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Forum Review

Role of Thioredoxin in Cell Growth Through Interactions with Signaling Molecules

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ABSTRACT

The thioredoxin system helps maintain a reducing environment in cells, but thioredoxin functions as more than simply an antioxidant. Thioredoxin functions depend on the protein's redox state, as determined by two conserved cysteines. Key biologic activities of thioredoxin include antioxidant, growth control, and antiapoptotic properties, resulting from interaction with target molecules including transcription factors. Mechanisms by which thioredoxin regulates cell growth include binding to signaling molecules such as apoptosis signal–regulating kinase-1 (ASK-1) and thioredoxin-interacting protein (Txnip). The molecular interplay between thioredoxin, ASK-1, and Txnip potentially influences cell growth and survival in diverse human diseases such as cancer, diabetes, and heart disease. In this review, we focus on the structure of thioredoxin and its functional regulation of cell growth through the interactions with signaling molecules. *Antioxid. Redox Signal.* 8, 2143–2151.

INTRODUCTION

A DELICATE PROOXIDANT AND ANTIOXIDANT BALANCE is essential for the living cell. Excessive production of reactive oxygen species leads to oxidative stress, potentially inducing cellular death through apoptosis or necrosis. Reactive oxygen species including superoxide, hydrogen peroxide, and nitric oxide can be toxic at high levels but can also regulate genes that control cell growth and differentiation. Thioredoxins, glutaredoxins, and DsbA protein disulfide isomerases are examples of redox-active proteins containing the Cys-X-Y-Cys motif that participate in maintaining the reduced state (38). Two isoforms of thioredoxin have been identified in mammalian cells: cytosolic thioredoxin (thioredoxin1) and mitochondrial thioredoxin (thioredoxin2). Here, we focus on the cytosolic thioredoxin (thioredoxin1), to which we refer simply as thioredoxin.

Thioredoxin is a small ubiquitous protein conserved through all species, from Archebacteria to humans. Despite sequence divergence of thioredoxins in different species, all thioredoxins have the same overall three-dimensional structure that consists of a central core of five β strands surrounded by four α helices (Fig. 1A). Thioredoxin has two highly conserved redox-active cysteine residues in the active catalytic center, and its activity is related to the thiol-disulfide redox chemistry of these two cysteine residues in the sequence Trp-Cys-Gly-Pro-Cys to reduce disulfide bonds in a variety of proteins, both intracellularly and extracellularly (Fig. 2). Oxidized thioredoxin (Trx-S_2) has a disulfide, and reduced thioredoxin [Trx-$(SH)_2$] has a dithiol. One of the most characterized functions of thioredoxin is the NADPH-dependent protein disulfide reduction, which shuttles electrons from NADPH, through thioredoxin reductase and thioredoxin, to the oxidized form of the substrate protein, resulting in a reduced disulfide bond in the substrate protein (Fig. 3) (26). These substrates include the essential enzyme ribonucleotide reductase, glutathione peroxidase, glutathione, peroxiredoxins, protein disulfide-isomerases (PDIs), oxytocin, fibrinogen, and insulin. Thus, thioredoxin can interact with a broad range of proteins by a redox-sensitive mechanism that regulates reversible oxidation of two cysteine thiol groups to a disulfide accompanied by the transfer of two electrons and two protons.

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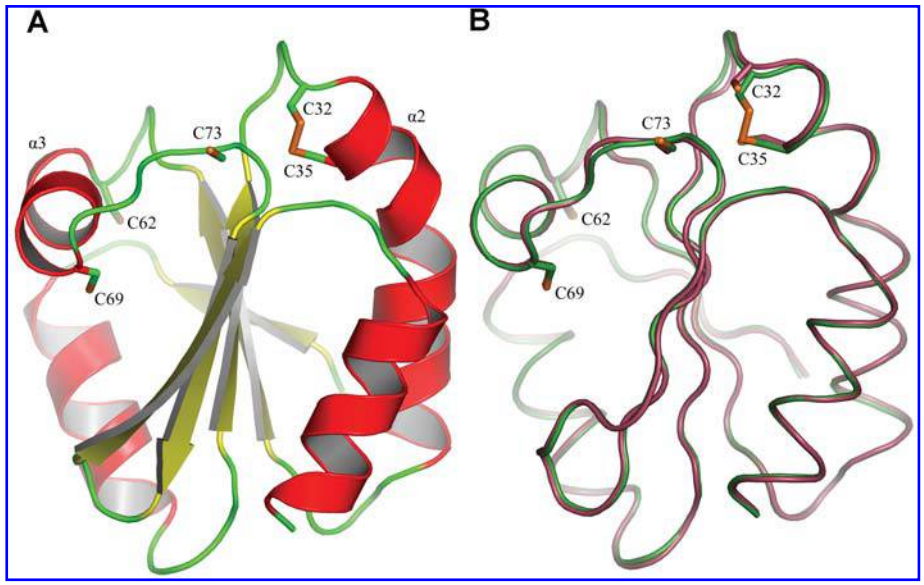

FIG. 1. (A) Structure of human thioredoxin1. Cartoon representation of the structure of human thioredoxin1 with cysteine residues shown as sticks. Cysteines and α-helices are labeled. (**B**) Reduced and oxidized thioredoxin1. Structural superposition of the fully reduced (purple) and oxidized (green) forms of human thioredoxin1. Cysteine residues are labeled. Note the overall similarity and minor conformational differences around the Cys32-Cys35 redox pair. Figure prepared from Protein Data Bank entries 1ERU (oxidized) and 1ERT (reduced), by using the program PyMOL (DeLano Scientific LLC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

In addition to its function as a scavenger of reactive oxygen species, thioredoxin has been implicated in a number of biologic processes including transcription factor modulation, autocrine stimulation of cell growth, potent cell-survival regulation, and inhibition of apoptosis through interacting with a variety of signaling molecules. Thioredoxin expression is increased in a variety of human primary cancers, where it is associated with aggressive cell growth and less cell apoptosis. Cancer-promoting effects include inhibiting tumor-suppressor proteins such as thioredoxin-interacting protein (Txnip) (17) and phosphatase and tensin homolog (PTEN) (42). Thioredoxin can bind to the amino-terminal portion of apoptosis signal-regulating kinase-1 (ASK-1), inhibiting the kinase activity of ASK-1 and ultimately protecting cells from apoptosis. Thioredoxin translocates from the cytosol into the nucleus and regulates some transcription factors by either direct or indirect interaction. Indeed, thioredoxin interacts with cysteines in nuclear factor (NF)-κB, and redox factor-1 (Ref-1). This review summarizes the current knowledge of structures of thioredoxin and its regulatory role in cell growth.

STRUCTURES OF THIOREDOXIN

The three-dimensional structure of thioredoxin has been revealed by x-ray crystallography and nuclear magnetic resonance (NMR), which showed that its active-site disulfide (Cys32 and Cys35) is located at the N-terminal end of helix α2 (Fig. 1A) (55, 78). The available structural data indicate that subtle conformational differences exist between the oxidized and reduced states of thioredoxin (Fig. 1B). High-resolution solution structures of both the reduced (dithiol) and oxidized (disulfide) forms have been determined by both x-ray crystallography (78) and multidimensional heteronuclear NMR (55). Although the global fold of the reduced and oxidized forms of human thioredoxin is very similar, a slight

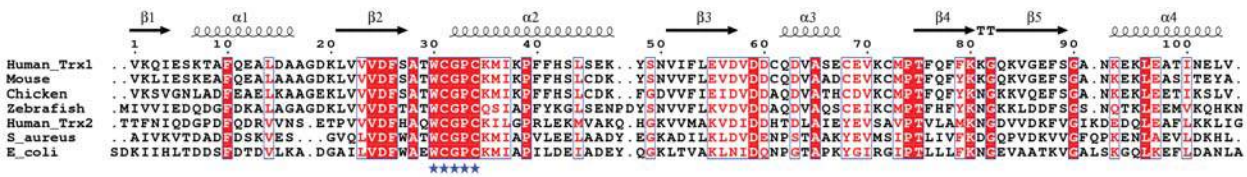

FIG. 2. Multiple alignment of thioredoxin sequences. Amino acid sequences downloaded from the Swiss-Prot or TrEMBL databases were aligned by using the default parameters in ClustalW (71) and displayed by using ESPript (19). Numbering is for human Trx1, and secondary structure above the alignment is from the crystal structure of reduced human Trx1 (PDB entry 1ERT). Positions with identity are highlighted in red; conserved positions are in red text. Blue stars below the alignment indicate the highly conserved WCGPC active-site motif containing the redox-active cysteine pair. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

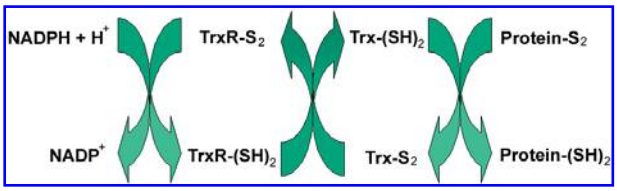

FIG. 3. Mechanism of NADPH-dependent protein disulfide reduction by the thioredoxin (Trx) system. The thioredoxin (Trx) active site alternates between a reduced [Trx-$(SH)_2$] form and an oxidized (Trx-S_2) form. Thioredoxin is maintained in the reduced state by thioredoxin reductase (TrxR) and NADPH. Reduced thioredoxin then directly reduces the disulfide in the substrate protein. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

shortening of the distance is found between the C_α positions of Cys32 and Cys35 on formation of the disulfide bond (Fig. 1B) (78). Additionally, higher mobility is observed within the active site in the reduced form (55).

Unlike thioredoxins from lower species or the mitochondrial thioredoxin2, human thioredoxin1 contains additional conserved cysteine residues at positions 62, 69, and 73. Weichsel et al. (78) reported that the crystal structure of human thioredoxin is dimeric in the crystal through a disulfide bond between Cys73 of each monomer (Fig. 4A and B). Interestingly, the thioredoxin active site is blocked by dimer formation. Wild-type thioredoxin rapidly loses its ability to stimulate cellular growth in the absence of reducing agents, whereas the C73S mutant does not, so dimerization of secreted thioredoxin in the oxidizing extracellular environment via Cys73 disulfide formation could limit its growth-stimulating effects (16). Cys62 and Cys69 are also thought to play a role in regulation of thioredoxin activity. They are located at either end of the α3 helix (Fig. 1A), and can also form a disulfide bond under more oxidizing conditions (77). This second dithiol/disulfide motif in thioredoxin can inhibit reduction of the active site of thioredoxin by thioredoxin reductase when oxidized, suggesting that reversible oxidation of the conserved nonactive site cysteine residues may regulate thioredoxin function under conditions of oxidative stress. Because Cys62 and Cys69 are separated by ~10Å in the available structures, a substantial structural rearrangement, likely involving local unfolding of helix α3, must take place for disulfide bond formation to occur (77). It has also been reported that *S*-nitrosylation at Cys69 is required for scavenging reactive oxygen species and contributes to the antiapoptotic function of thioredoxin (23).

The oxido-reductase activity of thioredoxin is dependent on its ability to interact with a variety of disulfide-containing protein targets. Interestingly, many thioredoxin substrates show little or no sequence homology around the site of interaction with thioredoxin (53). Currently, NMR structures of human thioredoxin1 complexed with 13-residue peptide fragments from NF-κB (54) and Ref-1 (55) provide the only structural views of thioredoxin interacting with its cellular targets. Both complexes were prepared by covalent cross-linking of the cysteine-containing peptide fragment to mutant thioredoxin protein containing only the nucleophilic Cys32. The structures reveal that target proteins interact with thioredoxin at a shallow, crescent-shaped groove in the protein sur-

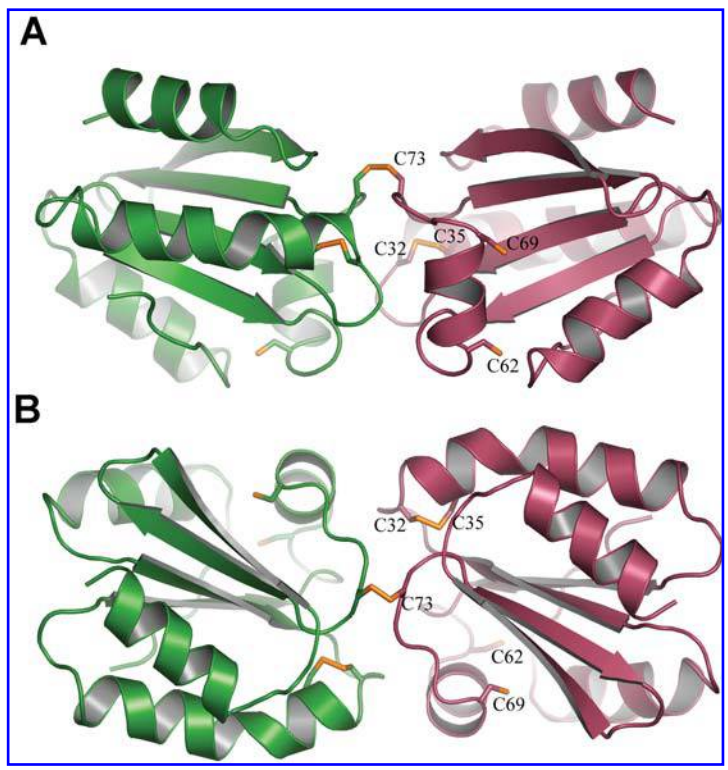

FIG. 4. The thioredoxin dimer. (**A**) Cartoon representation showing the interaction and intermolecular disulfide of the human thioredoxin1 dimer observed in crystal structures. For clarity, the cysteines of only the purple monomer are labeled. (**B**) Same as (A), except rotated by 90 degrees (view from the "top") relative to (**A**). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

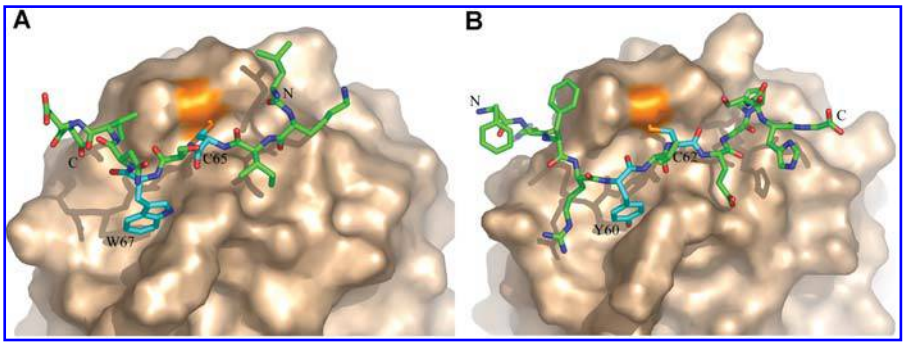

FIG. 5. Structural interaction of thioredoxin with target proteins. Thioredoxin (tan surface) is shown interacting with peptides derived from two physiologic target proteins, Ref-1 (**A**) and NF-κB (**B**), as determined by NMR. Target peptides, which were cross-linked to thioredoxin via an intermolecular disulfide to facilitate structure determination, are represented as sticks. The covalent heterodimer in the NMR structure was prepared by mutating Cys35 of thioredoxin and represents an artificially stabilized form of a transient intermediate in the disulfide exchange reaction catalyzed by wild-type thioredoxin. Note the opposite orientation of the target peptides in the two structures (N→C vs. C→N). The spatially equivalent tryptophan in Ref-1 (**A**) and a tyrosine in NF-κB (**B**) are highlighted in blue because of the proposed importance of a large, hydrophobic amino acid at this position for interaction with thioredoxin. The target cysteine is also colored blue. Figure prepared from Protein Data Bank entries 1CQG (Ref-1) and 1MDI (NF-κB). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

face (Fig. 5). Interestingly, NF-κB and Ref-1 bind in opposite orientations, demonstrating that thioredoxin-binding determinants do not need to be oriented in a specific linear sequence. These complexes are stabilized by several hydrogen bonds, as well as electrostatic and hydrophobic interactions, many of which are spatially conserved despite the opposite substrate peptide orientation. Thioredoxin has, therefore, balanced specificity with the need to interact with a large number of diverse protein substrates.

Recently, x-ray crystal structures of the reduced and oxidized forms of human thioredoxin2 (the mitochondrial thioredoxin) were published (65). As expected, they show that thioredoxin2 is structurally very similar to thioredoxin1, and the structure is also not affected significantly by disulfide formation or reduction. Interestingly, the same dimerization interface was present in thioredoxin2 crystals as was seen for thioredoxin1, although thioredoxin2 does not contain a Cys73 equivalent and runs as a monomer through a gel-filtration column.

THIOREDOXIN AS A GROWTH FACTOR

Thioredoxin was identified as a growth factor secreted by virus-transformed leukemic cell lines referred to as adult T-cell–derived leukemic factor (ADF) from HTLV-I transformed T cells (68). Recombinant thioredoxin promotes the growth of 3B6 cells without serum in a dose-dependent manner. Recombinant thioredoxin in the presence of thioredoxin reductase and NADPH can sustain 3B6 cell growth, which suggests that thioredoxin exerts its effects on cell growth and proliferation through its reducing activity (73). Exogenously added human thioredoxin is capable of stimulating growth in

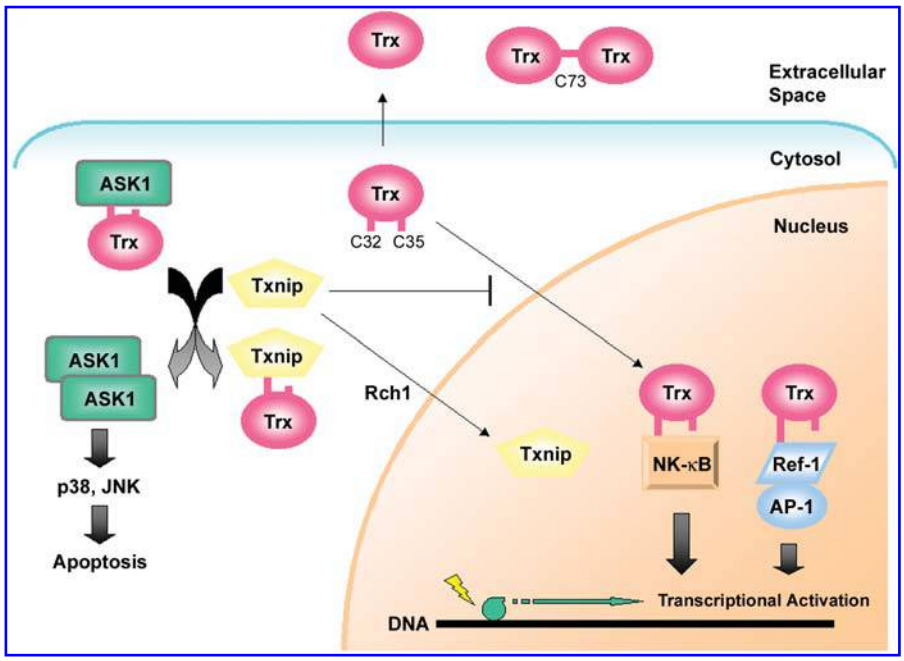

FIG. 6. Model figure. The molecular interplay between thioredoxin (Trx), apoptosis signal-regulating kinase-1 (ASK1) and thioredoxin-interacting protein (Txnip) influences cell growth and apoptosis. Nuclear thioredoxin regulates transcription factors including nuclear factor (NF)-κB, redox factor-1 (Ref-1), and activator protein-1 (AP-1). Note that precise binding mechanisms between thioredoxin and signaling molecules are still unknown. Disulfides do not form spontaneously in mammalian cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars.)

a number of solid-tumor–derived cell lines (15), and this requires a functional thioredoxin active site (49) but does not require a stable thioredoxin-receptor complex to be formed (15). A series of imidazolyl disulfide-containing compounds that inhibit thioredoxin and thioredoxin reductase can prevent cell-growth stimulation by thioredoxin in cell culture (50). Thus, the reducing activity of thioredoxin is necessary for its mitogenic activity (14). Exogenous thioredoxin also prevents apoptosis caused by depletion of glutathione and L-cysteine and protects lymphoid cells against TNF-α– or hydrogen peroxide–mediated cytotoxicity (28).

Although the precise secretory pathway is unknown, thioredoxin can be secreted by cells (11, 57, 58). Thioredoxin does not have a typical amino-terminal signal sequence that is usually present in proteins secreted via the endoplasmic reticulum. However, thioredoxin acts by a redox-sensitive export mechanism to increase cell proliferation in response to growth factors produced by the cell itself (15). Extracellular thioredoxin performs a variety of physiologic and pathophysiologic functions. For example, thioredoxin can potentiate the effect of other cytokines such as interleukins-1 and -2 (15, 73), suggesting proinflammatory effects of extracellular thioredoxin. Thioredoxin as an autocrine growth factor may synergize with other cytokines as a potent costimulatory molecule outside cells, because studies with ^{125}I-labeled thioredoxin show that the binding to the surface of MCF-7 cells is not saturable; this suggests the absence of binding of thioredoxin to a specific cell-surface receptor (15).

Thioredoxin is secreted by normal cells and a variety of cancer cells. Thioredoxin expression is increased in human primary cancer cells of acute lymphoblastic leukemia (64), lung (31), breast, colon (3), cervix (13), stomach (21), liver (44), pancreas (45), and in a number of tumor-derived transformed cell lines (58, 59). Increased levels of thioredoxin are related to resistance to antitumor drugs such as cisplatin, mitomycin C, doxorubicin, and etoposide (32, 62, 85), suggesting that thioredoxin leads to tumor growth via a decrease in the sensitivity of cancer cells to drug-induced apoptosis. Disulfides inhibit cell growth in a number of cancer cell lines (48). The thioredoxin redox inhibitors, 1-methylpropyl 2-imidazolyl disulfide and pleurotin, inhibit hypoxia-induced factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) formation, leading to decreased angiogenesis in cancer cells (79).

In atherosclerosis, the abnormal growth of vascular smooth muscle cells and immune cells in the plaque is promoted by oxidative stress. Thioredoxin expression is upregulated in the endothelial cells and macrophages of human atherosclerotic plaques and in balloon-injured rat arteries (69), indicating that thioredoxin and its cellular redox modification play a crucial role in arterial neointima formation. Thioredoxin and several other antioxidants participate in normal early embryonic development. Thioredoxin differentially regulates the embryonic stem cell transcription factor Oct-4 to maintain the totipotentiality of embryonic stem cells (22). Thioredoxin can also act as a neurotropic cofactor that augments the effect of nerve growth factor on neuronal differentiation and regeneration (40).

In contrast to its stimulatory activities, thioredoxin has also been implicated in cell growth inhibition. Genetic screening of possible tumor suppressor genes identified that growth arrest of HeLa cells by interferon-γ is mediated by human thioredoxin (8). As a major antioxidant, thioredoxin protects the heart against oxidative stress and can inhibit cardiac hypertrophy (81). In general, relatively low levels of H_2O_2 are associated with the activation of ERK1/2 and the stimulation of cell growth in cardiomyocytes. Conversely, a higher level of H_2O_2, although still activating ERK1/2, also activates the JNK and p38 MAPKs but induces apoptosis (33). Thus, thioredoxin has multiple functions as an antioxidant, an autocrine growth factor, and a growth inhibitor or promoter, depending on the specific cellular context.

THIOREDOXIN AND ITS INTERACTION WITH SIGNALING MOLECULES

Recent evidence indicates that reactive oxygen species can act as signaling molecules by promoting the formation of disulfide bonds between redox-sensitive proteins. Thioredoxin can interact with the various signaling molecules through the conserved active site containing Cys-X-Y-Cys motif. In addition to its role in DNA synthesis, by virtue of its acting as a hydrogen donor for the essential enzyme ribonucleotide reductase, thioredoxin has been implicated in a number of biologic processes including protein folding [by protein disulfide isomerase (10) and DsbA (39)] and transcription factor modulation.

Activator protein-1 (AP-1) is a ubiquitous transcription factor that regulates the expression of genes involved in cell growth in response to external stimuli such as growth factors, phorbol esters, and ionizing radiation (2). The stimulatory effect of thioredoxin on AP-1 activity is caused not by direct interaction between thioredoxin and AP-1, but by stepwise interaction from thioredoxin to Ref-1 to AP-1. Thioredoxin enhances the sequence-specific DNA-binding activity of the tumor-suppressor protein p53 directly and by enhancing Ref-1 mediation (72). P53-dependent p21 transactivation is regulated by the thioredoxin-Ref-1 cascade. In contrast, thioredoxin can also negatively regulate AP-1 activity through a direct interaction between thioredoxin and Jun activation domain–binding protein 1 (Jab1) (27).

Nuclear factor (NF)-κB is another target molecule of redox regulation by thioredoxin (Fig. 5B). NFκB can inhibit cell death and promote cancer growth. NF-κB regulates the expression of genes that antagonize cell death, such as caspase inhibitors cIAP-1, cIAP-2, xIAP, and survivin (52, 67, 74). NF-κB components p50 and p65/RelA have a well-conserved cysteine on the DNA-binding domain. Thioredoxin enhances DNA-binding of NF-κB by reduction of a disulfide bond involving Cys62 of the p50 subunit of NF-κB (41). Thioredoxin also activates c-Jun NH_2-terminal kinase (JNK)-signaling, which mediates degradation of I-κB to activate NF-κB (6). Hirota et al. (25) reported that thioredoxin translocates from the cytoplasm into the nucleus in response to NF-κB activation stimuli such as UVB irradiation and TNF-α treatment. In the cytoplasm, overexpression of thioredoxin suppresses the degradation of I-κB; however, in the nucleus, thioredoxin directly reduces the cysteines of NF-κB and allows NF-κB-dependent gene expression (25). Thus, thioredoxin facilitates protein–nucleic acid interactions by reducing cysteines in the DNA-binding loop of several transcription factors.

Through two-hybrid screening, apoptosis signal-regulating kinase (ASK)-1 was identified as an interacting partner of thioredoxin (60). ASK-1 is a MAP kinase kinase kinase that leads to stress-induced apoptosis. The oxidized form (intramolecular disulfide between Cys32 and Cys35) or redox-inactive form (the double-mutation at catalytic sites Cys32 and Cys35) of thioredoxin does not bind to ASK-1, but the reduced form or the single mutation at the redox-active site (C32S or C35S) retains the ability to bind ASK-1 (36). The association of thioredoxin via one of the Cys with ASK-1 appears to be necessary and sufficient to promote ASK-1 ubiquitination and degradation, leading to reduced ASK-1 apoptotic activity. Apoptotic stimuli such as reactive oxygen species and TNF-α induce the dissociation of thioredoxin from ASK-1, leading to the activation of ASK-1. Reactive oxygen species induce the dimerization of thioredoxin and its dissociation from ASK-1, followed by multimerization of ASK-1 and activation of its kinase activity (18, 35). Therefore, the thioredoxin–ASK-1 system could be one of the signaling mechanisms for reactive oxygen species–mediated apoptosis.

Thioredoxin also binds directly to protein kinase Cα, δ, ϵ, and χ (76), a component of phagocyte oxidase $p40^{phox}$ (48), and the cysteine protease inhibitor lipocalin (56) to regulate activity in a redox-dependent manner. Thioredoxin facilitates the induction of stress-response proteins such as heme oxygenase-1 (80) and manganese superoxide dismutase (6). Glucocorticoid receptors are maintained in a reduced steroid-binding state by a thioredoxin-mediated reducing system in the cytosol (20). Thus, thioredoxin can interact with a variety of signaling molecules.

THIOREDOXIN-INTERACTING PROTEIN (TXNIP) IS A NEGATIVE REGULATOR OF THIOREDOXIN

Thioredoxin-interacting protein (Txnip), also know as vitamin D_3 upregulated protein (VDUP-1) or thioredoxin-binding protein-2 (TBP-2), was originally reported as a gene of unknown function in HL-60 cells induced by 1α,25-dihydroxyvitamin D_3 (5). Txnip is a ubiquitously expressed gene in normal tissues. A series of studies has revealed that Txnip binds to thioredoxin and inhibits thioredoxin function (30, 48, 82). Compared with the regulation of thioredoxin gene expression, Txnip expression is more dramatically regulated by cellular stress. Oxidative or mechanical stress downregulates Txnip expression without affecting thioredoxin expression, leading to a net increase in thioredoxin activity in vascular endothelial cells, smooth muscle cells, and cardiomyocytes (63, 75, 83). This suggests that thioredoxin activity can be controlled by Txnip expression.

Txnip acts as a growth-suppressor gene. Txnip arrests cell-cycle progression at G_0/G_1, likely via its interaction with thioredoxin (46). Txnip expression is inversely related to lung cell proliferation during fetal lung development (12). Txnip overexpression inhibits osteoclastogenesis with decreased AP-1 binding activity in osteoclast precursors (1). In contrast to the upregulation of thioredoxin in tumor cells, Txnip expression is downregulated in various tumor cells from breast (4, 24, 84), lung (24), stomach (24), colon (4), lymph glands (7), pheochromocytoma (51), and melanoma (66). Txnip is located on chromosome 3 band F2.2 in mice, and is syntenic with a region of human chromosome 1q21 that is frequently mutated or lost in human cancers (37). Loss of Txnip is essential in oxidative stress–induced renal carcinogenesis (9), and severe lymphoid hyperplasia in the small intestine is observed in Txnip-knockout mice (34). Overexpression of Txnip suppresses tumor growth (24) and the formation of metastases (17). Anticancer and antiproliferative agents (5-fluoroucil and anisomycin) dramatically induce Txnip expression in cancer cells (70). The histone deacetylase inhibitor SAHA arrests cancer cell growth by inducing Txnip, followed by decreased levels of thioredoxin (4).

Txnip renders cells more susceptible to oxidative stress and induces apoptosis. Txnip overexpression suppresses cell growth and promotes apoptosis in vascular smooth muscle cells and cardiomyocytes (63, 75, 86). Txnip is associated with glucotoxicity and β-cell apoptosis in diabetes (43). Furthermore, insulin-like growth factor-1 (IGF-1), a potent growth and survival factor, suppresses Txnip expression through a PI3-kinase–dependent pathway in cerebellar granule neurons undergoing apoptosis (61). Because Txnip fails to bind to thioredoxin when the Cys32 and Cys35 are mutated, Txnip seems to interact with the thioredoxin active-site cysteines (30). Downregulation of Txnip by using RNA interference increases the association of thioredoxin with ASK-1 and thereby inhibits activation of p38 and JNK (83). Thus, binding of Txnip to the catalytic cysteines of thioredoxin inhibits both thioredoxin activity and ability to bind to ASK-1, leading to stress-induced apoptosis.

Whereas thioredoxin translocates from the cytoplasm to the nucleus, Txnip prevents nuclear translocation of thioredoxin by hydrogen peroxide and PDGF in vascular smooth muscle cells (63). Moreover, Txnip itself has been demonstrated to be translocated into the nucleus through interaction with importin α1 (Rch1) (47). Enhanced Txnip expression in the nucleus is associated with growth suppression in MCF-7 cells (46). Txnip blocks the Jab1-mediated translocation of p27(kip1) from the nucleus to the cytoplasm and increases p27(kip1) stability, which is a critical process in tumor progression (29). Because Jab1 is known to be a nuclear partner of thioredoxin, Txnip might be a part of a transcriptional complex with thioredoxin and Jab1. Finally, Txnip inhibits tumor cell growth by blocking cyclin A–promoter activity through the interaction with zinc-finger transcriptional corepressors (promyelocytic leukemia zinc-finger, Fanconi anemia zinc-finger, and histone deacetylase 1) (24). Thus, growth-control and transcription-factor modulation by thioredoxin may be regulated by Txnip as part of a nuclear transcriptional coactivation complex in the nucleus.

CONCLUSIONS

The mechanisms of thioredoxin-induced growth control are multifaceted. The functional activity of thioredoxin in promoting cellular growth is critically associated with the conserved disulfide motif. Direct antioxidant properties, transcription regulation, and ASK-1 inhibitory antiapoptotic effects are mechanisms by which thioredoxin can exert its growth control

(Fig. 6). Txnip, a negative regulator of thioredoxin, may regulate diverse cellular processes including growth signaling. Thus, further exploration of proteins that interact with thioredoxin will likely uncover new mechanisms by which the redox state of thioredoxin can exert growth control.

ABBREVIATIONS

AP-1, activator protein-1; ASK-1, apoptosis signal-regulating kinase-1; ERK, extracellular signal-regulated kinase; IAP, inhibitor of apoptosis protein; IκB, inhibitor-κB; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; PDGF, platelet-derived growth factor; PI3-kinase, phosphatidylinositol 3-kinase; Rch1, importin α1; Ref-1, redox factor-1; TNF, tumor necrosis factor; Trx, thioredoxin; Trx-S2, oxidized thioredoxin; Trx-$(SH)_2$, reduced thioredoxin; Txnip, thioredoxin-interacting protein.

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