Peroxiredoxins (Prxs) are a ubiquitous family of antioxidant enzymes that also control cytokine-induced peroxide levels which mediate signal transduction in mammalian cells. Prxs can be regulated by changes to phosphorylation, redox and possibly oligomerization states. Prxs are divided into three classes: typical 2-Cys Prxs; atypical 2-Cys Prxs; and 1-Cys Prxs. All Prxs share the same basic catalytic mechanism, in which an active-site cysteine (the peroxidatic cysteine) is oxidized to a sulfenic acid by the peroxide substrate. The recycling of the sulfenic acid back to a thiol is what distinguishes the three enzyme classes. Using crystal structures, a detailed catalytic cycle has been derived for typical 2-Cys Prxs, including a model for the redox-regulated oligomeric state proposed to control enzyme activity.

Peroxiredoxins (Prxs) [1,2] have received considerable attention in recent years as a new and expanding family of thiol-specific antioxidant proteins, also termed the thioredoxin peroxidases and alkyl-hydroperoxide-reductase-C22 proteins. Prxs exert their protective antioxidant role in cells through their peroxidase activity (ROOH + 2e− → ROH + H2O), whereby hydrogen peroxide, peroxynitrite and a wide range of organic hydroperoxides (ROOH) are reduced and detoxified [3–7]. Indeed, these enzymes are truly ubiquitous having been identified in yeast, plant and animal cells, including both protozoan and helminth parasites, and most, if not all, eubacteria and archaea.

Although located primarily in the cytosol, Prxs are also found within mitochondria, chloroplasts and peroxisomes, associated with nuclei and membranes, and, in at least one case, exported [3,8]. Prxs are produced at high levels in cells: they are among the ten most abundant proteins in Escherichia coli [9], the second or third most abundant proteins in the unicellular protozoan Tetrahymena thermophila [10], and compose 0.1–0.8% of the soluble protein in other mammalian cells [11]. Many organisms produce more than one isofrom of Prx, including at least six Prxs identified in mammalian cells (PrxI–PrxVI; Table 1). Recently, a range of other cellular roles have also been ascribed to mammalian Prx family members, including the modulation of cytokine-induced hydroperoxide levels, which have been shown to mediate signaling cascades leading to cell proliferation, differentiation and apoptosis [3,8,12,13]. The peroxidatic functions of Prxs probably overlap to some extent with those of the better known glutathione peroxidases and catalases, although it has been suggested that their moderate catalytic efficiencies (~10^5 M^−1 s^−1) compared with those of glutathione peroxidases (~10^6 M^−1 s^−1) [3] and catalases (~10^6 M^−1 s^−1) [14] makes their importance as peroxidases questionable [3]. Nonetheless, the high abundance of Prxs in a wide range of cells and a recent finding that a bacterial Prx [alkyl hydroperoxide reductase C22 (AhpC)] and not catalase is responsible for reduction of endogenously generated H2O2 [15] argue that Prxs are indeed important players in peroxide detoxification in cells.

Prx use redox-active cysteines to reduce peroxides and were originally divided into two categories, the 1-Cys and 2-Cys Prxs, based on the number of cysteinyi residues directly involved in catalysis [1]. Structural and mechanistic data now support the further division of the 2-Cys Prxs into two classes called the ‘typical’ and ‘atypical’ 2-Cys Prxs. The peroxidase reaction is composed of two steps centered around a redox-active cysteine called the peroxidatic cysteine. Based on existing data [16,17], all three Prx classes appear to have the first step in common, in which the peroxidatic cysteine (Cys−S=O) attacks the peroxide substrate and is oxidized to a cysteine sulfenic acid (Cys−SOH) (Fig. 1) [16,18]. The peroxide decomposition probably requires a base to deprotonate the peroxidatic cysteine as well as an acid to protonate the poor RO− leaving group, but these catalysts have yet to be identified. All Prxs to date conserve an active-site Arg, which would lower the pH of the peroxidatic cysteine somewhat by stabilizing its thiolate form (Fig. 1). The second step of the peroxidase reaction, the resolution of the cysteine sulfenic acid, distinguishes the three Prx classes.

The typical 2-Cys Prxs are the largest class of Prxs and are identified by the conservation of their two redox-active cysteines, the peroxidatic cysteine (generally near residue 50) and the resolving cysteine (near residue 170) [3]. Typical 2-Cys Prxs are obligate homodimers containing two identical active sites [19–22]. In the second step of the peroxidase reaction, the peroxidatic cysteine sulfenic acid (Cys−S=O) from one subunit is attacked by the resolving cysteine (Cys−S=R) located in the C terminus of the other subunit (Fig. 1). This condensation reaction results in the formation of a stable intersubunit disulfide bond, which is then reduced by one of several cell-specific disulfide

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**Structure, mechanism and regulation of peroxiredoxins**

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oxidoreductases (e.g. thioredoxin, AhpF, tryparedoxin or AhpD [23–25]), completing the catalytic cycle.

The second class of Prxs are the atypical 2-Cys Prxs, which have the same mechanism as typical 2-Cys Prxs but are functionally monomeric [26,27]. In these Prxs, both the peroxidatic cysteine and its corresponding resolving cysteine are contained within the same polypeptide, with the condensation reaction resulting in the formation of an intramolecular disulfide bond (Fig. 1). Although the resolving cysteines of typical and atypical 2-Cys Prxs are not conserved in sequence, they are functionally equivalent. To recycle the disulfide, known atypical 2-Cys Prxs appear to use thioredoxin as an electron donor [26].

The last class of Prxs, the 1-Cys Prxs, conserve only the peroxidatic cysteine and do not contain a resolving cysteine (Fig. 1) [17]. Their cysteine sulfinic acid generated on reaction with peroxides is presumably reduced by a thiol-containing electron donor, but the identity of this redox partner is not yet clear (although proposed electron donors have included glutathione, lipoic acid and cyclophilin [3,7,28,29]). By analogy, one donor thiol probably forms a transient mixed disulfide bond with the enzyme, followed by its reduction by a second donor thiol, thus recycling the enzyme.

Recently, studies of several typical 2-Cys Prxs have revealed dramatic changes in oligomeric state (dimers and decamers) linked to changes in redox state like those occurring during the catalytic cycle. A combination of biophysical techniques has been used to examine the various oligomeric forms of several these enzymes, revealing an intimate connection between the oxidation state of the peroxidatic cysteine and the preferred oligomeric state of the enzyme. Although we are at an early stage of understanding the link between the oligomeric state of Prxs and their function, the recent biophysical studies have provided some important insights to the field and are the subject of this article.

Table 1. Six subclasses of Peroxiredoxins (Prxs) from mammals

<table>
<thead>
<tr>
<th>Prx subtype</th>
<th>Prx (2-Cys)</th>
<th>PrxII (2-Cys)</th>
<th>PrxIII (2-Cys)</th>
<th>PrxIV (2-Cys)</th>
<th>PrxV (atypical 2-Cys)</th>
<th>PrxVI (1-Cys)</th>
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<tbody>
<tr>
<td>Previous nomenclature</td>
<td>TPx-A</td>
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<td>NKEF B</td>
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<td>Torin</td>
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<td>PAG</td>
<td>Band-8</td>
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<td>Polypeptide length</td>
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<td>199 aa</td>
<td>256 aa (cleaved at 63–64)*</td>
<td>271 aa (cleaved at 36–37)*</td>
<td>214 aa (cleaved at 52–53)*</td>
<td>224 aa</td>
</tr>
<tr>
<td>Human chromosomal location</td>
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<td>1q12</td>
<td>10q25–q26</td>
<td>10p22.13*</td>
<td>11q13</td>
<td>1q23.3</td>
</tr>
<tr>
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<td>Mitochondria</td>
<td>Cytosol, Golgi, secreted</td>
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<td>aopp_2_human</td>
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<td>Cyclophilin</td>
<td>Heparin</td>
<td>DNA</td>
<td>Cyclophilin</td>
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<tr>
<td></td>
<td>Presenilin-1</td>
<td>(stomatin)</td>
<td>Abrin A-chain</td>
<td>Cyclophilin</td>
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<td></td>
<td>Macroage migration inhibitory factor</td>
<td>Erythrocyte membrane</td>
<td>Cyclophilin</td>
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<tr>
<td></td>
<td>Cyclophilin</td>
<td></td>
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</tbody>
</table>
| Abbreviation: aa, amino acids. *These proteins are post-translationally processed.

Dimers, decamers and redox-dependent oligomerization

The first reports of Prx oligomerization came in the late 1960s, when transmission electron microscopy (TEM) studies of torin, an abundant protein isolated from human erythrocytes, revealed discrete complexes with apparent tenfold symmetry (Fig. 2a) [30]. Later, in the 1980s, bacterial and yeast Prxs were identified based on their antioxidant properties [31,32]. Torin has since been identified as mammalian PrxII, a typical 2-Cys Prx [33]. In the TEM reports, it was observed that under certain conditions, PrxII and the related PrxIII could also form higher-order multimers by stacking into columns of various lengths (Fig. 2b) [33,34]. The physiological relevance of these columns, if any, is not known. Recently, single-particle TEM analysis of negatively stained PrxII particles enabled the three-dimensional reconstruction of the toroid to ~20-A resolution (Fig. 2c) [33]. Importantly, the surface-rendered TEM reconstruction correlated well with the solvent-accessible surface revealed by the X-ray crystal structure of PrxII (Fig. 2d) [20].

The oligomeric properties of several typical 2-Cys Prxs in solution have been studied using gel filtration [35–39], light scattering [22,37] and analytical ultracentrifugation [20,22]. Factors shown to promote oligomerization in typical 2-Cys Prxs include high [37] or low [34,39] ionic strength, low pH [40], high magnesium [34] or calcium [41,42] concentrations, reduction of the redox-active disulfide center [20,22,26], and ‘overoxidation’ of the peroxidatic cysteine to a sulfonic acid (Cys–SO2H) [20]. Reduction of the active-site disulfide of typical 2-Cys Prxs is emerging as the primary factor in the stabilization of the decameric forms of these enzymes; a direct link between redox state and oligomerization state was recently established through analytical ultracentrifugation of several bacterial 2-Cys Prxs [22,43] (L.B. Poole, unpublished) and human PrxII [20], as well as earlier gel-filtration
studies of porcine PrxII [36]. Information gleaned from high-resolution crystal structures of various redox and oligomeric forms of Prxs is shedding light on the mechanisms controlling the redox-sensitive oligomerization.

The Prx classes have similar active sites
Since 1998, the crystal structures of six Prxs have been published, including four typical 2-Cys Prxs (PrxI, PrxII, TryP and AhpC [19–22]), one atypical 2-Cys Prx (PrxV [27]) and one 1-Cys Prx (PrxVI [17]) (Fig. 3). These structures reveal Prxs to be very similar, each containing a thioredoxin fold with a few additional secondary-structure elements present as insertions. The most striking differences involve their oligomeric states. The typical 2-Cys Prxs are monomeric enzymes, whereas both the typical 2-Cys and the 1-Cys Prxs are domain-swapped homodimers in which the C terminus of one subunit reaches across the dimer interface to interact with the other subunit. In the typical 2-Cys Prxs, the resolving cysteine (pink S_R) originate from different subunits and condense to form an intersubunit disulfide bond (black and pink striped bar). Reduction of typical and atypical 2-Cys Prxs involves one flavoprotein disulfide reductase and at least one additional protein or domain containing a CXXC motif, which is oxidized from a dithiol (2 RSH) to a disulfide (RSSR) state during Prx reduction (e.g. thioredoxin reductase and thioredoxin, AhpF, trypanothione reductase, trypanothione and tryparedoxin, or lipoxime dehydrogenase, SucB and AhpD [23–26]). Reductants of 1-Cys Prxs include low molecular weight thiols, but physiological partners are as yet unidentified.

Fig. 1. Peroxiredoxin (Prx) mechanism. (a) The common first step of peroxide reduction involving nucleophilic attack by the peroxidatic cysteine (S_P) and formation of the cysteine sulfenic acid intermediate (S.PO.H), probably shared by all Prxs. Both the catalytic base that deprotonates the peroxidatic cysteine and the catalytic acid that protonates the RO^− leaving group are labelled ‘B’, although this does not imply that they are the necessarily same entity. The guanidino group of the conserved arginine is presumed to stabilize the ionized peroxidatic cysteine. (b) The three mechanisms distinguishing the Prx classes, with peroxidatic cysteines and resolving cysteines in the reduced (SH) and sulfenic-acid (SPOH) or disulfide (S_P and S_R connected) state. In the case of dimeric 2-Cys Prxs, the peroxidatic cysteine (black S_P) and resolving cysteine (pink S_R) originate from different subunits and condense to form an intersubunit disulfide bond (black and pink striped bar). Reduction of typical and atypical 2-Cys Prxs involves one flavoprotein disulfide reductase and at least one additional protein or domain containing a CXXC motif, which is oxidized from a dithiol (2 RSH) to a disulfide (RSSR) state during Prx reduction (e.g. thioredoxin reductase and thioredoxin, AhpF, trypanothione reductase, trypanothione and tryparedoxin, or lipoxime dehydrogenase, SucB and AhpD [23–26]). Reductants of 1-Cys Prxs include low molecular weight thiols, but physiological partners are as yet unidentified.
The pyrrolidine ring of Pro44 limits the solvent and peroxide accessibility of the peroxidatic cysteine and shields the reactive cysteine sulfenic acid intermediate from further oxidation by peroxides. Although the side chain of residue 45 is not conserved, its main-chain conformation is, resulting in its peptide amide donating a hydrogen bond to the \( \text{S}^\cdot\text{O}^\cdot\text{H} \) of the peroxidatic cysteine (Cys51), whereas its carbonyl oxygen accepts a hydrogen bond from the O\( ^\cdot \text{C} \) (Cys51), whereas its carbonyl oxygen accepts a hydrogen bond to the S\( ^\cdot\text{O} \) of Thr48 in the reduced structures (PrxV). The C-terminal arm is disordered beyond the resolving cysteine for disulfide-bond formation. The resulting disulfide bond is solvent exposed, exposing the peroxidatic cysteine (Fig. 4b). The C-terminal arm is also unfolded in these structures, positioning the resolving cysteine for disulfide-bond formation. The resulting disulfide bond is solvent exposed, allowing the cysteine to be attacked by thiol-containing reductants [46]. In crystal structures of the disulfide-bonded enzymes (PrxI and AhpC), the C-terminal arm is disordered beyond the resolving cysteine, indicating a high degree of mobility for this locally unfolded segment.

**Catalytic cycle for typical 2-Cys Prxs**

Some typical 2-Cys Prxs from bacteria [15,34] (L.B. Poole, unpublished) and human and rat PrxII [13,27] undergo redox-sensitive oligomerization. These studies revealed that the reduced or overoxidized forms of the enzyme favored the decameric state, whereas the disulfide-bonded forms existed predominantly as dimers. The current ensemble of Prx structures forms the basis of a detailed catalytic cycle that includes the redox-sensitive oligomerization of these 2-Cys Prxs [22], although the precise nature of the link between oligomerization state and catalytic turnover is still uncertain. Two conserved sequence motifs have been identified that are necessary for decamer formation in 2-Cys Prxs: region I and region II (Fig. 4a). Region I is part of the conserved loop–helix active-site motif and plays a key role in decamer formation by forming a surface complementary to region II of
the adjacent dimer (Fig. 5a). The remaining portion of the loop–helix motif, the Cp loop (Fig. 4a), contains the peroxidatic cysteine and has been identified as the molecular switch responsible for the redox-sensitive oligomerization of 2-Cys Prxs [22].

In the reduced (thiol) state, the Cp loop forms a helix, positioning the peroxidatic cysteine in the active-site pocket and packed against region I, thus buttressing region I against region II in the interface (Fig. 5bi). During peroxide decomposition, the peroxidatic cysteine is oxidized to a cysteine sulfenic acid, burying the sulfur atom with the sulfenic-acid oxygen (Fig. 5bii). Local unfolding of the active site converts the Cp loop into a solvent-exposed loop, making the sulfenate sulfur accessible for disulfide-bond formation (Fig. 5biii). It is notable that such an unfolding in the absence of a suitable reductant could have deleterious consequences by making the sulfenate accessible to further oxidation by peroxide to yield inactive sulfinic (–SO2H) or sulfonic (–SO3H) acid forms (Fig. 5bvi). By maintaining a locally high concentration of reduced thiol (the resolving cysteine), 2-Cys Prxs can avoid this fate by forming a stable disulfide bond (Fig. 5biv).

Still, overoxidation of some yeast Prxs and mammalian Prxs I–IV and VI has been observed after exposure of cells to peroxides [35,47,48]. In the crystal structure of PrxII, the peroxidatic cysteine is present as cysteine sulfinic acid (Cys–SO2H), trapping this protein as a decamer owing to its inability to form a disulfide bond. Overoxidation of this protein might even promote further aggregation (Fig. 2b) [34]. In this structure, the Cp loop containing the peroxidatic cysteine sulfenic acid maintains a fold very similar to that of a reduced Prx and also buttresses the dimer–dimer interface. In addition, the folded conformation of the Cp loop in the PrxII structure might
be favored by the salt bridge between the sulfinic acid and the conserved Arg127 in the active site [20]. Interestingly, PrxII shows a well-ordered helix at its C terminus, which has been proposed to stabilize the C-terminal arm, reducing its mobility [20]. Because disulfide-bond formation requires the C-terminal arm to unfold, it was proposed that this added stability might slow down this step and allow further oxidation of the reactive Cys–SOH groups as diverse as bacteria and mammals have been reported to undergo redox-sensitive oligomerization, it is tempting to speculate that this might be a property of this class in general.

**Regulation of Prx activity**

Prxs have received a great deal of attention recently owing to their role in regulating levels of hydrogen peroxide, an intracellular signaling molecule common to many cytokine-induced signal-transduction pathways [3,8,12,13]. As noted above, some Prxs are themselves sensitive to inactivation by hydrogen peroxide and perhaps peroxynitrite through irreversible oxidation of their peroxidatic cysteine. Indeed, regulation of redox signaling through cysteine modification by peroxides and peroxynitrite has been reported for a growing number of enzymes and transcriptional regulators [50]. It was recently shown that the overoxidation of PrxII is likely to be physiologically relevant, in that its peroxidatic cysteine is oxidized to sulfinic (–SO₂H) or sulfonic (–SO₃H) acid forms in vivo upon exposure of Leydig cells to tumor necrosis factor [51]. It has been proposed that Prxs in mammalian cells act as a dam against oxidative stress, and that the ratio of active to inactive enzyme might play a role in whether cells are susceptible to cytokine-induced apoptosis [51]. In addition to overoxidation, Prx activity has also been shown to be regulated by phosphorylation and proteolysis [36,49,52,53].

Recently, phosphorylation of mammalian PrxI, PrxII, PrxIII and PrxIV at the conserved residue Thr89 (PrxII numbering) by cyclin-dependent kinases was shown to decrease the peroxidase activity of the Prxs [52]. In the case of PrxI, this phosphorylation was observed to occur in vivo during mitosis. The authors concluded that the phosphorylated Thr89 had an unfavorable electrostatic effect on the peroxidatic active site. However, analysis of the mammalian crystal structures show Thr89 to be solvent exposed and too distant (>16 Å) to interfere with the active site. An examination of the structure of the PrxII decamer reveals that a phosphorylated Thr89 would introduce unfavorable electrostatic interactions within the dimer–dimer interface by placing two negatively

stabilizes the decamer interface, whereas disulfide-bond formation traps the Cp loop in the unfolded state, in effect removing the Lynchpin supporting the interface and weakening the decamer (Fig. 5biv). Despite the instability of the disulfide-bonded form of the decamer, the high protein concentration during the crystallization of this form of AhpC favored decamer formation, allowing this important intermediate to be trapped [22]. At physiologically relevant concentrations, as more active sites in the decamer form disulfide bonds, the instability reaches a critical point and the decamer breaks down into free dimers (Fig. 5bv). In doing so, region I collapses into the active-site pocket vacated by the peroxidatic cysteine, restructuring the oligomerization interface. The catalytic cycle is completed with the reduction of the redox-active disulfide bond. Although the loop-helix structure of the active site is conserved in all Prxs, the region-I–Cp-loop and region-II sequence motifs have only been identified in typical 2-Cys Prxs (Fig. 4b). Given this sequence conservation and the observation that typical 2-Cys Prxs from groups as diverse as bacteria and mammals have been reported to undergo redox-sensitive oligomerization, it is tempting to speculate that this might be a property of this class in general.

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charged phosphates in close proximity (Fig. 5a). Indeed, a reasonable alternative interpretation is that phosphorylation of Thr89 attenuates the enzyme activity by disrupting the decameric structure (Fig. 5a). Several researchers have reported that dimeric forms of Prxs exhibit less activity than decameric forms [23,37,39]. This observation is supported by the crystal structures, which show that the active sites of the typical 2-Cys Prxs are adjacent to and stabilized by the dimer–dimer interface of the decamer. It is notable that these two control mechanisms, phosphorylation and overoxidation, probably favor different oligomeric states (dimer and decamer, respectively).

Another mechanism proposed to regulate peroxidase activity in vivo entails specific proteolysis of the C terminus of Prxs, preventing peroxide-mediated inactivation in response to rising levels of peroxide [49]. In studies of a typical 2-Cys Prx from yeast, a portion of the enzyme was found to have a truncated C-terminal following purification [49]. In follow-up mutagenesis studies, C-terminally truncated forms of the enzyme were found to be more resistant to peroxide overoxidation and inactivation than the sensitive wild-type enzyme [49]. A similar truncation of PrxII that removed the C-terminal 13 residues (including the last α helix) has also been observed during the isolation of the enzyme from erythrocytes [53]. Interestingly, the regulatory protease calpain is present in erythrocytes and will specifically cleave this region of PrxII in vitro [36]. Proteolysis would make the enzyme resistant to overoxidation but leave it susceptible to inactivation by phosphorylation.

Conclusions

The ubiquitous Prxs appear to be diverse in function, ranging from antioxidant enzymes to regulators of signal transduction. This diversity is reflected in slight evolutionary modifications in sequence and structure, built around a common peroxidatic active site. The literature within the Prx field is currently focused on their more recently identified roles as regulators of redox-sensitive signaling [3,8]. Although the precise relationship between the peroxidase activity and the oligomeric status of these enzymes is currently unclear, the two appear to be closely linked. Here, we have highlighted the current state of our understanding of Prx mechanism, structure and regulation.

† There are three classes of Prx, distinguished by the number and location of catalytic cysteines – the typical 2-Cys, atypical 2-Cys and 1-Cys Prxs.

† Despite differences in quaternary structure and catalytic cycle, all three classes share the same peroxidatic active-site structure.

† Some bacterial and mammalian typical 2-Cys Prxs undergo redox-sensitive oligomerization, and this might be a property of typical 2-Cys Prxs in general.

† Prx peroxidase activity might be regulated in vivo by cysteine oxidation, phosphorylation and limited proteolysis.

Future research should aim to improve our understanding of the influence of changes in oligomeric structure and post-translational modifications upon the peroxidatic and signaling activities of Prxs.
Acknowledgements

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