

The RNA-binding protein Secisbp2 differentially modulates UGA codon reassignment and RNA decay

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ABSTRACT

Dual-assignment of codons as termination and elongation codons is used to expand the genetic code. In mammals, UGA can be reassigned to selenocysteine during translation of selenoproteins by a mechanism involving a 3' untranslated region (UTR) selenocysteine insertion sequence (SECIS) and the SECIS-binding protein Secisbp2. Here, we present data from ribosome profiling, RNA-Seq and mRNA half-life measurements that support distinct roles for Secisbp2 in UGA-redefinition and mRNA stability. Conditional deletions of the *Secisbp2* and *Trsp* (tRNA^{Sec}) genes in mouse liver were compared to determine if the effects of Secisbp2 loss on selenoprotein synthesis could be attributed entirely to the inability to incorporate Sec. As expected, tRNA^{Sec} depletion resulted in loss of ribosome density downstream of all UGA-Sec codons. In contrast, the absence of Secisbp2 resulted in variable effects on ribosome density downstream of UGA-Sec codons that demonstrate gene-specific differences in Sec incorporation. For several selenoproteins in which loss of Secisbp2 resulted in greatly diminished mRNA levels, translational activity and Sec incorporation efficiency were shown to be unaffected on the remaining RNA. Collectively, these results demonstrate that Secisbp2 is not strictly required for Sec incorporation and has a distinct role in stabilizing mRNAs that can be separated from its effects on UGA-redefinition.

INTRODUCTION

The prevailing view is that the genetic code is mainly fixed and its readout predictable. However, for a subset of genes, mRNA decoding is dynamic and may be altered to regu-

late expression or to produce new proteins not predicted by standard decoding rules. Both aspects are illustrated by selenoproteins, which are the fundamental paradigm for expansion of the genetic code by codon reassignment in mammals. Selenoproteins contain the rare amino acid selenocysteine (Sec) that is incorporated during translation in response to an in-frame UGA codon (1) and its insertion is regulated by selenium (Se) availability (2). In mammals, there are up to 25 genes encoding selenoproteins (3) with known Sec-dependent oxidoreductase activities involved in redox regulation, protection from oxidative damage, thyroid hormone metabolism, protein folding and cellular Ca^{2+} homeostasis (1).

The incorporation of Sec during translation of a mRNA in eukaryotes requires the presence of a selenocysteine insertion sequence (SECIS element) located in the 3' untranslated region (UTR). The SECIS element consists of a kink-turn secondary structure with a central tandem non-Watson-Crick G-A tandem pair (4) and conserved apical AAR (R = G or A) motif. At least two other *trans*-acting factors, the SECIS binding protein 2 (Secisbp2), which interacts specifically with SECIS elements (5), and a specialized elongation factor Eefsec are thought to be required to complete redefinition of UGA to Sec (Figure 1A) (6,7). In addition, Secisbp2 has also been shown to interact with the ribosome (8) and Eefsec (9) leading to models in which Secisbp2 plays a physical role in the recruitment of the $\text{Sec-tRNA}^{\text{Sec}}$ to the ribosome. Layered on top of these core components of the Sec-incorporation machinery are other *trans*-acting factors, such as L30 (10), nucleolin (11,12) and Eif4a3 (13), that have been reported to modulate Sec incorporation efficiency.

Selenoprotein expression is regulated at multiple levels and depends on the bioavailability of Se. A hierarchy among selenoproteins has been observed, which means that upon Se shortage, some selenoproteins are made at normal rates, while the biosynthesis of others is greatly diminished (14–

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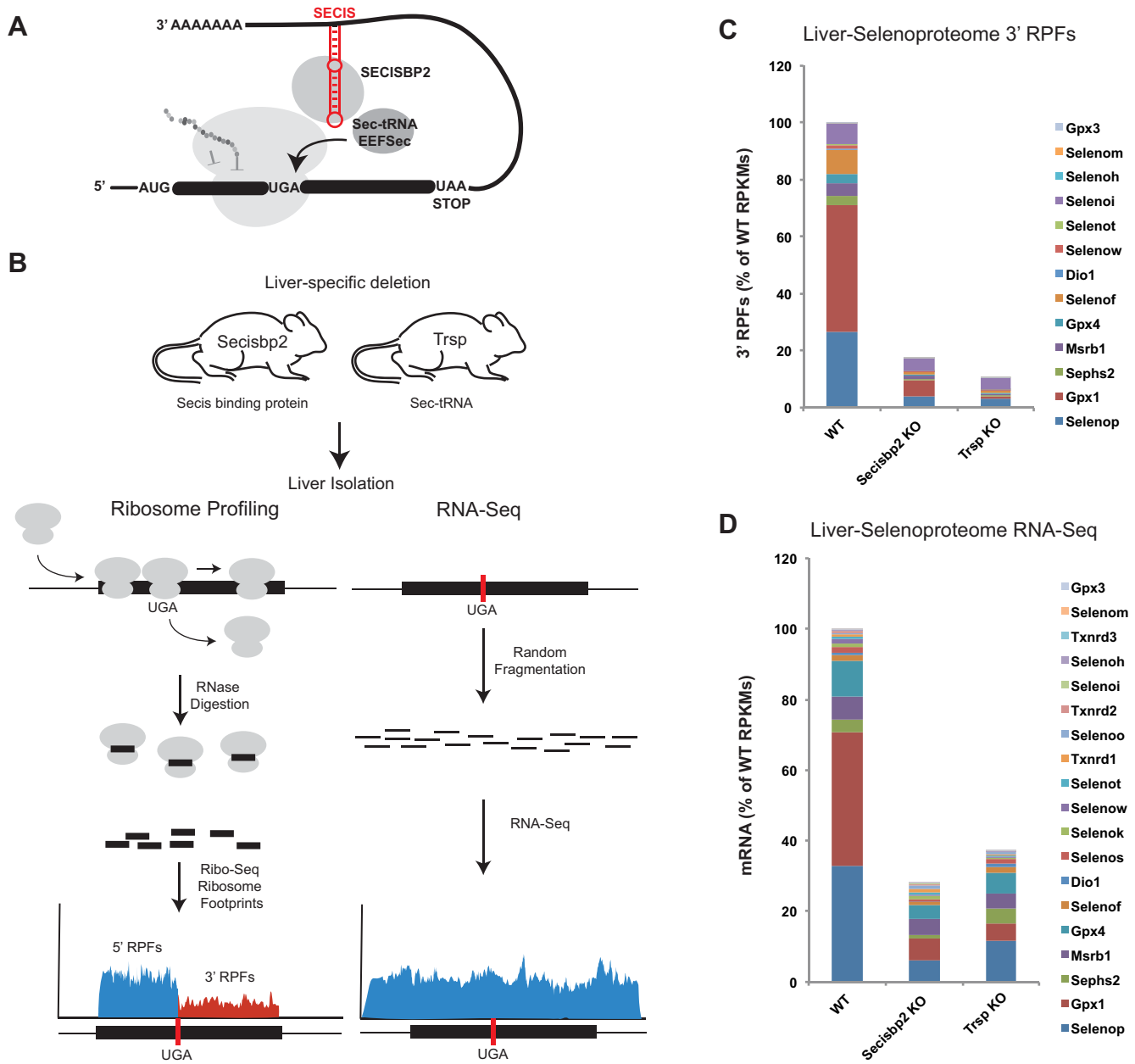


Figure 1. Experimental design and selenoproteome analysis. (A) Illustration of current models for selenocysteine (Sec) incorporation. Included are components of the Sec insertion machinery, selenocysteine insertion sequence (SECIS) (red), SECIS binding protein (Secisbp2), Sec-tRNA^{[Ser]^{Sec} (Sec-tRNA) and the elongation factor (Eefsec). (B) Schematic of ribosome profiling and RNA-Seq. For ribosome profiling, ribosome protected fragments (RPFs) are considered upstream (blue) and downstream (red) of UGA. (C) Overview of the effects of *Secisbp2* and *Trsp* deletions on translation of all selenoproteins in mouse liver. RPFs were determined and normalized for total mapped reads and length (reads/kb/million mapped reads – RPKM) from UGA to the termination codon. RPKMs were then summed and expressed as a percentage of the wild type. (D) Same as C for randomly sheared RNA fragments (RNA-Seq) with the exception that RPKMs were determined across the entire length of selenoprotein mRNAs.}

16). One factor affecting selenoprotein expression is the efficiency of Sec incorporation. Both *in vitro* and *in vivo* experiments demonstrate that the process of Sec incorporation is an inherently inefficient process that is subject to gene-specific regulation in response to Se availability (2,17,18). Several models including differential affinity for binding of Secisbp2 to SECIS elements, competition with accessory factors (13), and Se-responsive modification of tRNA^{[Ser]^{Sec} (19–21) have been proposed to account for the}

gene-specific translation efficiency and the effects of Se on UGA-redefinition efficiency.

Another factor implicated in selenoprotein hierarchy is differential mRNA stability (15,22). For example, glutathione peroxidase 1 (*Gpx1*) mRNA is sensitive to the nonsense-mediated decay (NMD) pathway (23). NMD initiates mRNA degradation if a termination codon is encountered >50 nts upstream of a splice junction complex. While the UGA-Sec codon in *Gpx1* mRNA conforms to this rule,

Gpx4 mRNA is relatively stable in Se-deficiency despite its homologous gene structure (24). Moreover, *Sephs2* mRNA is labile, although it is produced from an intron-less gene, and many selenoprotein mRNAs carry UGA close to their termination signal within the last exon and thus cannot be substrates of NMD. These observations raise questions as to whether other cellular mechanisms are involved in determining mRNA stability and imply gene-specific effects that are not readily explained by current NMD models.

While numerous studies indicate that *Secisbp2* is required for Sec incorporation *in vitro*, recent studies from our group have suggested that inactivation of the *Secisbp2* gene *in vivo* is less detrimental than loss of tRNA^{[Ser]Sec} and that *Secisbp2* may have a role in stabilizing a subset of selenoprotein mRNAs *in vivo* (25). Here, we have developed an experimental model to examine in detail the mechanisms by which *Secisbp2* affects expression of individual selenoproteins. Murine hepatocytes were chosen for conditional deletion of the *Secisbp2* gene as they express a high number of selenoproteins and the organ tolerates the complete loss of selenoprotein expression. We assess UGA-redefinition efficiency by deep sequencing of ribosome protected mRNA footprints (ribosome profiling) and comparisons of the densities of ribosome protected fragments (RPFs) located 5' and 3' of UGA. In addition, effects on mRNA levels and stability were measured by RNA-Seq and half-life experiments. The results unexpectedly reveal that *Secisbp2* is not absolutely required for UGA redefinition of all selenoprotein mRNAs, but rather has differential gene-specific effects on UGA-redefinition efficiency. We further propose that *Secisbp2* stabilizes mRNAs on which UGA-redefinition has failed.

MATERIALS AND METHODS

This manuscript adopts the new systematic nomenclature of selenoprotein names (26).

Mice

Generation and maintenance of mice carrying hepatocyte-specific conditional alleles of *Trsp* and *Secisbp2* were described previously (25,27). In brief, mice were kept in individually ventilated cages under a 12:12 h light-dark cycle and fed standard breeding chow according to local regulations. Whole livers were isolated from three young adult (5–8 weeks) male mice for each genetic background, *Secisbp2* KO, *Trsp* KO and matched male littermates carrying wild-type alleles. Livers were rapidly frozen in liquid nitrogen, pulverized and stored at -80°C . To assess the completeness of Cre-mediated recombination, we crossed a Cre reporter mouse strain, *ROSA26^{mT/mG}* into the conditional *Secisbp2* knockout line and examined liver sections from *Alb-Cre; Secisbp2^{fl/fl}* and respective control mice. Liver sections were analyzed by confocal microscopy and the fraction of non-recombined hepatocytes was 8/250 cells in control (3.2%) and 5/250 in *Secisbp2* knockout (2%; Supplementary Figure S7).

Ribosome profiling and RNA-Seq

For ribosome profiling, ~ 100 mg of material was suspended in 1.5 ml of lysis buffer (10 mM Tris-Cl (pH 7.5), 300 mM KCl, 10 mM MgCl₂, 200 $\mu\text{g/ml}$ cycloheximide, 1 mM DTT and 1% Triton X-100). Insoluble debris was removed by centrifugations at $12\,000 \times g$ at 4°C . Next 600 U of RNase1 (Ambion) were added and the sample was incubated at RT for 45 min. Monosomes were isolated by centrifugation through 50% sucrose. Ribosomes pellets were resuspended in Qiazol (Qiagen) and ribosome protected fragments were isolated using the miRNAeasy kit (Qiagen) with modification to retain small RNAs, as described by the manufacturer. For RNA-Seq, frozen pulverized liver was suspended in Qiazol, and total RNA was isolated as described by the manufacturer. PolyA mRNA was isolated using the Poly(A) Purist Mag kit (Ambion). Both RPFs and randomly fragmented PolyA enriched total RNA were electrophoresed on a 15% Tris-Borate-EDTA (TBE) Urea gel and RNA fragments between 20 and 40 nts in size were purified prior to the construction of libraries for deep-sequencing. Small RNA sequencing libraries were constructed using the Illumina TruSeq Small RNA Sample Prep kit (Illumina), according to the manufacturer's instructions. Libraries were subjected to 50-cycle single-end sequencing on the Illumina HiSeq 2000 Instrument. Data can be obtained from the NCBI GEO repository, entry GSE84112.

Bioinformatic analysis of deep-sequence data

Adapter sequences were trimmed from all sequences using the Hannon laboratory FastX toolkit. Sequences from ribosome profiling and polyA enriched libraries that aligned uniquely to selenoprotein mRNAs, allowing for two mismatches, were identified using bowtie. RefSeq entries used for this alignment are shown in Supplementary Table S2.

For RPFs, the 5' ends of RPFs were offset 15 nts to approximate the position of the A-site (see Supplementary Figure S1). Reads with the A-site mapping to the first and last 15 nts of the annotated coding sequence were excluded to avoid bias at the initiation and termination codon. Analysis of RPFs upstream of UGA-Sec codons excluded UGA and the five preceding codons. Total mapped reads used to derive RPKM calculations were determined by aligning sequences against RefSeq entries obtained from the UCSC genome browser in which all mRNAs derived from the same gene were reduced to a single entry corresponding to the longest isoform.

Western blot analysis

Liver protein lysates from wild-type, *Secisbp2* and *Trsp* KO mice were extracted with RIPA buffer, electrophoresed in a 12% acrylamide SDS-PAGE gel and transferred to a nitrocellulose membrane (GE Healthcare). After blocking, membranes were probed with antibodies and detected as described (25).

Hepatocyte culture and treatment

Livers from *Secisbp2*, *Trsp* KO and wild-type mice were perfused with collagenase buffer via the vena cava. After mechanical disaggregation, cells were passed through a

cell strainer, counted and plated on collagen-coated plates. After 4 h media was replaced and cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (GIBCO).

The following day, hepatocytes were treated with 2.5 $\mu\text{g/ml}$ actinomycin D or 2.5 $\mu\text{g/ml}$ actinomycin D and 3 $\mu\text{g/ml}$ cycloheximide (Panreac Applichem), respectively, for the times indicated in the Figure legends.

Northern blot of selenoproteins

Total RNA was extracted by Trizol (Invitrogen). A total of 5 μg of RNA sample were loaded on a denaturing agarose gel. 5S RNA and 18S RNA were used as loading controls. A 5S RNA probe was made as indicated in the tRNA Northern blot section. Membrane was exposed and radioactivity was detected by PhosphorImager (BAS-1800 II Fujifilm). Densitometry quantification was performed with AIDA Imager analyzer (raytest).

Northern blot of tRNA

Five micrograms of total RNA from mouse liver samples were electrophoresed using a 10% urea-acrylamide gel. Probes for tRNA^{[Ser]Sec} (5'- CGCCGAAAG-GTGGAAATTGAA -3'), tRNA^{Ser} (5'- CGTAGTCG-GCAGGATTTCGAA -3') and 5S RNA (5'- TCTCCCATC-CAAGTACTAACCA -3'), were labeled with [γ -³²P]ATP by T4 polynucleotide kinase (NEB). After washing, the membrane was exposed to a PhosphorImager screen.

Distinguishing tRNA^{[Ser]Sec} isoforms

One gram of liver from wild type or *Secisbp2* KO mice was used for total tRNA isolation. After aminoacylation with [³H]serine, seryl-charged tRNA was chromatographed twice on a RPC-5 column, initially in absence of Mg²⁺ and then in presence of Mg²⁺ as described previously (28). By two-step chromatography, tRNA^{[Ser]Sec} can be separated and quantified from total tRNA^{Ser} and distribution of two tRNA^{[Ser]Sec} isoforms (containing either mcm⁵U or mcm⁵Um at position 34) determined (29).

RESULTS

Global analysis of selenoprotein mRNA and translation

To test how selenoprotein expression is altered when *Secisbp2* and the tRNA^{[Ser]Sec} gene (*Trsp*) are removed *in vivo*, we took advantage of two hepatocyte-specific knock-out mouse models, *Alb-Cre; Secisbp2^{fl/fl}* (25) and *Alb-Cre; Trsp^{fl/fl}* (27,30), which are deficient in *Secisbp2* and tRNA^{[Ser]Sec}, respectively. As selenoprotein synthesis is proportional to the amount of mRNA and the rate of translation, we first sought to assess these parameters and analyze to what extent they are affected by the lack of *Secisbp2* or tRNA^{[Ser]Sec}. To this end, livers were harvested and ribosome profiling and RNA-Seq experiments were performed as described under Materials and Methods. In ribosome profiling, which entails deep sequencing of ribosome protected mRNA fragments (RPFs), the number of ribosome

footprints mapping to each mRNA provides a quantitative measure of translational activity across the mRNA (31). Assessment of RPFs aligned to all RefSeq mRNAs revealed that they were highly enriched in the coding sequences relative to the UTRs (Supplementary Figure S1), were ~30 nts in size, and were positioned with a strong triplet phasing corresponding to the expected step size (3 nts) of actively translating ribosomes. For selenoproteins, it is of special interest whether, and at what efficiency, UGA is translated. While RPFs located 5' of UGA (Figure 1B, 5' RPFs) reflect the number of ribosomes that initiated translation, RPFs 3' of UGA (Figure 1B, 3' RPFs) are proportional to the number of ribosomes that have successfully redefined UGA and are translating downstream codons. Triplet phasing and ~30 nt sizes were observed for the RPFs located both upstream and downstream of selenoprotein UGA-Sec codons (Supplementary Figure S2) demonstrating that the RPFs obtained from selenoprotein mRNAs have the expected features of footprints obtained from actively translating ribosomes. As UGA-redefinition efficiency is not 100%, we surmised that 3' RPFs, representing ribosomes that have successfully incorporated Sec, provide the most accurate measure of effective full-length selenoprotein synthesis rates.

To examine the aggregate effects of deleting *Secisbp2* on selenoprotein translation, 3' RPFs for all selenoproteins (excluding selenoproteins with UGA-Sec codons near the termination codon) were normalized for gene length and total mapped sequence reads in each sample (reads/kilobase/million mapped reads – RPKMs) and shown as a percentage of selenoprotein 3' RPFs found in wild-type liver samples (Figure 1C). *Secisbp2* inactivation results in an ~80% reduction of 3' RPFs in liver. We then analyzed livers in which selenoprotein expression is abrogated by inactivation of tRNA^{[Ser]Sec} (Figure 1C) and found a >90% reduction of 3' RPFs. In the absence of tRNA^{[Ser]Sec}, the remaining 3' RPFs may derive from liver cells that are not hepatocytes (e.g. endothelial cells, Kupffer and Ito cells) and/or low-level near-cognate decoding of UGA and continued translation to the termination codon. Since 3' RPFs are less abundant in tRNA^{[Ser]Sec}-deficient liver than in *Secisbp2*-deficient liver, our findings suggest that in-frame UGA-Sec codons within selenoprotein mRNAs can be successfully decoded in the absence of *Secisbp2*.

We then examined the abundance of selenoprotein mRNAs by RNA-Seq. RPKMs were calculated across the entire length of each selenoprotein, summed and shown as a percentage of wild type (Figure 1D). Most selenoprotein mRNAs were reduced in both mutants, however, in contrast to ribosome profiling data, deletion of *Secisbp2* had a greater effect on total selenoprotein mRNA abundance than deletion of tRNA^{[Ser]Sec} (70% and 60% reduction, respectively). Collectively, this global analysis of the selenoproteome suggests that UGA redefinition occurs at a reduced level in the absence of *Secisbp2*, and that *Secisbp2* may play a previously under-appreciated role in maintaining selenoprotein mRNA levels.

The effects of *Secisbp2* and tRNA^{[Ser]Sec}-depletion on 3' RPFs and mRNA levels are shown for each individual selenoprotein in Figure 2. The effects of *Secisbp2*-depletion on selenoprotein expression can be observed to fall into three categories (see Figure 2A), those in which mRNA

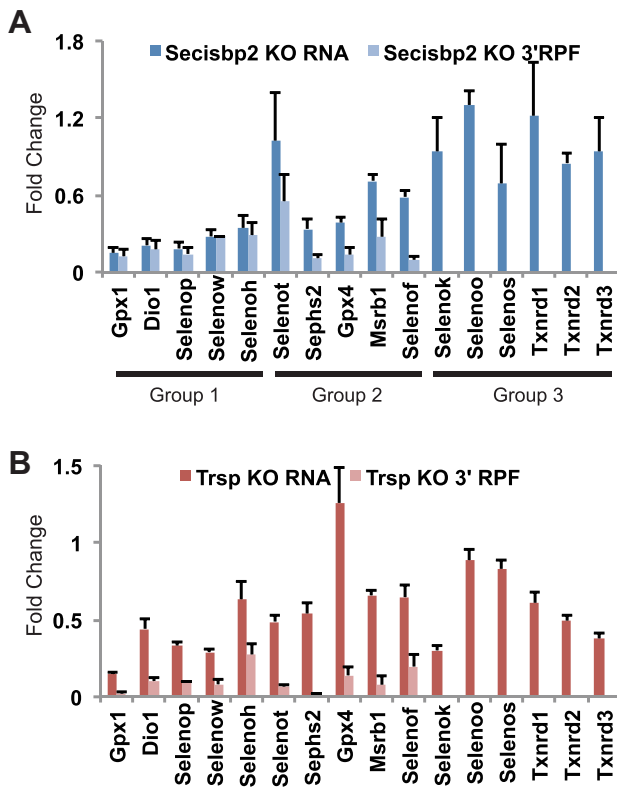


Figure 2. Comparison of the effects of *Secisbp2*-depletion on selenoprotein mRNA levels and translation. Fold change in selenoprotein mRNA levels and 3' RPFs between (A) *Secisbp2*-deficient, (B) $tRNA^{[Ser]Sec}$ -deficient and their corresponding wild-type samples were calculated. Three groups are indicated corresponding to selenoproteins in which mRNA levels and 3' RPFs were reduced to similar degrees (Group 1), 3' RPFs were reduced more than mRNA levels (Group 2) and selenoproteins in which Sec is located near the C-terminus (Group 3).

abundance and 3' RPFs are reduced to approximately equivalent amounts (*Gpx1*, *Dio1*, *Selenop*, *Selenow* and *Selenoh*), those in which 3' RPFs are reduced to a greater degree than mRNA levels (*Selenot*, *Sephs2*, *Gpx4*, *Msrb1* and *Selenof*), and the selenoproteins with UGA near the 3' end of the coding sequence that have relative preserved mRNA levels (*Selenok*, *Selenoo*, *Selenos*, *Txnrd1*, *Txnrd2* and *Txnrd3*). In contrast, 3' RPFs are dramatically reduced for all selenoproteins in the $tRNA^{[Ser]Sec}$ -depleted liver although mRNAs are affected to varying degrees (Figure 2B). Notably, *Gpx4* mRNA was preserved in the $tRNA^{[Ser]Sec}$ -deficient liver but strongly reduced by inactivation of *Secisbp2*. Further, several selenoprotein mRNAs with UGA codons near the end of the open reading frame were reduced in $tRNA^{[Ser]Sec}$ -deficient liver but somewhat less affected by the loss of *Secisbp2*.

Ribosome profiling measurements of *Secisbp2* effects on translation initiation and UGA redefinition

Assuming that selenoprotein translation downstream of UGA (3' RPFs) is proportional to not only the rate of UGA redefinition but also translation initiation, we sought to separately assess these parameters for each selenopro-

Table 1. Ratio of 3'RPF/5'RPF, in wild type control mice

Gene	<i>Secisbp2</i> wild type	<i>Trsp</i> wild type
<i>Gpx1</i>	0.76	0.52
<i>Dio1</i>	0.15	0.22
<i>Selenop</i>	0.39	0.59
<i>Selenoh</i>	0.51	0.78
<i>Sephs2</i>	0.43	0.58
<i>Gpx4</i>	0.16	0.24
<i>MsrB1</i>	0.56	0.55

tein and analyze to what extent they are affected by the lack of *Secisbp2* or $tRNA^{[Ser]Sec}$. Changes in translational efficiency can be separated from changes in mRNA abundance by normalizing RPFs to mRNA levels (RPF density). First, we examined the RPFs upstream of UGA (5' RPFs) for individual selenoproteins. We surmised that an increase in translation initiation would result in an increase in 5' RPF density. Conversely, a reduction in translation initiation would reduce 5' RPF density. The 5' RPF densities are shown for hepatic selenoproteins in Figure 3A and B. A comparison of selenoprotein 5' RPF density between wild type, *Secisbp2*- and $tRNA^{[Ser]Sec}$ -deficient livers revealed that for most selenoproteins, translation 5' of UGA was unaffected by the loss of *Secisbp2* or $tRNA^{[Ser]Sec}$. Exceptions were observed for *Selenok* and *Selenot* in which 5' RPF density was altered approximately 2- to 3-fold upon deletion of *Secisbp2*, and for *Txnrd1* and *Txnrd2*, where the loss of $tRNA^{[Ser]Sec}$ reduced 5' RPF density by approximately 1.5- to 2-fold.

To examine the effects of UGA redefinition and Sec incorporation on translation downstream of UGA, we compared selenoprotein 3' RPF density in *Secisbp2*-deficient, $tRNA^{[Ser]Sec}$ -deficient and wild-type livers (Figure 3C and D). In *Secisbp2*-deficient liver, 3' RPF density is reduced between 2- and 5-fold on *Selenot*, *Msrb1*, *Gpx4* and *Sephs2* mRNAs in agreement with the proposed role of *Secisbp2* in mediating UGA redefinition (Figure 3C). In contrast, the density of ribosomes translating downstream of UGA is preserved in *Secisbp2*-deficient liver for *Dio1*, *Selenop*, *Gpx1* and *Selenow* (see Discussion Figure 6). In $tRNA^{[Ser]Sec}$ -deficient liver, 3' RPF density was significantly reduced on all selenoprotein mRNAs, including those only moderately or not affected by *Secisbp2*-inactivation (Figure 3D).

We reasoned that the 5' and 3' RPF measurements from ribosome profiling data should allow an estimate of the impact of *Secisbp2* on UGA-redefinition efficiency for each selenoprotein. The ratios of 3' to 5' RPFs were calculated excluding mRNAs where UGA was too close to the 5' or 3' end of the coding sequence or had low expression values (Table 1). These ratios ranged from 0.1 to 0.8. While the numbers are qualitatively consistent with previous findings of inefficient UGA redefinition, care should be taken not to interpret these numbers as absolute redefinition efficiencies since any differences in translation elongation rates or library construction bias between the 5' and 3' regions might impact these ratios. In addition, reduced stability of

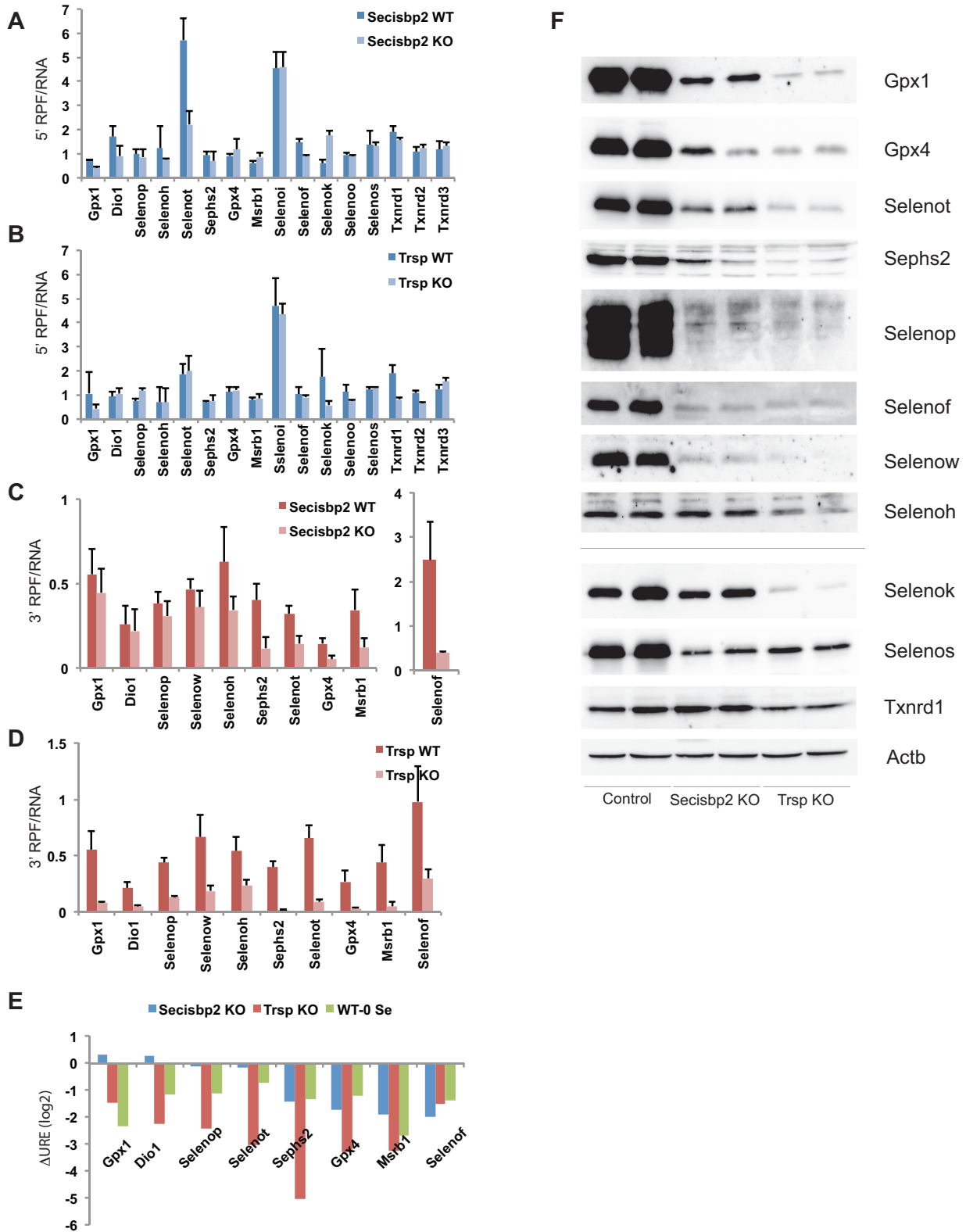


Figure 3. Ribosome profiling in *Secisbp2*- and *tRNA^{[Ser]Sec}*-deficient mice. 5' and 3' RPFs were quantified (RPKM) and normalized to the abundance of the corresponding mRNAs. Comparisons of 5' RPF density in *Secisbp2*- and *tRNA^{[Ser]Sec}*-deficient liver relative to the wild-type control are shown in (A) and (B), respectively. Likewise, 3' RPF density is shown in (C) and (D). For *Selenop*, ribosome densities were determined upstream and downstream of the first UGA-Sec codon only. (E) UGA-redefinition efficiency (URE) is shown as the ratio between 3' RPF and 5' RPF density. The changes in URE log₂ values are shown for selenoproteins in which both 5' and 3' RPF density could be determined. (F) Western blot analysis of selenoproteins from the liver of *Secisbp2* KO and *Trsp* KO mice (Alb-Cre; *Secisbp2*^{fl/fl} and Alb-Cre; *Trsp*^{fl/fl}, respectively).

mRNAs on which termination occurs at the UGA further influences this ratio (see Discussion Figure 6). Nevertheless, it should be possible to estimate changes in UGA-redefinition efficiency on stable mRNAs by comparing the differences in 3' RPF:5' RPF ratios for each mRNA between wild-type and mutant samples. In this way, the confounding factors of translation rates and library bias should be roughly equivalent for each mRNA revealing only the change in UGA-redefinition. Changes in UGA-redefinition efficiency (Δ URE; log₂) are shown in Figure 3E. The blue bars show that URE is unaffected for *Gpx1*, *Dio1*, *Selenop* and *Selenot* in *Secisbp2*-deficient liver, while *Sephs2*, *Gpx4*, *Msrbl* and *Selenof* UREs are reduced. These results are in contrast to the observed reduction in UREs found for all selenoproteins in tRNA^{[Ser]^{Sec}}-deficient liver (red bars). For further comparison, we calculated Δ URE for the same selenoprotein mRNAs from livers of mice fed Se-deficient diets analyzed previously (2) and plotted them into the same diagram (green bars). These data show that selenoproteins with URE values that were relatively unaffected by *Secisbp2*-deficiency change their URE under conditions of nutritional Se deficiency, when both factors are present, but aminoacylated Sec-tRNA^{[Ser]^{Sec}} is limiting. The impact of RNA stability on the apparent changes in URE is considered in detail in the Discussion.

To confirm that the abundance of 3' RPFs is a good measure of selenoprotein synthesis, hepatic selenoproteins were visualized by Western blot (Figure 3F). Comparisons between the fold changes in protein abundance between wild type and *Secisbp2*- or tRNA^{[Ser]^{Sec}}-deficient livers calculated from Western blots and 3' RPFs had a positive correlation coefficient of $R^2 = 0.47$ (data not shown), which is in agreement with the previously reported correlation between ribosome profiling and protein levels in yeast for the whole proteome of $R^2 = 0.42$ (31). However, comparisons of 3' RPFs normalized to mRNA abundance (Figure 3C and D) and Δ URE calculations (Figure 3E) reveal that the selenoproteins in which 3' ribosome density was least affected by *Secisbp2* deletion (e.g. *Selenop*, *Gpx1* and *Selenow*), were in fact significantly reduced in protein abundance (Figure 3F and Supplementary Figure S3). For these selenoproteins, as shown in Figure 2, reduced mRNA abundance is the primary effect of *Secisbp2* loss, with UGA redefinition remaining relatively unaffected during translation of the remaining mRNA. Finally, we performed ⁷⁵Se metabolic labelling in hepatocytes isolated from wild type and *Secisbp2* knockout mice as well as the human HAP1 cell line carrying a null mutation in *Secisbp2* to illustrate that Sec is incorporated in the absence of *Secisbp2* (Supplementary Figure S4). ⁷⁵Se labelling can clearly be seen for several selenoproteins, including Txnrd1 and *Gpx4*, in cells lacking *Secisbp2*.

Ribosome coverage analysis

Ribosome A-site coverage corrected for mRNA abundance is shown in Figure 4. *Selenop* is the only mammalian selenoprotein with multiple UGA-Sec codons. As a Se transport protein, it contains up to 10 Sec residues in mice, which are encoded by 10 UGA codons in the mRNA. Consistent with the results of Figure 3, it can be seen in Figure 4A that the distribution of RPFs density across the *Selenop* coding se-

quence is similar in wild type and *Secisbp2*-deficient liver. In contrast, in tRNA^{[Ser]^{Sec}}-deficient liver (Figure 4B), ribosome density upstream of the first UGA-Sec is comparable to wild type with the exception that ribosome density is increased immediately 5' to the first UGA. Downstream of the first UGA-Sec codon, RPFs are significantly lower in *Trsp* compared to *Secisbp2* mutants. In addition, the presence of two ribonuclease resistant fragments can be observed in *Selenop* that overlap with the first SECIS element (Figure 4A and B; see Supplementary Figure S5A for nucleotide level protection of SECIS 1). The size of the first ribonuclease resistant fragment (~30 nts) is consistent with a ribosome footprint, whereas the second ribonuclease resistant fragment is larger (~38 nts) (Supplementary Figure S5B and S5C).

In the case of *Dio1*, *Secisbp2* deletion has little effect on downstream RPF density (Figure 4C), whereas *Trsp* deletion severely reduces 3' RPF density (Figure 4D). Similar to *Selenop*, RPFs immediately upstream of the *Dio1* UGA-Sec codon show increased density when tRNA^{[Ser]^{Sec}} is deficient. In contrast to the above examples, *Sephs2* reveals a loss of RPFs 3' of UGA in the *Secisbp2* deletion (Figure 4E), although not to the same degree as is seen in the *Trsp* deletion (Figure 4F).

To further examine the effect of *Secisbp2* deficiency on ribosome pausing near UGA, the position of ribosome A-sites for all RPFs that were positioned with the ribosomal A-site codon located either at UGA or in the 5 preceding codons was determined as a percentage of all RPFs on the same mRNA (Supplementary Table S1). As observed for changes in dietary Se levels, the stress-related selenoproteins *Gpx1*, *Selenow*, *Selenoh* and *Msrbl* revealed the highest levels of ribosome pausing upstream of UGA with relatively few RPF A-sites residing on UGA. Pausing upstream of UGA was increased in the *Trsp* knockout and under conditions of Se deficiency for several of the stress related selenoproteins, compared with samples obtained from wild type or *Secisbp2* deletion mice.

Effects on selenoprotein mRNA half-life

We assessed selenoprotein mRNA stability in primary hepatocytes isolated from mice. Cells were exposed to actinomycin D to inhibit RNA polymerase II activity and decay of mRNAs was followed by Northern blot analyses over a period of 30 h. Messenger RNA half-lives were calculated from auto-radiographic images for *Secisbp2*-deficient and wild-type hepatocytes and normalized to ribosomal RNA levels. Selenoproteins like *Gpx1*, *Selenop* and *Selenof* displayed reduced mRNA levels in *Secisbp2* mutant cells, but their half-lives, in the range of days, were too long to be reasonably determined within 30 h of cell culture in the presence of actinomycin D (Figure 5A). *Txnrd1*, *Sephs2* and *Selenot* mRNA stability could be reasonably assessed (Figure 5B and C). *Txnrd1* a selenoprotein mRNA mildly affected by mRNA degradation displayed half-lives of 8.1 h and 7.2 h in wild-type control and *Secisbp2*-deficient cells, respectively. *Sephs2* mRNA half-life was reduced from 10.5 h in controls to 3.6 h in *Secisbp2*-deficient cells. Likewise, *Sephs2* mRNA half-life was reduced to 3.6 h in tRNA^{[Ser]^{Sec}}-deficient cells compared to 10.8 h in the wild-type con-

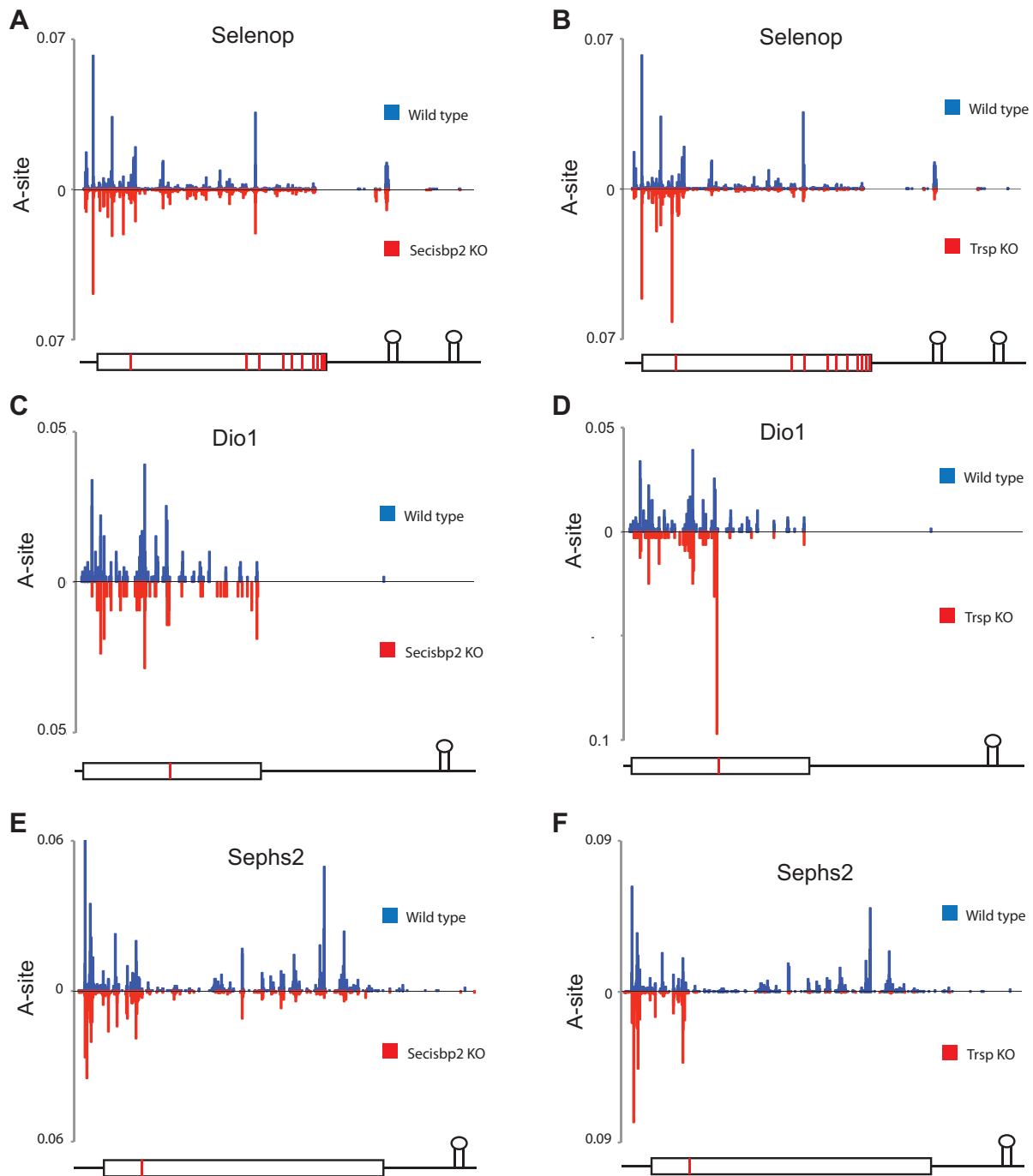


Figure 4. RPF A-site coverage map for *Selenop*, *Dio1* and *Sephs2*. The A-site position of each RPF was determined in wild type (blue-plotted above the x-axis) and Secisbp2- or tRNA^{[Ser]Sec}-deficient liver (red-plotted below the x-axis) for each gene, summed at each position and normalized to million mapped reads and RNA levels. (A and B) *Selenop* A-site plots. (C and D) *Dio1* A-site plots. (E and F) *Sephs2* A-site plots. The position of UTRs (thin lines), coding sequences (rectangular box), UGA-Sec codons (red vertical bars) and SECIS structures are indicated graphically below the plots for each gene.

trol culture. The *Txnrd1* mRNA half-life was 2.9 h in tRNA^{[Ser]Sec}-deficient cells compared to 5.1 h in controls. *Selenot* was the only selenoprotein mRNA with relatively unaffected URE that could be assessed for half-life. Its half-life in Secisbp2-deficient cells was 19.6 h, while it was 20.1 h in wild type. In comparison, *Selenot* half-life in the ab-

sence of tRNA^{[Ser]Sec} was 13.7 h versus 15.1 h in wild-type controls.

To test whether selenoprotein mRNA destabilization is translation-dependent (e.g. NMD or No-go decay), we assessed the stability of *Txnrd1* and *Sephs2* mRNA in the presence of cycloheximide. A dose-response study on wild-type cells was performed to determine the optimal concen-

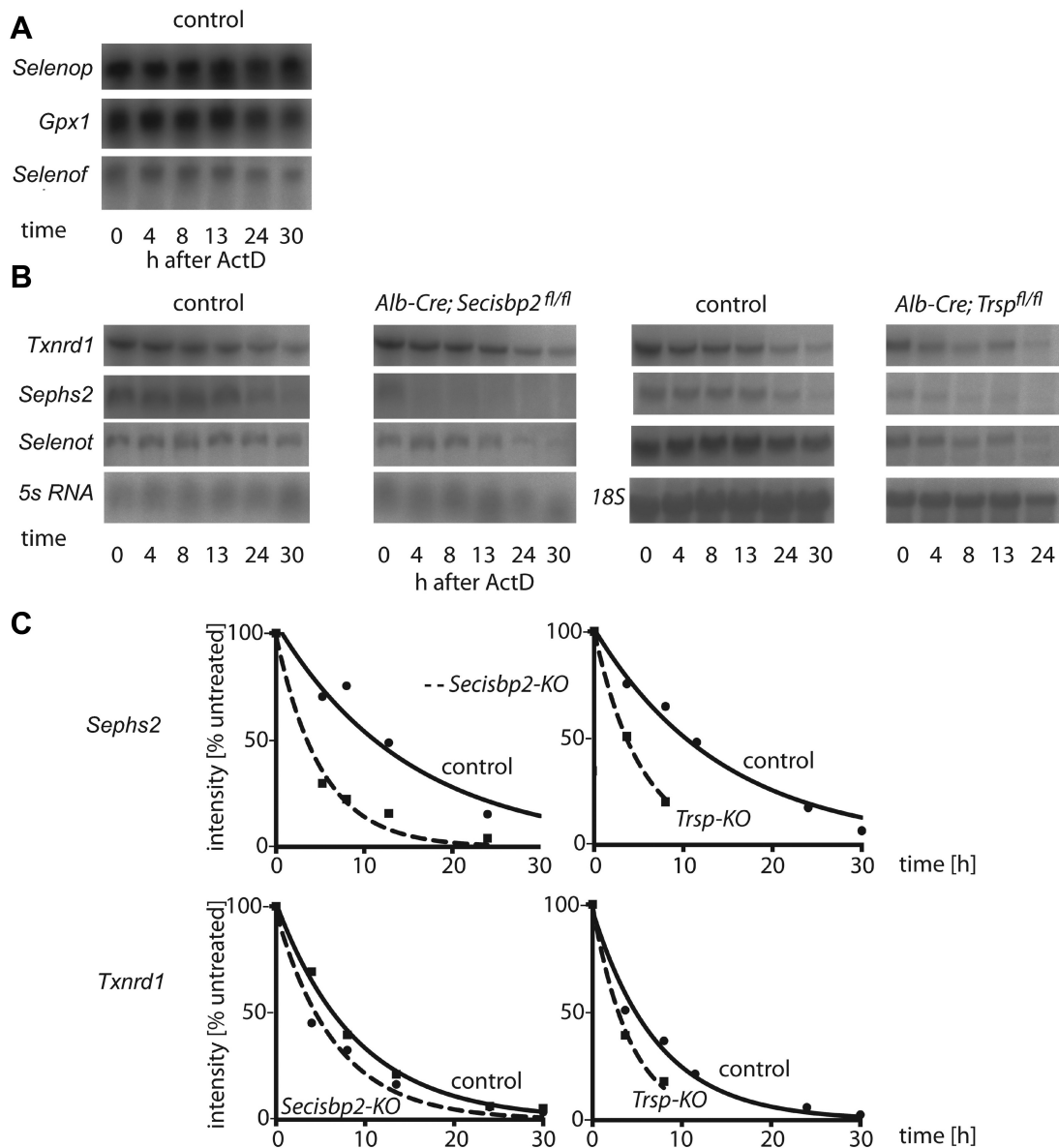


Figure 5. Selenoprotein mRNA stability in primary hepatocytes. (A) *Selenop*, *Gpx1* and *Selenof* mRNA levels were stable over 30 h of actinomycin D treatment such that half-lives could not be reliably estimated. (B) *Txnrd1*, *Sephs2* and *Selenot* mRNAs half-lives were determined in the presence of actinomycin D in *Secisbp2*-deficient, *tRNA*^{[Ser]^{Sec}-deficient and control cell cultures. (C) Graphs showing the degradation of *Sephs2* and *Txnrd1* mRNA in *Secisbp2*- and *tRNA*^{[Ser]^{Sec}-deficient cells. 5s RNA and 18s RNA were used as loading controls.}}

tration of cycloheximide (Supplementary Figure S6). The incubation over 30 h in the presence of both actinomycin D and cycloheximide stabilized both *Sephs2* and *Txnrd1* mRNAs. *Sephs2* showed half-lives of 7.9 h and 8.4 h in controls and *Secisbp2*-deficient cells, respectively (Supplementary Figure S6B). These data suggest that the shorter mRNA half-lives of *Sephs2* mRNA in *Secisbp2*-deficient cells depend on a translation-dependent mRNA degradation pathway.

***tRNA*^{[Ser]^{Sec}}** abundance and 2'-O methylation status

2'-O methylation of *tRNA*^{[Ser]^{Sec} at the wobble base U34 is responsive to bioavailability of Se (32,33). It may be ar-}

gued that the gene-specific response of selenoproteins to *Secisbp2* deficiency reflects *tRNA*^{[Ser]^{Sec} methylation status or abundance. To investigate this possibility, we isolated *tRNA*^{[Ser]^{Sec} from *Secisbp2*-deficient and wild-type liver, enriched *tRNA*^{[Ser]^{Sec}, charged the tRNAs with [³H]-Ser and assessed *tRNA*^{[Ser]^{Sec} profiles by chromatography on a RPC-5 column. As shown in Supplementary Figure S7A, *tRNA*^{[Ser]^{Sec} from both wild type and *Secisbp2*-deficient liver showed the characteristic double peak with the earlier eluting left peak representing U34 *tRNA*^{[Ser]^{Sec} and the later eluting right peak representing Um34 *tRNA*^{[Ser]^{Sec}. Thus, there is no indication that lack of *Secisbp2* interferes with *tRNA*^{[Ser]^{Sec} U34 2'-O-methylation or affects the abundance of *tRNA*^{[Ser]^{Sec} (Supplementary Figure S7B).}}}}}}}}}

DISCUSSION

Global analysis of selenoprotein expression suggests a model describing dual roles for Secisbp2 in UGA redefinition and gene-specific rates of RNA decay

The redefinition of UGA codons to Sec illustrates that the readout of the genetic code need not be fixed across the genome and, in the case of selenoproteins, is required for the insertion of the uncommon amino acid Sec that is utilized for selenoprotein mediated redox reactions. Since UGA redefinition is thought to depend on the SECIS-Secisbp2 interaction, we analyzed liver-specific Secisbp2-deficient mice in order to probe the role of Secisbp2 in UGA redefinition and selenoprotein biosynthesis. tRNA^{[Ser]Sec}-deficient mice were also used here as a negative control, because UGA redefinition and thus, selenoprotein expression is dependent on Sec-tRNA^{[Ser]Sec}. Ratios of RPFs upstream and downstream of UGA codons were utilized as a proxy for UGA-redefinition efficiency. The results demonstrate that UGA redefinition occurs with low efficiency, as had been suggested by studies with model and *in vitro* experimental systems (18,34–37).

By comparing changes in selenoprotein mRNA levels directly with 3' RPFs (Figure 2) in Secisbp2-deficient tissue, we find that the effects of loss of Secisbp2 can be categorized into three groups: (i) Selenoproteins in which RNA and 3' RPFs are reduced to equivalent levels (*Gpx1*, *Dio1*, *Selenop*, *Selenow* and *Selenoh*) suggesting that mRNAs on which UGA redefinition fails are rapidly degraded and that Sec incorporation on the remaining mRNA occurs with near wild-type efficiency. (ii) Selenoproteins in which 3' RPFs are reduced more than the mRNA levels (*Selenot*, *Sephs2*, *Gpx4*, *Msrbl* and *Selenof*), which is consistent with a partial reduction in mRNA stability and translation, but clearly shows that Secisbp2 affects the efficiency of UGA-redefinition; and (iii) selenoproteins with near terminal UGA codons in which RNA levels are mostly preserved (*Selenok*, *Selenoo*, *Selenos*, *Txnrd1*, *Txnrd2* and *Txnrd3*). The observation that a subset of selenoproteins are strongly reduced in mRNA abundance, but that 3' RPFs are preserved on the remaining RNAs in the absence of Secisbp2 demonstrates that, in contrast to the prevailing view, Secisbp2 is not absolutely essential for UGA recoding. In addition, the RNA levels for several mRNAs are reduced to a greater degree by the loss of Secisbp2 than by loss of Sec-tRNA^{[Ser]Sec} indicating that Secisbp2 protects these mRNAs from degradation in a manner that is not simply due to defects in Sec incorporation.

We introduce Δ URE (Figure 3) as a new parameter to assess the combined impacts of a biological condition or genetic mutation on both RNA stability and UGA-redefinition efficiency (Figure 6). As shown schematically in Figure 6, upon encountering a UGA codon (Figure 6A), the ribosome either redefines the UGA codon (Figure 6B) and continues translation or fails to incorporate Sec (Figure 6C) and is released from the mRNA. In the instances when redefinition fails, ribosomes may either terminate or perhaps be released by the No-go decay pathway, either of these outcomes may lead to RNA decay (Figure 6D and E). The ability to monitor 5' RPFs when Sec incorpora-

tion fails is dependent on the extent of RNA degradation. For example, when RNAs are stable, the 5' RPFs are fully accounted for and Δ URE is proportional to changes in UGA redefinition efficiency (URE). However, if the mRNA is rapidly degraded following failure to redefine UGA, then ribosomes 5' of the UGA will be lost and the corresponding 5' RPFs will be underestimated leading to an apparent increase in Δ URE. This observation is most clearly reflected in the group 1 selenoproteins (*Gpx1*, *Dio1*, *Selenot* and *Selenop*) where Δ URE is unchanged by *Secisbp2* deletion. We interpret these results to indicate that the failure to redefine UGA in these mRNAs leads to rapid RNA decay such that 5' and 3' RPFs are only measured for the subpopulation of mRNAs on which UGA redefinition was successful. Conversely, those mRNAs showing reductions in Δ URE have more stable mRNAs that escape RNA decay even when UGA redefinition fails. Due to the confounding effects of changes in RNA decay, Δ URE does not reflect actual changes in UGA redefinition, and thus, should only be used as a measure of changing URE if mRNA levels are unchanged between the conditions compared. Regardless, these findings, as well as the observation that many selenoprotein mRNAs are more stable in the absence of Sec-tRNA^{[Ser]Sec} than Secisbp2, indicate that Secisbp2 stimulates, but is not required for UGA redefinition, and may additionally affect the stability of mRNAs on which UGA redefinition failed.

Ribosomal profiling of selenoproteins as a tool to assess redefinition

We assume that UGA-redefinition is reasonably reflected by the abundance of 3' RPFs, since these correlate well with protein levels as judged by western blot ($R^2 = 0.47$). It may be assumed that read-through of UGA might be achieved by recruitment of near-cognate tRNAs (Cys in response to UGY codons, Trp in response to UGG codon) or even tRNAs misreading the second base (Ser for UCA, Leu for UUA). Insertion of any amino acid except Cys is expected to result in catalytically inactive selenoenzymes and Cys incorporation will reduce activity by orders of magnitude, e.g. as observed for the hepatocyte-specific selenoenzyme Dio1 (34,38). Dio1 activity is greatly reduced in primary hepatocytes isolated from liver-specific *Secisbp2* KO mice, but in proportion to its mRNA level (25). If Sec was replaced by Cys or any other amino acid in this enzyme, its residual activity would have been even lower. In addition, the mRNA level of *Txnrd1* was only marginally reduced in livers from these mice and activity in the Sec-specific *Txnrd1* assay of insulin reduction was almost 20% of wild type (25). Thus, enzymatic activity of two enzymes is supported by Sec incorporation in *Secisbp2* KO cells. In order to investigate whether Sec is still incorporated into selenoproteins, we have metabolically labeled primary hepatocytes derived from *Secisbp2* KO mice and found that ⁷⁵Se incorporation is greatly reduced, yet detectable, in particular in the more abundant selenoproteins *Txnrd1*, *Gpx4* and *Selenof* (Supplementary Figure S4A). The same has recently been observed in a human haploid HAP1 cell line engineered deficient in Secisbp2. Dubey and Copeland have metabolically labeled these cells with ⁷⁵Se and showed that

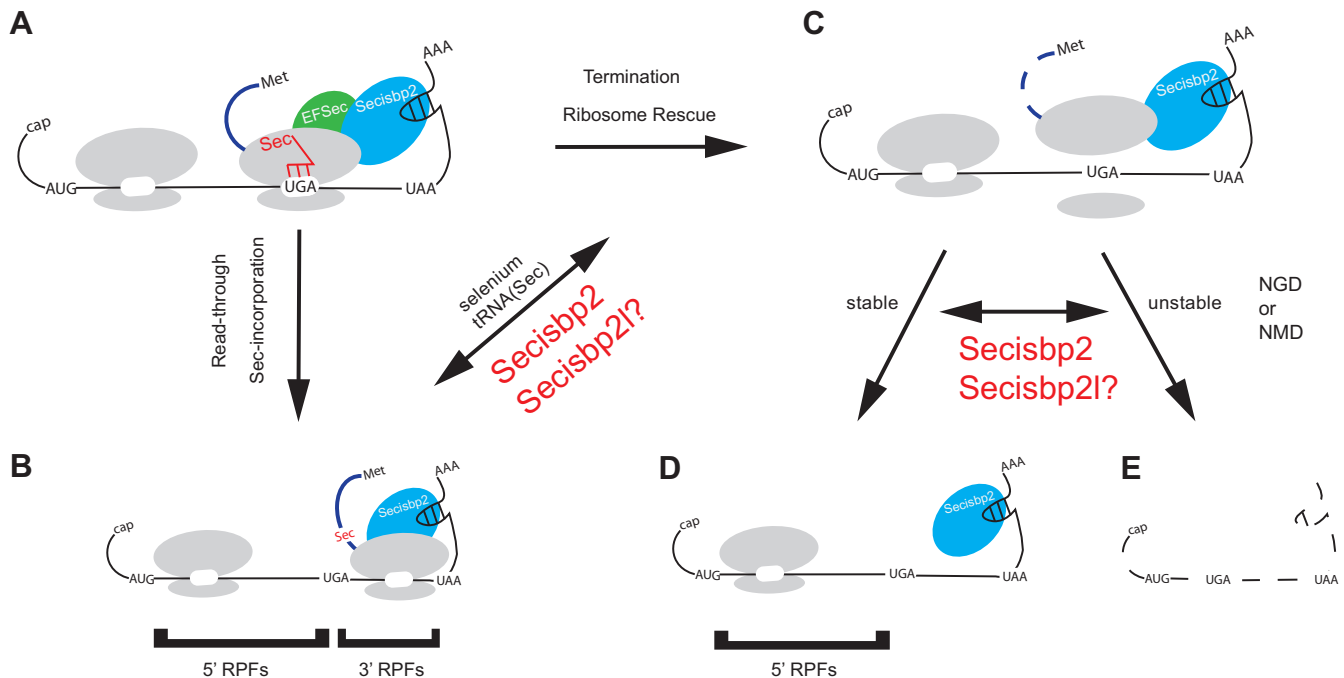


Figure 6. A model of UGA-redefinition and selenoprotein mRNA stability. Schematic of the roles of Secisbp2 in determining gene-specific Sec incorporation rates and RNA stability when Sec incorporation fails. When the ribosome encounters a UGA during translation of (A) selenoprotein mRNAs, either (B) Sec is incorporated or (C) ribosomes are released from the mRNA. As indicated, the efficiency of Sec incorporation is affected by selenium availability, Sec-tRNA^{[Ser]Sec} (tRNA^{Sec}) modification status and abundance or Secisbp2. It is possible that the Secisbp2 homolog Secisbp21 may have Sec incorporation stimulatory activity on a subset of selenoprotein mRNAs when Secisbp2 is absent. When Sec incorporation fails, the data presented here indicates that (D and E) Secisbp2 has an additional role to play in stabilizing the mRNA, which is gene specific and separable from its role in UGA redefinition. Again, Secisbp21 may bind and thus stabilize selenoprotein mRNAs. Since inhibition of translation with cycloheximide (i.e. A-site bound to tRNA) blocks mRNA decay in *Secisbp2* mutants, we propose that mRNA decay is translation-coupled.

⁷⁵Se-selenoprotein labeling is reduced, but clearly present (39). Similarly, we found significant ⁷⁵Se incorporation into TXNRD1 in *SECISBP2*-deficient HAP1 cells. Western blot analysis of TXNRD1, GPX1 and GPX4 showed that GPX4 is less affected than GPX1 in HAP1 *SECISBP2*-deficient cells (Supplementary Figure S4B).

While translation initiation rate estimated from 5' RPF density varies significantly between selenoproteins, it remains relatively unchanged upon *Secisbp2* inactivation for most selenoproteins, with the notable exception that loss of *Secisbp2* appears to reduce 5' RPFs of *Selenot*, which is not regulated on the level of URE or mRNA abundance. Overall, it is evident from the data that 3' RPF density is higher in *Secisbp2*-deficient livers than in tRNA^{[Ser]Sec}-deficient livers. These findings support our earlier results that suggested limited *Secisbp2*-independent translation of several selenoprotein mRNAs (25).

Coverage plots along the length of selenoprotein mRNAs show, at high resolution and for individual selenoproteins, how the lack of *Secisbp2* or tRNA^{[Ser]Sec} impacts the read-through of UGA-Sec codons. In the case of *Selenop*, the largest drop of coverage is seen after the first UGA, while at later UGAs the URE is relatively unchanged (Figure 4A). This is consistent with earlier findings (36,40,41) and suggests that a ribosome having successfully incorporated Sec can more efficiently read through subsequent UGA-Sec codons. While such a model explains the ability of ribosomes to produce full-length *Selenop* with its multiple

Sec residues, the data clearly indicate that incorporation of Sec during translation of selenoprotein mRNAs, including at least the first UGA-Sec of *Selenop*, is a rate-limiting step in selenoprotein translation that is subject to regulation.

Upon deeper analysis of the profiling data, two new ribonuclease resistant fragments were detected in the 3' UTR of *Selenop* mRNA overlapping SECIS1. As shown in Supplementary Figure S5, the two ribonuclease resistant fragments cover an extended region of SECIS1 outside the region known to be bound by *Secisbp2* (5). The experimental procedure involving purification of ribosomes suggests that the ribonuclease resistant fragments were either protected by a ribosome or by a complex that associates with ribosomes. Although ribosomes are greatly depleted in 3' UTRs, a recent study demonstrates that ribosomes do access the 3' UTR in certain circumstances and that components of the No-Go decay pathway, which can clear ribosomes that are unable to terminate normally, are involved in release of those ribosomes (42). The protected fragments cannot be due to protection by *Secisbp2*, because they were also found in *Secisbp2*-deficient livers.

For selenoproteins involved in stress-related responses, such as Gpx1, *Selenoh*, *Selenoh* and *Msrb1*, we see significant accumulation of ribosome footprints upstream of UGA. These results imply that a delay in translation occurs prior to the ribosome encountering UGA that is either unique or particularly pronounced for these mRNAs. Interestingly, the lack of *Secisbp2* does not lead to increased ribo-

somal stacking upstream of UGA-Sec codons compared to the wild type, while increased ribosome density is observed in livers lacking Sec-tRNA^{[Ser]Sec} (Figure 4 and Supplementary Table S1). Perhaps this delay is required in order for the ribosome to receive signals for Sec insertion that is dependent upon the Sec-tRNA^{[Ser]Sec} prior to encountering UGA. Whether a lack of ribosome stacking reflects rapid termination at the UGA codon when Sec incorporation is reduced, or perhaps clearing of paused ribosomes by translational quality control mechanisms such as No-go decay (43) deserves further study.

Secisbp2 stabilizes selenoprotein mRNAs

Selenoproteins Dio1, Selenot, Selenop and Gpx1 illustrate that regulation of selenoprotein expression can operate through different mechanisms. While their UREs remained unchanged on a subpopulation of mRNAs in Secisbp2-deficient liver, their mRNA levels were reduced to an extent that largely explained their reduced protein amounts. Reduced *Gpx1* and *Dio1* mRNA levels are consistent with earlier reports on dietary Se deficiency in rats, where these mRNAs were decreased and mRNA half-lives were reduced (44). Similar results have been found in *Secisbp2*-knockdown cells (45). One surprising observation was the finding that *Sephs2* and *Gpx4* mRNA levels were decreased in the *Secisbp2* mutant, and notably *Gpx4* was unaffected in the *Trsp* mutant. This implies that *Gpx4* and *Sephs2* mRNAs, which are resistant to changes in Se levels, are stabilized by a mechanism involving Secisbp2.

We have analyzed the role of Secisbp2 in regulating mRNA stability by determining the half-lives of selenoprotein mRNAs in primary hepatocytes in the presence of actinomycin D. The half-lives of *Txnrd1* (9 h) and *Selenot* (15 h) are near the median of 9 h found for mRNAs in murine 3T3 cells (46). *Gpx1*, *Selenop* and *Selenof* mRNAs had very long half-lives that were too long to be determined reliably (20–50 h). This finding is consistent with two of these transcripts being among the most abundant in liver. The half-life of *Sephs2* mRNA is clearly reduced from 10.5 to 3.6 h in Secisbp2-deficient hepatocytes. However, as inactivation of *Trsp* leads to the same change in mRNA stability, it remains unclear whether this effect is specific for Secisbp2 or related to the general inability to translate *Sephs2* mRNA. Similarly *Txnrd1* mRNA is destabilized by both mutations (half-lives reduced from 6–7 to 3–5 h).

NMD was suggested as a mechanism of *Gpx1* mRNA destabilization and UPF1 identified as a factor involved (23,47), but neither *Sephs2* nor *Txnrd1* are canonical NMD targets (48). By incubation of primary hepatocytes from the *Secisbp2* mutant and controls with actinomycin D and cycloheximide, we found that *Sephs2* and *Txnrd1* mRNAs are stabilized by inhibition of translation. These observations combined with evidence from ribosome profiling that ribosome pausing occurs prior to the ribosome encountering UGA in a subset of selenoproteins raises the question as to whether the No-Go decay translational control pathway (43) may, in addition to NMD, be involved in regulating selenoprotein mRNA levels. Consistent with this view is the observation that a mutation within the selenocysteine redefinition element of *Selenon* that decreases read-through of

UGA also destabilizes the respective mRNA in the muscle of a patient (49). It is also intriguing that ribosome pausing as well as the effects on RNA levels are more pronounced in the tRNA^{[Ser]Sec}-deficient tissue when Secisbp2 is present. Based on this, it is tempting to speculate that in the presence of Secisbp2, information is conveyed to the ribosome to decode UGA as Sec rather than terminate and that consequently, when Sec-tRNA^{[Ser]Sec} is absent, the ribosome stalls waiting for the Sec-tRNA^{[Ser]Sec} that never arrives, thus activating the No-Go Decay pathway.

Can Secisbp21 compensate for the lack of Secisbp2?

The low level of Sec redefinition in the absence of Secisbp2 in hepatocytes raises the question whether its paralog Secisbp21 may function at low level in selenoprotein expression. This question may be best answered in a *Secisbp2/Secisbp21* double mutant mouse model, provided that inactivation of *Secisbp2* is not lethal to the animal. The physiological role of Secisbp21, however, is not known, albeit *Secisbp21* genes are not present in plants and fungi, organisms that do not possess the selenoprotein biosynthesis machinery (50). The human gene was cloned owing to its sequence homology to *Secisbp2*, which is greatest in the RNA-binding domain and the selenocysteine insertion domain (50). Human Secisbp21 did not support Sec incorporation in an *in vitro* system of selenoprotein biosynthesis despite its ability to bind human SECIS elements with lower affinities (51). It is possible that Secisbp21 and Secisbp2 have diverged in structure and function during evolution, since Secisbp21 from the worm *Capitella*, that does not contain a *Secisbp2* gene, is able to substitute for rat Secisbp2 in the *in vitro* system (51). It is also possible, but has not been demonstrated, that in mouse, Secisbp21 is able to bind and protect selenoprotein mRNAs from degradation without promoting UGA redefinition. There may as well exist signals in some selenoprotein mRNAs, as e.g. selenocysteine redefinition elements (52), which might be able to protect certain selenoprotein mRNAs by increasing redefinition or by recruiting other general RNA-binding proteins.

Multiple mechanisms coordinate to achieve gene-specific regulation of selenoprotein expression

Based on the data presented here, selenoprotein synthesis (S) can be approximated by measuring mRNA abundance (R), translation initiation efficiency (T) and URE, or $S \propto R \times T \times URE$. Herein, we show that changes in each of these parameters can be measured using a combination of RNA-Seq (R = RNA RPKM) and ribosome profiling (T = (5' RPF RPKM/RNA RPKM); URE = (3' RPF RPKM)/(5' RPF RPKM)). As discussed above, variations in RNA decay rates should be considered carefully in applying this model. For analyses concerned only with estimating selenoprotein synthesis rates irrespective of mechanism, mathematical reduction of this formula leads to a simplified equation, $S \propto 3' RPF RPKM$. Using all these parameters, we find that the abundance of selenoprotein mRNAs is regulated in a gene-specific manner. For example, *Gpx1* is strongly regulated by mRNA decay. *Selenot* in contrast does not change its mRNA abundance or redefinition efficiency in Secisbp2-

deficient hepatocytes, but down-regulates translation initiation. *Gpx4* mRNA and UGA-redefinition are dependent on Secisbp2, but mRNA stability is remarkably unaffected by Se availability or tRNA^{[Ser]Sec} inactivation. Thus, in contrast to a widely held notion, there may not be one overarching mechanism that governs selenoprotein expression, but each selenoprotein has evolved a coordinated set of mechanisms that respond specifically in the context of its biological function within a given organ to determine expression levels. The power of the methods presented here is that the different parameters involved can now be directly addressed in a gene-specific manner and on a global scale.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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