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Selenium at the Redox Interface of the Genome, Metabolome and Exposome

Jolyn Fernandes[#], **Xin Hu**[#], **M. Ryan Smith**[#], **Young-Mi Go**[*], and **Dean P Jones**[*]

Department of Medicine, Emory University, Atlanta, GA 30322

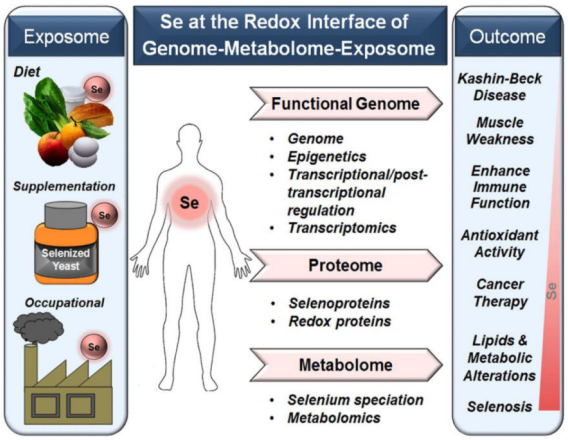
[#] These authors contributed equally to this work.

Abstract

Selenium (Se) is a redox-active environmental mineral that is converted to only a small number of metabolites and required for a relatively small number of mammalian enzymes. Despite this, dietary and environmental Se has extensive impact on every layer of omics space. This highlights a need for global network response structures to provide reference for targeted, hypothesis-driven Se research. In this review, we survey the Se research literature from the perspective of the responsive physical and chemical barrier between an organism (functional genome) and its environment (exposome), which we have previously termed the redox interface. Recent advances in metabolomics allow molecular phenotyping of the integrated genome-metabolome-exposome structure. Use of metabolomics with transcriptomics to map functional network responses to supplemental Se in mice revealed complex network responses linked to dyslipidemia and weight gain. Central metabolic hubs in the network structure in liver were not directly linked to transcripts for selenoproteins but were, instead, linked to transcripts for glucose transport and fatty acid β-oxidation. The experimental results confirm the survey of research literature in showing that Se interacts with the functional genome through a complex network response structure. The results imply that systematic application of data-driven integrated omics methods to models with controlled Se exposure could disentangle health benefits and risks from Se exposures and also serve more broadly as an experimental paradigm for exposome research.

Graphical abstract

[*]Corresponding authors: Dean P. Jones, Ph.D. Young-Mi Go, Emory University, 205 Whitehead Research Center, Atlanta, GA 30322. Tel: 404-727-5970. Fax: 404-712-2974. dpjones@emory.edu, ygo@emory.edu.



Keywords

dietary supplement; environmental toxicity; integrative omics; nutritional requirements; oxidative stress; selenium metabolism

I. Introduction

Selenium (Se) is a redox-active metalloid with complex roles in redox biology and medicine. Properties of Se allow stable structures with multiple oxidation states (−2, +2, +4, and +6), covalent bonding with non-metals (*e.g.*, carbon) and also strong coordination with metals [e.g., cadmium Cd)]. These properties provide biological value for Se in enzymes with relatively specific functions in detoxification and also in systems with very broad integrative functions. These uses of Se share a common characteristic of being components of the redox interface through which an individual utilizes environmental resources and responds to challenges to maintain cellular and molecular organization. The notion of a redox interface was introduced as a way to map out the multiple, and often overlapping, functional systems that serve to protect relatively reduced intracellular redox elements from relatively oxidizing (O_2-rich) environmental conditions [1]. This can be defined as the components of the physical, chemical, enzymatic and sequestration/chelation barrier system that protect redox elements of a living organism from environmental challenges. This is shown conceptually for Se in **Fig 1** based upon an earlier depiction of the redox interface between the exposome and functional genome [1]. In this schematic, Se is included as part of the exposome (left) because this is the primary exposure for most individuals. In addition, exposure from dietary supplements, occupational, therapeutic, consumer product and pollution sources can occur. On the right-hand side of this figure, the functional genome is represented by the omics layers, each of which requires or responds to Se exposure in the diet. Response at each level impacts multiple nodes within the network structure (Fig 1, center) which serves as the responsive physical and chemical barrier between the individual and his/her environment. Additional description of the roles of Se in this network structure are provided below. Delineation of this organizational structure will provide a systems level understanding of Se biology, including Se nutrition as well as Se toxicology, and ultimately lead to improved Se-related therapeutics in cancer and age-related disease.

The present review on Se at the redox interface is founded upon recent advances in redox theory, omics technologies and data-driven omics analyses, which have converged to create new opportunities to understand the organization and function of Se in complex systems. Redox theory was developed to complement genetics with four simple principles for molecular organization and function of complex living systems [2]. These principles provide a central logic to how cells maintain bioenergetics and metabolism and link these to macromolecular structure and function through reversible protein switches. Activation and deactivation of these protein switches in turn enables spatial and temporal organization within cells and cell populations. The collective function of these connections within an organism creates an adaptive network through which a genome utilizes environmental resources and protects against environmental threats (Fig 1).

Maturation of omics technologies has resulted in recognition that omics layers do not exist in isolation and that individual omics layers did not evolve in isolation. Indeed, data-driven omics integration now shows that transcripts can serve as central hubs for metabolites and metabolites can serve as central hubs for transcripts [3-5]. Thus, science has moved rapidly from reductionist models with a single cause and single effect to network models in which complex inter-omic interactions can be visualized and studied [6-9]. The reductionist and global approaches are complementary, with improved integrative tools being catalysts for transition in approaches and interpretations.

Consideration of Se at the redox interface offers an important prototype for broader consideration of genome-exposome interactions. Se is a component of the exposome, with human exposures ranging from nutritional deficiencies to excess environmental exposures which result in toxicities. Adaptation to variation in Se requires a broad range of compensatory responses. At the same time, only a very limited number of genes encode Se-containing proteins (25 selenoproteins in humans and 24 in mice), so only a small number of proteins are directly affected. The recent development of high-resolution metabolomics (HRM), with detection of tens of thousands chemical signals in biological extracts [10, 11], allows deep phenotyping of responses to variation in Se exposure [12]. HRM uses liquid chromatography (LC) or gas chromatography (GC) with ultra-high-resolution mass spectrometry and advanced data extraction algorithms to extend metabolic coverage beyond that obtained with commonly used targeted metabolomics platforms [11-13]. This detailed metabolic phenotyping enables connection of genetic responses to environmental Se exposures, *i.e.*, a genome × metabolome × exposome (G × M × E) analysis. Together with the extensive knowledgebase of Se nutrition, molecular biology, biochemistry and toxicology, an integrated systems view of Se biology is beginning to emerge, allowing greater understanding and precision for the nutritional requirements and toxicity of Se.

In this review, we start with an overview of Se as an integral component of the functional genome, follow with a summary of the Se metabolome, continue with a review of Se exposures through the human exposome, and finish with recent data-driven integration of the G × M × E of Se. The results show that an integrated omics approach can help define optimal Se intake. The results also indicate that integrated omics tools can help address complex questions of metal-metal interactions in nutrition and environmental health.

II. Se as an Integral Component of the Functional Genome

The functional genome is a global term encompassing the complex relationship between the genotype and phenotype in an organism. Epigenetic regulation, gene transcription, translation, and protein function are studied as components of functional genomics research in an attempt to understand biological outcome. Functional genomics thereby includes the integration of vast information obtained by high throughput methodologies, approaching a comprehensive view of the function of a cell. Se, an essential dietary metal, forms an integral component of this functional genome. As illustrated in Fig 2, the effect of Se is pronounced not only at the gene level but at multiple layers of functional genomics including pre-transcriptional, post-transcriptional and post-translational as well. This section highlights the role of Se at the different layers of the functional genome.

A. Selenoprotein genes

Se is co-translationally inserted in protein as the 21st amino acid, selenocysteine (Sec) and accounts for a vast majority of the biological activities of Se. The UGA codon in RNA serves as both a termination codon and, in selected contexts, as a Sec codon. Twenty-five selenoprotein genes have been identified in sequenced mammalian genomes [14]. These genes encode selenoproteins participating in multiple aspects of the cells such as redox signaling (GPX1, GPX3, GPX4, TRXRD1, TRXRD2) [15-18], protein folding and degradation (SEP15, SELS) and metabolism (SEPP1, SPS1, SPCS), which in turn alter regulation and gene expression [19, 20].

While Se incorporation into these proteins is essential for function, it is also well known that Se deficiency or excess regulates the transcription of these selenoproteins [21, 22]. Depending on Se dose, diverse effects of Se have been observed on different cellular functions. Hence Se affects several cellular and genetic functions associated with immunity [21], energy metabolism [23, 24] and cancer [25, 26]. Some of the diseases associated with selenoprotein gene polymorphisms include Kashin-Beck disease [27, 28], thyroid disease [29] and cancers, *e.g.*, colorectal [30, 31], prostate [32, 33] and breast [34]. These effects may be related to Se incorporation into protein when Se replaces sulfur in the biosynthesis of cysteine or methionine, thereby replacing these natural amino acids [35, 36].

The use of Se for glutathione peroxidase and thioredoxin reductase activities in redox homeostasis has been extensively studied [37-39], especially concerning the benefit in reactivity to incorporation of selenolate compared to the typical thiolate in biological systems [40-42]. Recent studies describe how factors such as the increased electrophilicity and reduced pKa of Sec (~5.2) versus Cys (~8.3) contribute to the effectiveness of Sec in enzyme catalysis [43-47]. In redox-regulated proteins, the use of Se instead of sulfur is thought to be favored to tune redox processes due to its orders of magnitude faster kinetics [48]. Ultimately this benefits the oxidoreductase catalysis activities that depend on the presence of Sec at the enzymatic active site (reviewed in [44]). Se is also incorporated into proteins as selenomethionine (SeMet), but this is generally believed to be a non-specific incorporation into protein. Alternatively, such Se-containing proteins could provide a way to sense excess Se and/or contribute to cellular dysfunction and toxicity.

B. Se affects epigenetic regulation

Se has long-term effects on gene expression through epigenetic mechanisms, such as acetylation/deacetylation of histone proteins (Fig 2, **Epigenetic Regulation**). Such epigenetic mechanisms modulate gene expression while the genetic sequence remains intact. Dysregulation of histone acetyltransferases and histone deacetylases have been implicated in a wide variety of diseases like cancer and inflammation [10–13]. Se supplementation in the form of selenite, which is a strong oxidant, in primary and immortalized macrophages, decreased histone acetylation in inflammation related genes such as NF-κB, COX-2 and TNF-α promoters. A similar decrease in histone acetylation by dietary levels of Se was also seen in colonic extracts from mice with inflammation induced by dextran sulfate [49]. The results suggest that the decrease in histone acetylation was due to inhibition in HAT activity (histone acetyltransferase). In addition to selenite, a number of other selenocompounds have been demonstrated as inhibitors of HDAC activity, as observed in human prostate cancer and melanoma, and in HeLa cell culture studies [50-52]. Long-term adaptation to dietary or environmental Se is also indicated by strong correlation of maternal Se levels with methylation patterns in offspring [53].

DNA methylation is another common epigenetic mechanism by which gene expression is regulated. Selenium in the form of SeMet increases global hepatic DNA methylation potentially by effects on one carbon metabolism (methyl donor for DNA methylation), including higher SAM/SAH ratio and serine hydroxymethyltransferase mRNA level [54]. In contrast, in a majority of cancer cells, Se compounds such as selenite and methylselininic acid (MSA) cause inhibition of DNMT activity or protein levels and decreased DNA methylation [49, 55-57]. The multiple types of Se compounds and differences in activities create a range of possible approaches so that epigenetic regulation by Se is an attractive approach for new cancer therapeutics. At the same time, these effects on DNA methylation suggest possible roles in cancer prevention which could be independent of previously studied antioxidant effects.

C. Se alters genomic stability

Low Se levels have been linked to cancer with several lines of evidence indicating an effect on genomic stability (Fig 2, **Genomic Stability**), and human interventional studies have suggested Se supplementation to be useful as a preventive strategy [58-60]. Lower Se levels have been associated with higher accumulated gene damage in high risk group for prostate cancer [61]. DNA damage measured by comet assay show a nonlinear dose response with increased damage induced by both low and high Se levels [62]. In further support of possible anti-cancer activity, Se protects against DNA damage from ionizing radiation [63] and UV light [64]. Selenite and SeMet are the most widely used compounds with extensive evidence for inhibition of mutagenesis, increase in excision capacity, increase in activity of DNA repair enzymes like glycosylases and reduction in DNA oxidation [65-67]. Collectively, the results show that Se influences the genome through maintenance of genomic stability. The complexity of the processes and forms of Se often preclude precise understanding of the mechanistic details. Application of integrated network approaches has the potential to improve this understanding.

D. Se modulates transcription factors

Se contributes to regulation of the functional genome through activation and repression of transcription factors controlling gene expression (Fig 2, **Transcription Factors**). Se at physiological levels blocks activation of the transcription factor NF-κB that regulates inflammatory gene expression [68, 69]. NF-κB, a cellular responder to oxidative stress, also regulates selenoprotein gene expression such as *SELENOS*, *GPX4* and *DIO2* [70]. This property of Se is widely being explored in cancer prevention [71]. Redox-active Se compounds modulate zinc finger motifs through oxidation. This influences gene expression and genomic stability because zinc finger motifs are an important component of multiple transcription factors, cell cycle and DNA repair proteins [72]. In the mouse liver, Se upregulates sterol regulatory element-binding transcription factor 1 (Srebf1) and thereby regulates lipid-associated genes [54]. While redox mechanisms are indicated, whether these occur by non-specific oxidative reactions of inorganic Se or specific biomolecular interactions of Se is difficult to resolve. It is noteworthy, however, that cellular studies suggest selenoproteins such as thioredoxins and GPX1 also regulate transcription factors, potentially through their antioxidant effect [73, 74].

E. Se alters the transcriptome

Transcriptomic analysis of selenoprotein genes has revealed extensive effects on differential gene expression pattern based on Se concentration and tissue of study (Fig 2, **Transcriptome**). Studies performed in 8 founder strains of mice, showed significant down-regulation of *GPX1*, *SELENOW*, and *SELENOH* transcripts in Se-deficient mice [75-78]. The regulation of selenoprotein transcripts by Se is not known, but for *GPX1* and *GPX4* mRNA, regulation is suggested to occur post-transcriptionally through nonsense-mediated decay, potentially due to the insufficiency of Sec-tRNA, but may occur through other factors as well [79, 80]. Global analysis of the seleno-transcriptome expression in human breast cancer MCF-7 and MDA-MB231 cell lines compared with healthy breast MCF-10A cells revealed downregulation of *GPX1*, *GPX4*, *GPX5* and *GPX7* and upregulation of *DIO2*, *GPX2* and *GPX3* genes [81].

While targeted transcriptomic pathways provide key information on Se-dependent responses, untargeted transcriptomics pathways reveal a comprehensive view of alterations in cellular patterns and insights into regulation of non-selenoprotein transcripts and related function. Transcriptomics analysis in human rectal mucosa in response to suboptimal Se levels showed cytoskeleton remodeling and reduction in inflammatory and immune responses. The differentially expressed genes belonged to cancer (80%), gastrointestinal diseases (58%), and inflammatory disease (39%) functional pathways [82]. Transcriptome analysis of goose T cells exposed to sodium selenite for 24 h showed that multiple selenoproteins involved in promotion of immune function and lysosome pathway related genes were promoted by Se stimulation, while the heat-shock proteins (HSP), interleukins (IL) and interferons were mainly down-regulated, suggesting weakened cytokine responses [22]. In relation to dietary Se and metabolism in juvenile rainbow trout, hepatic transcriptomic data further demonstrate that dietary Se increases the expression of networks for fatty acid synthesis and growth-related signaling cascades [23]. As discussed in more detail below, Se supplementation in mice to increase from an adequate intake (AI) to a four-

fold higher intake approaching the tolerable upper intake level (UL) showed similar effects on liver transcription.

Selenium is also known to mediate responses through post transcriptional regulation as noted by its influence on microRNAs (Fig 2, **Post-transcriptional regulation)**. Selenium deficiency in human colon adenocarcinoma cells (Caco-2) demonstrated altered expression of miR185, which in turn is responsible for expression of selenoprotein GPX2 and SEPSH2 [83]. Sodium selenite supplementation decreased miR328 levels (involved in cell apoptosis) in H9c2 cardiomyocyte cells [84]. In rat cardiomyocytes, miRNA microarray analysis showed highest expression change in miR374 in response to selenium deficiency [85]. Together with the transcriptome data above, these results show that variation in Se exposure has widespread effects on the functional genome. Such a range of molecular responses implies that effects occur through a global network structure response rather than through simple cause-effect relationships.

III. Se in the Metabolome

The quantitatively most important connection between the proteome and metabolome of animals occurs through two key Se-containing aminoacids, Sec and SeMet (Fig 3) [86]. Sec has the highest biologically activity and is different from Cys in several aspects. Sec contains Se in the selenol form; at physiologic pH, the selenol group is largely ionized to the selenolate, making it a more reactive nucleophile than the thiol of Cys. Selenols also have more negative redox potentials than thiols, and selanyl radicals are more easily produced than thiyl radicals. Free Sec exists at very low levels and at excess can cause toxicity to animal cells (IC_{50} in the micromolar range) via mechanisms involving oxidative stress, disruption in protein degradation, unfolded protein response and endoplasmic reticulum (ER) stress [87].

Unlike Sec, SeMet is relatively inactive and without apparent biomedical function distinct from Met because ribosomal machinery for protein synthesis does not distinguish between SeMet and Met. Consequently, the content of SeMet in proteins is proportional to the relative abundance of SeMet and Met in the organism. The dose of SeMet necessary to elicit toxicity in cultured cells varies vastly from micromolar to millimolar range and is highly dependent on the Met concentrations [59, 88]. Toxicity from SeMet is thought to arise from metabolism to other species of Se, most importantly Sec [87]. To gain a better understanding of the range of naturally occurring Se compounds in animals, a broader consideration of Se in microorganisms, plants and fungi is necessary.

A. Se in microorganisms, fungi and plants

The requirement for Se and distribution of selenoproteins are widespread in all forms of life. Sec-containing proteins have been identified in bacteria, archaea and eukaryotes, and the number of annotated selenoproteins in sequenced genome of diverse species has increased dramatically in recent years (provided in http://www.selenodb.org) [89]. The number and function of selenoproteins varies extensively across individual species [90-92]. Microorganisms require Se for Sec in selenoenzymes, mainly in glycine reductase, formate dehydrogenase and NiFeSe hydrogenase [93, 94]. The contribution of Se to support

functions of selenoproteins has received growing interest in the study of gut microbiome homeostasis in mammals [95]. Se intervention studies with germ-free mice show that dietary Se increases the diversity of the gastrointestinal microbiota and affects composition and colonization of the gastrointestinal microflora which in turn changes the host Se status and selenoproteome [96]. Oxidized forms of Se, *i.e.*, Se oxyanions, can also serve as electron acceptors in anaerobic respiration, forming nanoparticles of elemental Se [93] (Fig 3). An additional common metabolic transformation of Se in prokaryotes involves methylation, catalyzed by several methyltransferases [97].

Since the 1950s, the metabolism of Se in yeast has been extensively studied to elucidate molecular mechanisms of Se metabolism and toxicity. This research has yielded Se enriched yeast as both a model for Se research and an important form for dietary Se supplementation. In consideration of yeast for dietary supplementation, it is important to keep in mind that selenoproteins are completely absent in yeast and higher plants [91]. This means that yeast and plants do not have specific mechanisms to directly incorporate Sec into proteins. As a result, in yeasts and higher plants, inorganic selenium is used in the sulfur assimilation pathway to form selenoamino acids which are incorporated non-specifically into proteins in the place of methionine and cysteine. The differences of yeast and animal metabolism have been reviewed and also compared to plant metabolism [87]. In addition to the lack of specific mechanisms to incorporate Sec into proteins, differences exist in the trans-sulfuration (and trans-selenylation) pathway, with animals having unidirectional conversion of Met to Cys, plants having unidirectional conversion of Cys to Met and yeast having bidirectional interconversions. Other differences include de-selenylation mechanisms and in some plants, capacity to generate S-methyl-Sec.

Rao *et al*. [98] used high-resolution mass spectrometry to map out various oxidized and conjugated forms of Se metabolites in Se-enriched yeast. Results showed that the spectrum of selenocompounds includes many small molecules with Se replacing sulfur, such as analogues of glutathione, γ-glutamylcysteine, homocysteine and respective diseleno-compounds. Further high-resolution mass spectrometry analysis based upon selenium isotopic pattern demonstrated 64 Se metabolites, including selenoethers, Sec-containing di- and tripeptides, and selenoadenosine metabolites [99]. The results show that widespread substitution of Se for S occurs in yeast metabolism and raises the possibility that these Se metabolites may also be found in humans consuming Se-enriched yeast.

Higher plants and fungi do not require Se yet metabolize Se as part of mechanisms to protect against Se toxicity [86]. Plants can readily absorb the inorganic Se forms (*e.g.* selenate and selenite) (Fig 3) from soil and incorporate Se into Sec and SeMet. A detailed description of Se metabolism in plants was provided by Pilon-Smits [100]. Excess SeMet is converted to volatile dimethylselenide (DMSe), while Sec can be converted to elemental Se and Se-methylselenocysteine (SeMSC). The later may be converted to Se-methylselenomethionine and then to volatile dimethyldiselenide (DMDSe) for elimination. Se can also be accumulated to high levels by members of the genus *Astragalus* (Fabaceae). Animals that ingest these hyperaccumulator plants may convert methyl-Sec to Sec leading to Se poisoning in animals consuming these plants [101]. Other forms identified in plants include selenocystathionine (converted from Sec), γ-glutamyl-Se-methyl-Sec (from SeMSC), Se-

adenosyl-SeMet and Se-adenosyl-Sec (both from SeMet), γ-glutamyl-selenocystathionine, selenopeptides and selenohomocysteine [100]. A few of the forms are especially of interest, such as SeMSC and γ-glutamyl-Se-methyl-Sec, due to their therapeutic application in cancer treatment. However, despite the complex pathways of Se metabolism in plants, network analyses are not available and most of the understanding in Se metabolism is limited to the few major dietary forms (*e.g.* SeMet).

B. Metabolism of dietary Se in animals

Central reactions in the metabolism of Se in animal cells are well understood and have been extensively reviewed [87, 102-105]). The KEGG database provides a list of enzymes and selenocompounds that have been identified in these metabolic pathways (http://www.genome.jp/kegg-bin/show_pathway?map00450). Briefly, the predominant dietary form of Se, SeMet, is converted to Se-adenosylmethionine and undergoes methyl transfer to yield Se-adenosylhomocysteine (SeAH). SeAH is then hydrolyzed to selenohomocysteine (SeHCys) which is converted to Sec by the trans-sulfuration pathway. Excess SeMet is nonspecifically incorporated into body proteins in place of Met. Sec is obtained from the diet or from the catabolism of selenoproteins. Sec can release Se as hydrogen selenide (Fig 3). Inorganic Se forms, selenite and selenate, are reduced to hydrogen selenide through the glutathione and thioredoxin systems. For selenoprotein synthesis, hydrogen selenide is converted to selenophosphate, an active precursor of Sec. Hydrogen selenide can also be metabolized and excreted in the form of selenosugars and trimethylselenonium ions (TMSe). At extremely high levels of Se intake, volatile endproducts of Se are formed and exhaled as dimethyl selenide (known as the garlic odor) [105].

Compared to the selenoprotein biosynthesis precursors, less is known about the levels and other possible fates of hydrogen selenide and selenophosphate; a large gap has been consistently reported between the sum of identified urinary Se species and total urinary Se [102, 106], indicating a need for additional research in this area. Application of the high-resolution mass spectrometry methods used for yeast [98] could potentially improve understanding of the spectrum of selenometabolites and their nutritional and toxicological importance in mammalian systems.

C. Improved analytical methods for Se speciation

Despite the high-resolution mass spectrometry methods described above, the availability of analytical procedures for the determination of total Se and speciation in different environmental media [107], the complete speciation of Se remains a major challenge due to selenoamino acid incorporation and release during protein turnover. The mostly widely implemented analytical technique consists of a separation chromatography coupled with ICP-MS (inductively coupled plasma mass spectrometer), and often supplemented with other molecular mass spectrometry [107-110]. The compartmentalization of Se species can influence the serum Se levels for SeMet compared to inorganic Se supplement [86, 111]. Se in plasma is mainly present as selenoprotein P (SEPP1; 68 ± 7%), glutathione peroxidase (GPX3; 25 ± 4%) and associated to albumin (7 ± 4%). Sec and other selenols also undergo oxidation, so an additional challenge is avoidance of oxidation artifacts. Encinar *et al.* used a procedure where the protein-bound Sec was reduced and derivatized with iodoacetamide and

separated from SeMet. The species were then detected by ICP-MS equipped with a collision cell [112].

In serum, other low molecular weight Se compounds are minor and can be difficult to detect. Selenosugars are major urinary metabolites, and trimethylselenonium ion (TMSe) and selenite are present as minor products [102]. The responses of human urinary and serum Se metabolites are summarized in Table 1. Notably, selenosugar 1 responds most strongly to Se supplementation while TMSe only increases by a small amount suggesting that TMSe is only a byproduct of the methylation enzyme instead of a significant source of detoxification [106].

D. Metabolome-wide association study (MWAS) of Se

The development of orbitrap mass spectrometry provides capabilities to overcome analytical challenges for Se metabolism by providing improved sensitivity and specificity [87]. The application of orbitrap MS in yeast, described above, enabled mapping of minor species of Se metabolic pathways otherwise impossible to detect using traditional analytical procedures.

This approach has been expanded to metabolome-wide association study (MWAS) of Se in mice to provide information on metabolic pathways and functional networks which respond to variation in Se. Se supplementation (Na_2SeO_4, 20 μmol/L in drinking water) for 16 weeks in mice showed that many liver metabolites vary with Se supplementation [24]. These included acylcarnitines, triglycerides and glycerophospholipid, long-chain acyl-CoAs, phosphatidylcholines and sterols (**Supplemental Fig 1** and Fig 3 in [24]). Changes in lipid metabolism pathways were also observed in lung and plasma. Harup *et al.* [113] used urinary metabolomics to investigate the effects of Se nanoparticles and selenite in rats and also found that excess Se intake altered fatty acid metabolism.

E. The impact of Se intake on the whole metabolome: integrated omics

Mounting evidence suggests that Se intake over the range of adequate to excess levels significantly impacts the transcriptome and metabolome beyond effects on Se metabolism and selenoprotein abundance or activity. High doses of Se cause increased body weight and hyperinsulinemia, hyperglycemia, insulin resistance, glucose intolerance, and altered lipid metabolism in mice, rats and pigs [114-116]. Possible mechanisms involve a more reduced state and elevated glutathione peroxidase activity, with over-scavenging of H_2O_2, a second messenger for insulin signaling [75, 114, 117]. Research also shows, however, that plasma and tissue selenoprotein concentrations and activities reach a plateau after slightly exceeding adequate intake [75, 118, 119], and Se up to 20 times the required intake level does not change the transcript levels of 22 detected selenoproteins [119]. Thus, supplemental Se disrupts energy metabolism by mechanisms other than those controlling the abundance or activities of selenoproteins.

We recently developed integrated omics methods [120-122], which allow use of HRM data with other omics data to define central hubs in complex systems [123, 124]. In particular, transcriptome-metabolome wide association study (TMWAS) [120] provides an approach to

address complexity in nutrition and health, as this can show how specific nutrients interact with genes, proteins and metabolites [125]. By comparing Se intakes near the upper limit (UL) to those at adequate intake (AI) or recommended dietary allowance (RDA) levels, the central organizational structure of biologic responses to Se over an otherwise adequate range of intake was obtained [126] [120]. The research showed that Se supplementation induced changes in hepatic gene expression and associated metabolites in fatty acid metabolism, sterol and glucose homeostasis (**Supplemental Fig 1**) with no detectable differences in liver Se content as measured by ICP-MS, and no detectable differences in transcripts or activities for representative liver selenoproteins. Accumulation of acylcarnitines and other lipid metabolites and decreased bile acid metabolites suggested that Se supplementation altered fatty acid oxidation and created an imbalance with downstream energy metabolism dependent upon acetyl-CoA. Such a metabolic disruption could contribute to an increased body mass observed with supplemental Se [24].

IV. Se as a Ubiquitous Component of the Exposome

Such a systems level response is expected when one considers the natural history of the interactions of living systems with Se in the environment. Se is commonly found within the earth so living organisms have always had some level of exposure. In modern times, Se is used in commercial activities such as ore manufacturing and coal burning, with production of gaseous products such as hydrogen selenide and Se dioxide [127]. In non-occupational settings, individuals are exposed to Se through the diet, especially including the more organo-seleno compounds like Sec, SeMet, and seleno-phosphate [128] (Fig 3). The many biological roles, such as maintenance of antioxidant response, thiol/disulfide-like exchange, gut microbiome expression, and normal development, have occurred as a consequence of evolution of complex organisms with continual Se exposures [38, 44, 96].

As noted above, inorganic forms of Se from the soil are accumulated in plants and converted to more bioactive forms such as Sec or SeMet. In plants, Se also stimulates the synthesis of phytochelatins, oligomers of glutathione produced by phytochelatin synthase, which chelate Se and a range of metals for detoxification [129-131]. Se concentration in the soil varies widely upon geographic location, ranging anywhere from 0.01 mg/kg up to 1200 mg/kg [132, 133]. SeMet is the primary form of Se absorbed from plants. Highest intake of Sec occurs through consumption of animal tissues, especially organ meats [134, 135].

Importantly, the window between sufficient and toxic levels of Se intake in mammals is quite narrow (Fig 4). The minimum amount of dietary Se required for normal human function is 0.055 mg/d, while the majority of Americans tend to consume higher amounts, typically within the mean range of 0.108-0.151 mg/d depending on gender and Se supplement status [136, 137]. This range emphasizes the critical need to understand the adaptive systems which support normal function despite wide variation. This nutrient intake range also provides an excellent window to study Se in balancing the pro-oxidant and pro-reductant states within individuals, especially because either extreme state could cause deleterious effects. In principle, a large number of genetic and epigenetic variations could impact how an individual responds to this variation, either in terms of health benefit or disease.

With appropriate dietary intake, Se is sufficient to maintain cellular homeostasis through proper protein folding, cellular signaling, and maintenance of antioxidant defenses (Fig 4). Se deficiency, though rare in many countries, leads to changes in GSH metabolism, inactivation of several CYP-450's, and loss of many selenoproteins which ultimately leads to cellular dysregulation [138-144]. These cellular changes are often exhibited in symptoms which range from muscle weakness, cardiomyopathies, to more serious conditions such as Keshan disease and Kashin-Beck disease, which left untreated ultimately lead to death [145-148] (Fig 4).

Conversely, when the upper limit of Se levels of 0.4 mg/d is exceeded, Se toxicity can also occur. Pathologies such as selenosis, which involve factors such as increased oxidative stress and redox dysregulation can lead to cell growth inhibition [135] (Fig 4). Physical symptoms often include hair and nail loss, as well as nausea, skin lesions, and neurophathy [134]. To determine which species of Se is most toxic, yeast models have been used to examin toxicological profiles of seleno-compounds. These results showed that Sec and SeMet were both significantly less toxic than inorganic form of selenite [149].

Due to the interaction of Se with genome, transcriptome, proteome, metabolome, and microbiome, Se is an archetype for the study of genome-exposome interactions. The exposome represents the sum of all exposures for individuals and includes the cumulative biologic responses, both beneficial and noxious, as they relate to health [150, 151]. The inherent complexity of cumulative environmental exposures, in combination with the role of Se as an essential cog in eukaryotic cellular function, make Se attractive for a conceptual, "top-down", look at mechanisms of Sec in the function systems protecting living organisms from their environments.

V. Se at the Redox Interface of the Functional Genome, Metabolome and Exposome

The redox interface between a individual genome and respective exposome was presented as a collection of proteomic and metabolic pathways by which an organism responds to and protects against oxidative stress [1]. The original concept was founded upon the observation that the steady-state oxidation of cysteine residues in proteins mapped according to functional pathways rather than to subcellular compartments [126]. This led to consideration of possible mechanisms by which Cys residues in multiple proteins could be coordinately controlled [152]. Experimental studies with differential inhibition of thioredoxin reductase- and glutathione-dependent redox systems [153], as well toxicologic studies with cadmium [154], showed that either stimulated oxidation or impaired function of reductase systems resulted in pathway-specific changes in steady-state oxidation of Cys residues. A bilateral model to maintain stable steady-state oxidation of Cys residues contains Se-dependent systems at central control points for both reduction and oxidation (Fig 5). For reduction, the selenoenzymes, thioredoxin reductases, have a central role in reducing oxidized proteins. For oxidation, the selenoenzymes GSH peroxidases, as well as the thioredoxin-dependent peroxiredoxins, function to regulate the H_2O_2 supply for protein oxidation. The implication of this structure is that the entire redox proteome functions as a network with an inherent

stability to changes in oxidant burden [155]. This network is well suited to maintain cell functions at a relatively reducing redox poise despite the organism existing within a relatively oxidizing environment.

A. Energy extraction, bioenergetics; NAD, ATP and other energy currencies

The redox proteomics network structure is maintained by relatively low-flux redox pathways linked to NADP within an overall high-flux structure supporting energy metabolism. The high-flux pathways are near-equilibrium reactions linked to NAD [2]. In these high-flux reactions, metabolic fuels are oxidized to provide energy currencies, including transmembranal electrochemical gradients, ATP, acetyl-CoA and other chemicals used to support physical, chemical, electrical and osmotic work. NADPH is also used to support work, especially for reduction reactions in biosynthesis and detoxification; NADPH is coupled to the NAD system through the mitochondrial transhydrogenase. This organizational structure allows bioenergetics to be stabilized by fuel acquisition, storage and use for work while metabolic and macromolecular organization are maintained by kinetically controlled switches within the proteome [2, 156]. The central bioenergetics systems do not directly depend upon Se, but instead, depends upon an orthogonal system of redox regulation [157] which is critically dependent upon selenoenzymes. While all of the bioenergetic machinery is susceptible to oxidative stress, and selenoenzymes protect against oxidative stress, a more fundamental role for selenoenzymes may lie in maintenance of the metabolic and structural organization through reversible redox switches in proteins as described in the second principle of the redox code [2, 156]. In this operational structure (Fig 5), NADPH is used to generate H_2O_2 and other oxidants to maintain low-flux oxidation of sulfur switches in proteins and also to maintain thioredoxins and GSH to reduce the sulfur switches. With this stable Se-dependent redox structure in place, the organism has maximal capacity to tolerate variations in exogenous oxidative threats.

B. Se role in redox regulation; pro-oxidative and anti-oxidative redox properties

The four common oxidation states of Se (−2, +2, +4, +6) and the concentrations of chemicals with these oxidation states are important factors affecting cellular redox systems. Numerous studies show that Se has both anti-oxidative and pro-oxidative activities, depending on forms of the Se species [91, 158, 159]. Selenoproteins display anti-oxidative properties of Se, specifically via catalytically active Sec in their chemical structure [91]. Of the 25 human selenoproteins, reducing redox functions are shown by representative examples of redox reactions: *1)* Sec in GPX1 scavenges H_2O_2 [160] by reducing it to H_2O, and the redox state of Sec is restored in the active site by involving glutathione reductase which consumes NADPH as a source of reducing equivalents. *2)* Sec in TRXRD1 is present within the Gly-Cys-Sec-Gly redox active center and shows higher redox reactivity than Cys in thiol-disulfide exchange reactions [161]. *3)* Sec in methionine-R-sulfoxide reductase-1 attacks the sulfur atom of methionine-R-sulfoxide and releases reduced methionine by forming a seleninic acid [162]. These anti-oxidative properties of Se have provided strong justification for use of Se compounds for the prevention of cancer and protection against cardiovascular, infectious and neurological diseases.

In contrast to these antioxidant activities, selenite, selenocysteine, selenolates, hydrogen selenide and monomethylselenol (CH_3SeH), are highly reactive and have pro-oxidative properties (Fig 3). These compounds generate reactive oxidants, superoxide anion and H_2O_2 upon reaction with thiols [163, 164], and therefore are cytotoxic. Selenite (Se^{+4}) (Fig 3), a major inorganic Se compound, functions as an oxidant and is reduced to selenium dioxide and hydrogen selenide (Se^{-2}) by substrates such as NADPH [165]; hydrogen selenide functions as a potent reductant yielding elemental Se or seleno-phosphate in the presence of a proper electron acceptor [166, 167]. GSH and selenite spontaneously react to generate oxidants as well as several species of selenium intermediates including glutathioselenol, hydrogen selenide, selenodiglutathione, and elemental Se [166]. Selenite can similarly react with protein thiols and/or GSH to cause protein malfunction or misfolding as associated with cardiovascular disease, cancer, viral and bacterial infections, and other multiple diseases. The selenolates (RSe^-) and hydrogen selenide (HSe^-) (Fig 3) react with oxygen and thiols efficiently resulting in non-stoichiometric consumption of thiols and NADPH, increased levels of oxidants, and eventually stimulation of cytotoxic signaling and cell death mechanism [168]. In addition, selenium compounds, including SeMet and 1,4-anhydro-5-seleno-D-talitol (SeTal), react rapidly with oxidants to form selenoxides (SeMetO and SeTalO) [169].

Proteomics analyses have been used to study both beneficial and toxic effects of Se. In rice, lower doses of Se (2 and 6 mg/L sodium selenite) activated an anti-oxidative system and enhanced photosynthesis while higher Se treatment (10 mg/L sodium selenite) damaged photosynthesis apparatus and inhibited photosynthesis [170]. In embryonic lung fibroblasts, a strong correlation between Se and replicative senescence was observed, with increased oxidants and decreased antioxidant defense and cell metabolism in senescent cells [171]. In PC-3 prostate cancer cells treated with methaneseleninic acid (MSA) [172], MSA was rapidly reduced to methylselenol [173], a cytotoxic Se species suppressing tumorigenesis in animal models. A total of 194 proteins responding to MSA were identified as redox-sensitive. These proteins include p53, nestin and heat shock protein 70 (HSP70). HSP70 was one of the most redox sensitive in response to MSA from an early state (0.5 h) as well as late state (24 h). The results suggested that MSA and selenite were converted to the reactive monomethylated metabolite more efficiently than SeMet and resulted in rapid redox changes in proteins without affecting the expression levels [172].

C. Integration of signaling, control and responses to environmental challenges

The numbers of Se-containing metabolites and proteins with specific incorporation of Se are relatively small, yet Se broadly impacts all aspects of cell function. Effects on endogenous systems can be readily studied by targeted manipulations. In contrast, studies of interactions within the spectrum of environmental exposures are much more difficult because diets are complex and exposures are variable in frequency (continuous, repetitive, intermittent), variable intensity, and presence of co-exposures. Additionally, responses of individuals to exposures can be cumulative due to the irreversible nature of some biologic changes (e.g., mutations, cell selection in immunity, physical scarring). Thus, elaboration of a systems level understanding of Se will require development of a strategy for systematic study and development of a redox selenobiology atlas.

Practical aspects could include studies with varied Se exposure within a standardized grid of other exposures (Fig 6). For this, Se dosing could be standardized relative to Dietary Recommended Intakes (DRI) by using the Estimated Average Requirement (EAR), the Recommended Dietary Allowance (RDA), 3 × RDA and 10 × RDA (Fig 6). This would cover a range from marginally suboptimal to somewhat excessive levels. Temporal exposures could be continuous over lifespan and also sequentially varied for different exposure windows, *e.g.*, preconception, early embryogenesis, fetal development, early postnatal, childhood and sexual maturation, adult and aging (Fig 6). With these doses and time windows for exposure, application of available omics technologies to 36 dose-time windows would provide an initial look at the time-resolved integrated genome-metabolome-exposome network structure. This would identify central interactive hubs and communities of functional networks. The data would also provide a reference data set for more detailed time resolution and dose response studies.

This structure to map integrated Se network responses could be replicated with each of the nearly 40 essential nutrients, thereby elaborating the genome-metabolome-exposome network structure for all essential nutrients. For instance, Se level is known to impact functions of vitamin E, and levels of vitamin E impact function of Se [174, 175]. Vitamin E can at least partially compensate for loss of Gpx4 by protecting cells against deleterious lipid peroxidation [175]. Overlapping roles of Se and vitamin E show synergistic improvement of oxidative status of liver [176]. Some studies in animal models or cells report that most selenoprotein transcripts levels are altered by Se deficiency [177, 178]; whereas, in studies that supplement with vitamin E, the number of selenoprotein transcripts altered by Se status is considerably reduced [175, 179]. Thus, elaboration of the integrated network response structure for Se with other nutrients such as vitamin E will substantially improve understanding of system responses to oxidative stress.

This approach can be further used as a foundation to map the time-resolved interactions of Se with other essential nutrients during development and aging. The maps could also be extended to time-resolved interaction of Se with other exposures, such as exercise, environmental chemicals or infections. Such a structure naturally creates opportunity to evaluate any disease biomarkers, drug response or disease model in the context of Se exposures, thereby creating a universal structure to test responses to nutritional, pharmacologic and toxicologic challenges.

V. Summary and Perspective

Se is a widely distributed environmental mineral that is redox active and present in many forms in the human diet. Se is present in few metabolites and required for only a small number of selenoproteins, but every level of functional organization within mammals responds to Se exposure. Available research using metabolomics and transcriptomics shows that functional network responses to Se in mouse liver are linked to dyslipidemia and weight gain without detectable changes in Se content or selenoprotein transcript abundances or selenoenzyme activities. Application of such data-driven integrated omics methods to systematically study Se exposure will allow the biologic responses of Se to be disentangled from other exposures and enable precise understanding of health benefits and risks from

dietary and supplemental Se at an individual level. Such a research strategy would provide a prototype for molecular phenotyping of the integrated genome-metabolome-exposome structure for any dietary or environmental exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AI	adequate intake
Cys	cysteine
DNMT, DIO	iodothyronine deiodinase
DNA	methyltransferase
EAR	estimated average requirement
HDAC	histone deacetylase
HSP	heat shock protein
HRM	high-resolution metabolomics
ICP-MS	inductively coupled plasma mass spectrometer
G × M × E	genome × metabolome × exposome
GPX	glutathione peroxidase
KEGG	Kyto Encyclopedia of Genes and Genomes
Met	methionine
MSA	methaneseleninic acid
RDA	recommended dietary allowance
Se	selenium
Sec	selenocysteine
SeMet	selenomethionine
SEP	selenoprotein
SREBF	sterol regulatory element-binding transcription factor

TMSe	trimethylselenonium
TMWAS	transcriptome-metabolome-wide association study
TRXR	thioredoxin reductase
UL	tolerable upper intake level.

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Highlights

- ✓ Selenium (Se) is required for a small number of enzymes but dietary and environmental Se impacts every layer of omics space
- ✓ Metabolome and transcriptome interactions in mice reveal complex network responses linked to dyslipidemia and weight gain
- ✓ Central metabolic hubs in the network structure were not directly linked to selenoproteins but were linked to transcripts for glucose transport and fatty acid β-oxidation
- ✓ Global network response structures are needed to provide focus for targeted, hypothesis-driven Se research

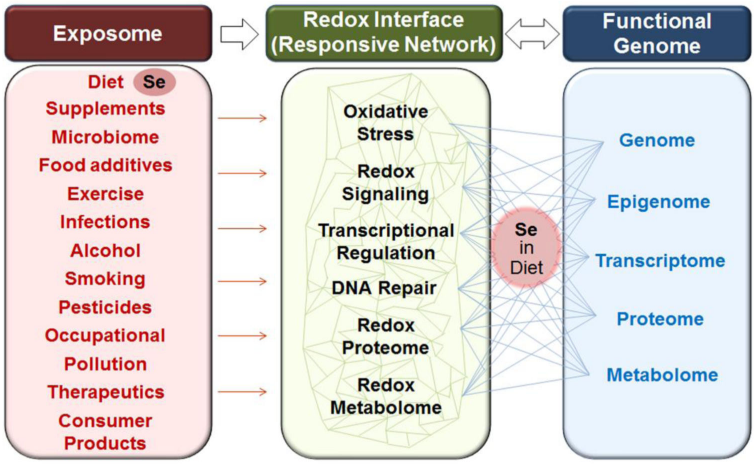


Fig. 1. Selenium at the Redox Interface.

The exposome (left) includes environmental exposures that complement the genome as determinants of health and disease. Se is an essential redox-active mineral present in mammalian food and environment. Se-dependent enzymes play a role in maintenance of macromolecular structure and function and also participate in the responsive physical and chemical barrier between an organism and its environment termed the redox interface. The redox interface (center) is a network of systems that function together to enable beneficial effects and protect against harmful environmental exposures. Se has a relatively limited number of metabolites and is required for only a small number of proteins, yet variation in Se impacts every level of omics space (right) and broadly impacts the redox interface network structure. Development of an understanding of this Se-related network structure will improve evaluation of health benefits and risks from Se and also serve as an example to more broadly develop systematic knowledge of the broad range of environmental exposures and also their interactions with variable Se intake.

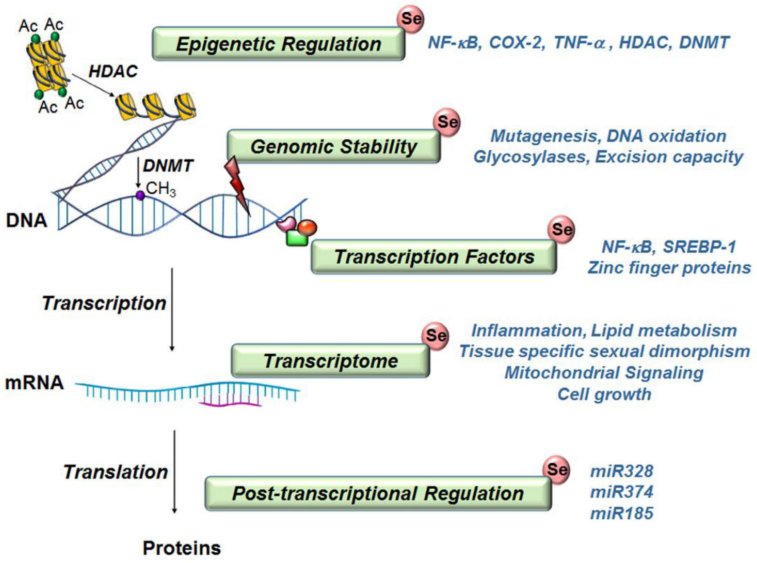


Fig. 2. Se has impacts on functional genomics.

Se mediates epigenetic regulation through changes in histone acetylation and DNA methylation potentially by alterations in histone deacetylases (HDAC) and DNA methyltransferases (DNMT). Low levels of Se lead to DNA damage and thereby impact genomic stability. Seleno-compounds decrease mutagenesis and DNA oxidation and increase DNA repair through glycosylases and excision repair. Se modulates transcription factors and thereby influences their function; examples include NF-κB for oxidative stress related pathways, zinc finger proteins for genomic stability and cell cycle, and SREBP-1 for lipid regulation. Extensive alterations in the global transcriptome occur in multiple tissue types and across multiple species in response to Se deficiency or excess selenium and affects immunity, cell growth and lipid metabolism. Post-transcriptional regulation involves influence of Se on multiple miRNAs responsible for protein synthesis and abundace in cell apoptosis or regulation of selenoproteins.

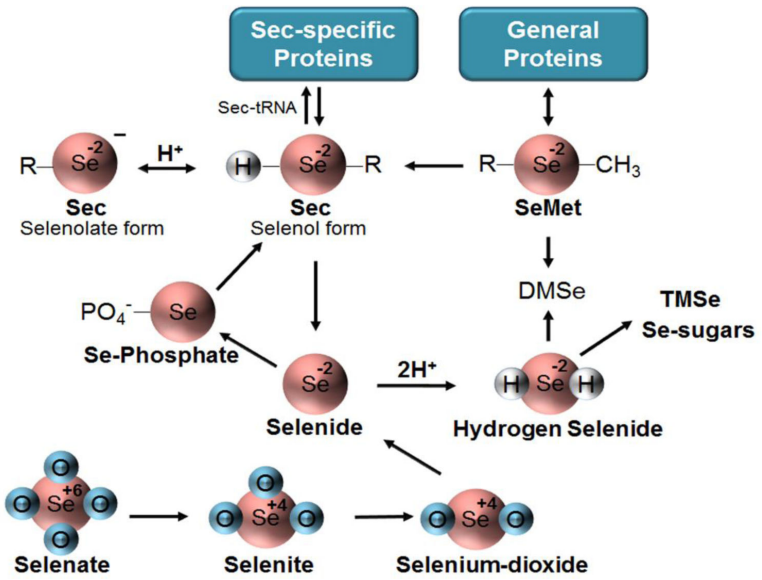


Fig. 3. Bioactive Selenochemicals.

Se is present in biological systems in many forms, both inorganic and organic, with Se at different oxidation states (shown inside circles) and the chemicals present at different ionization states (charge given outside circle). Inorganic selenooxyanions (bottom), such as selenate, selenite, and selenium dioxide, are reduced to hydrogen selenide through the glutathione and thioredoxin systems. Selenide is present as HSe^- at physiologic pH, converted to Se-phosphate and then to selenocysteine (Sec), existing mostly as the selenolate at physiologic pH. Sec is then incorporated into proteins (Sec-specific Proteins) via Sec-tRNA. Selenocysteine lysase rapidly converts Sec back to inorganic selenium quickly yielding selenide restarting the cycle. Selenomethionone (SeMet), a seleno-based nutrient absorbed from mammalian diets, can be incorporated into proteins in a non-specific manner (General Proteins), and can be converted to Sec or eliminated via conversion to dimethylselenide (DMSe). Hydrogen selenide also undergoes methylation to form either DMSe or trimethyl-selenide (TMSe) or can undergo conjugation with a sugar to be excreted from the biological system.

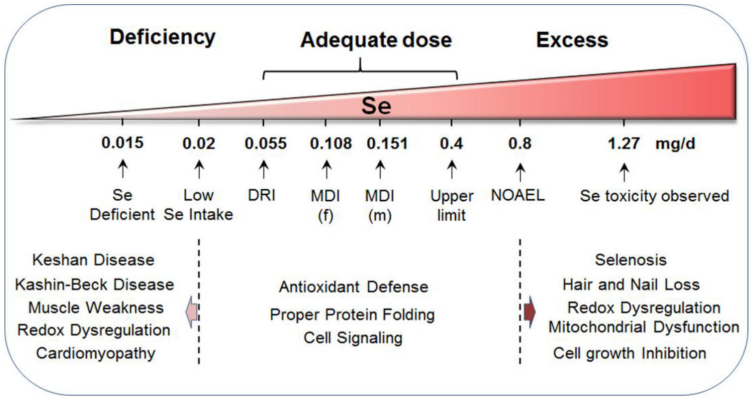


Fig. 4. Se dose range for individuals.

Se absorption is critical for normal cellular function and processes. Se-deficient individuals, clinically defined as people who have levels of Se lower than 0.015 mg/d, often undergo increased oxidative stress and are diagnosed with pathologies such as Keshan disease and Kashin-Beck disease as well as frequently experience moderate to severe muscle weakness and cardiomyopathy. The daily recommended intake (DRI) level for Se in the US is 0.055 mg/d, while other countries have differing ranges for basal and normal levels of Se status. In the US, the mean daily intake (MDI) has been found to be 0.108 mg/d for females and 0.151 mg/d for males after accounting for Se supplementation and Se in the diet. Within this range, normal cellular processes such as cell-signaling, protein folding, and anti-oxidant defense are performed. However, hyper-abundance of Se often is defined as being above the tolerable upper intake level (UL), which is set at 0.4 mg/d, while individuals with known cases of Se toxicity have Se serum levels of 1.27 mg/d. Se toxicity is found to dysregulate mitochondrial function and produce increased oxidative stress. Pathologies of Se toxicity, commonly defined as selenosis, involve symptoms such as the loss of hair and nails, halitosis, nausea, lesions and peripheral neuropathy.

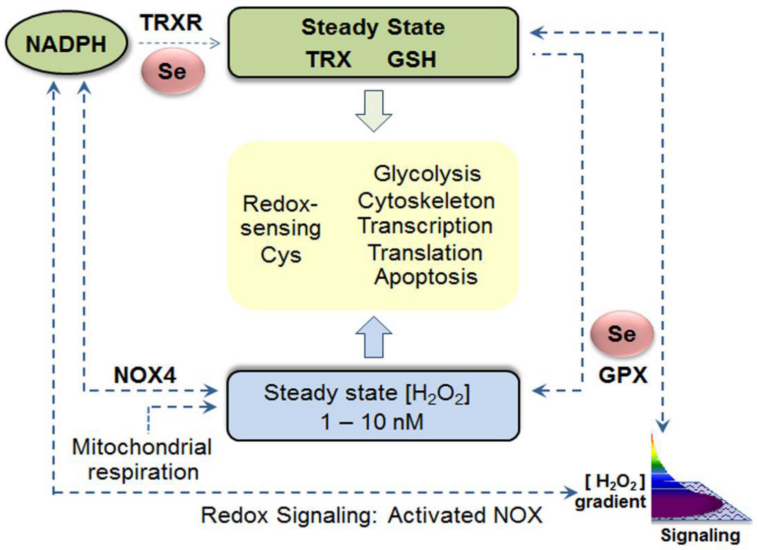


Fig. 5. Selenium-dependent enzymes support redox systems controlling the redox proteome.
The redox proteome includes reversible sulfur switches which serve to coordinate and regulate functional systems. The system operates with kinetic control through steady-state oxidation of protein Cys residues by H_2O_2 and other oxidants counterbalanced by steady-state reduction by thioredoxins (TRX) and GSH. The H_2O_2 availability is kinetically controlled by Se-dependent GSH peroxidases (GPX) and other control systems. The TRX system is maintained by Se-dependent thioredoxin reductases (TRXR). Figure based upon [2].

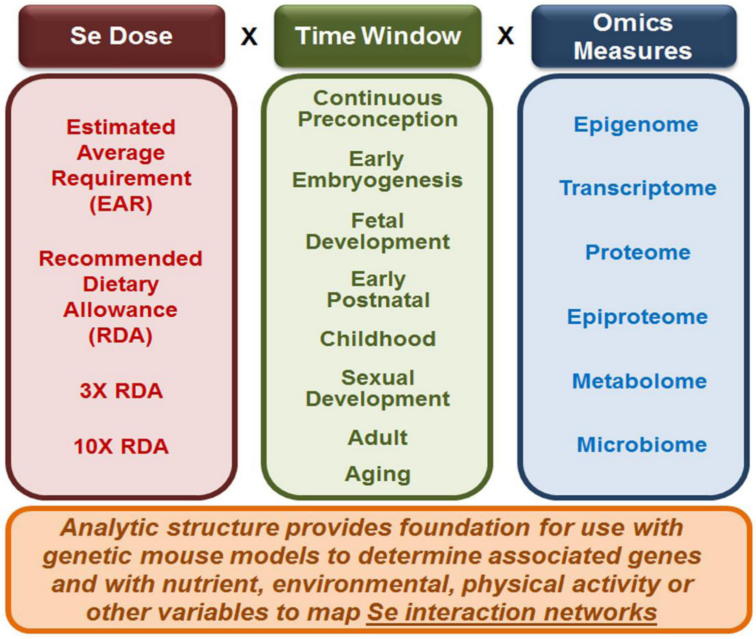
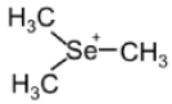
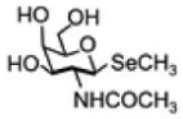
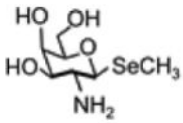
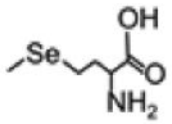
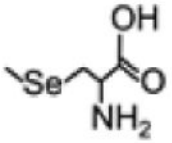
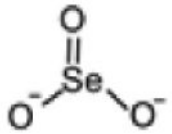


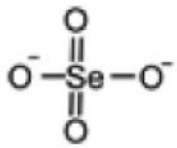
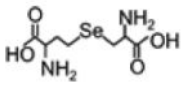
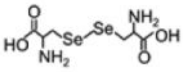
Fig. 6. Development of a data-driven foundation for redox systems biology of selenium.

A minimal analytic framework to develop a reference omics dataset for Se exposures includes systematic variation in dose of exposure (left), time window of exposures (middle) and omics measures of biologic responses (right). The Recommended Dietary Allowance (RDA), set at the amount of Se expected to be adequate in about 97% of the population, provides a useful starting point. The estimated average requirement (EAR) is a useful value for comparison because this level is expected to be inadequate in 50% of the population. A value 3X the RDA is useful to determine effects of excess Se within the adequate intake range, while a value of 10X the RDA is somewhat above the UL and expected to contribute to adverse outcomes. Omics data for continuous exposure to these levels would show the extent of epigenetic, transcriptomic, proteomic, metabolomic and microbiome responses to this spectrum of exposures from moderate deficiency to moderate excess exposures. This reference would provide a basis for comparison to effects from varied exposures in specific time windows (middle), with 1X RDA intake at other times. The continuous exposure data would also provide a reference to test for genetic differences in responses in genetic mouse models and to test for interactions of Se exposures with other dietary and environmental exposures (bottom).

TABLE 1.

Se metabolites idenfied from human urinary and serum.

Se metabolite	Structure	Monoisotopic mass	Baseline abundance	Response	Ref
TMSe (trimethylselenonium ion)		124.987	-Trace in urine and serum. -Varied between TMSe producers (major) and non-TMSe producers (minor or non-detectable) in urine	-Increase as a minor urinary product after SeMet supplement -Increase moderately in urine after selenite supplement -Minor product in urine	[106, 180, 181]
Selenosugar 1 (methyl-2-acetamido-2-deoxy-1-seleno-b-D-galactopyranoside)		299.027	-Small amount in urine and serum -Major metabolite in urine non-TMSe producers -Major urinary metabolite	-Increase to the major product after SeMet treatment -Respond strongly to SeMet treatment as the major metabolite	[102, 180, 181]
Selenosugar 3 (methyl-2-amino-2-deoxy-1-seleno-b-D-galactopyranoside)		257.0164	-Small amount in urine and serum -2nd major metabolite in non-TMSe producers -Major metabolite	-Increase to the 2nd major product after SeMet treatment -Increase moderately after selenite supplement	[106, 180, 181]
SeMet (selenomethionine)		196.9955	-Below detection limit in serum and urine	-Increase after L-SeMet supplement; -No response to other supplement	[180, 181]
SeMSC (Se-methyl-selenocysteine)		182.9799	-Expected but not detected	-Trace in serum. Increase in Se excreted in urine after supplementation with SeMSC	[182]
Selenite		127.9013	-Low amount in serum -Below detection limit in urine	-Unchanged in serum -Below detection limit in urine	[106, 180]

Se metabolite	Structure	Monoisotopic mass	Baseline abundance	Response	Ref
Selenate		143.8962	-Below detection limit in urine	-Increase moderately after selenite supplement in urine	[106]
Selenocystathionine		270.0119	-detected not quantified in the urine	-Increased in epithelial ovarian cancer	[183]
Selenocystine		335.9128	-detected and quantified in the serum	-decrease in cystic fibrosis patients compared to healthy controls	[184]