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Review

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Selenocysteine-Containing Proteins in Mammals

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Abstract
Since the recent discovery of selenocysteine as the 21st amino acid in protein, the field of selenium biology has rapidly expanded. Twelve mammalian selenoproteins have been characterized to date and each contains selenocysteine that is incorporated in response to specific UGA code words. These selenoproteins have different cellular functions, but in those selenoproteins for which the function is known, selenocysteine is located at the active center. The presence of selenocysteine at critical sites in naturally occurring selenoproteins provides an explanation for the important role of selenium in human health and development. This review describes known mammalian selenoproteins and discusses recent developments and future directions in the selenium field.

Selenium is an essential element for many forms of life. It is a component of naturally occurring selenium-containing proteins and transfer RNAs. In mammals, selenium is covalently bound in all selenoproteins described to date as the amino acid selenocysteine (Sec) [48]. In bacterial proteins, selenium also occurs as Sec [49], but in addition this element is present in some selenoproteins as a dissociable, catalytically essential cofactor [26, 27, 36, 37]. Selenium-containing tRNAs have been detected in mammals [19] and bacteria [20], but only bacterial seleno-tRNAs have been characterized [98]. The present review will discuss known selenoproteins in mammals and future developments in the selenium field. Additional information on the biology of selenium may be found in other recent reviews [14, 46, 48, 51, 62, 63, 66, 86].

The Past: The 21st Amino Acid in Protein

The discovery that the code word UGA has a dual function in the 'almost' universal genetic code [46] marks the first addition to the code [64] since it was deciphered [57, 75] and reported to be universal in the mid 1960s [70]. UGA serves as both a codon for termination of protein synthesis and as a codon for Sec. In this sense, UGA is analogous to AUG, which functions as both a codon for initiation of protein synthesis and a codon for methionine at internal positions of proteins. The role of UGA as a codon for Sec has been known for about 10 years [64]. However, the fact that Sec is the 21st naturally occurring amino acid in protein is completely accepted as Sec has its own tRNA, code word, translation factors and other com-

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ponents involved in the protein translation machinery [14, 48]. The presence of a Sec insertion sequence (SECIS) element downstream of the UGA codon that codes for this amino acid is necessary for its recognition as a signal for Sec insertion [66]. Mammalian SECIS elements are stem-loop structures located in the 3′-untranslated region of selenoprotein mRNAs [66], while the corresponding stem-loop structures in bacterial selenoproteins are immediately downstream of the UGA Sec codon [14].

An interesting feature of Sec biosynthesis is that this amino acid is synthesized on its tRNA. There are many common steps in Sec biosynthesis in mammalian and bacterial systems, but the biosynthesis of this amino acid has only been completely established in prokaryotes [14]. Sec tRNA is first aminoacylated with serine, the serine moiety is in turn modified to an aminoacrylyl intermediate on tRNA in bacterial systems by Sec synthase and then the intermediate serves as the acceptor for activated selenium. The selenium donor is selenophosphate which is synthesized by selenophosphate synthetase [86]. Many of these steps are apparently very similar in mammals with the exception that the intermediate in Sec biosynthesis has not been identified and may actually be phosphoseryl-tRNA [47, 48]. In any case, this unique pathway is highly specific for selenium as sulfur shares many chemical and physical properties with selenium, but sulfur cannot replace selenium in Sec biosynthesis. On the other hand, selenium can readily substitute for sulfur in sulfur pathways and be incorporated nonspecifically into proteins in the form of selenomethionine (replacing methionine), Sec (replacing cysteine) and selenium analogs of sulfur cofactors [reviewed in 48].

The Present: Selenoproteins, an Even Dozen Thus Far

Twelve mammalian selenoproteins have been described to date which are shown in table 1. They represent various protein families. Sec is present at the active centers of the characterized selenoenzymes which are the glutathione peroxidases, the iodothyronine deiodinases, thioredoxin reductase and selenophosphate synthetase. Although Sec occurs infrequently in protein compared to other amino acids, its presence at a critical site in these enzymes suggests that selenium is important for a variety of biological processes and for human health [63].

A common feature among mammalian selenoproteins is that they appear to regulate and/or be regulated by intra- and/or extracellular redox processes. Nature may have taken advantage of utilizing Sec at critical sites in certain enzymes due to the unique redox properties of selenium [16]. Although Sec is structurally similar to cysteine, selenium is a better nucleophile than sulfur and selenocysteines are ionized at physiological pH, while cysteines typically are protonated [48, 86]. The biological function of several selenoproteins, including selenoprotein P, selenoprotein W and the 15-kd selenoprotein, remains unknown. These proteins, however, contain putative Sec redox-active centers and it is therefore tempting to speculate that these centers serve a redox-dependent function.

The Glutathione Peroxidase Family

The glutathione peroxidase selenoenzyme family contains four members, GPX1, GPX2, GPX3 and GPX4. GPX1 is also called classical or cytosolic glutathione peroxidase, GPX2, gastrointestinal glutathione peroxidase, GPX3, plasma glutathione peroxidase, and GPX4, phospholipid hydroperoxide glutathione peroxidase. GPX1, GPX2, GPX3 and GPX4 have also been abbreviated as cGPX, GPX-GI, pGPX and PHGPX, respectively. Glutathione peroxidases are homologous enzymes, which share several common features such as they catalyze glutathione-dependent degradation of hydroperoxides and utilize Sec as a critical component of the enzyme active center. However, each member of this class of selenoenzymes has several distinct characteristics and our current knowledge of each is summarized below. Additional information on the glutathione peroxidase family of enzymes may be found in the following reviews [2, 22, 48, 88, 93, 94].

(1) Glutathione Peroxidase 1 (GPX1 or cGPX). GPX1 is one of the most thoroughly studied and best-characterized selenoproteins. It catalyzes glutathione-dependent reduction of hydrogen peroxide and various organic hydroperoxides. The enzyme is a homotetramer of ~22 kd subunits and it is located in the cytosol. The genes for mouse GPX1 and *Escherichia coli* formate dehydrogenase H were the first selenoprotein genes to be sequenced, which revealed that Sec is encoded by TGA [18, 103]. Bovine erythrocyte GPX1 has been crystallized and its three-dimensional structure determined [29]. Although GPX1 is considered, together with catalase and superoxide dismutase, as a major antioxidant enzyme, its role as an essential antioxidant has been questioned. Indeed, dramatic losses in GPX1 activity and expression level occur in selenium-deficient animals [89] without an apparent effect on cellular metabolism. Such observations led investigators to propose that GPX1 may serve as a selenium storage or selenium buffer protein [88]. In addition, when

Table 1. Mammalian selenocysteine-containing proteins

	Selenoprotein	Function	Expression	Chromosomal localization
1	Glutathione peroxidase 1 (GPXl or cGPX)	Glutathione-dependent hydroperoxide removal	Ubiquitous	h 3q11–13.1 and/or 3p21.3 m chr. 9
2	Glutathione peroxidase 2 (GPX2 or GPX-GI)	Glutathione-dependent hydroperoxide removal	Gastrointestinal tract	h 14q24.1 m chr. 12
3	Glutathione peroxidase 3 (GPX3 or pGPX)	Antioxidant (hydroperoxide removal)	Plasma	h chr. 5 m chr. 11
4	Phospholipid hydroperoxide glutathione peroxidase (GPX4 or PHGPX)	Phospholipid hydroperoxide removal	Ubiquitous	h 19p13.3
5	Thyroid hormone deiodinase 1 (D1)	Conversion of T_4 to T_3 Inactivation of T_3 and T_4	Thyroid gland, liver, kidney, central nervous system	h 1p32-p33
6	Thyroid hormone deiodinase 2 (D2)	Conversion of T_4 to T_3	Pituitary and thyroid glands, placenta, heart and skeletal muscles, central nervous system, brown fat	h 14q24.3
7	Thyroid hormone deiodinase 3 (D3)	Inactivation of T_3 and T_4	Placenta, central nervous system, skin	h 14q32 m 12F1
8	Thioredoxin reductase (TR1)	NADPH-dependent reduction of thioredoxin	Ubiquitous	h 12q23-q24.1
9	Selenophosphate synthetase 2 (SPS2)	Synthesis of selenophosphate	Ubiquitous	m chr. 7
10	Selenoprotein P (SelP)	Antioxidant? Selenium storage?	Plasma	h 5q31
11	Selenoprotein W (SelW)	Redox?	Ubiquitous	h 19q13.3
12	15-kd selenoprotein	Protein folding?	Ubiquitous	h 1p31

Chromosomal localization is shown for human (h) and mouse (m) selenoprotein genes.

mice lacking the GPX1 gene were generated, no phenotypic changes were observed in these knockout mice, even under hyperoxia conditions [54]. However, recent findings of the essential role of GPX1 in protection of mice from viral-induced myocarditis suggest that this enzyme may be important in rescuing cells from environmental stress [4], while its function under normal conditions may be compensated for by other cellular components.

Supplementation of the diet with selenium has been shown to reduce the risk of prostate, colon and lung cancers [23], and chemopreventive properties of this element have been associated with GPX1. Expression of this enzyme is often altered in malignant cells relative to controls [30]. Interestingly, GPX1 exhibits contrasting patterns of regulation with another antioxidant selenoenzyme, thioredoxin reductase, in several tested cancer systems [41]. Further research is necessary to determine which selenoprotein(s) are indeed responsible for chemopreventive properties of selenium.

(2) Glutathione Peroxidase 2 (GPX2 or GPX-GI). GPX2, like GPX1, is a tetramer consisting of identical 22-kd subunits and occurs in the cytosol. However, in contrast to GPX1, which is expressed in every cell type tested, GPX2 is mainly expressed in the epithelium of the

gastrointestinal tract [22]. In this organ, GPX2 activity accounts for about 50% of the total selenium-dependent glutathione peroxidase activity [31].

(3) Glutathione Peroxidase 3 (GPX3 or pGPX). GPX3 differs from other glutathione peroxidases in that it is a glycoprotein and it is the only extracellular selenium-containing glutathione peroxidase. GPX3 is able to detoxify hydroperoxides in the presence of glutathione. However, since the levels of glutathione in plasma are extremely low, an alternative reducing substrate has been sought. Indeed, it was found that thioredoxin, thioredoxin reductase or glutaredoxin are better electron donors than glutathione for GPX3 under physiological conditions [11]. These findings are reinforced by the lack of conservation of the glutathione-binding site in this glutathione peroxidase [94]. The crystal structure at 2.9 Å has been recently reported for GPX3 [81] providing an explanation for differences in substrate specificity and catalytic activities of plasma and intracellular glutathione peroxidases.

(4) Glutathione Peroxidase 4 (GPX4 or PHGPX). GPX4 is different from other selenium-dependent glutathione peroxidases in that it is capable of reducing phospholipid hydroperoxides. GPX4 is an ~19-kd monomeric enzyme and it occurs in both a soluble and a membrane-bound form. The subcellular localization of GPX4 is controlled by alternative transcription and translation sites [78]. Under conditions of limiting selenium, selenium is preferentially incorporated into GPX4 relative to GPX1. GPX4 is ubiquitously expressed and is one of the most abundant selenoproteins in mammals (the other most abundant intracellular selenoproteins are GPX1, thioredoxin reductase (TR) and the 15-kd selenoprotein). The dramatic elevation of GPX4 expression in testis after puberty suggests that this selenoenzyme may have a role in the male reproductive system [69]. Other selenoproteins may also be involved in mediating an essential role of selenium in spermatogenesis, particularly testis-specific selenoproteins [87].

The Iodothyronine Deiodinase Family

The iodothyronine deiodinases are a family of three oxidoreductases that regulate the activity of thyroxine by catalyzing removal of iodine from the inner (5-iodine) or outer (5′-iodine) ring of this hormone. Thyroxine is secreted by the thyroid gland and the deiodinases, designated type 1, type 2 and type 3 deiodinase or D1, D2 and D3, are primarily located in mammalian tissues peripherally to the thyroid. Although D1, D2 and D3 have different roles in the activation and inactivation of thyroxine, these deiodinases share several common features. All three are selenoenzymes containing the Sec residue in a highly homologous sequence that is located in the midregion of each protein. Sec occurs at the enzymes' active center and is most likely involved in the transfer of iodine. Each deiodinase is an integral membrane protein and thus contains, as would be expected, a hydrophobic sequence in the N-terminal portion of the enzyme. There are several distinct features of D1, D2 and D3 which are discussed below. It should also be noted that an excellent review on the deiodinase family has recently been published [62] and a more detailed account of the substrates, substrate specificities and primary structures of each deiodinase may be found in this review.

(5) Type 1 Deiodinase (D1). D1 is a 29-kd selenoprotein that is capable of deiodinating both the inner and outer ring of the prohormone, T_4. Thus, D1 can convert T_4 to the active hormone, T_3, as well as inactivate both T_4 and T_3. Its role is to provide T_3 to the plasma and to inactivate T_4 and T_3. It is located in the thyroid, liver, kidney and central nervous system and is a thyroid-responsive selenoenzyme.

D1 occupies a unique historical position in the elucidation of selenoprotein biosynthesis in mammals. The location of the SECIS element in mammalian selenoprotein mRNA and the role of the SECIS element in determining that a UGA codon designates Sec instead of termination was first demonstrated with D1 mRNA [9, 10].

(6) Type 2 Deiodinase (D2). D2 is a 30.5-kd selenoprotein that catalyzes the conversion of T_4 to T_3. The Km of D2 for T_4 is three orders of magnitude lower than that of D1 for T_4. This selenoenzyme is essential for providing a source of T_3-receptor-bound T_3 in the brain and pituitary gland. D2 activity may be regulated both pre- and post-translationally by T_4 [see 24 and references therein]. Interestingly, a second TGA codon is encoded in the gene for rat and human D2 eight codons from the termination codon that may signal Sec insertion into protein or termination of protein synthesis during transient expression [24, 85]. D2 is expressed in the pituitary gland, the central nervous system, placenta and brown fat of humans and rats and in the thyroid gland and skeletal and heart muscle of humans, but not rats.

(7) Type 3 Deiodinase (D3). D3 is a 31.5-kd selenoprotein that inactivates T_3 and T_4 by catalyzing the removal of iodine from the inner ring of either substrate. D3, like D1, is a thyroid-responsive selenoenzyme and it plays the major role in inactivating T_3 or T_4. It occurs in placenta, the central nervous system and skin. D3 is expressed in highest levels in placenta and functions to impair access of T_3 and T_4 to the fetus.

(8) Thioredoxin Reductase (TR)

Mammalian TR was first characterized in late 1970s [56], but it was not until 1996 that this enzyme was recognized as a selenoprotein [38, 91]. Although most selenoproteins contain Sec in the N-terminal portion or in the middle of the protein, TR contains Sec as a C-terminal penultimate residue [35, 38, 102], which is essential for enzyme activity [1, 42, 52]. TR is a homodimer consisting of two identical 55-kd subunits, each containing a flavin adenine dinucleotide cofactor. The major function of TR is to catalyze NADPH-dependent reduction of thioredoxin in the cytosol [83]. In addition, TR has been implicated in a variety of processes, including the reductions of glutathione peroxidase [11], disulfide isomerase (and related enzymes) [67, 68], dehydroascorbate [73], ascorbyl-free radical [74], selenite, selenodiglutathione [13], hydroperoxides [12] and other proteins and compounds. The broad substrate specificity of TR has been attributed to the presence of Sec in the enzyme. However, the puzzling location of Sec in a conserved C-terminal Gly-Cys-Sec-Gly tetrapeptide cannot be explained easily, since other members of the pyridine nucleotide-disulfide oxidoreductase family, such as glutathione reductase and lipoamide dehydrogenase, lack the C-terminal Sec-containing extension [38]. Relative to these other enzymes, TR has three orders of magnitude higher reactivity to several inhibitory gold, quinone and alkylating compounds [1, 28, 44, 60, 71, 72, 76, 95]. The high reactivity of TR to these compounds has also been attributed to Sec in the enzyme. The purified reduced TR can be inactivated by oxygen, while elevated levels of oxygen in the growth medium resulted in a lower specific activity of TR. These oxygen-dependent processes have been associated with the loss of selenium from the enzyme [42].

TR is often overexpressed in malignant tissues [34, 38, 41] and it has oncoprotein-like properties [59]. Products of oncogenes may stimulate cell proliferation and also sensitize cells to apoptosis [32]. A role of TR and its substrate thioredoxin in cell proliferation [3, 33, 34, 90] and apoptosis [25, 55, 84] has been reported. In addition, TR and thioredoxin are involved in redox regulation of cell signaling [87]. TR occurs in a variety of isoform and isoenzyme types [38, 42, 61, 82, 87, 91]. We recently discovered and characterized two new mammalian thioredoxin reductases TR2 and TR3 that contain a TGA-encoded Sec residue [87]. TR2 is preferentially expressed in testis, while TR3 contains a mitochondrial targeting signal and is expressed in various cell types [87].

(9) Selenophosphate Synthetase 2 (SPS2)

SPS2 is a recently discovered ~50-kd selenoenzyme [45]. It catalyzes activation of selenium in an ATP-dependent manner. The product, which is selenophosphate, donates selenium to the intermediate in the biosynthesis of Sec on tRNA. The natural selenoenzyme has not been isolated, which precluded its detailed characterization, but the selenide-dependent activity has been demonstrated for the overexpressed cysteine-for-Sec mutant of SPS2 [58]. SPS2 is homologous to mammalian selenophosphate synthetase 1 (SPS1), with the latter replacing the putative active center Sec with threonine [65]. Which of these two selenophosphate synthetases is the major protein involved in selenoprotein synthesis remains to be determined. Selenophosphate synthetase genes have been found in bacteria, archaea, *Drosophila melanogaster* and *Caenorhabditis elegans*. These genes encode either Sec-containing or homologous cysteine-containing enzymes. The finding of Sec in mammalian SPS2 is important in view of the possible autoregulation mechanism of selenoprotein synthesis, i.e., SPS2 is possibly involved in its own biosynthesis [45].

(10) Selenoprotein P (Sel P)

Sel P (P designates plasma) is a 57-kd glycoprotein and is the major selenoprotein in the plasma of mammals [51]. It contains 9–12 Sec residues depending on the organism in which it occurs and is the only known selenoprotein that contains more than one Sec residue [80]. All Sec residues in this selenoprotein are coded by UGA. The efficiency of Sec incorporation at each UGA codon in Sel P mRNA appears to be different which results in multiple product forms [21] that arise from termination at different UGA codons [53]. In particular, the second in-frame UGA codon dictates significant termination giving rise to a selenoprotein product that may have a separate function [53]. Sel P contains two types of SECIS elements in the 3′-untranslated region of its mRNA suggesting that both are required for Sec insertion in response to multiple and different UGA codons [50]. The protein is rapidly expressed following supplementation of selenium-deficient animals with selenium. It has been suggested that Sel P serves as an antioxidant [17] or selenium storage (or transport) protein. Further studies are required to establish the function of this interesting selenoprotein.

(11) Selenoprotein W (SelW)

SelW is a ~8-kd intracellular protein of unknown function [96]. A single Sec residue is present in this protein in an N-terminal putative active center redox motif

Cys-Gly-Ala-Sec-Gly [97], suggesting the possible involvement of the protein in a redox-related process. The isolated protein may occur in the glutathionylated form [8], which is consistent with a putative redox role for SelW. The protein is expressed in many tissues and in increased levels in muscle and brain. SelW expression, as well as expression of most other selenoproteins are regulated by selenium [101], i.e., selenium elevates selenoprotein expression.

(12) 15-kd Selenoprotein

The 15-kd protein is the most recently discovered selenoprotein. It contains a single Sec residue in the middle of the protein in a conserved Cys-Gly-Sec-Lys putative redox-active center [39]. The 15-kd protein does not share any sequence homology to previously characterized proteins and its function currently is not known. The 15-kd selenoprotein is expressed in a variety of tissues with increased levels in prostate and thyroid. It has been found in humans, mice and rats. *C. elegans* and *Brugia malayi* also contain homologs of this selenoprotein, but the homologs have cysteine in place of Sec.

Preliminary evidence suggests that the 15-kd selenoprotein may be involved in cancer [40]. Indeed, selenoprotein expression is altered in several tested cancers and two cancer-related polymorphisms have been found in the 3′-untranslated region of the human protein, including one in the SECIS element. These variations in sequence may potentially affect the expression level of the protein in malignant relative to normal tissue.

The 15-kd protein has been isolated from rat prostate and mouse liver in a complex with UDP-glucose glycoprotein glucosyltransferase (UGTR) [40]. Interestingly, UGTR is involved in quality control of protein folding by glucosylating misfolded proteins and directing them for degradation. If indeed the 15-kd protein is involved in this process, it will be an interesting new role for a selenoprotein.

The Future: Role of Sec in Health and Total Number of Selenoproteins

The role that selenium plays as an important component in the diet of mammals apparently changes with age. That is, selenium serves as an absolutely essential micronutrient in early mammalian development since removal of the Sec tRNA gene from the mouse genome is lethal to the embryo [15]. Furthermore, rats that are weaned at birth onto a selenium-deficient diet manifest dramatically slower rates of growth, abnormal muscle and skeletal development, develop cataracts and a variety of other disorders as compared to their siblings maintained on a selenium-sufficient diet. Adult rats and mice maintained on a selenium-deficient diet for extended periods of time appear to be less affected by the loss of selenium unless challenged by environmental stress. Although in studies involving selenium-deficient animals, selenoprotein expression has not been reduced as effectively as in Sec tRNA gene knockout studies, these nutritional experiments suggest that the role of selenium may change from an essential requirement to sustain life in embryonic development to one that is a requirement for adequate growth and development at birth to more of a chemopreventive or protective agent against environmental stress in later life. Studies involving conditional removal of the Sec tRNA gene from the genome of mice (i.e., conditional knockouts) at selected stages of development and in selected tissues [5, 77, 79] should provide further insight into defining the role of selenium throughout life. Experiments in which mice are generated carrying the Sec tRNA gene so that the gene can be conditionally removed from the genome of mice are in progress [Hatfield, unpubl. data].

Selenium deficiency in humans is associated with numerous health disorders, including increased cancer risk, AIDS mortality, heart disease and problems with the male reproductive system [23, 63, 69]. This may be explained by the fact that selenium deficiency results in decreased selenoprotein expression and, hence, in the disruption of processes that are maintained by these selenoproteins. Supplementation of the human diet with selenium therefore offers a potentially efficient and inexpensive means of preventing or delaying the onset of certain of these maladies. Future research will, no doubt, result in determining the precise mechanisms of how selenium benefits human health and in offering this micronutrient as a dietary supplement to people at risk of developing various types of cancer, AIDS and other diseases that are influenced by selenium. Most certainly, many, if not all, of the beneficial effects of selenium on human health are mediated by selenoproteins, but which selenoproteins are involved and their role is not well understood. It is also quite likely that many protective effects of selenium are mediated by yet-to-be characterized selenoproteins.

The total number of selenoproteins that are encoded in the genomes of mammals is difficult to predict. Various estimates range from 25 to 100. These estimates are based on ^{75}Se-labeling experiments, which detect selenoprotein spots on 2D gels [6, 7], and on the extrapolation of the current proportion of known selenoproteins among all

characterized proteins taking into account that many selenoproteins are expressed in low levels [Gladyshev, unpubl. data]. In fact, the growing interest in discovering novel selenoproteins is hampered in part by the difficulty involved in their identification due to low expression levels.

An important task in further developing the selenoprotein field and in elucidating the role of selenium in human health is the detection, isolation and characterization of additional selenoproteins. There are several approaches that may be used in identifying new selenoproteins. For example, isolation of labeled proteins from tissues and cells following labeling of animals and cell lines with ^{75}Se. Historically, this has been the most productive approach in identifying new selenoproteins and it still proves to be a useful technique for this purpose. More recently, identification of Sec-encoded TGA codons and of SECIS elements followed by determination of the selenoprotein open reading frame in nucleotide databases has been very useful in finding novel selenoproteins. Searches of databases utilizing the unique features of the Sec insertion machinery have been aided tremendously by recent developments in projects involving sequencing of entire genomes, and by the expressed sequence tag projects. For example, the approach of identifying SECIS elements in nucleotide databases was recently applied to search for potential SECIS elements in the HIV genome [43, 92]. SECIS elements are characterized by the presence of a highly conserved ATGAN[10–12 nucleotides]AAN[16–26 nucleotides]NGAN sequence, which forms stem-loop structures with AAN located in the loop, and TGAN and NGAN forming the non-Watson-Crick quartet interaction [66, 99]. Searching for SECIS elements in nucleotide databases is a unique tool for identifying new mammalian selenoproteins [Kryukov and Gladyshev, unpubl. data].

An alternative computer approach to search for novel selenoproteins involves an examination of nucleotide databases for Sec TGA codons. This approach takes advantage of the fact that many mammalian selenoproteins have homologs in lower eukaryotes, and these homologs incorporate cysteine in place of Sec. Thus, computer approaches may be utilized that search for in-frame TGA codons in the mammalian nucleotide sequences that correspond to TGC/T in lower organisms.

The above approaches of 'digital cloning' for discovering new selenoproteins are unlikely to result in the identification of all mammalian selenoprotein genes, but the development of these methods will be useful for future whole genome sequence analyses. Completion of the sequence of the human genome, which is scheduled to be finished in 2003 [100], as well as completion of the *C. elegans* and *D. melanogaster* genome projects will be invaluable in detecting all selenoprotein genes encoded in these genomes.

An additional area to be explored in the near future is high-throughput, comprehensive analysis of relative transcript expression levels [104]. The emerging technologies, such as serial analysis of gene expression, differential display approaches and various hybridization analyses (such as microchips and membranes) will not only reveal parallel changes in selenoprotein expression, but also determine how selenium affects expression of proteins that do not contain selenium.

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