Chapter 14
Selenium Regulation of the Selenoprotein and Non-selenoprotein Transcriptomes in a Variety of Species

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Abstract Selenium (Se) status regulates the expression of selenoproteins, not only by availability of Se for incorporation into these selenoproteins, but also by regulation of the selenoprotein transcriptome. Selenoprotein transcripts can be used as "molecular biomarkers" for assessment of Se deficiency in rodents, and we have expanded these studies to avian species and the nematode to better investigate the hierarchy of Se incorporation into selenoproteins. In addition, we are investigating the impact of high-Se levels on selenoprotein transcripts, and the impact of high-Se intake on the expression of non-selenoprotein transcripts. These studies indicate that increased reactive oxygen species are associated with Se deficiency and high-Se intake, and that panels of non-selenoprotein transcripts have potential as biomarkers of supranutritional and toxic-Se status.

Keywords Biomarker • *Caenorhabditis elegans* • Chicken • Collagen • Hierarchy • Mouse • Rat • Reactive oxygen species • Requirement • Turkey

14.1 Introduction

Selenium (Se) status regulates the expression of selenoproteins, not only by availability of Se for formation of the key cofactor, selenocysteine (Sec), in these selenoproteins, but also by regulation of the selenoprotein transcriptome. This regulation translates into use of selenoproteins and selenoenzymes as biomarkers of Se status and requirements, and also offers the "selenotranscriptome" as a "molecular biomarker" of Se status. The hierarchy of selenoenzyme biomarkers in rodents was reviewed in the third edition of this book [1].

Right after the discovery that glutathione peroxidase (GPX) was a selenoenzyme [2], dietary Se was shown to regulate the level of GPX activity [3]. Dietrich Behne was the first to describe the differential incorporation of ^{75}Se into selenoproteins in

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various tissues as a hierarchy of Se supply to tissues and selenoproteins [4]. Subsequent researchers also reported differential Se incorporation or expression of selenoproteins as new selenoproteins were discovered. Exploring the underlying mechanism for Se regulation of GPX activity, we found that GPX protein could also be used as a biomarker for Se status [5, 6]. That led to investigating *Gpx* mRNA levels, which were surprisingly found also to be regulated by Se status [7]. Relative to *Gpx* mRNA, differential regulation by Se status was reported for transcripts for selenoprotein P (*Sepp1*), 5′-deiodinase (*Dio*), *Gpx4*, *Gpx2*, and other known selenoproteins [8–12]. These additional selenoproteins expanded the array of both biochemical and transcript-based biomarkers of Se status in our studies [13–17]. The bioinformatics identification of the complete selenoproteome by Gladyshev and colleagues [18] allowed us to use selenoprotein transcripts as "molecular biomarkers" for assessment of Se deficiency in rodents [19–22]. These studies have now been expanded to avian species, such as turkeys and chickens [23–25], and nematodes [26, 27], to better investigate the hierarchy of Se incorporation into selenoproteins. In addition, this approach of investigating the effect of high-Se intake (supranutritional and toxic) on selenoprotein transcripts in these species was examined to investigate the impact of high-Se status on the expression of non-selenoprotein transcripts.

14.2 Animal Models for Se Regulation of Selenoprotein Transcripts

To study the effect of Se deficiency on selenoprotein expression, weanling rats and mice from Se-adequate dams were typically fed a basal 30 % torula yeast-based diet supplemented with 0.4 % additional methionine (Met) to meet the sulfur amino acid requirements, and supplemented with 100 mg/kg of vitamin E, which provides twice the vitamin E requirement [28]. This diet allowed adequate growth and prevented liver necrosis, so that the impact of Se status was studied without impaired growth or subsequent effects of disease. Since selenomethionine is an excellent analog of Met, it is readily aminoacylated to Met tRNA and incorporated in place of Met into the nascent polypeptide. Furthermore, dietary Met alters the incorporation of Se from selenomethionine and, therefore, these studies were usually conducted using inorganic Se [29, 30].

These basal torula yeast diets consistently contained 0.005–0.007 μg Se/g, which were then supplemented with Se as sodium selenite. For most studies, multiple, graded levels of supplemental Se were used so that the impact of Se status on the target Se biomarkers could be titrated. Importantly, the plateau levels for the biomarker could be better established, indicating that Se is no longer the rate-limiting factor controlling expression of the biomarker. The multiple levels of dietary Se within the plateau also helped establish the variability and effectiveness for detecting biologically-important differences. Specifically, a "Se-response curve" was constructed for each biomarker using sigmoidal or hyperbolic regression analysis including all individual values at each dietary Se treatment as described previously [13, 17, 19]. The "plateau break-

point" for each Se-response curve, defined as the intersection of the line tangent to the point of steepest slope and the plateau, was calculated to estimate the "minimum dietary Se requirement" necessary to obtain the plateau response.

14.3 Se Regulation of Conventional Se Biomarks

In rodents, Se-deficient diets as compared to Se-adequate diets, had no effect on animal growth. However, liver Se concentration fell to three and 10 % of Se-adequate levels in rats and mice fed these basal diets, respectively, demonstrating that the diets were in fact Se-deficient. Similarly, in rats and mice fed these diets for 4 weeks, liver GPX1 activity fell to two and three percent of Se-adequate levels, plasma GPX3 activity fell to two and 37 % of Se-adequate levels, but liver GPX4 activity fell to 47 and 55 % of Se-adequate levels, respectively [19]. Liver thioredoxin reductase (TXNRD) activity fell to 15 % of Se-adequate levels in Se-deficient rats [16]. Clearly, there is a differential effect of Se deficiency on the activity levels of these selenoenzymes.

In this context the minimal dietary Se required to achieve hepatic GPX1 plateau activity was ~0.1 μg Se/g diet, and was ~0.05 μg Se/g diet for liver GPX4, liver TXNRD activity, and plasma GPX3 activity in rats [19]. Similar minimal Se requirements were found in mice [21, 31]. These studies thus showed that there was a Se requirement hierarchy for these selenoenzyme activity biomarkers. This raised the question about underlying Se regulation of the selenoprotein transcriptome.

14.4 Se Regulation of Selenoprotein Transcripts in Rats

The discovery that Se deficiency dramatically down-regulated the level of *Gpx1* mRNA was the start of our focus on Se regulation of transcript levels [7]. Early studies reported differential down-regulation of selenoprotein transcripts in Se-deficient relative to Se-adequate rat liver to 36 % for *Dio* [8], to 67 % for *Sepp1* [8], but only to 90–110 % for *Gpx4* [32], clearly indicating that a hierarchy exists for Se regulation of transcript abundance. Subsequent studies using multiple graded levels of Se supplementation showed that liver *Gpx1* mRNA levels fell to 10–11 % of Se-adequate levels in both Se-deficient male and female rats [13, 14], even though Se-adequate female rat liver had twice the level of GPX1 activity and mRNA as male rat liver [33]. *Gpx4* mRNA levels, in contrast, only fell non-significantly to 61 % and 82 % of levels in 0.1 and 0.2 μg Se/g supplemented rats, respectively [9], illustrating the importance of fully assessing multiple plateau levels for transcript abundance. *Txnrd1* transcripts in Se-deficient rat liver were 70 % of Se-adequate levels [16].

In all of the above studies, the Se-response curves demonstrated that selenoprotein transcript levels reach well-defined plateaus with increasing Se supplementation. Furthermore, as expected, plateau levels of selenoprotein transcripts reached plateau levels at dietary Se concentrations roughly half or less than dietary Se concentrations

necessary for plateau levels of corresponding selenoprotein enzyme activity. Thus, some selenoprotein transcripts, such as *Gpx1,* were suggested to be used as "molecular biomarkers" to detect Se deficiency [34, 47], although resulting dietary Se levels would not provide sufficient Se to maximize selenoenzyme activity.

The report by Gladyshev and colleagues in 2003 [18] of a bioinformatics approach to identify the complete selenoproteome in species was a huge boost for Se research. Quantitative polymerase chain reaction (qPCR) was used to assess transcript abundance for all 24 selenoproteins in rats supplemented with deficient to supranutritional Se (0.007–0.8 μg selenite/g diet) and demonstrated that the majority of liver selenoprotein mRNAs were not significantly regulated by Se status [19]. In liver, *Gpx1* mRNA remained the most highly down-regulated transcript in Se deficiency, falling to 10 % of Se-adequate levels. *Sepw1*, *Selh*, and *Selk* mRNAs were also highly down-regulated to <25 % of Se-adequate levels, whereas *Sepp1* mRNA was only down-regulated to 50 %, while the *Gpx4* mRNA level was not significantly altered by Se deficiency [19]. This hierarchy of Se regulation is shown in Fig. 14.1.

The resulting Se-response curves showed that all regulated selenoprotein transcripts were down-regulated by Se deficiency and reached well-defined plateaus with increasing supplemental Se, and all had plateau breakpoints in a tight range of 0.04–0.07 μg Se/g diet [19]. This tight range suggested that one underlying mechanism

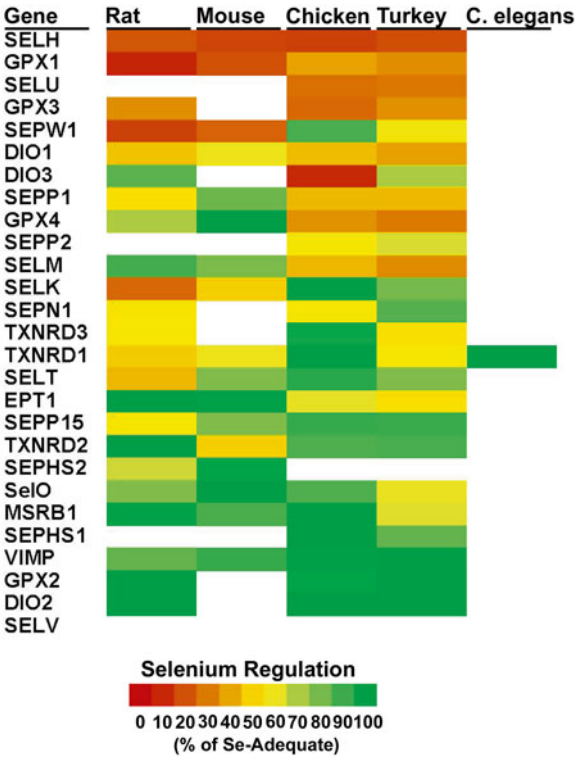

Fig. 14.1 Se regulation hierarchy. Heat map of the hierarchy of down-regulation of selenoprotein transcript abundance in Se-deficient liver, shown as percent of Se-adequate levels for rats [19], mice [21], chickens [25], turkeys [24], and *C. elegans* (total organism) [26], determined by qPCR or microarray analysis as reported in these references. *White bars* indicate missing data or genes. Chicken *DIO3* abundance is not significantly different from Se-adequate

was in play for regulation of selenoprotein mRNA levels [21]. In addition, supplementation of up to 0.8 μg Se/g diet showed that, above the plateau breakpoint, none of the liver selenoprotein mRNA levels were altered by such Se supplementation. This indicated that none of these selenoprotein mRNAs could be used as biomarkers for supranutritional or anti-carcinogenic levels of Se.

14.5 Se Regulation of Selenoprotein Transcripts in Mice

Similar studies were conducted in mice, assessing transcript levels by northern [31] and microarray analyses followed by qPCR confirmation [21]. The impact of Se deficiency on mouse liver selenoprotein mRNA levels is shown in Fig. 14.1. Microarray analyses showed that nine of the 17 selenoprotein mRNAs present on the array were significantly down-regulated by Se deficiency. In mouse liver, *Gpx1*, *Selh* and *Sepw1* mRNAs were highly down-regulated to ~20 % by Se deficiency, whereas *Sepp1* and *Gpx4* mRNA levels were not regulated, as confirmed by qPCR [21]. Se-response curves showed that the plateau breakpoint for mouse liver *Gpx1* mRNA was at 0.04 μg Se/g diet, with no further increase up to 0.5 μg Se/g [31]. However, *Gpx4* and *Sepp1* mRNA levels were not altered by Se supplementation level from deficient to supranutritional.

14.6 Se Regulation of Selenoprotein Transcripts in Turkeys

The Se requirement for turkeys is 2–3 times higher than that of rodents [35]. An early study indicated that some of the apparent GPX1 activity in turkeys might be due to GPX4 [36]. Thus, GPX1 was separated from GPX4, which demonstrated that 47 % of the H_2O_2 activity in Se-adequate turkey liver was due to GPX4, and also determined a factor for calculation of GPX4- and GPX1-specific enzyme activities [37]. Day-old male turkey poults fed torula yeast-based diets supplemented with 0–0.5 μg Se/g for 28 days were found to require 0.05 μg Se/g for maximum growth. Liver Se concentration, liver GPX1 activity, and liver GPX4 activity fell to 15 %, 3 % and 7 %, respectively, of Se-adequate levels, thus showing that Se biology in the turkey is distinctly different from that of rodents. Se-response curves using GPX1 and GPX4 activity in liver showed that the minimum dietary Se requirement in the growing male turkey was 0.3 μg Se/g diet, or three-times that for rodents. The level of Se-adequate liver GPX1 activity in turkeys was 10 % of the level in rat liver, and the level of GPX4 activity is six-times the level in rat liver [37]. In these studies, *GPX1* and *GPX4* transcripts were decreased to ~30 % of Se-adequate levels [37].

The sequence of the turkey selenoprotein transcriptome was completed recently, and expressed transcripts for 24 turkey selenoproteins were found [23]. Notably, *SELV* and *SEPHS2* appeared to be missing, but two additional selenoprotein transcripts, *SELU* and *SEPP2*, which are paralogs of *SELV* and *SEPP1*, with in-frame UGA codons and SECIS elements for Sec incorporation were also observed. *SEPHS1*, a homolog of

SEPHS1 in mammals and also a non-selenoprotein, was present. Importantly, these sequences averaged 96 % sequence identity (range 92–99 %) with the corresponding chicken sequences [23].

These sequences were used to assess Se regulation of selenoprotein transcripts in turkey poults fed 10 graded levels of Se from deficient to 1.0 μg Se/g diet [24]. qPCR analysis found that five of the 24 turkey selenoprotein transcripts were down-regulated, four significantly by Se deficiency to ≤35 % of Se adequate levels, *SELH, GPX4, GPX1, SELU,* and *GPX3*, whereas 6 of 24 were only decreased to 35–50 % and 11 more to >70 % (Fig. 14.1). Resulting Se-response curves showed that all down-regulated selenoprotein transcripts reached well-defined plateaus with increasing supplemental Se, and all had plateau breakpoints in a tight range of 0.05–0.09 μg Se/g diet. Furthermore, Se supplementation up to 1.0 μg Se/g showed that none of these selenoprotein mRNAs can be used as biomarkers for supranutritional Se status [24].

14.7 Se Regulation of Selenoprotein Transcripts in Chickens

In a parallel study in chicks [25], day-old male broilers were fed the torula yeast diet supplemented with 0–1.0 μg Se/g for 29 days. Only 0.025 μg Se/g were required for maximum growth, and liver GPX1 activity and liver GPX4 activity fell to 2 % and 10 %, respectively, of Se adequate levels, showing that Se biology in the chicken, like the turkey, is distinctly different from that of rodents. Se-response curves using GPX1 and GPX4 activity in liver showed that the minimum dietary Se requirement in the growing male turkey was 0.13 and 0.10 μg Se/g diet, respectively, or ~0.05 μg Se/g higher than that for rodents. The level of Se-adequate liver GPX1 and GPX4 activities in the chick were very similar to the levels in turkey poults [25].

qPCR analysis of chick liver selenoprotein transcript levels revealed a pattern of Se regulation similar to that of the turkey. The same five selenoprotein transcripts as in the turkey, *SELH, GPX3, SELU, GPX4,* and *GPX1*, were significantly down-regulated by Se deficiency to <40 % of Se adequate levels, whereas 6 of 24 were only decreased to 40–50 % and 13 more to >70 % (Fig. 14.1). Resulting Se-response curves further showed that all significantly-regulated selenoprotein transcripts reached well-defined plateaus with increasing supplemental Se, and all had plateau breakpoints in a tight range of 0.07–0.11 μg Se/g. Furthermore, Se supplementation up to 1.0 μg Se/g diet showed that none of these selenoprotein mRNAs can be used as biomarkers for supranutritional Se status [25].

14.8 Se Regulation of Selenoprotein Transcripts in *Caenorhabditis elegans*

The selenoproteome in *C. elegans* consists of a single selenoprotein, thioredoxin reductase (designated as *trxr-1*), as well as a Cys-containing paralog, *trxr-2. C. elegans* can be grown in axenic (bacteria-free) media, allowing careful control of Se supplementation.

Low media Se, however, had no effect on growth, and only decreased apparent TRXR activity to ~80 % of Se-supplemented levels [26]. Deletion of *trxr-1*, *trxr-2*, or both, in *C. elegans* was without phenotype under standard conditions [26, 38], and only blocked the increase in apparent TRXR activity associated with Se supplementation [26]. Low media Se had no effect on *trxr-1* mRNA levels relative to Se supplemented media (Fig. 14.1) [26].

14.9 Overall Selenoprotein Transcript Regulation

Figure 14.1 shows a similar hierarchy of a "Se-deficiency regulon" among the four animal species with respect to down-regulation by Se deficiency. The upper half of the heat maps, representing *SELH* to *SELK* transcript levels, shows *SELH*, *GPX1*, and *GPX3* as the transcripts most commonly down-regulated to <40 % of Se adequate levels. In avian species, this group includes *SELU*. *Sepw1* and *Selk* are also highly down-regulated by Se deficiency in rodents, but not in avian species, whereas *GPX4* and *SELM* are more highly regulated in avian species. Although not presented here, there are usually fewer selenoprotein transcripts down-regulated by Se deficiency in non-hepatic tissues of these animals.

The complete mechanism responsible for Se regulation of selenoprotein transcript abundance remains unclear. Nonsense-mediated decay clearly is involved, but the various proposed models to explain this regulation continue to fall short when the profile of Se regulation of the complete selenotranscriptome is examined in a whole animal. As previously discussed in detail, the position of the UGA codon adjacent to the splice junction does not match with the hierarchy of the Se-deficiency regulon [1, 21]. It is important to recognize that the studies reported here were all conducted in whole animals, not cultured cells. As elegantly shown by Maquat and colleagues [39], rodent *Gpx4* mRNA is highly regulated in cultured cells but not in rat liver, clearly demonstrating that other factors are in play in whole animals.

One notable feature of the Se-deficiency regulon from these studies is that, for highly regulated Se transcripts, the plateau breakpoints all resided in a tight group on the Se-response curves. Furthermore, these tight groups were all similar, 0.03–0.07 μg/g diet for the rat, 0.04 μg/g for the mouse, 0.05–0.09 μg Se/g for the turkey, and 0.07–0.11 μg Se/g for the chick, in spite of much larger differences in the level of dietary Se required for maximal selenoenzyme activity. This suggested that a common mechanism is present when Se status regulates selenoprotein mRNA levels in all species [21], and that other factors are also important which subsequently modulate Se availability for incorporation into proteins.

Relative transcript abundance may play a role. It was clear that *Gpx1* mRNA was highly abundant in Se-adequate rat liver, perhaps 20-fold higher than *Gpx4* mRNA and twice as high as *Sepp1* mRNA [40], providing more range for changes in abundance as well as saturation of Se incorporation rates. In contrast, *GPX4* mRNA is three-fold higher and *SEPP1* mRNA was 12-fold higher than *GPX1* mRNA in turkey and chick liver than in rodents. This variable Se-responsive transcript abundance would suggest that Se incorporation into SEPP1 protein and Se

export from liver would dominate over Se incorporation into resident hepatic selenoproteins in avian species.

Collectively, the above studies suggested that whole animal Se metabolism is likely to play a role in the differences between the minimum Se requirement for plateau transcript level and the requirement for plateau level of the selenoprotein. High levels of liver SEPP1 transcripts may out-compete levels with transcripts for GPX1 and other selenoproteins, facilitating export of Se to other tissues, and thus, extending the amount of dietary Se necessary to maximize Se incorporation into hepatic selenoproteins.

14.10 Se Regulation of Non-selenoprotein Transcripts in Rodents

Changes in nutrient levels as well as other factors can have a dramatic effect on the full transcriptome. For instance in yeast, changes in media amino acid concentration or other environmental factors can alter a third of the yeast transcriptome [41]. It was initially anticipated that modest changes in dietary Se supplementation, from deficient to twice the minimum Se requirement, might elicit Se-specific changes as an animal switches from Se retention to Se excretion. Surprisingly, feeding mice 0 vs. 0.2 μg Se/g had a negligible effect on the total transcriptome as determined by microarray analysis [22]. Only three and five transcripts (all selenoproteins) in mouse kidney and liver, respectively, were decreased significantly by Se deficiency, and only three non-selenoprotein transcripts in mouse liver and three in kidney were increased significantly by Se-deficiency; five of the six up-regulated transcripts were nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or NRF2) targets, indicating that increased reactive oxygen species is associated with Se deficiency [22].

Se toxicity is another aspect to consider in the above studies. When rats were fed a Se-deficient diet supplemented with 0, 0.08, 0.24, 0.8, 2.0 and 5.0 μg Se/g (0 to 50-times the dietary Se requirement), 5.0 μg Se/g, but not 2.0 μg Se/g diet, significantly altered growth (20 % decrease), indicating a mild onset of Se toxicity at 5.0, but not 2.0 μg Se/g. Liver GPX1, liver GPX4 and plasma GPX3 activities all reached plateau levels by 0.24 μg Se/g, and were not further increased by additional dietary Se [22]. When total liver RNA from these rats was subjected to microarray analysis, fewer than 10 out of >30,000 transcripts were significantly altered by 0, 0.08, 0.8 and 2.0 μg Se/g treatments relative to 0.24 μg Se/g-fed rats. As in mice, only selenoprotein transcripts were down-regulated by Se deficiency in rat liver, and Se deficiency increased only two non-selenoprotein transcripts significantly in rat liver [22].

Supplementation with 2.0 μg Se/g altered just six transcripts significantly in rat liver; none were selenoprotein transcripts, and none changed up or down more than fourfold. In contrast, supplementation with 5.0 μg Se/g significantly altered 1193 transcripts or four percent of the transcriptome. As shown in Fig. 14.2, filtering this 1193 high-Se transcript dataset against a calorie-restriction dataset plus a drug toxicity dataset resulted in a 715 “toxic-Se specific” transcript dataset [22]. Additional filtering against a dataset of 1076 NFE2L2-targeted genes [42, 43] showed that

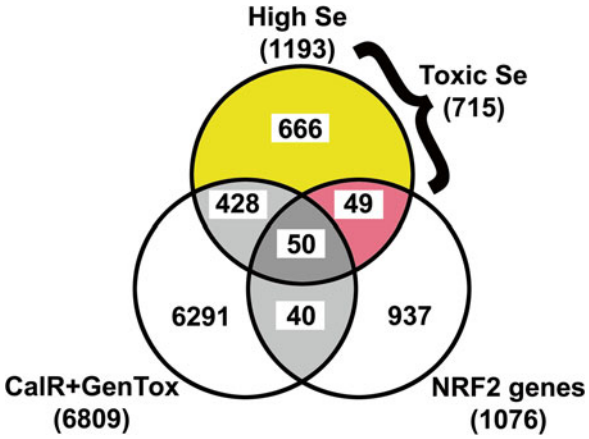

Fig. 14.2 Toxic-Se rat liver transcripts, including NFE2L2 (NRF2) targets. The 1193 high-Se transcripts changed significantly by 5.0 μg Se/g as compared to 0.24 μg Se/g [22] were filtered against a combined "CalR + GenTox" probe set (6809 transcripts changed significantly by caloric restriction (CalR) [51] combined with a general drug toxicity (RatTox FX 1.0, GenTox) probe set), and filtered against a "NRF2 genes" probe set (1076 transcripts for known NRF2-regulated genes [42, 43]). The result was the set of 715 "toxic-Se specific" transcripts, including 49 transcripts of NRF2 target genes (in *red*) plus 666 additional toxic-Se transcripts (in *yellow*)

8.3 % of the 1193 transcripts (99 of 1193) were known NFE2L2 targets, including 49 in the 715 toxic-Se specific dataset. The prevalence of NFE2L2-regulated genes in the dataset of genes significantly altered by Se toxicity as well as in the small set of genes up-regulated by Se deficiency indicated that Se excess as well as Se deficiency increases oxidative stress.

The 715 toxic-Se specific transcript dataset contained 667 unique transcripts, with 542 being up- vs. 125 down-regulated. GOMiner analysis identified 33 biological processes significantly enriched in a Se-specific filtered dataset. These were nearly all related to cell movement/morphogenesis, extracellular matrix, and development/angiogenesis, including a number of collagen-related genes [22].

Interestingly, four selenoprotein transcripts in the 1193 high-Se transcripts were significantly up-regulated by 5 μg Se/g diet. *Selm* and *Sepw1* mRNAs were up-regulated 2.5-fold relative to Se-adequate levels and *Txnrd1* and *Gpx3* were up-regulated 1.5-fold relative to Se-adequate levels [44]. Note that GPX3 activity, presumably primarily from kidney, was not elevated at 5.0 μg Se/g [22]. These four selenoprotein transcripts can thus be considered candidate molecular biomarkers for toxic-Se status, but it is unclear whether these increases were due to primary Se toxicity, or were changes secondary to mild toxicity.

Unsupervised hierarchical clustering of the Se-specific transcript in rats fed 5.0 and 2.0 μg Se/g vs. lower Se intakes was used to identify clusters of potential biomarkers of high-Se intake [22]. Three clusters containing 117 transcripts (72 genes) were up-regulated to some extent by 2.0 as well as 5.0 μg Se/g, and one distinct cluster containing 44 transcripts (25 genes) was down-regulated to some extent by 2.0 as well as 5.0 μg Se/g diet. Functional analysis revealed a set of genes within the

clusters that were up-regulated by 2.0 and 5.0 μg Se/g that are involved in glucose transport, insulin signaling, or glycoprotein biosynthesis.

14.11 Se Regulation of Non-selenoprotein Transcripts in *C. elegans*

Initial studies with *C. elegans* showed that culturing in 0.2 and 0.4 mM Se resulted in a significant delay in growth as compared to 0, 0.05, or 0.1 mM Se, indicating Se-induced toxicity [26]. A microarray study in *C. elegans* cultured in axenic media supplemented with five levels of Se from 0 to 0.4 mM selenite was used to characterize Se regulation of the full transcriptome. Worms were staged to mid-L4 larval stage to minimize developmental differences [27]. Relative to 0.1 mM Se treatment, culturing *C. elegans* at 0, 0.05, 0.2, and 0.4 mM Se resulted in 1.9, 9.7, 5.5, and 2.3 %, respectively, of the transcriptome being altered at least twofold. Filtering these datasets found 295 overlapping transcripts that were altered by both 0.2 and 0.4 mM Se, but not by other treatments. A "toxic-Se specific" dataset of 182 genes was then identified by filtering against gene sets for sulfur [45] and cadmium toxicity [46]. Genes in this toxic-Se dataset were significantly enriched in functions related to oxidoreductase activity, and significantly depleted in genes related to structural components of collagen and the cuticle [27]. These results suggest that Se toxicity in *C. elegans* also caused an increase in reactive oxygen species and stress responses, marked by increased expression of oxidoreductases and reduced expression of cuticle-associated genes, which together underlie impaired growth.

14.12 Se Regulation of Non-selenoprotein Transcripts in Avian Species

In supplemented chicks and turkeys with graded levels of Se up to 1.0 μg Se/g, 1.0 μg Se/g had no effect on growth [24, 25]. In a preliminary study, total RNA pooled from four chicks supplemented with Se-adequate and high Se (0.3 and 1.0 μg Se/g) diets was subjected to microarray analysis using the Affymetrix Chicken Genome Array (32,773 transcripts). Because an average of 96 % sequence identity was found between the turkey and chicken selenoprotein transcripts [23], total liver RNA pools from turkeys fed Se-adequate and high Se (0.4 and 1.0 μg Se/g) diets were also analyzed. Filtering expression to 20–100 % showed significant expression of 30,524 probe sets for chicken RNA, and 30,458 probe sets for turkey RNA, indicating that the chicken array could be used for turkey RNA analysis. As in the studies with rats fed ≤2.0 μg Se/g, there were very few changes associated with high-Se feeding. Only 78 liver transcripts (31 up, 47 down) were changed ≥2-fold in chicks fed 1.0 vs. 0.3 μg Se/g; only 55 liver transcripts (14 up, 41 down) were changed ≥2-fold in turkeys fed 1.0 vs. 0.4 μg Se/g. Along with lack of growth depression due to feeding 1.0 μg Se/g to these

young birds, the near complete lack of changes in transcript abundance clearly showed that this level of dietary Se was not toxic, and that homeostatic mechanisms not linked to changes in gene expression were sufficient to readily accommodate this level of Se ingestion.

14.13 Molecular Biomarker Panels

The finding that Se status regulates GPX1 mRNA levels [7] and that this transcript can be used as a "molecular biomarker" to assess Se status and requirements suggested that individual transcripts or panels of transcripts have potential as biomarkers of nutritional status [34, 47]. In rats, total RNA isolated from whole blood or erythrocytes has been used successfully for assessing Se status [48]. Attempts to extend this to humans, however, have not been successful; plasma Se concentration and plasma GPX3 activity as well as mRNA biomarkers from a European population, ingesting an average of 46 μg Se/day, all resided on the plateau region of the corresponding Se-response curves [49].

Better biomarkers for determining upper limits for Se intake are needed [50]. The lack of major changes in non-selenoprotein transcript abundance, as well as selenoprotein transcript abundance, in rats fed ≤2.0 μg Se/g, and in chickens and turkeys fed ≤1.0 μg Se/g, however, showed that homeostasis is readily able to manage the elevated Se intake. The small set of transcripts with altered abundance in rats fed 2.0 μg Se/g provides an example of how transcript panels might be used as biomarkers for establishing upper nutrient limits. In this rat study, when individual transcript levels for the six transcripts regulated by 2 μg Se/g plus the five transcripts regulated by Se deficiency were analyzed by step-wise multiple regression [34, 47], the resulting equation predicted liver Se concentration with an overall correlation coefficient of 0.9988 ($P < 10^{-6}$), accounting for 99 % of the variation in liver Se concentration over the full range from 0 to 5 μg Se/g. This example illustrated that panels of selenoprotein and non-selenoprotein transcripts clearly have potential as molecular biomarkers of nutrient status [34, 47].

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