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SELENOPROTEINS: MOLECULAR PATHWAYS AND PHYSIOLOGICAL ROLES

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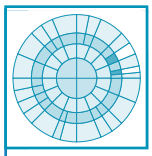

Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: Molecular Pathways and Physiological Roles. *Physiol Rev* 94: 739–777, 2014; doi:10.1152/physrev.00039.2013.—Selenium is an essential micronutrient with important functions in human health and relevance to several pathophysiological conditions. The biological effects of selenium are largely mediated by selenium-containing proteins (selenoproteins) that are present in all three domains of life. Although selenoproteins represent diverse molecular pathways and biological functions, all these proteins contain at least one selenocysteine (Sec), a selenium-containing amino acid, and most serve oxidoreductase functions. Sec is cotranslationally inserted into nascent polypeptide chains in response to the UGA codon, whose normal function is to terminate translation. To decode UGA as Sec, organisms evolved the Sec insertion machinery that allows incorporation of this amino acid at specific UGA codons in a process requiring a *cis*-acting Sec insertion sequence (SECIS) element. Although the basic mechanisms of Sec synthesis and insertion into proteins in both prokaryotes and eukaryotes have been studied in great detail, the identity and functions of many selenoproteins remain largely unknown. In the last decade, there has been significant progress in characterizing selenoproteins and selenoproteomes and understanding their physiological functions. We discuss current knowledge about how these unique proteins perform their functions at the molecular level and highlight new insights into the roles that selenoproteins play in human health.

I. INTRODUCTION

Selenium (Se) is an essential trace element in many organisms, including humans. This micronutrient supports various important cellular and organismal functions. Se deficiency has been recognized as a contributing factor to pathophysiological conditions, including heart disease (127), neuromuscular disorders (258), cancer (159), male infertility (351), and inflammation (168), as well as numerous other disorders. Se has been also implicated in mammalian development (181), immune function (292), inhibition of viral expression (19), and delaying the progression of AIDS in HIV-positive patients (40). Research on the physiological roles and health benefits of Se has made tremendous progress following the discoveries that the amino acid residue in proteins, selenocysteine (Sec), is a major form of Se in the cell and that Sec is encoded by the UGA codon. These findings suggested that proteins containing Sec, which is the 21st "naturally occurring" amino acid in the genetic code **(FIGURE 1)**, were largely responsible for the health benefits of Se.

Since the initial discovery of Sec, there have been significant developments in our understanding of how Sec is synthesized and inserted into selenoproteins. In addition, the identities of selenoproteins, which constitute the human selenoproteome (there are 25 genes encoding selenoproteins in humans), have been established. This information allowed researchers to study various aspects of Se biology and selenoprotein functions. In this review, we provide a brief overview of Sec biosynthesis and selenoprotein synthesis. We further discuss recent developments that provide mechanistic insights into the physiological functions of selenoproteins and their roles in human health. The identity of selenoproteins and their distribution are also discussed from an evolutionary perspective.

It should be noted that recent advances in the Se field have not only contributed to our better understanding of how Se functions at the molecular level, but also provided intricate details on general protein translation machinery and evolution of the genetic code, and allowed prediction of catalytic cysteine residues at the genome-wide level. Further functional characterization of selenoproteins and analyses of selenoproteomes may explain the various biological and

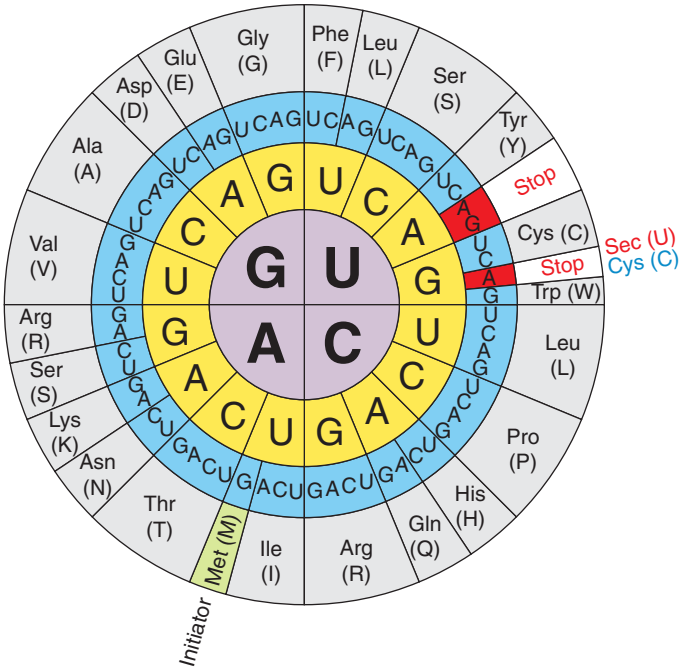

FIGURE 1. The genetic code illustrating the dual function of the UGA codon and that Sec is the 21st amino acid that is encoded by UGA.

biomedical effects of Se and help identify new molecular pathways and biological processes that are dependent on selenoproteins.

II. SEC BIOSYNTHESIS

Proteins containing Sec are present in all three evolutionary lines of descent: eukarya, archaea, and eubacteria, and they were also observed in viruses. Whereas the pathway for Sec biosynthesis in eubacteria has previously been established in great detail (108), for many years its biosynthesis in eukaryotes and archaea remained unknown. In fact, Sec was the last proteinogenic amino acid in eukaryotes for which biosynthesis was not resolved (372). The pathway for its biosynthesis was determined only in recent years using a combination of comparative genomic, molecular, and structural approaches (372, 380). Sec is unique among other amino acids in that it is the only known amino acid in eukaryotes whose biosynthesis occurs on its own tRNA, designated Sec tRNA$^{[Ser]Sec}$ (134, 209). tRNA$^{[Ser]Sec}$ is initially aminoacylated with serine in a reaction catalyzed by seryl-tRNA synthetase (SerRS) to form seryl-tRNA$^{[Ser]Sec}$ (209, 214), which provides the backbone for Sec biosynthesis.

A. Sec tRNA (tRNA$^{[Ser]Sec}$)

tRNA$^{[Ser]Sec}$ has been described as both the key molecule (27) and central component (134) of selenoprotein biosynthesis. It was initially identified as a nonsense suppressor seryl-tRNA in mammalian and avian tissues that decoded specifically the stop codon UGA (132). The sequence of tRNA$^{[Ser]Sec}$ revealed several unique features that distinguished it from all other tRNAs (81, 82). At 90 nucleotides, it was the longest tRNA that had been sequenced at that time and much longer than the ~75 nucleotides in other tRNAs; and tRNA$^{[Ser]Sec}$ has relatively few modified bases and an exceptionally long variable arm (81, 82). Two isoforms of tRNA$^{[Ser]Sec}$ were identified and sequenced, and both species formed phosphoseryl-tRNA$^{[Ser]Sec}$ (82, 130 and see below). Other novel features of tRNA$^{[Ser]Sec}$ included an atypically long acceptor stem and a long D-stem that may have up to 6 base pairs (bp) instead of 3 or 4 bp found in other tRNAs (330). Eukaryotic and archaeal tRNA$^{[Ser]Sec}$ have a 9/4 secondary structure, wherein the acceptor stem contains 9 bp and the T stem 4 bp (139, 148, 330). In contrast, bacterial tRNA$^{[Ser]Sec}$ has a 8/5 cloverleaf form, whereas all canonical tRNAs have 7/5 secondary structures (156, 157). The structures of tRNA$^{[Ser]Sec}$ from *Escherichia coli*, *Mathanococcus jannaschii*, and *Homo sapiens* are shown in clover leaf models in **FIGURE 2**.

The crystal structures of bacterial (157) and human (156) tRNA$^{[Ser]Sec}$ have been recently solved, which revealed unusual secondary structures and unique tertiary interactions. In addition, the crystal structure of tRNA$^{[Ser]Sec}$ demonstrated that the unusually long D stem containing 6 bp in this tRNA does not interact with the variable arm, resulting in an open cavity instead of the tertiary hydrophobic core (156). Two additional base pairs in the D stem of tRNA$^{[Ser]Sec}$ have been shown to be required for formation of enhanced secondary structure that compensates for the absence of canonical tertiary interactions (154).

The gene for tRNA$^{[Ser]Sec}$, designated *Trsp*, and its 5'- and 3'-flanking sequences that include the upstream regulatory elements and downstream Pol III termination signal, was initially sequenced from chickens (135) and subsequently from a variety of other eukaryotes, including a number of mammals, and *Xenopus*, *Drosophila*, *Caenorhabditis elegans*, *Taxoplasma gondii*, several species of *Plasmodium* (46 and references therein), and a variety of other eukaryotes (228 and references therein). With the use of a reverse transcription (RT)-PCR technique, tRNA$^{[Ser]Sec}$ was also sequenced from *Chlamydomonas* (290), *Dictyostelium discoideum*, and *Tetrahymena thermophila* (46). Interestingly, tRNAs$^{[Ser]Sec}$ are the longest tRNAs found in eukaryotes, archaea, and bacteria and vary in length from 90 to 93 nucleotides in eukaryotes (46, 228) to well over 100 nucleotides in bacteria (139, 228). Most of the extra nucleotides, which place these tRNAs in a class by themselves in terms of length, are found in the long extra arm. *Trsp* is a single copy gene in all organisms examined thus far that encode the selenoprotein synthesis machinery except zebrafish which contain two copies (133). Human *Trsp* has been localized on chromosome 19q13.2→q13.3 (257). Interestingly, mouse *Trsp* maps on chromosome 7 at the centromere proximal region sharing the conserved gene ar-

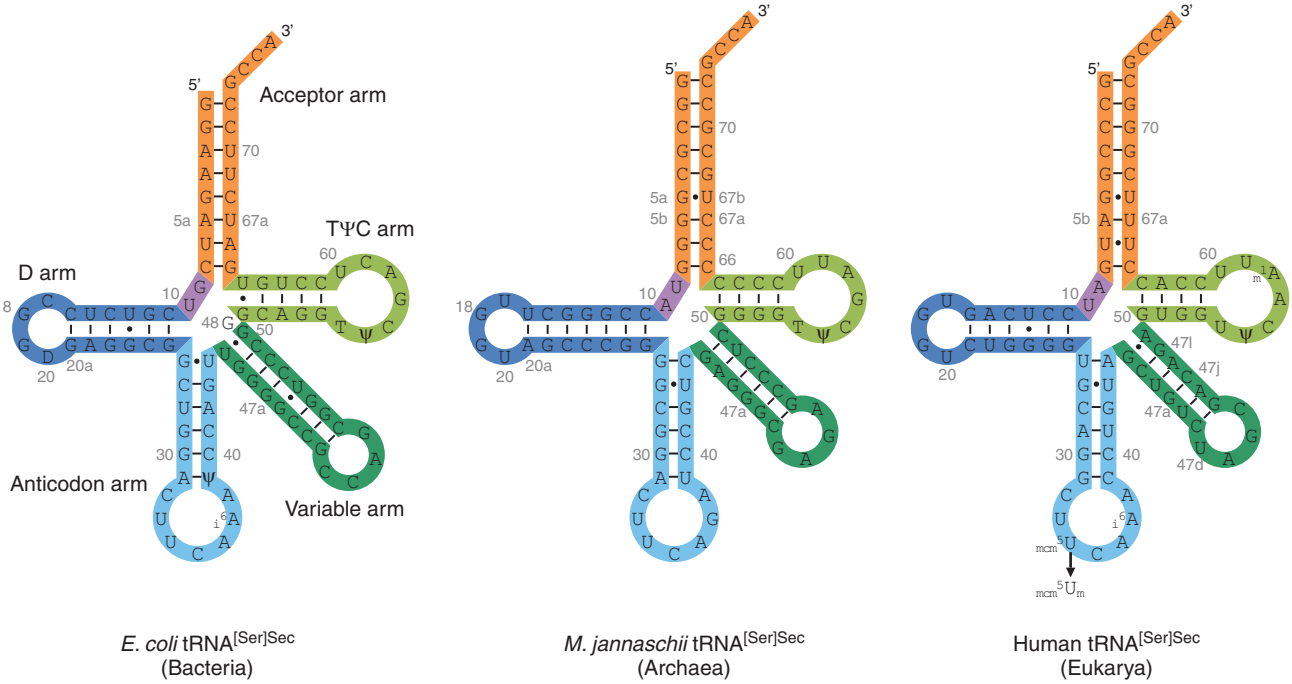

FIGURE 2. Cloverleaf models of eukaryotic, archaeal, and bacterial tRNAs[Ser]Sec.

rangements with human chromosome 19 (273) that represent an example of conserved mouse-human synteny.

There are several additional features of tRNA[Ser]Sec, which distinguish it from other tRNAs. It has a triphosphate moiety attached to its 5′-terminal guanosine nucleotide. This is due to an unusual transcription of tRNA[Ser]Sec, which begins at the first nucleotide within the coding sequence, whereas all canonical tRNAs have a 5′ leader sequence that must be processed (208). The regulation of *Trsp* transcription is also novel among tRNAs. *Trsp* expression, unlike other tRNAs, is governed by three upstream regulatory regions, a TATA box motif at nucleotide −30, a proximal sequence element at approximately −70, and a distal sequence element at approximately −200 (see 136 and references therein). The biosynthesis of the two tRNA[Ser]Sec isoforms including the modified bases have been reconstituted in *Xenopus* oocytes (62, 329). These modifications include 1-methyladenosine (m^1A) at position 58, pseudouridine (Ψ) at position 55, isopentenyladenosine (i^6A) at position 37, and either 5-methoxycarbonylmethyluridine (mcm^5U) or 5-methoxycarbonylmethyl-2′-*O*-methyluridine (mcm^5Um) at position 34 (82). Two of the modifications are located in the anticodon loop and may contribute to the stabilization of the codon-anticodon interaction on the ribosome. mcm^5U serves as a precursor of mcm^5Um, and Um34 methylation is the last step in the maturation of tRNA[Ser]Sec. The synthesis of Um34 requires the presence of other modifications and intact secondary and tertiary structures (178) and, in addition, that tRNA[Ser]Sec be aminoacylated (177). Moreover, two major tRNA[Ser]Sec isoforms, each containing either mcm^5U or mcm^5Um at position 34, exist in mammalian cells, and their relative distribution is influenced by Se status (58, 82, 131). Under conditions of Se deficiency, the level of mcm^5U is greater than mcm^5Um, and during Se supplementation, the ratio of the two isoform is reversed. Interestingly, housekeeping selenoproteins (e.g., thioredoxin reductases TR1 and TR3) are synthesized by the mcm^5U isoform, while stress-related selenoproteins (e.g., glutathione peroxidases GPx1 and GPx3, and selenoproteins MsrB1, SelT, and SelW) are synthesized by mcm^5Um (44). There are also several selenoproteins, such as GPx4 and selenoprotein P, that appear to be synthesized by both isoforms (42).

B. Seryl-tRNA synthetase

Aminoacylation of tRNA[Ser]Sec with Ser is the first step in the biosynthesis of Sec in both eubacteria and eukaryotes: the Ser moiety serves as the backbone for Sec **(FIGURE 3)**. The fact that tRNA[Ser]Sec is initially aminoacylated with serine by seryl-tRNA synthetase (SerS) suggests that it has identity elements for Ser, but not for Sec. These elements are located in the discriminator base and the long variable arm, both of which were essential for aminoacylation by SerS (274, 370). This has been further confirmed by recent crystallographic studies that revealed an interaction of the variable stem of tRNA[Ser]Sec with the NH_2-terminal domain of SerS (156, 157). Moreover, the orientation of the extended variable arm in the tertiary structure of tRNA[Ser]Sec is nearly identical to that of $tRNA^{Ser}$, suggesting that it is recognized by SerS in the same manner, and is distinguished from the other tRNAs (155). However, the overall contribution of SerS to charging tRNA[Ser]Sec accounts for only

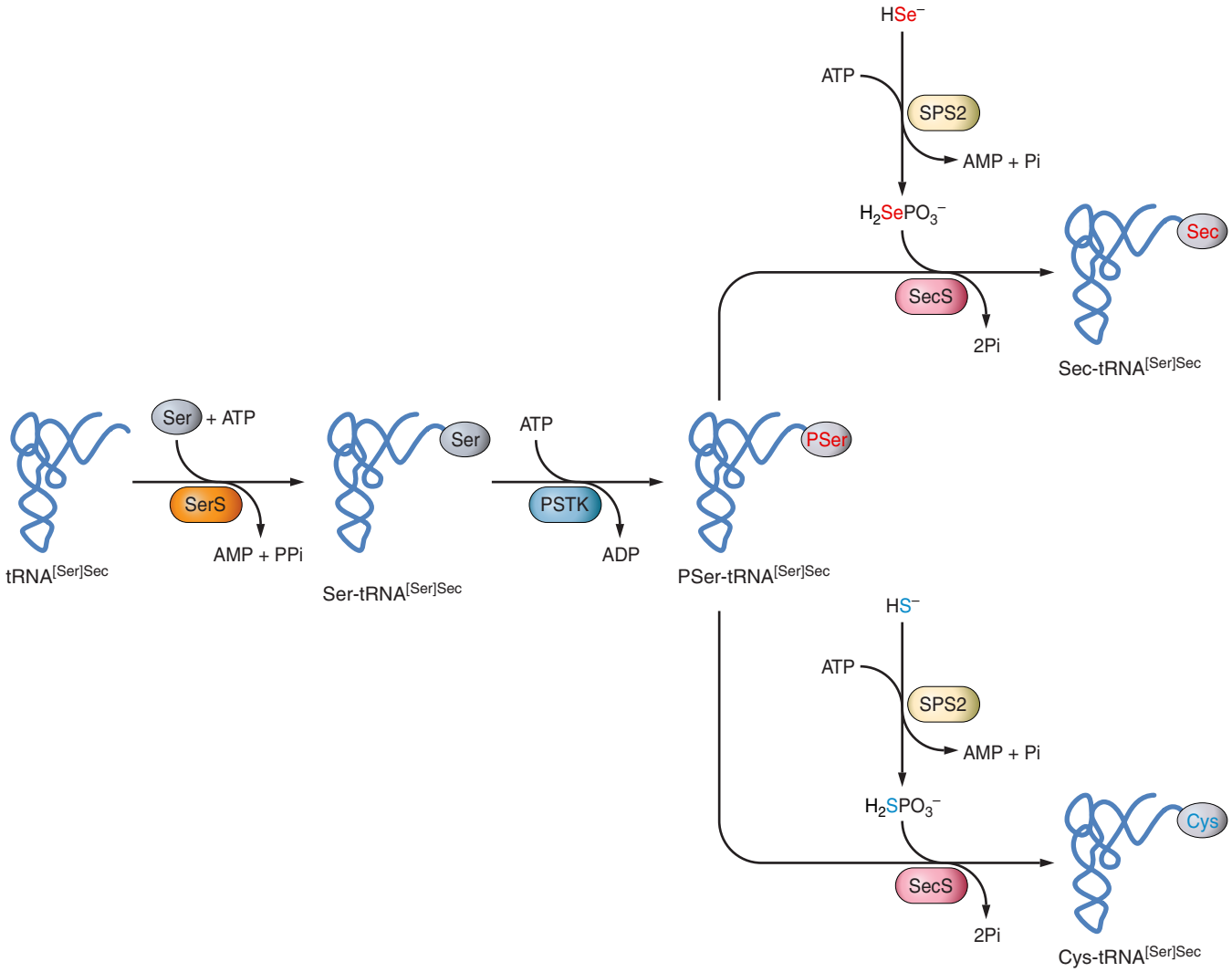

FIGURE 3. Mechanism of Sec biosynthesis in eukaryotes and the Sec machinery-based pathway for synthesis of Cys. The pathway for Sec biosynthesis in eukaryotes is shown, in which phosphoseryl-tRNA kinase (PSTK) provides the phosphorylated intermediate PSer-tRNA$^{[Ser]Sec}$ serving as a substrate for SecS. Selenophosphate ($H_2SePO_3^-$) generated by SPS2 from selenite and ATP is used as a donor of active Se for SecS (see *top right* portion of the figure for the final steps in Sec biosynthesis). The de novo synthesis of Cys using the Sec biosynthetic machinery is shown on the *bottom right* (see text for details).

~1% of that for charging serine tRNA in prokaryotes, reflecting the limited flux of Ser for the Sec biosynthesis pathway (18). Structural differences of tRNA$^{[Ser]Sec}$ from the SerS cognate tRNA acceptors were proposed to be responsible for this reduced acceptor activity (28). Alternatively, decreased utilization of tRNA$^{[Ser]Sec}$ by SerS might be attributed to a much higher intracellular concentration of the tRNA^{Ser} substrate.

C. Phosphoseryl-tRNA$^{[Ser]Sec}$ Kinase and Sec Synthase

The conversion of the serine moiety on tRNA$^{[Ser]Sec}$ to selenocysteyl-tRNA$^{[Ser]Sec}$ is catalyzed by Sec synthase (SecS), which incorporates selenophosphate, the active form of Se, into the amino acid backbone and forms Sec-tRNA. Bacterial SecS was identified in the early 1990s by Böck and collaborators (108–110). In *E. coli* SecS is a pyridoxal phosphate (PLP)-containing protein encoded by the *selA* gene. The reaction catalyzed by SelA involves the removal of the hydroxyl group from serine and the formation of an aminoacrylyl intermediate, which serves as an acceptor for activated Se. The active Se donor in bacteria has been suggested to be monoselenophosphate, which is synthesized from selenide and ATP by selenophosphate synthetase (SPS) (SelD protein in *E. coli*) (117). However, considering the low catalytic efficiency of SelD for selenide, it is likely that some other form of Se serves as the natural Se donor.

Recently, the details of Sec biosynthesis in archaea and eukaryotes have been established **(FIGURE 3)**, demonstrating that biosynthesis of Sec in these organisms occurs by a distinct mechanism. The major difference of the eukaryotic Sec biosynthesis pathway from that in eubacteria is the presence of an additional catalytic step leading to the synthesis of a *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ intermediate, which serves as a substrate for eukaryotic SecS. The formation of phosphoseryl-tRNA in rooster liver by a minor fraction of seryl-tRNA within the total seryl-tRNA population was reported by Maenpaa and Bernfield as early as 1970 (238), but the identities of the kinase that is responsible for phosphorylation of this seryl-tRNA and eukaryotic SecS remained unknown. It was later shown that this minor seryl-tRNA is a seryl-tRNA$^{[Ser]Sec}$ (209), and the kinase involved in its phosphorylation, designated phosphoseryl-tRNA kinase (PSTK), and SecS were finally identified using computational and comparative genomic approaches (372, 380). Selenoproteins occur in some, but not all, organisms, e.g., in about half of eukaryotes and ~10% of archaea (384, 385). This observation was utilized in a comparative analysis of completely sequenced genomes for Sec insertion machinery as proxy for Sec utilization and identification of additional components involved in Sec biosynthesis. This analysis revealed putative PSTK and SecS (45, 372), and the functions of these proteins were confirmed experimentally. Interestingly, SecS has previously been shown to coprecipitate with tRNA$^{[Ser]Sec}$ in cell extracts from patients with an autoimmune chronic hepatitis (209), and belongs to a family of PLP-dependent enzymes that is distinct from that of bacterial SecS (169). This protein was reported to form a complex with other proteins involved in the Sec insertion (373), being consistent with its SecS function (6, 133, 169, 319).

The crystal structures of human SecS in complex with tRNA$^{[Ser]Sec}$ (280) and that of the archaeal PSTK·Sec tRNA$^{[Ser]Sec}$ complex (57, 314) have been recently solved. The structures of the Sec machinery support a PLP-dependent mechanism of Sec-tRNA$^{[Ser]Sec}$ synthesis **(FIGURE 4)**. In humans, four SecS subunits form a tetramer, which binds two tRNA$^{[Ser]Sec}$ molecules through their long acceptor-TΨC arms. Interaction of tRNA$^{[Ser]Sec}$ with the active site of SecS induces enzyme's conformational change that allows binding of *O*-phosphoseryl-tRNA$^{[Ser]Sec}$, but not free phosphoserine, in order for the reaction to occur. Additional details of bacterial Sec formation were revealed by the recently reported structure of the SelA·tRNA$^{[Ser]Sec}$ complex (155). Although both bacterial and human SecS are type I PLP-dependent enzymes, their substrates are different (Ser-tRNA$^{[Ser]Sec}$ vs. *O*-phosphoseryl-tRNA$^{[Ser]Sec}$). In contrast to human SecS, the bacterial SecS structure consists of 10 subunits, which form a ring-shaped pentamer of dimers. Each homodecamer binds 10 tRNA$^{[Ser]Sec}$ molecules, with four SecS subunits interacting with each tRNA. This structure creates a large cleft between the two SecS dimers, where the 3′-terminal end of Ser-tRNA$^{[Ser]Sec}$ can fit. In addition, the NH_2-terminal domain of SecS is involved in the recognition of the unique D-arm structure of Ser-tRNA$^{[Ser]Sec}$ that distinguishes it from Ser-tRNA^{Ser}. Therefore, the difference between bacterial and eukaryotic/archaeal SecS structures may be explained by the need for the bacterial enzyme to differentiate between Ser-tRNA$^{[Ser]Sec}$ and Ser-tRNA^{Ser} (155). However, in eukaryotic and archaeal systems, SecS recognizes the phosphate moiety of *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ synthesized by PSTK (280), while PSTK discriminates Ser-tRNA$^{[Ser]Sec}$ from Ser-tRNA^{Ser} (57).

The functional role of SecS in biosynthesis of Sec on its tRNA has been supported by a recent finding of mutations in the SecS gene in Jewish populations of Iraqi and Moroccan descents (3). Two different homozygous mutations in SecS, which resulted in substitution of Tyr334 with Cys residue and replacement of Ala239 with Thr, were associated with progressive cerebello-cerebral atrophy in these patients. Although lack of tRNA$^{[Ser]Sec}$ as a result of the whole-body *Trsp* gene knockout in mice leads to embryonic lethality (368), patient mutations in SecS have a less dramatic phenotype. Based on the SecS structure, these mutations are expected to disrupt protein folding and catalytic activity of SecS. However, whether the patient mutations found in the Jewish population of Iraqi and Moroccan descent lead to complete loss of SecS function or only partially inhibit SecS catalyzed production of Sec-tRNA$^{[Ser]Sec}$ currently remains unknown. Particularly, it would be interesting to see the expression levels of which selenoproteins (or whether all of them) are affected in these individuals.

D. Selenophosphate Synthetase

Two proteins were identified in mammals with homology to SelD from *E. coli*, SPS1 (176, 232) and SPS2 (124). Both proteins were initially thought to function as SPSs. However, further analysis revealed that SPS2 could generate selenophosphate in vitro, whereas SPS1 could not (372). In addition, a Sec→Cys SPS2 mutant complemented SelD deficiency when expressed in *SelD*$^{-}$ *E. coli* cells (124, 175, 179), whereas SPS1 was able to complement SelD deficiency only when *E. coli* cells were cultured in the medium supplemented with L-Sec (340). These findings suggested that SPS2 is required for de novo synthesis of selenophosphate, while SPS1 may have a possible role in Sec recycling through a Se salvage system. Since SPS2 is itself a selenoprotein, it possibly serves as an autoregulator of selenoprotein synthesis (124, 175). Interestingly, some insects, including the red flour beetle *Tribolium castaneum*, the silkworm *Bombyx mori*, several fruit fly species (but not *Drosophila melanogaster*), and several other organisms, lack Sec biosynthesis, the insertion machinery and selenoproteins, but contain orthologs of SPS1 (227). Thus it is likely that SPS1 functions in a pathway unrelated to selenoprotein synthesis. Interestingly, knockdown of SPS1 in SL2 *Drosophila* cells leads to growth inhibition, increased intracellular glutamine levels,

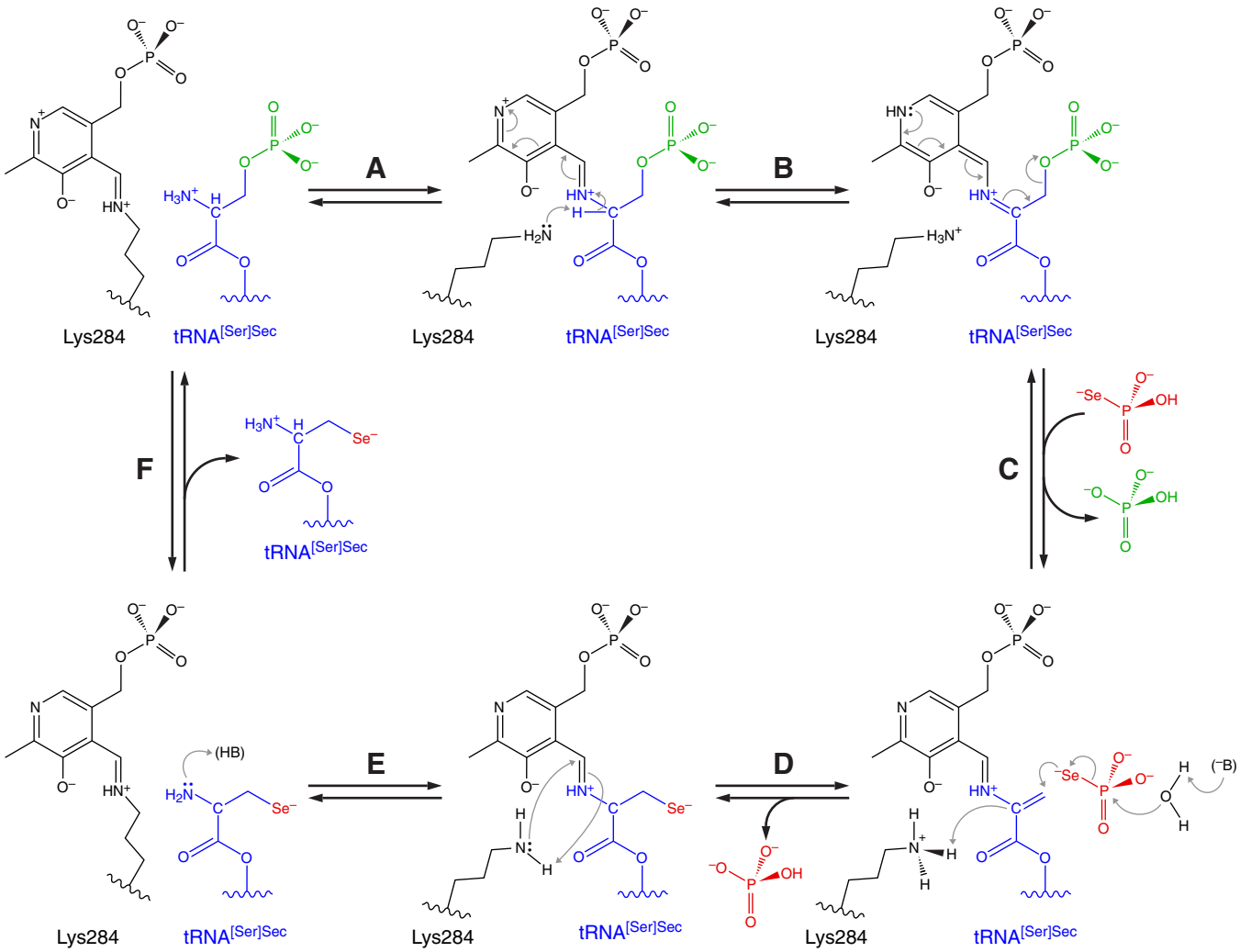

FIGURE 4. The PLP-dependent mechanism of eukaryotic SecS. *A*: amino group of *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ attacks the Schiff base (internal aldimine) between Lys284 and PLP to form an external aldimine. *B*: after the formation of the external aldimine, the side chain of Lys284 abstracts the Cα proton from phosphoserine. *C*: electron delocalization leads to β-elimination of phosphate and formation of dehydroalanyl-tRNA$^{[Ser]Sec}$. Free phosphate dissociates, whereas selenophosphate binds to the enzyme's active site. *D*: an unidentified base (-B) activates water that hydrolyzes selenophosphate. Se attacks the double bond of dehydroalanyl-tRNA$^{[Ser]Sec}$, and the second phosphate dissociates. Lys284 returns the proton to the Cα carbon leading to the formation of the selenocysteinyl moiety. *E*: Lys284 forms the Schiff base with PLP leading to the release of the oxidized form of Sec-tRNA$^{[Ser]Sec}$. *F*: the free amino group of Sec-tRNA$^{[Ser]Sec}$ is protonated, and the active site of SecS is regenerated.

megamitochondrial formation, and decreased expression of genes involved in PLP metabolism (210, 316). Because treatment of cells with 4-deoxypyridoxin (4-DPN), an inhibitor of PLP biosynthesis, also inhibits cellular growth and causes megamitochondrial formation, vitamin B_6 biosynthesis has been suggested as a primary target of SPS1 in *Drosophila*. As noted above, SecS is a PLP-dependent enzyme. Therefore, it is an attractive possibility that SPS1 may be involved in Se metabolism by regulating biosynthesis and/or uptake of vitamin B_6.

In addition to its role in Sec biosynthesis, SPS2 enzyme has been recently implicated in Cys biosynthesis (374). Cys mutants of selenoproteins partially preserve their activities, whereas other mutations disrupt their functions. Moreover, Cys was observed in place of Sec in a naturally occurring selenoprotein, TR1, in rodents that were subjected to Se deficiency (234). It was further found that Cys can be biosynthesized de novo by using the Sec biosynthetic machinery (374) and then inserted into selenoproteins. During Se deficiency, SPS2 can utilize sulfide instead of selenide and generate thiophosphate ($H_2SPO_3^-$) as an active donor for the SecS-catalyzed reaction **(FIGURE 3)**. SecS then makes Cys-tRNA$^{[Ser]Sec}$ from $H_2SPO_3^-$ and *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ intermediates, and Cys is further inserted into selenoproteins at the UGA-encoded sites instead of Sec. This replacement was demonstrated to occur both in vitro and in vivo using cells cultured in the presence of $H_2SPO_3^-$ and in mice

fed a Se-deficient diet (374). However, even in mice fed a Se-adequate diet, a fraction of TR was found to contain Cys at the UGA encoded Sec position. Since Cys is an essential amino acid in mammals, the synthesis of Cys on $tRNA^{[Ser]Sec}$ represents a novel de novo pathway for the synthesis of this amino acid. In a very recent study, Sec was also found to be replaced in selenoproteins by other amino acids, such as arginine (Arg) and tryptophan (Trp), in addition to Cys, when mammalian cells were treated with different antibiotics (344). However, the substitution of Sec in these cases occurred due to translational errors rather than Sec replacement with Cys through the Sec biosynthesis pathway. These findings highlighted vulnerability of Sec insertion into proteins in the presence of widely used antibiotics, such as Geneticin, doxycycline, and chloramphenicol, and suggested that prolonged treatment of infections with antibiotics may inhibit functions of essential selenoproteins in humans due to a high level of amino acid misincorporation at the Sec sites (344).

E. Sec Lyase

The mechanism by which Sec is decomposed was found fairly recently. It involves the enzyme Sec lyase (SCL), which catalyzes a PLP-dependent degradation of Sec to L-alanine and elemental Se (253). SCL activity was originally identified in a homogenate from rat liver (95), and the pig liver SCL protein was subsequently isolated and biochemically characterized (94). It was demonstrated that mammalian SCL contains a PLP cofactor and forms a homodimer. More recently, structural studies revealed the details of its catalytic mechanism and how this unique enzyme distinguishes Sec from Cys and Ser residues, which only differ from Sec by a single atom (66, 278). In addition to mammals, SCL homologs are widely present in organisms throughout the animal kingdom, including some unicellular eukaryotes. SCL activity was also found in several bacterial species, but, in contrast to the mammalian homodimeric protein, bacterial SCL is monomeric (60, 61). In mammals, SCL is expressed in a variety of tissues with the highest activity observed in liver and kidney; however, SCL activity was not detected in blood and fat tissues (94, 253).

The physiological function of SCL remains unknown. It was proposed that SCL may be involved in the recycling of the Se from Sec during degradation of selenoproteins. Therefore, SCL could be potentially used by cells to provide an alternative source of Se for Sec biosynthesis. Recently, SCL has been shown to be involved in selenoprotein synthesis in vivo in cell culture (193). In addition, in vitro experiments demonstrated that both SPS1 and SPS2 interact with SCL (343) and that bacterial SCL proteins could provide Se to SPS from free Sec, to produce selenophosphate (201, 324). These data implicate SCL in the recycling of Se and selenoprotein synthesis. However, further experiments are required to resolve details of its physiological function.

Recent advances in characterizing the physiological role of SCL have been provided by whole body knockout of the SCL gene in mice (309). Surprisingly, SCL knockout mice are characterized by disrupted glucose and lipid homeostasis and insulin signaling when fed a normal Se diet. Furthermore, these symptoms worsened when animals were subjected to Se deficiency leading to development of obesity and hypercholesterolemia (309). The data suggest that the metabolic perturbations caused by the lack of SCL might be due to defects in selenoprotein synthesis and are in line with the recently observed link between selenoprotein deficiency and disrupted glucose homeostasis in mice (198). It remains to be tested which selenoproteins are most involved in the development of this phenotype in mice lacking SCL.

III. MECHANISM AND REGULATION OF SEC INCORPORATION INTO PROTEINS

Cotranslational incorporation of Sec into proteins is dictated by in-frame UGA codons present in selenoprotein mRNAs. Sec is introduced into selenoproteins by a complex mechanism that requires special *trans*-acting protein factors, $Sec\text{-}tRNA^{[Ser]Sec}$ and a *cis*-acting Sec insertion sequence (SECIS) element **(FIGURE 5)**. When a ribosome encounters the UGA codon, which normally signals translation termination, Sec machinery interacts with the canonical translation machinery to augment the coding potential of UGA codons and prevent premature termination. SECIS elements serve as the factors that dictate recoding of UGA as Sec (23, 26, 348). In response to the SECIS element in selenoprotein mRNA, $Sec\text{-}tRNA^{[Ser]Sec}$, which has an anticodon complimentary to the UGA, translates UGA as Sec. At least two *trans*-acting factors are required for efficient recoding of UGA as Sec in eukaryotes: SECIS binding protein 2 (SBP2) and Sec-specific translation elongation factor (eEFSec). SBP2 is stably associated with ribosomes and contains a distinct L7Ae RNA-binding domain that is known to bind SECIS elements with high affinity and specificity (70, 71, 231). Aside from binding to ribosomes and SECIS elements, SBP2 also interacts with eEFSec, which recruits $Sec\text{-}tRNA^{[Ser]Sec}$ and facilitates incorporation of Sec into the nascent, growing polypeptide (348). Instead of SBP2 and eEFSec, bacteria have a Sec-specific translation elongation factor (SelB in *E. coli*) that directly recognizes SECIS and is required for binding and delivery of SECIS elements to the ribosome (96). Since the discovery of SBP2, additional SECIS-binding proteins were identified and their roles in selenoprotein synthesis were characterized, including ribosomal protein L30 (53), eukaryotic initiation factor 4a3 (eIF4a3) (32), and nucleolin (254). While ribosomal protein L30 has been predicted to constitute a part of the basal Sec insertion machinery, nucleolin and eIF4a3 serve as regulatory proteins that modulate synthesis of selenoproteins and may contribute to the hierarchy of selenoprotein expression. Consistent with the regulatory role of these proteins, a recent study has shown that the known core factors are

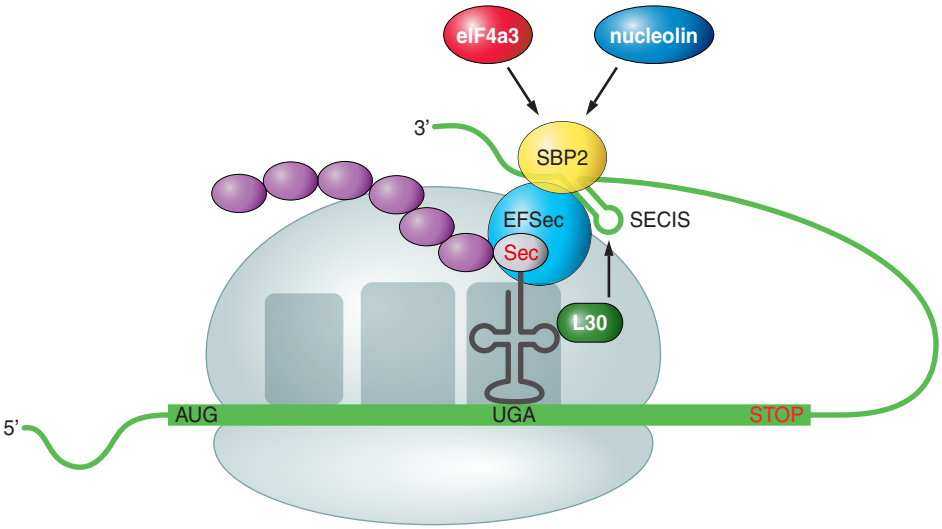

FIGURE 5. Mechanism of Sec insertion in eukaryotes. The figure shows known factors that are required for Sec incorporation into proteins in response to the UGA codon. In addition, the factors that may influence the efficiency of the Sec insertion are shown, including ribosomal protein L30, a eukaryotic translation initiation factor eIF4a3, and nucleolin (see text for details).

sufficient for Sec incorporation in a plant in vitro translation system (125).

A. SECIS Elements

SECIS elements are *cis*-acting stem-loop RNA structures that are found in the 3′-untranslated regions of all eukaryotic and archaeal selenoprotein mRNAs (230), although eukaryotic and archaeal SECIS elements have different sequences, motifs, and structures (296). In bacteria, SECIS elements are of a third type (different from those in eukaryotes and archaea) and are located immediately downstream of the UGA encoding Sec, within the coding region of selenoprotein genes (28). Several features distinguish SECIS elements from other functional mRNA stem-loop structures. Eukaryotic SECIS elements are formed by two helixes separated by an internal loop, a GA Quartet (SECIS core) structure, and an apical loop or bulge **(FIGURE 6)** (362). The GA Quartet is located at the base of helix II and is composed of four non-Watson-Crick interacting base pairs, including two tandem G·A/A·G base pairs, which are characteristic of kink-turn motifs (119, 249). The GA Quartet is the main functional element of the SECIS and is required for interaction with SBP2. RNA-footprinting studies revealed that SBP2 primarily binds to the SECIS core as well as 5′-strand of the internal loop and the upper part of helix I (100). Moreover, a large internal loop favors SBP2 binding, and the identity of nucleotides at positions 2 and 3 in the loop influences SBP2 affinity (65). In some SECIS elements, the apical loop forms an additional ministem, which is used to classify SECIS elements into two different types. SECIS elements whose apical loop lacks the ministem are classified as type 1, and those containing the ministem (bulge) belong to type 2 SECIS elements (97, 122). In addition to the SECIS core, a conserved AAR motif in the apical region of SECIS is required for Sec incorporation (247). The function of AAR motif remains unknown, and no AAR-sequence-specific binding proteins have been identified. Interestingly, some selenoproteins, such as mammalian selenoproteins M and O, contain SECIS elements with CC nucleotides instead of AA in this motif, suggesting that the apical loop is not involved in protein binding, but might

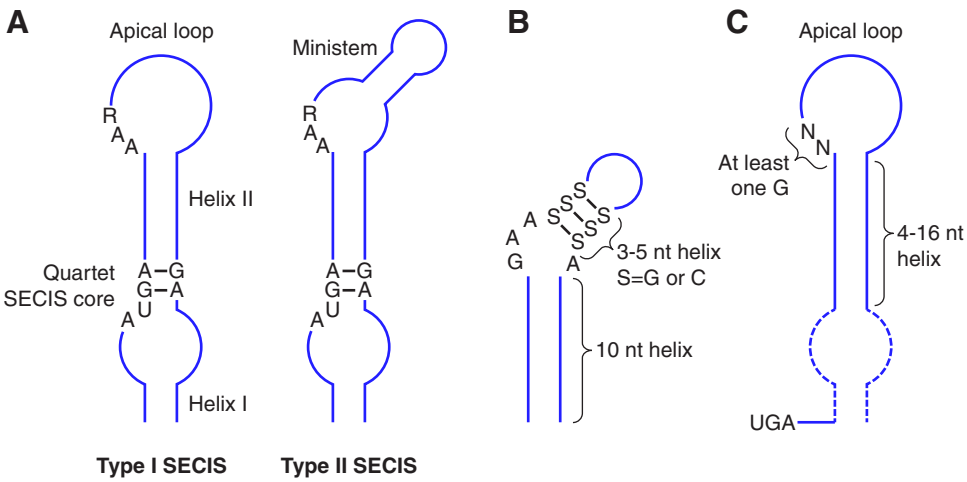

FIGURE 6. Structure of SECIS elements in eukaryotes (*A*), archaea (*B*), and bacteria (*C*). Consensus structures of SECIS elements with conserved nucleotides are shown. The indicated features and structural constraints represent the majority of SECIS elements found in selenoprotein mRNAs, but some additional exceptions may occur.

play a role on the ribosome, e.g., by assisting with binding of eEFSec to the ribosome or accommodation of Sec-tRNA$^{[Ser]Sec}$ (146, 183, 186).

B. SBP2

SBP2 contains three distinct domains: an NH_2-terminal domain whose function is not currently known, a Sec incorporation domain (SID) in the middle part of the protein, and a COOH-terminal RNA-binding domain (RBD), which belongs to a large family of L7Ae RNA-binding proteins known to interact with RNAs containing kink-turn motifs (e.g., rRNA, snRNA, and SECIS) (70, 71). The NH_2-terminal domain is not required for Sec incorporation and is completely absent in some organisms (e.g., insects and worms), suggesting that it might play a regulatory role (70, 86). Mutational analysis demonstrated that the RBD domain containing the L7Ae motif is required for SECIS binding activity of SBP2 (5, 71). However, this domain alone was not sufficient to mediate interaction with SECIS, and both RBD and SID were shown to be involved in SECIS binding (31, 339). Moreover, it was found that the SID and RBD domains, when expressed as separate proteins, were able to form a SECIS-dependent complex and supported Sec incorporation in vitro (85). Since SID itself does not have any detectable binding to SECIS, these observations suggest that it might be involved in enhancing SECIS-binding of RBD, and that the SID and RBD complex is induced by binding the SECIS element (85).

In addition to SBP2, vertebrates contain a paralog of SBP2 named the SBP2-like (SBP2L) protein. Biochemical characterization of SBP2L protein has been limited, and its function remains unknown. Despite the sequence similarity between SBP2 and SBP2L, mammalian SBP2L does not support Sec decoding using a rabbit reticulocyte lysate in vitro translation system (86). However, some invertebrates, e.g., the worm *Capitella teleta*, sea urchins, and ascidians, lack SBP2 protein and contain only a SBP2L homolog, which is able to support Sec incorporation (87). Moreover, both SBP2 and SBP2L can efficiently bind SECIS elements in mammals, but unlike SBP2, interaction of mammalian SBP2L protein with SECIS does not promote association of its SID and RBD domains. These observations suggest that SBP2L might act as a posttranscriptional regulator of selenoprotein expression or could direct incorporation of Sec into selenoproteins under specific conditions that allows stable interaction of the RBD with the SID domain (87). Alternatively, it is possible that SBP2L might require activation, e.g., by phosphorylation or binding of a currently unidentified protein partner or partners, to support Sec insertion.

SBP2 is a limiting factor for selenoprotein synthesis. Knockdown of SBP2 in mammalian cells using siRNA leads to decreased expression of selenoproteins (282), whereas overexpression of SBP2 has been shown to enhance Sec incorporation (231). Moreover, increased levels of SBP2 differentially influence insertion of Sec into individual selenoproteins, suggesting that efficiency of this process is likely determined by the structure of SECIS elements and their different affinity for SBP2 (231, 322). These findings are further supported by a recent discovery of mutations in the SBP2 gene in patients with hypothyroidism caused by defects in synthesis of type 2 deiodinase (DI2), a selenoprotein involved in thyroid hormone activation (14, 80, 90, 306). In one of the affected families, DI2 deficiency was caused by a homozygous missence mutation (R540Q) in the RNA-binding domain of SBP2 (90). This mutation was shown to decrease SBP2 binding to a subset of SECIS elements necessary for Sec insertion into selenoproteins leading to defective synthesis of some selenoproteins, including DI2, GPx1, and SelP (31) The relatively mild phenotype (i.e., delayed growth associated with thyroid hormone deficiency) in these patents can be explained by a selective SBP2 SECIS-binding defect as levels of other selenoproteins were not affected by the R540Q mutation in the SBP2 gene. In a separate study, characterization of patients with different SBP2 mutations revealed decreased production of most of the 25 known selenoproteins, leading to a more complex phenotype (306).

C. Sec-Specific Elongation Factor

A Sec-specific eukaryotic elongation factor (eEFSec) is responsible for recruiting tRNA$^{[Ser]Sec}$ and, together with SBP2, it inserts Sec into nascent protein chains in response to UGA codons (96, 348). Similar to the canonical elongation factor eEF1A, which is involved in incorporation of the other 20 amino acids, eEFSec has GTPase activity. But unlike eEF1A, it has high specificity for aminoacylated tRNA$^{[Ser]Sec}$ and does not bind phosphoseryl-tRNA$^{[Ser]Sec}$ or other aminoacylated tRNAs (45, 348). eEFSec has a "chalice-like" structure consisting of four domains (213). Domains I, II, and III, which form the cup of the chalice, have strong similarity to the elongation factor eEF1A. Compared with EF1A, eEFSec contains a unique COOH-terminal extension named domain IV, which is located at the base of the chalice. Domain IV was proposed to be involved in interactions with SBP2 and the long variable arm of tRNA$^{[Ser]Sec}$ (156, 381). eEFSec has been shown to form a complex with SBP2 (85, 348, 381). The formation of the complex was dependent on the presence of a SECIS element, but SBP2 and eEFSec could interact even in the absence of tRNA$^{[Ser]Sec}$ or GTP (85). Moreover, the RBD of SBP2 and the SECIS element together were sufficient for binding eEFSec, suggesting that RBD and SECIS could form a favorable interface for recruitment of eEFSec. It is likely that the complex between SBP2 and eEFSec is mediated by the SECIS element and may be further stabilized by tRNA$^{[Ser]Sec}$, as SBP2 and eEFSec interaction could be enhanced by overexpression of the tRNA$^{[Ser]Sec}$ gene (381). As discussed

above, in bacteria, the function of eEFSec and SBP2 is performed by a single protein factor SelB, which is involved in both SECIS binding and delivery of $tRNA^{[Ser]Sec}$ to the ribosome (28). The presence of two separate proteins in eukaryotes might be dictated by a distant location of SECIS (in the 3′-UTR), compared with bacteria, which have SECIS immediately downstream of Sec-encoding UGA codon. To prevent $tRNA^{[Ser]Sec}$ from acting as a suppressor tRNA, eEFSec, or SelB in bacteria, must be denied general access to the A-site on the translating ribosome. To decode UGA as Sec, these factors undergo conformational change upon binding a SECIS element, which stimulates functional interactions with the ribosome. Indeed, SelB was able to catalyze the ribosome-dependent GTP hydrolysis only in the presence of the SECIS element (151).

D. Ribosomal Protein L30

Ribosomal protein L30 has been identified as one of the proteins capable of binding SECIS in cells using a UV-cross-linking assay (53). L30 is a component of the large (60S) ribosomal subunit in eukaryotes and has been shown to bind a SECIS element through an L7Ae RNA-binding motif, which is also found in SBP2, at a site overlapping with the SBP2-binding site (38). L30 is abundantly expressed in a number of different tissues in mammals, and the majority of the cellular L30 protein is associated with ribosomes. However, a small fraction of L30 is present in a ribosome-free form, which autoregulates splicing of its transcript in the nucleus and is involved in pre-rRNA processing in the nucleolus (74, 359). Although the exact function of L30 in the selenoprotein synthesis pathway remains unknown, the following observations suggest that it might constitute a part of the basal Sec insertion machinery. L30 binds to SECIS elements both in vitro and in vivo, and overexpression of this protein in rat hepatoma cells stimulates Sec decoding (53). In vitro studies revealed that SBP2 preferentially binds to an open form of the SECIS element, whereas L30 can bind equally well to kinked and open SECIS structures. Moreover, L30 competes with SBP2 for SECIS binding under conditions of high magnesium, which is known to induce a kink-turn in SECIS. The latter finding has led to the proposal of a model in which SBP2 initially binds to the SECIS element, but during Sec decoding it is displaced by L30 that is involved in tethering SECIS to the large ribosomal subunit (53). However, the exact role of L30 in the synthesis of endogenous selenoproteins and whether L30 participates in promoting SBP2/ribosome interaction awaits further investigation.

E. Regulation of Selenoprotein Expression

Previous studies using both cell culture and animal models have shown that expression of selenoproteins is differentially regulated by Se availability (141, 211, 307, 335). While expression of some selenoproteins is significantly decreased under Se-deficient conditions, e.g., GPx1, MsrB1, SelW, and SelH (which we refer to as stress-related selenoproteins), expression of the other group of selenoproteins including TR1 and TR3 (referred to as housekeeping selenoproteins) is less regulated by dietary Se. This "hierarchical" regulation of selenoprotein expression occurs at both mRNA abundance and translational levels; however, the underlying mechanisms are not fully understood (335). The decreased mRNA abundance of stress-related selenoproteins under conditions of Se deficiency was attributed to altered rates of mRNA turnover due to nonsense-mediated decay (NMD) rather than transcriptional regulation (260, 366). In eukaryotes, NMD pathway is used to eliminate mRNA transcripts with premature stop codons. As all selenoproteins contain an in-frame UGA codon, the reason why some of the selenoprotein transcripts are more susceptible to NMD than others remains unclear. The position of UGA relative to the SECIS element, the local UGA context as well as the differential affinities of SECIS elements for SBP2 have been proposed to mediate the sensitivity of specific selenoproteins to NMD (322, 367). In addition, a number of factors have been shown to affect the efficiency of Sec incorporation into proteins and thus might influence mRNA stability (discussed below).

Recent studies have identified a eukaryotic translation initiation factor eIF4a3 as an important regulator of selenoprotein synthesis, which contributes to the preferential translation of essential selenoproteins during Se deficiency (32). eIF4a3 is a member of the DEAD box family of RNA-dependent ATPases, which are involved in splicing of pre-mRNA in the nucleus (217). As part of its canonical function, eIF4a3 binds spliced mRNA during the formation of the exon junction complex and participates in NMD (52, 112, 279). In addition to performing its canonical function, eIF4a3 has been shown to serve as a negative regulator of Sec incorporation (32). eIF4a3 exhibits preferential binding to a subset of SECIS elements that are located in the 3′-UTR of stress-related selenoproteins, e.g., GPx1 and MsrB1, but has low affinity to the SECIS elements in mRNA from essential (housekeeping) selenoproteins, e.g., TR1 and GPx4. Since eIF4a3 and SBP2 have overlapping binding sites, these two proteins compete for binding to the SECIS element. Also, it was shown that the level of eIF4a3 protein is dramatically induced by Se deficiency (31). Therefore, eIF4a3 has been proposed to selectively bind a subset of SECIS elements in the mRNA of nonessential genes and prevent Sec incorporation during Se limitation by masking the SBP2 binding site.

Another SECIS-binding protein that has been shown to influence the efficiency of UGA translation is nucleolin. Nucleolin is an abundant phosphoprotein located in the nucleolus, which is involved in rRNA synthesis and ribosome biogenesis. This protein also plays a role in regulation

of transcription and chromatin remodeling and contains four RNA recognition motifs (338). A search for SECIS-binding proteins by screening a cDNA library using a radiolabeled GPx1 SECIS as a probe identified nucleolin as a potential SECIS-binding protein (369). However, subsequent studies reported conflicting data on nucleolin's affinity for SECIS elements and its role in regulation of selenoprotein synthesis. In one study, nucleolin has been shown to selectively bind SECIS elements in selenoproteins that rank high in the hierarchy of selenoprotein expression, but it had low affinity for SECIS elements from nonessential selenoproteins (254). Using siRNA knockdown of nucleolin, this study demonstrated that nucleolin acts as a positive regulator for the translation of a subset of mRNAs that encode essential selenoproteins, but has no effect on nonessential selenoproteins. Since the activity of nucleolin is not regulated by Se, it was proposed that this protein may regulate selenoprotein expression by preferentially recruiting SBP2 or other factors of the Sec decoding machinery to selectively translate mRNA encoding essential selenoproteins (254). In contrast, in a separate study, nucleolin was shown to bind SECIS elements from a variety of selenoprotein genes with minimal differences, and likely does not contribute to the hierarchy of selenoprotein synthesis (322). In turn, this study proposed that SBP2 serves as a major regulator of differential selenoprotein mRNA translation and sensitivity to NMD by preferentially binding some SECIS elements with higher affinity compared with others (322). Nevertheless, conflicting data have been also reported showing overall lack of correlation between the SECIS element binding by SBP2 and Sec incorporation efficiency (85, 203). Considering that SECIS elements can be bound by a number of different proteins, including SBP2, SBP2L, eIF4a3, L30, nucleolin, and possibly others, it would not be surprising if multiple SECIS-binding proteins regulate the expression of selenoproteins in a combinatorial manner. Further studies are required to validate the physiological role of different SECIS-binding proteins in dictating the hierarchy of selenoprotein expression and to test their contribution in Sec insertion in different organs and tissues. Although the functions of core elements of the selenoprotein synthesis machinery have been established, the mechanism by which Sec is incorporated into proteins in eukaryotes is not completely understood, and it is not known whether other *trans*-acting factors can be involved in this process.

The idea that the decreased decoding efficiency of the UGA-Sec codon in stress-related selenoproteins may affect the susceptibility of selenoprotein transcripts to NMD under Se-deficient conditions has been further supported by recent ribosome profiling studies (145). At the translational level, Se deficiency results in decreased ribosome density downstream of the Sec-encoding UGA codon of several selenoprotein mRNAs, suggesting a slower rate of Sec incorporation (145). The genes whose mRNA abundance is affected by Se availability, i.e., *GPx1*, *MsrB1, SelW*, and *SelH*, demonstrated the greatest changes in translation under Se-deficient conditions. Moreover, it was shown that translation of these genes requires Um34 methylation of Sec-tRNA$^{[Ser]Sec}$ as a mutant Sec-tRNA$^{[Ser]Sec}$ (A37G), which prevents Um34 synthesis, also leads to decreased efficiency of Sec incorporation in this group of stress-related selenoproteins. Together, these data support the idea that Sec incorporation is a limiting step in selenoprotein biosynthesis, which, together with NMD, is primarily responsible for the regulation of selenoprotein expression by dietary Se.

F. Position-Dependent Incorporation of Sec Into Selenoproteins

Since the genetic code was deciphered in the 1960s, several variations in codon assignments were observed in nuclear and mitochondrial genomes. Later, expansion of the genetic code was made to include Sec and pyrrolysine as the 21st and 22nd naturally occurring amino acids that are encoded by termination codons UGA and UAG, respectively. A recent study demonstrated that in some organisms the UGA codon can also govern the insertion of two different amino acids (349). In the past, strict one-to-one correspondence between codons and amino acids was considered an integral part of the genetic code. Therefore, the observation that UGA can encode two amino acids challenged this paradigm. Genome analysis of the ciliate *Euplotes crassus* revealed that UGA can code for both Sec and Cys, and that identity of the inserted amino acid is governed by a specific 3′-untranslated region and location of the UGA within the mRNA. Moreover, it was found that this codon could serve its dual role in *E. crassus* even within the same mRNA, and that the structural properties of *Euplotes* selenoprotein mRNA were sufficient to preserve the location-dependent dual-function of UGA when expressed in a heterologous mammalian system.

How do organisms differentiate between the different functions of the UGA codon? The answer to this question appears to be dependent on the selenoprotein under consideration. The fact that the same UGA codon can code for both Sec and Cys in *E. crassus* even in the same protein with the choice of the inserted amino acid determined by the position of the codon within mRNA suggested that this organism tightly regulates insertion of Sec into proteins (349). In *Euplotes* TR1, the insertion of Sec is limited to its natural position despite the presence of multiple in-frame UGA codons. However, in mouse GPx1, Sec can be inserted into other places in the protein different from its natural position, although with lower efficiency (367). Furthermore, some selenoproteins contain multiple Sec residues. One such protein is SelP, which has 10 Sec residues in humans and also contains two SECIS elements within its 3′-UTR. The UGA codon encoding Sec can be located in various gene regions. Some selenoproteins contain Sec in the NH_2-terminal region, while others have Sec in the middle and

COOH-terminal regions, and the location of the SECIS elements and their distances from the Sec codon may vary greatly in mammals (122, 203). However, it remained unknown what factors determine whether Sec encoded by UGA is restricted to a specific site or can be inserted at any position. Structure and type of the SECIS element, its position, as well as the overall structure of selenoprotein mRNA have been proposed to mediate this decision. In a recent study, the SECIS element- and UGA position-dependent Sec insertion was examined in several mammalian selenoproteins (350). It was found that mammals also evolved the ability to limit Sec insertion into natural positions in some selenoproteins while in other selenoproteins the position at which Sec can be inserted is not restricted. In mammalian TR1 (TxnRd1) and TGR (TxnRd3), Sec insertion was UGA position dependent, but this was not the case in TR3 (TxnRd2), in which Sec could be inserted into different positions with similar efficiencies. Moreover, position-dependent Sec insertion was controlled by the SECIS element in the 3′-UTR and its structure, suggesting a possible role of interaction with SBP2 (350).

IV. SELENOPROTEINS AND THEIR DISTRIBUTION

During the early stages of selenoprotein research, identification of selenoproteins was largely dependent on experimental approaches, which included analyses of proteins for the presence of Se by mass spectrometry and detection of radioactive ^{75}Se that was metabolically incorporated into proteins in the form of Sec (16, 20). The first selenoprotein identified was mammalian glutathione peroxidase 1 (GPx1) (103), which was followed by the discovery of bacterial glycine reductase (353) and formate dehydrogenase (7) in 1973. Subsequently, a number of selenoproteins have been identified experimentally in all kingdoms of life (20, 115, 271). But it was not until bioinformatics approaches were developed, based on unique genomic features of selenoproteins (see below), that the genome-wide identification of selenoproteins was possible, and one of the first applications of these methods was the characterization of the entire human selenoproteome encoded by 25 selenoprotein genes (186). Due to further advances in bioinformatics tools and high-throughput sequencing technologies, the number of identified selenoproteins in diverse species has increased dramatically in recent years.

Since selenoproteins genes contain UGA codons, which are normally used as stop signals during protein translation, selenoproteins are often misannotated in sequence databases. Two major bioinformatics approaches have proven to be useful for identification of selenoproteins. The first approach is based on the fact that every selenoprotein gene possesses two characteristic genomic features, namely, an in-frame UGA codon encoding Sec residue and a SECIS element, which has a conserved sequence motif present only in selenoprotein mRNAs and possesses a specific stem-loop secondary structure. By finding candidate SECIS elements in completely sequenced genomes, it became possible to identify selenoprotein genes through analyses of upstream coding sequences containing UGA (49, 116, 246). The sensitivity of this technique was further improved by analyzing the genomes of closely related species for evolutionarily conserved SECIS elements belonging to orthologous selenoproteins in these organisms (186). Although this approach has been successfully used to predict a large number of selenoproteins, it also has some limitations due to different patterns of SECIS elements present in distant species. As such, parameters and sequence patterns had to be adjusted when newly sequenced genomes from distant species were analyzed. To overcome this limitation, new SECIS prediction tools have been developed that incorporate a scoring procedure based on secondary RNA structure predictions allowing to significantly improve the sensitivity of this approach (244).

In the second approach, selenoproteins are identified by searching for in-frame UGA codons through analyses of sequences adjacent to UGA in completely sequenced genomes (49, 186, 187, 383). Because almost all selenoproteins contain homologs in which Sec is replaced with Cys, this approach allowed identification of selenoproteins independent of searches for SECIS elements (104, 107). Recently, a new tool, Seblastian, was developed for selenoprotein gene prediction and analysis, which combines the improved SECIS prediction-based approach for identification of selenoproteins (SECISearch 3) with the SECIS-independent method that relies on Sec/Cys homologs (244). Seblastian is currently the method of choice for detection and analysis of eukaryotic selenoproteins. It can detect the absolute majority of selenoprotein genes in sequenced genomes and large sequence databases. It should be noted that, in addition to identification of new selenoproteins, the Sec/Cys homology approach can be used for identification of catalytic redox-active Cys residues in non-selenoprotein enzymes. The fact that Sec plays important catalytic functions in most of the known selenoproteins has been recently used to develop a bioinformatics procedure for high-throughput identification of redox-active Cys in proteins by searching for sporadic occurrence of Sec/Cys pairs in sequence databases (107).

Currently there are more than 50 selenoprotein families known, the majority of which have been identified by bioinformatics approaches **(FIGURE 7)**, and it is likely that many additional selenoproteins will be discovered by searching genomes and other sequences. The distribution of selenoproteins varies greatly among species. Although selenoproteins are widespread in all three domains of life, i.e., eukarya, archaea, and eubacteria, the number of selenoproteins in individual selenoproteomes (a full set of selenoproteins in an organism) can range from one, e.g., in the

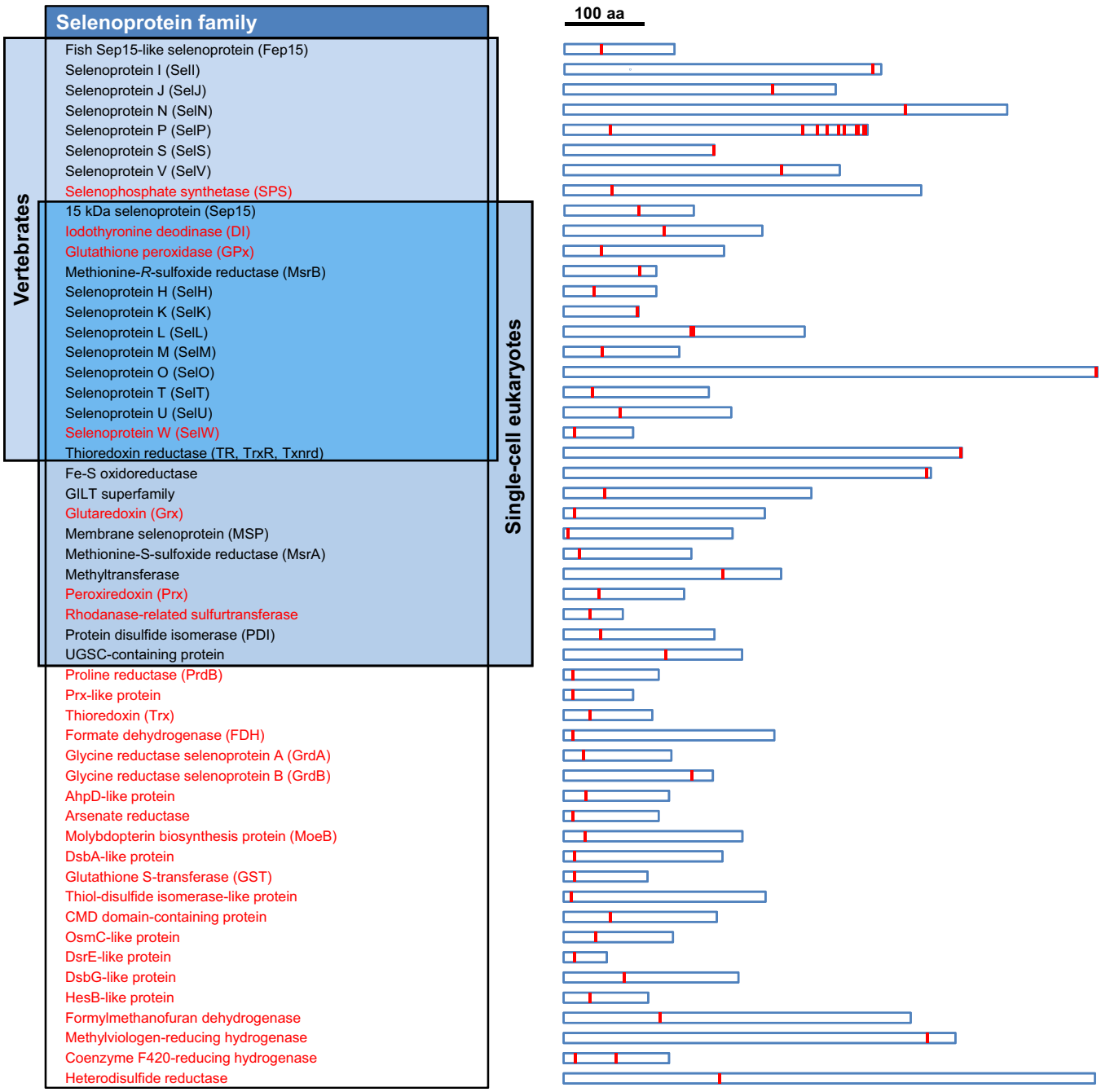

FIGURE 7. Selenoprotein families. Selenoproteins that are found in vertebrates or single-cell eukaryotes are indicated by shaded boxes, whereas selenoproteins that occur in prokaryotic organisms are highlighted in red. The relative size of each selenoprotein is shown on the *right*, and the location of Sec residues is indicated by red lines.

nematode *C. elegans* (341), to as many as 59 found in the pelagophyte *Aureococcus anophagefferens* (118). Moreover, selenoproteins were completely lost in fungi, higher plants, and some animal species, including beetles, silkworms, and several other insects (227). Analyses of available selenoproteomes can provide information about evolutionary trends regarding utilization of Sec. For example, comparative analyses of organisms containing large selenoproteomes with those lacking selenoproteins or containing a small number of selenoproteins revealed that several groups of terrestrial organisms reduced their utilization of Se by replacing selenoproteins with Cys homologs or completely losing some selenoproteins. In contrast, most aquatic organisms have large selenoproteomes, suggesting that environment plays a role in the evolution of selenoproteins (225). Interestingly, more than half of the identified selenoprotein families are present in both single-cell eukaryotes and vertebrates, indicating that they have an ancient origin. However, there are several selenoprotein families that are found only in vertebrates. Vertebrate-specific

selenoproteins include Fep15, SelI, SelJ, SelN, and SelP, whereas SelV evolved uniquely in placental mammals (245).

Analyses of vertebrate and mammalian selenoproteomes also demonstrated a trend toward reduced selenoprotein usage in mammals (226), but the specific evolutionary forces require further investigation (48). Indeed, all Cys/Sec replacements found in mammals were from Sec to Cys. Although some selenoprotein genes appeared in mammals (GPx6, SelV, GPx5, the latter immediately converted to a Cys version), they evolved by gene duplication. The number of Sec residues in SelP also varies dramatically, from 7 (e.g., naked mole rats) to 15 (e.g., dogs). Both the number of Sec moieties in SelP and the number of selenoproteins in mammalian selenoproteomes, however, are lower than those in fish. Thus all mammals appear to have reduced the use of Sec compared with fish and other vertebrates (245) and, in addition, some mammals further reduced Sec utilization. The most dramatic reduction was observed in naked mole rats, which reduced the levels of Se and the expression of GPx1 in liver and kidney (167a).

V. PHYSIOLOGICAL ROLES OF SELENOPROTEINS

One key feature common for all selenoproteins is the presence of Sec residues in their sequence. With a few exceptions, this amino acid is located in the enzyme active sites and was adopted to perform catalytic redox reactions **(FIGURE 8)** (11). Therefore, the physiological functions of selenoproteins strictly depend on the presence of Sec, and mutations of Sec to any other amino acid residue leads to enzyme inactivation. The human selenoproteome is encoded by 25 genes **(FIGURE 9)**. Although all functionally characterized selenoproteins have redox-active functions, Sec is utilized to serve diverse biological roles. We further discuss the known physiological functions of human selenoproteins and highlight recent insights into the roles of selenoproteins in human health and disease.

A. Glutathione Peroxidases

Selenoproteins of the glutathione peroxidase (GPx) family are widespread in all three domains of life (345). In mammals, there are eight GPx paralogs, from which five (GPx1, GPx2, GPx3, GPx4, and GPx6) contain a Sec residue in their active site. In the other three GPx homologs (GPx5, GPx7, and GPx8), the active-site Sec is replaced by Cys. Moreover, GPx6 homologs in some mammals are not selenoproteins and have a Cys in the active site (186). The Cys-containing GPx homologs also prevail in bacteria, protozoa, fungi, and terrestrial plants. GPxs play a wide range of physiological functions in organisms and are involved in hydrogen peroxide (H_2O_2) signaling, detoxification of hydroperoxides, and maintaining cellular redox homeostasis.

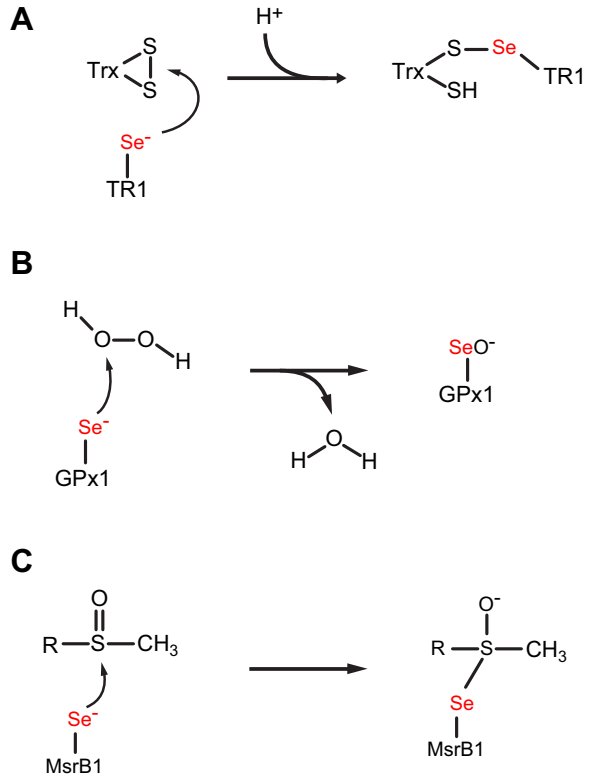

FIGURE 8. Redox reactions catalyzed Sec-containing oxidoreductases. First steps in the catalytic cycles are shown for thioredoxin reductase 1 (Trx1) (*A*), glutathione peroxidase 1 (GPx1) (*B*), and methionine sulfoxide reductase B1 (MsrB1) (*C*).

GPx1 is the most abundant selenoprotein in mammals. This cytosolic enzyme catalyzes glutathione (GSH)-dependent reduction of hydrogen peroxide to water (235). It is also the first mammalian protein whose gene was found to contain Sec-encoding UGA in the open reading frame (101). GPx1 received much attention as an intracellular antioxidant enzyme scavenging toxic H_2O_2 molecules (reviewed in Ref. 235). In recent years, H_2O_2 has been also implicated as an important signaling molecule that regulates a variety of biological processes and pathways, including cell proliferation, apoptosis, stress response, and mitochondria-related functions (73). As GPx1 degrades H_2O_2, its reduced activity or deficiency results in elevated intracellular H_2O_2 levels, whereas overexpression of GPx1 may lead to reductive stress and may disrupt H_2O_2 signaling. Therefore, many of the physiological roles of H_2O_2 are modulated by this enzyme.

Reduction of hydrogen peroxide to water consumes GSH. However, not all GPxs utilize GSH as originally predicted, as some GPx homologs have specificity for thioredoxin or other thiol oxidoreductases. Initially, reduction of H_2O_2 by GPx1 involves the conversion of the active-site Sec residue to the selenenic acid intermediate (-Se-OH) (103, 184, 355). During the second step, the selenenic acid is reduced by one molecule of GSH, leading to formation of a glutathiony-

Selenoprotein	Abbreviation	Sec location (protein size)	Function
Glutathione peroxidase 1	GPx1, GPX1	47 (201)	Cytosolic glutathione peroxidase
Glutathione peroxidase 2	GPx2, GPX2	40 (190)	Gastrointestinal glutathione peroxidase
Glutathione peroxidase 3	GPx3, GPX3	73 (226)	Plasma glutathione peroxidase
Glutathione peroxidase 4	GPx4, GPX4	73 (197)	Phospholipid hydroperoxide glutathione peroxidase
Glutathione peroxidase 6	GPx6, GPX6	73 (221)	Olfactory glutathione peroxidase
Iodothyronine deodinase 1	DI1, D1, DIO1	126 (249)	Thyroid hormone-activating iodothyronine deodinase
Iodothyronine deodinase 2	DI2, D2, DIO2	133, 266 (273)	Tissue-specific thyroid hormone-activating iodothyronine deodinase
Iodothyronine deodinase 3	DI3, D3, DIO3	144 (278)	Tissue-specific thyroid hormone-deactivating iodothyronine deodinase
Thioredoxin reductase 1	TR1, TrxR1, TXNRD1	498 (499)	Reduction of cytosolic thioredoxin
Thioredoxin/glutathione reductase	TGR, TR2, TrxR3, TXNRD3	655 (656)	Testis-specific thioredoxin reductase
Thioredoxin reductase 3	TR3, TrxR2, TXNRD2	522 (523)	Reduction of mitochondrial thioredoxin and glutaredoxin
Methionine-R-sulfoxide reductase	MsrB1, SelR, SelX, MSRB1	95 (116)	Reduction of oxidized methionine residues
Selenophosphate synthetase 2	SPS2, SEPHS2	60 (448)	Involved in synthesis of selenoproteins
Selenoprotein W	SelW, SEPW1	13 (87)	Unknown
Selenoprotein T	SelT	36 (182)	Unknown
Selenoprotein H	SelH	38 (116)	Unknown
Selenoprotein V	SelV	273 (346)	Unknown
Selenoprotein I	SelI, SEPI, EPT1	387 (397)	Unknown
15 kDa selenoprotein	Sep15	93 (162)	Putative role in quality control of protein folding in the ER
Selenoprotein M	SelM, SEPM	48 (145)	Unknown
Selenoprotein K	SelK	92 (94)	Putative role in ER-associated degradation
Selenoprotein S	SelS, SEPS1, VIMP	188 (189)	Putative role in ER-associated degradation
Selenoprotein O	SelO	667 (669)	Unknown
Selenoprotein N	SelN, SepN, SEPN1,	428 (556)	Putative role during muscle development
Selenoprotein P	SelP, SEPP1	59, 300, 318, 330, 345, 352, 367, 369, 376, 378 (381)	Se transport

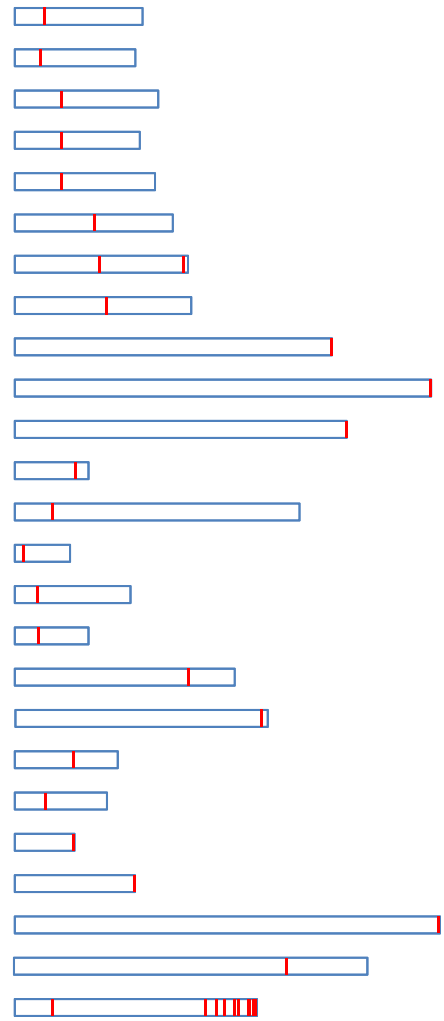

FIGURE 9. Human selenoproteome. The relative length of selenoproteins and location of Sec within different proteins are shown on the *right*.

lated selenol (-Se-SG) intermediate (103, 184). Then, this intermediate reacts with a second GSH molecule restoring the enzyme's active site Sec and producing a single molecule of oxidized glutathione (GSSG). GSSG is subsequently reduced by glutathione reductase (GR), which consumes NADPH as a source of reducing equivalents.

It has been demonstrated that mammalian GPx1 forms a homotetramer (13, 102). Structurally, GPx1 is characterized by a thioredoxin fold (202, 248) and consists of seven β-strands, five of which form a central β-sheet surrounded by four α-helixes on the surface of the protein **(FIGURE 10)**. Moreover, the crystal structure of the mammalian GPx1 revealed that Sec46 also occurs on the protein surface in a catalytic triad containing Gln81 and Trp136, which are required for enzyme-substrate interactions (92). These amino acid residues demonstrate strict conservation in all Sec containing GPxs, and their mutation disrupts GPx enzymatic activity (239, 240). In addition, an Asn residue (Asn137, numbering is based on human GPx4) was found to be necessary for enzymatic function (346). This amino acid is located in close proximity to catalytic Sec and was proposed to form a catalytic tetrad in the active site. GPx4 is structurally similar to other GPxs, but in contrast to GPx1, it is a monomeric protein. Structural comparison also showed that GPx4 lacks an internal loop (loop 1), which partially shields the active site in GPx1. It was proposed that the position of this loop in the structure prevents binding of bulky lipid hydroperoxide substrates by GPx1 (314).

Mammalian Sec-containing GPxs have overlapping substrate specificities and tissue distribution. GPx1 is a ubiquitous enzyme expressed in all cell types, with the highest expression levels observed in the liver and kidney. In contrast, expression of GPx2 and GPx3 is limited to certain tissues. GPx2 is primarily found in the epithelium of the gastrointestinal tract, whereas GPx3 is secreted primarily

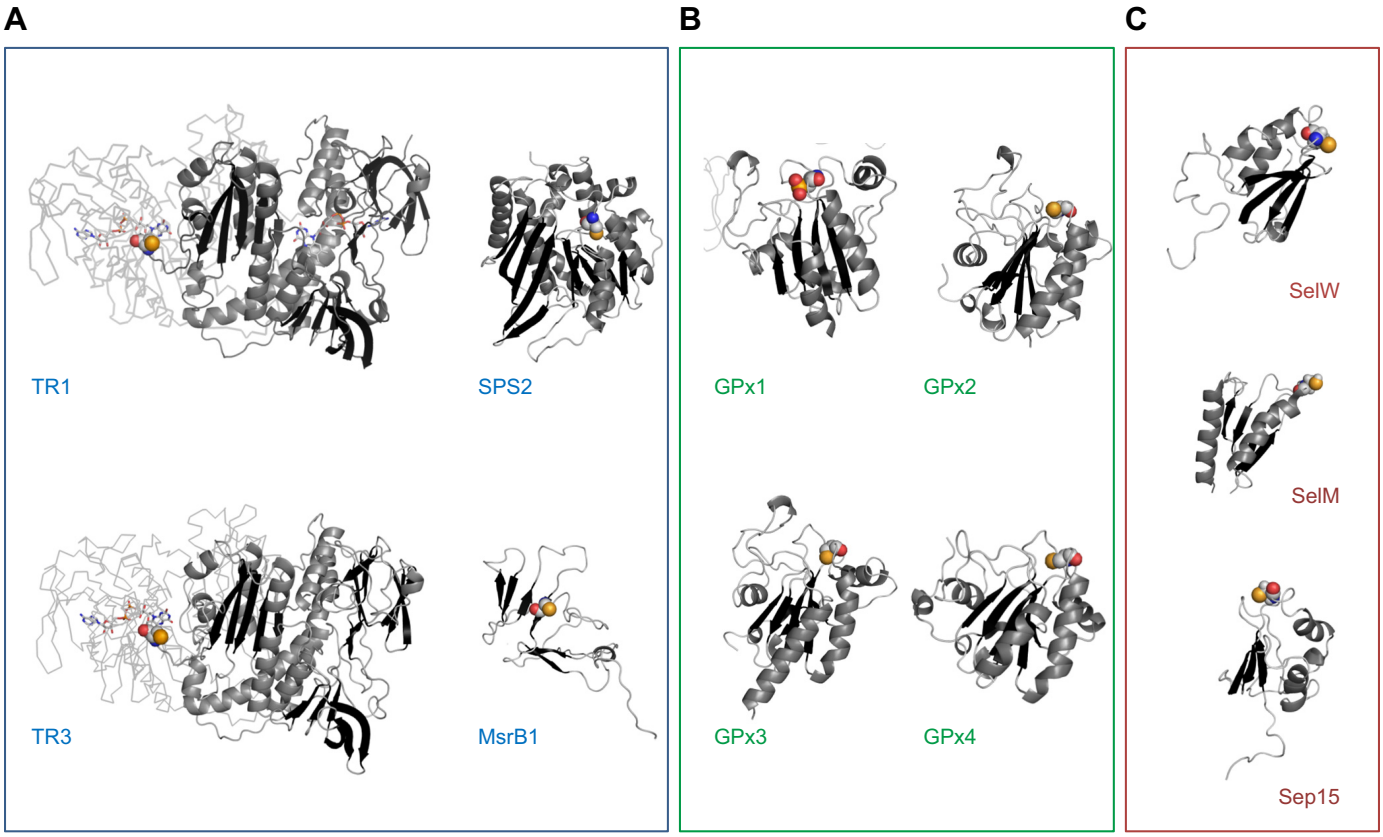

FIGURE 10. Structures of mammalian selenoproteins. *A*: non-thioredoxin-fold selenoproteins. *B*: thioredoxin-fold proteins of the glutathione peroxidase (GPx) family. *C*: other (i.e., non-GPx) thioredoxin-fold selenoproteins. Helices are shown in dark gray, strands in black, and coils in light gray. For each protein, the catalytic Sec/Cys are shown as spacefills. Both mammalian TR1 and TR3 are homodimers, with one monomer shown in a ribbon representation, and the other monomer is shown as a backbone structure. The COOH terminus of TR3 (pdb code 1zdl) reported in the figure has been modeled based on the structure of human TR1 (pdb code 3ean). GPx1, GPx2, and GPx3 are natural homotetramers (only one monomer is shown). GPx1 has been crystallized with its Sec overoxidized.

from kidney and is the major GPx form in plasma. GPx4 is expressed in a wide range of cell types and tissues, and GPx6 is only found in olfactory epithelium and during embryonic development (186). All tetrameric vertebrate GPxs, including GPx1, GPx2, GPx3, and possibly GPx6, have specificity for hydrogen peroxide and other soluble, low-molecular-weight hydroperoxides, e.g., *tert*-butyl hydroperoxide, cumene hydroperoxide, and fatty acid hydroperoxides. In turn, monomeric GPx4 is involved in the reduction of complex phospholipid hydroperoxides such as phospatidylcholine hydroperoxide and cholesterol hydroperoxide that are associated with membranes. As discussed above, specificity of GPx4 for phospholipid hydroperoxides is determined by the configuration of its active site, which lacks a loop structure present in GPx1 and other tetrameric GPxs and allows access for bulky phospholipid hydroperoxide molecules. The specificity of this enzyme for lipid hydroperoxides has also been attributed to the presence of positively charged residues near the active site that facilitate binding of the negatively charged phospholipids (322).

Being selenoproteins, GPx family proteins are also subject to regulation at posttranscriptional and translational levels. GPx1 belongs to a group of stress-related selenoproteins and is highly regulated by Se availability (15, 336). During Se deficiency, the level of GPx1 in the liver and kidney of mice is significantly reduced. In contrast, restoration of Se in the diet leads to a dramatic increase in GPx1 activity. However, GPx4 levels are less affected by Se availability, and this protein belongs to a subclass of housekeeping selenoproteins (22, 129, 365).

GPx1 has been considered as one of the major antioxidant enzymes, and together with catalases and peroxiredoxins, it has been implicated in protection of cells from oxidative damage by degrading toxic H_2O_2 (235). Knockout mouse models lacking GPx1 as well as overexpressing GPx1 have been developed. GPx1 knockout mice are viable, but are characterized by an increased susceptibility to oxidative stress (56, 111). Therefore, GPx1 appears to play a protective role under conditions of oxidative stress, but is dispensable under unstressed conditions, consistent with its low position in the

hierarchy of selenoprotein expression. In contrast, overexpression of GPx1 in mice and cell culture models is associated with impaired signaling through redox-sensitive signaling cascades, including those regulated by tumor necrosis factor (TNF)-α (185) and the epidermal growth factor (128) as well as insulin signaling (212). As H_2O_2 serves as an important signaling molecule, both GPx1 deficiency and its increased activity are expected to regulate H_2O_2-mediated responses. According to one of the models, H_2O_2 can directly oxidize target proteins (e.g., receptor proteins that activate downstream signaling cascades) (196). Oxidation of regulatory amino acids in such proteins can lead to alteration of their structure and augment protein function. In this situation, GPxs indirectly participate in H_2O_2 signaling by lowering the level of hydroperoxides. This mode of regulation is observed, for example, in tyrosine protein phosphatase 1B (300) and PTEN (194). According to the second model, GPx family proteins and other thiol-dependent peroxidases can serve as sensors of H_2O_2 levels in the cell, which then transfer oxidizing equivalents from H_2O_2 to target proteins (196). Support for this model was provided by a recent observation that deletion of all eight peroxidases in yeast (yeast encodes 3 GPxs and 5 peroxiredoxins) renders cells unable to sense H_2O_2 and activate transcriptional response to oxidative stress (105). Whether GPxs play a role in H_2O_2 sensing in mammals remains unknown. At least in the case of GPx4, this mode of regulation may be possible since mammalian GPx4 has been shown to oxidize thiols in proteins (126, 242). Although GPxs are perceived as antioxidant enzymes, several studies have demonstrated that GPx1 might also play a pro-oxidant role under certain conditions. For example, increased GPx1 activity in mice overexpressing GPx1 sensitized mice to acetaminophen hepatotoxicity and increased hepatic protein nitration by depleting intracellular GSH, which serves as a major scavenger of peroxynitrite ($ONOO^-$) (255). Consistently, knockout of GPx1 protected primary hepatocytes against peroxynitrite-induced cytotoxicity and apoptosis (387), suggesting that GPx1 does not prevent, but rather may cause oxidative stress by promoting accumulation of reactive nitrogen species.

An important role of GPx1 in regulation of metabolic processes has received much attention recently due to the finding of the development of type 2 diabetes-like phenotypes in GPx1-overexpressing mice (250). Paradoxically, increased levels of GPx1 in these mice were associated with increased insulin resistance, hyperinsulinemia, hyperglycemia as well as with the development of obesity. It was proposed that these changes in GPx1-overexpressing mice might be explained by elevated pancreatic β-cell mass and β-cell insulin secretion caused by disrupted reactive oxygen species (ROS) signaling (250, 285). By degrading H_2O_2, high GPx1 levels have been implicated in both promoting insulin secretion through activation of PDX1 transcription factor-dependent differentiation of pancreatic β-cells and increased insulin secretion due to lower levels of UCP2 protein (364). The development of type 2 diabetes-like phenotypes in GPx1-overexpressing mice might also be attributed to dysregulation of insulin signaling in insulin target tissues, which in turn reflects lower phosphorylation of insulin receptor and AKT following insulin stimulation as a result of decreased intracellular H_2O_2 levels. Consistent with the central role of GPx1 in metabolic regulation, GPx1 deficiency and consequently increased levels of H_2O_2 in GPx1 knockout mice were associated with improved insulin sensitivity and attenuated development of high-fat-diet induced obesity (229). In accordance with these observations, the results of several clinical trials that investigated the chemopreventive effects of dietary Se supplementation revealed that increased intake of Se above the nutrient requirements may raise the risk of type 2 diabetes mellitus in humans (222, 327). Although the exact mechanism of the diabetogenic effect of high Se intake remains unclear, increased expression of GPx1, SelP, MsrB1, and SelS has been implicated in the development of type 2 diabetes and insulin resistance (198, 256, 287, 382). At least some of these effects could be attributed to diminished intracellular H_2O_2 levels as a result of high GPx1 activity leading to suppression of insulin signaling and increased insulin secretion, similar to GPx1-overexpressing mice (386).

Another protein of the GPx family, GPx2, has been shown to play a role in the development of cancer (17). GPx2 is preferentially expressed in the gastrointestinal epithelium, but its elevated expression was also found in epithelium-derived tumors. However, whether GPx2 has a protective role against cancer and/or promotes cancer development still remains an open question (30). One line of evidence supporting a cancer preventive role of GPx2 comes from the finding that GPx2 gene expression is under control of the antioxidant response transcription factor Nrf2. In addition, mice lacking the GPx2 gene are characterized by an increased sensitivity to UV-induced skin cancer development (363), whereas the double GPx1/GPx2 knockout mice demonstrated the development of spontaneous colitis and intestinal cancer (64). However, expression of GPx2 has also been shown to be regulated by the Wnt pathway, which is involved in activation of cellular proliferation, and knockdown of GPx2 in rat and human cancer cells resulted in significant growth inhibition (263) and induction of apoptosis (375). Together, these observations indicate that GPx2 might have a dual role in cancer similar to thioredoxin reductase 1 and Sep15 (discussed below) by both preventing and promoting tumor cell growth at the later stages of cancer development.

Although the active site residues in all selenoprotein GPx enzymes are conserved, and their catalytic mechanisms are essentially identical, the physiological roles of GPx family proteins are far more diversified. Among other GPxs, GPx4 stands out because of its broad substrate specificity and

ability to reduce complex lipid hydroperoxides and use protein thiols as donors of electrons in addition to GSH (354, 355). Under normal physiological conditions, GSH is abundant in somatic cells and is primarily used by GPx4. As GPx4 is involved in the reduction of membrane-bound phospholipid and cholesterol hydroperoxides, inhibition of lipid peroxidation is considered to be its primary function in most tissues, except for testis (305). This enzyme, together with vitamin E, which slows down the oxidative chain reaction driven by lipid peroxyl radicals (R-OO$^{\cdot}$), plays an important protective role by preventing lipid decomposition (241). In addition to spontaneous lipid peroxidation, several physiological processes, e.g., recycling of mitochondria in reticulocytes, depend on enzymatic activity of 12/15-lipoxygenase (LOX) (291). By converting phospholipid hydroperoxides (R-OOH) to corresponding alcohols (R-OH), GPx4 prevents initiation of new chain reactions and serves as antagonist of 12/15-LOX preventing nonenzymatic lipid peroxidation (305). As knockout of the GPx4 gene in mice leads to embryonic lethality, it was proposed that the role of GPx4 in inhibiting lipid peroxidation is essential during early embryo development (152, 376). Moreover, GPx4 has been postulated to play an important role in preventing oxidative stress-induced apoptosis. Studies in which GPx4 was conditionally deleted revealed that GPx4 deficiency leads to increased lipid peroxidation and cell death in knockout cells (310). This lipid peroxide-induced apoptosis occurs through a caspase-independent mechanism and is dependent on functional 12/15-LOX lipoxygenase and activation of apoptosis inducing factor (AIF). The role played by GPx4 in regulating lipid peroxide-stimulated cell death has important physiological implications. Many neurodegenerative diseases are associated with low GSH status leading to inhibition of GPx4 activity (378). Therefore, this novel redox-regulated cell death pathway links GPx4 to preventing neurodegeneration.

Among other functions, GPx4 has also been implicated in regulating protein tyrosine phosphatases (PTPs), which represent well-studied in vivo targets of H_2O_2 signaling. Due to reversible oxidation of the Cys residue in the active site of PTPs, H_2O_2 has been shown to inhibit their function. A recent study demonstrated that, in addition to H_2O_2, PTPs are also subject to inactivation by lipid hydroperoxides (69). Therefore, similar to GPx1 and peroxiredoxin II (PrxII), which regulate PTPs by modulating levels of H_2O_2, GPx4 may participate in the control of PTP proteins by degrading lipid hydroperoxides.

Under conditions of low GSH levels, e.g., observed during sperm maturation, GPx4 can also use protein thiols as donors of electrons (354). In spermatids, during the early stages of sperm maturation, GPx4 is expressed at high levels and is catalytically active. But it becomes inactivated and serves a structural function in mature sperm cells. GPx4 was found to form cross-linked high-molecular-weight polymers due to formation of intramolecular selenenylsulfide bridges involving Cys and the active site Sec residues (167). These high-molecular-weight structures containing GPx4 and another protein named sperm mitochondrion-associated cysteine-rich protein (SMCP) form a protective capsule that surrounds tightly packed mitochondria in the midpiece of spermatozoa. SMCP contains a small unstructured motif, PPCCPP, which serves as a substrate of GPx4 (242). When GSH becomes limiting, GPx4 catalyzes formation of a disulfide bond between adjacent Cys-containing motifs in SMCP, which can be further reshuffled to cross-link other capsular proteins containing Cys residues. There are three different alternatively spliced *GPx4* mRNA isoforms that code for cytosolic (cGPx4), mitochondrial (mGPx4), and nuclear (nGPx4) proteins. The cytosolic (short) form of GPx4 is ubiquitously expressed both during embryonic development and in adult organs and tissues. However, nuclear and mitochondrial isoforms, which differ from the cytosolic form by their NH_2-terminal sequences, are expressed only in testes. Whereas isoform-specific knockout of cGPx4 leads to embryonic lethality (218), mGPx4$^{-/-}$ (304) and nGPx4$^{-/-}$ (68) mice are viable. In addition, nGPx4$^{-/-}$ mice do not demonstrate significant defects in sperm maturation and fertility (68). However, the lack of mitochondrial GPx4 causes male infertility in mice, consistent with its role during male gametogenesis (304).

B. Thyroid Hormone Deodinases

The iodothyronine deodinase family of selenoproteins consists of three paralogous proteins in mammals (DI1, DI2, and DI3), which are involved in regulation of thyroid hormone activity by reductive deodination. These proteins have distinct subcellular localizations and tissue expression. DI1 and DI3 are located on the plasma membrane, but DI2 is localized to the endoplasmic reticulum (ER). Surprisingly, homologs of mammalian deodinases occur not only in other vertebrates, but are also found in simple eukaryotes and bacteria. The function of deodinase homologs in these organisms is not known.

Deiodinases are integral membrane selenoproteins characterized by a thioredoxin fold. All three deiodinase selenoenzymes contain a single transmembrane domain and form a homodimer structure. The active-site Sec residue is located in the NH_2-terminal part of the protein. However, in DI2, an additional Sec, whose function is unknown, is present in the COOH-terminal region close to the end of the protein. This residue does not participate in the catalytic mechanism and is dispensable for the D2 functional activity (301). Despite low (<50%) sequence identity among the three deodinase members, they expectedly have similar overall topology and structural organization (39). Each protein contains a single transmembrane domain at the NH_2 terminus followed by $\beta\alpha\beta$ and $\alpha\beta\beta$ motifs commonly present in thioredoxin-fold proteins. The two parts of the thi-

oredoxin-fold domains in deodinases are separated by a large region that shares sequence homology with the active site of α-L-iduronidase (IDUA), a glycosidase involved in removal of α-L-iduronic acid residues during degradation of the glycosaminoglycans.

The majority of thyroid hormone that is produced by thyroid is secreted in its inactive form, thyroxine (T_4). This prohormone can be converted to active thyroid hormone, 3,3′,5-triiodothyronine (T_3), by outer ring deiodination in a reaction catalyzed by DI1 and DI2 (24). In turn, T_3 and T_4 can be inactivated by DI3, and under specific conditions by DI1, which catalyze the removal of the inner ring iodine leading to formation of inactive T_2 and reverse T3 (rT_3), respectively. Therefore, deiodinases play an important role in maintaining levels of thyroid hormone and its activity by both activating its prohormone and degrading the biologically active T_3. The circulating levels of thyroid hormone are primarily regulated by DI1 activity. However, DI2 and DI3 have been implicated in fine-tuning local intracellular T_3 concentrations in a tissue-specific manner, without changing overall serum levels of T_3 (114). This local regulation of thyroid hormone activity is important for a number of physiological processes, e.g., tissue regeneration after injury and in specific tissues during development. One of the major effects of DI2 deficiency is observed in skeletal muscle tissue. During normal development, DI2 expression reaches maximum levels in skeletal muscles just after birth and then decreases in the postnatal period. Moreover, activity of DI2 is increased in muscle following injury and is associated with enhanced transcription of T_3-dependent genes that are required for muscle differentiation and regeneration implicating DI2 in muscle regeneration process (79). The role of DI2-mediated increases in T_3 in muscle differentiation and regeneration was further confirmed by DI2 knockout studies. First, knockdown of DI2 by RNAi could block myoblast differentiation, whereas this inhibitory effect could be partially reversed by external supplementation of high concentrations of T_3. Moreover, DI2 knockout mice are characterized by delayed muscle repair after injury and decreased expression of many T_3-dependent muscle differentiation genes, including MyoD (a master regulator of muscle myogenesis), Myogenin, SERCA2, and Tropinin 2. The gene coding for DI2, *Dio2*, is itself under the control of the Forkhead box O3 (FoxO3) transcription factor that, together with Pax3/7, has been implicated in stimulation of muscle differentiation (79). Conversely, decreased levels of FoxO3 lead to decreased *Dio2* expression in myogenic precursor cells and inhibit their differentiation.

Another well-studied example of tissue-specific regulation of thyroid hormone by DI2 includes its effect on brown adipose tissue (BAT) during adaptive thermogenesis (318). In response to cold exposure, expression of DI2 in BAT of rats is induced up to 50-fold leading to activation of thyroid hormone in this tissue, whereas serum levels of T3 in cold-exposed rats do not significantly change. This local increase in T3 allows adaptation of animals to cold exposure by activating thyroid hormone receptor-mediated gene expression program that stimulates BAT thermogenesis (317). Consistent with the role of DI2 in regulating BAT thermogenesis, DI2 knockout mice are characterized by impaired adaptation to cold exposure (76).

In turn, DI3 exerts an opposite effect on tissue-specific thyroid hormone action by inactivating T_4 and T_3. For example, in basal cell carcinomas (BCCs), which are characterized by increased signaling of the sonic hedgehog pathway, sonic hedgehog-dependent overexpression of DI3 leads to inactivation of T_3 (78). As thyroid hormone signaling affects the balance between proliferation and differentiation, it appears that decreased T_3 levels caused by enhanced DI3 expression contribute to high proliferation rates of BCC tumors. In fact, knockdown of DI3 in BCC cells caused significant reduction of BCC growth in vivo. Importantly, sonic hedgehog-dependent decrease of active T_3 in BCC occurs independently from serum levels of thyroid hormone. Therefore, deodinases exert their physiological functions by controlling thyroid hormone concentrations in a precise spatio-temporal manner.

C. Thioredoxin Reductases

Thioredoxin reductases (TRs) are oxidoreductases that, together with thioredoxin (Trx), comprise the major disulfide reduction system of the cell. In mammalian cells, there are three TR isozymes, all of which are Sec-containing proteins. These proteins contain a Sec residue in the COOH-terminal penultimate position. Thioredoxin reductase 1 (TR1, also known as TrxR1 and TxnRd1) is primarily localized in the cytosol and nucleus. Although cytosolic thioredoxin (Trx1) serves as the major substrate for TR1, TR1 can also reduce a variety of low-molecular-weight compounds (12). There are at least six different TR1 isoforms in mammals, which are produced by alternative splicing and NH_2-terminal extension of the protein due to use of distinct transcription initiation sites (298, 331, 334). The second member of the TR protein family is thioredoxin reductase 3 (TR3, also designated TxnRd2 and TrxR2). TR3 is localized in the mitochondria, where it is involved in reduction of mitochondrial thioredoxin (Trx2) and glutaredoxin 2 (Grx2). Similar to TR1, multiple TR3 isoforms have been described (352). Both TR1 and TR3 are present in all vertebrates, and knockout of either of them leads to embryonic lethality in mice (29, 67, 160). The third TR homolog in mammals is thioredoxin/glutathione reductase (TGR, also designated TR2, TxnRd3, and TrxR3). This protein differs from TR1 and TR3 in that TGR contains an additional glutaredoxin (Grx) domain, which is located in the NH_2-terminal part of the protein (333). Due to the presence of the Grx domain, TGR displays Grx activity, suggesting that this protein is involved in both Trx and GSH systems. However, the phys-

iological function of TGR remains unknown. TGR is expressed at high levels in testis after puberty, and it was proposed that TGR might be involved in the formation/isomerization of disulfide bonds during sperm maturation (332). Two distinct TGR isoforms are found in mice, wherein the long form is produced by an alternative CUG start codon, as opposed to the normally used AUG (113).

The major physiological role of TR1 is the NADPH-dependent reduction of Trx1. Being a major protein disulfide reductase in the cell, Trx1 is involved in the control of many physiological processes (e.g., antioxidant defense, regulation of transcription factors, and apoptosis) (12, 266) and serves as an electron donor for redox-active enzymes, including ribonucleotide reductases, peroxiredoxins, and methionine sulfoxide reductases (51, 323, 328). Another less characterized protein substrate of TR1 is thioredoxin-related protein 14 (TRP14) (163). Similar to Trx1, TRP14 serves as a disulfide reductase, although it acts on a distinct set of protein targets. TRP14 has been shown to activate NF-κB by stimulating TNF-α signaling (162). In addition to Trx1 and Trp14, TR1 can reduce nondisulfide substrates such as hydroperoxides, vitamin C, and selenite. A second system capable of reducing protein disulfides in the cell consists of Grx and glutathione reductase (GR). Grx catalyzes reduction of disulfides in proteins using GSH as a reductant, whereas GR participates in reduction of GSSG using NADPH as an electron donor. Therefore, Trx and Grx systems have partially overlapping functions under conditions when one of the systems is disrupted, and it has been demonstrated that these reductive pathways can partially compensate for each other in bacteria (326, 360).

Crystal structures of rat cytosolic TR1 (55, 302) and mouse mitochondrial TR3 (25) have been described, which revealed the details of their catalytic mechanism. Structural analyses showed that mammalian TR1 and TR3 have very similar structures resembling more closely that of GR rather than prokaryotic TRs. TRs form a homodimeric structure that has two subunits oriented in a "head-to-tail" manner **(FIGURE 10)**. Each subunit consists of FAD-binding and NADPH-binding domains, and an interface domain located between two monomer subunits. Compared with GRs, mammalian TRs contain a highly conserved 16-residue extension at the COOH terminus of the protein with Sec present in a characteristic Gly-Cys-Sec-Gly motif. Structural studies revealed that the very end of this conserved extension is flexible, which allows transferring electrons from the buried NH_2-terminal redox-active site to the surface of the enzyme and supports interaction with Trx. Moreover, the crystal structure of the Sec-containing selenoprotein TR1 showed that Sec forms an unusual selenenylsulfide bond with the adjacent Cys residue in oxidized TR1 and that a closely located Tyr116 is involved in the electron transfer between two active sites (55).

Although TR1, together with Trx1, has been implicated in general antioxidant defense by serving as an electron donor for peroxiredoxins and methionine sulfoxide reductases, its multiple regulatory roles are not entirely understood. Recent studies have demonstrated that the Trx system might be directly involved in selective activation of target proteins. One such example includes PTPs, which modulate the activity of a wide range of intracellular signaling pathways. PTPs are subject to inhibitory oxidation by ROS produced by growth factor receptor stimulation, but specific mechanisms that can reverse the oxidation and restore the enzyme's activity remained unknown. Recently, TR1 has been shown to selectively reactivate oxidized PTP1B, via either Trx1 or TRP14 substrates, and modulate PDGF-β receptor tyrosine kinase signaling (75). Although the molecular basis and mechanism by which PTP oxidation is reversed by the Trx system remain unknown, these findings open up the possibility that the Trx system might control the oxidation state of a more broad set of PTPs (and possibly other regulatory proteins).

Among other physiological roles, TR1 has been implicated in DNA repair, maintaining redox homeostasis and regulation of cell signaling (12, 266, 297). In addition, this selenoprotein is known to activate the p53 tumor suppressor (251). These observations led to the proposition that TR1 may have a role in cancer prevention (137, 311). Several lines of evidence also demonstrated that TR1 can contribute to the development of cancer. TR1 is expressed at high levels in many cancer cell lines and cancers (311), and inactivation of TR1 in malignant cell lines led to reduced cell proliferation and decreased tumor progression, when mice were injected with the TR1 knockdown cells compared with the control cancer cell line (137, 379). Therefore, TR1 may have two opposing roles in cancer development. First, TR1 may serve to prevent cancer by maintaining cellular redox homeostasis and decreasing the rate of mutations that initiate tumor formation. At the same time, TR1 is required for tumor growth due to high susceptibility of cancer cells to oxidative stress (98, 243, 303). In a recent study, the dual role of TR1 in cancer has been further investigated using conditional knockout of TR1 in liver in a chemically induced model of hepatocarcinogenesis (47). In this study, TR1 deficiency was associated with dramatically increased tumor incidence compared with control mice, suggesting that TR1 protects against tumor formation in chemically induced liver cancer. Interestingly, lack of TR1 was associated with compensatory upregulation of another antioxidant selenoprotein, GPx2, and enzymes involved in GSH metabolism in tumors from TR1 hepatocyte-specific knockout mice. These enzymes, and TR1 itself, are targets of the Nrf2 transcription factor. Therefore, induction of Nrf2-dependent antioxidant responses may protect cancer cells from oxidative stress (30, 284). However, additional studies will be needed to elucidate interdependencies of different redox systems in the context of normal tissues and

during tumor development. TR1 has also been shown to regulate diverse drug detoxification and metabolic pathways. In addition to upregulation of enzymes for glutathione biosynthesis, liver-specific TR1 knockout mice are characterized by increased expression of other proteins involved in detoxification of xenobiotics, including glutathione-*S*-transferases, UDP-glucuronyl-transferases, and xenobiotic exporters. TR1 deficiency in these mice is also associated with repressed expression of genes involved in lipogenesis and increased accumulation of glycogen in the liver (158). To what extent these cytoprotective and metabolic changes contribute to both cancer preventive and tumor promoting functions of TR1 remains to be established. In addition to its role in cancer, TR1 has been recently implicated in mediating the important role of Se in immune function and prevention of HIV infection (166). TR1 has been proposed to negatively regulate the activity of the HIV-1 encoded transcriptional activator, Tat, in human macrophages by reducing disulfide bonds in a Cys-rich motif of Tat involved in HIV-1 transactivation.

Several research groups have used TR1 as a model to address the question of why Sec is utilized in proteins instead of Cys-containing homologs can have significant activity towards the substrates of selenoenzymes. As discussed above, Sec is present within the Gly-Cys-Sec-Gly redox-active center that is located at the COOH-terminal end of TR1. However, in TR1 homologs from several organisms, Sec is replaced with Cys, e.g., *D. melanogaster* TR1 (DmTR1) contains Ser-Cys-Cys-Ser COOH-terminal sequence. Strikingly, both human TR1 and DmTR1 have comparable catalytic efficiencies, whereas substitution of Sec with Cys in the human protein leads to almost complete inactivation of the enzyme, suggesting that the active site Cys residue in DmTR1 can be activated by the protein microenvironment, i.e., by lowering pK_a of the Cys (120). It was proposed that the Ser residues within Ser-Cys-Cys-Ser tetrapeptide in DmTR1 are required for activation of Cys. However, when the Ser-Cys-Cys-Ser motif was introduced into the mammalian ortholog instead of the Gly-Cys-Sec-Gly motif, the mutant protein had negligible enzymatic activity indicating that additional features of the DmTR1 enzyme might also play a role in active site Cys activation (164). The fact that Sec-containing human TR1 and Cys-containing DmTR1 have comparable catalytic efficiencies raises the question: What catalytic advantages are provided by energetically costly Sec in Se-containing enzymes? Although Sec has several unique chemical and physical properties that might have determined its evolutionarily selection over Cys in catalytic sites of certain thiol oxidoreductases, currently there is no clear consensus regarding the exact nature of this property. First, it was proposed that Sec provides an advantage in Se-containing proteins as it allows using a broader range of substrates and pH conditions in which an enzyme can function due to its lower pK_a (120). Second, the higher reactivity of Sec compared with Cys in thiol-disulfide-like exchange reactions could be attributed to inherent high nucleophilicity of Sec and its higher reactivity with electrophiles (11). At the same time, electrophilicity of the Se atom could also allow the enzyme to resist irreversible oxidative inactivation, in contrast to Cys whose overoxidized forms are not easily reduced (91, 320, 321). In addition, evidence was presented that thiol-disulfide exchange reactions could be accelerated by the presence of Se instead of sulfur as Se could serve as a better leaving group as well as the fact that it could stabilize a favorable enzyme conformation due to longer sulfur-selenium bond length compared with the sulfur-sulfur bond (200). Future studies are needed to determine which of the aforementioned properties (or their combination) are biologically relevant in the context of specific reactions catalyzed by TR1 and other Se-containing enzymes.

D. Methionine-*R*-Sulfoxide Reductase 1

An additional functionally characterized mammalian selenoprotein is methionine-*R*-sulfoxide reductase 1 (MsrB1). This is a zinc-containing selenoprotein that was initially identified as selenoprotein R (SelR) (189) and selenoprotein X (SelX) (215) by searching for putative SECIS element structures in EST databases using bioinformatic tools. Later, this protein was found to function as a stereospecific methionine-*R*-sulfoxide reductase, which catalyzes repair of the *R* enantiomer of oxidized methionine residues in proteins (190). Based on its functional similarity to methionine-*S*-sulfoxide reductase A (MsrA), which catalyzes reduction of the other isomer, this selenoprotein was renamed MsrB1. Although MsrB1 and MsrA are structurally different and have no sequence similarity, they have complementary functions, i.e., each protein acts on only one of the two stereoisomers. Interestingly, in some organisms, such as the unicellular eukaryotes, *Chlamydomonas reinhardtii* and *Aureococcus anophagefferens*, and an anaerobic bacterium, *Clostridium* sp. OhILA, MsrA is a selenoprotein and contains Sec residue in the active site (118, 170, 174). The Sec-containing *Chlamydomonas* and *Clostridium* MsrA proteins displayed more than 10–50 times higher activity than MsrA homologs naturally containing Cys, suggesting Sec provides catalytic advantages in these redox-active enzymes (170, 174).

The Sec-containing MsrB1 protein is the major MsrB in mammals, which is primarily localized in the cytosol and nucleus. It has the highest activity in mammalian liver and kidney among three known MsrB enzymes (172). Two additional MsrB homologs (MsrB2 and MsrB3) contain a Cys residue in place of Sec in the enzyme's active site and have different subcellular distributions. MsrB2 is localized in mitochondria, whereas MsrB3 is targeted to the ER. Although MsrB2 and MsrB3 show lower levels of expression than MsrB1, these Cys-containing homologs have catalytic efficiencies similar to that of MsrB1. This observation allowed

studying specific adjustments that evolved in the active sites of Cys-containing MsrBs to maximize their activity. The catalytic mechanism of MsrB1 involves three distinct steps (171, 205). Initially, the catalytic Sec residue attacks the sulfur atom of methionine-*R*-sulfoxide and forms a selenenic acid intermediate, thereby releasing reduced methionine as a product. During the second step, Sec selenenic acid forms a selenenylsulfide bond with the resolving Cys. The selenenylsulfide is then reduced by Trx or Grx restoring a catalytically active enzyme. Compared with MsrB1, Cys-containing MsrB2 and MsrB3 do not possess a resolving Cys. During the catalytic cycle, the active site Cys residue in these proteins undergoes oxidation to generate Cys sulfenic acid, which then can be directly reduced by Trx (173). Mutational studies revealed that MsrB2 and MsrB3 contain unique conserved residues that were essential for MsrB activity in this setting, including His77, Val/Ile81, and Asn97 (numbering is based on mouse MsrB1), but were not required for MsrB1. In contrast, Sec-containing MsrB1 activity was dependent on the presence of the conserved resolving Cys (170). Moreover, mutation of the active site Cys in MsrB2 and MsrB3 to Sec led to an increase in activity of more than 100-fold, but these selenoproteins could not be efficiently reduced by Trx. These studies demonstrated that Sec provides catalytic advantage to thiol oxidoreductases, but utilization of Sec imposes additional requirements for the environment of the active site residues so that Sec can be reduced by physiological electron donors such as a Trx.

Crystal structures of several Cys-containing bacterial MsrBs have been reported (171, 180, 233). In addition, solution NMR structures of mammalian MsrB1 and MsrB1 in complex with its natural electron donor, thioredoxin, have been also recently determined (2, 84). Structural studies revealed that MsrB has an overall β-fold consisting of two antiparallel β-sheets and a highly flexible NH_2-terminal region **(FIGURE 10)**. Although MsrB and MsrA proteins are structurally different, their active-site configurations display mirror symmetry reflecting the fact that these enzymes catalyze the reduction of two methionine sulfoxide stereoisomers. In addition to a conserved resolving Cys, which participates in the formation of a selenenylsulfide bond, MsrB1 has four conserved Cys residues organized in two CxxC motifs. These Cys residues are involved in coordination of a zinc atom, and substitution of any of these Cys residues with Ser results in loss of the enzyme activity. The structural studies further demonstrated that zinc metal cofactor is not directly involved in catalysis, but rather plays a structural role in MsrB enzymes. Moreover, upon oxidation of the enzyme, the highly disordered NH_2-terminal region of MsrB1, which contains the resolving Cys, is positioned over the protein active site. This observation suggests that the Cys residue can form a selenenylsulfide bond during the catalytic cycle that further confirms its role in the mechanism of MsrB1 (2).

In contrast to MsrA, which can catalyze the reduction of both free methionine-*S*-sulfoxide and its protein-based form, MsrB can reduce methionine-*R*-sulfoxide back to methionine only in proteins. Recently, in some organisms, an additional Msr was discovered, designated fRMsr, which can reduce free methionine-*R*-sulfoxide (204, 221). However, fRMsr has a very low activity with the protein-based form of methionine-*R*-sulfoxide, and its homologs are found only in unicellular organisms. Consistent with this observation, no fRMsr activity was detected in mammals (206).

Methionine is one of the two sulfur-containing amino acids (the other one is Cys) that are the most susceptible to oxidation (216). Oxidation of methionine residues in proteins may lead to a significant alteration of their structure and disrupt protein function. Since Msrs catalyze the reduction of methionine sulfoxide back to methionine, it was proposed that this class of enzymes can be involved in a protective mechanism against oxidative damage to proteins. Accordingly, MsrB1 was viewed as an important antioxidant enzyme that participates in protein repair. Previously, several proteins were identified as targets of Msr enzymes. For example, the ribosomal protein L12, calmodulin, and the *Shaker* potassium channel undergo inactivation upon oxidation of methionine residues, but their functions can be restored by MsrA (171). In addition, the TRPM6 Mg^{2+} channel was recently identified as a binding partner of MsrB1 (41). It was shown that inactivation of TRPM6 due to oxidation of one of its methionine residues, Met1755, during oxidative stress can be reversed by MsrB1. Because of widespread occurrence of methionine in proteins, it is expected that a number of additional MsrB1 targets exist. However, identification of MsrB1 target proteins at the genome-wide level has been limited due to methodological difficulties (because Msrs do not form a covalently linked intermediate with their substrates during catalysis) and the lack of anti-methionine sulfoxide antibodies.

In addition to repair of oxidized methionines in proteins, MsrB1 has been recently shown to play an important role in regulation of cellular functions by reversing oxidation of regulatory methionine residues in a stereospecific manner (207). Similar to other reversible posttranslational modifications, e.g., phosphorylation of tyrosine residues in proteins, methionine oxidation and reduction were proposed to play regulatory roles (144, 220). For example, methionine oxidation has been shown to activate the transcription factor, HypT, which is involved in the bacterial response to hypochlorite (88). Moreover, oxidation of methionine residues has been shown to activate calcium/calmodulin (Ca^{2+}/CaM)-dependent protein kinase II (CaMKII) in the absence of Ca^{2+}/CaM, whereas MsrA activity could reverse this effect (93). Methionine oxidation has also been shown to regulate actin assembly by inhibiting formation of F-actin filaments (342). Remarkably, oxidation of the methio-

nine residues in actin occurs through a targeted enzyme-based modification rather than through nonspecific oxidation by ROS. Monooxygenase Mical, which belongs to a family of flavin-containing monooxygenases, has been shown to selectively oxidize 2 out of the 16 methionines in actin, and this posttranslational modification leads to disassembly of F-actin (149, 150). However, whether oxidation of the methionines in actin could be reversed was not known. In a recent study, Mical has been shown to stereospecifically oxidize methionine in actin to methionine-*R*-sulfoxide, and MsrB1 to reverse the Mical induced oxidation, regulating actin assembly and microphage function (207). Thus Mical and MsrB1 serve as antagonists, further supporting the role of methionine oxidation/reduction in control of protein function. Similar to phosphorylation of tyrosine residues in proteins, reversible methionine oxidation could play a much broader role in control of cellular functions than previously anticipated. In this regard, the substrates of Mical and its paralogs (mammals contain three Micals and two additional Mical-like proteins) could include proteins other than actin, opening up the field to a broader investigation of processes regulated by MsrB1.

Finally, MsrB1 is a nonessential selenoprotein (106), whose expression is regulated by Se availability (267). Moreover, expression of this selenoprotein is reduced in mice as a function of age (269). One of the physiological consequences of MsrB1 deficiency is a reduced innate immunity due to disruption of actin polymerization-dependent processes in macrophages, including filopodia formation, macropinocytosis, and release of cytokines (207). Thus MsrB1 may contribute to age-related decline in immune function, and reduced MsrB1 levels might also explain a weakened immune response associated with Se deficiency (292). The relevance of MsrB1 in regulating other actin polymerization-dependent cellular processes, and whether MsrB1 can participate in the regulation of proteins other than actin by reversing oxidation of regulatory methionine residues, remain to be explored.

E. Selenophosphate Synthetase 2

Similar to bacterial selenophosphate synthetase SelD (325), selenophosphate synthetase 2 (SPS2) catalyzes the synthesis of the active Se donor selenophosphate that is necessary for Sec biosynthesis (371). All vertebrates possess Sec-containing SPS2, whereas in lower eukaryotes the active-site Sec residue in SPS2 is replaced with Cys. Because SPS2 is a selenoprotein in vertebrates, it was proposed to serve an autoregulatory role in selenoprotein synthesis (124, 175). Even though SPS2 is a Sec-containing protein and is present in a single copy in most vertebrate genomes, comparative genomics analyses revealed that in mammals the SPS2 gene was duplicated and the original multiexon gene (SPS2a) was replaced by an intronless gene (SPS2b) in placental mammals (245). However, in some species including marsupials (opossum and wallaby), both SPS2a and SPS2b copies are found.

F. Selenoproteins W, T, H, and V

Selenoproteins W (SelW), T (SelT), H (SelH), and V (SelV) belong to the Rdx family of selenoproteins (83). The members of this protein family possess a thioredoxin-like fold and are characterized by the presence of a conserved Cys-x-x-Sec motif. In addition, these proteins contain a conserved stretch of amino acids in the COOH-terminal portion of the protein with the tGxFEI(V) consensus sequence. Based on the presence of the thioredoxin fold and the Cys-x-x-Sec motif, it was proposed that the Rdx family proteins are thiol-based oxidoreductases, but the exact function of any of these proteins remains unknown.

SelW is one of the first identified Sec-containing proteins and is one of the most abundant selenoproteins in mammals. This small 9-kDa selenoprotein is localized in the cytosol and is expressed at high levels in muscles and brain (123, 356). SelW belongs to the stress-related group of selenoproteins as its expression is highly regulated by the availability of Se in the diet (145). When purified from rat muscle, native SelW was found to form a complex with glutathione (21). In addition, 14-3-3 protein (the ubiquitous signaling adapter protein) was identified as a target of mammalian SelW (83). The solution NMR structure of mouse SelW, in which the active-site Sec was mutated to Cys, has been recently reported **(FIGURE 10)** (1). The NMR spectroscopy further confirmed the interaction of SelW with 14-3-3 protein and identified two flexible external loops in SelW that are involved in its binding. It has been proposed that SelW could be involved in redox regulation of 14-3-3 protein (262) and/or interaction of 14-3-3 protein with its binding partners, CDC25B (283) and Rictor (161). However, the molecular details of regulation of 14-3-3 protein by SelW and its physiological function remain unknown.

SelT is one of the first Sec-containing proteins identified using bioinformatic tools (189). This selenoprotein is predominantly localized to the ER and Golgi and is ubiquitously expressed both during embryonic development and in adult tissues (83). Knockdown of SelT in mouse fibroblasts using RNAi leads to decreased expression of extracellular matrix genes involved in cell structure organization and alters cell adhesion properties (312). In addition, the loss of SelT resulted in the upregulation of several oxidoreductase genes, including another member of the Rdx family, SelW. In a recent study, SelT was identified as a target of the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) (121). Overexpression of SelT in PC12 cells resulted in an increase of the intracellular Ca^{2+} levels, whereas knockdown of SelT inhibited PACAP-stimulated release of Ca^{2+} from the ER and reduced growth

hormone secretion. These data suggested a role for SelT in the regulation of Ca^{2+} homeostasis and neuroendocrine function. More recently, SelT has been implicated in the regulation of pancreatic β-cell function and glucose homeostasis by using a conditional pancreatic β-cells SelT knockout in mice (289).

SelH was initially identified in fruit flies as BthD protein (246), and subsequently its homologs were found in mouse and human genomes (186). This 14-kDa selenoprotein contains a Sec residue within the Cys-x-x-Sec motif, and also has a conserved nuclear targeting RKRK motif in the N-terminal sequence. SelH has a unique subcellular localization pattern, and was found to localize specifically to the nucleoli. (270). Expression of SelH is relatively low in adult mouse tissues, but is elevated during embryonic development. Similar to SelW, SelH is sensitive to dietary Se intake (145). Consistent with its subcellular localization, SelH contains an AT-hook motif, which is present in DNA-binding proteins of the AT-hook family. With the use of an in vitro chromatin immunoprecipitation assay, it was found that SelH specifically binds to sequences containing heat shock and stress response elements (281). Moreover, SelH possesses glutathione peroxidase activity (270) and has been implicated in the regulation of transcription of a group of genes that are involved in de novo glutathione synthesis and phase II detoxification enzymes. Further studies are warranted to determine the mechanisms of such regulation.

SelV is one of the least characterized selenoproteins. It recently evolved, most likely by duplication from SelW, and is found only in placental mammals (245). However, it was specifically lost in some organisms including gorillas (357). SelV is closely related to SelW but is a larger protein due to the presence of an additional NH_2-terminal domain, which is not found in SelW. The function of this NH_2-terminal sequence is not known. In contrast to SelW, SelV expression is detected only in testes, and thus may be involved in male reproduction, but its specific function is not known.

G. Selenoprotein I

Selenoprotein I (SelI) is a recently evolved selenoprotein, which is found only in vertebrates (186). It is a transmembrane protein containing a highly conserved CDP-alcohol phosphatidyltransferase domain, which is present in choline (CHPT1) and choline/ethanolamine (CEPT1) phosphotransferases. CHPT1 and CEPT1 catalyze the last step in de novo synthesis of the two major phospholipids through the transfer phosphocholine and phosphoethanolamine groups to diacylglycerol from CDP-choline and CDP-ethanolamine, respectively. CHTP1 is involved in the synthesis of phosphatidylcholine from CDP-choline (140). In contrast, CEPT1 has a dual specificity for CDP-choline and CDP-ethanolamine and synthesizes both phosphatidylcholine and phosphatidylethanolamine. Similar to choline phosphotransferases, SelI contains seven transmembrane domains and has three conserved aspartic residues that are required for catalytic activity within a $DG(X)_2AR(X)_8G(X)_3D(X)_3D$ motif (245). The major distinction of SelI from CHPT1 and CEPT1 is the presence of the COOH-terminal extension-containing Sec residue, the function of which is currently not known. Truncated SelI protein recombinantly expressed in *E. coli*, which lacks the Sec residue, has been shown to possess ethanolamine phosphotransferase activity (143). However, the physiological function of the intact Sec-containing SelI and the role played by Sec residue have to be further examined.

H. The 15-kDa Selenoprotein and Selenoprotein M

The 15-kDa selenoprotein (Sep15) and selenoprotein M (SelM) are thioredoxin-like fold ER-resident proteins that form a distinct selenoprotein family. Sep15 was identified experimentally in 1998 as a 15-kDa selenoprotein of unknown function (115). Later this protein was proposed to mediate the cancer prevention effect of dietary Se (192) and regulation of redox homeostasis in the ER (182). SelM is a distant homolog of Sep15, which was identified by bioinformatics approaches (183). Sep15 and SelM share 31% sequence identity and demonstrate somewhat similar distribution, with homologs present from green algae to humans. Expression of these selenoproteins was detected in a wide range of mammalian tissues, although their tissue expression patterns differ. Sep15 showed the highest level of expression in prostate, liver, kidney, and testis, whereas SelM is highly expressed in brain. In addition to Sep15 and SelM, another member of the Sep15 protein family, designated Fep15 (for fish Sep15-like protein), was identified in fish (268). Fep15 is an ER-resident selenoprotein of unknown function that is found only in fish. The narrow distribution of Fep15 suggests that this selenoprotein has a specialized function that is distinct from those of Sep15 and SelM.

Comparative analyses of Sep15 and SelM sequences revealed that both proteins share a common thioredoxin-like domain and contain an NH_2-terminal signal peptide, consistent with their ER localization. In addition, SelM possesses a COOH-terminal extension with an ER retention signal, which is lacking in Sep15. In contrast, Sep15 has a distinct Cys-rich domain in the NH_2-terminal part of the protein. This domain is required for interaction of Sep15 with its binding partner UDP-glucose:glycoprotein glucosyltransferase (UGGT) (195). The lack of a typical ER retention signal in Sep15 suggests that it is maintained in the ER through its binding to UGGT rather than specific signal-mediated retrieval. The structures of the thioredoxin-like domain of *Drosophila* Sep15 and mouse SelM have been solved using NMR spectroscopy (99). Structural studies revealed that both proteins have an overall α/β-fold common for thioredoxin-like oxidoreductases with a four-

stranded β-sheet surrounded by three α-helices **(FIGURE 10)**. Moreover, similar to other oxidoreductases containing thioredoxin-like domains, Sep15 and SelM possess redox-active motifs in a loop between strand β1 and helix α1. The presence of the thioredoxin-fold domain and redox-active motifs suggested an oxidoreductase function for Sep15 and SelM. However, the consensus sequences of the redox motifs in Sep15 and SelM (CxU and CxxU, respectively) differ from those of functionally characterized oxidoreductases, e.g., thioredoxin and protein disulfide isomerase. The identity of residues in these redox motifs determines their redox potential, which in turn may influence the functional specificity. The redox potential of *Drosophila* Sep15 was found to be −225 mV, suggesting that Sep15 may be involved in the reduction or isomerization of disulfide bonds in proteins. In addition, the structural studies demonstrated that the COOH-terminal extension of SelM that is absent in Sep15 is highly flexible. This flexible region may plausibly participate in substrate binding or interaction with other protein factors. Collectively, the presence of redox-active motifs and structural similarities to other thioredoxin-fold oxidoreductases suggest that Sep15 and SelM may catalyze the reduction or rearrangement of disulfide bonds in the ER-localized or secretory proteins (197).

Biochemical characterization of Sep15 demonstrated that it forms a tight complex with UGGT in a 1:1 ratio (195). UGGT is an ER-resident chaperone involved in the regulation of the calnexin (CNX) cycle, a protein quality control pathway that assists in the folding of *N*-linked glycoproteins in the ER (138, 259). The presence of a thioredoxin-like fold and the association of Sep15 with UGGT suggests that Sep15 might have roles in disulfide bond formation and quality control of a restricted group of glycoproteins serving as UGGT substrates (197). Consistent with its function in protein folding, Sep15 expression is induced by conditions that are associated with accumulation of misfolded proteins in the ER (199). Further studies focused on identification of potential target proteins as well as endogenous donors/acceptors of reducing equivalents are needed to clarify the roles of Sep15 in quality control pathways in the ER. Expression of Sep15 is also subject to regulation by dietary Se, and it belongs to the group of stress-related selenoproteins. However, in some organs, including brain and testis, the decrease in Sep15 expression under Se-deficient conditions is less pronounced (99, 267). Interestingly, there are two polymorphic sites at nucleotide positions 811 (C/T) and 1125 (A/G) in the human Sep15 gene (192). The later polymorphism is located in the SECIS element and was shown to influence the efficiency of Sec incorporation into Sep15 in a Se-dependent manner (147, 191). It was shown that the A1125 variant is prevalent in African Americans and may be associated with increased incidence of breast, head, and neck tumors. In addition, Sep15 has been implicated in preventing liver (192), prostate (147), breast (264), and lung cancers (8). However, other studies demonstrated a role of Sep15 in promoting some types of cancer including colon cancer (153, 347). Taken together, these observations reveal complexities with regard to the possible role of Sep15 in mediating the chemopreventive effects of dietary Se. Sep15 knockout mice have been recently developed (167). These mice did not have any obvious phenotypes during development and growth, but were characterized by elevated levels of oxidative stress markers in the liver and cataract development. Further phenotypic characterization of $Sep15^{-/-}$ mice should provide clues into its physiological functions.

Because SelM is highly expressed in the brain, several studies investigated the possible role of SelM in neuroprotection. Overexpression of SelM in neuronal cells prevented oxidative damage induced by H_2O_2 treatment, whereas knockdown of SelM using shRNA caused decreased cell viability and a strong apoptotic cell death (294). Moreover, overexpression of SelM has been demonstrated to inhibit aggregation of β-amyloid peptide ($A\beta_{42}$), when $A\beta_{42}$ was cotransfected with SelM in HEK293T cells, suggesting a possible role of SelM in preventing Alzheimer's disease (54). In addition to its proposed antioxidant role, SelM has been implicated in regulating Ca^{2+} release from ER calcium stores in neuronal cultures in response to H_2O_2 (294). However, the exact function of SelM and its physiological roles in the brain as well as in other tissues remain unclear. Recently, SelM knockout mice have been developed (288). Phenotypic characterization of $SelM^{-/-}$ animals revealed that deletion of SelM does not affect neuronal and cognitive function, but leads to increased body weight and development of obesity in mice.

I. Selenoproteins K and S

Although selenoprotein K (SelK) and selenoprotein S (SelS) have no significant sequence similarity, they could be assigned to a single SelK/SelS family of related selenoproteins based on their topology, including a single transmembrane domain in the NH_2-terminal sequence; the presence of a glycine-rich (G-rich) segment that has an unusually high content of glycine, proline, and positively charged amino acids; and a characteristic location of Sec residues in the COOH-terminal end of the protein (313). This selenoprotein family includes SelK and SelS homologs as well as distantly related SelK/SelS-like Cys-containing proteins, such as Romo1 (reactive oxygen species modulator 1). Comparative genomics analyses revealed that the SelK/SelS-like protein family is the most widespread eukaryotic selenoprotein family, whose members are present in nearly all known Se-utilizing organisms ranging from unicellular eukaryotes to humans. In addition, Cys-containing SelK/SelS-like proteins were identified in a wide variety of species including fungi, insects, and plants. Both SelK and SelS are localized to the ER membrane and belong to a type III group of transmembrane proteins that contain a single transmem-

brane domain, with the COOH-terminal end of the protein facing the cytosol. This COOH-terminal region containing the G-rich domain is highly unstructured. Moreover, both SelK and SelS selenoprotein homologs contain Sec in the third or second position from the COOH terminus. Although very limited data on biochemical characterization of SelK and SelS is available, this group of selenoproteins differs from the thioredoxin-like fold ER-localized selenoproteins. In contrast to Sep15 and SelM, which have been proposed to facilitate protein folding in the ER, SelK and SelS have been recently implicated in ER-associated degradation (ERAD) of misfolded proteins (313, 377). The ERAD machinery consists of multiprotein complexes that are involved in recognition, ubiquitination, and retrotranslocation of protein substrates from the ER to the cytosol, and their subsequent degradation by the ubiquitin/proteasome system (219, 252). The retrotranslocation of misfolded proteins through the ER membrane requires their polyubiquitination followed by extraction of protein substrates to the cytosol by the p97 ATPase (also called VCP) found in a complex with the ubiquitin fusion degradation 1 (Ufd1) and nuclear protein localization 4 (Npl4) proteins. In addition, p97 interacts with a membrane protein complex containing Derlin-1 protein that facilitates attachment of p97 ATPase to the ER membrane. Recent studies identified SelS (also known as VIMP) as a binding partner of p97 and Derlin-1, which together with SelS form retrotranslocation channel (377). In higher eukaryotes, two additional Derlin-1-related proteins are encoded in the genome (Derlin-2 and Derlin-3). More recently, SelS was found to also bind human Derlin-2 and a long form of Derlin-3 (transcriptional variant Derlin-3b) (219, 272, 313). Strikingly, all three Derlin proteins were also found to associate with SelK, suggesting that this selenoprotein performs an analogous function to that of SelS (313). Although both SelS and SelK interact with Derlins, SelS preferentially binds Derlin-2, whereas SelK has the highest affinity for Derlin-1. The different binding affinities of SelS and SelK for Derlins were proposed to determine the substrate specificity and the nature of proteins that are translocated through the specific Derlin channel (313). In addition to binding p97 and Derlins, SelS and SelK have been shown to interact with each other (through their transmembrane domains, although the interaction might also be through additional proteins) as well as with ERAD substrates. Consistent with the role of SelS and SelK in ERAD, their genes contain functional ER stress response elements, and expression of both proteins is upregulated by the conditions promoting the accumulation of misfolded proteins in the ER. Moreover, knockdown of SelK using RNAi led to the accumulation of several glycosylated ERAD substrates, further supporting the idea that SelK might be involved in binding misfolded proteins and targeting them to the Derlin complex and subsequent proteasome-dependent degradation. It should be noted that SelK is involved in the retrotranslocation of only a subset of misfolded proteins from the ER, as knockdown of SelK affected degradation of some, but not all, tested misfolded glycoprotein substrates. SelS differs from SelK by the presence of an additional coiled-coil domain in the cytosolic portion of the protein, which has been proposed to mediate the interaction with other proteins or oligomerization of SelS (377). Further studies focused on the identification of SelS interacting proteins are expected to clarify the substrate specificity of this protein and provide insights into its physiological functions.

Although both SelK and SelS have been implicated in Derlin-dependent ERAD, the exact function of SelS and SelK and their Sec catalytic residues in this process remain unknown. Recent structural characterization of the recombinantly expressed cytosolic portion of human SelS (cSelS) (in which Sec188 was replaced with Cys) revealed that the cytosolic tail of SelS consists of two extended α-helixes followed by a highly disordered COOH-terminal region (63). The intrinsically disordered nature of the COOH-terminal end of SelS suggested the possibility that this region provides flexibility to access many structurally different substrates, e.g., misfolded proteins. Moreover, the cytosolic portion of recombinant SelS has been shown to contain a disulfide bond between Cys174 and Cys188, suggesting the presence of a selenenylsulfide bond in the native SelS. Recently, a Sec-containing cSelS protein has been biochemically characterized (223, 224). These studies further confirmed the formation of the selenenylsulfide bond in SelS and suggested a reductase function for SelS. The redox potential of SelS [-234 mV (223)] indicates that it might be involved in the reduction of disulfide bonds in ERAD substrates prior to the translocation from the ER using the Trx/TR system as the electron donor. Further studies are needed to clarify the possible role of SelS in the reduction of disulfides in glycoprotein substrates in vivo.

In addition to its possible role in ERAD, a large body of evidence implicated SelS and SelK in inflammation and the immune response. Genetic variants in the SelS gene were shown to regulate the circulating levels of proinflamatory cytokines including IL-6, IL-1β, and TNF-α (72) and have been linked to the development of coronary heart disease, ischemic stroke (4), preeclampsia (261), gastric (315) and colorectal (337) cancer, and other conditions that are associated with increased inflammation. Moreover, characterization of the recently developed SelK knockout mouse model demonstrated that SelK deficiency leads to deficient Ca^{2+} flux during immune cell activation and impaired immune response (358). These findings suggest that SelK and SelS might mediate the anti-inflammatory effects of Se and its role in the immune system (265, 361). However, the molecular mechanisms by which SelS and SelK affect immune function and inflammatory response await further investigation.

J. Selenoprotein O

Selenoprotein O (SelO) is one of the least characterized human selenoproteins. Despite its discovery more than a decade ago (186), no structural or biochemical characterization of this protein has been reported. Homologs of human SelO have been detected in a wide variety of species including bacteria, yeast, animals, and plants. SelO contains a single Sec residue located in the antepenultimate position at the COOH-terminal end of the protein. However, the majority of SelO homologs contains a Cys residue in place of Sec (Sec-containing SelO sequences are present only in vertebrates). Analysis of vertebrate SelO protein sequences revealed the presence of a mitochondrial targeting peptide and a putative protein kinase domain in this selenoprotein (89). The function of SelO and any of its homologs are thus far unknown.

K. Selenoprotein N

Selenoprotein N (SelN) was among the first selenoproteins that were identified through bioinformatic approaches (215). Mutations in the human SelN gene (also known as *SEPN1*) are associated with a group of early-onset muscle disorders known as SEPN1-related myopathies (10). SelN is an ER-resident transmembrane glycoprotein that is highly expressed during embryonic development and to a lesser extent in adult tissues including skeletal muscle (286). Recent studies using a zebrafish animal model revealed that SelN is required for early muscle development and differentiation in this organism (77, 165). More recently, *SelN*$^{-/-}$ mice were developed (50, 293). However, in contrast to what was observed in zebrafish, no abnormalities were found in the structure and the size of muscle fibers in SelN knockout mice. Moreover, no defects in muscle function or muscle contractility could be observed using functional exercise tests and in situ muscle contractility analyses, respectively. SelN knockout mice were healthy and fertile and otherwise indistinguishable from wild-type control mice. However, these mice displayed whole-body rigidity during the repeated forced swimming tests (FST) and a reduced mobility following these tests compared with wild-type mice. Strikingly, after a prolonged period of repeated FST, *SelN*$^{-/-}$ mice developed a severe kyphosis and hypotrophy similar to clinical symptoms observed in patients with SEPN1-related myopathies. In addition, muscles from SelN knockout mice were not able to regenerate after repeated injury caused by exposure to cardiotoxins due to a reduced number of muscle progenitor satellite cells that are involved in muscle repair (50). Collectively, these observations suggest that SelN plays an important role in the maintenance of satellite cells and is required for regeneration of skeletal muscle tissue following stress or injury.

Recently, ryanodine receptor (RyR) was identified as a binding partner of SelN (165). RyR forms a calcium release channel that mediates the release of Ca^{2+} from the sarcoplasmic reticulum during muscle contraction. Interaction of SelN with RyR suggested that this selenoprotein might serve as a cofactor of RyR and might be involved in regulation of intracellular calcium mobilization. Consistent with its possible role as a cofactor of RyR, SelN deficiency resulted in decreased binding affinity of RyR for ryanodine. In addition, in the absence of SelN, RyR became unresponsive to regulation by redox potential of the environment (165). However, in another study SelN deficiency has been implicated in increased susceptibility of myotubes to oxidative stress, whereas it had only a secondary effect on calcium homeostasis (9). Further studies are required to better understand the underlying mechanisms of SEPN1-related myopathies and contribution of SelN to normal muscle function. Also, the function of SelN in other organs and tissues remains unknown.

L. Selenoprotein P

Selenoprotein P (SelP) is an abundantly expressed secreted selenoprotein that accounts for almost 50% of the total Se in plasma (33). The SelP protein family has recently evolved, and SelP homologs are found predominantly in vertebrates. The unique feature of SelP is the presence of multiple Sec residues. For example, human SelP gene has 10 in-frame Sec-encoding TGA codons and two SECIS elements within its 3′-UTR. However, the number of Sec residues in vertebrate SelP sequences varies greatly from 7 in the naked mole rat to as many as 17 found in zebrafish SelP homologs (226). Moreover, zebrafish also contain a second SelP gene (SelPb) that lacks a Sec-rich COOH-terminal region (188), whereas several SelP isoforms have been described in rodents (33). In the latter case, in addition to the full-length protein containing 10 Sec residues, 3 shorter isoforms were identified that appeared to be produced by premature termination at the second, third, and seventh UGA codons in SelP mRNA (237). All four isoforms share the same sequence in the NH_2 terminus of the protein and originate from the same SelP mRNA transcript. SelP is also subject to posttranslational modifications. It has three *N*-glycosylation sites and one *O*-glycosylation site, and the glycosylation of SelP at these sites has been experimentally verified (236). SelP is synthesized predominantly in liver, which is the major source of plasma SelP (43), although SelP mRNA expression was detected in virtually all tissues.

The fact that SelP is secreted into the plasma and the presence of multiple Sec residues in its sequence suggested that this selenoprotein might function as a Se supplier to peripheral tissues (36, 299). Additional insights into the role of SelP in Se metabolism in mammals have been provided by whole body SelP knockout mouse models (142, 308). SelP knockout mice fed a normal Se diet are characterized by a sharp decrease in Se levels specifically in the brain and testis,

whereas Se content in the kidney and other organs and tissues was only moderately affected. Moreover, deletion of SelP gene resulted in an increase in Se in the liver, and it also increased urinary excretion of Se metabolites (34). These findings suggested that liver is a primary organ that is responsible for uptake of Se from the blood and synthesis/export of SelP, which in turn transports Se to peripheral tissues. Consistent with the very low Se concentrations in brain and testis, SelP knockout mice fed normal or low Se diets developed severe neurological problems and male infertility (142, 308). However, tissue-specific expression of SelP in the liver of *SelP*$^{-/-}$ mice restored Se transport and prevented infertility and neurological phenotypes (295). The fact that the largest decrease in Se levels was seen in the brain and testes of SelP knockout mice indicates that SelP has a more direct role in delivering Se to these tissues. Moreover, it was found that feeding a high Se diet prevented the development of neurological lesions (142), but did not prevent infertility in *SelP*$^{-/-}$ mice (277). These observations suggested that, in addition to SelP, other forms of Se can be utilized for its delivery to the brain and possibly other tissues, whereas testis strictly depends on the Se supply function of SelP. Additional studies also showed that the uptake of SelP by brain is induced by Se deficiency providing evidence that a yet unidentified receptor protein or proteins in the brain mediates its uptake (36).

Recently, two endocytic receptors, apolipoprotein E receptor-2 (ApoER2) (276) and megalin (275), have been implicated in the targeted tissue-specific uptake of SelP. ApoER2 was shown to bind SelP and facilitate its uptake by testis and brain (35, 276). Consistent with its role in Se metabolism in these tissues, ApoER2 knockout mice showed sharply decreased Se levels in testis and brain that were associated with neurological dysfunction and defective spermatogenesis similar to phenotypes observed in *SelP*$^{-/-}$ mice. However, the levels of Se in other tissues (including liver, kidney, and muscle) and the whole body of ApoER2 knockout mice did not change. Another SelP receptor recently identified by Olson et al. (275) is megalin, which have been shown to regulate the uptake of plasma SelP by proximal tubule epithelium in kidney. Selective loss of proximal tubule SelP uptake by kidney in megalin$^{-/-}$ mouse embryos suggested that different organs utilize distinct SelP receptor proteins for uptake of Se from plasma. Interestingly, both ApoER2 and megalin are expressed in the brain. However, Olson et al. (275) were not able to assess the Se uptake in this tissue as megalin$^{-/-}$ mice die before birth. In a more recent study, the role of megalin in Se metabolism in adult tissues was analyzed using megalin-mutant mice, in which function of megalin was disrupted by a missense mutation in its extracellular domain (59). This study further confirmed that megalin participates in Se uptake and regulates Se metabolism in kidney and brain. It would be important to test in future studies whether other members of the lipoprotein receptor family may also function in SelP uptake and contribute to the hierarchical retention of Se by different tissues. It should also be noted that, in addition to the receptor-dependent transport of SelP, this selenoprotein can be taken up by cells nonspecifically through pinocytosis (37). But this uptake mechanism appears to function mostly under Se-adequate conditions.

Together, the currently available evidence suggests that SelP plays an important role in transporting Se to peripheral tissues, particularly brain and testis, and preserving the function of these organs under conditions of limiting Se. Although the function of the NH_2-terminal portion of SelP is not known, it is likely related to the Se transport function of SelP. For example, it might serve as an oxidoreductase that acts on COOH-terminal Sec residues, supporting their oxidized state during protein secretion or transit to target organs.

Table 1. *Major unsolved questions*

Major Unsolved Questions
1) What are the specific functions and regulation of the remaining human selenoproteins?
2) Why is Sec used in proteins, and what unique properties of Se provide advantages for the specific functions of Sec-containing proteins?
3) How did the selenoprotein synthesis machinery evolve extending the genetic code beyond the canonical 20 amino acids?
4) What is the identity of the methylase involved in Um34 synthesis?
5) What is the regulation and stoichiometry of the proteins involved in the selenoprotein synthesis, including eEFSec, SBP2, L30, eIF4a3, and nucleolin in mammalian cells?
6) What roles do SPS1 and SBP2L proteins, paralogs of SPS2 and SBP2, respectively, play in selenoprotein synthesis?
7) What is the role of the highly conserved AAR motif in the apical region of the SECIS element?
8) What are the effects of different polymorphic variants of selenoprotein genes found in human populations, and could these selenoprotein allelic variants play a role in pathophysiological states?
9) Which specific human genotypes and segments of the human population can benefit from dietary Se supplementation?
10) What are the mechanisms for the uptake, detoxification, and excretion of the various chemical forms of Se?
11) What is the importance of low-molecular-weight Se compounds versus selenoproteins in cancer prevention and promotion, as well as in other effects of Se on human health?

VI. CONCLUSIONS AND PERSPECTIVES

In the past decade, significant progress has been made in elucidating the functions and physiological roles of human selenoproteins, new selenoprotein families have been identified, and new functions have been assigned to previously characterized selenoproteins. Moreover, recent advances in the field of selenoprotein research have provided insights into the mechanisms by which Sec is synthesized and incorporated into proteins. However, numerous aspects of selenoprotein synthesis and regulation of selenoprotein expression are not understood. Moreover, physiological roles of several selenoproteins have not been firmly established, and functions of several Se-containing proteins still remain unknown **(FIGURE 9)**. Despite the fact that identities of all proteins in the human selenoproteome were established more than a decade ago, biochemical characterization of some selenoproteins (e.g., TGR, SelW, SelM, SelT, SelH, SelV, SelI, SelO) has been limited. Although most of the well-characterized selenoproteins perform redox functions, which require the unique chemical properties of Sec, it has now become clear that selenoproteins do not simply function as antioxidant enzymes. The specific functions of selenoproteins involve thiol-based redox signaling, control of the reduced state of Cys residues in cytosolic and mitochondrial proteins, removal of hydrogen peroxide, repair of oxidatively damaged proteins, control of cytoskeleton/actin assembly, formation and quality control of structural disulfide bonds in proteins, hormone activation and inactivation, selenoprotein synthesis, Se transport, protein folding and ER-associated degradation, and other functions. The important role of micronutrient Se in human health and development is likely mediated by the combined action of selenoproteins constituting the human selenoproteome. As such, selenoproteins might serve as potential targets for the development of new therapies for a number of different diseases that are known to be affected by dietary Se and/or altered organismal Se status, including cancer, diabetes, viral infection, inflammation, AIDS, as well as neurodegenerative and cardiovascular diseases. Many questions regarding the biology of selenoproteins and roles of Se and selenoproteins in human health remain unanswered and new questions are emerging **(TABLE 1)**. Answers to these questions as well as further characterization of functions of selenoproteins should help further explain the biological roles of Se in human health.

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DISCLOSURES

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