

Peroxiredoxin 5: Structure, Mechanism, and Function of the Mammalian Atypical 2-Cys Peroxiredoxin

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Abstract

Peroxiredoxin 5 (PRDX5) was the last member to be identified among the six mammalian peroxiredoxins. It is also the unique atypical 2-Cys peroxiredoxin in mammals. Like the other five members, PRDX5 is widely expressed in tissues but differs by its surprisingly large subcellular distribution. In human cells, it has been shown that PRDX5 can be addressed to mitochondria, peroxisomes, the cytosol, and the nucleus. PRDX5 is a peroxidase that can use cytosolic or mitochondrial thioredoxins to reduce alkyl hydroperoxides or peroxynitrite with high rate constants in the 10^6 to 10^7 $\text{M}^{-1}\text{s}^{-1}$ range, whereas its reaction with hydrogen peroxide is more modest, in the 10^5 $\text{M}^{-1}\text{s}^{-1}$ range. PRDX5 crystal structures confirmed the proposed enzymatic mechanisms based on biochemical data but revealed also some specific unexpected structural features. So far, PRDX5 has been viewed mainly as a cytoprotective antioxidant enzyme acting against endogenous or exogenous peroxide attacks rather than as a redox sensor. Accordingly, overexpression of the enzyme in different subcellular compartments protects cells against death caused by nitro-oxidative stresses, whereas gene silencing makes them more vulnerable. Thus, more than 10 years after its molecular cloning, mammalian PRDX5 appears to be a unique peroxiredoxin exhibiting specific functional and structural features. *Antioxid. Redox Signal.* 15, 817–829.

Introduction

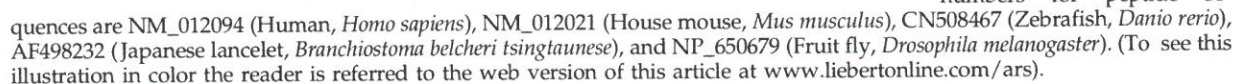
PEROXIREDOXINS represent a superfamily of thiol-dependent peroxidases that are able to reduce hydrogen peroxide, alkyl hydroperoxides, and peroxynitrite (30, 72, 98). As documented now by a large literature, these enzymes are highly conserved throughout evolution and are thus widely distributed among all phyla (42). Peroxidase activity of peroxiredoxins depends on cysteines (Cys). All peroxiredoxins exhibit a conserved peroxidatic Cys (Cp) in the N-terminal domain of the protein. During the peroxidase reaction, the Cp attacks the O-O bond of the peroxide and is subsequently oxidized to a Cys sulfenic acid, which is then reduced back during the resolution step. Based on this resolution mechanism and the existence or the lack of a resolving Cys (Cr) localized to the C-terminal region of the enzyme, peroxiredoxins were classified into three subfamilies referred as 1-Cys, typical 2-Cys, and atypical 2-Cys (80). In the 1-Cys peroxiredoxin subfamily, enzymes possess only the Cp, and the sulfenic acid formed during Cp oxidation is reduced by an external thiol. In typical 2-Cys peroxiredoxins, the Cr of one subunit attacks the sulfenic acid of a second subunit resulting

in the formation of a stable intermolecular disulfide bond, which can be then reduced by a thiol-dependent reductant. Finally, in atypical 2-Cys peroxiredoxins, the Cr is located within the same polypeptide chain, and the reaction with Cp results in the formation of an intramolecular instead of an intermolecular disulfide bond. This classification is based on the catalytic mechanism of the enzyme, but other classifications exist according to sequence homology (30, 42, 89) or primary sequence characteristics and structural data (13, 59, 74).

Peroxiredoxin 5 (PRDX5) (Fig. 1), also named PrxV, ACR1, PMP20, or AOEB166, is the only mammalian atypical 2-Cys peroxiredoxin (41, 80, 99). Partial amino acid sequences of PRDX5 were initially reported as sequences of an unknown liver or lung protein (29, 92). In 1999, three different articles reported the identification and the molecular cloning of a novel mammalian protein named ACR1 (44), PMP20 (99), or AOEB166 (41). Human ACR1 (Alu complex in gel retardation assays 1) was described as a DNA-binding protein potentially implicated in the repression of RNA-polymerase-III-driven transcription of the Alu-family retrotransposons (44). Human and murine PMP20 (peroxisomal membrane protein 20) were

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According to its amino acid sequence, PRDX5 is the most divergent isoform among mammalian peroxiredoxins (53, 72). Mature human PRDX5 (Fig. 2) shares only 28% to 30% sequence identity with human typical 2-Cys and 1-Cys peroxiredoxins (53). Interestingly, PRDX5 exhibits also a remarkably wide subcellular distribution compared with the other mammalian peroxiredoxins. These features, as well as other characteristics discussed later, suggest that this perox-

The human *PRDX5* gene is localized to chromosome 11q13 and is composed of six exons and five introns spanning ap-

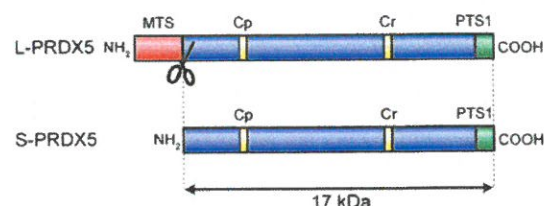


FIG. 2. Representation of the long (L-PRDX5) and short (S-PRDX5) forms of human PRDX5. L-PRDX5 and S-PRDX5 are produced by alternative use of two translation-initiation sites. L-PRDX5 possesses a mitochondrial targeting sequence (MTS) that is cleaved on translocation into mitochondria. Mature human L-PRDX5 and S-PRDX5 have the same molecular mass (17 kDa) and exhibit a SQL peroxisomal targeting sequence type 1 (PTS1) at their C-terminus. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

proximately 3.7 kb (41, 45, 46, 61). Interestingly, the number of exons and introns as well as the size of the corresponding protein-coding exons appears to be highly conserved among chordates, although intron lengths varied considerably throughout evolution (61). It must also be pointed out that human *PRDX5* and *HSPC152/TRM112* genes are in close proximity on chromosome 11 and positioned in opposite directions (61). This locus appears to be conserved in mammals. In humans, only 689 bp separate the two first ATGs corresponding to the translation start codons of each gene, suggesting that *PRDX5* and *HSPC152/TRM112* may share regulating sequences (61).

In silico analysis of the upstream sequence of the human *PRDX5* gene revealed features of the 5'-flanking region of a housekeeping gene. The 5'-flanking region of the human *PRDX5* gene and its murine counterpart contain CpG islands, and they lack TATA- and CAAT-boxes (45, 46, 61). Moreover, multiple transcription start sites were reported for the human *PRDX5* gene, also a characteristic of housekeeping genes (41, 61, 99). *PRDX5* has been reported to be constitutively expressed in virtually all mammalian tissues but at different levels, depending on the cell type (41, 46, 80). It was also shown that transcriptional or translational upregulation may occur in mammalian cells in pathophysiologic situations or in response to different kinds of stresses (see later). Accordingly, the analysis of the 5'-flanking region of human *PRDX5* revealed the presence of several putative response elements for transcription factors involved in the response of mammalian cells to oxidative stress, such as Nrf2 or NF- κ B (45, 46, 61). However, their implication in *PRDX5* regulation is still to be clearly demonstrated. Interestingly, Foxo3a, another transcription factor involved in a direct transcriptional regulation of antioxidant enzymes such as MnSOD/SOD2 or catalase, was also shown to interact with PGC-1 α to regulate *PRDX5* transcription in endothelial cells (64, 88). Moreover, it was reported that Ets1/2, known as transcription factors implicated in oxidative stress-inducible expression, were associated with HMGB1 to function as coactivators to upregulate *PRDX5* expression in human cancer cells exposed to hydrogen peroxide or hypoxia (82). Finally, it was reported recently that a specific compensatory increase of *PRDX5* appears in cells of *PRDX1*^{-/-} mice as the consequence of a fine-tuning mechanism to control increase of cytoplasmic and nuclear ROS (reactive oxygen species) (27). It was suggested that *PRDX5* expression may be under the transcriptional control of c-Myc and *PRDX1* complexes, therefore regulating ROS homeostasis in situations in which the peroxidase activity of *PRDX1* is compromised (27).

Upregulation of *PRDX5* expression was first described during acute inflammation induced by lipopolysaccharide (LPS) in rat lung (41). More recently, it was shown that this dramatically increased expression of *PRDX5* in the lung during airway inflammation was moderately induced by changes in *PRDX5* expression in epithelial cells but rather caused by migrating neutrophils and monocytes recruited by LPS and expressing high levels of this peroxiredoxin (10, 48). Accordingly, *PRDX5* expression is highly increased *in vitro* in primary macrophages exposed to LPS and IFN- γ (1, 19). Downstream of the LPS/TLR pathway, p38 and JNK were shown also to be major contributors to *PRDX5* upregulation (1). Moreover, up- or downregulation of *PRDX5* has been further reported in the literature in different pathophysiologic

situations that may have functional consequences in term of antioxidant protection or redox-dependent signaling. Upregulation was reported in cells with mitochondrial dysfunction (35), in the lungs of mice in an asthmatic model (56), in degenerative human tendons (90), in osteoarthritic cartilage (91), in human tendon cells exposed to H₂O₂ (101), in ovarian carcinoma (38), in malignant mesothelioma (40), in breast carcinoma (37), in different thyroid pathologies (26, 70), during keratinocyte differentiation (79), in astrocytes in multiple sclerosis (31), in endothelial cells during shear stress (60), and in retina during hyperglycemia (49). Downregulation was described during aging in colon epithelial cells (55), during aging of peripheral nerves (57), in bone marrow macrophages during ionizing radiation exposure (11), in corneal endothelial dystrophy (36), in prostate after androgen deficiency (66, 84), in the nucleus accumbens in the brains of rats addicted to cocaine (18), in corticobasal neurodegeneration (12), in failing myocardium (6), in adrenocortical carcinoma (24), and in rat epithelial cells of airways exposed to cigarette-smoke extract (81).

In an initial report on the characterization of *PRDX5* (41), it was noted that human *PRDX5* mRNAs contained two AUGs in the same reading frame that could be used as two potential translation-initiation sites. It was suggested that translation from the first AUG would result in the synthesis of a long 214-residue protein, whereas the use of the second AUG would result in the production of a short 162-residue polypeptide. Moreover, as reported in Nguyen-Nhu *et al.* (61), several transcription start sites were also identified in human from *PRDX5* transcripts expressed in liver, lung, and brain. These transcription start sites are positioned upstream and downstream of the first translation-initiation site (61), suggesting that the use of alternative transcription start sites or the alternative use of the two translation initiation sites or both could be indeed used by human cells to produce a long form (L-*PRDX5*) or a short form (S-*PRDX5*) of the protein (Fig. 2). Interestingly, the additional 52 residues at the amino-terminal part of human L-*PRDX5* exhibited features of a cleavable mitochondrial targeting sequence. This sequence was shown to be able to target a fusion of L-*PRDX5* and green fluorescent protein into mitochondria (41). Also, it was noted that a potential peroxisomal targeting sequence (SQL) was present at the carboxy-terminus of both S-*PRDX5* and L-*PRDX5* (41, 99). Further examinations of *PRDX5* in tissues or cell lines and subcellular fractionation revealed that the protein was detected at only one molecular size (17 kDa), which was identical to that of the short form (80). The first amino acid downstream of the predicted cleavage site for the mitochondrial presequence of L-*PRDX5* corresponds exactly to the first amino acid of S-*PRDX5* after the cleavage of its first methionine. It corresponds also to the first amino acid initially determined by amino acid sequencing of AOEB166 spot in two-dimensional electrophoresis analysis of human bronchoalveolar lavage fluid (41, 92). These observations and the identification of a functional mitochondrial presequence in L-*PRDX5* suggest that L-*PRDX5* is rapidly imported into mitochondria as a precursor protein where the mitochondrial presequence is cleaved, converting L-*PRDX5* to a mature form, indistinguishable from S-*PRDX5*. Accordingly, detection by immunoblotting of *PRDX5* in cells transfected with constructs coding for human L-*PRDX5* and human S-*PRDX5* revealed only one band corresponding to a protein of 17 kDa (4).

Alternative splicing variants expressed in human, baboon, and African green monkey have been also reported (61).

These alternative splicing variants were not detected in mouse, rat, or pig tissues (61). Although it was noted that coding sequences are in frame with the two AUGs in the primate mRNA variants, no translation products were so far detected with Western blotting either in human tissues (80, 90, 91) or in human cell lines (80). However, it was shown recently that among these alternative splicing variants in humans, two isoforms showed widespread expression in neoplastic and normal tissues but were unstable at the protein level (78). The peptides were detectable only after inhibition of proteasomal degradation and were shown to be part of the immune-mediated stress response implicated in the detection and elimination of damaged normal or neoplastic cells (78).

Subcellular Localization

PRDX5 is widely expressed in mammalian tissues, and similar to other peroxiredoxins, the amounts of PRDX5 were estimated at between 0.2 and 1.3 $\mu\text{g}/\text{mg}$ of soluble proteins in rat tissues (80). However, contrary to the other members of the family, PRDX5 exhibits a surprisingly wide intracellular localization. PRDX5 can be localized to mitochondria, peroxisomes, the cytosol, and the nucleus in mammalian cells. It was previously noted that L-PRDX5 possesses an N-terminal mitochondrial targeting sequence that is able to address a fusion protein of L-PRDX5 and green fluorescent protein to mitochondria (41) (Fig. 2). The mitochondrial localization of PRDX5 was confirmed by subcellular fractionation (15, 46), *in situ* immunolocalization (3, 4, 40, 54, 63), as well as in mouse mitochondrial proteome analysis (65). The L-PRDX5 also exhibits a C-terminal peroxisomal targeting sequence 1 (PTS1) SQL tripeptide, but it appeared that when both mitochondrial and peroxisomal targeting sequences coexist in the L-PRDX5, the enzyme is addressed preferentially to mitochondria (3, 4). S-PRDX5 possesses only the C-terminal SQL peroxisomal targeting sequence. The peroxisomal localization of PRDX5 was confirmed in peroxisomal fractions prepared from guinea pig liver or HeLa cells (80), by *in situ* immunodetection, and in peroxisomal fractions of HeLa cells expressing HA-tagged S-PRDX5 (99), as well as in human peroxisomal proteome analysis (75). Moreover, it has been shown that PRDX5 is also localized in the cytosol (4, 40, 80, 99) and, in several situations, in the nucleus of mammalian cells (26, 40, 44, 63, 70). It was proposed that peroxisomal, cytosolic, and nuclear PRDX5 corresponds to the S-PRDX5 form, based on the fact that C-terminal SQL in PRDX5 appears as a weak peroxisomal targeting sequence and that in cells overexpressing S-PRDX5, the enzyme may be localized to these three subcellular compartments (4, 46, 61, 99). It is clear that further work will be necessary to elucidate the mechanisms controlling the complex PRDX5 subcellular addressing.

Crystal Structure

The reduced form

The first available crystal structure of the atypical 2-Cys human PRDX5 concerned its reduced form, in which the side chains of all Cys residues, the peroxidatic (Cp) Cys⁴⁷, the resolving (Cr) Cys¹⁵¹, and the additional Cys⁷² were characterized by the presence of a sulfhydryl group (16, 17). The overall structure of the reduced form is presented in Fig. 3 as a topologic diagram and as a ribbon diagram in more or less

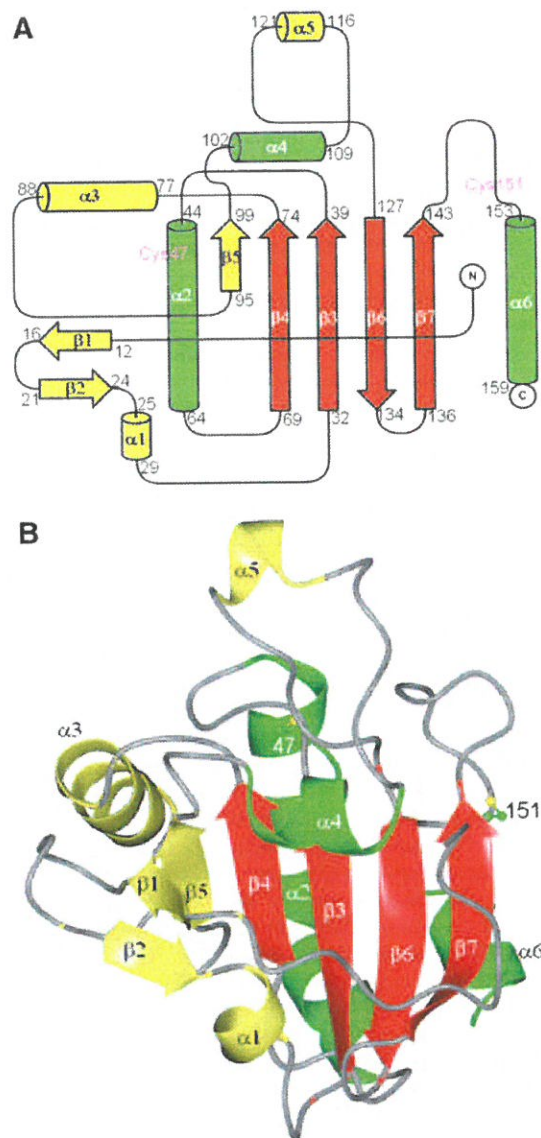


FIG. 3. Structure of the reduced form of human PRDX5. (A) Topologic diagram with indication of the secondary structural elements. The beginning and the end of each of them are labeled. The secondary elements belonging to the thioredoxin fold are colored green (helices) and red (β -strands). The remaining secondary elements are colored yellow. (B) Ribbon diagram colored as in A. The position of the peroxidatic (Cp) and resolving (Cr) Cys residues are indicated, and their side chains are represented as balls and sticks. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

similar orientations. This structure is characterized by the presence of a thioredoxin fold composed of a four-stranded β -sheet flanked by three α -helices. In addition to this thioredoxin fold, one can observe, in the N-terminal part, a short two-stranded β -sheet followed by a short α -helix. Furthermore, an additional β -strand (β 5) is associated with the thioredoxin β -sheet to form a five-stranded β -sheet. Two additional α helices

($\alpha 3$ and $\alpha 5$) are inserted in the sequence. The presence of this last $\alpha 5$ helix is unique, as it is not observed in other peroxiredoxin structures, and it replaces the usual loop joining $\alpha 4$ to $\beta 6$ in the thioredoxin fold. The complete structure is composed of one single domain. This situation is different from what is observed in 1-Cys and in typical 2-Cys peroxiredoxins, in which a second domain is usually present in the C-terminal part of the sequence.

The Cp residue Cys⁴⁷ of PRDX5 corresponds to a highly conserved Cys residue in all peroxiredoxins. In the reduced form, this residue is located in the N-terminal part of the $\alpha 2$ helix, which is kinked at the level of Ala⁵⁹. The direct surrounding of the S^γ atom of Cys⁴⁷ involves Arg¹²⁷, Thr⁴⁴, Val³⁹, and a benzoate ion. Arg¹²⁷ is probably responsible for the positively charged active-site pocket in which Cys⁴⁷ lies (Fig. 4). As shown in Fig. 5, the N^{η1} atom of Arg¹²⁷ is at a distance of 3.3 Å from Cys⁴⁷ S^γ. The threonine residue, Thr⁴⁴, forms a hydrogen bond between its oxygen atom O^{γ1} and Cys⁴⁷ S^γ at a distance of 3.0 Å. The presence of a benzoate ion in the vicinity of the active site was completely unexpected. One of the oxygen atoms is in direct contact with Cys⁴⁷ S^γ. The aromatic part of the benzoate is in contact with hydrophobic residues belonging to the $\alpha 5$ helix, which is present only in PRDX5. The presence of this ion was observed in all the crystal structures of the reduced forms of human PRDX5, as well as in the C47S mutant of this enzyme (23). It has disappeared in the oxidized forms. The origin and the exact role of this ion remain unknown, but it was speculated by Karplus and Hall (39) that it could bind as a substrate analogue, with the two carboxylate oxygens mimicking the two oxygen atoms of a peroxide substrate.

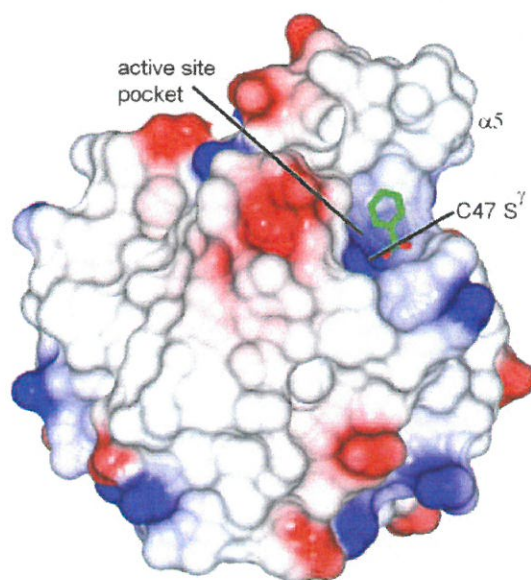


FIG. 4. Molecular surface of human PRDX5 showing the formation of the active-site pocket. The color represents the electrostatic potential, from red for the negatively charged regions to blue for the positively charged ones. The benzoate ion covers the access to the pocket. The position of C47 S^γ belonging to the peroxidatic Cys residue (Cp) is shown. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

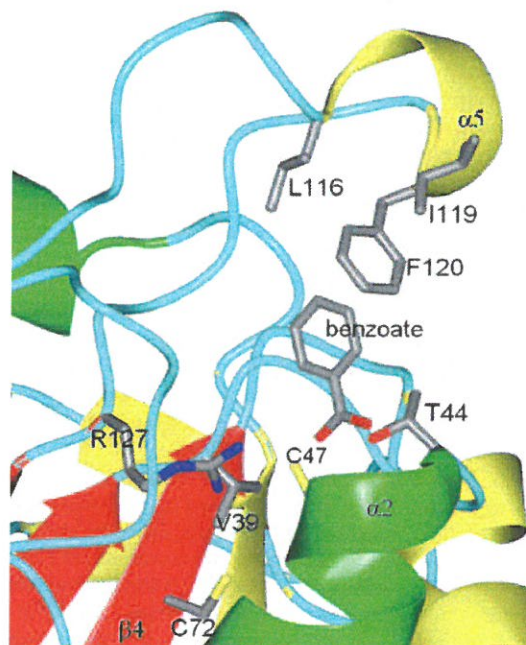


FIG. 5. Details of the region of the active site with the peroxidatic Cys (Cp) residue (Cys⁴⁷) in the N-terminal part of the $\alpha 2$ helix. The side chains of some residues discussed in the text are shown. The benzoate ion, via one of its oxygen atoms, is in direct contact with Cys⁴⁷ S^γ. The aromatic part of the benzoate is covered by hydrophobic residues belonging to the $\alpha 5$ helix. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

The other Cys residue implicated in the proposed mechanism of action of PRDX5 (80), the Cr residue Cys¹⁵¹, is located in the loop between $\beta 7$ and $\alpha 6$, and its side chain is exposed to the solvent. The proposed mechanism involves the formation of an intramolecular disulfide bond between these two Cys residues. However, the distance observed between the two S^γ atoms is 13.8 Å and the formation of the expected disulfide bond will thus not be possible without a large conformational rearrangement, as will be explained later in the description of the oxidized form of human PRDX5. This large conformational rearrangement is confirmed by the fact that freshly grown PRDX5 crystals are destroyed immediately in presence of hydrogen peroxide and survive only for a few days when they are simply exposed to air, whereas crystals of the C47S mutant in which the disulfide bond between Cys⁴⁷ and Cys¹⁵¹ cannot occur can survive several weeks, even in presence of hydrogen peroxide. An additional Cys, Cys⁷², is found in the C-terminal part of the $\beta 4$ strand of human PRDX5. It was shown (80) that this residue is not implicated in the mechanism, because its mutation does not modify the activity. It is thus surprising to find this Cys located close to the peroxidatic Cys, at the bottom of the active-site pocket.

The oxidized forms

From a historic point of view, the first crystal structure of the oxidized form of human PRDX5 showed unexpected intermolecular disulfide bonds between the Cp residue of one

monomer and the Cr residue of another monomer and vice versa (22). The formation of this kind of dimer involved a domain swapping in which the $\alpha 6$ helix of one chain comes in contact with the other chain and regenerates in an intermolecular way exactly the same hydrophobic contacts with $\alpha 2$ and $\beta 6$ that were observed in the reduced form in an intramolecular way. This movement associated with the unwinding of the N-terminal part of $\alpha 2$ helices brings Cys⁴⁷ of one chain in close proximity to Cys¹⁵¹ of the other chain, allowing the formation of the disulfide bond. Because it is not clear whether this dimeric oxidized form is physiologically relevant, it is not further discussed. The crystal structure of the oxidized form with the expected intramolecular disulfide bond was described a few years later (83). In this structure, the overall fold is very similar to that of the reduced form previously described. The only differences occur in the regions of the Cp and Cr residues. The N-terminal part (residues 44 to 51) of the $\alpha 2$ helix containing the Cp residue Cys⁴⁷ is completely unwound, allowing a movement of this residue in the direction of the Cr residue Cys¹⁵¹. An amplitude as large as 9.5 Å was estimated for this movement but clearly did not suffice for the formation of the disulfide bridge. It was also necessary to displace the region containing the Cr residue Cys¹⁵¹ in the direction of Cys⁴⁷. This second movement involved residues 145 to 156 and thus included the N-terminal part of the $\alpha 6$ helix. The amplitude of the movement of the Cr residue is about 4 Å. The combination of these two movements finally allows the formation of the intramolecular disulfide bond illustrated in Fig. 6. This disulfide bond appears at the surface of the molecule and is thus accessible for reduction by thioredoxin. The benzoate ion has disappeared, as well as the contacts of Cys⁴⁷ with Thr⁴⁴ and Arg¹²⁷. The large flexibility of

the two regions (44 to 51 and 145 to 156) involved in the formation of this disulfide bond can be estimated from a comparison of the main-chain conformational angles ϕ and ψ . This is illustrated by a multiple Ramachandran plot (Fig. 7), in which an arrow joins the locations of a residue in the reduced and the oxidized forms. As expected, the origin of many arrows appears in the α -helix region. It can also be seen that some angle modifications are close to 180 degrees, the maximum possible value.

Oligomerization

In typical 2-Cys and 1-Cys peroxiredoxins, the molecules are associated as dimers, known as type B dimers, in which two $\beta 7$ strands of two molecules are linked in an antiparallel mode to form a 10-stranded β -sheet. This kind of dimer was never observed for human PRDX5 in either the reduced or the oxidized form. As can be seen in Figs. 3B and 6A, the conformation of the $\beta 7$ - $\alpha 6$ loop presents a bump close to the C-terminal part of $\beta 7$, which does not allow the bringing of the $\beta 7$ strands of two molecules together for the formation of the large β -sheet. However, PRDX5 molecules are also associated as dimers, known as type A dimers, illustrated in Fig. 8, in which the $\alpha 3$ helix of one monomer comes in close contact with the $\alpha 5$ helix of the other one. These contacts also involve residues in the $\beta 3$ - $\alpha 2$ loop and in the $\beta 5$ - $\alpha 4$ loop. Most contacts are hydrophobic, except an intermolecular salt bridge Arg¹²⁴ to Asp⁷⁷. These dimers exist irrespective of the redox state, and the buried surface is approximately 730 Å² per monomer. This kind of type A interaction also is observed during the formation of toroid decamers in reduced or overoxidized forms of typical 2-Cys peroxiredoxins by the association of

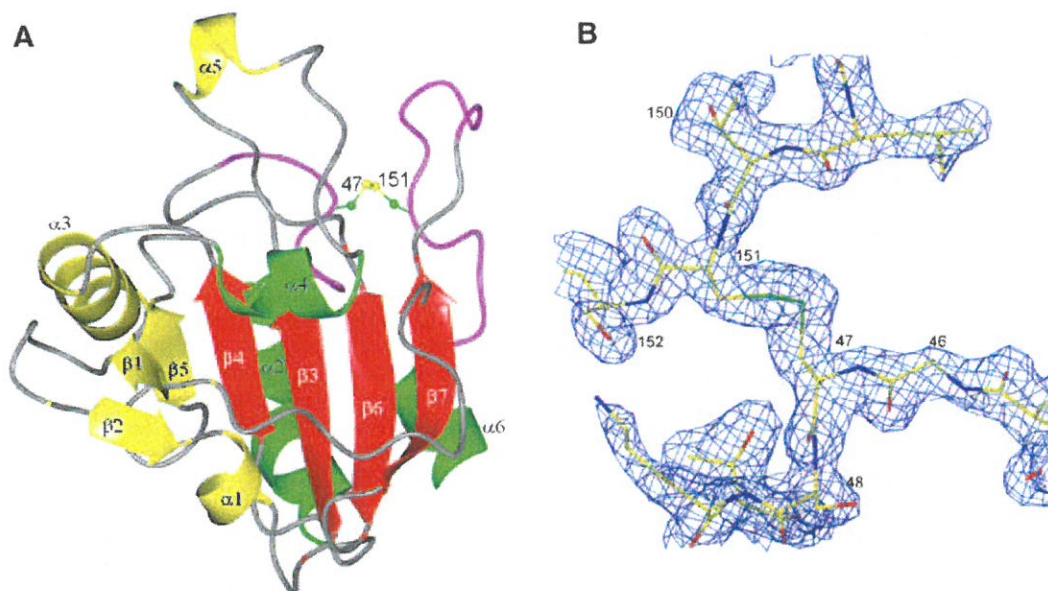


FIG. 6. Structure of the oxidized form of human PRDX5 with the formation of an intramolecular disulfide bond. (A) Ribbon diagram in which the orientation and the colors are similar to those in Fig. 3B, except that the flexible regions are colored magenta. (B) The region of the intramolecular disulfide bond in the oxidized PRDX5 molecule. The electron density corresponds to a sigma map contoured at a level of 1.1 σ . [Reprinted with permission from Smeets *et al.* (83)]. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

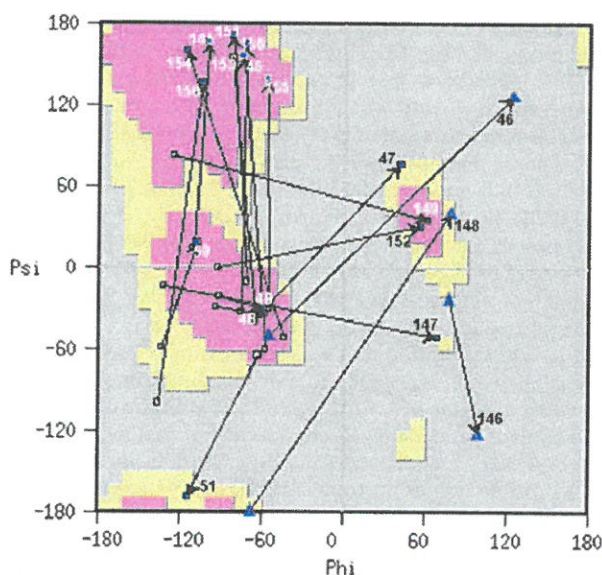


FIG. 7. Multiple-model Ramachandran plot of the mobile regions of a reduced and an oxidized molecule of human PRDX5. Arrows connect equivalent residues of the two redox states departing from the reduced one. The tips of the arrows are labeled and correspond to the oxidized state. Glycine residues are represented as triangles, and the other residues, as squares. [Reprinted with permission from Smeets *et al.* (83)]. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

five type B dimers (98). Of course, PRDX5 is unable to form this kind of decamer, because type B dimers do not exist.

Enzymatic Mechanism and Kinetics

Mammalian PRDX5 was the first characterized peroxiredoxin belonging to the atypical 2-Cys subfamily (80). Mutants of human PRDX5 in which Cys⁴⁷ (Cp), Cys⁷², and Cys¹⁵¹ (Cr) were replaced by a serine revealed that oxidized Cys⁴⁷ reacts with Cys¹⁵¹ to form an intramolecular disulfide bridge (Fig. 9), whereas in typical 2-Cys peroxiredoxins, the resolving mechanism leads to the formation of an intermolecular disulfide linkage between two monomers (80). The existence of an intramolecular disulfide bridge between Cp and Cr was confirmed in oxidized crystal forms (83). The reduction of the disulfide formed by the oxidation of PRDX5 was shown to be performed by cytosolic or mitochondrial thioredoxin but not by glutaredoxin or glutathione (20, 80, 87, 103). Interestingly, cyclophilin A was also reported as potential reductant for PRDX5 (51), but the physiologic relevance is unknown. It must be also noted that a PRDX5 reductant in peroxisomes remains to be identified with regard to the potential peroxidase activity of PRDX5 in this organelle.

Prokaryotic and eukaryotic peroxiredoxins catalyze the reduction of hydrogen peroxide, organic peroxides, and peroxynitrite with different efficiencies (87). Human PRDX5 was the first mammalian peroxiredoxin shown to react with high rate constants with peroxynitrite in the $10^7 \text{ M}^{-1}\text{s}^{-1}$ range (20, 87). Human PRDX5 reacts with hydrogen peroxide more slowly ($\sim 10^5 \text{ M}^{-1}\text{s}^{-1}$) than with organic peroxides ($\sim 10^6$

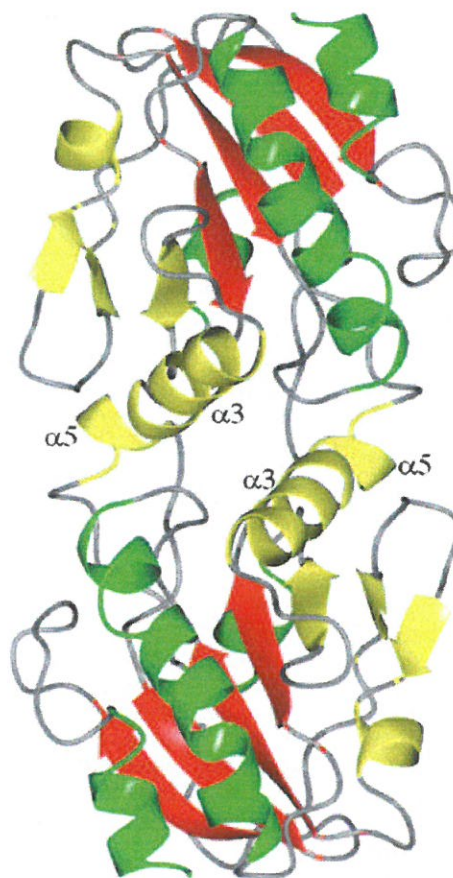


FIG. 8. Ribbon diagram of a dimer formed by reduced human PRDX5 molecules. The ribbon diagram shows the association of the $\alpha 3$ helix of one monomer with the $\alpha 5$ helix of the other one. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

$\text{M}^{-1}\text{s}^{-1}$) or peroxynitrite. This suggests that, in subcellular compartments where PRDX5 coexists with other peroxiredoxins (cytosol, nucleus, mitochondria), the mammalian atypical 2-Cys peroxiredoxin could be involved more specifically in the reduction of peroxynitrite or lipid peroxides instead of hydrogen peroxide (14, 87).

Peroxiredoxin enzymatic activities have been shown to be regulated by posttranslational modifications. Mammalian typical 2-Cys peroxiredoxins, for example, are prone to overoxidation of their Cp in presence of an excess of hydrogen peroxide, which results in a transient (sulfinic acid) or permanent (sulfonic acid) inactivation of the enzyme (94). The reduction of the sulfinic acid can be catalyzed by sulfiredoxin (9). It has been proposed that mammalian typical 2-Cys peroxiredoxins are more susceptible to hyperoxidation than are peroxiredoxins lacking a C-terminal YF motif that interacts with a GGLG motif in a loop neighboring the Cp. These motifs and other structural features would participate in the kinetic pause in disulfide bond formation, leading to overoxidation of Cp (97). These domains are lacking in prokaryotic peroxiredoxins but also in PRDX5 and PRDX6 in mammals. It appeared that mammalian PRDX5 is more resistant to the

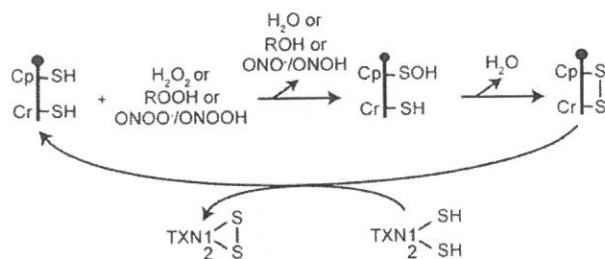


FIG. 9. Peroxidase reaction mechanism of human PRDX5. Hydrogen peroxide (H₂O₂), alkyl hydroperoxide (ROOH), or peroxynitrite (ONOO⁻/ONOOH) is reduced by the peroxidatic Cys (Cp), which is oxidized into sulfenic acid (-SOH). Sulfenic acid reacts with the thiol of the resolving Cys (Cr) to form an intramolecular disulfide bond. Oxidized PRDX5 is then reduced by cytosolic thioredoxin 1 (TXN1) or mitochondrial thioredoxin 2 (TXN2). Solid circles correspond to the amino terminus of the protein [modified with permission from (20, 53, 80)].

sulfenylation by hydrogen peroxide than are typical 2-Cys peroxidoredoxins (95). Accordingly, it was also shown that sulfiredoxin does not interact with PRDX5 (94). Although the activity of mammalian typical 2-Cys peroxidoredoxins may be modulated by different posttranslational modifications (72), little is known about such possible modifications of PRDX5. Interestingly, PRDX5 may undergo glutathionylation in rat hepatocytes exposed to oxidative stress (25), but the functional significance of such posttranslational modification is still to be investigated.

Functions

Cytoprotective antioxidant function of PRDX5 was first investigated after human PRDX5 was characterized as a peroxidase but also because of the presence of this enzyme in mitochondria and peroxisomes, two organelles that are major intracellular sources of ROS/RNS (reactive nitrogen species). Cellular peroxidase activity of human PRDX5 was first investigated in NIH3T3 and HeLa cells by overexpressing human PRDX5 that inhibited hydrogen peroxide accumulation induced by tumor necrosis factor (TNF)- α and PDGF (80) or p53 (103). Moreover, it was also reported that human PRDX5 expressed in mitochondria or in the cytosol of *Saccharomyces cerevisiae* protected yeast cells from oxidative stress induced by paraquat (85). Overexpression of human PRDX5 in the cytosol, in mitochondria, in the nucleus of CHO or HT-22 cells (4, 104), or in the cytosol of tendon cells (101) was shown also to reduce significantly cell death caused by exogenous peroxide exposure. Interestingly, exogenous administration of recombinant human PRDX5 was demonstrated to provide protection against ibotenate-induced excitotoxic stress in mouse brain (69). Also, PRDX5-expression modulation in mitochondria or in the nucleus of cell lines exposed to prooxidant drugs or peroxides revealed that the enzyme may confer protection against mitochondrial or nuclear DNA damage (3, 4, 45). Moreover, cells with reduced expression of PRDX5 were shown to be more prone to oxidative damage and apoptosis induced by etoposide, doxorubicin (Adriamycin), hydrogen peroxide, and the mitochondrial toxin MPP⁺ (2, 15, 43, 81). In *Xenopus* embryos, heterologous expression of

human PRDX5 protects cells against alcohol-induced ROS/RNS production (67, 68). Finally, orthologous PRDX5 in *Drosophila melanogaster*, that is expressed in different cellular compartments among which mitochondria, the nucleus and the cytosol, was demonstrated not only to protect against oxidative stress and apoptosis but also to promote longevity of the fly (71). It must be noted that yeast Pmp20, a 1-Cys peroxidoredoxin homologous to human PRDX5 and localized in peroxisomes of methylotrophic *Candida boidinii*, *Hansenula polymorpha*, or *Pichia pastoris*, has been reported to play a major antioxidant cytoprotective role, emphasizing the functional importance of peroxidoredoxins in these organelles (5, 32, 100).

It is now recognized that hydrogen peroxide produced in mammalian cells in response to different stimuli may serve as signaling molecule (73). Although PRDX5 was initially shown to modulate peroxide levels produced in cells after stimulation by PDGF, TNF- α , or p53 (80, 103), few data are available on a possible role of mammalian PRDX5 as a redox sensor. Moreover, it must be noted that mammalian PRDX5 seems to lack important features to be a good candidate as modulator of peroxide-dependent signal transduction. As discussed earlier, contrary to mammalian typical 2-Cys peroxidoredoxins, PRDX5 appears to be more resistant to overoxidation by hydrogen peroxide (95), and, so far, PRDX5 is not recognized as a good substrate for kinases, such as cyclin-dependent kinases regulating the peroxidase activity of typical 2-Cys peroxidoredoxins (8, 96). However, we cannot totally exclude that mammalian PRDX5 may act in redox signaling mechanisms as a peroxidase-based protein-oxidation relay (28) by using a mechanism similar to the one identified in yeast between peroxidase Orp1 and transcription factor Yap1 (86). It is tempting to hypothesize that the translocation of PRDX5 into the nucleus, as described for example in thyrocytes under pathophysiologic oxidative stresses (26, 70), may be involved in redox-dependent transcription regulation. Further studies will be necessary to investigate this possible function.

The existence of different enzymes able to decompose peroxides in the same subcellular compartment, such as the mitochondrion, the peroxisome, or the cytosol, obviously raises questions about the redundancy and thus the specific function of these antioxidant enzymes. In mitochondria, PRDX5 coexists with other peroxidases, namely PRDX3, GPX1, and GPX4 (14). Mitochondrial localization of PRDX5 seems to be quite well conserved throughout evolution, as predicted mitochondrial targeting sequences have been also identified in orthologous PRDX5 in invertebrates (71, 102) (see also Fig. 1). A very informative kinetic hierarchy of mitochondrial reductases of hydrogen peroxide was proposed recently, based on known rate constants and enzyme abundance (14). Obviously, the hierarchy must be adapted to cell types, as this ranking depends on abundance of each peroxidase and the availability of reducing equivalents for peroxidase recycling. In this analysis, mitochondrial PRDX5 would play a minor role in the reduction of hydrogen peroxide compared with PRDX3 or GPX1. However, in this organelle, PRDX5 high rate constants with alkyl hydroperoxides and peroxynitrite could confer to this peroxidase a more-specific function in peroxide reduction (15, 87). Moreover, one should also take into account a possible heterogeneous distribution of peroxidases inside mitochondria and consider also different susceptibilities to inactivation. It is clear that such speculations will need experimental validation.

In the peroxisomal matrix, PRDX5 may assist catalase in peroxide reduction. Catalase is a very efficient hydrogen peroxide reductase, whereas PRDX5 is an efficient alkyl hydroperoxide and peroxynitrite reductase (20, 87). In methylotrophic yeasts, Pmp20 peroxiredoxin appeared, as emphasized earlier, to play a major role as protective antioxidant enzyme when yeast cells grow with methanol that is metabolized by peroxisomal enzymes (5, 32, 100). Studies investigating the effects of human S-PRDX5 overexpression were not able to target the protein only to this organelle. Overexpression of the short form of human PRDX5 addresses the protein to the cytosol, the peroxisomes, and the nucleus (4, 46, 99, 101). Moreover, even if PRDX5 acts as a peroxidase in the peroxisomes, a reductant is still to be identified as, to our knowledge, no thioredoxin system has been described in peroxisomes.

In the cytosol of mammalian cells, PRDX5 may coexist with PRDX1, PRDX2, and PRDX6 (72). Thus, the specific role of PRDX5 in this subcellular compartment is not clear. However, according to enzyme kinetics (87) and on accumulating data reporting that PRDX1 and PRDX2 are involved in hydrogen peroxide signaling in mammalian cells (73, 96), cytosolic PRDX5 as well as PRDX6 should rather be considered cytoprotective antioxidant enzymes (2, 4, 58) (see also the article by A. Fisher in this Forum), although this view may be not generalized to all mammalian cell types.

Finally, the function of PRDX5 in the nucleus is still a matter of debate. Initially, PRDX5 was described as a DNA-binding protein able to repress transcription of retrotransposons in human cells (44) before its peroxidase activity was established. Also, consistent with a role as a protective antioxidant enzyme, overexpression of PRDX5 in the nucleus protects DNA from oxidative damage caused by peroxides (4), and silencing the gene stimulates formation of etoposide-induced double-strand DNA breaks (47). It is important to note that in yeast, peroxiredoxins appeared also to play a major role against oxidative attacks leading to DNA damage and genome instability (7, 33, 34, 93). However, PRDX5 is not expressed in the nucleus at high levels in all cell types in mammals (4). Interestingly, translocation into the nucleus under pathophysiologic situations, for example in thyrocytes (26, 70), suggests that PRDX5 may have a function in the nucleus either as a DNA-protective enzyme or as a redox-dependent signaling molecule acting possibly as a peroxidase-based protein oxidation relay (28).

An unexpected role of PRDX5 in immunogenicity in human metastatic melanoma appeared more recently with the identification of an antigen generated by a point mutation in PRDX5 in human melanoma lacking the wild-type allele (77). It was suggested that this tumor antigen could be an immunogenic target for T cell-specific immunotherapy (76). Moreover, as mentioned earlier, the alternative splicing variants of PRDX5 expressed in human cells appeared (61) to be involved in immune regulation (78). Through the generation of short antigenic peptides encoded by these spliced PRDX5 isoforms, it was proposed that an immune-mediated response to stress, including oxidative stress, may contribute to the detection and elimination of neoplastic cells or even damaged normal cells (78).

Conclusions and Perspectives

To conclude, PRDX5 is the last-discovered peroxiredoxin in mammals and the only mammalian atypical 2-Cys peroxir-

edoxin. In humans, PRDX5 exhibits a remarkably wide subcellular distribution with a localization in the cytosol, in mitochondria, in peroxisomes, and in the nucleus. PRDX5 is a peroxidase that uses cytosolic thioredoxin 1 or mitochondrial thioredoxin 2 as physiologic reductants to reduce alkyl hydroperoxides or peroxynitrite with high rate constants, whereas the PRDX5 reaction with hydrogen peroxide is slower. Although PRDX5 was shown to be constitutively and ubiquitously expressed in virtually all mammalian tissues, up- and downregulation have been reported in different pathophysiologic situations. Moreover, PRDX5 overexpression protects cells against nitrooxidative stress, whereas a decreased expression makes cells more vulnerable. These observations as well as biochemical and structural features suggest that in mammalian cells, PRDX5 may act mainly as a cytoprotective antioxidant enzyme like prokaryotic peroxiredoxins, whereas typical 2-Cys peroxiredoxins would rather play the role of redox sensors in hydrogen peroxide-dependent intracellular signaling. It is clear that further studies will be required to gain insights into the specific functions of PRDX5 in subcellular compartments to which other peroxidases are also targeted. Finally, although knockout mice exist for the five other peroxiredoxins, so far, PRDX5 knockout mice or transgenic mice overexpressing PRDX5 are lacking but would be necessary to understand better the functions of the unique mammalian atypical 2-Cys peroxiredoxin.

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Abbreviations Used

ACR1 = Alu complex in gel-retardation assays 1 protein
AOEB166 = antioxidant enzyme B166
CHO = Chinese hamster ovary cells

c-Myc = c-Myc transcription factor
Cp = peroxidatic cysteine
Cr = resolving cysteine
Ets1/2 = E-twenty-six transcription factor 1 and 2
Foxo3a = forkhead box transcription factor O3a
GPX1 = glutathione peroxidase 1
GPX4 = glutathione peroxidase 4
HeLa = HeLa tumor cells
HMGB1 = high-mobility-group protein B1
HSPC152 = *Homo sapiens* hypothetical protein 152
HT-22 = mouse hippocampal HT-22 cells
IFN- γ = interferon- γ
JNK = c-Jun N-terminal kinase
L-PRDX5 = long peroxiredoxin 5
LPS = lipopolysaccharide
MnSOD, SOD2 = manganese superoxide dismutase, superoxide dismutase 2
MPP⁺ = 1-methyl-4-phenylpyridinium ion
MTS = mitochondrial targeting sequence
NF- κ B = nuclear factor-kappa B
NIH3T3 = NIH/3T3 mouse fibroblast cell line
Nrf2 = nuclear factor erythroid 2-related factor 2
Orp1 = oxidant receptor peroxidase 1
p38 = mitogen-activated protein kinase p38
p53 = cellular tumor antigen p53
PDGF = platelet-derived growth factor
PGC-1 α = peroxisome proliferator activated receptor- γ coactivator 1- α
PMP20, Pmp20 = peroxisomal membrane protein 20
PRDX1 = peroxiredoxin 1
PRDX2 = peroxiredoxin 2
PRDX3 = peroxiredoxin 3
PRDX5, PrxV = peroxiredoxin 5
PRDX6 = peroxiredoxin 6
PTS1 = peroxisomal targeting sequence 1
RNS = reactive nitrogen species
ROS = reactive oxygen species
S-PRDX5 = short peroxiredoxin 5
TLR = Toll-like receptor
TNF- α = tumor necrosis factor alpha
TRM112 = tRNA methyltransferase 112
TXN1 = thioredoxin 1
TXN2 = thioredoxin 2
Yap1 = yeast AP-1-like transcription factor

