# Selenocysteine tRNA$^{[Ser]Sec}$ Levels and Selenium-Dependent Glutathione Peroxidase Activity in Mouse Embryonic Stem Cells Heterozygous for a Targeted Mutation in the tRNA$^{[Ser]Sec}$ Gene†

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ABSTRACT: To investigate the effect of a reduced level of selenocysteine (Sec) tRNA$^{[Ser]Sec}$ in selenoprotein biosynthesis, two mouse embryonic stem (ES) cell lines heterozygous for the corresponding gene were generated by homologous recombination of the host genome with targeting vectors encoding a deleted or a disrupted tRNA$^{[Ser]Sec}$ gene. The presence of a single functional gene in ES cells afforded us an opportunity to determine directly in the cell line the effect of reduced gene dosage on (1) the levels of the Sec tRNA$^{[Ser]Sec}$ population, (2) the distributions of the isoacceptors within the Sec tRNA population, and (3) selenoprotein biosynthesis. We therefore determined the amounts and distributions of the two major tRNA$^{[Ser]Sec}$ isoacceptors, designated mcm$^5$U and mcm$^5$Um, within the Sec tRNA population and determined the activity of the anti-oxidant, selenium-containing glutathione peroxidase (GPx) in the heterozygotes and in wild type cells grown in media with and without added selenium. The level of the Sec tRNA$^{[Ser]Sec}$ population in the heterozygotes was approximately 60% of that of wild type cells grown in media under normal conditions, while the ratio of the mcmU isoacceptor in wild type vs mutant cells was approximately 2:1 and of the mcmUm isoacceptor approximately 1:1. In the presence of media supplemented with selenium, the Sec tRNA$^{[Ser]Sec}$ population increased about 20% in wild type cells and virtually not all in heterozygous cells, and the level of the Sec tRNA$^{[Ser]Sec}$ population was, therefore, approximately 50% of that of wild type cells. GPx activity was indistinguishable among these cell lines in either selenium-supplemented or unsupplemented media, indicating that the resultant changes in tRNA$^{[Ser]Sec}$ levels did not have a measurable effect on GPx biosynthesis.

The selenocysteine (Sec)[1] tRNA [designated tRNA$^{[Ser]Sec}$ (Hatfield *et al*., 1994)] population in most mammalian cells consists primarily of two major isoacceptors which insert Sec into nascent polypeptides in response to UGA codons in selenoprotein mRNAs [see recent review by Lee *et al*. (1996)]. These two Sec tRNA$^{[Ser]Sec}$ isoacceptors differ from each other by a single methyl group attached to the 2′-*O*-ribose of the nucleoside in the wobble position of one of the tRNAs (Amberg *et al*., 1993; Diamond *et al*., 1993). The modified nucleoside in the wobble position is either 5-methylcarboxymethyluridine (mcm$^5$U) or 5-methylcarboxymethyluridine-2′-*O*-methylribose (mcm$^5$Um), respectively. The levels and distributions of the mcm$^5$U and mcm$^5$Um isoacceptors respond to exogenous selenium in mammalian cells grown in culture (Hatfield *et al*., 1991), in the tissues of rats maintained on selenium-deficient diets (Diamond *et al*., 1993), and in *Xenopus* oocytes maintained in cultural media (Choi *et al*., 1994). Selenoprotein levels in mammalian cells are also affected by exogenous selenium [reviewed in Sunde (1994, 1997)].

It has been questioned whether the Sec tRNA$^{[Ser]Sec}$ population is limiting in the biosynthesis of selenoproteins (Barry *et al*., 1992, 1994). One means of assessing if the Sec tRNA isoacceptors are limiting is to selectively reduce the amount of these tRNAs available for protein synthesis and then determine if selenoprotein biosynthesis is altered. In the present study, we took advantage of the fact that the effect of losing one tRNA$^{[Ser]Sec}$ gene copy on the Sec tRNA$^{[Ser]Sec}$ population and on selenoprotein biosynthesis could be determined in embryonic stem (ES) cells which provided the earliest opportunity of examining the effect of this mutation on selenium metabolism. Thus, the level of the tRNA$^{[Ser]Sec}$ population, the distribution of the Sec

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[1] Abbreviations: Sec, selenocysteine; ES cells, embryonic stem cells; mcm$^5$U, 5′-methylcarboxymethyluridine; mcm$^5$Um, 5′-methylcarboxymethyluridine-2′-*O*-methylribose; GPx, glutathione peroxidase; kb, kilobase(s); bp, base pair(s); pSETΔ1.9, targeting vector with a 1.9 kb deletion of the tRNA$^{[Ser]Sec}$ gene; pSETΔ0.059, targeting vector with a 59 bp deletion within the coding region of the tRNA$^{[Ser]Sec}$ gene; EF cells, embryonic fibroblast cells.

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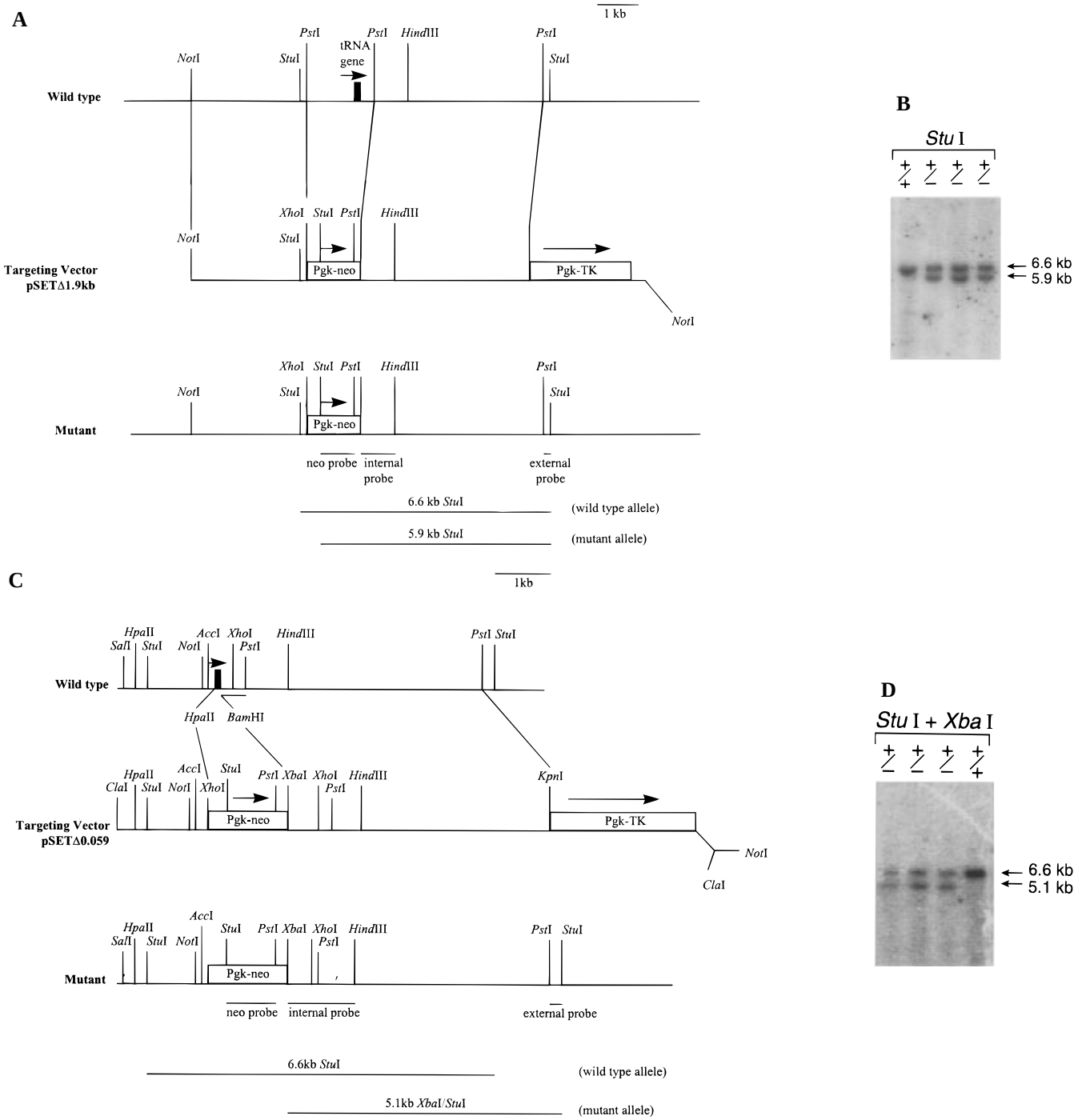


FIGURE 1: Targeted replacement of the gene encoding Sec tRNA[Ser]Sec. (A) Construction of the targeting vector pSETΔ1.9 (see Materials and Methods). The external, internal, and neo probes used for Southern blot analyses are shown. Wild type and mutant alleles designate wild type and mutant DNA fragments that hybridize with the external probe. (B) Southern blot analysis of ES cell clones transfected with pSETΔ1.9 (see text for details). (C) Construction of the targeting vector pSETΔ0.059 (see Materials and Methods). The probes and wild type and mutant DNA fragments are the same as described above in panel A. (D) Southern blot analysis of ES cell clones transfected with pSETΔ0.059 (see text for details).

tRNA[Ser]Sec isoacceptors, and the activity of the selenoprotein, glutathione peroxidase (GPx), were determined in wild type ES cells and in ES cell lines heterozygous for mutation of the tRNA[Ser]Sec gene. We find that selectively reducing the amount of the tRNA[Ser]Sec population by as much as 50% does not appear to affect the level of biosynthesis of GPx.

## MATERIALS AND METHODS

*Construction of the pSETΔ1.9 Targeting Vector*. The targeting vector, pSETΔ1.9, was generated using pPNT as the backbone (see Figure 1A). pPNT contained the genes for phosphoglucokinase (Pgk)-neomycin (neo) and Pgk-herpes simplex virus thymidine kinase (TK). A genomic clone of the Sec tRNA[Ser]Sec gene and flanking regions was isolated from a 129/Sv mouse genomic library as described (Ohama *et al.*, 1994; Bösl *et al.*, 1995). A *Not*I−*Pst*I fragment (∼3 kb) containing the upstream region of the tRNA[Ser]Sec gene and a *Pst*I fragment containing the downstream region of the gene (∼4 kb) were placed 5′ and 3′ to the neo gene, respectively. The neo and tRNA[Ser]Sec genes, therefore, have the same transcriptional orientation. The *Pst*I fragment was blunt-ended with T4 DNA polymerase and

cloned into the *Bam*HI site (also blunt-ended) of pPNT. The *Not*I−*Pst*I fragment encoding the 5′-flank of the tRNA$^{[Ser]Sec}$ gene was originally prepared by initially cloning the fragment into pBluescript KSII$^+$ (from Stratagene) and then subcloned into the *Not*I−*Xho*I cloning sites to generate pSETΔ1.9 (see Figure 1A).

*Construction of pSETΔ0.059 Targeting Vector*. To remove a portion of the coding sequence of the tRNA$^{[Ser]Sec}$ gene and replace it with the neo gene, the second targeting vector, pSETΔ0.059, was constructed using pPNT (see Figure 1C). The 5′-flanking region of the tRNA$^{[Ser]Sec}$ gene was prepared from a *Sal*I−*Hpa*II fragment spanning the 5′-end to 1.85 kb upstream of the gene. A *Sal*I−*Hpa*II fragement was originally cloned into pBluescript KSII$^+$ after digestion with *Sal*I−*Cla*I. The 3′-flanking region of the gene was prepared from a *Bam*HI−*Pst*I fragment spanning the 3′-end of the tRNA$^{[Ser]Sec}$ gene to approximately 5 kb downstream that was constructed by digesting a PCR product generated with primer 5′-CTAGGATCC*GTGGTTCAA-TTCCACCTTTCGGG*-3′, containing a *Bam* HI site (underlined) and the 3′-end of the Sec tRNA$^{[Ser]Sec}$ gene (italicized) and primer 5′-CCAGTCGGGTGGTCTAGGGGGTT-3′, located approximately 600 bp downstream of the 3′-end of the Sec gene. The resulting fragment (∼350 bp) was ligated to a *Xho*I−*Pst*I fragment (∼5 kb) located downstream of the gene (see Figure 1C). The 5′-flanking and 3′-flanking regions were ligated with the 5′-end and 3′-end of the neo gene, respectively, in pPNT to generate pSETΔ0.059 as shown in Figure 1C.

*Transfection and Selection of ES Cells*. ES cells were cultured on a feeder layer of embryonic fibroblast (EF) cells in HEPES-buffered Dulbecco's modified Eagle's media supplemented with 15% heat-inactivated fetal bovine serum (from Hyclone), 0.1 mM $\beta$-mercaptoethanol (from Sigma), nonessential amino acids (from Gibco), and antibiotics. The EF cells were derived from 14 day-old embryos and $\gamma$-irradiated with a total of 3000 rad before plating.

ES cells were electrophorated at 250 $\mu$F and 250 V with the linearized targeting vectors (pSETΔ1.9 was linearized with *Not*I and pSETΔ0.059 with *Cla*I), plated, incubated for 24 h at 37 °C, the resulting transfected ES cell clones were screened by adding G418 (350 mg/mL dry powder; from GIBCO) and ganciclovir (5 $\mu$M; from Syntex Research) to the culture media, and after an additional 8−12 days of incubation, ES clones were selected and further expanded for freezing and DNA preparation (Lee *et al*., 1995; Fernandez-Salguero *et al*., 1995; Fernandez-Salguero & Gonzalez, 1996).

*Screening for Homologous Recombination*. Clones were screened for homologous recombination by Southern hybridization. Genomic DNA isolation and Southern hybridization were performed by the methods described previously (Sambrook *et al*., 1989; Laird *et al*., 1991; Tybulewica *et al*., 1991). Genomic DNA was digested with *Stu*I. Both internal (inside the mouse DNA construct) and external (outside the mouse DNA construct and in the host genomic DNA) probes were used for hybridization. The external probe was prepared by digesting the plasmid containing the 3′-flanking region of the gene with *Pst*I and *Stu*I and isolating the 250 bp fragment (see Figure 1).

*Growth of ES Cells, Isolation, Aminoacylation, and Fractionation of tRNA, Ribosomal Binding Studies, and Assay of GPx Activity*. Wild type ES cells and ES cells carrying the targeted mutations were grown in the absence of feeder cells in standard medium containing 1000 units/mL of LIF, the cells were harvested, the tRNA was extracted and deacylated, tRNA was aminoacylated with L-[$^3$H]serine (29 Ci/mM from Amersham), and the resulting [$^3$H]seryl-tRNAs were chromatographed two successive times on a RPC-5 column (Kelmers & Heatherly, 1971) as described (Hatfield *et al*., 1991; Diamond *et al*., 1993). Ribosomal binding studies were carried out on fractionated [$^3$H]-aminoacyl-tRNAs by the procedure of Nirenberg and Leder (1964) as described (Hatfield *et al*, 1979). GPx activity was measured by a standard assay which spectrophotometrically measures the oxidation of NADPH in the coupled system containing reduced glutathione, glutathione reductase, cellular extracts and hydrogen peroxide as the substrate as described (Samuels *et al*., 1991). The rate of oxidation of NADPH was measured at 339 nm in a Beckman DU 540 spectrophotometer at 30 s intervals over 5 min. The background rate of oxidation was determined without lysate. GPx activity was expressed as the nmol of NADPH oxidized/min/mg of protein.

## RESULTS

*Targeting Vectors and Generation of Heterozygotes*. The two targeting vectors, designated pSETΔ1.9 and pSETΔ0.059, were designed to disrupt the functional tRNA$^{[Ser]Sec}$ gene by homologous recombination, and they were prepared as shown in Figure 1A and 1C, respectively. Homologous recombination of pSETΔ1.9 with genomic DNA resulted in a 1.9 kb deletion of the tRNA$^{[Ser]Sec}$ gene and flanking sequences, while homologous recombination of pSETΔ0.059 resulted in deletion of only 59 bp within the tRNA$^{[Ser]Sec}$ gene coding sequence.

Analysis of genomic DNA from mutant cells by Southern hybridization demonstrated that the targeted vector had homologously recombined with the host genome (see Figure 1B and D). As shown in Figure 1B, genomic DNA from wild type cells and from mutant cells carrying pSETΔ1.9 which was digested with *Stu*I and screened with the external probe resulted in a single 6.6 kb band in wild type DNA and in 6.6 and 5.9 kb bands in mutant DNA. Similarly, genomic DNA from wild type and from mutant cells carrying pSETΔ0.059 which was digested with *Stu*I−*Xba*I and screened with the external probe resulted in a single 6.6 kb band in wild type DNA and in 6.6 and 5.1 kb bands in mutant DNA (see Figure 1D).

*Coding Properties, Levels, and Distributions of tRNA$^{[Ser]Sec}$ Isoacceptors*. Sec tRNA is initially aminoacylated with serine by seryl-tRNA synthetase (Diamond *et al*., 1981; Hatfield *et al*., 1982). Therefore, tRNA extracted from mutant and wild type cell lines was aminoacylated with [$^3$H]-serine, and the resulting labeled, Sec tRNA$^{[Ser]Sec}$ isoacceptors were resolved from the seryl-tRNA population and from each other by reverse phase chromatography (Hatfield *et al*., 1991; Diamond *et al*., 1993). It is essential to resolve the Sec tRNA$^{[Ser]Sec}$ population from the seryl-tRNA population, as the former represents about 1−2% of the total seryl-tRNA population.

To confirm that the two tRNA species in the mutant cell lines, which were labeled with serine, were the Sec isoacceptors designated mcm$^5$U and mcm$^5$Um, [$^3$H]seryl-tRNAs were fractionated on a reverse phase chromatographic

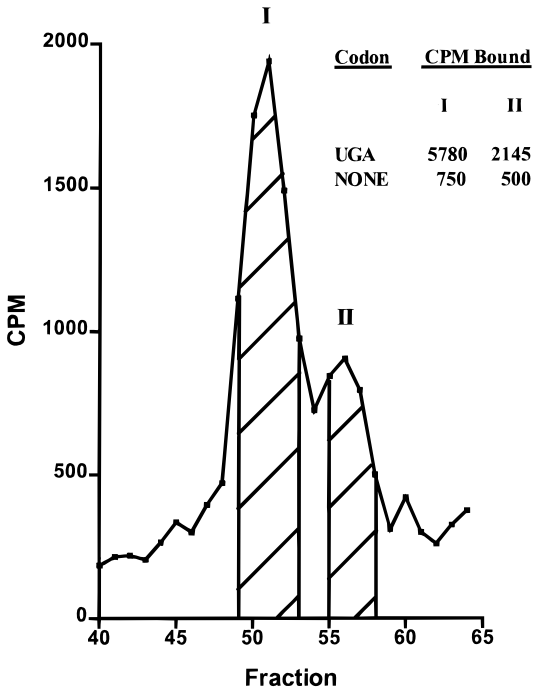


FIGURE 2: Coding responses of Sec tRNA[Ser]Sec isoacceptors. Transfer RNA was isolated from ES cells carrying the pSETΔ1.9 targeted vector, aminoacylated with [3H]serine, the resulting labeled tRNAs fractionated, the Sec tRNA[Ser]Sec isoacceptors collected, and ribosomal binding studies carried out as described in Materials and Methods.

column. Those isoacceptors predicted to be the Sec tRNA[Ser]Sec isoacceptors were isolated from the column and examined for their ability to decode the Sec codon, UGA [see Figure 2 and Hatfield *et al*. (1979, 1991) and Diamond *et al*. (1981, 1993)]. Both isoacceptors recognize UGA in a ribosomal binding assay (Figure 2). Their patterns of elution from the RPC-5 column correspond to those observed for mcm5U and mcm5Um (Hatfield *et al*., 1991; Diamond *et al*., 1993).

The amount of the Sec tRNA[Ser]Sec population was determined in wild type and the two heterozygotes as described previously (Hatfield *et al*., 1991; Diamond *et al*., 1993) following resolution of the Sec tRNA[Ser]Sec population by reverse phase chromatography. The levels of the Sec tRNA[Ser]Sec population in both heterozygotes were approximately 60% of that observed in wild type cells (see Figure 3 and Table 1).

The distributions of the mcm5U and mcm5Um isoacceptors in each cell line were also determined. The amount of mcm5U, which is the species that elutes initially from the column, was approximately 2-fold greater in wild type as compared to mutant cell lines. 77.1% of the Sec tRNA population in wild type cells was the mcm5U isoacceptor, while the relative amounts in the mutant cell lines represented 39.3% in the line carrying the pSETΔ1.9 targeted vector and 43.5% in the line carrying the pSETΔ0.059 targeting vector, respectively, compared to the wild type cells (see column 5 in Table 1). The amount of the second eluting species, mcm5Um, was present in approximately equal amounts in wild type and mutant cells. 22.9% of the Sec tRNA[Ser]Sec population in wild type cells was the mcm5U isoacceptor, and the relative amounts in the heterozygotes represented 21.4% in the line carrying the pSETΔ1.9 targeting vector and 16.6% in the line carrying the pSETΔ0.059 targeting vector, respectively, of that observed in wild type cells (see column 5 in Table 1).

*Effect of Selenium on the Sec tRNA[Ser]Sec Population.* The selenium status influences the levels and distributions of the Sec tRNA[Ser]Sec isoacceptors in mammalian cells grown in culture (Hatfield *et al*., 1991), in tissues from rats maintained on a selenium-adequate or selenium-deficient diet (Diamond *et al*., 1993), and in cultured *Xenopus* oocytes (Choi *et al*., 1994). In each of these cell lines or tissues, the Sec tRNA[Ser]Sec population increases by 20%−100% and the distribution of the Sec isoacceptors changes from a predominance of mcm5U to one of mcm5Um in response to selenium.

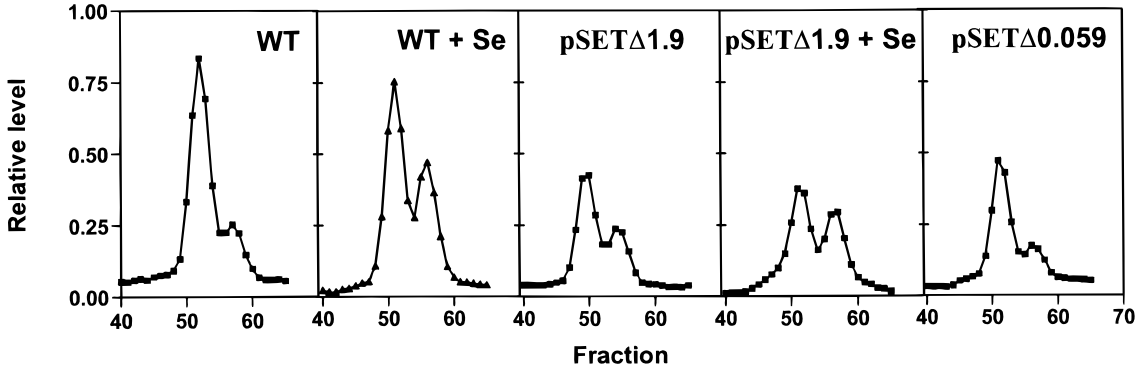


FIGURE 3: Reverse phase chromatography of the Sec tRNA[Ser]Sec isoacceptors from wild type ES cells and ES cells carrying the pSETΔ1.9 or pSETΔ0.059 targeting vector. Transfer RNA was isolated from wild type and mutant ES cells grown in standard media or media supplemented with 0.5 μM sodium selenite and aminoacylated with [3H]serine, and the resulting labeled tRNAs were fractionated as described in Materials and Methods.

Table 1: Levels and Distributions of Ser tRNA[Ser]Sec Isoacceptors[a]

| | −Se/+Se | | | |
|---|---|---|---|---|
| sample | percent mcm5U | percent mcm5Um | percent serine tRNA population | percent wild type −Se(mcm5U + mcm5Um)/ +Se(mcm5U − mcm5Um) |
| wild type | 77.1/58.0 | 22.9/42.0 | 1.63/1.95 | 100(77.1 + 22.9)/100(58.0 + 42.0) |
| pSETΔ1.9 | 64.7/54.7 | 35.3/45.3 | 0.99/1.04 | 60.7(39.3 + 21.4)/53.3(29.2 + 24.1) |
| pSETΔ0.059 | 72.4/ND | 27.6/ND | 0.98/ND | 60.1(43.5 + 16.6)/ND |

[a] Levels and distribution of isoacceptors were determined as described (Hatfield *et al*., 1991; Diamond *et al*., 1993).

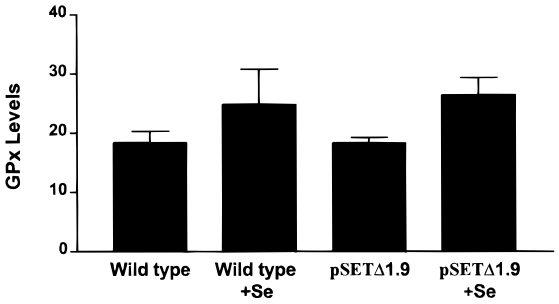


FIGURE 4: GPx activity in wild type ES cells and ES cells carrying the pSETΔ1.9 targeting vector. ES cells were incubated with or without added selenium. 0.5 $\mu$M sodium selenite was added to the culture media of the respective ES cell line for 7 days. GPx activity was measured as described in the Materials and Methods and presented as the average activity determined from three independent cultures, and error bars indicated the SEM. GPx activity is presented as nmol of NADPH oxidized/min/mg of protein.

We, therefore, tested the effect of supplementing the media with selenium on the Sec tRNA$^{[Ser]Sec}$ population in wild type and mutant ES cells.

In response to selenium added to the media, the Sec tRNA$^{[Ser]Sec}$ population increased about 20% in wild type cells, but only very slightly, if at all, in the mutant cell line carrying the pSETΔ1.9 targeting vector (column 4 in Table 1). It should be noted that since the tRNA$^{[Ser]Sec}$ population increased about 20% in wild type cells in response to selenium [see Hatfield *et al*. (1991)], the expected contribution to the increased level from each allele is approximately 10%. Thus the level of induction in mutant cells in response to selenium may actually be closer to 10% and therefore could not be reliably measured. The distribution of the Sec isoacceptors shifted dramatically in wild type cells and less so in the heterozygous cells carrying the pSETΔ1.9 mutation (columns 2 and 3). The relative amounts of the mcm$^5$U isoacceptor decreased in wild type cells from 77.1% to 58.0%, and the mcm$^5$Um isoacceptor increased from 22.9% to 42%. The mcm$^5$U isoacceptor decreased in the mutant cell line from 64.7% to 54.7%, and the mcm$^5$Um isoacceptor increased from 35.3% to 45.3%. The amount of mcm$^5$U and mcm$^5$Um in the mutant cell line harboring pSETΔ1.9 relative to that observed in the wild type cells was 29.2% and 24.1%, respectively, in cells supplemented with selenium in the growth media (column 5).

*Glutathione Peroxidase Activity*. The Sec tRNA$^{[Ser]Sec}$ isoacceptors are essential components of the translational machinery dedicated to synthesis of selenium-containing proteins. In order to determine if the quantitative changes described above for the tRNA$^{[Ser]Sec}$ population influence selenoprotein biosynthesis, the levels of GPx, a well-characterized Sec-containing enzyme, were examined in the wild type cell line and the heterozygous mutant cell line carrying the pSETΔ1.9 targeting vector. As shown in Figure 4, GPx activity was indistinguishable between these two cell lines. In addition, supplementation of the culture media with selenium has been shown to result in an increase of GPx activity to varying degrees in different cell types [see Sunde (1994, 1997) for review and Diamond *et al*. (1996a,b) and references therein]. Induction of GPx activity by selenium can occur post-transcriptionally [see Diamond *et al*. (1996b) and references therein] and at concentrations similar to those shown to induce changes in the tRNA$^{[Ser]Sec}$ population. It was therefore examined whether adding selenium to the media of wild type ES cells and ES cells heterozygous for the targeted mutation resulted in changes in GPx activity. As shown in Figure 4, adding 0.5 $\mu$M sodium selenite to the media of wild type and mutant cell lines resulted in a marginal increase in activity that was statistically indistinguishable from each other.

## DISCUSSION

Two different mouse ES cell lines, both heterozygous for mutation of the Sec tRNA$^{[Ser]Sec}$ gene, were generated by homologous recombination with different targeting vectors. Use of one targeting construct, designated pSETΔ1.9, resulted in an approximately 1.9 kb deletion of the Sec tRNA$^{[Ser]Sec}$ gene and flanking sequences. As the Sec tRNA$^{[Ser]Sec}$ gene is only 87 bp long and the flanking DNA involved in its function encompasses about another 250 bp [see review in Lee *et al*. (1996)], it is essential to be sure that any effects resulting from loss of the tRNA$^{[Ser]Sec}$ gene are due solely to its removal and not to the removal of extra delected DNA that might encode regions essential to other cellular functions. We therefore prepared a second targeting vector that disrupted tRNA$^{[Ser]Sec}$ gene function by removal of only 59 bp within the coding sequence of the gene. The latter targeting vector, designated pSETΔ0.059, was also incorporated into the mouse genome by homologous recombination.

Generation of ES cell lines heterozygous for the tRNA$^{[Ser]Sec}$ gene provided an opportunity to determine whether the Sec tRNA$^{[Ser]Sec}$ population could be selectively reduced or whether the remaining functional gene would compensate for the loss of the other gene. We found that both heterozygous cell lines contain approximately 60% of the Sec tRNA$^{[Ser]Sec}$ population observed in wild type ES cells. Therefore, they show only a slight increased expression of the tRNA$^{[Ser]Sec}$ gene over the expected 1:1 ratio contributed by each gene located at both loci. The data also show that the amount of the tRNA$^{[Ser]Sec}$ population can be selectively reduced by the approach described herein.

The Sec tRNA$^{[Ser]Sec}$ population is distributed largely between two major isoacceptors designated mcm$^5$U and mcm$^5$Um in most mammalian cells and tissues (Lee *et al*., 1996). The only difference in primary structure between the two species is the presence of a methyl group on the 2′-*O*-ribose of the nucleoside in the wobble position of one of the isoacceptors. In the present study, the heterozygotes were found to contain approximately as much of the mcm$^5$Um isoacceptor as wild type cells and therefore most of the reduction in the Sec tRNA$^{[Ser]Sec}$ population in heterozygous cells occurred at the expense of the mcm$^5$U isoacceptor. Thus, the ratio of mcm$^5$U to mcm$^5$Um is shifted in the heterozygous mutant such that the level of the 2′-*O*-methylated isoacceptor is similar to that observed in wild type cells. It is intriguing to speculate that the maintenance of similar levels of the mcm$^5$Um isoacceptor in mutant and wild type cells reflects a more significant role of this species, relative to the mcm$^5$U isoacceptor, in selenoprotein biosynthesis. However, the functional differences between these isoacceptors remain to be established.

The level of the Sec tRNA$^{[Ser]Sec}$ population and the distributions of the Sec tRNA$^{[Ser]Sec}$ isoacceptors in mammalian cells grown in culture (Hatfield *et al*., 1991), in

mammalian tissues (Diamond *et al*., 1993), and in *Xenopus* oocytes (Choi *et al*., 1994) are influenced by the presence of selenium. To our knowledge, this remains the only example of the relative abundances of a tRNA population being influenced by a trace element. In the present study, the effect of supplementing the growth media with selenium on the Sec tRNA[Ser]Sec population was examined. The Sec tRNA[Ser]Sec population in wild type cells increased approximately 20% in response to added selenium which was the same increment observed in HL60 and rat mammary tumor cells grown in media supplemented with selenium (Hatfield *et al*., 1991). On the other hand, the Sec tRNA[Ser]Sec population increased only slightly, if at all, in the heterozygote in response to selenium. The distributions, however, of both Sec tRNA[Ser]Sec populations responded to selenium added to the media. Approximately 20% of the Sec tRNA[Ser]Sec population shifted from the $mcm^5U$ to the $mcm^5Um$ isoacceptor in wild type cells. This change was less dramatic in the heterozygous cell line where about 10% of the Sec tRNA[Ser]Sec population shifted from $mcm^5U$ to $mcm^5Um$.

The selective reduction in the Sec tRNA[Ser]Sec population by approximately 50% in heterozygous mutant cells grown in media supplemented with selenium did not appear to affect the biosynthesis of the selenoprotein, GPx. In fact, GPx activity was virtually the same in both wild type and heterozygous cell lines. These studies suggest that the Sec tRNA[Ser]Sec population is not limiting in selenoprotein biosynthesis in ES cells, at least not for GPx.

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