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Forum Review

Thioredoxin-1 and Posttranslational Modifications

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ABSTRACT

Thioredoxin-1 is a 12 kDa protein that consists of a redox regulatory domain containing the active cysteine residues 32 and 35. These cysteines are conserved from bacteria to human. Unlike thioredoxins from lower species, mammalian thioredoxin-1 contains three additional nonactive cysteine residues at positions 62, 69, and 73 (for human thioredoxin-1). Key biological functions of thioredoxin-1 are antioxidative, anti-apoptotic, and pro-proliferative properties. Thioredoxin-1 is regulated by the ability of the thioredoxin reductase to reduce oxidized thioredoxin-1 at cysteines 32 and 35. However, posttranslational modifications of thioredoxin-1, including glutathionylation, thiol-oxidation, and *S*-nitros(yl)ation, at the nonactive cysteines importantly contribute to the regulation and functions of thioredoxin-1. This review focuses on the posttranslational modifications of the active and nonactive cysteines and their contribution for functional regulation of thioredoxin-1.
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INTRODUCTION

OXYGEN AND NITRIC OXIDE are physiologically relevant molecules. Both molecules act as double-edged swords. In high concentrations, oxygen and the resulting reactive oxygen species, as well as nitric oxide, are toxic, whereas in low concentrations these molecules play important roles in the regulation of a variety of cellular functions. Oxidant stress of the vascular wall is a hallmark of atherosclerosis (for review, see Ref. 21). To defend themselves from oxidative damage, including oxidative and nitrosative stress, cells possess antioxidative enzymes such as superoxide dismutase, catalase, and the thioredoxin system, to maintain intracellular levels of reactive oxygen species and reactive nitrogen species (41, 42).

The thioredoxin family includes three proteins, thioredoxin-1, thioredoxin-2, and Sp-thioredoxin (37, 47, 49). They contain a conserved -Cys–Gly–Pro–Cys- active site (cysteine 32 and cysteine 35 within thioredoxin-1), which is essential for the redox regulatory function of thioredoxins (26, 34). Thioredoxin-1 is a 12 kDa protein, which is ubiquitously expressed in mammalian cells (26). Thioredoxin-1 exerts its enzymatic activity as an oxidoreductase via the cysteines 32 and 35 in the active site of thioredoxin-1 (26, 34). This site is conserved among species from bacteria to humans (10, 26). The active site cysteines are accessible on the surface of the protein and are oxidized to a disulfide upon reduction of a target protein (27, 34, 44). Thioredoxin-1 itself is reduced by the thioredoxin reductase. These two oxidoreductases form the thioredoxin system in mammalian cells (Fig. 1). The fundamental importance of thioredoxin-1 has been demonstrated by the lethal phenotype of thioredoxin-1 deficiency in mice (35).

Posttranslational modifications of proteins are conserved during evolution and relate to all aspects of cell biology. These modifications include phosphorylation of serine, threonine, and tyrosine residues, modifications of cysteine thiols and transition metal centers by nitric oxide, and modifications of cysteine thiols by glutathione or oxygen. The latter three are so-called redox based protein modifications. All of these protein modifications are reversible and play an important role in cellular signaling and in human diseases.

With respect to the thioredoxin system, all redox-based modifications have been demonstrated to regulate thioredoxin-1. In contrast, phosphorylation of thioredoxin-1 has not been demonstrated. Oxidation has also been demon-

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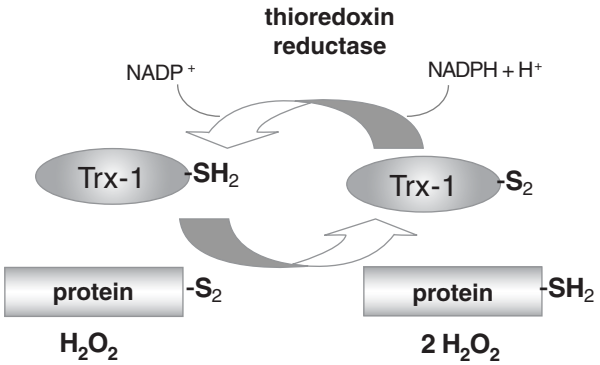

FIG. 1. The thioredoxin system. Cartoon represents the two oxidoreductases thioredoxin-1 and thioredoxin reductase, which form the thioredoxin system.

strated for thioredoxin-2 by different metals such as arsenic, cadmium, and mercury, resulting in a dissociation of thioredoxin-2 from apoptosis-signaling kinase 1 in cell culture (19). However, the impact of posttranslational modifications of thioredoxin-2 on cellular function remains to be elucidated. Therefore, this review will focus on the redox-based modifications of thioredoxin-1 and their influence for cellular signaling and human diseases.

CYSTEINES IN THIOREDOXIN-1

Thioredoxin-1 contains five cysteines. Cysteines 32 and 35 are in the redox-regulatory domain of thioredoxin-1, which is conserved throughout species (10, 26). Thioredoxin-1 exerts several functions via its redox-regulatory domain (43). Besides its enzymatic activity as an oxidoreductase in scavenging H_2O_2 and thereby reducing H_2O_2 to H_2O (28), thioredoxin-1 directly interacts with other proteins by forming disulfide bridges such as ribonucleotide reductase, protein disulfide isomerase, apoptosis signaling kinase I, and vitamin D_3-upregulated protein 1 (Txnip, also named VDUP-1) and interfering with the function of the proteins (9, 32, 33, 46, 55). Furthermore, thioredoxin-1 translocates into the nucleus under specific stimuli and directly binds different transcription factors and, thereby, modulates their DNA-binding activity, for example, p53, NFκB, and AP-1 via Ref-1 (8, 20, 23, 24, 29, 52). Besides the two cysteines at positions 32 and 35 in the redox-regulatory domain, thioredoxin-1 contains three cysteines at positions 62, 69, and 73, which are believed to be structurally important (1, 53) and are also conserved in mammals. These three cysteines have been implicated in redox-based protein modifications and will be described in more detail in the following sections of the review.

GLUTATHIONYLATION OF THIOREDOXIN-1

Glutathionylation describes the formation of mixed disulfides between proteins and glutathione. Glutathionylation of proteins has been shown to occur under physiological conditions, providing evidence that this protein modification does not only occur after oxidative stress (3, 4). Of note, the amount of glutathionylated proteins increased with oxidative stress up to 20–50% of the total glutathione content (15), given a rationale for the importance of this protein modification under physiological and pathophysiological conditions. However, the mechanism by which glutathione reacts with proteins is not understood. Several mechanisms have been proposed, ranging from an oxidation including an oxidant such as diazenecarbonyl derivate diamide (30) to a thiol/disulfide exchange (16). However, it is not clear which of these mechanisms occurs *in vivo*. Up to now it is only evident that glutathionylation is important as a reversible protein modification that can be detected *in vivo* under physiological and pathophysiological conditions. Several proteins have been described to undergo glutathionylation, including thioredoxin-1. However, glutathionylation of thioredoxin-1 could only been demonstrated under conditions of oxidative stress in eukaryotic cells and in plants (7, 36) (Fig. 2). In both cases glutathionylation of thioredoxin occurred not at cysteines spanning the redox regulatory domain, but at cysteine 73 in eukaryotes and at cysteine 60 in plants. Like cysteine 73, glutathionylated cysteines in plant thioredoxin are located in the flexible α3–β4 loop (11, 36). Glutathionylation of thioredoxin led to a reduction in the enzymatic activity of thioredoxin under conditions of oxidative stress (Fig. 2). Further studies are needed to address whether glutathionylation has an impact on the other known thioredoxin functions, such as altering its affinity for interacting proteins or its subcellular localization. Nevertheless, a possible crosstalk between the glutathione and thioredoxin systems under conditions of oxidative stress may act as an indicator of the redox status of the cell.

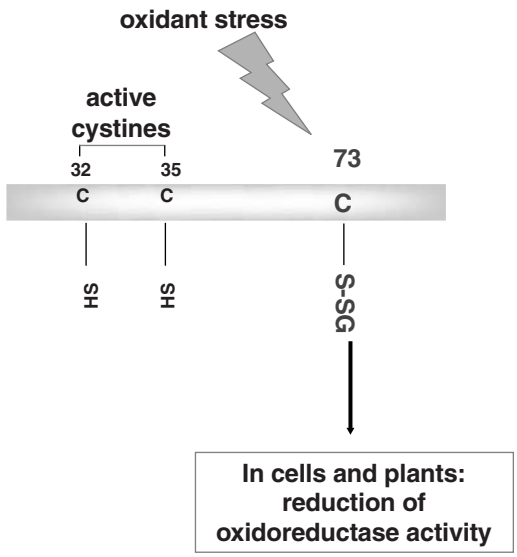

FIG. 2. Regulation of thioredoxin by glutathionylation. Glutathionylation of cysteine 73 occurs under conditions of oxidative stress in mammals and plants, which leads to inactivation of thioredoxin-1 activity.

THIOL-OXIDATION OF THIOREDOXIN-1

Thioredoxin-1 reduces disulfide bridges in oxidized target proteins via cysteines 32 and 35. The active site cysteines become oxidized. This disulfide is cycled back under NADPH consumption to the dithiol by thioredoxin-1 reductase (see Fig. 1). In the reduced form, thioredoxin-1 can bind to different proteins and thereby regulate their protein functions. The regulatory function of thioredoxin-1 on different proteins via the active cysteines is described by Yoshioka *et al*. in Part II of this Forum in more detail (56). Interestingly, a second dithiol/disulfide motif was found within thioredoxin-1 form by the cysteines 62 and 69 (51). Watson *et al*. discovered that upon oxidative stress induced by diamide first the oxidation of the active cysteines and the cysteine 62 and cysteine 69 occurred. Of note, the disulfide between cysteines 62 and 69 inhibited the reduction of the active site cysteines of thioredoxin-1 by thioredoxin reductase (51). Structural modeling of the disulfide cysteine 62 and cysteine 69 predicted a profound effect on the tertiary structure of thioredoxin-1 by disrupting the helical structure of the α3 helix. These data suggested that the interaction between thioredoxin-1 and thioredoxin reductase is sensitive to the presence of a nonactive site disulfide in the α3-helix. Thus, it is tempting to speculate that thioredoxin reductase binds to thioredoxin-1 at the α3-helix region. This interpretation is further supported by the finding that homodimerization of thioredoxin-1 via cysteine 73, which is in close proximity to the α3-helix, made the active site cysteines inaccessible for thioredoxin reductase (14). Taken together, these data support the concept that reversible oxidation of the conserved nonactive site cysteines of mammalian thioredoxin-1 play an important regulatory role for the function of thioredoxin-1. One may speculate that the nonactive site cysteines, thereby, also influence the binding proteins of thioredoxin-1 and thereby their functions. However, further studies are needed to fully elucidate the influence of the oxidation of the nonactive site cysteines on the regulation of thioredoxin-1 binding proteins.

S-NITROS(YL)ATION OF THIOREDOXIN-1

Nitric oxide (NO) is a crucial factor for the integrity and function of the endothelium (2, 40). Besides its role in blood pressure regulation, NO exerts anti-inflammatory, antithrombotic, and anti-apoptotic effects in the cardiovascular system (2, 5, 13, 22, 54). The initial discovery of endothelium-derived NO-mediated cell signaling was the reaction with the heme–iron center of the guanylate cyclase, leading to increased cyclic guanosine monophosphate (cGMP) accumulation and thus, to vasorelaxant activity (12, 13, 39). Over the past 10 years, it became clear that NO can also function in a cGMP-independent manner by reacting with cysteine residues in target proteins, a reaction named *S*-nitros(yl)ation (48). *S*-nitros(yl)ation has since then been implicated in the control of a wide array of protein functions. Indeed, a head-to-head comparison with phosphorylation revealed that both posttranslational modifications play a role in regulation of almost all cellular functions (for review, see Ref. 31).

Recent studies demonstrated that thioredoxin-1 is also a target for *S*-nitros(yl)ation (Fig. 3). In endothelial cells, thioredoxin-1 has been shown to be *S*-nitros(yl)ated at cysteine 69. *S*-nitros(yl)ation of thioredoxin-1 at cysteine 69 increased the enzyme activity of thioredoxin-1. Interestingly, *S*-nitros(yl)ation of thioredoxin-1 at cysteine 69 in part accounts for the anti-apoptotic capacity of thioredoxin-1 in endothelial cells, whereas mutation of cysteine 69 had no protective effect in human embryonic kidney cells, which do not produce NO endogenously, underscoring the importance for *S*-nitrosylated thioredoxin-1 in NO-producing cells (17). Moreover, the most important physiological stimulus for the activation of the endothelial nitric oxide synthase and thus, for the synthesis of nitric oxide in endothelial cells, is laminar flow in the blood vessel (6). Recently, it has

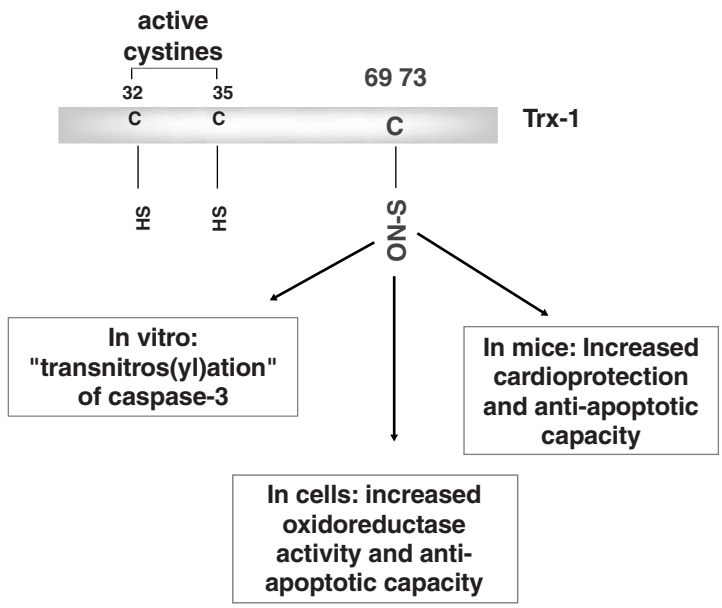

FIG. 3. Regulation of thioredoxin-1 by *S*-nitros(yl)ation. *S*-nitrs(yl)ation of thioredoxin-1 can occur at cysteine 69 and 73. *S*-nitros(yl)ation at cysteine 69 in nitric oxide producing cells leads to increased thioredoxin-1 activity and to enhanced anti-apoptotic properties. *S*-nitros(yl)ation of cysteine 73 can "transnitros(yl)ate" caspase-3 *in vitro*. In animals, *S*-nitros(yl)ated thioredoxin-1 demonstrated enhanced cardioprotective and anti-apoptotic functions.

been demonstrated that laminar flow regulated *S*-nitros(yl)ation of different target proteins (25). *S*-nitros(yl)ation of the caspase-3 subunit p17, the small GTPase $p21^{ras}$, and thioredoxin-1 were significantly increased, which resulted in a deactivation of caspase-3 and an increase of the activity of the small GTPase $p21^{ras}$ and of thioredoxin-1 (25). These data suggest that *S*-nitros(yl)ation might be one signal transduction pathway for mediating the response to laminar flow. Recently, a novel antioxidative effect of HMG-CoA reductase inhibitors (statins), which are lipid-lowering drugs that also exert pleiotropic vasculoprotective effects via activation of the endothelial NO synthesis, has been demonstrated via increasing *S*-nitros(yl)ation of thioredoxin-1 in endothelial cells (18). Furthermore, Mitchell *et al*. has demonstrated that thioredoxin-1 can transfer NO to caspase-3 and thereby inactivating the enzyme, further suggesting an important anti-apoptotic function for *S*-nitros(yl)ation of thioredoxin-1 (38). Mitchell *et al*. found that purified thioredoxin-1 is *S*-nitrosylated at cysteine 73 and not at cysteine 69 (38). Thus, one may hypothesize that cysteine 69 and cysteine 73 are both required for *S*-nitros(yl)ation and transferring nitric oxide to several target proteins in cells. Based on the recent finding that thioredoxin-1 contains a second dithiol/disulfide motif spanning cysteine 62 and cysteine 69, which was predicted to have a profound effect by disrupting the helical structure of the α3 helix of thioredoxin-1 (51) and that cysteine 73 is responsible for homodimerization of thioredoxin-1 (45), a second hypothesis arises that *S*-nitros(yl)ation of thioredoxin-1 at cysteine 69 and/or 73 depends on the redox-status of the cell and thus, on the availability of a reduced cysteine 69 and/or cysteine 73. Strikingly, in a mouse model for myocardial ischemia and reperfusion, the cardioprotective and anti-apoptotic effects of thioredoxin-1 were significantly potentiated by thioredoxin-1, which was *S*-nitrosylated prior to i.p. injection (50). Taken together, *S*-nitros(yl)ation of thioredoxin-1 importantly contributes to the cardioprotective and anti-apoptotic functions of thioredoxin-1 in cells and in mice. Thus, *S*-nitros(yl)ation of thioredoxin-1 could offer a potential approach to increase endogenous protective mechanisms and could be a potential therapeutic tool to improve cardiovascular diseases.

CONCLUSION

The active cysteines 32 and 35 of the redox-regulatory domain within thioredoxin are required for reduction of oxidized proteins and H_2O_2 as well as for the interaction and regulation of target proteins. However, the nonactive cysteines of thioredoxin-1 importantly contribute to the key biological activities. Posttranslational modifications of the nonactive cysteines not only interfere with the oxidoreductase activity of thioredoxin-1, but also with the regulation of its interacting proteins. Thus, further studies are needed to fully understand the impact of the nonactive cysteines within thioredoxin-1, which may uncover new signaling mechanisms of thioredoxin-1.

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ABBREVIATIONS

cGMP, cyclic guanosine monophosphate; NO, nitric oxide; Txnip, vitamin D_3-upregulated protein 1.

REFERENCES

1. Akamatsu Y, Ohno T, Hirota K, Kagoshima H, Yodoi J, and Shigesada K. Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues. *J Biol Chem* 272: 14497–14500, 1997.
2. Berk BC, Abe JI, Min W, Surapisitchat J, and Yan C. Endothelial atheroprotective and anti-inflammatory mechanisms. *Ann NY Acad Sci* 947: 93–109; discussion 109–111, 2001.
3. Brigelius R, Lenzen R, and Sies H. Increase in hepatic mixed disulphide and glutathione disulphide levels elicited by paraquat. *Biochem Pharmacol* 31: 1637–1641, 1982.
4. Brigelius R, Muckel C, Akerboom TP, and Sies H. Identification and quantitation of glutathione in hepatic protein mixed disulfides and its relationship to glutathione disulfide. *Biochem Pharmacol* 32: 2529–2534, 1983.
5. Busse R and Fleming I. Endothelial dysfunction in atherosclerosis. *J Vasc Res* 33: 181–194, 1996.
6. Busse R and Fleming I. Pulsatile stretch and shear: physiological stimuli determining the production of endothelial-derived relaxing factor. *J Vasc Res* 35: 73–84, 1998.
7. Casagrande S, Bonetto V, Fratelli M, Gianazza E, Eberini I, Massignan T, Salmona M, Chang G, Holmgren A, and Ghezzi P. Glutathionylation of human thioredoxin: a possible crosstalk between the glutathione and thioredoxin systems. *Proc Natl Acad Sci USA* 99: 9745–9749, 2002.
8. Das KC. c-Jun NH2-terminal kinase-mediated redox-dependent degradation of IkappaB: role of thioredoxin in NF-kappaB activation. *J Biol Chem* 276: 4662–4670 Epub 2000 Nov 4663, 2001.
9. Edman JC, Ellis L, Blacher RW, Roth RA, and Rutter WJ. Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin. *Nature* 317: 267–270, 1985.
10. Eklund H, Gleason FK, and Holmgren A. Structural and functional relations among thioredoxins of different species. *Proteins* 11: 13–28, 1991.
11. Forman–Kay JD, Clore GM, Wingfield PT, and Gronenborn AM. High-resolution three-dimensional structure of reduced recombinant human thioredoxin in solution. *Biochemistry* 30: 2685–2698, 1991.
12. Furchgott RF and Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373–376, 1980.
13. Furchgott RF and Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J* 3: 2007–2018, 1989.
14. Gasdaska JR, Kirkpatrick DL, Montfort W, Kuperus M, Hill SR, Berggren M, and Powis G. Oxidative inactivation of thioredoxin as a cellular growth factor and protection by a Cys73→Ser mutation. *Biochem Pharmacol* 52: 1741–1747, 1996.

15. Gilbert HF. Redox control of enzyme activities by thiol/disulfide exchange. *Methods Enzymol* 107: 330–351, 1984.
16. Gilbert HF. Thiol/disulfide exchange equilibria and disulfide bond stability. *Methods Enzymol* 251: 8–28, 1995.
17. Haendeler J, Hoffmann J, Tischler V, Berk BC, Zeiher AM and Dimmeler S. Redox regulatory and anti-apoptotic functions of thioredoxin depend on *S*-nitrosylation at cysteine 69. *Nat Cell Biol* 4: 743–749, 2002.
18. Haendeler J, Hoffmann J, Zeiher AM, and Dimmeler S. Antioxidant effects of statins via S-nitrosylation and activation of thioredoxin in endothelial cells. A novel vasculoprotective function of statins. *Circulation* 110: 856–861, 2004.
19. Hansen JM, Zhang H, and Jones DP. Differential oxidation of thioredoxin-1, thioredoxin-2, and glutathione by metal ions. *Free Radic Biol Med* 40: 138–145 Epub 2005 Oct 2019, 2006.
20. Harper R, Wu K, Chang MM, Yoneda K, Pan R, Reddy SP, and Wu R. Activation of nuclear factor-kappa b transcriptional activity in airway epithelial cells by thioredoxin but not by N-acetyl-cysteine and glutathione. *Am J Respir Cell Mol Biol* 25: 178–185, 2001.
21. Harrison D, Griendling KK, Landmesser U, Hornig B, and Drexler H. Role of oxidative stress in atherosclerosis. *Am J Cardiol* 91: 7A–11A, 2003.
22. Harrison DG. Endothelial dysfunction in atherosclerosis. *Basic Res Cardiol* 1: 87–102, 1994.
23. Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, and Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc Natl Acad Sci USA* 94: 3633–3638, 1997.
24. Hirota K, Murata M, Sachi Y, Nakamura H, Takeuchi J, Mori K, and Yodoi J. Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. *J Biol Chem* 274: 27891–27897, 1999.
25. Hoffmann J, Dimmeler S, and Haendeler J. Shear stress increases the amount of S-nitrosylated molecules in endothelial cells: Important role for signal transduction. *FEBS Lett* 551: 153–158, 2003.
26. Holmgren A. Thioredoxin and glutaredoxin systems. *J Biol Chem* 264: 13963–13966, 1989.
27. Holmgren A. Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. *Structure* 3: 239–243, 1995.
28. Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. *Antioxid Redox Signal* 2: 811–820, 2000.
29. Hwang CY, Ryu YS, Chung MS, Kim KD, Park SS, Chae SK, Chae HZ, and Kwon KS. Thioredoxin modulates activator protein 1 (AP-1) activity and p27Kip1 degradation through direct interaction with Jab1. *Oncogene* 23: 8868–8875, 2004.
30. Kosower NS and Kosower EM. Formation of disulfides with diamide. *Methods Enzymol* 143: 264–270, 1987.
31. Lane P, Hao G, and Gross SS. *S*-nitrosylation is emerging as a specific and fundamental posttranslational protein modification: head-to-head comparison with O-phosphorylation. *Sci STKE* 2001: RE1, 2001.
32. Liu H, Nishitoh H, Ichijo H, and Kyriakis JM. 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: 2198–2208, 2000.
33. Liu Y and Min W. Thioredoxin promotes ASK1 ubiquitination and degradation to inhibit ASK1-mediated apoptosis in a redox activity-independent manner. *Circ Res* 90: 1259–1266, 2002.
34. Martin JL. Thioredoxin—a fold for all reasons. *Structure* 3: 245–250, 1995.
35. Matsui M, Oshima M, Oshima H, Takaku K, Maruyama T, Yodoi J, and Taketo MM. Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev Biol* 178: 179–185, 1996.
36. Michelet L, Zaffagnini M, Marchand C, Collin V, Decottignies P, Tsan P, Lancelin JM, Trost P, Miginiac–Maslow M, Noctor G, and Lemaire SD. Glutathionylation of chloroplast thioredoxin f is a redox signaling mechanism in plants. *Proc Natl Acad Sci USA* 102: 16478–16483 Epub 12005 Nov 16471, 2005.
37. Miranda–Vizuete A, Ljung J, Damdimopoulos AE, Gustafsson JA, Oko R, Pelto–Huikko M, and Spyrou G. Characterization of Sptrx, a novel member of the thioredoxin family specifically expressed in human spermatozoa. *J Biol Chem* 276: 31567–31574, 2001.
38. Mitchell DA and Marletta MA. Thioredoxin catalyzes the S-nitrosation of the caspase-3 active site cysteine. *Nat Chem Biol* 1: 154–158 Epub 2005 Jul 2010, 2005.
39. Moncada S and Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med* 329: 2002–2012, 1993.
40. Nerem RM, Harrison DG, Taylor WR, and Alexander RW. Hemodynamics and vascular endothelial biology. *J Cardiovasc Pharmacol* 21: S6–10, 1993.
41. Nordberg J and Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31: 1287–1312, 2001.
42. Orr WC and Sohal RS. Extension of lifespan by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 263: 1128–1130, 1994.
43. Powis G and Montfort WR. Properties and biological activities of thioredoxins. *Annu Rev Biophys Biomol Struct* 30: 421–455, 2001.
44. Qin J, Clore GM, and Gronenborn AM. The high-resolution three-dimensional solution structures of the oxidized and reduced states of human thioredoxin. *Structure* 2: 503–522, 1994.
45. Ren X, Bjornstedt M, Shen B, Ericson ML, and Holmgren A. Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione. *Biochemistry* 32: 9701–9708, 1993.
46. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
47. Spyrou G, Enmark E, Miranda–Vizuete A, and Gustafsson J. Cloning and expression of a novel mammalian thioredoxin. *J Biol Chem* 272: 2936–2941, 1997.

48. Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, Singel DJ, and Loscalzo J. *S*-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc Natl Acad Sci USA* 89: 444–448, 1992.
49. Taniguchi Y, Taniguchi–Ueda Y, Mori K, and Yodoi J. 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. *Nucleic Acids Res* 24: 2746–2752, 1996.
50. Tao L, Gao E, Bryan NS, Qu Y, Liu HR, Hu A, Christopher TA, Lopez BL, Yodoi J, Koch WJ, Feelisch M, and Ma XL. Cardioprotective effects of thioredoxin in myocardial ischemia and reperfusion: role of *S*-nitrosation [corrected]. *Proc Natl Acad Sci USA* 101: 11471–11476 Epub 12004 Jul 11426, 2004.
51. Watson WH, Pohl J, Montfort WR, Stuchlik O, Reed MS, Powis G, and Jones DP. Redox potential of human thioredoxin 1 and identification of a second dithiol/disulfide motif. *J Biol Chem* 278: 33408–33415 Epub 32003 Jun 33419, 2003.
52. Wei SJ, Botero A, Hirota K, Bradbury CM, Markovina S, Laszlo A, Spitz DR, Goswami PC, Yodoi J, and Gius D. Thioredoxin nuclear translocation and interaction with redox factor-1 activates the activator protein-1 transcription factor in response to ionizing radiation. *Cancer Res* 60: 6688–6695, 2000.
53. Weichsel A, Gasdaska JR, Powis G, and Montfort WR. Crystal structures of reduced, oxidized, and mutated human thioredoxins: evidence for a regulatory homodimer. *Structure* 4: 735–751, 1996.
54. Wolin MS, Xie YW, and Hintze TH. Nitric oxide as a regulator of tissue oxygen consumption. *Curr Opin Nephrol Hypertens* 8: 97–103, 1999.
55. Yamanaka H, Maehira F, Oshiro M, Asato T, Yanagawa Y, Takei H, and Nakashima Y. A possible interaction of thioredoxin with VDUP1 in HeLa cells detected in a yeast two-hybrid system. *Biochem Biophys Res Commun* 271: 796–800, 2000.
56. Yoshioka J, Schreiter ER, and Lee RT. Role of thioredoxin in cell growth through interactions with signaling molecules. *Antioxid Redox Signal* 2006 (in press).

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2. Ying Xiong , Joachim D. Uys , Kenneth D. Tew , Danyelle M. Townsend . 2011. S-Glutathionylation: From Molecular Mechanisms to Health Outcomes. *Antioxidants & Redox Signaling* **15**:1, 233-270. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
3. Kenneth D. Tew, Yefim Manevich, Christina Grek, Ying Xiong, Joachim Uys, Danyelle M. Townsend. 2011. The role of glutathione S-transferase P in signaling pathways and S-glutathionylation in cancer. *Free Radical Biology and Medicine* **51**:2, 299-313. [CrossRef]
4. Kenneth D. Tew, Danyelle M. Townsend. 2011. Regulatory functions of glutathione S -transferase P1-1 unrelated to detoxification. *Drug Metabolism Reviews* **43**:2, 179-193. [CrossRef]
5. Matteo Landriscina , Francesca Maddalena , Gabriella Laudiero , Franca Esposito . 2009. Adaptation to Oxidative Stress, Chemoresistance, and Cell Survival. *Antioxidants & Redox Signaling* **11**:11, 2701-2716. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Alberto Bindoli, Maria Pia Rigobello, Guido Scutari, Chiara Gabbiani, Angela Casini, Luigi Messori. 2009. Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs. *Coordination Chemistry Reviews* **253**:11-12, 1692-1707. [CrossRef]
7. Isabella Dalle-Donne, Ranieri Rossi, Graziano Colombo, Daniela Giustarini, Aldo Milzani. 2009. Protein S-glutathionylation: a regulatory device from bacteria to humans. *Trends in Biochemical Sciences* **34**:2, 85-96. [CrossRef]
8. Kathryn F. Tonissen, Giovanna Di Trapani. 2009. Thioredoxin system inhibitors as mediators of apoptosis for cancer therapy. *Molecular Nutrition & Food Research* **53**:1, 87-103. [CrossRef]
9. Sanghwa Han. 2008. Force field parameters for S-nitrosocysteine and molecular dynamics simulations of S-nitrosated thioredoxin. *Biochemical and Biophysical Research Communications* **377**:2, 612-616. [CrossRef]
10. N MAULIK, D DAS. 2008. Emerging potential of thioredoxin and thioredoxin interacting proteins in various disease conditions. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1780**:11, 1368-1382. [CrossRef]
11. Melissa Kemp, Young-Mi Go, Dean P. Jones. 2008. Nonequilibrium thermodynamics of thiol/disulfide redox systems: A perspective on redox systems biology. *Free Radical Biology and Medicine* **44**:6, 921-937. [CrossRef]
12. Martin Keller, Andreas Rüegg, Sabine Werner, Hans-Dietmar Beer. 2008. Active Caspase-1 Is a Regulator of Unconventional Protein Secretion. *Cell* **132**:5, 818-831. [CrossRef]
13. S HAN. 2007. Molecular dynamics simulations of thioredoxin with S-glutathiolated cysteine-73. *Biochemical and Biophysical Research Communications* **362**:2, 532-537. [CrossRef]
14. Toshimitsu Niwa. 2007. Protein glutathionylation and oxidative stress#. *Journal of Chromatography B* **855**:1, 59-65. [CrossRef]
15. A BARTHEL, E OSTRAKHOVITCH, P WALTER, A KAMPKOTTER, L KLOTZ. 2007. Stimulation of phosphoinositide 3-kinase/Akt signaling by copper and zinc ions: Mechanisms and consequences. *Archives of Biochemistry and Biophysics* **463**:2, 175-182. [CrossRef]
16. Alberto Jiménez, Laura Mateos, José R. Pedrajas, Antonio Miranda-Vizuete, José L. Revuelta. 2007. The txl1 + gene from Schizosaccharomyces pombe encodes a new thioredoxin-like 1 protein that participates in the antioxidant defence against tert -butyl hydroperoxide. *Yeast* **24**:6, 481-490. [CrossRef]
17. Junichi Sadoshima . 2006. Redox Regulation of Growth and Death in Cardiac Myocytes. *Antioxidants & Redox Signaling* **8**:9-10, 1621-1624. [Citation] [Full Text PDF] [Full Text PDF with Links]