The function of the chloroplast 2-cysteine peroxiredoxin in peroxide detoxification and its regulation

K.J. Dietz¹, F. Horling, J. König and M. Baier²

University of Bielefeld, Department of Plant Physiology and Biochemistry W5, 33501 Bielefeld, Germany

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Abstract

The Arabidopsis genome contains nine open reading frames with homology to members of the peroxiredoxin (prx) family: one 1-Cys-prx, two 2-Cys-prx, five type II-prx, and one peroxiredoxin Q. The function of the peroxiredoxins in plant metabolism is only slowly emerging. They are assumed to reduce toxic peroxides to their corresponding alcohols with a rather broad substrate specificity. The 2-Cys peroxiredoxins (2-CP) were recently identified as members of the antioxidant defence system of chloroplasts. Knock-out mutants of Synechocystis and antisense mutants of Arabidopsis have provided insight into the function of 2-CPs in the photosynthetic antioxidant network. This review summarizes present knowledge on the enzymatic mechanism, the physiological context and the genetic regulation of the 2-CPs in plants and cyanobacteria. In addition, an extrapolation on the metabolic role of the chloroplast 2-CP is attempted based on the molecular features of 2-CPs from other organisms.

Key words: Antioxidants, Arabidopsis, chloroplast peroxiredoxin, peroxide detoxification.

Peroxiredoxins in non-photosynthetic bacteria, fungi and animals

Peroxiredoxins have been identified in all groups of organisms and constitute a phylogenetically old group of enzymes with catalytic function in the detoxification of cell-toxic peroxides (Chae et al., 1993; Prosperi et al., 1993; Storz et al., 1989; Lim et al., 1998). Related to the history of detection, the enzyme has received various names, for example alkyl hydroperoxide reductase (Ahp), protector protein, thiol-specific antioxidant (TSA) and, recently, peroxiredoxins (Prx). Within the peroxiredoxins, four clusters of related proteins are distinguished: 1-Cys peroxiredoxins (1-CP), 2-Cys peroxiredoxins (2-CP), YLR109-related peroxiredoxins (type II-Prx), and bacteriferritin-associated proteins (Prx Q). Biochemical data on type II-Prx and Prx Q are still scarce (Verdoucq et al., 1999). All Prx are characterized by one or two cysteine residue(s), respectively, located in a conserved structural environment of the protein and are central for the catalytic reaction (Chae et al., 1993; Baier and Dietz, 1996c; Verdoucq et al., 1999). The first peroxiredoxins were cloned from bacteria such as Salmonella typhimurium and E. coli (Storz et al., 1989). Since then, peroxiredoxin genes have been isolated from many organisms. For example, at least five 2-CP isoforms are present in the genomes of mouse and man (Prx I, II, III, IV, V; Seo et al., 2000).

Sites of high levels of expression coincide with sites of active oxygen metabolism such as brain and erythrocytes. For example, in erythrocytes, with their main function in oxygen transport, the protein fraction represented by the 2-CP is the third most dominant protein with 14 x 10⁶ copies in each red blood cell (Schroeder et al., 2000).

The functional 2-CP is a homodimer with two reduced Cys residues per subunit. During the catalytic cycle, the peroxide substrate is reduced to the corresponding alcohol and the Cys residues oxidized to a disulphide bridge (Fig. 1). The regeneration of catalytically active 2-CP depends on reduction of the disulphide bridge. In most eukaryotic organisms, thioredoxin (Chae et al., 1999) or thioredoxin-related proteins such as trypathionin (in trypanosomes: Nogoeeke et al., 1997; Montemartini et al., 1999; Lopez et al., 2000) act as electron donors. Recently, it was shown that the thioredoxin system also regenerates the mitochondrial
1-CP of *Saccharomyces* (Pedrajas et al., 2000) and the YLR109-related peroxiredoxins (type II-Prx) (Verdoucq et al., 1999).

The substrate specificity of 2-CPs is rather broad and catalyses not only detoxification of $\text{H}_2\text{O}_2$ and alkyl hydroperoxides but also reduction of reactive nitrogen peroxides (Lim et al., 1993; Bryk et al., 2000). Nitric oxide reacts with superoxide to form peroxynitrite which disproportionates to form nitrate and highly reactive hydroxyl radical and nitrogen dioxide radical (equations (1) and (2)).

\[
\text{NO} + \text{O}_2^{-} \rightarrow \text{OONO}^{-} \quad (1)
\]
\[
2 \text{OONO}^{-} + \text{H}^{+} \rightarrow \text{NO}_3^{-} + \text{OH}^{-} + \text{NO}_2 \quad (2)
\]

Both, the hydroxyl radical and the nitrogen dioxide radical are extremely reactive compounds and oxidize organic compounds such as DNA and proteins (Bryk et al., 2000). Reductive detoxification of peroxynitrite is important to avoid destructive oxidation of cellular constituents.

**Peroxiredoxins in plants and their subcellular localization**

In the genome of *Arabidopsis thaliana* there are nine open reading frames with sequence similarity to peroxiredoxins (Table 1; Fig. 1). As stated above, peroxiredoxins can be grouped in 1-Cys Prx, 2-Cys Prx, type II Prx, and Prx Q. Members of each group have recently been identified in plants also. The first cDNA-sequence of a plant 1-cysteine peroxiredoxin (1-CP PER1) was identified as a ‘dormancy-related protein’ being expressed in the embryo and the aleurone layer of the barley caryopsis (Aalen et al., 1994). Immunocytochemistry revealed that the PER1 1-CP is preferentially localized in the nucleus, and there within the nucleolus (Stacy et al., 1996, 1999). Homologous sequences of PER1 were also isolated from *Arabidopsis*, rice, buckwheat, and wheat (Haslekas et al., 1998; Lewis et al., 2000; Majoul et al., 2000). The authors suggest a role of the 1-CP in protecting macromolecules from oxidative destruction during the period of drying and reswelling of the seeds and speculate on a role in maintaining dormancy.

Plant 2-CPs were first cloned from barley and spinach (Baier and Dietz, 1996a, b), and afterwards from *Arabidopsis thaliana* (Baier and Dietz, 1997), chinese cabbage (Cheong et al., 1999), rye (Berberich et al., 1998), and the liverwort *Riccia fluitans* (Horling et al., 2001). In the genome of *A. thaliana* there are two genes annotated encoding 2-CPs and one gene encoding a 1-CP (Table 1).

**Fig. 1.** (A) Simplified scheme of peroxide reduction by Prx and regeneration of oxidized Prx through an appropriate electron donor like Trx or GSH. (B) Unrooted tree of relatedness of the 9 prx genes of the *Arabidopsis thaliana* genome based on amino acid homology. The type II prx genes and the 2-CP prx-genes form distinct branches, and also the prxQ and 1CP prx gene are separated. The sequences were aligned and compared in order to construct a phylogenetic tree using the programs CLUSTAL W (http://www2.ebi.ac.uk/clustalw/) and Treecon (Van de Peer and De Wachter, 1994) (http://bio-www.uia.ac.be/yvdp/Userman/treeconv.html).

**Table 1. The nine peroxiredoxin genes in the Arabidopsis thaliana genome**

<table>
<thead>
<tr>
<th>No.</th>
<th>prx Group</th>
<th>MATDB entry</th>
<th>Targeting</th>
<th>Length (aa)/molecular mass (kDa)</th>
<th>Isoelectric point</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1CP-prx</td>
<td>AT1g48130</td>
<td>Nucleus</td>
<td>216/24.0</td>
<td>6.14</td>
<td>Stacy et al., 1996, 1999</td>
</tr>
<tr>
<td>2</td>
<td>2CP-prxA</td>
<td>AT3g11630</td>
<td>Chloroplast</td>
<td>266/29.0</td>
<td>6.91 (4.97)</td>
<td>Baier and Dietz, 1996, 1997; Cheong et al., 1999</td>
</tr>
<tr>
<td>3</td>
<td>2CP-prxB</td>
<td>AT5g06290</td>
<td>? Peroxisome</td>
<td>271/29.6</td>
<td>5.55 (4.68)</td>
<td>Choi et al., 1999; Verdoucq et al., 1999</td>
</tr>
<tr>
<td>4</td>
<td>type II prxA</td>
<td>AT1g65990</td>
<td>? Peroxisome</td>
<td>553/62.7</td>
<td>6.04</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>type II prxB</td>
<td>AT1g65980</td>
<td>? Peroxisome</td>
<td>162/17.4</td>
<td>5.04</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>type II prxC</td>
<td>AT1g65970</td>
<td>? Peroxisome</td>
<td>162/17.4</td>
<td>5.22</td>
<td></td>
</tr>
<tr>
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<td>AT1g60740</td>
<td>? Peroxisome</td>
<td>174/19.2</td>
<td>6.10</td>
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<tr>
<td>8</td>
<td>type II prxE</td>
<td>AT3g52960</td>
<td>? Peroxisome</td>
<td>234/24.7</td>
<td>9.46</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>prx Q</td>
<td>AT3g26060</td>
<td>? Chloroplast predicted</td>
<td>215/23.6</td>
<td>9.7</td>
<td>Kong et al., 2000</td>
</tr>
</tbody>
</table>
The function of 2-CPs

The catalytic activity of peroxiredoxins

Peroxiredoxins reduce peroxide substrates to the corresponding alcohol. In the 1-CP, 2-CP, type II-prx, and prx Q, a Cys-residue in conserved structural environment constitutes the amino acid residue essential for the catalytic activity with the peroxide substrates. In addition to Cys64 (numbers as counted in the mature barley 2-CP: Baier and Dietz, 1996), members of the 2-CP group contain an additional Cys residue at a more amino-terminal position (C185). During the catalytic cycle with two peroxides two disulfide bonds are successively formed with intermediate modification of one Cys residue to cysteine-sulphenic acid (Cys-OH) and subsequent release of H₂O₂. Thus, three different conformational states of the 2-CP exist and can be detected in non-reducing denaturing gels as distinct bands which correspond to (i) the fully reduced 2-CP, the 2-CP with (ii) a single disulfide bridge and (iii) two disulfide linkages (Gommel et al., 1997; Baier and Dietz, 1998b). The shift in electrophoretic mobility under non-reducing conditions represents a convenient assay for reactivity with peroxide substrates (Fig. 2). Oxidation of the 2-CP also occurs during exposure to dissolved oxygen. The fully oxidized 2-CP loses its enzymatic activity and must be regenerated. For many peroxiredoxins it has been established that thioredoxins serve as the electron donor for the regeneration of the active form. In chloroplasts, thioredoxin f and m function in the regulation of redox-sensitive target proteins (Schürmann and Jacquot, 2000) and could be involved in the regeneration of oxidized 2-CP.

The substrate specificity of the plant peroxiredoxins has not been investigated in any detail yet. The reconstitution of a functional redox cascade can be achieved for 2-CP with NADPH, E. coli thioredoxin reductase, E. coli thioredoxin, and peroxide substrates such as H₂O₂ (Fig. 3), and butyl hydroperoxide or cumene hydroperoxide (data not shown). At 250 μM, the barley 2-CP reduced all three substrates at a similar rate (data not shown). The H₂O₂-reducing activity at 25 and 10 μM concentration was as high as in the presence of 50 and 100 μM H₂O₂ (Fig. 3). From kinetic analysis the Kₘ(H₂O₂) was estimated to be below 2 μM (J König, unpublished data). At high H₂O₂ concentrations (≥500 μM), the 2-CP was inactivated (Fig. 3). H₂O₂ and butyl hydroperoxide were also shown to be substrates of the cyanobacterial 2-CP. In the absence of other electron acceptors, the addition of either peroxide induced oxygen evolution in wild-type Synechocystis (Yamamoto et al., 1999). The onset of peroxide-induced oxygen liberation in photosynthesis was accompanied by quenching of chlorophyll a fluorescence, which indicated peroxide-dependent relief of thylakoid energization and oxidation of thylakoid electron transport carriers. The 2-CP-cDNA (C2C-Prx) was isolated from chinese cabbage and the protein characterized following recombinant production of the protein (Cheong et al., 1999). C2C-Prx protected DNA from oxidative damage, and glutamine synthase from inactivation in the presence of a mixed functional oxidation system consisting

(From: The Arabidopsis Genome Initiative, 2000. Compared to cytosolic animal and fungal 2-CPs and bacterial 2-CPs, all 2-CPs from higher plants cloned so far show an amino-terminal extension. Three pieces of evidence have proven that the pre-peptide mediates import into chloroplasts and, consequently, that plant 2-CP is a chloroplast enzyme: (1) 2-CP translated in a cell-free system was post-translationally imported by isolated intact chloroplasts. Following the import the targeting signal was cleaved off resulting in a mature protein similar to animal and bacterial 2-CP (Baier and Dietz, 1997). (2) During fractionation of mesophyll protoplasts, the 2-CP co-purified with chloroplasts. (3) In immuno-histochemical studies of leaf cross-sections, the 2-CP was detected in chloroplasts where it was preferentially attached to the thylakoid membrane (M Baier, Kahmann, KJ Dietz, unpublished data).

The third class of plant peroxiredoxin-like proteins (type II peroxiredoxins) was identified in a screen for proteins binding to a modified thioredoxin h, first in yeast (YLR109) and, by means of sequence homology, in plants (in A. thaliana: AtTPX2) and animals (Verdoucq et al., 1999; Choi et al., 1999). This third class of peroxiredoxins is suggested to be targeted to the peroxisomes of eukaryotic cells and to be involved in detoxifying H₂O₂ that escapes from detoxification by catalase, or alkyl hydroperoxide produced by reactions of lipids with reactive oxygen species in the peroxisomal membrane. Alternatively, they may remain in the cytosol. The genome of Arabidopsis contains five genes coding for type II peroxiredoxins (Table 1). Recently, the fourth type of peroxiredoxin, a homologue of the bacteri ferritin co-migratory protein of E. coli, was identified in Sedum lineare (Kong et al., 2000). One gene for a homologous protein is encoded in the A. thaliana genome (Table 1). In the comparatively small Prx Q-proteins the two catalytic Cys residues are spaced by only four amino acids. It has been suggested that the protein exists as a functional monomer and the disulfide bridge is formed between the two Cys-residues (Cys44 and Cys49) (Kong et al., 2000).

Homologues of the higher plant 2-CP were also found in the red algae Porphyra purpurea and the cyanobacterium Synechocystis PCC6803. The high similarity of the algal and cyanobacterial 2-CP with the plant 2-CPs suggests that the plastidic form was introduced into the plants via the photosynthetic endosymbi ont which later on developed into the chloroplast (Baier and Dietz, 1997). This hypothesis concurs with the genomic organization of the 2-CP gene. The chloroplast transit sequence is encoded by a separate exon.

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of Fe$^{2+}$, O$_2$ and thiols. The activity of peroxiredoxins towards H$_2$O$_2$ is well established. However, an analysis of the peroxiredoxin-activity towards lipid peroxides with physiological importance is not available for plants. Such detailed studies have only been performed for the trypanothionine-dependent tryparedoxin peroxidase of trypanosomatids (Nogoceke et al., 1997) and the bacterial alkyl hydroperoxide reductase (AhpC; Hillas et al., 2000). The tryparedoxin peroxidase reduced linoleic acid hydroperoxide and phosphatidylcholine hydroperoxide with similar rate constants. It is likely, that the plant 2-CPs have a similar substrate specificities, but experimental proof needs to be provided.

### Structure of the 2-CP

The human 2-CPs Tpx-B from erythrocytes (Schröder et al., 2000) and the ‘proliferation associated gene’ pag (HBP 23, Hirotsu et al., 1999) were structurally resolved at 1.7 and 2.6 Å atomic resolution, respectively. These studies demonstrated that the functional 2-CP is a dimer. During catalysis, the more amino-terminally located conserved Cys-residue is oxidized followed by disulphide bridge formation and release of water. Under highly oxidizing conditions, the amino-terminally located Cys residue is further oxidized to sulphinic acid. In this ‘over-oxidized’ state the two catalytically interacting Cys residues, which are located on the different subunits of the dimer, are separated by more than 10 Å and the disulphide bridge cannot be formed. As a consequence, the enzyme is trapped in a conformation that cannot be reactivated by reduction. Concomitantly, the 2-CP aggregates as a decamer. Decamerization has been described for the human erythrocyte 2-CP (Schröder et al., 2000) and the *Trypanosomas* peroxiredoxin (Nogoceke et al., 1997).

The peroxide detoxification system was reconstituted with 2-CP of chinese cabbage and yeast thioredoxin/ thioredoxin reductase and the peroxide reduction measured at a concentration of 1 mM H$_2$O$_2$ (Cheong et al., 1999). It will have to be established whether such high H$_2$O$_2$ concentrations are suitable for activity tests or lead to permanent inactivation by modification of the sulphhydryl group to sulphinic acid. Figure 3 shows a decrease in the activity of the heterologously expressed barley 2-CP by elevated concentrations of H$_2$O$_2$. In this
reaction, the peroxide reduction was coupled to NADPH, thioredoxin reductase and thioredoxin of *E. coli*.

In addition, the atomic structure at 1.7 Å resolution allowed refinement of the model of catalytic action. The midpoint potential of the barley 2-CP was determined to be more negative than −310 mV (J König, M Baier, KJ Dietz, unpublished results). Apparently, the specific molecular environment activates the Cys-residue. It was shown for the tryparedoxin peroxidase of *Crithidia fasciculata* that a Trp- and an Arg-residues are of critical importance for maintenance of the peroxiredoxin activity (Montemartini et al., 1999). Mutation of the corresponding amino acids (Arg 140 and Trp 100) of the barley 2-CP showed their importance for maintaining the catalytic activity.

**The physiological function of the 2-Cys peroxiredoxin**

The physiological function of 2-CPs in photosynthetic organisms has been investigated in insertion mutants of *Synechocystis* PCC 6803 (Δ2-CP) (Klughammer et al., 1998; Yamamoto et al., 1999) and in antisense mutants of *A. thaliana*. (Baier and Dietz, 1999b; Baier et al., 2000). The knock-out mutant strains of *Synechocystis* grew at the same rates as the wild type at low light intensities, but showed considerably decreased growth rates at high light intensities (Klughammer et al., 1998). Wild-type *Synechocystis* evolved O2 upon the addition of peroxides to the suspension medium, concomitantly, the chlorophyll *a* fluorescence was quenched. Both responses were not seen in the Δ2-CP cells indicating that the 2-CP is the only enzyme that scavenges H2O2 and alkyl hydroperoxides in *Synechocystis* (Yamamoto et al., 1999). Furthermore, wild type-like fast growth was restored for Δ2-CP cells when the Fe and trace element concentration was lowered in the nutrient medium to 1/10th (Klughammer et al., 1998). Transition metals such as Fe and Cu, but also other heavy metals cause oxidative stress either by direct transfer of electrons to oxygen or reactive oxygen species, and by means of the inhibition of metabolic reactions (Dietz et al., 1999). Thus micronutrients are both essential and toxic elements. Optimum growth at micromolar concentrations of Fe and Cu are intimately coupled to an efficient detoxification of reactive oxygen species by antioxidants. Elimination of the 2-CP as the main H2O2-detoxifying enzyme in *Synechocystis* can impair growth even at low metal concentrations.

The situation is considerably more complicated in higher plants. The site of most active oxygen metabolism in the light is the chloroplast. O2 is photo-reduced at photosystem I (PSI) and H2O2 is produced at appreciable rates. Attached to PSI is a scavenging complex for H2O2 consisting of CuZn superoxide dismutase (SOD), ascorbate peroxidase (Apx), ferredoxin (Fd), and Fd-dependent monodehydroascorbate reductase (Asada et al., 1998). The activity of this complex is believed to exceed the rate of O2-photoreduction more than 100-fold and, therefore, appears to be sufficiently active to detoxify H2O2 originating from the Mehler reaction (water/water cycle). In addition to detoxification of reactive oxygen species, the water/water cycle via ascorbate peroxidase serves the dissipation of excessively absorbed energy. The Prx-mediated detoxification pathway represents an alternative water/water cycle (Fig. 4). Similar to the Apx-dependent water/water cycle, the 2-CP-mediated detoxification of H2O2 is not only a scavenging mechanism for reactive oxygen species, but also allows dissipation of excess excitation energy and protection of the photosystems from photo inhibition.

Chloroplastic Apx is highly sensitive to inactivation by reactive oxygen species. In tobacco, activity of chloroplast Apx was lost during a 48 h drought treatment at high light (Shikanai et al., 1998). Simultaneously, the rates of photosynthetic CO2-fixation and activities of other oxidation-sensitive enzymes such as phosphoribulokinase (PRK) and glutathione reductase (GSHR) declined in the wilted and light-stressed leaves, however, to a lesser extent than Apx activity. Heterologous expression of a bacterial catalase in the chloroplast protected photosynthesis and PRK and GSHR from oxidative inhibition. In a converse manner, Apx activity also disappeared in these transgenic lines. Shikanai et al. concluded from their study that the ascorbate-mediated antioxidative system is insufficient to protect the photosynthetic apparatus from photoinhibition under conditions of (severe) oxidative stress (Shikanai et al., 1998). The 2-CP-dependent water/water cycle may be an important alternative metabolic pathway to detoxify H2O2 under normal conditions as well as under oxidative stress.

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![Diagram](image-url)  
**Fig. 4.** Ascorbate- and 2-CP-dependent reduction of hydrogen peroxide as alternative metabolic pathways of the water/water cycle.
Antisense suppression of 2-CP activity causes oxidation of ascorbate pool

Circumstantial evidence for the role of 2-CP in H$_2$O$_2$ reduction and sparing of the ascorbate peroxidase system was obtained from Arabidopsis plants with decreased levels of 2-CP expression. The light stress sensitivity of the 2-CP-antisense plants was increased and their early seedling development disturbed (Baier and Dietz, 1998c, 1999b). However, the most striking observation was an increased oxidation state of the ascorbate pool in the transgenic lines with decreased 2-CP levels (Baier et al., 2000). Concomitantly, expression and activities of stromal and thylakoid bound Apx and monodehydroascorbate reductase (MDHAR) were stimulated. Apparently, insufficient amounts of 2-CP directly fed back to the ascorbate system of the leaves. A possible explanation for that observation is the direct involvement of the 2-CP in H$_2$O$_2$ reduction as an alternative to the water/water pathway. Reduced activities of the 2-CP pathway would shift the burden of H$_2$O$_2$ reduction to the Apx-dependent water/water cycle and oxidation state of the ascorbate pool would increase.

The role of 2-CP in alkyl hydroperoxide reduction

Peroxisiredoxins reduce a broad range of alkyl hydroperoxide substrates including short- and long-chain alkyl hydroperoxide, phospholipid peroxides and cholesterol peroxide (Nogoecke et al., 1997; Hillas et al., 2000). For the time being, it can be assumed that plant Prx also shows little discrimination between various alkyl hydroperoxide substrates and reduce diverse peroxides. Alkyl hydroperoxides are produced by spontaneous chemical reactions of unsaturated organic substrates with reactive radicals such as OH$^-$, or by enzymatic reaction of polyunsaturated lipids with lipoxygenases. Subsequently, alkyl hydroperoxides may initiate radical chain reactions and cause membrane destruction. Peroxides need to be eliminated to avoid severe damage of the cells. In addition to detoxification by non-enzymatic antioxidants, two types of enzymes exist in the chloroplasts which are suggested to catalyse the reduction of alkyl hydroperoxides (Baier and Dietz, 1999a, b): (i) The phospholipid hydroperoxide glutathione peroxidase (PHGPx) (Mullineaux et al., 1998) which reacts with peroxide substrates by concomitant oxidation of a cysteine residue. PHGPx is reactivated with reduced glutathione (Eshdat et al., 1997). (ii) The 2-CPs which have a similar substrate spectrum as PHGPxs and are regenerated by thioredoxin. Analysis of transgenic Arabidopsis with reduced 2-CP contents suggest that PHGPx cannot substitute for decreased 2-CP activity (Baier and Dietz, 1999b; Baier et al., 2000).

(i) Antisense suppression of 2-CP caused increased damage of chloroplast proteins and impaired photosynthesis. (ii) Increased oxidation of the ascorbate pool and up-regulation of Apx and MDAR transcript level and activity indicated a major disturbance of antioxidant metabolism in the mutant plants, despite the fact that (iii) the PHGPx-transcript level, which was at least 100-fold lower than the 2-CP transcript level, was unchanged in the antisense mutants. A distinct sub-organelar compartmentalization may provide the explanation for the lack of interchangeability of PHGPx and 2-CP in the chloroplast. The PHGPx has been reported to be a soluble enzyme located in the stroma (Mullineaux et al., 1998) whereas the 2-CP is preferentially attached to the thylakoid membrane.

Regulation of 2-CP gene expression

The expression of the 2-CP genes is under developmental and redox control. The steady-state mRNA amount decreased with tissue age in barley (Baier and Dietz, 1996), A. thaliana (Baier and Dietz, 1999b) and R. fluitans (Horling et al., 2001). Irrespective of the declining message abundance, the protein accumulated with tissue maturation and was unchanged during further ageing under normal growth conditions. However, the developmental responses of both, the 2-CP-mRNA and protein, still need to be tested under conditions of oxidative stress.

Redox-dependent changes in 2-CP gene expression were studied in the liverwort R. fluitans which was taken as a model plant due to the simple application protocol for effectors. The amount of 2-CP transcripts decreased rapidly and strongly upon application of ascorbate to the medium. A severe drop of transcript abundance was observed as early as 2 h after the start of treatment. 24 h after transfer of the liverwort to medium supplemented with 10 mM ascorbate, the 2-CP transcript was undetectable (Horling et al., 2001). The ascorbate-induced decline in 2-CP-mRNA was prevented after pretreating the thalli with the protein kinase inhibitor staurosporin. Apparently, ascorbate triggers a redox-signal transduction pathway which involves a protein phosphorylation step and suppresses 2-CP gene expression. Supplementing the medium with GSH caused a decrease in transcript amount at concentrations as low as 0.1 mM, but no further enhancement of the response at elevated GSH concentrations. In contrast to the liverwort, thios fed to barley leaves through the petiole decreased the 2-CP transcript level of barley leaves in a concentration-dependent manner (Baier and Dietz, 1997). Establishing oxidizing conditions, for example by the administration of paraquat, stimulated 2-CP expression only slightly. The small response to oxidizing agents is interesting since the bacterial homologue of the 2-CP AhpC was initially identified by its strong response upon exposure to butyl hydroperoxide and H$_2$O$_2$ (Morgan et al., 1985).
From all these studies it is concluded that the 2-CP is a constitutive enzyme of the chloroplast expressed at a high level under normal growth conditions. Although up-regulation of expression is not observed under stress, down-regulation is triggered under highly reducing conditions.

**Perspectives: 2-CP, signalling and future work**

Reactive oxygen species and peroxides are cell toxic on the one hand but they also provide valuable information on the state and metabolic performance of the cells on the other hand (Baier and Dietz, 1998a). The reactive oxygen species \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) have been proposed as components of signalling pathways (Noctor et al., 2000). Also alkylhydroperoxides are metabolic intermediates involved in the synthesis of signal molecules, for example, in the jasmonic acid pathway (Farmer et al., 1998). Hydroperoxides produced by lipoxygenase activity from linolenic or linoleic acid can either be cleaved by hydroperoxide lyase, are processed by allene oxide synthase or reduced by peroxygenase and reductases (Weichert et al., 1999). Prx with its broad substrate specificity may serve as reductase. The relative activity of the enzymes will determine the channelling of the substrate to the various pathways. Thus, the activity of peroxiredoxins in general, and chloroplast 2-CP in particular, will affect fatty acid signalling by compounds such as traumatin and jasmonic acid.

For animal cells, the relationship between the activity of peroxiredoxins and cellular signal transduction has been worked out in a series of publications. Overexpression of the peroxiredoxin Prx I suppressed the activation of the nuclear transcription factor NF-KB by externally applied tumour necrosis factor (Kang et al., 1998). The activation of the apoptosis programme via the p53-pathway was inhibited in cells over-expressing Prx V (Zhou et al., 2000). In another study, the expression level and the subunit composition of the 2-CP dimer correlated with the propensity of the cells to activate the NF-KB-pathway (Jin et al., 1997). The authors of these studies concluded that peroxiredoxins participate in regulating intracellular redox signals and in antioxidative defence. Thus, the central role of peroxiredoxins in peroxide metabolism has unequivocally been established in bacteria and heterotrophic eukaryotes (Flohé, 1998; Rhee et al., 1999). Similar functions in accentuating or suppressing signalling pathways can be hypothesized for plant Prx.

Despite the progress made over the last seven years in understanding the activity and principal functions of peroxiredoxins in plants, important questions need to be addressed in the future, particularly, concerning physiological roles and regulation. Some topics are listed below and show the perspectives for the next years.

(1) The predicted chloroplast localization of the 2-CP-Prx encoded on chromosome 5 will have to be demonstrated unequivocally, similar to the proven location of 2-CP-Prx encoded on chromosome 3 (Baier and Dietz, 1997). The subcellular localization needs to be established for the type II Prx and PrxQ.

(2) Subunit oligomerization has been shown for Prx from other organisms such as trypanosomatids, mammals and yeast and reversible membrane association for the Prx of erythrocytes (Schröder et al., 2000). Both mechanisms represent intriguing possibilities of redox-dependent regulation of Prx activity and should be investigated in plants.

(3) Although the reconstitution of the redox chain with plant Prx, *E. coli* or yeast thioredoxin and thioredoxin reductase was successful in coupling NADPH oxidation to peroxide reduction *in vitro* (Cheong et al., 1999; Verdoucq et al., 1999; Fig. 3), the authentic endogenous electron donors of all plant peroxiredoxins still need to be identified and analysed, as well as their substrate spectrum with respect to physiologically relevant alkylhydroperoxides and peroxinitrite.

(4) Up to now, the physiological context of Prx activity has only been worked out for transgenic *Arabidopsis* with reduced levels of 2-CP and knockout mutants of *Synechocystis* (Baier and Dietz, 1999; Klughammer et al., 1998; Yamamoto et al., 1999). Similar studies will have to be performed for the various types of Prx and should include knockout mutants in order to separate completely the Prx function from similar metabolic reactions in antioxidant defence and signalling.

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**Note added in proof**

The genome of *Arabidopsis thaliana* contains a tenth Prx gene denominated Prx II F (Acc. no. At3g06050) whose gene product is predicted to be targeted to the mitochondrion.

**References**


Lewis ML, MiKi K, Ueda T. 2000. FePer 1, a gene encoding an evolutionarily conserved 1-Cys peroxiredoxin in buckwheat (Fagopyrum esculentum Moench), is expressed in a seed-specific manner and induced during seed germination. Gene 248, 81–91.


