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Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implications for diseases in the cardiovascular system

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Berndt C, Lillig CH, Holmgren A. Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implications for diseases in the cardiovascular system. *Am J Physiol Heart Circ Physiol* 292: H1227–H1236, 2007. First published December 15, 2006; doi:10.1152/ajpheart.01162.2006.—Reactive oxygen species (ROS) and the cellular thiol redox state are crucial mediators of multiple cell processes like growth, differentiation, and apoptosis. Excessive ROS production or oxidative stress is associated with several diseases, including cardiovascular disorders like ischemia-reperfusion. To prevent ROS-induced disorders, the heart is equipped with effective antioxidant systems. Key players in defense against oxidative stress are members of the thioredoxin-fold family of proteins. Of these, thioredoxins and glutaredoxins maintain a reduced intracellular redox state in mammalian cells by the reduction of protein thiols. The reversible oxidation of Cys-Gly-Pro-Cys or Cys-Pro(Ser)-Tyr-Cys active site cysteine residues is used in reversible electron transport. Thioredoxins and glutaredoxins belong to corresponding systems consisting of NADPH, thioredoxin reductase, and thioredoxin or NADPH, glutathione reductase, glutathione, and glutaredoxin, respectively. Thioredoxin as well as glutaredoxin activities appear to be very important for the progression and severity of several cardiovascular disorders. These proteins function not only as antioxidants, they inhibit or activate apoptotic signaling molecules like apoptosis signal-regulating kinase 1 and Ras or transcription factors like NF- κ B. Thioredoxin activity is regulated by the endogenous inhibitor thioredoxin-binding protein 2 (TBP-2), indicating an important role of the balance between thioredoxin and TBP-2 levels in cardiovascular diseases. In this review, we will summarize cardioprotective effects of endogenous thioredoxin and glutaredoxin systems as well as the high potential in clinical applications of exogenously applied thioredoxin or glutaredoxin or the induction of endogenous thioredoxin and glutaredoxin systems.

oxidative stress; thiol-disulfide oxidoreductases; apoptotic signaling; myocardial remodeling; glutathione

THE CELLULAR THIOL REDOX STATE is a crucial mediator of multiple metabolic, signaling, and transcriptional processes in cells, and a fine balance between oxidizing and reducing conditions is essential for the normal function and survival of cells. Reactive oxygen species (ROS), particularly H_2O_2 , are now known to be important second messengers in intracellular signaling. With respect to proteins, the thiol group of cysteinyl side chains is susceptible to a number of oxidative modifications, for instance, the formation of inter- or intramolecular disulfides between protein thiols [protein (P)-S-S-P] or between protein thiols and low-molecular-weight thiols such as glutathione (P-S-SG), the oxidation to sulfenic (P-SOH),

sulfinic (P-SO₂H), and sulfonic (P-SO₃H) acid and S-nitrosylation (P-S-NO) (see Fig. 1). These modifications can alter the function of numerous proteins that contain cysteines of structural importance, within their catalytic centers or as part of protein-protein interaction interfaces. To a great extent, the redox state of these cysteinyl residues is controlled by the thioredoxin (TRX) and the glutaredoxin (GRX) systems.

The TRX Family of Proteins

Both TRXs and GRXs were first discovered as hydrogen donors for *Escherichia coli* ribonucleotide reductase (48, 68), an enzyme essential for the synthesis of deoxyribonucleotides from ribonucleotides. Ribonucleotide reductase is universally present, and the mammalian enzyme is essential for DNA replication and repair. The catalytic mechanism of ribonucleotide reductase involves formation of a disulfide for each deoxyribonucleotide synthesized. This is reduced by TRX or

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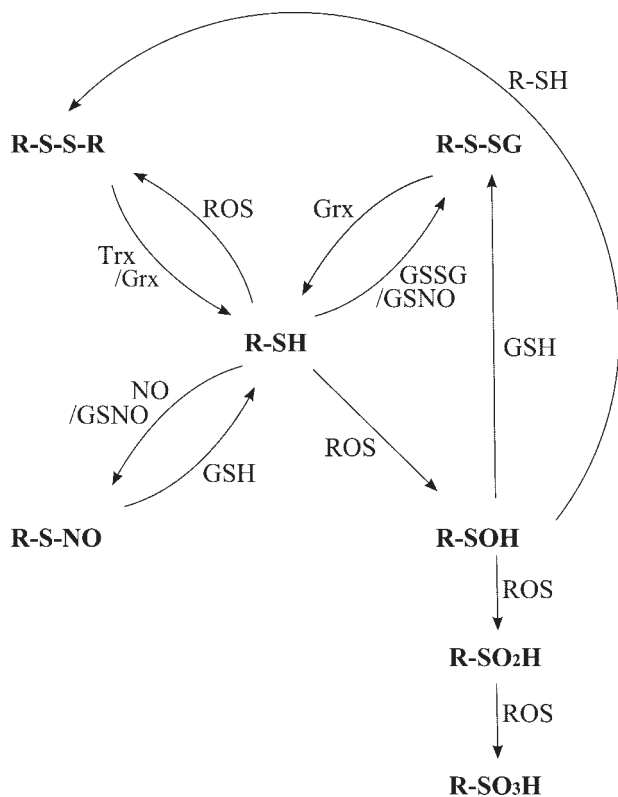


Fig. 1. Stable modifications of protein thiol groups and their interconversion. Under oxidizing conditions, protein thiols (R-SH) can form intra- and inter-molecular disulfides (R-S-S-R) that can be re-reduced by thioredoxin (TRX) or glutaredoxin (GRX). Single protein thiols can also undergo glutathionylation (R-S-SG), which can be reversed specifically by GRX. In the presence of reactive nitrogen species (ROS), thiols can be *S*-nitrosylated (R-S-NO), for instance by *S*-nitrosoglutathione (GSNO). Oxidation of the thiol group may also lead to the formation of sulfenic (R-SOH), sulfinic (R-SO₂H), and sulfonic (R-SO₃H) acid. Whereas the formation of sulfenic acid can be reversed, i.e., by GSH, the two latter species are with very few exceptions irreversible. NO, nitric oxide.

GRX. Today, we know TRXs and GRXs as general disulfide reductases maintaining cellular thiol redox homeostasis (51).

TRXs and GRXs comprise the TRX family of thiol-disulfide oxidoreductases that is characterized by the TRX fold (81) and a common dithiol/disulfide active site motif, Cys-X-X-Cys, located at the end of a β -strand and in the beginning of an α -helix (51, 81). TRXs catalyze the reversible reduction of disulfides utilizing both cysteinyl residues in their Cys-Gly-Pro-Cys active site. The NH₂ terminally located active site Cys residue has a low pK_a value of around 7 (61) and acts as nucleophile attacking the target disulfide to form a covalent-mixed disulfide intermediate (Fig. 2A, 1), which in turn is reduced by the COOH-terminally located, now deprotonated, active site thiolate (Fig. 2A, 2). This leaves a reduced or dithiol target protein and a disulfide in the active site of TRX. This disulfide is reduced by TRX reductase (TRXR) using electrons from NADPH (Fig. 2A, 3 and 4). GRXs, which are identical to thioltransferases, catalyze reactions in two distinct but functionally connected reaction mechanisms (15, 37, 49, 78, 121, 147). The reduction of protein disulfides by GRXs requires both cysteinyl residues in their Cys-Pro-Tyr-Cys active site and results in a disulfide between the two thiol groups similar to TRXs (Fig. 2B, 1 and 2). However, in the case of GRXs, this

disulfide is reduced by one molecule of GSH, leading to a mixed disulfide between GSH and the NH₂-terminal active site cysteine (Fig. 2B, 3). This mixed disulfide is subsequently reduced by a second GSH molecule (Fig. 2B, 4). The reduction of protein-GSH-mixed disulfides (deglutathionylation) by GRXs requires only the NH₂ terminally located active site Cys residue (Fig. 2B, 5). Finally, glutathione disulfide (GSSG) is regenerated by glutathione reductase at the expense of NADPH.

The TRX System

Mammalian cells contain two TRX systems, the cytosolic TRX1/TRXR1 and the mitochondrial TRX2/TRXR2. The mammalian TRXs are homodimeric selenoenzymes containing a FAD and a penultimate COOH-terminal selenocysteine residue in their Gly-Cys-SeCys-Gly active site, a feature that clearly distinguishes them from their smaller bacterial counterparts (35, 79, 154). Mammalian TRX1 was first described as an electron donor for enzymes that form a disulfide during their catalytic cycle, like ribonucleotide reductase (28), methionine sulfoxide reductases (14), and peroxiredoxins (106). TRX1 is normally a cytosolic protein, but certain stimuli like mild oxidative stress can induce its translocation to the nucleus or its leaderless export from the cell (82, 109). Today, numerous proteins have been identified to be redox regulated by the TRX system. For instance, for the binding of NF- κ B subunit p50 to its target sequence in DNA, reduction of a single cysteinyl residue by TRX1 in the nucleus is required (42, 83). Other examples of the large number of transcription factors activated by TRX1-catalyzed reduction include hypoxia-inducible factor 1- α (137), the tumor suppressor p53 (130), the glucocorticoid receptor (39), the estrogen receptor (41), polyoma virus enhancer-binding protein 2 (3), and c-Fos/c-Jun complexes (43). Although the structures of oxidized and reduced TRX are very similar, the protein undergoes small conformational changes mainly located in the active site upon reduction (52). These changes, which include increased mobility and structural sub-states in reduced TRX, have a dramatic effect on the binding activity of reduced TRX to other proteins. Apoptosis signal-regulating kinase 1 (ASK-1) is a mitogen-activated protein (MAP) kinase kinase kinase required, for instance, for tumor necrosis factor- α -induced apoptosis (54). Only reduced TRX forms a complex with ASK-1, suppressing its kinase activity, whereas oxidation of TRX leads to dissociation of the complex and activation of ASK-1 (112). Thus the redox state of TRX1 itself serves as a regulatory switch for the induction of apoptosis induction via the JNK and p38 MAPK pathways. TRX binding protein-2 [TBP-2, also called vitamin D₃-upregulated protein 1 (VDUP1) or TRX-interacting protein (Txnip)] was identified as a TRX binding partner using two hybrid experiments (60, 97, 143). TBP-2 binds specifically to reduced TRX and can thereby serve as a negative regulator of TRX function (60). The exact biochemical mechanism of the TBP-2 interaction with TRX has not yet been studied in vitro.

Next to TRX1, mammalian cells contain a series of additional homologous proteins. TRX2 is located in mitochondria and essential for embryonic development and actively respiring cells (98). The testis/sperm-specific TRXs, which do not exhibit any activity as thiol-disulfide oxidoreductases, were subject to a review previously (87). In contrast to these pro-

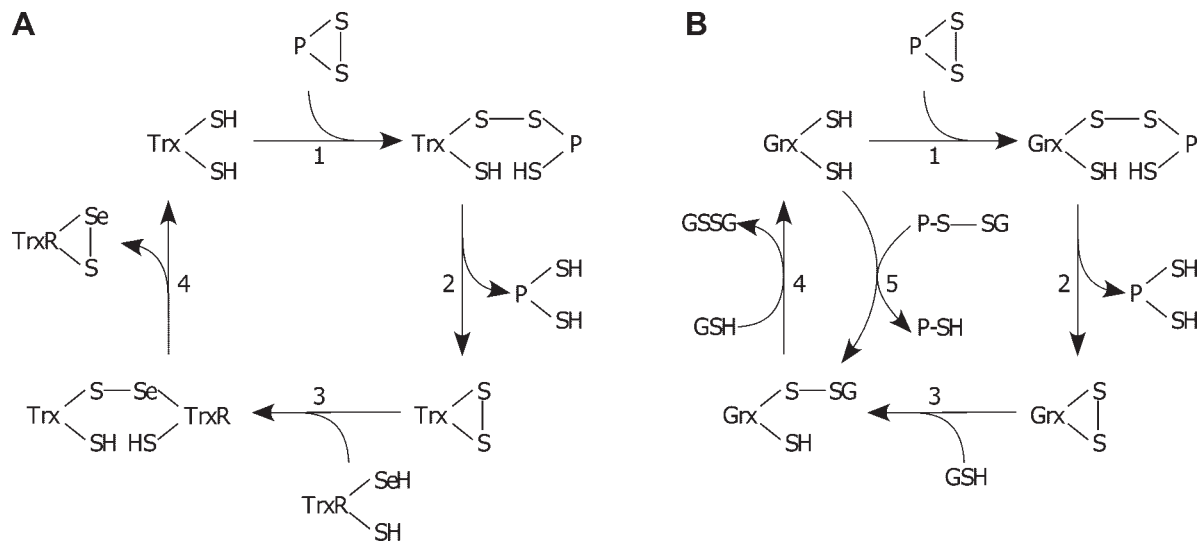


Fig. 2. *A* and *B*: redox cycles and reaction mechanisms of TRXs (*A*) and GRXs (*B*). TRXs catalyze the reversible reduction of disulfides utilizing both cysteinyl residues in their Cys-Gly-Pro-Cys active site, reducing the target disulfide to form a covalent-mixed disulfide intermediate (*A*, 1), which in turn is reduced by the COOH-terminal active site thiolate (*A*, 2). TRX is in turn reduced by TRX reductase (TRXR) in a similar manner (*A*, 3 and 4). GRXs catalyze reactions in two distinct but functionally connected reaction mechanisms. The mechanism of protein disulfides reduction by GRXs is similar to those of TRXs (*B*, 1 and 2). GRXs are reduced by GSH leading to a mixed disulfide (*B*, 3). The reduction of protein-GSH-mixed disulfides (deglutathionylation) leads also to the mixed disulfide between GSH and the NH₂-terminal active site cysteine (*B*, 4). P, protein; HS, thiol.

teins, TRX, like TRX1, and nucleoredoxin are active disulfide reductases (44, 66, 69). Protein kinase C-interacting cousin of TRX (PICOT) was identified as an interaction partner of protein kinase C- θ . PICOT bridges the TRX and GRX family, because it is composed of a NH₂-terminal TRX domain, which is required for the interaction with protein kinase C, but lacks both active site cysteines and two COOH-terminal monothiol GRX domains that both contain a Cys-Gly-Phe-Ser active site. Overexpression of PICOT in T cells inhibited the activation of c-Jun NH₂-terminal kinase and the transcription factors activator protein-1 as well as NF- κ B (139). Additional GRX domains have been identified as NH₂-terminal domains of a predominantly testis-specific splice variant of TRXR1 and of the TRXR homologue TRX glutathione reductase.

The GRX System

The GRX system consists of the glutathione redox couple (GSH/GSSG), glutathione reductase, and GRX. The GSH-to-GSSG ratio in the cell is an important indicator for the redox environment and the major determinant of the cellular redox potential. This potential seems to correlate with the biological status of the cell with, for instance, proliferation occurring at -240 mV, differentiation at -200 mV, and apoptosis at -170 mV (115). Human cells contain three GRXs: the cytosolic GRX1 with the active site Cys-Pro-Tyr-Cys; the primary mitochondrial GRX2 containing the active site Cys-Ser-Tyr-Cys (36, 77); and a second mitochondrial GRX, named GRX5, due to its homology to yeast GRX5, with only one Cys residue in its active site, i.e., Cys-Gly-Phe-Ser (138).

GRX1 supports ribonucleotide reductase with electrons and is involved in general disulfide-dithiol exchanges (47), dehydroascorbate reduction (136), cellular differentiation (122), regulation of transcription factors (6, 44, 95), and apoptosis (17, 20). GRX1 is upregulated in pancreatic cancer cells (93), and increased expression is related to doxorubicin/adriamycin-

resistance in MCF-7 breast tumor cells (86). GRX1 can protect cerebellular granule neurons from dopamine-induced apoptosis by activating NF- κ B via regulation factor 1 (20), involving the Ras-phosphoinositide 3-kinase and Jun NH₂-terminal kinase pathways (19). In general, there is upcoming evidence that the formation of mixed disulfides of protein thiols with glutathione is a key event in the regulation of the cellular response to oxidative stress. GRXs can catalyze both the formation and the reduction of mixed disulfides between protein thiols and GSH (37, 149). In general, the reduction of these mixed disulfides is favored, but under conditions where the concentration of GSH is decreased and where oxidized glutathione (GSSG) is increased, i.e., oxidative stress, mixed disulfides are formed rather than reduced (110). It was shown that glutathionylation can be induced by oxidative stress and alters the biological function of several important proteins like actin (134), glyceraldehyde 3-phosphate dehydrogenase (75), protein tyrosine phosphatase 1B (7), creatine kinase (105), c-Jun (64), NF- κ B subunit p50 (103), caspase-3 (152), and human immunodeficiency virus protease (24). Several studies indicate that GRX can catalyze the reduction of the mixed disulfides, regenerating the activities of these proteins (7, 24, 75, 134).

GRX2 is an efficient catalyst of protein de-glutathionylation; its catalytic efficiency (k_{cat}/K_m) is about 1.5- to 3-fold higher compared with GRX1 (59). GRX2 catalyzes the reversible glutathionylation of mitochondrial complex I, the major source of ROS in the cell, and this modification of two critical thiol groups regulates the production of superoxide by the complex (8, 126). The oxidized active site of GRX2, which in all other eukaryotic GRXs is exclusively reduced by GSH, is also a substrate for TRXR (59). At sufficiently oxidizing conditions involving the cumulation of GSSG, the active site in GRXs cannot be reduced by GSH (50); the direct reduction via TRXR enables GRX2 to reduce protein disulfides, glutathionylated proteins, and a series of low-molecular-weight substrates even

during conditions of oxidative stress (59). HeLa cells, in which the expression of GRX2 was silenced by RNA interference, were dramatically sensitized to cell death induced by oxidative stress-inducing apoptotic agents (74). The EC₅₀ for doxorubicin/adriamycin decreased 60-fold and the one for phenylarsine oxide, which reacts specifically with vicinal dithiols, 40-fold. Corroboratively, overexpression of GRX2 decreased the susceptibility of HeLa cells to apoptosis induced by doxorubicin or 2-deoxy-D-glucose by preventing the loss of cardiolipin, inhibition of cytochrome *c* release, and, subsequently, caspase activation, i.e., apoptosis (29). GRX2 was described as the first iron-sulfur protein from the TRX family of proteins (73). Spectroscopic analysis revealed a [2Fe-2S] cluster bridging two molecules of GRX2. This iron-sulfur cluster is complexed by the two NH₂-terminal active site thiols of two GRX2 monomers and two molecules of glutathione that are bound noncovalently to the proteins and in equilibrium with glutathione in solution (9). Because holo-GRX2 was enzymatically inactive, it was proposed to serve as a redox sensor for the activation of the protein during oxidative stress (9, 73), when the function of the protein seems to be crucial for the protection against apoptotic stimuli.

The emerging group of GRXs, which contain the active site sequence Cys-Gly-Phe-Ser, has been shown to be involved in iron homeostasis in *E. coli*, yeast, and mammals (31, 107, 138). Knockout of yeast mitochondrial GRX5 (yGRX5) led to constitutive oxidative damage, iron accumulation in the cell, and inactivation of iron-sulfur cluster containing enzymes. Thus a function of yGRX5 in iron-sulfur cluster synthesis was suggested (107). Depletion of yGRX5 increased the amount of iron bound to the iron-sulfur cluster scaffold Isu1, indicating that the protein might be required in a step following [Fe-S] cluster synthesis on Isu1 when the [Fe-S] clusters are inserted into apo proteins (91). Recent studies indicate that this function might be conserved for mammalian GRX5 as well (90, 138).

TRX and GRX in the Cardiovascular System

The heart is one of the most prominent oxygen-consuming organs, and oxidative stress is associated with most of the cardiovascular diseases and heart failures, e.g., chronic heart failure (32, 38, 55, 71). Zweier et al. (156) measured free radical generation followed by reperfusion in a direct manner. The molar ratio of free radicals increased directly after cardiopulmonary bypass (CPB) surgery with a peak after 4 h (23). Myocardial infarction after ischemia-reperfusion is a consequence not only of necrosis but also apoptosis, and ROS are most likely the signaling molecules (4, 85). Blocking the signal transduction that leads to apoptosis significantly reduces myocardial infarct size and ameliorates cardiac function (27). To prevent ROS-induced disorders, the heart needs effective antioxidant and antiapoptotic systems.

Physiological Effects of Endogenous TRX1

TRX1 was demonstrated by immunohistochemistry for the first time in 1978 in calf hearts (53). Later, measurements of enzymatic activity indicated the presence of TRX1 in cardiac tissues (102) and in plasma (12). Today, we know that TRX1 is ubiquitously expressed in endothelial and vascular smooth muscle cells and in fibroblasts of the adventitia (99). Endogenous TRX1 has the potential to decrease reperfusion-induced

arrhythmias (5) and is a central mediator of cardiomyocyte growth (148). TRX1 is upregulated and/or activated in spontaneous age-related myocarditis (88) in infiltrating cells and damaged myocytes in rats with giant cell myocarditis during the acute state (118) and in acute immune-mediated myocarditis (89). Acute myocarditis is most often caused by a virus (65). In acute coxsackievirus B3 myocarditis in inbred strains of mice, upregulation of TRX expression correlated with the severity of the disease. Myocarditis induced by immunization of porcine cardiac myosin in rats resulted in upregulation of TRX1 in the acute stage but not in the chronic state (89). TRX is upregulated by doxorubicin-adriamycin-induced oxidative stress in association with 8-hydroxy-2'-deoxyguanosine, an oxidative stress marker, and has a protective role against doxorubicin-adriamycin-induced cardiotoxicity (117). In atherosclerotic lesions such as hypercellular lesions, infiltrating macrophages highly express TRX (99). A central feature in the development of an atherosclerotic plaque is the accumulation of large amounts of cholesterol in macrophages residing in the arterial wall. Low-density lipoprotein is believed to be the major source of cholesterol in macrophages, and high plasma levels of lipoprotein increase the risk of atherosclerosis. The cellular uptake of oxidized lipoprotein leads to the generation of ROS (113). Macrophages respond to oxidized low-density lipoprotein by activating the TRX system (40a). Regions in the vasculature that are exposed to steady laminar blood flow are protected from atherosclerosis and show a higher TRX activity compared with regions where flow was disturbed (127, 146). Plasma/serum levels of TRX are upregulated in most of the cardiovascular diseases investigated so far, except in patients with stable and effort angina (63). Plasma levels were significantly elevated 4 h after angioplasty and returned to baseline after 24 h (133). TRX1 serum levels in patients with acute coronary syndrome, dilated cardiomyopathy, and chronic heart failure were significantly elevated compared with those in control patients. Moreover, there was an association between TRX concentrations and severity of heart failures (63, 57). During CPB, total antioxidant capacity decreases and oxidative stress as well as apoptosis increases (80, 104). In cardiac surgical patients, preoperative plasma levels of TRX were comparable with those of healthy controls, whereas TRX levels during surgery significantly increased (94). In contrast to CPB, which induces a generalized oxidative stress, ischemia-reperfusion may represent a localized oxidative stress (40). After ischemia-reperfusion, TRX is downregulated in isolated rat hearts (129).

Overexpression and Exogenous Application of TRX1

The highly beneficial effects of TRX in cardiovascular disorders call for clinical applications of exogenously applied TRX or the induction of endogenous TRX. For instance, treatment with TRX protects against reperfusion-induced arrhythmias in an isolated rat heart model (5), appears to attenuate age-induced cardiac hypertrophy in mice, and suppresses progression of cardiac fibrosis (2). Myocardial apoptosis and infarct size in mice were reduced by treatment with TRX 10 min before reperfusion (124). Overexpression of TRX inhibits oxidative modification of Ras and therefore inhibited the α -adrenergic receptor-stimulated hypertrophy (67). Mice with heart-specific expression of human TRX1 exhibited reduced levels of hypertrophy and oxidative stress in

response to pressure overload and were protected against ischemic-reperfusion injury. Heart-specific expression of the enzymatically inactive TRX1C32,35S but dominant negative mutant inhibited endogenous TRX and resulted in an increase of oxidative stress and induced cardiac hypertrophy under basal conditions (129, 142). Today, preconditioning remains to be one of the most powerful techniques for cardioprotection (21, 22, 72, 92). Brief periods of ischemia can prevent or reduce cytotoxic damage caused by a subsequent prolonged period of ischemic-reperfusion in the heart. Around 50 genes with altered expression have been identified in the preconditioned heart, but most of them have not been shown to be involved in the preconditioning mechanism (100). Whereas TRX is downregulated in isolated rat hearts after ischemia-reperfusion, the protein is upregulated in adapted hearts. Inhibition of the TRX system with the specific inhibitor CDDP abrogated cardioprotective effects of ischemic adaption and enhances oxidative stress in adapted myocardium (129). TRX protects against experimental autoimmune myocarditis after preconditioning through specific upregulation by temocapril treatment. Knockdown of TRX1 by short-hairpin RNA attenuated the cardioprotective effect of preconditioning, i.e., TRX works not only as an extracellular mediator but also as an intracellular mediator of preconditioning (111).

Other TRXs and GRXs in Cardioprotection

Mitochondrial TRX2 is present in heart tissues (13), and its expression and distribution in general are correlated with the metabolic activity of the tissue (120). Both GRX1 and GRX2 are localized in heart tissues (Ref. 102 and unpublished data from C. H. Lillig). Like TRX1, GRX1 is expressed ubiquitously in endothelial and vascular smooth muscle cells and in fibroblasts of the adventia (99). GRX1 can also be found in

plasma (12, 76, 94), whereas GRX2 is not excreted from cells (76). GRX1 levels are increased during CPB surgery and correlate with plasma hemoglobin levels (76). Hemolysis is a regular event during CPB surgery (62); therefore, GRX1 levels may be explained by increased hemolysis (76). GRX1 seems to be involved in protection against atherosclerosis. In atherosclerotic lesions, GRX1 is highly expressed by the infiltrating macrophages (99). Angiotensin II induces vascular smooth muscle cell hypertrophy via ROS-mediated Ras activation that may contribute to atherosclerosis or restenosis (10). Overexpression of GRX1 caused effective deglutathionylation of Ras and inhibited Ras activation (1). PICOT is upregulated in response to hypertrophic stimuli, and the inhibition of protein kinase C has been implicated in the development of cardiac hypertrophy (101). This suggested that PICOT may act as an endogenous negative feedback regulator of cardiac hypertrophy (Fig. 3) (58). PICOT overexpression enhances the inotropic property of cardiomyocytes (58).

Signaling Pathways

Two pathways are strongly affected by TRXs and GRXs in cardioprotection: the Ras/Raf/ERK and the ASK-1/JNK/p38 pathways (Fig. 3). α -Andrenic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated by ROS-dependent activation of the Ras-Raf-MEK1/2-ERK1/2 signaling pathway via oxidative modification of Ras thiols (140, 141). Both TRX1 and GRX1 seemed to be responsible for these modifications (1, 67). The Ras-Raf-ERK pathway was activated in mice with inhibited endogenous TRX. These effects were prevented after injection of *N*-2-mercaptopropionyl glycine, an antioxidant (142). ASK-1 activation leads to cardiomyocyte apoptosis and is involved in several diseases (96). As outlined above, ASK-1 is negatively regulated by binding of reduced

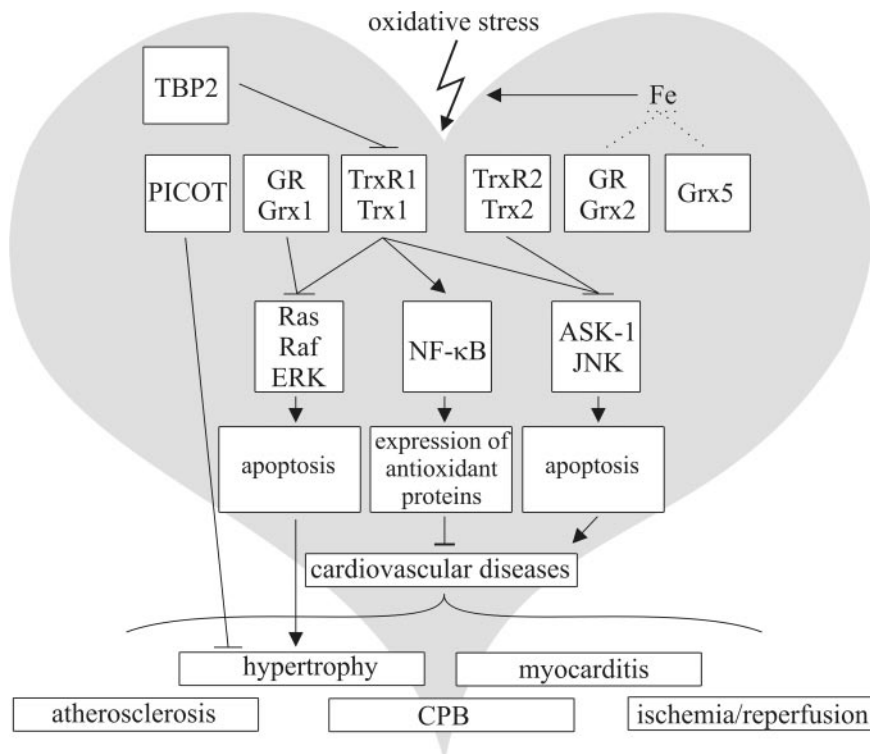


Fig. 3. Protective effects of TRX and GRX systems as well as other members of the TRX family of proteins in cardiovascular diseases. TRX1, TRX2, and GRX1 inhibit ROS-dependent diseases via inhibition of activation of the Ras/Raf/ERK and apoptosis signal-regulating kinase 1 (ASK-1)/JNK pathways. TRX1 activates antioxidant gene transcription by NF- κ B. Activity of TRX1 is regulated by TRX-binding protein 2 (TBP-2). Protein kinase C interacting cousin of TRX (PICOT) is involved in protection against hypertrophy, whereas mitochondrial GRXs 2 and 5 may be involved in protection against iron-catalyzed oxidative stress. GR, glutathione reductase; CPB, cardiopulmonary bypass.

TRX1, TRX2, and/or GRX1 (112, 119, 153). TRX-mediated inhibition of the ASK-1 pathway is one possible mechanism by which steady laminar flow is atheroprotective (144). Pathogenic features of atherosclerosis are oxidative stress and inflammation characterized by endothelial expression of the vascular cell adhesion molecule 1 (VCAM1) (108). Steady laminar flow increased TRX activity (135) and decreased VCAM1 expression by inhibiting JNK and p38 (145). Preconditioning, leading to increased levels of TRX1, blocked ischemia-reperfusion-mediated increase in JNK1, p38, and c-Jun activities (114). The preconditioning induced activation of NF- κ B, which has an essential role in the cardioprotective properties of ischemic preconditioning (84, 85). Moreover, extracellular TRX1 inhibits interleukin-1 β mRNA and protein synthesis in monocyte-derived macrophages in a dose-dependent manner. This effect was partly mediated through a reduction of NF- κ B activation, which was the result of the reduction of both p50 and p65 subunit mRNA and protein synthesis on one hand and of the induction of inhibitor- κ B α mRNA and protein expression on the other hand. In contrast, endogenous TRX1 activates NF- κ B (Fig. 3; see Ref. 11).

So far, the most important endogenous inhibitor of TRX1 known is TBP-2/VDUP1/Txnip (60, 97). Several reports suggest a pivotal role of TBP-2 during cardiovascular disorders. Overexpression of TBP-2 attenuates cardiac hypertrophy in response to mechanical strain, phenylpyrine, or angiotensin II (148) and causes apoptotic cell death (135). In vascular smooth muscle cells, hyperglycemia increased oxidative stress by inducing TBP-2 and inhibiting TRX function (116). Expression of TBP-2 in cardiac myocytes is rapidly suppressed by hypertrophic stimuli, such as physiological fluid shear stress, H₂O₂, and pressure overload (135, 148). TRX1 activity increases in rat neonatal cardiomyocytes with suppressed TBP-2 expression (135). Besides ROS, reactive nitrogen species may also contribute to the severity of ischemia-reperfusion injury (30, 155). Treatment with *S*-nitrosoglutathione-preincubated TRX1 enhanced cardioprotective effects on myocardial infarct size and apoptosis by reduction of p38 MAPK activity (123). Cardiomyocyte apoptosis induced by the peroxydinitrite donor 3-morpholinodisodionimine (SIN-1) was decreased by treatment with TRX1. Nitrosotyrosine content was reduced, but no inhibition of inducible nitric oxide synthase expression or nitric oxide production was seen (124). TRX1 is irreversibly inhibited by nitration at Y49 through SIN-1 treatment. In ischemic-reperfused cardiac tissues, a significant amount of TRX was found to be nitrated and therefore inactivated. Treatment with nitrated TRX does not protect cardiac tissue and cannot bind ASK-1, suggesting the activation of ASK-1 and, thereby, the activation of apoptosis (125).

The Whole TRX and GRX Systems Are Important for Cardiac Protection

Glutathione reductase activity is decreased in the blood of patients with myocardial infarction after reperfusion (26) but upregulated in endothelial cells by fluid shear stress, inhibiting JNK activation by ROS (46). TRXR1 is upregulated in human atherosclerotic plaques induced by low-density lipoproteins (33). Inactivation of TRXR1 in knockout mice causes early embryonic lethality, whereas heart-specific inactivation had no effect on development, suggesting that TRXR1 is dispensable

for cardiac development (56). Knockout of mitochondrial TRXR2 led to embryonic lethality as well; however, heart-specific inactivation of TRXR2 resulted in fatal cardiomyopathy and death shortly after birth (18). Mitochondria from heart failure produce more oxygen radicals than normal mitochondria in the presence of NADPH, indicating that ROS are produced primarily in the mitochondria during ischemia and heart failure (128). Therefore, it remains to be investigated to what extent the two TRX systems contribute to the protection against ROS-induced cardiovascular diseases. Moreover, no investigations have addressed the potential roles of GRX2 and GRX5 in cardiac disorders. Both of these GRXs are localized in mitochondria and involved in iron homeostasis (74, 90, 138). Iron-catalyzed oxidative reactions are implicated in myocardial damage (Fig. 3) (16, 25, 34, 70), i.e., atherosclerosis. Iron deposition was found to be high in atherosclerotic lesions (132), and low-density lipoprotein oxidation can be effectively inhibited by the iron-chelator desferrioxamine (151). Moreover, elevated levels of the iron sequester ferritin are associated with an increased risk of atherosclerotic coronary artery disease (150). Cardiac hypertrophy is a hallmark of Friedreich ataxia, an autosomal recessive disease caused by deficiency of the mitochondrial iron donor protein frataxin. Heterozygous mutations in frataxin may mimic or modify hypertrophic cardiomyopathy (131). Further investigation of the roles of proteins with redox and iron-regulating activities like GRX2 and GRX5 in the cardiovascular system is necessary to evaluate their potential in therapy or diagnosis.

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