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Review Article

Selenium and GPX4, a vital symbiosis

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ABSTRACT

Selenium has transitioned from an environmental poison and carcinogen to an essential micronutrient associated with a broad array of health promoting effects. These beneficial effects are now accepted to be linked to its incorporation into selenoproteins, a family of rare proteins utilizing a specialized translation machinery to integrate selenium in the form of selenocysteine. Despite this recognized role, much less is known regarding the actual role of selenium in these proteins. Here, we will provide the reader with an overview of the essential role of specific selenoproteins and their link to pathology based on mouse studies and relevant mutations discovered in humans. Additionally, we will cover recent insights linking a non-interchangeable role for selenium in glutathione peroxidase 4 and its function in suppressing ferroptosis. This critical dependency ultimately generates a strong reliance on metabolic pathways that regulate selenium metabolism and its incorporation into proteins, such as the mevalonate pathway.

1. Introduction

In the year of 2017, we celebrated the 200 years' discovery of the trace element selenium (Se) by the Swedish scientist Jöns Jacob Berzelius [1]. It is now clear that selenium exerts its essential function as integral part of the 21st amino acid selenocysteine (Sec). Selenium incorporation into proteins entails a complex machinery both, for the synthesis of the Sec-specific tRNA (Sec-tRNA$^{[Ser]Sec}$) and its co-translational incorporation at the opal codon UGA. Yet, despite these cumbersome requirements, it is not clear why some organisms opt to use this complex biological set up. In light of the notion that selenoproteins are mostly accredited for their role in redox reactions, it is further intriguing to consider why not using cysteine instead of Sec since it has been shown that for many organisms cysteine-containing homologs of selenoproteins do exist [2].

Nonetheless, recent findings have helped us to provide a better understanding of the uniqueness of this trace element and its importance for tissues homeostasis [3,4]. Therefore, we will present the reader with some of the recent advances in understanding the importance of selenium-based enzyme catalysis with particular focus on human and animal studies linking selenium to disease conditions. Particular emphasis is given to the emerging role of the critical function of one distinct member of the selenoprotein family, glutathione peroxidase 4 (GPX4), linking selenium to cell homeostasis by suppressing ferroptosis.

2. Sec biosynthesis

A remarkable feature of Sec incorporation is that it is translated by the recoding of the STOP codon UGA [5]. UGA recoding of mRNAs encoding Sec-containing proteins in eukaryotes requires a *cis*-acting Sec insertion sequence (SECIS) element at the 3`UTR, which forms a stem-loop like structure and that serves as a recognition signal and platform for factors to allow Sec incorporation at its cognate anticodon. Upon recognition of the SECIS element at least two trans-acting factors, SECIS binding protein 2 (SECISBP2) and Sec-specific translation elongation factor (eEFSec), are recruited and are necessary for efficient recoding [6,7].

Moreover, the incorporation of Sec into proteins is a highly complex and energetically demanding process. Specifically, Sec, and in some specific cases cysteine, is distinct from other amino acids in that it is the only amino acid in eukaryotes whose biosynthesis occurs on its own tRNA, designated tRNA$^{[Ser]Sec}$. The synthesis of the tRNA is initiated by the amino acylation of serine on the tRNA$^{[Ser]Sec}$ in a reaction that is catalysed by seryl-tRNA synthetase (SARS) generating Ser-tRNA$^{[Ser]Sec}$ (Fig. 1). This is subsequently phosphorylated by phosphoseryl-tRNA kinase (PSTK), yielding P-Ser-tRNA$^{[Ser]Sec}$ thus providing the scaffold for Se loading on the tRNA [8]. Loading of Se on the P-Ser-tRNA$^{[Ser]Sec}$ requires first the conversion of selenide (HSe^-) into selenophosphate ($H_2SePO_3^-$), a reaction that is catalysed by SEPHS2 (selenophosphate synthetase 2) [9]. $H_2SePO_3^-$ is then used by SEPSECS (O-phosphoseryl-tRNA(Sec) selenium transferase) in a pyridoxal phosphate-dependent

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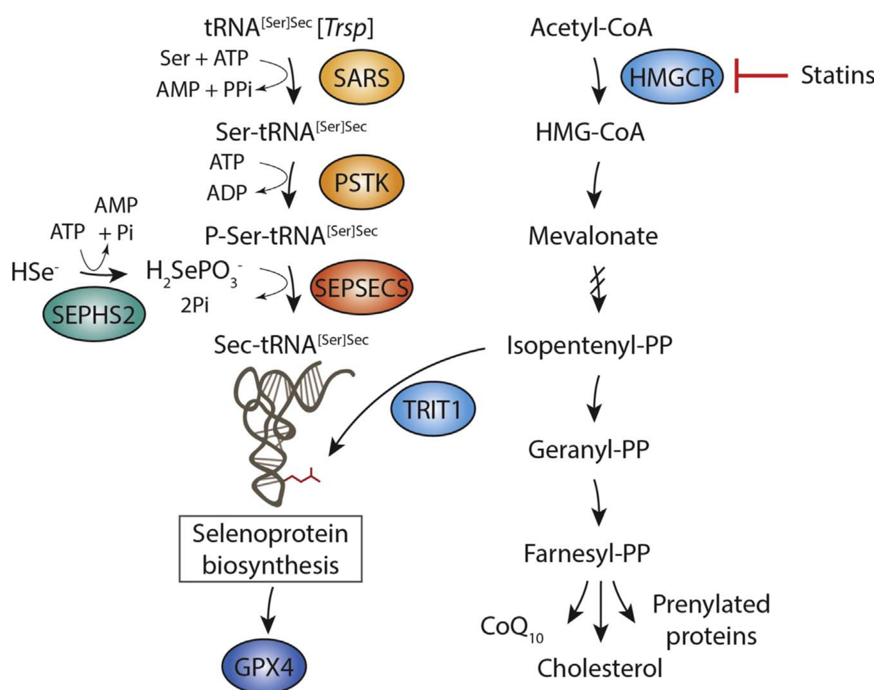

Fig. 1. Cross-talk between the tRNA[Ser] Sec and cholesterol biosynthesis pathways. The tRNA[Ser]Sec synthesis pathway is shown on the left, and the initial step of the mevalonate pathway on the right. For more details the reader is referred to the text (Abbreviations: GPX4, glutathione peroxidase 4; HMG-CoA, 3-Hydroxy-3-Methylglutaryl-Coenzym-A; HMGCR, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; PSTK, phosphoseryl-tRNA kinase; SARS, seryl-tRNA synthetase; SEPHS2, selenophosphate synthetase 2; SEPSECS, O-phosphoseryl-tRNA(Sec) selenium transferase; TRIT1, tRNA isopentenyltransferase 1; *Trsp*, synonym for nuclear encoded tRNA selenocysteine 2 (anticodon TCA)).

manner to incorporate Se in P-Ser-tRNA[Ser]Sec to yield the final product Sec-tRNA[Ser]Sec (Fig. 1) [10]. Interestingly, SEPHS2 is promiscuous and can also utilize sulfide (HS^-) leading to the formation of thiophosphate ($H_2SPO_3^-$), which consequently leads to the incorporation of cysteine in place of Sec, thereby generating Cys-tRNA[Ser]Sec [11]. This appears to be of relevance because it was already shown in mice that a fraction of thioredoxin reductase 1 (TXNRD1) in fact contains cysteine in the place of Sec, particularly under selenium-deprived conditions [12].

The efficiency of UGA recoding to Sec is also influenced by chemical modification of the Sec-tRNA[Ser]Sec. Among the most relevant modifications found in the Sec-tRNA[Ser]Sec are the modifications located in the anticodon loop. For instance, the Sec-tRNA[Ser]Sec population consists of two isoforms differing by the 2′-O-methylribose modification at position 34, designated Um34. This isoform appears to play an essential role in the expression of stress-responsive selenoproteins and is subject to regulation by Se concentrations. The synthesis of Um34 is dependent on the selenium status, and the resulting isoform, 5-methoxycarbonylmethyluracil-2′-O-methylribose (mcm5Um), is enriched under conditions of selenium sufficiency, whereas the unmethylated form, mcm5U, is abundant under selenium deficiency. Another critical modification is the N6-isopentyladenosine formation at position 37. Specifically, the formation of N6-isopentyladenosine at position 37 appears to influence the modification at Um34 (Fig. 1) [13].

3. Selenium, selenoprotein and disease

In humans, the micronutrient Se had been initially considered as a highly toxic micronutrient associated with hair loss, diarrhoea and emesis or even as a carcinogenic compound [14]. Yet, animals studies have later shown that Se is an essential trace element in mammals by preventing liver necrosis caused by vitamin E deficiency in rats [15]. Since then, it is recognized that Se plays most, if not all, of its biological functions through its use as an integral part of selenoproteins, which was first recognized in glutathione peroxidase 1 by two groups independently [16,17]. Later studies have shown that the human proteome consists of 25 distinct selenoproteins (24 in mice). Approximately half of them is allocated to members of the glutathione peroxidase (GPX), thioredoxin reductase (TXNRD), iodothyronine deiodinase family of proteins and others, which are unique in terms of structure and function [18]. Additionally, due to the inherent nucleophilicity of selenium-containing selenoproteins, it is believed that most of the selenoenzymes are involved in oxidoreductase reactions.

Up until now, several human diseases have been linked to defects in selenium metabolism and specific selenoproteins [4], and are thus briefly presented below (Fig. 2):

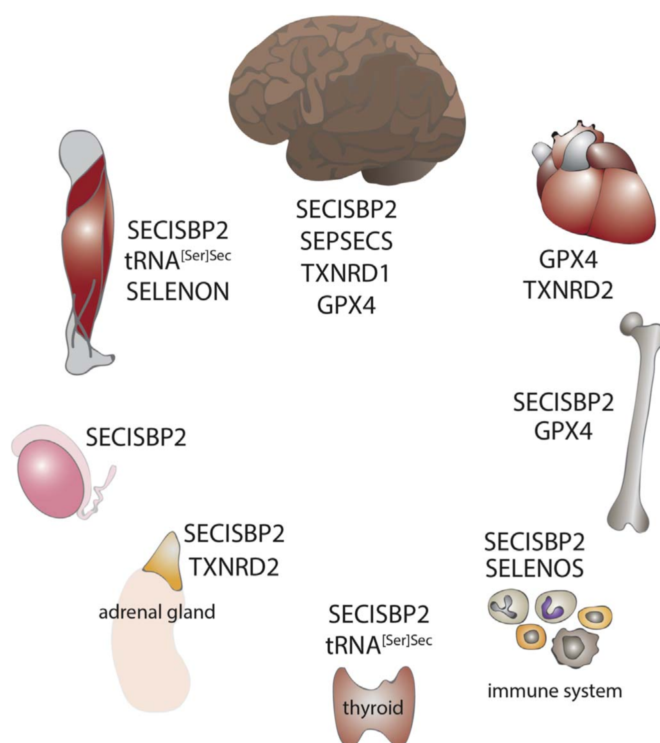

Fig. 2. Mutations in human genes involved in Sec metabolism and distinct selenoproteins. For details the reader is referred to the text (Abbreviations: GPX4, glutathione peroxidase 4; SECISBP2, SECIS binding protein 2; SELENON, selenoprotein N; SELENOS, selenoprotein S; SEPSECS, O-phosphoseryl-tRNA(Sec) selenium transferase; TXNRD1, thioredoxin reductase 1; TXNRD2, thioredoxin reductase 2).

a) *SELENON*
The first characterized inborn error in Se metabolism was identified in patients presenting rigid spine muscular dystrophy; the symptoms were then shown to be caused by mutations in selenoprotein N (SELENON) [19,20]. Later on, *SELENON* mutations have been characterized in a broader spectrum of disorders, now known as SELENON-related myopathy. Specifically, based on structural insights it is believed that SELENON is able to bind Ca^{2+} in the ER membrane, thus tentatively linking muscular selenium status and SELENON in Ca^{2+} handling [21]. The mutations so far identified in SELENON patients are complex and encompass mutations in the UGA codon as well as in the SECIS and SRE elements. These mutations are associated with a complete loss of selenium dependent activity. Supporting this, a mouse model lacking SELENON recapitulates the muscular phenotype of patients, particularly when "challenged" with physical exercise [22].

a) *SECISBP2*
Mutations in *SECISBP2* (previously called SBP2) were first reported with compound heterozygous defects in the *SECISBP2* gene [23]. Because of generalized impaired selenoprotein synthesis, these patients present a complex phenotype including azoospermia and sperm defects during later developmental stages of spermatogenesis, axial muscular dystrophy, impaired T lymphocyte proliferation, perturbed cytokine secretion by mononuclear cells and an increased sensitivity to UV light-induced skin damage. Remarkably, augmented systemic insulin sensitivity was another characteristic, which is in accordance with what was observed in *Gpx1* knockout mice [24]. Patients carrying mutations in SECISBP2 were also linked to resistance to thyroid hormones [25]. Since then, a series of patients have been identified, which have shown a broad spectrum of pathologies involving neurodegeneration and muscular impairments. The discrepancies in phenotypes observed for these patients is mostly accredited to different types of mutations [4]. Mouse studies using *Secisbp2* deficient animals uncovered its essential function during embryogenesis suggesting that the point mutations observed in patients affect specific functions of SECISBP2 by generating hypomorphic variants [26].

a) *SEPSECS*
Mutations in *SEPSECS* have been initially characterized in non-consanguineous Sephardic Jewish families of Moroccan or Iraqi ancestry [27,28]. Characteristics of SEPSECS deficiency involves a complex neurodevelopmental phenotype involving, but not limited to, microcephaly, delayed motor and intellectual development. Since its initial discovery mutations in *SEPSECS* have been described in other ethnicities. In the study of Anttonen et al., the authors described a marked laminar necrosis and a severe loss of myelin leading to neuronal loss and astrogliosis in patients carrying missense or nonsense mutants of SEPSECS. These mutants did not completely abolish SEPSECS function but were sufficient to impair the expression of the selenoproteins GPX1, GPX4, TXNRD1 and thioredoxin reductase 2 (TXNRD2) [29]. Currently, no mouse model of SEPSECS deficiency exists.

b) *tRNA$^{[Ser]Sec}$*
A patient carrying a C65G transversion in tRNA$^{[Ser]Sec}$ was recently described [30]. This mutation was mapped to the TΨC-arm and was associated with variety of symptoms, including abdominal pain, fatigue, muscle weakness, and low plasma levels of selenium. Further analysis revealed that this patient still sustained expression of selenoproteins but presented a decreased expression of stress-inducible selenoproteins, a notion consistent with the impairment of the 29-O methylation at ribose 34 of the tRNA.

c) *TXNRD1*
Mouse studies have identified TXNRD1 to be one of the most critical selenoproteins for embryogenesis as the targeted knockout of *Txnrd1* causes early embryonic lethality around E8.0 and E9.5 [31,32]. Intriguingly, a recent study has identified through whole-exosome sequencing a family with genetic generalized epilepsy to carry mutations in *TXNRD1*. The Pro190Leu substitution was shown to affect the highly conserved Pro. Characterization of fibroblasts and biopsies of these patients revealed a marked decrease in TXNRD1 activity and a strongly reduced Se incorporation [33]. In depth enzyme analysis demonstrated that the Pro190Leu mutant still presented approximately 30% of residual TXNRD1 activity. Hence, this suggests that partial activity of TXNRD1 might suffice for proper embryogenesis but not for specific activity of certain neuronal populations.

d) *TXNRD2*
Carriers of a mutant allele of TXNRD2 (Ala59Thr, Gly375Asp) have been associated with dilated cardiomyopathy – the respective mutations have been proposed to disrupt the FADH binding site [34]. Consistent with this, earlier studies showed that animals lacking TXNRD2 present dilated cardiomyopathy and perinatal mortality [35]. Despite this, functional analysis of these mutants in reconstitution experiments in *Txnrd2* knockout mouse embryonic fibroblasts showed that they functionally behave as null or even dominant-negative mutants as the patients carrying the mutant TXNRD2 alleles were heterozygous for these mutant forms. Moreover, a stop gain mutation in TXNRD2 (p. Y447X), leading to the complete absence of the TXNRD2 protein, was reported to cause familial glucocorticoid deficiency [36]. This indicates that patients null for TXNRD2 are viable, whereas mice lacking the *Txnrd2* gene invariably die during midgestation [35]. Hence, it seems that there are modifier genes that allow survival of given null mutations perhaps on mixed genetic backgrounds (as evident in humans), whereas on a congenic background the phenotypic manifestation is more severe as observed in *Txnrd2*$^{-/-}$ embryos. In fact, *Txnrd2*$^{-/-}$ embryos on a mixed genetic background (C57BL/6j × 129/Ola) develop until E18.5, while on a C57BL/6 background they die around E13.5 (Conrad, unpublished observation). A similar finding was recently reported for a targeted mutation of the active site Cys of GPX4 (see below).

e) *GPX4*
Mutations in *GPX4* leading to truncated GPX4 versions and thus to null variants were identified in two families affected with Sedaghatian-type spondylometaphyseal dysplasia (SSMD). SSMD is a neonatal lethal form of spondylometaphyseal dysplasia characterized by severe metaphyseal chondrodysplasia with mild limb shortening, platyspondyly, cardiac conduction defects, and central nervous system abnormalities [37]. Curiously, *Gpx4* deletion in mice leads to early embryonic lethality [38,39]; therefore, it is striking that the mutant patients still proceeded through embryogenesis with a truncated and inactive form of GPX4. Similarly, mice expressing a Sec to Cys variant in the active site of GPX4 are born and die at the pre-weaning stage (on a mixed C57BL/6J × 129S6SvEv genetic background), whereas they die during midgestation on a congenic C57BL/6J genetic background [40]. Whether this is due to a known mutation in the gene encoding the mitochondrial NAD(P) transhydrogenase, which is important for NADPH metabolism and thus GSH metabolism, on the C57BL/6 background remains to be investigated [41,42]. All these dichotomies suggest that genetic or environmental factors can have a strong impact on the severity of phenotypes related to the loss of GPX4 (and likely other selenoproteins as evident for TXNRD2).

4. Selenoproteins are essential for life

The vital role of Se for mammalian life as part of selenoproteins has been initially demonstrated by the landmark study of Bösl et al. [43], who generated a knockout mouse lacking *Trsp* (n-TUtca2; nuclear encoded tRNA selenocysteine 2 (anticodon TCA)), the gene encoding

tRNA[Ser]Sec. Thereby, the authors demonstrated that heterozygous mutants failed to generate viable homozygous mutant offspring. A detailed analysis showed that homozygous mutants die shortly after implantation and are resorbed, maximally reaching embryonic day 6.5 (E6.5). Interestingly, cultures of pre-implantation embryos demonstrated that trophoectoderm cells were able to grow and proliferate. Based on these findings, the authors rationalized the lethality observed in *Trsp* deficient animals was due to a cell non-autonomous effect; however, as it will be discussed later this appears to be not the case. Moreover, due to early embryonic lethal phenotype further studies on the specific role of selenoproteins had been precluded for some years.

Further insights into the role of selenoproteins in tissue development and homeostasis were gained with the generation of the first *Trsp* conditional knockout model by the group of Dolph Hatfield [44]. The generation of this mouse model provided an invaluable tool to perturb selenoprotein synthesis in a spatio-temporal manner. An initial assessment using this model focused on the specific deletion of *Trsp* in mammary epithelial cells using MMTV (mammary tumor virus) and WAP (*Whey acidic promoter*) driven Cre lines. Surprisingly, contrasting with the severe phenotype observed during embryogenesis loss of *Trsp* in mammary epithelial cells did not provoke any noticeable phenotype [44]. Following the generation of this conditional mouse line, several studies further investigated the role of selenoprotein in different tissues and allowed their implication in a wide array of biological processes that encompass immunity, carcinogenesis, stress adaptation and cell death [45–48]. In another model of conditional *Trsp* deletion, upregulation of Nrf2 (Nuclear Factor, Erythroid 2 Like 2, NFE2L2)–dependent target genes including NAD(P)H:quinone oxidoreductase 1 and glutathione S-transferase P1 was reported [49]. Consequently, the simultaneous deletion of *Trsp* and *Nrf2* severely hampers cell and overall mouse survival in macrophages and liver, respectively, indicating that loss of selenoprotein expression is in part counteracted by the Nrf2-dependent system. Such a relationship between Trps and Nrf2 also seems to apply for the erythroid lineage, causing anemia in respective knockout mice [50].

5. GPX4 and the pro-survival role of selenium

Despite the well-accepted role of selenium promoting its beneficial effects through distinctive selenoproteins, much less was known regarding the specific activity of certain selenoproteins. In this particular respect, the generation of knockout models for specific selenoproteins was instrumental for the understanding of the health promoting effects of selenium. For a comprehensive review of the knockout studies available, we direct the reader to recent reviews covering this in more detail [51–53].

Particularly important was the notion that apart from GPX4 no other selenoprotein closely resembled the phenotypes observed in *Trsp* knockout animals. For instance, as it was initially pointed out *Trsp*-deficient animals die at E6.5 [43], which is close to the early embryonic lethal phenotype observed in *Gpx4* knockout animals that die at E7.5 [38,39] (Table 1). This is particularly relevant since among all the other selenoproteins the only one that presents an equally early embryonic lethality is selenoprotein T (SELENOT) [54], and perhaps TXNRD1 depending on the genetic background [31,32]. Mice lacking TXNRD2 also die during embryogenesis, albeit significantly later (E13.5) [35].

Interestingly, conditional knockout models have provided strong evidence that GPX4 is essential to translate the beneficial effects of Se [55]. For instance, the study of Wirth et al. [56] compared the phenotypes resulting from the conditional deletion of *Trsp* and *Gpx4* using *CamKIIα-Cre* deleter strains, which restricts the gene loss to cortical and hippocampal neurons (Table 1). Using these models the authors demonstrated that *Trsp* and *Gpx4* knockout animals presented a marked neurological phenotype characterized by hyperexcitability, spontaneous seizures and ataxia. This phenotype was attributed to the selective loss of parvalbumin-positive interneurons, albeit the phenotype of *Trsp*-deficient animals was shown to be more severe.

The aggravated phenotype is justified by the potential additive effect of the loss of other selenoproteins beyond the loss of GPX4. In a similar approach, the authors used another neuron-specific Cre deleter strain under the control of the *tubulin-α1* promotor, namely *Tα1-Cre* transgenic mice, which restricts the genetic deletion to neurons, but is not restricted to any specific type of neurons. Thereby, the authors described marked cerebellar hypoplasia, associated with Purkinje cell death and decreased granule cell proliferation in both, neuron-specific *Trsp* and *Gpx4* knockout animals [57].

This critical role of Se to allow proper GPX4 functions does not appear to be solely restricted to neurons. Studies analysing the role of selenoprotein in liver have also indicated a similar critical relationship. Specifically, when comparing liver-specific *Trsp* and *Gpx4* knockout animals the authors presented an interesting finding. The liver-specific *Gpx4* deletion showed a more severe phenotype than *Trsp* knockout mice, dying 1–2 days after birth, while *Trsp*-deficient survived longer and died over a period of more than 3 months [58]. This observation is consistent with the concept that *Trsp* deletion would lead to a delayed loss of function of GPX4 compared to the knockout of *Gpx4*, a notion supported by the finding that indeed the loss of GPX4 is not as pronounced in *Trsp* knockout compared to *Gpx4* knockout mice. Therefore, the slower kinetic of deletion allows hepatocytes to build up an adaptive response, which was demonstrated to be through the up-regulation of several Nrf2 target genes [58].

Moreover, several additional conditional knockout models of GPX4 have been generated, which have helped us to further substantiate our knowledge on its in vivo importance. For instance, knockout of GPX4 in T-cells showed that CD8+ T cells from GPX4 null mice presented a marked defect in maintaining immune cell homeostasis in the periphery. Additionally, both antigen-specific CD8+ and CD4+ T cells lacking GPX4 were not able to expand upon acute lymphocytic choriomeningitis virus and *Leishmania major* parasite infections. This defect could be rescued with dietary α-tocopherol supplementation [59]. Similarly, *Trsp*-deficient T-cells presented a similar phenotype by being unable to mount an immunological response upon T cell receptor

Table 1
Overlapping phenotypes of *Trsp* and *Gpx4* knockout mouse models.

Tissue	Phenotype	Cre-line	Refs.
Embryogenesis	*Gpx4*, embryonic lethal at E7.5, whereas *Trsp* is lethal at E6.5	–	[38,43]
T-cells	T-cells are unable to proliferate and sustain an immune response	*Gpx4* (*Cd4-Cre*); *Trsp* (*LckCre*)	[59,60]
Keratinocytes	Epidermal hyperplasia, dermal inflammatory infiltrate, dysmorphic hair follicles, and alopecia in perinatal mice – *Trsp* KO are not viable	*K14-Cre*	[62]
Cortical neurons	Hyperexcitability, spontaneous seizures and ataxia due to the selective loss of parvalbumin-positive interneurons	*CamKIIa-Cre*	[56,64]
Cerebellar neurons	Cerebellar hypoplasia, associated with Purkinje cell death and decreased granule cell proliferation	*Tα1-Cre*	[57]
Liver	Marked liver necrosis; more pronounced in GPX4 KO	*Alb-Cre*	[58]
Hematopoietic system	Anemia caused by death of erythroid precursor cells	*Mx1-Cre*	[50,63]

Abbreviation: KO, knockout.

stimulation [60].

Studies of the role of GPX4 in skin epidermis using a *K14-cre* deleter strain have also revealed marked similarities between loss of GPX4 and *Trsp*. Both animals showed epidermal hyperplasia, dermal inflammatory infiltrate, dysmorphic hair follicles, and alopecia in perinatal mice. Nonetheless, unlike *Gpx4* knockout mice, which were able to recover and reach reproductive age, the *Trsp* knockout animals die a few days after birth via a still unsolved mechanism [61,62]. Furthermore, other studies have also highlighted a critical role of GPX4 and selenium in the physiology of the hematopoietic system. Specifically, mice lacking GPX4 in the hematopoietic compartment were shown to develop anemia due to an increase of cell death rates of erythroid precursor cells [63]. The anemic phenotype was also observed in mice lacking *Trsp* in the hematopoietic compartment [50]. Thus, it has become evident from in vivo mouse studies that the targeted loss of *Gpx4* and *Trsp* provoked phenotypes that share several similarities, hinting to the potential role of GPX4 to convey the pro-survival role of Se in a myriad of cell and tissue types [50].

6. GPX4 and ferroptosis

Initial studies on the mechanism of cell death induced by GPX4 loss/inactivation relied on the use of mouse embryonic fibroblasts derived from GPX4 conditional animals. These cells were genetically manipulated in order to express Cre-ERT2, allowing the disruption of GPX4 in cell culture at will upon the addition of 4-hydroxytamoxifen (TAM) [64]. Death of cells induced by GPX4 loss was shown to be unique as it occurred independently of classical proteins involved in cell death, such as caspases [64], and receptor interacting protein kinases [65], and is now known as ferroptosis [66]. Characterization of the events leading to cell death upon loss of GPX4 identified that it requires the specific oxidation of phosphatidylethanolamine (PE) containing polyunsaturated fatty acids (PUFAs), such as arachidonic and adrenic acid [67,68]. Ferroptosis execution downstream of GPX4 inactivation is driven by the specific oxidation of arachidonoyl-PE, a process that has been tentatively suggested to be carried out by the non-heme iron-containing lipoxygenases that insert stereospecifically molecular oxygen in free or esterified PUFAs [71]. Adding to that, it was recently proposed that phosphatidylethanolamine binding protein 2 (PEBP2) plays a critical role in regulating the activity of lipoxygenases, making them competent to accept esterified PUFAs as substrates [69]. Intriguingly, α-tocopherol has been proposed to directly inhibit lipoxygenases by outcompeting arachidonic acid access to the lipoxygenase active site [68]. Yet, despite providing a potential answer for the initial observation of the beneficial effect of selenium in the α-tocopherol dependent liver necrosis, unequivocal proof for the role of lipoxygenase in the ferroptosis process is still scant, and has been recently questioned. The specific oxidation of PE has been also associated with the mitochondrial damage observed during ferroptosis. This event appears to be required for the release of mitochondrial proteins such as apoptosis inducing factor 1 (AIFM1), which is observed upon GPX4 inactivation [64], or other ferroptotic triggers [70]. Nonetheless, the contribution of the release of mitochondrial factors to ferroptosis is still far from being clear. How the currently described mechanisms are involved in the pathological conditions therefore still remain open.

7. Se protects GPX4 from irreversible inactivation

Despite several hints indicating that GPX4 is in fact the limiting enzyme conferring the pro-survival role of selenium in most cases, unequivocal evidence was still missing. Therefore, we have recently developed a novel mouse model, where Sec in GPX4 was altered to cysteine [40]. It is interesting to note that, while some organisms like higher plants and fungi use the readily available sulfur to express Cys-homologs, mammals, fish, birds, nematodes, and bacteria still keep the energetically costly and inefficient process of selenoprotein expression. Therefore, this mouse model should provide us with the tools and means to answer what the actual advantage of selenolate- over thiolate-based redox catalysis is. As such, by using this mouse model, we were able to show that the *Gpx4*$^{cys/cys}$ mouse, unlike the full knockout, is viable and does not show any overt phenotype until approximately two weeks after birth, whereupon they had to be sacrificed. *Gpx4*$^{cys/cys}$ animals showed severe spontaneous seizures and were hyperexcitable, which could be explained in part by the selective loss of parvalbumin-positive interneurons. Remarkably, the same neurons were previously reported to be affected by the conditional deletion of *Trsp* [56]. Therefore, it emerges that selenium metabolism is critical for the survival of this specific neuronal subtype, and hence for mammalian life. Yet, it remains to be elucidated what makes this specific type of neurons so dependent on selenium-containing GPX4. On the molecular level, we could also demonstrate that the major advantage of selenolate- versus thiolate-based catalysis resides on the fact that Sec is inherently resistant to overoxidation, whereas the cysteine variant rapidly undergoes irreversible oxidation upon increased peroxide exposure [40]. This sustained peroxide exposure was sufficient to inactivate the cysteine variant and trigger ferroptosis. Even more intriguing was the fact that it was possible to generate cells deficient for *Trsp* in a GPX4-Cys background, whereas this was not feasible in the wildtype GPX4 background. Therefore, our findings refute the initial concept proposed by Bösl and co-workers [43], where they propose that loss of *Trsp* leads to cell death due to cell non-autonomous functions. Our findings provide evidence, that at least in proliferating cells, Se is exclusively necessary for the anti-ferroptotic role of GPX4. Moreover, we provide unequivocal evidence for a direct and essential requirement for selenium in one defined selenoprotein [40].

8. Cholesterol metabolism regulates Sec insertion and sensitivity to ferroptosis

An important feature that has recently re-emerged is the impact of cellular metabolic pathways impinging on the function of selenoproteins. Among the most relevant ones that deserves mentioning is the role of the mevalonate pathway, also known as the isoprenoid pathway. The mevalonate pathway utilizes carbons from acetyl-CoA to generate isopentenyl pyrophosphate (IPP) (Fig. 1). IPP is a metabolic intermediate used for the biosynthesis of a variety of biomolecules including cholesterol, heme, coenzyme Q and vitamin K just to name a few [72]. Additionally, in a subsequent step IPP is isomerized to dimethylallyl diphosphate (DMADP). DMADPP is a substrate for the tRNA isopentenyltransferase 1 (TRIT1). TRIT1 is responsible for the formation of the N6-isopentyladenosine at position 37 in a subset of tRNAs, including tRNA$^{[Ser]Sec}$ [73], as outlined above. This modification appears to be important for the maturation of tRNA$^{[Ser]Sec}$. Consistent with the relevance of the cross-talk between the mevalonate pathway with the selenoprotein coding machinery are findings indicating that the long term use of statins, known inhibitors of the mevalonate pathway, leads to a systemic repression of the translation of selenoproteins [74]. This long term use of statins has been associated with some of the phenotypes observed in animals models deficient for selenoprotein synthesis, such as myopathy [75] and hepatotoxicity [76]. Therefore, the use of ferroptosis inhibitors in combinations with statins might emerge as a potential beneficial combination in order to avoid unwanted side effects of the long-term usage of this class of compounds. This is supported by the notion that CoQ supplementation has a beneficial effect on statin-induced myopathies by a mechanism that could be partly related to ferroptosis suppression [77,78]. Particularly interesting to note is that in case of the hepatotoxic effects of statins a variable pattern of injury is present that might relate, among other causes, to differential levels of α-tocopherol recapitulating the findings observed in *Trsp* and GPX4 null livers [58]. Additionally, a recent report has demonstrated that tumors in a mesenchymal state acquire an unforeseen sensitivity to GPX4 inhibition and statin treatment, further supporting the notion that the

mevalonate pathway converges at the level of selenoprotein, in this particular case GPX4, to provide a pro-survival role [79].

9. Concluding remarks

After 200 years of its discovery, we are still learning and discovering intriguing aspects regarding the chemistry and biology of selenium and selenoproteins. The discovery that the selenoprotein GPX4 controls a novel and highly relevant cell death modality has further spurred the interest in the metabolism and function of new players regulating the biology of selenoproteins. Yet, further studies are still needed to characterize the importance and relevance of selenium in specific selenoproteins and why, and if, selenium is indeed required for their function. Essentially, this is still unresolved for many selenoproteins, but at least in the context of cell survival convincing evidence points to the absolute requirement of Se for the function of GPX4 [41]. We foresee that the next few years of research will bring more exciting insights into the function and regulation of selenoproteins in (patho)physiological contexts.

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