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Multiple functions of 2-Cys peroxiredoxins, I and II, and their regulations via post-translational modifications

Sue Goo Rhee[a,b,*], Hyun Ae Woo[c]

[a] Yonsei Biomedical Research Institute, Yonsei University College of Medicine, Seoul, 120-749, South Korea
[b] The Biochemistry and Biophysics Center, NHLBI, National Institutes of Health, Bethesda, MD, 20892, USA
[c] College of Pharmacy and College of Natural Sciences, Ewha Womans University, Seoul, 120-750, South Korea

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ABSTRACT

Peroxiredoxins (Prxs) are an unusual family of thiol-specific peroxidases that possess a binding site for H_2O_2 and rely on a conserved cysteine residue for rapid reaction with H_2O_2. Among 6 mammalian isoforms (Prx I to VI), Prx I and Prx II are mainly found in the cytosol and nucleus. Prx I and Prx II function as antioxidant enzymes and protein chaperone under oxidative distress conditions. Under oxidative eustress conditions, Prx I and Prx II regulate the levels of H_2O_2 at specific area of the cells as well as sense and transduce H_2O_2 signaling to target proteins.

Prx I and Prx II are known to be covalently modified on multiple sites: Prx I is hyperoxidized on Cys^{52}; phosphorylated on Ser^{32}, Thr^{90}, and Tyr^{194}; acetylated on Lys^{7}, Lys^{16}, Lys^{27}, Lys^{35}, and Lys^{197}; glutathionylated on Cys^{52}, Cys^{83}, and Cys^{173}; and nitrosylated on Cys^{52} and Cys^{83}, whereas Prx II is hyperoxidized on Cys^{51}; phosphorylated on Thr^{89}, Ser^{112}, and Thr^{182}; acetylated on Ala^{2} and Lys^{196}; glutathionylated on Cys^{51} and Cys^{172}; and nitrosylated on Cys^{51} and Cys^{172}. In this review, we describe how these post-translational modifications affect various functions of Prx I and Prx II.

1. Introduction

Peroxiredoxins (Prxs) are a large family of peroxidases that reduce peroxides such as hydrogen peroxide (H_2O_2), alkyl hydroperoxides, and peroxynitrite [1–4]. These peroxidases are ubiquitously expressed, with multiple isoforms present in most organisms. Unlike other peroxidases that contain prosthetic groups (such as metals, heme, flavin) or selenocysteine. Prxs rely on the sulphur atom of a conserved Cys residue, termed the peroxidatic Cys (C_P), to cleave the peroxyl (–O–OH) bond. Most, but not all, Prx enzymes contain an additional conserved Cys residue, termed the resolving Cys (C_R). On the basis of the presence or location of the C_R residue, Prxs are classified into 2-Cys, atypical 2-Cys, and 1-Cys subfamilies [1,5]. Mammalian cells express six Prx isoforms: four 2-Cys Prx isoforms (PrxI to PrxIV), one atypical 2-Cys isoform (PrxV), and one 1-Cys Prx isoform (PrxVI) [6–9].

The basic structural unit of all Prxs (not only mammalian Prxs but also Prxs of other species) is a homo-dimer, which gives rise to a high-affinity peroxide binding site that is lacking in other peroxide-eliminating enzymes such as catalase and glutathione peroxidases (GPxs) and generates a specific environment that renders the sulfhydryl group of C_P (C_P–SH) highly sensitive to oxidation by peroxides [4,10,11]. The C_P–SH reacts with peroxide to form cysteine sulfenic acid (C_P–SOH) with the release of water. In 2-Cys Prxs, the unstable sulfenic intermediate forms an intersubunit disulfide with a resolving cysteine thiol (C_R–SH) residue located in the COOH-terminal region of the other subunit in the Prx dimer, in which two subunits are arranged in an antiparallel orientation with domain swapping. The disulfide is subsequently reduced by the thioredoxin system comprising thioredoxin (Trx), thioredoxin reductase (TR) and NADPH to complete the catalytic cycle (Fig. 1).

The C_P–SOH produced as an intermediate during catalysis occasionally undergoes further oxidation to cysteine sulfinic acid (C_P–SO_2H) [12], a reaction that cannot be reversed by Trx (Fig. 1). This hyperoxidation, which is mainly observed with 2-Cys Prx enzymes from eukaryotic but not prokaryotic cells occurs when disulfide formation between C_P–SOH and C_R–SH is slow enough to allow the reaction of C_P–SOH with another peroxide. Because the sulphur atom of disulfide state is resistant to further oxidation and becomes liable again to hyperoxidation only when it is in the sulfenic state, hyperoxidation of C_P–SOH occurs only when Prx is engaged in the catalytic cycle [12].

The hyperoxidation of C_P–SOH to C_P–SO_2H is reversible, with the reduction being catalyzed by sulfiredoxin (Srx) [13,14]. Srx defines a

* Corresponding authorYonsei Biomedical Research Institute, Yonsei University College of Medicine, Seoul, 120-749, South Korea.
E-mail address: rheesg@nih.gov (S.G. Rhee).

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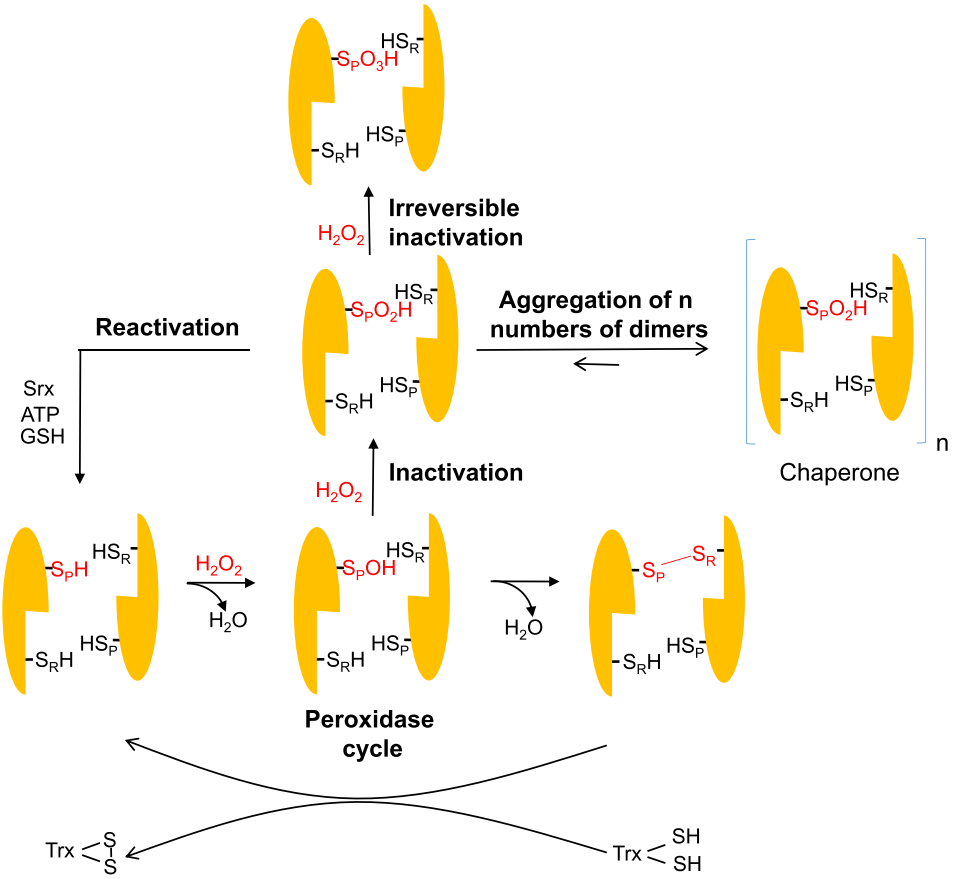

Fig. 1. Catalytic cycle of peroxidase reaction and hyperoxidiation of 2-Cys Prxs.

conserved family of proteins in lower and higher eukaryotes, all of which share a conserved Cys residue. The Srx-catalyzed reduction requires ATP and a thiol such as GSH or Trx [14,15]. Sulfinic Prx is further oxidized to sulfonic form (C_P–SO_3H) in cells exposed to higher concentrations of H_2O_2 and the sulfonic Prx cannot be reduced by Srx [15]. Oxidation of cysteine to sulfinic acid is not restricted to 2-Cys Prx enzymes. Atypical 2-Cys Prx (Prx V) and 1-Cys Prx (Prx VI) also undergo hyperoxidation, albeit more slowly than 2-Cys Prx [16]. Furthermore cysteine residues of many other proteins are also oxidized to sulfinic acid. However, Srx binds specifically to the 2-Cys Prxs and the Srx-mediated reduction is specific to 2-Cys Prx isoforms. Not only prokaryotic 2-Cys Prx enzymes are insensitive to oxidative inactivation, but also prokaryotes do not express Srx [14,17]. Reversible inactivation of 2-Cy Prxs through hyperoxidation was therefore proposed to be a eukaryotic adaptation that allows H_2O_2 to accumulate to substantial levels under certain circumstances for signaling purposes in the floodgate hypothesis [17] (see below). Subsequently, hyperoxidation was found to confer a new function on 2-Cys Prxs, namely that of a protein chaperone, which protects cells against stress-induced protein unfolding [18,19].

The functional switch of 2-Cys Prxs from peroxidase to chaperone involves changes in their quaternary structure. In the reduced state, 2-Cys Prx dimers associate into donut-shaped ring-like decamers, stabilized by hydrophobic interactions between dimer–dimer interface residues. Upon oxidation of C_P–SH to disulfide, the dimer-dimer interaction becomes weak, thus leading to the dissociation of decamers into the disulfide-linked dimers [17,20,21]. Hyperoxidation of C_P–SH causes the formation of oligomeric structures of filamentous or spherical shapes with molecular weight higher than that of decamers. These higher oligomeric structures bring about the peroxidase-independent chaperone function.

It has been well accepted that H_2O_2 is generated in response to the stimulation of many cell surface receptors and involved in redox-regulation of signaling pathways in various cell types [22–25]. The mechanism of redox-regulation is based mostly on the reversible oxidation of cysteine residues of effector proteins [26], which include protein tyrosine phosphatases (PTPs) [27–31], protein tyrosine kinases (PTKs) [32–34], mitogen activated protein kinsases (MAPKs) [35,36], AGC protein kinases [cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), protein kinase C (PKC)} [37–39], transcription factors [40,41], and ion channels [42–46].

Mammalian cells express three types of H_2O_2-eliminating enzyme: catalase, GPxs, and Prxs [47]. Catalase is localized exclusively in peroxisomes, whereas GPx and Prx isoforms are found in various organelles. Prxs are much more abundant than GPxs, typically constituting 0.1–0.8% of total soluble protein in cells. Prx isoforms are therefore prime candidates for regulators of H_2O_2 signaling initiated by cell-surface receptors. Kinetic analysis revealed that the second-order rate constants for the oxidation of C_P–SH to C_P–SOH are in the range of 1×10^6–$1 \times 10^8\ M^{-1}\,s^{-1}$ and that the target thiol groups of H_2O_2 effector proteins react with H_2O_2 several orders of magnitude more slowly than does C_P–SH of Prx [2,4,48–50]. Furthermore, Prxs are abundant proteins that are present in various compartments of the cell, and H_2O_2 molecules bind to Prx with submicromolar affinity [6,48,51]. Thus cysteine residues of H_2O_2 target proteins are at a competitive disadvantage for reaction with H_2O_2 that is produced only transiently.

It has been shown recently that this kinetic disadvantage can be circumvented when 2-Cys Prx molecules near target molecules are transiently inactivated via post-translational modification such as phosphorylation and hyperoxidation of catalytic cysteine [52–54]. Such localized inactivation of 2-Cys Prx thus allows H_2O_2 to accumulate in specific regions of the cell without global redox disturbance. In another way, H_2O_2 target proteins are not directly oxidized by H_2O_2, and the oxidation of their cysteine residues are instead catalyzed by a member

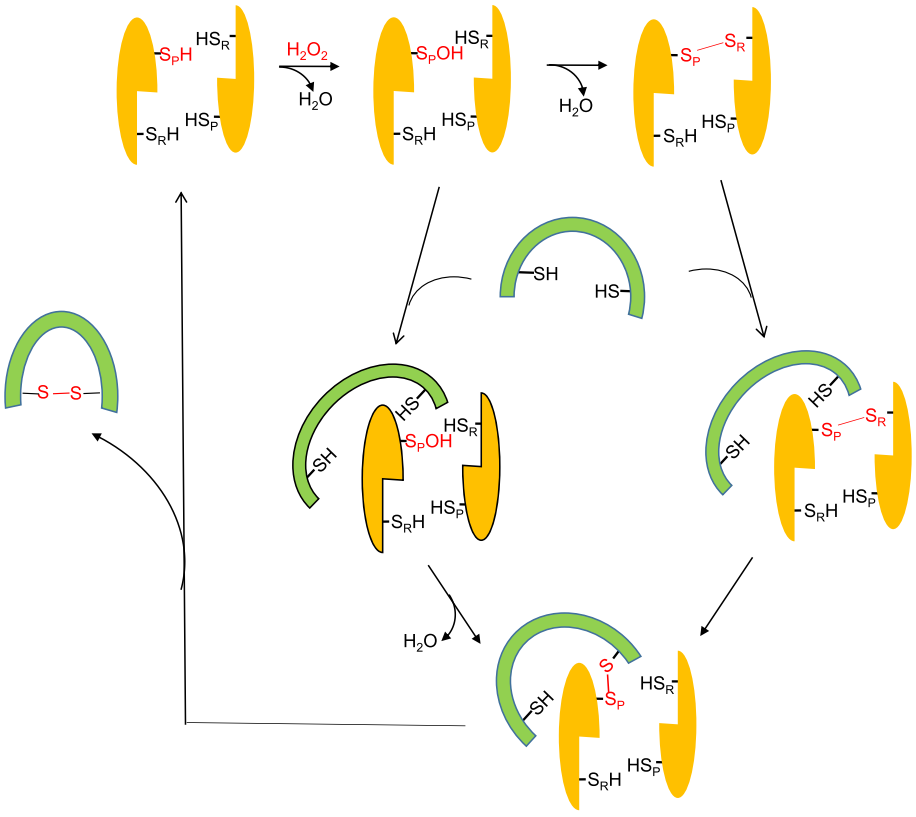

Fig. 2. Sensor and transducer of H_2O_2 signaling by 2-Cys Prxs. The sulphur atom (S_P) of the peroxidatic Cys (C_P) of 2-Cys Prx is rapidly and selectively oxidized by H_2O_2. The oxidized 2-Cys Prx enzyme, in either the sulfenic or disulfide state, forms an intermolecular disulfide-linked intermediate with a bound effector protein (shown as the green curved molecule). Resolution of the disulfide by reaction with another Cys–SH of the effector results in regeneration of reduced Prx and oxidation of the effector protein, the latter process leading to a change in effector function.

of 2-Cys Prxs [26,55–57]. In this scenario, 2-Cys Prx is first oxidized by H_2O_2 and then transfers its oxidation state to a H_2O_2 target protein, thus serving as both a sensor and transducer of H_2O_2 signaling (Fig. 2)

It is clear that 2-Cys Prx enzymes are not simply for the elimination of peroxides to relieve oxidative distress. In cells exposed to high levels of peroxides, 2-Cys Prxs are transformed into peroxidase-independent protein chaperone. In cells that produce H_2O_2 for eustress as in the case of the propagation of receptor signaling, 2-Cys Prxs function as local regulators of H_2O_2 concentration and sensors and transducers of H_2O_2 signaling. In this review, we describe how these various functions of 2-Cys Prxs are regulated via post-translational modifications, such as phosphorylation, acetylation, glutathionylation, and nitrosylation. The sites of such modifications are schematically shown in Fig. 3.

1.1. Regulation of Prx I via phosphorylation

Ligation of the receptors for growth factors such as platelet-derived growth factor (PDGF) or epidermal growth factor (EGF) induces the production of H_2O_2 through activation of NADPH oxidase present in lipid rafts [22,23,58]. This generation of oxidative eustress is necessary for the propagation of signaling because activation of those receptor protein tyrosine kinases is not sufficient to increase the steady-state level of protein tyrosine phosphorylation in cells, and concurrent inhibition of protein tyrosine phosphatases (PTPs) through H_2O_2-dependent oxidation of their active site Cys is required to prevent a futile cycle of phosphorylation and dephosphorylation [24,59]. For H_2O_2 to be able to oxidize PTP Cys, it must be protected from elimination by the abundant and efficient Prx enzymes until it has completed the relatively slow oxidation reaction. The protection of H_2O_2 is achieved when Prx I associated with the

Plasma membrane at lipid rafts is selectively phosphorylated at Tyr^{194} by a member of Src PTK family and thereby inactivated in cells stimulated by PDGF or EGF [52]. Similar tyrosine phosphorylation-dependent inactivation occurs in cell activated via immune receptors such as the T cell receptor (TCR) or B cell receptor (BCR) [52].

Although Prx I is localized predominantly to the cytosol, a small

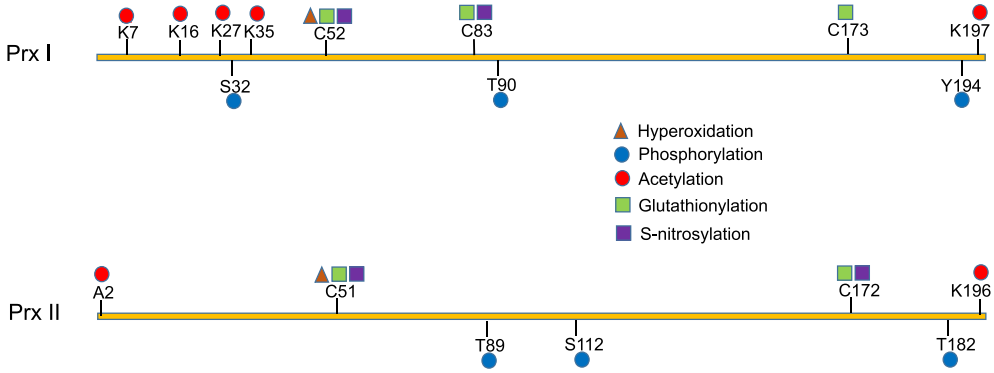

Fig. 3. Post-translational modification of Prx I and Prx II. Peroxidase activity, localized H_2O_2 regulatory role, and chaperone function of Prx I and Prx II are extensively modulated via various covalent modifications. The modified amino acid residues and the types of modification are indicated.

fraction of the enzyme is associated with the lipid rafts [52]. Tyrosine-phosphorylated Prx I, which accounts for ~0.3% of the total Prx I in the cell, is exclusively confined to lipid rafts and is not detected in the cytosol. The localized inactivation of Prx I thus allows H_2O_2 to accumulate around lipid rafts, where signaling proteins such as receptors, NADPH oxidase, PTPs, and Src family kinases are concentrated, while preventing the toxic accumulation of H_2O_2 elsewhere. The physiological relevance of this reversible inactivation is supported by that the Tyr^{194} phosphorylation of Prx I also occurs at the wound edge during repair of a cutaneous wounds in mice, with growth factors such as EGF, PDGF, fibroblast growth factor, keratinocyte growth factor, and insulin-like growth factor–1 being known to act as key inducers of the proliferation of keratinocytes and fibroblasts at the wound margin [52].

In another example, localized accumulation of H_2O_2 is achieved via phosphorylation of Prx I through on Thr^{90}, which is located within a consensus sequence, (S/T)PX (K/R), for phosphorylation by cyclin-dependent kinases (Cdks) [54,60]. Prx I phosphorylation by Cdk1–Cyclin B complex occurs during mitosis and is virtually undetectable during interphase, and the phosphorylation inactivates peroxidase function. The amount of phosphorylated Prx I in mitotic HeLa cells is ~0.4% of total Prx I. As in the case of Prx I tyrosine phosphorylation around lipid rafts, PrxI threonine phosphorylation is a localized at the centrosome during early stages of mitosis (prophase, prometaphase, and metaphase) but not during interphase or late mitotic stages (anaphase, telophase, and cytokinesis) [54].

Increases in mitochondrial respiration and arachidonic acid metabolism as well as the activation of NADPH oxidase elevate the intracellular level of H_2O_2 during G2-M phase [54]. The centrosome is expected to be shielded from the high tide of H_2O_2 during the G2 phase of the cell cycle by Prx I physically associated with the organelle, exposed to H_2O_2 during early mitosis as Prx I becomes phosphorylated, and shielded again as a result of Prx I dephosphorylation during late mitosis by protein phosphatase 2A (PP2A), which is also concentrated at the centrosome [61].

Many proteins that function in the mitotic entry network—such as CycB and the kinases polo-like kinase 1(Plk1) and Aurora A (AurA)—are specifically recruited to the centrosome in the G2 phase of the cell cycle, resulting in the generation of high local concentrations of these mitotic activators [62]. Activation of theCdk1–CycB complex occurs first at the centrosome during prophase, and its amplification through multiple feedback loops involving CycB, Plk1, and AurA also occurs at this organelle [63]. Successful cell cycle progression from metaphase to anaphase also requires that Cdk1 activity be turned off through timely degradation of CycB, Plk1, and AurA. Degradation of these regulators by the 26S proteasome results from their ubiquitination by the multisubunit ubiquitin ligase APC (anaphase-promoting complex/cyclosome), which requires association with Cdc20 homolog 1 (Cdh1) as an activator protein [61]. Cdh1 is prevented from efficient interaction with APC by Cdk-mediated phosphorylation, with the dual specificity phosphatase cell division cycle 14B (Cdc14B) being responsible for dephosphorylation of Cdk-phosphorylated Cdh1 [61].

A fraction of each of APC, Cdh1, and Cdc14B is present at the centrosome [64], as are.

Cdk1, CycB, Plk1, and AurA. At the onset of mitosis, the steady activation of Cdk1 requires protection of CycB, Plk1, and AurA from APC–Cdh1. The low level of incipient Cdk1 activity is likely insufficient to maintain Cdh1 in its phosphorylated state in the absence of concurrent suppression of the activity of Cdc14B. Given that Cdc14B is highly sensitive to H_2O_2-dependent reversible inactivation via oxidation of its catalytic Cys residue, inactivation of Cdc14B as a result of Prx I phosphorylation by Cdk1 and consequent exposure of the centrosome to H_2O_2 likely provide a means to block the association of Cdh1 with APC. In the absence of such suppression of centrosomal Cdc14B activity, further activation of Cdk1 would not be expected to occur at the onset of mitosis because CycB, Plk1, and AurA would be degraded prematurely. Consistent with this scenario, downregulation of centrosomal H_2O_2 abundance by forced expression of a centrosome-targeted form of catalase was found to markedly delay mitotic entry and to lower the centrosomal levels of CycB, Plk1, and AurA [54].

When Thr^{90} of Prx I is substituted with Asp to mimic the phosphorylated status of the protein, the mutation not only reduces peroxidase activity markedly, but also changes its structure from low molecular weight species to high molecular weight complexes and enhances its chaperone activity by six fold, suggesting that phosphorylation of Prx I on Thr^{90} is likely to affect its protein structure and chaperone function [65]. This observation allows speculate that the increased chaperone activity is designed to protect cells from denaturation of centrosomal proteins that might occur as the result of sustained elevation of H_2O_2 around centrosome at the onset of mitosis.

CDKs are best recognized for their role in regulating cell cycle progression in the nucleus [66]. However, some specialized members like Cdk5 do not use cyclins to promote activation, and there is little evidence for involvement of cdk5 in classical cell cycle regulation. Instead, cdk5 is considered a key element in neuronal development and function [67,68]. There is also evidence suggesting a pathogenic role of cdk5 in neurodegenerative disorders, such as Parkinson's disease (PD) [69] and Alzheimer's disease (AD) [70]. Cdk5 phosphorylate Prx II at the Thr^{89} in the cytosol and thereby inactivates its peroxidase activity [71,72]. This peroxidase inactivation and the resulting cytosolic accumulation of H_2O_2 are thought to contribute to neurodegeneration in multiple models of ischemic injury or Parkinson's disease [71,72].

Prx I is also phosphorylated on Ser^{32} by T-cell-originated protein kinase (TOPK), a serine-threonine kinase member of the mitogen-activated protein kinase kinase (MAPKK) family [73]. TOPK is minimally expressed in differentiated cells, and its overexpression is a pathophysiological feature of many tumors including skin cancer cells. Studies with a human melanoma cells indicated that TOPK binds with Prx I, phosphorylates Prx1 at Ser^{32}, and co-localizes with Prx1 in the nucleus upon irradiation of the cells with UVB [73]. In contrast to the Thr^{90} phosphorylation of Prx I, Ser^{32} phosphorylation increases its peroxidase activity and does not affect its chaperone activity. The TOPK-mediated phosphorylation of Prx I contributes to the resistance of melanoma cells to UVB-induced apoptosis by blocking intracellular H_2O_2 accumulation [73].

Prx I phosphorylation on Ser^{32} was detected by large-scale phosphoproteomic characterizations of nine mouse tissues [74] and human liver tissue [75]. The proteomics studies also detected Prx II phosphorylated on Ser^{112} and Thr^{182}. But there have been no other reports on the Prx II phosphorylation on Ser^{112} and Thr^{182}. It is worth to note that despite the fact that phosphorylation of Prx I on Tyr^{194} and Thr^{90} clearly occurs, no phosphoproteomic studies have detected it. It is probably because both phosphorylation events are restricted to a small population of Prx I localized in a specific subcellular compartment under a specific cellular condition.

1.2. Regulation of Prx I and Prx II via acetylation

Proteins are typically acetylated on lysine residues with acetyl-coenzyme being the acetyl group donor. Lysine acetylation is a reversible process regulated by competition of two enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs), both of which act on histone proteins and non-histone proteins as well [76]. Eighteen HDACs are present in mammalian cells, categorized into two groups: zinc-dependent enzymes (HDAC1–11) and NAD^+-dependent enzymes (sirtuins 1–7). Among Zn-dependent HDACs, HDAC6 is unique, with both nuclear and cytoplasmic localization, two catalytic sites, and a ubiquitin-binding site [77]. Unlike other deacetylases, HDAC6 has unique substrate specificity for nonhistone proteins including α-tubulin and heat shock protein 90.

Prx I and Prx II were found to be HDAC6 substrates as the result of studies involving a human prostate cancer cell, LAPC4, which does not express HDAC6 protein while all other zinc-dependent HDACs are

expressed [78]. LAPC4 cells accumulated acetylated α-tubulin and other acetylated proteins including Prx I and Prx II. Inhibition of HDAC6 with the HDAC6-selective inhibitor tubacin or its down-regulation with siRNA in several transformed as well as normal cells resulted in an accumulation of acetylated Prx I and Prx II. The sites of acetylation were identified to be Lys^{197} in Prx I and Lys^{196} in Prx II by mutational studies, which did not rule out that there may be other lysines in each protein that are acetylated [78]. Acetylated Prx I and Prx II were more resistant to the hyperoxidation of C_P-SH by H_2O_2 and to the aggregation to high molecular weight complexes than were the non-acetylated forms [78]. Acetylation also slightly increased peroxidase activity. This observation led several laboratories to investigate the role of the HDAC6-mediated acetylation of Prx I and Prx II in the pathogenesis of neurological and myocardial disease due to oxidative stress.

The major pathological features of Alzheimer's disease are extracellular accumulation of amyloid-beta peptide (Aβ). Cytotoxic effects induced by Aβ include uncontrolled elevation of intracellular reactive oxygen species (ROS) and Ca2+. It has been shown that the levels and activity of HDAC6 are increased in the brains of Alzheimer's disease patients and that inhibition of HDAC6 in Alzheimer's disease model mice results in improved memory [79]. In addition, Aβ-induced impairment of mitochondrial axonal transport is rescued by HDAC6 inhibitor in primary neurons [80]. As expected, Prx1 acetylation is decreased in the hippocampus and cortex of AD patients compared to age- and sex-matched normal controls. Furthermore, acetylation of endogenous Prx I in primary hippocampal neurons and mouse hippocampal neuronal (HT22) cell line is decreased in response to Aβ treatment and recovered by tubacin, suggesting that acetylation of Prx I might be one of the crucial factors that modulate AD pathology [79]. The site of deacetylation on Prx I by HDAC6 was confirmed as Lys^{197} using a sequence-specific antibody to acetylated Prx I synthetic peptide SKEYFSK^{197}(Ac)QK [79].

The role of HDAC6-mediated Prx acetylation was also investigated using Parkinson mouse model induced by 6-hydroxydopamine [81]. Upon injury of dopaminergic neurons by 6-hydroxydopamin, the level of HDAC 6 expression increased and acetylation levels of Prx I and Prx II decreased. Pharmacological inhibition with tubacin increased acetylation of Prx I and Prx II, reduced ROS production and ameliorated dopaminergic neurotoxicity.

Diabetes patients are known to be more vulnerable to myocardial ischemia reperfusion injury, which is associated with excessive oxidative stress. Rat diabetic hearts express excessive HDAC6 activity and reduced levels of acetylated Prx I [82]. Treatment of the diabetes rat with tubacin dramatically improved cardiac function, reduced oxidative stress, and increased acetylated Prx I levels in diabetic rats with myocardial ischemia reperfusion injury [82].

Mass spectral analysis of tryptic peptides obtained from three human cell lines (MV4-11 myeloid leukemia cell, A549 lung carcinoma, and Jurkat leukaemic T-cell) identified 3600 lysine acetylation sites on 1750 proteins [83]. These include acetylation of Prx I on Lys^{7}, Lys^{16}, Lys^{27}, and Lys^{35}. But the same acetylome analysis revealed no acetylated Prx II peptide.

The significance of Lys^{27} acetylation of Prx I was investigated, using the genetic code expansion method, which allows N-acetyllysine to be incorporated in cells in a homogeneous and site-specific manner [84]. Glutamine, although used frequently as a mimic of acetyllysine, cannot fully recapitulate the properties of acetyllysine. It was found that acetylation of Lys^{27} increases Prx I oligomerization and greatly enhances its chaperone activity. This is likely because Lys^{27} lies at the interface of two Prx I dimers, and its acetylation facilitates Prx I oligomerization by decreasing the positive charge of the interface [84]. The roles of Prx I acetylation on Lys^{7}, Lys^{16} and Lys^{35} remain to be studied.

A majority of mammalian proteins are co-translationally acetylated at N-terminal residue, catalyzed by specific N-terminal acetylases. Prx II is acetylated at the N-terminal alanine whereas the N-terminal Ser of Prx I is not acetylated [85]. The differential N-terminal acetylation has to do with the reversibility of C_P–SH hyperoxidation. When HeLa cells are exposed to H_2O_2, the C_P–SH of Prx II is hyperoxidized to sulfinic acid (C_P–SO_2H) faster than that of Prx I. Nevertheless, the majority of the hyperoxidized Prx II formation is reversible, whereas the hyperoxidized Prx I shows much less reversibility because it is further oxidized to sulfonic (C_P–SO_3H) derivative [85]. Evidence suggests that the structural maintenance provided by N-terminal acetylation of Prx II does not affect the reactivity of C_P–SH towards H_2O_2 but prevents irreversible overoxidation of C_P–SH to C_P–SO_3H. The large scale acetylome analysis revealed N-terminal acetylation of Prx II but not of Prx I [86].

1.3. Regulation of Prx I and Prx II via glutathionylation

Protein S-glutathionylation, the reversible attachment a glutathione to a protein via the formation of disulfide with a protein thiol, occurs generally in cells under oxidative stress. Redox proteomic studies identified Prx I as a glutathionylated protein in oxidatively stressed human T lymphocytes and hepatocytes [87,88]. Prx I is also glutathionylated in A549 and HeLa cells after treatment with micromolar concentration of H_2O_2 [89]. Subsequent studies with purified recombinant human Prx I revealed that among its four Cys residues (Cys^{52}, Cys^{71}, Cys^{83}, and Cys^{173}), Cys^{52} (C_P), Cys^{83}, and Cys^{173} (C_R) can be glutathionylated. Cys83 is conserved in mammalian Prx I but absent in Prx II. When HeLa cells are exposed to low levels of H_2O_2, Prx I is preferentially glutathionylated, because glutathionylation of Cys ^{83} is more favorable over that of the two catalytic C_P and C_R residues.

Cys83 is known to be located at the dimer–dimer interface and stabilizes the hydrophobic interaction required for the decamer formation [90]. Analytical ultracentrifugation-sedimentation studies using various Cys mutant proteins revealed that glutathionylation of Prx I promotes changes in its quaternary structure from decamers to dimers and that monoglutathionylation of Cys^{83} is sufficient to induce the dissociation of decameric Prx I [91]. Prx I decamers exhibit chaperone activity which is lost if the decamers are dissociated into dimers. Indeed glutathionylation of Prx I, both the wild-type and mutant in which Cys^{52} and Cys^{173} are replaced by Ser, greatly reduced their chaperone activity [91].

Deglutathionylation is efficiently catalyzed by the thiodisulfide oxidoreductase, glutaredoxin (Grx). The mammalian Grx I, the best characterized from the Grx group, catalyzes the deglutathionylation of multiple protein substrates with widely varying degrees of efficiency, suggesting that substrate recognition plays an important role in deglutathionylation process and other enzymes may also catalyze the deglutathionylation [92]. In fact, the levels of protein glutathionylation induced by nitric oxide (NO) in A549 cells decrease upon overexpression of Srx and purified Srx protein catalyzes deglutathionylation of glutathionylated actin and protein tyrosine phosphatase 1B (PTP1B) [93].

When Prx I glutationylated *in vitro* is used as substrate, the deglutathionylation of Cys^{173} and Cys^{83} is preferentially catalyzed by Srx, whereas Grx I is more favorable for deglutathionylating Cys^{52} [89]. Mechanistic studies revealed that glutathionylated Srx at Cys^{99}, the catalytic residue for the reduction of hyperoxidized 2-Cys Prx enzymes, is an intermediate for the deglutathionylation catalyzed by Srx [89]. Srx binds tightly to 2-Cys Prxs and amino acid residues of Srx and Prx 1 involved in their association have been identified [94]. Mutations of those residues (Tyr^{92} of Srx; and Glu^{123}, Arg^{128}, Pro^{174}, and Trp^{177} of Prx I) drastically reduced deglutathionylation activity, supporting the notion that Srx deglutathionylates Prx I specifically due to its high affinity interaction with Prx I [89].

Proteomic analysis identified Prx II as a protein released in glutathionylated form by macrophages treated with lipopolysaccharide (LPS). The released Prx II was shown to trigger the production of TNF-α [95]. Inflammation is associated with increased oxidative stress and

causes various forms of protein oxidation [96]. Oxidation (glutathionylation) of intracellular Prx II and its release appear to serve as an inflammatory signal [95]. Interestingly, glutathionylated Prx II is released only as a disulfide-linked homodimers [95]. In addition to C_P and C_R, mammalian Prx II contains additional Cys residue Cys^{70}. It is likely, therefore that in the released Prx II, Cys^{70} is the glutathionylated residue while C_P and C_R are involved in disulfide formation. Alternatively, one C_P is glutationylated while the other C_P is used for disulfide formation with C_R.

The active site Cys residues, C_P and C_R, of Prx II become glutathionylated when its disulfide-linked dimers are incubated with GSH *in vitro* [97]. Prx II become glutathionylated also when its reduced protein is treated with H_2O_2 and GSH. The latter reaction occurs via the C_P-SOH intermediate, which reacts sufficiently rapidly for physiological concentrations of GSH to inhibit the formation of C_P-S-S-C_R and protect against hyperoxidation to C_P-SOH [97]. Glutathionylation is reversed by Grx1. Thus, GSH plus Grx1 is able to support the peroxidase activity of Prx II, albeit much slower than that supported by Trx and TR. Glutathionylated Prx II is detected in erythrocytes from Grx1 knock-out mice after exposure to H_2O_2 [97]. These results suggest that GSH/Grx1 system provides an alternative mechanism to Trx and TR for Prx II catalytic recycling, which may be more prominent in situations such as in the erythrocyte where Prx II is the third most abundant protein but the amount of TR is extremely low [97].

1.4. Regulation of Prx I and Prx II via S-nitrosylation

S-nitrosylation, the covalent attachment of an NO moiety to sulfhydryl residues of proteins, can provide protection of cells against irreversible oxidation of critical Cys residues of a variety of proteins [98]. Conversely, dysregulation of S-nitrosylation has been implicated in the pathogenesis of various cardiovascular and neurodegenerative diseases.

S-nitrosylation of Prx II was detected with the use of the ascorbate-dependent biotin switch method in both human neuroblastoma SH-SY5Y cells and primary neuronal cultures after treatment with the NO donor S-nitrosocysteine and in HEK-293 cells stably expressing neuronal NO synthase (nNOS) following exposure to calcium ionophore to activate nNOS [99]. In addition, studies with primary neurons from wild-type or nNOS knockout mice following treatment with *N*-methyl-D-aspartic acid (NMDA) showed that Prx II is S-nitrosylated in an NOS-sensitive fashion [99]. Excessive activation of glutamate receptors by excitatory amino acids like NMDA leads to excitotoxic damage, which is thought to be critical in neurodegenerative disorders such as Parkinson's disease through production of ROS including NO. Mammalian Prx II has three Cys residues, Cys^{51} (C_P), Cys^{70}, and Cys^{172} (C_R). Nitrosoproteomic analysis of various Cys mutants revealed that C_P and C_R are major targets for S-ntrosylation. Thus, S-nitrosylation of Prx II during Parkinson's disease inactivates its peroxidase activity and is believed to contribute to the neurodegeneration process by worsening oxidative stress [99].

Prx II is abundantly nitrosylated in mouse embryonic stem (ES) cells briefly exposed to S-nitrosoglutathione [100]. The resulting inhibition of Prx II peroxidase activity caused a transient accumulation of H_2O_2 and subsequent activation of phosphatidylinositol 3-kinase pathway to drive differentiation of the ES cells to cardiomyocytes. Exposure of A549 and NCI–H1299 human lung cancer cells to S-nitrosoglutathione also resulted in nitrosylation of Prx II at Cys51 and Cys172, causing the accumulation of endogenous H_2O_2 and the apoptosis of the cells by triggering the 5′ AMP-activated protein kinase (AMPK)/Sirtuin1 pathway [101].

Denitrosylation (removal of –NO) of nitrosoproteins is carried out by protein disulfide reductases like thioredoxin, glutathione-NO reductase, and protein disulfide isomerases [102]. Denitrosylation is also achieved by GSH or intramolecularly by a thiol located in the nitosylated protein. Srx was shown to denitrosylate Prx II in an ATP-dependent manner [103]. Accordingly, overexpression of Srx protects dopaminergic neural cells from NO-induced oxidative stress by reducing S-nitrosylated Prx II. This denitrosylation activity of Srx toward Prx II is also likely attributable to its high affinity interaction with Prx II.

Prx I is also found to be S-nitrosylated in several cultured cells upon exposure to NO donors such as S-nitrosocysteine and S-nitrosoglutathione [104]. Nitrosylation is observed mainly at Cys^{52} and Cys^{83} but also at low frequency at Cys^{71} and Cys^{173}. Instead of being a nitrosylation site, Cys^{173} rather promotes denitrosylation through the formation of a disulfide bond with the nitrosylated Cys^{52}. As such, the NO donors bring about disulfide formation between Cys^{52} (C_P) and Cys^{172} (C_R) and inactivation of peroxidase activity [104]. The inactivation is, however, transient, because the disulfide bond is reduced by Trx-TR system. Accordingly, the rate of reactivation is dependent on the rate of denitrosylation. Because the auto-denitrosylation C_P-SNO by C_R-SH is a slow process compared with the dehydration reaction between C_P-SOH and C_R-SH, nitrosylation temporarily withdraws Prx I from the peroxidase catalytic cycle, resulting in reduced activity. In addition to the direct effects of NO on Prx I, NO exerts a marked inhibitory effect on the catalytic cycle Prx I by inhibiting the Trx/TrxR system [104].

Bacillus anthracis expresses bacterial nitric oxide synthase (bNOS) and produces NO. The NO production by bNOS occurs at early stage of Bacillus infection, while the host NO production occurs at later time points. Nitrosoproteomic analysis identified Prx I as a predominant target for S-nitrosylation during toxigenic *Bacillus anthracis Sterne* infection of human small airway epithelial cells (HSAECs) [105]. The S-nitrosylation of Prx1 in the infected HSAECs leads to a decrease in its peroxidase activity while enhancing its chaperone function [105]. The enhanced chaperone activity is attributed to high molecular weight species of Prx I corresponding to the decamer and dodecamer forms that are not present uninfected cells. Infected cells showed increased levels of high molecular weight species as well as disulfide-linked dimers of Prx I [105]. It appears therefore that S-nitrosylation first promotes dimerization of Prx I subunits through the formation of disulfide between C_P and C_R and then causes oligomerization of the disulfide-linked dimers by linking Cys^{83} of one dimer with Cys^{83} of another dimer. The presence of the Cys^{83}– Cys^{83} disulfide bond at the dimer-dimer interface of decameric Prx1 has been reported previously [90]. It is suggested, therefore, that nitrosylation of Prx I elevates its chaperone activity in the early stage of *Bacillus anthracis* infection and that the elevated chaperone activity contributes to enhanced cell viability within the infected lung.

Recently, the role of S-nitrosylation as a ubiquitous, stable post-translational modification that directly regulates many proteins, has been challenged [106]. Studies using rat smooth muscle cells as a model indicate that S-nitrosylation predominantly serves as a transient intermediate in the formation of disulfide either with other cysteines in the protein or with GSH, leading to a proposal that protein disulfide formation is likely to be the dominant end effector resulting from protein S-nitrosylation.

2. Conclusions and future directions

Prxs are not just simple peroxidases that relieve cells from oxidative distress by reducing H_2O_2. They function as protein chaperone to stabilize proteins unfolded under distress. They also contribute to the management of eustress by regulating local concentrations of H_2O_2 and by serving as the sensor and tranducer of H_2O_2 signaling. Among 6 mammalian Prxs, Prx I to IV belong to 2-Cys Prx subfamily. Prx I and Prx II are mainly in the cytosol and nucleus, while Prx III is exclusively localized in the mitochondria and Prx IV in the endoplasmic reticulum. Various functions of Prx I and Prx II are extensively regulated via covalent modifications such as phosphorylation, acetylation, glutathionylation and S-nitrosylation (see Table 1). Though Prx I and Prx II show 78% identity and more than 90% homology in their amino acid

Table 1
Covalent modifications of Prx I and Prx II and their effect on peroxidase activity, hyperoxidability and chaperone function.

	Amino acid	Modification	Effects on Prx function	Ref
Prix I	Lys^{7}	Acetylation	Unknown	[83]
	Lys^{16}	Acetylation	Unknown	[83]
	Lys^{27}	Acetylation	Increases oligomerization and chaperone function	[83,84]
	Ser^{32}	Phosphorylation	Increases peroxidase activity but no effect on chaperone function	[73–75]
	Lys^{35}	Acetylation	Unknown	[83]
	Cys^{52}	Hyperoxidation	Inactivates peroxidase activity and increases chaperone function	[13,18,19]
	Cys^{52}	Glutathionylation	Inactivates peroxidase activity	[87,88]
	Cys^{52}	Nitrosylation	Inactivates peroxidase activity	[104]
	Cys^{83}	Glutathionylation	Reduces chaperone function	[87,88,91]
	Cys^{83}	Nitrosylation	Enhances chaperone function	[104,105]
	Thr^{90}	Phosphorylation	Inactivates peroxidase activity and increases chaperone function	[54,60,65]
	Cys^{173}	Glutathionylation	Inactivates peroxidase activity	[87,88]
	Tyr^{194}	Phosphorylation	Inactivates peroxidase activity	[52]
	Lys^{197}	Acetylation	Increases peroxidase activity and reduces sensitivity to hyperoxidation	[78,79]
Prx II	Ala^{2}	Acetylation	Prevents irreversible hyperoxidation of C_P-SH to C_P-SO_3H	[85,86]
	Cys^{51}	Hyperoxidation	Inactivates peroxidase activity and increases chaperone function	[13,18,19]
	Cys^{51}	Glutathionylation	Inactivates peroxidase activity	[97]
	Cys^{51}	Nitrosylation	Inactivates peroxidase activity	[99–101]
	Thr^{89}	Phosphorylation	Inactivates peroxidase activity	[71,72]
	Ser^{112}	Phosphorylation	Unknown	[74,75]
	Cys^{172}	Glutathionylation	Inactivates peroxidase activity	[97]
	Cys^{172}	Nitrosylation	Inactivates peroxidase activity	[99–101]
	Thr^{182}	Phosphorylation	Unknown	[74,75]
	Lys^{196}	Acetylation	Increases peroxidase activity and reduces sensitivity to hyperoxidation	[78]

sequences, Prx1 and Prx2 appear to possess unique functions and regulatory mechanisms, with Prx I having significantly more modification sites than Prx II.

Not discussed in this review is the regulation of Prx I and Prx II functions through interaction with other proteins. Prx I and Prx II interact with a variety of proteins [107], with such interaction being likely to modulate the function of the binding partners in a reciprocal manner. Many of the binding proteins are likely the effector molecules of H_2O_2 signaling. The roles of interacting proteins are gradually emerging. In one recent example, oxidation resistance 1 (Oxr1), a protein known to be neuroprotective against oxidative stress, interacts with Prx II and acts as a functional switch for Prx II activity, from a peroxidase to a high molecular weight chaperone complex, through the modulation of its hyperoxidation and S-nitrosylation [108]. In another example, Prx I interacts with a histone H2A variant (H2AX) in nucleus, while Prx II interacts with cellular inhibitor of apoptosis protein-1 (cIAP1) in cytosol to affect H_2O_2-mediated apoptosis signaling pathways differently in cells exposed to tumor necrosis factor α (TNFα) [109]. Many Prx-interacting proteins have been identified through advanced proteomic studies without much information on its significance. Further detailed studies on the binding proteins would be rewarding to understand how Prxs manage oxidative distresses and eustresses.

The sulphur atom (S_P) of the peroxidatic Cys (C_P) attacks the peroxide substrate and is oxidized to sulfenic acid (-S_POH). The S_P-OH in the NH_2-terminal region of one subunit of the homodimer is attacked by the sulphur atom (S_R) of the resolving Cys (C_R) located in the COOH-terminal region of the second subunit, resulting in the formation of an intersubunit disulfide bond that is subsequently reduced to S_PH by thioredoxin (Trx) to complete peroxidase catalytic cycle. Alternatively, the S_POH can enter the second pathway to undergo further oxidation by H_2O_2 to sulfinic acid (S_PO_2H), resulting in catalytic inactivation. The inactivated, hyperoxidized Prx is reactivated through a reduction reaction catalyzed by sulfiredoxin (Srx), which requires ATP and a cellular thiol like GSH. The hyperoxidation of S_POH to S_PO_2H promotes the formation of high molecular weight oligomeric structures that exhibit chaperone activity. The S_PO_2H can undergo further oxidation to sulfonic acid (S_PO_3H), which cannot be reduced in cells.

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Appendix A. Supplementary data

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References

[1] S.G. Rhee, H.Z. Chae, K. Kim, Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling, Free Radic. Biol. Med. 38 (2005) 1543–1552.
[2] L.B. Poole, A. Hall, K.J. Nelson, Overview of peroxiredoxins in oxidant defense and redox regulation, Curr. protoc. toxicol./editor. board, Mahin D (2011) 1–15 Maines (Chapter 7).
[3] A. Perkins, K.J. Nelson, D. Parsonage, L.B. Poole, P.A. Karplus, Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling, Trends Biochem. Sci. 40 (2015) 435–445.
[4] S.G. Rhee, I.S. Kil, Multiple functions and regulation of mammalian peroxiredoxins, Annu. Rev. Biochem. 86 (2017) 749–775.
[5] S.G. Rhee, S.W. Kang, T.S. Chang, W. Jeong, K. Kim, Peroxiredoxin, a novel family of peroxidases, IUBMB Life 52 (2001) 35–41.
[6] H.Z. Chae, H.J. Kim, S.W. Kang, S.G. Rhee, Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin, Diabetes Res. Clin. Pract. 45 (1999) 101–112.
[7] M.S. Seo, S.W. Kang, K. Kim, I.C. Baines, T.H. Lee, S.G. Rhee, Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate, J. Biol. Chem. 275 (2000) 20346–20354.
[8] S.W. Kang, I.C. Baines, S.G. Rhee, Characterization of a mammalian peroxiredoxin that contains one conserved cysteine, J. Biol. Chem. 273 (1998) 6303–6311.
[9] S.G. Rhee, Overview on peroxiredoxin, Mol. Cell 39 (2016) 1–5.
[10] H.Z. Chae, S.J. Chung, S.G. Rhee, Thioredoxin-dependent peroxide reductase from yeast, J. Biol. Chem. 269 (1994) 27670–27678.
[11] H.Z. Chae, T.B. Uhm, S.G. Rhee, Dimerization of thiol-specific antioxidant and the essential role of cysteine 47, Proc. Natl. Acad. Sci. U. S. A 91 (1994) 7022–7026.
[12] K.S. Yang, S.W. Kang, H.A. Woo, S.C. Hwang, H.Z. Chae, K. Kim, S.G. Rhee, Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation of the catalytic site cysteine to cysteine-sulfinic acid, J. Biol. Chem. 277 (2002) 38029–38036.
[13] H.A. Woo, H.Z. Chae, S.C. Hwang, K.S. Yang, S.W. Kang, K. Kim, S.G. Rhee, Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation, Science 300 (2003) 653–656.
[14] B. Biteau, J. Labarre, M.B. Toledano, ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin, Nature 425 (2003) 980–984.
[15] T.S. Chang, W. Jeong, H.A. Woo, S.M. Lee, S. Park, S.G. Rhee, Characterization of mammalian sulfiredoxin and its reactivation of hyperoxidized peroxiredoxin

through reduction of cysteine sulfinic acid in the active site to cysteine, J. Biol. Chem. 279 (2004) 50994–51001.
[16] H.A. Woo, S.W. Kang, H.K. Kim, K.S. Yang, H.Z. Chae, S.G. Rhee, Reversible oxidation of the active site cysteine of peroxiredoxins to cysteine sulfinic acid. Immunoblot detection with antibodies specific for the hyperoxidized cysteine-containing sequence, J. Biol. Chem. 278 (2003) 47361–47364.
[17] Z.A. Wood, E. Schroder, J. Robin Harris, L.B. Poole, Structure, mechanism and regulation of peroxiredoxins, Trends Biochem. Sci. 28 (2003) 32–40.
[18] H.H. Jang, K.O. Lee, Y.H. Chi, B.G. Jung, S.K. Park, J.H. Park, J.R. Lee, S.S. Lee, J.C. Moon, J.W. Yun, Y.O. Choi, W.Y. Kim, J.S. Kang, G.W. Cheong, D.J. Yun, S.G. Rhee, M.J. Cho, S.Y. Lee, Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function, Cell 117 (2004) 625–635.
[19] J.C. Moon, Y.S. Hah, W.Y. Kim, B.G. Jung, H.H. Jang, J.R. Lee, S.Y. Kim, Y.M. Lee, M.G. Jeon, C.W. Kim, M.J. Cho, S.Y. Lee, Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H2O2-induced cell death, J. Biol. Chem. 280 (2005) 28775–28784.
[20] Z.A. Wood, L.B. Poole, R.R. Hantgan, P.A. Karplus, Dimers to doughnuts: redox-sensitive oligomerization of 2-cysteine peroxiredoxins, Biochemistry 41 (2002) 5493–5504.
[21] A. Hall, K. Nelson, L.B. Poole, P.A. Karplus, Structure-based insights into the catalytic power and conformational dexterity of peroxiredoxins, Antioxidants Redox Signal. 15 (2011) 795–815.
[22] M. Sundaresan, Z.X. Yu, V.J. Ferrans, K. Irani, T. Finkel, Requirement for generation of H2O2 for platelet-derived growth factor signal transduction, Science 270 (1995) 296–299.
[23] Y.S. Bae, S.W. Kang, M.S. Seo, I.C. Baines, E. Tekle, P.B. Chock, S.G. Rhee, Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation, J. Biol. Chem. 272 (1997) 217–221.
[24] S.G. Rhee, Y.S. Bae, S.R. Lee, J. Kwon, Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation, Sci. STKE : Signal Transduct. Knowl. Environ. (2000) 1–6 e1.
[25] H. Sies, Role of metabolic H2O2 generation: redox signaling and oxidative stress, J. Biol. Chem. 289 (2014) 8735–8741.
[26] S.G. Rhee, H.A. Woo, D. Kang, The role of peroxiredoxins in the transduction of H2O2 signals, Antioxidants Redox Signal. 28 (2018) 537–557.
[27] S.R. Lee, K.S. Kwon, S.R. Kim, S.G. Rhee, Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor, J. Biol. Chem. 273 (1998) 15366–15372.
[28] J. Kwon, S.R. Lee, K.S. Yang, Y. Ahn, Y.J. Kim, E.R. Stadtman, S.G. Rhee, Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 16419–16424.
[29] T.C. Meng, T. Fukada, N.K. Tonks, Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo, Mol. Cell 9 (2002) 387–399.
[30] N.K. Tonks, Redox redux: revisiting PTPs and the control of cell signaling, Cell 121 (2005) 667–670.
[31] A. Salmeen, D. Barford, Functions and mechanisms of redox regulation of cysteine-based phosphatases, Antioxidants Redox Signal. 7 (2005) 560–577.
[32] E. Giannoni, M.L. Taddei, P. Chiarugi, Src redox regulation: again in the front line, Free Radic. Biol. Med. 49 (2010) 516–527.
[33] T.H. Truong, K.S. Carroll, Redox regulation of epidermal growth factor receptor signaling through cysteine oxidation, Biochemistry 51 (2012) 9954–9965.
[34] D.H. Kang, D.J. Lee, K.W. Lee, Y.S. Park, J.Y. Lee, S.H. Lee, Y.J. Koh, G.Y. Koh, C. Choi, D.Y. Yu, J. Kim, S.W. Kang, Peroxiredoxin II is an essential antioxidant enzyme that prevents the oxidative inactivation of VEGF receptor-2 in vascular endothelial cells, Mol. Cell 44 (2011) 545–558.
[35] A. Barajas, A. Basye, M.G. Angelos, C.-A. Chen, Modulation of p38 kinase by DUSP4 is important in regulating cardiovascular function under oxidative stress, Free Radic. Biol. Med. (2015) 170–181.
[36] P.J. Nadeau, S.J. Charette, J. Landry, REDOX reaction at ASK1-Cys250 is essential for activation of JNK and induction of apoptosis, Mol. Biol. Cell 20 (2009) 3628–3637.
[37] K.M. Humphries, M.S. Deal, S.S. Taylor, Enhanced dephosphorylation of cAMP-dependent protein kinase by oxidation and thiol modification, J. Biol. Chem. 280 (2005) 2750–2758.
[38] J.R. Burgoyne, M. Madhani, F. Cuello, R.L. Charles, J.P. Brennan, E. Schroder, D.D. Browning, P. Eaton, Cysteine redox sensor in PKGIa enables oxidant-induced activation, Science 317 (2007) 1393–1397.
[39] D. Lin, D.J. Takemoto, Oxidative activation of protein kinase Cgamma through the C1 domain. Effects on gap junctions, J. Biol. Chem. 280 (2005) 13682–13693.
[40] R.M. Whittal, C.C. Benz, G. Scott, J. Semyonov, A.L. Burlingame, M.A. Baldwin, Preferential oxidation of zinc finger 2 in estrogen receptor DNA-binding domain prevents dimerization and, hence, DNA binding, Biochemistry 39 (2000) 8406–8417.
[41] R. Rainwater, D. Parks, M.E. Anderson, P. Tegtmeyer, K. Mann, Role of cysteine residues in regulation of p53 function, Mol. Cell Biol. 15 (1995) 3892–3903.
[42] P. Aracena-Parks, S.A. Goonasekera, C.P. Gilman, R.T. Dirksen, C. Hidalgo, S.L. Hamilton, Identification of cysteines involved in S-nitrosylation, S-glutathionylation, and oxidation to disulfides in ryanodine receptor type 1, J. Biol. Chem. 281 (2006) 40354–40368.
[43] K. Kolbe, R. Schonherr, G. Gessner, N. Sahoo, T. Hoshi, S.H. Heinemann, Cysteine 723 in the C-linker segment confers oxidative inhibition of hERG1 potassium channels, J. Physiol. 588 (2010) 2999–3009.
[44] R. Bhardwaj, M.A. Hediger, N. Demaurex, Redox modulation of STIM-ORAI signaling, Cell Calcium 60 (2016) 142–152.
[45] N. Ogawa, T. Kurokawa, K. Fujiwara, O.K. Polat, H. Badr, N. Takahashi, Y. Mori, Functional and structural divergence in human TRPV1 channel subunits by oxidative cysteine modification, J. Biol. Chem. 291 (2016) 4197–4210.
[46] E.T. Chouchani, L. Kazak, M.P. Jedrychowski, G.Z. Lu, B.K. Erickson, J. Szpyt, K.A. Pierce, D. Laznik-Bogoslavski, R. Vetrivelan, C.B. Clish, A.J. Robinson, S.P. Gygi, B.M. Spiegelman, Mitochondrial ROS regulate thermogenic energy expenditure and sulfenylation of UCP1, Nature 532 (2016) 112–116.
[47] H. Sies, Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: oxidative eustress, Redox biology 11 (2017) 613–619.
[48] B. Manta, M. Hugo, C. Ortiz, G. Ferrer-Sueta, M. Trujillo, A. Denicola, The peroxidase and peroxynitrite reductase activity of human erythrocyte peroxiredoxin 2, Arch. Biochem. Biophys. 484 (2009) 146–154.
[49] A.G. Cox, A.V. Peskin, L.N. Paton, C.C. Winterbourn, M.B. Hampton, Redox potential and peroxide reactivity of human peroxiredoxin 3, Biochemistry 48 (2009) 6495–6501.
[50] A.V. Peskin, F.M. Low, L.N. Paton, G.J. Maghzal, M.B. Hampton, C.C. Winterbourn, The high reactivity of peroxiredoxin 2 with H(2)O(2) is not reflected in its reaction with other oxidants and thiol reagents, J. Biol. Chem. 282 (2007) 11885–11892.
[51] D. Parsonage, K.J. Nelson, G. Ferrer-Sueta, S. Alley, P.A. Karplus, C.M. Furdui, L.B. Poole, Dissecting peroxiredoxin catalysis: separating binding, peroxidation, and resolution for a bacterial AhpC, Biochemistry 54 (2015) 1567–1575.
[52] H.A. Woo, S.H. Yim, D.H. Shin, D. Kang, D.Y. Yu, S.G. Rhee, Inactivation of peroxiredoxin I by phosphorylation allows localized H(2)O(2) accumulation for cell signaling, Cell 140 (2010) 517–528.
[53] I.S. Kil, S.K. Lee, K.W. Ryu, H.A. Woo, M.C. Hu, S.H. Bae, S.G. Rhee, Feedback control of adrenal steroidogenesis via H2O2-dependent, reversible inactivation of peroxiredoxin III in mitochondria, Mol. Cell 46 (2012) 584–594.
[54] J.M. Lim, K.S. Lee, H.A. Woo, D. Kang, S.G. Rhee, Control of the pericentrosomal H2O2 level by peroxiredoxin I is critical for mitotic progression, J. Cell Biol. 210 (2015) 23–33.
[55] R.M. Jarvis, S.M. Hughes, E.C. Ledgerwood, Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells, Free Radic. Biol. Med. 53 (2012) 1522–1530.
[56] M.C. Sobotta, W. Liou, S. Stocker, D. Talwar, M. Oehler, T. Ruppert, A.N. Scharf, T.P. Dick, Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling, Nat. Chem. Biol. 11 (2015) 64–70.
[57] H. Nassour, Z. Wang, A. Saad, A. Papaluca, N. Brosseau, B. Affar el, M.A. Alaoui-Jamali, D. Ramotar, Peroxiredoxin 1 interacts with and blocks the redox factor APE1 from activating interleukin-8 expression, Sci. Rep. 6 (2016) 29389.
[58] M. Ushio-Fukai, R.W. Alexander, Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase, Mol. Cell. Biochem. 264 (2004) 85–97.
[59] S.G. Rhee, Cell signaling. H2O2, a necessary evil for cell signaling, Science 312 (2006) 1882–1883.
[60] T.S. Chang, W. Jeong, S.Y. Choi, S. Yu, S.W. Kang, S.G. Rhee, Regulation of peroxiredoxin I activity by Cdc2-mediated phosphorylation, J. Biol. Chem. 277 (2002) 25370–25376.
[61] C. Wurzenberger, D.W. Gerlich, Phosphatases: providing safe passage through mitotic exit, Nat. Rev. Mol. Cell Biol. 12 (2011) 469–482.
[62] J. Bonnet, P. Coopman, M.C. Morris, Characterization of centrosomal localization and dynamics of Cdc25C phosphatase in mitosis, Cell Cycle 7 (2008) 1991–1998.
[63] A. Lindqvist, V. Rodriguez-Bravo, R.H. Medema, The decision to enter mitosis: feedback and redundancy in the mitotic entry network, J. Cell Biol. 185 (2009) 193–202.
[64] J.M. Peters, The anaphase promoting complex/cyclosome: a machine designed to destroy, Nat. Rev. Mol. Cell Biol. 7 (2006) 644–656.
[65] H.H. Jang, S.Y. Kim, S.K. Park, H.S. Jeon, Y.M. Lee, J.H. Jung, S.Y. Lee, H.B. Chae, Y.J. Jung, K.O. Lee, C.O. Lim, W.S. Chung, J.D. Bahk, D.J. Yun, M.J. Cho, S.Y. Lee, Phosphorylation and concomitant structural changes in human 2-Cys peroxiredoxin isotype I differentially regulate its peroxidase and molecular chaperone functions, FEBS Lett. 580 (2006) 351–355.
[66] J. Pines, Cyclins and cyclin-dependent kinases: take your partners, Trends Biochem. Sci. 18 (1993) 195–197.
[67] R. Dhavan, L.H.A. Tsai, Decade of CDK5, Nat. Rev. Mol. Cell Biol. 2 (2001) 749–759.
[68] B.S. Li, M.K. Sun, L. Zhang, S. Takahashi, W. Ma, L. Vinade, A.B. Kulkarni, R.O. Brady, H.C. Pant, Regulation of NMDA receptors by cyclin-dependent kinase-5, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 12742–12747.
[69] P.D. Smith, S.J. Crocker, V. Jackson-Lewis, K.L. Jordan-Sciutto, S. Hayley, M.P. Mount, M.J. O'Hare, S. Callaghan, R.S. Slack, S. Przedborski, H. Anisman, D.S. Park, Cyclin-dependent kinase 5 is a mediator of dopaminergic neuron loss in a mouse model of Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13650–13655.
[70] J.C. Cruz, L.H. Tsai, Cdk5 deregulation in the pathogenesis of Alzheimer's disease, Trends Mol. Med. 10 (2004) 452–458.
[71] D. Qu, J. Rashidian, M.P. Mount, H. Aleyasin, M. Parsanejad, A. Lira, E. Haque, Y. Zhang, S. Callaghan, M. Daigle, M.W. Rousseaux, R.S. Slack, P.R. Albert, I. Vincent, J.M. Woulfe, D.S. Park, Role of Cdk5-mediated phosphorylation of Prx2 in MPTP toxicity and Parkinson's disease, Neuron 55 (2007) 37–52.
[72] J. Rashidian, M.W. Rousseaux, K. Venderova, D. Qu, S.M. Callaghan, M. Phillips, R.J. Bland, M.J. During, Z. Mao, R.S. Slack, D.S. Park, Essential role of cytoplasmic cdk5 and Prx2 in multiple ischemic injury models, in vivo, J. Neurosci. : Off. J. Soc.Neurosci. 29 (2009) 12497–12505.
[73] T.A. Zykova, F. Zhu, T.I. Vakorina, J. Zhang, L.A. Higgins, D.V. Urusova,

A.M. Bode, Z. Dong, T-LAK cell-originated protein kinase (TOPK) phosphorylation of Prx1 at Ser-32 prevents UVB-induced apoptosis in RPMI7951 melanoma cells through the regulation of Prx1 peroxidase activity, J. Biol. Chem. 285 (2010) 29138–29146.
[74] E.L. Huttlin, M.P. Jedrychowski, J.E. Elias, T. Goswami, R. Rad, S.A. Beausoleil, J. Villen, W. Haas, M.E. Sowa, S.P. Gygi, A tissue-specific atlas of mouse protein phosphorylation and expression, Cell 143 (2010) 1174–1189.
[75] Y. Bian, C. Song, K. Cheng, M. Dong, F. Wang, J. Huang, D. Sun, L. Wang, M. Ye, H. Zou, An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome, J Proteomics 96 (2014) 253–262.
[76] H. Lehrmann, L.L. Pritchard, A. Harel-Bellan, Histone acetyltransferases and deacetylases in the control of cell proliferation and differentiation, Adv. Canc. Res. 86 (2002) 41–65.
[77] C. Hubbert, A. Guardiola, R. Shao, Y. Kawaguchi, A. Ito, A. Nixon, M. Yoshida, X.F. Wang, T.P. Yao, HDAC6 is a microtubule-associated deacetylase, Nature 417 (2002) 455–458.
[78] R.B. Parmigiani, W.S. Xu, G. Venta-Perez, H. Erdjument-Bromage, M. Yaneva, P. Tempst, P.A. Marks, HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 9633–9638.
[79] H. Choi, H.J. Kim, J. Kim, S. Kim, J. Yang, W. Lee, Y. Park, S.J. Hyeon, D.S. Lee, H. Ryu, J. Chung, I. Mook-Jung, Increased acetylation of Peroxiredoxin1 by HDAC6 inhibition leads to recovery of Abeta-induced impaired axonal transport, Mol. Neurodegener. 12 (2017) 23.
[80] C. Kim, H. Choi, E.S. Jung, W. Lee, S. Oh, N.L. Jeon, I. Mook-Jung, HDAC6 inhibitor blocks amyloid beta-induced impairment of mitochondrial transport in hippocampal neurons, PloS One 7 (2012) e42983.
[81] W. Jian, X. Wei, L. Chen, Z. Wang, Y. Sun, S. Zhu, H. Lou, S. Yan, X. Li, J. Zhou, B. Zhang, Inhibition of HDAC6 increases acetylation of peroxiredoxin1/2 and ameliorates 6-OHDA induced dopaminergic injury, Neurosci. Lett. 658 (2017) 114–120.
[82] Y. Leng, Y. Wu, S. Lei, B. Zhou, Z. Qiu, K. Wang, Z. Xia, Inhibition of HDAC6 activity alleviates myocardial ischemia/reperfusion injury in diabetic rats: potential role of peroxiredoxin 1 acetylation and redox regulation, Oxid Med Cell Longev (2018), https://doi.org/10.1155/2018/9494052 9494052; 2018.
[83] C. Choudhary, C. Kumar, F. Gnad, M.L. Nielsen, M. Rehman, T.C. Walther, J.V. Olsen, M. Mann, Lysine acetylation targets protein complexes and co-regulates major cellular functions, Science 325 (2009) 834–840.
[84] Y. Pan, J.H. Jin, Y. Yu, J. Wang, Significant enhancement of hPrx1 chaperone activity through lysine acetylation, Chembiochem.: Eur. J. Chem. Biol. 15 (2014) 1773–1776.
[85] J.H. Seo, J.C. Lim, D.Y. Lee, K.S. Kim, G. Piszczek, H.W. Nam, Y.S. Kim, T. Ahn, C.H. Yun, K. Kim, P.B. Chock, H.Z. Chae, Novel protective mechanism against irreversible hyperoxidation of peroxiredoxin: nalpha-terminal acetylation of human peroxiredoxin II, J. Biol. Chem. 284 (2009) 13455–13465.
[86] S. Gauci, A.O. Helbig, M. Slijper, J. Krijgsveld, A.J. Heck, S. Mohammed, Lys-N and trypsin cover complementary parts of the phosphoproteome in a refined SCX-based approach, Anal. Chem. 81 (2009) 4493–4501.
[87] M. Fratelli, H. Demol, M. Puype, S. Casagrande, I. Eberini, M. Salmona, V. Bonetto, M. Mengozzi, F. Duffieux, E. Miclet, A. Bachi, J. Vandekerckhove, E. Gianazza, P. Ghezzi, Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 3505–3510.
[88] M. Fratelli, H. Demol, M. Puype, S. Casagrande, P. Villa, I. Eberini, J. Vandekerckhove, E. Gianazza, P. Ghezzi, Identification of proteins undergoing glutathionylation in oxidatively stressed hepatocytes and hepatoma cells, Proteomics 3 (2003) 1154–1161.
[89] J.W. Park, J.J. Mieyal, S.G. Rhee, P.B. Chock, Deglutathionylation of 2-Cys peroxiredoxin is specifically catalyzed by sulfiredoxin, J. Biol. Chem. 284 (2009) 23364–23374.
[90] W. Lee, K.S. Choi, J. Riddell, C. Ip, D. Ghosh, J.H. Park, Y.M. Park, Human peroxiredoxin 1 and 2 are not duplicate proteins: the unique presence of CYS83 in Prx1 underscores the structural and functional differences between Prx1 and Prx2, J. Biol. Chem. 282 (2007) 22011–22022.
[91] J.W. Park, G. Piszczek, S.G. Rhee, P.B. Chock, Glutathionylation of peroxiredoxin I induces decamer to dimers dissociation with concomitant loss of chaperone activity, Biochemistry 50 (2011) 3204–3210.
[92] H.Z. Chae, H. Oubrahim, J.W. Park, S.G. Rhee, P.B. Chock, Protein glutathionylation in the regulation of peroxiredoxins: a family of thiol-specific peroxidases that function as antioxidants, molecular chaperones, and signal modulators, Antioxidants Redox Signal. 16 (2012) 506–523.
[93] V.J. Findlay, D.M. Townsend, T.E. Morris, J.P. Fraser, L. He, K.D. Tew, A novel role for human sulfiredoxin in the reversal of glutathionylation, Canc. Res. 66 (2006) 6800–6806.
[94] T.J. Jonsson, L.C. Johnson, W.T. Lowther, Structure of the sulphiredoxin-peroxiredoxin complex reveals an essential repair embrace, Nature 451 (2008) 98–101.
[95] S. Salzano, P. Checconi, E.M. Hanschmann, C.H. Lillig, L.D. Bowler, P. Chan, D. Vaudry, M. Mengozzi, L. Coppo, S. Sacre, K.R. Atkuri, B. Sahaf, L.A. Herzenberg, L.A. Herzenberg, L. Mullen, P. Ghezzi, Linkage of inflammation and oxidative stress via release of glutathionylated peroxiredoxin-2, which acts as a danger signal, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12157–12162.
[96] B.S. Berlett, E.R. Stadtman, Protein oxidation in aging, disease, and oxidative stress, J. Biol. Chem. 272 (1997) 20313–20316.
[97] A.V. Peskin, P.E. Pace, J.B. Behring, L.N. Paton, M. Soethoudt, M.M. Bachschmid, C.C. Winterbourn, Glutathionylation of the active site cysteines of peroxiredoxin 2 and recycling by glutaredoxin, J. Biol. Chem. 291 (2016) 3053–3062.
[98] J.S. Stamler, S. Lamas, F.C. Fang, Nitrosylation. the prototypic redox-based signaling mechanism, Cell 106 (2001) 675–683.
[99] J. Fang, T. Nakamura, D.H. Cho, Z. Gu, S.A. Lipton, S-nitrosylation of peroxiredoxin 2 promotes oxidative stress-induced neuronal cell death in Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 18742–18747.
[100] B. Wu, H. Yu, Y. Wang, Z. Pan, Y. Zhang, T. Li, L. Li, W. Zhang, L. Ge, Y. Chen, C.K. Ho, D. Zhu, X. Huang, Y. Lou, Peroxiredoxin-2 nitrosylation facilitates cardiomyogenesis of mouse embryonic stem cells via XBP-1s/PI3K pathway, Free Radic. Biol. Med. 97 (2016) 179–191.
[101] Y. Zhang, C. Sun, G. Xiao, H. Shan, L. Tang, Y. Yi, W. Yu, Y. Gu, S-nitrosylation of the Peroxiredoxin-2 promotes S-nitrosoglutathione-mediated lung cancer cells apoptosis via AMPK-SIRT1 pathway, Cell Death Dis. 10 (2019) 329.
[102] R. Sengupta, S.W. Ryter, B.S. Zuckerbraun, E. Tzeng, T.R. Billiar, D.A. Stoyanovsky, Thioredoxin catalyzes the denitrosation of low-molecular mass and protein S-nitrosothiols, Biochemistry 46 (2007) 8472–8483.
[103] C.R. Sunico, A. Sultan, T. Nakamura, N. Dolatabadi, J. Parker, B. Shan, X. Han, J.R. Yates 3rd, E. Masliah, R. Ambasudhan, N. Nakanishi, S.A. Lipton, Role of sulfiredoxin as a peroxiredoxin-2 denitrosylase in human iPSC-derived dopaminergic neurons, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E7564–E7571.
[104] R. Engelman, P. Weisman-Shomer, T. Ziv, J. Xu, E.S. Arner, M. Benhar, Multilevel regulation of 2-Cys peroxiredoxin reaction cycle by S-nitrosylation, J. Biol. Chem. 288 (2013) 11312–11324.
[105] M.C. Chung, F. Alem, S.G. Hamer, A. Narayanan, K. Shatalin, C. Bailey, E. Nudler, R.M. Hakami, S-nitrosylation of peroxiredoxin 1 contributes to viability of lung epithelial cells during Bacillus anthracis infection, Biochim. Biophys. Acta Gen. Subj. 1861 (2017) 3019–3029.
[106] I. Gusarov, E. Nudler, Protein S-nitrosylation: enzymatically controlled, but intrinsically unstable, post-translational modification, Mol. Cell 69 (2018) 351–353.
[107] S.G. Rhee, H.A. Woo, Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger H(2)O(2), and protein chaperones, Antioxidants Redox Signal. 15 (2011) 781–794.
[108] D.M. Svistunova, J.N. Simon, E. Rembeza, M. Crabtree, W.W. Yue, P.L. Oliver, M.J. Finelli, Oxidation resistance 1 regulates post-translational modifications of peroxiredoxin 2 in the cerebellum, 130 (2019) 151–162.
[109] S. Lee, J.Y. Lee, E.W. Lee, S. Park, D.H. Kang, C. Min, D.J. Lee, D. Kang, J. Song, J. Kwon, S.W. Kang, Absence of cytosolic 2-cys Prx subtypes I and II exacerbates TNF-alpha-induced apoptosis via different routes, Cell Rep. 26 (2019) 2194–2211 e2196.