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# PROPERTIES AND BIOLOGICAL ACTIVITIES OF THIOREDOXINS

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■ **Abstract** The mammalian thioredoxins are a family of small (approximately 12 kDa) redox proteins that undergo NADPH-dependent reduction by thioredoxin reductase and in turn reduce oxidized cysteine groups on proteins. The two main thioredoxins are thioredoxin-1, a cytosolic and nuclear form, and thioredoxin-2, a mitochondrial form. Thioredoxin-1 has been studied more. It performs many biological actions including the supply of reducing equivalents to thioredoxin peroxidases and ribonucleotide reductase, the regulation of transcription factor activity, and the regulation of enzyme activity by heterodimer formation. Thioredoxin-1 stimulates cell growth and is an inhibitor of apoptosis. Thioredoxins may play a role in a variety of human diseases including cancer. An increased level of thioredoxin-1 is found in many human tumors, where it is associated with aggressive tumor growth. Drugs are being developed that inhibit thioredoxin and that have antitumor activity.

## INTRODUCTION

Thioredoxin (Trx) was first described in 1964 by Laurent et al (1) as a small redox protein from *Escherichia coli*. Trx family members have subsequently been shown to be present in a wide number of eukaryotic and prokaryotic species (for reviews see 2, 3, 4). The first report of a mammalian Trx appeared in 1967 by Moore (5), who described and later purified a redox protein from Novikoff hepatoma (6). Human Trx was subsequently identified by other investigators under other names: as an interleukin-1 (IL-1)-like cytokine produced by Epstein-Barr virus (EBV)-infected B-lymphoblastoid cells (7); as adult T cell leukemia-derived factor, an interleukin-2 (IL-2) receptor inducing factor produced by human T lymphotrophic virus type 1-infected T cells (8); and as early pregnancy factor, part of a complex in the serum of pregnant animals that increases the complement-dependent inhibition of lymphocytes binding to heterologous blood cells (rosette formation) (9). The proteins have the same predicted amino acid sequence as Trx (10, 11) and are all now referred to by this name. Trxs have been implicated in a number of mammalian cell functions. Activity has been found outside the cell (cell growth stimulation

and chemotaxis), in the cytoplasm (as an antioxidant and a reductant cofactor), in the nucleus (regulation of transcription factor activity), and in the mitochondria. The focus of this review is the properties and functions of mammalian Trxs.

## REDOX BIOCHEMISTRY OF TRX

Trxs are a family of proteins that have a conserved catalytic site (-Trp-Cys-Gly-Pro-Cys-Lys-) that undergoes reversible oxidation to the cystine disulfide (Trx-$S_2$) through the transfer of reducing equivalents from the catalytic site cysteine residues (Cys) to, typically, a disulfide substrate (X-$S_2$). The oxidized Trx is then reduced back to the Cys form [Trx-$(SH)_2$] by the NADPH- dependent flavoprotein thioredoxin reductase. The reactions that Trx undergoes are:

$$\text{Trx-}(SH)_2 + \text{X-}S_2 \overset{\leftarrow}{\rightarrow} \text{Trx-}S_2 + \text{X}(SH)_2$$

$$\text{Trx-}S_2 + \text{NADPH} \overset{\text{TrxR}}{\underset{\leftarrow}{\rightarrow}} \text{Trx-}(SH)_2 + \text{NADP}^+$$

## TRX REDUCTASES

A discussion of the role of Trx is not complete without consideration of the role of thioredoxin reductases, the only enzymes that, to date, are known to be able to reduce the active site of Trx (for reviews see 12, 13). Mammalian thioredoxin reductases are homodimeric, flavin adenine dinucleotide–containing proteins with a penultimate C-terminal selenocysteine residue (14, 15). The Cys residues of the conserved redox catalytic site of mammalian thioredoxin reductases, -Cys-Val-Asn-Val-Gly-Cys-, undergo reversible oxidation reduction in much the same way as Trx. The selenocysteine is essential for the activity of mammalian thioredoxin reductases (16), although human Trx can be relatively efficiently reduced by the nonselenocysteine-containing bacterial thioredoxin reductase (17). To date two full-length human thioredoxin reductases have been cloned, a 54.4 kDa thioredoxin reductase-1, which is predominantly cytosolic (18), and a 56.2 kD thioredoxin reductase-2, which has a 33-amino-acid N-terminal extension identified as a mitochondrial import sequence (19–21). A third, incomplete thioredoxin reductase sequence has been reported (22).

## MULTIPLE FORMS OF TRX

### Cloned Forms

The structures of the known mammalian Trx family members are shown in Figure 1. Human thioredoxin-1 is a 104-amino-acid protein with a molecular weight of 12

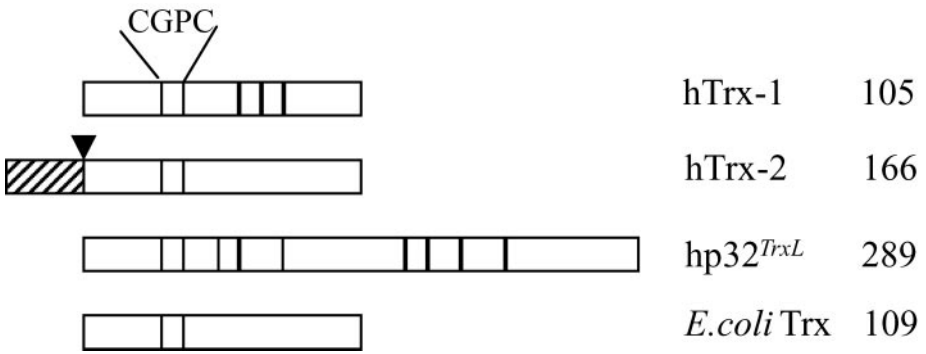



**Figure 1** Trx family members. Human thioredoxin-1 (hTrx-1), human thioredoxin-2 (hTrx-2), human Trx-like p32 protein (hp32$^{TrxL}$), and for comparison *E. coli* Trx. The dark lines show the position of Cys residues. The shaded area is a mitochondrial import sequence on thioredoxin-2 and the arrow shows the cleavage site for the sequence.

kDa (10, 11). Chicken, mouse, rat, and bovine Trxs have also been cloned (23, 24). Trxs exists both with and without the N-terminal methionine, and numbering is from the N-terminal methionine. Human and other mammalian thioredoxin-1s contain, in addition to the two catalytic site Cys residues -Trp-Cys$^{32}$-Gly-Pro-Cys$^{35}$-Lys, three other Cys residues, Cys$^{62}$, Cys$^{69}$, and Cys$^{73}$ (all positions are for human thioredoxin-1), not found in bacterial Trxs (10, 11). These Cys residues may impart unique biological properties to mammalian thioredoxin-1 (25, 26). The gene for human thioredoxin-1 is located on chromosome 9 at bands 9q32 (27). An earlier report placed the thioredoxin-1 gene on chromosome 3 at bands 3p11-p12 (28); however, this may represent a pseudogene (29). A human thioredoxin-1 pseudogene has been mapped to human chromosome 10q25.2-q25.3 (30). The gene for mouse thioredoxin-1 is found on the second proximal quarter of chromosome 4, which is homologous to the human chromosomal 9q32 location of the thioredoxin-1 gene, and a pseudogene is found on mouse chromosome 1 (31).

The presence of mutant forms of thioredoxin-1 has been suggested but not confirmed. There is a report of a human thioredoxin-1 cloned from EBV transformed human lymphoid B cell line with two alterations in the predicted amino acid sequence of Lys$^{39}$→Asn and Met$^{74}$→Thr (32). These mutations may represent cloning artifacts. A novel isoform of Trx in MP6 T lymphocyte cells has been reported with Lys→Arg replacements determined by protein sequencing at positions 3, 8, 21, and 96 (33). However, other researchers have been unable to detect the corresponding Arg-specific codons in Trx mRNA from the same cell line (34). Our studies with over 50 human cancer cell lines have failed to find evidence for expression of mutant forms of thioredoxin-1 (11, 35).

A second, slightly larger Trx based on electrophoretic mobility was identified in pig heart mitochondria (36). This second Trx, thioredoxin-2, was cloned from a rat heart library and found to encode a 166 amino acid, 18 kDa protein that had a

conserved Trx catalytic site but that lacked the other Cys residues found in mammalian thioredoxin-1 (37). A 60-amino-acid N-terminal extension of thioredoxin-2 exhibited characteristics consistent with a mitochondrial translocation signal, and Western blotting has confirmed the mitochondrial localization of thioredoxin-2. A putative cleavage site gives a 12.2 kDa protein, and recombinant truncated thioredoxin-2 that contains the catalytic site has been shown to reduce insulin and to be reduced by thioredoxin reductase-1 and NADPH (37). Human thioredoxin-2 has also been cloned (38).

A 32-kDa thioredoxin-like cytosolic protein ($p32^{TrxL}$) has been cloned from a human testis cDNA library (39). The predicted protein sequence is 289 amino acids with an N-terminal Trx domain of 105 amino acids, a conserved Trx active site (-Cys-Gly-Pro-Cys-), and a high degree of homology to human thioredoxin-1 (39) (Figure 1). The sequence of the remaining 184 C-terminal amino acids showed no homology to other proteins in the database. $p32^{TrxL}$ is ubiquitously expressed in human tissues, with the highest levels appearing in stomach, testis, and bone marrow. Neither the full length $p32^{TrxL}$ protein nor the N-terminal 105 or 107 amino acid Trx-like fragments were reduced by thioredoxin reductase and NADPH (39, 40). However, when reduced by dithioethreitol, the N-terminal 107 amino acid fragment of $p32^{TrxL}$ was able to reduce insulin (40).

A 435-amino-acid redox protein with similarity to Trx but with a -Trp-Cys-Pro-Pro-Cys- catalytic site (instead of -Trp-Cys-Gly-Pro-Cys-) has been cloned from a mouse YAC library and localized to the nucleus (41). The protein has been called nucleoredoxin and localized to mouse chromosome 11 (41).

### Processed Forms of Trx

A shorter, 10-kDa, form of thioredoxin-1 has been reported to be secreted and bound to the outer plasma membrane of human U937 cells (42) and MP6 cells (33). The shorter form has the same N-terminal amino acid sequence as thioredoxin-1 and is immunoreactive with human anti-thioredoxin-1 polyclonal antibody but has enhanced eosinophil cytotoxic activity (42). Although they still have the conserved active site, truncated mutant forms of human thioredoxin-1 lacking the C-terminal 16 or 24 amino acids similarly showed increased eosinophil cytotoxic activity and, surprisingly, are without insulin disulfide reductase activity (43). Alternatively spliced forms of thioredoxin-1 mRNA have been isolated, but with in-frame stop codons (34). It appears, therefore, that the truncated forms of Trx are formed by proteolytic cleavage.

## STRUCTURE OF TRX

Extensive structural data exist for human thioredoxin-1. The solution structures of oxidized and reduced mutant ($Cys^{62} \rightarrow Ala$, $Cys^{69} \rightarrow Ala$, $Cys^{73} \rightarrow Ala$) thioredoxin-1 (44) and crystal structures of oxidized and reduced wild-type thioredoxin-1, as well as solution and crystal structures of various mutant forms of thioredoxin-1

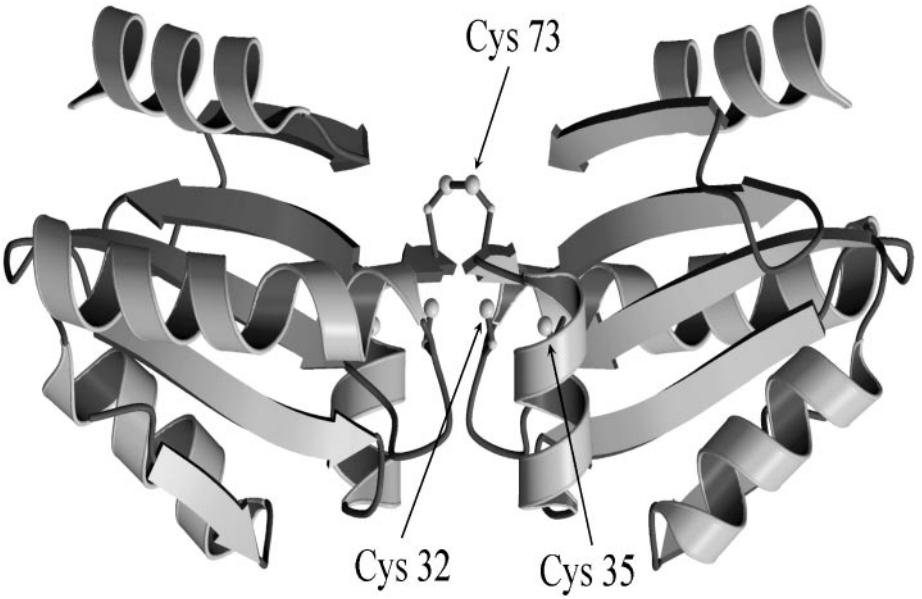



**Figure 2** Structure of the thioredoxin-1 dimer showing the position of the $Cys^{32}$ and $Cys^{35}$ catalytic site residues and the cross-linked $Cys^{73}$ residues.

(44, 45), have been reported. These studies and earlier structure studies with *E. coli* Trx (46–48) show that Trx is a compact globular protein with a five-stranded beta sheet forming a hydrophobic core surrounded by four alpha helices on the external surface. The conserved active site amino acids, -Trp-Cys-Gly-Pro-Cys-, link the second beta strand to the second alpha helix and form the first turn of the second helix. This stable tertiary structure is known as the Trx fold (49). The mechanism for the reducing action of Trx is that substrate $X\text{-}S_2$ binds to a conserved hydrophobic surface and in the hydrophobic environment of the complex, the thiolate of $Cys^{32}$ acting as a nucleophile, combines with the protein substrate to form a covalently linked mixed disulfide (-$Cys^{32}$-S-S- protein). Finally, the now deprotonated $Cys^{35}$ attacks the -$Cys^{32}$-S-S-protein disulfide bond, releasing the reduced protein substrate and forming Trx-$Cys^{32}$-$Cys^{35}$-disulfide, which is then reduced by thioredoxin reductase (50). X-ray crystallography has shown small redox-dependent conformational change in the active site and an unusual thiol-thiol hydrogen bond that may provide an explanation for the putative depressed pKa of the active site $Cys^{32}$ (45). However, the true pKa of $Cys^{32}$ remains in dispute (51).

## Homo- and Heterodimer Formation by Trx

Human thioredoxin-1 forms covalently linked homodimers in solution (45, 52) especially in the presence of a strong oxidant or when stored at high concentrations

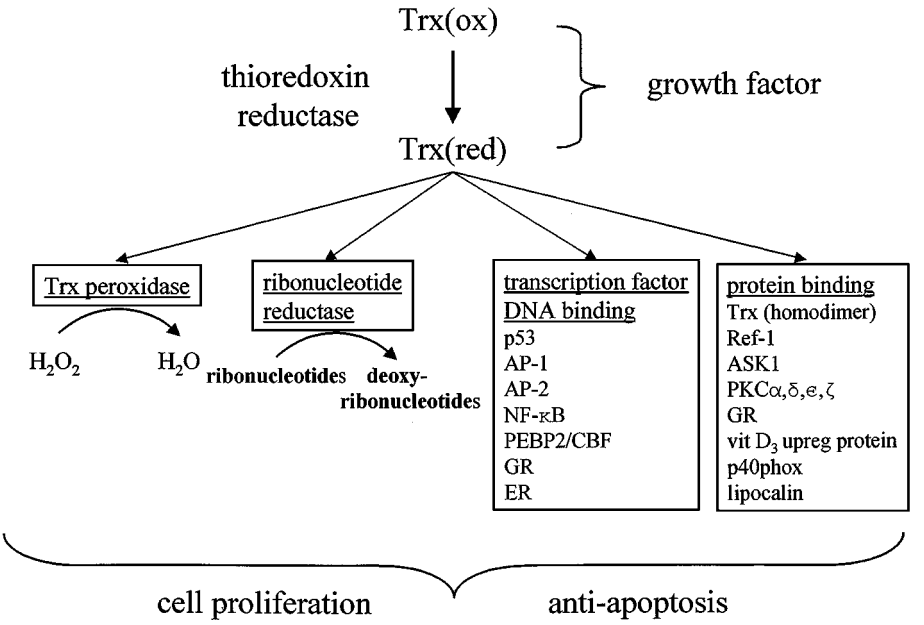



**Figure 3** Actions of Trx in the cell.

(26). The crystal structure of human thioredoxin-1 revealed a dimeric structure in which monomers are disulfide-linked through $Cys^{73}$ from each subunit, and active site residues are reduced and buried in the dimer interface (45) (Figure 2). The crystal structure of the $Cys^{73}\rightarrow$ Ser mutant revealed a dimeric form of the protein nearly identical to that of the wild-type protein, only without the disulfide linkage, suggesting the dimer may represent a naturally occurring form of the protein. The dimer contains an 1100 $Å^2$ largely hydrophobic interface with five hydrogen bonds in addition to the disulfide bond. In the dimer interface are 12 amino acids from each monomer, 10 of which are invariant in higher animals, including $Cys^{73}$ (45, 49). The apparent dissociation constant for non-covalent dimer formation under reducing conditions varied between 6 and 166 $\mu$M at pHs of 3.8 to 8.0, respectively, using a chemical modification assay and gel filtration (53). A study using analytical ultracentrifugation and NMR spectroscopy failed to find evidence of dimer formation in solution (54). However those experiments were done under conditions where active site residues $Cys^{32}$ and $Cys^{35}$ were oxidized (disulfide linked), which interferes with dimer formation (DAR Sanders & WR Montfort, unpublished data), and dimer formation of the reduced protein can be detected by ultracentrifugation (RR Sotelo-Mundo & WR Montfort, unpublished data). It is not yet clear if dimer formation occurs under physiological conditions, since the association is relatively weak, nor is it clear what role thioredoxin-1 dimers might play, since the active site becomes buried on dimer formation, is

not a substrate for reduction by thioredoxin reductase, and does not stimulate cell growth (26, 52). The local concentration of human thioredoxin-1 in tissues has not yet been accurately determined but overall is about 2 to 12 $\mu$M in bovine tissues (55) and possibly higher in tumor tissues that over express thioredoxin-1, suggesting the protein may reach concentrations where dimer formation of the reduced protein is appreciable.

Human thioredoxin-1 also forms heterodimers with numerous protein redox partners, as is described in more detail later. Two such interactions have been studied by NMR (56, 57) with a $Cys^{35} \rightarrow Ala$ mutant form of human thioredoxin-1 and peptides from p50 NFkB and Ref-1. Both p50 NFkB and Ref-1 have redox sensitive Cys residues that must be in the reduced state for the proteins to participate in transcriptional regulation. Stable complexes were achieved through disulfide bond formation between the thioredoxin-1 $Cys^{32}$ and $Cys^{62}$ of p50 NFkB or $Cys^{65}$ of Ref-1. Both peptides formed specific complexes with human thioredoxin-1 through a boot-shaped cleft on the thioredoxin-1 surface that coincides with part of the homodimer interface. In addition to the disulfide bond, both complexes were found to be stabilized by numerous hydrogen-bonding, electrostatic and hydrophobic interactions, however, the orientation of the peptides in the binding cleft differs between the two complexes in that they run in opposite directions ($N \rightarrow C$ vs $C \rightarrow N$). The numerous interactions between the peptides and thioredoxin-1 suggest there is specificity to the interactions, but dissociation constants for the full heterodimers have not been reported.

## REGULATION OF TRX EXPRESSION

The coding region of the human thioredoxin-1 gene spans 1.3 kb and is organized into 5 exons (28). The promoter region contains many possible regulatory binding motifs compatible with constitutive expression, including GCF, SP1, and WT-ZFP (28); with inducible expression, including AP-1, AP-2, NF-$\kappa$B, Oct-1, PEA-3, and Myb (28); and with an oxidative stress response element (58, 59). A variety of stress stimuli increase thioredoxin-1 expression in cells including hypoxia (60, 61), *Staph. aureus* protein (62), lipopolysaccharide (63), $O_2$ (64, 65), $H_20_2$ (66–68), phorbol ester, viral infection, photochemical oxidative stress (69), X-radiation, and UV irradiation (67, 70–75). Thioredoxin-1 is transcriptionally induced by activation of heat shock factor 2 during hemin-induced differentiation of K562 erythroleukemia cells (76) and by retinol (vitamin A) in monkey tracheobronchial epithelial cells (77). Estradiol increases thioredoxin-1 expression in primary cultures of human endometrial stromal cells (78), whereas estradiol, although not progesterone and testosterone, increases the expression of thioredoxin-1 in bovine artery endothelial cells (79). Estradiol and testosterone increase thioredoxin-1 expression in uterus of ovarectomized rats, whereas there is no effect on the levels of hepatic thioredoxin-1 (80). The expression of thioredoxin-1 also shows cell cycle dependency (60, 81).

## SUBCELLULAR LOCALIZATION

Thioredoxin-1 is predominantly a cytosolic protein, and although it has no known nuclear localization sequence, it has been detected in the nucleus of normal endometrial stromal cells (78), tumor cells (82–84), and primary solid tumors (73, 82, 84, 85). Treatment of cells with $H_2O_2$ (86), PMA (83), UV irradiation (59), hypoxia (61, 87), and the cancer drug cisplatin (88) has been reported to cause the translocation of thioredoxin-1 from the cytoplasm to the nucleus. The mechanism for this translocation is not known, but it may be the consequence of thioredoxin-1 being carried along bound to another protein with a nuclear import sequence (89). Thioredoxin-1 is also found associated with plasma and cell membranes, presumably on the outside surface of cells (90, 91). As previously noted thioredoxin-2 is found in the mitochondria (37) while $p32^{TrxL}$ is a cytosolic protein (39).

## BIOLOGICAL ACTIVITIES

Trx has many biological activities. These are summarized in Figure 3 and are discussed below.

### Growth Factor

Thioredoxin-1 acts as a growth factor, and it is produced by a variety of cells including EBV-transformed B cells (7, 92), MP6 T-cell hybridoma (93), and hepatoma cells (25, 72). Thioredoxin-1 is secreted by lymphocytes, hepatocytes, fibroblasts, and a variety of cancer cells (60, 62, 94–96). The mechanism by which secretion occurs is not known, but since thioredoxin-1 has no leader sequence, the mechanism appears to involve a pathway independent of the endoplasmic-Golgi secretory pathway (94, 97). Thioredoxin-1 stimulates the growth of lymphocytes (92), normal fibroblasts (98), and a variety of tumor cell lines (25, 72). Neither the catalytic site mutant $Cys^{32} \rightarrow Ser/Cys^{35} \rightarrow Ser$ thioredoxin-1 nor *E. coli* Trx at concentrations 50-fold higher than human thioredoxin-1 are able to stimulate cell growth (98). The mechanism responsible for growth stimulation by thioredoxin-1 is atypical for a growth factor. No evidence has been found of saturable binding of $^{125}$I-labeled human thioredoxin-1 to the surface of MCF-7 breast cancer cells that would indicate the presence of a thioredoxin-1 receptor, and thioredoxin-1 appears to sensitize cells to growth factors produced by the cell itself (25). Not all growth factors are enhanced by thioredoxin-1. For example, in MCF-7 breast cancer cells, the effects of insulin and epidermal growth factor are not potentiated (25), while IL-2 is enhanced up to a 1000-fold and basic fibroblast growth factor (bFGF) up to a 100-fold (15). The mechanism that stimulates the effects of IL-2 may include an increase in the alpha subunits of the IL-2 receptor (8, 99). Thioredoxin-1 also increases the cell expression of a variety of cytokines, including IL-1, IL-2, IL-6, IL-8, and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (100), as well as of the TNF-$\alpha$ induced expression of IL-6 and IL-8 by human rheumatoid arthritis

fibroblasts (101). However, thioredoxin-1 transfection of murine L929 cells down-regulates the expression of IL-6, which is associated with inhibition of p38 MAP kinase activity in the cells (102). Thioredoxin-1 prevents the inhibition of cdc25 phosphatase activity that leads to impairment of $p34^{cdc2}$ dephosphorylation during the second cell cycle of mouse embryonic development in vitro (103).

## Antioxidant

The direct antioxidant properties of thioredoxin-1 include removal of hydrogen peroxide (104). Thioredoxin-1 has also been reported to be an efficient electron donor to human plasma glutathione peroxidase (105). Thioredoxin-1 is present in plasma at concentrations up to 6 nM (see below) and has been suggested to have an antioxidant role in plasma (3). However, plasma contains considerably higher levels of reduced glutathione, around 1 $\mu$M (106, 107), and reduced plasma proteins provide additional thiol buffering capacity (108). It seems unlikely that the relatively low concentration of thioredoxin-1 in plasma would contribute much antioxidant activity.

Thioredoxin-1 appears to exert most of its antioxidant properties in cells through thioredoxin peroxidase. The thioredoxin peroxidases belong to a conserved family of antioxidant proteins, the peroxiredoxins (PRDXs), which use thyl groups as reducing equivalents to scavenge oxidants (109). The reduced form of thioredoxin peroxidase scavenges oxidant species such as $H_2O_2$ and alky peroxides and, in the process, homo or heterodimerize with other family members through disulfide bonds formed between conserved Cys residues (109, 110). Trx reduces the oxidized thioredoxin peroxidase to the monomeric form. Several human thioredoxin peroxidases have been cloned: thioredoxin peroxidase-1 (22 kDa monomer), also known as natural killer enhancing factor-B (111); thioredoxin peroxidase-2 (22-kDa monomer), also known as natural killer enhancing factor-A (112), proliferation associated gene (113), or heme-binding protein 23 (114); thioredoxin peroxidase-3 (21.6 kDa monomer), also known as human murine erythroleukemia-associated 5, AOP1, or SP-22, which is primarily mitochondrial (115, 116); and thioredoxin peroxidase-4 (31 kDa monomer), or human AOE372, identified as a cytosolic and secreted protein (110, 117). A factor purified from bovine adrenal cortex, identified as mitochondrial thioredoxin-2, is necessary for the peroxidase activity of thioredoxin peroxidase-4 (116). Thioredoxin peroxidase-4 has also been identified as TRANK (thioredoxin peroxidase-related activator of NF-$\kappa$B and c-Jun N-terminal kinase), which when added to U-937 human myeloid cells increases the binding of the trancription factor NF-$\kappa$B to DNA and increases NF-$\kappa$B transactivation (118). However, transfection of cells with thioredoxin peroxidase-2 (110, 115) or thioredoxin peroxidase-4 (110) inhibits NF-$\kappa$B activation measured by gel shift mobility assays, reporter constructs, and nuclear immunohostochemical localization. The inhibition of NF-$\kappa$B activation by thioredoxin peroxidase-4 may be caused by decreasing the phosphorylation of the cytosolic NF-$\kappa$B inhibitor, I$\kappa$B, which leads to its decreased degradation (110). A fifth member of the PRDX family, PRDX-5, contains only one of the essential Cys residues and reduces $H_2O_2$

in the presence of dithioethreitol, but not in the presence of thioredoxin-1 (119). PRDX-6 has been cloned from a human liver library (120) but appears to be a nonselenium containing glutathione peroxidase (121, 122).

Thioredoxin peroxidases are found in human red blood cells as antioxidants protecting red blood cells from oxidant injury (123, 124). Transfection of human endothelial cells with thioredoxin peroxidase-1 protects the cells from $H_2O_2$-induced cytotoxicity and inflammation-induced monocyte adhesion (124). Thioredoxin peroxidase-2 is found abundantly expressed in rapidly growing and transformed cells but is present at low levels in quiescent cells (113, 125). Transfection of Molt-4 leukemia cells with thioredoxin peroxidase-2 protects the cells from apoptosis induced by serum deprivation, ceramide, and etoposide and inhibits the release of cytochrome c from mitochondria during apoptosis (124, 126). Thioredoxin peroxidase-2 binds to the SH3-binding domain of c-Abl tyrosine kinase and inhibits kinase activity (127). Thioredoxin peroxidase-2 protein is increased as cells enter S-phase and by agents that induce oxidative stress (128). The crystal structure of rat thioredoxin peroxidase-2 has been reported (114).

Another mechanism for removing $H_2O_2$ in the cell is through selenocysteine-containing glutathione peroxidases that use reduced glutathione as a source of reducing equivalents (129). As far as is known the glutathione and Trx redox systems are not coupled in the cell. Selenium has different effects on the Trx and glutathione peroxidase systems, increasing the activity of thioredoxin reductase (130) but not of thioredoxin peroxidase, and increasing the activity of glutathione peroxidase but not glutathione reductase (129; Figure 4). Thus, in the presence of an excess of peroxide, an increase in available selenium would predictably increase the levels of reduced Trx relative to reduced glutathione.

## Cofactor

One of the earliest functions ascribed to bacterial Trx was as a source of reducing equivalents for ribonucleotide reductase (1), which catalyzes the conversion of nucleotides to deoxynucleotides, the first unique step of DNA synthesis and an essential step for cellular proliferation (131). The importance of thioredoxin-1 for eukaryotic ribonucleotide reductase is less understood, and there may be other sources of reducing equivalents (132). However, irreversible inhibition of thioredoxin reductase by some antitumor quinones has been associated with a decrease in cellular ribonucleotide reductase activity (133). Thioredoxin-1 has also been suggested to be a source of reducing equivalents for vitamin K epoxide reductase, which is necessary for the biosynthesis of plasma clotting factors (134, 135), although other studies have shown that inhibitors of the Trx system and antibodies against thioredoxin-1 do not inhibit vitamin K epoxide reduction (136).

## Transcription Factor Regulation

Thioredoxin-1 selectively activates the DNA-binding of a number of transcription factors. This includes the transcription factor NF-$\kappa$B that is important for

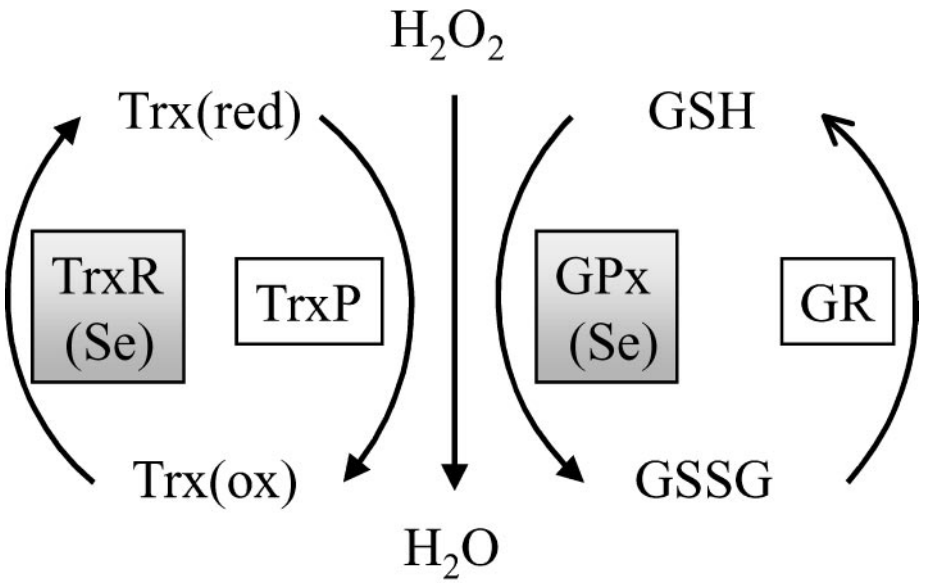



**Figure 4** Effects of selenium on thioredoxin peroxidase (TrxP) and glutathione peroxidase cycles. Trx is thioredoxin, reduced (red) and oxidized (ox), GSH is reduced glutathione and GSSG is oxidized glutathione. The enzymes are the selenoproteins (shown shaded) thioredoxin reductase (TrxR) and glutathione peroxidase, and the non-selenoproteines are thioredoxin peroxidase and glutathiore reductase.

the cell response to oxidative stress, apoptosis, and tumorigenesis (137, 138). The binding of NF-$\kappa$B to DNA measured by gel shift assays is inhibited under oxidizing conditions (139, 140) and by oxidized thioredoxin-1 (141). Thioredoxin-1 increases the DNA binding of NF-$\kappa$B to DNA and is more active than L-cysteine, reduced glutathione, and nonphysiological reducing agents such as N-acetyl cysteine, 2-mercaptoethanol, or dithiothreitol (140, 142). The binding of NF-$\kappa$B to DNA requires that the $Cys^{62}$ of the NF-$\kappa$B p50 subunit be reduced (143). If the $Cys^{62}$ of one subunit is linked in a disulfide bridge with the $Cys^{62}$ of the other in uncomplexed NF-$\kappa$B, the DNA can no longer gain access to the binding surface of the p50 homodimer. If the p50 $Cys^{62}$ is disulfide-linked to DNA, it can no longer be released from the complex (144, 145). NMR has been used to show a disulfide-bonded complex between a catalytically inactive mutant human thioredoxin-1 ($Cys^{35}\rightarrow$Ala,$Cys^{62}\rightarrow$Ala, $Cys^{69}\rightarrow$Ala,$Cys^{73}\rightarrow$Ala) and a 13-residue peptide comprising residues 56–68 of the p50 subunit of NF-$\kappa$B that encompasses the critical $Cys^{62}$ residue (56). Both stable and transient transfection of cells with human thioredoxin-1 have been found to increase NF-$\kappa$B transactivation measured with a reporter construct (143, 146). However, other studies have found that transient transfection with thioredoxin-1 or addition of thioredoxin-1 to the

medium results in a dose-dependent inhibition of constitutive as well as phorbol ester–stimulated NF-$\kappa$B DNA binding and transactivation (147). This difference may be due to the use of reporter constructs that contain different NF-$\kappa$B promoter elements or to the differing roles thioredoxin-1 plays in the cytosol (inhibition of I $\kappa$B degradation) and in the nucleus (NF-$\kappa$B activation).

A second transcription factor whose activity is regulated by Trx is the glucocorticoid receptor, which is subject to redox modulation through critical cysteine residues, whose oxidation leads to decreased ligand binding activity (148) and decreased DNA binding (74). Transfection of cells with thioredoxin-1 has been shown to increase the expression of glucocorticoid receptor mediated genes and glucocorticoid receptor reporter activity in oxidant treated cells, whereas transfection with antisense thioredoxin-1 decreases the expression of the genes and glucocorticoid receptor reporter activity (86, 149–151). Thioredoxin-1 associates with the DNA binding domain of the glucocorticoid receptor in the nucleus, which may account for the increased glucocorticoid receptor DNA binding activity caused by thioredoxin-1 (86).

The transcription factor AP-1 (Fos and Jun homo- and heterodimers), whose activation is closely correlated with increased cell growth, is redox regulated (147, 152). DNA binding of AP-1 is increased by the reduction of a single conserved Cys residue in the DNA binding domain of each of the homodimers (131). Mutant Fos and Jun proteins in which this Cys residue is replaced by Ser show constitutive DNA binding (131). Cells transfected with human thioredoxin-1 show an increase in AP-1 activity measured with a reporter construct (146). Thioredoxin-1 does not reduce AP-1 directly but does so through another nuclear redox protein Ref-1, (153). Ref-1 is a 37-kDa protein that also has an apurine/apyrimidine endonuclease repair activity and a core domain that is highly conserved in a family of prokaryotic and eukaryotic DNA repair enzymes (154). Sequences in the N-terminal domain of Ref-1 are required for redox activity while C-terminal sequences are necessary for DNA repair activity (154).

Other transcription factors whose binding to DNA is increased by thioredoxin-1 are AP-2 (155), the estrogen receptor (156), and transcription factor PEBP2/CBF (157). Cotransfection experiments have shown that thioredoxin-1 and Ref-1 increase the transactivating activity of the C-terminal activation domain of hypoxia-inducible factor 1$\alpha$ (HIF1$\alpha$), which contains a specific cysteine residue essential for the hypoxia inducible interaction with the CREB binding protein that leads to increased expression of genes such as erythropoietin (87).

p53 is a tumor suppressor protein and transcription factor found to be deleted in a large number of human cancers (158). p53 is often referred to as the gatekeeper of the genome, and it induces $G_1$ arrest to allow cells time to repair DNA damage or, if the damage is too great, to induce apoptosis. The binding of p53 to DNA is enhanced by phosphorylation by specific kinases in response to DNA damage leading to the regulation of cell cycle-related genes (159, 160). p53 binding to DNA is also redox sensitive and is inhibited by oxidizing conditions (161). The redox regulation of p53 DNA binding occurs through critical cysteine residues in

the DNA binding domain of p53, the mutation of which markedly decreases DNA binding (127). When wild-type but not mutant forms of human p53 are expressed in the fission yeast *Saccharomyces pombe*, strong growth inhibition occurs (162). This was used as a model system to screen for genes whose function is required for normal activity of p53, and a mutant yeast strain partially resistant to the effects of human p53 expression was found that contained a recessive mutation in a novel gene, *trr1*, which has strong homology to thioredoxin reductase (163). The levels and localization of the p53 protein were unchanged in the mutant yeast strain, suggesting that it was not p53 expression that was altered. Loss of *trr1* function resulted in yeast with an increased sensitivity to the toxic effects of $H_2O_2$ and a 100% oxygen atmosphere. Studies in the budding yeast *Saccharomyces cerevisiae* have shown that deletion of the *trr1* gene inhibits the ability of human p53 to stimulate reporter gene expression (164, 165). The effect of the *trr1* gene on p53 transactivation, but not DNA binding, was exerted through the negative regulatory domain rather than the DNA binding or oligomerization domains of p53 (165). Human thioredoxin-1 at concentrations as low as 10 nM has been shown to increase the sequence-specific binding of p53 to DNA measured by a gel shift assay and to increase p53 transactivating activity measured with a p21 reporter construct and p21 protein levels in cells transiently transfected with thioredoxin-1 (88). The redox inactive mutant thioredoxin-1 C32S/C35S partly blocks the increase in p53 transactivation caused by cisplatin treatment of cells. The DNA binding of p53 and transactivating activity in cells is also increased by the nuclear redox protein Ref-1 (88, 166). Thioredoxin-1, which as previously noted, associates with and reduces Ref-1, increases the effects of p53 on DNA binding and cotransfection of cells with the redox inactive mutant thioredoxin-1. C32S/C35S decrease the Ref-1 mediated p53 transactivation, suggesting a role for both thioredoxin-1s in the Ref-1-mediated transactivation of p53 (88). Thioredoxin peroxidase 5 inhibits p53-induced apoptosis, raising the possibility that part of the effect of thioredoxin-1 on p53 activity is mediated through scavenging of $H_2O_2$ (69).

## Protein Binding

Thioredoxin-1 binds to a variety of cellular proteins (Table 1). Protein binding occurs only with the reduced but not the oxidized or mutant redox-inactive C32S/C35S forms of thioredoxin-1 (167–170). The mechansim of thioredoxin-1 binding has not been elucidated but may involve mixed disulfide formation between a catalytic site cysteine residue and a cysteine on the other protein. A protein to which thioredoxin-1 binds and that has received attention is apoptosis signal-regulating kinase 1 (ASK1). ASK1 is an activator of the c-Jun N-terminal kinase (JNK) and p36 MAP kinase pathways and is required for TNF-$\alpha$-induced apoptosis (171, 172). Reduced but not oxidized or redox inactive C32S/C35S mutant thioredoxin-1 binds to the N-terminal portion of ASK1 to inhibit its activity, and thioredoxin-1 also inhibits ASK1-dependent apoptosis when transiently transfected into MvILu containing an inducible ASK1 (167). Deletion of specific

**TABLE 1** Thioredoxin-1 binding to proteins

| Protein | Comments | Method | Ref |
|---|---|---|---|
| Trx-1 | Homodimerization | X-ray | (26, 45, 228, 22, 9) |
| Ref-1 | Trx reduces Ref-1 which then reduces AP-1 and p53 | Mammalian two hybrid assay | (88) |
| | Ref-1 peptides bind to a boat shaped cleft in Trx-1 | NMR | (57) |
| ASK1 | (Apoptosis signal-regulated kinase) binds reduced but not oxidized or redox inactive mutant Trx and is inhibited. TNF-$\alpha$ causes ROS mediated dissociation of Trx and ASK1 activation | Yeast two hybrid assay | (167) (168) |
| PKC$\alpha$,$\delta$,$\epsilon$ and $\zeta$ | Trx binding inhibits autophosphorylation and histone phosphorylation by PKC | Phage display panning | (230) |
| NF-$\kappa$B | NF-$\kappa$B peptides bind to a boat shaped cleft in Trx-1 | NMR | (56) |
| vit D(3) up regulated protein-1 | Binds reduced but not oxidized or redox inactive mutant Trx, in cells vit D(3) upregulated protein-1 expression inhibits Trx expression | Yeast two hybrid assay | (231) |
| Glucocorticoid receptor | Trx associates with the GR receptor in the nucleus under oxidizing conditions | — | (86) |
| p40 phox | Cytosolic component of phagocyte oxidase, does not bind mutant redox Trx | Yeast two hybrid assay | (170) |
| Lipocalin | Lipid binding protein and cystein proteinase inhibitor from human tears and other tissues | Phage display panning | (232) |

N-terminal residues renders ASK1 constitutively active and no longer influenced by thioredoxin-1 (167). Oxidation of thioredoxin-1 by the TNF-$\alpha$ or stress- induced generation of reactive oxygen species leads to dissociation of thioredoxin-1 and the activation of ASK1, which may contribute to TNF-$\alpha$ induced apoptosis. A role for the release of thioredoxin-1 bound to ASK-1 in mediating TNF-$\alpha$ induced apoptosis has been proposed (167, 168). However, whether Trx binding to other proteins has physiological significance remains to be determined. The Trx-related protein $p32^{TrxL}$ has also been found to bind to the catalytic fragment of mammalian

STE-20-like (MST) kinase (40). MST is proteolytically activated by caspases during CD95 (FAS, Apo-1) induced apoptosis and has been suggested to generate apoptotic signals downstream of caspase activation (173).

## Inhibition of Apoptosis

Thioredoxin-1 added to culture medium prevents apoptosis of lymphoid cells induced by L-cysteine and glutathione depletion (174) and also protects B-type chronic lymphocytic leukemia cells against apoptosis associated with an increase in TNF-$\alpha$ secretion, which is an autocrine growth factor for these cells (175). We have shown that stable transfection of mouse WEHI7.2 lymphoid cells with human thioredoxin-1 inhibits apoptosis induced by a variety of agents, including dexamethasone, staurosporine, thapsigargin, and etoposide (176). The inhibition of apoptosis caused by transfection with thioredoxin-1 is similar to the pattern of inhibition of apoptosis caused by transfection of the cells with the anti-apoptosis oncogene *bcl-2*. When innoculated into *scid mice*, the thioredoxin-1-transfected cells form tumors that grow more rapidly and show less spontaneous apoptosis than do vector-alone or *Bcl-2* transfected cells. The tumors are also resistant to growth inhibition by dexamethasone. Thus, thioredoxin-1 offers a survival as well as a growth advantage to tumors in vivo, unlike *Bcl-2*, which offers only a survival advantage and requires other genetic changes to stimulate tumor growth (177). The mechanism for the anti-apoptotic effects of thioredoxin-1 is unknown. As noted above a role for thioredoxin-1 binding to to ASK1 in mediating TNF-$\alpha$ induced apoptosis has been proposed (167, 168).

## Noncatalytic Site Activities of Trx

While most attention has focused on the redox-dependent biological activities of Trx that require the catalytic site Cys residues, there may be activities that are independent of this redox activity, although they could still require other reduced cysteine residues. Precedents exist with *E. coli* Trx where catalytic site redox inactive mutants are as active as the wild-type protein in supporting in vivo F1 and M13 phage assembly (178) and the activation of phage T7 DNA polymerase (179). A catalytic site mutant human thioredoxin-1 in which both catalytic site Cys residues are converted to Ser ($Cys^{32} \rightarrow Ser/Cys^{35} \rightarrow Ser$) retains its ability to cause an increase in the rosette inhibition titre of serum (180). Changing $Cys^{73}$ but not $Cys^{69}$ to Ser completely abolished the activity of thioredoxin-1 in the rosette inhibition assay. Because $Cys^{73}$ is needed for stable dimer formation by thioredoxin-1 (45), it is possible that dimerization or association of thioredoxin-1 with another protein is necessary for the rosette modifying activity. Truncated forms of human thioredoxin-1 retaining the conserved active site but lacking the C-terminal 16 or 24 amino acids are without insulin disulfide reductase activity and show increased eosinophil cytotoxic activity compared to wild-type thioredoxin-1 (43).

## Cancer Drug Resistance

Several lines of evidence suggest that Trxs may play a role in resistance to the cell-killing effects of anticancer drugs. The sensitivity of adult T-cell leukemia cell lines to doxorubicin is lowest in those cell lines with the highest levels of thioredoxin-1 (181). In contrast, leukemia cell lines, which overall have lower thioredoxin-1 levels than do epithelial cancer cell lines, together with colon and renal cancer cell lines show the greatest sensitivity to thioredoxin-1 inhibiting disulfide drugs with antitumor activity and other inhibitors of thioredoxin-1 reductase (182). Human hepatoma cells with increased thioredoxin-1 show a decreased sensitivity to cell killing by cisplatin but not by doxorubicin or mitomycin C (85). It has been reported that bladder and prostate cancer cell lines made resistant to cisplatin have four- to sixfold increases in levels of thioredoxin-1 mRNA and thioredoxin-1 protein (183). This resistance to cisplatin could be reversed by lowering thioredoxin-1 levels with a thioredoxin-1 antisense expression plasmid, which also increases the sensitivity of the cells to doxorubicin, mitomycin C, etoposide, $H_2O_2$, and UV irradiation but not to vincristine and colchicine. Gastric and colon cancer cell lines resistant to cisplatin have also been reported to have up to 2.5-fold increases in levels of thioredoxin-1 protein and up to twofold increases in Trx reductase activity, whereas a positive correlation between cisplatin resistance and Trx-1 levels was found in a panel of 11 ovarian cancer cell lines (184). In a follow-up study by the same group, stable transfection of ovarian and colon cancer cell lines with thioredoxin-1 cDNA giving a 2- to 2.5-fold increase in thioredoxin-1 levels failed to increase the resistance to cisplatin, doxorubicin, and mitomycin C (185). However, others have found that stable transfection of fibrosarcoma cells with thioredoxin-1 giving a 2-fold increase in thioredoxin-1 levels gave a 3-fold increase in resistance to cisplatin (186). In the cisplatin resistant cell lines, a relatively small increase in thioredoxin-1 expression of two- to threefold was associated with a cisplatin resistance of 4- to 20-fold (85, 184, 187). Thioredoxin-1 levels are also increased in mitoxantrone resistant cells (188). Thus, thioredoxin-1 appears to be necessary but not sufficient for the anticancer drug resistance.

## Reperfusion Injury

Recombinant human thioredoxin-1 has been shown to exert a protective effect against reperfusion-induced arrhythmias in an isolated rat heart model, with a concentration of 0.1 $\mu$M thioredoxin-1 being the most effective (189). In a rat in vivo model of lung ischemia reperfusion injury, recombinant human thioredoxin-1 improved lung function and lessened the histological signs of damage (190). In a rabbit in vivo model of lung reperfusion injury, human recombinant thioredoxin-1 had no effect on lung function or on lipid peroxides in the lung but attenuated the signs of lung damage including intra-alveolar exudation, interstitial thickening, and cellular infiltration (191). In the same rabbit in vivo model, N-acetyl cysteine did not provide any protection against lung damage. In a dog lung transplantation model, human recombinant thioredoxin-1 as well as N-acetyl cysteine protected against loss of pulmonary function and prevented histological signs of damage

(192). Thioredoxin-1 expression is increased in rat brain after transient and permanent cerebral occlusion (193). Thioredoxin-1 transgenic mice showed smaller infarct sizes after middle cerebral artery occlusion (194). Thioredoxin-1 protects endothelial cells against reperfusion injury (169) and protects rats against retinal ischemia reperfusion injury (195).

## Transgenic and Knockout Animals

The many biological properties of Trx make it of considerable interest to have animals showing increased or decreased expression of Trx. Mice with targeted disruption of the thioredoxin-1 gene show that homozygous animals die shortly after implantation whereas heterozygous animals are viable, fertile, and in other respects apparently normal (196). The lethal effects of thioredoxin-1 in early development is a finding consistent with the one that thioredoxin-1 is widely distributed in different organs and tissues during fetal development (197). Transgenic mice with human thioredoxin-1 under the control of a $\beta$-actin promoter have been reported (194). Heart showed the highest level of increased expression of human—relative to mouse—thioredoxin-1, with kidney, brain, lung, skin, and liver showing lower levels. The mice were functionally normal, and no histological abnormalities were observed. There was no change in the expression of Cu Zn superoxide dismutase, Mn superoxide dismutase, or glutathione peroxidase. The thioredoxin-1 transgenic mice showed smaller infarct sizes after middle cerebral artery occlusion and a subsequent, larger increase in C-fos expression, observations that lead to the suggestion that thioredoxin-1 may have a neuroprotective function through the activation of AP-1. Selective expression of human thioredoxin-1 under the control of a human insulin promoter in the $\beta$-islet cells of the pancreas protected the mice against spontaneous diabetes and against streptozotocin-induced apoptosis (198).

## Other Activities

Thioredoxin-1 can act as a catalyst of protein folding because of relatively weak protein disulfide bond isomerizing activity (199). Thioredoxin-1 directly reactivates proteins that have been inactivated by oxidative stress, including glyceraldehyde-3-phosphate dehydrogenase (200), iodothyronine 5′-deiodinase (201), and ornithine decarboxylase (202). Protein disulfide isomerase, which has two regions homologous to the Trx catalytic site with -Trp-Cys-Gly-His-Cys-Lys- (203), is several-fold more efficient than thioredoxin-1 at catalyzing the isomerization, and because it is abundant in the endoplasmic reticulum lumen, where most protein disulfides are formed, it is the major physiological catalyst of protein disulfide isomerization (199). It is interesting that the gonadotrophic hormone subunit $\beta$-lutropin has a Trx- like -Cys-Gly-Pro-Cys- sequence and a $\beta$-follitropin Cys-Gly-Lys-Cys sequence, and both are several-fold more active as protein disulfide isomerases than Trx (204).

Thioredoxin-1 was identified by a random screening of antisense expressed genes as a component of the pathway signaling the interferon-gamma growth arrest of HeLa cells (10). Thioredoxin-1 is also a chemotaxic factor for cells of

leukocyte lineage (205). Chemotaxis is not exhibited by redox inactive mutant thioredoxin-1, and the effects are seen in the nM range, which is comparable to other known chemokines.

## TRX IN PLASMA

Plasma levels of thioredoxin-1 in normal individuals are between 10 and 80 ng/ml (0.8–6.6 nM) (188, 206–209). Studies have been conducted to determine whether plasma thioredoxin-1 is elevated in human disease. Serum thioredoxin-1 has been reported to be elevated almost twofold in patients with hepatocellular carcinoma, and after surgery to remove the tumor, the levels drop (188). Serum thioredoxin-1 is not elevated in patients with chronic hepatitis or liver cirrhosis (188). Plasma thioredoxin-1 is also elevated more than twofold in patients with pancreatic ductal carcinoma compared to normal individuals (84). Serum thioredoxin-1 has been reported to be elevated in patients with HIV (206), rheumatoid arthritis, and Sjogren's syndrome (101, 210).

## TRX IN HUMAN DISEASE

### Skin Damage

Normal skin shows thioredoxin-1 immunohistochemical staining in the sebaceous glands, secretory components of sweat glands, and the outer root sheath of the hair follicle, but not in the interfollicular epidermis (211). Enhanced expression of thioredoxin-1 has been demonstrated by immunohistochemical staining in the epidermal cells of sun-exposed skin (67) and may be a protective response to oxidative damage.

### Atherosclerosis

NO and peroxynitrite contribute to the damage to smooth muscle and endothelial cells seen during atherosclerotic plaque formation (212). Thioredoxin-1 prevents the NO-dependent inhibition of purified NO-synthase (213), and when human thioredoxin-1 was transiently transfected into porcine pulmonary artery endothelial cells, it protected against a loss of NO-synthase activity (214). Thioredoxin-1-transfected L929 murine fibrosarcoma cells show resistance to peroxynitrite-induced cytotoxicity, although the effect is small (215). Thioredoxin-1 mRNA and thioredoxin reductase mRNA are increased in endothelial cells and macrophages of human atherosclerotic plaques, leading to the suggestion that thioredoxin-1 plays a role in the pathogenesis of atherosclerosis (215).

### Immune Function

Thioredoxin-1 is a component of early pregnancy factor in serum that provides immune protection of the developing embryo (29). Thioredoxin-1 is initially

produced by maternal tissues, but the fetus begins thioredoxin-1 production after implantation (216). Eosinophils are inflammatory cells associated with inflammatory responses, allergic diseases, and tumor cytotoxicities. Thioredoxin-1 increases eosinophil migration, cytotoxicity, and the release of major basic protein from eosinophils (217–219). Thioredoxin-1 expression is increased in infiltrating B cells and epithelial cells of patients with Sjogren's syndrome, an EBV-associated autoimmune disease (220).

## Alzheimers Disease

The brains of subjects with Alzheimer's disease show decreased levels of thioredoxin-1, particularly in the amygdala and hippocampus, while at the same time the thioredoxin reductase activity is increased (221). It was suggested that these changes may contribute to the increased oxidative stress and subsequent neurodegeneration observed in Alzheimer's disease.

## HIV

Trx-expressing cells are absent from the lymph nodes of patients with AIDS and AIDS related complex (222). However, plasma levels of thioredoxin-1 are significantly elevated in patients with AIDS (206). Approximately 25% of AIDS patients had thioredoxin-1 levels higher than the highest level found in plasma of control subjects and tended to have lower overall CD4 counts. Furthermore, increased plasma thioredoxin-1 corresponded with decreased cellular thiols and altered cell surface antigen expression (CD62L, CD38 and CD20) that occurs in the later stages of HIV infection.

Added thioredoxin-1 has been reported to inhibit the expression of HIV in human macrophages as measured by p24 antigen production and integration of the provirus as well as expression of the integrated virus in chronically infected cells (223). Thioredoxin-1 was considerably more potent than N-acetyl cysteine in inhibiting HIV production. Surprisingly a truncated form of thioredoxin-1, human eosinophil cytotoxicity factor, potentiated HIV production.

## Cancer

Studies with a variety of human primary tumors have shown that thioredoxin-1 is overexpressed in the tumor compared to levels in the corresponding normal tissue (Table 2). We have shown by immunohistochemical studies using paraffin embedded tissue sections that thioredoxin-1 expression is increased in more than half of human primary gastric cancers. The thioredoxin-1 levels showed a highly significant positive correlation ($p < 0.001$) with cell proliferation measured by nuclear proliferation antigen and a highly significant negative correlation ($p < 0.001$) with apoptosis measured by the terminal deoxynucleotidyl transferase (Tunel) assay. In a recent immunohistochemical study of thioredoxin-1 levels in human colon cancer, thioredoxin-1 protein was not increased compared to normal colonic mucosa in precancerous colon polyps but was increased sixfold in

**TABLE 2** Thioredoxin overexpression by human primary cancers

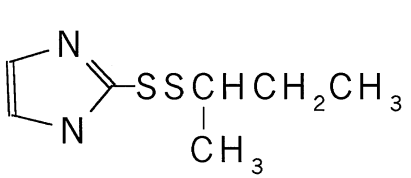
| Tumor | Type | Number of subjects | Percent overexpressed | Reference |
|---|---|---|---|---|
| Lung | mRNA | 10 | 50[a] | (11) |
| Colon | mRNA | 10 | 60[a] | (60) |
| | protein | 18 | 55[a] | (224) |
| Cervix | protein | 79 | 78[b] | (73) |
| Hepatoma | mRNA | 20 | 85 | (85) |
| | protein | 25 | 52 | (72) |
| Gastric | protein | 10 | 50 | (82) |
| Pancreatic | protein | 58 | 41 | (84) |
| | | 29 | 7 (differentiated) | |
| Squamous cell carcinoma | protein | 6 metastatic | 6 | (211) |
| Myeloma | protein | 10 | 9 | unpublished |
| Non–Hodgkins lymphoma | protein | 20 | 7 (advanced) | unpublished |
| Acute lymphocytic leukemia | protein | 33 | 15 | unpublished |

[a]Compared to corresponding normal tissue from the same subject.
[b]Immunohistochemistry and comparison to normal tissue from the same subject.

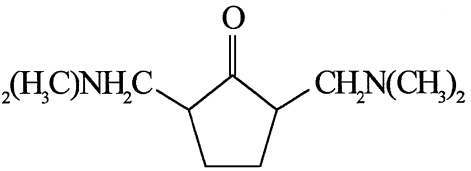
primary colon cancers and almost ninefold in metastatic colon tumors in adjacent lymph glands (224). Furthermore, high levels of thioredoxin-1 in the tumor appear to be associated with decreased patient survival (J Raffel, AK Bhattacharyya & G Powis unpublished observations).

## DRUGS THAT INHIBIT TRX

The growth-stimulating effects of thioredoxin-1, together with the finding that it is overexpressed by a number of human primary tumors, raise the intriguing possibility that thioredoxin-1 contributes to aggressive tumor growth and poor patient prognosis (25). Furthermore, because thioredoxin-1 also inhibits apoptosis caused by a number of anticancer drugs and is a cause of resistance to the cytotoxic effects of some anticancer drugs, it is possible that increased thioredoxin-1 could be a cause of resistance to chemotherapy. These findings make thioredoxin-1 an attractive target for the development of drugs to inhibit cancer cell growth. Several such compounds have been identified. They include 1-methylhydroxypropyl 2-imidazoloyl disulfide (PX-12, Figure 5), which was identified as an inhibitor of thioredoxin-1 by binding to the $Cys^{73}$ residue (225). The median $IC_{50}$ for growth inhibition of a variety of cell lines by PX-12 is 8.1 $\mu$M (177). PX-12 has been shown to have in vivo antitumor activity against human tumor xenografts in *scid* mice (226). The growth inhibition by compound PX-12 in a 60 human tumor panel was significantly




PX-12




NSC 131233

**Figure 5** Structures of PX-12 and NSC 131233.

correlated with the expression of thioredoxin-1 mRNA (182). Several other inhibitors of thioredoxin-1 have been identified by the COMPARE program, from over 50,000 compounds tested by the Naional Cancer Institute in the 60 human tumor cell line panel, as having a pattern of cell-killing activity similar to PX-12 (227). One of these compounds, 2,5-bis[dimethylamino)methyl]cyclopentanone (NSC 131233), is an irreversible inhibitor of thioredoxin-1 with a Ki of 1.0 $\mu$M.

## SUMMARY AND CONCLUSIONS

The mammalian Trxs have been known for many years. However, only recently has the multiplicity of biological functions of Trx become apparent. The most studied Trx, thioredoxin-1, is found in the cytosol and the nucleus and acts as a cofactor that provides reducing equivalents to other redox enzymes. It can directly reduce cysteine groups on proteins to alter protein binding activity, for example of transcription factors, and it can bind to proteins to alter their enzymatic activity. Thioredoxin-1 is a secreted protein, and it acts outside the cell to stimulate cell growth and inside the cell to stimulate cell growth and inhibit apoptosis. Whether these extracellular and intracellular activities of Trx-1 are related is not clear at this time; however, redox activity is necessary for both. The ability of thioredoxin-1 to homodimerize, leading to a loss of redox activity, is conserved among mammalian species. Whether homodimerization occurs in cells and is of biological significance remains to be established. A new mitochondrial Trx family member, thioredoxin-2, has recently been identified, but its function remains unknown. Thioredoxin-1 has multiple biological activities including antioxidant, growth stimulation, and inhibition of apoptosis. However, the mechanisms responsible for most of these activities have not been established, and their identification provides a challenge for future work. Thioredoxin-1 may play a role in a number of human diseases but particularly cancer, in which increased levels of thioredoxin-1 are found in many tumors and are associated with aggressive tumor growth. Thus, thioredoxin-1 presents a target for drugs to inhibit cancer cell growth. The first generation of drugs that inhibit thioredoxin-1 are currently in development. The challenges for the future are to identify a unifying mechanism for the various biological

activities of the Trxs and to determine exactly how redox control of protein function by Trx can occur in the highly reducing intracellular environment of the cell.

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## LITERATURE CITED

1. Laurent TC, Moore EC, Reichard P. 1964. Enzymatic synthesis of deoxyribonucleotides VI. Isolation and characterization of thioredoxin, the hydrogen donor from *Escherichia coli* B. *J. Biol. Chem.* 239: 3436–44
2. Holmgren A. 1985. Thioredoxin. *Annu. Rev. Biochem.* 54:237–71
3. Nakamura H, Nakamura K, Yodoi J. 1997. Redox regulation of cellular activation. *Annu. Rev. Immunol.* 15:351–69
4. Follmann H, Haberlein I. 1995. Thioredoxins: Universal, yet specific thiol-disulfide redox cofactors. *BioFactors* 5:147–56
5. Moore EC. 1967. A thioredoxin-thioredoxin reductase system from rat tumor. *Biochem. Biophys. Res. Commun.* 29:264–68
6. Herrmann EC, Moore EC. 1973. Purification of thioredoxin from rat Novikoff ascites hepatoma. *J. Biol. Chem.* 248:1219–23
7. Wakasugi H, Rimsky L, Mahe Y, Kamel AM, Fradelizi D, et al. 1987. Epstein-Barr virus-containing B-cell line produces an interleukin 1 that it uses as a growth factor. *Proc. Natl. Acad. Sci. USA* 84:804–808
8. Tagaya Y, Okada M, Sugie K, Kasahara T, Kondo N, et al. 1988. IL-2 receptor(p55)/TAC-inducing factor purification and characterization of adult T cell leukemia-derived factor. *J. Immunol.* 140:2614–20
9. Clarke FM, Orozco C, Perkins AV, Cock I, Tonissen KF, et al. 1991. Identification of molecules involved in the 'early pregnancy factor' phenomenon. *J. Reprod. Fert.* 93: 525–39
10. Deiss LP, Kimchi A. 1991. A genetic tool used to identify thioredoxin as a mediator of a growth inhibitory signal. *Science* 252:117–20
11. Gasdaska PY, Oblong JE, Cotgreave IA, Powis G. 1994. The predicted amino acid sequence of human thioredoxin is identical to that of the autocrine growth factor human adult T-cell derived factor (ADF): Thioredoxin mRNA is elevated in some human tumors. *Biochim. Biophys. Acta* 1218:292–96
12. Holmgren A. 1989. Thioredoxin and glutaredoxin systems. *J. Biol. Chem.* 264: 13963–66
13. Mustacich D, Powis G. 2000. Thioredoxin reductase. *Biochem. J.* 346:1–8
14. Gladyshev VN, Jeang K, Stadtman TC. 1996. Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. *Proc. Natl. Acad. Sci. USA* 93:6146–51
15. Powis G, Gasdaska JR, Gasdaska PY, Berggren M, Kirkpatrick DL, et al. 1997. Selenium and the thioredoxin redox system: effects on cell growth and death. *Oncol. Res.* 9:303–12
16. Gasdaska JR, Harney JW, Gasdaska PY, Powis G, Berry MJ. 1999. Regulation of human thioredoxin reductase expression and activity by 3′-untranslated region selenocysteine insertion sequence and mRNA instability elements. *J. Biol. Chem.* 274:25379–85

17. Zhong L, Arner ESJ, Ljung J, Aslund F, Holmgren A. 1998. Rat and calf thioredoxin reductase are homologous to glutathione reductase with a carboxyl-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. *J. Biol. Chem.* 273:8581–91
18. Gasdaska PY, Gasdaska JR, Cochran S, Powis G. 1995. Cloning and sequencing of human thioredoxin reductase. *FEBS Lett.* 373:5–9
19. Gasdaska PY, Berggren MM, Berry MJ, Powis G. 1999. Cloning, sequencing and functional expression of a novel human thioredoxin reductase. *FEBS Lett.* 442:105–11
20. Lee S, Kim J, Kwon K, Yoon HW, Levine RL, et al. 1999. Molecular cloning and characterization of a mitochondrial selenocysteine-containing thioredoxin reductase from rat liver. *J. Biol. Chem.* 274:4722–34
21. Miranda-Vizuete A, Damdimopoulos AE, Pedrajas JR, Gustafsson J-A, Spyrou G. 1999. Human mitochondrial thioredoxin reductase: cDNA cloning, expression and genomic organization. *Eur. J. Biochem.* 261:405–11
22. Miranda-Vizuete A. 1999. TrxR3 a novel human thioredoxin reductase. GenBank Accession *AF133519*:bp 1–2165
23. Gotoh S, Terashima H, Yagi K, Mizoguchi T. 1998. Cloning and sequence of bovine thioredoxin cDNA. GenBank Accession *AF104105*:bp 1–550
24. Matsui M, Taniguchi Y, Hirota K, Taketo M, Yodoi J. 1995. Structure of the mouse thioredoxin-encoding gene and its processed pseudogene. *Gene* 152:165–71
25. Gasdaska JR, Berggren M, Powis G. 1995. Cell growth stimulation by the redox protein thioredoxin occurs by a novel helper mechanism. *Cell Growth Differ.* 6:1643–50
26. Gasdaska JR, Hill SR, Kirkpatrick L, Montfort W, Weichsel A, et al. 1996. Oxidative inactivation of thioredoxin as a cellular growth factor and protection by a $Cys^{73>}$ Ser mutation. *Biochem. Pharmacol.* 52:1741–47
27. Heppell-Parton A, Cahn A, Bench A, Lowe N, Lehrach H, et al. 1995. Thioredoxin, a mediator of growth inhibition, maps to 9q31. *Genomics* 26:379–81
28. Kaghad M, Dessarps F, Jacquemin-Sablon H, Caput D, et al. 1994. Genomic cloning of human thioredoxin-encoding gene: mapping of the transcription start point and analysis of the promoter. *Gene* 140:273–78
29. Tonissen KF, Wells JRE. 1991. Isolation and characterization of human thioredoxin-encoding genes. *Gene* 102: 221–28
30. Miranda-Vizuete A. 1999. Human thioredoxin 1 pseudogene 2. GenBank Accession *AF146024*:bp 1–1210
31. Taketo M, Matsui M, Rochelle JM, Yodoi J, Seldin MF. 1994. Mouse thioredoxin gene maps on chromosome 4, whereas its pseudogene maps on chromosome 1. *Genomics* 21:251–53
32. Wollman EE, d'Auriol L, Rimsky L, Shaw A, Jacquot J, et al. 1988. Cloning and expression of cDNA for human thioredoxin. *J. Biol. Chem.* 263:15506–12
33. Rosen A, Lundman P, Carlsson M, Bhavani K, Srinivasa BR, et al. 1995. A $CD4^+$ T cell line-secreted factor, growth promoting for normal and leukemic B cells, identified as thioredoxin. *Inter. Immunol.* 7:625–33
34. Hariharan J, Hebbar P, Ranie J, Philomena Sinha AM, Datta S. 1996. Alternative forms of the human thioredoxin mRNA: identification and characterization. *Gene* 173:265–70
35. Berggren M, Mustacich D, Powis G. 2000. Absence of mutant forms of thioredoxin-1 (TRX-1) in human tumor cell lines and evidence for an alternatively spliced TRX-1 MRNA. *Amer. Assoc. Cancer Res.* 41:151 (Conf.)

36. Bodenstein J, Follmann H. 1991. Characterization of two thioredoxins in pig heart including a new mitochondrial protein. *Z. Naturforsch.* 46c:270–79 (In German)
37. Spyrou G, Enmark E, Miranda-Vizuete A, Gustafsson J. 1997. Cloning and expression of a novel mammalian thioredoxin. *J. Biol. Chem.* 272:2936–41
38. Miranda–Vizuete A, Gustafsson J–A, Spyrou G. 1999. Human mitochondrial thioredoxin. GenBank Accession *HSU78678.1*:bp 1–756
39. Miranda-Vizuete A, Gustafsson J, Spyrou G. 1998. Molecular cloning and expression of a cDNA encoding a human thioredoxin-like protein. *Biochem. Biophys. Res. Commun.* 243:284–88
40. Lee K, Murakawa M, Takahashi S, Tsubuki S, Kawashima S, et al. 1998. Purification, molecular cloning, and characterization of TRP32, a novel thioredoxin-related mammalian protein of 32 kDa. *J. Biol. Chem.* 273:19160–66
41. Kurooka H, Kato K, Minoguchi S, Takahashi Y, Ikeda J, et al. 1997. Cloning and characterization of the nucleoredoxin gene that encodes a novel nuclear protein related to thioredoxin. *Genomics* 39:331–39
42. Balcewicz-Sablinska MK, Wollman EE, Gort R, Silberstein DS. 1991. Human eosinophil cytotoxicity-enhancing factor II. Multiple forms synthesized by U937 cells and their relationship to thioredoxin/adult T cell leukemia-derived factor. *J. Immunol.* 147:2170–74
43. Silberstein DS, McDonough S, Minkoff MS, Balcewicz-Sablinska MK. 1993. Human eosinophil cytotoxicity-enhancing factor. *J. Biol. Chem.* 268:9138–42
44. Qin J, Clore G, Gronenborn A. 1994. The high-resolution three-dimensional solution structures of the oxidized and reduced state of human thioredoxin. *Structure* 2: 502–22
45. Weichsel A, Gasdaska JR, Powis G, Montfort WR. 1996. Crystal structures of reduced, oxidized, and mutated human thioredoxins: evidence for a regulatory homodimer. *Structure* 4:735–51
46. Katti SK, LeMaster DM, Eklund H. 1990. Crystal structure of thioredoxin from *Escherichia coli* at 1.68 Å resolution. *J. Mol. Biol.* 212:167–84
47. Holmgren A, Soderberg B-O, Eklund H, Branden C-I. 1975. Three-dimensional structure of *Escherichia coli* thioredoxin-S2 to 2.8 Å resolution. *Proc. Natl. Acad. Sci. USA* 72:2305–309
48. Jeng MF, Campbell A, Begley T, Holmgren A, Case D, et al. 1994. High-resolution solution structures of oxidized and reduced *Escherichia coli* thioredoxin. *Structure* 2:853–68
49. Eklund H, Gleason F, Holmgren A. 1991. Structural and functional relations among thioredoxins of different species. *Proteins* 11:13–28
50. Holmgren A. 1995. Thioredoxin structure mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. *Structure* 3:239–43
51. Takahashi N, Creighton TE. 1996. On the reactivity and ionization of the active site cysteine residues of *Escherichia coli* thioredoxin. *Biochem.* 35:8342–53
52. Ren X, Bjornstedt M, Shen B, Ericson ML, Holmgren A. 1993. Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione. *Biochem.* 32:9701–705
53. Andersen JF, Sanders DAR, Gasdaska JR, Weichsel A, Powis G, Montfort WR. 1997. Human thioredoxin homodimers: regulation of pH, role of Aspartate 60, and crystal structure of the Aspartate 60 > Asparagine mutant. *Biochem.* 36:13979–88
54. Gronenborn AM, Clore GM, Louis JM, Wingfield PT. 1999. Is human thioredoxin monomeric or dimeric? *Protein Sci.* 8: 426–29
55. Holmgren A, Luthman M. 1978. Tissue distribution and subcellular localization of bovine thioredoxin determined by

radioimmunoassay. *Biochem.* 17:4071–77
56. Qin J, Clore GM, Kennedy WP, Hutch JR, Gronenborn AM. 1994. Solution structure of human thioredoxin in a mixed disulfide intermediate complex with its target peptide from the transcription factor NF–kappaB. *Structure* 3:289–97
57. Qin J, Clore GM, Kennedy WP, Kuszewski J, Gronenborn AM. 1996. The solution structure of human thioredoxin complexed with its target from Ref-1 reveals peptide chain reversal. *Structure* 4:613–20
58. Taniguchi Y, Taniguchi-Ueda Y, Mori K, Yodi J. 1996. A novel promoter sequence is involved in the oxidative stress-induced expression of the adult T-cell leukemia derived factor (ADF)/human thioredoxin (Trx) gene. *Nucl. Acids Res.* 24:2748–52
59. Masutani H, Hirota K, Sasada T, Ueda-Taniguchi Y, Taniguchi Y, et al. 1996. Transactivation of an inducible anti-oxidative stress protein, human thioredoxin by HTLV-I tax. *Immunol. Lett.* 54:67–71
60. Berggren M, Gallegos A, Gasdaska JR, Gasdaska PY, Warneke J, Powis G. 1996. Thioredoxin and thioredoxin reductase gene expression in human tumors and cell lines, and the effects of serum stimulation and hypoxia. *Anticancer Res.* 16:3459–66
61. Denko N, Schindler C, Koong A, Laderoute K, Green C, and Giaccia A. 2000. Epigenetic regulation of gene expression in cervical cancer cells by the tumor microenvironment. *Clin. Cancer Res.* 6:480–87
62. Ericson ML, Hörling J, Wendel-Hansen V, Holmgren A, and Rosen A. 1992. Secretion of thioredoxin after *in vitro* activation of human B cells. *Lymphokine Cytokine Res.* 11:201–207
63. Ejima K, Koji T, Nanri H, Kashimura M, and Ikeda M. 1999. Expression of thioredoxin and thioredoxin reductase in placentae of pregnant mice exposed to lipopolysaccharide. *Placenta* 20:561–66
64. Das KC, Guo XL, White CW. 1999. Hyperoxia induces thioredoxin and thioredoxin reductase gene expression in lungs of premature baboons with respiratory distress and bronchopulmonary dysplasia. *Chest* 116:101s
65. Das KC, Guo X, White CW. 1999. Induction of thioredoxin and thioredoxin reductase gene expression in lungs of newborn primates by oxygen. *Amer. J. Physiol.* 276:L530–39
66. Hayashi T, Ueno Y, Okamoto T. 1993. Oxidoreductive regulation of nuclear factor kappa B. *J. Biol. Chem.* 268:11380–88
67. Sachi Y, Hirota K, Masutani H Toda K, Takahashi O, et al. 1995. Induction of ADF/TRX by oxidative stress in keratinocytes and lymphoid cells. *Immunol. Lett.* 44:189–93
68. Higashikubo A, Tanaka N, Noda N, Maeda I, Yagi K, et al. 1999. Increase in thioredoxin activity of intestinal epithelial cells mediated by oxidative stress. *Biol. Pharm. Bull.* 22:900–903
69. Zhou Y, Kok KH, Chun AC, Wong CM, Wu HW, et al. 2000. Mouse peroxiredoxin V is a thioredoxin peroxidase that inhibits p53-induced apoptosis. *Biochem. Biophys. Res. Commun.* 268:921–27
70. Nakamura H, Matsuda M, Furuke K, Kitaoka Y, Iwata S, et al. 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
71. Tomimoto H, Akiguchi I, Wakita H, Kimura J, Hori K Yodoi J. 1993. Astroglial expression of ATL–derived factor, a human thioredoxin homologue, in the gerbil brain after transient global ischemia. *Brain Res.* 625:1–8
72. Nakamura H, Masutani H, Tagaya Y, Yamauchi A, Inamoto Tet al. 1992. Expression and growth-promoting effect of adult T-cell leukemia-derived factor. A human thioredoxin homologue in hepatocellular carcinoma. *Cancer* 69:2091–97

73. Fujii S, Nanbu Y, Nonogaki H, Konishi I, Mori T, et al. 1991. Coexpression of adult T-cell leukemia-derived factor, a human thioredoxin homologue, and human papillomavirus DNA in neoplastic cervical squamous epithelium. *Cancer* 68:1583–91
74. Hutchison KA, Matic G, Meshinchi S, Bresnick EH, Pratt WB. 1991. Redox manipulation of DNA binding activity and BuGR epitope reactivity of the glucocorticoid receptor. *J. Biol. Chem.* 266:10505–509
75. Hoshi Y, Tanooka H, Miyazaki K, Wakasugi H. 1997. Induction of thioredoxin in human lymphocytes with low-dose ionizing radiation. *Biochim. Biophys. Acta* 1359:65–70
76. Leppa S, Pirkkala L, Chow SC, Eriksson JE, Sistonen L. 1997. Thioredoxin is transcriptionally induced upon activation of heat shock factor 2. *J. Biol. Chem.* 272:30400–404
77. An G, Wu R. 1992. Thioredoxin gene expression is transcriptionally up-regulated by retinol in monkey conducting airway epithelial cells. *Biochem. Biophys. Res. Commun.* 183:170–75
78. Maruyama T, Sachi Y, Furuke K, Kitaoka Y, Kanzaki H, et al. 1999. Induction of thioredoxin, a redox-active protein, by ovarian steroid hormones during growth and differentiation of endometrial stromal cells in vitro. *Endocrinol.* 140:365–72
79. Ejima K, Nanri H, Araki M, Uchida K, Kashimura M, Ikeda M. 1999. 17beta-estradiol induces protein thiol/disulfide oxidoreductases and protects cultured bovine aortic endothelial cells from oxidative stress. *Eur. J. Endocrinol.* 140:608–13
80. Sahlin L, Wang H, Masironi B, Holmgren A, Eriksson H. 1999. Regulation of thioredoxin mRNA in the rat uterus by gonadal steroids. *J. Steroid Biochem. Mol. Biol.* 68:203–209
81. U-Taniguchi Y, Furuke K, Masutani H, Nakamura H, Yodoi J. 1995. Cell cycle inhibition of HTLV-1 transformed T cell lines by retinoic acid: the possible therapeutic use of thioredoxin reductase inhibitors. *Oncol. Res.* 7:183–189
82. Grogan TM, Fenoglio-Prieser C, Zeheb R, Bellamy W, Frutiger Y, et al. 2000. Thioredoxin a putative oncogene product is overexpressed in gastric carcinoma and associated with increased proliferation and increased cell survival. *Hum. Pathol.* 31:475–81
83. Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, Yodoi J. 1997. AP–1 transcriptional activity is regulated by a direct association between thioredoxin and Ref–1. *Proc. Natl. Acad. Sci. USA* 94:3633–38
84. Nakamura H, Bai J, Nishinaka Y, Ueda S, Sasada T, et al. 2000. Expression of thioredoxin and glutaredoxin, redox-regulating proteins, in pancreatic cancer. *Cancer Detect. Prev.* 24:53–60
85. Kawahara N, Tanaka T, Yokomizo A, Nanri H, Ono M, et al. 1996. Enhanced coexpression of thioredixin and high mobility group protein 1 genes in human hepatocellular carcinoma and the possible association with decreased sensitivity to cisplatin. *Cancer Res.* 56:5330–33
86. Makino Y, Yoshikawa N, Okamoto K, Hirota K Yodoi J, et al. 1999. Direct association with thioredoxin allows redox regulation of glucocorticoid receptor function. *J. Biol. Chem.* 274:3182–88
87. Ema M, Hirota K, Mimura J, Abe H, Yodoi J, et al. 1999. Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *EMBO J.* 18:1905–14
88. Ueno M, Masutani H, Jun Arai R, Yamauchi A, Hirota K, et al. 2000. Thioredoxin-dependent redox regulation of p53-mediated p21 activation. *J. Biol. Chem.* 274:35809–15
89. Imamoto N, Kamei Y, Yoneda Y. 1998. Nuclear transport factors: function, behavior and interaction. *Eur. J. Histochem.* 42:9–20

90. Martin H, Dean M. 1991. Identification of a thioredoxin-related protein associated with plasma membranes. *Biochem. Biophys. Res. Commun.* 175:123–28
91. Wollman EE, Kahan A, Fradelizi D. 1997. Detection of membrane associated thioredoxin on human cell lines. *Biochem. Biophys. Res. Commun.* 230:602–606
92. Wakasugi N, Tagaya Y, Wakasugi A, Mitsui M, Maeda M, et al. 1990. Adult T-cell leukemia–derived factor/Thioredoxin produced by both human T–lymphotropic virus type 1 and Epstein-Barr virus-transformed lymphocytes, acts as an autocrine growth factor and synergized with interleukin-1 and interleukin-2. *Proc. Natl. Acad. Sci. USA* 87:8282–86
93. Ericson ML, Horling J, Wendel-Hansen V, Holmgren A, Rosen A. 1992. Secretion of thioredoxin after in vitro activation of human B cells. *Lymphokine Cytokine Res.* 11:201–207
94. Rubartelli A, Bajetto A, Allavena G, Wollman E, Sitia R. 1992. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J. Biol. Chem.* 267:24161–64
95. Hori K, Katayama M, Sato N, Ishii K, Waga S, Yodoi J. 1994. Neuroprotection by glial cells through adult T cell leukemia-derived factor/human thioredoxin (ADF/TRX). *Brain Res.* 652:304–10
96. Rubartelli A, Bonifaci N, Sitia R. 1995. High rates of thioredoxin secretion correlate with growth arrest in hepatoma cells. *Cancer Res.* 55:675–80
97. Rubartelli A, Sitia R. 1991. Interleukin I$\beta$ and thioredoxin are secreted through a novel pathway of secretion. *Biochem. Soc. Trans.* 19:255–59
98. Oblong JE, Berggren M, Gasdaska PY, Powis G. 1994. Site-directed mutagenesis of active site cysteines in human thioredoxin produces competitive inhibitors of human thioredoxin reductase and elimination of mitogenic properties of thioredoxin. *J. Biol. Chem.* 269:11714–20
99. Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, et al. 1989. ATL-derived factor (ADF) an IL-2 receptor/Tac inducer homologous to thioredoxin: possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO J.* 8:757–64
100. Schenk H, Vogt M, Droge W, Schulze-Osthoff K. 1996. Thioredoxin as a potent costimulus of cytokine expression. *J. Immunol.* 156:765–71
101. Yoshida S, Katoh T, Tetsuka T, Uno K, Matsui N, Okamoto T. 1999. Involvement of thioredoxin in rheumatoid arthritis: its costimulatory roles in the TNF-alpha-induced production of IL-6 and IL-8 from cultured synovial fibroblasts. *J. Immunol.* 163:351–58
102. Hashimoto S, Matsumoto K, Gon Y, Furuichi S Maruoka S, et al. 1999. Thioredoxin negatively regulates p38 MAP kinase activation and IL-6 production by tumor necrosis factor-alpha. *Biochem. Biophys. Res. Commun.* 258:443–47
103. Natsuyama S, Noda Y, Yamashita M, Nagahama Y, Mori T. 1993. Superoxide dismutase and thioredoxin restore defective $p34^{cdc2}$ kinase activation in mouse two-cell block. *Biochim. Biophys. Acta* 1176:90–94
104. Spector A, Yan G-Z, Huang R-RC, McDermott MJ, Gascoyne PRC, Pigiet V. 1988. The effect of $H_2O_2$ upon thioredoxin-enriched lens epithelial cells. *J. Biol. Chem.* 263:4984–90
105. Bjornstedt M, Xue J, Huang W, Akesson B, Holmgren A. 1994. The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. *J. Biol. Chem.* 269:29382–84
106. Hogarth L, English M, Price L, Wyllie R, Pearson ADJ, Hall AG. 1996. The effect of treatment with high dose melphalan, cisplatin or carboplatin on levels of glutathione in plasma, erythrocytes, mononuclear cells and urine. *Cancer Chemother. Pharmacol.* 37:479–85

107. Berntsen H, Lonning PE, Ekse D, Netteland B, Johannessen DC, et al. 1998. Influence of treatment with aminoglutethimide on plasma and red-blood-cell glutathione status in breast cancer patients. *Cancer Chemother. Pharmacol.* 42:46–52
108. Cotgreave IA, Gerdes RG. 1998. Recent trends in glutathione biochemistry—glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? *Biochem. Biophys. Res. Commun.* 242:1–9
109. Chae HZ, Chung SJ, Rhee SG. 1994. Thioredoxin-dependent peroxide reductase from yeast. *J. Biol. Chem.* 269:27670–78
110. Jin D, Zoon HJ, Rhee SG, Jeang K. 1997. Regulatory role for a novel human thioredoxin peroxidase in NF-kappaB activation. *J. Biol. Chem.* 272:30952–61
111. Shau H, Butterfield LH, Chiu R, Kim A. 1994. Cloning and sequence analysis of candidate human nautral killer-enhancing factor genes. *Immunogenetics* 40:129–34
112. Pahl P, Berger R, Hart I, Chae HZ, Rhee SG, Patterson D. 1995. Localization of TDPX1, a human homologue of the yeast thioredoxin-dependent peroxide reductase gene (TPX) to chromosome 13q12. *Genomics* 26:602–606
113. Prosperi M, Ferbus D, Karczinski I, Goubin G. 1993. A human cDNA corresponding to a gene overexpressed during cell proliferation encodes a product sharing homology with amoebic and bacterial proteins. *J. Biol. Chem.* 268:11050–56
114. Hirotsu S, Abe Y, Okada K, Nagahara N, Hori H, et al. 1999. Crystal structure of a multifunctional 2-Cys peroxiredoxin heme-binding protein 23 kDa/proliferation-associated gene product. *Proc. Natl. Acad. Sci. USA* 96:12333–38
115. Kang SW, Chae HZ, Seo MS, Kim K, Baines IC, Rhee SG. 1998. Mammalian peroxidoxin isoforms can reduce hydrogen peroxide generated in response to growth factors and tumor necrosis factor-$\alpha$. *J. Biol. Chem.* 273:6297–302
116. Watabe S, Kohno H, Kouyama H, Hiroi T, Yago N, Nakazawa T. 1994. Purification and characterization of a substrate protein for mitochondrial ATP-dependent protease in bove adrenal cortex. *J. Biochem.* 115:648–54
117. Matsumoto A, Okado A, Fujii T, Fujii J, Egashira M, et al. 1999. Cloning of the peroxiredoxin gene family in rats and characterization of the fourth member. *FEBS Lett.* 443:246–50
118. Haridas V, Ni J, Meager A, Su J, Yu G-L, et al. 1998. Cutting edge: TRANK, a novel cytokine that activates NF-kappaB and c-Jun N-terminal kinase. *J. Immunol.* 161:1–6
119. Kang SW, Baines IC, Rhee SG. 1998. Charaterization of a mammaliam peroxiredoxin that contains one conserved cysteine. *J. Biol. Chem.* 273:6303–11
120. Lyu MC, Rhee SG, Chae HZ, Lee TH, Adamson MC, et al. 1999. Genetic mapping of six mouse peroxiredoxin genes and fourteen peroxiredoxin related sequences. *Mamm. Genome* 10:1017–19
121. Yanagawa T, Ishikawa T, Ishii T, Tabuchi K, Iwasa S, et al. 1999. Peroxiredoxin I expression in human thyroid tumors. *Cancer Lett.* 145:127–32
122. Ichimiya S, Davis JG, O'Rourke DM, Katsumata M, Greene MI. 1997. Murine thioredoxin peroxidase delays neuronal apoptosis and is expressed in areas of the brain most susceptible to hypoxic and ischemic injury. *DNA Cell Biol.* 16:311–21
123. Shau H, Gupta RK, Golub SH. 1993. Identification of a natural killer enhancing factor (NKEF) from human erythroid cells. *Cell. Immunol.* 147:1–11
124. Shau H, Kim AT, Hedrick CC, Lusis AJ, Tompkins C, et al. 1997. Endogenous natural killer enhancing factor-B increases cellular resistance to oxidative stresses. *Free Rad. Biol. Med.* 22:497–507

125. Prosperi M, Apiou F, Dutrillaux B, Goubin G. 1994. Organization and chromosomal assignment of two human PAG gene loci: PAGA encoding a functional gene and PAGB a processed pseudogene. *Genomics* 19:236–41
126. Zhang P, Liu B, Kang SW, Seo MS, Rhee SG, Obeid LM. 1997. Thioredoxin peroxidase is a novel inhibitor of apoptosis with a mechanism distinct from that of Bcl-2. *J. Biol. Chem.* 272:30615–18
127. Ruppitsch W, Meisslitzer C, Hirsch-Kauffmann M, Schweiger M. 1998. Overexpression of thioredoxin in fanconi anemia fibroblasts prevents the cytotoxic and DNA damaging effect of mitomycin C and diepoxybutane. *FEBS Lett.* 422:99–102
128. Prosperi M, Ferbus D, Rouillard D, Goubin G. 1998. The pag gene product, a physiological inhibitor of c-abl tyrosine kinase is overexpressed in cells entering S phase and by contact with agents inducing oxidative stress. *FEBS Lett.* 423:39–44
129. Stadtman TC 1996 Selenocysteine. *Annu. Rev. Biochem.* 65:83–100
130. Berggren MM, Mangin JF, Gasdaska JR, Powis G. 1999. Effect of selenium on rat thioredoxin reductase activity: increase by supranutritional selenium and decrease by selenium deficiency. *Biochem. Pharmacol.* 57:187–93
131. Jordan A, Reichard P. 1998. Ribonucleotide reductases. *Annu. Rev. Biochem.* 67:71–98
132. Hopper S, Iurlano D. 1983. Properties of a thioredoxin purified from rabbit bone marrow which fails to serve as a hydrogen donor for the homologous ribonucleotide reductase. *J. Biol. Chem.* 258:13453–57
133. Mau B, Powis G. 1992. Inhibition of cellular thioredoxin reductase by diaziquone and doxorubicin. *Biochem. Pharmacol.* 43:1621–26
134. Silverman RB, Nandi DL. 1988. Reduced thioredoxin: a possible physiological cofactor for vitamin K epoxide reductase. Further support for an active site disulfide. *Biochem. Biophys. Res. Commun.* 155:1248–54
135. Soute BAM, Groenen-van Dooren MCL, Holmgren A, Lundström J, Vermeer C. 1992. Stimulation of the dithiol-dependent reductases in the vitamin K cycle by the thioredoxin system: strong synergistic effects with protein disulphide-isomerase. *Biochem. J.* 281:255–59
136. Preusch PC. 1992. Is thioredoxin the physiological vitamin K epoxide reducing agent? *FEBS Lett.* 305:257–59
137. Gilmore TD, Koedood M, Piffat KA, White DW 1997. Rel/NF-kappaB/ IkappaB proteins and cancer. *Oncogene* 11: 1367–78
138. Moore BE, Bose HRJ. 1988. Expression of the *v-rel* oncogene in reticuloendotheliosis virus-transformed fibroblasts. *Virology* 162:377–87
139. Tolendano MB, Leonard WJ. 1991. Modulation of transcription factor NF-kappaB binding activity by oxidation-reduction *in vitro*. *Proc. Natl. Acad. Sci. USA* 88:4328–32
140. Hayashi T, Ueno Y, Okamoto T. 1993. Oxidoreductive regulation of nuclear factor kappaB: Involvement of a cellular reducing catalyst thioredoxin. *J. Biol. Chem.* 268:11380–88
141. Galter D, Mihm S, Dröge W. 1994. Distinct effects of glutathione disulfide on the nuclear transcription factors kappaB and the activator protein-1. *Eur. J. Biochem.* 221:639–48
142. Sorachi K, Sugie K, Maekawa N, Takami M, Kawabe T, et al. 1992. Induction and function of FcERII on YT cells: possible role of ADF/thioredoxin in FcERII expression. *Immunobiology* 185:193–206
143. Matthews JR, Wakasugi N, Virelizier J-L, Yodoi J, Hay RT. 1992. Thioredoxin regulates the DNA binding activity of NF-kappaB by reduction of a disulphide bond involving cysteine 62. *Nucl. Acids Res.* 20:3821–30

144. Ghosh G, Van Duyne G, Ghosh S, Sigler PB. 1995. The structure of NFkappaB p50 homodimer bound to a kappaB site. *Nature* 373:303–10
145. Muller GW, Rey FA, Sodeoka M, Verdine GL, Harrison SC. 1995. Structure of the NF–kappaB homodimer bound to DNA. *Nature* 373:311–17
146. Freemerman AJ, Gallegos A, Powis G. 1999. NF–kappaB transactivation is increased but not involved in the proliferative effects of thioredoxin overexpression in MCF–7 breast cancer cells. *Cancer Res.* 59:4090–94
147. Schenk H, Klein M, Erdbrügger W, Dröge W, Schulze-Osthoff K. 1994. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappaB and AP-1. *Proc. Natl. Acad. Sci. USA* 91:1672–76
148. Chakraborti PK, Garabedian MJ, Yamamoto KR, Simons SS Jr. 1992. Role of cysteines 640, 656, and 661 in steroid binding to rat glucocorticoid receptors. *J. Biol. Chem.* 267:11366–73
149. Makino Y, Okamoto K, Yoshikawa N, Aoshima M, Hirota K, et al. 1996. Thioredoxin: a redox-regulating cellular cofactor for glucocorticoid hormone action. Cross talk between endocrine control of stress response and cellular antioxidant defense system. *J. Clin. Invest.* 98:2469–77
150. Grippo JF, Tienrungroj W, Dahmer MK, Housley PR, Pratt WB. 1983. Evidence that the endogenous heat-stable glucocorticoid receptor-activating factor is thioredoxin. *J. Biol. Chem.* 258:13658–64
151. Tanaka H, Makino Y, Okamoto K. 1999. Thioredoxin in the endocrine response to stress. *Vitam. Horm.* 57:153–75
152. Meyer M, Schreck R, Baeuerle PA. 1993. $H_2O_2$ and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J.* 12:2005–15
153. Abate C, Patel L, Rauscher III FJ, Curran T. 1990. Redox regulation of fos and jun DNA-binding activity *in vitro*. *Science* 249:1157–61
154. Xanthoudakis S, Miao GG, Curran T. 1994. The redox and DNA-repair activities of ref-1 are encoded by nonoverlapping domains. *Proc. Natl. Acad. Sci. USA* 91:23–27
155. Huan Y, Domann FE. 1998. Redox modulation of AP-2 DNA binding activity in vitro. *Biochem. Biophys. Res. Commun.* 249:307–12
156. Hayashi S, Hajiro-Nakanishi K, Makino Y, Eguchi H, Yodoi J, Tanaka H. 1997. Functional modulation of estrogen receptor by redox state with reference to thioredoxin as a mediator. *Nucl. Acids Res.* 25:4035–40
157. Akamatsu Y, Ohno T, Hirota K, Kagoshima H, Yodoi J, Shigesada K. 1997. Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues. *J. Biol. Chem.* 272:14497–500
158. Lane DP. 1994. p53 and human cancers. *Brit. Med. Bull.* 50:582–99
159. Banin S, Moyal L, Shieh S, Taya Y, Anderson CW, et al. 1998. Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* 281:1674–77
160. Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, et al. 1998. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* 281:1677–79
161. Hainaut P, Milner J. 1993. Redox modulation of p53 conformation and sequence-specific DNA binding *in vitro*. *Cancer Res.* 53:4469–73
162. Bischoff JR, Casso D, Beach D. 1999. Human p53 inhibits growth in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* 12:1405–11
163. Casso D, Beach D. 1996. A mutation in a thioredoxin reductase homolog suppresses p53-induced growth inhibition

in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* 252:518–29
164. Pearson GD, Merrill GF. 1998. Deletion of the *Saccharomyces cerevisiae TRR1* gene encoding thioredoxin reductase inhibits p53-dependent reporter gene expression. *J. Biol. Chem.* 273:5431–34
165. Merrill GF, Dowell P, Pearson GD. 1999. The human 53 negative regulatory domain mediates inhibition of reporter gene transactivation in yeast lacking thioredoxin reductase. *Cancer Res.* 59:3175–79
166. Jayaraman L, Murthy KG, Zhu C, Curran T, Xanthoudakis S, Prives C. 1997. Identification of redox/repair protein Ref-1 as a potent activator of p53. *Genes Develop.* 11:558–70
167. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, et al. 1998. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *The EMBO* 17:2596–606
168. Liu H, Nishitoh H, Ichijo H, Kyriakis JM. 2000. Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. *Mol. Cell. Biol.* 20:2198–208
169. Isowa N, Yoshimura T, Kosaka S, Liu M, Hitomi S, et al. 2000. Human thioredoxin attenuates hypoxia-reoxygenation injury of murine endothelial cells in a thiol-free condition. *J. Cell. Physiol.* 182:33–40
170. Nishiyama A, Ohno T, Iwata S, Matsui M, Hirota K, et al. 1999. Demonstration of the interaction of thioredoxin with p40phox, a phagocyte oxidase component, using a yeast two-hybrid system. *Immunol. Lett.* 68:155–59
171. Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, et al. 1997. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275:90–94
172. Tobiume K, Inage T, Takeda K, Enomoto S, Miyazono K, Ichijo H. 1997. Molecular cloning and characterization of the mouse apoptosis signal-regulating kinase 1. *Biochem. Biophys. Res. Commun.* 239:905–10
173. Lee K, Murakawa M, Nishida E, Tsubuki S, Kawashima S, et al. 1998. Proteolytic activation of MST/Krs, STE20-related protein kinase, by caspase during apoptosis. *Oncogene* 16:3029–37
174. Iwata S, Hori T, Sato N, Hirota K, Sasada T, et al. 1997. Adult T cell leukemia (ATL)-derived factor/human thioredoxin prevents apoptosis of lymphoid cells induced by L-cystine and glutathione depletion: possible involvement of thiol-mediated redox regulation in apoptosis caused by pro-oxidant state. *J. Immunol.* 158:3108–17
175. Nilsson J, Soderberg O, Nilsson K, Rosen A. 2000. Thioredoxin prolongs survival of B-type chronic lymphocytic leukemia cells. *Blood* 95:1420–26
176. Baker A, Payne CM, Briehl MM, Powis G. 1997. Thioredoxin, a gene found overexpressed in human cancer, inhibitis apoptosis *in vitro* and *in vivo*. *Cancer Res.* 57:5162–67
177. McDonnell TJ, Korsmeyer SJ. 1991. Progression from lymphoid hyperplasia to high–grade malignant lymphoma in mice transgenic for the t(14:18). *Nature* 349:254–56
178. Russel M, Model P. 1986. The role of thioredoxin in filamentous phage assembly. Construction, isolation, and characterization of mutant thioredoxins. *J. Biol. Chem.* 261:14997–5005
179. Huber HE, Russel M, Model P, Richardson CC. 1986. Interaction of mutant thioredoxin of *Escherichia coli* with the gene 5 protein of phage T7: The redox capacity of thioredoxin is not required for stimulation of DNA polymerase activity. *J. Biol. Chem.* 261:15006–5012
180. Tonissen K, Wells J, Cock I, Perkins A, Orozco C, Clarke F. 1993. Site-directed mutagenesis of human thioredoxin.

Identification of cysteine 74 as critical to its function in the "early pregnancy factor" system. *J. Biol. Chem.* 268:22485–89

181. Wang J, Kobayashi M, Sakurada K, Imamura M, Moriuchi T, Hosokawa M. 1997. Possible roles of an adult T-cell leukemia (ATL)-derived factor/thioredoxin in the drug resistance of ATL to adriamycin. *Blood* 89:2480–87
182. Kunkel M, Kirkpatrick DL, Johnson G, Johnson JI, Paul KT, et al. 1997. Cell line-directed screening assay for inhibitors of thioredoxin reductase signalling as potential anticancer drugs. *Anti-Cancer Drug Des.* 12:659–70
183. Yokomizo A, Ono M, Nanri H, Makino Y, Ohga T, et al. 1995. Cellular levels of thioredoxin associated with drug sensitivity to cisplatin, mitomycin C, doxorubicin and etoposide. *Cancer Res.* 55:4293–96
184. Yamada M, Tomida A, Yoshikawa H, Taketani Y, Tsuruo T. 1996. Increased expression of thioredoxin/adult T-cell leukemia-derived factor in cisplatin-resistant human cancer cell lines. *Cancer Res. Clin.* 2:427–32
185. Yamada M, Tomida A, Yoshikawa H, Taketani Y, Tsuruo T. 1997. Overexpression of thioredoxin does not confer resistance to cisplatin in transfected human ovarian and colon cancer cell lines. *Cancer Chemother. Pharmacol.* 40:31–37
186. Sasada T, Iwata S, Sato N, Kitaoka Y, Hirota K, et al. 1996. Redox control of resistance to cis-diamminedichloroplatinum (II) (CDDP): protective effect of human thioredoxin against CDDP-induced cytotoxicity. *J. Clin. Invest.* 97:2268–76
187. Sasada T, Nakamura H, Ueda S, Sato N, Kitaoka Y, et al. 1999. Possible involvement of thioredoxin reductase as well as thioredoxin in cellular sensitivity to cis-diamminedichloroplatinum (II). *Free Rad. Biol. Med.* 27:504–14
188. Miyazaki K, Noda N, Okada S, Hagiwara Y, Miyata M, et al. 1998. Elevated serum level of thioredoxin in patients with hepatocellular carcinoma. *Biotherapy* 11:277–88
189. Aota M, Matsuda K, Isowa N, Wada H, Yodoi J, Ban T. 1996. Protection against reperfusion-induced arrhythmias by human thioredoxin. *J. Cardiovasc. Pharmacol.* 27:727–32
190. Yokomise H, Fukuse T, Hirata T, Olikubo K, Go T, et al. 1994. Effect of recombinant human adult T cell leukemia-derived factor on rat lung reperfusion injury. *Respiration* 61:99–104
191. Okubo K, Kosaka S, Isowa N, Hirata T, Hitomi S, et al. 1997. Amelioration of ischemia-reperfusion injury by human thioredoxin in rabbit lung. *J. Thor. Cardiovasc. Surg.* 113:1–9
192. Yagi K, Liu C, Bando T, Yokomise H, Inui K, et al. 1994. Inhibition of reperfusion injury by human thioredoxin (adult T-cell leukemia-derived factor) in canine lung transplantation. *J. Thor. Cardiovasc. Surg.* 108:913–21
193. Takagi Y, Horikawa F, Nozaki K, Sugino T, Hashimoto N, Yodoi J. 1998. Expression and distribution of redox regulatory protein, thioredoxin during transient focal brain ischemia in the rat. *Neurosci. Lett.* 251:25–28
194. Takagi Y, Mitsui A, Nishiyama A, Nozaki K, Sono H, et al. 1999. Overexpression of thioredoxin in transgenic mice attenuates focal ischemic brain damage. *Proc. Natl. Acad. Sci. USA* 96:4131–36
195. Shibuki H, Katai N, Kuroiwa S, Kurokawa T, Yodoi J, Yoshimura N. 1998. Protective effect of adult T-cell leukemia-derived factor on retinal ischemica-reperfusion injury in the rat. *Investig. Opthalmol. Vis. Sci.* 39:1470–77
196. Matsui M, Oshima M, Oshima H, Takaku K, Maruyama T, et al. 1996. Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Devel. Biol.* 178:179–85

197. Fujii S, Nanbu Y, Konishi I, Mori T, Masutani H, Yodoi J. 1991. Immunohistochemical localization of adult T-cell leukaemia-derived factor, a human thioredoxin homologue, in human fetal tissues. *Virchows Arch. Pathol. Anat. & Histopathol.* 419:317–26
198. Hotta M, Tashiro F, Ikegami H, Niwa H, Ogihara T, et al. 1998. Pancreati beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. *J. Exp. Med.* 188:1445–51
199. Hawkins HC, Blackburn EC, Freedman RB. 1991. Comparison of the activities of protein disulphide-isomerase and thioredoxin in catalyxing disulphide isomerization in a protein substrate. *Biochem. J.* 275:349–53
200. Fernando MR, Nanri H, Yoshitake S, Nagata-Kuno K, Minakami S. 1992. Thioredoxin regenerates proteins inactivated by oxidative stress in endothelial cells. *Eur. J. Biochem.* 209:917–22
201. Bhat GB, Iwase K, Hummel BCW, Walfish PG. 1989. Kinetic characteristics of a thioredoxin-activated rate hepatic and renal low-$K_m$ iodothyronine 5′-deiodinase. *Biochem. J.* 258:785–92
202. Flamigni F, Marmiroli S, Caldarera CM, Guarnieri C. 1989. Involvement of thiol transferase- and thioredoxin-dependent systems in the protection of 'essential' thiol groups of ornithine decarboxylase. *Biochem. J.* 259:111–15
203. Freedman RB, Hirst TR, Tuite MF. 1994. Protein disulphide isomerase: building bridges in protein folding. *Trends Biochem. Sci.* 19:331–36
204. Boniface JJ, Reichert LE Jr. 1990. Evidence for a novel thioredoxin-like catalytic property of gonadotropic hormones. *Science* 247:61–64
205. Bertini R, Howard OM, Dong HF, Oppenheim JJ, Bizzarri C, et al. 1999. Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes, and T cells. *J. Exp. Med.* 189:1783–89
206. Nakamura H, DeRosa S, Roedere M, Anderson MT, Dubs JG, et al. 1996. Elevation of plasma thioredoxin levels in HIV infected individuals. *Int. Immunol.* 8:603–11
207. Nakamura H, Vaage J, Valen G, Padilla CA, Bjornstedt M, Holmgren A. 1998. Measuremnts of plasma glutaredoxin and thioredoxin in healthy volunteers and during open-heart surgery. *Free Rad. Biol. Med.* 24:1176–86
208. Das KC, White CW. 1998. Detection of thioredoxin in human serum and biological samples using a sensitive sandwich ELISA with digoxigenin-labeled antibody. *J. Immunol. Methods* 211:9–20
209. Kogaki H, Fujiwara Y, Yoshiki A, Kitajima S, Tanimoto T, et al. 1996. Sensitive enyme-linked immunosorbent assay for adult T-cell leukemia-derived factor and normal value measurement. *J. Clin. Lab. Anal.* 10:257–61
210. Kumagi S. 1998. ADF/thioredoxin as an indicator of oxidative stress. *Jpn. J. Clin. Pathol.* 46:574–80
211. Wakita H, Yodoi J, Masutani H, Toda K, Takigawa M. 1992. Immunohistochemical distribution of adult T-cell leukemia-derived factor/thioredoxin in epithelial components of normal and pathologic human skin conditions. *J. Invest. Dermatol.* 99:101–7
212. White CR, Brock TA, Chang LY, Crapo J, Briscoe P, et al. 1994. Superoxide and peroxynitrite in atherosclerosis. *Proc. Natl. Acad. Sci. USA* 91:1044–48
213. Patel JM, Zhang J, Block ER. 1996. Nitric oxide-induced inhibition of lung endothelial cell nitric oxide synthase via interaction with allosteric thiols: role of thioredoxin in regulation of catalytic activity. *Amer. J. Respir. Cell Mol. Biol.* 15:410–19

214. Zhang J, Li YD, Jawaharlal MP, Block ER. 1998. Thioredoxin overexpression prevents NO-induced reduction of NO synthase activity in lung endothelial cells. *Amer. J. Physiol.* 275:L288–93
215. Takagi Y, Gon Y, Todaka T, Nozaki K, Nishiyama A, et al. 1998. Expression of thioredoxin is enhanced in atherosclerotic plaques and during neointima formation in rat arteries. *Lab. Invest.* 78:957–66
216. Morton H, Rolfe BE, Cavanagh AC. 1982. Early pregnancy factor: biology and clinical significance. In *Pregnancy Proteins*, ed. JG Grudzinskas, B Teisner, M Seppala, pp. 391–405, Sydney: Academic
217. Hori K, Hirashima M, Ueno M, Matsuda M Wage S, et al. 1993. Regulation of eosinophil migration by adult T cell leukemia-derived factor. *J. Immunol.* 151:5624–30
218. Tagaya Y, Wakasugi H, Masutani H, Nakamura H, Iwata S, et al. 1990. Role of ATL-derived factor (ADF) in the normal and abnormal cellular activation: involvement of dithiol related reduction. *Mol. Immunol.* 27:1279–89
219. Koyanagi H, Wakasugi N, Yoshimatsu K, Takashima Y, Yodoi J, et al. 1995. Role of ADF/TRX and its inhibitor on the release of major basic protein from human eosinophils. *Biochem. Biophys. Res. Commun.* 213:1140–47
220. Saito I, Shimuta M, Terauchi K, Tsubota K, Yodoi J, Miyasaka N. 1996. Increased expression of human thioredoxin/adult T cell leukemia-derived factor in Sjogren's syndrome. *Arthrit. Rheum.* 39:773–82
221. Lovell MA, Chengsong X, Gabbita SP, Markesbery WR. 2000. Decreased thioredoxin and increased thioredoxin reductase levels in alzheimer's disease brain. *Free Rad. Biol. Med.* 28:418–27
222. Masutani H, Naito M, Takahashi K, Hattori T, Koito A, et al. 1992. Dysregulation of adult T-cell leukemia-derived factor (ADF)/thioredoxin in HIV infection: loss of ADF high-producer cells in lymphoid tissues of AIDS patients. *AIDS Res. Hum. Retrovir.* 8:1707–15
223. Newman GW, Balcewicz-Sablinska MK, Guarnaccia JR, Remold HG, Silberstein DS. 1994. Opposing regulatory effects of thioredoxin and eosinophil cytotoxicity-enhancing factor on the development of human immunodeficiency virus 1. *J. Exp. Med.* 180:359–63
224. Gallegos A, Raffel J, Bhattacharyya AK, Powis G. 2000. Increased expression of thioredoxin in human primary and metastatic colon cancer. *Amer. Assoc. Cancer Res.* 41:189
225. Oblong JE, Chantler EL, Gallegos A, Kirkpatrick DL, Chen T, et al. 1994. Reversible inhibition of human thioredoxin reductase activity by cytotoxic alkyl 2-imidazolyl disulfide analogues. *Cancer Chemother. Pharmacol.* 34:434–8
226. Powis G, Kirkpatrick DL, Angulo M, Gasdaska JR, Powell MB, et al. 1996. Thioredoxin redox signalling: A novel target for anticancer drug development. *Anticancer Drugs* 7(Suppl. 3):121–6
227. Bootman MD, Pay GF, Rick CE, Tones MA. 1990. Two sulphonated dye compounds which compete for inosital 1,4,5-trisphosphate binding to rat liver microsomes: effects on 5′-phosphatase activity. *Biochem. Biophys. Res. Commun.* 166: 1334–39
228. Guerri C, Godfrey W, Grisolia S. 1976. Protection against toxic effects of formaldehyde in vitro, and of methanol or formaldehyde in vivo, by subsequent administration of SH reagents. *Physiol. Chem. Phys.* 8(6):543–550
229. Schlager JJ, Hoerl BJ, Riebow J, Scott DP, Gasdaska P, et al. 1993. Increased NADPH: (quinone-acceptor) oxidoreductase (DT-diaphorase) activity is associated with density dependent growth inhibition of normal but not transformed cells. *Cancer Res.* 53:1338–42
230. Watson JA, Rumsby MG, Wolowacz RG. 1999. Phage display identifies

thioredoxin and superoxide dismutase as novel protein kinase C-interacting proteins: Thioredoxin inhibits protein kinase C-mediated phosphorylation of histone. *Biochem. J.* 343:301–5

231. Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, et al. 1999. Identification of thioredoxin-binding protein-2/vitamin D (3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. *J. Biol. Chem.* 274:21645–50
232. Redl B, Merschak P, Abt B, Wojnar P. 1999. Phage display reveals a novel interaction of human tear lipocalin and thioredoxin which is relevant for ligand binding. *FEBS Lett.* 460:182–86