

Peroxiredoxin Functions as a Peroxidase and a Regulator and Sensor of Local Peroxides*

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Peroxiredoxins (Prxs) contain an active site cysteine that is sensitive to oxidation by H₂O₂. Mammalian cells express six Prx isoforms that are localized to various cellular compartments. The oxidized active site cysteine of Prx can be reduced by a cellular thiol, thus enabling Prx to function as a locally constrained peroxidase. Regulation of Prx via phosphorylation in response to extracellular signals allows the local accumulation of H₂O₂ and thereby enables its messenger function. The fact that the oxidation state of the active site cysteine of Prx can be transferred to other proteins that are less intrinsically susceptible to H₂O₂ also allows Prx to function as an H₂O₂ sensor.

An unusual antioxidant protein, now called peroxiredoxin (Prx),² was initially identified on the basis of its capacity to protect proteins from oxidative damage induced by reactive oxygen species (ROS) produced in the presence of DTT. It was named “protector protein” or “thiol-specific antioxidant” before being renamed Prx (1–5). The ROS were generated as the result of the reduction of molecular oxygen by DTT to superoxide and H₂O₂, which were further reduced to hydroxyl radicals in the presence of trace amounts of contaminating metal (iron or copper) ions (6). Analysis of purified yeast Prx revealed that it did not contain conventional redox centers such as metals, heme, flavin, or selenocysteine. Prx therefore did not resemble any antioxidant known at the time. It was subsequently found that (i) Prx is present in all biological kingdoms from bacteria to mammals; (ii) two cysteine residues, corresponding to Cys⁴⁷ and Cys¹⁷⁰ of yeast Prx, are highly conserved among Prx family members; (iii) Prxs are homodimers arranged in a head-to-tail orientation; and (iv) Cys⁴⁷-SH of Prx is specifically oxidized by H₂O₂ to cysteine sulfenic acid (Cys-SOH), which is resolved by reaction with Cys¹⁷⁰-SH of the adjacent monomer, resulting in the formation of a disulfide linkage, Cys⁴⁷-S-S-Cys¹⁷⁰ (7). The antioxidant function of Prx was attributed to the fact that the disulfide could be reduced by

DTT, resulting in completion of a catalytic cycle. Thioredoxin (Trx) was eventually shown to be the biological donor of reducing equivalents for the catalytic function of Prx (8), with peroxynitrite (ONOO⁻) in addition to H₂O₂ and lipid peroxides also being found to be reduced by Prx enzymes (9). The conserved Cys residue corresponding to Cys⁴⁷ of yeast Prx was later referred to as the peroxidatic Cys (C_P) to reflect its sensitivity to oxidation by peroxides, and the conserved Cys residue corresponding to Cys¹⁷⁰ was designated the resolving Cys (C_R) (10).

Given that H₂O₂ reacts with the thiolate (deprotonated) form of cysteine, oxidation of cysteine is enhanced by a microenvironment that lowers the normally high pK_a (~8.6) of cysteine thiols to a value below neutral pH. The pK_a values of the C_P residue of members of the Prx family are in the range of 5–6 (11–16). However, whereas most deprotonated thiols react with H₂O₂ with a rate constant of ~20 M⁻¹ s⁻¹ (17), the C_P residue of Prxs reacts with H₂O₂ with rate constants of 1 × 10⁵ to 1 × 10⁷ M⁻¹ s⁻¹ (14, 17). The low pK_a alone thus appears to be insufficient to account for the high reactivity of Prx with peroxide. The unusually high activity was recently attributed to the fact that Prx enzymes activate not only the thiolate of C_P but also the peroxide substrate via a hydrogen bonding network formed by four amino acids (Pro, Thr, Arg, and Glu, Gln, or His) conserved in the active site of all Prx enzymes (18).

Mechanism of Peroxidase Reaction

On the basis of the location or absence of the C_R residue, Prxs are classified into 2-Cys, atypical 2-Cys, and 1-Cys Prx subfamilies (4, 5, 19). Mammalian cells express six isoforms of Prx: four 2-Cys Prx isoforms (Prxs I–IV), one atypical 2-Cys Prx isoform (Prx V), and one 1-Cys Prx isoform (Prx VI). These isoforms vary in subcellular localization, with Prx I, II, and VI being localized mainly in the cytosol; Prx III being restricted to mitochondria; Prx IV being found predominantly in the endoplasmic reticulum (ER); and Prx V being present in the cytosol, mitochondria, and peroxisomes. As discussed below, however, Prx localization is multifarious, being dependent on the cell type and environment.

The peroxidase reaction mechanisms of mammalian Prx enzymes are shown in Fig. 1. As demonstrated first with yeast Prx, the conserved C_P-SH of 2-Cys Prx is selectively oxidized by H₂O₂ to the C_P-SOH intermediate. An intermolecular disulfide is then formed with C_R-SH and is ultimately reduced by Trx (Fig. 1A). Eukaryotic members of the 2-Cys Prx subgroup are distinct from most prokaryotic members in that they contain two structural motifs (GGLG and YF) whose interaction restricts the ability of C_P-SOH to approach C_R-SH and thereby hinders disulfide formation (10, 18, 20). As a result, C_P-SOH of eukaryotic 2-Cys Prxs can react with a second H₂O₂ molecule and become hyperoxidized to cysteine sulfinic acid (C_P-SO₂H) before it is able to react with C_R-SH (21, 22). Such hyperoxidation results in the inactivation of peroxidase activity and in the formation of higher order molecular aggregates that exhibit protein chaperone function (23–25). The C_P-SO₂H moiety of 2-Cys Prxs can be reduced in an ATP-de-

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² The abbreviations used are: Prx, peroxiredoxin; ROS, reactive oxygen species; Trx, thioredoxin; C_P, peroxidatic Cys; C_R, resolving Cys; ER, endoplasmic reticulum; Srx, sulfiredoxin; KO, knock-out; PTP, protein-tyrosine phosphatase; PTK, protein-tyrosine kinase; Nox, NADPH oxidase; PDI, protein-disulfide isomerase; PLA₂, phospholipase A₂.

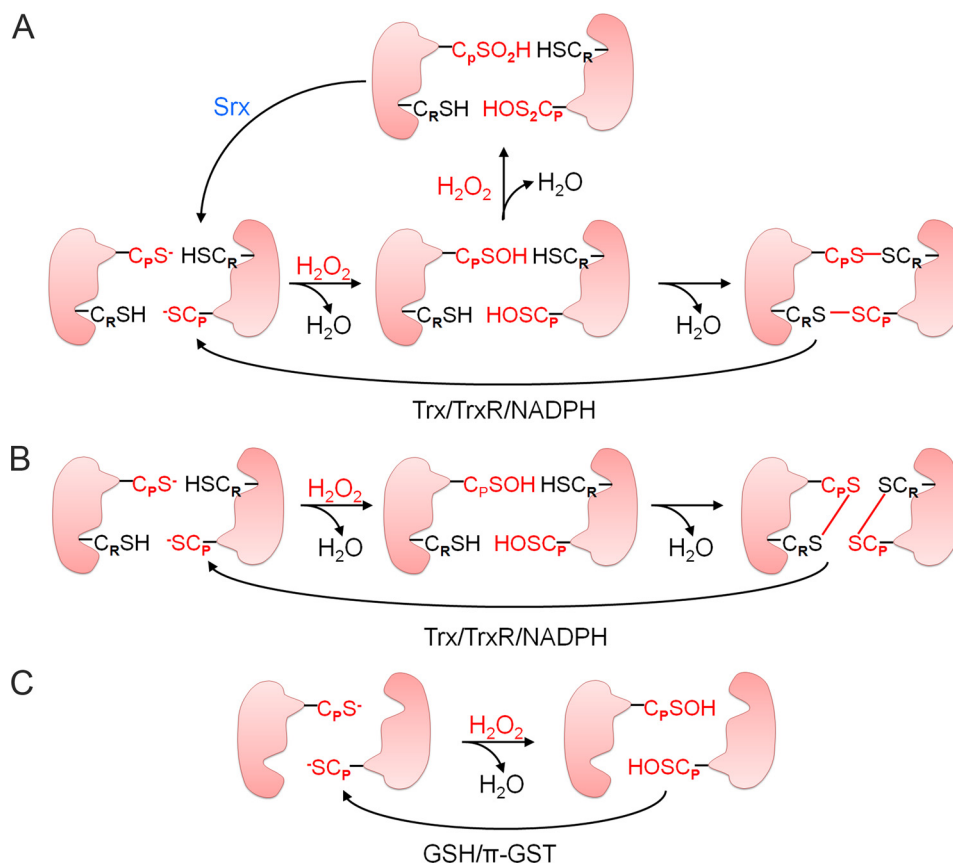


FIGURE 1. Reaction mechanisms of Prx enzymes. The reaction mechanisms of 2-Cys Prx (A), atypical 2-Cys Prx (B), and 1-Cys Prx (C) enzymes are shown. *TrxR*, Trx reductase.

pendent reaction catalyzed by sulfiredoxin (Srx) (Fig. 1A) (26). Recent evidence has shown that 2-Cys Prxs from several prokaryotic organisms, including cyanobacteria, contain the GGLG and YF motifs and undergo hyperoxidation at the C_p residue (27) and that cyanobacteria express a eukaryote-like Srx protein (28).

Prxs V and VI, the mammalian members of the atypical 2-Cys and 1-Cys Prx subgroups, respectively, also exist in an antiparallel dimeric form (29–33). During the catalytic cycles of Prxs V and VI (Fig. 1, B and C), the C_p -SH residue is first oxidized by peroxides, as with 2-Cys Prxs. For Prx V, the resulting C_p -SOH then reacts with C_r -SH of the same subunit to form an intramolecular disulfide linkage, which is also reduced by Trx as in 2-Cys Prx (Fig. 1B). In the catalytic cycle of Prx VI, Cys-SOH does not form a disulfide because of the unavailability of another Cys-SH nearby. C_p -SOH of oxidized Prx VI is reduced by GSH in the presence of the Pi isoform of GST but not by Trx or glutaredoxin (29, 30, 34–36). Prxs V and VI are less sensitive to hyperoxidation than are 2-Cys Prxs, and hyperoxidized forms of Prxs V and VI are not reduced by Srx (37).

Reduction of Locally Produced Peroxides by Concerted Action of 2-Cys Prx and Srx

The role of Prx enzymes as peroxidases has been demonstrated by increasing or decreasing their expression levels in various cell lines and evaluating the sensitivity of the cells to oxidative insults. Prx-deficient mice have also been generated by targeting the gene for each isoform. These mice develop to

adulthood with no overt phenotype when maintained under normal laboratory conditions. Prx II knock-out (KO) mice subsequently develop hemolytic anemia (38), and ablation of Prx I resulted in spontaneous tumor formation in aging mice in one study (39). These phenotypes of Prx KO mice may not be related to the peroxidase activity of the targeted protein, however. As described below, certain Prx enzymes also function as local regulators of H_2O_2 , which serves as an intracellular messenger. The tumor suppressor function of Prx I was attributed to such regulation of H_2O_2 (40, 41).

An *in vivo* antioxidant function of Prx I was recently demonstrated in the livers of ethanol-fed mice (42). The production of ROS and oxidative stress play a central role in the pathogenesis of alcoholic liver disease. Ethanol consumption induces the accumulation of CYP2E1 (cytochrome P450 2E1), a major contributor to ethanol-induced ROS production in the liver. CYP2E1 catalyzes the oxidation of a wide variety of low molecular weight compounds, including ethanol (43). Elevated CYP2E1 levels alone result in oxidative stress because electron transfer from the donor system (NADPH and NADPH-dependent cytochrome P450 reductase) to CYP2E1 is not perfectly coupled and is therefore leaky (44). Such leaked electrons react with O_2 to produce superoxide, which is converted to H_2O_2 or reacts with nitric oxide to produce peroxynitrite. Ethanol feeding in mice was found to markedly increase the abundance of Srx protein and mRNA in the liver. However, it had no significant effect on the hepatic abundance of the six Prx isoforms.

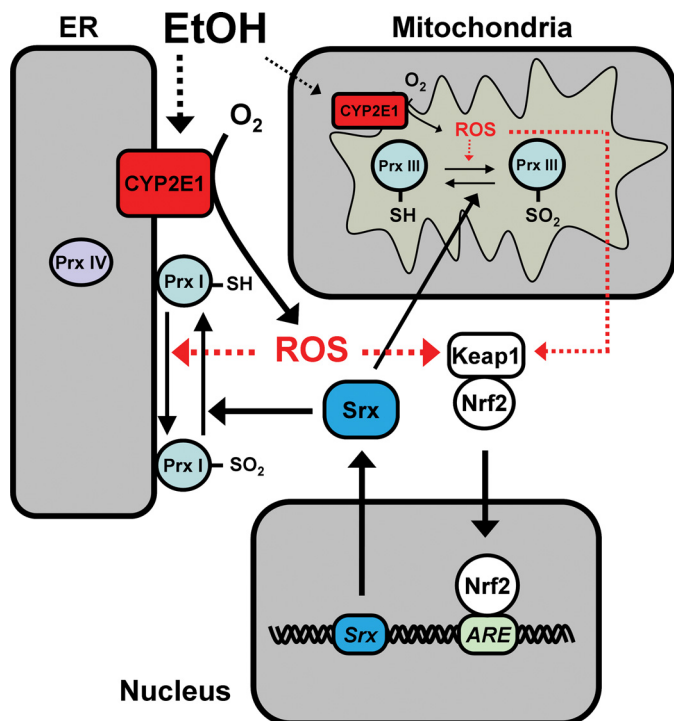


FIGURE 2. Antioxidant roles of Prx I, Prx III, and Srx in ethanol-fed mouse liver. EtOH feeding increases the abundance of CYP2E1 in the ER. A large proportion of Prx I is present at the cytosolic side of the ER membrane, where CYP2E1 is located. Prx I is therefore preferentially engaged in the reduction of ROS produced by CYP2E1 and becomes hyperoxidized. Reactivation of such hyperoxidized Prx I requires the action of Srx, the expression of which is greatly increased via an Nrf2- and antioxidant regulatory element (ARE)-dependent pathway in the livers of ethanol-fed mice. Ethanol feeding also increases the abundance of CYP2E1 and the production of ROS in mitochondria, likely resulting in hyperoxidation of Prx III that is reversed in part by translocation of Srx from the cytosol into mitochondria.

Analysis of Nrf2 KO mice indicated that the ethanol-induced up-regulation of Srx expression is mediated mainly via a pathway dependent on the transcription factor Nrf2 (42).

During elimination of peroxides, all 2-Cys Prxs undergo unavoidable hyperoxidation. Among Prxs I-IV, however, only Prx I was found to be hyperoxidized (to a moderate extent) in the livers of ethanol-fed mice, with this effect being markedly enhanced in Srx KO mice (Fig. 2) (42). These observations suggested that Prx I is the most active 2-Cys Prx in elimination of ROS in the livers of ethanol-fed mice and that, despite the elevated expression of Srx in such mice, the capacity of Srx is not sufficient to counteract the hyperoxidation of Prx I that occurs during ROS reduction. The preferential hyperoxidation of Prx I also occurs despite the fact that both Prxs I and II are cytosolic proteins and that Prx II is more prone to hyperoxidation than is Prx I in most cell types (41, 45). A protease protection assay showed that a large fraction of Prx I, but not of Prx II, is located at the cytoplasmic side of the ER membrane, where CYP2E1 is embedded (42). The selective burden of ROS removal borne by Prx I is thus likely attributable to its proximity to the ROS-generating CYP2E1 (Fig. 2). In addition to inducing the expression of Srx in the liver, ethanol feeding elicited the translocation of some Srx molecules to microsomes. However, the capacity of Srx located near the surface of the ER was not sufficient to fully counteract the hyperoxidation of Prx I, with consequent accu-

mulation of a small amount of Prx I-SO₂. Chronic ethanol feeding in Srx KO mice resulted in hyperoxidation of 30–50% of total Prx I, which likely represents virtually all ER-bound Prx I molecules (42).

Ethanol feeding also increases the abundance of CYP2E1 and the production of ROS in mitochondria (46), likely resulting in the hyperoxidation of Prx III, a member of the 2-Cys Prx sub-family that is specifically localized to mitochondria (Fig. 2). However, Prx III-SO₂ was not detected in the livers of ethanol-fed wild-type mice, probably because increased oxidative stress resulted in the translocation of Srx into mitochondria (47), and the capacity of mitochondrial Srx was then sufficient to counteract the hyperoxidation of Prx III. A small proportion of Prx III was hyperoxidized in the livers of ethanol-fed Srx-deficient mice, suggesting that Prx III is vulnerable to hyperoxidation by ethanol-induced ROS and that Prx III-SO₂ accumulates only in the absence of Srx. Mammalian cells also contain a mitochondrion-specific Trx system (48), suggesting that Prx III together with mitochondrial Trx might provide a primary line of defense against H₂O₂ produced by the mitochondrial respiratory chain.

Ethanol-induced oxidative damage in the liver includes protein modification such as carbonylation, addition of 4-hydroxynonenal, and nitration of tyrosine to yield 3-nitrotyrosine (49). The pivotal roles of Srx and Prx I in protection of the liver against ethanol-induced oxidative stress were apparent in mice deficient in Srx or Prx I. Subjection of such mice to chronic ethanol feeding thus resulted in more severe oxidative damage to the liver, as revealed by protein carbonylation and by the formation of 4-hydroxynonenal and 3-nitrotyrosine adducts, compared with that observed in ethanol-fed wild-type mice (42).

Regulation by Prx I of H₂O₂ Produced Locally at Plasma Membrane

Many mammalian cell types produce H₂O₂ for the purpose of intracellular signaling in response to stimulation through various cell surface receptors (50, 51). For example, in cells stimulated with growth factors such as PDGF or EGF, the production of H₂O₂ is required for propagation of growth factor signaling (52, 53). Hydrogen peroxide is distinct from other messenger molecules in that it acts not by binding to effectors but by oxidizing their critical residues, most notably cysteine, as exemplified by the inhibition of protein-tyrosine phosphatases (PTPs) and the tumor suppressor PTEN (phosphatase and *ten*-sin homolog) as a result of oxidation of their catalytic Cys by H₂O₂ produced in response to growth factor stimulation (19, 54). Indeed, the activation of protein-tyrosine kinases (PTKs) is not sufficient to increase the steady-state level of protein tyrosine phosphorylation in cells; the concurrent inhibition of PTPs by H₂O₂ is also required. It had remained unclear, however, how a toxic molecule like H₂O₂ is able to oxidize effector proteins selectively without inflicting damage on other proteins and lipids. One possible explanation was localized production of H₂O₂. Evidence indicates that NADPH oxidase (Nox) is largely responsible for receptor-dependent H₂O₂ production (55, 56) and that Nox1 and Nox2 are activated within lipid rafts, which serve as a platform for the assembly of various signaling proteins, including receptor PTKs and Src family kinases (57).

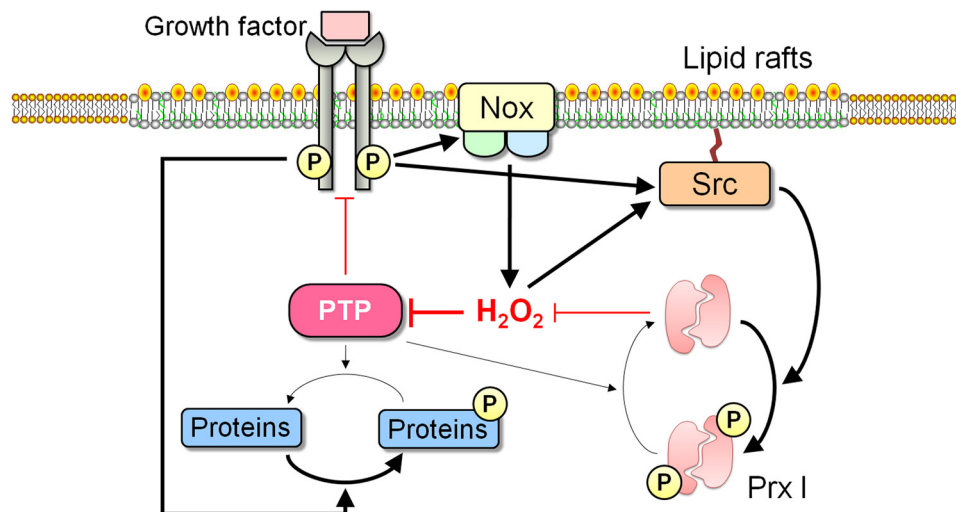


FIGURE 3. **Model for mechanism underlying H₂O₂ accumulation around lipid rafts and its role in intracellular signaling.** See text for details.

Proteins such as PTPs and PTEN have a low pK_a thiol that can be selectively oxidized by H₂O₂ with a rate constant of $\sim 20 \text{ M}^{-1} \text{ s}^{-1}$ (17). Given such a moderate rate of oxidation, for H₂O₂ to serve as a signaling molecule, its concentration must increase rapidly above a certain threshold (10–100 μM) and remain elevated long enough for it to oxidize effector molecules (58). However, in most cells, H₂O₂-eliminating enzymes are present at large concentrations in the cytosol to ensure that the toxic molecule remains at low intracellular levels ($< 0.1 \mu\text{M}$) (58). The localized production of H₂O₂ might therefore not be sufficient to support its messenger function without protection from local H₂O₂-eliminating enzymes.

Although Prxs I and II are present mostly in the cytosolic fraction, a small proportion of these enzymes was shown to be constitutively associated with detergent-resistant lipid rafts (41). Prx I, but not Prx II, was also found to be phosphorylated (at Tyr¹⁹⁴) by PTKs of the Src family in several cell types stimulated via growth factor receptors, including those for PDGF and EGF, or via immune receptors such as the T cell and B cell receptors. This phosphorylation induced inactivation of the peroxidase activity of Prx I and was confined to Prx I molecules associated with lipid rafts; it was not observed with Prx I present in the cytosol. The spatially confined inactivation of Prx I thus provides a means for the generation of favorable H₂O₂ gradients around lipid rafts, where signaling proteins are concentrated, while minimizing the general accumulation of H₂O₂ to toxic levels and disturbance of global redox potential. Prx I phosphorylation was enhanced by increasing Nox1 expression, supporting the notion that Prx I phosphorylation occurs in the cellular microdomains where H₂O₂ is produced. The phosphorylation of Prx I at Tyr¹⁹⁴ was also detected at the wound edge during repair of a cutaneous injury in mice. This finding likely reflects the fact that growth factors such as EGF, PDGF, FGF, TGF- α , keratinocyte growth factor, and insulin-like growth factor-1 act as key inducers of the proliferation of keratinocytes and fibroblasts at the wound edge (59).

A model depicting the mechanism underlying H₂O₂ accumulation around lipid rafts and its role in intracellular signaling is shown in Fig. 3. Engagement of a growth factor receptor or an

immune receptor thus induces the production of H₂O₂ through activation of Nox present in lipid rafts. The activated receptor also induces the phosphorylation of raft-associated Prx I at Tyr¹⁹⁴ through activation of a Src family PTK, resulting in inactivation of Prx I, which would otherwise destroy the newly generated H₂O₂. Inactivation of Prx I allows the accumulation of H₂O₂ around lipid rafts, which in turn promotes further phosphorylation and inactivation of Prx I both by activating Src kinases and by inactivating PTPs. These two positive feedback loops allow the sustained H₂O₂ signaling necessary for the regulation of biological responses. The inhibition of PTPs by H₂O₂ is critical for growth factor signaling because activation of receptor PTKs alone is not sufficient to achieve the levels of protein tyrosine phosphorylation necessary for such signaling, with the concurrent inhibition of PTPs by H₂O₂ preventing the futile cycle of phosphorylation and dephosphorylation (52–54). Reversible inactivation of the inositol lipid phosphatase PTEN by H₂O₂ is also necessary for signaling mediated by phosphoinositide 3-kinase (60, 61).

In contrast to Prx I, Prx II was found to be inactivated not via tyrosine phosphorylation but rather via hyperoxidation of its peroxidatic cysteine and only under conditions of sustained oxidative stress. Hyperoxidized Prx I or II could not be detected even when the extent of PDGF-induced H₂O₂ generation was increased by overexpressing the PDGF receptor or Nox1 (together with two additional proteins, Nox1 and Nox1, which are necessary for activation of Nox1) (41). Although both Prxs I and II are associated with lipid rafts, inactivation of Prx I, which is more efficient as a peroxidase than is Prx II, might be sufficient to allow local accumulation of H₂O₂ for signal propagation. The role of raft-associated Prx II remains unclear. Given that the accumulation of a small amount of H₂O₂ at lipid rafts can trigger rapid signal amplification through the action of the positive feedback loops shown in Fig. 3, the presence of both Prxs II and I might provide a safeguard to prevent inappropriate activation of signaling cascades by spurious production of H₂O₂ at rafts. Functional differences between Prxs I and II are also apparent in the phenotypes of the corresponding KO mice: mice lacking Prx I thus manifest an increased susceptibility to

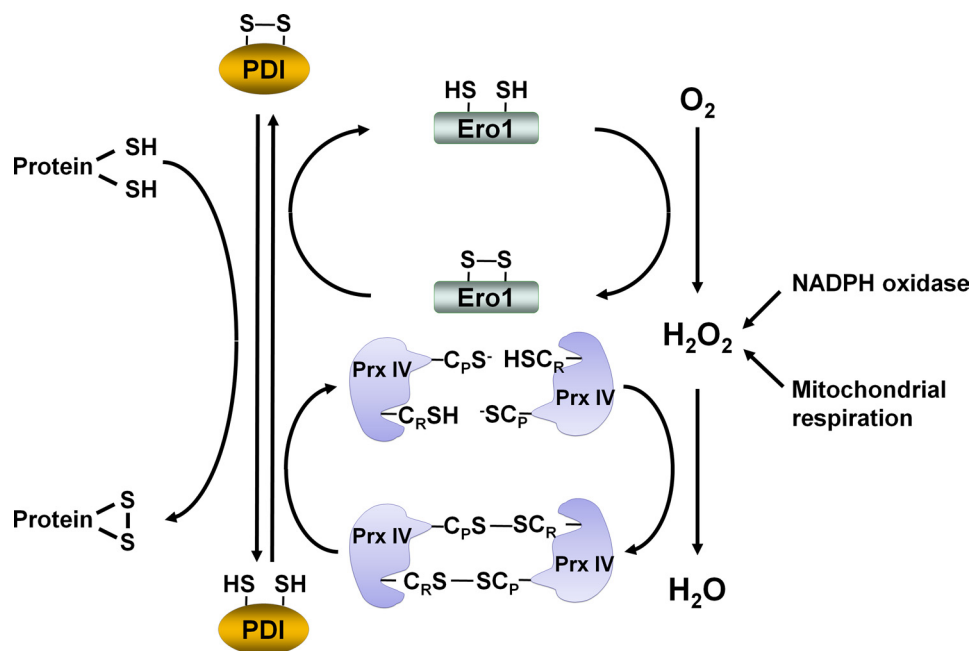


FIGURE 4. **Role of Prx IV as H₂O₂ sensor for protein folding in ER.** Oxidation of thiols to disulfide linkages during protein folding in the ER is achieved with the use of H₂O₂ produced by oxidant-generating sources such as Ero1, NADPH oxidase, and mitochondria. Ero1 produces H₂O₂ by transferring electrons from reduced PDI to O₂. In the proposed model, C_P-SH and C_R-SH of Prx IV are first oxidized by H₂O₂ to form a disulfide. The oxidized state of Prx IV is then transferred to PDI thiols, thereby converting them to a disulfide and finally resulting in the formation of a disulfide in the protein to be folded. Prx IV in the ER physically interacts with PDI. It is thus thought to function as a sensor of H₂O₂ in PDI-mediated protein folding.

spontaneous cancer, whereas those deficient in Prx II do not (39, 40). Prx I has thus been suggested to play a tumor suppressor role, and this suggestion was further supported by the observation that Prx I deficiency in mice increases the susceptibility to Ras- or ErbB2-induced breast cancer (40).

The model shown in Fig. 3 also exemplifies a double-negative feedback loop, in which PTPs suppress receptor PTK activation, and H₂O₂ mediates coupling of receptor PTK activation with PTP inhibition. Computer simulation has recently been applied to analyze the signaling system involving a receptor PTK, a PTP, Prx I, and H₂O₂ (62). It was suggested previously that the plasma membrane is partitioned into 50–300-nm-wide domains by the combined action of actin-based membrane skeleton “fences” and anchored-transmembrane protein “pickets” (63), with proteins and lipids being highly mobile within these membrane domains. However, the hopping of signaling proteins across the fences is thought to occur with low probability and therefore becomes the rate-limiting factor in lateral spread of receptor signaling. The computer simulation indicated that the propagation of growth factor signaling initiated by a local source is possible as a result of the tight H₂O₂-dependent coupling between the receptor PTK and PTP. In addition, the simulation predicted that the phosphorylation-dependent regulation of Prx I activity contributes to lowering the threshold for later propagation of signaling but not substantially to its axial propagation in the cytoplasm (62).

Role of Prx IV as H₂O₂ Sensor for Protein Folding in ER

Prx IV is an ER-resident protein (64). The ER is responsible for maintaining redox conditions that facilitate the formation of disulfide bonds, which involves the transfer of electrons from the reduced cysteines of unfolded proteins to oxidoreductases

of the protein-disulfide isomerase (PDI) family. Completion of the catalytic cycle requires re-formation of the intramolecular disulfide of PDI. Ero1 (ER membrane-associated oxidoreductin 1) was found to participate in PDI reoxidation and disulfide bond formation (65). During the PDI oxidation reaction, Ero1 mediates the flavin-dependent transfer of electrons directly to molecular oxygen, producing one molecule of H₂O₂ for every disulfide bond formed (Fig. 4). The H₂O₂ produced by Ero1 can be reduced by Prx IV. Given that the ER does not contain Trx, it has been unclear, however, how the inevitable formation of the disulfide C_P-S-S-C_R within Prx IV can be reversed to support peroxidase activity. A novel solution to this problem has been provided by the finding that PDI physically interacts with Prx IV and serves as the disulfide reductase of oxidized Prx IV (66, 67). Indeed, PDI itself was oxidized more efficiently by Prx IV than by Ero1 (67). In addition to providing a pathway for maintaining the catalytic activity of Prx IV, the coupling of the oxidation of Prx IV by H₂O₂ to the formation of a disulfide ensures that two disulfide bonds are formed for every oxygen molecule reduced (Fig. 4).

Despite its key role in protein folding, Ero1 was found not to be essential in mice (68), suggesting the existence of alternative sources of H₂O₂ molecules for protein folding. Nox4, which is present in the ER, was proposed as a candidate for such a source. ER-associated mitochondria also can provide H₂O₂ to support ER function. It was proposed that H₂O₂ molecules provided by these sources are used to oxidize Prx IV and that the oxidized state of Prx IV is then transferred to PDI thiols, thereby converting them to a disulfide and finally resulting in the formation of a disulfide in the protein to be folded (Fig. 4) (66, 67). Prx IV thus functions as a sensor of H₂O₂ in PDI-

mediated protein folding. This sensor function of Prx IV is possible because the active site of Prx is configured to favor oxidation by peroxides but not for reaction with other oxidants or disulfide-containing proteins. The peroxide sensor function of thiol-based peroxidases has been demonstrated previously for the oxidation of Yap1 (a transcription factor that controls anti-oxidant gene expression) by Orp1 (a Prx-related glutathione peroxidase) in *Saccharomyces cerevisiae* (69, 70). Similarly, in *Schizosaccharomyces pombe*, Tpx1 (a 2-Cys Prx) serves as a peroxide sensor for Pap1, the homolog of Yap1 (71, 72). In plants, the nuclear-encoded chloroplast 2-Cys Prx was shown to regulate the transcription activity of Rap2.4 (73).

Other Localized Functions of Prx

A somewhat unusual disulfide reductase activity of Prx I has been described recently in relation to regulation of the six-transmembrane protein GDE2 (74). GDE2, which controls the onset and progression of the differentiation of spinal motor neurons through its extracellular glycerophosphodiester phosphodiesterase activity, is normally inactive because of the presence of an intramolecular disulfide bond between Cys²⁵ and Cys⁵⁷⁶. However, GDE2 associates with Prx I through its N-terminal 38 amino acids, resulting in the activation of GDE2 through Prx I-catalyzed reduction of the intramolecular disulfide bond. This finding thus uncovered a novel disulfide reductase activity of Prx I. Prx I-dependent activation of GDE2 should result in the formation of the intermolecular disulfide C_P-S-S-C_R within Prx I, suggesting that the progression of neuronal differentiation relies on the effective recycling of Prx I to the active reduced form by Trx.

Prx VI is the prototype and the only mammalian 1-Cys member of the Prx family. Major differences from other Prxs include the use of GSH instead of Trx as the physiological reductant, heterodimerization with Pi GST as part of the catalytic cycle, and the ability to hydrolyze phospholipids as a result of Ca²⁺-independent phospholipase A₂ (PLA₂) activity (35, 36, 75–77). Prx VI is thus a bifunctional protein and has separate active site residues for peroxidase (Cys⁴⁷, the C_P residue) and PLA₂ (Ser³²) activities. Prx VI is found in both cytosolic and lysosomal compartments in lung alveolar epithelium. It is capable of reducing a broad spectrum of peroxides and provides protection against oxidative stress through its peroxidase activity (78), and it participates in the turnover of lung surfactant phospholipids through its PLA₂ activity (79). Given that the pH optimum for the PLA₂ activity of the enzyme is in the acidic range (80) and that the peroxidase activity is expressed at cytosolic pH, the subcellular localization of Prx VI is likely to affect the balance between the two activities. The localization of Prx VI to lysosome-like organelles in lung epithelial cells was shown recently to require the activity of the mitogen-activated protein kinases ERK and p38 but not to involve Prx VI phosphorylation. Rather, ERK and p38 appear to regulate the subcellular localization of Prx VI through activation of 14-3-3ε as a chaperone protein that binds to Prx VI, resulting in entry of Prx VI into the vesicular pathway and its further targeting to lysosomal compartments (81).

Concluding Remarks

The structure of Prx provides a specific environment that renders C_P highly sensitive to oxidation by H₂O₂ while at the same time protecting it from reaction with other cellular oxidants. Six different Prx isozymes are found in various locations of mammalian cells, allowing Prx to function as a peroxidase as well as a sensor of H₂O₂ in specialized cellular compartments. As exemplified by the tyrosine phosphorylation-dependent inactivation of Prx I, the peroxidase function of Prx can be regulated via various post-translational modifications. Such modifications also include threonine and serine phosphorylation (82, 83), lysine acetylation (84), cysteine glutathionylation (85, 86), cysteine nitrosylation (87), and limited proteolysis (88), although the biological relevance of these reactions remains unclear at the present time. Prxs also interact with numerous proteins, which might direct their cellular localization and sensitivity to oxidation (5).

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