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Typical 2-Cys peroxiredoxins – modulation by covalent transformations and noncovalent interactions

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2-Cys peroxiredoxins are peroxidases devoid of prosthetic groups that mediate in the defence against oxidative stress and the peroxide activation of signaling pathways. This dual capacity relies on the high reactivity of the conserved peroxidatic and resolving cysteines, whose modification embraces not only the usual thiol–disulfide exchange but also higher oxidation states of the sulfur atom. These changes are part of a complex system wherein the cooperation with other post-translational modifications – phosphorylation, acetylation – may function as major regulatory mechanisms of the quaternary structure. More importantly, modern proteomic approaches have identified the oxyacids at cysteine residues as novel protein targets for unsuspected post-translational modifications, such as phosphorylation that yields the unusual sulfi(o)nic–phosphoryl anhydride. In this article, we review the biochemical attributes of 2-Cys peroxiredoxins that, in combination with complementary studies of forward and reverse genetics, have generated stimulating molecular models to explain how this enzyme integrates into cell signaling *in vivo*.

Redox chemistry plays an important regulatory function in an intricate web of interconnecting signals that serve in a wide range of cellular events, such as differentiation, development, adaptation and death. Three decades since the description of a thiol–disulfide cascade in higher plants [1], the concept of redox signaling has pervaded into physiology, genetics and biochemistry, embracing the molecular mechanisms involved in cellular adaptations to obnoxious metabolites, i.e. reactive oxygen species [2–5]. In this context, peroxiredoxins (Prxs) constitute a large family of peroxidases found in archaea, prokaryotes and eukaryotes which, in the latter, are specifically targeted to the cytosol and the organelles. Beyond the difficulties of establishing their role in a cellular context, and even more in a living organism, Prxs are highly redundant in cells. Indeed, there are three isoforms in *Escherichia coli* (bacteria) [6], five in *Saccharomyces cerevisiae* (yeast) [7], six in *Homo sapiens* (mammal) [8,9] and 10 in *Arabidopsis thaliana* (plant) [10] (Table 1). In contrast with major peroxidases that contain prosthetic groups tightly bound to their active site, Prxs rely on

Abbreviations
AhpC, alkyl hydroperoxide reductase C; CDK, cyclin-dependent kinase; Cys_P, peroxidatic cysteine residue; Cys_R, resolving cysteine residue; E_m, midpoint reduction potential; Fd, ferredoxin; NTR, NADP–thioredoxin reductase; PDOR, protein-disulfide oxidoreductase; Prx, peroxiredoxin; Srx, sulfiredoxin; Trx, thioredoxin.

Table 1. Prx subfamilies in different organisms. The subfamilies of Prx were grouped according to [11,12]. However, their study raised a plethora of conventional names and abbreviations which were merged into the 'usual designation' based on the peroxidatic mechanism and the primary structure. The scientific literature employs Arabic (roman) numbers and capital letters to designate mammal and plant Prxs, respectively. In addition, eukaryote isoforms occupy a definite intracellular compartment including the cytosol and the organelles suited to particular forms of metabolism. Higher plants (*Arabidopsis*): two 2-Cys Prxs, PrxQ and Type II E are located at the chloroplast, 1-Cys Prx is present in the nucleus and Type II distributes between the cytosol (A–D) and the mitochondrion (F) [10]. Mammals: the six Prx isoforms of mammals are allocated to the cytosol (2-Cys Prx1, 2-Cys Prx2, 1-Cys Prx6), the nucleus (2-Cys Prx1), the mitochondria (2-Cys Prx3, 1-Cys Prx6), the peroxisomes (2-Cys Prx5) and potentially secreted (2-Cys Prx4) [12,105]. Yeasts (*S. cerevisiae*): the yeast cytosol contains two typical 2-Cys Prxs (cTPx1 and cTPx2) and one atypical 2-Cys Prx (cTPx3), whereas the nucleus holds one similar to the plant PrxQ (bacterioferritin co-migratory protein, nTPx) and the mitochondria contains a 1-Cys Prx (mTPx) [7]. In prokaryotes, the Prx isoforms are also distributed in different compartments. Bacteria (*E. coli*): of three proteins showing thiol-dependent antioxidant activities, two are located at the cytosol and the third resides in the periplasmic space [106,107].

Subfamily	Usual designation	Higher plants (*Arabidopsis - thaliana*)		Mammals		Yeasts (*S. cereviseae*)	Bacteria (*E. coli*)
A	Typical 2-Cys Prx	2-Cys Prx	A	Prx	1 (I)	cPrx1 (cTPx 1, Tsa 1p, YML028W),	AhpC
					2 (II)	cPrx2 (cTPx II, Tsa 2p, YDR453C)	
			B		3 (III)		
					4 (IV)		
B	1-Cys Prx	1-Cys Prx		Prx6 (VI)		mTPx (Prx1p, YBL064C)	NO
C		PrxQ		NO		nTPx (bacterio ferritin co-migratory protein, YIL010W, Dot5p)	Ec BCP (bacterio ferritin co-migratory protein)
D	Atypical 2-Cys Prx	Type II	A	Prx5 (V)		cPrx3 (cTPx III, Ahp1p,	NO
			B			PMP 20, YLR109W)	
			C				
			D				
			E				
			F				
E (bacterial periplasmic thiol peroxidases)		NO		NO		NO	*Ec* Tpx

the sulfur atom of a conserved Cys residue, termed the peroxidatic Cys (Cys_P), to cleave the peroxyl –O–OH bond. According to the presence or absence of a second Cys residue, called the resolving Cys (Cys_R), Prxs were originally grouped into two subfamilies: 2-Cys Prx and 1-Cys Prx, respectively. Later, the underlying mechanism of catalysis provided the basis to further divide 2-Cys Prxs into two groups, named 'typical', which form homodimers through an intersubunit disulfide bond, and 'atypical', which form an intramolecular cystine in the same polypeptide [11,12]. However, each Prx uses a combination of strategies to achieve its cellular functions, as revealed recently by Prx6 from *Arenicola marina* (annelid worm), whose primary structure is 63% identical to the mammalian 1-Cys Prx, but the presence of an intermolecular disulfide bond leads to its classification among typical 2-Cys Prxs [13]. Differences in the amino acid sequence, the mechanism of oligomerization and the catalytic cycle provided the basis for the broad clustering of this protein family into five different subfamilies that apparently appeared at different times in evolution (Table 1) [11,12,14]. In this context, the field of typical 2-Cys Prxs has reached a certain level of pre-eminence, mainly as a result of the conservation of the primary structure in distant phyla and the participation in disparate, and even opposing, metabolisms [15]. Therefore, this review attempts to provide a synoptic overview of the structures and functions of the ubiquitous typical 2-Cys Prxs, focusing on the use of cysteine sulfur oxidation states in post-translational modifications and noncovalent interactions that trigger the appropriate response to oxidative stress. At the forefront of this goal is the demand for biochemical mechanisms to identify protein targets and to guide the rational design of drugs with pharmacokinetic properties.

Structure

An extensive literature on 2-Cys Prxs from microorganisms, plants and animals has revealed the existence of homodimers in which the Cys_P from one monomer is linked via a redox-active disulfide to the Cys_R located at a complementary polypeptide. On formation of this basic unit, the boundary interface between monomers aligns parallel to the plane of the central β-sheet (B-type interface), where the intermolecular disulfide bond buries completely Cys_P, whereas Cys_R is partially

exposed to the surrounding solvent [16,17]. Almost all homodimers of 2-Cys Prx (α_2) associate noncovalently to a doughnut-shaped decamer $(\alpha_2)_5$, wherein boundary interfaces between homodimers stand perpendicular to the central β-sheet (A-type interface) (cf. fig. 2 in [18]). Although all 2-Cys Prxs form a pentamer of homodimers, the quaternary structure of mammalian 2-Cys Prx1 is further stabilized by additional intercatenary disulfide bonds at the dimer–dimer interface [19].

As expected for the noncovalent association of proteins, the relative proportion of 2-Cys Prx oligomers in solution is dictated not only by the concentration of the protein and the composition of the surrounding solvent (pH, ionic strength, metabolites), but also by the primary structure and the state of amino acid side-chains [20–22]. Recently, the analysis of rat, human and plant 2-Cys Prxs has revealed many species with a propensity to the decameric form, not only by increasing the protein concentration and decreasing the ionic strength, but also by preventing the formation of the intercatenary disulfide bond [23–25]. Among the studies on the morphology of 2-Cys Prxs, transmission electron microscopy has further revealed an increasingly sophisticated array of protein aggregates [26,27]. Negatively stained structures of yeast 2-Cys Prx1 show three different configurations in electron micrographs: spherical- and ring-shaped structures, as well as irregularly shaped small particles [26,28]. Very probably, there is a continuum of intermediates between small oligomers and higher order assemblies because the rings are organized as dodecamers in the 3.3 Å crystal structures of C168S 2-Cys Prx3 and C176S alkyl hydroperoxide reductase C (AhpC) from *Mycobacterium tuberculosis* [29,30].

Redox dependence of oligomerization

As it is increasingly becoming evident that the sulfur atom of Cys residues can adopt many oxidation states in the response of stressed cells to environmental insults [31], a large number of studies have examined the intimate relationship between these post-translational modifications and the oligomerization of 2-Cys Prx. The structural evidence indicates that the fully reduced active site strengthens the A-type interface which supports the formation of the decamer, whereas the intersubunit disulfide locks the active site into place promoting the destabilization of the decamer [32,33]. If the particular geometry of the peroxidatic active site governs the oligomerization of 2-Cys Prxs, it should be expected that the overoxidation of Cys_P to sulfinic ($R–SO_2H$) and sulfonic ($R–SO_3H$) acids will prevent the formation of the intersubunit disulfide, thereby enhancing the propensity to aggregate into decameric species. As predicted, not only does the overoxidation of Cys_P stabilize the decameric form [34–37], but also the exposure of leaf chloroplasts and mouse lung epithelial cells to the oxidative stimulus drives 2-Cys Prxs to different quaternary structures [38–41].

Peroxidase activity

In the peroxidase activity of 2-Cys Prxs (EC 1.11.1.15), Cys_P starts the catalytic cycle, yielding the sulfenic acid derivative ($–Cys_P–SOH$), with the concurrent reduction of the peroxide (ROH) (reaction 1, Fig. 1A). Given that protonated sulfhydryls (R–SH) do not react with hydroperoxides, multiple structural factors around Cys_P stabilize the thiolate anion ($R–S^-$) required for nucleophilic attack on the terminal oxygen of the peroxyl bond (RO–OH) [42]. To close the catalytic cycle, two successive reactions bring the simplest oxyacid of organic sulfur back to the thiol form [32]. First, the sulfenic acid reacts with the sulfhydryl group of Cys_R provided by a partner subunit, forming an intersubunit disulfide bond and concurrently releasing water (reaction 2, Fig. 1A). Second, a complementary reductant system closes the catalytic cycle through a thiol–disulfide exchange with specific cysteines of protein-disulfide oxidoreductases (PDOR) (reaction 3, Fig. 1A). Essentially, the functional entities for a complete turn of the peroxidase cycle reside in monomers for peroxidation and in dimers for the posterior dehydration and disulfide bond reduction.

A surprising innovation in the formation of the sulfenic acid was recently reported using the C207S mutant of 2-Cys Prx from the aerobic hyperthermophilic archaeon *Aeropyrum pernix* K1. Both the refined crystal structure and quantum chemical calculations are in good agreement with the formation of the sulfenic acid derivative of the peroxidatic Cys50 via a hypervalent sulfur intermediate (sulfurane) (Fig. 1B) [43]. In this model, the sulfur atom of Cys_P50 is covalently linked to (a) the $N^{\delta1}$ atom of the imidazole moiety of the neighboring His42 and (b) the oxygen atom whose electronegativity at the apical position of the sulfur atom stabilizes the sulfurane. Next, the Arg149 contributes to the protonation of the imidazole moiety, cleaving the sulfur–nitrogen bond for the formation of the sulfenic acid at Cys_P50. Whether the hypervalent sulfur is a faithful intermediate in the oxidation of sulfhydryl groups remains an unresolved issue in the subfamily of 2-Cys Prxs, because the reported structures of the mammalian orthologues (PDB: 1qmv, 1qq2) do not hold a histidine residue close to the sulfur atom of Cys_P.

One of the most interesting features of 2-Cys Prxs concerns the mechanism by which a cycle of three distinct

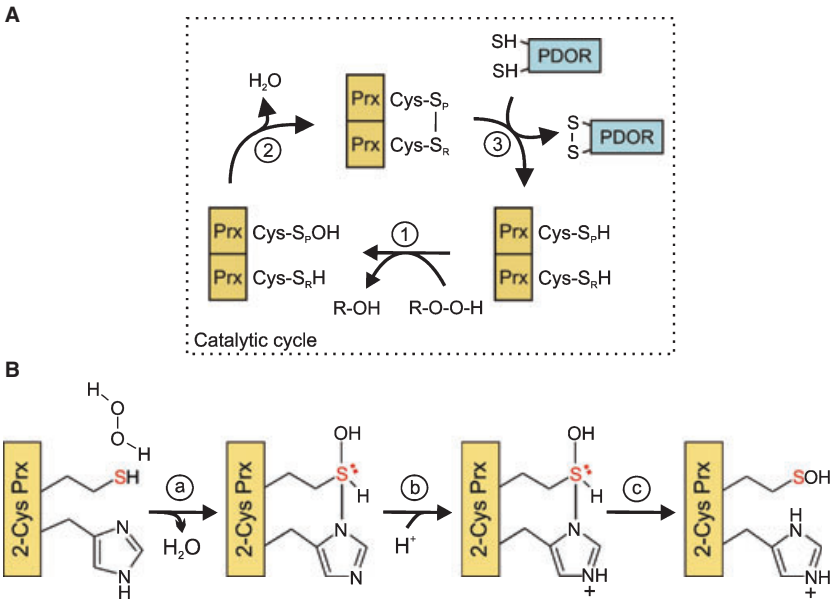

Fig. 1. The peroxidase activity of 2-Cys Prx. (A) The catalytic cycle. The hydroperoxide (R–O–O–H) oxidizes the thiol of Cys_P53 to the sulfenic acid form (reaction 1) which, after reacting with the reduced Cys_R175, yields the homodimer linked through a disulfide bond (reaction 2) (numbering of Cys residues refers to rapeseed 2-Cys Prx). Subsequently, the reduced form of a PDOR closes the catalytic cycle, returning the oxidized 2-Cys Prx to the activated (reduced) state (reaction 3). (B) Proposed mechanism for the formation of sulfenic acid through a sulfurane intermediate [43]. The addition of H_2O_2 brings His42 of the typical 2-Cys Prx from *Aeropyrum pernix K1* (ApTPx) close to Cys_P50, but the side-chain of the latter residue remains reduced (preoxidation). At this stage, nucleophilic attack of the sulfur atom on one of the peroxyl oxygens may cause the formation of the –S–O– bond assisted by the nitrogen atom of His42, yielding the hypervalent sulfur (sulfurane) intermediate (reaction a). After protonation of the imidazole moiety (reaction b), the hypervalent sulfur intermediate splits into –Cys–SOH and histidine (reaction c).

redox transformations occurs within a single active site. As shown in Fig. 2A, the sulfur oxidation state of Cys_P goes through three different stages in the peroxidatic cycle: sulfhydryl (−2) → sulfenic acid (0) → disulfide (−1) → sulfhydryl (−2). Among the elementary steps of the catalytic mechanism (Fig. 2B), two involve the transfer of $2H^+ + 2e^-$ (reactions 1 and 3), whereas the remaining single dehydration (reaction 2) brings about the one-electron reduction and oxidation of Cys_P and Cys_R, respectively. In essence, 2-Cys Prxs employ three different sulfhydryl groups as reductants to complete the catalytic cycle: (a) Cys_P cleaves the peroxyl bond RO–OH yielding the sulfenic acid, (b) Cys_R reduces the sulfenic group of Cys_P, leading to the formation of the intercatenary disulfide bond, and (c) the –CXXC– motif of PDORs restores the thiol group to Cys_P.

The oxidation of the peroxidatic cysteine

The refinement of the peroxidase assay revealed that the 2-Cys Prxs from mammals (Prx2), yeast (*S. cerevisiae* cTPx I and cTPx II) and bacteria (*Salmonella typhimurium* AhpC) react with H_2O_2 at rates (*c.* 10^7 $M^{-1}{\cdot}s^{-1}$) comparable with those of catalases (*c.* 10^7 $M^{-1}{\cdot}s^{-1}$) and glutathione peroxidases (*c.* 10^8 $M^{-1}{\cdot}s^{-1}$) [22,44,45]. The precise measurement of the kinetic constants of *Salmonella typhimurium* AhpC, a well-known model of bacterial 2-Cys Prxs, provided recently a better understanding of the substrate specificity for H_2O_2, ethyl-, *t*-butyl- and cumene-hydroperoxide. The most affected catalytic constant is K_m for the oxidant hydroperoxide, whereas the overall k_{cat} and K_m values for the reductant thioredoxin (Trx) are virtually independent of the hydroperoxide substrate. As a consequence, the specificity constants, k_{cat}/K_m, for small hydroperoxides (H_2O_2, ethyl-hydroperoxide) are almost two orders of magnitude higher than for larger ones (*t*-butyl-, cumene-hydroperoxide), clearly indicating that the active site discriminates between the substituents linked to the –O–OH moiety [46].

In addition to the capacity to reduce alkyl hydroperoxides, the thiolate anion of Cys_P has the ability to cleave the peroxyl bond of the peroxynitrite formed when the superoxide anion reacts with the nitric oxide ($O_2^- + NO \rightarrow O\text{–}ONO^-$). Given that the homolytic decomposition readily converts peroxynitrite into two radical species ($HO\text{–}ONO \rightarrow HO^- + NO_2^-$] [47], 2-Cys Prx removes efficiently this toxic nitro-oxidant in a two-electron process that yields nitrite [NO_2^-] and sulfenic acid [48–50]. By contrast, chloramines – impor-

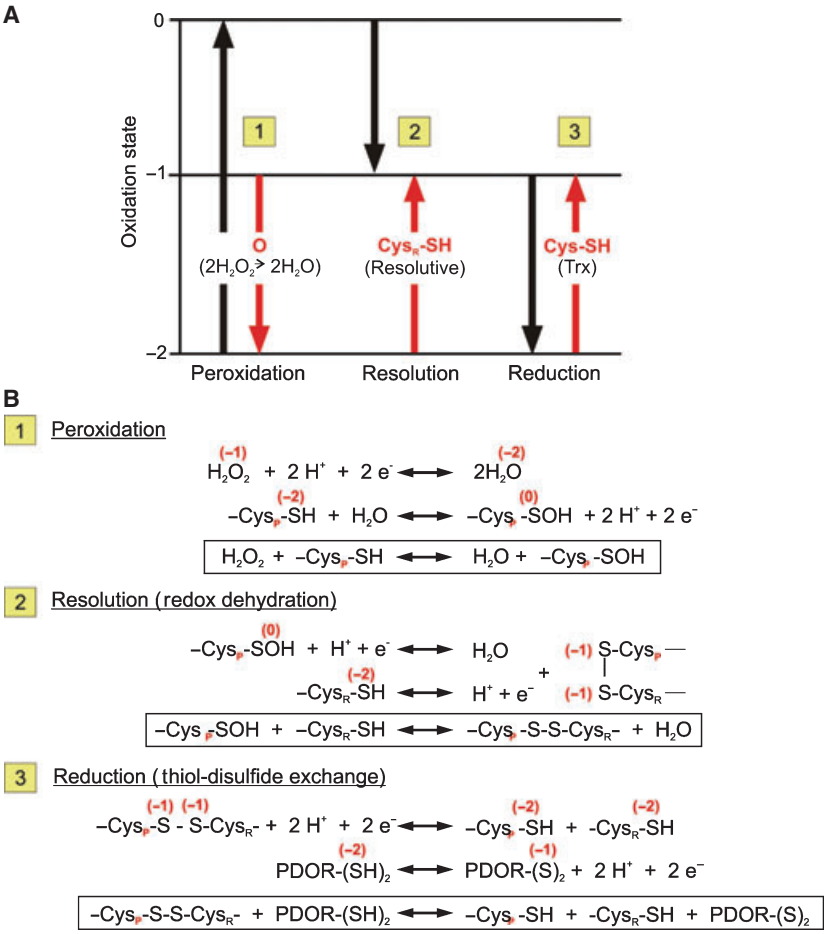

Fig. 2. The redox cycle in peroxidase activity. (A) Oxidation states of Cys_P and the complementary redox couple. The arrows illustrate the oxidation (up) and reduction (down) of the sulfur atom in the peroxidatic $–Cys_P$ (black) and respective atom in the complementary redox couple (red) [Peroxidation: oxygen atom in H_2O_2; Resolution: resolving $–Cys_R$; Reduction: Cys pair of PDOR (–Cys–SH)]. Numbering in yellow squares reflects the reactions of the catalytic cycle described in Fig. 1A. (B) Half-reactions and atom oxidation states of the peroxidase cycle. Sections 1–3 describe every redox couple in the peroxidase cycle of 2-Cys Prx, wherein the oxidation state of the atoms that participate in the elementary redox reactions are depicted in red between parentheses. Of note, the formation of the disulfide bond in the second stage (Resolution, reaction 2) implies the monoelectronic reduction of the sulfenic acid at Cys_P and the concurrent oxidation of Cys_R in the complementary subunit.

tant oxidants produced via myeloperoxidase in inflammatory processes ($R–NH_2 + HOCl \rightarrow R–N(H)Cl$] – and alkylating agents are much less reactive than hydroperoxides [45,51]. Hence, Cys_P resides in an active site whose tertiary structure considerably improves the reactivity with hydroperoxides, but restricts the interaction with other sulfhydryl reagents.

The reduction of sulfenic acid back to the active sulfhydryl group

Resolution (redox dehydration)

The nucleophilic and electrophilic reactivity of the sulfur atom in sulfenic acid enables the protein to react with another sulfenate or sulfhydryl group to form a thiosulfinate [–S–S(O)–] or a disulfide, respectively [52,53]. Although the former reaction is unusual in biological systems, compelling data indicate that the latter proceeds with (a) protein sulfhydryl groups forming intercatenary or intracatenary disulfide bonds or (b) small thiols leading to the thiolation of the proteins [54]. In typical 2-Cys Prxs, the sulfenic acid moiety reacts with the sulfhydryl group of Cys_R located at another subunit, releasing water and concurrently forming an intermolecular disulfide. Consistent with the need for a complementary thiol to support full peroxidase activity, C169S 2-Cys Prx from yeast (Tpx1) scavenges H_2O_2 in the presence of the small thiol dithiothreitol, whereas the C48S counterpart is completely inactive [55,56].

Thiol–disulfide exchange

The reduction of the intermediate disulfide bond by physiological electron donors restores ultimately the fully folded conformation at Cys_P, enabling 2-Cys Prx to react with another molecule of hydroperoxide (Fig. 3). In addressing the thiol–disulfide exchange mechanism, a large number of studies support the notion that the complex set of reductants, PDORs and associated reductases varies largely with the organism, intracellular location, stage of development and response to environmental cues. Thus, the NADP–Trx reductase (NTR) was found initially to catalyze

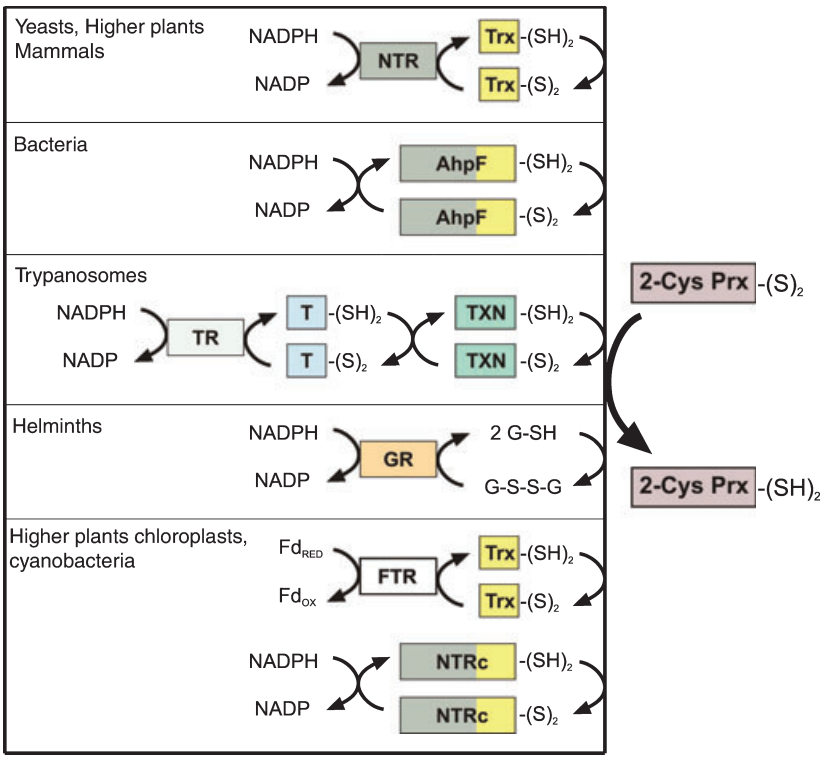

Fig. 3. Reduction of the 2-Cys Prx disulfide bond. In all organisms, cellular compartments that rely on reduced carbon skeletons as the main source of energy (e.g. cytosol, mitochondria) use NADPH as the ultimate reductant for cleaving the unique disulfide bond of 2-Cys Prxs. Chloroplasts of higher plants and cyanobacteria are a notable exception because NADPH and an iron–sulfur protein, reduced Fd, are electron acceptors in the photosynthetic electron transport system triggered by light. Next, extremely diverse PDOR systems, often proteins or domains containing the –CXXC– motif, mediate the transfer of the reducing power to oxidized 2-Cys Prxs. Yeasts, higher plants, mammals: the reducing power of NADPH cleaves the unique disulfide bond of Trx assisted by NTR, a member of the superfamily of flavo-PDORs. Although NTRs are widely distributed, two forms have evolved: (a) in yeasts and higher plants, a homodimer (*c.* 35 kDa subunit) harboring a redox-active disulfide [–CA(V/T)C–] between the FAD and NADPH domains, and (b) in mammals, a homodimer (55 kDa subunit) characterized by a much longer C-terminal domain exhibiting a highly reactive selenocysteine residue as a redox center. Bacteria: in bacteria, a specialized flavoprotein, AhpF, mediates efficiently the transfer of reducing power from NADPH to the alkyl hydroperoxide reductase AhpC, a member of the typical 2-Cys Prx subfamily. Trypanosomes: the concerted action of NADPH and trypanothione reductase (TR) delivers electrons and $2H^+$ to the oxidized form of trypanothione [bis(glutathionyl)spermidine] [T–$(S)_2$], which, in turn, spontaneously reduces the oxidized form of tryparedoxin [TXN–$(S)_2$], a member of the Trx fold superfamily in trypanosomes. At this stage, reduced tryparedoxin [TXN–$(SH)_2$] transfers the reducing power to the oxidized form of 2-Cys Prx. Helminths: although reduced glutathione generally does not cleave the disulfide bond of 2-Cys Prxs, it reduces efficiently two isoforms from *Schistosoma mansoni* using a flavoprotein reductase (GR) for recycling the oxidized form. Higher plant chloroplasts, cyanobacteria: in illuminated chloroplasts, two different reductants, Fd and NADPH, participate in the reduction of 2-Cys Prxs. The former and two external protons reduce the disulfide bond of the iron–sulfur Fd-Trx reductase (FTR), which, in turn, reduces the cystine of oxidized Trx–$(S)_2$ via thiol–disulfide exchange. Complementary NADPH is used in chloroplasts, assisted by the single polypeptide of NTRc, which supports the functioning of a complete NADP–Trx system using the NTR and Trx domains located at the N-terminal and C-terminal regions, respectively.

the cleavage of the Trx disulfide bond by NADPH [midpoint reduction potential (E_m) = −340 mV] [$NADPH + H^+ + Trx(S)_2 \rightarrow NADP^+ + HS–Trx–SH$] in yeast and mammals [55]. Later, the trypanothione/tryparedoxin couple in trypanosomes [57], the alkyl hydroperoxide reductase flavoprotein in bacteria (i.e. AhpF) [58] and the reduced glutathione in helminths [59] appeared as cognate partners of the couple NTR–Trx. However, two different systems provide reducing equivalents for the reduction of 2-Cys Prx in illuminated higher plant chloroplasts and cyanobacteria. First, the product of the photosynthetic electron transport system activated by light, reduced ferredoxin (Fd_{red}) (E_m = −420 mV), regenerates thiol groups of Trx [$2Fd_{red} + 2H^+ + Trx(S)_2 \rightarrow 2Fd_{ox} + HS–Trx–SH$] assisted by the Fd-Trx reductase. Second, it has been found recently that photochemically generated NADPH reduces 2-Cys Prx through a new flavoprotein containing an NTR domain complemented by a C-terminal region that bears the canonical Trx motif,

–CGPC– [60–64]. The fact that Trx constitutes the specialized protein dedicated to the reduction of many eukaryotic 2-Cys Prxs should introduce an additional level of complexity, because most organisms contain different isoforms [65]. Therefore, not surprisingly, the chloroplast 2-Cys Prx is efficiently regenerated in higher plants by Trx-x [66,67] and a Trx-like protein CDSP32 [68], whereas the other 18 Trxs are much less effective. Moreover, *Drosophila melanogaster* Trx2, but not Trx1, reduces the cognate 2-Cys Prx (Trx peroxidase1) [69,70].

In addition to the reductants described above, functional data point to a large family of proteins possessing peptidyl–prolyl *cis–trans*-isomerase activity, cyclophilins, as key players in the reduction of the disulfide bond of 2-Cys Prxs [71]. Following the activation of various isoforms of human 2-Cys Prxs by mammalian cyclophilins [72,73], studies in higher plants confirmed the breadth of this seminal finding [67,74]. Given that the E_m value of *Arabidopsis* cyclophilin 20-3 (−319 mV) is more negative than that of pea 2-Cys Prx (−307 mV), the catalytic role of the former in the reductive activation of the latter probably occurs under an excess of electron pressure in photosynthesis [39]. However, it is important to establish whether the cleavage of the disulfide bond in 2-Cys Prxs proceeds with reduced cyclophilins as electron donors or enhancers of Trx activity, because the activity of peptidyl–prolyl *cis–trans*-isomerase is null in the oxidized state and resumes after Trx-mediated reduction [75].

Overoxidation of cysteines

The conversion of sulfhydryl groups to sulfenic acid occurs occasionally in proteins from healthy cells, but increases markedly in response to low concentrations of hydroperoxides [76]. During the course of probing the role of Cys_P in the catalytic activity of 2-Cys Prx, it was found that the initial formation of sulfenic acid is mechanistically necessary, but the striking versatility of sulfur oxidation states may drive to sulfinic and sulfonic acids which, in turn, abrogate catalysis. The notion that 2-Cys Prxs must be engaged in the catalytic cycle to be overoxidized is corroborated by the finding that H_2O_2 converts $–Cys_P–SH$ to $–Cys_P–SO_2H$ only when all the catalytic participants (NADPH, NTR, Trx) are present [77]. Indeed, other candidates may be integrated with H_2O_2 in the overoxidation of 2-Cys Prxs because lipid hydroperoxides, produced via lipoxygenase and cyclooxygenase, are able to oxidize human 2-Cys Prx1, 2-Cys Prx2 and 2-Cys Prx3 to the respective $–Cys_P–SO_2H$ and $–Cys_P–SO_3H$ forms [78].

To participate in the cell response to peroxide stress [79], the overoxidized sulfur atom should return to the initial state when the stimulus disappears. For many years, the inability of the NADP–Trx system to reduce the sulfinic acid relegated the overoxidation of 2-Cys Prxs to a wasteful process. However, the discovery that the reduction of sulfinic acid proceeds via the novel protein sulfiredoxin (Srx) sparked a key advance towards an understanding of how 2-Cys Prxs sort out the response to increasing levels of peroxide stress [80,81]. The mechanism of the Srx-dependent reversal of 2-Cys Prx overoxidation starts with the transfer of the ATP γ-phosphate to the sulfinic acid moiety of 2-Cys Prx ($Prx–Cys_P–SO_2^-$), yielding the sulfinic acid–phosphoryl anhydride [$Prx–Cys_P–S(O)OPO_3^{2-}$] (reaction b, Fig. 4) which, in turn, forms with Srx a thiosulfinate [$Prx–Cys_P–S(O)–S–Srx$] with the concomitant release of phosphate (reaction c, Fig. 4). At this stage, a reductant first cleaves the covalently linked heterocomplex (2-Cys Prx–Srx), reinstating the sulfenic acid form of 2-Cys Prx in the catalytic cycle (reaction d, Fig. 4), and subsequently rescues Srx for the recovery of additional molecules of overoxidized 2-Cys Prx (reaction e, Fig. 4) [82]. The lack of experimental details regarding the isolation of the intermediate anhydride [$Prx–Cys_P–S(O)OPO_3^{2-}$] is cast in sharp relief by two recent studies [82,83]. First, the superposition of the crystal structure of the Srx–ATP complex onto the Srx–Prx complex uncovers that the unfolding of the 2-Cys Prx active site places the γ-phosphate of ATP in close proximity to the Sγ atom of Cys_P (3.0 Å) and to the Sγ atom of Srx-Cys99 (3.5 Å), making plausible the inline attack of the peroxidatic $–Cys_P–SO_2^-$ by the γ-phosphate. Second, Srx appears to function as the reductase, whose active site Cys forms the intermolecular thiolsulfinate with the peroxidatic Cys_P of 2-Cys Prx when the latter is activated by phosphorylation.

Although overoxidized oxyacids are apparently absent when 2-Cys Prx2 dampens signaling via interactions with the receptor of platelet-derived growth factor [84], additional studies in different organisms entail the function of a 'floodgate' by which overoxidation promotes H_2O_2 signaling (cf. [18]). Studies with the fission yeast *S. pombe* and rat neurons show that the higher oxidation states of 2-Cys Prxs function as the molecular switch that regulates the activation of specific transcription factors [85–87].

Chaperone activity

Increasingly, the biochemical analyses of different pathways have revealed that many proteins fulfil more

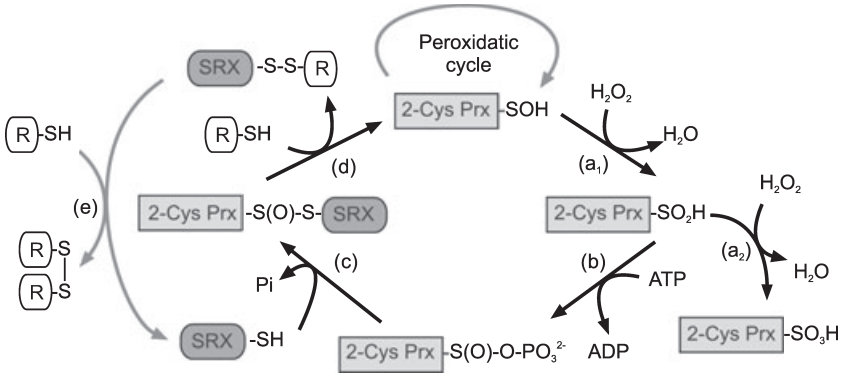

Fig. 4. The catalytic mechanism for the reduction of the overoxidized (sulfinic) 2-Cys Prx by Srx. The sulfenic acid formed as an intermediate in the peroxidatic cycle occasionally undergoes further oxidation to sulfinic and sulfonic forms (reactions a_1 and a_2, respectively), halting the reduction of H_2O_2. The autophosphorylation or Srx-catalyzed phosphorylation of the sulfinic form of the peroxidatic Cys_P (reaction b) yields the sulfinic–phosphoryl anhydride that subsequently reacts with the conserved Cys residue of Srx, forming the thiosulfinate intermediate that links covalently 2-Cys Prx with Srx (reaction c). An external physiological thiol (R–SH) (e.g. Trx) cleaves the heterocomplex releasing the sulfenic derivative of 2-Cys Prx, which returns to the peroxidatic cycle, and the Srx-reductant heterodisulfide (reaction d). A complementary thiol–disulfide exchange between the heterodisulfide and the physiological reductant closes the catalytic cycle of Srx and brings 2-Cys Prx back to the peroxidase function (reaction e).

than one function [88,89]. One of the most interesting aspects of these proteins, designated as moonlighting proteins, is the use of covalent post-translational modifications and noncovalent interactions to switch between different functions in order to respond accordingly to environmental stimuli. The presence of additional functions in the 2-Cys Prx subfamily was first revealed by the identification of 2-Cys Prx1 (formerly Pag) as the protein that inhibits the intrinsic tyrosine kinase activity of the oncoprotein c-Abl [90]. Later, the study of the cytosolic yeast 2-Cys Prxs, cPrx1 and cPrx2, was extremely helpful in elucidating that, complementary to the reduction of hydroperoxides, a chaperone activity is associated with transitions of the oligomerization state [26,91]. Separate studies in eukaryotes and prokaryotes soon confirmed that the exposure of cells to oxidative stress or heat shock shifts the quaternary structure of 2-Cys Prxs to large molecular assemblies with the loss of peroxidase activity and the concurrent appearance of molecular chaperone capacity [20,23,92]. Hence, it quickly become apparent that some 2-Cys Prxs may be converted to high-molecular-mass species to prevent the misfolding or unfolding of proteins under short-term stress conditions, but, if the oxidative stress is too severe, all the protein may be switched to molecular chaperones for the salvage of unfolded proteins. Consistent with pleiotropic effects, a recent analysis of actively translating *S. cerevisiae* ribosomes revealed that a severe oxidative stress releases the ribosome-associated 2-Cys Prx and concurrently promotes ribosomal protein aggregation, increasing translation defects [93].

If 2-Cys Prx can interact noncovalently with partner proteins, it might be expected that specific functions of the latter could be fine-tuned by the former, for example, to modulate enzyme activity. The study of chloroplast fructose-1,6-bisphosphatase from rapeseed leaves, a key enzyme in the Benson–Calvin cycle for photosynthetic CO_2 assimilation, has been particularly informative in this respect [94]. The oxidized form of chloroplast 2-Cys Prx from rapeseed leaves enhances the activity of chloroplast fructose-1,6-bisphosphatase without using the redox activity of the former. This noncovalent stimulation of the hydrolytic activity, absent with reduced 2-Cys Prx, seems to be sufficient to promote a catalytically competent enzyme which functions when chloroplasts set up reductants to cope with the oxidative stress caused by intense illumination.

Post-translational modifications of 2-Cys Prx

Phosphorylation

The identification of additional functions changed the classical view of 2-Cys Prx from a catalyst in the reduction of hydroperoxides to a key modulator of important biological processes. As the complicated interactions that synchronize the alternation between different activities are largely unknown, post-translational modifications may be put forward as the plausible mechanism. Early studies found that several cyclin-dependent kinases (CDKs), including CDK1

(formerly Cdc2), catalyze the specific incorporation of the radioactive label from [^{32}P]ATP[γP] to human 2-Cys Prx1 at the consensus site for CDKs (-*Thr90*-Pro-Lys-Lys-) [95]. The introduction of a negative charge at position 90 yields significant alterations of surface hydrophobicity and regions that surround aromatic amino acids, which, in turn, promote the formation of high-molecular-mass complexes [20]. These global changes markedly lower the capacity to reduce H_2O_2 and greatly enhance the chaperone activity. Apart from direct effects of CDKs on 2-Cys Prx obtained from *in vitro* experiments, a functional linkage is observed in cell cycles of HeLa, HepG2 and NIH 3T3, where the phosphorylation of 2-Cys Prx1 parallels the activation of CDK1 during the mitotic phase of the cell cycle, but not in the interphase [95]. These findings suggest that the cytosolic location of 2-Cys Prx1 probably prevents the interaction with activated CDKs until the rupture of the nuclear envelope during mitosis, when CDK1 is fully active. Significantly, a potent and selective inhibitor of CDKs, roscovitine, abrogates the phosphorylation of 2-Cys Prx1 both *in vitro* and *in vivo*. Much in accord with these studies, drugs that induce Parkinson's disease in the dopaminergic neurons of mice elicit the phosphorylation of 2-Cys Prx2 at Thr89, which, in turn, reduces the peroxidase activity and concurrently increases the levels of H_2O_2 [96].

Although Ser, Thr and Tyr residues are phosphorylated in most proteins involved in signal transduction, covalently bound phosphoryl moieties at His, Cys and Asp residues have been found mainly as phosphoenzyme intermediates and much less frequently as stable post-translational modifications. During the last 5 years, two different lines of research have supported the notion that ATP phosphorylates Cys_P and Cys_R of 2-Cys Prx. The postulated mechanism initially described in *S. cerevisiae* for the Srx-dependent conversion of sulfinic acid back to sulfhydryl involves the phosphorylation of Cys_P as an essential step, even though the sulfinic–phosphoryl anhydride was not isolated. To add yet another complexity, recent experiments have implicated Cys_R in the autophosphorylation of 2-Cys Prx [97], despite the prevailing view which restricts the function of this particular residue to the target for the formation of the disulfide bond in closing the peroxidatic cycle [98,99]. As revealed by mass spectroscopy, the incorporation of the phosphoryl moiety requires the overoxidized forms of Cys_R to yield the sulfinic–phosphoryl [Prx–Cys_R–$S(O)OPO_3^{2-}$] and the sulfonic–phosphoryl [Prx–Cys_P–$S(O_2)OPO_3^{2-}$] anhydrides. Although the postulated mechanism for these modifications is rooted in the model for anhydride formation in the Srx-mediated reduction of sulfinic acid [80], the autophosphorylation contrasts with the other mechanisms of 2-Cys Prx phosphorylation with regard to two prominent features; neither requires a catalyst such as CDK or Srx, nor proceeds via Thr91 or Cys_P. Notably, the overoxidation of Cys_R takes place in redox environments (e.g. quinones; $E_m \sim -0.15$ V) markedly milder than those usually employed in the examination of oxidative stress (i.e. H_2O_2, $E_m = 1.76$ V). Although the precise roles of the putative phosphorylation of Cys_P and the autophosphorylation of Cys_R are not yet understood, they might constitute an important platform to mediate signal transduction. Certainly, the covalent incorporation of the phosphoryl moiety into oxyacids integrates at a single amino acid residue the nonredox chemistry of ATP with multiple oxidation states of the sulfur atom, providing a versatile mechanism for perceiving changes in the energy and redox status of the cell (Fig. 5). As 2-Cys Prxs process a wide spectrum of stimuli into different cellular responses, a deeper understanding of the components and mechanisms implied in the regulation mediated by phosphorylation will require approaches that include precise biochemical analyses and the finding of new partners and complexes.

Acetylation

A recent finding has added another twist to the consideration of 2-Cys Prx as a exclusive target for redox stimuli. The observation of human esophageal squamous cells has shown that the expression profile of 2-Cys Prx1 is significantly up-regulated in a microarray

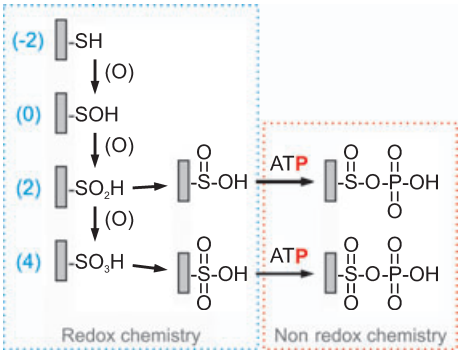

Fig. 5. The dual chemistry of the sulfur atom at Cys_R. Hydroperoxides oxidize Cys_R to oxyacids increasing, as a consequence, the oxidation state of the sulfur atom (blue parentheses). This redox chemistry (blue broken square) is linked to the nonredox chemistry (red broken square) of the phosphoryl moiety via the formation of the mixed anhydrides sulfinic–phosphoryl and sulfonic–phosphoryl by autophosphorylation.

analysis of cancer lines that have been challenged with FK228, a potent antitumor drug [100]. To enhance gene expression, FK228 promotes the acetylation of histones H3 and H4 at the promoter site of 2-Cys Prx1 via the inhibition of the histone deacetylase. Furthermore, the suppression of gene expression by RNA interference reduces the antitumor activity of FK228, supporting the antiproliferative effect of the drug through the activation of the 2-Cys Prx1 gene. Although histone acetylation is a likely candidate for controlling 2-Cys Prxs at the transcriptional level, very recent studies have shown that this peculiar post-translational modification of proteins is yet another biochemical mechanism that can selectively alter the functioning of 2-Cys Prxs [101]. The 22 kDa proteins are not acetylated in three human prostate cancer cells that express a particular histone deacetylase, HDAC6. By contrast, cell lines that lack HDAC6, LAPC4 and normal counterparts deprived of histone deacetylase activity by a specific inhibitor (vorinostat) accumulate acetylated 22 kDa proteins. Although mass spectroscopy analyses have identified 2-Cys Prx1 and 2-Cys Prx2 as the 22 kDa proteins immunoprecipitated by LAPC4 cells, two subsequent analyses have confirmed the acetylation of both isoforms. Lys197 and Lys196 appear as the acetylation sites when 2-Cys Prx1 and 2-Cys Prx2, respectively, are (a) incubated *in vitro* with histone acetyltransferase and acetyl-CoA or (b) characterized in LAPC4 cells transfected with site-directed mutants of 2-Cys Prxs. However, more importantly, protein acetylation enhances not only the peroxidase activity but also the resistance to overoxidation.

Nucleotide/Mg^{2+}-dependent modulation of 2-Cys Prx functions via noncovalent interactions

At a time when ATP was found to be the substrate for the phosphorylation of 2-Cys Prx, Aran *et al.* [97] noted that the concerted action of a nucleotide and Mg^{2+} impaired the reduction of H_2O_2, whereas only the latter decreased the capacity to prevent the thermal aggregation of citrate synthase. These findings reveal two novel features for nucleotides and bivalent cations in the modulation of 2-Cys Prx function: (a) the kinetic regulation of peroxidase activity as a process easily discernible from the thermodynamic control originating from the availability of the peroxide substrate and (b) the differential regulation of peroxidase and chaperone activities by modulators devoid of redox capacity. In contrast with the phosphorylation of human 2-Cys Prx mediated by the CDK1–cyclin B complex [95], the capacity of the nucleotide/Mg^{2+} couple to inhibit peroxidase activity rapidly, reversibly and without exogenous catalysts is congruent with a noncovalent mechanism that perhaps complements other post-translational modifications. Not only are purine nucleotides more potent inhibitors than pyrimidine derivatives, but also the response of peroxidase activity to increasing concentrations of ATP exhibits three well-defined stages: (a) a monotonic decay up to 0.9 mM, (b) a stabilization at half of the maximal activity from 0.9 to 1.2 mM, and (c) a sharp decrease to undetectable levels beyond 1.5 mM (cf. fig. 1 in [97]). In line with previous studies [97], recent dynamic and static light scattering experiments have revealed that the presence of both ATP and Mg^{2+} drives the quaternary structure of 2-Cys Prx to assemblies of larger size (hydrodynamic radius of *c.* 69 nm), which return to the decameric form after the removal of any modulator (hydrodynamic radius of *c.* 13.8 nm) (M. Aran, unpublished results). Notably, the concerted action of the metabolite linked to the energy status of the cell and a highly mobile bivalent cation converts the rather stable decamer to higher order assemblies approximating to the dodecahedron $[(a_2)_5]_{12}$ observed in electron microscopic preparations of the erythrocyte counterpart [27]. The preceding data convey the concept that the allosteric regulation of 2-Cys Prx through ATP/Mg^{2+} invokes a wide variety of assemblies, which, in turn, ensure a multiplicity of functional features. In this type of protein, designated as morpheeins [102], the alternation between many quaternary structures provides the appropriate shift of the specific activity in response to protein concentration, allosteric regulation, cooperativity and hysteresis. Although noncovalent interactions of 2-Cys Prx with ATP are not sufficient to account for the functional regulation, the participation of this nucleotide in the post-translational modification of specific amino acid residues is an unusual example of a modulator that plays more than one role in controlling the functions of 2-Cys Prx (Fig. 6).

Concluding remarks

The response of 2-Cys Prxs to oxidative insults is contingent not only on the dose and duration of the oxidative stress, but also on the subcellular localization relative to the target to be protected. Particularly illustrative in this respect is a proteomic analysis performed in C4 plants, whose special photosynthetic trait relies on the differential functioning of chloroplasts in two different types of leaf cell: bundle sheath and mesophyll cells [103]. Notably, 2-Cys Prxs are 2.5-fold more abundant in mesophyll than in bundle sheath chloroplasts. This preferential expression is not

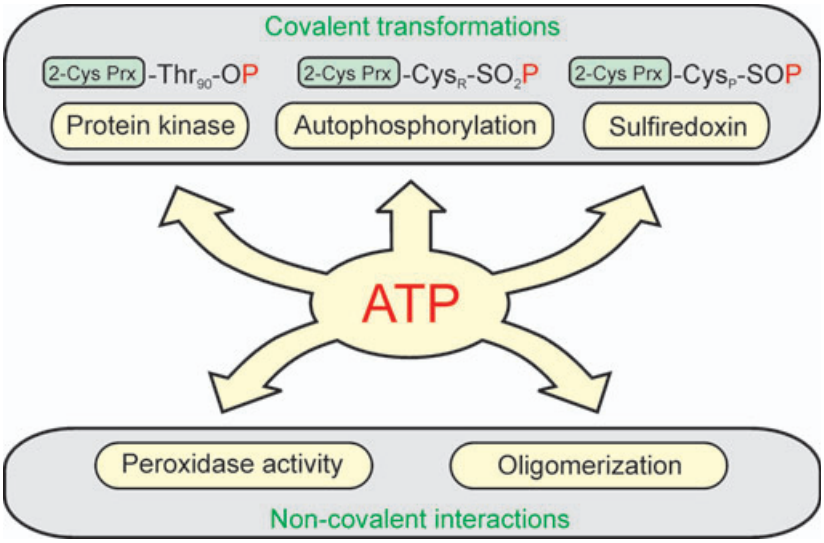

Fig. 6. Pivotal role of ATP in the structural modifications of 2-Cys Prx. The diagram summarizes the participation of ATP in covalent transformations of (a) Thr90 catalyzed by CDK1, (b) Cys_R by autophosphorylation and (c) Cys_P assisted by Srx, and noncovalent interactions that drive the quaternary structure to the formation of large assemblies.

surprising because the former chloroplasts generate reactive oxygen species during the functioning of photosystems II and I, whereas the decreased activity of photosystem II in the latter chloroplasts produces less oxidative stress. On this basis, it should be expected that detailed analyses of transgenic organisms will contribute to elucidate the role played by the temporal and spatial expression of 2-Cys Prx genes in response to specific situations.

The central role of 2-Cys Prx in redox perception provides a strong impetus to learn more about how it is regulated. Although the diversity of phosphorylation sites and novel acetylation may have important implications in the regulation of 2-Cys Prx, the associated activities can be further modulated by small molecules. Accordingly, it is becoming increasingly clear that ATP participates not only as a substrate for post-translational modifications, but also as a modulator of the quaternary structure. Indeed, this dual capacity to use the nucleotide for covalent transformations and noncovalent interactions may be at the root of mechanisms by which 2-Cys Prxs harness nonredox chemistry to cope with situations of oxidative stress. In the coming years, similar approaches will be crucial to unravel whether 2-Cys Prxs rely on noncovalent interactions to control the functions of other, as yet unknown, components or auxiliary proteins, mainly enzyme activity. The elucidation of complexes with target proteins *in vitro* and *in vivo*, their hierarchy of importance and the dynamics of the association are all goals of future research.

Although we cannot yet foresee the complete set of biological processes that require 2-Cys Prx, it is becoming clear that the peroxidase activity poises the low oxidative stimuli while post-translational modifications serve in signal transduction when the level of oxidative stress exceeds that which can be successfully handled. These tunable functions alert the cell to stimulate the expression of antioxidant or, eventually, apoptotic genes. Therefore, the transition from antioxidant enzyme to regulatory signal acts as a rheostat that prevents an overreaction in response to low levels of environmental stimuli. However, more experimental work is needed to characterize the regulatory elements involved in the optimization of the gradual switch. Accordingly, ongoing methodological innovations are providing novel approaches to answer the question of how biological systems integrate the sulfenic acid chemistry into signal transduction [104]. In this context, the redox status of the cell milieu should be clearly defined to determine how 2-Cys Prxs coordinate the associated functions with (a) cell redox components milder than harsh oxidants often used in studies of oxidative stress, (b) upstream and downstream redox proteins, such as Trxs and cyclophilins, and (c) other antioxidant enzymes with similar activities (e.g. glutathione peroxidase, ascorbate peroxidase). Experimentally, proteomic techniques will be helpful to determine how the thiol–disulfide regulatory network, including glutathionylation, crosstalks with the formation of *S*-oxyacids and *S*-nitroso moieties in proteins.

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References

1 Buchanan BB, Wolosiuk RA & Schurmann P (1979) Thioredoxin and enzyme regulation. *Trends Biochem Sci* **4**, 93–96.

2 Cooper CE, Patel RP, Brookes PS & Darley-Usmar VM (2002) Nanotransducers in cellular redox signaling: modification of thiols by reactive oxygen and nitrogen species. *Trends Biochem Sci* **27**, 489–492.

3 Allen JF (1993) Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J Theor Biol* **165**, 609–631.

4 Paget MS & Buttner MJ (2003) Thiol-based regulatory switches. *Annu Rev Genet* **37**, 91–121.

5 Neill S, Desikan R & Hancock J (2002) Hydrogen peroxide signalling. *Curr Opin Plant Biol* **5**, 388–395.

6 Zhou Y, Wan XY, Wang HL, Yan ZY, Hou YD & Jin DY (1997) Bacterial scavengase p20 is structurally and functionally related to peroxiredoxins. *Biochem Biophys Res Commun* **233**, 848–852.

7 Park SG, Cha MK, Jeong W & Kim IH (2000) Distinct physiological functions of thiol peroxidase isoenzymes in *Saccharomyces cerevisiae*. *J Biol Chem* **275**, 5723–5732.

8 Zhou Y, Kok KH, Chun AC, Wong CM, Wu HW, Lin MC, Fung PC, Kung H & Jin DY (2000) Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. *Biochem Biophys Res Commun* **268**, 921–927.

9 Seo MS, Kang SW, Kim K, Baines IC, Lee TH & Rhee SG (2000) Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate. *J Biol Chem* **275**, 20346–20354.

10 Dietz KJ, Horling F, Konig J & Baier M (2002) The function of the chloroplast 2-cysteine peroxiredoxin in peroxide detoxification and its regulation. *J Exp Bot* **53**, 1321–1329.

11 Trivelli X, Krimm I, Ebel C, Verdoucq L, Prouzet-Mauleon V, Chartier Y, Tsan P, Lauquin G, Meyer Y & Lancelin JM (2003) Characterization of the yeast peroxiredoxin Ahp1 in its reduced active and overoxidized inactive forms using NMR. *Biochemistry* **42**, 14139–14149.

12 Knoops B, Loumaye E & Van Der Eecken V (2007) Evolution of the peroxiredoxins. *Subcell Biochem* **44**, 27–40.

13 Loumaye E, Andersen AC, Clippe A, Degand H, Dubuisson M, Zal F, Morsomme P, Rees JF & Knoops B (2008) Cloning and characterization of *Arenicola marina* peroxiredoxin 6, an annelid two-cysteine peroxiredoxin highly homologous to mammalian one-cysteine peroxiredoxins. *Free Radic Biol Med* **45**, 482–493.

14 Copley SD, Novak WR & Babbitt PC (2004) Divergence of function in the thioredoxin fold suprafamily: evidence for evolution of peroxiredoxins from a thioredoxin-like ancestor. *Biochemistry* **43**, 13981–13995.

15 Flohe L & Harris JR (2007) Introduction. History of the peroxiredoxins and topical perspectives. *Subcell Biochem* **44**, 1–25.

16 Hirotsu S, Abe Y, Okada K, Nagahara N, Hori H, Nishino T & Hakoshima T (1999) Crystal structure of a multifunctional 2-Cys peroxiredoxin heme-binding protein 23 kDa/proliferation-associated gene product. *Proc Natl Acad Sci USA* **96**, 12333–12338.

17 Sarma GN, Nickel C, Rahlfs S, Fischer M, Becker K & Karplus PA (2005) Crystal structure of a novel *Plasmodium falciparum* 1-Cys peroxiredoxin. *J Mol Biol* **346**, 1021–1034.

18 Hall A, Karplus PA & Poole LB (2009) Typical 2-Cys peroxiredoxins – structures, mechanisms and functions. *FEBS J* **276**, doi:10.1111/j.1742-4658.2009.06985.x.

19 Lee W, Choi KS, Riddell J, Ip C, Ghosh D, Park JH & Park YM (2007) Human peroxiredoxin 1 and 2 are not duplicate proteins: the unique presence of CYS83 in Prx1 underscores the structural and functional differences between Prx1 and Prx2. *J Biol Chem* **282**, 22011–22022.

20 Jang HH, Kim SY, Park SK, Jeon HS, Lee YM, Jung JH, Lee SY, Chae HB, Jung YJ, Lee KO *et al.* (2006) Phosphorylation and concomitant structural changes in human 2-Cys peroxiredoxin isotype I differentially regulate its peroxidase and molecular chaperone functions. *FEBS Lett* **580**, 351–355.

21 Konig J, Lotte K, Plessow R, Brockhinke A, Baier M & Dietz KJ (2003) Reaction mechanism of plant 2-Cys peroxiredoxin. Role of the C terminus and the quaternary structure. *J Biol Chem* **278**, 24409–24420.

22 Parsonage D, Youngblood DS, Sarma GN, Wood ZA, Karplus PA & Poole LB (2005) Analysis of the link between enzymatic activity and oligomeric state in AhpC, a bacterial peroxiredoxin. *Biochemistry* **44**, 10583–10592.

23 Matsumura T, Okamoto K, Iwahara S, Hori H, Takahashi Y, Nishino T & Abe Y (2008) Dimer–oligomer interconversion of wild-type and mutant rat 2-Cys peroxiredoxin: disulfide formation at dimer–dimer interfaces is not essential for decamerization. *J Biol Chem* **283**, 284–293.

24 Barranco-Medina S, Kakorin S, Lazaro JJ & Dietz KJ (2008) Thermodynamics of the dimer–decamer transition of reduced human and plant 2-cys peroxiredoxin. *Biochemistry* **47**, 7196–7204.

25 Cao Z, Bhella D & Lindsay JG (2007) Reconstitution of the mitochondrial PrxIII antioxidant defence pathway: general properties and factors affecting PrxIII activity and oligomeric state. *J Mol Biol* **372**, 1022–1033.

26 Jang HH, Lee KO, Chi YH, Jung BG, Park SK, Park JH, Lee JR, Lee SS, Moon JC, Yun JW *et al.* (2004)

Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. *Cell* **117**, 625–635.
27 Meissner U, Schroder E, Scheffler D, Martin AG & Harris JR (2007) Formation, TEM study and 3D reconstruction of the human erythrocyte peroxiredoxin-2 dodecahedral higher-order assembly. *Micron* **38**, 29–39.
28 Gourlay LJ, Bhella D, Kelly SM, Price NC & Lindsay JG (2003) Structure–function analysis of recombinant substrate protein 22 kDa (SP-22). A mitochondrial 2-Cys peroxiredoxin organized as a decameric toroid. *J Biol Chem* **278**, 32631–32637.
29 Cao Z, Roszak AW, Gourlay LJ, Lindsay JG & Isaacs NW (2005) Bovine mitochondrial peroxiredoxin III forms a two-ring catenane. *Structure* **13**, 1661–1664.
30 Guimaraes BG, Souchon H, Honore N, Saint-Joanis B, Brosch R, Shepard W, Cole ST & Alzari PM (2005) Structure and mechanism of the alkyl hydroperoxidase AhpC, a key element of the *Mycobacterium tuberculosis* defense system against oxidative stress. *J Biol Chem* **280**, 25735–25742.
31 Kemp M, Go YM & Jones DP (2008) Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. *Free Radic Biol Med* **44**, 921–937.
32 Wood ZA, Schroder E, Robin Harris J & Poole LB (2003) Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* **28**, 32–40.
33 Wood ZA, Poole LB, Hantgan RR & Karplus PA (2002) Dimers to doughnuts: redox-sensitive oligomerization of 2-cysteine peroxiredoxins. *Biochemistry* **41**, 5493–5504.
34 Schroder E, Littlechild JA, Lebedev AA, Errington N, Vagin AA & Isupov MN (2000) Crystal structure of decameric 2-Cys peroxiredoxin from human erythrocytes at 1.7 Å resolution. *Structure* **8**, 605–615.
35 Rabilloud T, Heller M, Gasnier F, Luche S, Rey C, Aebersold R, Benahmed M, Louisot P & Lunardi J (2002) Proteomics analysis of cellular response to oxidative stress. Evidence for in vivo overoxidation of peroxiredoxins at their active site. *J Biol Chem* **277**, 19396–19401.
36 Wagner E, Luche S, Penna L, Chevallet M, Van Dorsselaer A, Leize-Wagner E & Rabilloud T (2002) A method for detection of overoxidation of cysteines: peroxiredoxins are oxidized in vivo at the active-site cysteine during oxidative stress. *Biochem J* **366**, 777–785.
37 Immenschuh S & Baumgart-Vogt E (2005) Peroxiredoxins, oxidative stress, and cell proliferation. *Antioxid Redox Signal* **7**, 768–777.
38 Dietz KJ, Jacob S, Oelze ML, Laxa M, Tognetti V, de Miranda SM, Baier M & Finkemeier I (2006) The function of peroxiredoxins in plant organelle redox metabolism. *J Exp Bot* **57**, 1697–1709.
39 Konig J, Baier M, Horling F, Kahmann U, Harris G, Schurmann P & Dietz KJ (2002) The plant-specific function of 2-Cys peroxiredoxin-mediated detoxification of peroxides in the redox-hierarchy of photosynthetic electron flux. *Proc Natl Acad Sci USA* **99**, 5738–5743.
40 Foyer CH & Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell* **17**, 1866–1875.
41 Phalen TJ, Weirather K, Deming PB, Anathy V, Howe AK, van der Vliet A, Jonsson TJ, Poole LB & Heintz NH (2006) Oxidation state governs structural transitions in peroxiredoxin II that correlate with cell cycle arrest and recovery. *J Cell Biol* **175**, 779–789.
42 Salsbury FR Jr, Knutson ST, Poole LB & Fetrow JS (2008) Functional site profiling and electrostatic analysis of cysteines modifiable to cysteine sulfenic acid. *Protein Sci* **17**, 299–312.
43 Nakamura T, Yamamoto T, Abe M, Matsumura H, Hagihara Y, Goto T, Yamaguchi T & Inoue T (2008) Oxidation of archaeal peroxiredoxin involves a hypervalent sulfur intermediate. *Proc Natl Acad Sci USA* **105**, 6238–6242.
44 Ogusucu R, Rettori D, Munhoz DC, Netto LE & Augusto O (2007) Reactions of yeast thioredoxin peroxidases I and II with hydrogen peroxide and peroxynitrite: rate constants by competitive kinetics. *Free Radic Biol Med* **42**, 326–334.
45 Peskin AV, Low FM, Paton LN, Maghzal GJ, Hampton MB & Winterbourn CC (2007) The high reactivity of peroxiredoxin 2 with H_2O_2 is not reflected in its reaction with other oxidants and thiol reagents. *J Biol Chem* **282**, 11885–11892.
46 Parsonage D, Karplus PA & Poole LB (2008) Substrate specificity and redox potential of AhpC, a bacterial peroxiredoxin. *Proc Natl Acad Sci USA* **105**, 8209–8214.
47 Hughes MN (2008) Chemistry of nitric oxide and related species. *Methods Enzymol* **436**, 3–19.
48 Bryk R, Griffin P & Nathan C (2000) Peroxynitrite reductase activity of bacterial peroxiredoxins. *Nature* **407**, 211–215.
49 Wong CM, Zhou Y, Ng RW, Kung HF & Jin DY (2002) Cooperation of yeast peroxiredoxins Tsa1p and Tsa2p in the cellular defense against oxidative and nitrosative stress. *J Biol Chem* **277**, 5385–5394.
50 Dubuisson M, Vander Stricht D, Clippe A, Etienne F, Nauser T, Kissner R, Koppenol WH, Rees JF & Knoops B (2004) Human peroxiredoxin 5 is a peroxynitrite reductase. *FEBS Lett* **571**, 161–165.
51 Pattison DI & Davies MJ (2006) Reactions of myeloperoxidase-derived oxidants with biological substrates: gaining chemical insight into human inflammatory diseases. *Curr Med Chem* **13**, 3271–3290.

52 Claiborne A, Mallett TC, Yeh JI, Luba J & Parsonage D (2001) Structural, redox, and mechanistic parameters for cysteine-sulfenic acid function in catalysis and regulation. *Adv Protein Chem* **58**, 215–276.
53 Allison WS (1976) Formation and reactions of sulfenic acids in proteins. *Acc Chem Res* **9**, 293–299.
54 Kiley PJ & Storz G (2004) Exploiting thiol modifications. *PLoS Biol* **2**, e400.
55 Chae HZ, Chung SJ & Rhee SG (1994) Thioredoxin-dependent peroxide reductase from yeast. *J Biol Chem* **269**, 27670–27678.
56 Jara M, Vivancos AP, Calvo IA, Moldon A, Sanso M & Hidalgo E (2007) The peroxiredoxin Tpx1 is essential as a H_2O_2 scavenger during aerobic growth in fission yeast. *Mol Biol Cell* **18**, 2288–2295.
57 Nogoceke E, Gommel DU, Kiess M, Kalisz HM & Flohe L (1997) A unique cascade of oxidoreductases catalyses trypanothione-mediated peroxide metabolism in *Crithidia fasciculata*. *Biol Chem* **378**, 827–836.
58 Poole LB, Reynolds CM, Wood ZA, Karplus PA, Ellis HR & Li Calzi M (2000) AhpF and other NADH: peroxiredoxin oxidoreductases, homologues of low Mr thioredoxin reductase. *Eur J Biochem* **267**, 6126–6133.
59 Sayed AA & Williams DL (2004) Biochemical characterization of 2-Cys peroxiredoxins from *Schistosoma mansoni*. *J Biol Chem* **279**, 26159–26166.
60 Serrato AJ, Perez-Ruiz JM, Spinola MC & Cejudo FJ (2004) A novel NADPH thioredoxin reductase, localized in the chloroplast, which deficiency causes hypersensitivity to abiotic stress in *Arabidopsis thaliana*. *J Biol Chem* **279**, 43821–43827.
61 Moon JC, Jang HH, Chae HB, Lee JR, Lee SY, Jung YJ, Shin MR, Lim HS, Chung WS, Yun DJ *et al.* (2006) The C-type Arabidopsis thioredoxin reductase ANTR-C acts as an electron donor to 2-Cys peroxiredoxins in chloroplasts. *Biochem Biophys Res Commun* **348**, 478–484.
62 Perez-Ruiz JM, Spinola MC, Kirchsteiger K, Moreno J, Sahrawy M & Cejudo FJ (2006) Rice NTRC is a high-efficiency redox system for chloroplast protection against oxidative damage. *Plant Cell* **18**, 2356–2368.
63 Spinola MC, Perez-Ruiz JM, Pulido P, Kirchsteiger K, Guinea M, Gonzalez M & Cejudo FJ (2008) NTRC new ways of using NADPH in the chloroplast. *Physiol Plant* **133**, 516–524.
64 Stenbaek A, Hansson A, Wulff RP, Hansson M, Dietz KJ & Jensen PE (2008) NADPH-dependent thioredoxin reductase and 2-Cys peroxiredoxins are needed for the protection of Mg-protoporphyrin monomethyl ester cyclase. *FEBS Lett* **582**, 2773–2778.
65 Mora-Garcia S, Stolowicz F & Wolosiuk RA (2005) Redox signal transduction in plant metabolism. In *Control of Primary Metabolism in Plants* Vol. 22 (Plaxton W & McManus M, eds). Blackwell Publishing, Oxford.
66 Collin V, Lamkemeyer P, Miginiac-Maslow M, Hirasawa M, Knaff DB, Dietz KJ & Issakidis-Bourguet E (2004) Characterization of plastidial thioredoxins from Arabidopsis belonging to the new y-type. *Plant Physiol* **136**, 4088–4095.
67 Bernier-Villamor L, Navarro E, Sevilla F & Lazaro JJ (2004) Cloning and characterization of a 2-Cys peroxiredoxin from *Pisum sativum*. *J Exp Bot* **55**, 2191–2199.
68 Broin M, Cuine S, Eymery F & Rey P (2002) The plastidic 2-cysteine peroxiredoxin is a target for a thioredoxin involved in the protection of the photosynthetic apparatus against oxidative damage. *Plant Cell* **14**, 1417–1432.
69 Radyuk SN, Klichko VI, Spinola B, Sohal RS & Orr WC (2001) The peroxiredoxin gene family in *Drosophila melanogaster*. *Free Radic Biol Med* **31**, 1090–1100.
70 Bauer H, Kanzok SM & Schirmer RH (2002) Thioredoxin-2 but not thioredoxin-1 is a substrate of thioredoxin peroxidase-1 from *Drosophila melanogaster*: isolation and characterization of a second thioredoxin in *D. melanogaster* and evidence for distinct biological functions of Trx-1 and Trx-2. *J Biol Chem* **277**, 17457–17463.
71 Barik S (2006) Immunophilins: for the love of proteins. *Cell Mol Life Sci* **63**, 2889–2900.
72 Jaschke A, Mi H & Tropschug M (1998) Human T cell cyclophilin 18 binds to thiol-specific antioxidant protein Aop1 and stimulates its activity. *J Mol Biol* **277**, 763–769.
73 Lee SP, Hwang YS, Kim YJ, Kwon KS, Kim HJ, Kim K & Chae HZ (2001) Cyclophilin a binds to peroxiredoxins and activates its peroxidase activity. *J Biol Chem* **276**, 29826–29832.
74 Laxa M, Konig J, Dietz KJ & Kandlbinder A (2007) Role of the cysteine residues in *Arabidopsis thaliana* cyclophilin CYP20-3 in peptidyl–prolyl cis–trans isomerase and redox-related functions. *Biochem J* **401**, 287–297.
75 Motohashi K, Koyama F, Nakanishi Y, Ueoka-Nakanishi H & Hisabori T (2003) Chloroplast cyclophilin is a target protein of thioredoxin. Thiol modulation of the peptidyl–prolyl cis–trans isomerase activity. *J Biol Chem* **278**, 31848–31852.
76 Saurin AT, Neubert H, Brennan JP & Eaton P (2004) Widespread sulfenic acid formation in tissues in response to hydrogen peroxide. *Proc Natl Acad Sci USA* **101**, 17982–17987.
77 Yang KS, Kang SW, Woo HA, Hwang SC, Chae HZ, Kim K & Rhee SG (2002) Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation of the catalytic site cysteine to cysteine-sulfinic acid. *J Biol Chem* **277**, 38029–38036.
78 Cordray P, Doyle K, Edes K, Moos PJ & Fitzpatrick FA (2007) Oxidation of 2-Cys-peroxiredoxins by

arachidonic acid peroxide metabolites of lipoxygenases and cyclooxygenase-2. *J Biol Chem* **282**, 32623–32629.
79 Wood ZA, Poole LB & Karplus PA (2003) Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* **300**, 650–653.
80 Biteau B, Labarre J & Toledano MB (2003) ATP-dependent reduction of cysteine-sulphinic acid by *S. cerevisiae* sulphiredoxin. *Nature* **425**, 980–984.
81 Jeong W, Park SJ, Chang TS, Lee DY & Rhee SG (2006) Molecular mechanism of the reduction of cysteine sulfinic acid of peroxiredoxin to cysteine by mammalian sulfiredoxin. *J Biol Chem* **281**, 14400–14407.
82 Roussel X, Bechade G, Kriznik A, Van Dorsselaer A, Sanglier-Cianferani S, Branlant G & Rahuel-Clermont S (2008) Evidence for the formation of a covalent thiosulfinate intermediate with peroxiredoxin in the catalytic mechanism of sulfiredoxin. *J Biol Chem* **283**, 22371–22382.
83 Jonsson TJ, Johnson LC & Lowther WT (2008) Structure of the sulphiredoxin–peroxiredoxin complex reveals an essential repair embrace. *Nature* **451**, 98–101.
84 Choi MH, Lee IK, Kim GW, Kim BU, Han YH, Yu DY, Park HS, Kim KY, Lee JS, Choi C *et al.* (2005) Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. *Nature* **435**, 347–353.
85 Bozonet SM, Findlay VJ, Day AM, Cameron J, Veal EA & Morgan BA (2005) Oxidation of a eukaryotic 2-Cys peroxiredoxin is a molecular switch controlling the transcriptional response to increasing levels of hydrogen peroxide. *J Biol Chem* **280**, 23319–23327.
86 Vivancos AP, Castillo EA, Biteau B, Nicot C, Ayte J, Toledano MB & Hidalgo E (2005) A cysteine-sulfinic acid in peroxiredoxin regulates H_2O_2-sensing by the antioxidant Pap1 pathway. *Proc Natl Acad Sci USA* **102**, 8875–8880.
87 Papadia S, Soriano FX, Leveille F, Martel MA, Dakin KA, Hansen HH, Kaindl A, Sifringer M, Fowler J, Stefovska V *et al.* (2008) Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat Neurosci* **11**, 476–487.
88 Jeffery CJ (1999) Moonlighting proteins. *Trends Biochem Sci* **24**, 8–11.
89 Jeffery CJ (2004) Molecular mechanisms for multitasking: recent crystal structures of moonlighting proteins. *Curr Opin Struct Biol* **14**, 663–668.
90 Wen ST & Van Etten RA (1997) The PAG gene product, a stress-induced protein with antioxidant properties, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity. *Genes Dev* **11**, 2456–2467.
91 Moon JC, Hah YS, Kim WY, Jung BG, Jang HH, Lee JR, Kim SY, Lee YM, Jeon MG, Kim CW *et al.* (2005) Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H_2O_2-induced cell death. *J Biol Chem* **280**, 28775–28784.
92 Chuang MH, Wu MS, Lo WL, Lin JT, Wong CH & Chiou SH (2006) The antioxidant protein alkylhydroperoxide reductase of *Helicobacter pylori* switches from a peroxide reductase to a molecular chaperone function. *Proc Natl Acad Sci USA* **103**, 2552–2557.
93 Trotter EW, Rand JD, Vickerstaff J & Grant CM (2008) The yeast Tsa1 peroxiredoxin is a ribosome-associated antioxidant. *Biochem J* **412**, 73–80.
94 Caporaletti D, D'Alessio AC, Rodriguez-Suarez RJ, Senn AM, Duek PD & Wolosiuk RA (2007) Non-reductive modulation of chloroplast fructose-1,6-bisphosphatase by 2-Cys peroxiredoxin. *Biochem Biophys Res Commun* **355**, 722–727.
95 Chang TS, Jeong W, Choi SY, Yu S, Kang SW & Rhee SG (2002) Regulation of peroxiredoxin I activity by Cdc2-mediated phosphorylation. *J Biol Chem* **277**, 25370–25376.
96 Qu D, Rashidian J, Mount MP, Aleyasin H, Parsanejad M, Lira A, Haque E, Zhang Y, Callaghan S, Daigle M *et al.* (2007) Role of Cdk5-mediated phosphorylation of Prx2 in MPTP toxicity and Parkinson's disease. *Neuron* **55**, 37–52.
97 Aran M, Caporaletti D, Senn AM, Tellez de Inon MT, Girotti MR, Llera AS & Wolosiuk RA (2008) ATP-dependent modulation and autophosphorylation of rapeseed 2-Cys peroxiredoxin. *Febs J* **275**, 1450–1463.
98 Budde H, Flohe L, Hofmann B & Nimtz M (2003) Verification of the interaction of a tryparedoxin peroxidase with tryparedoxin by ESI-MS/MS. *Biol Chem* **384**, 1305–1309.
99 Jonsson TJ, Ellis HR & Poole LB (2007) Cysteine reactivity and thiol–disulfide interchange pathways in AhpF and AhpC of the bacterial alkyl hydroperoxide reductase system. *Biochemistry* **46**, 5709–5721.
100 Hoshino I, Matsubara H, Hanari N, Mori M, Nishimori T, Yoneyama Y, Akutsu Y, Sakata H, Matsushita K, Seki N *et al.* (2005) Histone deacetylase inhibitor FK228 activates tumor suppressor Prdx1 with apoptosis induction in esophageal cancer cells. *Clin Cancer Res* **11**, 7945–7952.
101 Parmigiani RB, Xu WS, Venta-Perez G, Erdjument-Bromage H, Yaneva M, Tempst P & Marks PA (2008) HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation. *Proc Natl Acad Sci USA* **105**, 9633–9638.
102 Jaffe EK (2005) Morpheeins – a new structural paradigm for allosteric regulation. *Trends Biochem Sci* **30**, 490–497.

103 Majeran W, Cai Y, Sun Q & van Wijk KJ (2005) Functional differentiation of bundle sheath and mesophyll maize chloroplasts determined by comparative proteomics. *Plant Cell* **17**, 3111–3140.
104 Poole LB & Nelson KJ (2008) Discovering mechanisms of signaling-mediated cysteine oxidation. *Curr Opin Chem Biol* **12**, 18–24.
105 Hofmann B, Hecht HJ & Flohe L (2002) Peroxiredoxins. *Biol Chem* **383**, 347–364.
106 Cha MK, Kim HK & Kim IH (1995) Thioredoxin-linked 'thiol peroxidase' from periplasmic space of *Escherichia coli*. *J Biol Chem* **270**, 28635–28641.
107 Jeong W, Cha MK & Kim IH (2000) Thioredoxin-dependent hydroperoxide peroxidase activity of bacterioferritin comigratory protein (BCP) as a new member of the thiol-specific antioxidant protein (TSA)/alkyl hydroperoxide peroxidase C (AhpC) family. *J Biol Chem* **275**, 2924–2930.