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Review

Redox Control of Cellular Function by Thioredoxin; a New Therapeutic Direction in Host Defence

YUMIKO NISHINAKA, HAJIME NAKAMURA, HIROSHI MASUTANI and JUNJI YODOI*

Department of Biological Responses, Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaharacho, Sakyo, Kyoto 606-8507, Japan

Abstract. Compelling evidence has suggested that oxidative stress mediates various cellular responses, and control of reduction/oxidation (redox) is important in maintaining the homeostasis of an organism. The thioredoxin (TRX) system, as well as the glutathione system, is one of the key systems in controlling cellular redox status. TRX is a small ubiquitous protein with the redox-active site sequence -Cys-Gly-Pro-Cys-. It has been demonstrated to be a multifunctional protein, which has regulatory roles in cellular signaling and gene transcription in addition to cytoprotective activities through the quenching of reactive oxygen species. Various oxidative stimuli, such as UV irradiation, cytokines and some chemicals, promptly induce the expression of TRX. Overexpression of TRX correlates with a wide variety of oxidative stress conditions and, in some cases, TRX has shown promising effects for clinical use, for instance in the attenuation of tissue injury in ischemia reperfusion models. The modulation of TRX functions in association with other redox-regulatory molecules should give us a new therapeutic strategy in the treatment of oxidative stress-mediated disorders and diseases.

Key words: redox regulation; thioredoxin; reactive oxygen species; therapy.

Introduction

Oxygen is indispensable for the maintenance of life in aerobic organisms, from bacteria to mammals. Reactive oxygen species (ROS) are naturally generated from oxygen during respiration for energy metabolism, or in response to various stimuli, such as UV irradiation, X-ray, inflammatory cytokines and chemical carcinogens. ROS include superoxide, singlet oxygen, H_2O_2 and the highly reactive hydroxyl radical. ROS can alter or disrupt the balance of redox status in cells, which causes various dysfunctions and diseases[36, 44]. Recently, ROS have been shown to act as signal-transduction components in regulating cellular responses[1, 44]. Accordingly, homeostatic control of ROS is thought to be one of the key determinants in maintaining cell growth pathways, which include proliferation, apoptosis and senescence. Living cells have evolved several systems to maintain intracellular redox status by scavenging ROS. These systems include the glutathione (GSH)[10] and the thioredoxin (TRX) systems[13]. Oxidative conditions may produce conformational changes and dimer/multimer formation of redox sensitive proteins, resulting in the functional modulation of those proteins. The GSH and TRX systems reverse the disulfide formation of proteins. Change of the intracellular GSH level is highly sensitive to oxidative stress. Expression of TRX is also promptly induced by a variety of oxi-

* Correspondence to: Junji Yodoi, M.D. Ph.D., Department of Biological Responses, Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaharacho, Sakyo, Kyoto 606-8507, Japan, tel.: +81 75 751 4024, fax: +81 75 761 5766, e-mail: yodoi@virus.kyoto-u.ac.jp

dative stimuli. Thus, both GSH and TRX are considered as redox sensors reflecting the intracellular redox status. Besides the systems mentioned above, a number of endogenous proteins associated with redox control, such as superoxide dismutase, catalase, glutathione S-transferase and heat shock proteins, are induced in response to oxidative stress[22]. These redox-sensitive proteins interact with one another to regulate cellular redox status. It seems reasonable for cells to have such redundant mechanisms to ensure the optimal redox status for various biological states. Indeed, accumulating evidence has suggested that a proper balance between oxidants and antioxidants is crucial for maintaining health and longevity, and antioxidants may be useful in the prevention and/or the treatment of a variety of diseases[36, 44]. Accordingly, an overall understanding of redox regulation and its clinical application are of great importance.

This review summarizes the current understanding of the mechanisms contributing to redox-mediated cellular responses, in particular in view of TRX and its associated molecules.

Thioredoxin and the Thioredoxin Superfamily

TRX is a small ubiquitous protein having oxidoreductase activity via its redox-active disulfide/dithiol site within the conserved active sequence, -Cys-Gly-Pro--Cys-[13]. The TRX system consists of TRX, TRX reductase and nicotinamide adenine dinucleotide phosphate (NADPH). The reduced form of TRX reduces a target protein by giving hydrogen in its active site and, as a result, TRX assumes its oxidized form. Oxidized TRX is reduced by NADPH-dependent TRX reductase (Fig. 1). TRX was originally purified from *Escherichia coli* as a hydrogen donor for ribonucleotide reductase, essential for DNA synthesis, and it was later isolated and characterized from a wide variety of prokaryotic and eukaryotic species[3, 13]. In 1989, human TRX was identified as adult T cell leukemia-derived factor, which we originally defined as an IL-2 receptor α chain inducer produced by human T cell leukemic virus type I (HTLV-I)-transformed T cells[49, 51, 59, 60]. Since then, extensive studies have shown that TRX has multiple roles in cellular responses, in addition to its reducing activity[14, 30].

Protein disulfide-isomerases (PDIs), which play a pivotal role for native disulfide formation during protein folding, also have similar active sites -Cys-Gly--His-Cys-[34]. Recently, several molecules having a similar active site sequence have been found and categorized as the TRX superfamily. Table 1 summerizes the members of the human TRX superfamily. Glutaredoxin (GRX), known as thioltransferase, has GSH-disulfide oxidoreductase activity with the redox-active site -Cys-

Table 1. Thioredoxin superfamily

	kDa	Localization	Active site sequence
Thioredoxin	12	cytosol	-Cys-Gly-Pro-Cys-
Thioredoxin 2	12	mitochondria	-Cys-Gly-Pro-Cys-
TRX related protein 32 (TRP32)	32	cytosol	-Cys-Gly-Pro-Cys-
Glutaredoxin (GRX)	12	cytosol	-Cys-Gly-Tyr-Cys-
Nucleoredoxin	48	nucleus	-Cys-Pro-Pro-Cys-
Protein disulfide isomerase (PDI)	55	ER	-[Cys-Gly-His-Cys]$_2$-
Ca binding protein 1 (CaBP1)	49	ER	-[Cys-Gly-His-Cys]$_2$-
Ca binding protein 2 (ERp72)	72	ER	-[Cys-Gly-His-Cys]$_3$-
Phospholipase Cγ 12	61	ER	-[Cys-Gly-His-Cys]$_2$-

ER – endoplasmic reticulum.

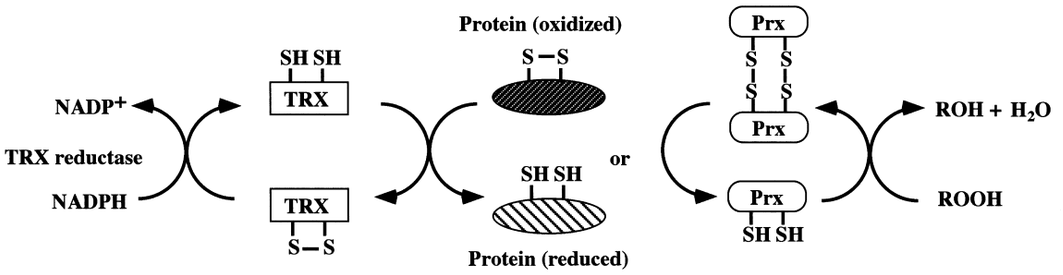

Fig. 1. The thioredoxin (TRX) system and peroxiredoxin (Prx)

-Gly-Tyr-Cys-[58]. GRX reduces low molecular weight disulfides and proteins in concert with NADPH and GSH reductase. TRX2 is a new member of TRX with a mitochondria-specific signal peptide, indicating a specific localization in mitochondria[48]. The biological functions of these members have not yet been clarified.

TRX-dependent peroxidases defined as the peroxiredoxin (Prx) family, also play important roles in the regulation of cellular redox status and cell signaling. So far, six members of Prx have been identified in humans, all of which utilize TRX as the electron donor, except for Prx VI (Fig. 1). The features and functions of the Prx family were well described in a recent review by others[5].

Redox Regulation of Intracellular Signaling by TRX

The transcriptional regulation of gene expression is influenced by the cell's redox status through the oxido--reductive modification of transcription factors. Several important transcription factors have been shown to be modulated by reducing agents[2, 8, 44]. TRX is one of the key mediators in modulating the activities of transcription factors (Fig. 2). The localization of TRX is mainly in the cytoplasm, however, by a variety of stimuli, it translocates to the nucleus[21]. Ref-1, a DNA-repair (A/P) endonuclease, enhances the DNA-binding activities of many nuclear transcription factors, such as AP-1, NF-κB, ATF/CREB, Myb, HIF-1α[7] and p53, through a redox-dependent mechanism. The reduction of Ref-1 by TRX is required for its activity[11, 39]. Thus, TRX regulates many transcriptional events through Ref-1.

TRX can also directly enhance the DNA-binding activities of several factors. Recent studies by UENO et al.[53] suggested that TRX facilitates p53-mediated p21 activation through Ref-1-independent and/or -dependent mechanisms. The enhancement of DNA-binding activity by TRX was also reported in nuclear receptors, such as the glucocorticoid receptor[20] and the estrogen receptor[9]. NF-κB has been most extensively investigated with respect to regulation by TRX[25, 35, 43]. Treatment of NF-κB with oxidants, such as diamide or hydrogen peroxide, renders the protein incapable of DNA-binding, while reduction of the protein by TRX as well as thiol reductants, such as DTT, enhances its DNA-binding activity. This action is associated with the reduction of Cys62 in the DNA-binding loop of NF-κB p50 by TRX[25]. Interestingly, studies on the targeted overexpression of TRX, either in the cytosol or in the nucleus, have indicated that TRX has, depending on its localization, dual and opposing roles in the regulation of NF-κB[12]. In the cytosol, TRX interferes with the activities of NF-κB by blocking the dissociation of I-κB from NF-κB, whereas TRX enhances its DNA-binding activities in the nucleus. Although there is compelling evidence that oxidative stress promptly induces the translocation of TRX into the nucleus, where TRX controls transcriptional events, how TRX translocates from the cytoplasm to the nucleus (and *vice versa*) remains to be elucidated.

Structural analysis of TRX complexed with Ref-1 or NF-κB was performed by QIN et al.[37, 38] and revealed that these complexes represent kinetically stable mixed disulfide intermediates in the same binding location of TRX, but in opposing orientations. This indicates the TRX has the potential to target a wide range of proteins by balancing versatility in substrate recognition.

Immunological Functions of TRX

TRX is secreted from various types of cells, including normal lymphocytes and virus-transformed cell lines, by a leaderless pathway. As human TRX was first identified as a cytokine with IL-2 receptor-inducing activity, secreted TRX exhibits several cytokine--like activities distinct from its reducing activity[30, 60]. HORI et al.[15] reported that TRX enhances the chemotactic activity of eosinophil cell lines. In 1989, SILBERSTEIN et al.[45] reported a cytokine, named eosinophil cytotoxicity-enhancing factor (ECEF), which was later proved to be homologous to TRX. ECEF is a truncated form of TRX produced by the activated macrophage cell line U937 and it augments the cytotoxic activity of eosinophil. ECEF/truncated TRX has strong cytotoxicity-enhancing activity, but no dithiol reductase activity[46]. It is interesting that truncated TRX, cleaved by an unknown protease, acquires a new biological function. Recently, the chemoattractant activity of TRX has received much attention. BERTINI et al.[4] have shown that TRX is chemotactic for monocytes, polymorphonuclear leukocytes, and T lymphocytes both *in vitro* and *in vivo*. The potency of the chemotactic action of TRX is comparable with that of known chemokines, such as MCP-1, IL-8 and RANTES. It should be noted that those chemokines have two cysteine residues in their conserved active sites, -CC-, -CXC-, or -CX_3C-[19], while the active site sequence of TRX also includes two cysteine residues, -CXXC-. Despite the similarity of active site sequences, the signaling mechanism of TRX is distinct from that of the known chemokines. All the known members of the chemokine family are G-protein dependent, whereas the chemotactic activity of TRX is

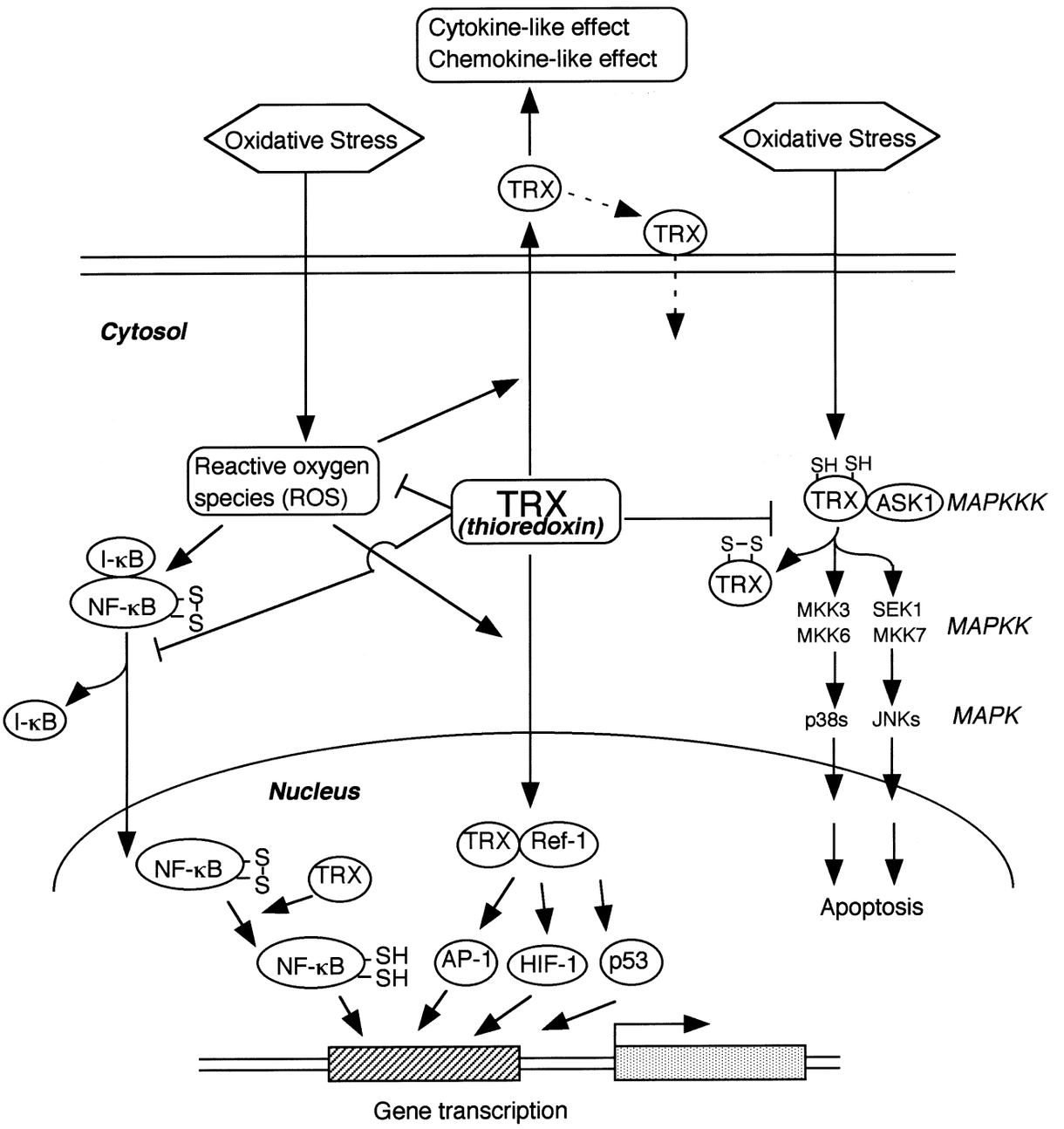

Fig. 2. Oxidative stress and signal transduction; intracellular and extracellular functions of thioredoxin

G-protein independent[4]. Further investigation is needed for understanding the detailed mechanism of the chemokine-like activity of TRX. It is still controversial whether a specific receptor for TRX on cell membrane exists. However, the discovery of a specific target/receptor for TRX on cell membrane would be a breakthrough in elucidating the mechanisms of the immunological functions of TRX.

Cytoprotective Effects of TRX *inVitro* and *in Vivo*

TRX has been shown to play crucial roles in cytoprotection against a variety of types of oxidative stress. Exogenous TRX can protect cells from anti-FAS antibody-induced apoptosis and cytotoxicity induced by TNF-α, hydrogen peroxide and activated neutrophils[23, 29].

TRX is also a potent costimulator of the expression of various cytokines[42, 56]. Recently, NILSSON et al.[31] reported that TRX induces the secretion of TNF-α and maintains the expression of Bcl-2, whereby it prolongs the survival of B-type chronic lyphocytic leukemia.

Overexpression of TRX has been observed in a wide variety of oxidative conditions, such as viral infection, diabetes, ischemic reperfusion and malignant tissues[30, 59, 60]. During viral infection, a considerable amount of ROS is generated and causes tissue damage and DNA breaks. As TRX was first purified from HTLV--I-transformed cells[59], TRX is induced and/or secreted from transformed cells related to infection of viruses such as Epstein-Barr virus (EBV)[55], hepatitis C virus and papilloma virus. An elevated TRX level in serum was also reported in late-stage HIV patients[27]. Recently, SONO et al.[47] have reported that TRX suppressed the lytic replication of EBV induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) and prevented the cell death evoked by lytic induction. These observations suggest that TRX is closely related to virus infection and its prognosis.

Oxidative stress has been suggested to be involved in the pathogenesis of diabetes. HOTTA et al.[16] have observed that the incidence of both autoimmune and drug-induced diabetes was markedly reduced in TRX--overexpressing transgenic mice originating from non-obese diabetic mice. This strongly suggests that TRX overexpression can attenuate the oxidative stress-induced diabetes. TAKAGI et al.[50] reported that ischemic neuronal injury was markedly attenuated in TRX transgenic mice compared with control mice, indicating that TRX plays a crucial role in brain damage during stroke. In other *in vivo* studies, TRX showed potent protective activities against reperfusion injury of the lung in rabbit and dog[54]. Interestingly, TRX transgenic mice have shown about a 30–35% increase in life span (MITSUI et al., in preparation). This observation suggests the relevance of oxidative stress to longevity. On the other hand, a homozygous mutant gene carrying a targeted disruption of TRX was lethal after implantation, suggesting that TRX expression is essential for the early differentiation and morphogenesis of the mouse embryo[24].

TRX Binding Proteins

To date, several TRX-binding proteins have been isolated. Mitogen-activated protein kinase (MAPK) is one of the signaling pathways activated by a variety of types of oxidative stress[17]. Apoptosis signal-regulating kinase (ASK1), which mediates the apoptosis signal by activating the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways, was shown to bind to reduced TRX, whereby it becomes inactive[40]. When TRX is oxidized under oxidative stress, it dissociates from ASK1 and the apoptosis signal is transduced (Fig. 2). NISHIYAMA et al.[32] have reported another TRX-binding protein (TBP-2) which is identical to vitamin D_3-upregulated protein (VDUP1). Although the biological function of TBP-2/VDUP1 has not yet been determined, overexpression of TBP-2 decreases the reducing activity of TRX, suggesting that TBP-2 is a negative regulator of TRX. The redox active site in TRX is required for the binding to both TBP-2 and ASK1. The conformation of TRX adjacent to the active site may be critical for the binding between these molecules. A phagocyte oxidase component, p40phox, was also reported to have a TRX binding property[33]. These three proteins were all cloned by a yeast two-hybrid system. WATSON et al.[57] identified TRX as a protein kinase C (PKC)-interacting protein using a phage display system and showed that the exogenously added TRX inhibits autophosphorylation of PKC and PKC-mediated phosphorylation of histone *in vitro*. Further studies should be performed to know whether TRX interacts with PKC in cells. Cloning of other TRX-binding proteins using cDNA libraries from different sources is in progress in our laboratory. Our preliminary data suggest that there are tissue specific TRX-interacting molecules (MATSUMOTO et al. unpublished data). Finding new binding proteins would give us new insights into the roles of TRX, and these proteins can be good candidates for redox regulatory agents.

Redox Status and Cancer

Because of the variety of cytoprotective activities of TRX, one might be interested in using TRX for a redox-based therapy. As we described above, maintenance of intracellular redox status is a key for the improvement of oxidative stress-induced diseases. For cancer therapy, however, a different consideration may be required. Overexpression of TRX is frequently observed in malignant tissues, including adult T cell leukemia[51], hepatocellular carcinoma[28] and pancreatic cancer[26]. *cis*-Diamminedichloroplatinum (II) (CDDP) is one of the most effective chemotherapeutic agents for a variety of human malignant tumors, and its cytotoxicity is attributed to its generation of ROS. Recent studies have shown that an elevated expression of TRX appears to attenuate the cytotoxicity of CDDP, thus

developing resistance to chemotherapy[41]. In this case, TRX may protect tumor cells from ROS generated by CDDP as well as normal cells. Similar observations have been reported on the correlation between antioxidants and drug-resistance[18]. Thus, a strategy for the inhibition of cellular antioxidant activity and down-regulation of antioxidant proteins have to be investigated to overcome such chemotherapeutic drug-resistance and to achieve maximum efficacy in the treatment of cancer. TRX-binding proteins and their derivative peptides or compounds, which can modulate TRX activity, may be good candidates for this purpose.

It is well accepted that the induction of apoptosis in tumor cells is preferable to that of necrosis for cancer therapy. One of the mechanisms by which oxidative stress causes apoptosis is the imbalance of the intracellular redox status elicited by the dysregulation of antioxidant systems[6, 44]. UEDA et al.[52] have reported that caspase-3, one of the key determinants for the process of apoptosis, is redox-sensitive and requires an intracellular reducing condition for its activity. When cell death was triggered by the modulation of thiols by thiol-oxidizing agents such as diamide, the release of cytochrome *c* from the mitochondria occurred in the initial stage. The subsequent process, either apoptosis or necrosis was determined by the degree of intracellular stress condition caused by diamide[52]. Excess generation of ROS by diamide led to cell necrosis. These findings suggest that the induction of apoptosis by oxidative stimuli requires the precise control of intracellular redox balance to an apoptosis-inducible level, where caspase-3 or other determinants for apoptosis can be activated. This might be critical for a redox-based cancer therapy.

Conclusion

In the last decade or so, the field of redox regulation research has rapidly expanded and the importance of redox regulation in cellular functions has been widely accepted. As we described in this review, TRX, a stress-inducible antioxidant protein, and its network play important roles in regulating cellular signaling and gene expression. Given that oxidative stress is involved in a variety of diseases, our current knowledge points out the potential for redox-based therapy. Further studies will elucidate the whole picture of redox regulatory mechanisms by TRX and other molecules, and increasing knowledge in the redox regulation systems will provide us a new aspect in the development of strategies for diagnosing and treating diseases.

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