September 2007

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JBC Papers in Press. Published on September 11, 2007 as Manuscript M707036200
The latest version is at http://www.jbc.org/cgi/doi/10.1074/jbc.M707036200

SELECTIVE RESTORATION OF THE SELENOPROTEIN POPULATION IN A MOUSE HEPATOCYTE SELENOPROTEINLESS BACKGROUND WITH DIFFERENT MUTANT SELENOCYSTEINE tRNAs LACKING Um34

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Running Title: Partial Restoration of Selenoproteins in Liver

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Novel mouse models were developed in which the hepatic selenoprotein population was targeted for removal by disrupting the selenocysteine (Sec) tRNA$^{[Ser]Sec}$ gene (*trsp*) and then selenoprotein expression was restored by introducing wild type or mutant *trsp* transgenes. The selenoprotein population was partially replaced in liver with mutant transgenes encoding mutations at either position 34 (T34->A34) or 37 (A37->G37) in tRNA$^{[Ser]Sec}$. The A34 transgene product lacked the highly modified 5-methoxycarbonylmethyl-2' *O*-methyluridine and its mutant base A was converted to I34. The G37 transgene product lacked the highly modified N^6-isopentenyladenosine. Both mutant tRNAs lacked the 2'-methylribose at position 34 (Um34), and both supported expression of housekeeping selenoproteins (e.g., thioredoxin reductase 1) in liver, but not stress-related proteins (e.g., glutathione peroxidase 1). Thus, Um34 is responsible for synthesis of a select group of selenoproteins rather than the entire selenoprotein population. The ICA anticodon in the A34 mutant tRNA decoded Cys codons, UGU and UGC, as well as the Sec codon, UGA. However, metabolic labeling of A34 transgenic mice with ^{75}Se revealed that selenoproteins incorporated the label from the A34 mutant tRNA, whereas other proteins did not. These results suggest that the A34 mutant tRNA did not randomly insert Sec in place of Cys, but specifically targeted selected selenoproteins. High copy numbers of *A34* transgene, but not *G37* transgene, were not tolerated in the absence of wild type *trsp* further suggesting insertion of Sec in place of Cys in selenoproteins.

There are 24 known selenoproteins in rodents and 25 in humans (1). The targeted removal of specific selenoproteins has shown that some are essential in development whereas others appear to be non-essential. For example, the loss of selenoproteins glutathione peroxidase 4 (GPx4; 2), or thioredoxin reductase 1 (TR1 or Txnrd1; 3) or 2 (TR3 or Txnrd2; 4) is embryonic lethal, while the loss of glutathione peroxidase 1 (GPx1; 5) or 2 (GPx2; 6) appears to be of little or no consequence. Other studies, however, suggest that

those selenoproteins whose loss results in little or no phenotypic change may function in protective mechanisms against certain environmental stresses (see 6 and references therein). There are selenoproteins whose removal or mutation results in dramatic effects on health. For example, knockout of selenoprotein P (SelP) causes neurological problems (7,8) and knockout of type 2 iodothyronine deiodinase results in a variety of defects including an impaired adaptive thermogenesis and hypothermia in cold-exposed mice (see 9 and references therein), retarded cochlear development and hearing loss (10) and a pituitary resistance to T_4 (11). Mutations affecting selenoprotein N (SelN) result in several muscle disorders (12,13).

LoxP-Cre technology, which allows the removal of embryonic lethal genes in specific tissues and organs (3,4,14), has been used to examine the roles of essential selenoprotein genes in development and health. Such studies have elucidated key roles of TR1 in embryogenesis of numerous tissues and organs, except heart (3), and of TR3 in hematopoiesis and in heart development and function (4). The targeted removal of the nuclear form of GPx4 (designated snGPx4) results in viable and completely fertile animals, although the overall structural stability of sperm chromatin is diminished (14). Loss of SelP in liver, achieved by targeted knockout of the selenocysteine (Sec) $tRNA^{[Ser]Sec}$ gene (designated *trsp*), implicated SelP in transport functions in plasma, and substantiated its essential role in brain (15).

Selenoprotein synthesis is dependent on the presence of Sec $tRNA^{[Ser]Sec}$. Given this dependence, selenoprotein expression can be modulated by perturbing Sec $tRNA^{[Ser]Sec}$ expression, providing a means of elucidating the role of selenoproteins and selenium in development and health (16). The Sec $tRNA^{[Ser]Sec}$ population in higher vertebrates consists of two isoforms that differ by a single 2'*O*-methyl group. One isoform contains 5-methoxycarbonylmethyluridine (mcm^5U) at position 34 and the other is methylated on the ribosyl moiety at that position generating 5-methoxycarbonylmethyl-2'*O*-methyluridine (mcm^5Um; 17). The presence of this 2'-methyl ribose modification (designated Um34) confers several unique properties on mcm^5Um. For example, Um34 affects Sec $tRNA^{[Ser]Sec}_{mcmUm}$ secondary and tertiary structure (18). Um34 addition is dependent on the prior synthesis of the four modified bases found in $tRNA^{[Ser]Sec}$ and on an intact tertiary structure (19). Synthesis of all other modified nucleosides of Sec $tRNA^{[Ser]Sec}$, including mcm^5U, is less stringently associated with primary and tertiary structure. In addition, synthesis of Um34 is dependent on the selenium status of the organism, with increased dietary selenium increasing Um34 levels (17).

Removal of *trsp* is embryonic lethal (20,21). Therefore, to alter the Sec $tRNA^{[Ser]Sec}$ population, techniques of influencing Sec $tRNA^{[Ser]Sec}$ levels other than the sole removal of *trsp* must be employed. We previously generated transgenic mice with extra copies of wild type or mutant Sec $tRNA^{[Ser]Sec}$ transgenes (22), and mice with a conditional knockout of *trsp* (21), and then rescued selenoprotein expression in *trsp* null mice with wild type or mutant Sec $tRNA^{[Ser]Sec}$ transgenes (23,24). Consistent with reports that the Sec $tRNA^{[Ser]Sec}$ population is not limiting in selenoprotein biosynthesis (20,22,25), we found little or no effect of extra copies of wildtype transgenes on selenoprotein expression in the tissues and cells examined (22). In contrast, multiple copies of a mutant *trsp* transgene can lead to specific altercations in the selenoprotein population (22). For example, transgenes with a mutation at position 37 (*A37->G37*) produce a tRNA gene product that not only lacks isopentenyladenosine (i^6A) at this site, but also lacks Um34 (19). Selenoprotein synthesis was affected in mice carrying the *G37* Sec $tRNA^{[Ser]Sec}$ transgene in a protein- and tissue-specific manner (22). Rescue of selenoprotein expression in *trsp* null mice with the *G37* Sec $tRNA^{[Ser]Sec}$ transgene results in the recovery of housekeeping selenoproteins, while numerous stress-related selenoproteins that are non-essential to survival are either not rescued, or are poorly rescued (23,24).

Although the wild type and mutant *trsp* transgenic models and transgenic-*trsp* rescue models have provided considerable insight into selenoprotein expression and the hierarchy of selenoprotein expression (21-24), they have limitations. For example, when expressing mutant *trsp* transgenes in mice carrying the endogenous allele of *trsp*, expression from the wildtype *trsp* can confound the studies. Studies with rescue

models like that described above, with a germline conditional *trsp* allele, focus on the selenoprotein population in the whole animal. The targeted removal of floxed *trsp* in defined cell types using transgenic mice with tissue-specific expression of Cre recombinase permitted some study of the effects of selenoprotein loss in specific tissues and organs in the absence of endogenous *trsp*. However, the resulting animals have a variety of defects including embryonic mortality or early adult death (26) restricting the use of these models for studying the role of selenium and selenoproteins in health.

In the present study, we generated a mouse model that targets the removal of *trsp* in liver for use in elucidating the role of selenium and selenoproteins and the contributions of housekeeping and stress-related selenoproteins in health. *trsp* *lox*P-albumin *Cre* mice (27) were crossed with i^6A-Um34 deficient Sec $tRNA^{[Ser]Sec}$ transgenic mice (designated herein as *G37* transgenic mice; (22)) or with another mutant *trsp* (*T34->A34*) transgenic mouse described herein that lacks mcm^5U and consequently the Um34 modification (designated herein as *A34* transgenic mice). The resulting mouse lines lacks *trsp* in liver and are dependent on the *A34* mutant transgene or the *G37* mutant transgene for selenoprotein expression. These new mouse models provide us with novel experimental systems for investigating the role of numerous stress-related selenoproteins in health in a specifically targeted organ.

EXPERIMENTAL PROCEDURES

Materials - ^{75}Se-selenium (specific activity 1000 Ci/mmol) was obtained from the Research Reactor Facility, University of Missouri, Columbia, MO, ^{3}H-serine (specific activity 29 Ci/mmol) from Amersham and ^{32}P-α-dCTP (specific activity ~6000 Ci/mmol) from Perkin Elmer. Hybond Nylon N+ membranes were purchased from Amersham, NuPage 10% polyacrylamide gels, polyvinylidene difluoride (PVDF) membranes, Superscript II reverse transcriptase and SeeBlue Plus2 protein markers from Invitrogen, SuperSignal West Dura Extended Duration Substrate from Pierce, Universal Reference RNA from Stratagene, ADP Sepharose 4B resin from GE Healthcare and anti-rabbit-HRP and anti-chicken-HRP conjugated secondary antibodies from Sigma. Reagents for the TR1 assay (22) were purchased from Sigma Aldrich. Antibodies against GPx1 were obtained from Abcam and antibodies against GPx4, TR1, TR3, SelR, and SelT were from our laboratories (1,24,27). All other reagents were obtained commercially and were products of the highest grade available.

Animals and genotyping of mice – Homozygous floxed *trsp* C57BL/6 mice ($trsp^{fl}$) that were also homozygous for albumin *Cre* (*AlbCre*) were designated *Δtrsp* after $trsp^{fl}$ was removed by the Cre recombinase (23,24,27). *Δtrsp* C57BL/6-FVB/N transgenic mice were homozygous for one of three types of *trsp* transgene ($trsp^t$) alleles as follows: 1) wild type transgene encoding 10 copies of wild type $trsp^t$/allele (22); 2) *G37* low or high copy transgene encoding either 1 (low) or 8 (high) copies of the *A37->G37* mutant $trsp^t$/allele; or 3) *A34* transgene encoding one copy of the *T34->A34* mutant $trsp^t$/allele. The product of the *G37* transgene lacks the highly modified base, i^6A, at position 37 and also Um34. The single copy *G37* transgenic mouse was generated specifically for this study (22) to compare to the effects of the single copy *A34* transgene. The product of the *A34* transgene lacked the highly modified base, mcm^5U, and also lacked Um34. *T34->A34* transgenic mice were generated exactly as described (22) except that the transgene construct contained an A at position 34 instead of a T and the base at position 37 was the wild type A base; and three founders that were heterozygous for 1, 4 and 6 transgene copies were obtained. *A34* transgenic mice were in strain FVB/N and founders were bred to obtain the corresponding homozygous mice (designated *A34*-2, *A34*-8 and *A34*-12, respectively).

Genotype designations and definitions are given in the legend of Table 1. All mice used in this study were males. Matings to obtain mouse lines carrying wild type, *A34* and *G37* transgenes and *Δtrsp* in their liver are summarized in Table 1.

Primers used for detecting *trsp*, $trsp^{fl}$, *Δtrsp*, $trsp^t$, *G37* or *A34* by PCR are designated CKNO2 (forward primer) and RES1 and VP1 (reverse primers) (see Fig. 1 and ref 27). Primers used for detecting *AlbCre* are designated LIV1 (forward primer) and LIV2 (reverse primer) (see Fig. 1 and 28 and references therein). The care of animals was in accordance with the National Institutes of

Health institutional guidelines under the expert direction of Dr. Kyle Stump (National Cancer Institute, National Institutes of Health, Bethesda, MD).

^{75}Se-Labeling of selenoproteins - Mice were injected intraperitoneally with 50 μCi of ^{75}Se/g and sacrificed 48 hrs after injection as described (see 23,27 and references therein). Tissues and organs were excised, immediately frozen in liquid nitrogen and stored at –80^{o}C. Tissues were homogenized and 40 μg of protein were electrophoresed on NuPage 10% polyacrylamide gels. Gels were stained with Coomassie blue, dried and exposed to a PhosphorImager as described (23,27 and references therein). To further assess ^{75}Se-labeling of TR1, the labeled protein was purified from crude extracts of tissue using ADP Sepharose 4B prior to gel electrophoresis as described (22). TR3 is also enriched by the ADP-Sepharose procedure, but the amounts of TR3 relative to TR1 are only about 10% and likely not to influence overall levels of the TR population (e.g., in Fig. 3B).

Northern and Western blot analyses - Total RNA isolated from liver and kidney was analyzed by Northern blot hybridization using ^{32}P-labeled probes. Membranes were analyzed with a PhosphorImager as described (23,27). Deiodinase-1 (D1), GPx1, GPx4, SelK, SelP, SelR, SelW, Sep15, selenophosphate synthetase-2 (SPS2), and TR1 probes were used (23). The remaining probes were generated by RT-PCR using Superscript II reverse transcriptase and Universal Reference RNA or mouse liver RNA (23,27).

Protein extracts were prepared from liver and kidney and electrophoresed on NuPage 10% polyacrylamide gels. Proteins were transferred to PVDF membranes as previously described with the exception that 40 μg of each protein extract were loaded onto gels (21-23,27) and immunoblotted with antibodies against GPx1 (1:1000 dilution), GPx4 (1:2000), SelR (1:1000) and SelT (1:400). Anti-rabbit-HRP conjugated secondary antibody (1:30000) was used in all Western blots. Membranes were washed with 0.1% TBS-T, incubated in SuperSignal West Dura Extended Duration Substrate and exposed to X-ray film.

GPx and TR1 activities and selenium assays - Total GPx activity was measured using a standard assay with hydrogen peroxide as substrate (23,27 and references therein). TR1 activity was determined in cytosol-enriched protein extracts using the insulin reduction method (8).

The amount of selenium in extracts of liver, kidney, testes, brain and plasma was determined by Oscar E. Olsen Biochemistry Laboratories at South Dakota State University as described (23,27).

Isolation, aminoacylation, fractionation, and sequencing of tRNA and coding studies - Total tRNA was isolated from liver of each mouse line. The tRNA was aminoacylated with [^{3}H]serine (29) and the resulting aminoacylated tRNA fractionated on a RPC-5 column (30) as described (22,23,27). Sec tRNAs that were synthesized intracellularly from the *A34* mutant transgene encoding a base change were sequenced using an RT-PCR technique (31) in which individual fractions from the RPC-5 columns were used as designated in Fig. 6. Codon recognition studies were carried out on ^{3}H-seryl-tRNA$^{[Ser]Sec}$ fractions from the RPC-5 column using the ribosomal binding technique of Nirenberg and Leder (32) as described (29). Trinucleoside diphosphates AGA, GGA, CGA, UGA, UGU and UGC were the gift of Marshall Nirenberg or were prepared as described (29).

RESULTS AND DISCUSSION

Characterization of transgenic mice carrying the A34 mutant trsp transgene – Founder mice containing 1, 4 and 6 copies (heterozygous animals) of the *A34* mutant transgene were generated as described in the Experimental Procedures. Each mouse line was bred to yield homozygous *trsp* transgenic animals and the resulting lines were characterized in parallel with transgenic mice carrying low and high copy numbers of wild type or *G37 trsp^{t}* transgenes (22). To determine the effects of *A34* transgenes on selenoprotein biosynthesis, wild type and *A34* transgenic mice carrying 4 or 12 copies of mutant transgenes were labeled with ^{75}Se and the resulting labeled selenoprotein population analyzed (Fig. 1). The highest *A34* transgene copy number inhibited GPx1 expression, while TR1 expression showed little change in liver and kidney of both *A34* transgenic mice. Thus, the low copy *A34* transgene number appeared to have only a minor effect on overall synthesis of selenoproteins. Similar observations were reported previously for the *G37*

transgene carrying similar *trsp*^{t} copy numbers (22). These data suggested that the mutations at position 34 and 37 in Sec tRNA$^{[Ser]Sec}$, which both result in loss of Um34, had similar effects on the expression of selected selenoproteins.

Replacement of housekeeping selenoproteins in liver – Mouse lines carrying the targeted removal of *trsp* in liver (Δ*trsp*) and carrying either *A34*, low or high copy *G37* transgenes, or the wild type (*trsp*^{t}) transgenes were generated (See Table 1). Importantly, *trsp* is not expressed in liver, while it, along with each transgene, is expressed in all other organs and tissues. Thus, the effects of the transgene products on selenoprotein expression and function occur independently of the wild type gene only in the *trsp* targeted organ. Since the *trsp* and *trsp*^{t} mouse lines express wild type Sec tRNA$^{[Ser]Sec}$ and over-expression of *trsp* has little or no effect on selenoprotein synthesis (17,20,21,22,37), they both were considered as controls. Genotypes were determined in liver and kidney, as the affected and control organs, respectively, and the expected genotypes were found as shown in Fig. 2.

Attempts to restore the housekeeping selenoprotein population in liver of Δ*trsp* mice by mating with *A34* transgenic mice carrying a higher copy number of *A34* transgenes than the 2 copies used in replacing selenoprotein expression in these mice were unsuccessful. In addition, we were not able to rescue *trsp* null mice with any *A34* transgenic mice regardless of the transgene copy number. Furthermore, the number of matings to obtain restored selenoprotein expression in Δ*trsp* liver with the *A34* transgene exceeded, by more than three-fold, those required to obtain the *A37* and *trsp*^{t} selenoprotein liver replacement mice. These findings and their implications are further discussed below in the section *Consequences of A34 Sec tRNA$^{[Ser]Sec}$ in selenoprotein synthesis*.

^{75}Se-Labeling - The expression of selenoproteins in liver, kidney, testis, brain, heart and plasma of the six mouse lines was examined by labeling animals with ^{75}Se and analyzing labeled proteins in tissue extracts following gel electrophoresis. Coomassie blue-stained gels of total proteins (Fig. 3A, lower panels) from the different tissues served as loading controls and similar patterns and amounts of total proteins from the same tissues were observed with the possible exception of an enriched band indicated with an arrow in the liver Δ*trsp* extract. Identification of this band as glutathione *S*-transferase and its possible significance were reported elsewhere (27). Another protein band which migrated near 20 kDa was observed to vary in amounts in liver of mutant Sec tRNA$^{[Ser]Sec}$ mouse lines (see arrow with a question mark in lower liver panel in Fig. 3). This band did not vary reproducibly in livers from mutant *trsp* mice and therefore we have not identified it.

Variations were observed in the selenoprotein labeling patterns within organs and tissues of mice with the six different genotypes, particularly in liver between the two control mice containing wild type *trsp* and *trsp*^{t} and the four mouse lines containing the defective tRNAs$^{[Ser]Sec}$ (Fig. 3). Some ^{75}Se-labeled bands have been previously identified, including TR1, GPx1, GPx4 and Sep15, which are indicated in the liver panel (23,27,33). SelW, indicated with a question mark, has been tentatively identified (see 27 and references therein). The band that migrates just below TR1 is likely selenophosphate synthetase 2 (SPS2), although SelP also migrates at this position (1). GPx3 and SelP, which are indicated in the plasma panel, have been characterized in plasma (27 and references therein).

The selenoprotein labeling patterns from *trsp* and *trsp*^{t} control mice (lanes 1 and 2, respectively, in each panel of Fig. 3) were similar with some minor differences. For example, GPx1 and GPx4 appeared to be more enriched in kidney (control tissue), and liver of *trsp*^{t} mice than in the corresponding tissues in *trsp* mice. Comparison of mice encoding wild type *trsp* to those lacking *trsp* in liver (Δ*trsp*) showed that, as expected, most of the selenoprotein population is absent in Δ*trsp* liver. The minor selenoprotein bands observed in Δ*trsp* mice are likely proteins from liver cell types other than hepatocytes (27) which is the only cell type in which *trsp* deletion is targeted (28).

The presence of the *A34* or *G37* mutant transgenes in Δ*trsp* mice resulted in TR1 and GPx4, and possibly SelP and/or selenophosphate synthetase 2 (SPS2), being restored in liver. The selenoprotein-labeled population in kidney appeared to be similar in the four tRNA$^{[Ser]Sec}$ defective mouse lines with the possible exceptions of a reduced level of GPx1 in the high copy *G37* transgene line (Fig. 6) and the elevated levels of

GPx1 and 4 in Δ*trsp*. The higher number of transgenes in high copy *G37* than *A34* would also seem to account for the reduced amounts of GPx1 observed in the other tissues. The *G37* low copy number and *A34* transgenes resulted in similar effects in labeling in the tissues examined except plasma. SelP, which is synthesized largely in the liver and transported to other tissues (see 15 and references therein), is reduced in plasma of *A34* and *G37* mice and possibly in testes of these mouse lines compared to control mice. SelP is also reduced in plasma in Δ*trsp* mice compared to the two control mice, *trsp* and *trsp*^{t}. These observations are further considered below in the section on *Consequences of A34 Sec tRNA*$^{[Ser]Sec}$ *in selenoprotein synthesis*.

GPx4 also appeared to be slightly enriched in testes of several mouse lines compared to *trsp* mice. GPx levels were further examined by measuring GPx activity in each tissue of the six mouse lines (see GPx assays below and in Table 1).

To examine the ^{75}Se-labeling of TR1 in liver and kidney in more detail, TR1 was enriched from these tissues by passing tissue extracts over an ADP-Sepharose 4B affinity column (22). As shown in Fig. 4B, similar amounts of TR1 were present in both tissues of each mouse line with the exception of liver from Δ*trsp* mice which expressed TR1 poorly. In addition, TR1 appeared to be slightly enriched in kidney of Δ*trsp* mice and possibly in both tissues of *trsp*^{t} mice.

Northern blot analysis - Analysis of mRNA levels is an alternative means of examining the status of selenoprotein expression. The presence of UGA in the coding region targets some selenoprotein mRNAs for nonsense mediated decay (NMD; 34,35). As shown previously (34,35), NMD of some selenoprotein mRNAs is dependent on selenium status (see also reviews in 17,36). GPx1 mRNA was present in low, but detectable levels, in liver of the four tRNA$^{[Ser]Sec}$ defective mice compared to those of the two control mouse lines (Fig. 4). The levels of SelW mRNA were reduced substantially in liver of the four tRNA$^{[Ser]Sec}$ defective mice and in reduced levels in kidney of *A34* and high copy *G37* transgenic mice. SelT mRNA levels were reduced in liver of the four tRNA$^{[Ser]Sec}$ defective mice, but present in similar levels as control mice in kidney. SelR mRNA was reduced in liver of high copy *G37* transgenic mice, but remained at similar levels in liver and kidney of the other mice with only slightly lower amounts in liver of the low copy *G37* transgenic and Δ*trsp* mice. The level of SelK mRNA was similar in the mouse lines examined with the exception of a slightly reduced level in liver of Δ*trsp* mice. The mRNA levels of the other selenoproteins examined appeared to be present in similar amounts in both tissues of the six mouse lines or to vary only slightly in the four tRNA$^{[Ser]Sec}$ defective mice as compared to those of the controls.

Selenium status - The selenium levels were determined in liver, kidney, testes, brain and plasma (Table 2). The amounts of selenium were similar in each tissue of the *trsp* and *trsp*^{t} control mice with the exception of liver and kidney that appeared to have a somewhat higher selenium level in mice carrying the *trsp*^{t} transgene. The four defective tRNA$^{[Ser]Sec}$ mice had lower, but similar levels in liver, kidney and plasma compared to the two control mice, while *A34* and the low copy *G37* transgenic mice had similar levels as the two controls in testes and brain. The high copy *G37* transgenic and Δ*trsp* mice had similar, but slightly lower selenium levels than the two other transgenic defective mice in testes.

Western blot analysis – The expression levels of several selenoproteins were further examined by Western blot analyses. Because GPx1, SelR and SelT were not rescued in an earlier study involving the *G37* transgene (23,24), as assessed by Western blot analysis, we focused on these selenoproteins to determine whether they might be restored in liver by the *A34* transgene. GPx4 expression was also examined since its ^{75}Se-labeling patterns varied in liver and kidney within the different mouse lines (Fig. 3).

GPx1 was not detected in liver of Δ*trsp* mice, or in liver of mice carrying either the *A34* or *G37* transgenes (Fig. 5). GPx1 was present in lower levels in kidney of Δ*trsp*, *A34* and low copy *G37* transgenic mice than in the other mice, and was not detected in kidney from high copy *G37* transgenic mice. The relative amounts of GPx1 were similar in kidney of Δ*trsp* and *A34* mice, but less than observed in the low copy *G37* transgenic mouse. Possible reasons for these differing levels of GPx1 expression are further considered below.

GPx4 was present in liver of low copy *G37* transgenic mice and was virtually absent in liver from the other three tRNA$^{[Ser]Sec}$ defective mice. This selenoenzyme appeared to be reduced, but present in kidney of Δ*trsp*, *A34* and low copy *G37* transgenic mice and virtually absent in high copy *G37* transgenic mice. SelR was poorly expressed in liver of both *G37* transgenic mouse lines relative to control mice, but slightly better expressed in *A34* mice. SelR was also not expressed in kidney of the high copy *G37* mice and weakly expressed in the other tRNA$^{[Ser]Sec}$ defective mice (Fig. 5), although SelR mRNA was expressed in this tissue (Fig. 4). Interestingly, SelT was not expressed in kidney of high copy *G37* mice and partially or poorly expressed in kidney or liver of the tRNA$^{[Ser]Sec}$ defective mice (Fig. 5) even though its mRNA appeared to be synthesized in sufficient levels within these tissues for adequate translation (Fig. 4).

The combination of reasonable mRNA levels with low protein levels strongly suggests that the defect in stress-related selenoprotein synthesis in the mutant *trsp^t* mice is in translation rather than via effects on mRNA stability. More specifically, these findings raise the possibility that NMD of selenoprotein mRNAs (34,35) results from a block to translation due to the absence of the Um34 isoform. The corresponding mRNA with the encoded NMD criteria are then degraded. The site of regulation of mRNA decay may therefore be the Um34 methylation step that is known to be sensitive to selenium status (37). In fact, the Um34 isoform is dramatically reduced during selenium deficiency leading us to speculate that phenotypes displayed by the A34 and G37 transgene lines (Northern data shown in Fig. 4 and Western data shown in Fig.5) likely mimic those provoked by selenium-deficient conditions, thus pinpointing this phenomenon as a failure of mRNA translation by the Um34 isoform.

Glutathione peroxidase and TR1 activities – Since the labeling of GPx1 and GPx4 with ^{75}Se appeared to be enhanced in kidney of *trsp^t*, Δ*trsp*, *A34* and low copy *G37* mice (Fig. 3), but their levels were diminished in this tissue as assessed by Western blotting (Fig. 5), we examined the cytosolic GPx activities in kidney and several other tissues from the six mouse lines (Table 3, Expt I). The assay did not distinguish between the different peroxidases but reveals whether total GPx activity was increased or decreased. In liver, where most of the GPx activity is due to GPx1, the activities were similar in the two control mice, *trsp* and *trsp^t*, but extremely low in Δ*trsp* mice and Δ*trsp* mice carrying either the *A34* or *G37* transgene. In the other tissues examined, GPx levels were also similar in the two control mice. However, non-liver tissues of the four tRNA$^{[Ser]Sec}$ defective mouse lines had variable amounts of GPx activity. For example, in kidney, GPx activities were reduced in the Δ*trsp* mice or mice carrying *A34* and low copy *G37* transgenes, but were even lower in mice carrying the high copy *G37* transgene. Testes and brain had normal GPx activities in the Δ*trsp* mice, whereas mice encoding the *A34* and low copy *G37* transgenes had reduced activities, and those encoding the high copy *G37* transgene had even lower levels. Plasma had low and similar activity levels in the four defective tRNA$^{[Ser]Sec}$ mouse lines, although the levels in low copy *G37* mice appeared to be slightly higher. Thus, the mutations in *trsp* did not support full GPx1 activities. In particular, the high copy *G37* transgene apparently exerted dominant negative effects in kidney and brain even in the presence of wildtype *trsp* alleles, since GPx activities in high copy *G37* mutants were below the levels in Δ*trsp* mutants. These observations appear to exclude simple effects due to impaired selenium transport to these tissues.

Interestingly, GPx activities in kidney of Δ*trsp* mice were lower than in control mice even though kidney was not the targeted tissue (Table 3). We have proposed that this is a result, at least in part, of reduced selenoprotein P (SelP) expression and thus impaired selenium transport of SelP from Δ*trsp* liver to kidney (15). The selenium levels observed in kidney in the present study were lower in Δ*trsp* mice than control mice (Table 2). This result seems to conflict with a previous study with the same mouse lines (27), but gender differences likely explain this apparent conflict. In the present report, male mice were analyzed, while in the earlier study, female mice were studied (27). We have recently described lower selenium levels and GPx activities in mutant kidneys from male Δ*trsp* mice (15). Since gender-differences were observed in these experiments and female Δ*trsp* mice were less affected than male mutants (U.Schweizer, unpublished data), the gender-specific differences

in expression of prototype selenoenzymes in major organs of the mouse were systematically explored (38,39). GPx and deiodinase type I activities were higher in female kidney compared to male kidney, while hepatic deiodinase type I activity was higher in male compared to female mice. Since GPx activity is a good indicator of tissue selenium content, it would seem that the reduced selenium level detected in kidney of Δ*trsp* mice compared to that of control mice (Table 2) may account in part for the lower GPx activities found in Δ*trsp* mice, while an additional dominant negative effect of high copy G37 further reduced kidney GPx activity. It should also be noted that the observation of gender differences with respect to selenium metabolism in rodents is not a recent one. For example, Burk and collaborators found that male rats were more affected by selenium deficiency than female rats and the nutritional requirement for selenium is higher in males than females (40).

The Western blot analysis of GPx1 and GPx4 in liver and kidney (Fig. 5) were in closer agreement with the direct GPx enzyme assays (Table 1, Expt 1) than with the ^{75}Se-labeling data (Fig. 3). This discrepancy almost certainly reflects variations in both selenoprotein pool sizes and turnover rates that would influence patterns of ^{75}Se-labeling. In addition, the A34 tRNA$^{[Ser]Sec}$ isoform may also insert Sec in place of Cys in response to certain Cys codons, UGU and UGC, that would also affect labeling patterns as well as the activity and Western blot analysis (see section below on *Consequences of A34 Sec tRNA$^{[Ser]Sec}$ on selenoprotein synthesis*).

TR1 assays were also carried out in liver and kidney of the six mouse lines (Table 3, Expt II). TR1 activities were similar in kidney of each mouse line except in high copy *G37* which was about half that of controls. In liver, Δ*trsp* had low, detectable activity which was likely due, for the most part, to liver cell types other than hepatocytes (27). TR1 activity was recovered with the three mutant transgenes, but low copy *G37* was slightly less than controls, high copy *G37* about half that of controls and *A34* slightly less than that of low copy *G37*.

Fractionation, sequencing and codon recognition of T34->A34 Sec tRNA$^{[Ser]Sec}$ - The Sec tRNA$^{[Ser]Sec}$ population was examined in liver from five of the six mouse lines. The low copy *G37* transgenic mouse was excluded because it had been examined previously (27). The endogenous wild type Sec tRNA$^{[Ser]Sec}$ was absent in liver of Δ*trsp* mice (data not shown) which allowed us to examine the A34 mutant tRNA population without any influence of host wild type tRNA. Total tRNA was isolated from liver of the five mouse lines, aminoacylated with ^{3}H-serine and the resulting ^{3}H-seryl-tRNA isoforms chromatographed over a RPC-5 column. The elution profile of the tRNA$^{[Ser]Sec}$ population from the *A34* replacement mouse is shown in Figure 6. The mutant tRNA eluted from the column as two major peaks. A small aliquot of two fractions from each peak was taken for sequencing, while the remainder of each peak was pooled for coding studies. The codon recognition properties of Peak I demonstrated that it decoded UGU, UGC and UGA (Fig. 6) suggesting that the anticodon was ICA. Peak II decoded UGU suggesting that its anticodon was ACA. Sequences of two separate fractions of Peak I showed that the base in the wobble position was G, which corresponds to I in the actual sequence (31). The anticodon was therefore ICA. Sequences of the two fractions of Peak II demonstrated that the base in the wobble position was A and the anticodon was ACA. The distributions of Peaks I and II were 66.6 and 33.4%, respectively. The elution profiles of ^{3}H-seryl-tRNA$^{[Ser]Sec}$ from the other mouse lines, with the exception of that from the Δ*trsp* mouse line, are shown in the inset in Figure 6.

Um34 is important for stress-related selenoprotein expression – The A34 and G37 mutations result in the loss of highly modified, but very different bases, mcm^{5}U in A34 and i^{6}A in G37. The common feature of these mutant tRNAs is that they lack Um34. The two tRNAs are clearly capable of decoding Sec UGA codons as both support selenoprotein synthesis in mouse liver lacking wild type Sec tRNA$^{[Ser]Sec}$ (see Figs. 3 and 5), but neither is able to restore stress-related selenoprotein synthesis in liver following *trsp* knockout (Fig. 5). Although minor differences were observed in mRNA stability of certain selenoprotein mRNAs and in levels of certain selenoproteins, the overall effects of both mutant tRNAs were similar providing strong evidence that the Um34 Sec tRNA$^{[Ser]Sec}$ isoform is responsible for stress-related selenoprotein synthesis. The minor differences in the effects of the two tRNAs

on selenoprotein synthesis are likely due to the loss of the large, highly modified base in each mutant tRNA. Furthermore, the fact that selenium deficiency in rodents mimics the effects of the two Um34 lacking tRNAs in that the level of the Um34 Sec tRNA$^{[Ser]Sec}$ isoform and the expression of stress-related selenoproteins are reduced (reviewed in reference 17) also provides strong support that this isoform is indeed responsible for stress-related selenoprotein synthesis.

How does the Um34 Sec tRNA$^{[Ser]Sec}$ isoform regulate stress-selenoprotein synthesis? We examined many of the more likely features that might be expected to play a role in the selective expression of selenoproteins by the Um34 isoform (37). These included nucleotide context of the UGA Sec codon, the total length of the cDNA coding region, the number of exons within the gene, the exon within the gene wherein the UGA resides, the number of nucleotides between the UGA Sec codon and the stop codon, the number of nucleotides between the stop codon and the highly conserved AUGA sequence within the SECIS element, and the number of nucleotides between the highly conserved AUGA sequence within the SECIS element and the downstream poly A signal. We concluded that none of these components are likely involved. Remaining candidates for mediating the effects of Um34 include the uncharacterized Um34 methylase and/or different SECIS-binding proteins. Interestingly, a new SECIS-binding protein that preferentially binds to different selenoprotein mRNAs has been detected (D. Driscoll, personal communication). We are currently working to identify and characterize the Um34 methylase.

Consequences of A34 Sec tRNA$^{[Ser]Sec}$ on selenoprotein synthesis – Although the anticodon in the A34 tRNA$^{[Ser]Sec}$ was changed to decode Sec as well as Cys codons, the ^{75}Se-labeling studies showed that only natural selenoproteins were labeled with ^{75}Se. The two tRNA$^{[Ser]Sec}$ isoforms, tRNA$^{[Ser]Sec}_{ICA}$ and $_{ACA}$, apparently do not replace Cys in the general protein population, likely because tRNA$^{[Ser]Sec}$ associates with a specific elongation factor (EFsec) rather than EF-1alpha. However, both mutant tRNAs have the potential to translate Cys codons, UGU and UGC, with a preference for UGU (see Fig. 6). The demonstrated ability of tRNA$^{[Ser]Sec}_{ICA}$ to translate UGA (Figs. 3 and 5) indicates that it utilizes the Sec decoding machinery (i.e., SECIS elements (41) and EFsec (42,43)) which are required for incorporation of Sec into protein. These mutant tRNAs probably insert Sec at some Cys codons in selenoprotein mRNAs with insertion governed by the same criteria that control Sec insertion at UGA (location of the Sec UGA codon relative to the SECIS element, for example, 44,45). These tRNAs would likely compete with tRNA^{Cys} for decoding specific Cys codons.

Replacement of Cys with Sec in selenoproteins would most likely result in lower enzymatic activity as is found in the GPxs in the mutant tRNA lines (Table 3). That the level of SelP was severely reduced in the presence of the *A34* transgene (see plasma panel in Fig. 3) supports the idea of competition, but also may indicate that a protein with multiple amino acid replacements is more rapidly degraded and/or poorly transported. Clearly, SelP has multiple Sec and Cys residues and the repeated use of a Sec tRNA$^{[Ser]Sec}_{ICA}$ and/or $_{ACA}$ would likely result in reduction in overall SelP expression.

Competition of Cys and Sec codons for tRNA$^{[Ser]Sec}$ may explain the inability to rescue *trsp* null mice with any *A34* transgenic mouse regardless of the transgene copy number even though the same breeding scheme successfully rescued *trsp* null mice using wild type or *G37* transgenic mice (23,24). We were able to restore selenoprotein expression in liver of mice targeted for removal of Δ*trsp* with *A34* transgenic mice carrying two copies of the mutant transgene, but not with higher copy numbers employing the same breeding scheme as that which replaced selenoprotein expression with *trsp^t* and *G37* transgenes. Sec tRNA$^{[Ser]Sec}$ isoforms with anticodons ACA and ICA may compete more effectively with Cys tRNA in decoding selenoprotein mRNAs and insert Sec in place of Cys disrupting function and resulting in lethality. Although high copy numbers were tolerated by *A34* transgenic mice, these animals also had wild type *trsp*.

Significance of developing novel mouse models – Selenium is reported to have many health benefits including roles in preventing cancer, heart disease and other cardiovascular diseases, in delaying the aging process and the onset of AIDS in HIV positive patients, male fertility, immune function, mammalian development and viral inhibition (46).

Numerous large-scale, human clinical trials have been undertaken to examine the effect of selenium in preventing the onset of disease with most focusing on the effects of selenium in cancer prevention. These trials are very costly and were designed with little understanding of how selenium acts at the molecular level. Development of animal models to elucidate the metabolic roles of selenium, selenoproteins and low molecular weight selenocompounds is essential to understanding roles of selenium in health and development and in designing better human clinical trials. For example, recent evidence suggests that selenium may be ambivalent in its metabolic action in cancer in that it has cancer chemopreventive activity through some selenoproteins, but once a malignancy begins, selenium also promotes growth through selenoprotein TR1 (47,48). Furthermore, it is possible that the outcome of selenium supplementation at the doses used in human clinical trials may depend on individual genotypes, disease states and other factors that can be elucidated through animal models.

We have therefore generated several mouse models to provide a better understanding of the role of selenium, selenoproteins and low molecular weight selenocompounds in health and development. Our transgenic mouse model employing high copy *G37* transgenic mice has been used to show that both selenoproteins and low molecular weight selenocompounds have a role in preventing colon cancer (49) and selenoproteins have a role in preventing prostate cancer (50). This model has also been used to examine other aspects of the role of selenoproteins in health (51,52). Our floxed *trsp* model using *loxP/Cre* technology allowing targeted removal of *trsp* in specific tissues or organs has been used to show that selenoproteins play a role in endothelial development and heart disease prevention (26), proper liver function (27), neuronal function (Schweizer et al, submitted for publication) and in skin function and development (unpublished data).

The above useful models are surpassed by the one presented here which allows alteration of the selenoprotein population in liver with wild type or mutant *trsp* transgenes. These mice are phenotypically normal, allowing study of the role of selenium, housekeeping selenoproteins and stress-related selenoproteins as well as the entire selenoprotein population in resistance to various factors, such as toxic metabolites, hepatocarcinogens and liver cancer driver genes. The approach of targeting specific tissues for *trsp* removal can be used to generate other model systems for studying the role of selenium and selenoproteins in tissues of interest. The possibility that the expression of G37 and A34 mutant $tRNAs^{[Ser]Sec}$ in all tissues and organs may hinder experimentation seems not to be an issue. The high copy *G37* transgenic mouse, which expresses G37 tRNA in all tissues and organs, has been used to show that selenoproteins and low molecular weight selenocompounds have a role in colon cancer prevention (49) and selenoproteins in prostate cancer prevention (50). The major advantage of the current model is that we can target the removal of *trsp* and then replace or partially replace the selenoprotein population. The targeted mouse models presented in this study are amongst the most sophisticated mouse models developed to date for studying the role of selenium, selenoproteins and low molecular weight selenocompounds in health and development.

ACKNOWLEDGEMENTS

This research was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research, by NIH grants to V.N.G. and by a grant from the Korea Research Foundation (C00086) awarded to B.J.L.

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FIGURE LEGENDS

Fig. 1. ^{75}Se-Labeling of selenoproteins in *A34* transgenic mice. Two of the *A34* transgenic mouse lines encoding four copies of mutant transgenes (heterozygous animals containing a single allele with four copies) and encoding 12 copies of the mutant transgenes (homozygous animals containing two alleles with six copies/allele) were labeled with ^{75}Se, protein extracts prepared from liver and kidney and electrophoresed. Gels were stained with Coomassie blue to assess the total protein population (see lower panels) and ^{75}Se-labeled proteins detected using a PhosphorImager (see upper panels). Molecular weights of the protein markers are shown on the left of the panels and selenoprotein identity is indicated on the right by arrows (see also references 22, 27 and 33 for identification of selenoproteins, and Fig. 3 and its legend). Details of the ^{75}Se-labeling experiments are given in Experimental Procedures.

Fig. 2. Genotyping of mouse strains. DNA was isolated from liver and kidney of the 6 mouse lines (Table 1) and PCR products (indicated on the left of the figure) were generated with the primers (indicated on the right) as described in Experimental Procedures. PCR of *trsp*^{fl} (wild type floxed gene) yielded a 1180 bp fragment, of *trsp,* (wild type gene) a 980 bp fragment, of Δ*trsp* (knocked out gene), a 500 bp fragment, of *trsp*^{t}*, A34* or *G37* (either wild type, *A34* or *G37* transgene), a 1072 bp fragment, and of *AlbCre* (*Alb* promoter controlling expression of the *Cre* recombinase gene), a 370 bp fragment (see also 27).

Fig. 3. ^{75}Se-Labeling of selenoproteins. A) The six mouse lines were labeled with ^{75}Se. Protein extracts were prepared from liver, kidney, testes, brain, heart and plasma, and treated as described in the legend to Figure 1. Molecular weights of the protein markers are shown on the left of the panels and selenoproteins are indicated on the right by arrows. Selenoprotein identifications are based on references 22, 27 and 33. The arrows in the Coomassie Blue stained gel in the lower liver panel indicate glutathione *S*-transferase, GST, identified in an earlier study (27) and an unidentified protein (indicated with a question mark) that varied inconsistently in amounts in liver of the mutant *trsp*^{t} transgenic mice (see text). During preparation of plasma from *trsp* and Δ*trsp* mice, greater hemolysis of the red blood cells occurred which accounted for the globin observed in the Commassie Blue stained gels (see lanes 1 and 6 in the Plasma panel). B) ^{75}Se-labeled TR1 was purified from crude extracts of liver and kidney using ADP Sepharose 4B prior to gel electrophoresis as described in Experimental Procedures. Partially purified ^{75}Se-labeled TR1 from the two organs is shown.

Fig. 4. Northern blot analysis. RNA was extracted from liver and kidney of the six mouse strains and electrophoresed. RNA was then transblotted onto the appropriate membrane and hybridized with the indicated probes. Relative labeling was assessed using a PhosphorImager as described in Experimental Procedures. Staining of developed gels with ethidium bromide showed that identical amounts of 18s and 28s rRNA were present in all tissue extracts (loading control, data not shown). Each of the Northerns shown was carried out separately on two occasions with tissues from different mice with similar results. SelK mRNA was not examined in liver and kidney of low copy *G37* mice as the levels of SelK mRNA from both *A34* or high copy *G37* mice were very similar.

Fig. 5. Western blot analysis. Protein extracts were prepared from liver and kidney from the six mouse lines and electrophoresed. Protein was then transblotted onto the appropriate membrane and treated with the appropriate antibodies as described in Experimental Procedures. Selenoproteins are labeled on the left of each panel.

Fig. 6. Fractionation, sequencing and codon recognition studies. Transfer RNA was isolated from five mouse lines (*A34*, high copy *G37*, and *trsp*^{t} transgenic mice, and *trsp* and Δ*trsp* mice), aminoacylated with ^{3}H-serine and the resulting ^{3}H-seryl-tRNAs chromatographed on a RPC-5 column as described in Experimental Procedures. The graph shows ^{3}H-seryl-tRNA$^{[Ser]Sec}$ from the A34 mouse line, the arrows indicate the fractions from which small aliquots of the two peaks were taken for sequencing and ICA and

ACA show the anticodon sequences determined from sequencing these samples. The hatched areas show the fractions pooled for coding studies which were carried out using 10,000 total cpm of Peak I/assay wherein the cpm bound to ribosomes in the absence of codon were 1,170, and using 10,000 total cpm of Peak II/assay wherein the cpm bound to ribosomes in the absence of codon were 1,452. CPM bound to ribosomes in the absence of codon were subtracted from the cpm bound in the presence of codon and given as ΔCPM Bound. The inset shows the corresponding ^{3}H-seryl-tRNA$^{[Ser]Sec}$ isoforms from the other four mouse lines and the relative cpm at the highest point of each peak. The fraction numbers of each highest point shown in brackets were: G37, 14,010 cpm [19]; *trsp*, 5,065 cpm [49]; and *trsp*^{t}, 37,805 cpm [44]. The distributions of the two isoforms were determined as described previously (22): ICA and ACA were present at 66.6% and 33.4% respectively.

TABLE 1. Summary of breeding schemes for generating experimental mouse lines[a,e]

Matings[a]	Wild type[b,c]	Wild type replacement [b,c] (20 copies)	*A34* transgenic[c] (2 copies)	*G37* transgenic[c] (2 or 16 copies)[d]	Δ*trsp* knockout[c]
1st breeding	$trsp^{+/+}$ X $trsp^{fl/+}$-$AlbCre^{+/+}$	$trsp^{t/t}$ X $trsp^{fl/+}$-$AlbCre^{+/+}$	$trsp^{+/+}$-$A34^{t/t}$ X $trsp^{fl/+}$-$AlbCre^{+/+}$	$trsp^{+/+}$-$G37^{t/t}$ X $trsp^{fl/+}$-$AlbCre^{+/+}$	$trsp^{fl/fl}$ X $trsp^{+/+}$-$AlbCre^{+/+}$
F1 (2nd breeding)	$trsp^{+/+}$-$AlbCre^{+/-}$ X $trsp^{+/+}$-$AlbCre^{+/-}$	$trsp^{fl/+}$-$AlbCre^{+/-}$-$trsp^{t/-}$ X $trsp^{fl/+}$-$AlbCre^{+/-}$-$trsp^{t/-}$	$trsp^{fl/+}$-$AlbCre^{+/-}$-$A34^{t/-}$ X $trsp^{fl/+}$-$AlbCre^{+/-}$-$A34^{t/-}$	$trsp^{fl/+}$-$AlbCre^{+/-}$-$G37^{t/-}$ X $trsp^{fl/+}$-$AlbCre^{+/-}$-$G37^{t/-}$	$trsp^{fl/+}$-$AlbCre^{+/-}$ X $trsp^{fl/+}$-$AlbCre^{+/-}$
F2 (experimental mice)[e]	$trsp^{+/+}$-$AlbCre^{+/+}$	$trsp^{fl/fl}$-$AlbCre^{+/+}$-$trsp^{t/t}$	$trsp^{fl/fl}$-$AlbCre^{+/+}$-$A34^{t/t}$	$trsp^{fl/fl}$-$AlbCre^{+/+}$-$G37^{t/t}$	$trsp^{fl/fl}$-$AlbCre^{+/+}$
Designation[f]	*trsp*	$trsp^{t}$	*A34*	*G37*	Δ*trsp*

[a]Matings to obtain each mouse line used in this study are shown in the table.
[b]Both wild type mice, designated *trsp* in the text, and wild type selenoprotein replacement mice, designated $trsp^{t}$ in the text, were used as control mice.
[c]Genotype designations denote the following: $trsp^{+}$ (wild type Sec $tRNA^{[Ser]Sec}$ gene), $trsp^{fl}$ (floxed Sec $tRNA^{[Ser]Sec}$ gene); $trsp^{t}$ (Sec $tRNA^{[Ser]Sec}$ transgene); Δ*trsp* (liver *trsp* knockout); *AlbCre* (albumin Cre gene); *A34* (*A34* mutant transgene); and *G37* (*G37* mutant transgene). Homozygous genotypes and the corresponding heterozygous genotypes are designated as follows: $trsp^{+/+}$ and $trsp^{+/-}$ (wild type Sec $tRNA^{[Ser]Sec}$ gene); $trsp^{fl/fl}$ and $trsp^{fl/+}$ (floxed Sec $tRNA^{[Ser]Sec}$ gene); $trsp^{t/t}$ and $trsp^{t/-}$ (wild type Sec $tRNA^{[Ser]Sec}$ transgene); $AlbCre^{+/+}$ and $AlbCre^{+/-}$ (albumin Cre gene); $A34^{t/t}$ and $A34^{t/-}$ (*A34* transgene); and $G37^{t/t}$ and $G37^{t/-}$ (*G37* transgene).
[d]*G37* transgenic mice carrying 2 or 16 copies of the transgene are referred to in the text as *G37* low copy number or *G37* high copy number, respectively.
[e]Experimental mice are those animals generated from the matings for use in the study.
[f]The genotypes shown in the F2 experimental mice are designated in the text as: *trsp* or wild type control mouse; $trsp^{t}$ or wild type replacement mouse which is also a control mouse; *A34* transgenic mouse; *G37* transgenic mouse; Δ*trsp* or liver knockout mouse.

TABLE 2. Selenium levels in tissues of liver replacement mice

	Selenium levels (ppm)[a]					
Tissue	*trsp*	*trsp*^{t}	*A34*	*G37* low	*G37* high	*Δtrsp*
Liver	1.40	1.66	0.37	0.43	0.43	0.41
Kidney	1.27	1.50	0.86	1.02	0.63	0.95
Testes	0.81	0.85	0.80	0.78	0.69	0.69
Brain	0.19	0.17	0.16	0.15	0.09	0.16
Plasma	0.40	0.34	0.10	0.17	0.10	0.09

[a]Selenium levels were measured as described in Experimental Procedures. Each assay was carried out in duplicate and values represent the average of at least two different mice of each genotype.

TABLE 3. Glutathione peroxidase and TR1 activities

Expt	Enzyme	Tissue	Enzyme activities[a]					
			trsp	*trsp*^{t}	*A34*	*G37* low	*G37* high	*Δtrsp*
I	GPx	Liver	468.4 ±48.1	438.4 ±54.4	3.8 ±1.2	65.3 ±11.7	29.2 ±2.0	29.0 ±10.2
	GPx	Kidney	364.7 ±27.2	373.6 ±12.5	118.2 ±38.9	194.6 ±33.8	61.5 ±10.3	163.4 ±18.7
	GPx	Testes	20.3 ±1.2	21.8 ±0.5	14.3 ±2.0	10.4 ±0.7	7.6 ±0.5	20.9 ±1.4
	GPx	Brain	15.5 ±2.0	18.7 ±0.2	11.5 ±3.1	16.2 ±0.8	6.6 ±0.4	14.8 ±0.9
	GPx	Plasma	1369.0 ±133.3	1594.0 ±135.8	521.1 ±7.6	731.6 ±173.3	405.0 ±55.8	490.4 ±105.0
II	TR1	Liver	1.05 ± 0.12	1.02 ±0.05	0.74 ±0.07	0.90 ±0.11	0.46 ±0.08	0.25 ±0.08
	TR1	Kidney	1.11 ± 0.07	1.19 ± 0.18	1.10 ± 0.03	1.01 ± 0.07	0.57 ± 0.08	1.25 ± 0.06

[a]Glutathione peroxidase (Experiment I) and TR1 activities (Experiment II) were measured in mice as given in Experimental Procedures. Each assay was carried out in triplicate. Values expressed are nmol NADPH/min/mg protein or nmol NADPH/min/ml plasma for glutathione peroxidase activity and ΔA_{412}/mg protein for TR1 activity.

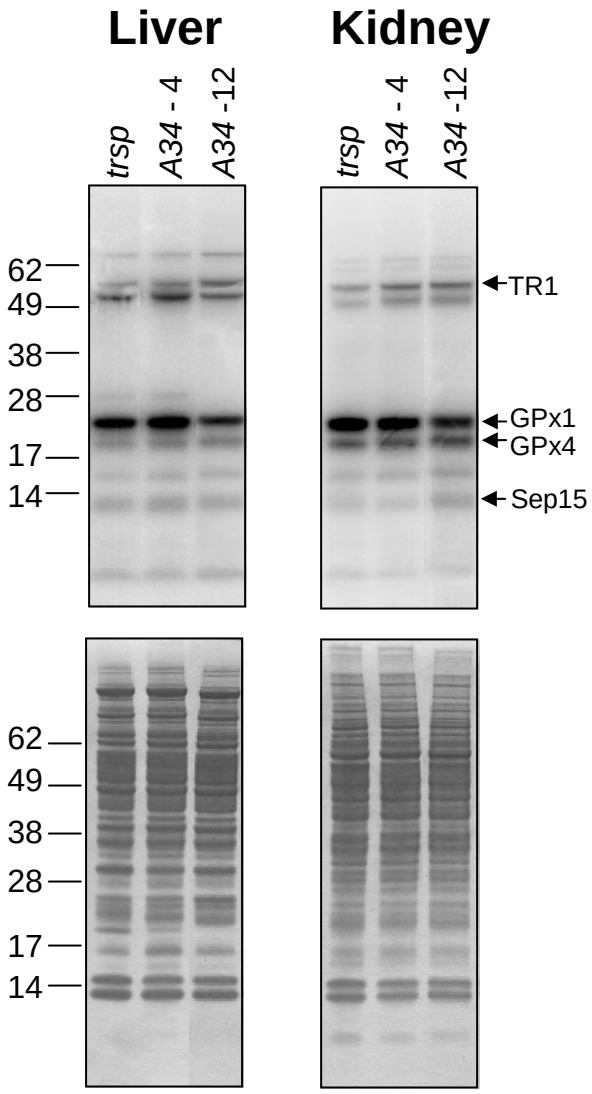
Liver
Kidney
trsp
A34 - 4
A34 -12
trsp
A34 - 4
A34 -12
62
49
38
28
17
14
TR1
GPx1
GPx4
Sep15
62
49
38
28
17
14

Figure 1

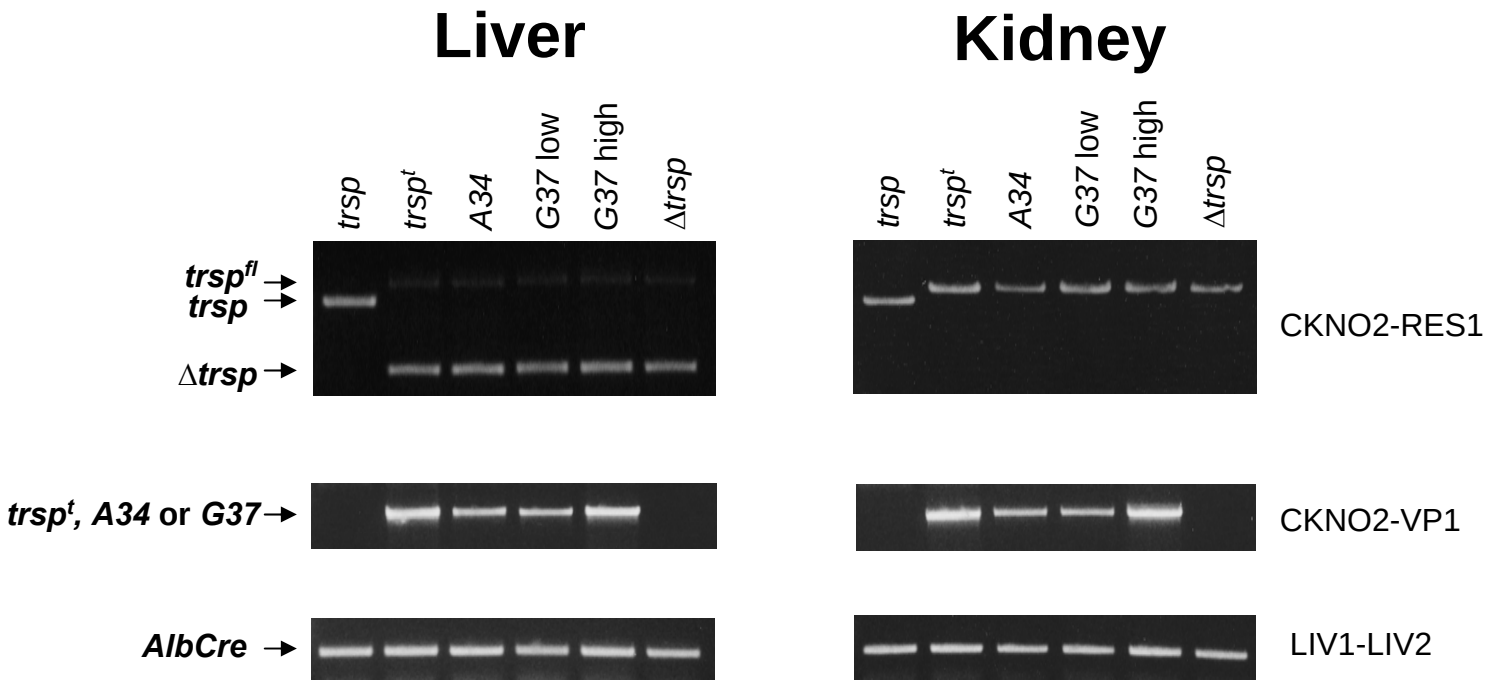
Liver
Kidney
trsp
trspt
A34
G37 low
G37 high
Δtrsp
trspfl
trsp
Δtrsp
CKNO2-RES1
trspt, A34 or G37
CKNO2-VP1
AlbCre
LIV1-LIV2

Figure 2

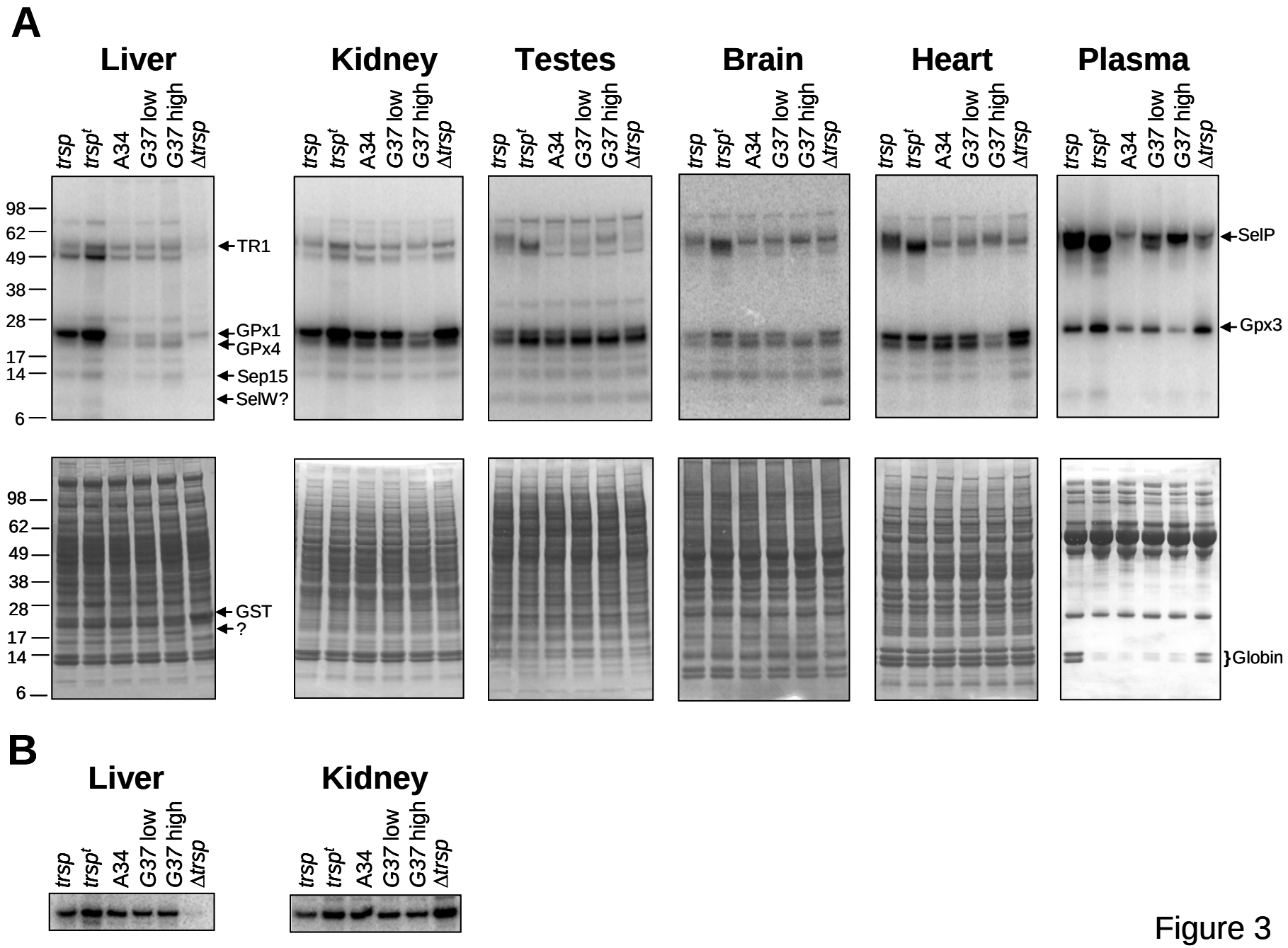
A
Liver
Kidney
Testes
Brain
Heart
Plasma
trsp
trspt
A34
G37 low
G37 high
Δtrsp
98
62
49
38
28
17
14
6
TR1
GPx1
GPx4
Sep15
SelW?
SelP
Gpx3
GST
?
}Globin
B
Liver
Kidney

Figure 3

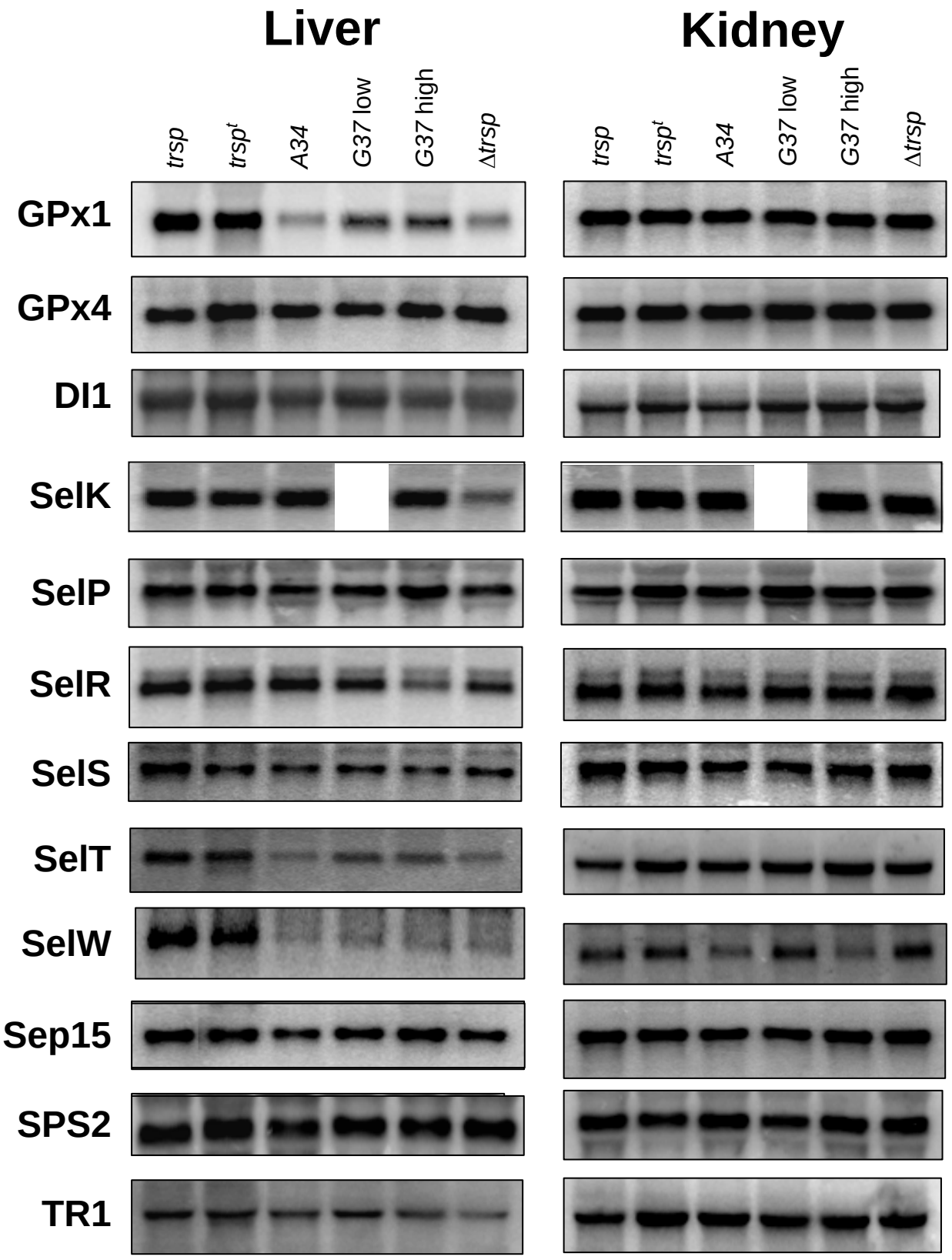
Liver
Kidney
trsp
trsp^t
A34
G37 low
G37 high
Δtrsp
GPx1
GPx4
Dl1
SelK
SelP
SelR
SelS
SelT
SelW
Sep15
SPS2
TR1

Figure 4

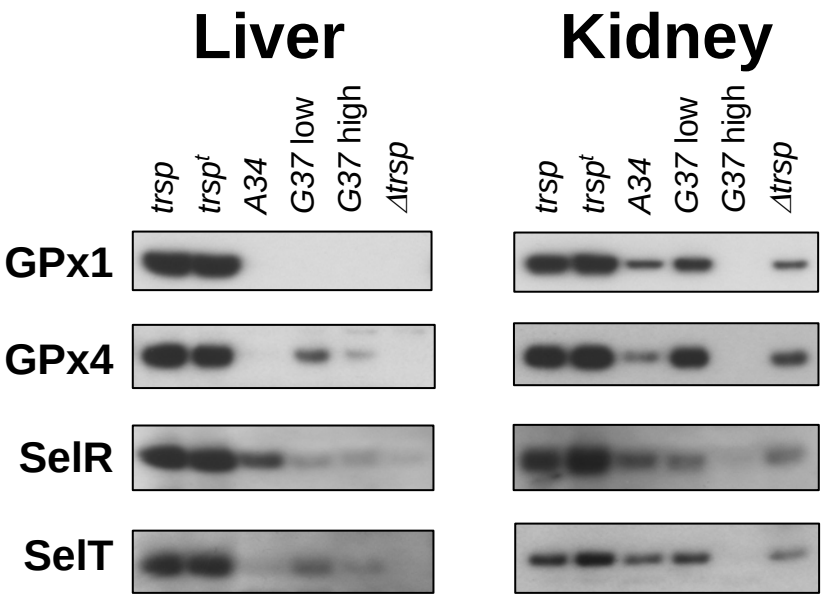
Liver
Kidney
trsp
trsp^t
A34
G37 low
G37 high
Δtrsp
GPx1
GPx4
SelR
SelT

Figure 5

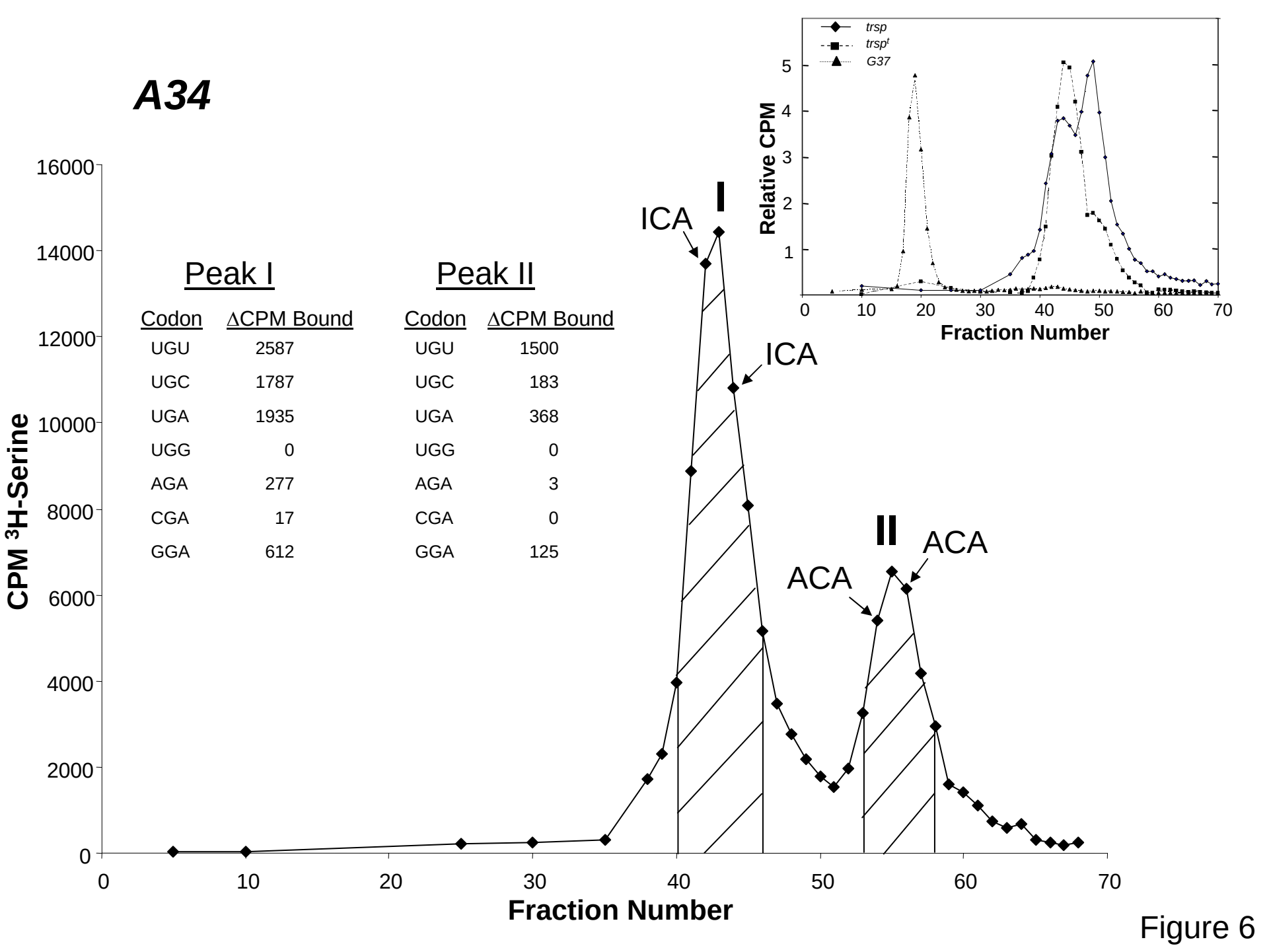
A34
Peak I
Codon ΔCPM Bound
UGU 2587
UGC 1787
UGA 1935
UGG 0
AGA 277
CGA 17
GGA 612
Peak II
Codon ΔCPM Bound
UGU 1500
UGC 183
UGA 368
UGG 0
AGA 3
CGA 0
GGA 125
ICA
I
ICA
ACA
II
ACA
CPM 3H-Serine
Fraction Number
trsp
trsp^t
G37
Relative CPM
Fraction Number

Figure 6