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Review

Emerging roles of thioredoxin cycle enzymes in the central nervous system

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Abstract. The thioredoxins (Trxs) constitute a family of enzymes which catalyze the reduction of protein disulfide bonds. Recent animal studies have revealed the importance of the Trx superfamily in various experimental systems. For example, the homozygous disruption of the genes encoding cytoplasmic (*TRX1*) or mitochondrial Trx (*TRX2*) in mice generates lethal embryonic phenotypes. In contrast, transgenic mice overexpressing *TRX1* show an extended life span and are relatively resistant to ischemia-mediated brain damage. In addition to their capacity to detoxify peroxides in concert with peroxiredoxins and Trx reductases, Trx isozymes perform multiple redox signaling functions mediated by their specific interaction with various proteins, including redox-regulated kinases and transcription factors. Recent studies indicate that specific isoforms of Trx cycle enzymes, targeted to different cell compartments, are key regulators of fundamental processes, such as gene expression, cell growth and apoptosis. The present review is primarily focused on the emerging neuroprotective role of these proteins in the central nervous system.

Key words. Thioredoxin; antioxidants; brain; neurodegeneration; mitochondria; peroxiredoxins; apoptosis; oxidative stress.

Introduction

The central nervous system (CNS) and neurons in particular are vulnerable targets of oxidative injury and oxidative stress-mediated cell death [1–4]. Neurons are highly aerobic cells, whose capacity to withstand oxidative stress is limited because of the following features: (i) high content of easily oxidizable substrates such as polyunsaturated fatty acids and catecholamines; (ii) mitochondrial production of superoxide and downstream reactive oxygen species (ROSs), which can be dramatically enhanced under conditions of high energy demands or dysfunction of electron transport complexes [5–7]; (iii) relatively low antioxidant capacity, reflected by relatively low levels of glutathione (GSH) [8] and GSH peroxidase (GPx) [9]. Oxidative stress may be a major cause of normal physiological aging, as first proposed 50 years ago by Harman in his 'free radical theory of aging' [10]. Although still controversial, this theory has gained support from many studies [11]. Oxidative stress and deficits in mitochondrial function are two major factors involved in the etiology of age-related neurodegenerative conditions, including Alzheimer's (AD) and Parkinson's diseases (PD) (reviews: [5, 6, 12]). Several studies have shown that postmortem brain tissues isolated from aged healthy patients and particularly those affected by AD and/or PD display

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increased levels of oxidative markers, including protein carbonyl groups and nitrotyrosine, fatty acid oxidation products and oxidized DNA bases, and loss of protein sulfhydryl groups (reviewed in [1, 2, 12–14]). The different extents of oxidative damage reported in distinct human brain regions are thought to reflect the oxidative stress resulting from localized imbalance between ROS production and antioxidant and/or repair activities. That oxidative stress is a direct cause of age-related neurodegeneration was elegantly demonstrated in *Drosophila* [15].

Brain antioxidant defenses involve low molecular weight redox compounds and several families of antioxidant enzymes [16]. The redox compounds include GSH, nicotinamide adenine dinucleotides (NADPH, NADH), tocopherols (vitamin E), ascorbic acid (vitamin C), ubiquinone (Q10) and lipoic acid. The antioxidant enzymes include those which dismutate superoxide ($O_2^{\cdot -}$), such as cytosolic (Cu/Zn-SOD or SOD-1) and mitochondrial superoxide dismutases (MnSOD or SOD-2); those which reduce hydrogen peroxide (H_2O_2 e.g. produced by SODs), such as catalase and peroxidases that can also reduce fatty acid and lipid hydroperoxides; and various disulfide reductases, such as thioredoxin (Trx) isozymes [17] (see below), and glutaredoxins [18]. Although peroxide reduction in the CNS is usually assumed to rely mainly on the GSH system via the GSH peroxidases GPx-1 [19–23] and phospholipid-hydroperoxide glutathione peroxidase (GPx-4), it can also be achieved by Trx peroxidases called peroxiredoxins (Prxs) (see below). Genetic manipulations of the expression of specific antioxidant enzymes in cultured cells and animal models of neurodegenerative diseases have provided indirect evidence for the involvement of mitochondria and oxidative stress in various neurodegenerative conditions. For example, overexpression of Cu/ZnSOD in transgenic mice reduced the damage caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration to the nigrastriatal dopaminergic pathway, a form of damage similar to that observed in Parkinson's disease [24]. Conversely, mice deficient in the expression of Cu/ZnSOD or GPx-1 were more sensitive to MPTP treatment than normal animals [25, 26]. These results suggest that some of the deleterious effects of MPTP were caused by $O_2^{\cdot -}/H_2O_2$-derived radicals. The similarity between the MPTP model and the mitochondrial complex I functional deficits commonly found in PD further suggests that oxidative stress of mitochondrial origin may play a significant role in the etiology of this neurodegenerative disorder. Functional disruption of complex I by 1-methyl-4-phenylpyridinium (MPP^+, the active metabolite of MPTP) or by pesticides, such as rotenone, induce ROS formation and aggregation of α-synuclein, leading to demise of dopamine neurons [13]. Complex I impairment may be the central cause of sporadic PD [13]. Another case in point is the early mitochondrial dysfunction and ROS production involved in motor neuron degeneration characterizing amyotrophic lateral sclerosis (ALS), and in transgenic mouse models of ALS that overexpress mutant SOD1 (e.g. G93A) genes (review: [27]). Extents of mitochondrial dysfunction and death of mouse NSC-34 motor neuron-like cells triggered by mutant SOD1 could be reduced by transgenic overexpression of antioxidant enzymes [28], or exposure to the antioxidant N-acetylcysteine [29]. A recent study [30] demonstrated a direct binding interaction between wild- type or mutant SOD1 and the anti-apoptotic protein Bcl-2, which was detected in vitro (Neuro2A cells) and in vivo in mouse and human spinal cords. Entrapment of mitochondria-anchored Bcl-2 by SOD1 aggregates was proposed to deplete motor neurons of this anti-apoptotic protein [30]. Whether the selective recruitment of mutant SOD1 to spinal mitochondria, suggested to account for their dysfunction and selective toxicity in ALS [31], does occur in motor neurons mitochondria is not known. All these examples support the attractive concept that impairment of mitochondrial functions, oxidative stress and deficits in energy production are critical events contributing to neurodegenerative conditions [6, 13, 32–34].

In contrast to GSH, which is thought to be largely responsible for low redox potential and free thiol levels inside cells and organelles due to its high intracellular concentration (1–10 mM) [35], the Trx systems may play a critical role in the redox regulation of protein thiols involved in signal transduction and gene regulation. The Trx proteins constitute a family of enzymes which utilize the reducing power of NADPH to reduce disulfide bonds in proteins. Thioredoxins are abundant proteins found in several isoforms in different species, which carry out essential biosynthetic reactions and regulate many biological functions. In addition to Trx proteins, which, in conjunction with Trx reductases (TrxR), have the capacity to reduce various protein disulfides with variable efficiencies [17, 36, 37], this system includes a family of Prxs, which contribute to the detoxification of various peroxides [38] (fig. 1). Each type of enzyme is expressed in distinct isoforms, which are localized in specific cell compartments such as the cytosol and mitochondria. Their expression can be modulated by changes in the cellular and molecular redox environment, and various stress factors. Of note, several studies reported increased Trx1 expression in many human primary cancers and tumor cell lines [39–44] including astrocytomas [45] (see below). Elevated Trx levels may contribute to increased cancer cell growth and resistance to chemotherapy by several mechanisms, including stimulation of DNA synthesis, activation of redox-modulated transcription factors that regulate cell growth and inhibition of apoptosis [43].

The crucial role of mitochondria in energy production and cell survival regulation in the CNS is currently rais-

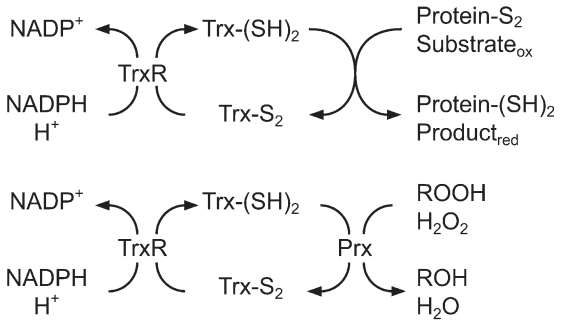

Figure 1. Trx cycle system. The diagram summarizes the fundamental oxido-reduction reactions catalyzed by the thioredoxin (Trx) cycle enzymes. Trx reductase (TrxR) uses NADPH as a source of electrons to reduce the active site disulfide of oxidized Trx ($Trx\text{-}S_2$) and a variety of oxidized antioxidant molecules [38]. Reduced Trx [$Trx\text{-}(SH)_2$] is highly efficient in directly reducing protein/peptide disulfides. Trx [$Trx\text{-}(SH)_2$] also provides the electrons for the reduction of peroxides, which is catalyzed by peroxiredoxins (Prx).

ing particular interest in the function of the mitochondrial Trx cycle isozymes. A rapidly growing number of studies suggest that the Trx cycle enzymes perform important functions in the CNS, including neurotrophic and neuroprotective actions. This review will mainly focus on the emerging role of these antioxidant enzymes in the CNS. For basic knowledge on the structure and function of Trx system enzymes, the reader is referred to excellent previous reviews [17, 37, 38, 46–53].

The Trxs

Trx

The Trxs constitute a family of proteins all of which have a conserved catalytic site (Cys-Gly-Pro-Cys) which undergoes reversible oxidation of the cysteine pair while reducing disulfide bridges of various proteins. Trx was originally identified in *Escherichia coli*, in 1964, as a hydrogen donor for ribonucleotide reductase required for DNA synthesis [54]. The first report on a mammalian Trx-Trx reductase system was published in 1967 [55]. The disulfide state of Trx is regenerated by a Trx reductase (TrxR), which is the only type of enzyme known so far to reduce back the inactive disulfide form of Trx [46, 56]. Acting together, these two enzymes constitute a highly efficient system for reducing cystine disulfide bridges to cysteines [17, 36, 37]. Another family of proteins acting in conjunction with Trx is the peroxide scavenger Prx [50, 57]. Because of their unique properties and capacity to quench singlet oxygen and scavenge hydroxyl radicals [36, 58], the Trxs are considered to be crucial antioxidant proteins.

The early embryonic lethal phenotype resulting from the homozygous deletion of the cytoplasmic Trx1 gene (*TRX1*) highlights the importance of this protein in mammalian development [59]. Moreover, Trx1 was found to play an important role in various signaling events, which are redox-regulated and known to participate in the control of numerous physiological processes including growth, cell survival/apoptosis, development, differentiation and proliferation [38, 47]. For example, in response to various stress conditions, Trx1 is translocated to the nucleus and increases the activity of several transcription factors, such as nuclear factor (NF)-κB, AP-1 and p53, and related coactivators, such as Ref-1, which depend on reduced sulfhydryl groups for their function [60–63]. In regard to cell survival control, Trx1 acts as a negative regulator of apoptosis signal-regulating kinase I (ASK1), a function suppressed by Trx1 oxidation and consequent release from ASK1, which triggers apoptosis [64]. Trx1 can also perform extracellular functions upon leaderless secretion from various cells [65]. Extracellular Trx1 was proposed to function as an autocrine growth factor and co-cytokine [17, 38, 43, 47]. In addition to Trx1, the most extensively studied eukaryotic thioredoxin, several other Trx isoforms have been identified in mammalian cells [47]. Of interest, a mitochondrial isoform (Trx2) was discovered in various organisms, a protein, which is transported into mitochondria using an N-terminal protein-targeting sequence [66–68]. *TRX2* inactivation studies in DT-40 chicken cells suggest that this mitochondrial Trx isozyme is essential for cell survival [69]. The homozygous disruption of *TRX2* generates a lethal embryonic phenotype in mice [70]. In support of Trx2 protective function(s) against oxidative stress, transfection of Trx2 reduced the sensitivity of human osteosarcoma and embryo kidney cells to cell death induced by etoposide [68] or *tert*-butylhydroperoxide [71]. In addition, identification of a distinct microtubule-associated Trx (Txl-2) and a transmembrane isoform (TMX) was also recently reported [72, 73].

Basal expression of Trxs in the CNS

Trx1 and TrxR were first localized immunohistochemically in the rat sciatic nerve [74]. Trx1 was then found widely expressed in rat brain, especially in regions of high metabolic activity, such as substantia nigra and the subthalamic nucleus [75]. An ADF/TRX immunohistochemistry study in gerbil brain during reperfusion following transient cerebral ischemia showed widespread immunoreactivity in non-ischemic control brain regions, including the ependyma, tanycytes, endothelial cells as well as subcommisural organs, and weak staining in the neuronal cell bodies [76]. During reperfusion, and in contrast to non-ischemic controls, ADF/TRX was expressed in glial cells in the CA1 and dentate hilus of the hippocampus [76]. Weak staining was observed in the striatum and vascular endothelial cells, with no staining detected in astroglia and microglia in control gerbil and rat

[76–78]. Immunohistochemical analysis of Trx1 in human brain [79] showed positive Trx1-like staining in white matter astrocytes. Trx1 expression, assessed by semiquantitative reverse transcription polymerase chain reaction (RT-PCR), was also found to be more intense in white matter than in grey matter [79]. This study also detected Trx1 expression in peripheral nerves and Schwann cells, in agreement with a previous report [74].

The mitochondrial isoform, Trx2, is abundant and widely distributed in rat brain [80]. Brain regions showing highest expression at the RNA and protein levels include the olfactory bulb, frontal cortex, some hypothalamic and thalamic nuclei, cerebellum and the brainstem nuclei [80]. The expression pattern of Trx2 appears to be associated with brain regions producing high levels of ROSs [80].

Trx induction in the CNS

A variety of hormones and non-toxic chemicals, and stress conditions including infectious agents have been reported to induce Trx1 expression, as reviewed elsewhere [47, 48]. A growing number of studies report a striking association between Trx1 up-regulation in the CNS during neuron survival following various injuries resulting in oxidative stress (see table1). Mechanical nerve injury and transient focal brain ischemia induce Trx1 in various brain areas and cell types, with induction patterns suggesting Trx1 function(s) in neuroprotection or regeneration of the brain following injury and oxidative stress [74–78, 81]. Neuronal survival was associated with Trx1 up-regulation in response to ischemic stress [77, 78, 82]. Conversely, the vulnerability of cortical neurons isolated from stroke-prone spontaneously hypersensitive rats to transient hypoxia/reoxygenation was associated with inefficient up-regulation of Trx1 and Trx2 as compared to normal Wistar rats [83]. Hypoxia-ischemia treatments of neonatal rats caused a reduction of Trx immunoreactivity and stimulation of peroxynitrite production associated with neuronal damage in the infarct brain region, while surviving neurons of the peri-infarct cortex showed Trx1 induction, again suggesting neuroprotective function(s) of Trx [82].

The molecular mechanisms leading to induction of Trx1 in brain lesions appear to involve ROSs. The promoter region of Trx1 contains various transcription factor binding sites, such as SP1, GCF and WT-ZFP conferring constitutive expression, inducible expression elements such as AP-1, AP-2, NF-κB, Oct-1, PEA-3, Myb and the antioxidant-responsive element (ARE) [84–87]. AREs are required for the induction of Trx1 in the SH-SY5Y neuronal cell line in response to hemin [88] or the phase II enzyme inducer, *tert*-butylhydroquinone (*t*-BHQ) in K562 cells [84]. ARE-mediated Trx1 induction involves the transcription factor Nfr2 [84, 88]. Nuclear translocation of Nfr2 and Trx1 expression in SH-SY5Y cells were shown to be dependent on phosphatidylinositol 3-kinase (PI3K) [88].

Trx1 induction was also proposed to contribute to a mechanism of pre-conditioning neuroprotection. Preconditioning of SH-SY5Y neuronal cells by transient serum depletion produces a hormesis phenomenon characterized by an enhanced tolerance to subsequent lethal oxidative stress, associated with increased expression of several proteins, including antioxidant enzymes (Trx1, Prx1, MnSOD) and antiapoptotic Bcl-2 [89]. The involvement of Trx1 in preconditioning-induced neuroprotection was indicated by the demonstration that antisense-mediated inhibition of Trx1 expression reduced the hormesis effect [89].

Little is known of the regulation of *TRX2* gene expression in the CNS. The induction patterns of Trx1 and Trx2 appear to be similar in cortical neurons exposed to hypoxia-reoxygenation, suggesting that *TRX2* is an oxidative stress-regulated gene, which may share common regulatory elements with *TRX1* [83]. However, the regulation of *TRX2* is probably more complex since Trx1 and Trx2 messenger RNA (mRNA) expression patterns were found to be quite distinct in mouse lens following exposure to photochemical oxidative stress [90]. Trx2 expression can be induced by dexamethasone in rat brain, but exclusively in paraventricular hypothalamic and reticular thalamic nuclei [80]. The basis for this region-specific Trx2 induction, which would be unrelated to oxidative stress, remains to be determined.

Glial secretion of Trx1

Astroglial cells are the main cell type that display induced expression of Trx1 during ischemia [76]. Exposure of astrocytoma U251 cells to low concentrations of H_2O_2 was shown to release Trx1 in the culture medium, and the addition of this conditioned medium to neuron cultures isolated from embryonic mouse cortex and striatum was found to promote their survival in the absence of serum [91]. These observations support the view that glial cells provide a neurotrophic and antioxidant support for the neurons.

Neuroprotective effects of transgenic Trx1 expression

Transgenic mice have been generated, which overexpress human Trx1 (hTrx1) in various tissues, including brain (fivefold increase) [92]. hTrx1 expression was localized in pyramidal neurons, hippocampus, cortex, vascular endothelial cells and glial cells. These transgenic mice display extended life span, an important observation linking increased longevity to the expression of an antioxidant enzyme in mammals [93]. In addition, these mice are more resistant to focal brain ischemia [92] or kainate-me-

Table 1. Expression of Trx cycle enzymes in the CNS.

Protein (p) Activity (a) mRNA (r)	Pathology, experimental system, stress insult	Oxidative stress[a]	Expression		Species	References
			Localization	Level[b]		
Trx /TrxR (p)	sciatic nerve crush	+	dilatated axons both proximally and distally to the crush	+	rat	[74]
Trx1 (p)	3-NP (systemic injection)	+	hippocampal (CA3), dentate gyrus, lateral striatum	+	rat	[211]
TrxR (p/a)	cortical astrocyte cultures exposed to t-BHQ	-	cortical astrocytes	+	mouse	[153]
TrxR (p/a)	cortical neuron cutures exposed to t-BHQ	-	cortical neurons	=	mouse	[153]
Trx1 (p)	spinal cord fetal neuron cultures exposed to GGA	-	spinal cord fetal neurons culture	+	rat	[212]
Trx1 (p)	SH-SY5Y cells exposed to 17-β-estradiol	-	SH-SY5Y cell line	+	human	[213, 214]
Trx1 (r,p)	middle cerebral artery occlusion	+	perifocal ischemic region	+	rat	[77, 78]
Trx1 (r,p)	middle cerebral artery occlusion	+	core region	–	rat	[77, 78]
Trx1 (r)	partial unilateral hemitransection	+	cortical hemisphere (lesion)	+	rat	[75]
Trx1, Trx2 (r)	hypoxia/ reoxygenation of culture cortical neurons	+	cortical neuron	+	rat	[83]
Trx2 (r)	dexamethasone (ip injection)	ND	paraventricular hypothalamic and reticular thalamic nucleus	+	rat	[80]
Trx1 (r,p)	Alzheimer's disease	+	white matter astrocytes (preferentially)	+	human	[79]
Trx1 (p)	Alzheimer's disease	+	amygdala hippocampus / parahippocampal gyrus	–	human	[109]
TrxR (a)	Alzheimer's disease	+	amygdala / cerebellum	+	human	[109]
Trx1 (p)	MPP+ exposure	+	PC12 cell line	–	rat	[215]
Trx1 (p)	hypoxia-ischemia	+	injured region (cortex, striatum)	–	rat	[82]
Trx1 (p)	hypoxia-ischemia	+	around injured region	+	rat	[82]
Trx1 (r)	hypoxia-ischemia	+	cerebral hemisphere ipsilateral to the carotid ligation	+	rat	[82]
Prx1 (p)	blood intracranial injection	+	around hemorrhagic region microglia (acute) astrocytes (chronic)	+	rat	[195]
Prx1 (p)	blood intracranial injection	+	neurons and oligodentrocytes	=	rat	[195]
Prx1 (p)	Alzheimer's	+	temporal and occipital cortex, thalamus	+	human	[198]
Prx1 (p)	Down's Syndrome	+	thalamus	+	human	[198]
Prx2 (p)	Alzheimer's disease	+	thalamus	+	human	[198]
Prx2 (p)	Down's Syndrome	+	cerebellum, temporal cortex, thalamus	+	human	[198]
Prx3 (p)	Alzheimer's disease	+	occipital cortex, thalamus	–	human	[198]
Prx3 (p)	Down's Syndrome	+	frontal cortex	–	human	[198]
Prx1 (p)	Alzheimer's, Down's Syndrome, Pick's disease	+	frontal cortex (study limited to frontal cortex and cerebellum)	=	human	[199]
Prx2 (p)	Alzheimer's, Down's Syndrome, Pick's disease	+	frontal cortex (study limited to frontal cortex and cerebellum)	+	human	[199]
Prx3 (p)	Down's Syndrome, Pick's disease	+	frontal cortex (study limited to frontal cortex and cerebellum)	–	human	[199]
Prx6 (p)	Pick's disease	+	frontal cortex (study limited to frontal cortex and cerebellum)	+	human	[199]
Prx3 (p)	aging	+	cerebellum (study limited to frontal cortex and cerebellum)	–	human	[199]
Prx2 (p)	aging	+	brain	–	human	[200]
Prx3 (r,p)	ibotenate microinjection in hyppocampus	+	hippocampus	–	rat	[197]
Prx6 (r)	intracerebral prion injection in mouse	+	(preferentially in astrocytes)	+	mouse	[203]
Prx1/ Prx3 (r)	intracerebral prion injection	+	brain	=	mouse	[203]
Prx1 (p)	CJD (sporadic)	+	frontal cortex (study limited to frontal cortices)	–	human	[202]
Prx2/Prx3 (p)	sCJD	+	frontal cortex, cerebellum (study limited to frontal cortices)	=	human	[202]
Prx6 (p)	sCJD	+	frontal cortex (study limited to frontal cortices)	+	human	[202]
Prx1 (p)	kainate, ip injection	+	cortex, cerebellum, hippocampus, basal ganglia	–	rat	[194]

[a] Oxidative stress: +, increased or –, decreased as determined in the study or as previously established; ND, not determined or not known.
[b] Expression levels compared to controls: +, increased; –, decreased; =, no change; ND, not determined.

diated excitotoxic stress in the hippocampus [94] than their wild-type littermates. The increase in protein carbonyl content after 1-h focal ischemia, a marker of protein oxidation, was suppressed in the brain of Trx1 transgenic mice, suggesting an antioxidant neuroprotective role for Trx1 [92]. In addition, *c-fos* induction by ischemia, an effect thought to attenuate ischemic injury, was enhanced as compared to non-transgenic animals. Moreover, the neurological deficit caused by ischemic treatment was improved in the transgenic mice as compared with their wild-type littermates. All these observations point to neuroprotective functions of Trx1 in the CNS [92, 93]. To what extent these Trx-mediated effects resulted from enhanced ROS detoxification [36, 58] or from modulation of signaling functions through multiple Trx1 interactions with various protein partners [38, 47] is not known.

Neuroprotective effects of systemic Trx1 administration

Intravenous administration of recombinant human Trx1 (rhTrx1) in mice was recently reported to decrease brain damage consequent to transient focal cerebral ischemia [95]. The administration of rhTrx1 reduced protein carbonyl content and activation of the stress related-MAPK p38 in response to ischemia. Of interest, rhTrx1 was able to pass through the blood brain barrier into the ischemic brain hemisphere ipsilateral to the transient middle cerebral artery occlusion, but not into the contralateral hemisphere. The entry of rhTrx was related to increased permeability of endothelial cells during the early reperfusion period after transient focal brain ischemia [95].

Trx1 as neurotrophic cofactor

Nerve growth factor (NGF) is a prototypic neurotrophic factor playing an essential role in the development, maintenance and function of the CNS (for a recent review see: [96, 97]). NGF activates several signaling pathways that culminate in the transcriptional activation of crucial genes, such as *c-fos*, which are required in many aspects of neuronal function [98, 99]. Recent work now suggests that Trx1 is an important player in NGF signaling pathways [100]. Since the neurotrophic cofactor properties of Trx1 have been recently reviewed by Masutani et al. [101], this topic will be discussed here only briefly. A neurotrophic activity associated with a factor identified as Trx1 was first reported by Endoh et al. [102]. More recently, Trx1 was implicated in neurite outgrowth in PC12 cells [100]. NGF-mediated differentiation of PC12 cells was shown to be dependent on catalytically active Trx1 since neurite outgrowth was suppressed by overexpression of an inactive Trx mutant (C32S/C35S). NGF was shown to activate Trx1 expression via cyclic AMP (cAMP)-response elements (CREs) present in the *TRX1* gene promoter, and also to induce nuclear translocation of Trx1 [100]. Trx1 is thought to sustain activation of transcription factors and co-activators such as AP-1 and Ref-1 [100], which are redox-regulated by Trx1 [62, 103, 104] and involved in the differentiation process [105, 106].

Trx1 upregulation in astrocytomas

Little is known of the potential role of Trx1 in CNS malignancies. Trx1 and TrxR1 levels are frequently increased in astrocytomas [45]. Diffuse astrocytomas display a more intense immunostaining for Trx than pylocytic astrocytomas, and this staining was significantly associated with increased malignancy grade. The rise in Trx expression was also correlated with a higher tumor mitotic activity and a worse survival prognosis [45]. These data corroborate previous studies suggesting a link between increased Trx1 expression and cancer progression (review [43]). Multiple neurotrophic and anti-apoptotic signals regulated by Trx1 may favor cancer progression if Trx1 is upregulated. For example, Trx1 is an inhibitor of the tumor suppressor PTEN [107], whose function is often lost in many forms of cancer. PTEN is a negative regulator of AKT kinases, which are involved in the control of several survival pathways and are frequently upregulated in tumors. Loss of PTEN is frequently observed in high-grade astrocytomas and associated with PI3K/AKT signaling upregulation in these tumors (review: [108]).

Trx1 in AD

Among the very few studies available on the expression of Trx cycle enzymes in neurodegenerative processes (see table 1), one report indicated increased Trx1 protein and RNA levels in the grey and white matter of AD brains, this increase being most pronounced in white matter astrocytes [79]. The significance of these observations, however, is not convincing, since only three out of five AD samples analyzed showed increased Trx1 expression as compared to three healthy controls. In contrast, another study involving 10 AD and 10 control subjects reported decreased Trx1 levels in the AD brain, which were observed in various regions including the amygdala and hippocampus/parahippocampal gyrus, where the difference from control subjects reached statistical significance [109]. Interestingly, this study also showed that treatments of primary rat hippocampal cell cultures with exogenous Trx or TrxR from *Escherichia coli* enhanced their survival against β-amyloid cytotoxicity, a pro-oxidant peptide thought to contribute to plaque formation in AD brain. Thus, it was suggested that Trx might play a protective role in AD, and Trx1 deficit [109] might even-

tually contribute to increased oxidative stress and subsequent neurodegeneration in AD. Assessment of Trx1 expression in AD clearly warrants further investigations on a larger scale. The significance of any aberrant expression pattern of Trx cycle enzymes reported in various neurodegenerative conditions, including AD, is not evident. Although most of the reported observations appear to reflect a protective role of the investigated enzyme, recent observations suggest that, in some cases at least, the Trx-related protein may also induce neuronal toxicity [110–112]. It would, in fact, not be surprising that deregulation of any of these enzymes could have either beneficial or detrimental effects on brain cell survival and function, depending on the brain region, cell type and pathology investigated. It is likely that the expression of the Trx cycle enzymes must be tightly regulated in order to maintain optimal brain cell function and to mount appropriate defenses in response to stress conditions.

Trx and prion conformational transformation

The transmissible spongiform encephalopathies are a group of neuropathologies putatively related to the conformational transformation of the soluble α-helix-rich normal cellular prion protein (PrP^{C}) into the pathologic, insoluble β-pleated-sheet-enriched form (PrP^{Sc}). The latter form aggregates and can further propagate the transformation of normal PrP^{C} into PrP^{Sc} in cells. The conversion to the β-pleated-sheet involves the reduction of disulfide bridges, a reaction that can be carried out in vitro by Trx1 [110]. In fact, the Trx1/TrxR1/NADPH system reduces prion protein 7000-fold more efficiently than dithiothreitol (DTT) [110, 111]. However, the Trx1-mediated reduction of PrP^{C} was not sufficient to produce neurotoxic PrP^{Sc}, suggesting the requirement for additional modifications and cofactors for the complete transformation to the scrapie form [110]. Such modifications could be triggered by specific chaperones such as GroEL [113], which normally ensures proper protein folding in bacteria. This chaperone operates in concert with Trx in bacteria [114], suggesting that in mammalian cells Trx1 may also act in concert with similar chaperones in the transformation of PrP^{C} to the neurotoxic PrP^{Sc} form.

Trx1, protein aggregation and PD

The evidence that Trx can play a role in determining the state of prion protein conformation raises the important question of the potential implication of the Trx system in the phenomenon of protein aggregation toxicity underlying many neurodegenerative diseases, including PD, AD and ALS. A common feature of these diseases is the formation of intracytoplasmic proteinaceous inclusions associated with oxidative damage and neuronal death [2]. In PD, various proteins aggregate and produce inclusions called Lewy bodies. The main component of Lewy bodies, α-synuclein, is encoded by a gene, which is mutated in one familial form of PD [115]. The cause of α-synuclein aggregation is not fully understood, but oxidative and nitrative stress was suggested to contribute to this process [13]. Moreover, this process was shown to have a role in the dysfunction of the ubiquitin-proteasome system (UPS) [116]. UPS deregulation was proposed to be involved in PD, as further supported by the identification of PD-associated mutations in UPS genes, such as parkin [117] and DJ-1 [118]. Proteasome inhibition could, in turn, block the degradation of misfolded proteins and thus amplify the neurotoxic aggregation phenomenon [13]. This model is further supported by the protective effects of chaperones observed against α-synuclein neurotoxicity [119].

There is currently no direct evidence that Trx1 can prevent the aggregation process found in PD or other neurodegenerative diseases. Trx1 could potentially prevent protein aggregation by acting in concert with a recently reported multicomponent redox-chaperone network, which controls protein folding/unfolding in prokaryotes [114]. A similar role of Trx in eukaryotes would not be unexpected since Trx1 has a protein-refolding activity on scrambled (mispaired disulfide-containing) RNase A [36], can release the oxidative inhibition of several enzymes [120–122] and has weak protein disulfide isomerase activity [123]. A critical issue will be to find out whether impairment of the Trx system would worsen protein aggregation involved in PD etiology [13], and, conversely, whether boosting Trx systems may prevent it.

Trx reductases

The Trx reductases (TrxRs) belong to the flavoprotein family of pyridine nucleotide disulfide oxydo-reductases [38, 46]. They are homodimeric proteins in which each monomer includes two prosthetic flavin adenine dinucleotide (FAD) groups and a NADPH binding site. All the TrxR isoforms contain a redox center consisting of two cysteines adjacent to the flavin ring of FAD in the N-terminal part of the protein. In contrast to the prokaryotic yeast and *Drosophila* TrxR, which do not contain selenium, the mammalian TrxR isozymes carry a second redox-active center formed by a cysteine-selenocysteine (Cys-Sec) located in the C-terminal part of the protein. These two redox centers are both essential for the catalytic activity of mammalian TrxR toward reduction of oxidized Trx [56, 124–126], and various antioxidant molecules such as lipoic acid, ascorbic acid and ubiquinol [127–130]. In addition, mammalian TrxR enzymes have the capacity to reduce lipid hydroperoxides [131], and peroxynitrite in the presence of Sec [132]. So far, three different isoforms of TrxR have been identified in mam-

malian cells. TrxR1 (also called TRα or TR1) is expressed mainly in the cytosol [133–135]. TrxR2 (also called TRβ or TR3) is a mitochondrial-specific isoform, which is localized to this organelle by an N-terminal targeting sequence [134, 136–140]. TGR (also called TR2 or TrxR3) is a TrxR which has the unusual capacity to reduce both oxidized Trx and GSSG, and is highly expressed in testis [141–143]. In contrast to yeast and mammalian systems, in which cytosolic and mitochondrial TrxR isozymes are encoded by separate genes, a single gene (*Trxr-1*) encodes both TrxR isoforms in the fruitfly, *Drosophila melanogaster* [144]. Targeted mutations leading to separate dysfunction of each isoform demonstrated that both (non-seleno) isozymes perform essential and non-interchangeable biological functions in the fruitfly [144]. Yeast studies have shown that strains lacking the genes encoding cytosolic (*TRR1)* or mitochondrial (*TRR2*) TrxR are viable but become sensitized to oxidative stress induced by H_2O_2 [66, 145]. So far, the effects of loss of TrxR functions in mammalian systems are not known. Transfection of cytosolic TrxR1 in mammalian cells was shown to increase the DNA binding activity of transcription factors, such as AP-1, in response to ionizing radiation [146], and NF-κB in response to tumor necrosis factor-alpha (TNF-α) [147]. Human TrxR1 and TrxR2 both have the capacity of reducing cytochrome c, a process suggested to provide an alternative mechanism for electron transport during oxidative phosphorylation [148]. The physiological significance of this process remains to be determined. The effects of overexpressing TrxR1 or TrxR2 are poorly documented. Reduced growth rates are a characteristic feature of transfectant cells overexpressing TrxR1 [149] or TrxR2 [149–151]. Enhanced TrxR2 activity was recently reported to protect human embryo kidney HEK-293 cells against cytotoxicity induced by antimycin A-mediated mitochondrial complex III inhibition [148]. However, such an effect was not observed in other human, mouse or monkey cell lines, which overexpressed TrxR2 and/or Trx2 [150, 151].

Expression of TrxRs in the CNS

Little information is available on the expression of TrxR isoforms in the CNS. Strong TrxR and Trx1 immunoreactivity was observed in the cytoplasm of neuronal rat cells [152], e.g. Schwann cells and at the nodes of Ranvier [74]. TrxR1 and Trx1 accumulated in the dilated axon upon mechanical nerve injury, and this accumulation could be blocked by microtubule destabilization agents, suggesting anterograde and retrograde transport of these proteins in axons [74] (see table 1). This was the first study suggesting synthesis of Trx and TrxR in nerve cell bodies and their axoplasmic transport in the sciatic nerve. A recent study has documented a multiplex and real-time PCR analysis of the complex patterns of Trx1, Trx2, TrxR1, TrxR2 and glutaredoxin mRNA expressions in various mouse organs [143]. Although brain was one of the organs showing the lowest expression of the three TrxR isoforms, TrxR1 was expressed at the highest level [143]. An in vitro study showed that exposure to the phase II enzyme inducer t-butylhydroquinone induced both cytosolic and mitochondrial TrxR activities and protein contents in cortical astrocytes, but not in cortical neurons isolated from newborn mice [153].

Selenium, TrxRs and brain function

The importance of selenium to human health [154] and its emerging role in brain function have been recently reviewed [155]. Incorporated into proteins as biologically active selenocysteine (Sec), Se is an essential element required for the enzymatic activity and function of 25–30 selenoproteins, which include key antioxidant enzyme isoforms of TrxR and GPx [156, 157]. The unusual redox activities of these selenoproteins result from the unique chemical properties of selenium, which is a better nucleophile than sulfur, and of Sec residues, which are more reactive and have lower redox potentials than Cys residues [158]. The availability of selenium in situ can limit the synthesis of selenoproteins to different levels in various tissues. Brain was found to be the last organ displaying signs of selenium deficit under conditions of dietary selenium deficiency (for review, [159]), suggesting the importance of maintaining a critical minimal level of selenoproteins in the nervous system. The main selenoproteins characterized in mammalian brain include TrxR1 and TrxR2, GPx1 and GPx4, iodothyronine deiodinases 2 and 3, and selenoprotein P. Selenoprotein P was reported to have both an antioxidant activity and a function required for selenium delivery to the brain [160]. At limiting Se concentrations, the selenoproteins are found to be hierarchized with respect to the extent their synthesis/turnover is affected by Se deficiency. For example, TrxR activity in brain is least compromised in conditions of selenium deficiency, in contrast to GPx activity and other organs [161]. This suggests that high-ranking selenoproteins, such as TrxR and GPx4, play a more critical role in the CNS than low-ranking selenoproteins, a suggestion supported by GPx4 gene disruption studies [162]. Dietary supplementation studies have demonstrated various neuroprotective effects of selenium. For example, selenium supplementation was reported to counteract intractable seizures of children presenting systemic selenium deficiency [163, 164], and to reduce the toxicity of methamphetamines [165] and 6-hydroxydopamine [166] in mice. Because selenium supplementation up-regulates the synthesis of several selenoproteins, notably several TrxR and GPx isoforms, such studies are obviously not suitable to assess the contribution of a specific selenoprotein to any observed effect. Alterna-

tive approaches based on models in which a single selenoprotein-encoding gene is either disrupted or up-regulated in vivo or in cultured cells, are a priori more suitable to address the role of a specific selenoprotein. For example, disruption of the gene for selenoprotein P (*Sepp*$^{-/-}$), which caused weight deficits and poor development of motor coordination in the mouse, produced a dramatic decrease of Se levels in brain with a strong concomitant reduction of both GPx and TrxR activities in this organ [167]. This study strongly suggests that selenoprotein P is required to maintain adequate Se levels in brain for downstream synthesis of other critical antioxidant selenoproteins such as GPx and TrxR isoforms. Little is known about the neuroprotective potential of TrxR isoforms in the CNS. Because targeted inactivation of mitochondrial Trx2 causes massive apoptosis, exencephaly and early embryonic lethality in mice [70], we have investigated whether gains of TrxR2 function may inhibit the onset of mitochondria-dependent apoptosis [150]. Unexpectedly, *TrxR2* transfection of mouse neuroblastoma Neuro2A cells, which increased TrxR activity up to sixfold in mitochondria, had no significant effects on their viability and on responses to various pro-oxidant and non-oxidant classic apoptotic inducers, and similar results were obtained with co-transfections of TrxR2 and Trx2 in Neuro2A, human HeLa and monkey Cos-7 cells [150, 151]. The lack of protective effects of TrxR2/Trx2 excess on mitochondria-dependent apoptosis may be due to non-limiting activity of these enzymes in these cells, or that their cofactor NADPH was depleted at the onset of apoptosis [150, 151]. Alternatively, the key determinant of redox-dependent regulation of mitochondrial apoptosis may be another antioxidant enzyme, such as the mitochondrial thioredoxin peroxidase Prx3 [168], the activity of which presumably depends on Trx2/TrxR2 cooperation [50, 52]. It will be of interest to assess the effects of TrxR2 down-regulation in primary neural cell cultures and animal models in which conditional *TrxR2* gene inactivation is targeted to specific neural cell types, including neurons and glial cells.

TrxR in Alzheimer's disease

In contrast to low Trx1 protein levels, TrxR activity was significantly elevated in the amygdala and cerebellum of AD brain [109]. Concomitant with enhanced TrxR activity, other main antioxidant enzymatic activities such as GPx, GSSG reductase, catalase and SOD1 were also found elevated in several regions of AD brain, where lipid peroxidation was most pronounced [169]. This elevation of antioxidant enzyme status was suggested to reflect a compensatory response to counteract increased oxidative stress characterizing this pathology [109, 169]. An analysis of the distinct expression of TrxR1 and TrxR2 isoforms in AD brain has not yet been reported.

The Prxs

The Prxs, also called thioredoxin peroxidases, are a relatively newly discovered family of antioxidant enzymes, most of which use the reducing activity of Trx (or other electron donors) to catalyze the reduction of peroxides, including H_2O_2 and alkyl peroxides [49–53, 57]. The first Prx was identified in yeast [170]. Until now, six Prx subtypes have been identified in mammalian cells on the basis of their amino acid sequence [50]. In addition to their common peroxide scavenging activity, each of them may serve divergent functions associated with various biological processes, including cell proliferation, differentiation and gene expression (reviewed in [49–53, 57]; for a comprehensive description of the Prx nomenclature, see Wang et al. [171] and Wood et al. [53]). All Prxs share the same basic catalytic mechanism, which involves a peroxide-mediated oxidation of an active (peroxidatic) Cys into its sulfenic acid form, and recycling of the sulfenic acid back to a thiol using one of several cell-specific disulfide oxido-reductases, and Trx as a reducing co-factor. Five different Prx isoenzymes have been cloned (Prx 1–5), all of which contain two catalytically active Cys residues and constitute a '2-Cys Prx' class. In contrast, the newly described Prx6 has only one Cys residue involved in peroxidatic activity, thus belonging to a '1-Cys Prx' class. In addition, this second class of Prx cannot use thioredoxin as reducing agent, but can function with other physiological electron donors, such as cyclophilin A, but apparently not with glutathione [172, 173]. Interestingly, in addition to scavenging peroxides, this 1-Cys Prx can also reduce peroxidized membrane phospholipids and was thus suggested to contribute to protect cells against oxidant-induced plasma membrane damage [174].

The Prx1, Prx2 and Prx6 isoforms are mainly localized in the cytosol, but are also found in the nucleus [135, 173, 175, 176], while Prx3 is exclusively localized in the mitochondria [139]. Prx4, which contains an N-terminal secretion signal, is expressed in the endoplasmic reticulum and exported into the extracellular space [177]. Prx5 is found in mitochondria as a long form and in peroxisomes as a short variant [178]. The in vivo antioxidant functions of Prx1, 2 and 6 have been recently highlighted by targeted inactivation of these genes in the mouse. Homozygous mice deficient for these isoforms (–/–) are viable and fertile but develop sensitivity to oxidative stress and show increased oxidative markers in their tissues [171, 179, 180]. Prx1 null mice (–/–) display reduced life span, apparently due to the development of severe hemolytic anemia and appearance of malignant tumors in aging mice, both of which are also observed at increased frequency in heterozygotes as compared to normal Prx1 +/+ mice [179]. Similar to Prx1, Prx2 also appears to play a major role in protecting red blood cells from oxidative stress, since Prx2 null mice also develop hemolytic ane-

mia [179, 180]. Originally named PAG (for proliferation associated gene), Prx1 was identified as a heme-binding protein [181], whose expression is induced in response to serum or oxidative stress [182, 183]. In proliferating cells, the level of Prx1 was found to peak at the entry of S phase, suggesting that Prx1 may be regulated during the cell cycle [183]. Prx1 is inhibited by cyclin-dependant kinase 2-mediated phosphorylation [184], and it inhibits the c-Abl tyrosine kinase by interacting with its SH3-domain, thereby suppressing its cytostatic effect [185]. In addition, Prx1 may play a tumor suppressor role by inhibiting the transcriptional activity of c-Myc and preventing c-Myc-mediated transformation [186].

The mitochondrial Trx-dependent peroxidase Prx3 was recently shown to play a major role in the control of cell survival under stress conditions. Its overexpression was first shown to confer resistance of cancer cells against prooxidant-induced cell death, suggesting that this enzyme has the potential to inhibit H_2O_2-dependent apoptosis [187]. This suggestion was recently confirmed and extended by the finding that depletion of Prx3 by RNA interference in HeLa cells increased intracellular levels of H_2O_2 and sensitized these cells to induction of apoptosis by staurosporine or TNF-α [168]. This work also showed that Prx3 is about 30-fold more abundant than GPx1 in HeLa cell mitochondria and thus could be the most important H_2O_2-detoxifying enzyme in this organelle. Whether this abundance of Prx3 is a general feature of normal mammalian cells, or a peculiarity associated with the cancer phenotype [188, 189] of HeLa cells is not clear. Of interest, this study also showed indirectly that H_2O_2 can be a critical mediator of apoptosis in response to two agents, TNF-α and etoposide, known to stimulate peroxide production in mitochondria.

Expression of Prxs in the CNS

The expression patterns of the six different Prx isozymes show distinct distribution profiles in different brain regions and different cell types. A feature of interest is the cell type-specific expression of Prx1 and Prx6 in astrocytes and glial cells, and of Prx2 in neurons [171, 176, 190, 191]. Prx2 is highly expressed in mouse brain regions, which correspond to human brain regions sensitive to hypoxic and ischemic injury [191]. Expression of Prx3, 4 and 5 isoforms was also detected in the CNS [178, 192, 193], but their relative distribution in different brain regions and cell types has not been documented yet.

A number of studies have shown that several Prx isoforms can be induced in brain in response to various insults, which has suggested neuroprotective function(s) for these proteins in the CNS (see table 1). Prx1 levels were increased specifically in glial cells in response to hemorrhagic and excitotoxic stress [194, 195]. Exposure to hemin, a strong prooxidant, was also shown to induce Prx1 in SH-SY5Y neuronal cells [88]. As for Trx1, the induction of Prx1 appears to involve the transcription factor Nfr2 [88, 196].

Mitochondrial Prx3 mRNA levels were decreased in response to ibotenate-mediated excitotoxicity, in contrast to Cu/ZnSOD and catalase mRNA levels, which increased [197]. Prx3 expression was also reported to be decreased in brain regions known to be specifically affected in AD, Down's syndrome (DS) or PD [198, 199]. Prx3 protein levels in the cerebellum were found to be inversely related to human age [199]. Similarly, a proteomic comparison of old versus young human brain samples revealed decreased Prx2 protein levels in old individuals [200]. These apparent deficits in Prx antioxidant defenses might contribute to increased oxidative stress in the aging brain.

Altered expression of Prxs in neurodegenerative diseases

Recent studies have revealed aberrant patterns of Prx expression in the CNS of patients affected by neurodegenerative disorders (see table 1). A proteomic study reported brain region- and disease-specific increases of Prx2 and Prx1, and, in contrast, decreases of mitochondrial Prx3 in AD and DS brains [198]. A second proteomic study found that Prx2 was significantly increased in frontal cortex of DS, AD and PD, whereas Prx3 was decreased in frontal cortex of DS and PD; Prx6 displayed a significant increase only in PD frontal cortex [199]. Prx2 and Prx6 were also found elevated in Pick's disease [199]. In contrast to Prx2, Prx3 levels were decreased in AD, DS and Pick's disease [198, 199]. The decrease was significant in the thalamus and occipital cortex in AD, and in the frontal cortex in DS [198], and in whole brain of Pick's disease patients [199]. The interpretation of these data is at best speculative. The elevated levels of these Prx subtypes were suggested to reflect an antioxidant defense upregulation in response to increased ROS production, or glial cell proliferation in the case of Prx1. Whether the Prx3 deficits reflect mitochondrial impairment, downregulation of Prx3 expression or simply cell loss is not known. Prx2 was recently found co-localized with GPx1 and SOD-1 in neuronal Lewy body-like hyaline inclusions of spinal cord of familial ALS patients bearing a SOD-1 gene mutation, as well as of transgenic rats expressing the same SOD1 mutation [201]. The observed co-aggregation of these antioxidant enzymes was speculated to amplify oxidative stress and toxicity by causing a breakdown of the redox system [201].

Two-dimensional gel and Western blot analysis of Prx proteins in brains of patients with prion-associated sporadic Creutzfeldt-Jacob disease (sCJD) displayed decreased levels of Prx1 but increased levels of Prx6 in the

frontal cortex, with no significant changes for Prx2 and Prx3 isoforms [202]. In agreement with these observations in sCJD, an increased expression of Prx6 RNA was also observed in mouse brain spongiform degeneration induced by intracerebral prion injection [203]. Prx6 up-regulation was limited to astrocytes in the affected tissues, and no significant changes in Prx1 and Prx3 expression were detected. Whether Prx6 up-regulation reflects increased oxidative stress associated with prion infection, and whether it may play a role in CJD pathogenesis remains to be determined.

Prx1-presenilin-1 interactions

Prx1 (PAG) was recently shown to interact with presenilin-1 (PS-1) [112], a transmembrane protein involved in the cleavage of other intramembranous proteins, such as the β-amyloid precursor protein, Notch and Ire1p. PS-1 is mutated in about 50% of cases of familial AD. Overexpression of Prx1 by plasmid microinjection was found to induce apoptosis in primary cultures of superior cervical ganglion sympathetic (SCG) neurons, and to accelerate SCG neuronal death due to NGF deprivation [112]. Both effects were blocked by co-expression of wild-type PS-1 but not truncated PS-1 mutant lacking exon 10 (PS1dE10), a region which interacts with Prx1 and is mutated in some forms of FAD. However, it is not clear how excess Prx1 causes apoptosis in SCG and how PS-1 protects against Prx1-mediated neuronal death [112].

Prx-mediated neuroprotection

Prx2 overexpression was reported to protect cultured neuronal cells from cell death induced by growth factor depletion [191]. Recently, systemic administration of recombinant Prx5 or adenoviral gene transfer of Prx3 was shown to provide protection against ibotenate-induced excitotoxic stress in the mouse [197, 204]. In the latter model, Prx3 completely inhibited protein nitration and markedly reduced gliosis, a post-neuronal cell death event [197]. In contrast to Prx5-mediated protective effects against ibotenate excitotoxicity, Prx5 provided no protection against excitotoxic stress induced by the AMPA receptor activator S-bromowillardiine [204]. The authors suggested that H_2O_2 could be the dominant ROS produced by NMDA receptor activation, while AMPA-kainate receptor might generate non-peroxide ROS that would not be detoxified efficiently by Prx enzymes. This interpretation is supported by the observation that superoxide rather than peroxide production is associated with apoptotic cell death upon selective activation of AMPA receptors in hippocampal cultures [205].

Concluding remarks and perspectives

A dominant feature of the current state of knowledge is the emerging evidence for neuroprotective roles of Trx in the CNS. A striking example is the neurotrophic activity of Trx1 as a cofactor capable of enhancing NGF effects, a mechanism suspected to help maintain neuronal cholinergic activity and neuronal survival in AD brain [100]. An interesting issue to be investigated is whether Trx could modulate the activities of some other neurotrophic factors such as BDNF and related neurotrophins, since these factors play a crucial role in the maintenance, survival and selective vulnerability of various neuronal populations within the normal and diseased brain [206, 207]. The finding that intravenous administration of recombinant human Trx1 can protect mice against brain damage induced by transient focal cerebral ischemia is of potential interest for the treatment of ischemic stroke [95]. The protective effect of Trx1 in ischemic stress was related to its antioxidant and anti-apoptotic functions, and to suppression of neutrophil chemotaxis and adhesion to endothelial cells [95, 208]. A remarkable observation is that circulating Trx1 is able to permeate the affected ischemic cerebral region through the blood-brain barrier, the permeability of which is increased during ischemic stress [209], as well as in other neurodegenerative conditions (reviewed in: [210]). Although administration of recombinant Trx may offer a new tool for the treatment of pathogen-free disorders involving leukocyte infiltration, therapeutic use of Trx would require caution because it may worsen pathogen-mediated inflammation [208].

Animal models of conditional gene expression are powerful tools for studying the effects of gene inactivation, or overexpression, at specific stages of development and of neurodegenerative diseases. The generation of mouse models in which the expression of cytosolic Trx1 or mitochondrial Trx2 is conditionally suppressed (e.g. by knockout or interference RNA inhibition) or overexpressed (by transgenesis), and targeted to specific brain cells such as motor neurons or glial cells, should help delineate the respective function of each of the Trx isoforms in these cells. In addition, crossings of conditional Trx expression mice with established transgenic mouse models of a given human neurodegenerative disease (e.g. those carrying the G93A SOD1 mutation for ALS) offer an important approach for investigating the role of each Trx isoform in the etiology and evolution of the neurodegenerative process.

Similar to their respective partners Trx1 and Trx2, both cytosolic and mitochondrial TrxR isoforms are likely to be essential for CNS development and function in mammals, although no TrxR1 or TrxR2 knockout studies have yet been reported to prove it. In addition to reducing oxidized Trx1, TrxR1 can regenerate a variety of other antioxidant, and probably neuroprotective, molecules such

as dehydroascorbic acid (DHA), ubiquinone, lipoic acid, and vitamin E via ascorbic acid [127-130]. These beneficial properties warrant further investigations to evaluate relative neuroprotective effects of each TrxR isoform, in relation to the abundance or deficits of these antioxidant molecules. Here again, conditional TrxR inactivation or overexpression targeted to specific brain cells in vivo or ex vivo is expected to help characterize and distinguish the function(s) of each TrxR isoform.

There are good indications that several Prx isoforms such as Prx2, 3 and 5, can contribute additional neuroprotective functions [191, 197, 204]. Of particular interest, the mitochondrial Prx3 protein was shown to be a key regulator of apoptosis [168]. These first observations should be followed up in relevant transgenic or knockout animal models to further assess the respective functions of these Prx isozymes in the CNS. The recently generated Prx1, 2 or 6 knockout mice, which are viable, should be useful tools to investigate the potential roles of these Prx isoforms in neurodegenerative processes [171, 179, 180]. In addition, novel models of cell-type targeted conditional Prx gene inactivations should also help determine the consequences of deregulating the expression of Prx isoforms at different developmental stages or at different stages of a neurodegenerative process. However, the interpretation of potential observations made in such experiments may be limited by the complication of multiple interactions of Prx isoforms with themselves [49, 51–53, 193] and with other protein partners such as c-Abl and PS-1 [51–53, 112, 185, 193].

Elucidation of the multiple protein-partner interactions of the Trx cycle enzymes involved in the redox regulation of fundamental biological processes such as gene expression, cell cycling and survival should help in the understanding of their potential roles in the pathogenesis of neurodegenerative diseases. In the long range, the neuroprotective properties of these redox-sensitive versatile proteins might be used to develop novel treatments for reducing oxidative stress in the CNS, delaying the onset of age-related neurological disorders and retarding their progression.

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