CHAPTER 2

EVOLUTION OF THE PEROXIREDOXINS

Taxonomy, homology and characterization

BERNARD KNOOPS, ELÉONORE LOUMAYE
AND VALÉRIE VAN DER EECKEN

Laboratory of Cell Biology, Institut des Sciences de la Vie, Université catholique de Louvain, Place Croix du Sud, 5, B-1348 Louvain-la-Neuve, Belgium

Abstract: Peroxiredoxins compose a superfamily of peroxidases ubiquitously found throughout evolution in prokaryotes, archaea and eukaryotes. These enzymes contain a conserved catalytic peroxidatic cysteine (Cp) in the N-terminal region of the protein. The residues surrounding Cp and the catalytic site appear also to be well conserved. Peroxiredoxins can be classified either into three subfamilies according to their catalytic mechanism or into five subfamilies according to sequence homology. Notably, the number of peroxiredoxin genes increased during evolution. In eukaryotes, the higher number of genes coding for peroxiredoxin family members is due to the existence of different isoforms targeted to different subcellular compartments but is probably due also to the acquisition of new functions. Indeed, it has been postulated that the antioxidant protective role of peroxiredoxins, which is particularly critical in prokaryotes, in yeasts and in parasitic eukaryotes, may have evolved to a modulatory role in hydrogen peroxide signaling in plants and animals

Keywords: Peroxidase, Antioxidant enzyme, Peroxide, Bacteria, Archaea, Eukaryotes

1. INTRODUCTION

The first characterized peroxiredoxin (Prx or, as imposed by International Nomenclature Committees, PRDX for human/bovine peroxiredoxins or Prdx for murine counterparts) has been identified in the yeast *Saccharomyces cerevisiae* (Kim *et al*., 1988). Subsequently, it appeared that this novel antioxidant enzyme, named at that time TSA for thiol-specific antioxidant, was a member of an emerging superfamily of proteins conserved throughout the evolution in all kingdoms of life (Prosperi *et al*., 1993; Chae *et al*., 1994a). Indeed, numerous members of the Prx superfamily have been later identified and characterized in prokaryotes, archaea and eukaryotes (see Table 1; Hofmann *et al*., 2002). It appeared afterwards that Prxs are ancestral thiol-dependent selenium- and heme-free peroxidases highly expressed

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Table 1. Classification of Prxs from different kingdoms of life. Prxs are classified according to their enzymatic mechanism (typical 2-Cys, atypical 2-Cys or 1-Cys) and sequence homology (geometric forms in the table but see also phylogenetic tree of Fig. 2). Circles (PrxI/PRDX1 subfamily), triangles (PrxV/PRDX5 subfamily), inversed triangles (BCP-PrxQ subfamily), squares (PrxVI/PRDX6 subfamily) and hexagons (Tpx subfamily) represent the different clusters based on sequence homology. The phylogenetic tree is shape-coded accordingly. *E. coli: Escherichia coli; A. pernix: Aeropyrum pernix; S. cerevisiae: Saccharomyces cerevisiae; P. falciparum: Plasmodium falciparum; A. thaliana: Arabidopsis thaliana; D. melanogaster: Drosophila melanogaster; H. sapiens: Homo sapiens*

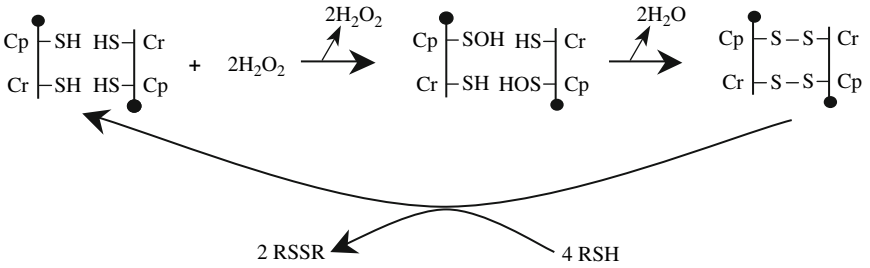
			Enzymatic mechanism		
			Typical 2-Cys	Atypical 2-Cys	1-Cys
Bacteria					
	E. coli	AhpC	O		
		Tpx		⬡	
		BCP			∇
Archaea					
	A. pernix	Prx	□		
Eukaryotes					
Yeasts		Tsa1p	O		
	S. cerevisiae	Tsa2p	O		
		Ahp1p	Δ		
		Prx1p			□
		Dot5p		∇	
Protozoa		TPx1	O		
	P. falciparum	TPx2	O		
		AOP			Δ
		1-Cys-Prx			□
Plants		2-Cys PrxA	O		
	A. thaliana	2-Cys PrxB	O		
		PrxIIB	Δ		
		PrxIIC	Δ		
		PrxIID	Δ		
		PrxIIE	Δ		
		PrxIIF	Δ		
		1-Cys Prx			□
		PrxQ		∇	
Animals		Prx4156	O		
	D. melanogaster	Prx4783	O		
		Prx5037	O		
		PrxV		Δ	
		Prx6005			□
		Prx2540			□
		PRDX1	O		
	H. sapiens	PRDX2	O		
		PRDX3	O		
		PRDX4	O		
		PRDX5		Δ	
		PRDX6			□

in virtually all living species including anaerobic organisms (Wood *et al*., 2003a; Rhee *et al*., 2005). In *Escherichia coli*, Prxs are among the ten most expressed proteins (Link *et al*., 1997) and in mammalian cells they represent 0.1 to 0.8% of soluble proteins (Seo *et al*., 2000). Moreover, it must be noted that Prxs are now very often detected as major spots in proteomic analyses using two-dimensional gel electrophoresis.

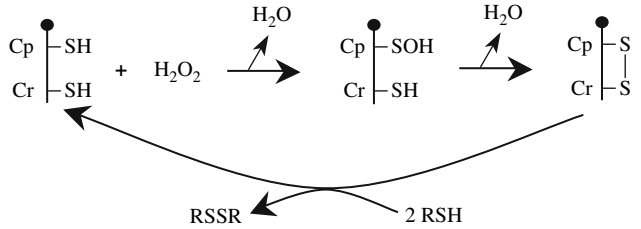
Interestingly, Prxs show no significant sequence homology with catalases, superoxide dismutases or other peroxidases such as selenocysteine-containing glutathione peroxidases, although they may be structurally close to them (see below and Chapter **3**). Prxs do not contain any redox cofactors such as heme, flavin or metal ions. The presence of a conserved and catalytically essential cysteine residue in the N-terminal domain of all Prxs, termed now the peroxidatic cysteine (Cp), was noted earlier (Chae *et al*., 1994b). Not only the residues surrounding the Cp but also the entire peroxidatic catalytic site appear to be well conserved among Prxs (Wood *et al*., 2003a and see Chapter **3** and **4**). The N-terminal Cp is the primary site of enzyme oxidation (Figure 1 and see Chapter **3** and **4**). Indeed, the Cp attacks the peroxide and is subsequently oxidized to a cysteine sulfenic acid (Cp-SOH) (Wood *et al*., 2003a and Chapter **4**). The high reactivity of the conserved Cp in Prxs with peroxides is due to its low pK_a in its catalytic site. Indeed, the Cp environment promotes ionization of the thiol group to the thiolate anion (Wood *et al*., 2003a and Chapter **4**). The reduction of the cysteine sulfenic acid is the second step of the peroxidase reaction and differs according to the type of Prx (Figure 1 and Chapter **4**) but probably also depends on the availability of the electron-donor substrate for certain Prxs. Based on the resolution mechanism and the existence or the lack of a resolving cysteine (Cr) localized to the C-terminal region of the enzyme, Prxs were divided into three subgroups referred to as typical 2-Cys, atypical 2-Cys and 1-Cys Prxs (Seo *et al*., 2000). Finally, at the end of the catalytic cycle, the enzyme is reduced by a thiol-containing reductant. Thus, the conservation and the success of Prxs throughout evolution may be due to their apparently simple catalytic mechanism that does not require heme, flavin or reactive heteroatoms. It could also be due to their ability to use, depending on the type of Prx, different electron donor substrates such as thioredoxins, tryparedoxin, glutathione or glutaredoxins for their reduction. However, the drawback of this simple mechanism would be their moderate catalytic efficiencies towards peroxides ($\sim 10^5\,M^{-1}s^{-1}$) compared to selenocysteine-containing glutathione peroxidases ($\sim 10^8\,M^{-1}s^{-1}$) or even heme-containing catalases ($\sim 10^6\,M^{-1}s^{-1}$).

Functionally, it has been proposed that certain Prxs (typical 2-Cys Prxs) have evolved from antioxidant protective enzymes in bacteria or in parasites to regulators of peroxide-mediated signaling cascades in organisms such as yeasts, plants and mammals (Wood *et al*., 2003, see also Chapter **12**). Indeed, many cell types are known to produce hydrogen peroxide in response to extracellular stimuli and the generated peroxide may affect the function of several proteins including transcription factors or protein kinases and phosphatases (Rhee *et al*., 2005). In mammals, typical 2-Cys Prxs have been shown to be more sensitive to inactivation than prokaryotic Prxs by hydrogen peroxide through overoxidation of the Cp into

(a) typical 2-Cys Prx



(b) atypical 2-Cys Prx



(c) 1-Cys Prx

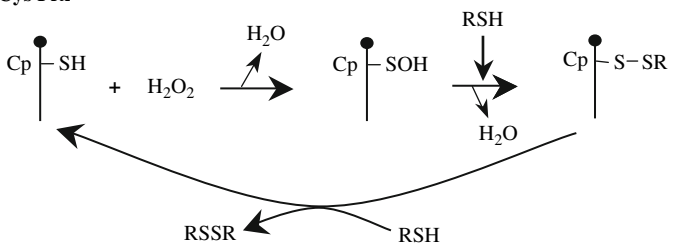


Figure 1. Peroxidase reaction mechanisms of (a) typical 2-Cys Prxs; (b) atypical 2-Cys Prxs; (c) 1-Cys Prxs (modified from Chae *et al*., 1994b; Kang *et al*., 1998; Seo *et al*., 2000). Hydrogen peroxide (H_2O_2) or organic hydroperoxides (ROOH) are reduced by the Cp, which is oxidized in sulfenic acid (-SOH). This later reacts immediately with a thiol group to form an intermolecular (typical 2-Cys Prxs) or an intramolecular (atypical 2-Cys Prxs) disulfide bond, which is subsequently reduced by thiol-containing electron donors (RSH) that are thioredoxins for mammalian typical and atypical 2-Cys Prxs and glutathione for mammalian 1-Cys Prx. Cp and Cr refer to peroxidatic and resolving cysteines respectively. Closed circles correspond to the amino-terminus of each protein

sulfinic (Cp-SO_2H) or sulfonic (Cp-SO_3H) acid forms. As such, Prxs would act as a dam against peroxides. The ratio of active to inactive enzymes would determine the signaling cascade and the oxidation of transcription factors or signaling effectors by peroxides (Wood *et al*., 2003b; Rhee *et al*., 2005). Interestingly, this mechanism is probably finely regulated as overoxidation of the Cp into sulfinic acid may be reduced by sulfiredoxin in yeasts, plants and mammals (Biteau *et al*., 2003; Jeong *et al*., 2006; Liu *et al*., 2006), and also by sestrins (Budanov *et al*., 2004). Moreover, phosphorylation of mammalian typical 2-Cys Prxs by cyclin-dependent kinases was also demonstrated to modulate their peroxidase activity, showing that other post-translational mechanisms in addition to overoxidation may regulate peroxide reduction by mammalian Prxs (Chang *et al*., 2002).

As a matter of fact, the number of *Prx* genes increased throughout evolution depending also on the phyla and species. For example, there are three genes coding for Prxs in the bacterium *Escherichia coli*, five in the yeast *Saccharomyces cervisiae*, but six in *Homo sapiens* and even nine in the plant *Arbidopsis thaliana* (Table 1). In eukaryotes, the higher number of genes coding for family members is partly explained by the compartmentalization and the existence of mitochondrial, nuclear, peroxisomal and chloroplast isoforms. However, Prx functions in metazoa could be more complex than thought previously. Indeed, knocked-out mice for *Prdx1* (Neumann *et al*., 2003), *Prdx2* (Lee *et al*., 2003) and *Prdx6* (Wang *et al*., 2003) have revealed that mutants are more sensitive to certain oxidative stresses.

2. CLASSIFICATION OF PEROXIREDOXINS

As mentioned above, all Prxs exhibit a conserved Cp residue in their N-terminal region that attacks peroxides but also peroxynitrite at least for some members of the family (Bryk *et al*., 2000; Dubuisson *et al*., 2004, Jaeger *et al*., 2004). Originally in mammals, Prxs were divided into two subfamilies (or subgroups), the 1-Cys and the 2-Cys Prxs, based on the number of cysteine residues directly involved in the enzymatic mechanism and the conservation of surrounding residues around catalytic cysteines (Rhee *et al*., 2005). Later, a third subfamily emerged and now mammalian Prxs are divided into three subfamilies referred to as typical 2-Cys, atypical 2-Cys and 1-Cys Prxs (Rhee *et al*., 2005; Figure 1 and Table 1). This classification is based on catalytic mechanisms and has been extended to all Prxs from all biological kingdoms. In the typical 2-Cys subfamily, the resolving cysteine, corresponding to the second redox-active cysteine, is localized to the C-terminal region of the enzyme. During the peroxidase reaction, the cysteine sulfenic acid from one subunit is attacked by the resolving cysteine of another subunit resulting in the formation of a stable intersubunit disulfide bond which can then be reduced by thioredoxin in mammalian Prxs. In atypical 2-Cys Prxs, the C-terminal resolving cysteine is located within the same polypeptide chain and the reaction with the peroxidatic cysteine results in the formation of an intramolecular disulfide bond. The mammalian atypical 2-Cys Prx uses thioredoxin to reduce the disulfide bond. Finally, in 1-Cys Prxs, only the N-terminal peroxidatic cysteine is present and the resolving cysteine is

missing. Nevertheless, in mammalian 1-Cys Prx, the peroxidatic cysteine sulfenic acid formed upon reaction with peroxides is reduced by glutathione (Manevich *et al*., 2004). Thus, it appears that this mechanistic classification may be extended to all Prxs only when the enzymatic mechanism is clearly characterized for novel Prx members first identified on homology criteria by alignment of their amino acid sequences. Indeed, sequence alignment of Prxs from prokaryotes, archaea and eukaryotes, and construction of phylogenetic trees (Verdoucq *et al*., 1999; Hofmann *et al*., 2002; Figure 2) revealed clusters or subfamilies that may include

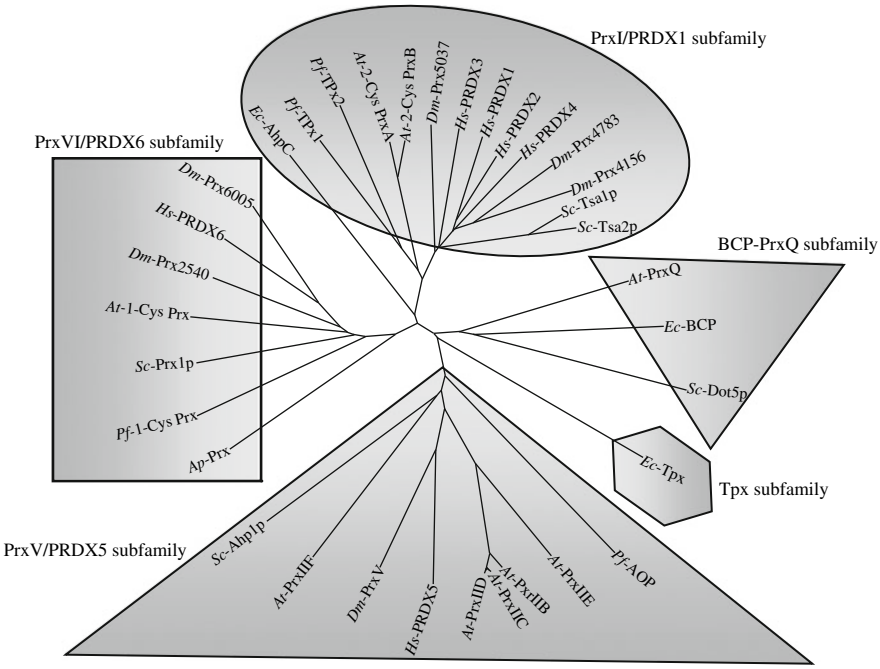


Figure 2. Phylogenetic tree of the peroxiredoxin family. Protein alignment was performed with ClustalX 1.81 program (Higgins and Sharp, 1988). Tree drawing was achieved with the neighbor-joining method (Saitou and Nei, 1987). The unrooted tree was drawn with Treeview, and has been divided into five clusters (subfamilies) represented by the different shapes. *Ec: Escherichia coli; Ap: Aeropyrum pernix; Sc: Saccharomyces cerevisiae; Pf: Plasmodium falciparum; At: Arabidopsis thaliana; Dm: Drosophila melanogaster; Hs: Homo sapiens*. GenBank™ accession numbers of the peptide sequences are as follows: *Ec*-AhpC (NP_415138); *Ec*-Tpx (NP_415840); *Ec*-BCP (NP_416975); *Ap*-Prx (NP_148509); *Sc*-Tsa1p (NP_013684); *Sc*-Tsa2p (NP_010741); *Sc*-Prx1p (NP_009489); *Sc*-Dot5p (NP_012255); *Sc*-Ahp1p (NP_013210); *Pf*-TPx1 (AAF67110); *Pf*-TPx2 (AAK20024); *Pf*-1-Cys-Prx (AAG14353); *Pf*-AOP (1XIYA); At-PrxIIB (NP_176773); *At*-PrxIIC (NP_176772); *At*-PrxIID (NP_564763); *At*-PrxIIE (NP_190864); *At*-PrxIIF (NP_566268); *At*-2-Cys PrxA (NP_187769); *At*-2-Cys PrxB (NP_568166); *At*-1-Cys Prx (NP_175247); *At*-PrxQ (NP_189235); *Dm*-Prx4156 (NM_080263); *Dm*-Prx4783 (NM_167359); *Dm*-Prx5037 (NM_079663); *Dm*-PrxV (NM_176513); *Dm*-Prx6005 (NM_078739); *Dm*-Prx2540 (NM_165769); *Hs*-PRDX1 (NM_002574); *Hs*-PRDX2 (NM_005809); *Hs*-PRDX3 (NM_006793); *Hs*-PRDX4 (NM_006406); *Hs*-PRDX5 (NM_012094); *Hs*-PRDX6 (NM_004905)

Prxs mechanistically classified as 1-Cys or 2-Cys Prxs (Figure 2 and Table 1). For example, human PRDX5, which is the prototype of an atypical 2-Cys, belongs to the phylogenetic cluster of *S. cerevisiae* Ahp1p Prx, although this one is mechanistically classified among typical 2-Cys Prxs (Park *et al*., 2000). Interestingly, biochemical characterization of a novel Prx of *Toxoplasma gondii* has also revealed that a Prx initially classified among 1-Cys Prxs may present mechanistic features of a typical 2-Cys Prx (Deponte and Becker, 2005). As illustrated in Figure 2 and based on sequence alignment of Prxs from all biological kingdoms in agreement with Hofmann *et al*. (2002), five major clusters can be distinguished among Prxs.

More recently, several authors have proposed a third type of Prx classification based on primary sequence characteristics and structural data (Copley *et al*., 2004; Sarma *et al*., 2005; Mizohata *et al*., 2005). Notably, according to these classifications, Prxs could be separated into four (Copley *et al*., 2004) or even up to seven subfamilies (Mizohata *et al*., 2005). However, more structural data of Prxs from various species are needed to validate these classifications. It appears also from these studies that in remote species, especially in archaea, Prxs may evolve by elongating their C-terminal domain containing additional catalytic cysteines (Mizohata *et al*., 2005).

3. PEROXIREDOXINS IN PROKARYOTES

Historically, before the eukaryotic peroxiredoxin TSA from *Saccharomyces cerevisiae* was characterized (Kim *et al*., 1988), another antioxidant enzyme from the prokaryotes *Salmonella typhimurium* and *Escherichia coli* had been identified as a peroxidase of the alkyl hydroperoxide reductase system and was named AhpC for alkyl hydroperoxide reductase subunit C (Jacobson *et al*., 1989). This alkyl hydroperoxide reductase system was shown to be composed of the 21-kDa AhpC and the 57-kDa flavoprotein AhpF. Moreover, mechanistically, the alkyl hydroperoxide reductase system involved peroxide reduction by AhpC and subsequent reduction of AhpC for regeneration by AhpF, coupled to NAD(P)H oxidation (Jacobson *et al*., 1989). Interestingly, homology between bacterial AhpC and *S. cerevisiae* TSA became clear (Chae *et al*., 1994). Subsequently, two additional Prxs, the thiol peroxidase (Tpx also known as p20 and scavengase) and the thioredoxin-dependent bacterioferritin-comigratory protein (BCP), were identified and characterized in bacteria including *E. coli* (Cha *et al*., 1995; Jeong *et al*., 2000). Contrary to AhpC, Tpx and BCP use reducing equivalents from thioredoxin to reduce peroxides. In addition, it must be noted that novel hybrid Prx proteins with a fused glutaredoxin domain were also found recently in pathogenic and anaerobic bacteria (Vergauwen *et al*., 2001; Kim *et al*., 2003; Pauwels *et al*., 2003). Functionally, prokaryotic Prxs appear to play an important role in antioxidant protection in non-pathogenic species but also in pathogenic species to defend against peroxides and peroxynitrite produced by inflammatory cells (Seaver and Imlay, 2001).

4. PEROXIREDOXINS IN ARCHAEA

Genome sequencing projects as well as biochemical studies have identified Prxs in aerobic and anaerobic archaea such as *Aeropyrum pernix* (Jeon and Ishikawa, 2003; Mizohata *et al*., 2005; see also Table 1 and Figure 2), *Sulfolobus solfataricus* (Limauro *et al*., 2006) and *Pyrococcus horikoshii* (Kawakami *et al*., 2004). Interestingly, as mentioned previously, data suggest also that in *A. pernix*, the new Prx could define a novel structural subfamily of Prx (Mizohata *et al*., 2005). Indeed, this archaeal Prx presents a primary sequence homologous to mammalian PRDX6 classified in 1-Cys Prxs (see Table 1) although with a longer C-terminal extension. However, mechanistically, *A. pernix* Prx possesses at least two catalytically active cysteines that classify it among classical 2-Cys Prxs (Mizohata *et al*., 2005). Also, the expression of archaeal Prx is induced by exogenous exposure to hydrogen peroxide in *A. pernix* and in *S. solfataricus* and by exogenous oxygen in anaerobic *P. horikoshii*, suggesting that archaeal Prx may *in vivo* act indeed as protective antioxidant enzyme (Jeon and Ishikawa, 2003; Kawakami *et al*., 2004; Limauro *et al*., 2006).

5. PEROXIREDOXINS IN EUKARYOTES

In eukaryotes, the number of genes coding for Prxs increased compared to the number of genes identified in prokaryotes and archaea (Hofmann *et al*., 2002; see Table 1). The higher number of Prxs in eukaryotic species may be explained both by the subcellular compartmentalization but also by the acquisition of new functions like that of a modulator of hydrogen peroxide signaling. In eukaryotic cells, Prxs are located in the cytosol, in mitochondria, in chloroplasts, in peroxisomes, and in some cases they are secreted (Hofmann *et al*., 2002; Wood *et al*., 2003; Leyens *et al*., 2003).

5.1. Yeast (*saccharomyces cerevisiae*)

Referring to the introduction, TSA from *S. cerevisiae* was the first Prx to be characterized (Kim *et al*., 1988). It appeared later that four additional Prxs are encoded by distinct *S. cerevisiae* genes (Park *et al*., 2000). Interestingly, the characterization of the five Prxs showed that depending on the isoform, they may be localized to the cytoplasm, the mitochondria, the peroxisomes or the nucleus (Park *et al*., 2000). *S. cerevisiae* Prxs are reduced during the catalytic cycle by electrons provided by thioredoxins (Park *et al*., 2000). Functionally, *S. cerevisiae* Prx-deficient mutants are viable but more sensitive to various oxidants and Prx-null yeast cells for the five Prxs have been reported to show an increased rate of spontaneous nuclear DNA mutations (Wong *et al*., 2004). Interestingly, a chaperone function has recently been reported for yeast for Prxs (Jang *et al*., 2004).

5.2. Protozoa

In protozoa, Prxs have been extensively studied in *Plasmodium falciparum*, the causative agent of malaria, but also in the pathogens of the genera *Trypanosoma* and

Leishmania among others (Hofmann *et al*., 2002). These parasites are challenged by reactive oxygen and nitrogen species during their life stages in humans and consequently their redox systems, including Prxs, have been thought to be essential for their pathogenicity. Interestingly, in *P. falciparum*, catalase and selenocysteine-containing glutathione peroxidases are lacking (Rahfls *et al*., 2002). Therefore, Prxs appear as a major defense line against oxidative and nitrosative attacks in these organisms, and also as potential targets for therapeutic strategies (Hofmann *et al*., 2002; Sarma *et al*., 2005). In *P. falciparum*, four Prxs have been characterized and classified mechanistically among typical 2-Cys and 1-Cys Prxs (Sarma *et al*., 2005; see Table 1). Moreover, among the four characterized *P. falciparum* Prxs, one is mitochondrial and three appear to be cytosolic (Sarma *et al*., 2005; Yano *et al*., 2005).

5.3. Plants

In *A. thaliana*, the first higher plant whose genome has been completely sequenced, phylogenetic and biochemical analyses have confirmed the existence of nine expressed Prxs (Rouhier and Jacquot, 2005; Dietz *et al*., 2006). More recently, the analysis of the genome of *Oryza sativa* (rice) has also shown the presence of nine Prxs demonstrating that plants possess more members of the Prx family compared to prokaryotes, archaea, yeasts, protozoa or animals (Dietz *et al*., 2006; see below and Chapter 13). In higher plants, it appeared that Prxs evolved into four distinct classes (Rouhier and Jacquot, 2005; Dietz *et al*., 2006) corresponding to four subfamilies, as illustrated in Table 1 and Figure 2. These Prxs include typical and atypical 2-Cys Prxs and one 1-Cys Prx. Interestingly, in *A. thaliana*, four Prxs are localized to chloroplasts, three to the cytoplasm, one to mitochondria and one to the nucleus/cytoplasm. Thus, *A. thaliana* Prxs are localized to different subcellular compartments where they may act either as protective antioxidant enzymes, as modulator of peroxide- or peroxynitrite-mediated signal transduction or as redox sensors (Dietz *et al*., 2006; Rouhier and Jacquot, 2005).

5.4. Animals

In animals and especially in mammals, Prxs have been known under very different names (Wood *et al*., 2003a; Leyens *et al*., 2003; see also Chapter 1) although their homology with yeast and bacterial Prxs was noted more than a decade ago (Prosperi *et al*., 1993; Chae *et al*., 1994). Functionally, the role of animal Prxs as protective antioxidant enzymes has been questioned in view of the enzymatic efficiency with peroxides of selenocysteine-containing glutathione peroxidases and catalase. These latter are expressed in the same subcellular compartments of animal cells (Hofmann *et al*., 2002). Indeed, at least some animal Prxs may be acting more specifically as modulators of hydrogen peroxide-mediated signal transduction (Wood *et al*., 2003; Rhee *et al*., 2005).

5.4.1. Invertebrates

Relatively few data exist on invertebrate Prxs except for insects and certain parasitic metazoa such as nematodes.

5.4.1.1. Nematodes In parasitic nematodes, Prxs have been studied because of their possible implication in the protection of the parasites against oxidative attacks by the host phagocytes (Henkle-Dührsen and Kampkötter, 2001). Interestingly, parasitic nematodes are able to reduce peroxides but catalase or selenocysteine-containing glutathione peroxidases are expressed at low levels in contrast to high levels of Prxs, suggesting that Prxs could be essential antioxidant enzymes in these animals (Chandrashekar *et al*., 2000). 2-Cys and 1-Cys Prxs have been identified in nematodes such as *Onchocerca volvulus, Onchocerca ochengi, Dirofilaria immitis* or *Brugia malayi* but more biochemical and structural characterizations are needed (Henkle-Dührsen and Kampkötter, 2001).

5.4.1.2. Insects In *Drosophila melanogaster*, six distinct Prxs encoded by six distinct genes have been identified and characterized (Radyuk *et al*., 2001; Radyuk *et al*., 2003; Peterson and Luckhart, 2006). Moreover, their orthologs have been found also in the genome of *Anopheles gambiae* by *in silico* homology search in databases (Peterson and Luckhart, 2006). *D. melanogaster* Prx members are either classified among typical and atypical 2-Cys Prxs as well as 1-Cys Prxs (Table 1 and Figure 2). Three *D. melanogaster* Prxs are localized to the cytosol, two are targeted to the mitochondria and one was found to be secreted (Radyuk *et al*., 2001; Peterson and Luckhart, 2006). Moreover, overexpression of insect Prxs conferred increased resistance to toxicity induced by hydrogen peroxide, parquat or peroxynitrite (Radyuk *et al*., 2001; Peterson and Luckhart, 2006).

5.4.2. Vertebrates

In vertebrates, orthologs of mammalian Prxs have been identified in databases of fish, amphibian and bird species but so far, except of course for mammalian Prxs, there are few biochemical and functional studies available in the literature.

5.4.2.1. Mammals Mammalian Prxs have been extensively studied (for review see Hofmann *et al*., 2002; Wood *et al*., 2003a; Leyens *et al*., 2003; Rhee *et al*., 2005). Six distinct genes encode six different Prxs in man, rat, mouse and cattle (Leyens *et al*., 2003; Rhee *et al*., 2005). Mammalian Prxs have been classified mechanistically into three subfamilies. Indeed, in mammals, PRDX1 to PRDX4 are classical 2-Cys Prxs, PRDX5 is an atypical 2-Cys Prx and PRDX6 is a 1-Cys Prx (Seo *et al*., 2000). Prxs are expressed constitutively in virtually all mammalian tissues although at different levels of expression (Seo *et al*., 2000; Leyens *et al*., 2003). PRDX1, PRDX2 and PRDX6 are cytosolic enzymes (Rhee *et al*., 2005). PRDX3 is a mitochondrial Prx addressed to this organelle by an N-terminal mitochondrial presequence and PRDX4 exhibits an N-terminal signal sequence for its secretion

(Rhee *et al*., 2005). Finally, PRDX5 presents a more complex subcellular distribution, as it has an N-terminal mitochondrial presequence, a C-terminal peroxisomal targeting sequence and it has been localized also to the cytosol and the nucleus (Knoops *et al*., 1999; Banmeyer *et al*., 2004; Rhee *et al*., 2005). Human Prxs reduce peroxides by the use of reducing equivalents derived from cytosolic thioredoxin for PRDX1, PRDX2 and cytosolic PRDX5, from mitochondrial thioredoxin for PRDX3 and mitochondrial PRDX5 and finally from glutathione for PRDX6 (Manevich *et al*., 2004; Rhee *et al*., 2005). As mentioned before, it has been shown that the activity of certain mammalian Prxs can be modulated by posttranslational modification such as phosphorylation of a threonine residue (Chang *et al*., 2002) or reversible overoxidation of the Cp (Chang *et al*., 2004), suggesting that in mammals, Prxs may serve as components of hydrogen peroxide-mediated signal transduction (Wood *et al*., 2003; Rhee *et al*., 2005; see Chapter **15**).

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