to be important for additional metabolic performances and pathogenicity. A better understanding of such unique pathways in clinically relevant microorganisms might provide a rational basis for therapeutic intervention.

### Metabolic Function of Mammalian Selenoproteins

Glutathione peroxidases are found in all mammalian tissues in which oxidative processes occur. By reduction of hydroperoxides to the corresponding alcohols, these enzymes can prevent the production of reactive oxygen radicals and thus may contribute to the protection of the

**Table 2** Selenoproteins in Prokaryotes<sup>a</sup>.

Selenoprotein (gene name)	Function	References
Glycine reductase	Formation of a selenoether	Arkowitz and Abeles, 1990
Glycine/sarcosine/betaine reductase Selenoprotein A ( <i>grdA</i> )	Redox function, transfer of a selenoether	Andreesen <i>et al.</i> , 1999
Glycine reductase selenoprotein B ( <i>grdB</i> )	Formation of a selenoether	Wagner <i>et al.</i> , 1999
Sarcosine reductase selenoprotein B ( <i>grdF</i> )	Formation of a selenoether	Andreesen <i>et al.</i> , 1999
Betaine reductase selenoprotein B ( <i>grdH</i> )	Formation of a selenoether	Andreesen <i>et al.</i> , 1999
Proline reductase ( <i>prdB</i> )	Redox function, formation of a selenoether	Kabisch <i>et al.</i> , 1999
Heterodisulfide reductase ( <i>HdrA</i> )	Redox function	Wilting <i>et al.</i> , 1997
Seleno-peroxiredoxin ( <i>prxU</i> )	Redox function (peroxidase)	Andreesen <i>et al.</i> , 1999
Putative redox active selenoprotein ( <i>prpU</i> )	Redox function	Andreesen <i>et al.</i> , 1999
Formate dehydrogenase ( <i>fdhF</i> )	Hydrogen donor	Boyington <i>et al.</i> , 1997
Formylmethanofuran dehydrogenase ( <i>fwuB</i> )	Redox function	Vorholt <i>et al.</i> , 1997
NiFeSe-hydrogenase ( <i>hydV</i> )	Hydrogen donor	Garcin <i>et al.</i> , 1999
F <sub>420</sub> non-reducing hydrogenase ( <i>vhuU</i> , <i>vhuD</i> )	Redox function	Pfeiffer <i>et al.</i> , 1998
F <sub>420</sub> reducing hydrogenase ( <i>fruA</i> )	Redox function	Wilting <i>et al.</i> , 1997
Selenophosphate synthetase ( <i>seld</i> )	Formation of key metabolite for selenoprotein synthesis	Lacourciere and Stadtman, 1999
CO dehydrogenase ( <i>coxL</i> )	Formation of a carbon oxide selenide	Dobbek <i>et al.</i> , 1999
Nicotinic acid hydroxylase	Unknown	Gladyshev <i>et al.</i> , 1996
Xanthine dehydrogenase	Unknown	Schräder <i>et al.</i> , 1999

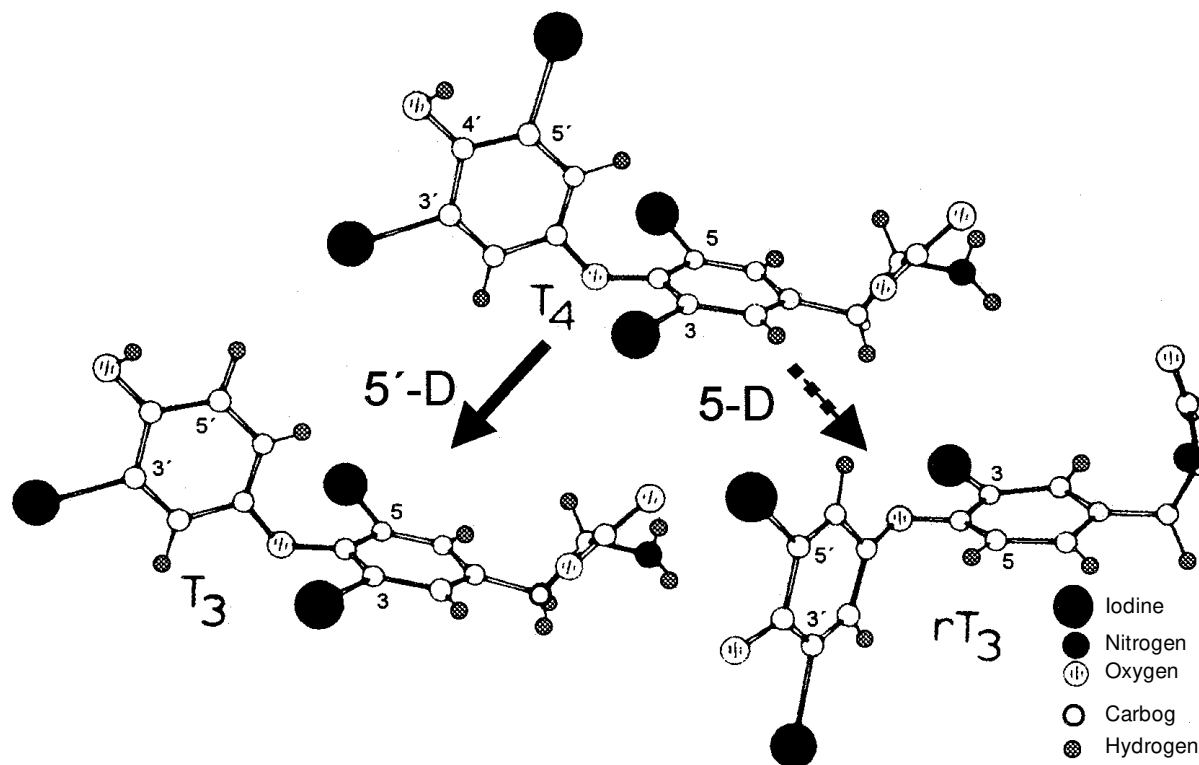
<sup>a</sup> Adapted from Flohé *et al.* (2000).

organism's macromolecules and biomembranes against oxidation (Sies *et al.*, 1972; Flohé, 1989; Ursini *et al.*, 1995). The role of the cytoplasmic GPx as an 'emergency enzyme' to fight oxidative stress was verified by reverse genetics (Ho *et al.*, 1997; Cheng *et al.*, 1998; de Haan *et al.*, 1998; Fu *et al.*, 1999; Jaeschke *et al.*, 1999) and, in this role, cGPx cannot be substituted by any of the other selenoproteins. Glutathione peroxidases, in particular the less ubiquitously distributed isozymes, are engaged in redox regulation of many metabolic processes (Brigelius-Flohé, 1999) and appear to be involved in peroxynitrite scavenging (Sies *et al.*, 1997). PHGPx may, *e. g.*, regulate the biosynthesis of leukotrienes, thromboxanes and prostaglandins and thus modulate inflammatory processes (Smith and Lands, 1972; Haurand and Flohé, 1988; Schnurr *et al.*, 1996; Weitzel and Wendel, 1993; Imai *et al.*, 1998). Glutathione peroxidases, in particular PHGPx, have further been shown to dampen cytokine-induced transcriptional gene activation (Brigelius-Flohé *et al.*, 1997), *e. g.* by inhibiting phosphorylation of I $\kappa$ B (Kretz-Remy *et al.*, 1996).

All of the three deiodinase isoenzymes identified up to now appear to contain selenocysteine (Figure 2). These enzymes catalyze the activation of the prohormone thyroxine (T4), which is secreted by the thyroid, to the active thyroid hormone 3,3',5-triiodothyronine (T3) (type I and type II 5'-deiodinase) or the deiodination of T4 and T3 to metabolites (type III 5-deiodinase) that are not hormonally active. These three isozymes are encoded by different

genes and show distinct specificities, tissue- and development-specific expression patterns and regulation. Essentially they control the local availability and concentration of the highly active thyroid hormone T3 (reviewed in Köhrle, 1999a, b, 2000a, b).

In contrast to prokaryotic homologs, thioredoxin reductase of mammals was identified as a selenoprotein (Tamura and Stadtman, 1996). It needs selenocysteine as the penultimate amino acid residue for its appropriate enzymatic function (Marcocci *et al.*, 1997; Gromer *et al.*, 1998; Lee *et al.*, 2000; Gorlatov and Stadtman, 2000). Recently, two more tissue-specifically expressed isoenzymes were also identified as selenoproteins (Gasdaska *et al.*, 1999; Lee *et al.*, 1999; Watabe *et al.*, 1999) and related proteins were identified by '*in silico*' cloning (Lescure *et al.*, 1999). Various natural and synthetic compounds, apart from disulfide groups in peptides and proteins, can be reduced by TrxR (Holmgren and Björnstedt, 1995; Björnstedt *et al.*, 1995). The natural substrate of TrxR, thioredoxin (Trx), is a central regulator of the cellular redox status (Follmann and Häberlein, 1995). It is, *e. g.*, required for the redox-regulated function of transcription factors and hormonally-regulated nuclear receptors. Furthermore, ribonucleotide reductase needs reduced Trx for the production of deoxynucleotides. Thus, TrxR enables a basic metabolic process and regulates multiple metabolic events in eukaryotic cells (Hayashi *et al.*, 1993; Björnstedt *et al.*, 1997; Makino *et al.*, 1999; Holmgren, 2000). Likely, it is the pivotal role of selenium in TrxR that explains why a knock-out of the seleno-



**Fig. 2** Metabolism of Thyroid Hormone by 5' and 5-Deiodinases.

Reductive 5'-(phenolic ring) deiodination of the prohormone L-thyroxine (3,3',5,5'-tetraiodo-L-thyronine,  $T_4$ ) to thyromimetically active 3,3',5-triiodo-L-thyronine ( $T_3$ ) is catalyzed by the two deiodinase selenoenzymes, type I and type II 5'-deiodinase ( $\longrightarrow$  5'D). Type III 5-deiodinase (5-D  $\dashrightarrow$ ) removes iodide in 5-position from the tyrosyl ring of  $T_4$  and forms thyromimetically inactive 3,3',5'-triiodo-L-thyronine (reverse  $T_3$ ,  $rT_3$ ). Figure 2 illustrates the conformational aspects of thyroid hormones and the bulky iodine atoms (black) of the non-planar diphenylether aromatic ring system (modified from Cody *et al.*, 1986). Whereas  $T_4$  and  $T_3$  occupy a skewed conformation, the inactive metabolite  $rT_3$ , lacking the iodine atom in the 5-position, has an antiskewed orientation of the two phenolic rings. The physiological cofactor(s) of these deiodinase selenoenzymes is (are) unknown.

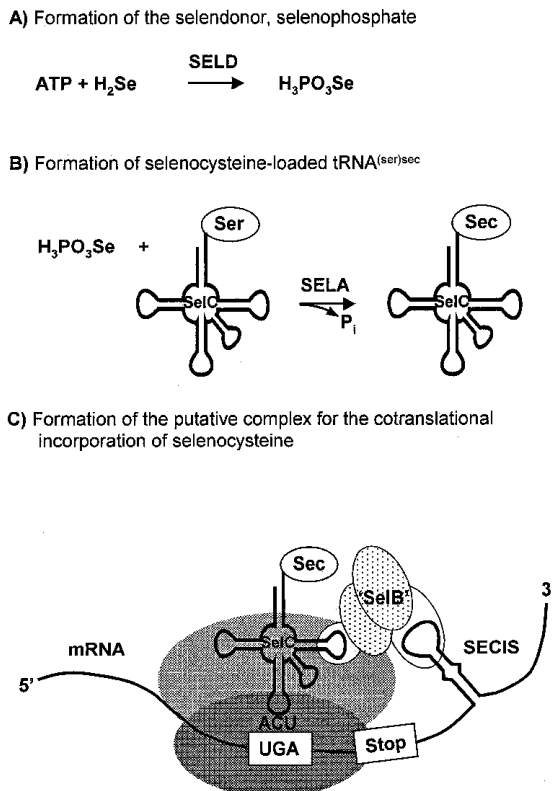
cysteyl-tRNA gene is lethal in mice (Bösl *et al.*, 1997), since targeted disruption of the thioredoxin gene proved to be equally lethal (Matsui *et al.*, 1996).

Approximately 60–70% of the plasma selenium is bound in the selenoprotein P. The function of this protein is not yet clear. It is assumed to be an extracellular antioxidative protein which contributes to the decomposition of peroxynitrite (Arteel *et al.*, 1998; 1999) or might exhibit some *in vitro* peroxidase activity (Sies *et al.*, 1999) like many natural and synthetic selenocysteine-containing proteins (Haring and Schreier, 1999). However, it could equally well bind heavy metals (Burk and Hill, 1994). The role of the selenoprotein P as a transport protein of selenium in plasma has meanwhile been questioned, because selenocysteine could only be released by destroying the protein (Burk and Hill, 1994). Selenoprotein P is presumed to exert its antioxidative effect particularly in the vascular system, where it is bound to endothelial cells with high affinity (Hill and Burk, 1997), probably *via* its histidine-rich domains as shown by *in vitro* interaction with heparin (Arteel *et al.*, 2000). The inhibition of the activity of the human selenoprotein P promoter and the protein expression through pro-inflammatory cytokines and TGF $\beta$  in liver cells characterizes selenoprotein P as a negative acute phase protein (Dreher *et al.*, 1997, Mostert *et al.*, 1999).

Recently, selenoprotein P has further been reported to promote neuron survival *in vitro* (Yan and Barrett, 1998).

### The Biosynthesis of Selenoproteins

The biosynthesis of selenoproteins in prokaryotes has been largely clarified (Böck, 2000; Figure 3). Related studies were mainly conducted with formate dehydrogenase in *E. coli*, which also contains selenium as a selenocysteine residue integrated in the peptide chain. The insertion of the selenocysteine is encoded by the triplet TGA, which usually functions as a stop codon (Zinoni *et al.*, 1986; Böck and Stadtman, 1988; Böck *et al.*, 1991a). Recoding of TGA as a selenocysteine codon requires an mRNA secondary structure called SECIS (for selenocysteine-insertion sequences), which, in bacteria, is localized immediately downstream of the UGA codon. This secondary structure is recognized by a special translation factor, SelB (Baron *et al.*, 1993), which directs a special tRNA<sup>(Ser)Sec</sup>, encoded by the SelC gene, to the ribosome, where the latter enables the incorporation of selenocysteine by means of a UGA anticodon (Leinfelder *et al.*, 1988; Forchhammer *et al.*, 1991). The selenocystyl-loaded tRNA<sup>(Ser)Sec</sup> is synthesized from a seryl-loaded tRNA<sup>(Ser)Sec</sup> by means of selenophos-



**Fig. 3** Model of Selenocysteine Incorporation in Eukarya. Decoding of the UGA codon as selenocysteine and its subsequent incorporation requires a complex multicomponent system and several reactions. These include: (A) the formation of the selenium donor, selenophosphate, from ATP and selenide, catalyzed by the selenophosphate synthetase-2 (SELD), which is a selenoenzyme by itself; (B) a specific tRNA, called tRNA<sup>(Ser)Sec</sup> or SelC, which is first loaded with serine and then converted into selenocysteine (Sec) by selenocysteine synthase (SELA) using selenophosphate as selenium source; (C) a putative translation complex in analogy to the established complex in prokarya. The mRNA forms a characteristic secondary structure in the 3' non-translated region, the selenocysteine incorporating sequence (SECIS). The selenocysteine-loaded tRNA<sup>(Ser)Sec</sup> is transferred to the ribosome A site and recognizes the UGA codon by its anticodon ACU. The complex is stabilized via the interaction of putative SECIS-binding proteins ("SelB") with the SECIS and the tRNA. These components of the translational complex remain to be identified.

phate (Leinfelder *et al.*, 1990), which is generated from H<sub>2</sub>Se and ATP (Lacourciere, 1999; Lacourciere and Stadtman, 1999).

The eukaryotic system of selenoprotein synthesis appears to be homologous in part (Figure 3). The selenocysteine codon is TGA (UGA) again; the tRNA<sup>(Ser)Sec</sup> (Lee *et al.*, 1989) and the selenophosphate synthetase (Low *et al.*, 1995; Guimaraes *et al.*, 1996; Lacourciere and Stadtman, 1999) are closely related. The essential difference of the eukaryotic selenocysteine biosynthesis system consists in the position of the SECIS element. In eukarya, it is located in the 3' nontranslated region and thus decoding from an appreciable distance is necessary (Berry *et al.*, 1993; Low and Berry, 1996). In this respect the archaeal seleno-

protein biosynthesis is more similar to the eukaryotic system than to the bacterial system (Wilting *et al.*, 1997). Considering the similarity of the systems, a SelB-orthologous translation factor is also postulated for eukarya, which should have affinity to both SECIS and tRNA<sup>Sec</sup> (Flohé *et al.*, 1997), and should control the correct decoding of the distant UGA codon by competing with the termination factor (Nasim *et al.*, 2000). Up to now, the eukaryotic SelB-ortholog could not yet be identified. Using gel shift experiments, various proteins that bind to eukaryotic SECIS structures were detected. The bands, however, differ in molecular masses (from 48–120 kDa) and their affinities to SECIS elements of a given selenoprotein mRNA could usually not be compared to that of other SECIS elements (Shen *et al.*, 1995, 1998; Yamada, 1995; Hubert *et al.*, 1996; Lesoon *et al.*, 1997). A protein of 120 kDa was reported to be required for eukaryotic selenoprotein biosynthesis (Copeland *et al.*, 2000). It does not, however, display any homology with bacterial SelB and appeared to be only part of a larger complex. Most of these SelB candidates can therefore equally be considered as nucleic acid-binding proteins that regulate, *e.g.* tissue-specific expression of certain selenoproteins or mRNA stability and the functions of bacterial SelB, *i.e.* binding of the SECIS element and tRNA<sup>(Ser)Sec</sup>, as well as selenocysteine incorporation, might be exerted by two (or more) different proteins in archaea and mammalia (Rother *et al.*, 2000).

## Regulation of Selenoprotein Expression

### Tissue-Specific Expression

The more common selenoproteins display an expression pattern that reflects the metabolic activity of the tissue, whereas others are more selectively distributed.

Type I 5'-deiodinase is found primarily in thyroid, liver, kidney and pituitary, type II 5'-deiodinase in brown fat tissue of rodents, in placenta, thyroid, pituitary, and in the central nervous system, type III 5-deiodinase in skin, placenta and also in the central nervous system (Köhrle, 2000b). The testicle is the tissue containing the highest amount of PHGPx (Maiorino *et al.*, 1998). In *Schistosoma mansoni*, PHGPx is found in the female genitals (Maiorino *et al.*, 1996). GI-GPx is expressed only in the gastrointestinal tract (Chu *et al.*, 1993, Mörk *et al.*, 1998; Wingler and Brigelius-Flohé, 2000), whereas pGPx is secreted and preferentially formed at metabolic surfaces (proximal tubulus of the kidney, intestinal epithelium, skin, lung, epididymis, vas deferens, chorioidal plexus; Brigelius-Flohé, 1999). The extracellular selenoprotein P displays a similar expression pattern but is highly expressed in liver and, surprisingly, also in testicular Leydig cells and the Purkinje layer of the cerebellum (Steinert *et al.*, 1998). Among the selenoproteins, which are not yet defined functionally or structurally, selenoprotein W is found in heart and skeletal muscle, other poorly characterized selenoproteins in testis, prostate and pancreas (Behne *et al.*, 1997).

The sometimes unusual expression pattern certainly points to highly-specialized roles. In none of the cases has the molecular basis for tissue-specific expression so far been elucidated and, apart from PHGPx and the deiodinases (see below), the tissue-specific biological role of the selenoproteins remains obscure.

### Hormone-Dependent Regulation

In fetal osteoblast-like cells (hFOB), human thioredoxin reductase is rapidly induced, like an immediate-early gene, through 1,25-dihydroxy vitamin D<sub>3</sub>, certain cytokines and growth factors; here also the selenium status modulates the expression of TrxR (Schütze *et al.*, 1998a, b). The three deiodinase isoenzymes are regulated by thyroid hormones, retinoids, sexual hormones, gluco- and corticosteroids and a series of growth factors and cytokines, as verified by promoter studies for individual deiodinase isoenzymes (Jakobs *et al.*, 1997; Schmutzler *et al.*, 1998, for an overview see Köhrle, 2000b).

Hormone-dependent expression of PHGPx, which had been assumed to be mediated by putative hormone-responsive elements in the promoter or in the introns (Brigelius-Flohé *et al.*, 1994), could not be verified by reporter gene constructs. PHGPx rather is preferentially expressed in round spermatids. These cell's development in turn depends on testosterone (Maiorino *et al.*, 1998). The androgen-dependent expression of pGPx in epididymis might be based on the same principle of hormone-mediated growth and differentiation of the producing cell type (Schwaab *et al.*, 1998).

### Regulation by Oxidative Stress

An induction of GPx genes by oxidative stress was often postulated but has never been convincingly demonstrated *in vivo*. The induction of cGPx through an oxygen-responsive element has only been described *in vitro* (Cowan *et al.*, 1993; for a review see Flohé *et al.*, 1997).

### Selenium-Dependent Regulation

Biosynthesis of selenoproteins, of course, depends on the bioavailable selenium. Their biosynthesis, however, follows a strict hierarchy in the case of limited selenium supply (Burk and Hill, 1993; Sunde, 1994; Gross *et al.*, 1995; Flohé *et al.*, 1997). Even within the family of glutathione peroxidases there are enormous differences in the expression in response to selenium (Brigelius-Flohé, 1999). In all examined cases the PHGPx activity in selenium deficiency was stable for a long time, whereas the activity of cGPx declined quickly and substantially (Lei *et al.*, 1995). This loss of activity upon selenium deprivation is not only caused by reduced protein synthesis, but also by enhanced degradation of the pertinent mRNA. The mRNA stability of the cGPx is the lowest, that of the PHGPx is relatively unchanged and reduced only under severe selenium deficiency. That of GI-GPx is extremely high. The mRNA even increases under poor selenium provision (Wingler *et al.*, 1999). The reasons for this phenomenon

are not clear at all. It is likely that the mRNA stability is regulated by selenium-responsive RNA-binding proteins in an analogous way as has been demonstrated for mRNA of proteins involved in iron metabolism (Hentze, 1991). It has not yet been examined in detail whether mRNA stability responds to selenium in a tissue-specific manner.

### Medical Implications

The characteristics of selenium deficiency, but also of selenium toxicity, are largely known from epidemiological studies. At present, a daily intake of 70 µg for adults is considered normal, while daily intakes below 20 µg are rated as insufficient. The toxicity limit is estimated at 800 µg per day (Bähr *et al.*, 1999).

Comprehensive analyses of selenium deficiency in livestock production and agriculture were conducted in the US, New Zealand and other regions with varying selenium supply, as reviewed extensively by the National Research Council (1983) of the United States. As a consequence, selenium supplementation of animal food or the use of mineral fertilizers has become routine in many selenium-deficient countries, although the basic biochemical processes causing the deficiency symptoms remain to be worked out. Human selenium supply relies on such animal-mediated enrichment of the food chain in many regions of the world, where selenium, like iodine, has been eluted from agriculturally-used soils and earth surfaces and is no longer supplied to the food chain *via* assimilation by plants.

### Manifest Selenium Deficiency

While typical selenium deficiency syndromes of livestock, like white muscle disease in cattle, mulberry heart disease in pig, and exsudative diathesis in poultry, have been recognized in many countries, manifest selenium deficiency is commonly not a problem for humans living in developed countries, where people have access to varied food derived from diverse sources. Human diseases unambiguously attributed to, or associated with, selenium deficiency have been reported to occur only in remote rural areas with extreme selenium deficiency.

In a cooperation between Chinese and American groups, the pathophysiology of the exemplary Chinese selenium deficiency syndrome, Keshan disease, was largely clarified: against the background of a selenium deficiency, coxsackie viruses become virulent. This etiology of Keshan disease has recently been corroborated by compelling animal experiments. In both selenium-deficient and cGPx(−/−) mice, avirulent Coxsackie strains mutated into virulent ones (Beck *et al.*, 1998). Selenium deficiency, thus, is a necessary but not a sufficient condition to cause disease manifestation.

Detailed concepts for the pathogenesis of the myxoedematous cretinism have been developed from epidemiological and intervention studies by Dumont's team in Brussels in cooperation with Central African and Asian



teams. The resulting hypotheses are now being tested in animal experiments. This disease occurs in the case of a serious simultaneous selenium and iodine deficiency, whereas iodine deficiency alone leads to a neurological type of cretinism (Dumont *et al.*, 1994; Köhrle 1999a). Whether the Kashin Beck osteoarthropathy is also caused by selenium deficiency or combined selenium and iodine deficiency is still the topic of scientific controversy. In any event, further pathogenic factors, *e.g.* mycotoxins, appear to contribute to this peculiar syndrome (Ge and Young, 1993; Moreno-Reyes *et al.*, 1998).

### Selenium and Tumor Incidence

The relationship between selenium supply and cancer incidence is supported by epidemiological surveys, and cohort and case control studies, as well as by retrospective and prospective prevention or intervention studies (National Research Council, 1983). Different approaches from several regions (*e.g.* China, USA, Scandinavia) almost unanimously demonstrated low selenium intake to be associated with a higher incidence of mammary, thyroid, prostate, lung and colorectal carcinoma (for recent overviews see Combs *et al.*, 1997; Combs and Gray, 1998; Knekt *et al.*, 1998). Animal experiments on initiation, promotion and proliferation of tumors tended to support a beneficial effect of selenium. In animal experiments, however, individual selenium compounds differed in their efficacy (Ip and Ganther, 1990; Ip, 1998). The protective effect of selenium is not readily explained in terms of prevention of oxidative DNA damage, since, as a rule, higher dosages of selenium than those optimizing peroxide metabolism are required (Combs *et al.*, 1997; Combs and Gray, 1998; Ganther, 1999). Accordingly, topical discussions presume a direct antiproliferative effect of pharmacological selenium concentrations, be it due to enhancement of apoptosis by excess selenium supply or to distinct inhibition of proliferation-enabling enzymatic processes by certain selenium metabolites (Ganther, 1999).

A pivotal prospective, placebo-controlled, double-blind study conducted with more than 11 00 patients over 4.5 years on average and a selenium supplementation of 200  $\mu\text{g}/\text{d}$  in the form of selenium-enriched yeast remained ambiguous in failing the main endpoint, *i.e.* lowering the recurrence of non-melanoma skin cancer, but surprised in meeting secondary endpoints beyond expectations: total cancer incidence and cancer mortality were significantly lowered by supplementation. Particularly the incidence of colorectal, pancreatic and lung tumors decreased during the intervention and the 6.5-year follow up-period in the selenium-supplemented group (Clark *et al.*, 1996). To comply with biometric rules, however, the observations have to be verified in dedicated examinations.

### Selenium and Intensive Care Medicine

Critically ill patients, above all those with systemic inflammatory response syndrome (SIRS), have lower serum selenium levels and GPx activity already when they are ad-

mitted to intensive care units (Hawker *et al.*, 1990; Gärtner *et al.*, 1997). In SIRS patients, the abundant  $\text{H}_2\text{O}_2$  formation necessitates adequate protection by glutathione peroxidases (Prabha *et al.*, 1991). Low serum selenium levels correlate with the low GPx activity and are almost consistently found during the hyperdynamic septic phase of shock, after surgery, or in patients with liver diseases (Robinson *et al.*, 1992). Also, serum selenium levels correlate inversely with mortality (Forceville *et al.*, 1998). The reason is still not clear. Selenium-deficient nutrition is discussed as a predisposing factor for SIRS (Richard *et al.*, 1991; Ringstad *et al.*, 1993). But also a rapid redistribution of selenium in the body or an excretion through the kidney at the beginning of the disease has been supposed, but could not yet be analytically verified (Forceville *et al.*, 1998). During longer parenteral nutrition and continuation of the disease the selenium level further decreases (Hawker *et al.*, 1990; Sando *et al.*, 1992). It is equally unclear how a selenium supplementation might interfere with the septic syndrome. Normalization of peroxide metabolism is most frequently discussed. But a mechanistically unclear improvement of the immune response that is observed upon selenium supplementation (McKenzie *et al.*, 1998), comprising *inter alia* enhanced activity of natural killer cells (Dimitrov *et al.*, 1986), might be of particular relevance in the late phases of SIRS.

First pilot studies have addressed the effect of selenium on septic diseases with promising results (Gärtner *et al.*, 1997; Zimmermann *et al.*, 1997; Angstwurm *et al.*, 1999). In these conditions, the usual parenteral nutrition, which contains low dosages of selenium (35  $\mu\text{g}/\text{day}$ ), neither leads to a normalization of serum selenium levels nor to an increase of GPx activity. Based on the preliminary data, controlled prospective studies examining the efficiency of selenium at different dosages in an adequate number of patients appear mandatory, because SIRS still has one of the highest mortality rates (40% on average) among the acute diseases.

### Selenium and Thyroid Function

The human thyroid contains a high amount of selenium because it produces  $\text{H}_2\text{O}_2$  for oxidative thyroid hormone synthesis and has to protect itself from oxidative damage by the expression of selenoperoxidases. Moreover, it needs selenium for activating the prohormones of T4 to T3 catalyzed through the 5'-deiodinases. As mentioned above, serious selenium deficiency combined with iodine deficiency leads to a myxedematous type of cretinism. In iodine deficiency, the production of  $\text{H}_2\text{O}_2$  is stimulated by pituitary thyrotropin (Dumont *et al.*, 1994). In this condition, the thyroid is postnatally damaged and becomes fibrotic under the influence of TGF $\beta$  (Contempre *et al.*, 1996). But there are also hints that the selenium status influences the progress of auto-immune diseases of the thyroid (1/6 of all women have thyroid auto-antibodies) by unclear mechanisms (Schmidt *et al.*, 1998; Köhrle, 1999a). Also, lower selenium concentrations can be found in thy-

roid tumor tissue (Köhrle, 1999a). In a large-scale Norwegian study, a significantly higher incidence of thyroid tumors correlated with prediagnosed low selenium levels (Glattre *et al.*, 1989). The role of the individual selenoproteins in pathologically altered thyroid tissue is, however, as unclear as their contribution to auto-immune diseases and tumor development. Neither is it evident whether there is a direct link between the low selenium status in critically ill patients (*e. g.* SIRS) and the simultaneously observed low T<sub>3</sub>-production due to inefficiently expressed hepatic selenoprotein type I 5' deiodinase in the 'Low-T<sub>3</sub>-Syndrome' (Köhrle *et al.*, 2000b).

### Selenium and Male Fertility

The necessity of adequate selenium provision for male fertility is known particularly from veterinary medicine (see National Research Council, 1983, and Flohé, 1989 for older literature). Yet the precise role of selenium in male fertility remained an enigma for decades. In sperm, selenium is largely associated with the keratin-like material that embeds the helix of mitochondria in the midpiece of spermatozoa. A protein derived from this so-called mitochondrial capsule was reported to contain selenium and accordingly termed 'mitochondrial capsule selenoprotein (MCS)' (Pallini and Bacci 1979; Calvin *et al.*, 1981). The pertinent genes of rats and mice, however, did not contain any TGA codons within the translated regions (Adham *et al.*, 1996; Cataldo *et al.*, 1996). Accordingly, MCS could no longer be considered the selenoprotein essential to sperm function. More recent studies showed that PHGPx is abundantly expressed in spermatogenic cells (Maiorino *et al.*, 1998, 1999; Mizuno *et al.*, 2000) but exists as an enzymatically-inactive structural protein in mature sperm, where it contributes to the formation of the mitochondrial capsule (Ursini *et al.*, 1999). In spermatozoa, PHGPx thus replaces MCS, which had mistakenly been considered a selenoprotein. Because the morphological defects of sperm under selenium deficiency primarily affect the mitochondrial capsule, reduced fertility can conceivably be explained by insufficient PHGPx synthesis. The relevance of further selenoproteins that are specifically expressed in the male genitalia (Behne *et al.*, 1997) has yet to be clarified.

### Selenium and Atherogenesis

Fatty acid and cholesterol ester hydroperoxides, as present in oxidized LDL (oxLDL), are believed to initiate atherogenesis (Steinberg, 1997). In atherosclerotic lesions, hydroperoxides as well as the 15-lipoxygenase were found (Ylä-Herttua, 1991), and lipid hydroperoxides can induce adhesion molecules in cultivated endothelium cells (Friedrichs *et al.*, 1999). Both oxLDL-induced foam cell formation and smooth muscle cell proliferation and hydroperoxide-induced presentation of adhesion molecules are considered to synergize in the initiation of atherogenesis.

A prophylactic role of selenium in the prevention of cardiovascular disturbances was observed in epidemiologi-

cal studies (National Research Council, 1983; Salonen, 1987). In view of the oxidative processes implicated in early atherogenesis, the selenium effect is tentatively attributed to the optimization of glutathione peroxidase activities. Possible candidates would particularly be the pGPx as an extracellular enzyme and PHGPx, which efficiently reduces hydroperoxides in oxidized LDL. This seemingly straightforward hypothesis, however, suffers from at least two inconsistencies: pGPx lacks a sufficient supply of reducing capacity in the extracellular compartment (Brigelius-Flohé, 1999) and does not have the optimum specificity (Yamamoto and Takahashi, 1993), PHGPx does display the optimum specificity to reduce all kinds of hydroperoxy groups in oxLDL (Ursini *et al.*, 1982, 1995) but is not present extracellularly, where LDL is oxidized, and does not readily decline in moderate selenium deficiency. Clearly, the link between selenium and atherogenesis, if real, has still to be identified. It is likely that the redox regulation of lipid mediator synthesis and cytokine-dependent signaling is more relevant to the initiation of atherogenesis than the mere antioxidant potential of selenoperoxidases (Brigelius-Flohé, 1999).

### Conclusions

The exponential progress in selenium biochemistry over the last two decades led to the identification, cloning and functional characterization of more than a dozen selenoenzymes with widely varied catalytic potential, and the key events of selenoprotein biosynthesis have been elucidated. This review is, however, not primarily meant to celebrate these achievements, it rather aims at underscoring that most of the roles of the essential trace element in biology still remain as obscure as they have been for most of the last century. This is because the trace element typically hides in 'trace enzymes'. In fact, the present state of the art could not have been achieved without advanced molecular biology techniques and most sophisticated physicochemistry.

For a long time, the selenium biochemistry was misinterpreted as the search for biological curiosities that might detract serious scientists from central biological problems. This view can no longer be maintained: as an integral moiety of the thioredoxin reductases it is relevant to basic steps of nucleic acid synthesis; it proved to be essential for male fertility in mammals; and the knock-out of the selenocystyl-tRNA gene in mice was lethal (Bösl *et al.*, 1997) and thus revealed that at least one of the selenoproteins must be indispensable for mammalian life in general.

Most of the known selenoproteins were detected in either bacteria and archaea or in mammals, but selenoproteins are definitely also present in lower eukarya (Maiorino *et al.*, 1996). Little is known about the role of selenium in the remaining living kingdom. Plants appear not to depend on selenoenzymes, yet they contain numerous low molecular weight selenocompounds of nutritional,

pharmacological, and toxicological interest (Läuchli, 1993; Neuhierl *et al.*, 1999). Together with bacteria, which may also 'dissimilate' bioavailable selenite to elementary selenium (Garbisu *et al.*, 1995), plants, by assimilation of inorganic selenium, certainly contribute to the maintenance of the geo-ecological selenium homeostasis that is equally important for livestock and human health (National Research Council, 1983).

As a rule the catalytic potential of known bacterial selenoenzymes is not paralleled by mammalian selenoproteins and *vice versa*, selenophosphate synthetase being the only known exception. Our fragmentary knowledge does not, however, exclude the possibility that the highly complex catalytic centers comprising selenocysteine, pterine cofactor-bound molybdenum and flavin or selenocysteine-coordinated iron/nickel/sulfur clusters are not used in eukaryotes. New sequences of mammalian selenoproteins are being detected at high speed by 'in silico cloning' (Lescure *et al.*, 1999; Kryukov *et al.*, 1999), and pulse-label experiments with radioactive selenium suggest the existence of another dozen or two, giving ample room for further surprises when the catalytic potentials and structures of these proteins emerge. It can also not be taken for granted that selenium exerts its catalytic role in mammals exclusively as a selenocysteine residue that is integrated into proteins. The chemical nature of the 'acid-volatile selenium' (Diplock *et al.*, 1973) of rat liver has never been worked out. Certainly, H<sub>2</sub>Se eventually could be generated from acid-denatured selenocysteine-containing proteins by  $\beta$ -elimination, but liberation of H<sub>2</sub>Se by acid from a selenanyl-bond, as present in bacterial CO dehydrogenase, or from iron/selenide clusters, must be rated as more likely.

As to the medical implications of selenium research, we may safely state that severe selenium deficiency requires supplementation, as is suggested from the eradication of Keshan disease in supplemented areas in China and the prevention of analogous symptoms in patients on supplemented parenteral nutrition. As evident from the experiments with cGPx(-/-) mice, the cardiac complication in selenium deficiency likely results from a disturbed hydroperoxide metabolism. Evidence is also emerging that selenium deficiency specifically complicates iodine deficiency by increasing the oxidative challenge to the thyroid. In all other pathological conditions for which selenium supplementation is currently discussed, neither a satisfying rationale nor compelling clinical data are available. Nevertheless, the beneficial effects of selenium inferred from softer data, *e.g.* epidemiological surveys on cancer incidence and cardiovascular disease, small-scale clinical trials with critically ill patients, the unexpected accidental observations in large-scale cancer prevention trials, or veterinary experience with selenium responsiveness of inflammatory diseases, should not be ignored. They certainly demand validation by dedicated prospective studies. In chronically developing multifactorial diseases like oncogenesis and cardiovascular disease such trials are hard to design, if they are feasible at all. Rather, these problems

may be rationally approached by studying the potential relevance of individual selenoproteins and their dependency on the selenium status. Thereby, selenium-responsive disease-related surrogate endpoints could be defined, which then may be tested in short-term clinical trials with manageable sample sizes.

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