Selenium Regulation of the Selenoprotein and Nonselenoprotein Transcriptomes in Rodents1,2

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ABSTRACT

This review discusses progress in understanding the hierarchy of selenoprotein expression at the transcriptome level from selenium (Se) deficiency to Se toxicity. Microarray studies of the full selenoproteome have found that 5 of 24 rodent selenoprotein mRNA decrease to <40% of Se adequate levels in Se deficient liver but that the majority of selenoprotein mRNA are not regulated by Se deficiency. These differences match with the hierarchy of selenoprotein expression, helping to explain these differences and also showing that selenoprotein transcripts can be used as molecular biomarkers for assessing Se status. The similarity of the response curves for regulated selenoproteins suggests one underlying mechanism is responsible for the downregulation of selenoprotein mRNA in Se deficiency, but the heterogeneity of the UGA position in regulated and nonregulated selenoprotein transcripts now indicates that current nonsense mediated decay models cannot explain which transcripts are susceptible to mRNA decay. Microarray studies on the full liver transcriptome in rats found only <10 transcripts/treatment were significantly down- or upregulated by Se deficiency or by supernutritional Se up to 2.0 μg Se/g diet (20X requirement), suggesting that cancer prevention associated with supernutritional Se may not be mediated by transcriptional changes. Toxic dietary Se at 50 μg Se/g diet, however, significantly altered ~4% of the transcriptome, suggesting number of transcriptional changes itself as a biomarker of Se toxicity. Finally, panels of Se regulated selenoprotein plus nonselenoprotein transcripts predict Se status from deficient to toxic better than conventional biomarkers, illustrating potential roles for molecular biomarkers in nutrition.

Introduction

Selenium status and selenoprotein expression. The enzyme glutathione peroxidase (Gpx)3 in 1972 was the first discovered biochemical role for the essential nutrient, selenium (Se) (1). The activity of this enzyme decreases dramatically in Se deficiency and increases during Se repletion, thus making Gpx1 a useful biochemical biomarker for Se status (2). This was followed by the identification of selenocysteine (Sec) as the Se cofactor present and incorporated into the peptide backbone of these selenoproteins (3,4). Over the next 30 yr, these discoveries were followed grudgingly by the identification of 17 additional selenoproteins in higher animals, including plasma Selenoprotein P (Sepp1) containing 10 Sec, all 3 mammalian deiodinases (Dio), all 3 mammalian thioredoxin reductases (Txnrd), and 3 additional Gpx with unique tissue distribution or substrate utilization (5,6).

Unique Se biochemistry and molecular biology. Characterization of this growing superfamily of selenoproteins revealed that the Sec in selenoproteins is encoded by a nonsense codon, UGA (7), that a unique Sec specific tRNA<Sup>Sec</Sup> is used to recognize Sec encoding UGA, that a unique 3′-untranslated region (UTR) stemloop structure called a selenocysteine insertion sequence (SECIS) element is required to reinterpret UGA for Sec insertion rather than termination of translation (8,9), and that a series of unique gene products specific for Se biology are required for synthesis of an activated form of Se and conversion of serine attached to the tRNA<Sup>Ser</Sup> -> Sec to Sec (5,10). Via this cellular machinery, Se in the form of Sec is incorporated cotranslationally into the growing polypeptide chain of selenoproteins at the position specified by the UGA codon. Details of these discoveries and processes are described in detail in an accompanying article (11).

Human supernutritional Se supplementation. The current RDA for Se is 55 μg Se/d for adult men and women...
and the tolerable upper intake level is 400 μg Se/d for adults (12). In addition to prevention of Se deficiency, higher intakes of Se are associated with prevention of cancer and other diseases (5,13,14), and large human trials have been conducted to confirm and further extend these associations. And yet the issue of optimum Se intake is now further complicated, because a number of recent original studies and meta analyses indicate that Se supplementation may have adverse effects on human health (15–19); this includes the SELECT trial, with over 35,000 participants, which was stopped after 5 y in 2008 because Se and vitamin E supplements were not preventing prostate cancer and because there were suggestions of adverse effects due to Se or vitamin E supplementation, including a suggestion that there was a higher incidence of diabetes in participants ingesting Se (200 μg/d) (20). Similarly, analysis of the 12-y data from the Nutritional Prevention of Cancer Trial full study also found a significantly higher diabetes incidence (HR 1.55) in Se supplemented participants (200 μg/d of Se as selenized yeast) compared with placebo participants and a significant HR of 2.7 for selenium supplementation in participants in the highest tertile of baseline plasma Se level (19). Lastly, over-the-counter formulation and misformulation is a real issue for the American public (21–23). Thus, biomarkers that could accurately assess high-Se status associated with supranutritional Se intakes, especially under all conditions, have the potential to better prescribe appropriate levels of beneficial Se intake, to better protect the public against Se toxicity, and to be a useful tool in clinical studies assessing supranutritional effects of Se supplementation.

Biomarkers. The studies described below were conducted in part to take advantage of nutrient regulation of the transcriptome to develop molecular biomarkers for assessment of nutrient status. A molecular biomarker can be defined as an mRNA transcript that indicates the (nutrient) status of an organism or tissue, as distinguished from biochemical biomarkers, such as enzyme activity, or chemical biomarkers, such as the concentration of an element, vitamin, or metabolite (24). These biomarkers offer early and more accurate prediction and diagnosis of disease and disease progression and the ability to identify individuals at risk. The use of microarrays also offers the opportunity to identify orthogonal (uncorrelated) biomarkers not known to be linked with conventional biomarkers. This is especially the case for assessing high-Se status, because conventional chemical and biochemical biomarkers for Se status fail to predict Se status under all conditions (24).

Selenoprotein hierarchy. Long before most of these selenoproteins were identified, Behne (25) recognized a “hierarchy” in the distribution of 75Se between tissues and furthermore recognized that within a tissue there is a “molecular hierarchy” such that Gpx1 is less labeled than other selenoproteins in Se deficiency. Hill and Burk (26) reported that Sepp1 and Dio1 mRNA levels decrease less than Gpx1 mRNA levels in Se deficiency, suggesting that this regulation might underlie this hierarchy. With the cloning of Gpx4, we reported that Gpx4 activity in Se deficient rat liver only falls to ~40% of Se adequate levels, whereas Gpx1 activity falls to 1% of Se adequate levels, that liver Gpx4 mRNA is not downregulated by Se deficiency whereas Gpx1 mRNA falls to ~10% of Se adequate levels and suggested that there are detailed underlying molecular mechanisms resulting in this differential regulation of selenoprotein expression (27). This review will discuss our progress in understanding the hierarchy of selenoprotein expression and nonselenoprotein expression at the transcriptome level across the spectrum from Se deficiency to Se toxicity.

Selenoproteome and Se requirements

Biochemical biomarkers of Se status. When weaning rats are fed a Se deficient diet (0.005 μg Se/g), liver Se concentrations fall to <5% of levels in Se adequate (0.24 μg Se/g diet) rats (Fig. 1A). Se supplementation results in a sigmoidal response in liver Se concentration, with a plateau breakpoint at 0.08 μg Se/g diet (Table 1), and a plateau in liver Se extending from 0.08 to 0.24 μg Se/g diet. With supranutritional Se supplementation at 0.4 and 0.8 μg Se/g diet, however, liver Se levels increase above the plateau and are 70% higher at 0.8 μg Se/g than in rats fed 0.08–0.24 μg Se/g. Liver Gpx1 activity in Se deficient rat liver falls similarly to 2% of Se adequate levels. With graded Se supplementation, liver Gpx1 activity rises sigmoidally to a defined plateau with a breakpoint at ~0.09 μg Se/g diet (Fig. 1B). These studies and many preceding studies (28–34) have established a Se requirement of 0.1 μg Se/g diet (1X requirement) as the minimum dietary Se necessary to achieve plateau levels of Gpx1 activity in rodent liver. In contrast to liver Se, supranutritional Se supplementation above this requirement does not further elevate liver Gpx1 activity. Similar Se response curves are also found for plasma Gpx3 activity (30), liver Txnrd activity (Fig. 1D) (31), Sepp1 levels (32), Dio activity (33), and selenoprotein W (Sewp1) levels (34), indicating that the minimum dietary Se requirement is 0.1 μg Se/g diet. Unlike the preceding selenoproteins, however, liver Gpx4 activity (Fig. 1C) only decreases in Se deficiency to ~25–40% of Se adequate levels and reaches the plateau at ~0.05 μg Se/g diet (27). Collectively, the regulation of these traditional biomarkers by Se status has provided excellent tools for assessing Se status and requirements over the range from Se deficient to Se adequate.

We reported in 1988 (35) that Gpx1 mRNA levels also drop dramatically in Se deficiency in rats, increase sigmoidally with increasing dietary Se, and reach well defined plateaus (27–29,36), providing a molecular biology based biomarker for Se status. Dietary Se requirements based on hepatic Gpx1 mRNA levels are ~0.05 μg Se/g diet for both male and female rats, even though female rats have twice the level of liver Gpx1 mRNA as well as Gpx1 activity compared with male rats (28). In contrast, Gpx4 mRNA levels (27) and Sepp1 mRNA levels (28,29) are not regulated by Se status in rats, blocking use of Gpx4 and Sepp1 mRNA as molecular biomarkers for assessing Se requirements.
Effects of Se status on mRNA levels for several other seleno-proteins have also been reported, including Sepp1, Trxrd1, and Dio1 (27–29,31,36,37), but these mRNA do not fall as dramatically in liver and other tissues as does Gpx1 mRNA. Requirements based on these molecular biomarkers are reported to be near 0.05 μg Se/g diet and generally less than levels required for maximal enzyme activities (36).

The selenoproteome. The complete sequencing of numerous genomes led Gladyshev et al. (38) to develop elegant computational methods, based on the in-frame UGA codon and SECIS element required for Se incorporation into all selenoproteins, that were used to identify the complete selenoprotein proteome or selenoproteome in humans, rodents, and other species. They found that the complete human selenoproteome consists of 25 selenoproteins, including 7 newly discovered selenoproteins, and that the complete rodent selenoproteome consists of 24 selenoproteins (Gpx6 is a Sec containing selenoprotein in humans and swine but a cysteine containing protein in rodents). These discoveries thus provided us with the blueprint to

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1 Table adapted with permission from (40) and (41). Detailed plots and ANOVA are provided for some biomarkers in Figures 1, 2.

2 Minimum dietary Se requirement for the growing rat as determined for each indicated biomarker. Requirements are the minimum dietary Se necessary for the indicated parameter to reach plateau levels when Se adequate weanling rats are fed these diets from weaning, as determined by breakpoint analysis. Shown are all conventional biomarkers assessed in these studies and all molecular biomarkers that were significantly (p < 0.05) regulated by Se status.

3 Kidney Sephs2 mRNA was significantly upregulated by Se deficiency.
investigate Se regulation of the full selenoproteome with the hypothesis that we would uncover additional selenoprotein mRNA that are highly regulated by Se status, thus finding new potential molecular biomarkers for Se status and perhaps leading to a better understanding of the underlying mechanism for Se regulation of mRNA stability (39).

**Dietary forms of Se.** The following studies were conducted using selenite (Na₂SeO₃) as the supplementary form of Se. Both inorganic Se (selenite, selenate) and organic [selenomethionine (SeMet), selenized yeast] forms are highly bioavailable, but there are distinct differences between inorganic Se and organic Se [reviewed in (6)]. SeMet cannot be synthesized from inorganic Se in higher animals, but dietary SeMet mixes with the methionine pool and is nonspecifically incorporated into all proteins where it has no Se specific role until the protein and the SeMet are degraded to inorganic Se, which then can be used for synthesis of Sec and incorporation into selenoproteins. Thus, in these rodent studies, provision of Se as selenite eliminates SeMet metabolism and keeps the Se in the inorganic Se pool (6).

**Se regulation of selenoprotein transcriptome.** To evaluate the complete selenoproteome for Se regulation of mRNA levels, we recently determined the selenoprotein transcript levels in total RNA from liver and kidney of Se deficient, Se marginal, and Se adequate mice (0.00, 0.05, and 0.2 mg Se/g diet, respectively) using microarray analysis and quantitative real-time PCR analysis (39). Analysis using the Affymetrix Mouse Genome 430 2.0 array identified 17 liver selenoprotein mRNA as "present," including 9 that were significantly downregulated in Se deficient liver (Gpx1, Selh, Sepw1, Txnrd1, Txnrd2, Selk, Dio1, Selt, and Sep15). Follow-up quantitative real-time PCR showed that Gpx1 mRNA levels in Se deficient liver decreased to 26% of Se adequate levels (0.20 mg Se/g) but also found that Sepw1 and Selh mRNA levels in Se deficient liver decreased to 26 and 33%, respectively, of levels in Se adequate liver, thus identifying 2 potential additional molecular biomarkers for assessment of Se status. In mice with marginal Se status (0.05 mg Se/g diet), however, none of the 9 Se regulated transcripts in liver remain significantly decreased relative to levels in Se adequate mice, suggesting that this regulation is mediated by one underlying mechanism (39).

We next conducted follow-up quantitative real-time PCR assessment of Se regulation of mRNA levels in weanling rats fed 10 graded levels of dietary Se from 0 to 0.8 μg Se/g diet for 28 d (40,41). Initial screening for all 24 rodent selenoprotein mRNA for potential regulation by Se status found that the majority of selenoprotein transcripts are not regulated by Se status in liver, kidney, muscle, or testes. Detailed quantitative real-time PCR analysis of the regulated selenoprotein mRNA found that Gpx1, Sepw1, and Selh mRNA levels (Fig. 2A–C) in Se deficiency are reduced to 10, 16, and 19%, respectively, of Se adequate levels in liver, reach plateau breakpoints at 0.07, 0.07, and 0.06 μg Se/g diet, respectively, and are not further increased by supernutritional Se supplementation (up to 8× requirement). In addition, liver Gpx3 and Selk mRNA are decreased to 20–40% of Se adequate levels, and Dio1, Sepn1, Sepp1, Selt, Sep15, and Txnrd3 mRNA levels are decreased significantly to 40–70% of Se adequate levels. In contrast, however, the majority of detected selenoprotein transcripts in liver, including Gpx4 mRNA (Fig. 2D), are not significantly affected by Se status across the full range from Se deficient to 0.8 μg Se/g, and similar patterns are observed for kidney and muscle, although the extent of Se regulation is further dampened in these tissues (40).

**Minimum dietary Se requirement hierarchy.** In this study, we used response curve breakpoints of the molecular
biology biomarkers to estimate minimum dietary requirements (40). This results in a hierarchy of requirements (Table 1) that range, where breakpoints are found, from 0.06 to 0.13 μg Se/g diet for conventional biomarkers and from 0.02 to 0.07 μg Se/g diet for molecular biomarkers depending on biomarker and tissue examined. Thus, requirements based on the molecular biology biomarkers are generally slightly lower than the requirements based on biochemical markers and slightly higher than the earlier reported requirements of 0.04 and 0.05 μg Se/g diet based on prevention of disease or maintenance of growth (42,43). Unlike the early studies, the Se requirement today for growth is <0.01 μg Se/g diet using pups from Se adequate dams with diets supplemented with vitamin E and sulfur amino acids. Notably, the present study did not identify any biomarkers with response curves that plateaued only at super-nutritional levels of dietary Se. The current NRC requirement of 0.15 μg Se/g diet (44) is well above the minimum requirements based on the molecular biomarkers, well above the usual biochemical biomarkers, and much higher than the minimum requirements based on growth and prevention of disease (42,43).

The data in Table 1 also nicely illustrate the tissue hierarchy and molecular hierarchy first observed by Behne (25). The minimum requirements listed in Table 1, all determined in the same animals, illustrate the molecular hierarchy such that in the same tissue with increasing dietary Se, Gpx1 is generally the last selenoprotein to reach plateau levels and this is accompanied by parallel mRNA response curves such that no selenoprotein mRNA requirement is higher than the Gpx1 mRNA requirement in this tissue. The tissue hierarchy is similarly observed at the mRNA level as the selenoprotein transcript based requirements in liver and kidney are grouped from 0.04 to 0.07 μg Se/g diet, whereas these mRNA based requirements in muscle are slightly lower, ranging between 0.03 and 0.05. Higher breakpoints of up to 0.13 μg Se/g diet were observed in this young, rapidly growing rat model based on kidney and muscle Gpx1 activity; the basis for these higher breakpoints is unclear but may be related to a role of Gpx1 as a Se store (45) such that these storage pools are not fully saturated in the young developing rodent. Studies with adult rats now indicate that the dietary Se requirement decreases in mature rodents relative to young rodents (36), suggesting that kidney and muscle Gpx1 activity should not be the basis for a higher Se requirement but rather illustrate the targeted transport via Sepp1 and Sepp1 receptors (46–48) to selected tissues such as testes. Thus, the requirement for testes Gpx1 and Gpx4 activities are 0.04 and <0.01 μg Se/g diet (Table 1) (41); requirements for selenoprotein mRNA levels in testes are lower than other tissues for Sepw1, similar for Sepp1, and higher than other tissues for Gpx1, apparently reflecting the targeted delivery of Se to testes and the heterogeneous transport of Se within the testes (41).

Species differences. Minimum Se requirements appear to be remarkably similar across species (49), presumably reflecting common underlying molecular mechanisms. This statement differentiates between minimum Se requirements—the minimum level of dietary Se necessary to raise a biomarker to plateau levels—compared with requirements that often include a safety factor, or that are based on studies that start with Se depleted animals or studies that lack sufficient graded levels of dietary Se such that the plateau breakpoint cannot be accurately determined. One notable exception is the turkey, where minimal Se requirements are twice those for other higher animals (50,51). We recently found that levels of both Gpx4 and Gpx1 activities fall dramatically in Se deficient turkeys in multiple tissues and that Se requirements in male turkey pouls based on liver Gpx1, gizzard Gpx4, and gizzard Gpx1 activities are ~0.3 μg Se/g diet. Cloning of turkey Gpx1 and Gpx4 cDNA revealed that both mRNA are regulated by Se status and fall to ~36% of Se adequate levels (50). These results indicate that the differences in dietary Se requirements are associated by differences in the underlying regulatory mechanisms.

In mammalian species, Lei et al. (52) recently conducted a study in swine that examined levels of 12 selenoprotein transcripts in pigs that had previously been fed a Se deficient diet for 4 wk and were then supplemented with 0.3, and 3.0 μg Se/g diet as Se enriched yeast for 8 wk. Of the 12 studied selenoprotein transcripts in liver, Sepw1, Gpx1, and Txnrd1 were significantly decreased in Se deficiency to 38, 46, and 45%, respectively, of levels in pigs fed 0.3 μg Se/g diet, but Gpx4, Sep1, Sep15, Gpx2, Dio3, Selk, Dio1, Sepp1, and Seph2 transcripts were not significantly changed by Se deficiency. Liver Sepw1 and Gpx1 transcripts were highly and significantly regulated by Se deficiency in our rat study, but Txnrd1 was not regulated significantly, and liver Sep1, Sep15, Selk, Dio1, and Sepp1 transcripts were significantly and at least moderately (to <70%) decreased by Se deficiency in our rat study but were not significantly regulated in the swine study (52). Some of these differences are likely due to use of a practical corn-soy diet (0.02 μg Se/g diet) compared with 0.005 μg Se/g diet in our rat studies, but species differences certainly also play a role in relative expression levels and susceptibility to Se deficiency.

Blood selenoprotein transcripts. We have also found that selenoprotein mRNA levels in rat blood are abundant and regulated by Se status and can be used to assess Se requirements (53,54). Thus, we have attempted to extend this analysis to using these molecular biomarkers for assessing human Se status (55). This study, however, found that a Reading, UK population consuming <50% of the daily U. S. Se dietary intake had plasma Se and Gpx3 activity levels on the plateau of the Se response curves. In whole blood RNA from these participants, we were readily able to detect mRNA levels for Gpx1 and other selenoproteins that are regulated in rodents, but there was no clear indication of less than plateau levels for any of these potential molecular biomarkers of Se status (55).

Implications of Se deficient transcriptome regulation. The first general observation from these studies is that

142 Sunde and Raines
Gpx1, Sepw1, and Selh mRNA are biomarkers that dramatically decrease in vivo and thus have potential as molecular biomarkers for Se status. Downregulation of Sepw1 in muscle has been previously reported (34), but regulation of Selh mRNA in rats by Se status had not. The present studies in intact rats, however, are inconsistent with studies on regulation of Sepw1 and Selh in genetic mouse models. In Se adequate Sepp1 knockout mice, changes in Se distribution were reported not to dramatically affect Selh mRNA levels in heart, lung, brain, or testes and were reported to not affect Sepw1 mRNA levels in heart, to raise Sepw1 mRNA levels in lung, and to dramatically decrease Sepw1 mRNA levels in brain and testes (56). In the present study, both Sepw1 and Selh mRNA reached well defined plateaus with breakpoints at 0.05–0.07 μg Se/g diet. The second important observation from these studies is that supernutritional dietary Se levels up to 0.8 μg Se/g diet (8× requirement) do not further increase any of the biochemical or molecular parameters measured in this study (Figs. 1, 2), with the exception of RBC and muscle Gpx1 activity (Table 1), nor do any of these selenoprotein transcripts have incisive breakpoints well above 0.1 μg Se/g diet that thus could be used as biomarkers for supernutritional or anticarcinogenic levels of Se. Because rodent anticancer studies often use 2 μg Se/g diet (20× requirement) (57) and human anticancer studies often use supplements of 200 μg Se/d (4× requirement) (20), these studies further reinforce the idea that the anticarcinogenic activity of Se is mediated by effects not directly related to selenoprotein activity (58,59), although cancer cells may respond differently than what was observed in these healthy rodent tissues (see below).

One overall striking feature of the Se response curves in this study (Fig. 2), just as in the mouse study, is that a marginal level of dietary Se (0.05 μg Se/g diet) raises selenoprotein mRNA levels to Se adequate levels; the dramatic impact on selenoprotein mRNA stability of these Se regulated mRNA occurs at <0.05 μg Se/g diet. In rats fed intermediate levels of dietary Se to better define Se regulation, the response curves for Gpx1, Selh, and Sepw1 mRNA are hyperbolic, all overlap, and reach plateau responses near 0.05 μg Se/g diet (40). This strongly suggests that there is one underlying mechanism at play in Se regulation of selenoprotein mRNA levels, but further studies will be required to unravel this mechanism.

Mechanisms underlying Se regulation of the selenoprotein transcriptome nonsense mediated decay

The mechanism(s) underlying the Se regulation of selenoprotein transcript levels is not understood. For Gpx1 and Gpx4 mRNA, at least, it is clear that this regulation is not due to transcriptional regulation nor to mRNA processing and export from the nucleus (60,61). Rather, the degradation of selenoprotein mRNA levels in Se deficiency appears to be due to nonsense mediated decay (NMD) (62,63). NMD occurs in eukaryotic cells as a mechanism for eliminating mRNA in which translation prematurely terminates due to a mutation resulting in an inframe nonsense codon. For NMD, the nonsense codon must be positioned >50–55 nucleotides (nt) upstream from a postsplicing exon-exon junction, and NMD depends on the presence of an exon junction complex (EJC) of protein, deposited during pre-mRNA splicing and located ~20–25 nt upstream of the exon-exon junction (64–66). Under these conditions, the translation complex stalls at the nonsense codon before it can sweep the EJC off the mRNA, allowing the EJC and related proteins to recruit mRNA degrading activities (65,66).

Selenoproteins and NMD. For selenoproteins under Se adequate conditions, the concentration of Sec-tRNA is sufficiently high such that NMD does not occur, because translation continues past the UGA, sweeping the EJC off the mRNA and thus preventing mRNA decay. Under Se deficient conditions, however, the hypothesis is that insufficient Sec-tRNA concentrations result in stalling of translation at the UGA; when the UGA is >50–55 nt upstream of an exon-exon junction, such as for rodent Gpx1 mRNA with its UGA located 105 nt from the exon-exon splice-junction, the EJC is not dislodged from the mRNA during the first round of translation, resulting in Gpx1 mRNA decay in Se deficiency. In contrast, when the UGA codon is located closer to the exon-exon junction, such as for rodent Sepx1 with its UGA located 34 nt from the exon-exon splice-junction, the translation complex also stalls at the UGA in Se deficiency but is close enough to dislodge the EJC, thus preventing NMD. This hypothesis is supported by studies showing that positioning of an in-frame UGA codon sufficiently upstream of a splice-junction in β-globin plus a SECIS element in the 3′-UTR will confer Se regulation onto β-globin mRNA (62).

Limitations of the NMD hypothesis. Selh fits the >55 nt rule, with its UGA 136 nt upstream of the exon-exon junction, but Sepw1’s UGA lies only 15 nt upstream and yet this mRNA is dramatically degraded in Se deficiency. For moderately regulated mRNA identified in this study, the UGA positions for Txnrd1 and Txnrd2 fit the >55 nt rule, but the UGA for Sepn1, Selk, Gpx3, and Sep15 are 1, 5, 22, and 28 nt, respectively, upstream of the closest exon-exon junction. The mechanism is further complicated, because transcripts with multiple exons downstream of the UGA can result in additional EJC, separated by >55 nt from the UGA, that could signal NMD (65,66). Thus, moderate Se regulation of transcript levels might be facilitated via additional downstream EJC for Sepn1, Gpx3 and Sep15, but this cannot be the case for highly regulated Selk with its UGA located 5 nt upstream of the exon-exon junction and with no additional downstream exon-exon junctions. Most importantly, the NMD hypothesis as currently stated is completely disproved by the stability of Gpx4 mRNA in Se deficient animals, as the Gpx4 UGA codon lies 105 nt upstream of the nearest exon-exon junction and 257, 282, and 342 nt upstream of its additional exon-exon junctions. Thus, Gpx4 transcripts should be targeted for NMD, but they are not,
clearly showing that the downregulation of selenoprotein transcripts in Se deficiency cannot be explained by NMD as currently hypothesized. These studies do provide a number of new selenoprotein mRNA, however, that can be used in future studies to better understand this process.

**Cell culture models vs. intact animals.** Our understanding of the mechanism underlying the Se regulation of selenoprotein mRNA level is further complicated, because there is a huge discrepancy between Se metabolism in intact animals compared with cultured cell models. Overexpression of selenoprotein mRNA in cultured cells typically results at best in a doubling of the selenoprotein itself when conducted in cells with modest or higher endogenous levels of the selenoprotein (62). At the same time, the extent of Se regulation of Gpx1 mRNA in cultured cells by Se deficiency, however, is much smaller than in the whole animal (2-fold vs. up to 10-fold) (62). More importantly, Gpx4 mRNA levels fall dramatically in Se deficient cultured cells and appear to be regulated by NMD (67), whereas Gpx4 transcripts are not regulated in intact animals (37,62), indicating that additional factors are likely to be involved. Thus, extrapolations and models based on studies conducted in cultured cells need to be made with caution unless confirmed in intact animals or tissues.

**Alternative hypotheses.** A number of hypotheses have been proposed to explain the hierarchy of susceptibility of selenoprotein mRNA to degradation (8,62,63,68–71). Some studies using chimeric constructs expressed in cultured cells suggest that regions in both the coding region and in the 3′-UTR are involved in making selenoprotein mRNA susceptible to decay in Se deficient cells (68). A number of additional features have been hypothesized to play a role in the hierarchy of Se regulation of selenoprotein expression both at the level of mRNA stability and at the level of translation (see below). The UGA position itself relative to the start codon or to the SECIS element, or the local UGA context all can affect translational efficiency and thus might affect mRNA sensitivity to NMD (72). Differential affinities of SECIS elements for isoforms of the Sec-tRNA also influence translational efficiency and are accompanied by changes in mRNA levels and so may influence mRNA stability (69); these shifts in relative levels of Sec-tRNA isoforms, however, are modest in Se deficient mice (73), making it unlikely that this alone regulates selenoprotein mRNA stability in intact animals. Lastly, the presence of a putative second SECIS stemloop immediately downstream of the UGA in the coding region in some selenoprotein mRNA is thought to influence translational efficiency (74), but only Sepn1 mRNA has been studied so far.

**Translational regulation.** Early studies with chimeric mRNA in cultured cells or oocytes found that SECIS elements differ in their ability to facilitate Sec incorporation in translation (8); thus, varied translational efficiency due to differences in SECIS elements might confer differential sensitivity to NMD (8,75). Similarly, differing affinities of SECIS binding proteins, such as SBP2, for the SECIS element have also been proposed to explain the hierarchy of sensitivity to NMD (70,71). These studies in cultured cells may explain differential effects of translation of selenoprotein mRNA, but studies with chimeric constructs have shown that SECIS elements from selenoprotein mRNA not susceptible to NMD are as sufficient as those from NMD susceptible mRNA in conferring NMD sensitivity, thus indicating that Se regulation of NMD involves more than the SECIS element and SECIS binding proteins (62). More recent studies, again in culture, have found 1000-fold differences in the ability of different selenoprotein SECIS elements to foster Sec incorporation at a UGA codon, but these differences were not explained by SBP2 binding affinity (76). Levels of another SECIS binding protein, nucleolin, also have differential effects on translation of UGA/SECIS containing recombinant constructs in cultured cells, but nucleolin does not seem to affect transcript levels, only translation (77). Even a eukaryotic initiation factor, elf4a3, has been shown to differentially bind to Gpx4 compared with Gpx1 SECIS elements in cultured cells and differentially inhibit translation (78). Thus, there are multiple levels when differential expression of selenoproteins occurs, including efficiency of translation, but the details of the mechanism(s) involved in Se regulation of selenoprotein transcript (mRNA) levels remain unclear.

**Se regulation of nonselenoprotein expression**

**High-Se biomarkers.** The conundrum of whether to recommend supernutritional Se supplementation is a real issue for nutritionists today and this is compounded because we lack good biomarkers for assessing supernutritional Se status. The 2000 Institute of Medicine DRI report (12) in reviewing the literature with regard to setting tolerable upper intake levels for Se found that the levels of serum Se in the NHANES III participants (means of 1.55 and 1.58 µmol/L for women and men, 31–50 y, respectively) indicate that at least 99% of these participants should have maximal concentrations of plasma selenoproteins; thus, the Institute of Medicine concluded that measurement of selenoproteins is not a useful biomarker for assessing higher levels of Se status or potential Se toxicity (12).

This current state of high-Se biomarkers is nicely summarized by the 2006 study of Burk et al. (79), who reviewed the area and identified 3 potential biomarkers in plasma for assessment of Se status and prediction of toxicity: Sepp1, Gpx3, and plasma Se, plus urinary Se. They found, however, that in 16 wk of supplementation of U.S. participants with 200, 400, or 600 µg Se/d, neither Sepp1 nor Gpx3 increased with any form of supplemental Se, and plasma Se increased with SeMet and selenized yeast but not with selenite. The level of urinary Se at the end of the study, however, was increased and dramatically dependent on the form of Se provided: 60% of dose for SeMet, 52% for Se yeast, and 41% for selenite, with the percentages unaffected by level of Se supplementation (79). This study clearly illustrates that current
biochemical (Sepp1, Gpx3) as well as chemical (plasma Se) biomarkers cannot effectively be used for assessing high-Se status in humans. Thus, the first listed research recommendation for Se by the DRI Panel (12) is: “Biomarkers for use in assessment of Se status are needed to prevent Se deficiency and Se toxicity... Plasma Se levels (and other measurements of the element) have to be carried out in participants fed levels of Se (both organic and inorganic forms) up to the Tolerable Upper Intake Level (UL).”

**Studies in rats fed supernutritional and toxic Se levels.**

To characterize Se regulation of the transcriptome, we recently conducted microarray studies in rats fed diets supplemented with graded levels of Se from Se deficient to 5 µg Se/g diet, or 50 times the requirement. Our objectives were to determine the transcriptional response to Se deficiency, super-nutritional, and toxic Se intakes in our well characterized rodent model to identify candidates for genes regulated by high-Se status, to provide insight into the molecular mechanisms underlying how animals homeostatically adapt to high-Se status, and to determine whether identified Se regulated genes could be used as molecular biomarkers of high-Se status.

Rats were fed 0, 0.08, 0.24, 0.8, 2.0, and 5.0 µg Se/g diet for 28 d, liver RNA was isolated, and the effect of Se status on the entire transcriptome was determined using Affymetrix Rat Genome 230 2.0 arrays (80). This microarray analysis of >30,000 transcripts using robust multichip averaging (RMA), with P-values adjusted for multiple comparisons, found that an intake of 5 µg Se/g is required to significantly alter the expression of a large set of liver transcripts, in this case 1193 transcripts, but still only 4% of the transcriptome (Fig. 3). This shows that a large and distinct transcriptional response profile can be induced by Se toxicity. In contrast, Se intakes < 5 µg Se/g diet significantly changed <10 transcripts compared with a Se adequate intake within an experiment. The vastly expanded number of gene expression changes observed in this study thus is a newly identified marker of Se toxicity. Importantly, as many as one-half of the 1193 transcripts altered by the 5-µg Se/g diet treatment may be Se specific (see below).

**Se deficiency.** Se deficiency downregulated (P < 0.05) 4 genes in rat liver (Selh, Gpx1, Selt, and Txnrd3), which are all selenoproteins we previously identified as highly regulated by Se status (39,40), plus 2 additional selenoprotein genes (Txnrd2, Selk) that were marginally downregulated (P < 0.07) by Se deficiency (Fig. 3). Notably, there were no nonselenoprotein transcripts significantly downregulated by Se deficiency. Similarly, there were only 2 upregulated liver transcripts [Ugt2b7 (UDP glucuronosyltransferase 2 family, polypeptide B7) and Gsta5 (glutathione S-transferase Yc2 subunit)], which were significantly upregulated in Se deficient rat liver 2- and 3-fold, respectively, relative to rats fed the 0.24-µg Se/g diet. Additionally, follow-up quantitative real-time-PCR analyses found 2 additional genes [Abcc3 (ATP-binding cassette, subfamily C (CFTR/MRP), member 3) and Nqo1 [NADPH dehydrogenase, quinone 1]] were upregulated 4- and 2-fold, respectively, by Se deficiency. It is should be noted that Abcc3 and Nqo1 are established Nrf2 upregulated genes. Importantly, both microarray and quantitative real-time-PCR expression of the genes upregulated in Se deficiency show that, like selenoprotein mRNA, these genes are restored to adequate levels in the mice fed a Se marginal diet (0.05 µg Se/g diet) (80). Overall, this analysis of the effect of Se deficiency on the transcriptome reinforces the specificity of Se regulation of selenoproteins in Se deficiency.

**Supernutritional Se (0.8 and 2.0 µg Se/g diet) status.** Surprisingly, there were no significant gene expression changes associated with increasing Se status from 0.08 to 0.8 µg Se/g diet (Fig. 3). For rats fed 2.0 µg Se/g diet (20× requirement), only 6 transcripts (5 genes and 1 EST) were significantly changed by 2 µg Se/g diet, with 5 additional transcripts nearly significant (P < 0.2). The genes upregulated at the 2.0-µg Se/g diet were: Rgs4, a regulator of g-protein signaling; Ccdd80, which may be involved in extracellular matrix (ECM) organization; RGD1560666, a gene of unknown function that contains putative signaling domains; and Oxct1, a member of the 3-oxoacid CoA transferase family. The lone downregulated gene at 20 times the requirement was Cirbp, which contains RNA binding domains and is thought to stabilize mRNA and enhance translation (80). Importantly, the microarray data presented here indicate that supernutritional Se intakes 8–20 times the Se requirement are not sufficient to cause a large transcriptional response. This is important because levels used in cancer prevention trials are typically 200 µg/d (4× requirement), which in the US will bring total Se intake to 5–8 times the requirement depending on dietary Se intake from foods. These data thus suggest that cancer prevention associated with
supernutritional Se supplementation may not be mediated by transcriptional changes.

**Toxic Se status (5.0 μg Se/g diet).** Feeding 5 μg Se/g diet to weanling rats for 28 d significantly reduced growth relative to all other groups starting at d 10 and resulted in a 23% reduction in final body weight. Plasma alanine and aspartate aminotransferase levels were both modestly increased 2-fold in rats fed the 5 μg Se/g diet, providing biochemical evidence of Se toxicity. In livers of rats fed the 5 μg Se/g diet, however, there were 1193 transcripts significantly altered at 50 times the requirement. Because the rats fed the 5 μg Se/g diet had reduced growth and elevated markers of liver damage, it is possible that many of these transcripts could be responding to general toxicity and/or caloric restriction. Therefore, the 1193 Se toxicity transcripts that overlapped with Affymetrix’s RatToxFX 1.0 array (218 of 2073 probe sets) and with 10-d caloric restriction data from a recent study (338 of 5391 probe sets) (81) were removed, along with redundant transcripts. After this filtering, 715 Se specific transcripts remained in this Se specific dataset. This 715-transcript dataset contained 48 duplicate transcripts, yielding 667 unique transcripts, which correspond to 437 unique genes for subsequent gene ontology analyses. Most of the unique transcripts were upregulated, with 542 up- and 125 downregulated. Within the 1193 Se toxicity set of transcripts significantly altered by the 5 μg Se/g diet, 99 genes were known Nrf2 targets, including 49 Nrf2 targets in the set of 437 Se specific genes (80). This clearly suggests that Se toxicity results in increased reactive oxygen species, perhaps more so than in Se deficiency.

**Gene ontology analysis.** GOMiner analysis identified 33 biological processes significantly enriched in the Se specific dataset in processes such as cell movement/morphogenesis, ECM, and development/angiogenesis (80). For example, collagen fibril organization was enriched by a factor of 6.72 (6 of 23 total genes present in the Se specific set). The genes in this category included 4 collagen genes (Col12a1, Col5a2, Col1a2, Col11a1), 1 serine proteinase inhibitor (Serpinh1), and 1 annexin (Anxa2). Further searching of the Se specific dataset found a total of 7 collagen genes. In addition, many other ECM related genes were present in the 5 μg Se/g dataset. Genes related to glucose metabolism were also enriched in the clusters that were upregulated to some extent by the 2 and 5 μg Se/g diets. There is some evidence that high-Se status is related to diabetes, and a selenosugar is one of the major excretory metabolites for Se (19,82,83). Se regulation of glucose related genes may be another piece of the puzzle linking Se and glucose metabolism.

**Supernutritional and toxic Se regulation of the selenoprotein transcriptome**

These microarray analyses also provide insight into the Se regulation of the selenoprotein expression across the full spectrum from deficient to toxic. **Figure 4** shows the RMA expression levels for all 22 selenoprotein genes present on the Rat Genome 230 2.0 array. Columns represent liver expression from individual rats fed a 0, 0.08, 0.24, 0.8, 2, and 5 μg Se/g diet. Rows represent the probe sets. Also shown for comparison are expression values for 2 control transcripts (Gapdh and Rsp14) and for 6 transcripts for cysteine containing paralogs of selenoproteins. RMA gene expression is shown using the indicated pseudo color scale from −2× (green) to +2× (red) relative to values for rats fed 0.24 μg Se/g.
Selenium and Sepw1 were upregulated 2.5-fold relative to Se adequate levels and Txnrd1 and Gpx3 were upregulated 1.5-fold relative to Se adequate levels. Sepw1 mRNA levels have been reported to be increased 21% in muscle of rats fed a 4 mg Se/g diet compared with levels in Se adequate rats (34). Interestingly, studies looking at 12 selenoprotein transcripts in pigs, including Txnrd1, did not identify any transcripts that were significantly altered by a 3.0 compared with 0.3 mg Se/g diet (52). These 4 selenoprotein transcripts thus are candidate molecular biomarkers for toxic Se status; it should be noted, however, that only Txnrd1 mRNA appears to be a potential biomarker for supernutritional Se status. Note that in this analysis of just the 22 selenoprotein genes, P-values were not adjusted for multiple testing, which explains why Txnrd1 was significantly upregulated by the 2.0 mg Se/g diet in this analysis but not identified in the analysis of the full transcriptome.

Se transcriptomics and cancer. Studies on the transcriptional effects of Se in various cancer cell lines and cancerous tissue have identified relatively few transcripts that are altered by high Se; these studies to date have produced variable results, with little similarity between studies (84–88). Previous microarray studies have investigated the transcriptional effects of Se supplementation up to 1.0 mg Se/g diet in rodents, of up to 200 μg Se/d in human participants, and of up to 22 μmol/L Se in cultured cells. A search (80) of the Se regulated genes in 9 previous Se microarray studies (84–92) found only a few genes that were identified in >1 study, and only selenoprotein genes were reported to be regulated in >3 studies. Unfortunately, many of these studies only compared high-Se to Se deficient treatments, so that the reported transcriptional changes are likely to be the result of Se deficiency instead of Se supplementation. The lack of Se specific regulation in these previous studies thus appears to be due to use of insufficient Se to produce the large, significant transcriptional responses that we observed with a 5 mg Se/g diet.

Biomarkers/panels of Se status. To illustrate the potential of molecular biomarkers for predicting Se status across the full spectrum of Se status, we previously evaluated the ability of 4 liver traditional biomarkers and 13 liver selenoprotein mRNA levels to predict liver Se concentration (93). Multiple regression analysis against liver Se concentration, with stepwise single elimination of biomarkers that did not significantly contribute, was used to identify biomarker panels with significant (P < 0.05) regression coefficients. The
resulting 4 selenoprotein mRNA biomarker panel predicted liver Se concentration with a correlation of 0.948, which was nominally higher and statistically the same as the correlation of 0.909 for the panel based on Gpx1 activity (data not shown). This panel, however, predicted an essentially flat Fl response curve after a ~0.2 µg Se/g diet compared with the continued but slow further increase in measured liver Se between 0.24 and 0.8 µg Se/g diet, clearly illustrating that additional, orthogonal biomarkers for sup Smartphone etiologic status are needed to more accurately predict this increase in tissue Se (93).

Thus, we recently (80) used the individual expression values for the 6 transcripts regulated by 2 µg Se/g plus the 6 transcripts regulated by Se deficiency in the rat microarray study to develop a molecular biomarker panel for predicting the measured liver Se concentrations. This analysis resulted in a panel of 11 significant transcripts (6 regulated by 2 µg Se/g and 5 regulated by Se deficiency), with an overall correlation coefficient of 0.9988 (P < 10^-6) that accounted for 99% of the variation in liver Se concentration over the full range from 0 to 5 µg Se/g diet (Fig. 6). In contrast, panels based on conventional liver selenoenzyme activity biomarkers (Gpx1 plus Gpx4 activity) or blood selenoenzyme biomarkers (RBC Gpx1 plus plasma Gpx3 activity) only predicted 57% or 80%, respectively, of the variability in liver Se concentration (80). This exercise clearly demonstrates the potential efficacy of using molecular biomarkers in panels for predicting Se status across the full spectrum from deficiency to toxicity.

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Selenium regulation of the transcriptome 149


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