Chapter 18
Glutathione Peroxidase 4

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Abstract Glutathione peroxidase 4 (GPx4) is a selenocysteine (Sec)-containing glutathione peroxidase. GPx4 catalyzes the reduction of hydroperoxides and the oxidation of thiols through a ping-pong mechanism in which the redox transitions are faster than the formation of enzyme-substrate complexes; thus, K_m and V_{max} are infinite. The formation of a charge separation in the redox center accounts for this extremely fast reaction. In the absence of reducing substrate, the oxidized selenium is stabilized, forming a bond with a nitrogen atom in the backbone. This reaction, which protects the enzyme from inactivation, is particular of Sec and does not take place when Cys substitutes for Sec. The glutathione (GSH)-dependent reduction of phospholipid hydroperoxides accounts for the vital function of GPx4 and links the peroxidase to a new subroutine of cell death, named ferroptosis. This reaction is also related to protection from cardio-metabolic disorders and promotion of viral spread and infectivity. Finally, GPx4 is also competent for the oxidation of specific protein thiols when GSH is permissively low. This reaction accounts for midpiece stability and chromatin compaction in spermatozoa.

Keywords Ferroptosis • Glutathione peroxidase 4 • Glutathione • Lipid hydroperoxides • Lipid peroxidation • Quantum chemistry • Spermatogenesis • Vitamin E

18.1 Introduction

Glutathione peroxidase 4 (GPx4, E.C. 1.11.1.12) is a selenocysteine (Sec)-containing GPx (Se-GPx). GPx4 was first purified through chromatographic steps following an activity present in cell sap that leads to inhibition of membrane lipid peroxidation [1]. This “peroxidation-inhibiting protein” was named “phospholipid

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hydroperoxide glutathione peroxidase" (PHGPx) to account for its peroxidase activity on hydroperoxy derivatives of phosphatidyl choline [2]. The systematic name GPx4 was introduced later, following the discovery (in addition to the previously described tetrameric GPx1) of the other tetrameric homologs GPx3 [3, 4] and GPx2 [5]. The most remarkable functional difference between GPx4 and GPx1, and seemingly also GPx2 and GPx3, is the failure of the tetrameric enzymes to reduce hydroperoxide (ROOH) groups in complex lipid substrates in membranes [6].

18.2 Gene Structure

The *GPx4* gene produces three distinct mRNAs that differ at the 5′ ends, encoding mitochondrial (mGPx4), cytosolic (cGPx4), and nuclear (nGPx4) proteins [7]. Although the first two mRNAs result from a longer or shorter transcript of the first 1a exon including an upstream or a downstream translational start, respectively, the mRNA encoding nGPx4 results from an alternate promoter allowing transcription of an alternate 1b exon located within the first intron of the gene. As a consequence, mGPx4 and nGPx4 contain an N-terminal extension that is completely cleaved from the mitochondrial protein and only partially in the nuclear protein. Although mature c- and mGPx4 are identical, and thus, indistinguishable, mature nGPx4 has variable N-terminal extensions. These forms coexist in the nuclei of epididymal spermatozoa [8]. Although the cGPx transcript is found in both somatic and germ cells, mGPx4 is expressed at high levels only in male germ cells, and nGPx4 is expressed at relatively low levels in male germ cells and at even lower levels in somatic cells (see also Sect. 18.9)

18.3 Protein Structure

GPx4, as all other GPxs, shares a thioredoxin fold with members of several families of oxidoreductases [9, 10]. The Trx fold has a typical secondary structure pattern given by four β-strands flanked by three α helices, yielding two layers of an α/β/α sandwich in the final structural scaffold. In the GPx fold, there is an additional α-helix and a small β-sheet between β2 and α2.

Multiple sequence alignments and structural comparative modeling analyses of a large number of homologous GPxs revealed that the monomeric pattern is much more diffuse in nature than the tetrameric pattern. The latter is apparently restricted to vertebrata and descends from the insertion of the additional α-helix generating the inter-subunit interface [11].

18.4 Enzymatic Activity

GPx4 lacks specificity toward the ROOH substrate and accepts either small ROOHs, such as H_2O_2, or more complex ones, such as phosphatidylcholine hydroperoxide (PCOOH) or cholesterol and cholesterol ester hydroperoxides [12, 13]. In the

standardized routine assay for measuring GPx4 activity, the substrate is PCOOH dispersed in Triton X-100 micelles [14].

The interfacial character of the GPx4 reaction on membranes has never been analyzed in depth, although our unpublished evidence indicates that the enzyme binds to the membrane surface by electrostatic interactions and that the ROOH group of esterified fatty acids is exposed to water. The possibility of accommodating large ROOH substrates results from the absence of the loop containing the tetrameric interface, which is indeed a late achievement in evolution of the family [11]. It is tempting to speculate that the formation of tetrameric peroxidases provided a specific advantage, separating the control of ROOH content in the membrane or water phases.

With respect to reducing substrate, GPx4 is unusual in that it accepts several structurally unrelated low-molecular-weight thiols and specific protein thiols when the GSH concentration is low. Protein thiol oxidation by GPx4 has been observed as a critical redox event during sperm maturation [15], but could also be relevant for other, still unexamined, condition of redox signaling, when the ROOH flux exceeds the reducing capacity limited by GSH concentration. Notably, a similar protein thiol oxidation capacity controlled by the amount of GSH was also described for the Cys homolog GPx7 in the endoplasmic reticulum, where protein disulfide isomerase competes with GSH as reducing substrate [16].

18.5 Kinetics

Despite the distinct substrate specificity described above, the kinetic mechanisms of GPx1 and GPx4 are identical with their strictly conserved identical redox center [17, 18]. Steady-state kinetic analysis of the reaction fits the model of a ping-pong mechanism, in which the enzyme and substrate interaction and the release of products are much slower than the redox transitions. This prevents the accumulation of enzyme-substrate complexes and leads to infinite V_{max} and K_m values. For further discussion of GPx kinetics, see Chap. 17.

18.6 Catalytic Mechanism

The catalytic cycle of Se-GPx, in comparison to the Cys-containing GPx (S-GPx), was recently studied using a quantum chemistry approach based on density functional theory (DFT) methods [19]. This computational mechanistic investigation explored the potential energy surface of the system and identified the intermediates and transition states of the reaction energy profile. The complete calculated catalytic cycle for Se-GPx is shown in Fig. 18.1. A stable cluster of seven amino acids (Sec46, Gln81, Trp136, Asn137, Phe138, and Gly47, plus a second Gly residue to mimic a peptide bond linked to Gly47) (numbering is for rat GPx4) was selected as a prototype of the stable catalytic pocket.

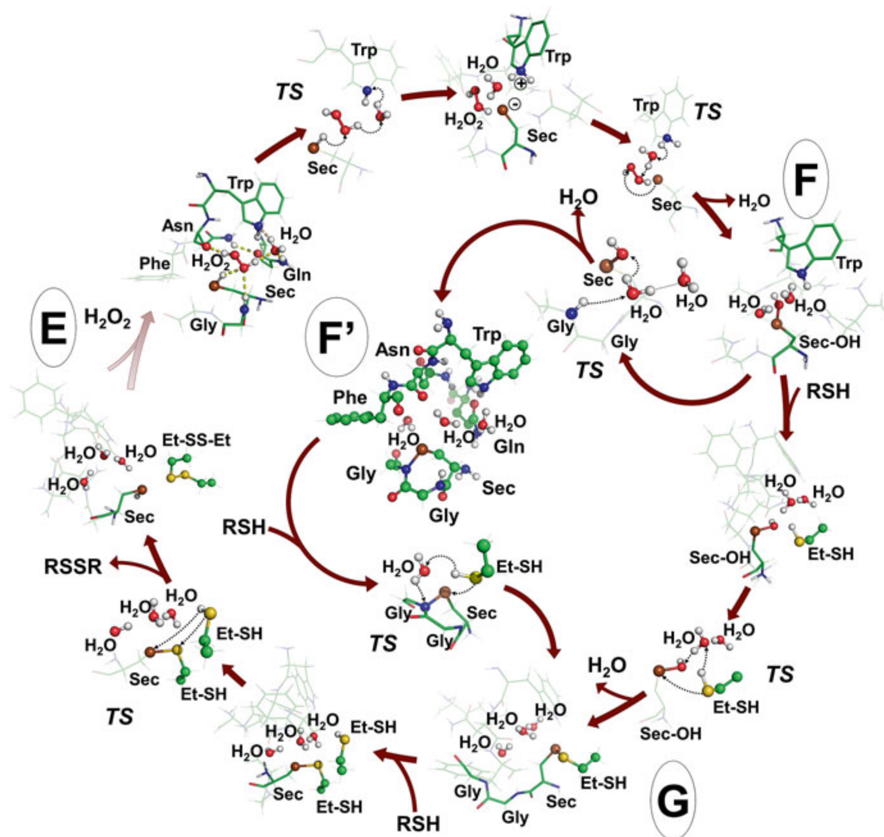

Fig. 18.1 Catalytic cycle of GPx4, focusing on the catalytic center studied using quantum level theory with the transition states (**TS**) of each step. Sec46, Gln81, Trp136, Asn137, Phe138, and Gly47, plus a second Gly to mimic a peptide bond linked to Gly47, are shown (numbering is for rat GPx4). The starting point is the reduced enzyme, **E**, followed by **F**, the enzyme containing Sec oxidized to selenenic acid, and **G**, the selenenylsulfide/disulfide intermediate. In the center of the figure, the formation of the eight-membered ring generated by the interaction of selenenic acid with the amide in the backbone (**F′**) is highlighted. **GSH**, modeled for simplicity as ethanethiol (Et-SH), is the reducing substrate, whereas **GSSG**, modeled for simplicity as Et-S-S-Et, is the corresponding disulfide

The coordination of a hydrogen peroxide molecule (H_2O_2 in Fig. 18.1) is thermodynamically favored and is stabilized by a hydrogen bonding network involving the selenol (or thiol in S-GPx) proton, the peroxide itself, a water molecule, and the indole of the Trp residue. Selenol (or thiol) deprotonation occurs via long-range proton transfer, leading to the formation of Sec^- (or Cys^-) and $TrpH^+$. This charge-separated intermediate is largely destabilized with respect to the reduced enzyme (**E**) and is converted, without appreciable activation energy, to the enzyme form in which Sec is oxidized to selenenic acid (or Cys to sulfenic acid) (**F**). The

reductive part is a two-step process involving two molecules of GSH, modeled for simplicity as ethanethiol (Et-SH), to form the selenenylsulfide (or disulfide) intermediate (**G**), which evolves in the presence of the second molecule of reducing substrate to regenerate **E** and the oxidized form of the reducing substrate (Et-SS-Et). The second reductive step has the highest activation energy and is thus the rate-limiting reaction of the cycle. The complete energy profile of Se-GPx is flatter than that of S-GPx, which influences the turn-over frequency ratio (TOF). TOF is also affected by the endothermicity of the last step, which is much slower when Cys substitutes for Sec (TOF Sec/Cys > 10^2). In Se-GPx, DFT calculations also support the mechanistic path in which the absence of reducing substrate leads to an oxidized intermediate identified by mass spectrometry as 2 amu lighter that **E**. This intermediate, **F′**, is an eight-membered ring generated by the interaction of selenenic acid with the amide in the backbone, one residue downstream of Sec. Thiolysis of the Se-N bond of the selenenylamide in **F′** (when the reducing substrate becomes available again) leads to the formation of **G**, which continues the cycle. The formation of **F′** seemingly protects Se-GPx from inactivation by over-oxidation and/or beta-cleavage leading to dehydroalanine (Dha). Notably, the formation of Dha from **F′** can only take place when the structure of the enzyme is disrupted, supporting the conclusion that the structure of the active site contributes to the stability of the oxidized form.

Evidence demonstrating that dimedone derivatizes selenium only when the reagent is present during the trypsinization of **F′** is fully consistent with and confirms the above mechanism (Zaccarin, M., unpublished). The selenenylamide bond is hydrolyzed only when the structure is disrupted, leading to the release of selenenic acid to react with dimedone. As opposed to Se-GPx, in the absence of reducing substrate, DFT calculations support a further oxidation of the sulfenic acid derivative for S-GPx, first to sulfinic and then to sulfonic acid, indicating that a S-GPx in the absence of a resolving Cys lacks protective mechanisms preventing oxidative inactivation.

18.7 Functions

GPx4 is needed for embryo development, cell survival, inflammation control, and male fertility. Recent evidence suggests roles in metabolism and viral infections, as well.

18.8 Genetic Diseases and Polymorphisms

In mice, ablation of *GPx4* leads to embryonic lethality, with hallmarks of programmed cell death at the gastrula level [20]. In humans, three *GPX4* mutations leading to truncation of GPx4 have been reported in a rare form of spondylometaphyseal dysplasia [21], a lethal neonatal disease characterized by severe

metaphyseal chondrodysplasia with mild limb shortening, platyspondyly, cardiac conduction defects, and severe central nervous system abnormalities. Interestingly, all three mutations, which are predicted to cause premature termination of GPx4, affect the enzyme downstream of the catalytic Sec.

A T/C variation at nucleotide 718 in the *GPX4* gene that corresponds to the 3′-UTR of the mRNA in Asian and Caucasian populations is apparently functional. In a cohort of patients with either adenomatous polyps or colorectal adenocarcinomas, a higher proportion of individuals with the CC genotype was observed in the cancer group [22]. Furthermore, this polymorphism was described as a predictor of stroke in patients with hypertension [23].

18.9 Male Fertility

GPx4 is necessary for survival of the immature male germ cell, stabilization of the spermatozoa midpiece, and chromatin condensation. Each of these functions is mediated by one of the *GPx4* transcripts, which target their products to discrete compartments, namely, the cytosol (cGPx4), mitochondria (mGPx4), or nucleus (nGPx4) (see also Sect. 18.2). Comparison of the phenotypes observed in genetic studies conclusively demonstrated that: i) cGPx4 accounts for the general vital function of GPx4 in cells, including immature male germ cells [24, 25]; ii) mGPx4, although not vital for cells, plays a major role in male fertility, generating the network of oxidized, cross-linked proteins that stabilize the midpiece of spermatozoa [24, 26]; and iii) nGPx4 is neither vital for cells nor critical for male fertility, but contributes to compaction of nuclear chromatin in maturing spermatozoa by oxidizing protamine thiols [27].

The vital function of cGPx4 is accounted for by the inhibition of lipid peroxidation when lipid hydroperoxides (LOOHs) are reduced and GSH is oxidized (see Sect. 18.11). The function of m- and nGPx4 in maturing spermatozoa involves the oxidation of protein substrates rather than GSH. In the final phase of male sperm maturation, mGPx4 indeed does oxidize and polymerize specific proteins of the mitochondrial capsule (i.e., the keratin-like amorphous sheath that surrounds the elongated mitochondria in the midpiece of mature spermatozoa), whereas nGPx oxidizes proteins of nuclear chromatin. This functional shift is seemingly triggered by massive GSH depletion, which occurs during sperm maturation [28]. This implies a physiologic role for ROOHs, albeit of a still unknown nature and source, in GPx4-catalyzed oxidation of protein substrates. As a result of the oxidative polymerization of proteins, mGPx4 eventually becomes entrapped in the oxidized network of the capsule, where it functions as a catalytically inactive structural component [15, 29].

Because the fine structures of both the mature mitochondrial capsule and the chromatin of mature spermatozoa have yet to be resolved, and whether a catalyzed reshuffling of mixed disulfides or selenodisulfides is also required remains unknown. In this respect, it has been suggested that the selenoenzyme, thioredoxin glutathione

reductase, which is the product of the *TXNRD3* gene, plays a role in protein disulfide proofreading [30].

18.10 Inflammation

GPx4 dampens inflammation by inhibiting lipoxygenase (LOX) and cyclooxygenase (COX) activity [31–34]. A comparative study showed that the activities of platelet 12-LOX, 15-LOX, and COX-2 are the most sensitive to GPx4, whereas that of 5-LOX is the least sensitive [33]. Inhibition is seemingly due to removal of LOOH required for the activation of the LOX and COX enzymes [35, 36].

A conditional knockout (KO) model of *GPx4* in neurons, in which mice develop astrocyte inflammation associated with reactive astrogliosis and neurodegeneration [37], confirmed the anti-inflammatory function of GPx4 in vivo. Moreover, transformed cells bearing an increased 12/15-LOX activity resulting from *GPx4* ablation, which surprisingly survive once implanted into mice yield, increased tumor angiogenesis [38]. This suggests that inhibition of eicosanoid production by GPx4 plays a role in biology of cancer progression.

Recent evidence indicates that the control of inflammation linked to the regulation of eicosanoid biosynthesis is a routine function of GPx4 and that regulation of GPx4 expression is part of the complex host-pathogen interaction. It was recently shown that *Salmonella typhimurium* modulates the activity of the host antioxidant machinery by dampening GPx4 expression in intestinal epithelial cells [39]. This occurs via a bacterial Type III secreted effector, SipA, and primes enterocytes to apically produce the pro-inflammatory 12-LOX product hepoxilin A_3, which governs the trans-epithelial migration of polymorphonuclear leucocytes associated with enteric infection. Suppression of GPx4 expression is discussed as a central mechanism governing the ability of *S. typhimurium* to evoke enteritis. Whether this effect is shared with GPx1, the expression of which is also decreased by *Salmonella*, requires further investigation.

18.11 Cell Death and Survival

Consistent with its vital function, GPx4 is also the essential component of a novel subroutine of cell death, designated ferroptosis by Dixon et al. [40, 41]. As it was recently reviewed [42] and is discussed in Chap. 43, ferroptosis will not be addressed in detail here. It is briefly noted that membrane peroxidation is the critical event leading to cell death by ferroptosis and that the series of biochemical events recapitulates the mechanisms of initiation and inhibition of lipid peroxidation, as widely studied in vitro beginning in the 1970s. Along with polyunsaturated lipids and oxygen, lipid peroxidation requires a reduced iron complex and yields, besides LOOH, reactive aldehydes, including malondialdehyde, 4-hydroxynonenal (HNE), and

truncated fatty acid chains. GPx4 and GSH, by reducing LOOH, prevent lipid peroxidation, indicating that initiation of the peroxidative chain reaction requires sparking amounts of preformed LOOH. Notably, the reduction of LOOH also inactivates LOX, because LOOH, seemingly by oxidizing catalytic iron, maintains these enzymes in an active state. Vitamin E (VE) slows down the rate of membrane lipid peroxidation by intercepting the peroxidation-driving lipid hydroperoxyl radicals (LOO•). In this case, the product of the antioxidant reaction of VE is LOOH, and thus, the activity of GPx4 is essential for the antioxidant effect of VE.

Given the pivotal role of traces of LOOH for the initiation of enzymatic and non-enzymatic lipid peroxidation, a relevant question emerges about the mechanism of their formation. A single mechanism has never been unambiguously defined. The most likely candidates involve hydroxyl radical, singlet oxygen, or iron-oxygen complexes. The practical outcome is that aerobic life continuously produces, by different mechanisms, traces of LOOH, from which enzymatic or non-enzymatic lipid peroxidation may be initiated by ferrous iron complexes or LOX. For this reason, we may conclude that the critical event initiating the lipid peroxidation chain reaction is a missed inhibition rather than an increased initiation rate.

Until its roles in specific aspects of cell biology and experimental pathology were brought to light by reverse genetic studies involving *Gpx4* KO, lipid peroxidation was primarily appreciated from a toxicological perspective. Cell death due to membrane lipid peroxidation primed by loss of GPx4 activity (i.e., ferroptosis) is seen today as the common mechanism of several degenerative diseases, including neurodegeneration [37, 43, 44], kidney failure [45], altered hematopoiesis [46], and possibly, ischemia-reperfusion injury [47]. Notably, another example of what today is known as ferroptosis was described in the early 1980s when a study involving single-photon counting in a perfused rat heart model showed that GSH depletion leads to severe contractile failure associated with lipid peroxidation [48].

The critical role of interplay between VE and GPx4 was demonstrated by evidence showing that VE slows down, but does not prevent, iron-induced lipid peroxidation in vitro in the absence of GPx4 and GSH [49]. In contrast, in cellular models, VE was shown to rescue death by ferroptosis [37], at least in the time frame of the experimental conditions. This possible discrepancy requires further consideration about the actual mechanism of VE in vivo. It is indeed worth investigating the hypothesis that VE prevents the formation of the first traces of LOOH by reducing the hydroperoxyl radical (HOO•) produced by protonation of superoxide anion, which may, as the species producing the traces of LOOH, be required for initiation. Notably, inhibition of a specific LOX could also explain the prevention of ferroptosis by VE. In summary, although VE and possibly other free-radical scavengers prevent or delay cell death by ferroptosis under some experimental conditions, the crucial anti-peroxidant mechanism is the reduction of LOOH, which relies exclusively on GPx4 and its substrate, GSH.

Ferroptosis is an appealing target for therapeutic strategies aimed at preventing cancer development and progression or halting degenerative diseases. Indeed, some cancer cells are particularly sensitive to GSH depletion brought about by cystine deprivation [50]. These cells are specifically sensitive to erastin, a compound identi-

fied by a high-throughput screening of small molecules as an inhibitor of the cystine/glutamate antiporter, x_c^-. In addition, an intracellular inhibitor of GPx4, (1S, 3R)-RSL3 (from oncogenic-RAS-synthetic lethality), triggers ferroptosis independent of the intracellular GSH concentration (see also Chap. 43). On the other hand, in a study involving kidney and an ischemia/reperfusion-induced tissue injury model, the spiroquinoxalinamine derivative liproxtatin-1 was shown to rescue cell death by GPx4 depletion by preventing lipid peroxidation; thus, liproxtatin-1 could be useful in preventing GPx4-mediated cellular degeneration [45]. Other compounds interfering with ferroptosis are currently being examined [42].

18.12 Metabolic Diseases

Although the role of GPx4 in ferroptotic cell death was revealed by complete *Gpx4* KO, a haplo-insufficiency transgenic model suggested a protective role in cardio-metabolic disorders related to obesity [51]. Also this effect of GPx4 is related to the inhibition of lipid peroxidation. When fed a high-fat, high-sucrose (HFHS) diet, *Gpx4*$^{+/-}$ mice exhibit much higher amounts of HNE adducts and protein carbonylation in the liver and heart compared with controls. Moreover, severe glucose intolerance, dyslipidemia, liver steatosis, and cardiac hypertrophy and fibrosis were observed [51].

The formation of reactive aldehydes, seemingly from decomposition of LOOH, was indicated as the primary agent responsible for cardio-metabolic derangements related to obesity. The observation that *Gpx1*$^{-/-}$ mice are protected from insulin resistance and liver steatosis, despite exhibiting increased systemic oxidative stress associated with diet-induced obesity [52], highlights the relevance of the peroxidase activity of GPx4 in preventing metabolic disorders. Moreover, as a HFHS diet induces transcriptional activation of *Gpx4* in *Gpx4*$^{+/-}$ mice, the presence and activation of a compensatory response mechanism can be conceived. This mechanism would account for the maintenance of oxidative/anti-oxidative homeostasis until the efficiency of GPx4 declines, leading to a pathologic alteration of the redox steady state [53].

The apparently paradoxical observation that haplo-insufficient *Gpx4*$^{+/-}$ mice live longer than wild type littermates [54], which is ascribed to an increased 'apoptosis', is consistent with evidence that GPx4 plays a role in controlling intracellular reactive aldehyde production. Seemingly, in *Gpx4*$^{+/-}$ mice, increased amounts of aldehydes derived from lipid peroxidation could play a protective hormetic function as nuclear factor-E2–related factor 2 activators [55].

18.13 Viral Diseases

A new avenue for GPx4 and VE biology research was uncovered recently in virology. The genomes of positive-stranded RNA viruses such as hepatitis C virus (HCV) are synthesized by a multiprotein replicase complex that assembles in association

with host intracellular membranes known as ‘the membranous web’ [56, 57]. The integrity of these membranes is vital for viral replication and infectivity, and therefore, endogenous lipid peroxidation is detrimental to viral replication [58]. Most HCV strains replicate poorly in cell culture. However, strain JFH1, a genotype 2a virus recovered from a patient with fulminant hepatitis, was shown to replicate efficiently in cell culture, thus attracting the attention of investigators. In Huh-7 hepatoma cells, JFH1 infectivity and replication are not restricted by oxidative damage or increased by VE [58]. This suggests that the replication and infectivity of strain JFH1 are not affected by lipid peroxidation and supports the hypothesis that the virus manipulates the host antioxidant capacity, inducing resistance. Consistent with these observations, it was recently shown that an adapted JFH1 strain induces both *GPX1* and *GPX4* transcription, although only GPx4 is required for HCV infection. Interestingly, *GPX4* mRNA is indeed elevated in biopsies from chronic hepatitis C patients and returns to baseline upon virus eradication [59]. Nevertheless, it was recently disclosed that in cell culture, replication of pan-genotypic HCV requires the product of the *SEC14L2* gene, tocopherol-associated protein 1, which enhances VE accumulation in cells [60]. In summary, emerging data indicate that VE and GPx4 are essential host components for HCV replication and spread. Whether this is the case for other positive-stranded RNA viruses and whether GPx4 and/or the enzymes of the GSH biosynthetic pathway and/or *SEC14L2* are suitable targets for antiviral therapies are stimulating hypotheses worthy of further investigation.

18.14 Conclusions, Unresolved Questions, and Perspectives

In the more than three decades since the “peroxidation inhibiting protein” was identified, several aspects of the intriguing enzymology of peroxidation have been elucidated, and major insights into the multifaceted physiology of GPx4 have been revealed. GPx4 is an oxidoreductase that reduces LOOHs in membranes at a very fast rate by taking advantage of the unusual chemistry of selenium. The catalyst is regenerated when the oxidized redox center is reduced back by GSH in a reaction in which the unusual reactivity of selenium again maximizes the rate. If GSH is limiting, the oxidizing capability of GPx4 can be directed toward alternative substrates. To date, this remarkable activity has been identified only in the last phase of sperm maturation.

The evolutionary acquisition of Sec in the redox center of GPx provided an unambiguous advantage by lowering the activation energy of each step of the catalytic cycle, leading to a much higher turnover frequency of the catalytic cycle for Sec- versus Cys-supported catalysis. However, although relevant, this is not the unique advantage of selenium versus sulfur. When the reducing substrate is limiting, the sulfenic acid derivative of Cys undergoes further oxidation, first to a sulfinic acid and then to a sulfonic acid derivative. Instead, selenenic acid produced in the catalytic cycle, if not promptly reduced by GSH, rather than undergoing further oxidation, binds to an amide group of the protein backbone, forming a selenenylamide. This species is stable in the active site until the cycle is reactivated by

GSH. An irreversible inactivation by beta-cleavage, which transforms Sec into Dha, may also take place with Sec linked to the amide, but this appears to occur only when the native conformation is lost. Determining whether this inactivation has a pathologic or physiologic significance will be a relevant issue for future studies.

Two aspects of the biochemistry of GPx4 suggests a physiologic scenario: i) inhibition of enzymatic and non-enzymatic lipid peroxidation, which protects cells from death by ferroptosis; and ii) the oxidation of some specific protein thiols, which imparts fertilizing capability to mammalian spermatozoa. Both functions have been independently verified in animal models. Determining whether these functions of GPx4 converge in a coherent physiologic context will be challenging. The most plausible sparking event is the decrease in cellular GSH, at least in the area surrounding GPx4. The overall scenario is that of a redox signal transduction pathway, specifically starting at the surface of membranes—certainly a challenging area for further studies on the "peroxidation inhibiting protein".

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