14

SELENOPROTEINS IN CELLULAR REDOX REGULATION AND SIGNALING

ARJUN V. RAMAN AND MARLA J. BERRY

Department of Cell and Molecular Biology, University of Hawaii, Biomedical Sciences Building, Honolulu, HI, USA

14.1 INTRODUCTION

In 1817 the Swedish chemist Jöns Jacob Berzelius discovered selenium (Se), a chemical element with the atomic number 34 and an atomic weight of 78.96 g/mol. It is a trace nonmetal of the chalcogen group that includes oxygen and sulfur and is present in the Earth's crust and in ocean water at abundances ranging from 0.05 to 2.0 ppm. Sulfur has physical and chemical properties similar to those of Se; however, their noninterchangeable nature in biological systems highlights the substantial differences between these two elements. Se exists naturally as structural analogs of sulfurous molecules, including inorganic selenides (Se^{2-}), selenites (SeO_3^{2-}), and selenates (SeO_4^{2-}), and the organic amino acids selenocysteine and selenomethionine. Although Se is a dietary antioxidant and is required in nanomolar concentrations by organisms in all three domains of life, it is simultaneously toxic, having a median lethal dose in humans of 1.5–6.0 mg/kg body weight with the inorganic salts being more acutely toxic than organic forms [1]. In vertebrates, Se is obtained almost exclusively by ingestion of plants and animals, and the amount of Se in dietary sources depends on the concentration of environmental Se. Currently, the recommended daily allowance of dietary Se for healthy adults is 55 μg/day and the upper tolerable intake level is 400 μg/day [2].

Low-molecular-weight selenocompounds are bioactive, but most cellular biological functions of Se are attributed to genetically encoded selenoproteins. Selenoproteins are defined by incorporation of the 21st amino acid selenocysteine (Sec), which is an analog of cysteine with the sulfur-containing side chain replaced by a Se-containing side chain. Although Se and sulfur have similar chemical and physical properties, key differences generate divergent character between Sec and cysteine, with Sec being a much more efficient catalyst. The cause of the higher catalytic capacity of Sec is uncertain, but a few ideas have been proposed. For example, Sec has been described as having stronger nucleophilic and electrophilic character than cysteine, potentially aiding in catalysis. Additionally, the selenol of Sec has a lower acid dissociation constant than the thiol of cysteine (pKa of 5.2 vs. 8.3), rendering the selenol a superior leaving group because of its higher acidity. This also means that at physiological pH, Sec tends toward ionization whereas cysteine tends toward protonation, and at low pH Sec retains much higher reactivity than cysteine. The biological rationale for Sec utilization is enigmatic, but rapid recycling of oxidized Sec to the reduced form as well as resisting irreversible oxidation to selenonic acid could potentially explain the biological pressure to use Sec over cysteine in proteins during evolution [3].

Sec is synthesized from serine after aminoacylation to a Sec-specific tRNA ($tRNA^{Sec}$) that recognizes the UGA "stop" codon, which typically signals to terminate mRNA translation. Recognition of the UGA codon as a Sec insertion site, as opposed to a termination site, requires a Sec insertion sequence (SECIS) element in selenoprotein mRNA molecules, which is a stem-loop structure that is

Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling, First Edition.
Edited by Tahira Farooqui and Akhlaq A. Farooqui.

typically downstream of the UGA codon [4]. Although selenoproteins are present in eukarya, archaea, and bacteria, the mechanism of Sec incorporation differs between domains in several respects. For example, the bacterial SECIS element is located immediately following the Sec UGA codon, whereas the eukaryotic and archaeal SECIS elements are typically located much farther downstream in the 3′ untranslated region (UTR). Additionally, the archaeal genus *Methanococcus* has a 5′ UTR SECIS element, while eukaryotes have two distinct forms of 3′ UTR SECIS elements [5, 6]. An elongation factor specific to Sec-tRNA^{Sec} has been identified as selB in bacteria, whereas the coordinated function of a SECIS binding protein (SBP2) and the eukaryotic Sec-tRNA^{Sec} elongation factor (EFSec) allows the cotranslational incorporation of Sec instead of termination [7]. Biosynthesis of Sec in eukarya and archaea is accomplished by the sequential actions of *O*-phosphoseryl-tRNA kinase (PSTK) and *O*-phosphoserine-tRNA:Sec-tRNA synthase (SEPSECS, SLA), which convert seryl-tRNA^{Sec} to selenocysteinyl-tRNA^{Sec} with a phosphoseryl-tRNA^{Sec} intermediate. Thus Sec formation in archaea and eukarya is a two-step process, whereas bacterial selenocysteine synthase (selA) can synthesize Sec directly from seryl-tRNA^{Sec}. The Se donor utilized by both SEPSECS and selA is selenophosphate, which is generated from selenide (H_2Se) by selenophosphate synthetase (SPS) enzymes. Selenophosphate is first transferred to *O*-phosphoseryl-tRNA^{Sec} by SEPSECS while displacing the phosphoseryl moiety, and subsequent hydrolysis of the phosphate group yields Sec charged to its cognate tRNA [8]. Bacteria have one SPS enzyme termed selD, while two (SPS1, SPS2) have been identified in eukarya, of which SPS2 is itself a selenoprotein. Selenocysteine lyase (SCLY), a putative Se recycling enzyme, is able to catalyze the hydrolysis of Sec into selenide and alanine and potentially delivers Se to SPS enzymes for phosphorylation and subsequent reinsertion into nascent selenoproteins [9]. Pyridoxal phosphate is a prosthetic group derived from vitamin B6 and is required by enzymes involved in metabolism of amino acids, glucose, lipids, and neurotransmitters. The enzymatic activity of both SCLY and SEPSECS is pyridoxal phosphate dependent, implying that Sec synthesis shares regulatory elements with standard metabolic pathways [8, 10, 11].

Several steps in selenoprotein synthesis are regulated by Se availability (dietary or environmental depending on the organism), as well as by the level of oxidative stress. For example, in addition to being a limiting substrate for SPS2, Se levels affect different SECIS elements differentially, potentially regulating selenoprotein synthesis efficiency at the level of translation [7]. Oxidation of the cysteine-rich, redox-sensitive domain of SBP2 masks the nuclear-export signal (NES), causing importation into the nucleus. Subsequently, it is either sequestered there during high oxidative burden or else reduced by nuclear-specific isoforms of selenoproteins, unmasking the NES for binding and exportation by the nuclear export receptor CRM-1 [12]. As discussed below, individual selenoprotein expression also responds differentially to Se availability and oxidative stress, providing another level of regulatory control for selenoprotein synthesis. Thus there is a complex, nonlinear interaction between Se status and oxidative burden that coordinates the synthesis of the numerous selenoproteins. Despite a low environmental availability of Se and the various elaborate mechanisms for Sec incorporation, selenoproteins are widespread in organisms, with certain plants and fungi being the only major exceptions that lack selenoproteins and an essential biological function for Se [13].

14.2 OXIDATIVE STRESS AND SELENOPROTEINS

Oxidative stress is a broadly used term, but it generally signifies an imbalance in prooxidants and antioxidants in cells and their environment, causing disruption of redox control and macromolecular damage. Here we consider free radicals and nonradical oxidants separately as prooxidants, and distinguish macromolecular damage as a mechanistically different outcome from disrupted redox control. Macromolecular damage to DNA, proteins, and lipids is caused by redox reactions with free radicals, which contain an unpaired electron and are scarce in biological systems because of enzymes and chemicals that rapidly scavenge them and typically generate nonradical oxidants [14]. For example the superoxide radical (O_2^{-}) is rapidly converted by superoxide dismutase to the nonradical oxidant hydrogen peroxide (H_2O_2). Superoxide can also react with nitric oxide (NO), another free radical, to produce the nonradical oxidant peroxynitrite (ONO_2^{-}). Although free radical-mediated macromolecular damage is a common end point observed in aging and disease, the rest of this chapter focuses on the role of selenoproteins in redox systems control and signaling.

Because of such rapid conversion in organisms, free radicals are quantitatively miniscule compared to nonradical two-electron oxidants such as hydrogen peroxide, fatty acid hydroperoxides, disulfides, peroxynitrite, aldehydes, and quinones. In a prototypical redox control system, these nonradical oxidants reversibly react with protein thiols to elicit a redox signal that functions in physiological regulation. Details on the mechanisms of reversible thiol oxidation as a biological control system are beyond the scope of this chapter, but they generally include reaction with an active site cysteine,

modification of a distal allosteric cysteine, and alteration of macromolecular interactions [15]. If the relevant cysteine is in an active site such modifications may serve as a simple binary on-off "switch" to activate or inactivate the protein. Another scenario in which the cysteine residue is not in the active site allows for allosteric regulation, which can throttle protein function along a continuous gradient, akin to a "dimmer." Finally, oxidation of cysteine thiols is known to control intra- and intermolecular protein interactions by formation of disulfides. Among other things, disulfide cross-linking is well established in maintaining proper protein structure, regulating the viscosity of mucus, and connecting actin filaments and tethering proteins to the cytoskeleton [16, 17].

Selenoproteins are closely linked with the cellular thiol-disulfide couples, particularly the glutathione (GSH) and thioredoxin (TXN) couples. GSH is a tripeptide made of glycine, cysteine, and glutamate and is the most abundant thiol in cells, present at millimolar concentrations. Oxidation of the cysteine thiol links two molecules of GSH to form glutathione disulfide (GSSG). This reaction can be spontaneous in the presence of electrophiles or, alternatively, can be catalyzed by a number of enzymes that utilize GSH as an electron donor, including glutathione peroxidase (GPX), glutaredoxin, and glutathione *S*-transferase enzymes. Reduction of GSSG, producing two molecules of GSH, is performed by the homodimeric flavoenzyme glutathione reductase.

All of the characterized selenoproteins that function as enzymes are oxidoreductases that catalyze thiol-disulfide oxidation-reduction (redox) reactions and contain Sec in the active site. In the early 1970s the first Se-dependent enzyme discovered was glutathione peroxidase 1 (GPX1), which catalyzes the reduction of hydrogen peroxide (H_2O_2) by oxidation of two molecules of GSH to GSSG [18]. Cytosolic and mitochondrial forms of the GPX1 enzyme are transcribed from the same gene containing one Sec-encoding TGA. The enzyme functions as a homotetramer utilizing four Se atoms per active enzyme [19]. GPX1 is the most abundant glutathione peroxidase and the most abundant selenoprotein in rats, representing a significant fraction of the total circulating Se pool [20].

Four homologous GPX selenoproteins have subsequently been identified in humans. GPX2, also known as gastrointestinal GPX, is a cytosolic enzyme that is specific to epithelial cells and is abundant in the gut [21]. The extracellular GPX3 has broad substrate specificity and is found in most extracellular compartments but is abundant in kidney and blood plasma [22]. GPX6 is closely related to GPX3 but is only expressed in the olfactory system and exists as a cysteine homolog in rodents [23]. GPX4 is structurally and functionally different from the other GPX enzymes because it is active as a monomer rather than a tetramer and can directly reduce membrane lipid hydroperoxides and free fatty acid hydroperoxides. Alternative splicing and transcription initiation generates three distinct isoforms of GPX4 that localize to the cytosol, mitochondria, and nucleus. Additionally, GPX4 translation is regulated by the mRNA binding protein guanine-rich sequence-binding factor 1 [24]. Genetic deletion of GPX4 in mice causes embryonic lethality, and knockdown of GPX4 in cells leads to rapid lipoxygenase-mediated lipid peroxidation and subsequent apoptosis, suggesting that removal of lipid hydroperoxides by GPX4 is essential for cell viability [25, 26]. There are three additional GPX enzymes (GPX5, GPX7, and GPX8) that are not selenoproteins in humans.

TXN is a small protein of ~12 kDa and is present at concentrations several orders of magnitude below GSH. It contains an active site dithiol that is highly conserved in evolution and widely distributed among the TXN superfamily. Through oxidation of the dithiol to a disulfide, TXN can directly reduce cysteine sulfenic acids and control the state of dithiol-disulfide motifs in target proteins, and can also serve as an electron-donating cofactor for enzymes such as ribonucleotide reductase, peroxiredoxins, and methionine sulfoxide reductases [27]. In turn, reducing oxidized TXN is mediated exclusively by the thioredoxin reductase (TXNRD) family of selenoproteins [28]. At least four selenoproteins (GPX3, GPX4, SEPP1, SEPX1) can utilize TXN as a cofactor for enzymatic reduction, and it is possible that others do as well [29, 30]. There are also numerous thioredoxin-like proteins that may depend on TXN or act in parallel to provide additional substrate specificity beyond that provided by TXN. Several selenoproteins contain a TXN-like fold, which is a well-described secondary/tertiary structure pattern with a conserved Cys-X-X-Cys or Cys-X-X-Ser/Thr active-site motif characteristic of oxidoreductases [31], where X is any amino acid. While it is tempting to speculate that these selenoproteins operate similarly to TXN, namely, by controlling the redox state of cysteine residues and dithiol motifs, there is at present little evidence to support or deny this notion.

Three mammalian thioredoxin reductases are selenoenzymes encoded by individual genes. TXNRD1, TXNRD2, and TXNRD3 encode homodimeric flavoproteins that localize to the cytosol, mitochondria, and testes, respectively. They are members of the pyridine nucleotide-disulfide oxidoreductase family and contain two redox-sensitive sites in the N- and C-termini that interact in a head to tail dimer conformation of the active enzyme. These enzymes are capable of reducing a number of substrates, but they depend on NADPH for donating electrons, which are first transferred to the FAD group, then passed to the N-terminal dithiol of one subunit and

subsequently to the C-terminal selenenyl-sulfide of the other subunit. The highly conserved Sec-containing C-terminal motif is absolutely critical for catalytic function of TXNRD enzymes [27, 32]. The main substrate for TXNRDs is the small redox-sensitive protein TXN, which is integral to physiological processes such as cell communication, metabolism, proliferation, and apoptosis. In general, the reactive dithiol of TXN will become oxidized to a disulfide during reduction of an oxidized target protein. Regeneration of reduced TXN proteins requires TXNRD, and thus the TXN/TXNRD system is completely dependent on Se in mammals. The importance of this system is highlighted by the fact that knockout of either TXNRD1 or TXNRD2 is embryonic lethal in mice [33, 34]. It is worth noting that TXNRDs from mammals differ from the Se-independent enzymes of archaea, bacteria, yeast, and plants.

Reactions between target proteins and TXN can be spontaneous, but several enzymes can catalyze the reduction of target proteins using TXN as an electron-donating cofactor. The human genome codes for four methionine sulfoxide reductase (MSR) enzymes that reduce oxidized methionine residues in proteins utilizing TXN as a cofactor. There is now considerable evidence that, like cysteine, reversible methionine oxidation can regulate protein function [35]. For example, calcium/calmodulin-dependent protein kinase II and the phosphatase calcineurin, among many other proteins, are regulated by methionine redox status [36, 37]. A single MSRA and three MSRB enzymes stereospecifically reduce S- and R-sulfoxidated methionines, respectively. MSRB1 is a selenoprotein also known as Selenoprotein R and SEPX1, while MSRA as well as MSRB2 and MSRB3 are Se-independent enzymes. SEPX1 is a zinc-containing protein present in the cytosol and nucleus and exhibits the highest methionine-R-sulfoxide reductase activity because of the presence of Sec in its active site [38]. Interestingly, redox status of the cysteine-rich metallothionein/thionein couple dictates zinc loading in that reduced thionein binds zinc and oxidation of metallothionein releases it. Moreover, thionein can reduce nonselenoprotein MSRB3 in the presence of TXN, TXNRD, and NADPH more efficiently than without thionein [39]. Therefore regulation of specific kinases, phosphatases, and other proteins by methionine-R-sulfoxide reduction is mediated by two selenoproteins (MSRB1, TXNRD1) and NADPH.

14.3 SELENOPROTEINS AND REDOX SYSTEMS

The GSH and TXN couples are central to a global system of connected redox elements involving reversible oxidation of proteins containing cysteine, methionine, and Sec residues. GPX and TXNRD selenoproteins, being critical effectors of peroxide and thioredoxin reduction, have a direct role in the redox systems biochemistry of organisms (Fig. 14.1A). Oxidative stress in the form of disrupted redox control arises when cysteine (or methionine) residues become abnormally oxidized or irreversibly modified, stripping the reversible redox reactivity that is required for physiological processes. Several covalent and noncovalent modifications to distal or active site cysteine residues modify protein structure and function. A redox-active cysteine residue can become reversibly oxidized to sulfenic acid, which can be reduced stepwise by two molecules of reduced GSH. Additionally, cysteinyl-*S*-glutathione conjugates are semistable in certain proteins, and *S*-glutathionylation can lead to altered protein function as observed with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and caspase 3 [40, 41]. If two neighboring reduced cysteine residues (dithiol) are reactive, the pair can be reversibly oxidized to a form a cysteine disulfide bridge, as with TXN. The gaseous molecules NO and hydrogen sulfide (H_2S) also react with cysteine residues to produce modified derivatives that are functionally relevant. For example, *S*-nitrosylation of Cys118 in Ras activates the protein by stimulating guanine nucleotide exchange, while sulfhydration, or the addition of a sulfhydryl group from hydrogen sulfide gas to a cysteine thiol, has recently been demonstrated to augment the activity of GAPDH [42, 43]. Proteins often contain multiple cysteine residues that can be reversibly modified (e.g., dithiol-disulfide, *S*-glutathionylation, *S*-nitrosylation, *S*-sulfhydration, etc.) to affect protein function and/or interactions.

From a systems perspective, cellular redox status is best understood in terms of redox potential. The redox potential (E_h), or electron motive force, for an oxidation/reduction couple depends on the inherent tendency of the molecule to accept/donate electrons relative to the standard hydrogen electrode and the concentrations of the acceptor and donor (oxidized and reduced species of the couple). In biological systems the redox potential of the major thiol/disulfide couples (GSH and TXN) is maintained at stable nonequilibrium conditions, or in other words, the mean E_h of the GSH and TXN couples in subcellular compartments is nonzero. These redox couples, along with the Cys/CySS (cysteine/cystine) couple and protein Cys residues involved in redox signaling, are in equilibrium neither with each other nor with the NADPH/$NADP^+$ couple. In addition to nonequilibrated redox potentials between couples within a subcellular compartment, redox potential is also not equilibrated across subcellular and extracellular compartments. The mitochondria and nucleus tend to be highly reduced (redox potential farther from zero),

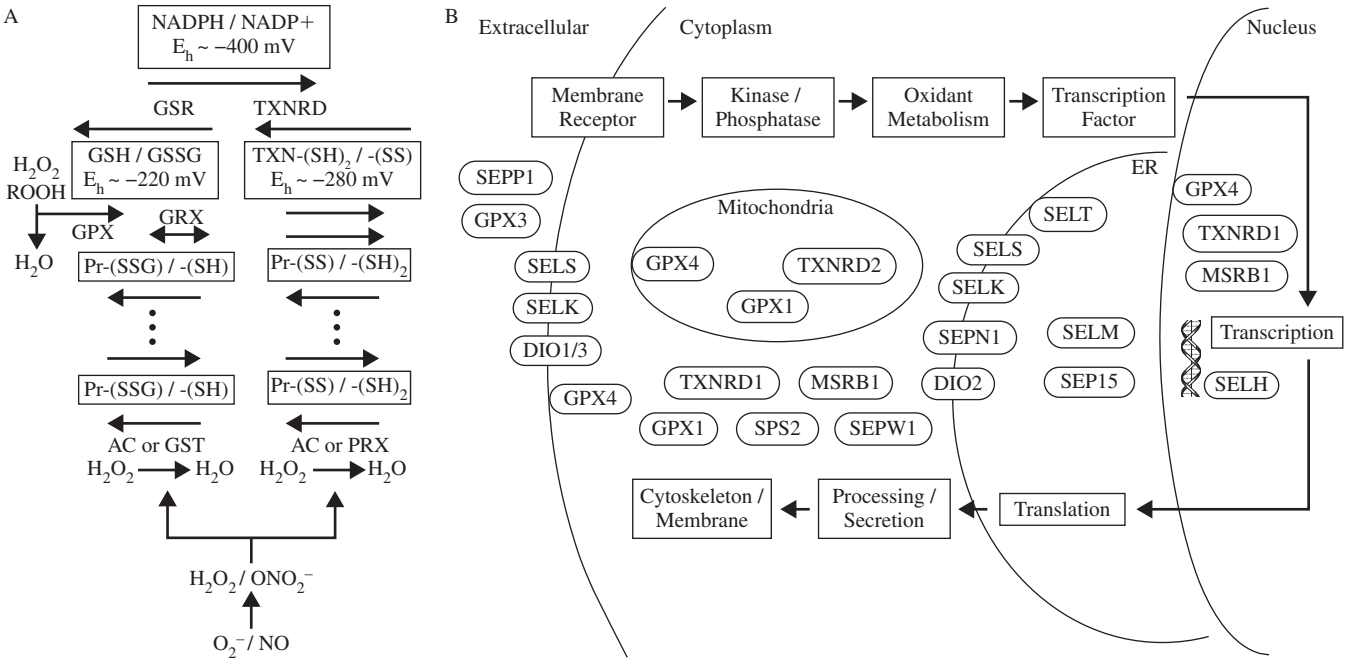

Fig. 14.1 Selenoproteins modulate redox control networks and cellular signaling pathways. (A) Thioredoxin (TXN) and glutathione (GSH) are central hubs of redox control networks. Both electron transport pathways ultimately rely on NADPH as an electron donor. GSH reductase (GSR) and TXN reductase (TXNRD) reduce the GSH and TXN pools, respectively, to maintain a nonequilibrium steady-state redox potential (E_h). E_h values shown are approximate cytoplasmic redox potentials and differ between subcellular compartments. Hypothetical GS-ylation of a cysteine sulfur switch [Pr-(SH)] can be autocatalytic (AC) or catalyzed by GSH-transferase (GST) enzymes. Glutaredoxin (GRX) enzymes can theoretically catalyze GS-ylation or removal of the GSH moiety depending on the local concentration of GSSG and GSH. GPX selenoenzymes oxidize the GSH pool to GSSG in the presence of organic and inorganic peroxides. A similar pathway is drawn for the TXN network sulfur switches, which are depicted here as protein dithiols [Pr-$(SH)_2$]. Vertebrate TXNRD enzymes are selenoproteins, making the entire TXN network dependent on selenium. The TXN-dependent switches can be autocatalytic (AC) or substrates for catalysts that are oxidized by prooxidants such as peroxiredoxins (PRX) and methionine sulfoxide reductases (not shown). Thus oxidation of a switch is determined by its proximity to the source of oxidant generation or activity of the catalyst. The ellipsis between hypothetical proteins illustrates that series of redox switches can be arranged in parallel circuits that depend on GSH or TXN, and ultimately NADPH. (B) Selenoproteins and redox elements affect regulation of cell signaling. An idealized receptor-mediated signaling cascade involves several steps that are redox sensitive and may be modulated by selenoproteins. Conserved Cys-rich domains of receptors allow for redox regulation, e.g., the EGF and NMDA receptors are known to be regulated by extracellular redox-dependent mechanisms [115, 116]. An array of kinases and phosphatases are redox sensitive and fine-tune intracellular signaling responses [117]. Downstream generation and clearance of oxidants within localized domains (redox-active endosome, mitochondrion, etc.) can differentially propagate and terminate signaling cascades [118]. Translocation of transcription factors and transcriptional activation are under GSH- and TXN-mediated control, while TXN interacts with TXN-like domains of elongation initiation factors to regulate translation [15]. Secretory and transmembrane proteins undergo extensive redox-dependent processing and trafficking in the secretory pathway [119]. Finally, redox-active cysteine residues in receptors, cell adhesion molecules, and cytoskeletal proteins determine receptor function, cytoskeletal structure, and cell surface interactions [120, 121]. Compartmentalization of selenoproteins may provide a functional mechanism for differential control of signaling steps within subcellular regions. Note that six human selenoproteins (GPX2, GPX6, TXNRD3, SELI, SELO, and SELV) have been omitted because of uncertain subcellular and/or restricted tissue distribution.

while the extracellular compartment is relatively oxidized (redox potential closer to zero), with the cytoplasm, endoplasmic reticulum, and lysosomes displaying intermediate values. The steady-state E_h values for thiol-disulfide couples range from −400 mV for NADPH/$NADP^+$ to −60 mV for plasma Cys/CySS [44].

With an intracellular reduced GSH concentration of 1 mM, oxidation of just 18 μM GSH to GSSG will lower the GSH/GSSG redox potential (ΔE_h) by ~60 mV. This relatively shallow redox potential gradient is sufficient to distinguish between proliferating and apoptotic cells, and can theoretically drive a 100-fold change in the dithiol-to-disulfide ratio in proteins with a reactive dithiol motif [45]. Therefore the current through a redox circuit need only be a fraction of the total electron transfer in cells, provided that spatial or catalytic

mechanisms are able to control reaction rates. Under aerobic conditions peroxide and oxygen are always present in cells; therefore coupling of electron transfer to peroxidase (e.g., GPX) or oxidase reactions can provide an additional energetic driving force to maintain function of low-current redox control circuits. Kinetic and spatial insulation of reactive thiol couples allows biosystems to be highly responsive and dynamic, and concurrently provides specificity because noncatalyzed oxidation-reduction and thiol-disulfide exchange reactions are slow in the cellular environment [44].

S-glutathionylated Sec and diselenide bridges have not been described in vivo, but transient and stable selenenyl-sulfide linkages are observed in selenoproteins as either active intermediates or structural features, respectively. For example, the reaction mechanisms for GPX and TXNRD enzymes involve a transient mixed selenenyl-sulfide bond, while rat selenoprotein P (SEPP1) has two such linkages that appear to be a structural feature [32, 46]. Transient nitrosylation and sulfhydration of Sec is thermodynamically possible, but its existence is speculative at present. Free Sec is virtually absent from cells, making a selenocysteine/selenocystine couple unlikely, but selenoproteins are present and could be maintained in a nonequilibrium steady state favoring the reduced form. Kinetic (reaction rate and substrate specificity) and spatial (subcellular and tissue distribution) insulation of the selenoproteins has been described; however, whether selenoproteins represent kinetically *limiting* sites of redox control remains an important question. The standard reduction potential (E_0) of selenoproteins is unexplored, and determining whether the mean E_0 of selenoproteins, particularly the TXN-like selenoproteins, is maintained in a nonequilibrium steady state will help determine whether they are central redox control points. Given the known kinetic advantages of the Sec amino acid compared to cysteine in catalysis, as well as the pivotal positions of GPX and TXNRD selenoproteins in respect to redox circuitry (Fig. 14.1A), it seems possible that one or more selenoproteins could be identified as kinetically limiting redox control points that are rapidly disrupted during oxidative stress. Additionally, several selenoproteins have unknown function, but, given the unique reactivity of Sec, they are well suited to transduce oxidant signals by formation of disulfide bonds in downstream targets even in the reducing cellular environment.

14.4 SELENOPROTEINS IN VERTEBRATE SIGNALING

Specific selenoproteins function at the intersection of cellular and organism metabolism by modulating insulin and thyroid hormone signaling. The iodothyronine deiodinases (DIO) function in activation and deactivation of thyroid hormone and were the second family of enzymes determined to be Sec-containing selenoproteins in the early 1990s [47]. Thyroid hormone metabolism at the level of both production in the thyroid and local hormone activity in the periphery is reliant on the DIO family of selenoenzymes. Most vertebrates have three DIO enzymes that can deiodinate thyroid hormones to control local availability. These integral membrane protein enzymes are thiol-requiring oxidoreductases that remove iodine atoms from the aromatic rings of thyroxine (T_4), triiodothyronine (T_3), and reverse triiodothyronine (rT_3) [48]. DIO1 is a plasma membrane protein found mainly in cells of the liver and kidney, is capable of deiodinating both the inner and outer rings, and produces most of the circulating T_3. DIO2 is found in the endoplasmic reticulum of cells in several tissues including the thyroid, heart, skeletal muscle, fat, and the central nervous system and selectively removes the outer ring iodine, making it the primary tissue activator of thyroid hormone by converting T_4 to T_3 DIO3 is also a plasma membrane protein; however, it is mainly found in fetal tissue and the placenta, selectively removes the inner ring iodine, and thus contributes to thyroid hormone inactivation.

A specific role for a selenoprotein in redox regulation of insulin signaling was established when it was found that overexpression of GPX1 causes hyperinsulinemia and insulin resistance in mice [49]. Moreover, genetic deletion of GPX1 promotes glucose tolerance and insulin sensitivity in mice on a high-fat diet by enhancement of insulin-induced PI3K/Akt signaling [50]. Dietary studies in humans have further suggested that supranutritional levels of Se are associated with type 2 diabetes, while animal studies confirm that both excessive dietary Se and GPX1 overexpression lead to hyperinsulinemia and insulin resistance [51–54]. Peroxide-induced oxidation of PTEN and *S*-glutathionylation of protein tyrosine phosphatase 1B are affected by GPX1 activity, which thereby modulates insulin receptor activation and insulin resistance [55].

Selenoprotein P (SEPP1) is a unique selenoprotein that contains multiple Sec residues and is also implicated in insulin resistance. Diabetic patients display an increase in hepatic SEPP1 mRNA and serum SEPP1 protein, and purified SEPP1 administered to mice is able to induce insulin resistance and glucose intolerance. Furthermore, knockdown or knockout of SEPP1 in mice improves glucose tolerance and insulin sensitivity, and SEPP1 knockout mice are protected against glucose intolerance and insulin resistance even when on an obesity-inducing diet [56]. Since SEPP1 expression can dictate expression of other selenoproteins including GPX1, its effect on insulin resistance may be direct or indirect. Primate and rodent

SEPP1 contains up to 10 Sec residues, while as many as 17 Sec residues are present in zebrafish. SEPP1 is the most abundant selenoprotein in blood and accounts for as much as 65% of plasma Se in rats [57]. Aside from the multiple Sec residues and high Se content, SEPP1 is also distinct in that it is one of only two extracellular selenoproteins (the other being GPX3 mentioned above), and it appears to be a vertebrate adaptation as it has not been found in the genome of *Caenorhabditis elegans* or *Drosophila melanogaster*. SEPP1 is abundantly produced by the liver and secreted into blood; however, local production and secretion in nearly all tissue systems has been described [58]. Bodily transport of Se to extrahepatic tissues, particularly the brain, testes, and kidneys, appears to be facilitated by receptor-mediated uptake of SEPP1 by the low-density lipoprotein receptor family members ApoER2 (LRP8) and Megalin (LRP2) [59]. The abundant Sec residues of SEPP1 are divided into two regions, with the bulk being located in the C-terminal domain that is required for the Se transport function. SEPP1 also contains an N-terminus Cys-X-X-Sec motif and catalyzes the reduction of lipid hydroperoxides in vitro, utilizing TXN as a cofactor [30, 60]. In addition to a Se transport function and peroxidase activity, SEPP1 exhibits pH-dependent heparin binding and heavy metal binding that likely also function in redox-dependent processes.

The examples of GPX1, SEPP1, and the DIO enzymes modulating insulin and thyroid hormone signaling highlight the fact that vertebrate metabolism is tightly integrated with selenoprotein function. This connection between Se and metabolism in multicellular organisms suggests that cell-autonomous regulation of redox systems and signaling may similarly depend on one or more selenoproteins. The regulatory control of cellular redox signaling by Selenoprotein W is discussed next as an example, bearing in mind that several selenoproteins with uncertain functions could similarly have a role in regulating target protein oxidation state.

Selenoprotein W (SEPW1) was purified in the early 1990s but putatively identified much earlier because of its absence in Se-deficient lambs suffering a myopathy called white muscle disease [61]. Mammalian SEPW1 is a highly conserved cytosolic protein of just 87 amino acids, and SEPW1 orthologs are among the most widely distributed selenoproteins in all species including prokaryotes [6, 62]. The expression level of SEPW1 in vertebrates is very sensitive to dietary Se intake as well as the expression level of SEPP1 [63–65]. Abundant SEPW1 expression is observed in muscle, and SEPW1 transcription during myocyte differentiation is maintained by binding of the myogenic transcription factor MyoD to the SEPW1 promoter [66]. A putative metal-response element in the promoter of the SEPW1 gene was probed in vitro with the use of a luciferase reporter fusion construct, and luciferase specific activity was found to be stimulated by copper and zinc but not cadmium [67]. Although a bona fide enzymatic activity has not been attributed to SEPW1, the presence of a Cys-X-X-Sec motif in a thioredoxin-like fold may indicate thioredoxin-like redox activity [31].

Recently, SEPW1 was shown to pull down and coimmunoprecipitate with the β and γ isoforms of 14-3-3 protein. This interaction was further confirmed by NMR spectroscopy and extended to identify three loops of SEPW1 that interact with 14-3-3 proteins [68]. 14-3-3 β and γ proteins are scaffolding proteins derived from the YWHAB and YWHAG genes, respectively, and bind a diverse assortment of proteins including kinases, phosphatases, and receptors. In this way 14-3-3 proteins coordinate molecular interactions and participate in cell cycle regulation, metabolism, apoptosis, protein trafficking, and gene transcription [69]. A computational study of SEPW1/14-3-3 interaction suggests that a conserved cysteine of 14-3-3 β and γ (Cys191 and Cys195, respectively) can be reversibly oxidized, with SEPW1 acting as a reducing agent [70]. The oxidized cysteine sulfenic acid of 14-3-3 can putatively react with Sec of SEPW1, producing a mixed complex. Subsequently, the formation of an intramolecular selenenyl-sulfide within SEPW1 would result in 14-3-3 being fully reduced. Oxidized SEPW1 can then migrate away and likely be reduced to its parent state by GSH, which is supported by evidence that a SEPW1 cysteine residue conserved from rodents to primates can be *S*-glutathionylated [71, 72]. Redox regulation of 14-3-3 proteins by SEPW1 could serve several cellular functions, but an intriguing possibility is presented by the in vitro finding that SEPW1 expression is regulated by the cell cycle and knockdown of SEPW1 induces cell cycle arrest [73]. Therefore SEPW1, through redox regulation of 14-3-3 proteins, may coordinate Se availability and oxidative burden with cellular proliferation, differentiation, and death. This redox-regulated functionality may serve as the basis for the association of SEPW1 with myopathies in livestock and multiple myeloma in humans [74].

The example of SEPW1 is meant to highlight the fact that selenoproteins with unknown roles can impact the cellular response to environmental changes, particularly in relation to growth and stress. In the following section a brief summary of what is known of the remaining selenoproteins is presented, with the biological functions described possibly owing to the activity of the selenoproteins in undefined redox circuits.

14.5 THE SELENOPROTEIN FAMILY

The human genome codes for 25 selenoproteins, most of which have been identified recently by bioinformatics

approaches looking for SECIS elements downstream of in-frame UGA codons [23]. The various members display wide subcellular and tissue distribution, and several are known to have multiple transcript variants and protein isoforms. Selenophosphate-synthetase 2 (SPS2) is a eukaryotic selenoprotein that is required for the synthesis of all selenoproteins including itself. Selenophosphate is generated by SPS2 in the presence of selenide and ATP [75]. A related protein called SPS1 contains a cysteine residue in place of Sec, but its involvement in Sec and selenoprotein biosynthesis is uncertain [76]. It has been suggested that SPS2 assimilates selenite, whereas SPS1 recycles Sec in a Se-salvage pathway [77]. Intriguingly SPS2, but not SPS1, is required for selenoprotein synthesis in NIH3T3 mouse fibroblasts [78]. However, an in vivo requirement of SPS2 and specificity of SPS1 and SPS2 with different Se substrates have not been reported.

Oxidative stress can trigger dysfunction of organelles including the mitochondria, nucleus, and endoplasmic reticulum. A subset of selenoproteins is observed in mitochondrion to combat against electron leak during oxidative respiration and phosphorylation. Mitochondrion-specific isoforms of GPX1, GPX4, and TXNRD2 regulate peroxide metabolism and oxidative tone within this organelle. The selenoproteins GPX4, TXNRD1, MSRB1, and SELH have been shown to exhibit varying degrees of nuclear localization (Fig. 14.1B). Apart from SELH, the other three selenoproteins are presumably involved in reduction of lipid peroxides, oxidized TXN, and sulfoxidized methionine residues within the nuclear envelope.

SELH is the only DNA-binding selenoprotein described and has a role in regulation of gene expression. Similar to SEPW1, Selenoprotein H (SELH) is a small selenoprotein that is highly expressed during development and is sensitive to dietary Se intake [79, 80]. Like several selenoproteins, it contains a Cys-X-X-Sec sequence within a thioredoxin-like fold, but unlike any other selenoprotein described to date, it is a DNA-binding protein of the AT-hook family. SELH is primarily located in the nucleus and is implicated in redox-sensitive transcription of genes whose products are involved in de novo glutathione synthesis and phase II detoxification [81]. Multiple metal-response elements are present in the SELH gene [82], and one group has confirmed in vivo that SELH mRNA and protein are upregulated under conditions of elevated copper in mouse liver [83]. Although it is a nuclear protein, mitochondrial biogenesis and function are also linked with SELH. Overexpression of SELH in a transformed neuronal cell line attenuates the UVB-induced increase of p53 protein and caspase-mediated apoptosis [84]. Additionally, SELH overexpression increases mitochondrial size, cytochrome c content, and expression of mitochondrial biogenesis proteins while boosting respiration [85]. Collectively these findings suggest that SELH is a Se- and metal-regulated selenoprotein that is able to transduce oxidant signals by modulating gene expression in conjunction with other redox-sensitive transcription factors. Further investigation is warranted to determine whether SELH modifies cysteine *S*-glutathionylation or disulfide formation in target proteins such as p53 to regulate gene expression.

The endoplasmic reticulum (ER) regulates the synthesis, folding, and transport of proteins and additionally constitutes the main intracellular store for calcium ions, which are integral in cell signaling. Seven selenoproteins are enriched in the ER (Fig. 14.1B), and some are postulated to have a role in protein folding and ER calcium handling, since oxidative mechanisms within the ER are known to regulate these processes [86]. The ER is a relatively oxidizing environment compared to other intracellular organelles and contains oxidase enzymes to facilitate the formation of disulfide bonds in proteins destined for export. Simultaneously, GSH and TXN system components are transported into the ER, providing both oxidation and reduction mechanisms for dynamic redox regulation associated with protein processing and secretion. Redox state affects calcium homeostasis by modulating ER calcium channels and chaperones, and oxidative stress and ER stress are intimately related in signaling for apoptosis [87]. Similar to the ER, the secretory and endosomal/lysosomal pathways are also more oxidized than other subcellular compartments [88, 89]. Ligand binding to various receptors stimulates endocytosis of redox-active endosomes whose luminal redox activity directs spatiotemporally regulated signaling and prevents nonspecific redox reactions [90, 91]. Therefore redox-mediated processes are vital for secretory and endocytic function, and the ability of selenoproteins to transmit oxidative signals from reactive intermediates to disulfide bonds of target proteins may help to explain the enrichment of selenoproteins in the ER.

Selenoprotein T (SELT) is an ER- and Golgi-localized selenoprotein that is ubiquitously expressed from development through adulthood and shares some sequence similarity with SEPW1 and SELH including the thioredoxin-like fold containing a Cys-X-X-Sec motif [31]. Deficiency of SELT in murine fibroblasts causes an upregulation of SEPW1, in addition to altering cell adhesion and redox regulation [92]. A biological role for SELT in neuroendocrine secretion and calcium mobilization in vitro has also been presented. SELT was identified as a target gene of the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP), and manipulation of SELT expression altered PACAP-induced intracellular Ca^{2+} changes and growth hormone secretion [93].

Two of the more abundant and ubiquitous ER selenoproteins, Selenoprotein M (SELM) and 15-kDa selenoprotein (SEP15), are both 15-kDa proteins that share 31% sequence homology and have Cys-X-X-Sec and Cys-X-Sec redox motifs, respectively. SELM and SEP15 have thioredoxin-like tertiary structure and homology to protein disulfide isomerases that suggest oxidoreductase activity, but direct evidence in support of this notion is lacking [94]. SEP15 associates with UDP-glucose:glycoprotein glucosyltransferase (UGTR), a protein involved in protein conformation quality control, and has been suggested to facilitate proper protein folding [95]. A role for SELM in protein folding has been proposed, and recent evidence suggests that SELM may also be involved in regulating the flux of calcium ions. Overexpression of SELM in a neuronal cell line in vitro reduced peroxide-induced calcium influx, whereas knockdown of SELM increased the baseline intracellular calcium concentration [96].

Selenoprotein N (SEPN1) is a large (70 kDa) single-spanning transmembrane protein localized to the ER membrane with two known isoforms generated by alternative splicing of exon 3 [97]. Several congenital muscular dystrophy syndromes such as multiminicore disease, rigid spine muscular dystrophy, and desmin-related myopathy with Mallory body-like inclusions are directly associated with mutations in the SEPN1 gene and have been classified as SEPN1-related myopathies [98]. Interestingly, mutations in SEPN1 leading to congenital fiber type disproportion are associated with insulin resistance [99]. To date, SEPN1 is the only selenoprotein gene in which mutations are directly and causatively linked to human disease. An in vivo study using zebrafish determined that SEPN1 associates with ER/SR ryanodine receptors, and that this interaction is necessary for the calcium-induced release of calcium from intracellular stores [100]. Ryanodine receptor channels are homotetramers with several redox-regulated cysteine residues, and SEPN1 contains a Cys-Sec-Gly-Ser motif, suggesting that SEPN1 regulates ryanodine receptor-mediated calcium flux in muscle by redox-dependent signaling. Finally, SEPN1 is integral to the generation and/or maintenance of skeletal muscle satellite cells, which are an adult stem cell population involved in muscle growth and regeneration [101].

Selenoprotein K (SELK) and Selenoprotein S (SELS) are also predominantly ER-localized single-spanning transmembrane proteins; however, they are much smaller than SEPN1 and also show some localization to the plasma membrane [23, 102, 103]. Both are widely expressed in a variety of tissues and have been implicated in the cellular response to ER stress. Specifically, ER stress agents regulated the expression of SELK in HepG2 hepatoma cells, and knockdown of SELK exacerbated cell death when challenged with ER stress [104]. Genetic deletion of SELK in mice decreases receptor-mediated calcium flux in immune cells, impairs calcium-dependent immune cell function, and increases West Nile virus-induced lethality [105]. An interesting link between metabolism and inflammation is presented in the case of SELS, which was originally identified as a glucose-regulated protein in a rodent model of diabetes [106]. The relationship between SELS and type 2 diabetes was confirmed in humans, and there is evidence that SELS can be secreted from liver and identified in blood sera, where it associates with LDL [107, 108]. SELS is now known to also be regulated by inflammatory cytokines [109], and reciprocally, reduced expression of SELS, due to polymorphisms in the gene promoter, influences the levels of IL-1, TNF-α, and IL-6 [110]. SELS participates in removal of misfolded proteins from the ER lumen [103, 111] and was demonstrated to prevent ER stress and have antiapoptotic function in macrophages and astrocytes [112, 113].

Three selenoproteins remain largely unexplored, with very little published data currently available. The sequences of Selenoprotein I (SELI), Selenoprotein O (SELO), and Selenoprotein V (SELV) were identified in the human genome several years ago; however, almost no information is available on the cellular localizations or physiological functions of these selenoproteins. SELI mRNA is known to be expressed in several tissues, and it is a putative transmembrane protein hypothesized to function in phospholipid biosynthesis based on the presence of a CDP-alcohol phosphatidyltransferase motif that is conserved in phospholipid synthases [23, 114]. SELO is predicted to be a 669-amino acid selenoprotein containing a Cys-X-X-Sec motif, but experimental data demonstrating a redox function are unavailable [23]. SELV appears to be a testes-restricted protein with a predicted thioredoxin-like fold housing a Cys-X-X-Sec motif and also has some sequence homology with SEPW1, SELH, and SELT [23].

14.6 CONCLUSION AND FUTURE PERSPECTIVES

The implications of selenoproteins functioning as part of the circuits mediated by central couples for redox signaling are widespread. These circuits control networks that integrate and coordinate cellular processes through a series of parallel redox elements, and selenoproteins are positioned in key places to drastically affect circuit function. The GPX enzymes rapidly consume peroxide to limit the distance of locally generated oxidant signals, and in doing so impact the redox potential of the GSH/GSSG couple. TXNRD selenoproteins reduce TXN to

maintain the nonequilibrium steady-state redox potential of the couple and thus are the basis of a functionally diverse array of signaling processes mediated by TXN. Reducing sulfoxidized methionines is one such process that is partially realized by another selenoprotein, MSRB1. DIO enzymes and SEPP1 modulate vertebrate metabolism, while SEPW1 and SELH modulate cellular metabolism. At least four ER-localized selenoproteins (SELT, SELM, SEPN1, SELK) are involved in regulating intracellular calcium flux and consequent cellular signaling, and another two (SEP15, SELS) participate in protein folding. SPS2 is a selenoprotein that regulates synthesis of all selenoproteins including itself, while SELI, SELO, and SELV still have unknown functions. The interaction of selenoproteins with thiol-disulfide pools in humans highlights their importance in redox regulation and signaling, particularly under conditions of oxidative stress. Oxidative stress is associated with diverse disease processes, and redox-sensing selenoproteins may provide a mechanistic link between sources of oxidative stress and the fundamental cell signaling that contributes to disease pathology. Specific selenoproteins could optimize target protein function by modifying reversible reactions (i.e., oxidation, nitrosylation, acylation, sulfhydration or metal binding) that affect macromolecular structure, activity, interactions, and trafficking. Elucidation of specific targets and circuits for selenoprotein activity will create new strategies for targeting relevant redox systems that go awry during disease pathology. Almost two hundred years since Berzelius named Se after the Greek moon goddess Selene, this unique trace element is poised to illuminate a world of redox systems biology.

REFERENCES

1. Koller LD and Exon JH. The two faces of selenium—deficiency and toxicity—are similar in animals and man. *Can J Vet Res* 1986; 50(3): 297–306.
2. Monsen ER. Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids. *J Am Diet Assoc* 2000; 100(6): 637–40.
3. Hondal RJ and Ruggles EL. Differing views of the role of selenium in thioredoxin reductase. *Amino Acids* 2011; 41(1): 73–89.
4. Berry MJ, Banu L, Chen YY, Mandel SJ, Kieffer JD, Harney JW, and Larsen PR. Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3′ untranslated region. *Nature* 1991; 353(6341): 273–6.
5. Wilting R, Schorling S, Persson BC, and Bock A. Selenoprotein synthesis in archaea: identification of an mRNA element of Methanococcus jannaschii probably directing selenocysteine insertion. *J Mol Biol* 1997; 266(4): 637–41.
6. Kryukov GV and Gladyshev VN. The prokaryotic selenoproteome. *EMBO Rep* 2004; 5(5): 538–43.
7. Papp LV, Lu J, Holmgren A, and Khanna KK. From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxid Redox Signal* 2007; 9(7): 775–806.
8. Palioura S, Sherrer RL, Steitz TA, Soll D, and Simonovic M. The human SepSecS–tRNASec complex reveals the mechanism of selenocysteine formation. *Science* 2009; 325(5938): 321–5.
9. Esaki N, Nakamura T, Tanaka H, and Soda K. Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. Mammalian distribution and purification and properties of pig liver enzyme. *J Biol Chem* 1982; 257(8): 4386–91.
10. Lacourciere GM, Mihara H, Kurihara T, Esaki N, and Stadtman TC. Escherichia coli NifS-like proteins provide selenium in the pathway for the biosynthesis of selenophosphate. *J Biol Chem* 2000; 275(31): 23769–73.
11. Mihara H, Kurihara T, Watanabe T, Yoshimura T, and Esaki N. cDNA cloning, purification, and characterization of mouse liver selenocysteine lyase. Candidate for selenium delivery protein in selenoprotein synthesis. *J Biol Chem* 2000; 275(9): 6195–200.
12. Papp LV, Lu J, Striebel F, Kennedy D, Holmgren A, and Khanna KK. The redox state of SECIS binding protein 2 controls its localization and selenocysteine incorporation function. *Mol Cell Biol* 2006; 26(13): 4895–910.
13. Lobanov AV, Hatfield DL, and Gladyshev VN. Eukaryotic selenoproteins and selenoproteomes. *Biochim Biophys Acta* 2009; 1790(11): 1424–8.
14. Jones DP. Redefining oxidative stress. *Antioxid Redox Signal* 2006; 8(9–10): 1865–79.
15. Jones DP. Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol* 2008; 295(4): C849–68.
16. Farah ME and Amberg DC. Conserved actin cysteine residues are oxidative stress sensors that can regulate cell death in yeast. *Mol Biol Cell* 2007; 18(4): 1359–65.
17. Moriarty-Craige SE and Jones DP. Extracellular thiols and thiol/disulfide redox in metabolism. *Annu Rev Nutr* 2004; 24: 481–509.
18. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, and Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 1973; 179(73): 588–90.
19. Esworthy RS, Ho YS, and Chu FF. The Gpx1 gene encodes mitochondrial glutathione peroxidase in the mouse liver. *Arch Biochem Biophys* 1997; 340(1): 59–63.
20. Hawkes WC, Wilhelmsen EC, and Tappel AL. Abundance and tissue distribution of selenocysteine-containing proteins in the rat. *J Inorg Biochem* 1985; 23(2): 77–92.
21. Chu FF, Esworthy RS, Ho YS, Bermeister M, Swiderek K, and Elliott RW. Expression and chromosomal mapping of mouse Gpx2 gene encoding the gastrointestinal form of glutathione peroxidase, GPX-GI. *Biomed Environ Sci* 1997; 10(2–3): 156–62.

22. Olson GE, Whitin JC, Hill KE, Winfrey VP, Motley AK, Austin LM, Deal J, Cohen HJ, and Burk RF. Extracellular glutathione peroxidase (Gpx3) binds specifically to basement membranes of mouse renal cortex tubule cells. *Am J Physiol Renal Physiol* 2010; 298: F1244–F1253.
23. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, and Gladyshev VN. Characterization of mammalian selenoproteomes. *Science* 2003; 300(5624): 1439–43.
24. Ufer C, Wang CC, Fahling M, Schiebel H, Thiele BJ, Billett EE, Kuhn H, and Borchert A. Translational regulation of glutathione peroxidase 4 expression through guanine-rich sequence-binding factor 1 is essential for embryonic brain development. *Genes Dev* 2008; 22(13): 1838–50.
25. Seiler A, Schneider M, Forster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Radmark O, Wurst W, Bornkamm GW, Schweizer U, and Conrad M. Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab* 2008; 8(3): 237–48.
26. Yant LJ, Ran Q, Rao L, Van Remmen H, Shibatani T, Belter JG, Motta L, Richardson A, and Prolla TA. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic Biol Med* 2003; 34(4): 496–502.
27. Arner ES and Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 2000; 267(20): 6102–9.
28. Zhong L, Arner ES, and Holmgren A. Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. *Proc Natl Acad Sci USA* 2000; 97(11): 5854–9.
29. Bjornstedt M, Xue J, Huang W, Akesson B, and Holmgren A. The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. *J Biol Chem* 1994; 269(47): 29382–4.
30. Takebe G, Yarimizu J, Saito Y, Hayashi T, Nakamura H, Yodoi J, Nagasawa S, and Takahashi K. A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein P. *J Biol Chem* 2002; 277(43): 41254–8.
31. Dikiy A, Novoselov SV, Fomenko DE, Sengupta A, Carlson BA, Cerny RL, Ginalski K, Grishin NV, Hatfield DL, and Gladyshev VN. SelT, SelW, SelH, and Rdx12: genomics and molecular insights into the functions of selenoproteins of a novel thioredoxin-like family. *Biochemistry* 2007; 46(23): 6871–82.
32. Hatfield DL, *Selenium: Its Molecular Biology and Role in Human Health*. 2nd ed. New York: Springer. 2006, xxii, 419 p.
33. Conrad M, Jakupoglu C, Moreno SG, Lippl S, Banjac A, Schneider M, Beck H, Hatzopoulos AK, Just U, Sinowatz F, Schmahl W, Chien KR, Wurst W, Bornkamm GW, and Brielmeier M. Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. *Mol Cell Biol* 2004; 24(21): 9414–23.
34. Jakupoglu C, Przemeck GK, Schneider M, Moreno SG, Mayr N, Hatzopoulos AK, de Angelis MH, Wurst W, Bornkamm GW, Brielmeier M, and Conrad M. Cytoplasmic thioredoxin reductase is essential for embryogenesis but dispensable for cardiac development. *Mol Cell Biol* 2005; 25(5): 1980–8.
35. Levine RL, Moskovitz J, and Stadtman ER. Oxidation of methionine in proteins: roles in antioxidant defense and cellular regulation. *IUBMB Life* 2000; 50(4–5): 301–7.
36. Agbas A and Moskovitz J. The role of methionine oxidation/reduction in the regulation of immune response. *Curr Signal Transduct Ther* 2009; 4(1): 46–50.
37. Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham AJ, Weiss RM, Spitz DR, Shea MA, Colbran RJ, Mohler PJ, and Anderson ME. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell* 2008; 133(3): 462–74.
38. Kim HY and Gladyshev VN. Methionine sulfoxide reduction in mammals: characterization of methionine-R-sulfoxide reductases. *Mol Biol Cell* 2004; 15(3): 1055–64.
39. Sagher D, Brunell D, Hejtmancik JF, Kantorow M, Brot N, and Weissbach H. Thionein can serve as a reducing agent for the methionine sulfoxide reductases. *Proc Natl Acad Sci USA* 2006; 103(23): 8656–61.
40. Sykes MC, Mowbray AL, and Jo H. Reversible glutathiolation of caspase-3 by glutaredoxin as a novel redox signaling mechanism in tumor necrosis factor-alpha-induced cell death. *Circ Res* 2007; 100(2): 152–4.
41. Mohr S, Hallak H, de Boitte A, Lapetina EG, and Brune B. Nitric oxide-induced S-glutathionylation and inactivation of glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* 1999; 274(14): 9427–30.
42. Lander HM, Milbank AJ, Tauras JM, Hajjar DP, Hempstead BL, Schwartz GD, Kraemer RT, Mirza UA, Chait BT, Burk SC, and Quilliam LA. Redox regulation of cell signalling. *Nature* 1996; 381(6581): 380–1.
43. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, and Snyder SH. H2S signals through protein S-sulfhydration. *Sci Signal* 2009; 2(96): ra72.
44. Kemp M, Go YM, and Jones DP. Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. *Free Radic Biol Med* 2008; 44(6): 921–37.
45. Schafer FQ and Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 2001; 30(11): 1191–212.
46. Ma S, Hill KE, Burk RF, and Caprioli RM. Mass spectrometric determination of selenenylsulfide linkages in rat selenoprotein P. *J Mass Spectrom* 2005; 40(3): 400–4.
47. Berry MJ, Banu L, and Larsen PR. Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* 1991; 349(6308): 438–40.

48. Bianco AC and Kim BW. Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest* 2006; 116(10): 2571–9.
49. McClung JP, Roneker CA, Mu W, Lisk DJ, Langlais P, Liu F, and Lei XG. Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. *Proc Natl Acad Sci USA* 2004; 101(24): 8852–7.
50. Loh K, Deng H, Fukushima A, Cai X, Boivin B, Galic S, Bruce C, Shields BJ, Skiba B, Ooms LM, Stepto N, Wu B, Mitchell CA, Tonks NK, Watt MJ, Febbraio MA, Crack PJ, Andrikopoulos S, and Tiganis T. Reactive oxygen species enhance insulin sensitivity. *Cell Metab* 2009; 10(4): 260–72.
51. Labunskyy VM, Lee BC, Handy DE, Loscalzo J, Hatfield DL, and Gladyshev VN. Both maximal expression of selenoproteins and selenoprotein deficiency can promote development of type 2 diabetes-like phenotype in mice. *Antioxid Redox Signal* 2011; 14(12): 2327–36.
52. Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, and Reid ME. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. *Ann Intern Med* 2007; 147(4): 217–23.
53. Wang XD, Vatamaniuk MZ, Wang SK, Roneker CA, Simmons RA, and Lei XG. Molecular mechanisms for hyperinsulinaemia induced by overproduction of selenium-dependent glutathione peroxidase-1 in mice. *Diabetologia* 2008; 51(8): 1515–24.
54. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, Parsons JK, Bearden JD 3rd, Crawford ED, Goodman GE, Claudio J, Winquist E, Cook ED, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella RM, Albanes D, Taylor PR, Probstfield JL, Jagpal TJ, Crowley JJ, Meyskens FL Jr., Baker LH, and Coltman CA, Jr. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2009; 301(1): 39–51.
55. Mueller AS, Bosse AC, Most E, Klomann SD, Schneider S, and Pallauf J. Regulation of the insulin antagonistic protein tyrosine phosphatase 1B by dietary Se studied in growing rats. *J Nutr Biochem* 2009; 20(4): 235–47.
56. Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, Ishikura K, Ando H, Takeshita Y, Ota T, Sakurai M, Yamashita T, Mizukoshi E, Yamashita T, Honda M, Miyamoto K, Kubota T, Kubota N, Kadowaki T, Kim HJ, Lee IK, Minokoshi Y, Saito Y, Takahashi K, Yamada Y, Takakura N, and Kaneko S. A liver-derived secretory protein, selenoprotein P, causes insulin resistance. *Cell Metab* 2010; 12(5): 483–95.
57. Burk RF and Hill KE. Selenoprotein P. A selenium-rich extracellular glycoprotein. *J Nutr* 1994; 124(10): 1891–7.
58. Burk RF and Hill KE. Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annu Rev Nutr* 2005; 25: 215–35.
59. Burk RF and Hill KE. Selenoprotein P-expression, functions, and roles in mammals. *Biochim Biophys Acta* 2009; 1790(11): 1441–7.
60. Saito Y, Hayashi T, Tanaka A, Watanabe Y, Suzuki M, Saito E, and Takahashi K. Selenoprotein P in human plasma as an extracellular phospholipid hydroperoxide glutathione peroxidase. Isolation and enzymatic characterization of human selenoprotein *J Biol Chem* 1999; 274(5): 2866–71.
61. Vendeland SC, Beilstein MA, Chen CL, Jensen ON, Barofsky E, and Whanger PD. Purification and properties of selenoprotein W from rat muscle. *J Biol Chem* 1993; 268(23): 17103–7.
62. Zhang Y, Fomenko DE, and Gladyshev VN. The microbial selenoproteome of the Sargasso Sea. *Genome Biol* 2005; 6(4): R37.
63. Hoffmann PR, Hoge SC, Li PA, Hoffmann FW, Hashimoto AC, and Berry MJ. The selenoproteome exhibits widely varying, tissue-specific dependence on selenoprotein P for selenium supply. *Nucleic Acids Res* 2007; 35(12): 3963–73.
64. Vendeland SC, Beilstein MA, Yeh JY, Ream W, and Whanger PD. Rat skeletal muscle selenoprotein W: cDNA clone and mRNA modulation by dietary selenium. *Proc Natl Acad Sci USA* 1995; 92(19): 8749–53.
65. Yeh JY, Beilstein MA, Andrews JS, and Whanger PD. Tissue distribution and influence of selenium status on levels of selenoprotein W. *FASEB J* 1995; 9(5): 392–6.
66. Noh OJ, Park YH, Chung YW, and Kim IY. Transcriptional regulation of selenoprotein W by MyoD during early skeletal muscle differentiation. *J Biol Chem* 2010; 285(52): 40496–507.
67. Amantana A, Vorachek WR, Butler JA, Costa ND, and Whanger PD. Effect of copper, zinc and cadmium on the promoter of selenoprotein W in glial and myoblast cells. *J Inorg Biochem* 2002; 91(2): 356–62.
68. Aachmann FL, Fomenko DE, Soragni A, Gladyshev VN, and Dikiy A. Solution structure of selenoprotein W and NMR analysis of its interaction with 14-3-3 proteins. *J Biol Chem* 2007; 282(51): 37036–44.
69. Fu H, Subramanian RR, and Masters SC. 14-3-3 proteins: structure, function, and regulation. *Annu Rev Pharmacol Toxicol* 2000; 40: 617–47.
70. Musiani F, Ciurli S, and Dikiy A. The interaction of selenoprotein W with 14-3-3 proteins: a computational approach. *J Proteome Res* 2011; 10(3): 968–76.
71. Beilstein MA, Vendeland SC, Barofsky E, Jensen ON, and Whanger PD. Selenoprotein W of rat muscle binds glutathione and an unknown small molecular weight moiety. *J Inorg Biochem* 1996; 61(2): 117–24.
72. Gu QP, Beilstein MA, Barofsky E, Ream W, and Whanger PD. Purification, characterization, and glutathione binding to selenoprotein W from monkey muscle. *Arch Biochem Biophys* 1999; 361(1): 25–33.
73. Hawkes WC, Wang TT, Alkan Z, Richter BD, and Dawson K. Selenoprotein W modulates control of cell cycle entry. *Biol Trace Elem Res* 2009; 131(3): 229–44.

74. Ria R, Todoerti K, Berardi S, Coluccia AM, De Luisi A, Mattioli M, Ronchetti D, Morabito F, Guarini A, Petrucci MT, Dammacco F, Ribatti D, Neri A, and Vacca A. Gene expression profiling of bone marrow endothelial cells in patients with multiple myeloma. *Clin Cancer Res* 2009; 15(17): 5369–78.
75. Guimaraes MJ, Peterson D, Vicari A, Cocks BG, Copeland NG, Gilbert DJ, Jenkins NA, Ferrick DA, Kastelein RA, Bazan JF, and Zlotnik A. Identification of a novel selD homolog from eukaryotes, bacteria, and archaea: is there an autoregulatory mechanism in selenocysteine metabolism? *Proc Natl Acad Sci USA* 1996; 93(26): 15086–91.
76. Low SC, Harney JW, and Berry MJ. Cloning and functional characterization of human selenophosphate synthetase, an essential component of selenoprotein synthesis. *J Biol Chem* 1995; 270(37): 21659–64.
77. Tamura T, Yamamoto S, Takahata M, Sakaguchi H, Tanaka H, Stadtman TC, and Inagaki K. Selenophosphate synthetase genes from lung adenocarcinoma cells: Sps1 for recycling L-selenocysteine and Sps2 for selenite assimilation. *Proc Natl Acad Sci USA* 2004; 101(46): 16162–7.
78. Xu XM, Carlson BA, Irons R, Mix H, Zhong N, Gladyshev VN, and Hatfield DL. Selenophosphate synthetase 2 is essential for selenoprotein biosynthesis. *Biochem J* 2007; 404(1): 115–20.
79. Novoselov SV, Kryukov GV, Xu XM, Carlson BA, Hatfield DL, and Gladyshev VN. Selenoprotein H is a nucleolar thioredoxin-like protein with a unique expression pattern. *J Biol Chem* 2007; 282(16): 11960–8.
80. Kipp A, Banning A, van Schothorst EM, Meplan C, Schomburg L, Evelo C, Coort S, Gaj S, Keijer J, Hesketh J, and Brigelius-Flohe R. Four selenoproteins, protein biosynthesis, and Wnt signalling are particularly sensitive to limited selenium intake in mouse colon. *Mol Nutr Food Res* 2009; 53(12): 1561–72.
81. Panee J, Stoytcheva ZR, Liu W, and Berry MJ. Selenoprotein H is a redox-sensing high mobility group family DNA-binding protein that up-regulates genes involved in glutathione synthesis and phase II detoxification. *J Biol Chem* 2007; 282(33): 23759–65.
82. Stoytcheva ZR, Vladimirov V, Douet V, Stoychev I, and Berry MJ. Metal transcription factor-1 regulation via MREs in the transcribed regions of selenoprotein H and other metal-responsive genes. *Biochim Biophys Acta* 2010; 1800(3): 416–24.
83. Burkhead JL, Ralle M, Wilmarth P, David L, and Lutsenko S. Elevated copper remodels hepatic RNA processing machinery in the mouse model of Wilson's disease. *J Mol Biol* 2011; 406(1): 44–58.
84. Mendelev N, Witherspoon S, and Li PA. Overexpression of human selenoprotein H in neuronal cells ameliorates ultraviolet irradiation-induced damage by modulating cell signaling pathways. *Exp Neurol* 2009; 220(2): 328–34.
85. Mendelev N, Mehta SL, Witherspoon S, He Q, Sexton JZ, and Li PA. Upregulation of human selenoprotein H in murine hippocampal neuronal cells promotes mitochondrial biogenesis and functional performance. *Mitochondrion* 2011; 11(1): 76–82.
86. Shchedrina VA, Zhang Y, Labunskyy VM, Hatfield DL, and Gladyshev VN. Structure-function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. *Antioxid Redox Signal* 2010; 12(7): 839–49.
87. Gorlach A, Klappa P, and Kietzmann T.The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. *Antioxid Redox Signal* 2006; 8(9–10): 1391–418.
88. Hwang C, Sinskey AJ, and Lodish HF. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* 1992; 257(5076): 1496–502.
89. Austin CD, Wen X, Gazzard L, Nelson C, Scheller RH, and Scales SJ. Oxidizing potential of endosomes and lysosomes limits intracellular cleavage of disulfide-based antibody-drug conjugates. *Proc Natl Acad Sci USA* 2005; 102(50): 17987–92.
90. Li Q, Harraz MM, Zhou W, Zhang LN, Ding W, Zhang Y, Eggleston T, Yeaman C, Banfi B, and Engelhardt JF. Nox2 and Rac1 regulate H_2O_2-dependent recruitment of TRAF6 to endosomal interleukin-1 receptor complexes. *Mol Cell Biol* 2006; 26(1): 140–54.
91. Oakley FD, Abbott D, Li Q, and Engelhardt JF. Signaling components of redox active endosomes: the redoxosomes. *Antioxid Redox Signal* 2009; 11(6): 1313–33.
92. Sengupta A, Carlson BA, Labunskyy VM, Gladyshev VN, and Hatfield DL. Selenoprotein T deficiency alters cell adhesion and elevates selenoprotein W expression in murine fibroblast cells. *Biochem Cell Biol* 2009; 87(6): 953–61.
93. Grumolato L, Ghzili H, Montero-Hadjadje M, Gasman S, Lesage J, Tanguy Y, Galas L, Ait-Ali D, Leprince J, Guerineau NC, Elkahloun AG, Fournier A, Vieau D, Vaudry H, and Anouar Y. Selenoprotein T is a PACAP-regulated gene involved in intracellular Ca2+ mobilization and neuroendocrine secretion. *FASEB J* 2008; 22(6): 1756–68.
94. Ferguson AD, Labunskyy VM, Fomenko DE, Arac D, Chelliah Y, Amezcua CA, Rizo J, Gladyshev VN, and Deisenhofer J. NMR structures of the selenoproteins Sep15 and SelM reveal redox activity of a new thioredoxin-like family. *J Biol Chem* 2006; 281(6): 3536–43.
95. Labunskyy VM, Ferguson AD, Fomenko DE, Chelliah Y, Hatfield DL, and Gladyshev VN. A novel cysteine-rich domain of Sep15 mediates the interaction with UDP-glucose:glycoprotein glucosyltransferase. *J Biol Chem* 2005; 280(45): 37839–45.
96. Reeves MA, Bellinger FP, and Berry MJ. The neuroprotective functions of selenoprotein M and its role in cytosolic calcium regulation. *Antioxid Redox Signal* 2010; 12(7): 809–18.
97. Moghadaszadeh B, Petit N, Jaillard C, Brockington M, Roy SQ, Merlini L, Romero N, Estournet B, Desguerre I, Chaigne D, Muntoni F, Topaloglu H, and Guicheney P. Mutations in SEPN1 cause congenital muscular dystrophy

with spinal rigidity and restrictive respiratory syndrome. *Nat Genet* 2001; 29(1): 17–8.

98. Ferreiro A, Ceuterick-de Groote C, Marks JJ, Goemans N, Schreiber G, Hanefeld F, Fardeau M, Martin JJ, Goebel HH, Richard P, Guicheney P, and Bonnemann CG. Desmin-related myopathy with Mallory body-like inclusions is caused by mutations of the selenoprotein N gene. *Ann Neurol* 2004; 55(5): 676–86.
99. Clarke NF, Kidson W, Quijano-Roy S, Estournet B, Ferreiro A, Guicheney P, Manson JI, Kornberg AJ, Shield LK, and North KN. SEPN1: associated with congenital fiber-type disproportion and insulin resistance. *Ann Neurol* 2006; 59(3): 546–52.
100. Jurynec MJ, Xia R, Mackrill JJ, Gunther D, Crawford T, Flanigan KM, Abramson JJ, Howard MT, and Grunwald DJ. Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle. *Proc Natl Acad Sci USA* 2008; 105(34): 12485–90.
101. Castets P, Bertrand AT, Beuvin M, Ferry A, Le Grand F, Castets M, Chazot G, Rederstorff M, Krol A, Lescure A, Romero NB, Guicheney P, and Allamand V. Satellite cell loss and impaired muscle regeneration in selenoprotein N deficiency. *Hum Mol Genet* 2011; 20(4): 694–704.
102. Chen CL, Shim MS, Chung J, Yoo HS, Ha JM, Kim JY, Choi J, Zang SL, Hou X, Carlson BA, Hatfield DL, and Lee BJ. G-rich, a *Drosophila* selenoprotein, is a Golgi-resident type III membrane protein. *Biochem Biophys Res Commun* 2006; 348(4): 1296–301.
103. Ye Y, Shibata Y, Yun C, Ron D, and Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* 2004; 429(6994): 841–7.
104. Du S, Zhou J, Jia Y, and Huang K. SelK is a novel ER stress-regulated protein and protects HepG2 cells from ER stress agent-induced apoptosis. *Arch Biochem Biophys* 2010; 502(2): 137–43.
105. Verma S, Hoffmann FW, Kumar M, Huang Z, Roe K, Nguyen-Wu E, Hashimoto AS, and Hoffmann PR. Selenoprotein k knockout mice exhibit deficient calcium flux in immune cells and impaired immune responses. *J Immunol* 2011; 186(4): 2127–37.
106. Walder K, Kantham L, McMillan JS, Trevaskis J, Kerr L, De Silva A, Sunderland T, Godde N, Gao Y, Bishara N, Windmill K, Tenne-Brown J, Augert G, Zimmet PZ, and Collier GR. Tanis: a link between type 2 diabetes and inflammation? *Diabetes* 2002; 51(6): 1859–66.
107. Gao Y, Pagnon J, Feng HC, Konstantopolous N, Jowett JB, Walder K, and Collier GR. Secretion of the glucose-regulated selenoprotein SEPS1 from hepatoma cells. *Biochem Biophys Res Commun* 2007; 356(3): 636–41.
108. Karlsson HK, Tsuchida H, Lake S, Koistinen HA, and Krook A. Relationship between serum amyloid A level and Tanis/SelS mRNA expression in skeletal muscle and adipose tissue from healthy and type 2 diabetic subjects. *Diabetes* 2004; 53(6): 1424–8.
109. Gao Y, Hannan NR, Wanyonyi S, Konstantopolous N, Pagnon J, Feng HC, Jowett JB, Kim KH, Walder K, and Collier GR. Activation of the selenoprotein SEPS1 gene expression by pro-inflammatory cytokines in HepG2 cells. *Cytokine* 2006; 33(5): 246–51.
110. Curran JE, Jowett JB, Elliott KS, Gao Y, Gluschenko K, Wang J, Abel Azim DM, Cai G, Mahaney MC, Comuzzie AG, Dyer TD, Walder KR, Zimmet P, MacCluer JW, Collier GR, Kissebah AH, and Blangero J. Genetic variation in selenoprotein S influences inflammatory response. *Nat Genet* 2005; 37(11): 1234–41.
111. Ye Y, Shibata Y, Kikkert M, van Voorden S, Wiertz E, and Rapoport TA. Recruitment of the p97 ATPase and ubiquitin ligases to the site of retrotranslocation at the endoplasmic reticulum membrane. *Proc Natl Acad Sci USA* 2005; 102(40): 14132–8.
112. Fradejas N, Pastor MD, Mora-Lee S, Tranque P, and Calvo S. SEPS1 gene is activated during astrocyte ischemia and shows prominent antiapoptotic effects. *J Mol Neurosci* 2008; 35(3): 259–65.
113. Kim KH, Gao Y, Walder K, Collier GR, Skelton J, and Kissebah AH. SEPS1 protects RAW264.7 cells from pharmacological ER stress agent-induced apoptosis. *Biochem Biophys Res Commun* 2007; 354(1): 127–32.
114. Horibata Y and Hirabayashi Y. Identification and characterization of human ethanolaminephosphotransferase1. *J Lipid Res* 2007; 48(3): 503–8.