Molecular biology of selenium with implications for its metabolism[1]

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ABSTRACT **Selenium has a highly specific metabolism centered around its incorporation as selenocysteine into selenoproteins. An outline of this metabolism has emerged from recent molecular biological and biochemical studies of bacteria and animals. A unique tRNA, designated $tRNA^{[Ser]Sec}$, is charged with L-serine, which is then converted through at least two steps to selenocysteine. With the aid of a unique translation factor, the selenocysteinyl-$tRNA^{[Ser]Sec}$ recognizes specific UGA codons in mRNA to insert selenocysteine into the primary structure of selenoproteins. Turnover of selenoproteins presumably liberates selenocysteine which is toxic in its free form. Selenocysteine β-lyase catabolizes free selenocysteine and makes its selenium available for reuse. Proteins contain almost all the selenium in animals. Of the known selenoproteins, the glutathione peroxidases contain the most selenium. Cellular and plasma glutathione peroxidases are products of different genes but have 44% identity of amino acid sequence. There is evidence for other proteins of this family. Selenoprotein P is an unrelated protein with multiple selenocysteines in its primary structure. It contains most of the selenium in rat plasma. Studies of the regulation of cellular glutathione peroxidase by selenium have yielded conflicting results, but there is a strong suggestion that mRNA levels of the rodent liver glutathione peroxidase decrease in selenium deficiency. This could be a mechanism for directing selenium to the synthesis of other selenoproteins. Although present knowledge allows construction of an outline of selenium metabolism, several steps have not been characterized and little is known about mechanisms of its regulation.—Burk, R. F. Molecular biology of selenium with implications for its metabolism. *FASEB J.* 5: 2274-2279; 1991.**

Key Words: selenocysteines • selenoproteins • glutathione peroxidase • selenoprotein P

THE ESSENTIALITY OF SELENIUM in biological systems has been recognized since the 1950s (1, 2). Deficiency of the element has been implicated in a number of naturally occurring and laboratory-produced pathological conditions in animals. Keshan disease, a cardiomyopathy of children reported from China, is a selenium-responsive human condition (3). There have been claims that selenium also plays a role in common diseases such as cirrhosis, cancer, and coronary heart disease. Assessment of these claims will require detailed knowledge of the biochemical functions of the element and its homeostasis.

Remarkable progress in elucidation of selenium metabolism has been made in the last 5 years. Several groups have presented results outlining the mechanisms of selenocysteine synthesis and incorporation into protein. Regulation of selenoprotein synthesis has been examined at the protein and mRNA levels, and new selenoproteins have been characterized. This brief review will consider a few of these advances. Other reviews have appeared recently (4, 5).

SYNTHESIS OF SELENOCYSTEINE AND ITS INCORPORATION INTO SELENOPROTEINS

In 1976 the form of selenium in the primary structure of selenoprotein A of *Clostridium sticklandii* was demonstrated to be selenocysteine (6). After the same form of the element was found in glutathione peroxidase (7) and in hydrolysates of tissues (8), it seemed likely that this amino acid was the major form of physiologically active selenium in animals.

It was difficult to envision how selenocysteine was incorporated into proteins. All 64 possible nucleic acid codons had been accounted for as coding for the 20 known protein amino acids or for termination of translation, so it seemed unlikely that a particular codon could specify selenocysteine. Nevertheless, one group presented evidence for a tRNA in rat liver with selenocysteine bound to it (9). They did not identify the anticodon of this tRNA, however, and their work was not followed up for several years. Meanwhile, Sunde and colleagues (10) reported on studies which suggested that inorganic forms of selenium were more easily incorporated into the selenocysteine of glutathione peroxidase than was selenium administered as selenocystine. They then demonstrated by administering labeled amino acids that the carbon skeleton of the selenocysteine in glutathione peroxidase was derived from serine and not from selenocysteine or cysteine (11). Their results suggested to many that selenocysteine arose from a posttranslational modification of a serine residue. Thus, two views were held about the incorporation of selenocysteine into selenoproteins. One suggested direct incorporation mediated by a tRNA and the other suggested modification of a serine residue.

The sequencing in 1986 of a cloned cDNA to mouse cellular glutathione peroxidase (12) was the first step toward reconciling these two mechanisms. The implied amino acid sequence of the mouse enzyme had 86% identity with bovine glutathione peroxidase. Coding for residue 47, which was selenocysteine in the bovine enzyme, was the triplet TGA. Soon thereafter, a TGA was found to correspond to selenocysteine in a formate dehydrogenase of *Escherichia coli* (13). These observations suggested that TGA (the UGA

[1]From the Symposium *Molecular Biology of Selenium* presented by the American Institute of Nutrition at the 74th Annual Meeting of the Federation of American Societies for Experimental Biology, Washington, D.C., April 4, 1990.

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codon in mRNA) specified incorporation of selenocysteine into the primary structure of these selenoproteins. However, UGA was known to function as a stop codon, so an explanation of its additional usage as a codon for selenocysteine was required. Studies by Böck and colleagues have provided considerable insight into this process.

This group characterized four gene products in *E. coli* required for incorporation of selenocysteine into protein. Their studies were carried out using mutants that had each been characterized as unable to make formate dehydrogenase (14, 15). *E. coli* contains two distinct formate dehydrogenases (16) and both are selenoenzymes. In complementarity studies using the mutants, four genes, designated *selA, selB, selC,* and *selD* (**Fig. 1**), were identified, isolated, and sequenced. The steps of selenocysteine synthesis and incorporation into selenoproteins which require these gene products will be discussed individually.

tRNA$^{[Ser]Sec}$

The selC gene product is a tRNA that differs substantially from other tRNAs of *E. coli* (17). Its anticodon sequence directly matches the UGA codon in mRNA; it is larger than other tRNAs; and it varies in some residues formerly considered invariant in tRNAs of *E. coli.* Charging experiments with this unique tRNA showed that L-serine is the amino acid that is charged and that seryl-tRNA ligase carries out this reaction (step 1, Fig. 1). Free selenocysteine did not compete with serine in this reaction, indicating that it cannot be charged directly to this tRNA. However, selenocysteine bound to this same tRNA was demonstrated in *E. coli* grown with selenite as the selenium source (18). This suggested that selenocysteine is made from serine while it is attached to this tRNA. Involvement of this tRNA with these two amino acids led one group to designate it as tRNA$^{[Ser]Sec}$ (19) and that designation will be used here.

An animal tRNA that recognized the UGA codon had been described in 1981 (20). This tRNA is charged with serine which then is phosphorylated in vivo. This opal suppressor tRNA had been characterized in detail but a function could not be demonstrated (21). After the UGA codon was shown to specify selenocysteine in glutathione peroxidase, tRNA$^{[Ser]Sec}$ was suspected to be involved and studies to assess this possibility were performed. tRNA$^{[Ser]Sec}$ was isolated from cells with selenocysteine attached (22). Thus this tRNA is the animal counterpart to the tRNA specified by *selC.* Hybridization studies have shown that this tRNA is widespread in the animal kingdom (19) and in enterobacteriaceae (23). Although studies of plants have not been reported, these findings imply that specifically incorporated selenocysteine occurs in bacterial and animal life forms.

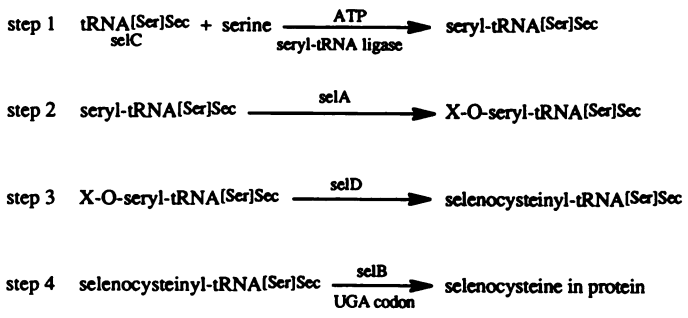

Figure 1. Proposed scheme for selenocysteine synthesis and incorporation into proteins. The four steps are based on *E. coli* genes *selA-D* as explained in the text. Isolation of a tRNA$^{[Ser]Sec}$ and of the products of the steps indicate that a similar mechanism functions in animal cells.

Activation of serine bound to tRNA$^{[Ser]Sec}$

Evidence has been presented that the side-chain oxygen of serine is activated (step 2, Fig. 1) before its replacement by selenium. *SelA* mutants of *E. coli* are unable to convert seryl-tRNA$^{[Ser]Sec}$ to selenocysteinyl-tRNA$^{[Ser]Sec}$ (24). Incubation of seryl-tRNA$^{[Ser]Sec}$ with *SelA* protein led to the consumption of the acylated serine and appearance of a compound which was not selenocysteine. Addition of *selD* protein and a source of selenide to the incubation caused disappearance of the unidentified compound and the appearance of selenocysteine.

Identification of phosphoseryl-tRNA$^{[Ser]Sec}$ in animal cells provides a strong indication that the initial activation step is carried out by a kinase in them (22). Thus, it seems likely that activation of the acylated serine occurs by different mechanisms in bacteria and animals (24).

Formation of selenocysteinyl-tRNA$^{[Ser]Sec}$

The *selD* gene product is required, along with a source of selenide, for conversion of the activated X-O-seryl-tRNA$^{[Ser]Sec}$ to selenocysteinyl-tRNA$^{[Ser]Sec}$ (step 3, Fig. 1). The gene codes for a protein of 347 amino acids (24). Expression studies confirmed the size of the protein and allowed its production for the in vitro experiments described in the preceding section. This conversion also occurs in animal cells (22), suggesting the presence in animals of a protein analogous to the *selD* protein.

Gene disruption studies demonstrated that *selD* is also necessary for the incorporation of selenium into certain tRNAs (24). Replacement of sulfur by selenium, yielding the modified nucleoside 5-methylaminomethyl-2-selenouridine in tRNA, had been demonstrated in bacteria (25) and plants (26). When this nucleoside, which contains selenium instead of sulfur, is present in the wobble position it affects codon-anticodon interaction (27).

A *Salmonella typhimurium* mutant also defective in incorporation of selenium into selenocysteine of formate dehydrogenases and into tRNAs has been isolated (28) and designated *selA1.* Complementarity studies of the *selA1* and *selD* genes indicate functional identity (29). Neither is required for uptake of selenite by the cell or for incorporation of selenium into selenomethionine and selenocysteine in a nonspecific way (29). This occurs presumably through sulfur metabolic pathways.

Because *selD* is necessary for two functions, it has been suggested that additional proteins, which have not yet been detected, are needed for each function (24). *SelD* might be solely a selenide carrier as it does not specify where selenium is incorporated. If this is the case, *selD* could be located at a major branch point of selenium metabolism. Perhaps it is the source of selenium for methylation and subsequent excretion in animals as well (30).

Incorporation of selenocysteine into protein

The protein encoded by the *selB* gene is a translation factor necessary for incorporation of selenocysteine into bacterial selenoproteins (step 4, Fig. 1). No evidence of an analogous translation factor has been reported for animal cells. *SelB*

mutants of *E. coli* accumulate selenocysteinyl-tRNA$^{[Ser]Sec}$ and fail to insert selenocysteine into protein in a specific manner (31). The deduced amino acid sequence of this protein exhibits extensive homology with EF-Tu (an elongation factor needed for binding amino acyl-tRNAs to the ribosome) in its initial 244 amino acids but is 68.8 kDa compared with the smaller 43-kDa EF-Tu. This difference in size suggests that the *selB* protein has more functions than EF-Tu.

EF-Tu serves for 20 amino acids and the *selB* protein apparently serves only for one: selenocysteine. EF-Tu has a much lower affinity for seryl-tRNA$^{[Ser]Sec}$ and for selenocysteinyl-tRNA$^{[Ser]Sec}$ than for other tRNAs, presumably because of the unique structural features of tRNA$^{[Ser]Sec}$ (32). The *selB* protein complexes with selenocysteinyl-tRNA$^{[Ser]Sec}$ but not with seryl-tRNA$^{[Ser]Sec}$ (31). This indicates that the *selB* protein recognizes the acylated selenocysteine. This set of circumstances mandates the conversion of seryl-tRNA$^{[Ser]Sec}$ to selenocysteinyl-tRNA$^{[Ser]Sec}$ before interaction with mRNA can occur. It is likely that the *selB* protein also plays a role in distinguishing the UGA codons which specify incorporation of selenocysteine from those which terminate translation.

Significance

The work reviewed in this section points to a highly specific metabolism of selenium in bacteria and animals. This can be contrasted with the metabolism in plants where selenium appears to follow sulfur metabolic pathways and its incorporation into proteins lacks specificity. It will be important to search for elements of the specific metabolic pathway in plants. Its presence would suggest that selenium is required by them. There is evidence of nonspecific selenium metabolism in bacteria (29) alongside the specific metabolism considered here, but there is little evidence of its occurrence in animals except under a few circumstances. The most important such circumstance occurs when selenomethionine is administered. Its metabolism in animals appears similar to that of methionine.

The major known fates of selenium in animals are incorporation into protein as selenocysteine, incorporation into certain modified tRNAs, and excretion as methylated compounds. The pathway reviewed here has been shown to be involved in the first two of these and likely contains elements involved in selenium homeostasis. *SelD* is a candidate for such a role. It will be important to determine which steps of selenium metabolism are regulated and how that regulation is exerted.

CATABOLISM OF SELENOCYSTEINE BY SELENOCYSTEINE β-LYASE

In 1981 Soda's group (33) demonstrated the conversion of selenomethionine to selenocysteine in rat liver by the transsulfuration pathway. They noted that selenocysteine did not accumulate and discovered that an enzyme was responsible for this. In subsequent work they purified and characterized this enzyme, which they named selenocysteine β-lyase (34). This activity occurs in most animal tissues and in bacteria as well. The bacterial enzyme is a different protein from the animal enzyme but has similar kinetic properties (35): both catalyze the β-elimination of L-selenocysteine into L-alanine. The selenium product is H_2Se when the reaction is carried out in the presence of dithiothreitol and is elemental selenium when it is carried out anerobically in the absence of thiols. L-Cysteine is a nonsubstrate competitive inhibitor. The presence of this enzyme implies that free selenocysteine does not persist in the cell.

When it was first described in 1982, selenocysteine β-lyase was difficult to integrate into contemporary schemes of selenium metabolism. However, with new insights into selenocysteine synthesis, this activity can be better understood. Not only might it supply a reduced form of selenium for the *selD* protein, it could serve as a detoxification mechanism for the reactive molecule selenocysteine. Free selenocysteine is much more toxic than selenomethionine (36). Selenocysteine β-lyase allows the organism to acquire selenium in the form of selenomethionine (a major form in plants) while avoiding the toxicity of the metabolite selenocysteine. It likely serves also to catabolize selenocysteine liberated by the turnover of endogenous selenoproteins and the catabolism of ingested selenoproteins.

Figure 2 shows a proposed scheme of selenium metabolism which incorporates new information on selenocysteine synthesis and selenocysteine β-lyase. This scheme is presented as being consistent with present knowledge and as a framework for conjecture. The position of selenocysteine β-lyase in this scheme is consistent with the enzyme's kinetic characteristics. It has a high K_m and a high V_{max}, making it suitable for detoxification. No studies of the regulation of selenocysteine β-lyase have appeared. However, organisms have been shown to adapt to excess selenium intake and an increase in selenocysteine β-lyase activity might accomplish this.

CHARACTERIZATION OF THE PLASMA SELENOPROTEINS GLUTATHIONE PEROXIDASE AND SELENOPROTEIN P

Plasma glutathione peroxidase

Glutathione peroxidase in red cells was demonstrated to be a selenoenzyme in 1973 (37). In the years that followed, this

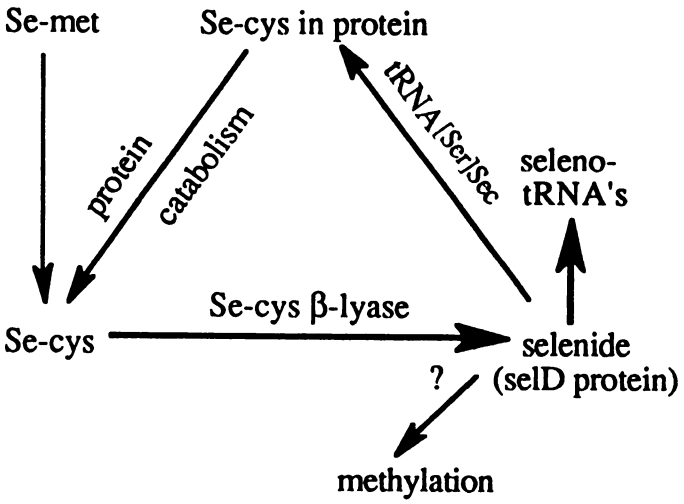

Figure 2. Proposed scheme of selenium metabolism showing the dual functions of selenocysteine β-lyase in detoxifying selenocysteine (Se-cys) and in providing selenide for *selD* protein. Sources of Se-cys include metabolism of selenomethionine (Se-met) via the transsulfuration pathway and catabolism of endogenous and ingested proteins. This scheme features selenide bound to *selD* protein as the source of selenium for its major metabolic fates. Inorganic selenium is not shown in the scheme but can be reduced to selenide by reaction with GSH.

observation was extended to an enzyme in other cells and one in plasma. Characterization studies have suggested that the cellular glutathione peroxidases are closely related because they exhibit similar kinetics, and polyclonal antibodies raised to them generally react across organs and species. Moreover, deduced amino acid sequences of some of them generally have a similarity of 80–96% (38). Plasma glutathione peroxidase has characteristics not shared with the cellular form.

Polyclonal antibodies raised against human plasma glutathione peroxidase do not cross-react with human cellular glutathione peroxidases (39). This has been explained recently by the finding that these proteins share only 44% amino acid sequence identity based on sequences deduced from cloned cDNA (40). Also, plasma glutathione peroxidase is glycosylated and the cellular enzyme is not (41). Cohen's group (42) has demonstrated that cultured HepG2 cells, a liver-derived cell line, secrete plasma glutathione peroxidase into the medium and synthesize the cellular enzyme (which is not secreted) as well. Thus, plasma and cellular forms of glutathione peroxidase are related but are different gene products. They share the property of decreasing in selenium deficiency.

Selenoprotein P

The other known plasma selenoprotein is also a glycoprotein secreted by the liver (43, 44). The presence of this protein was suspected in the mid-1970s (45), but it was first purified from rat plasma by immunoaffinity chromatography in 1987 (43). Deglycosylation of selenoprotein P reduces its M_r on sodium dodecyl sulfate-polyacrylamide gel electrophoresis from 57 to 43 kDa (46). Amino acid analysis indicates that there are 7.5 ± 1.0 selenocysteines per molecule and this accounts for all the selenium in the protein. Peptide fragmentation studies indicate that selenoprotein P has selenium-rich regions. Sequencing of cloned cDNA reveals multiple TGA codons in the open reading frame which presumably code for selenocysteine (47).

The concentration of selenoprotein P in rat plasma is 26 μg/ml as determined by radioimmunoassay (46). Removal of selenoprotein P by passing serum over an immunoaffinity column did not remove glutathione peroxidase activity but did remove more than 60% of the selenium. Thus, selenoprotein P accounts for most of the selenium in rat serum and is estimated to contain 8% of the selenium in the rat. Selenium-deficient rat serum has 5–10% of control selenoprotein P concentration as measured by radioimmunoassay. However, the selenium concentration of that serum is lower than would be predicted by the selenoprotein P concentration. This raises the possibility that selenium-poor forms of selenoprotein P are produced when the element is in short supply.

Injection of physiological amounts of selenium into selenium-deficient rats leads to a rapid increase in selenoprotein P concentration. By 6 h it increases to 35% and by 24 h to 75% of control (48). Plasma and liver glutathione peroxidase activities increase to only 6% of control at 24 h, indicating that the synthesis of selenoprotein P takes precedence over that of the glutathione peroxidases under conditions of limiting selenium supply.

The functions of these two plasma selenoproteins are not known. Glutathione peroxidase requires reduced glutathione for its catalytic activity, but reduced glutathione has a concentration of 20 μM in rat plasma, which is less than 1% of the concentration inside cells. Therefore the capacity of plasma glutathione peroxidase to remove H_2O_2 under physiological conditions can be questioned, which leaves open the possibility of other functions for this enzyme. Selenoprotein P is selenol- and thiol-rich, containing about 4 μM concentration of each in rat plasma. Thus, it might play a redox role, although evidence to support this is limited (49). Another proposed function is in the transport of selenium (50). Further work will be required to clarify the function of the plasma selenoproteins.

REGULATION OF CELLULAR GLUTATHIONE PEROXIDASE

More than 80% of the selenium in the rat (excluding selenium nonspecifically incorporated as selenomethionine) is present as selenocysteine (8). Approximately half of this selenocysteine, or 40% of whole-body selenium, is in glutathione peroxidase (51). In the absence of exogenous selenium supply, turnover of proteins containing selenium provides virtually all the selenide to *selD* protein for subsequent utilization (see Fig. 2). Thus, control of individual selenoprotein synthesis could be vital to efficient utilization of limited amounts of the element for its most essential functions. Selenoprotein concentrations decrease in selenium deficiency, and there is evidence that differential regulation of them occurs when selenium supply is limiting (52). Because of its quantitative significance, several laboratories have examined the effect of selenium deficiency on cellular glutathione peroxidase. They have measured its activity, immunoreactive protein, and mRNA to determine the mechanism of its decrease. Decreased synthesis of glutathione peroxidase would make selenium available for synthesis of other selenoproteins and thus might serve as a buffer against adverse effects. In this sense it would function as a storage form of the element.

There is unanimous agreement that glutathione peroxidase activity and immunoreactive glutathione peroxidase protein fall in selenium deficiency. However, Sunde's group (53) demonstrated by ELISA the presence of a small amount of an immunoreactive polypeptide in rat liver when no glutathione peroxidase activity could be detected. This result is compatible with synthesis of inactive glutathione peroxidase protein, perhaps not containing selenium. In similar experiments using immunoblotting, Reddy's group (54) was unable to detect glutathione peroxidase protein. These studies indicate that selenium deficiency does not cause accumulation of large amounts of enzymatically inactive glutathione peroxidase protein, but there is evidence for the presence of small amounts of such a protein as there is for a selenium-poor selenoprotein P (46).

Because the major effect of selenium deficiency did not appear to be posttranslational, several groups assessed the effect of selenium deficiency on glutathione peroxidase mRNA levels. Most studies indicate that liver glutathione peroxidase mRNA is decreased in selenium deficiency. Yoshimura et al. (55) performed Northern analysis on rat liver total RNA using an 867-base pair probe that contained the entire open reading frame. They found a strong signal in control liver but a negligible one in selenium-deficient liver. A control chicken β-actin probe revealed a striking increase of this mRNA in selenium-deficient liver. Saedi et al. (56) used a 700-base pair mouse liver glutathione peroxidase probe and found that selenium-deficient rat liver had 7–17% the level of mRNA in controls using total RNA and poly A-selected RNA. Chicken β-actin mRNA was unaffected by selenium deficiency in their experiments.

Unaccountably different results were obtained by Reddy's group (38). They used a 1539-base pair probe of rat liver enzyme which contained the entire open reading frame and a very long (888 base pairs) 3' noncoding region (38). Northern analysis of poly A-selected RNA gave a signal two- to threefold stronger in selenium-deficient liver than in control (54). In vitro translation by a rabbit reticulocyte lysate system showed greater production of immunoreactive glutathione peroxidase protein when selenium-deficient mRNA was used than when control mRNA was used. These results are not in harmony with those of other groups who used animals, although results with cultured cells have been similar (57, 58).

Time course experiments have been reported in which the responsiveness of glutathione peroxidase mRNA levels to selenium supply has been demonstrated. Imura's group (59) measured glutathione peroxidase activity and mRNA level in mice fed a selenium-deficient diet for 6 wk and then fed a control diet. They demonstrated a decrease to very low levels of enzyme activity and mRNA concentration in liver and kidney. Upon selenium refeeding, mRNA levels increased to control in 2 days. Glutathione peroxidase activity reached control only after 7 days. This lag between mRNA appearance and enzyme activity confirms results of Sunde's group (5). These results, while strengthening the argument that selenium supply regulates liver mRNA level for glutathione peroxidase, provide another example of lack of agreement of mRNA levels and enzyme activity (the lag). This suggests that the regulatory mechanisms for glutathione peroxidase are not simple and straightforward. Several different cDNAs related to glutathione peroxidase have been cloned (58, 60), and it is possible that some of the discordant results reviewed here are related to cross-hybridization of cDNA probes with more than one mRNA species.

Little is known about transcriptional control of glutathione peroxidase. Imura's group (61) has reported that nuclear run-on experiments indicate similar rates of transcription in selenium-deficient and control mouse livers which have different glutathione peroxidase mRNA levels. This suggests that the level of glutathione peroxidase control is posttranscriptional. Obtaining an accurate picture of the regulation of glutathione peroxidase by selenium requires further research. FJ

Supported in part by National Institutes of Health grants ES 02497 and HL 36371. The author is grateful to Kristina E. Hill for reading the manuscript.

Note added in proof: New information on the function of *selA* protein in bacteria has been reported in two papers (a, b). The gene product of *selA* is a pyridoxal phosphate-dependent enzyme which binds seryl $tRNA^{[Ser]Sec}$ and releases selenocysteinyl-$tRNA^{[Ser]Sec}$ The replacement of oxygen by selenium occurs while the substrate is bound to the enzyme. The *selA* protein has been designated selenocysteine synthase.

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