ANTIOXIDANTS & REDOX SIGNALING
Volume 21, Number 6, 2014

DOI: 10.1089/ars.2013.5358

ORIGINAL RESEARCH COMMUNICATION

Secisbp2 Is Essential for Embryonic Development and Enhances Selenoprotein Expression

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Abstract

Aims: The selenocysteine insertion sequence (SECIS)-binding protein 2 (Secisbp2) binds to SECIS elements located in the 3′-untranslated region of eukaryotic selenoprotein mRNAs. Selenoproteins contain the rare amino acid selenocysteine (Sec). Mutations in *SECISBP2* in humans lead to reduced selenoprotein expression thereby affecting thyroid hormone-dependent growth and differentiation processes. The most severe cases also display myopathy, hearing impairment, male infertility, increased photosensitivity, mental retardation, and ataxia. Mouse models are needed to understand selenoprotein-dependent processes underlying the patients' pleiotropic phenotypes. ***Results:*** Unlike $tRNA^{[Ser]Sec}$-deficient embryos, homozygous *Secisbp2*-deleted embryos implant, but fail before gastrulation. Heterozygous inactivation of *Secisbp2* reduced the amount of selenoprotein expressed, but did not affect the thyroid hormone axis or growth. Conditional deletion of *Secisbp2* in hepatocytes significantly decreased selenoprotein expression. Unexpectedly, the loss of Secisbp2 reduced the abundance of many, but not all, selenoprotein mRNAs. Transcript-specific and gender-selective effects on selenoprotein mRNA abundance were greater in Secisbp2-deficient hepatocytes than in $tRNA^{[Ser]Sec}$-deficient cells. Despite the massive reduction of *Dio1* and *Sepp1* mRNAs, significantly more corresponding protein was detected in primary hepatocytes lacking Secisbp2 than in cells lacking $tRNA^{[Ser]Sec}$. Regarding selenoprotein expression, compensatory nuclear factor, erythroid-derived, like 2 (Nrf2)-dependent gene expression, or embryonic development, phenotypes were always milder in *Secisbp2*-deficient than in $tRNA^{[Ser]Sec}$-deficient mice. ***Innovation:*** We report the first *Secisbp2* mutant mouse models. The conditional mutants provide a model for analyzing Secisbp2 function in organs not accessible in patients. ***Conclusion:*** In hepatocyte-specific conditional mouse models, *Secisbp2* gene inactivation is less detrimental than $tRNA^{[Ser]Sec}$ inactivation. A role of Secisbp2 in stabilizing selenoprotein mRNAs *in vivo* was uncovered. *Antioxid. Redox Signal.* 21, 835–849.

Introduction

NATURALLY OCCURRING EXPANSIONS to the genetic code rely on the recoding of stop codons. Selenocysteine (Sec) is the 21st proteinogenic amino acid and is present in all three domains of life. Its incorporation into the protein requires a complex machinery of *cis*- and *trans*-acting factors (24). Messenger RNAs coding for selenoproteins contain the opal codon UGA at the position of Sec insertion and a stem-loop structure called the selenocysteine insertion sequence (SECIS) element (3, 61). In a bioinformatic genome-wide search for SECIS elements associated with in-frame UGA

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Innovation

Interest in the selenocysteine insertion sequence (SECIS)-binding protein 2 (Secisbp2) is manifold. Since Secisbp2 plays a central role in UGA/Sec recoding and selenoprotein expression, the study of its molecular biology will ultimately lead to a better insight into translation in general and selenoprotein translation in particular. Second, congenital *SECISBP2* deficiency causes a syndrome of growth retardation that has been defined as an atypical form of resistance to the thyroid hormone. We provide the first mouse models that allow us to dissect the roles of Secisbp2 *in vivo*. Finally, mutations in *SECISBP2* demonstrate the fundamental effects of selenoproteins on human health, including immunological, metabolic, and neurological processes.

codons, 25 and 24 genes encoding selenoproteins were identified in the human and mouse genomes, respectively (29). In eubacteria, the SECIS element is located immediately downstream to the UGA in the coding region, while in eukaryotes, it is located in the 3′-untranslated region. In bacteria, a specific translation elongation factor, SelB, binds Sec-tRNA$^{[Ser]Sec}$ and interacts with the SECIS element (2). These two functions are served by two proteins in mammals, elongation factor Sec (EF-Sec) and SECIS-binding protein 2 (Secisbp2) (17, 22). In all domains of life, Sec biosynthesis occurs on its tRNA$^{[Ser]Sec}$ (31, 57, 60).

Secisbp2 was purified and cloned based on its ability to bind to SECIS elements *in vitro* (17). The protein contains an RNA-binding module, the L7Ae domain, which interacts with kink-turn RNA structures such as the one found in the SECIS core that contains a pair of non-Watson–Crick base pairs (30, 54). Secisbp2 facilitates Sec incorporation by binding to the SECIS element, promoting recruitment of EF-Sec and interacting with the ribosome (10, 18). Several other SECIS-binding proteins have since been identified. These include L30 (Rpl30), which is a component of the UGA recoding machinery (14), YB1 (48), NSEP1 (47), eIF4a3 (9), and nucleolin (33, 56), which play roles in regulating UGA recoding. In addition, Secisbp2L binds to SECIS elements, but does not support Sec incorporation (19) [reviewed in Seeher *et al.* (44)].

Mutations in *SECISBP2* were first identified in a family with several children exhibiting a delay in linear growth and bone age. In addition, these patients manifested slightly elevated total thyroxine (T_4), low tri-iodothyronine (T_3), high reverse T_3, and high plasma thyroid-stimulating hormone (TSH) as well as abnormal TSH suppression tests that suggested impaired thyroid hormone metabolism (21). Thyroid hormones are subject to deiodinating reactions catalyzed by deiodinases, a family of selenoenzymes (4). By comparison, deiodinase gene (*Dio1*) targeted mice show increased reverse T_3 (39), while *Dio2* deficiency leads to high T_4 and TSH (38). Indeed, Dio deficiency, along with reduced expression of selenoprotein P (Sepp) and glutathione peroxidases (Gpx), pointed to a general defect in selenoprotein mRNA translation, since *Dio2* mRNA levels were normal in fibroblasts from these patients (21). Similarly, abnormal thyroid function tests led to the identification of new patients carrying several homozygous and compound heterozygous mutations in *SECISBP2* (20). Other mutations are apparently more disruptive for selenoprotein biosynthesis and lead to additional symptoms like myopathy [similar to selenoprotein N deficiency (34)], abnormal gait (1), bilateral hearing loss, male infertility, increased photosensitivity, shortened telomere length, and compromised immune function along with abnormal glucose metabolism (40). Mutations in *SECISBP2* affect SECIS binding, underlining the importance of SECISBP2 for selenoprotein biosynthesis *via* SECIS interactions (1, 8).

The many roles of selenoproteins in mammalian health are revealed by phenotypes of mouse models deficient for individual selenoproteins or with inefficient selenoprotein mRNA translation, as well as by human congenital disorders of selenoprotein expression (16, 42). To study the role of Secisbp2 in tissues, organs, and whole organisms, mouse models are needed that allow for biochemical investigations in tissues not accessible in human patients. We describe here the first constitutive and conditional mouse models for *Secisbp2* and compare selenoprotein expression in these with our earlier work on similar mouse models lacking tRNA$^{[Ser]Sec}$.

Results

Constitutive Secisbp2 gene targeting

Secisbp2 gene targeting followed a knockout-first strategy (Fig. 1A) allowing for both classical gene knockout and *Cre* recombinase-mediated conditional gene ablation. Intercrosses of *Secisbp2*$^{+/-}$ mice yielded no live *Secisbp2*$^{-/-}$ offspring, but many resorbed conceptuses (Table 1). Dissection of *Secisbp2*$^{-/-}$ embryos demonstrated developmental retardation by embryonic day 8 (E8) (Fig. 1B). Whereas control embryos had progressed to TS12 (somites 1–7, first branchial arch develops, beginning of heart formation), *Secisbp2*$^{-/-}$ embryos are arrested in TS7, which is characterized by implantation of the conceptus and formation of the egg cylinder. Thus, *Secisbp2*$^{-/-}$ embryos failed after implantation, but developed further than tRNA$^{[Ser]Sec}$-deficient embryos that only reached the morulae or blastocyst stage (E3.5; TS2-3) and failed to implant (6).

SECISBP2-mutations in humans are associated with delayed bone development, growth defects, and abnormal thyroid hormone constellations. Heterozygous *Secisbp2*$^{+/-}$ mice of both sexes exhibit the same body weight and tail length as *Secisbp2*$^{+/+}$ litter mates (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/ars). Serum T_4 (Fig. 1C), T_3 (Fig. 1D), or TSH (Fig. 1E) remained normal. Secisbp2 protein levels are substantially reduced in the liver, kidney, brain, and testis (Fig. 1F). Serum Se, which is mainly determined by hepatically released Sepp, was significantly diminished in *Secisbp2*$^{+/-}$ mice (Fig. 1G). Hepatic cytosolic Gpx and thioredoxin reductase (Txnrd) activities were not changed (Fig. 1H, I), while the testicular Gpx4 protein was significantly reduced (Fig. 1J). The highest levels of the Secisbp2 protein are found in the testis, where it is believed to support high Gpx4 expression, a sperm structural protein. Even in the heterozygous mutants, we did not observe reduced fertility of *Secisbp2*$^{+/-}$ males consistent with earlier findings in heterozygous *Gpx4*$^{+/-}$ mice (37). It may be of future interest to explore whether *Secisbp2*$^{+/-}$ mice are vulnerable to dietary Se restriction.

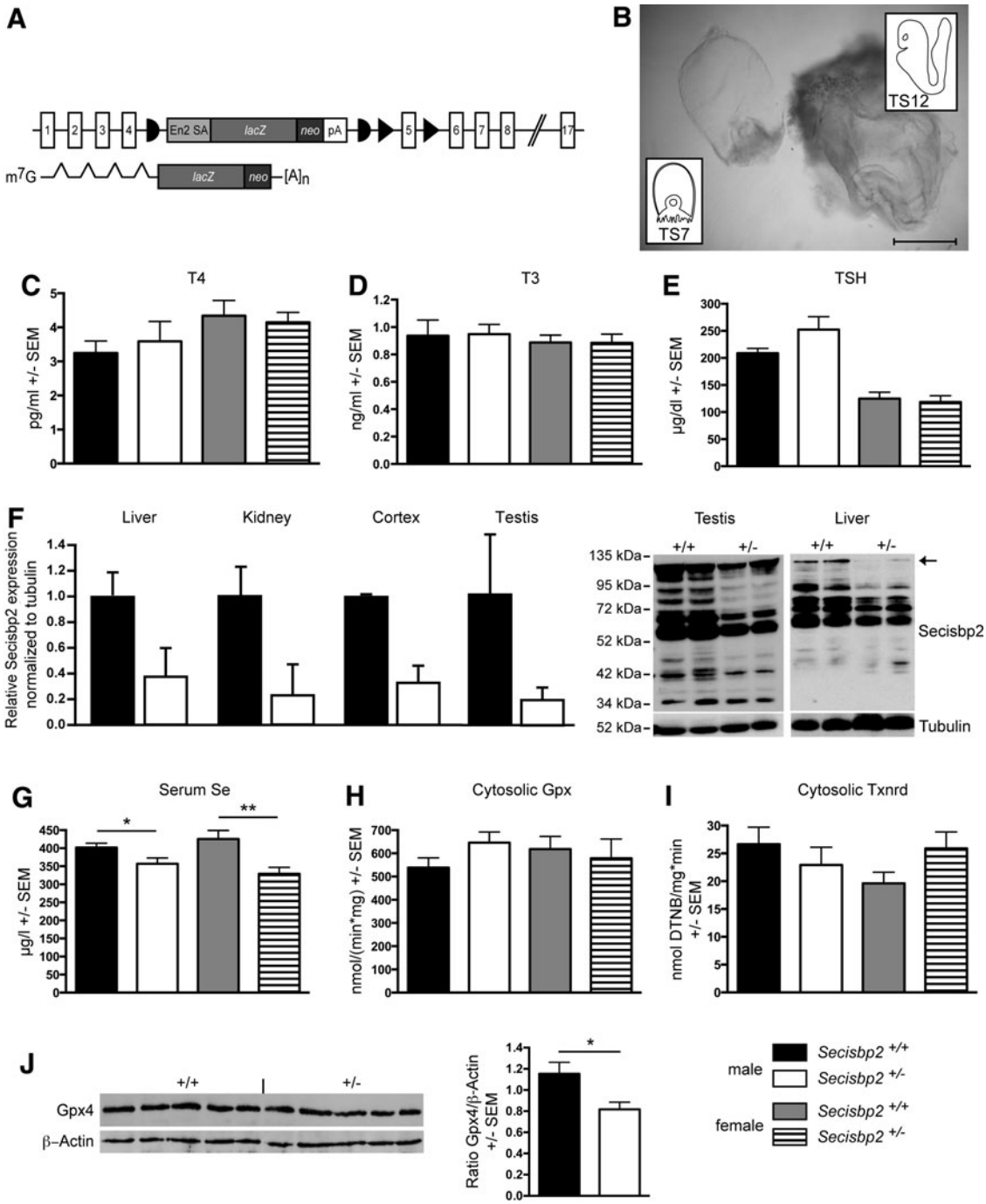

FIG. 1. Gene targeting of *Secisbp2*. **(A)** Structure of the targeted *Secisbp2* allele and mRNA after splicing. Insertion of a FRT-flanked gene-trap cassette containing an En2 splice acceptor (En2 SA), in-frame *lacZ/neo* reporter/selection cassette, and polyadenylation signal. The targeted allele produces a fusion transcript that terminates after exon 4. FRT sites are denoted as half circles and loxP sites as triangles. **(B)** Embryos at embryonic day 8.5. The homozygous *Secisbp2*$^{-/-}$ embryo was developmentally arrested at the Theiler stage 7 (TS7). The control embryo reached the appropriate TS12. *Insets:* Schematic drawings of TS7 and TS12 embryos. **(C)** Serum total T_4 levels, **(D)** total T_3 levels, **(E)** serum TSH were not altered in *Secisbp2*$^{+/-}$ mice. **(F)** Western blot for Secisbp2 shows reduced protein levels in several organs of *Secisbp2*$^{+/-}$ mice. **(G)** Serum Se was diminished in *Secisbp2*$^{+/-}$ mice. **(H)** Hepatic cytosolic Gpx activity was not significantly changed in *Secisbp2*$^{+/-}$ mice. **(I)** Hepatic cytosolic Txnrd activity determined with the DTNB assay. **(J)** Testicular Gpx4 protein expression was reduced in *Secisbp2*$^{+/-}$ mice. *Secisbp2*$^{+/+}$ and *Secisbp2*$^{+/-}$ mice were analyzed at the age of 2 to 5 months. $*p < 0.05$; $**p < 0.01$; Student's *t*-test. ($n = 4$–6 for all groups.). Scale bar = 500 μm. DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Gpx, glutathione peroxidase; Secisbp2, selenocysteine insertion sequence-binding protein 2; T_3, tri-iodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone; Txnrd, thioredoxin reductase.

TABLE 1. GENOTYPE ANALYSIS OF *SECISBP2*$^{+/-}$ AND *SECISBP2*$^{+/\Delta 5}$ INTERCROSS PROGENY

	Genotype[a]			
Age	*+/+*	*+/−*	*−/−*	*Resorption*
3 weeks	32	75	0	N/A
13.5 dpc	15	35	3	15
9.5 dpc	7	18	7	7
	Genotype[b]			
	+/+	*+/Δ5*	*Δ5/Δ5*	
3 weeks	26	45	0	N/A

[a]Genotyping of *Secisbp2*$^{+/-}$ intercrosses 3 weeks after birth yielded no live *Secisbp2*$^{-/-}$ offspring. Analysis of embryos from day 9.5 *post coitum* (dpc) detected several resorbed structures, which were identified as *Secisbp2*$^{-/-}$. Genotyping resorptions on 13.5 dpc was more complicated because of the more advanced stage of resorption. Most potential *Secisbp2*$^{-/-}$ embryos were likely assigned to *Secisbp2*$^{+/-}$ due to contamination with maternal tissue.
[b]Genotyping of *Secisbp2*$^{+/\Delta 5}$ intercrosses 3 weeks after birth yielded no live *Secisbp2*$^{\Delta 5/\Delta 5}$ offspring. *Secisbp2*$^{+/fl}$ heterozygous mice were crossed with a germline *Cre* deleter mouse strain to remove the floxed exon 5. The resulting genotype lacking exon 5 in one allele is denoted *Secisbp2*$^{+/\Delta 5}$.
N/A, not applicable; Secisbp2, selenocysteine insertion sequence-binding protein 2.

Conditional inactivation of Secisbp2 in liver

Embryonic lethality of the knockout mice required the study of selenoprotein biosynthesis in conditional *Secisbp2* mice. Germline expression of FLP recombinase removed the gene-trap cassette leaving a conditional (floxed) exon 5 (Fig. 2A). Sequencing of the mutant transcript confirmed that removal of exon 5 causes a frameshift resulting in a premature stop codon in exon 6 (not shown). Homozygous germline deletion of floxed exon 5 in *Secisbp2* (*Secisbp2*$^{\Delta 5/\Delta 5}$) yielded the same embryonic lethal phenotype as the knockout-first allele indicating that the *Secisbp2*$^{\Delta 5}$ allele is not functional (Table 1). We then targeted hepatocytes using a *Cre* transgene under control of the albumin promoter (*Alb-Cre*), which we had previously used to inactivate tRNA$^{[Ser]Sec}$ (gene symbol: *Trsp*) in hepatocytes. The liver is a good model for the study of selenoprotein expression, since targeted ablation of selenoprotein biosynthesis using the conditional *Trsp* in our hands does not lead to liver failure or other diseases (43, 46, 52). To test whether liver damage occurs upon *Secisbp2* deletion in hepatocytes, we determined liver transaminase activities in serum. No significant changes according to genotype of the animals were noted ($n = 6$–9; alanine-aminotransferase (ALAT, GPT) activity in U/ml ± standard error of the mean (S.E.M.): controls 0.23 ± 0.09 *versus* mutants 0.24 ± 0.12; aspartate-aminotransferase (ASAT, GOT) activity in U/ml: controls 0.36 ± 0.09 *versus* mutants 0.43 ± 0.13) indicating that no apparent liver damage occurred in *Alb-Cre; Secisbp2*$^{fl/fl}$ mice. Furthermore, the liver not only expresses many selenoproteins that can be measured reliably, but also provides sufficient material to do so.

Western blot analysis of control mouse liver extracts using an antibody directed against the C-terminus of Secisbp2 revealed a prominent band of 120 kDa that represents the full-

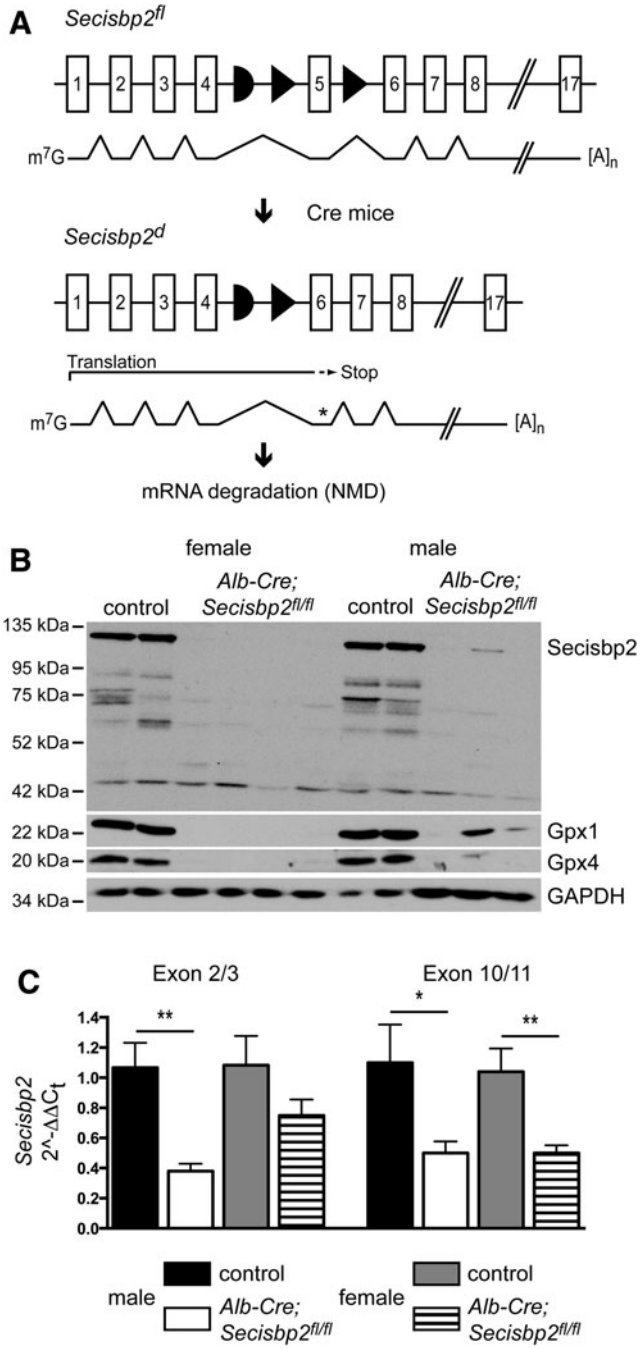

FIG. 2. Conditional inactivation of *Secisbp2* in hepatocytes. (A) *Cre*-mediated recombination removed exon 5 of *Secisbp2* generating a frameshift and premature stop (*asterisk*) in exon 6. **(B)** Hepatocyte-specific *Secisbp2* inactivation substantially reduced Secisbp2, Gpx1, and Gpx4 protein as assessed by western blotting. Forty-five micrograms (Secisbp2) or 25 μg (Gpx1, Gpx4, GAPDH) protein of liver extract was loaded per lane from 2–4 individual mice. An antibody directed against the C-terminus detected full-length Secisbp2 at 120 kDa. GAPDH served as loading control. **(C)** *Secisbp2* mRNA levels were reduced after removal of exon 5. Quantitative polymerase chain reaction (qPCR) targeting exons 2/3 and exons 10/11 showed that transcript levels are reduced (*$p < 0.05$; **$p < 0.01$;Student's *t*-test. $n = 6$).

length protein (Fig. 2B, lanes 1, 2, 7, 8). We also observed several minor bands ranging in size from 60 to 90 kDa, which presumably represent degradation products generated during tissue workup, since they varied in pattern and intensity depending on the extraction method. The Secisbp2 protein is quantitatively removed from the livers of *Alb-Cre; Secisbp2*$^{fl/fl}$ female and male mice (Fig. 2B; lanes 3–6 and 9–11, respectively). Selenoproteins, Gpx1 and Gpx4, were significantly reduced in the Secisbp2-deficient liver samples confirming the dependence of selenoprotein expression on Secisbp2. In only one male mouse, incomplete recombination occurred leaving residual Secisbp2 protein, which accordingly supported partial Gpx1 and Gpx4 expression (Fig. 2B, lane 10). This shows that all phenotypes observed are caused by *Secisbp2* inactivation and do not depend on another trait genetically coupled to the transgene. The mouse in which the *Cre* transgene was apparently nonfunctional was omitted from subsequent analyses except Figure 5C. Quantitative polymerase chain reaction (qPCR) using primers specific for exons 2/3 and exons 10/11 revealed a reduction of *Secisbp2* mRNA levels (Fig. 2C). The mutant transcript perhaps is degraded due to the premature stop codon in exon 6. Some of the remaining amounts of *Secisbp2* mRNA and protein may arise from endothelial cells and Kupffer cells that are not targeted by *Alb-Cre*.

Impact of Secisbp2 inactivation on selenoprotein mRNA levels

Selenoproteins carry in-frame UGA codons in their mRNAs, which could lead to the transcripts being degraded by nonsense-mediated decay (NMD) when Sec incorporation is impaired. Both Se deficiency and hepatic *Trsp* deficiency impair selenoprotein biosynthesis and thus reduce the amounts of several selenoprotein mRNAs in mouse livers (13, 53). We therefore tested whether selenoprotein mRNA levels were also impacted in *Alb-Cre; Secisbp2*$^{fl/fl}$ livers (Fig. 3). We were able to group selenoprotein transcripts into four categories according to their response to *Secisbp2* inactivation. Selenoproteins that carry the UGA codon in an upstream exon are in the first group, which potentially fall subject to NMD. Accordingly, many prototypic selenoprotein mRNAs (*e.g., Gpx1, Dio1*) were significantly reduced in mutant livers (Fig. 3A). The second group comprises selenoprotein mRNAs that also carry the UGA codon in an upstream exon, but in contrast to the first group, the reduction of mRNA is smaller and mostly limited to the male liver (Fig. 3B). In the third group are selenoprotein mRNAs carrying the UGA codon in the last exon. These transcripts are generally not subject to NMD and comprise prototypic examples like *Txnrd1* (Fig. 3C). One notable exception, *Seps* mRNA, falls into the fourth group. This mRNA was reduced in male and female *Secisbp2* mutant livers, despite its UGA being located close to the C-terminus in the last exon (Fig. 3D). Accordingly, a clear reduction of Seps protein levels in response to *Secisbp2* mutation was apparent (Fig. 4). In contrast, Sepk was expressed at normal levels. Interestingly, in liver homogenate from mice with tRNA$^{[Ser]Sec}$ inactivation, we found undetectable Sepk levels. This finding suggests that lack of *Secisbp2* may be mechanistically different from a mere blockade of selenoprotein mRNA translation.

SECIS elements are classified as being type 1 or type 2, which contain either an apical loop or an apical bulge. The differential response of selenoprotein mRNAs did not correlate with the type of SECIS element (Table 2). There was also no correlation between the loss of a particular selenoprotein mRNA and its predicted ability to serve as a substrate for NMD. The current rule for NMD requires that the premature stop codon is more than 50 nucleotides upstream of an exon–exon junction. As shown in Table 2, *Sps2* and *Seps* mRNAs were reduced in spite of the fact that these transcripts have only one exon or contain the UGA in the last exon, respectively. Likewise, several selenoprotein mRNAs are reduced even though the UGA codon is too close to the next downstream exon (*e.g., Sepp, Sepw, Sepr, Sep15,* and *Sepm*). Interestingly, all of the selenoprotein mRNAs that are not affected by Secisbp2 deficiency (*e.g., Txnrd1*, *Sepi*, *Sepk*, and *Sepo*) do not follow the rules for NMD (Table 2). Unexpectedly, several selenoprotein mRNAs that showed a reduction in response to Secisbp2 inactivation were not decreased in the livers of *Trsp*-deficient or Se-deficient male mice, specifically *Dio1*, *Sps2*, *Gpx4*, *Sepr*, *Sep15*, and *Seps* (11, 53), although *Dio1* mRNA was significantly reduced in another study (52). Thus, with respect to hepatic selenoprotein mRNA levels, *Trsp* deficiency and selenium deficiency more closely mimic each other, whereas *Secisbp2* deficiency gives a distinctively different profile.

Impact of Secisbp2 inactivation on hepatic selenoprotein expression

Sepp is a plasma Se transport protein primarily secreted from the liver by hepatocytes (12, 25, 36, 41, 43). In serum, significant Sepp remained in *Alb-Cre; Secisbp2*$^{fl/fl}$ mice compared with controls (Fig. 5A), which corresponded to about 30% serum Se remaining in the mutants (Fig. 5B). This is two times higher than in *Alb-Cre; Trsp*$^{fl/fl}$ mice as reported previously (43). In comparison, serum Se in *Alb-Cre; Sepp*$^{fl/fl}$ mice was reduced to 9% (25). We then analyzed liver extracts for remaining Sepp biosynthesis by western blot analysis. We found several bands corresponding to the full-length protein at 41 kDa and a series of increasingly glycosylated forms up to the >55 kDa secreted protein forms. Again, more Sepp proteins are detected in the *Alb-Cre; Secisbp2*$^{fl/fl}$ liver than in *Alb-Cre; Trsp*$^{fl/fl}$ controls (Fig. 5C). We therefore analyzed again Gpx1 and Gpx4 expression in liver extracts, but this time applied more protein (100 μg per lane). Under these conditions, small amounts of residual Gpx1 and Gpx4 proteins were detectable in the *Alb-Cre; Secisbp2*$^{fl/fl}$ liver, but not in *Alb-Cre; Trsp*$^{fl/fl}$ (Fig. 5C). Interestingly, the Txnrd1 protein, which carries Sec as the penultimate amino acid, was apparently not reduced (Fig. 5C). However, SDS-PAGE analysis cannot exclude the possibility that a Txnrd1 protein lacking the last two amino acids was synthesized due to premature termination at the UGA/Sec codon.

Activity assays are superior to western blots for determination of selenoenzymes, because activity usually critically depends on Sec incorporation. The cellular Gpx activity was reduced by 85% and 90% in male and female *Alb-Cre; Secisbp2*$^{fl/fl}$ livers, respectively (Fig. 5D). The cytoplasmic Txnrd activity as measured with the 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) assay was significantly reduced in the *Alb-Cre; Secisbp2*$^{fl/fl}$ liver (about 50% not shown) nicely corresponding to our findings in the *Alb-Cre; Trsp*$^{fl/fl}$ liver (52). The insulin-based Txnrd assay is considered more

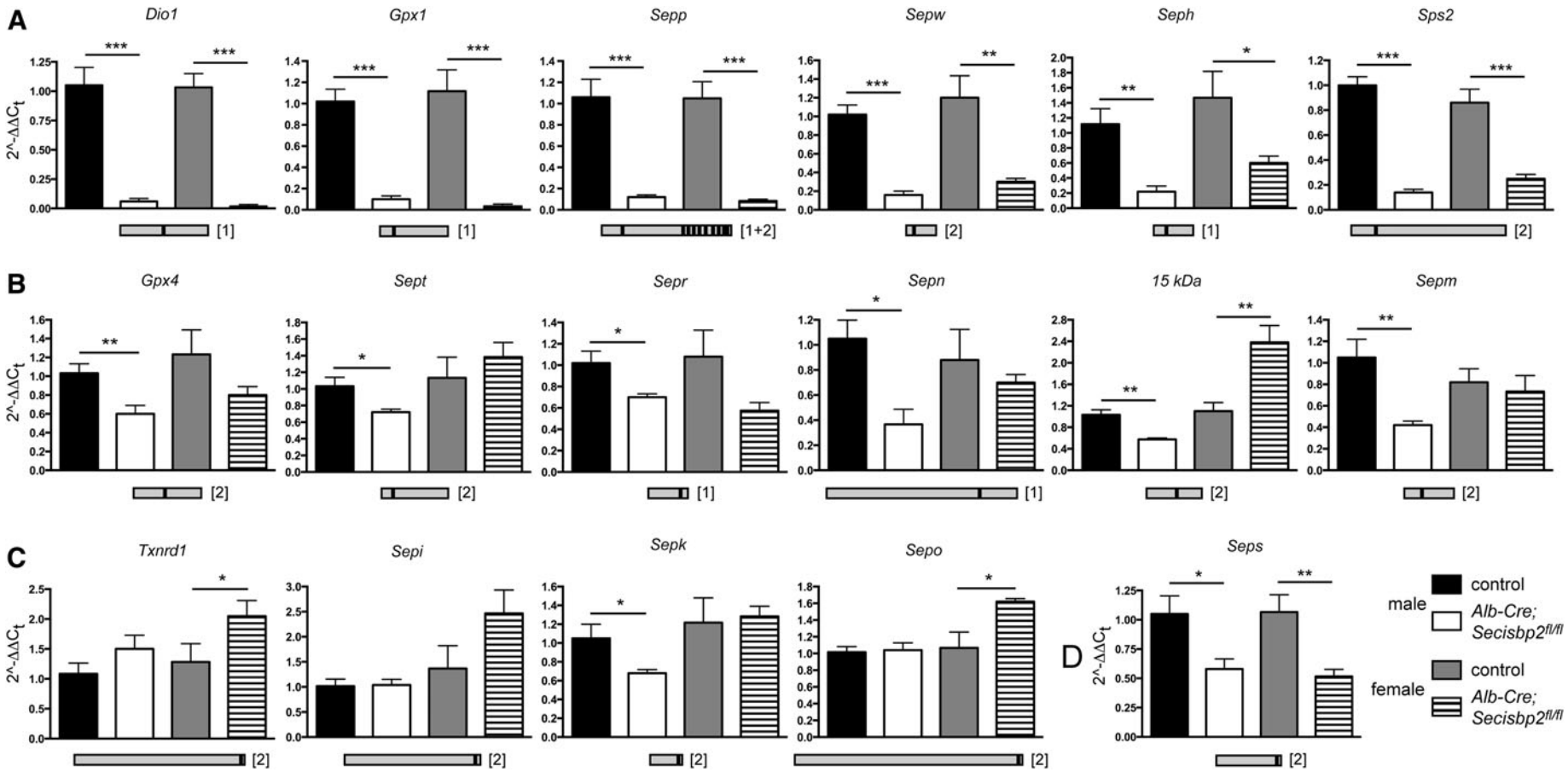

FIG. 3. Impact of *Secisbp2* inactivation on selenoprotein mRNA. Quantitative PCRs were performed with four to six animals per group. The selenoprotein mRNA is schematically shown as a gray bar with the position of the Sec/UGA marked as a black bar. The SECIS type is shown in brackets. **(A)** Group of selenoprotein mRNAs (*Dio1*, *Gpx1*, *Sepp*, *Sepw*, *Seph*, *Sps2*), which was markedly affected in male and female *Alb-Cre; Secisbp2*$^{fl/fl}$ mice and carries the UGA in an upstream exon. *Sepp* carries 10 UGAs wherein the initial one is in the first exon. **(B)** Group of selenoproteins (*Gpx4*, *Sept*, *Sepr*, *Sepn*, *Sep15*, *Sepm*) whose mRNAs were less or not affected and carry the UGA in an upstream exon. For some transcripts, sexual dimorphism was observed in response to *Secisbp2* inactivation. **(C)** Selenoprotein mRNAs within this group (*Txnrd1*, *Sepi*, *Sepk*, *Sepo*) carry UGA in the last exon and were not reduced in *Alb-Cre; Secisbp2*$^{fl/fl}$ mice. **(D)** *Seps* mRNA comprises its own group being moderately reduced in *Secisbp2*-deficient liver, although the UGA is located in the last exon (*$p<0.05$; **$p<0.01$; ***$p<0.001$, Student's *t*-test). Sec, selenocysteine; Sepp, selenoprotein P.

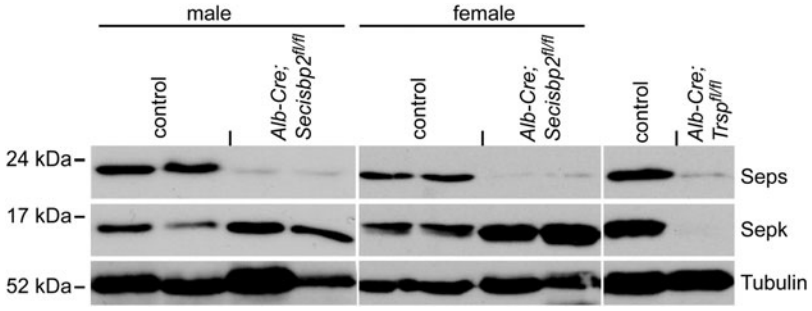

FIG. 4. Impact of *Secisbp2* inactivation on Seps and Sepk expression in liver. Seps was significantly reduced in *Alb-Cre; Secisbp2$^{fl/fl}$* and *Trsp$^{fl/fl}$* mice compared with wild-type controls as assessed by western blotting. Sepk expression was similar in control and in *Alb-Cre; Secisbp2$^{fl/fl}$* mice. In contrast, Sepk was not detectable in the *Alb-Cre; Trsp$^{fl/fl}$* liver. Forty-five micrograms of total protein extracts was loaded per lane. Tubulin was used as loading control. Representative image of two experiments.

sensitive to the presence of the penultimate Sec residue. Employing this assay, we found an even larger reduction in the *Secisbp2* mutants to about 40% of control activity (Fig. 5E).

Since the liver is not only composed of hepatocytes, our analysis may be complicated by proteins from endothelial cells and Kupffer cells. We therefore measured the activity of type I deiodinase (Dio1), which is specific for hepatocytes. Whereas *Dio1* mRNA was significantly reduced in the *Alb-Cre; Secisbp2$^{fl/fl}$* liver (Fig. 3A), it was obvious that a small fraction of Dio1 activity remained in the *Secisbp2*-targeted liver (Fig. 5F).

Selenoprotein expression in primary hepatocytes

To investigate the possibility further that hepatocytes lacking *Secisbp2* can still express small amounts of selenoproteins, we isolated primary hepatocytes from *Alb-Cre; Secisbp2$^{fl/fl}$* mice and litter mate controls thus removing background signals derived from endothelium or Kupffer

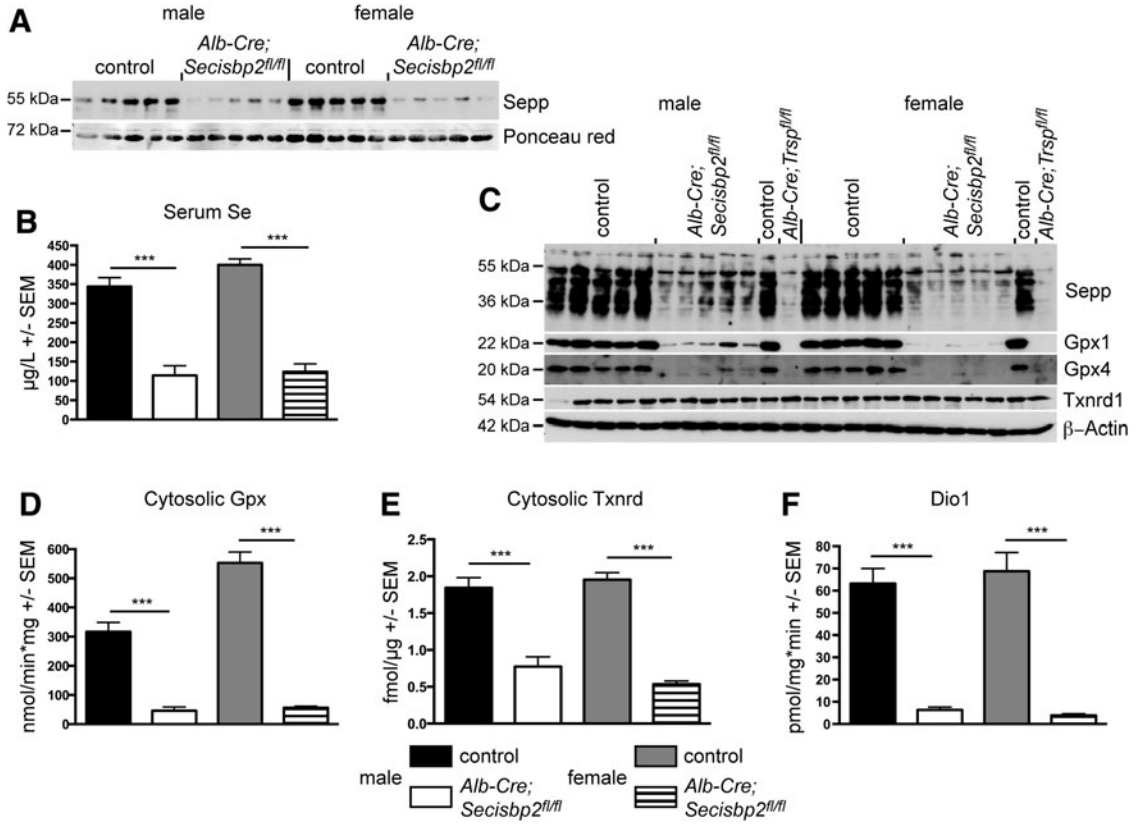

FIG. 5. Impact of *Secisbp2* inactivation on hepatic selenoprotein expression. (A) Sepp expression in serum was significantly decreased in *Alb-Cre; Secisbp2$^{fl/fl}$* mice compared with controls as assessed by western blotting. **(B)** The Se content in serum was reduced in mutated mice. **(C)** Western blot analysis of selenoprotein expression in liver. Significant Sepp remained in *Alb-Cre; Secisbp2$^{fl/fl}$* mice compared with the wild-type controls. Bands of different sizes represent the full-length peptide (41 kDa) and increasing glycosylated forms. More Sepp protein of all sizes was detected in *Alb-Cre; Secisbp2$^{fl/fl}$* than in *Alb-Cre; Trsp$^{fl/fl}$* liver. For Gpx1 and Gpx4, small amounts of residual protein could be detected in the mutant mice. One hundred micrograms of protein from individual mice was applied per lane. **(D)** Activity of cytosolic Gpx was significantly decreased in *Alb-Cre; Secisbp2$^{fl/fl}$* mice (by 85% and 90% in males and females, respectively). **(E)** Cytosolic Txnrd1 activity was significantly reduced, although the protein level was not reduced. **(F)** Dio1 activity was markedly reduced. For Se content in serum and enzyme activity assays, five to six animals per group were analyzed (***$p < 0.001$ Student's *t*-test).

Table 2. mRNA Features of Selenoprotein Classes Differentially Affected by *Secisbp2* Mutations

mRNA response to hepatic Secisbp2 *deficiency*	*Gene*	*Type of SECIS*	*Follows rules for NMD(UGA > 50 nt upstream of exon/exon junction)*	*mRNA response to hepatic* Trsp *deficiency in males*[a]
Greatly reduced in males and females	*Dio1*	1	Yes	Reduced
	Gpx1	1	Yes	Reduced
	Sepp	1 and 2	No (distance is 26 nt)	Reduced
	Sepw	2	No (distance is 15 nt)	Reduced
	Seph	2	Yes	Not tested
	Sps2	2	No (only one exon)	No change
Modestly reduced in males only	*Gpx4*	2	Yes	No change
	Sept	2	Yes	Reduced
	Sepr	1	No (distance is 34 nt)	No change
	Sepn	1	Yes	Not tested
	15 kDa	2	No (distance is 28 nt)	No change
	Sepm	2	No (distance is 21 nt)	Not tested
Modestly reduced in males and females	*Seps*	2	No (UGA in last exon)	No change
Not reduced in males and females	*Txnrd1*	2	No (UGA in last exon)	No change
	Sepi	2	No (UGA in last exon)	Not tested
	Sepk	2	No (distance is 5 nt)	Reduced
	Sepo	2	No (UGA in last exon)	Not tested

[a]Based on (11) and (12) in which only male mice were analyzed.
NMD, nonsense-mediated decay; nt, nucleotides.

cells. In parallel, we isolated hepatocytes from *Alb-Cre; Trsp*$^{fl/fl}$ mice. Comparing *Secisbp2*-deficient hepatocytes with *Trsp*-deficient cells, in the same analyses, allows one to separate effects simply related to impaired selenoprotein mRNA translation from effects specific for *Secisbp2* inactivation. Messenger RNA levels for *Secisbp2* were only reduced in *Alb-Cre; Secisbp2*$^{fl/fl}$ cells, but not in *Trsp*-deficient hepatocytes, consistent with the premature termination codon in exon 6 of the *Secisbp2* mutant (Fig. 6A). *Gpx1* mRNA levels were almost undetectable in both *Secisbp2* and *Trsp* mutant hepatocytes, consistent with its established sensitivity to NMD (Fig. 6B). *Gpx4* was reduced in both mutants, but more pronounced in the *Secisbp2* mutant (Fig. 6C). *Txnrd1* was not affected by either gene deletion (Fig. 6D). The hepatocyte-specific mRNA for *Sepp* was reduced in both mutants, but again more severely affected in *Secisbp2*-deleted cells (Fig. 6E). As in whole liver extracts, we observed Sepp biosynthesis in *Secisbp2*-deleted cells at a low level, this time however, definitely derived from hepatocytes (Fig. 6G). At close inspection, the Sepp species with the highest molecular weight are not visible in *Trsp* mutant cells, but it is expressed in *Secisbp2* mutant cells (arrow). Low residual expression of Gpx4 was not observed, possibly because of its much lower expression level than Sepp. A remarkable finding was the detection of low Dio1 activity in *Secisbp2*-deleted cells, but none in *Trsp* mutant cells (Fig. 6H). This was in stark contrast to higher *Dio1* mRNA in *Trsp* mutants than in *Secisbp2* mutants (Fig. 6F). This circumstantial evidence suggests that a very low level of translation of at least some selenoprotein mRNAs may occur in the absence of Secisbp2.

Expression of other known SECIS-binding proteins

Given the possible persistence of an inefficient mechanism of selenoprotein expression in hepatocytes, we speculated that one of the other SECIS-binding proteins may potentially compensate for the loss of Secisbp2 in hepatocytes. Alternatively, their expression levels may change when *Secisbp2* or selenoprotein transcript levels are reduced. We therefore investigated whether *Secisbp2* gene targeting changed mRNA expression of any of the other SECIS-binding proteins. Based on qPCR, neither *nucleolin* (Fig. 7A), *Ybx1* (Fig. 7B), *Rpl30* (Fig. 7C), or *Secisbp2l* (Fig. 7D) mRNAs were significantly changed in the livers of male *Alb-Cre; Secisbp2*$^{fl/fl}$ mice, although a significant increase in *nucleolin* mRNA was noted in female mutants (Fig. 7A). The transcript encoding the inhibitory SECIS-binding protein, *eIF4a3*, which represses *Gpx*1 mRNA translation in Se deficiency (9), did not change (Fig. 7E). Based on western blotting, the protein levels of L30, nucleolin, and eIF4a3 were not significantly different between the control and *Alb-Cre; Secisbp2*$^{fl/fl}$ female mice (Fig. 7F; Supplementary Fig. S2).

Induction of Nrf2 target genes in livers of Secisbp2 mutant mice

In *Alb-Cre; Trsp*$^{fl/fl}$ mice, which are globally defective in hepatic selenoprotein expression, nuclear factor, erythroid-derived, like 2 (Nrf2)-dependent gene expression was induced in response to the lack of antioxidant selenoproteins (46). We therefore tested mRNA expression of Nrf2-dependent genes, *Nqo1, Cd36, Hmox1, Gstm1, Gstm3, Gsta1, and Gsta4.* As expected, we found several-fold induction of most tested genes in male and female mutant mice (Fig. 8A). Interestingly, female *Alb-Cre; Secisbp2*$^{fl/fl}$ mice showed higher induction in more genes than males. Staining for proteins modified by the Nrf2-inducing mediator 4-hydroxynonenal (4-HNE) by western blotting did not reveal increased signals in the *Secisbp2* mutant liver suggesting efficient detoxification by Nrf2 pathway genes (Fig. 8B).

Discussion

Previous studies *in vitro* and in cell culture suggested that Secisbp2 is essential for translation of selenoprotein mRNAs

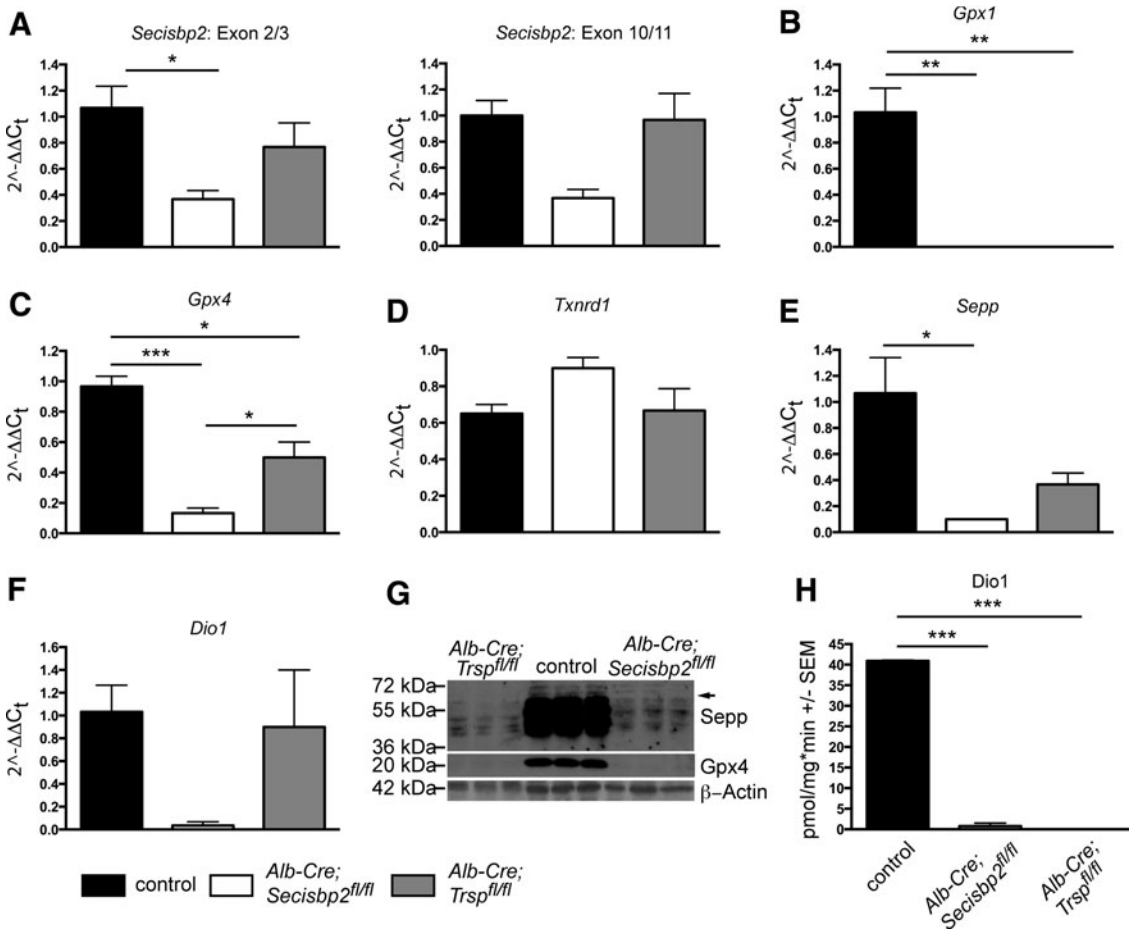

FIG. 6. Selenoprotein expression in primary hepatocytes. **(A)** *Secisbp2* mRNA levels were reduced in *Alb-Cre; Secisbp2*$^{fl/fl}$ hepatocytes, but not in *Alb-Cre; Trsp*$^{fl/fl}$ cells. **(B)** *Gpx1* mRNA was completely lost in both mutant cell types. **(C)** *Gpx4* mRNA levels were more strongly affected in *Alb-Cre; Secisbp2*$^{fl/fl}$ than in *Alb-Cre; Trsp*$^{fl/fl}$ hepatocytes. **(D)** *Txnrd1* mRNA was not changed. **(E)** *Sepp* mRNA was more affected in *Secisbp2*-deleted hepatocytes than in *Trsp*-deleted cells. **(F)** Only a small amount of *Dio1* mRNA was detected in *Alb-Cre; Secisbp2*$^{fl/fl}$ hepatocytes, whereas *Dio1* mRNA was not significantly reduced in *Alb-Cre; Trsp*$^{fl/fl}$. **(G)** Western blot analysis of selenoprotein expression. Sepp was present in several forms representing the full-length peptide (41 kDa), partially glycosylated and the fully glycosylated form (55 kDa, *arrow*). **(H)** *Secisbp2* targeting reduced Dio1 activity in primary hepatocytes more than 90%, but targeting of *Trsp* was even more effective despite higher mRNA levels ($*p<0.05$; $**p<0.01$; $***p<0.001$; one-way ANOVA with the Bonferroni's *post hoc* test).

(17). Our novel mouse mutants now permit us to investigate Secisbp2 function in embryonic development and in any specific cell type *in vivo* at any chosen developmental time point, provided a suitable *Cre* driver is chosen. In summary, our data support that *Secisbp2* is required for efficient selenoprotein expression and that it may play a role both in mRNA translation and mRNA stability. Comparison with mice lacking tRNA$^{[Ser]Sec}$ revealed residual selenoprotein expression and a less severe phenotype in *Secisbp2*-deficient mice, suggesting the existence of a compensatory or Secisbp2-independent molecular mechanism, which needs to be elucidated in future studies.

We chose hepatocytes as a model system, because, unlike mouse embryonic fibroblasts (28, 45), these cells survive abrogation of selenoprotein biosynthesis (43). A reason for this may be efficient transcriptional induction of Nrf2-responsive antioxidant proteins like glutathione-S-transferases (GST) (46). Hepatocytes allowed us to investigate a large number of selenoproteins at the level of protein expression or enzyme activity. We further took advantage of a conditional *Trsp* allele and compared the effects of *Secisbp2* inactivation with tRNA$^{[Ser]Sec}$ inactivation. Throughout, we observed a low level of selenoprotein expression remaining after *Secisbp2* inactivation. Similarly, quantification of Nrf2-dependent *Gst* mRNAs revealed significant induction of these genes in the *Secisbp2* mutant liver, but much lower than in mice harboring a conditional deletion of *Trsp* (46). For example, we found a 1.3-fold induction (normalized to *18S*-rRNA) of *Gstm1* in our male mutants compared with 3.2-fold in *Trsp* mutants [normalized to *Gusb* mRNA in (45)]. Likewise, *Gsta1* is induced 3.2-fold in male *Secisbp2* mutants compared with 23.4-fold in *Trsp* mutants. Except for one male (Fig. 2B, lane 10), incomplete gene inactivation is not a likely reason for the milder effect, since we used the same *Alb-Cre* driver for both *Secisbp2* and *Trsp*. Cre expression starts during embryonic stages, and the animals were analyzed at postnatal day 35 allowing ample time for *Secisbp2* recombination, Secisbp2 protein turnover, and loss of

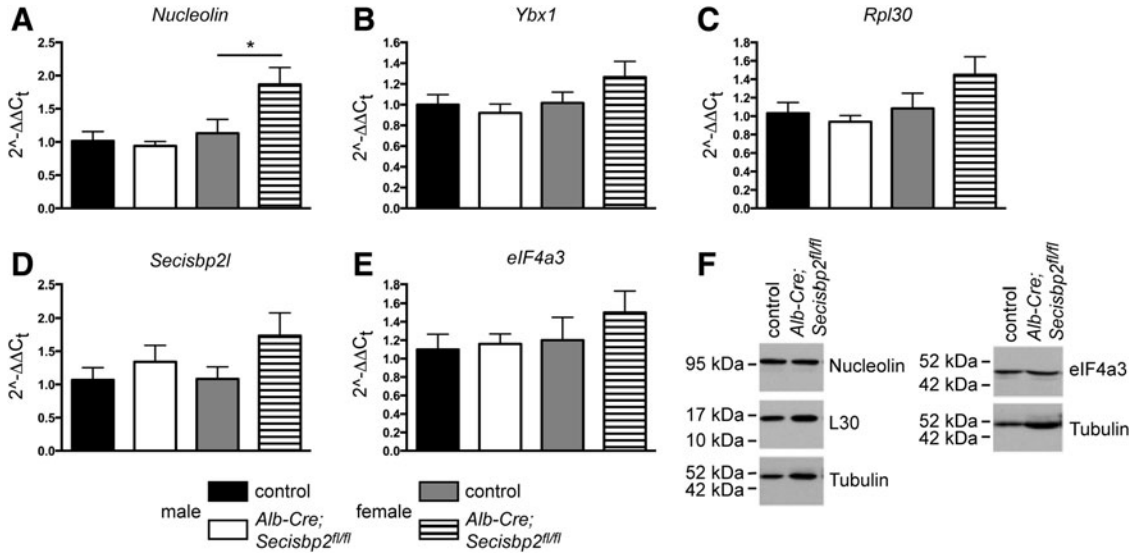

FIG. 7. Analysis of other SECIS-binding proteins. (A–E) Transcript levels of alternative SECIS-binding proteins were analyzed. None was significantly affected in livers of *Alb-Cre; Secisbp2*$^{fl/fl}$ mice compared with the wild-type controls. One exception: in females, *nucleolin* mRNA is slightly increased, five to six animals per group (*$p < 0.05$; Student's *t*-test). **(F)** Representative western blots for L30 (10 μg), nucleolin (10 μg), and eIF4a3 (60 μg per lane). The expression levels of these proteins were not significantly different between control and *Alb-Cre; Secisbp2*$^{fl/fl}$ mice based on two independent analyses ($n = 3$ in each group; Supplementary Fig. S2).

Secisbp2-dependent selenoproteins. Cell types not targeted by *Alb-Cre*-like endothelial cells or Kupffer cells may account for a minor fraction of selenoprotein expression, but should contribute the same background in both *Secisbp2* and *Trsp* mutant liver. Finally, within the liver, Dio1 and Sepp are selenoproteins specific for hepatocytes and residual expression of these proteins thus cannot stem from other cell types. This interpretation is supported by our hepatocyte culture experiments, in which *Secisbp2*- and *Trsp*-deficient cells were analyzed in parallel. Again, both the Dio1 activity and Sepp expression were higher in cells lacking *Secisbp2* than in cells lacking *Trsp*. Compared with *Trsp* mutants, a higher Dio1 activity in *Secisbp2*-deficient hepatocytes was found despite lower *Dio1* mRNA levels. Whereas these considerations are limited by measurements close to the detection limits of the methods, a comparison of embryonic development lends further support to the notion that *Secisbp2* inactivation is less detrimental than *Trsp* inactivation, that is, *Secisbp2*$^{-/-}$ embryos fail before gastrulation (clearly after implantation), while *Trsp*$^{-/-}$ embryos fail before implantation (6). This is consistent with the phenotypes of embryos lacking single essential selenoproteins. *Gpx4*$^{-/-}$ embryos die before E7.5 (27, 45, 59). *Txnrd1*$^{-/-}$ embryos die around E10.5 (5, 28). Developmental failure of *Secisbp2*$^{-/-}$ embryos precedes failure in mice lacking glutathione biosynthesis (*Gclc*$^{-/-}$), which do not develop mesoderm during gastrulation (49).

What could be the mechanism involved in the maintenance of a minor fraction of selenoprotein expression? One possibility is the low level expression of a truncated, hypomorphic Secisbp2 protein that initiates from Met302 in exon 6 after the *Cre*-induced frameshift. It is, however, inherently difficult to exclude the existence of a molecule that remained below the detection limit of our western blot. A second potential mechanism for functional compensation of *Secisbp2* inactivation may be the activity of another SECIS-binding protein. The expression of other SECIS-binding proteins does not change at the protein level, although *nucleolin* mRNA levels were increased in female mutant mice. More importantly, none of the known SECIS-binding proteins have been shown to substitute for Secisbp2 in Sec incorporation, including SECISBP2L (19).

A third possibility is incorporation of another amino acid in place of Sec, ultimately a mechanism of nonsense suppression. Examples of nonsense suppression are already known with regard to selenoproteins. For example, the aminoglycoside G418 can stimulate the incorporation of Arg by a near cognate tRNA into Gpx1 (23). Dietary Se deficiency results in incorporation of Cys at the penultimate UGA codon of Txnrd1 leading to a variant with about 10% activity compared with the Sec-containing enzyme (32, 58). All these mechanisms of nonsense suppression would ultimately lead to full-length, but inactive or minimally active, selenoenzymes. In the case of Txnrd, a significant activity is preserved in male and female *Secisbp2* mutants with 41% and 27% of wild-type activity, respectively. Considering that the amount of *Txnrd1* mRNA is not reduced, a fourth, although hypothetical, possibility arises: a low-efficiency pathway of Sec incorporation that is independent of Secisbp2 analogous to pyrrolysine insertion (51). It will require future studies in carefully designed systems to uncover such a mechanism.

Finally, an intriguing novel finding of our study is the major role that Secisbp2 plays in selenoprotein mRNA abundance. We did not expect such a massive reduction of selenoprotein mRNAs in the absence of Secisbp2. Clearly, it was expected that failure to translate selenoprotein mRNAs may subject these transcripts to NMD, although to date, only the Gpx1 mRNA has been shown to undergo NMD (35). Our experiments revealed that various selenoprotein mRNAs react differentially to the absence of Secisbp2. Moreover, direct

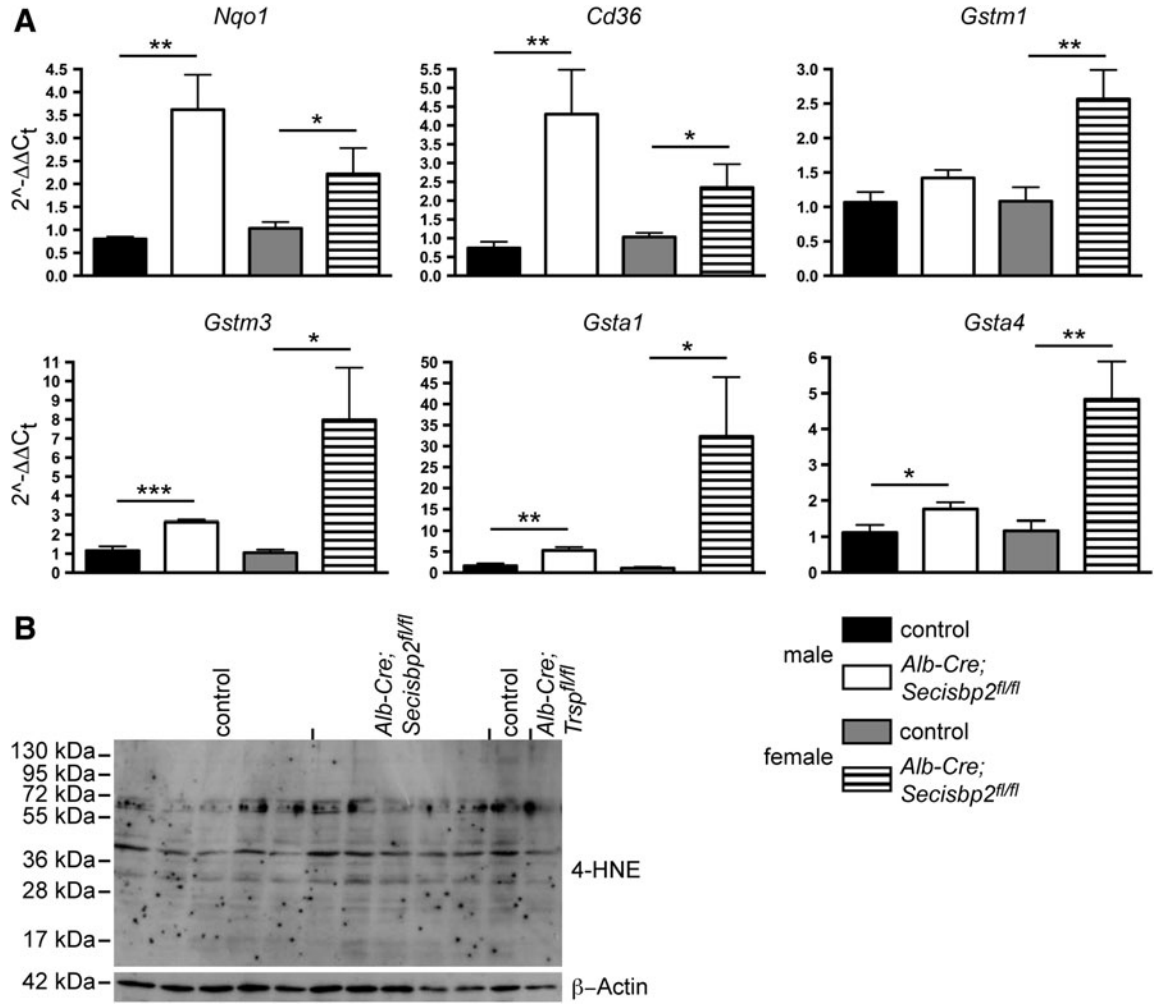

FIG. 8. Induction of Nrf2-dependent gene expression in liver. (A) Transcript levels of several Nrf2 target genes were increased in livers of *Alb-Cre; Secisbp2*$^{fl/fl}$ mice compared with wild-type controls. *18S*-rRNA served as the reference gene. **(B)** Western blot analysis for 4-HNE revealed no differences between *Secisbp2*-depleted livers and controls ($*p<0.05$; $**p<0.01$; $***p<0.001$; Student's *t*-test). 4-HNE, 4-hydroxy nonenal; Nrf2, nuclear factor, erythroid derived, like 2.

comparison with cells deficient for tRNA$^{[Ser]Sec}$ or cells lacking adequate Se demonstrates that lack of translation and subsequent NMD alone cannot account for reduced selenoprotein mRNA levels. Furthermore, several transcripts that showed a decrease in Secisbp2 deficiency do not follow the canonical rules for NMD (Table 2). Interestingly, some mRNAs were more strongly reduced upon *Secisbp2* inactivation than upon *Trsp* inactivation. Whereas not explored in detail here, these findings point to a potential role of Secisbp2 in the stabilization of a subset of selenoprotein mRNAs *in vivo*. A previous study showed that stable knockdown of SECISBP2 in a malignant mesothelioma cell line reduced the levels of a different group of selenoprotein mRNAs (50). The differences between the two studies may be due to the cell type, *in vitro versus in vivo*, or knockdown *versus Secisbp2* inactivation.

Differential binding of selenoprotein mRNAs by Secisbp2 has been implicated in establishing the hierarchy among selenoproteins and is sensitive to mutations in the lysine-rich domain of Secisbp2 (8). Patients carrying the R540Q mutation in *SECISBP2* exhibit a selective deficiency of selenoprotein expression (20). If patients carry more severe mutations, they suffer from a more global selenoprotein deficiency associated with a more severe clinical syndrome (40). Fibroblasts from these patients show changes of mRNA levels in the same way as in *Secisbp2*-deficient hepatocytes (40). Nevertheless, phenotypes of patients with mutations in *SECISBP2* are still weaker than complete *Secisbp2* gene inactivation afforded in our model. This suggests that point mutations observed in patients target specific functions of SECISBP2 or lead to hypomorphic, but not entirely inactive, alleles.

Materials and Methods

Nomenclature

Secisbp2 and SECISBP2 refer to rodent and human proteins, respectively. Names in italics indicate the respective genes.

Mouse models

Transgenic mice harboring the knockout-first allele of *Secisbp2* were obtained from the European Conditional

Mouse Mutagenesis Consortium (EUCOMM) after the gene was prioritized upon our request. The construct encompasses a *FRT*-flanked gene-trap cassette in intron 4 leading to an in-frame protein fusion with exon 4 followed by polyadenylation (Secisbp2$^{tm1a(EUCOMM)Wtsi}$). The gene-trap cassette was removed by intercross with a germline deleter *FLPe* transgenic mouse resulting in a conditional allele with exon 5 flanked by *loxP* elements (*Secisbp2*^{fl}). Deletion of conditional exon 5 in the germline using a deleter *Cre* transgenic mouse strain resulted in a *Secisbp2*$^{\Delta 5}$ allele, a functional knockout. The breeding colony was maintained on breeding diets (Ssniff, Soest, Germany) containing on average 0.2–0.3 ppm Se. Upon weaning, animals that were to be examined by western blotting and used for serum Se analysis were maintained on a low Se diet (Altromin, Lage, Germany; diet C1045 containing 0.08 ppm Se) supplemented with selenite to an adequate Se concentration (0.15 ppm Se, a dose defined as the recommended dietary allowance for mice, designated RDA) as in earlier experiments (36). The Se content of the diets used in the experiments were determined by total reflection X-ray fluorescence (TXRF) analysis that verified the intended Se content. Animal experiments were approved by the local governmental authorities (Landesamt für Gesundheit und Soziales, LAGeSo Berlin, Germany). Genotyping was done by PCR using primers Secisbp2-wt(fwd): GGTTCTGAGTTCCACTTAAAG, Secisbp2-rev2: GGTAT GCAAGGGCCACCTTTG and Secisbp2-loxP(rev): GGTA TGCAAGGGCCACCTTTG. *Alb-Cre* and *Trsp* genotyping was done as described (43). For *Secisbp2*$^{\Delta 5}$ genotyping, primers Secisbp2-rev2 (described above) and Secisbp2-fwd1: TCTGCTTCTGCCTCCTAAATG were used.

Primary hepatocytes

Primary hepatocytes were isolated as described (55) from female mice. Forty-eight hours after isolation, cells were harvested for analysis.

Hormone measurements

TSH and total serum T_4 and T_3 were measured by radioimmunoassays adapted to mice as described before (7).

Selenoenzyme analysis

Gpx assays were carried out with *tert*-butyl hydroperoxide and Txnrd activities were assessed with the DTNB assay in tissue homogenates as described (41). The protein concentration was determined by the Bradford method using IgG as a standard. The Dio1 activity was determined as described with ^{125}I-rT_3 as the substrate (52). Fifteen micrograms of membrane fraction protein was used, with 1 μM unlabeled rT_3. The reaction time was 60 min and the reaction temperature 37°C. Five to six male and female animals were analyzed for each genotype. The assay was done in triplicate.

Insulin-dependent Txnrd assay

Since DTNB may be reduced by GSTs (which were found increased in the livers of *Secisbp2*-deficient mice), the insulin-dependent fluorescent Txnrd assay from IMCO (Stockholm, Sweden) was also used to determine Txnrd activities in 35 μg of protein from liver cytosols (26). We followed the manufacturer's recommendations with one minor modification, that is, the Txnrd standards were adjusted to match the range of our samples (in nM): 0.125, 0.25, 0.375, 0.50, 0.625 and results were given in fmol Txnrd per μg cytosolic protein.

Liver transaminase determination

ALAT and ASAT measurements in serum were performed according to standard coupled tests involving 2-ketoglutarate and NADH plus alanine and lactate dehydrogenase (for ALAT) or aspartate and malate dehydrogenase (for ASAT). The decrease in A_{340} was followed over 3 min and from the slope of the linear part of the curve, the activity was calculated as 1 U = 1 μmol/min. The number of animals tested: male controls (6), female controls (9), male mutants (6), and female mutants (9).

Western blot analysis

An antiserum directed against the C-terminal 349 amino acids of mouse Secisbp2 was generated by the Protein Tech Group, Chicago, IL (used at 1:1000). For Gpx1 (1:10,000; rabbit antiserum from Abcam, Cambridge, United Kingdom), 25–100 μg protein from cytosolic fraction was separated on SDS/12% polyacrylamide gels. For Gpx4 (1:10,000; rabbit antiserum against amino acids 32–43 of rat protein made by Biosynthesis, Lewisville), 40 μg of whole testis homogenate and 25 or 100 μg of liver homogenate was used. The antiserum directed toward Sepp (1:400) has been generated in rabbits by immunization with a synthetic C-terminal peptide (ImmunoGlobe, Himmelstadt, Germany), and its specificity was verified using wild-type and *Sepp*-knockout mice (15, 43). For serum Sepp quantification, 0.2 μl serum was applied per lane. For Txnrd1 (1:1000; mouse monoclonal antibody; Abcam), 100 μg of protein from cytosolic fraction was used for western blot analysis. Antibodies against Sepk, Seps, eIF4a3, and L30 were from Sigma (Munich, Germany; ATLAS Prestige antibodies, rabbit polyclonal) used at 1:1000, 1:20,000, 1:1000, and 1:5000 dilution, respectively. After electrotransfer, nitrocellulose membranes were stained with Ponceau Red, photographed, and blocked with 5% BSA for 1 h at 25°C. As loading controls, the following antibodies were used: rabbit polyclonal β-actin antiserum (Sigma) at 1:2000 dilution, mouse monoclonal against beta I and II tubulin (Abcam) at 1:100,000, or mouse monoclonal 6C5 against GAPDH (Abcam) at 1:300,000.

Selenium measurements

Se was determined by TXRF using a Picofox™ S2 instrument (Bruker nano, Berlin, Germany) (15). Gallium was used as the internal standard for quantification and reference samples for serum (Sero, Billingstad, Norway) were included in all analyses and results were always within the reference range. All samples were measured in duplicate.

qPCR analysis

Total RNA was isolated from powdered mouse tissue according to the TRIzol protocol (Invitrogen, Darmstadt, Germany). Samples were treated with RQ1 RNase-Free DNase (Promega, Madison, WI). Total RNA from primary hepatocytes was isolated with the Aurum Total RNA Minikit (Biorad, München, Germany). cDNA was synthesized using the iScript cDNA synthesis kit (Biorad) according to the

manufacturer's protocol. qPCR was performed using SYBR Green from Abgene (Thermo Scientific, Epsom, United Kingdom) on an iCycler (Biorad). Primers used for qPCR detection of Nrf2 target genes were as described previously (46). Primers specific for selenoproteins and Nrf2 target genes are described in Supplementary Table S1. *18S* rRNA was used as reference gene for mRNA quantification.

Statistics

For all computations, GraphPad Prism software was used for the tests indicated in the figure legends. Data are expressed as mean ± S.E.M. Statistical significance was determined and indicated as $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$.

Acknowledgments

The authors thank Antje Kretschmer, Anja Fischbach, Vartitér Seher, and Ursula Reuter for excellent technical assistance. Dr. Markus Schupp, Charité-Universitätsmedizin Berlin helped with primary hepatocyte cultures, Dr. Carmen Birchmeier provided *FLPe* and *Cre* deleter mice, Dr. Wolfgang Wurst prioritized *Secisbp2* in EUCOMM. The study was funded by Deutsche Forschungsgemeinschaft DFG Schw914/2-1 and GK1208 to the U.S. and National Institutes of Health grants R01DK085391 and R01DK07859 to D.M.D.

Author Disclosure Statement

All authors stated that no competing financial interests exist.

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Date of first submission to ARS Central, April 9, 2013; date of final revised submission, November 15, 2013; date of acceptance, November 25, 2013.

Abbreviations Used

4-HNE = 4-hydroxynonenal
Alb-Cre = phage P1 Cre recombinase expressed from murine albumin promoter in transgenic mice
Dio = deiodinase
DTNB = 5,5′-dithiobis(2-nitrobenzoic acid)
Floxed = flanked by *loxP* elements (from phage P1)
FLPe = saccharomyces cerevisae FLP recombinase (enhanced) transgenic mice
Gpx = glutathione peroxidase
GST = glutathione-S-transferase
NMD = nonsense-mediated decay
Nrf2 = nuclear factor, erythroid derived, like 2
Sec = selenocysteine
SECIS = selenocysteine insertion sequence
$Secisbp2^{\Delta 5}$ = exon 5-deleted *Secisbp2* allele
Secisbp2 = SECIS-binding protein 2 (murine)
$Secisbp2^{fl}$ = conditional (exon 5 floxed) *Secisbp2* allele
Sepp = selenoprotein P
Trsp = $tRNA^{[Ser]Sec}$ gene symbol (murine)
TSH = thyroid-stimulating hormone
Txnrd = thioredoxin reductase
TXRF = total reflection X-ray fluorescence