February 2005

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Bradley A. Carlson  
_National Institutes of Health, Bethesda, Maryland_

Xue-Ming Xu  
_National Institutes of Health, Bethesda, Maryland_

Vadim N. Gladyshev  
_University of Nebraska-Lincoln, vgladyshev1@unl.edu_

Dolph L. Hatfield  
_National Institutes of Health, Bethesda, Maryland_

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Selective Rescue of Selenoprotein Expression in Mice Lacking a Highly Specialized Methyl Group in Selenocysteine tRNA*

Received for publication, October 14, 2004, and in revised form, December 13, 2004
Published, JBC Papers in Press, December 17, 2004, DOI 10.1074/jbc.M411725200

Bradley A. Carlson‡, Xue-Ming Xu‡, Vadim N. Gladyshev§, and Dolph L. Hatfield‡‡

From the §Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, Center for Cancer Research, NCI, National Institutes of Health, Bethesda, Maryland 20892 and the ‡Department of Biochemistry, University of Nebraska, Lincoln, Nebraska 68588

Selenocysteine (Sec) is the 21st amino acid in the genetic code. Its tRNA is variably methylated on the 2'-O-hydroxyl site of the ribosyl moiety at position 34 (Um34). Herein, we identified a role of Um34 in regulating the expression of some, but not all, selenoproteins. A strain of knock-out transgenic mice was generated, wherein the Sec tRNA gene was replaced with either wild type or mutant Sec tRNA transgenes. The mutant transgene yielded a tRNA that lacked two base modifications, N6-isopentenyladenosine at position 37 (i^6A37) and Um34. Several selenoproteins, including glutathione peroxidases 1 and 3, SelR, and SelT, were not detected in mice rescued with the mutant transgene, whereas other selenoproteins, including thioredoxin reductases 1 and 3 and glutathione peroxidase 4, were expressed in normal or reduced levels. Northern blot analysis suggested that other selenoproteins (e.g. SelW) were also poorly expressed. This novel regulation of protein expression occurred at the level of translation and manifested a tissue-specific pattern. The available data suggest that the Um34 modification has greater influence than the i^6A37 modification in regulating the expression of various mammalian selenoproteins and Um34 is required for synthesis of several members of this protein class. Many proteins that were poorly rescued appear to be involved in responses to stress, and their expression is also highly dependent on selenium in the diet. Furthermore, their mRNA levels are regulated by selenium and are subject to nonsense-mediated decay. Overall, this study described a novel mechanism of regulation of protein expression by tRNA modification that is in turn regulated by levels of the trace element, selenium.

The mechanism of selenocysteine (Sec) incorporation into protein as the 21st amino acid was elucidated in Escherichia coli by Böck (reviewed in Ref. 1). In mammals, the mechanism of Sec insertion into protein is not as completely understood (reviewed in Refs. 2 and 3). However, both prokaryotes and eukaryotes use the stop codon, UGA, to dictate the incorporation of Sec after the tRNA is initially aminoacylated with serine, and the biosynthesis of Sec occurs on its tRNA. The tRNA has therefore been designated Sec tRNA_{Sec}[Sec] (2). The presence of a stem-loop structure that occurs downstream of UGA in selenoprotein mRNA, known as a Sec insertion sequence (SECIS) element (4), is responsible for dictating UGA as Sec instead of the cessation of protein synthesis. In mammals, a specific SECIS-binding protein, designated SECIS-binding protein 2 (5), recognizes the SECIS element, and a specific elongation factor, designated EFsec (6, 7), recognizes selenocysteyl-tRNA_{Sec}[Sec] and the resulting complex guides Sec into the nascent polypeptide in response to UGA (2, 3).

In higher vertebrates, there are two Sec tRNA_{Sec}[Sec] isoforms that differ from each other by a single nucleoside modification at position 34, which is the wobble position of the anticodon (2). One isoform contains methylcarboxymethyluridine (mcm5U) at this site, and the other contains methylcarboxymethyluridine-2'-O-methylribose (mcm5Um). Several lines of evidence suggest that methylation of the ribosyl moiety at the 2'-O-hydroxyl site (designated Um34) is a highly specialized event. It is the last step in the maturation of Sec tRNA_{Sec}[Sec]. This modification step is dependent on the prior synthesis of the four modified bases found in Sec tRNA_{Sec}[Sec] and on an intact tertiary structure, whereas synthesis of the other modified nucleosides, including mcm5U, is less stringently connected to primary and tertiary structure (8). The methylation step is influenced by selenium status, whereby the levels of mcm5U are enriched and mcm5Um reduced under conditions of selenium deficiency, and the ratio of the two isoforms is reversed under conditions of selenium sufficiency (2). The presence of Um34 dramatically affects Sec tRNA_{Sec}[Sec] secondary and tertiary structure (9). The presence of Um34 on Sec tRNA_{Sec}[Sec] correlated with the expression of certain selenoproteins (e.g. GPx1) (10, 11), whereas enrichment of the isoform lacking Um34 correlated with the expression of other selenoproteins (e.g. TR3) (11). In addition, a specialized role of the selenium-induced, Um34 tRNA_{Sec}[Sec] in selenoprotein translation was recently reported (12). However, the specific role of this isoform in selenoprotein synthesis has not been elucidated.

The selenoprotein population in rodents is composed of 24 members, and there are 25 members in humans (13). The function of many of these selenoproteins is not known. However, several approaches have been used in assessing their function and to provide better insights into their possible roles in health. Direct assays of selenoproteins such as glutathione peroxidase 1 (GPx1) and thioredoxin reductase 1 (TR1) demonstrate that they can function as antioxidants (14, 15), whereas genetic and biochemical characterization of selenoproteins such as Sep15 (16, 17) and GPx1 (18, 19) suggests that these members may in addition have roles in cancer prevention.

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Selective Rescue of Selenoproteins

Gene knock-out studies involving selenoproteins in mice show that some, such as glutathione peroxidase 4 (GPx4) (20), selenoprotein P (SelP) (21, 22), thyroid hormone deiodinase 2 (23), and mitochondrial thioredoxin reductase (TR3) (24) have essential roles in cellular function, since their removal is lethal or results in an abnormal phenotypic change. Other selenoproteins, such as GPx1 (25) and glutathione peroxidase 2 (GPx2) (26), probably have nonessential roles, since their removal manifests little or no phenotypic change. Exposing animals lacking a nonessential selenoprotein to stress, however, demonstrates that the animal may not cope with certain stresses compared with their wild type counterparts (27, 28). Thus, some selenoproteins that are nonessential to life of the organism probably provide protection from environmental stress.

Several studies have examined the effect of altering Sec tRNA\[^{Sec}\] expression on selenoprotein biosynthesis, which provided another means of elucidating the function of selenoproteins and their roles in health. Removal of \(\text{trs}p\) in knock-out mice is embryonic lethal, demonstrating that selenoproteins are essential to mammalian development (29, 30). Generation of a conditional knock-out of \(\text{trs}p\) using flox-P-Cre technology has shown that Sec tRNA\[^{Sec}\] levels can be reduced in mammary tissue by about 80%, resulting in an altered selenoprotein expression in a selenoprotein-specific manner (30), whereas complete removal of \(\text{trs}p\) in liver demonstrated that selenoprotein expression is required for proper function of this organ (31). Overexpression of Sec tRNA\[^{Sec}\] in transgenic mice carrying extra copies of the wild type transgene resulted in little or no effect on selenoprotein expression (11). However, transgenic mice carrying extra copies of a Sec tRNA\[^{Sec}\] mutant transgene that produces a tRNA gene product lacking N^6-isopentenyladenosine (i\(^A\)37) at this site and Ums34 (8) affect selenoprotein synthesis in a selenoprotein- and tissue-specific manner (11).

In the present study, transgenic mice possessing \(\text{trs}p\) wild type or mutant transgenes (11) were used to rescue \(\text{trs}p\) null mice (30). Mice dependent on the wild type transgene for survival manifested little or no change in selenoprotein expression, whereas mice dependent on the i\(^A\)37A mutant transgene rescued only some selenoproteins. Rescued selenoproteins included TR1 and TR3, whereas those that were poorly rescued included GPx1, GPx3, SelR, SelT, and SelW. Other selenoproteins, such as GPx2, GPx4, SelP, and Sep15, appeared to be partially rescued. These studies not only show that expression of some selenoproteins in mammals is highly dependent on Ums34, but generating genetically altered mice that express wild type transgenes (strain FVB/N) wherein each allele carried 10 copies of the Sec tRNA\[^{Sec}\] transgene (designated \(\text{trs}p\)) or for the mutant Sec tRNA\[^{Sec}\] genes whose product lacked i\(^A\)37 and Ums34, wherein each allele carried 20 copies of the mutant transgene (designated \(\text{trs}p\)) were obtained (11). Antibodies against GPx1 were obtained from Qichang Shen, antibodies against GPx2 were from Regina Brigelius-Flohe, and antibodies against GPx4 were from Donna Driscoll, whereas antibodies against TR1, TR3, SelR, SelT, and Sep15 were from our laboratories (13, 32). The care of animals was in accordance with the National Institutes of Health institutional guidelines and the expert direction of Dr. Kyle Stump (NCI, National Institutes of Health, Bethesda, MD).

Genotyping Mice—DNA was extracted from mouse tail clippings and the presence or absence of \(\text{trs}p\), \(\text{trs}p\), and \(\text{trs}p\) determined by PCR with the appropriate primers. The forward and reverse primers designated SFPNOA and CKNO12, respectively, that complement bases beginning at –2137 bp and –295 bp upstream of the gene (30, 31) were used to detect \(\text{trs}p\) (yielding a 1.9-kb PCR fragment). CKNO2, a forward primer that complements bases beginning at –442 bp upstream of \(\text{trs}p\) (30), was used to detect \(\text{trs}p\). Two new reverse primers, designated RES1 (5'-cctgttgagacgaccttctatg-3') and VP1 (5'-tggtaattgtgaggcata-3'), where RES1 complements bases beginning at +538 bp downstream of \(\text{trs}p\) (the 5'-end of \(\text{trs}p\) is base +1) and VP1 corresponds to that region of the vector sequence (Bluescript II) recognized by for monitoring its insertion into the genome (11), were used to monitor, along with the forward primer, CKNO2, the presence or loss of \(\text{trs}p\) (CKNO2-RES1) or \(\text{trs}p\) (CKNO2-VP1). CKNO2-RES1 yields a 980-bp PCR fragment and a 500-bp PCR \(\Delta\text{trs}p\) fragment, and CKNO2-VP1 yields a 1072-bp PCR \(\text{trs}p\) or \(\text{trs}p\) fragment.

Isolation, Aminoacylation, Fractionation, and Quantification of tRNA and Primer Extension—Total RNA was isolated from mouse liver, aminoacylated with [\(^{14}\)C]serine and 19 unlabeled amino acids in the presence of rabbit reticulocyte synthetases (33), the resulting aminoacylated tRNA fractionated on a RPC-5 column (34) in the absence and subsequently in the presence of Mg\(^{2+}\) as given (11, 30). The amount of Sec tRNA\[^{Sec}\] expressed from the host \(\text{trs}p\) or from wild type (\(\text{trs}p\)) or mutant (\(\text{trs}p\)) tRNA\[^{Sec}\] transgenes relative to the total Ser tRNA population and the distributions of mmc\(^{14}\)U and mmc\(^{15}\)Um has been detailed elsewhere (11, 30). The presence of U at position 9 in host Sec tRNA\[^{Sec}\] and of a C at position 9 in the transgene Sec tRNA\[^{Sec}\] provided a means of distinguishing host from transgene-generated tRNA\[^{Sec}\] by primer extension using either ddG or ddA in the reaction and the appropriate primer as given (see Ref. 11 and references therein).

Labeling of Selenoproteins and GPx Activity Assays and Selenium Assay—Mice were injected intraperitoneally with 50 μCi of \(^{75}\)SeGly and sacrificed 48 h after injection. Tissues and organs were excised, immediately frozen in liquid nitrogen and stored at ~80 °C until ready for use. Tissues were homogenized, the extracts were electrophoresed on 10% polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and immunoblotted as described (see Ref. 31 and references therein).

GPx1–3 activities were measured using a standard assay with hydrogen peroxide as substrate as described previously (11, 30, 31). The amount of selenium in extracts of liver, heart, brain, and testes was determined by Oscar E. Olsen Biochemistry Laboratories at South Dakota State University as described (31).

Northern and Western Blot Analyses—Total RNA was isolated from liver, kidney, intestine, and brain tissues, and selenoprotein synthesis were quantified and loaded onto gels, and transblotted onto a nylon membrane; the membrane was hybridized with \(^{32}\)P-labeled probe; and the Northern blot was analyzed with a PhosphorImager as described (11). GPx1, GPx4, SPDS2, D1, TR1, and SelP probes were prepared and used as described (11). The SeV 733-bp fragment was isolated from a pcDNA3 plasmid, and expressed in a PhosphorImager as described (see Ref. 31 and references therein).

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Selective Rescue of Selenoproteins

**Fig. 1. Sec tRNA\[^{[S]er\[Sec]}\] levels.** Transfer RNA was isolated from liver of trsp\[^{[S]er\[Sec]}\], trsp\[^{i\[6\]A}\], and trsp\[^{i\[6\]A}\] mice, aminoacylated with \[^{[3]H}\]serine, and fractionated on an RPC-5 column as described under “Experimental Procedures.” A single isoform was present in liver of trsp\[^{i\[6\]A}\] mice and both mcm\[^{U}\] and mcm\[^{Um}\] isoforms were present in liver of trsp and trsp\[^{i\[6\]A}\] mice.

(1:1000), Gpx4 (1:2000), SelR (1:1000), SelT (1:400), Sep15 (1:1000), TR1 (1:1000), and TR3 (1:1000). Anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:30,000) was used in all Western blots with the exception of that with TR3, in which anti-chicken horseradish peroxidase-conjugated secondary antibody (1:10,000) was used. Following the attachment of the secondary antibody, membranes were washed with 0.1% TBS-T, incubated in SuperSignal West Dura Extended Duration Substrate and exposed to x-ray film.

**RESULTS**

**Generation of Rescued Mice**—To determine the role of the mutant Sec tRNA\[^{[S]er\[Sec]}\], mice were generated wherein the wild type Sec tRNA\[^{[S]er\[Sec]}\] gene (trsp) was replaced with several copies of the mutant transgene (trsp\[^{i\[6\]A}\]-), and consequently the animal’s dependence on trsp for survival was replaced with a dependence on trsp\[^{i\[6\]A}\]. This mouse carried 20 copies of a mutant trsp transgene (trsp\[^{i\[6\]A}\]) and synthesized elevated amounts of a single form of Sec tRNA\[^{[S]er\[Sec]}\] that lacked \[^{i\[6\]A}\]A37 and, importantly, Um34 (8, 11). The control mouse encoded 20 copies of the wild type transgene (trsp\[^{[S]er\[Sec]}\]) and synthesized elevated amounts of wild type Sec tRNA\[^{[S]er\[Sec]}\]. These knock-out trsp\[^{[S]er\[Sec]}\] mice were designated as rescued mice. To maintain rescued mice with the same number of wild type or mutant transgenes, matings were carried out in the following manner. Mice in which selenoprotein expression was rescued with the mutant transgene were generated by mating parents wherein one parent contained the genotype trsp/trsp-trsp\[^{i\[6\]A}\]/trsp\[^{i\[6\]A}\]-, and the other parent contained the genotype trsp/Δtrsp/Δtrsp\[^{i\[6\]A}\]-/Δtrsp/Δtrsp\[^{i\[6\]A}\]-. Offspring with genotype Δtrsp/Δtrsp-trsp\[^{i\[6\]A}\]- were selected and used for analysis. Mice in which selenoprotein expression was rescued with the wild type transgene were generated by mating parents wherein both parental strains contained the genotype trsp/Δtrsp-trsp\[^{i\[6\]A}\]-/trsp\[^{i\[6\]A}\]-. The same approach was used for mice lacking both parental strains of the Δtrsp/Δtrsp-trsp\[^{i\[6\]A}\] genotype. Genotype of the rescued mice were determined as described under “Experimental Procedures.”

**Sec tRNA\[^{[S]er\[Sec]}\] Expression**—Transfer RNA was isolated from the livers of mice with genotype trsp, Δtrsp-trsp\[^{i\[6\]A}\]- (hereafter designated trsp\[^{i\[6\]A}\]-) and Δtrsp-trsp\[^{i\[6\]A}\]- (hereafter designated trsp\[^{i\[6\]A}\]-). Genotypes of the rescued mice were determined as described under “Experimental Procedures.”

**RPC-5 chromatography** (Fig. 1). The Sec tRNA\[^{[S]er\[Sec]}\] population in liver of trsp mice consisted of two isoforms. The earlier eluting tRNA contained mcm\[^{U}\], and the latter eluting form contained mcm\[^{Um}\] (see Refs. 11 and 30 and references therein). The distribution of the two isoforms and the percentage of the Sec tRNA\[^{[S]er\[Sec]}\] population relative to the total seryl-tRNA population were consistent with previous data for the Sec tRNA\[^{[S]er\[Sec]}\] population in liver of trsp mice (see Refs. 11 and 30 and references therein). The distributions of the isoforms synthesized from trsp\[^{i\[6\]A}\], which contained much more of the mcm\[^{U}\] isoform (Fig. 1), were similar to those observed in previous studies employing transgenic mice carrying 20 copies of trsp\[^{i\[6\]A}\] (11). Since the wild type transgene carries a pyrimidine transition at position 9 (U → C), we confirmed that this mouse synthesized only Sec tRNA\[^{[S]er\[Sec]}\] from trsp\[^{i\[6\]A}\] by primer extension (data not shown). The \[^{i\[6\]A}\]- isoform is less hydrophobic than its \[^{i\[6\]A}\]-containing counterparts, and it elutes much earlier from the column (11). There was no evidence of synthesis of wild type Sec tRNA\[^{[S]er\[Sec]}\] nor of the expression of any other modified isoform of the \[^{i\[6\]A}\]-deficient species in mice carrying the \[^{i\[6\]A}\]- mutant transgene (see also Ref. 8). Furthermore, Um34 cannot be synthesized in \[^{i\[6\]A}\]- tRNA\[^{[S]er\[Sec]}\] (8), and the peak of Sec tRNA\[^{[S]er\[Sec]}\] from liver of mice with genotype trsp\[^{i\[6\]A}\]- shown in Fig. 1 consists of a single isoform lacking this methyl modification (11, 30). The amount of this isoform relative to the seryl-tRNA population in liver was consistent with that observed previously in mice carrying the same number of trsp\[^{i\[6\]A}\]- transgenes (11). Overall, these data established that only the mutant transgene lacking \[^{i\[6\]A}\]- and Um34 was expressed in mice with genotype trsp\[^{i\[6\]A}\]-, whereas a mixture of the wild type Sec tRNA\[^{[S]er\[Sec]}\] forms that were either methylated or unmethylated on the ribosyl moiety at position 34 were expressed in mice with genotype trsp\[^{i\[6\]A}\]. Thus, the lack of \[^{i\[6\]A}\]- and Um34 in mice carrying trsp\[^{i\[6\]A}\]- did not result in lethality.

**\[^{75}\]Se Labeling** — The expression of selenoproteins in several tissues of mice with genotype trsp, trsp\[^{i\[6\]A}\], or trsp\[^{i\[6\]A}\]- was analyzed by labeling animals with \[^{75}\]Se and examining the resulting labeled proteins following gel electrophoresis. Coomassie Blue-stained gels of total proteins from each tissue were compared with their same-tissue counterparts and found to be similar (Fig. 2), suggesting that mice encoding null trsp and
Selective Rescue of Selenoproteins

Dramatic differences in selenoprotein labeling patterns were observed between mice carrying trsp (both wild type and those carrying trsp') and mice with genotype trsp/i6A'. GPx1 was poorly rescued and was essentially undetectable in the tissues examined, and GPx3 was absent in plasma. Expression of two other selenoproteins was significantly reduced as observed from 75Se labeling. The 75Se-labeled band at approximately 9 kDa (most likely SelW, based on its size (13)) in liver, testis, lung, and spleen is absent in mice carrying trsp/i6A'. It was designated as SelW with a question mark in the liver panel. The band at ~32 kDa was absent in testes of mice with genotype trsp/i6A'. This band was rescued with trsp' or trsp/i6A' did not exhibit major differences in protein synthesis as a whole (see also Refs. 11, 30, 31).

Other selenoproteins, namely GPx4, SelP, and Sep15, are only partially rescued as demonstrated by 75Se-labeling and/or Western blot analysis in certain tissues in mice carrying trsp'. The observation that some mRNA levels appeared to be unaffected is interesting in light of the fact that the corresponding selenoproteins, namely GPx4, SelP, and Sep15, are only partially rescued as demonstrated by 75Se-labeling and/or Western blot analysis (see above). In addition, some selenoprotein mRNAs, such as SelM, SelT, and TR1, appeared to be enriched in mice carrying trsp' (Fig. 4).

Rescue of selenoproteins was also examined by Western blotting (Fig. 3). Further confirmation that GPx1 was virtually absent in mice carrying trsp/i6A', as observed in the 75Se-labeling patterns, was obtained by examining GPx1 levels in liver and kidney by Western analysis. Clearly, GPx1 was not expressed in mice carrying trsp/i6A'. SelH and SelT were also not rescued, whereas GPx2, GPx4, and Sep15 appeared to be only partially rescued in mice carrying trsp/i6A'. The Western blot of GPx4 in testis was overexposed in order that the slower migrating nuclear GPx4 could be visualized. It was not rescued in mice carrying trsp/i6A'.

Other selenoproteins examined by Western analysis showed that TR1 and TR3 were expressed in normal amounts in liver, kidney, and brain of trsp/i6A' mice. Taken together, the Western data established that the expression of several selenoproteins was inhibited, whereas that of several others was not affected by replacing trsp with trsp/i6A'.

**Northern Blot Analyses**—The levels of several mRNAs, such as SelH and SelS, and possibly SelM, SelV, and SelW, were reduced in mice carrying trsp/i6A'. Levels of other selenoprotein mRNAs, such as SelH and SelS, and possibly SelM, SelV, and SelW, were reduced in mice carrying trsp/i6A'. TR1 and TR2 appeared to be down in mice carrying trsp/i6A', suggesting that these proteins, along with GPx2, GPx3, SelR, SelT, and SelW, may not be rescued or may be only partially rescued. DI1, GPx4, SelK, SelP, and Sep15 appeared to have similar amounts of mRNA in mice carrying trsp and trsp/i6A'.

**Glutathione Peroxidase Activities**—It was surprising to find that GPx1, GPx2, and GPx3 appeared to be slightly enriched as observed by 75Se-labeling and/or Western blot analysis in certain tissues in mice carrying trsp' (see above), since previous studies suggested that Sec tRNA^Sel^ was not limiting in...
selenoprotein biosynthesis (11, 29, 36). To further assess the enrichment of the glutathione peroxidases, we assayed their activity in various tissues (Table I). The assay did not distinguish between the different peroxidases but did assess whether GPx activity was increased or decreased. GPx (most likely GPx1) activity appeared to be only slightly increased in liver and heart, but not in kidney. The highest increase in GPx activity (most likely GPx2) was observed in intestine. Although

### Table I

<table>
<thead>
<tr>
<th>Tissue</th>
<th>trsp</th>
<th>trsp&lt;sup&gt;1&lt;/sup&gt;</th>
<th>trsp&lt;sup&gt;1A&lt;/sup&gt;</th>
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<td>3.6</td>
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<tr>
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</table>

<sup>a</sup> GPx activities are expressed as nmol of NADPH/min/mg of protein for liver, kidney, heart, and intestine. Plasma values are expressed as nmol of NADPH/min/ml.

S<sup>75</sup>Se labeling of selenoproteins suggested that GPx3 might be enriched in plasma of mice carrying trsp<sup>1</sup> (see Fig. 2), its activity was increased only slightly. Further studies are needed, however, to examine increases in GPx expression and activity due to overexpression of Sec tRNA<sup>[Sec]</sup><sub>i6A</sub>

**Selenium Status**—Selenium levels in liver, heart, brain, and testes of mice carrying trsp<sup>1</sup>, trsp<sup>1</sup>, and trsp<sup>1A</sup> were determined. The amounts of this element were reduced in these tissues of mice carrying trsp<sup>1A</sup> as compared with the corresponding tissues for wild type mice as shown in Fig. 5. The greatest loss in selenium content, as well as relative overall loss, was in liver of mice carrying trsp<sup>1A</sup>. The relative loss in the brain and testes was surprisingly greater than that in the heart, since the former two tissues are known to maintain their selenium levels in virtually the same amounts even when the animal is subjected to selenium-deficient diets (37, 38). On the other hand, the levels of this element in heart and liver are responsive to selenium status and manifest reduced levels during conditions of selenium deficiency (37, 38).

**Litter Sizes and Male Fertility**—Matings between wild type mice and mice carrying trsp<sup>1</sup> resulted in normal litter sizes of about eight offspring. Matings between mice carrying trsp<sup>1A</sup>, however, resulted in few pregnancies and in only one or two offspring when the mating was successful. Therefore, the sperm from two male mice carrying trsp<sup>1A</sup> was analyzed and compared with sperm from mice carrying trsp<sup>1</sup> and trsp<sup>1</sup>. The sperm from wild type males and males carrying trsp<sup>1</sup> had greater than 90% normal morphology (data not shown). A value of ~10% abnormal morphology in sperm is not unusual for the wild type mice used in this study. SpERM from the trsp<sup>1A</sup>-mice, however, had a high percentage of cells with distorted morphology that probably accounted for their low fertility (see Table II). Matings between females rescued with the mutant transgene and males carrying one copy of trsp and heterozygous or homozygous for trsp<sup>i6A</sup> yielded smaller litter sizes with an average of four mice/litter. Analysis of sperm showed normal morphology (data not shown), suggesting that females rescued with the mutant transgene had some aberration with fertility and/or pregnancy to term. The observation regarding the reduced number of pregnancies and litter sizes was not further examined. Otherwise, the phenotypes of mice rescued with the mutant transgene appeared to be normal.

<sup>3</sup> R. Awasthi, personal communication.
Selective Rescue of Selenoproteins

**DISCUSSION**

Removal of trsp from the mouse genome is embryonic lethal (29, 30). However, as shown in the present study, mice can be rescued with either wild type or mutant transgenes without significantly affecting animal viability. Surprisingly, mice deficient in both the Um34 isoform and GPx1 were examined. Sperm from all males had greater than 90% normal morphology except those of rescued mice with genotype trsp/i6A as shown in the table.

![Selenium levels in tissues of mice carrying trsp, trsp\(^{-}\), and trsp/i6A.](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age</th>
<th>Sperm count</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>trsp/i6A-</td>
<td>132</td>
<td>45 (\times) 10⁶</td>
<td>41</td>
</tr>
<tr>
<td>trsp/i6A-</td>
<td>398</td>
<td>38 (\times) 10⁶</td>
<td>39</td>
</tr>
</tbody>
</table>

\(a\) Bent tails designates aberrant sperm with abnormal and bent tails with cytoplasmic droplets.

Mammalian selenium-containing proteins respond to changes in selenium status in a selenoprotein- and tissue-specific manner, but the mechanisms for this hierarchy (2, 3) and specific roles of many selenoproteins (13) have not been resolved. Our data suggest that the biosynthesis of GPx1, GPx3, SelR, SelT, and SelW is dependent on tRNA\[^{Sec} Sec\] \[^{Sec} Sec\]\_unm and whereas that of others, such as TR1 and TR3, is not. The group

selenoproteins (reviewed in Ref. 2) identified in this study. This hierarchy in selenoprotein synthesis (see Refs. 2 and 3 and this study) correlates with the presence of Um34, but not i6A. In addition, the synthesis of the methyl group on the cm^3^Um isoform is a highly specialized event in selenoprotein expression as noted in the Introduction. It is of significance to emphasize three earlier studies that show a direct correlation between the levels of Um34 on tRNA\[^{Sec} Sec\] and GPx1. Replenishment of selenium-deficient rats with this element showed a direct correlation between increasing amounts of tRNA\[^{Sec} Sec\] and GPx1 (10). Transgenic mice encoding the Sec tRNA\[^{Sec} Sec\] i6A37 minus transgene manifested a pronounced decrease in both the Um34 isoform and GPx1 (11). More recently, a study by Jameson and Diamond (12) demonstrated a preferential use of tRNA\[^{Sec} Sec\] um34 in selenoprotein synthesis when GPx1 was over-expressed. Therefore, the evidence supports a role of this methyl group in the synthesis of several selenoproteins that are down-regulated in the rescue mouse described in this study. Caution must be exercised in the interpretation that Um34 is solely responsible for the observed effects, since we cannot rule out a possible influence of i6A37. However, the Um34 modification clearly has a dramatic influence on selenoprotein expression as evidenced by the occurrence of similar selenoprotein levels detected during selenium deficiency and in the loss of Um34 on Sec tRNA\[^{Sec} Sec\] in the present study.

How then can the absence of Um34 play a major role in the expression of some, but not all, selenoproteins? An examination of many different parameters that might be involved in decoding efficiency of the selenoproteins identified in the present study as being poorly translated in rescued mice (GPx1, GPx3, SelR, SelT, and SelW), partially translated (GPx2, GPx4, SelP, and Sep15), and efficiently expressed (TR1 and TR3) as well as the 13 other selenoproteins in mice (13) did not reveal any specific pattern that would appear to influence translation. Other features such as the possibility of specific SECIS-binding proteins that could act specifically on different selenoproteins will have to await further investigation. The finding in the present study, however, that the presence of two Sec isoforms differing by a methyl group are utilized differently in selenoprotein biochemistry is highly significant and demonstrates a unique manner in which Sec machinery has evolved in higher animals to express this class of proteins under conditions of selenium deprivation.

**FIG. 5. Selenium levels in tissues of mice carrying trsp, trsp\(^{-}\), and trsp/i6A.** The amount of selenium was determined in duplicate in liver, heart, brain, and testes, and the values represent the average of assays from two separate animals of the same genotype.
of selenoproteins that are not rescued in the trsp1^pA- mouse includes stress-related selenoproteins, GPx1, GPx3, and SelR, whereas SelIT and SelW also probably fall into this category. Expression of several of these proteins is known to be affected by selenium deficiency, which also results in decreased levels of their mRNAs due to nonsense-mediated decay (40, 41). The data presented herein also suggest that some selenoproteins such as GPx4, Sep15, and SelP are partially rescued, but their mRNA levels were not affected. Determination of whether selenoprotein hierarchy may be regulated by Um34 in Sec tRNA^{Ser}{^}{\text{Sec}} and whether the corresponding mRNAs may not be translated and subject to nonsense-mediated decay, and therefore whether the second event is the result of the first, must await further study.

The group of selenoproteins that were fully rescued in the trsp1^pA- mice included TR1 and TR3 that are less affected by selenium deficiency (2, 3). TR1 and TR3 control the redox state of cytosolic and mitochondrial thioredoxins, respectively, which are essential mammalian proteins.

Nucleoside modifications within the tRNA anticodon loop have been shown to influence ribosomal frame shifting (43) and suppression of nonsense codons (44, 45). Although we have known for many years that the methyl modification at position 34 in Sec tRNA^{Ser}{^}{\text{Sec}} is a highly specialized event (see Introduction), this study provides the first example of translation of a number of mRNAs being dependent on the recoding of an in-frame nonsense codon by Um34 and describes a new and novel role of tRNA modification in the recoding process.

Acknowledgments—We thank Drs. R. Brigelius-Flohe, D. Driscoll, and Q. Shen for the antibodies to GPx2, GPx4, and GPx1, respectively; Roackie Awasthi for the sperm analysis; and Dr. James Weaver for the SelV fragment.