ANTIOXIDANTS & REDOX SIGNALING
Volume 2, Number 4, 2000
Mary Ann Liebert, Inc.

Review Article

The Mitochondrial Thioredoxin System

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ABSTRACT

Eukaryotic organisms from yeast to human possess a mitochondrial thioredoxin system composed of thioredoxin and thioredoxin reductase, similar to the cytosolic thioredoxin system that exists in the same cells. Yeast and mammalian mitochondrial thioredoxins are monomers of approximately 12 kDa and contain the typical conserved active site WCGPC. However, there are important differences between yeast and mammalian mitochondrial thioredoxin reductases that resemble the differences between their cytosolic counterparts. Mammalian mitochondrial thioredoxin reductase is a selenoprotein that forms a homodimer of 55 kDa/subunit; while yeast mitochondrial thioredoxin reductase is a homodimer of 37 kDa/subunit and does not contain selenocysteine. A function of the mitochondrial thioredoxin system is as electron donor for a mitochondrial peroxiredoxin, an enzyme that detoxifies the hydrogen peroxide generated by the mitochondrial metabolism. Experiments with yeast mutants lacking both the mitochondrial thioredoxin system as well as the mitochondrial peroxiredoxin system suggest an important role for mitochondrial thioredoxin, thioredoxin reductase, and peroxiredoxin in the protection against oxidative stress. Antiox. Redox Signal. 2, 801–810.

INTRODUCTION

The cytosolic thioredoxin system

THIOREDOXINS (Trx) are small, globular proteins of 12 kDa that participate in many different cellular functions that are dependent on thiol-disulfide interchange reactions catalyzed by their conserved active site WCGPC, the common characteristic of all thioredoxins (Holmgren, 1989). Thioredoxins are maintained in their active reduced form by the flavoenzyme thioredoxin reductase (TrxR) at the expense of NADPH, which constitutes the so-called thioredoxin system (Holmgren, 1989).

Before the identification of the mitochondrial thioredoxin system, all organisms studied so far, from lower prokaryotes to humans, were shown to contain at least one active thioredoxin system. For example, *Escherichia coli*, a bacterial model, contained one thioredoxin (Trx1) and one thioredoxin reductase. Trx1 and TrxR from this organism constitute the first thioredoxin system characterized as electron donor for the essential enzyme ribonucleotide reductase that converts ribonucleotides to deoxyribonucleotides (Jordan and Reichard, 1998). Apart from being an electron donor for ribonucleotide reductase, Trx1 can also function as electron donor for adenosine 3′-phosphate 5′-phosphosulfate (PAPS) reductase and methionine-sulfoxide reductase and is necessary for the life cycles of some bacteriophages such a T7, M13, and f1 (Chamberlin, 1974; Tsang and Schiff, 1976; Ejiri *et al.*, 1989; Lim *et al.*, 1985; Russel and Model, 1985). Thirty three years later, a second *E. coli* thioredoxin (Trx2) with an extension at the amino terminus was discovered and demonstrated to be responsible

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for the maintenance of the reduced environment of the cytoplasm (Miranda-Vizuete *et al.*, 1997a; Stewart *et al.*, 1998).

The lower eukaryote *Saccharomyces cerevisiae* also contains two thioredoxins (Trx1 and Trx2) and one thioredoxin reductase, all present in the cytoplasm. Trx1 and Trx2 have been implicated in vacuole inheritance, decreasing the rate of DNA synthesis, increasing cell size and generation time, and making the yeast cells auxotrophic for methionine/cysteine (Gan, 1991; Muller, 1991, 1994, 1995; Xu and Wickner, 1996). *E. coli* Trx1 and *S. cerevisiae* Trx1 and Trx2 are very similar in structure and have no additional cysteines other than those at the active site. However, *E. coli* Trx2 contains four cysteine residues at the amino terminus that might modulate its function as regulator of the cytosolic redox environment (Miranda-Vizuete *et al.*, 1997a). The picture becomes more complicated when we go higher in the evolutionary tree. In photosynthetic organisms three types of thioredoxins have been identified: two forms in chloroplasts (f and m) that are involved in regulatory systems in oxygenic photosynthesis and one form in cytosol and endoplasmic reticulum (h) (Besse and Buchanan, 1997). However, mammalian cells have only one thioredoxin and one thioredoxin reductase located in the cytosol (Holmgren and Björnstedt, 1995). Mammalian Trx1 differs from the yeast and bacteria thioredoxins by the presence of additional (structural) cysteine residues that can regulate its enzymatic activity by oxidation and dimer formation (Weichsel *et al.*, 1996).

Many functions have been ascribed to mammalian thioredoxin, including those already described for *E. coli* or yeast protein like electron donor for ribonucleotide reductase or methionine-sulfoxide reductase (Holmgren, 1989). Among the additional functions for mammalian thioredoxins are the modulation of transcription factors DNA binding activity, regulation of cell growth, and apoptosis and antioxidant properties (Schallreuter and Wood, 1986; Spector *et al.*, 1988; Abate *et al.*, 1990; Matthews *et al.*, 1992; Nakamura *et al.*, 1994; Baker *et al.*, 1997; Hirota *et al.*, 1997; Saitoh *et al.*, 1998; Ueno *et al.*, 1999). Mammalian thioredoxin reductases are homodimers (55 kDa/subunit) and contain selenocysteine as the penultimate residue. (Gladyshev *et al.*, 1996; Gromer *et al.*, 1998). This residue, together with the immediate anterior cysteine, constitutes an additional active redox center that has been shown to be necessary for enzymatic activity (Gorlatov and Stadtman, 1998; Gromer *et al.*, 1998; Nordberg *et al.*, 1998; Zhong *et al.*, 1998). Selenocysteine is encoded by an UGA codon that normally works as stop codon. This alternative reading of the genetic code is driven by a palindromic sequence in the 3′-untranslated region (UTR) of the mRNA named SECIS and it is essential to obtain active enzyme (Stadtman, 1996). Indeed, inactivation of this residue by selective alkylation or peptidase treatment abolishes enzymatic activity (Gorlatov and Stadtman, 1998; Gromer *et al.*, 1998; Nordberg *et al.*, 1998; Zhong *et al.*, 1998). A hypothesis of the catalytic mechanism of thioredoxin reductase has been proposed in which the reducing power from NADPH is sequentially transferred to FAD, the active site, and the Cys-Secys pair. This hypothesis also proposes that this last redox center is located in a flexible carboxy-terminal arm of the protein, thus explaining the broad range of substrates of thioredoxin reductase (Gromer *et al.*, 1998). Furthermore, mammalian thioredoxin reductase can also reduce thioredoxin but it can reduce lipid hydroperoxides, the cytotoxic peptide NK-lysin, and other low-molecular-weight metabolites like selenite, selenocysteine, selenoglutathione, vitamin K_3, and alloxan (Björnstedt *et al.*, 1995; Holmgren and Björnstedt, 1995; Andersson *et al.*, 1996).

THE MITOCHONDRIAL THIOREDOXIN SYSTEM

Mammalian mitochondrial thioredoxin

We used degenerate oligonucleotides based on the sequence of the human Trx1 active site for screening of a mammalian cDNA library in an attempt to isolate novel thioredoxins or thioredoxin-like proteins. This approach allowed us to isolate a rat cDNA that coded for a protein with the typical conserved active site of thioredoxins and an amino-terminal extension with high content of positively charged residues and a potential α-helix followed by β-

sheets, both typical features of mitochondrial targeting sequences (MTS) (Spyrou *et al.*, 1997). The prediction that this cDNA codes for a mitochondrial thioredoxin was also supported by evidence reported in 1990 by Bodenstein and Follman. They described a slightly larger form of Trx present in pig heart mitochondria with different electrophoretic mobilities compared with cytosolic Trx1 (Bodenstein and Follman, 1990). We named this protein Trx2 to distinguish it from the cytosolic Trx1 and confirmed its mitochondrial localization by GFP (A.E. Damdimopoulos, in preparation) and Western blot analysis (Spyrou *et al.*, 1997).

So far, homologues of Trx2 have been cloned from human (A.E. Damdimopoulos, in preparation), mouse, and bovine cDNA libraries (Miranda-Vizuete *et al.*, 1997b; Watabe *et al.*, 1997). The mature proteins are very similar (only two amino acids are different); however, the MTS of the four homologues contains a higher number of nonconserved residues (~30%). A potentially important difference between the mitochondrial and the cytosolic thioredoxin is the absence of structural cysteines in Trx2 (Trx1 has two or three depending on the organism). Thus, preincubation with dithiothreitol (DTT) is required to obtain fully active Trx1 by reduction of these structural cysteines, whereas Trx2 activity is independent of DTT treatment (Spyrou *et al.*, 1997). Trx2 mRNA is ubiquitously expressed with higher expression in tissues with a high metabolic rate like testis, skeletal muscle, cerebellum, or heart (Spyrou *et al.*, 1997).

Mammalian mitochondrial thioredoxin reductase

The discovery of a mitochondrial thioredoxin predicted the existence of a thioredoxin reductase in mitochondria to maintain Trx2 in its reduced active form. Several groups identified almost simultaneously human, mouse, rat, and bovine mitochondrial thioredoxin reductase, which we named TrxR2 to distinguish it from the previously characterized TrxR1 (Gasdaska *et al.*, 1999; Lee *et al.*, 1999; Miranda-Vizuete *et al.*, 1999a,b; Sun *et al.*, 1999; Watabe *et al.*, 1999). Similar to Trx2, mitochondrial thioredoxin reductase has an amino-terminal extension with all the above commented features of a MTS. The cleavage of this presequence would result in a 55-kDa mature protein containing the conserved FAD, NADPH, and interface domains. Furthermore, rat and bovine TrxR2 were shown to be homodimers, which is probably also true for the other homologues (Lee *et al.*, 1999; Watabe *et al.*, 1999). A SECIS element found in all TrxR2 homologues identified suggests that all TrxR2 are selenoproteins. So far only the bovine TrxR2 amino acid sequence has shown the presence of selenocysteine (Watabe *et al.*, 1999). TrxR2 mRNA is expressed in all human, mouse, and rat tissues tested with TrxR2 highly expressed in tissues with an intense metabolism like testis or skeletal muscle and thus is similar to mitochondrial thioredoxin (Lee *et al.*, 1999; Miranda-Vizuete *et al.*, 1999a,b).

Genomic organization and chromosomal localization of human mitochondrial Trx2 and TrxR2

The logarithmic increase of entries in the sequence databases as a consequence of large-scale projects to sequence different genomes including the human, has greatly facilitated the determination of the genomic organization of many genes. When we first attempted to identify the genomic organization and the chromosomal localization of human Trx2, this information was not available. Using specific primers for human Trx2 cDNA we amplified the Trx2 gene by PCR from human genomic DNA. The gene spans approximately 13 kb and consists of three exons separated by two introns of 4.2 and 9 kb, respectively. By fluorescence *in situ* hybridization (FISH) analysis, we identified the human Trx2 gene at chromosome 22q12.1–q13 (A.E. Damdimopoulos, in preparation). More recently, genomic clones (accession number HS1119A7) that contained the human Trx2 gene have become available in public databases and they confirm our data.

The genomic organization of the human TrxR2 gene was solved with the help of public sequence databases. Using the full-length human TrxR2 cDNA as template, we identified two overlapping cosmid clones (accession numbers AC000090 and AC000078) with high homology to TrxR2. Computer-assisted analysis confirmed that the whole genomic sequence of

human TrxR2 was contained in these two clones. The human TrxR2 gene consists of 18 exons spanning about 67 kb and, similarly to Trx2, also maps at chromosome 22 but at position 22q11.2 (Miranda-Vizuete *et al.*, 1999a). This synthenic group is maintained also in mouse and rat, thus allowing us to map the mouse TrxR2 gene to chromosome 16 at 11.2 centimorgans from the top linkage group and the rat TrxR2 gene at chromosome 11q23 (Miranda-Vizuete *et al.*, 1999b). Chromosome 22 is the second smallest among the human chromosomes, with the exception of the sex chromosome Y, and is the only one fully sequenced to this date (Dunham *et al.*, 1999). Many genes responsible for different diseases have been mapped at chromosome 22, including several types of tumors, leukemias, neurological and behavioral disorders, *etc.* (Dunham *et al.*, 1999). In particular, the velocardiofacial/DiGeorge syndrome locus is located at position 22q11 and is characterized by cardiovascular malformations, thymic hypoplasia, hypocalcemia due to hypoparathyroidism, and cranofacial and palatal abnormalities (Lindsay and Baldini, 1998). Human TrxR2 maps in the DiGeorge syndrome region and partially overlaps with the catechol-*o*-methyltransferase (*comt*) (Grossman *et al.*, 1992) gene, although in opposite orientation (Miranda-Vizuete *et al.*, 1999b). A *comt* gene knockout mouse lacking exons 2–4 displays a severe behavioral and neurological disorder phenotype (Gogos *et al.*, 1998). It is likely that this construct might also remove part of the putative promoter region of TrxR2 gene. Therefore, it is plausible to think that any of the phenotypes ascribed to the lack of *comt* gene could be in part due to a disregulation of TrxR2, although this hypothesis requires further investigation.

Saccharomyces cerevisiae *mitochondrial thioredoxin system*

Yeast, in particular *Saccharomyces cerevisiae*, is one of the most used living models for the study of cellular processes. In addition to the advantage of simple bacteria-like laboratory protocols, it offers all the advantages of a complete eukaryotic system with all its particular subcellular organelles and metabolism. Furthermore, its genomic sequence has been determined, making the identification of novel genes straightforward. We decided to investigate whether *S. cerevisiae* contained a mitochondrial thioredoxin system similar to the one found in mammalians, with the main objective of finding other functions of the system. We identified two open reading frames (ORF) in *S. cerevisiae* (YCR083W and YHR106W) that displayed high homology with the two yeast cytosolic thioredoxins and thioredoxin reductase (Gan, 1991); they were named Trx3 and Trr2, respectively (Pedrajas *et al.*, 1999). The presence of a MTS at their amino terminus suggested that both proteins are located in the mitochondria, which was later confirmed by Western blot analysis. The yeast mitochondrial Trx3 contained two structural cysteines whereas the yeast cytosolic homologues had none, opposite to the mammalian where mitochondrial Trx2 lacks structural cysteines. Surprisingly, yeast Trx3 activity was not dependent on DTT preincubation and therefore not regulated by redox (Pedrajas *et al.*, 1999). Yeast mitochondrial thioredoxin reductase, Trr2, is closer to yeast Trr1 and prokaryotic homologues than to mammalian ones. It is also a FAD-containing homodimer, but each monomer is smaller than the mammalian, 37 kDa versus 55 kDa, because mammalian thioredoxin reductases have a longer interface domain. More importantly, it does not contain any selenocysteine residue, indicating a different catalytic mechanism compared to mammalian TrxR (Pedrajas *et al.*, 1999).

Yeast mutants lacking both mitochondrial proteins have been studied for their resistance to oxidative stress and, surprisingly, only the mitochondrial thioredoxin reductase mutant was more sensitive to hydrogen peroxide treatment whereas the Trx3 mutant remained unaffected. Furthermore, treatment with CDNB (2,4-dinitrobenzene, reduces glutathione levels) was more toxic in the Trr2 mutant than the wild type or Trx3 mutant (Pedrajas *et al.*, 1999). This scenario resembles the one that predicted the existence of Trx2 in *E. coli* (Derman *et al.*, 1993). A screening of *E. coli* mutants that had a more oxidized cytoplasm than the wild type identified only thioredoxin reductase as the gene responsible for this phenotype, whereas the Trx1 mutant had no effect on the cytoplasmic redox status. Considering that *E. coli* thioredoxin

reductase only reduces thioredoxins, the presence of a novel thioredoxin responsible for the maintenance of a reduced cytosol was proposed. We discovered this new thioredoxin (Trx2) when searching *E. coli* genome for the mitochondrial thioredoxin ancestor (Miranda-Vizuete *et al.*, 1997a) and J. Beckwith's laboratory finally demonstrated its *in vivo* function (Stewart *et al.*, 1998). It is tempting to speculate that, in a similar way to what happens in bacteria, yeast and perhaps higher eukaryotes might contain a second mitochondrial thioredoxin-like protein, which could explain the sensitivity of the Trr2 mutant. Alternatively, mammalian cystolic thioredoxin reductase can act as antioxidant in a thioredoxin-independent way, *e.g.*, as electron donor for plasma glutathione peroxidase, and is able to reduce lipid hydroperoxides (Björnstedt *et al.*, 1994, 1995). This could also be the case for the mitochondrial thioredoxin reductase.

Functions of the mitochondrial thioredoxin system

The majority of ROS (hydrogen peroxide, superoxide anion, hydroxyl radical, and singlet oxygen) are produced in mitochondria because the mitochondrial electron-transport system consumes 85–90% of the oxygen used by the cell (Shigenaga *et al.*, 1994). An abnormal increase of these metabolites promotes cellular damage in several molecular components such as DNA, proteins, and lipids. However, ROS are also regarded as signaling molecules that can modulate the activity of several transcription factors, thus regulating gene expression (Schulze-Osthoff *et al.*, 1993). Therefore, an exquisite regulatory mechanism is present in this organelle to avoid production of ROS in an uncontrolled fashion. The mitochondrial antioxidant defense is comprised of enzymatic systems like glutathione peroxidase, glutathione reductase, manganese-superoxide dismutase (Mn-SOD) (Fridovich 1997; Roveri *et al.*, 1994), and low-molecular weight antioxidants (tocopherols, ascorbate, lipoates, uric acid, and glutathione). SOD dismutates the superoxide radical and produces hydrogen peroxide (H_2O_2), which is removed by catalase. Although catalases remove H_2O_2 in the cytosolic compartment, there has been no report of the presence of catalase in mitochondria, except in heart mitochondria (Radi *et al.*, 1991). Recently, a peroxidase named SP-22, which can reduce H_2O_2 and alkylhydroperoxides, has been identified in bovine mitochondria. SP-22 is a Trx-dependent peroxidase that uses electrons from Trx to remove H_2O_2 and plays an important role in the antioxidant defense in the cardiovascular system (Araki *et al.*, 1999). A mitochondrial thioredoxin-dependent peroxidase (Prx1p) has also been identified and characterized in *S. cerevisiae*, and its function in antioxidant defenses has been studied using yeast PRX1 null strains (Pedrajas *et al.*, 2000; Sung *et al.*, 2000). In experiments using sudden heat shock, which in yeast generates ROS and induces cell death (Davidson *et al.*, 1996), wild-type yeast cells showed a higher survival compared to the isogenic Prx1p null strain. In addition, this strain was more sensitive to H_2O_2 compared to the isogenic wild-type control. Furthermore, the *PRX1* null strain, expressing Prx1p from an episomal plasmid, was considerably more resistant to sudden heat shock as well as H_2O_2. The presence of a mitochondrial thioredoxin-dependent peroxidase/mitochondrial thioredoxin system in both yeast and mammalian cells suggests an important role in the removal of H_2O_2 and a protective role in defense against oxidative stress and cell death and, thus, adds these enzymes to the list of mitochondrial antioxidant enzymatic defenses.

Mitochondria play a pivotal role in the apoptotic process, a form of controlled cell suicide that regulates the number of cells in an organism as well as removal of aged or damaged cells (Kroemer *et al.*, 1998). Although the pathways leading to apoptosis are not fully understood, it is well established that ROS generated in mitochondria participate in this process (Tan, 1998). One mechanism by which ROS induces apoptosis is by opening the mitochondrial permeability transition pore (PT), a dynamic multiprotein complex formed at the contact site between the inner an the outer mitochondrial membranes and implicated in the Ca^{2+} efflux from mitochondria (Zoratti and Szabo, 1995; Crompton, 1999). The opening of this complex has been shown to be modulated by a redox-sensitive dithiol group as well as the redox state

of pyridine nucleotides (Petronilli *et al.*, 1994; Costantini *et al.*, 1996) and mitochondrial thioredoxin reductase seems to play a key role in its regulation (Kim *et al.*, 1996; Rigobello *et al.*, 1998).

However, the idea that the mitochondrial thioredoxin system might have other functions in this organelle is enticing. This speculation can be based on the particular mitochondrial metabolism and several parallels with the different functions of the cytosolic thioredoxin system. For example, formation/reduction of disulfide bonds is a major regulatory mechanism for the function of several proteins and, despite the presence of several antioxidative defense systems in mitochondria, Trx2 is the only known protein that can reduce protein disulfides. Another function of the mitochondrial thioredoxin system could be the regulation of the F0-F1 ATP synthase, a member of the respiratory chain. F0-F1 ATP synthase is organized in two components: the F0 membrane factor, integrated in the inner mitochondrial membrane, and the F1 ATPase, facing the mitochondrial matrix (Hatefi, 1985). A similar enzymatic complex has been described in the inner membrane of plant chloroplasts, and its activity is regulated by thioredoxin (Mills *et al.*, 1995). Several lines of evidence indicate that the mitochondrial ATPase F0-F1 complex is also redox regulated, so, it might be possible that mitochondrial thioredoxin is involved in this mechanism (Falson *et al.*, 1986; Yagi and Hatefi, 1987).

In an attempt to identify more functions of the mitochondrial thioredoxin system, we performed yeast two-hybrid screening using rat Trx2 as bait (A.E. Damdimopoulos, unpublished results) to identify Trx2 interacting proteins. Among the isolated interacting clones, we found two coding for known mitochondrial proteins—serinehydroxy-methyltransferase (SHMT) and cytochrome *c*-oxidase. SHMT is a mitochondrial matrix enzyme that participates in the metabolism of one-carbon units and its activity requires DTT *in vitro* (Garrow *et al.*, 1993). Therefore, it seems plausible that mitochondrial thioredoxin could replace DTT activation *in vivo*. Cytochrome-*c*-oxidase is a member of the respiratory chain, and its activity has also been reported to be regulated by a redox mechanism (Hatefi, 1985). Nitric oxide (NO) and its toxic metabolites can nitrosylate components of the mitochondrial respiratory chain, leading to a cellular energy deficiency state (Ghafourifar and Ritchter, 1997; Heales *et al.*, 1999). Nanomolar concentrations of NO bind to and inactivate reversibly the reduced form of cytochrome *c*-oxidase. Trx2 is likely to revert this nitrosylation as well as nitrosylation of other mitochondrial proteins since such a function *in vitro* for Trx1 was demonstrated (Nikotovic *et al.*, 1998). Immunoelectron microscopy analysis identifies Trx2 as a mitochondrial matrix protein attached to the inner membrane (M. Pelto-Hiukko *et al*, in preparation). This location strengthens the idea of Trx2 being a regulator of cytochrome *c*-oxidase and thus involved in the respiratory process.

Neurodegenerative diseases have widely disparate genetic aetiologies but share mitochondrial dysfunction as a final common pathway (Flint Beal *et al.*, 1997). Indirect evidence for an important role of the mitochondrial thioredoxin system in the pathophysiology of these diseases is provided from studies of Trx2 distribution in the rat brain using *in situ* hybridization and immunohistochemistry (Rybnikova *et al.*, 2000). Trx2 mRNA and protein were highly expressed in the neurons in several brain regions, including the olfactory bulb, the frontal cortex, the hippocampus, some hypothalamic and thalamic nuclei, the cerebellum, and numerous brainstem nuclei. Thus, the most prominent Trx2 expression in the brain was seen in areas with most intensive free radical production. The susceptibility of cells to NO toxicity is dependent on their antixoidant capacity, including the intracellular GSH concentration (Walker *et al.*, 1995). Therefore, it has been assumed that neurons are essentially more vulnerable to the toxic effects of NO because GSH is mostly found in glia, but not in neurons (Raps *et al.*, 1989; Philbert *et al.*, 1991). The presence of Trx2 in neurons, suggests that it might provide the major neuronal defense against NO toxicity.

Expression of Trx2 is high in the substantia nigra (Rybnikova *et al.*, 2000), a region with elevated concentrations of monoamine oxidase and transition metals (iron and copper), that also contribute to the production of free radicals (Riederer *et al.*, 1989; Jellinger, 1999). There are several reports that Parkinson's disease

(PD), which involves loss of dopaminergic neurons in the substantia nigra, is caused by an increase in oxidative stress and subsequent impairment of mitochondrial respiration (Shapira, 1994; Tritschler *et al.*, 1994; Zeevalk *et al.*, 1998). Thus, it is tempting to speculate that Trx2 plays a role in PD. Redox control provides the cell with a mechanism by which it can respond to changes in its environment through the modulation of the activity of certain genes, ultimately leading to an alteration in cell proliferation or cell death. The thioredoxin system plays a central role in this control and in protection against oxidative stress.

A new thioredoxin system has been identified in mitochondria of lower eukaryotes and in mammalian cells. Understanding its mechanism of action will provide insights for protection against oxidative stress induced cytotoxicity and tissue damage.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council (Project 13X-10370), Karolinska Institutet, Åke Winbergs Stiftelse, the TMR Marie Curie Research Training Grants (contract ERBFMBICT972824), Spanish Ministerio de Educación y Cultura, and the Södertörns Högskola.

ABBREVIATIONS

FISH, Fluorescence *in situ* hybridization; MTS, mitochondrial targeting sequence; ORF, open reading frame; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; ROS, reactive oxygen species; SECIS, selenocysteine insertion sequence; SHMT, serine-hydroxymethyltransferase; TPA, 12-O-tetradecanoyl-1,2-phorbol 13 acetate; Trx, thioredoxin; TrxR, thioredoxin reductase; UTR, untranslated region.

REFERENCES

ABATE, C., PATEL, L., RAUSCHER, F.J., and CURRAN, T. (1990). Redox regulation of fos and jun DNA-binding activity in vitro. Science **249,** 1157–1161.

ANDERSSON, M., HOLMGREN, A., and SPYROU, G. (1996). NK-lysin, a disulfide containing effector peptide of T-lymphocytes is reduced and inactivated by human thioredoxin reductase: Implication for a protective mechanism against NK-lysin cytotoxicity. J. Biol. Chem. **271,** 10116–10120.

ARAKI, M., NANRI, H., EJIMA, K., MURASATO, Y., FUJIWARA, T., NAKASHIMA, Y., and IKEDA, M. (1999). Antioxidant function of the mitochondrial protein SP-22 in the cardiovascular system. J. Biol. Chem. **274,** 2271–2278.

BAKER, A., PAYNE, C.M., BRIEHL, M.M., and POWIS, G. (1997). Thioredoxin, a gene found overexpressed in human cancer, inhibits apoptosis *in vivo* and *in vitro*. Cancer Res. **57,** 5162–5167.

BESSE, I., and BUCHANAN, B.B. (1997). Thioredoxin-linked plant and animal processes: The new generation. Botanical Bulletin of Academia Sinica (Taipei). **38,** 1–11.

BJÖRNSTEDT, M., XUE, J., HUANG, W., AKESSON, B., and HOLMGREN, A. (1994). The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. J. Biol. Chem. **269,** 29382–29384.

BJÖRNSTEDT, M., HAMBERG, M., KUMAR, S., XUE, J., and HOLMGREN, A. (1995). Human thioredoxin reductase directly reduces lipid hydroperoxides by NADPH and selenocystine strongly stimulates the reaction via catalytically generated selenols. J. Biol. Chem. **270,** 11761–11764.

BODENSTEIN, J., and FOLLMAN, H. (1990). Characterization of two thioredoxins in pig heart including a new mitochondrial protein. Z. Naturforsch. **46c,** 270–279.

CHAMBERLIN, M. (1974). Isolation and characterization of prototrophic mutants of Escherichia coli unable to support the intracellular growth of T7. J. Virol. **14,** 509–516.

COSTANTINI, P., CHERNYAK, B.V., SCORRANO, L., PETRONILLI, V., and BERNARDI, P. (1996). Modulation of the mitochondrial permeability transition pore by pyridine nucleotides and dithiol oxidation at two separates sites. J. Biol. Chem. **271,** 6746–6751.

CROMPTON, M. (1999). The mitochondrial permeability transition pore and its role in cell death. Biochem. J. **341,** 233–249.

DAVIDSON, J.F., WHYTE, B., BISSINGER, P.H., and SCHIESTL, R.H. (1996). Oxidative stress is involved in heat-induced cell death in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **93,** 5116–5121.

DERMAN, A.I., PRINZ, W.A., BELIN, D., and BECKWITH, J. (1993). Mutations that allow disulfide bond formation in the cytoplasm of *Escherichia coli*. Science **262,** 1744–1747.

DUNHAM, L., SHIMIZU, N., ROE, B.A., and CHISSOE, S. (1999). The DNA sequence of human chromosome 22. Nature **402,** 489–495.

EJIRI, S.L., WEISSBACH, H., and BROT, N. (1979). Reduction of methionine sulfoxide to methionine by Escherichia coli. J. Bacteriol. **139,** 161–164.

FALSON, P., DI PIETRO, A., and GAUTHERON, D.C. (1986). Chemical modification of thiol groups of mito-

chondrial F1-ATPase from the yeast *Schizosaccharomyces pombe*. Involvement of alpha- and gamma-subunits in the enzymatic activity. J. Biol. Chem. **261,** 7151–7159.

FLINT BEAL, M., HOWELL, N., and BODIS-WOLLNER, I. (1997). *Mitochondria and Free Radicals in Neurodegenerative Diseases*. (John Wiley & Sons, New York).

FRIDOVICH, I. (1997). Superoxide anion radical (O2-.), superoxide dismutases, and related matters. J. Biol. Chem. **22,** 18515–18517.

GAN, Z.R. (1991). Yeast thioredoxin genes. J. Biol. Chem. **266,** 1692–1696.

GARROW, T.A., BRENNER, A.A., WHITEHEAD, V.M., CHEN, X.N., DUNCAN, R.G., KORENBERG, J.R., and SHANE, B. (1993). Cloning of human cDNAs encoding mitochondrial and cytosolic serine hydroxymethyltransferase and chromosomal localization. J. Biol. Chem. **268,** 11910–11916.

GASDASKA, P.Y., BERGGREN, M.M., BERRY, M.J., and POWIS, G. (1999). Cloning, sequencing and functional expression of a novel human thioredoxin reductase. FEBS Lett. **422,** 105–111.

GHAFOURIFAR, P., and RITCHTER, C. (1997). Nitric oxide synthase activity in mitochondria. FEBS Lett. **418,** 291–296.

GLADYSHEV, V.N., JEANG, K.-T., and STADTMAN, T.C. (1996). Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, correspond to TGA in the human placental gene. Proc. Natl. Acad. Sci. USA **93,** 6146–6151.

GOGOS, J.A., MORGAN, M., LUINE, V., SANTHA, M., OGAWA, S., PFAFF, D., and KARAYIORGOU, M. (1998). Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc. Natl. Acad. Sci. USA **95,** 9991–9996.

GORLATOV, S.N., and STADTMAN, T.C. (1998). Human thioredoxin reductase from HeLa cells: selective alkylation of selenocysteine in the protein inhibits enzymatic activity and reduction with NADPH influences affinity to heparin. Proc. Natl. Acad. Sci. USA **95,** 8520–8525.

GROMER, S., WISSING, J., BEHNE, D., ASHMAN, K., SCHIRMER, R.H., FLOHÉ, L., and BECKER, K. (1998). A hypothesis on the catalytic mechanism of the selenoenzyme thioredoxin reductase. Biochem. J. **332,** 591–592.

GROSSMAN, M.H., EMANUEL, B.S., and BUDARF, M.L. (1992). Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1-q11.2. Genomics **12,** 822–825.

HATEFI, Y. (1985). The mitochondrial electron transport and oxidative phosphorylation system. Annu. Rev. Biochem. **54,** 1015–1069.

HEALES, S.J., BOLANOS, J.P., STEWART, V.C., BROOKES, P.S., LAND, J.M., and CLARK, J.B. (1999). Nitric oxide, mitochondria and neurological disease. Biochim. Biophys. Acta **1410,** 215–228.

HIROTA, K., MATSUI, M., IWATA, S., NISHIYAMA, A., MORI, K., and YODOI, J. (1997). AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. Proc. Natl. Acad. Sci. USA **94,** 3633–3638.

HOLMGREN, A. (1989). Thioredoxin and glutaredoxin systems. J. Biol. Chem. **264,** 13963–13966.

HOLMGREN, A., and BJÖRNSTEDT, M. (1995). Thioredoxin and thioredoxin reductase. Methods Enzymol. **252,** 199–208.

JELLINGER, K.A. (1999). The role of iron in neurodegeneration: prospects for pharmacotherapy of Parkinson's disease. Drugs Aging **14,** 115–140.

JORDAN, A., and REICHARD, P. (1998). Ribonucleotide reductases. Annu. Rev. Biochem. **67,** 71–98.

KIM, K.J., JANG, Y.Y., HAN, E.S., and LEE, C.S. (1996). Modulation of brain mitochondrial membrane permeability and synaptosomal Ca^{2+} transport by dopamine oxidation. Mol. Cell Biochem. **20,** 89–98.

KROEMER, G., DALLAPORTA, B., and RESCHE-RIGON, M. (1998). The mitochondrial death/life regulator in apoptosis and necrosis. Annu. Rev. Physiol. **60,** 619–642.

LEE, S.-R., KIM, J.-R., KWON, K.-S., YOON, H.W., LEVINE, R.L., GINSBURG, A., and RHEE, S.G. (1999). Molecular cloning and characterization of a mitochondrial selenocysteine-containing thioredoxin reductase from rat liver. J. Biol. Chem. **274,** 4722–4734.

LIM, C.J., HALLER, B., and FUCHS, J.A. (1985). Thioredoxin is the bacterial protein encoded by fip that is required for filamentous bacteriophage f1 assembly. J. Bacteriol. **161,** 799–802.

LINDSAY, E.A., and BALDINI, A. (1998). Congenital heart defects and 22q11 deletions: which genes count? Mol. Med. Today **4,** 350–357.

MATTHEWS, J.R., WAKASUGI, N., VIRELIZER, J.L., YODOI, J., and HAY, R.T. (1992). Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulphide bond involving cysteine 62. Nucleic Acids Res. **20,** 3821–3830.

MILLS, J.D., AUSTIN, P.A., and TURTON, J.S. (1995). Thioredoxin and control of F0F1: function and distribution. Biochem. Soc. Trans. **23,** 775–780.

MIRANDA-VIZUETE, A., DAMDIMOPOULOS, A.E., GUSTAFSSON, J.-Å., and SPYROU, G. (1997a). Cloning, expression and characterization of a novel *Escherichia coli* thioredoxin. J. Biol. Chem. **272,** 30841–30847.

MIRANDA-VIZUETE, A., GUSTAFSSON, J.-A., and SPYROU, G. (1997b). NCBI database entry no. MMU85089.

MIRANDA-VIZUETE, A., DAMDIMOPOULOS, A.E., PEDRAJAS, J.R., GUSTAFSSON, J.-Å., and SPYROU, G. (1999a). Human mitochondrial thioredoxin reductase. cDNA cloning, expression and genomic characterization. Eur. J. Biochem. **261,** 405–412.

MIRANDA-VIZUETE, A., DAMDIMOPOULOS, A.E., and SPYROU, G. (1999b). cDNA cloning, expression and chromosomal localization of the mouse mitochondrial thioredoxin reductase. Biochem. Biophys. Acta **1447,** 113–118.

MULLER, E.G.D. (1991). Thioredoxin deficiency in yeast prolongs S phase and shortens the G1 interval of the cell cycle. J. Biol. Chem. **266,** 9194–9202.

MULLER, E.G.D. (1994). Deoxyribonucleotides are maintained at normal levels in a yeast thioredoxin mutant defective in DNA synthesis. J. Biol. Chem. **269,** 24466–24471.

MULLER, E.G.D. (1995). A redox-dependent function of thioredoxin is necessary to sustain a rapid rate of DNA synthesis in yeast. Arch. Biochem. Biophys. **318,** 356–361.

NAKAMURA, H., MATSUDA, M., FURUKE, K., KITAOKA, Y., IWATA, S., TODA, K., INAMOTO, T., YAMAOKA, Y., OZAWA, K., and YODOI, J. (1994). Adult T cell leukemia-derived factor/human thioredoxin protects endothelial F-2 cell injury caused by activated neutrophils or hydrogen peroxide. Immunol. Lett. **42,** 75–80.

NIKITOVIC, D., HOLMGREN, A., and SPYROU, G. (1998). Inhibition of AP-1 DNA binding by nitric oxide involving conserved cysteine residues in Jun and Fos. Biochem. Biophys. Res. Commun. **242,** 109–112.

NORDBERG, J., ZHONG, L., HOLMGREN, A., and ARNER, E.S.J. (1998). Mammalian thioredoxin reductase is irreversibly inhibited by dinitrohalobenzenes by alkylation of both the redox active selenocysteine and its neighboring cysteine residue. J. Biol. Chem. **273,** 10835–10842.

PEDRAJAS, J.R., KOSMIDOU, E., MIRANDA-VIZUETE, A., GUSTAFSSON, J.-Å., WRIGHT, A.P.H., and SPYROU, G. (1999). Identification and functional characterization of a novel mitochondrial thioredoxin system in *Saccharomyces cerevisiae*. J. Biol. Chem. **274,** 6366–6373.

PEDRAJAS, J.R., MIRANDA-VIZUETE, A., JAVANMARDY, N., GUSTAFSSON, J.-Å., and SPYROU, G. (2000). Mitochondria of *Saccharomyces cerevisiae* contain one-conserved cysteine type peroxiredoxin with thioredoxin peroxidase activity. J. Biol. Chem. **275,** 16296–16301.

PETRONILLI, V., COSTANTINI, P., SCORRANO, L., COLONNA, R., PASSAMONTI, S., and BERNARDI, P. (1994). The voltage sensor of the mitochondrial permeability transition pore is tuned by the oxidation-reduction state of vicinal thiols. Increase of the gating potential by oxidants and its reversal by reducing agents. J. Biol. Chem. **269,** 16638–16642.

PHILBERT, M.A., BEISWANGER, C.M., WATERS, D.K., REUHL, K.R., and LOWNDES, H.E. (1991). Cellular and regional distribution of reduced glutathione in the nervous system of the rat: histochemical localization by mercury orange and *o*-phthaldialdehyde-induced histofluorescence. Toxicol. Appl. Pharmacol. **107,** 215–227.

RADI, R., TURRENS, J.F., CHANG, L.Y., BUSH, K.M., CRAPO, J.D., and FREEMAN, B.A. (1991). Detection of catalase in rat heart mitochondria. J. Biol. Chem. **266,** 22028–22034.

RAPS, S.P., LAI, J.C.K., HERTZ, L., and COOPER, A.J.L. (1989). Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. Brain Res. **493,** 398–401.

RIEDERER, P., SOFIC, E., RAUSCH, W.D., SCHMIDT, B., REYNOLDS, G.P., JELLINGER, K., and YOUDIM, B.H. (1989). Transition metals, ferritin, glutathione, and ascorbic acid in Parkinsonian brains. J. Neurochem. **52,** 515–520.

RIGOBELLO, M.P., CALLEGARO, M.T., BARZON, E., BENETTI, M., and BINDOLI, A. (1998). Purification of mitochondrial thioredoxin reductase and its involvement in the redox regulation of membrane permeability. Free Rad. Biol. Med. **24,** 370–376.

ROVERI, A., MAIORINO, M., and URSINI, F. (1994). Purification and characterization of phospholipid hydroperoxide glutathione peroxidase from rat testis mitochondrial membranes. Biochim. Biophys. Acta **1208,** 211–221.

RYBNIKOVA, E., DAMDIMOPOULOS, A.E., GUSTAFSSON, J.-Å., SPYROU, G., and PELTO-HUIKKO, M. (2000). Expression of novel antioxidant thioredoxin-2 in the rat brain. Eur. J. Neuroscience **12,** 1669–1678.

RUSSEL, M., and MODEL, P. (1985). Thioredoxin is required for filamentous phage assembly. Proc. Natl. Acad. Sci. USA **82,** 29–33.

SAITOH, M., NISHITOH, H., FUJII, M., TAKEDA, K., TOBIUME, K., SAWADA, Y., KAWABATA, M., MIYAZONO, K., and ICHIJO, H. (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J. **17,** 2596–2606.

SCHALLREUTER, K.U., and WOOD, J.M. (1986). The role of thioredoxin reductase in the reduction of free radicals at the surface of the epidermis. Biochem. Biophys. Res. Commun. **136,** 630–637.

SCHULZE-OSTHOFF, K., BEYAERT, R., VANDEVOORDE, V., HAEGEMAN, G., and FIERS, W. (1993). Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO J. **12,** 3095–3104.

SHAPIRA, A.H.V. (1994). Evidence for mitochondrial dysfunction in Parkinson's disease—a critical appraisal. Mov. Disord. **9,** 125–138.

SHIGENAGA, M.K., HAGEN, T.M., and AMES, B.N. (1994). Oxidative damage and mitochondrial decay in aging. Proc. Natl. Acad. Sci. USA **91,** 10771–10778.

SPECTOR, A., YAN, G.-Z., HUANG, R.-R.C., McDERMOT, M.J., GASCOYNE, P.R.C., and PIGIET, V. (1988). The effect of H2O2 upon thioredoxin-enriched lens epithelial cells. J. Biol. Chem. **263,** 4984–4900.

SPYROU, G., ENMARK, E., MIRANDA-VIZUETE, A., and GUSTAFSSON, J.-Å. (1997). Cloning and expression of a novel mammalian thioredoxin. J. Biol. Chem. **272,** 2936–2941.

STADTMAN, T.C. (1996). Selenocysteine. Annu. Rev. Biochem. **65,** 83–100.

STEWART, E.J., ASLUND, F., and BECKWITH, J. (1998). Disulfide bond formation in the Escherichia coli cytoplasm: an in vivo role reversal for the thioredoxins. EMBO J. **17,** 5543–5550.

SUN, Q.A., WU, Y., ZAPPACOSTA, F., JEANG, K.T., LEE, B.J., HATFIELD, D.L., and GLADYSHEV, V.N. (1999). Redox regulation of cell signaling by selenocysteine in mammalian thioredoxin reductases. J. Biol. Chem. **274,** 24522–24530.

SUNG GOO PARK, MEE-KYUNG CHA, WOOJIN JEONG, and IL-HAN KIM. (2000). Distinct Physiolog-

ical Functions of Thiol Peroxidase Isoenzymes in Saccharomyces cerevisiae. J. Biol. Chem. **275,** 5723–5732.

TAN, S. (1998). The regulation of reactive oxygen species production during programmed cell death. J. Cell Biol. **141,** 1423–1432.

TRITSCHLER, H.J., PACKER, L., and MEDORI, R. (1994). Oxidative stress and mitochondrial dysfunction in neurodegeneration. Biochem. Mol. Biol. Int. **34,** 169–181.

TSANG, M.L., and SCHIFF, J.A. (1976). Sulfate reducing pathway in Escherichia coli involving bound intermediates. J. Bacteriol. **125,** 923–933.

UENO, M., MASUTANI, H., ARAI, R.J., YAMAUCHI, A., HIROTA, K., SAKAI, T., INAMOTO, T., YAMAOKA, Y., YODOI, J., and NIKAIDO, T. (1999). Thioredoxin-dependent redox regulation of p53-mediated p21 activation. J. Biol. Chem. **274,** 35809–35815.

WALKER, M.W., KINTER, M.T., ROBERTS, D.R., and SPITZ, D.R. (1995). Nitric oxide-induced cytotoxicity: involvement of cellular resistance to oxidative stress and the role of glutathione in protection. Pediatr. Res. **37,** 41–49.

WATABE, S., HIROI, T., YAMAMOTO, Y., FUJIOKA, Y., HASEGAWA, H., and YAGO, N. (1997). SP-22 is a thioredoxin-dependent peroxide reductase in mitochondria. Eur. J. Biochem. **249,** 52–60.

WATABE, S., MAKINO, Y., OGAWA, K., HIROI, T., YAMAMOTO, Y., and TAKAHASHI, S.Y. (1999). Mitochondrial thioredoxin reductase in bovine adrenal cortes its purification, nucleotide/amino acid sequences, and identification of selenocysteine. Eur. J. Biochem. **264,** 74–84.

WEICHSEL, A., GASDASKA, J.R., POWIS, G., and MONTFORT, W.R. (1996). Crystal structures of reduced, oxidized and mutated human thioredoxins: evidence for a regulatory homodimer. Structure **4,** 735–751.

XU, Z., and WICKNER, W. (1996). Thioredoxin is required for vacuole inheritance in Saccharomyces cerevisiae. J. Cell. Biol. **132,** 787–794.

YAGI, T., and HATEFI, Y. (1987). Thiols in oxidative phosphorylation: thiols in the F0 of ATP synthase essential for ATPase activity. Arch. Biochem. Biophys. **254,** 102–109.

ZEEVALK, G.D., BERNARD, L.P., and NIKLAS, W.J. (1998). Role of oxidative stress and the glutathione system in loss of dopamine neurons due to impairment of energy metabolism. J. Neurochem. **70,** 1421–1430.

ZHONG, L., ARNER, E.S.J., LJUNG, J., ÅSLUND, F., and HOLMGREN, A. (1998). Rat and calf thioredoxin reductase are homologous to glutathione reductase with a carboxy-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. J. Biol. Chem. **273,** 8581–8591.

ZORATTI, M., and SZABO, I. (1995). The mitochondrial permeability transition. Biochem. Biophys. Acta **1241,** 139–176.

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Received for publication March 31, 2000; accepted May 2, 2000.

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3. Ivan Dimauro, Timothy Pearson, Daniela Caporossi, Malcolm J. Jackson. 2012. In vitro susceptibility of thioredoxins and glutathione to redox modification and ageing-related changes in skeletal muscle. *Free Radical Biology and Medicine* . [CrossRef]
4. Alexandra S. Bause, Marcia C. Haigis. 2012. SIRT3 regulation of mitochondrial oxidative stress. *Experimental Gerontology* . [CrossRef]
5. Briseida Cacho-Valadez , Fernando Muñoz-Lobato , José Rafael Pedrajas , Juan Cabello , Juan Carlos Fierro-González , Plácido Navas , Peter Swoboda , Chris D. Link , Antonio Miranda-Vizuete . 2012. The Characterization of the Caenorhabditis elegans Mitochondrial Thioredoxin System Uncovers an Unexpected Protective Role of Thioredoxin Reductase 2 in #-Amyloid Peptide Toxicity. *Antioxidants & Redox Signaling* **16**:12, 1384-1400. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
6. Fei Yin , Harsh Sancheti , Enrique Cadenas . Mitochondrial Thiols in the Regulation of Cell Death Pathways. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Chengwen Song, Zhaoxia Cui, Yuan Liu, Shuangyan Wang, Qianqian Li. 2012. First report of two thioredoxin homologues in crustaceans: Molecular characterization, genomic organization and expression pattern in swimming crab Portunus trituberculatus. *Fish & Shellfish Immunology* **32**:5, 855-861. [CrossRef]
8. Tat'yana Trendeleva, Evgeniya Sukhanova, Ludmila Ural'skaya, Nils-Erik Saris, Renata Zvyagilskaya. 2011. Effect of prooxidants on yeast mitochondria. *Journal of Bioenergetics and Biomembranes* . [CrossRef]
9. Marika Lindahl , Alejandro Mata-Cabana , Thomas Kieselbach . 2011. The Disulfide Proteome and Other Reactive Cysteine Proteomes: Analysis and Functional Significance. *Antioxidants & Redox Signaling* **14**:12, 2581-2642. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
10. Damon A. Lowes, Helen F. Galley. 2011. Mitochondrial protection by the thioredoxin-2 and glutathione systems in an in vitro endothelial model of sepsis. *Biochemical Journal* **436**:1, 123-132. [CrossRef]
11. Johanna Engelhard, Brooke E. Christian, Lars Weingarten, Gabriele Kuntz, Linda L. Spremulli, Tobias P. Dick. 2011. In situ kinetic trapping reveals a fingerprint of reversible protein thiol oxidation in the mitochondrial matrix. *Free Radical Biology and Medicine* **50**:10, 1234-1241. [CrossRef]
12. Douglas B. Murray, Ken Haynes, Masaru Tomita. 2011. Redox regulation in respiring Saccharomyces cerevisiae. *Biochimica et Biophysica Acta (BBA) - General Subjects* . [CrossRef]
13. Yuan Liu, Zhaoxia Cui, Chengwen Song, Shuangyan Wang, Qianqian Li. 2011. Multiple isoforms of immune-related genes from hemocytes and eyestalk cDNA libraries of swimming crab Portunus trituberculatus. *Fish & Shellfish Immunology* . [CrossRef]
14. Victor Okoh, Alok Deoraj, Deodutta Roy. 2011. Estrogen-induced reactive oxygen species-mediated signalings contribute to breast cancer. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* **1815**:1, 115-133. [CrossRef]
15. Manabu Tsuda, Ryousuke Ootaka, Chiaki Ohkura, Yoshihito Kishita, Ki-Hyeon Seong, Takashi Matsuo, Toshiro Aigaki. 2010. Loss of Trx-2 enhances oxidative stress-dependent phenotypes in Drosophila. *FEBS Letters* **584**:15, 3398-3401. [CrossRef]
16. Yves Meyer, Bob B. Buchanan, Florence Vignols, Jean-Philippe Reichheld. 2009. Thioredoxins and Glutaredoxins: Unifying Elements in Redox Biology. *Annual Review of Genetics* **43**:1, 335-367. [CrossRef]
17. Anna#Maria G. Psarra, Stefan Hermann, George Panayotou, Giannis Spyrou. 2009. Interaction of mitochondrial thioredoxin with glucocorticoid receptor and NF-#B modulates glucocorticoid receptor and NF-#B signalling in HEK-293 cells. *Biochemical Journal* **422**:3, 521-531. [CrossRef]

18. Subhankar Dolai, Rajesh K. Yadav, Swati Pal, Subrata Adak. 2008. Leishmania major ascorbate peroxidase overexpression protects cells against reactive oxygen species-mediated cardiolipin oxidation. *Free Radical Biology and Medicine* **45**:11, 1520-1529. [CrossRef]
19. N MAULIK, D DAS. 2008. Emerging potential of thioredoxin and thioredoxin interacting proteins in various disease conditions. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1780**:11, 1368-1382. [CrossRef]
20. Eun Sun Yang, Jin Hyup Lee, Jeen-Woo Park. 2008. Ethanol induces peroxynitrite-mediated toxicity through inactivation of NADP+-dependent isocitrate dehydrogenase and superoxide dismutase. *Biochimie* **90**:9, 1316-1324. [CrossRef]
21. F. Auchere, R. Santos, S. Planamente, E. Lesuisse, J.-M. Camadro. 2008. Glutathione-dependent redox status of frataxin-deficient cells in a yeast model of Friedreich's ataxia. *Human Molecular Genetics* **17**:18, 2790-2802. [CrossRef]
22. Yusuke Demizu , Ryohei Sasaki , Dunyaporn Trachootham , Helene Pelicano , Justin A. Colacino , Jinsong Liu , Peng Huang . 2008. Alterations of Cellular Redox State During NNK-Induced Malignant Transformation and Resistance to Radiation. *Antioxidants & Redox Signaling* **10**:5, 951-962. [Abstract] [Full Text PDF] [Full Text PDF with Links]
23. S SHIN, I KIL, J PARK. 2008. Silencing of mitochondrial NADP+-dependent isocitrate dehydrogenase by small interfering RNA enhances heat shock-induced apoptosis. *Biochemical and Biophysical Research Communications* **366**:4, 1012-1018. [CrossRef]
24. Jolanta Sroka, Agata Antosik, Jaros#aw Czy#, Ivan Nalvarte, Jerker M. Olsson, Giannis Spyrou, Zbigniew Madeja. 2007. Overexpression of thioredoxin reductase 1 inhibits migration of HEK-293 cells. *Biology of the Cell* **99**:12, 677. [CrossRef]
25. L LI, W SHOJI, H TAKANO, N NISHIMURA, Y AOKI, R TAKAHASHI, S GOTO, T KAIFU, T TAKAI, M OBINATA. 2007. Increased susceptibility of MER5 (peroxiredoxin III) knockout mice to LPS-induced oxidative stress. *Biochemical and Biophysical Research Communications* **355**:3, 715-721. [CrossRef]
26. Malin J. Svensson, Jan Larsson. 2007. Thioredoxin-2 affects lifespan and oxidative stress in Drosophila. *Hereditas* **144**:1, 25-32. [CrossRef]
27. Nicolas Navrot, Nicolas Rouhier, Eric Gelhaye, Jean-Pierre Jacquot. 2007. Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiologia Plantarum* **129**:1, 185-195. [CrossRef]
28. Stéphane Duvezin-Caubet, Andreas S. Reichert, Walter Neupert19 Analysis of Gene Function of Mitochondria **36**, 445-489. [CrossRef]
29. Sabine Urig, Katja Becker. 2006. On the potential of thioredoxin reductase inhibitors for cancer therapy. *Seminars in Cancer Biology* **16**:6, 452-465. [CrossRef]
30. V KAGAN, Y TYURINA, H BAYIR, C CHU, A KAPRALOV, I VLASOVA, N BELIKOVA, V TYURIN, A AMOSCATO, M EPPERLY. 2006. The “pro-apoptotic genies” get out of mitochondria: Oxidative lipidomics and redox activity of cytochrome c/cardiolipin complexes. *Chemico-Biological Interactions* **163**:1-2, 15-28. [CrossRef]
31. Ian W. Boucher, Paul J. McMillan, Mads Gabrielsen, Susan E. Akerman, James A. Brannigan, Claudia Schnick, Andrzej M. Brzozowski, Anthony J. Wilkinson, Sylke Muller. 2006. Structural and biochemical characterization of a mitochondrial peroxiredoxin from Plasmodium falciparum. *Molecular Microbiology* **61**:4, 948-959. [CrossRef]
32. H. Bayir, B. Fadeel, M.J. Palladino, E. Witasp, I.V. Kurnikov, Y.Y. Tyurina, V.A. Tyurin, A.A. Amoscato, J. Jiang, P.M. Kochanek, S.T. DeKosky, J.S. Greenberger, A.A. Shvedova, V.E. Kagan. 2006. Apoptotic interactions of cytochrome c: Redox flirting with anionic phospholipids within and outside of mitochondria. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1757**:5-6, 648-659. [CrossRef]
33. Alberto Jiménez, Markku Pelto-Huikko, Jan-Åke Gustafsson, Antonio Miranda-Vizuete. 2006. Characterization of human thioredoxin-like-1: Potential involvement in the cellular response against glucose deprivation. *FEBS Letters* **580**:3, 960-967. [CrossRef]
34. Antonio Miranda-Vizuete, Juan Carlos Fierro González, Gabriele Gahmon, Jan Burghoorn, Plácido Navas, Peter Swoboda. 2006. Lifespan decrease in a Caenorhabditis elegans mutant lacking TRX-1, a thioredoxin expressed in ASJ sensory neurons. *FEBS Letters* **580**:2, 484-490. [CrossRef]

35. Aude Smeets, Christine Evrard, Marie Landtmeters, Cécile Marchand, Bernard Knoops, Jean-Paul Declercq. 2005. Crystal structures of oxidized and reduced forms of human mitochondrial thioredoxin 2. *Protein Science* **14**:10, 2610-2621. [CrossRef]
36. John ShoffnerOxidative Phosphorylation Disease **20050942**, 247-300. [CrossRef]
37. Kerstin Fella, Matthias Glückmann, Jürgen Hellmann, Michael Karas, Peter-Jürgen Kramer, Michaela Kröger. 2005. Use of two-dimensional gel electrophoresis in predictive toxicology: Identification of potential early protein biomarkers in chemically induced hepatocarcinogenesis. *PROTEOMICS* **5**:7, 1914-1927. [CrossRef]
38. Alberto Jiménez, María J. Prieto-Álamo, Carlos A. Fuentes-Almagro, Juan Jurado, Jan-#ke Gustafsson, Carmen Pueyo, Antonio Miranda-Vizuete. 2005. Absolute mRNA levels and transcriptional regulation of the mouse testis-specific thioredoxins. *Biochemical and Biophysical Research Communications* **330**:1, 65-74. [CrossRef]
39. Valerian E. Kagan, Grigory G. Borisenko, Yulia Y. Tyurina, Vladimir A. Tyurin, Jianfei Jiang, Alla I. Potapovich, Vidisha Kini, Andrew A. Amoscato, Yasu Fujii. 2004. Oxidative lipidomics of apoptosis: redox catalytic interactions of cytochrome c with cardiolipin and phosphatidylserine. *Free Radical Biology and Medicine* **37**:12, 1963-1985. [CrossRef]
40. Ivan Nalvarte, Anastasios E. Damdimopoulos, Giannis Spyrou. 2004. Human mitochondrial thioredoxin reductase reduces cytochrome c and confers resistance to complex III inhibition. *Free Radical Biology and Medicine* **36**:10, 1270-1278. [CrossRef]
41. Dhananjaya V. Kalvakolanu. 2004. The GRIMs: a new interface between cell death regulation and interferon/retinoid induced growth suppression. *Cytokine & Growth Factor Reviews* **15**:2-3, 169-194. [CrossRef]
42. Kumuda C. Das . 2004. Thioredoxin System in Premature and Newborn Biology. *Antioxidants & Redox Signaling* **6**:1, 177-184. [Abstract] [Full Text PDF] [Full Text PDF with Links]
43. Stephan Gromer, Sabine Urig, Katja Becker. 2004. The thioredoxin system?From science to clinic. *Medicinal Research Reviews* **24**:1, 40-89. [CrossRef]
44. Jung H Suh, Shi-Hua Heath, Tory M Hagen. 2003. Two subpopulations of mitochondria in the aging rat heart display heterogenous levels of oxidative stress. *Free Radical Biology and Medicine* **35**:9, 1064-1072. [CrossRef]
45. Brian Speake, Nick Sparks, Peter SuraiComparative Aspects of Lipid Peroxidation and Antioxidant Protection in Avian Semen . [CrossRef]
46. F. Missirlis, S. Rahlfs, N. Dimopoulos, H. Bauer, K. Becker, A. Hilliker, J.P. Phillips, H. Jäckle. 2003. A Putative Glutathione Peroxidase of Drosophila Encodes a Thioredoxin Peroxidase That Provides Resistance against Oxidative Stress But Fails to Complement a Lack of Catalase Activity. *Biological Chemistry* **384**:3, 463-472. [CrossRef]
47. Alberto Jiménez, Catrine Johansson, Johanna Ljung, Johan Sagemark, Kurt D Berndt, Bin Ren, Gudrun Tibbelin, Rudolf Ladenstein, Thomas Kieselbach, Arne Holmgren, Jan-Åke Gustafsson, Antonio Miranda-Vizuete. 2002. Human spermatid-specific thioredoxin-1 (Sptrx-1) is a two-domain protein with oxidizing activity. *FEBS Letters* **530**:1-3, 79-84. [CrossRef]
48. KIICHI HIROTA, HAJIME NAKAMURA, HIROSHI MASUTANI, JUNJI YODOI. 2002. Thioredoxin Superfamily and Thioredoxin-Inducing Agents. *Annals of the New York Academy of Sciences* **957**:1, 189-199. [CrossRef]
49. Yves Balmer, Bob B. Buchanan. 2002. Yet another plant thioredoxin. *Trends in Plant Science* **7**:5, 191-193. [CrossRef]
50. Jonas Nordberg, Elias S.J. Arnér. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system1 1This review is based on the licentiate thesis “Thioredoxin reductase—interactions with the redox active compounds 1-chloro-2,4-dinitrobenzene and lipoic acid” by Jonas Nordberg, 2001, Karolinska Institute, Stockholm, ISBN 91-631-1064-4. *Free Radical Biology and Medicine* **31**:11, 1287-1312. [CrossRef]
51. Christine M. Sadek, Anastasios E. Damdimopoulos, Markku Pelto-Huikko, Jan-Ake Gustafsson, Giannis Spyrou, Antonio Miranda-Vizuete. 2001. Sptrx-2, a fusion protein composed of one thioredoxin and three tandemly repeated NDP-kinase domains is expressed in human testis germ cells. *Genes to Cells* **6**:12, 1077-1090. [CrossRef]
52. John F. Engelhardt , Chandan K. Sen , Larry Oberley . 2001. Redox-Modulating Gene Therapies for Human Diseases. *Antioxidants & Redox Signaling* **3**:3, 341-346. [Abstract] [Full Text PDF] [Full Text PDF with Links]