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Peroxiredoxins and Neurodegeneration

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Abstract: Peroxiredoxins (Prxs) are a family of novel antioxidant proteins that are found in a variety of species and participate in a number of vital biological processes such as proliferation, differentiation, response to oxidative stress and intracellular signaling. It has been proposed that they might participate in these cellular processes by playing a role in eliminating or regulating the intracellular concentration of peroxides produced during metabolism as well as in the signaling cascades of growth factors and cytokines. Mammalian cells express six isoforms of Prx (Prx I to VI), which are classified into three subgroups (typical 2-Cys, atypical 2-Cys and 1-Cys) based on the number and position of cysteine (Cys) residues that participate in catalysis and on amino acid sequences and the immunological reactivity. Members of the typical 2-Cys subgroup include Prx I through Prx IV and contain an additional conserved cysteine in the carboxyl-terminal region, whereas Prx V and Prx VI, members of the atypical 2-Cys and 1-Cys subgroups, respectively, do not contain this second conserved Cys. On the other hand, Prxs activity can be regulated by phosphorylation and proteolysis processes in addition to overoxidation. Taken together, this study suggest that the generation of the oxidative stress which caused neurodegeneration may couple with produced Prxs and the reverse is true. However, this argument is still unclear on account of the difficulties of the direct observation of the reactive oxygen species due to their biological lifetime is short. Thus, experiments will be required to solve these problems and to comprehend the actual role of Prxs in neurodegeneration.

Key words: Peroxiredoxins, neurodegeneration

Introduction

Oxidative stress is caused as the result of a variety of physiological and pathophysiological conditions (Matsumoto *et al*., 1999). Oxidative modifications of biological molecules such as proteins, lipids and nucleic acids are potentially harmful to living organisms and may contribute to aging (Stadtman, 1992). Therefore, thiol groups are highly sensitive to oxidation, proteins in which thiol groups play a key role represent an important target of oxidative stress. Certain antioxidative enzymes such as the peroxiredoxins (Prxs), superoxide dismutases (SODs) and glutathione peroxidases (GPxs) and antioxidant molecules such as glutathione and thioredoxin serve to protect essential molecules against oxidative modification (Matsumoto *et al*., 1999). Prxs are distinct from other peroxidases in that they have no cofactors, such as metals or prosthetic groups (Hirotsu *et al*., 1999). Prxs generally have two conserved cysteines (Cys-52 and Cys-173 of Heme-binding protein 23 kDa (HBP23)) at the N- and C-terminal regions (Chae *et al*., 1994a) and their antioxidant effects are coupled with the physiological electron donor activity of the thioredoxin system (Chae *et al*., 1994a; Kang *et al*. 1998a). Moreover, Prxs are a family of novel antioxidant proteins that are found in a variety of species and which has been shown to participate in a number of vital biological processes

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(Chae *et al*., 1993; Ishii *et al*., 1993; Shau *et al*., 1993; Chae *et al*., 1994a; Watabe *et al*., 1994) such as antioxidant defense, redox signaling, apoptosis control and kinase modulation (Isermann *et al*., 2004).

Numerous studies have demonstrated that Prxs can protect cells against a variety of nitrosative stresses (Peterson and Luckhart, 2005). In addition, brain cells also display higher sensitivity to oxidative stress than cells of other tissues due to the abundance of iron and oxidizable lipids (Halliwell, 1992; Beal, 1996; Castagne *et al*., 1999). Reactive Oxygen Species (ROS) toxicity is a common underlying feature of neuropathological states, including Pick's Disease (PD), Alzheimer's Disease (AD) and Down Syndrome (DS) and proteins of the Prx gene and other antioxidant gene families have been documented to exert antioxidant and cytoprotective effects (Butterfield *et al*., 1999). Characterizing the patterns of Prx protein expression in normal and diseased brain could thus yield significant insight into the neurochemical mechanisms of defense and pathology. Furthermore, Prxs provide a valuable tool for elucidating the role of antioxidant pathways in the pathological processes. Therefore, it is imperative to describe the backgrounds and recent findings which provide specific suggestions for explore the relation between peroxiredoxins and neurodegeneration:

Peroxiredoxins

The peroxiredoxins (Prxs) are a recently discovered family of antioxidant peroxidases that can reduce hydrogen peroxide and alkyl hydroperoxides to water and the corresponding alcohols, respectively and can protect cells from widely divergent organisms against a variety of nitrosative stress challenges (Bryk *et al*., 2000; Wong *et al*., 2002; Dubuisson *et al*., 2004; Trujillo *et al*., 2004). Prxs have also been implicated in oxidative signaling mechanisms regulating apoptosis (Kim *et al*., 2000), cell differentiation (Rabilloud *et al*., 1995; Jin *et al*., 1997; Butterfield *et al*., 1999). The peroxiredoxins define an emerging family of peroxidases able to reduce hydrogen peroxide and alkyl hydroperoxides with the use of reducing equivalents derived from thiol-containing donor molecules such as thioredoxin, glutathione and trypanothione (Declercq *et al*., 2001). Prxs are a family of peroxidases widely distributed in eukaryotes and prokaryotes (Plaisant *et al*., 2003). In higher plants they are involved in balancing hydroperoxide production during photosynthesis, while in higher animals peroxiredoxins appear to be involved in the redox-regulation of cellular signaling and differentiation, displaying in part opposite effects (Hofmann *et al*., 2002). Indeed, these enzymes are truly ubiquitous having been identified in yeast, plant and animal cells, including both protozoan and helminth parasites and most, if not all, eubacteria and archaea. Although located primarily in the cytosol, Prxs are also found within mitochondria, chloroplasts and peroxisomes, associated with nuclei and membranes and, in at least one case, exported (Jin and Jeang, 2000; Hofmann *et al*., 2002). Prxs are produced at high levels in cells; in addition, proteins in *Escherichia coli* (Link *et al*., 1997), the second or third most abundant protein in erythrocytes (Moore *et al*., 1991) and compose 0.1-0.8% of the soluble protein in other mammalian cells (Chae *et al*., 1999). Many organisms produce more than one isoform of Prx, including at least six Prxs identified in mammalian cells (Prx I-Prx VI). Furthermore, the Prxs are an emerging family of multifunctional enzymes that exhibit peroxidase activity *in vitro* and *in vivo* participate in a range of cellular processes known to be sensitive to reactive oxygen species (Schroder *et al*., 2000). An immediate physiological electron donor for the peroxidase catalysis for five Prx proteins (Prx I-V) has been identified as thioredoxin (Trx), but that for Prx VI (1-Cys Prx) is still unclear (Lee *et al*., 2001). They appear to be fairly promiscuous with respect to the hydroperoxide substrate; the specificities for the donor substrate vary considerably between the subfamilies, comprising GSH, thioredoxin and tryparedoxin (Hofmann *et al*., 2002).

Crystal structures of Prx I, II, V and VI have revealed that Prxs are novel members of the double-stranded RNA production and conditions for RNA interference (RNAi) (Peterson and Luckhart, 2005). 2-Cys (2-cysteine) peroxiredoxins are a large and diverse family of peroxidases which, in addition to their antioxidant functions, regulate cell signaling pathways, apoptosis and differentiation (Wood *et al*., 2002). These enzymes are obligate homodimers (alpha(2)), utilizing a unique intermolecular redox-active disulfide center for the reduction of peroxides and are known to

form two oligomeric states: individual alpha(2) dimers or doughnut-shaped (alpha(2))(5) decamers. Wood *et al*. (2002) suggest that the enzymatic and signaling activities of all 2-Cys Prxs are regulated by a redox-sensitive dimer to decamer transition. Plaisant *et al*. (2003) found that Prx V is a recently discovered mammalian member of this family of antioxidant enzymes that is able to reduce hydrogen peroxide and alkyl hydroperoxides (Plaisant *et al*., 2003). In doing so, a reactive cysteine in the peroxiredoxin active site is weakly oxidized (disulfide or sulfenic acid) by the destroyed peroxides and the cellular thiols (e.g., thioredoxin) are used to regenerate the peroxiredoxins to their active state (Rabilloud *et al*., 2002).

Also, Peroxiredoxins constitute a novel family of antioxidant proteins, which specifically prevent enzymes from metal-catalyzed oxidation (Chuchalin *et al*., 2003). Prx family members in the presence of some thiols can specifically prevent biopolymers (proteins, nucleic acids, lipids) from damage by reactive oxygen species (Chae *et al*., 1994b). Recently, a novel family of proteins which function as antioxidants, in the protection of thiol groups in proteins, has been reported (Chae and Rhee, 1994). They also exhibit peroxidase activity in a thioredoxin (Trx)-dependent manner, they are referred to as Prxs and constitute a system which is similar to the glutathione/glutathione-peroxidase system (Chae *et al*., 1994b). Peroxiredoxin exhibits thioredoxin-dependent peroxidase activity and constitutes a family of proteins (Matsumoto *et al*., 1999). Thioredoxin peroxidase (TPx), one of the Prx gene superfamily, was first reported from *Saccharo-myces cerevisiae* (Kim *et al*., 1988 and 1989). TPx functions as an antioxidant to remove the reactive oxygen species (ROS) O_2^- and H_2O_2 (Docampo, 1995) derived from normal cellular metabolism (Gutteridge and Halliwell, 1989) using thioredoxin as the electron donor (Henkle-Duhrsen and Kampkotter, 2001). High concentrations of ROS can have serious deleterious effects on membrane lipids, nucleic acids and proteins.

Prxs exert their protective antioxidant role in cells through their peroxidase activity (Wood *et al*., 2003):

$$ROOH + 2e^{\sim} \longrightarrow ROH + H_2O$$

$$H_2O_2 + Prx \xrightarrow{Thioredoxin} water$$

whereby hydrogen peroxide, peroxynitrite and a wide range of organic hydroperoxides (ROOH) are reduced and detoxified (Jacobson *et al*., 1989; Poole and Ellis, 1996; Peshenko and Shichi, 2001; Hofmann *et al*., 2002).

Mammalian Prxs and Their Reaction Mechanisms

The Prx antioxidant protein family is found in a wide variety of species and plays important roles in protecting cells against oxidants and in regulating signaling by hydrogen peroxide (Yanagawa *et al*., 2005). Also, the role of Prx in protection from hydroxyl radicals (Lim *et al*., 1993), thiyl radicals and oxidized thiyl radical anions (Yim *et al*., 1994) in the *in vitro* system is due at least in part to its peroxidase activity (Netto *et al*., 1996). The peroxiredoxin protein is expressed widely in animal tissues and serves an antioxidant function associated with removal of cellular peroxides (Sarafian *et al*., 1999). At least six Prx enzymes are present in mammals (Prx I-VI) (Rhee *et al*., 2001). However, Chae *et al*. (1999) recorded that twelve mammalian Prx members have been previously identified in association with various cellular functions apparently unrelated to peroxidase activity. Also, Prxs are a recently described superfamily of nonseleno-peroxidases that catalyze the reduction of a broad spectrum of peroxides (Wang *et al*., 2004). These Prx iso-forms are found in the cytosol, mitochondria, peroxisomes and plasma, all of which are potential sites of ROS production (Kang *et al*., 1998b; Matsumoto *et al*., 1999; Mizusawa *et al*., 2000; Oberley *et al*., 2001; Fujii and Ikeda, 2002).

The mammalian Prxs family contain six different isoforms that are divided into three subtypes according; 1) The number of cysteine residues that are directly involved in catalysis and peroxidase reactions: 2-Cys (Prx I-IV), atypical 2-cys (Prx V) and 1-Cys (Prx VI) (Wood *et al*., 2003); 2) The amino acid sequences (Jin *et al*., 1997); and 3.) The immunological reactivity (Jin *et al*., 1997; Kang *et al*., 1998a). Some Prxs possess reductase activity for hydrogen peroxide and alkyl

hydroperoxides depend on; 1) Their ability to reduce these substances to H_2O_2 or alcohols, respectively (Bruchhaus *et al*., 1997) 2) Their involvement in protection against phospholipid peroxidation (Manevich *et al*., 2002). Moreover, Prxs act as peroxynitrite reductases and participate in the detoxification of reactive nitrogen species (Bryk *et al*., 2000). The Prx isoforms share a common reactive cysteine residue in their N-terminal region, which is oxidized to either cysteine sulfinic acid or disulfide, both of which are then readily reduced back to the sulfhydryl state by various cellular reductants (Rhee *et al*., 2005b). Wood *et al*. (2003) reported that the peroxidase reaction is composed of two steps centered around a redox-active cysteine called the peroxidatic cysteine. All Prxs to date conserve an active-site Arg, which would lower the pKa of the peroxidatic cysteine somewhat by stabilizing its thiolate form. The second step of the peroxidase reaction, the resolution of the cysteine sulfenic acid, distinguishes the three Prx classes (Fig. 1). Thus, these Prxs use redox-active cysteines to reduce peroxides (Chae *et al*., 1994a).

Mammalian cells express six Prx isoforms (Prx I-VI), which can be divided into three subgroups according the number and position of the active Cys-Prxs residues as a following:

e.g.,Typical 2-Cys Prxs	Atypical 2-Cys Prxs	1-Cys Prxs
They are domain-swapped homodimers in which the C-terminus of one subunit reaches across the dimer interface to interact with the other subunit (Rhee *et al*., 2005a). Also, the 2-Cys Prx enzymes are obligate homodimers (Chae *et al*., 1994a, b; Hirotsu *et al*., 1999; Schroder *et al*., 2000).	They are monomeric enzymes (Rhee *et al*., 2005a).	They are domain-swapped homo dimers in which the C-terminus of one subunit reaches across the dimer interface to interact with the other subunit (Rhee *et al*., 2005a).
They contain both the N- and C-terminal -conserved Cys residues and require both of them for catalytic function (Seo *et al*., 2000; Rhee *et al*., 2001). Two identical active sites were found (Schroder *et al*., 2000).	They contain only the N-terminal Cys but require one additional, non-conserved Cys residue for catalytic activity (Seo *et al*., 2000; Rhee *et al*., 2001)	They contain only the N-terminal Cys and require only the conserved one for catalytic function (Seo *et al*., 2000 and Rhee *et al*., 2001).
Four (Prx I-IV) of the six mammalian Prxs belong to this subgroup (Hirotsu *et al*., 1999; Alphey *et al*., 2000; Schroder *et al*., 2000; Wood *et al*., 2002)	One (Prx V) belongs to this subgroup (Gommel *et al*., 1997; Rhee *et al*., 2005a).	One (Prx VI) belongs to this sub-group (Ishii *et al*., 1993; Rhee *et al*., 2005a). It reduces a broad spectrum of hydroperoxides (Pak *et al*., 2005).
They are the largest class of Prxs and are identified by the conservation of their two redox-active cysteines, the peroxidatic cysteine (generally near residue 50) and the resolving cysteine (near residue 170) (Hofmann *et al*., 2002). It is a novel cellular peroxidase that reduces peroxides in the presence of thioredoxin, thioredoxin reductase and nicotinamide adenine dinucleotide phosphate (NADPH) and that functions in H_2O_2 -mediated signal transduction (Kim *et al*., 2005).	They are the same mechanism as typical 2-Cys Prxs but are functionally monomeric (Seo *et al*., 2000; Declercq *et al*., 2001). In these Prxs, both the peroxidatic cysteine and its corresponding resolving cysteine are contained within the same polypeptide, with the conden sation reaction resultingin the formation of an intra molecular disnlfide bond (Fig. 1).	They conserve only the peroxidatic cysteine and do not contain a resolving cysteine (Fig. 1) (Choi *et al*., 1998). Their cysteine sulfenic acid generated on reaction with peroxides is presumably reduced by a thiol-containing electron donor, but the identity of this redox partner is not yet clear (although proposed electron donors have included glutathione, lipoic acid and cyclophilin (Fisher *et al*., 1999; Lee *et al*., 2001; Peshenko and Shichi 2001; Hofmann *et al*., 2002).
In the second step of the peroxidase reaction, the peroxidatic cysteine sulfenic acid (Cys-S_pOH) from one subunit is attacked by the resolving cysteine (Cys- S_RH) located in the C-terminus of the other subunit (Fig. 1). This condensation reaction results in the formation of a stable intersubunit disulfide bond, which is then reduced by one of several cell-specific disulfide oxidoreductases (e.g., thioredoxin or tryparedoxin (Nogoceke *et al*., 1997; Poole, *et al*., 2000; Bryk *et al*., 2002), completing the catalytic cycle.	Although, the resolving cyste-eines of typical and atypical 2-Cys Prxs are not conserved in sequence, they are functionally equivalent. To recycle the disnlfide, known atypical 2-Cys Prxs appear to use thioredoxin as an electron donor (Seo *et al*., 2000).	By analogy, one donor thiol probably forms a transient mixed disulfide bond with the enzyme, followed by its reduction by a second donor thiol, thus recycling the enzyme (Wood *et al*., 2003).

Prx I	1. Classification	1. It belongs to typical 2-Cys (Wood *et al.*, 2002).
	2. Previous names	2. TPx-A, NKEF A, PAG, MSP23, OSF3, HBP23 (Rhee *et al.*, 2005a).
	3. Polypeptide length	3. 199 amino acids (Rhee *et al.*, 2005a)
	4.Cellular location	4. It exists in cytosol and nucleus (Wood *et al.*, 2003)
	5. Interaction with proteins and other ligands	5. c-Abl, Presenilin-1, Heme, Macrophage migration inhibitory factor and Cyclophilin (Wood *et al.*, 2003)
	6. Reductant agent	6. It utilizes thioredoxin (Rhee *et al.*, 1999).
	7. Important	7. a.) Prx I has heme binding capacity (Ishii *et al.*, 1993) and this protein also acts as a natural killer cell-enhancing factor (NKEF-A) (Shau *et al.*, 1994). b.) It contains a consensns site (Thr(90)-Pro-Lys-Lys) for phosphorylation by cyclin-dependent kinases (CDKs) (Chang *et al.*, 2002). c.) Prx I is an antioxidant protein expressed in proliferating cells (Yanagawa *et al.*, 2005). It is a key cytoplasmic peroxidase that reduces intracellular hydroperoxides in concert with thioredoxin (Uwayama *et al.*, 2006). d.) Prx I is a thioredoxin peroxidase that is involved in the regulation of proliferation and differentiation of mammalian cells (Hess *et al.*, 2003). e.) Prx I plays a role in protecting tissue from Fe-catalyzed oxidative damage and in reducing the free radical and contributes to the prevention of tissue damage caused by Fe-NTA through, at least in part, the eliminate H_2O_2 (Uwayama *et al.*, 2006).
Prx II	1. Classification	1. It belongs to typical 2-Cys (Alphey *et al.*, 2000).
	2. Previous names	2. TPx-B, NKEF B, Calpromotin, Torin, Band-8 and TSA (Wood *et al.*, 2003).
	3. Polypeptide length	3. 199 (Rhee *et al.*, 2005a) or 198 amino acids (Wood *et al.*, 2003).
	4. Cellular location	4. It exists in cytosol (Rhee *et al.*, 2005a) membrane (Wood *et al.*, 2003) and highly expressed in red blood cells (RBCs) (Rhee *et al.*, 2001).
	5. Interaction with proteins and other ligands	5. Protein 7.2b (stomatin), Presenilin-1, RBCs, membrane and Cyclophilin (Wood *et al.*, 2003).
	6. Reductant agent	6. It utilizes thioredoxin (Rhee *et al.*, 1999).
	7. Important	7. a.) Han *et al.* (2005) suggest that Prx II may function as an enzymatic antioxidant to prevent cellular senescence. b.) Prx II, is also able to act as a natural killer cell-enhancing factor (NKEF-B) and is often referred to as a thiol-specific antioxidant (TSA) (Chae *et al.*, 1993; Lim *et al.*, 1994). c.) Prx II not only plays a protective role against oxidative damage, but also inhibits the immune cell responsiveness, which may be regulated by scavenging the low amount of reactive oxygen species (ROS) (Moon *et al.*, 2005).
Prx III	1. Classification	1. It belongs to typical 2-Cys (Schroder *et al.*, 2000).
	2. Previous names	2. AOP-1, SP22 and MER5 (Wood *et al.*, 2003).
	3. Polypeptide length	3. 256 amino acids (Wood *et al.*, 2003).
	4.Cellular location	4. It is restricted to mitochondria (Hofmann *et al.*, 2002).
	5. Interaction with proteins and other ligands	5. Cyclophilin and Abrin A-chain (Wood *et al.*, 2003).
	6. Reductant agent	6. It utilizes thioredoxin (Rhee *et al.*, 1999).
	7. Important	7.a.) The expression of Prx III was abundant in tissues that are rich in mitochondria, such as heart, kidney and skeletal muscle and whose oxygen consumption is relatively high (Matsumoto *et al.*, 1999). Since mitochondria are the main intracellular organelles which consume molecular oxygen by respiration. b.) Prx III may function in concert with Mn-SOD to scavenge reactive oxygen species (ROS) which leak from the electron transport chain (Matsumoto *et al.*, 1999). c.) Prx III together with the mitochondria-specifcic thioredoxin (Trx) (Spyrou *et al.*, 1997) and thioredoxin reductase (TrxR) (Lee *et al.*, 1999) provide a primary line of defense against H_2O_2.
Prx IV	1. Classification	1. It belongs to typical 2-Cys (Wood *et al.*, 2002).
	2. Previous names	2. AOE372, TRANK (Jin *et al.*, 1997).
	3. Polypeptide length	3. 271 amino acids (Wood *et al.*, 2003).
	4. Cellular location	4. It is present in the endoplasmic reticulum and the extracellular space (Kim *et al.*, 1989).
	5. Interaction with proteins and other ligands	5. Heparin and Cyclophilin (Wood *et al.*, 2003).
	6. Reductant agent	6. It utilizes thioredoxin (Rhee *et al.*, 1999).
	7. Important	7. a.) Prx IV contains the N-terminal signal sequence for secretory proteins and found in culture medium (Matsumoto *et al.*, 1999). b.) Prx IV would be able to function as a Trx-dependent peroxidase within the extracellular space in a manner similar to other antioxidant enzymes, such as plasma glutathione-peroxidase (GPx) and extracellular SOD (Matsumoto *et al.*, 1999).

Prx V	1. Classification	1. It belongs to atypical 2-Cys (Gommel *et al*., 1997).
	2. Previous names	2. AOEB166, PMP20 and AOPP (Wood *et al*., 2003).
	3. Polypeptide length	3. 214 amino acids (Wood *et al*., 2003).
	4.Cellular location	4. It localized intracellularly to cytosol, mitochondria and peroxisomes (Knoops *et al*., 1999).
	5. Interaction with proteins and other ligands	5. DNA and Cyclophilin (Wood *et al*., 2003).
	6. Reductant agent	6. It utilizes thioredoxin (Rhee *et al*., 1999).
	7. Important	7. a.) It may have a broader activity against ROS compared with other isoforms of Prxs and other antioxidant enzymes (Declercq *et al*., 2001). It is participating directly in eliminating hydrogen peroxide and neutralizing other ROS (Yuan *et al*., 2004). b.) It has been implicated in antioxidant protective mechanisms as well as in signal transduction in cells (Declercq *et al*., 2001).
Prx VI	1. Classification	1. It belongs to 1-Cys (Ishii *et al*., 1993).
	2. Previous names	2. ORF06, LTW4 and AOP2 (Wood *et al*., 2003).
	3. Polypeptide length	3. 224 amino acids (Wood *et al*., 2003).
	4.Cellular location	4. It exists in cytosol (Rhee *et al*., 2005a).
	5. Interaction with proteins and other ligands	5. Cyclophilin (Wood *et al*., 2003).
	6. Reductant agent	6. It utilizes GSH to catalyze the reduction of H_2O_2 and organic hydroperoxides including phospholipid hydroperoxides (Fisher *et al*., 1999).
	7. Important	7. a.) It is a bifunctional 25-KDa protein and has been called a moonlighting (Chen *et al*., 2000). b.) Prx VI functions in antioxidant defense mainly by facilitating repair of damaged cell membranes via reduction of peroxidized phospholipids (Manevich and Fisher, 2005). Thus, Prx VI, a unique mammalian peroxiredoxin, is an important antioxidant enzyme and has a major role in lung phospholipid metabolism.

Regulation of Prxs Activity

Prxs have received a great deal of attention recently owing to their role in regulating levels of hydrogen peroxide, an intracellular signaling molecule common to many cytokine-induced signal-transduction pathways (Jin *et al*., 2000; Hofmann *et al*., 2002). Indeed, regulation of redox signaling through cysteine modification by peroxides and peroxynitrite has been reported for a growing number of enzymes and transcriptional regulators (Claiborne *et al*., 1999). It was recently shown that the overoxidation of Prx II is likely to be physiologically relevant, in that its peroxidatic cysteine is oxidized to sulfinic ($-SO_3H$) or sulfonic ($-SO_2H$) acid forms *in vivo* upon exposure of Leydig cells to tumor necrosis factor (Rabilloud *et al*., 2002). It has been proposed that Prxs in mammalian cells act as a dam against oxidative stress and that the ratio of active to inactive enzyme might play a role in whether cells are susceptible to cytokine-induced apoptosis (Rabilloud *et al*., 2002). In addition to overoxidation, Prx activity has also been shown to be regulated by phosphorylation and proteolysis (Schroder *et al*., 1998; Cha *et al*., 2000; Chang *et al*., 2002; Koo *et al*., 2002).

Phosphorylation Process

Recently, phosphorylation of mammalian Prx I, Prx II, Prx III and Prx IV at the conserved residue Thr89 (PrxTT numbering) by cyclin-dependent kinases was shown to decrease the peroxidase activity of the Prxs (Chang *et al*., 2002). In the case of Prx I, this phosphorylation was observed to occur *in vivo* during mitosis. The authors concluded that the phosphorylated Thr89 had an unfavorable electrostatic effect on the peroxidatic active site. An examination of the structure of the Prx II decamer reveals that a phosphorylated Thr89 would introduce unfavorable electrostatic interactions within the dimer-dimer interface by placing two negatively charged phosphates in close proximity (Wood *et al*., 2003). In fact, a reasonable alternative interpretation is that phosphorylation of Thr89 attenuates the enzyme activity by disrupting the decameric structure (Wood *et al*., 2003). Several researchers have

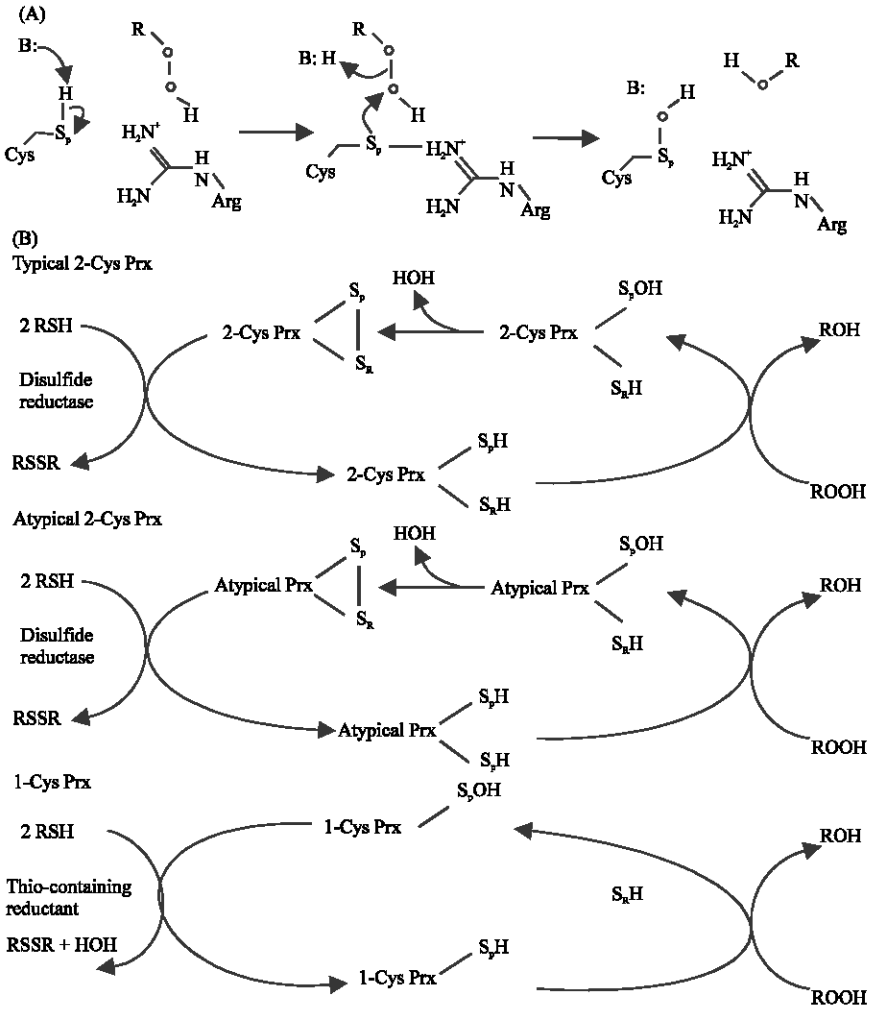

Fig. 1: Peroxiredoxin (Prx) mechanism (Wood *et al*., 2003). (a) The common first step of peroxide reduction involving nucleophilic attack by the peroxidatic cysteine (S_p) and formation of the cysteine sulfenic acid intermediate (S_pOH), probably shared by all Prxs. Both the catalytic base that deprotonates the peroxidatic cysteine and catalytic acid that protonates the RO^- leaving group are labeled B, although this does not imply that they are the necessarily same entity. The guanidino group of the conserved arginine is presumed to stabilize the ionized peroxidatic cysteine. (b) The three mechanisms distinguishing the Prx classes, with peroxidatic cysteine and resolving cysteines in the reduced (S_pH and S_RH, respectively), sulfenic acid (S_pOH) or disulfide (S_p and S_R, connected) state. In the case of dimeric 2-Cys Prxs, the peroxidatic cysteine (black S_p) and resolving cysteine (pink S_R) originate from different subunits and condense to form an intersubunit disulfide bond (black and pink striped bar). Reduction of typical and atypical 2-Cys Prxs involves one flavoprotein disulfide reductase and at least one additional protein or domain containing a CXXC motif, which is oxidized from a dithiol (2 RSH) to a disulfide (RSSR) state during Prx reduction (e.g., thioredoxin reductase and thioredoxin, AhpF, trypanothione reductase, trypanothione and tryparedoxin, or lipoamide dehydrogenase, SucB and AhpD (Nogoceke *et al*., 1997; Poole *et al*., 2000 and Bryk *et al*., 2002). Reductants of 1-Cys Prxs include low molecular weight thiols, but physiological partners are as-yet unidentified.

reported that dimeric forms of Prxs exhibit less activity than decameric forms (Nogoceke *et al*., 1997; Chauhan and Mande, 2001). This observation is supported by the crystal structures, which show that the active sites of the typical 2-Cys Prxs are adjacent to and stabilized by the dimmer-dimer interface of the decamer. It is notable that the phosphorylation mechanism probably favors different oligomeric states (dimer and decamer, respectively).

Like many enzymes involved in the metabolism of intracellular messengers, the peroxidase activity of Prx I and Prx II is regulated by phosphorylation, which in this instance is mediated by cyclin-dependent kinases (Cdks) (Chang *et al*., 2002). Phosphorylation at Thr90 by several Cdks, including Cdc2, *in vitro*, results in inhibition of its peroxidase activity. Experiments with HeLa cells arrested at various stages of the cell cycle showed that phosphorylation of Prx I on Thr90 occurs in parallel with the activation of Cdc2 (Rhee *et al*., 2005a). Prx I phosphorylation was observed in cells in the M phase but not in interphase cells, despite the fact that Prx I can be phosphorylated by other Cdks *in vitro*. This specificity likely reflects the facts that Prx I is a cytosolic protein and therefore only encounters activated Cdks after the breakdown of the nuclear envelope during mitosis and that Cdc2 is the Cdk that is activated in the M phase (Rhee *et al*., 2005a). These results suggest that Cdc2-dependent phosphorylation of Prx I/Prx II and the resulting accumulation of H_2O_2 might be a critical step in the progression of normal cell division.

Proteolysis Process

Another mechanism proposed to regulate peroxidase activity *in vivo* entails specific proteolysis of the C termini of Prxs, preventing peroxide-mediated inactivation in response to rising levels of peroxide (Koo *et al*., 2002). In studies of a typical 2-Cys Prx from yeast, a portion of the enzyme was found to have a truncated C-terminal following purification (Koo *et al*., 2002). In follow-up mutagenesis studies, C-terminally truncated forms of the enzyme were found to be more resistant to peroxide overoxidation and inactivation than the sensitive wild-type enzyme (Koo *et al*., 2002). A similar truncation of Prx II that removed the C-terminal 13 residues (including the last a helix) has also been observed during the isolation of the enzyme from erythrocytes (Cha *et al*., 2000). Interestingly, the regulatory protease calpain is present in erythrocytes and will specifically cleave this region of Prx II *in vitro* (Schroder *et al*., 1998). Proteolysis would make the enzyme resistant to overoxidation but leave it susceptible to inactivation by phosphorylation.

Neurodegeneration

The inherent biochemical and physiological characteristics of the brain, with its high unsaturated phospholipid content and energy requirements, make it particularly susceptible to free radical mediated injury (Krapfenbauer *et al*., 2003). Furthermore, the brain has the ability to readily accumulate iron, which promotes the production of the damaging hydroxyl radical and is relatively poorly endowed with protective antioxidant enzymes or antioxidant compounds (Halliwell and Gutteridge, 1999). The reactive oxygen intermediates (including superoxide and hydroxyl radicals as well as hydrogen peroxide) can cause direct cellular injury by including lipid and protein peroxidation and damage to nucleic acid (Takeda *et al*., 1984; Richard *et al*., 1990). Moreover, Betteridge (2000) reported that the free radicals can be produced by several different biochemical processes within the body including: (1) reduction of the molecular oxygen during aerobic respiration yielding superoxide and hydroxyl radicals; (2) by products of chemical reactions such as oxidation of catecholamme and activation of the arachidonic acid cascade product electrons, which can reduce molecular oxygen to superoxide; (3) production of superoxide and hypochlorous acid (HOC1), a powerful oxidant, by activated phagocytes and (4) nitric oxide production by vascular endothelium and other cells. ROS can cause widespread damage to biological macromolecules and are believed to play a causal role in many degenerative diseases (Demple and Amabile-Cuevas, 1991; Neumann *et al*., 2003). Lipid peroxidation was

previously reported in other neurodegenerative diseases, including Alzheimer's disease and epilepsy (Halliwell and Gutteridge, 1999). Also, Oxidative stress induced by Reactive Oxygen Species (ROS) such as hydrogen peroxide has been implicated in the pathogenesis of several neurodegenerative diseases (Kim *et al*., 2001a). Thus, the oxidative stress is one of the most important factors in the pathogenesis of idiopathic Parkinson's disease (Beal, 2002; Mouradian, 2002).

On the other hand, Sarafian *et al*. (1999) revealed a clear segregation of expression of Prx I and Prx II gene products in different brain cell types. In the cerebral cortex, cerebellum, basal ganglia, substantia nigra and spinal cord, Prx I was expressed primarily in astrocytes, while Prx II was expressed exclusively in neurons. Prx I was also prominently expressed in ependymal cells and subependymal matrix of substantia nigra and basal ganglia. Prx II was not expressed at uniform density in all neurons. In general, small neurons such as cerebellar granule neurons displayed little or no staining, while large neurons, such as hippocampal pyramidal and Purkinje neurons were heavily stained (Sarafian *et al*., 1999). The absence of expression of Prx I in neurons and the selective expression of Prx II in large neurons suggest that these antioxidant enzymes serve distinct functional roles that may reflect the different functions and biochemical activities of these cell types. Restricted expression of these genes may also contribute to the selective vulnerability of these cells to a wide variety of neuropathologic conditions. In addition, increased protein levels of Prx I and Prx II could provide protection against neuronal cell death induced by hydrogen peroxide, while decreased protein levels of Prx III could be caused by mitochondrial damage shown in Alzheimer's disease (AD) and Down syndrome (DS) (Kim *et al*., 2001a). Thus, upregulated Prx protein levels provides evidence for the involvement of ROS in the pathogenesis of AD and DS. Hence, increased expression of Prx II in DS, AD and PD frontal cortex could represent a cellular response initiated against apoptosis, as enhanced apoptosis is demonstrated in these disorders (Engidawork *et al*., 2001; Gulesserian *et al*., 2001b). In addition, Prx I reduces hydrogen peroxide, the widespread glial expression of Prx I indicates that it may play an important protective role against oxidative damage in the nervous system (Mizusawa *et al*., 2000).

On the other word, in human brain, Prx I was primarily expressed in astrocytes, whereas Prx II was expressed exclusively in neurons (Sarafian *et al*., 1999). Prx I, II and VI are localized predominantly to the cytosol, while Prx III and V are largely detected in the organdies (Seo *et al*., 2000) and Prx IV is secreted outside of the cells (Okado-Matsumoto *et al*., 2000). Normal aging is associated with increased oxidative stress, which in turn increases the probability of age-dependent neurodegenerative brain pathology (Squire, 2001). Oxidative stress-associated cell damage has also been described in age-related neurodegenerative disorders, including Pick's Disease (PD), Alzheimer's disease (AD) and Down syndrome (DS), as DS has a neurodegenerative component later in life (Lubec, 1996; Multhaup *et al*., 1997). The region-dependent differences in expression of antioxidant proteins between DS and AD brain were reported previously (Gulesserian *et al*., 2001c), explaining, at least in part, the variation observed in morphology and distribution of lesions (Mann, 1997). Moreover, abnormal phosphorylation of proteins, a shared characteristic feature of DS, AD and PD, is viewed as a consequence of oxidative stress (Gerst *et al*., 1999). It appears therefore likely that oxidative stress would contribute to the development of neurodegenerative pathologies in these disorders.

Previously, Prx II is exclusively expressed in neurons (Sarafian *et al*., 1999) and neuronal loss, in which apoptosis is implicated as a likely mechanism (Caims, 1999; Dickson, 2001), is one characteristic feature of DS, AD and PD. Taking this into account, one would expect decreased expression of Prx II. One possible explanation for this paradox could be that a compensatory mechanism might be initiated by surviving neurons to protect themselves from apoptosis. Oxidative stress in fetal DS is proposed to be linked to decreased expression of Prx proteins (Gulesserian *et al*., 2001a). The switch in direction of change in adult life with disease progression most likely supports that such compensatory response is operative. This indeed can be taken as evidence for the possible existence of a neuronal apoptosis decision cascade in neurodegenerative disorders in which a

competitive decision making process balances cell death mechanisms against protective and reparative processes (Cotman, 1998). Prx II may also be attributed to difference in regional sensitivity to apoptosis. Indeed, the previous studies in DS and AD (Engidawork *et al*., 2001; Gulesserian *et al*., 2001b) suggest that cerebellum is less vulnerable to apoptosis than frontal cortex. Although over-expression of Prx I is also shown to inhibit apoptosis, lipid peroxidation and the release of cytochrome c from mitochondria in different cells (Kim *et al*., 2000), no detectable changes in expression of Prx I were observed in both regions between the experimental subjects. This lends support for the notion that compensatory response initiated by neurons indeed accounts for the change in Prx II, as Prx I is predominantly expressed in glial cells (Sarafian *et al*., 1999).

Prx III is mainly found in the mitochondria (Krapfenbauer *et al*., 2003). Since there is extensive damage to mitochondria in neurodegenerative disorders (Kim *et al*., 2001b), leakage could be an important factor in the disease groups but not in controls. Decreased Prx III levels in the cytosol of DS and PD as well as AD (though insignificant) patients despite leakage suggest that all the mitochondrial Prx III is used up and what was detected is nascent Prx III (Krapfenbauer *et al*., 2003). Alternatively, Prx III detected in the cytosol could represent both nascent and leaked Prx III. In both cases, decreased Prx III could indicate that there is impaired synthesis of Prx III and/or enhanced Prx III instability in neurodegenerative diseases, this being more severe in DS and PD.

Prx VI has several unique characteristics, including the absence of a second cysteine residue that is conserved in all other Prxs, the presence of a unique carboxy-terminal domain and a demonstrated phospholipase activity (Krapfenbauer *et al*., 2003). Furthermore, Prx VI shows conservation of several amino acids important in dimer formation and active site configuration that are not found in other family members (Phelan, 1999). Recently, definitive evidence has been provided for the presence of both peroxidase and phospholipase activities in Prx VI (Chen *et al*., 2000). Accordingly, the enzyme can reduce hydrogen peroxide and short chain organic, fatty acid and phospholipid hydroperoxides. Prx VI expression did not show any apparent change in DS and AD, reflecting the protein may not have significant contribution to cellular defense against oxidative stress in the context of DS and AD. However, its elevation in PD may suggest that Prx VI could be useful in discriminating PD from DS/AD. Oxidative signals are shown to stimulate expression of regulatory factors that bind to stress response element of the Prx VI gene, thereby activating its expression (Fatma, *et al*., 2001). Increased expression of Prx VI in PD might therefore be caused by certain transcription factor and/or activating factor that is selectively upregulated in PD. Prx VI protects from ROS-mediated DNA fragmentation (Fatma, *et al*., 2001), which results in neuronal death by apoptosis or other mechanism (Gleckman *et al*., 1999). Thus, upregulation of Prx VI might be linked to greater degree of neuronal loss in PD (Dickson, 2001) although the technique of proteomics does not distinguish the cell (neuron/glia) that elaborates Prx VI. Generally, the oxidative stress and damage play a role in the pathogenesis of a number of diseases associated with neurodegeneration and Prxs play an important role against this damage.

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