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TrxR1 as a Potent Regulator of the Nrf2-Keap1 Response System

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Abstract

Significance: All cells must maintain a balance between oxidants and reductants, while allowing for fluctuations in redox states triggered by signaling, altered metabolic flow, or extracellular stimuli. Furthermore, they must be able to rapidly sense and react to various challenges that would disrupt the redox homeostasis. ***Recent Advances:*** Many studies have identified Keap1 as a key sensor for oxidative or electrophilic stress, with modification of Keap1 by oxidation or electrophiles triggering Nrf2-mediated transcriptional induction of enzymes supporting reductive and detoxification pathways. However, additional mechanisms for Nrf2 regulation are likely to exist upstream of, or in parallel with, Keap1. ***Critical Issues:*** Here, we propose that the mammalian selenoprotein thioredoxin reductase 1 (TrxR1) is a potent regulator of Nrf2. A high chemical reactivity of TrxR1 and its vital role for the thioredoxin (Trx) system distinguishes TrxR1 as a prime target for electrophilic challenges. Chemical modification of the selenocysteine (Sec) in TrxR1 by electrophiles leads to rapid inhibition of thioredoxin disulfide reductase activity, often combined with induction of NADPH oxidase activity of the derivatized enzyme, thereby affecting many downstream redox pathways. The notion of TrxR1 as a regulator of Nrf2 is supported by many publications on effects in human cells of selenium deficiency, oxidative stress or electrophile exposure, as well as the phenotypes of genetic mouse models. ***Future Directions:*** Investigation of the role of TrxR1 as a regulator of Nrf2 activation will facilitate further studies of redox control in diverse cells and tissues of mammals, and possibly also in animals of other classes. *Antioxid. Redox Signal*. 23, 823–853.

Introduction—Redox Control Through Nrf2 or TrxR1

MODIFICATIONS OF REDOX-SENSITIVE protein moieties by reactive oxygen species (ROS) and reactive nitrogen species have emerged as major post-translational mechanisms for regulation of protein function and downstream cellular events. These modifications can be reversed by reductive systems, of which the glutathione (GSH) and thioredoxin (Trx) systems are the most prominent in mammalian cells. These systems rely on NADPH-dependent disulfide reductases that, in turn, propel the reduction of a wide range of downstream targets. Both oxidative and reductive pathways are tightly controlled and ensure cellular redox homeostasis while also allowing regulation of redox signaling pathways. These redox processes are typically sensitive to reactive exogenous and endogenous molecules that easily modify critical redox-sensitive residues in proteins (24, 91, 121, 254).

Mammalian cells possess the transcription factor Nrf2 (Nuclear factor (erythroid-derived 2)-like 2) as a major regulator to coordinate cellular responses to oxidative and electrophilic stress (32, 34, 147, 208, 282, 287). Nrf2, when activated, binds to the antioxidant/electrophile responsive element (ARE/EpRE) in the promoter region of genes expressing enzymes that directly or indirectly promote cell survival and restoration of redox homoeostasis. Its portfolio of target genes includes, among others, phase 2 detoxification enzymes, proteins that promote the regeneration and synthesis of glutathione, antioxidant, and redox regulatory enzymes, including proteins of the Trx system, and enzymes that specialize in DNA and protein repair (11, 25, 32, 116, 144). Nrf2 is usually sequestered in the cytosol and constantly targeted for proteasomal degradation *via* Keap1 (Kelch-like ECH-associated protein 1), which is generally considered the main cellular sensor for oxidative and electrophilic stress (168, 169, 229).

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In this review, we wish to summarize and highlight the importance of the Trx system as a modulator of the Keap1-Nrf2 response pathway. The selenoprotein TrxR1, in particular, seems to operate in concert with Keap1 in detecting cellular stress and modulating appropriate Nrf2-dependent responses. We base our proposal on results from animal models and cell culture studies, strongly suggesting a direct causal relationship between TrxR1 inhibition or depletion and profound Nrf2 activation (36, 43, 44, 52, 61, 92, 192, 207, 221, 237, 242, 284, 285, 294). Inhibition of cellular TrxR1 activity is, compared with targeting of other redox-active enzymes, a likely scenario that has a major impact on numerous cellular events (11, 66, 127, 201, 292). We propose that electrophilic compounds that activate Nrf2 by targeting Keap1 also, if not predominantly, inhibit TrxR1 due to the highly reactive and accessible active site selenocysteine (Sec) residue of this enzyme. Some reactive molecules that target TrxR1 may furthermore not only inhibit the enzyme but also transform the protein to pro-oxidant SecTRAPs (selenium compromised thioredoxin reductase-derived apoptotic proteins) having NADPH oxidase activity (5, 6, 49), thus further promoting activation of Nrf2 in any cells that survive such an oxidative challenge. These links between TrxR1 targeting and Nrf2 activation will be discussed in detail next, but first, we give a brief general introduction of the closely intertwined redox systems in mammalian cells.

The Functions of TrxR1 in Relation to the Many Roles of the GSH and Trx Systems in Mammalian Redox Control

Mammalian cells utilize a variety of low-molecular-weight antioxidants, antioxidant enzymes, and repair systems not only to protect against oxidative damage but also to reverse oxidative modifications in order to regulate signaling pathways (16, 90, 141, 162, 187, 214, 215, 232, 240). The composition of antioxidants varies between tissues and is affected by nutrition and cellular redox states. Some of the well-known nonenzymatic antioxidants include Vitamin A and E, ascorbate, lipoic acid, ubiquinone, and GSH (110). Their redox properties and intracellular localization vary, and they may scavenge radical species, chelate transition metal ions, or promote oxidative stress, depending on concentration, cellular context, and oxygen tension. They may also be, either directly or indirectly, regenerated by various antioxidant enzymes, where the enzymes of the GSH and Trx systems are considered the most important (Fig. 1). These systems will briefly be introduced here, with the reader being referred to more comprehensive reviews on detailed discussions about the different players of these diverse redox systems. It is important to note that the different redox systems of cells constitute a complex redox milieu within which Keap1- and Nrf2-linked signaling must occur.

The tripeptide glutathione (GSH; γ-Glu-Cys-Gly) is present in low millimolar concentrations in cells and is thus their most abundant low-molecular-weight antioxidant (232, 246). It can scavenge electrophilic and oxidizing compounds either directly or as catalyzed by glutathione-S-transferases (GSTs), which have also been shown to be important in modulation of signaling pathways (116, 172). GSH is also utilized by glutathione peroxidases (GPxs) to reduce hydroperoxides or by glutaredoxins (Grxs) that operate as disulfide reductases and de-glutathionylation enzymes.

The GPx family contains eight isoforms that are expressed in various tissues and with different subcellular localizations. GPx1-4 and GPx6 in humans have a peroxidatic Sec residue, whereas the other isoforms are Cys dependent. GPx4 is unique in reducing peroxides of complex lipids such as phospholipids or cholesterol within the hydrophobic core of membranes. Of the GPx proteins for which mouse models have been made, only the GPx4 knockout is lethal (326), which might reflect particularly detrimental consequences of lipid peroxidation. In addition to being important antioxidant enzymes, GPxs are also discussed in redox signaling and regulation of

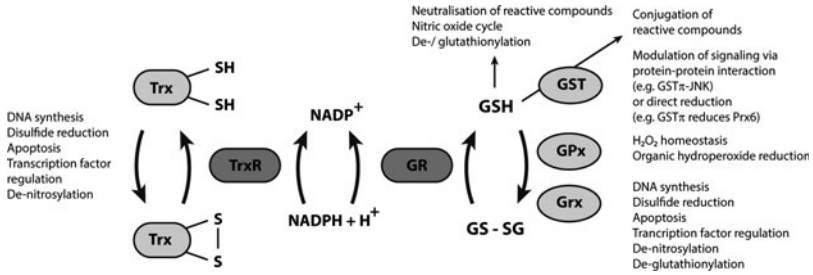

FIG. 1. Summarizing scheme of the complementary glutathione (GSH) and thioredoxin (Trx) systems. The GSH and Trx systems are two major complementary reductive systems in mammals, as illustrated here in a highly schematic manner. This scheme summarizes overall functions of the GSH and Trx system proteins, thus not considering compartmentalization effects that can further modulate their activities. General and vital functions such as DNA synthesis (through ribonucleotide reductase) and protein disulfide reduction can efficiently be supported by both systems. The key enzymes of the Trx system are the TrxRs that use electrons from NADPH to reduce Trx isoforms, as well as a number of other protein or nonprotein substrates. The GSH system fulfills similar functions, with GSH propelling enzymes such as GST, GPx, and Grx, and also directly participating in a number of processes. Oxidized GSH (glutathione disulfide, GSSG) is regenerated to its reduced form by glutathione reductase (GR) utilizing NADPH. Importantly for the purpose of this review, cells and animals generally display a significant cross-talk as well as functional overlap between the GSH and Trx systems. However, there is a major difference between the two NADPH-dependent enzymes in these reductive pathways; GR is a highly dedicated enzyme for GSSG reduction and is neither easily targeted nor inhibited by electrophiles or oxidative stress, while TrxRs are exceptionally reactive enzymes, the inhibition of which yield major effects on cellular redox control. In this review, we propose that the particular characteristics of the cytosolic TrxR1 isoform, in particular, renders it a status of a sensor communicating with the Nrf2 system, as further discussed in the text.

physiological processes. The GPx family of proteins was recently reviewed in detail by Brigelius-Flohé and Maiorino (33). The Grxs may also modulate many signaling events, and they have been thoroughly reviewed elsewhere (90, 187, 272).

After having donated their electrons, two GSH molecules form a glutathione disulfide (GSSG) *via* an intermolecular disulfide bridge, which, in turn, is reduced by glutathione reductase (GR) using NADPH as the electron donor (291). GSH also influences redox signaling events *via* glutathionylation of reactive thiol-groups in key cysteine residues, which can protect them from oxidative modifications and electrophilic compounds (102). The effect of GSH depletion on Nrf2 activation is, in contrast to TrxR1 inhibition, less clear. Some studies report Nrf2 activation on GSH depletion (59, 158, 176), whereas this is less clear in other studies (85, 183). An explanation for different results between these reports might possibly be different degrees of GSH depletion. With GSH being the most abundant low-molecular-weight antioxidant present in low millimolar concentrations (232, 246), a depletion of 80%–90% could be considered as having major effects, although the signs are less than those seen on TrxR1 depletion. Using mouse embryonic fibroblasts, it was shown that Nrf2 is required for antioxidant gene induction on GSH depletion and oxidative stress (176) and it was shown that Nrf2 activation on GSH depletion is associated with oxidative stress (158, 183). However, this is not necessarily the case with TrxR1 depletion, which can also promote an oxidative stress-independent activation of Nrf2 (284), as will be further discussed next.

The thioredoxin system is, in addition to the GSH-dependent enzymes, a key redox regulatory system in mammals that contributes to defence against oxidative stress (108, 198), cell proliferation and viability (14, 205), as well as protein folding and signal transduction (188, 211). It consists of isoenzymes of thioredoxin reductase (TrxR) that use NADPH as the electron donor to reduce their main substrates, isoforms of thioredoxin (Trx), and related proteins (16, 127, 201), which, in turn, sustain a number of pathways by providing redox enzymes either with electrons or *via* protein–protein interactions (198, 205). Substrates of Trxs that are likely of major importance in relation to signaling are the peroxiredoxins (Prxs). The Prx isoforms (Prx1-6) differ in cellular localization, substrate specificity, and reaction mechanism but all of them are highly reactive with peroxides. As such, they were initially recognized for their roles in prevention of oxidative stress, by direct reduction of hydrogen peroxide, organic hydroperoxides, lipid hydroperoxides, and peroxinitrite. Prxs are also currently recognized in the context of signal transduction, as they may transfer oxidative modification to specific target proteins *via* protein-protein interactions. The Prxs have also been discussed in detail in recent reviews (247, 256) but will be specifically discussed later in relation to targeting of TrxR1. More comprehensive discussions of the whole Trx system with regards to physiologic functions are provided by recent reviews of Mahmood *et al.* (205) and Lu and Holmgren (198).

Giving a full presentation of the GSH and Trx systems in mammals is beyond the scope of this review. Here, we shall only conclude that GSH, with all GSH-dependent enzyme systems, and the Trx system, including many Trx-dependent enzymes, support a wide range of reductive pathways in cells that strive to obtain redox homeostasis, while simultaneously allowing for fluctuations in redox control to enable redox signaling events. This occurs through an important interplay with Nrf2 regulation (Fig. 2). For the purpose of specifically introducing TrxR1 in relation to Nrf2 signaling, we first need

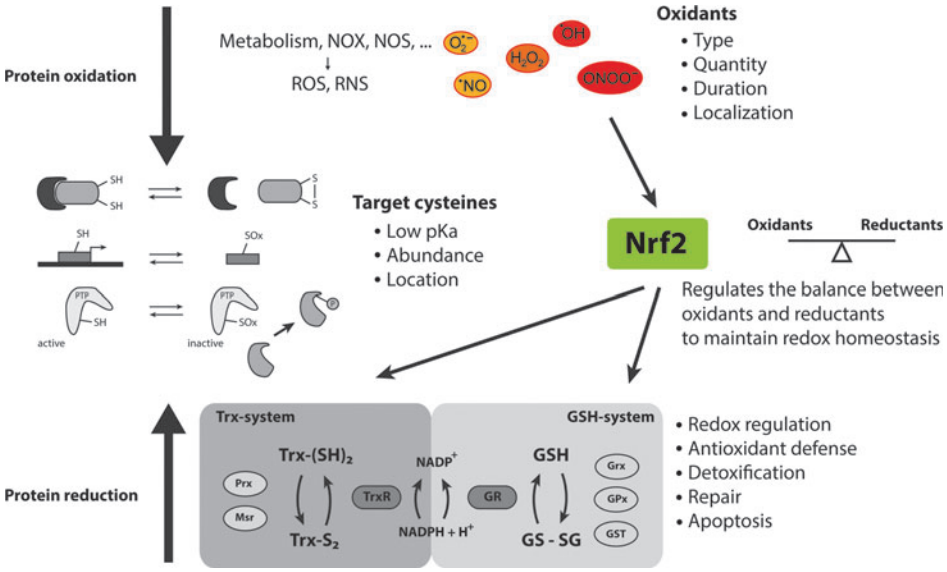

FIG. 2. The main principles of mammalian redox homeostasis. Reduction-oxidation (redox) reactions modify cellular components, particularly thiol groups of key cysteine residues that have a low pKa, by either increasing or decreasing their oxidation states, which, in turn, modulates their respective functions. Oxidative modifications, in turn, are reduced by various complex enzyme systems, of which the two most prominent are the GSH and the Trx systems (Fig. 1). A balance and tight regulation between protein oxidation and reduction is essential to maintain redox homeostasis and to enable redox signaling. The transcription factor Nrf2 is in this an essential regulator of redox homeostasis, as it induces transcription of various antioxidant enzymes in case of imbalances. In the remaining parts of this review, we discuss how Nrf2 activity, in turn, can be directly modulated by the Trx system and especially activated on a specific targeting of TrxR1. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

to underscore the key importance of TrxR1 for the Trx system, which will initially be done here through a few illustrative examples. For a full review of the enzymatic properties and physiological functions of TrxR1 as well as other TrxR isoenzymes, please see earlier reviews on the topic (13, 16, 26). It should be noted that mammalian TrxR variants, in contrast to most nonmammalian orthologs (13, 313), are larger enzymes utilizing a Sec residue in their active sites. The functional implications of the mammalian TrxR biochemical features for signaling are discussed later in detail. Mammals have three genes encoding three separate isoenzymes of TrxR (cytosolic TrxR1, mitochondrial TrxR2, and TGR in testis) that, furthermore, are subject to extensive splicing that results in expression of several different isoforms (16, 261, 279, 280, 295). It is possible that specific isoforms of TrxR have dedicated unique roles in signaling. However, in this review, most, if not all, of the discussed functional links to Nrf2 signaling are likely related to the classical cytosolic TrxR1 isoform, although very few studies have hitherto explicitly analyzed which specific isoform(s) of TrxR may be involved in the observed signaling effects.

With TrxR1 being the main enzyme propelling the whole Trx system, its inhibition will naturally impair important downstream functions of this system. That includes regulation of H_2O_2 homeostasis *via* peroxiredoxins (Prx) (255) or modulation of signaling pathways *via* reduction of protein tyrosine phosphatases (PTPs) (65, 113)—particularly these processes are also known to directly modulate activation of Nrf2 (198, 205). The Trx system is furthermore not only controlling intracellular ROS levels and redox events but also itself regulated by redox processes (76, 114, 128, 197, 198, 270, 311, 320). TrxR1 can, for instance, be directly inhibited by high ROS levels through an oligomerization process that seems to be promoted by oxidation of its surface exposed Tryptophan-114 residue (322). Nitrosylation events can also inhibit TrxR1 when occurring in the presence of Trx and Prxs (82), and denitrosylation through Trx was proposed to be a prerequisite for apoptotic signaling through caspases (27). Furthermore, Trx can easily be inhibited by its over-oxidation in absence or inhibition of TrxR1, in a process that is promoted by oxidized Prxs (76). In this context, it should be noted that Prxs are increasingly recognized as mediators of oxidation states as a mechanism of redox signaling (257, 276).

These short examples serve to illustrate that TrxR1 may be targeted and inhibited not only by treatment of cells or animals with electrophilic agents but also under conditions of normal physiological signaling. Before discussing in detail how specific targeting of TrxR1 is likely to be intimately linked to Nrf2 signaling, we shall briefly introduce the Nrf2/Keap1 system and its characteristics that may be particularly important in view of its links to the Trx system and the status of TrxR1.

The Keap1-Nrf2 Response Pathway

Nrf2, a ubiquitously transcribed member of the cap-n-collar subfamily of bZIP transcription factors, is clearly one of the most important regulators of detoxification and oxidative stress responses in mammalian cells. The underlying mechanisms that determine its activation are complex and have been extensively discussed in other recent reviews (32, 34, 147, 208, 282, 287). Here, we just wish to briefly summarize its main cellular roles and the overall mechanisms underlying its regulation.

Activation of Nrf2 is typically mediated by a variety of exogenous and endogenous stressors such as electrophilic agents and ROS. When activated, Nrf2 transits to the nucleus, heterodimerizes with one of several other ubiquitous BZIP family members, and binds to ARE(s) in the promoter region of its target genes. These genes encode proteins that collectively promote cell survival, such as several detoxifying enzymes, antioxidant enzymes (including several key proteins of both the GSH and Trx systems), receptors, transcription factors, metabolic enzymes, proteases, and more (11, 25, 32, 116, 144).

Under normal conditions, Nrf2 is bound to its inhibitor Keap1, a ubiquitin E3 ligase accessory protein that constantly targets Nrf2, *via* Cul3-mediated ubiquitination, for proteasomal degradation. Keap1 is also a sensor for Nrf2-activating compounds, with oxidation or electrophile targeting of key Cys residues in Keap1 causing the protein to undergo conformational changes (168, 229). As a consequence, Nrf2-Keap1 binding is partly disrupted so that Nrf2 ubiquitination and degradation is blocked. Nrf2 is, however, likely not released but instead occupies the now inactive Keap1, so that

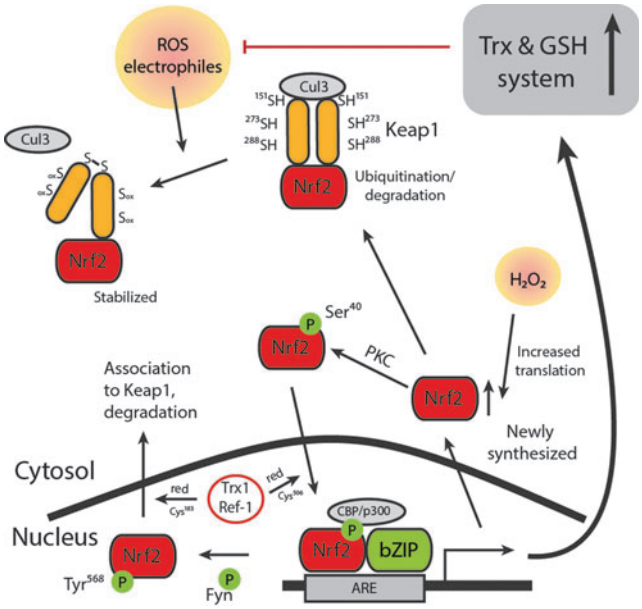

FIG. 3. Scheme of Nrf2 regulation. Nrf2 is bound to its inhibitor Keap1, which targets it for proteasomal degradation. Keap1 serves as a redox sensor as it changes conformation in response to oxidation or alkylation of crucial Cys residues in the protein. The Keap1/Nrf2 complex is stabilized and degradation is prevented on Keap1 targeting, whereby newly synthesized Nrf2 can bypass Keap1 and instead translocate to the nucleus where it activates specific ARE sequences. Nuclear Trx1/Ref-1 is important for reduction of critical Cys residues in Nrf2: one important for DNA binding, and the other being involved in nuclear export, as illustrated in the scheme. In addition, Nrf2 is subjected to phosphorylation, which further modulates its activation. Among the Nrf2 target genes are enzymes of the Trx and GSH systems. Upregulation of these counteracts the initial Nrf2 activating conditions and will facilitate downstream detoxification and antioxidant defense. This figure is modified from a figure in a review by Brigelius-Flohé and Flohé (32). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

newly synthesized Nrf2 can translocate to the nucleus where it forms heterodimers with bZIP transcription factors such as Mafs (predominantly), c-Jun, or ATF4 before binding ARE and activating target genes (146) (Fig. 3). It has been, and still is, debated whether Nrf2 also dissociates from Keap1 (32). However, a recent study convincingly showed that Nrf2 is indeed not released from Keap1 (22). Interestingly, it was also demonstrated that increased *de novo* translation of Nrf2 occurs very rapidly as a response to low (12.5 μM) H_2O_2 concentrations, with a rate that exceeds nuclear translocation of Nrf2 (64, 208). This may possibly be another sign that there can be additional potent, but yet unknown, H_2O_2 sensors in cells, in addition to Keap1 that are linked to Nrf2 activation (64, 208).

Keap1 is a homodimer that functions as adaptor of the Cullin-3-based E3 ligase. Each Keap1 subunit contains 27 (human) or 25 (mouse) Cys residues, of which 9 have been predicted to be overly reactive due to a basic microenvironment (71). Considering the broad chemical heterogeneity of Nrf2 activators, it was also suggested that the Cys residues of Keap1 should be targeted differently by different electrophiles, which may translate into specific cellular responses (169). Particularly Cys151, Cys273, and Cys288 of Keap1 were identified as good candidates for specific targeting. Cys151 was shown to be important for H_2O_2-, spermine nononate (NO donor)-, and HOCl-mediated Nrf2 activation, by forming an intermolecular disulfide with Cys151 of a second Keap1 molecule, leading to subsequent release of Cullin-3 (92, 252). The Cys273 and Cys288 residues are furthermore Zn-coordinated and essential for the response to many Nrf2 activators. A modification of those Cys residues disrupts the Zn-stabilized conformation of Keap1, thus inhibiting degradation (329) (Fig. 3).

Not only Keap1 but also Nrf2 itself is subject to redox regulation. Nrf2 has at least two redox-sensitive Cys residues within its nuclear localization signal (NLS) and nuclear export signal (NES) sequences. Oxidation of Cys183 in the NES site was proposed to interfere with Crm1 (chromosome region maintenance 1; exportin) binding and thus retain Nrf2 in the nucleus (184, 185). A similar effect was reported for nuclear Keap1, which would further prevent the nuclear export of Nrf2 (301). Such oxidations may be reversed by nuclear GSH or Trx systems. Trx1 was, for example, shown to promote nuclear export of Nrf2 (112). Reduction of Cys506 in the NLS region may be catalyzed by Trx1 together with redox factor-1 (Ref-1) as a part of activator protein 1 (AP1)-mediated activation (112, 125) and is important for interaction with the transcriptional coactivators CBP/p300 as well as for DNA binding of Nrf2 (30) (Fig. 3).

Nrf2 is also regulated by phosphorylation—certain events promote Nrf2 activation by phosphorylation, whereas others seem to diminish it. The Ser40 residue is, for example, phosphorylated by the redox-sensitive protein kinase C (PKC), which prevents binding to Keap1 and promotes nuclear translocation (231). On the other hand, Nrf2 can be phosphorylated by Fyn at Tyr568 in the nucleus, which may promote its Crm1 interaction and thus nuclear export. Activation and nuclear translocation of Fyn can be detected several hours after Nrf2 activation and is redox regulated, as it involves an H_2O_2-activated phosphorylation cascade, which may thus also be part of the final Nrf2 regulation (150) (Fig. 3). Processes that involve phosphatases and kinases are furthermore susceptible to cross-talk between different signaling pathways, which is an aspect that has elsewhere been thoroughly discussed by others in the context of Nrf2 (32, 193).

The different events of Keap1/Nrf2 regulation that are redox sensitive, as briefly summarized here, will naturally be affected by the overall redox status of cells. Perturbations of redox homeostasis may be triggered through a myriad of events, but as we propose in this review the selenoprotein TrxR1 may be uniquely positioned as a sensitive redox ''sensor'' that is functionally linked to the Keap1/Nrf2 system. This role of TrxR1 is due to a combination of its position as an important master regulator of the Trx system and its unique chemical reactivity.

Unique Biochemistry and Chemical Reactivity of TrxR1

TrxR1 is not absolutely required to keep Trx1 reduced in cells

As stated earlier, the thioredoxin system is one of the two key redox regulatory systems in mammalian cells and is, as such, important for defense against oxidative stress (108, 198), cell proliferation and viability (14, 205), as well as protein folding and signal transduction (188, 211). The main ''engine'' of the Trx system is TrxR that under normal conditions uses NADPH to reduce its main substrate thioredoxin (Trx) (127, 201), which, in turn, sustains a number of pathways by providing enzymes with either electrons or *via* protein–protein interactions (198, 205). It should, however, be noted that cytosolic Trx1 may also be maintained in a reduced form by the GSH system through the action of glutaredoxins (Grxs) (75, 331). This fact should explain how Trx1 is kept reduced in mouse embryonic fibroblasts lacking TrxR1, unless cells are further challenged such as with high glucose concentrations (244). Importantly, although the bulk of Trx1 is kept reduced in cells lacking TrxR1 and without overt signs of oxidative stress in the absence of TrxR1, Nrf2 still becomes robustly activated on knockout of TrxR1 (149, 244, 249, 284). This suggests that loss of TrxR1 activity can signal Nrf2 activation even in the absence of a general oxidative stress. In relation to its links to Nrf2 activation, it may possibly be important that mammalian TrxR1 also catalyzes reduction of various additional proteins beyond Trx1, as well as several redox-active low-molecular-weight compounds and therefore displays a broad functional spectrum (11) (Fig. 4).

The catalytic mechanism of TrxR1 involves an accessible and highly reactive Sec residue

The catalytic mechanism of mammalian TrxR enzymes has been extensively studied, as reviewed elsewhere (16, 129). It involves a transfer of electrons from NADPH *via* the enzyme-bound flavin adeninedinucleotide co-factor to a disulfide motif in the N-terminal domain of one subunit in the homodimeric enzyme. The reduced dithiol motif exchanges these electrons with the selenenylsulfide in the C-terminal active site motif of the other subunit, which in the form of a reduced selenolthiol motif catalyzes reduction of most substrates of the enzyme, such as Trx, or the artificial substrate 5,5′-dithiobis(2-nitrobenzoic) acid (DNTB) (56, 336) (Fig. 5A). Several of its substrates, however, including many quinone compounds, do not require an intact Sec-residue and may be directly reduced *via* the N-terminal Cys59/Cys64 dithiol motif (50, 129, 194, 195).

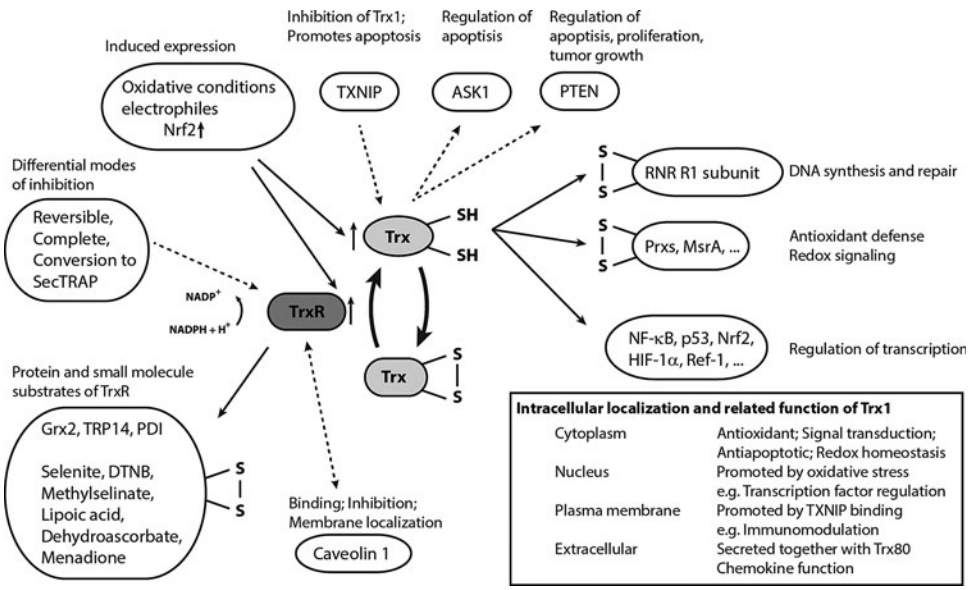

FIG. 4. Substrates and principle functions of the thioredoxin system. This scheme summarizes in greater detail the diverse functions of the Trx system, as well as the possible direct reactions involving TrxR. *Dotted lines* indicate direct protein–protein binding or modification, whereas *solid lines* denote redox activity and thiol–disulfide exchange reactions. Expression of both Trx and TrxR is induced *via* Nrf2 under various stress conditions (Fig. 2). Trx1 is predominantly located in the cytosol, where it provides ribonucleotide reductase (RNR) with electrons and supports the activity of Prxs (255) and Msrs (175). Trx1 can also translocate to the nucleus, where it regulates gene expression by modulating transactivation of various transcription factors, including NFκB, HIF, p53, Nrf2, AP-1, and the glucocorticoid receptor (8, 93, 99, 103, 112, 125, 126, 298). Furthermore, reduced Trx1 directly binds PTEN, a major tumor suppressor that prevents survival signaling by deactivating the PI3K/Akt pathway. Trx1 binding inhibits the phosphatase activity of PTEN and thus promotes cell proliferation and tumor growth while also inhibiting apoptosis (217). Trx1 is also an important regulator of apoptosis signal-regulating kinase 1 (ASK1). In its reduced form, Trx1 binds and thus inhibits ASK1. However, high levels of reactive oxygen species (ROS) promote oxidation of Trx1 and thus ASK1 release, leading to subsequent apoptosis (264). ASK1 release may also be promoted by the Trx1-interacting protein (TXNIP), an endogenous inhibitor of Trx1 that binds to reduced Trx1 and thus competes with ASK1 (327). Interestingly, TXNIP binding also mediates Trx1 translocation to the plasma membrane, which is proposed to enable inflammation in endothelial cells by promoting cell survival and vascular endothelial growth factor signaling during oxidative stress (319). In addition, Trx1 together with a truncated variant (Trx80) can be found in the extracellular environment where it exhibits an oxidoreductase-independent chemokine-like activity (232, 243). TrxR1 also catalyzes the reduction of various additional thiol-proteins and low-molecular-weight compounds and is a prime target for many electrophilic drugs (11). This figure is a modified version of a figure in a review from Lu and Holmgren (197).

With the main enzymatic activities of TrxR1 being dependent on its active site Sec residue (19, 78, 79, 98, 100, 178, 195, 233, 336–338), it is interesting to note that this residue is highly accessible and exposed to solvent in the reduced enzyme (28, 56, 79, 94, 95, 266). This should have importance for the roles of TrxR1 in signaling. Selenol groups have unique biochemical properties, including a high nucleophilicity and very low pK_a, which typically renders Sec several orders of magnitude more reactive in redox reactions compared with the thiol counterpart of Cys (17, 47, 139, 227, 312). For GPxs, the Sec-containing enzymes typically display 3–5 orders of magnitude higher rate constants than Cys-containing counterparts, although this difference cannot be the sole explanation for high catalytic efficiency seen in the selenoproteins (293). In general, Cys residues of proteins are believed to show reaction rates with peroxides at $\approx$1–500 $M^{-1}s^{-1}$, while catalytic Cys residues of Prxs display reaction rates of $\approx 10^5$–10^7 $M^{-1}s^{-1}$; the latter agrees very well with the idea that Prxs may also be the first targets of peroxides during oxidative signaling events (314, 315). In this context, it is interesting to note that electrophilic agents known to activate Nrf2 typically react with low-molecular-weight thiol compounds with second-order rate constants of only about $2–100\times10^3$ $M^{-1}s^{-1}$ (72). Reaction rates with Cys residues in pure Keap-1 were reported to be in the range of 140 $M^{-1}s^{-1}$ (92, 208), which may be compared with electrophiles that react with TrxR1. This includes 1-chloro-2,4-dinitrobenzene that targets the Sec residue of TrxR1 with a second-order rate constant in excess of 200 M^{-1} s^{-1} (18) and the acetaminophen metabolite NAPQI that displayed a second-order rate constant with TrxR1 of 2.37×10^3 M^{-1} min^{-1} (149). The gold-containing Nrf2 activating drug auranofin is also a highly potent TrxR1 inhibitor (106). Its reaction with TrxR1 occurs efficiently at stoichiometric amounts and is difficult to determine experimentally, but the second-order rate constant was found to be in excess of 1.6×10^6 M^{-1} min^{-1} (248). Thus, TrxR1 is exceptionally susceptible to attack by electrophilic compounds. This can lead to diverse effects with regards to TrxR1 function in a cellular context that, as argued here, will include Nrf2 activation. The molecular mechanisms for the relationship between TrxR1 and Nrf2 activation are likely complex and multifaceted, as will be discussed next. It should also be noted that if the effects of TrxR1 targeting are mild, they may be transient because TrxR1 is itself an Nrf2-induced

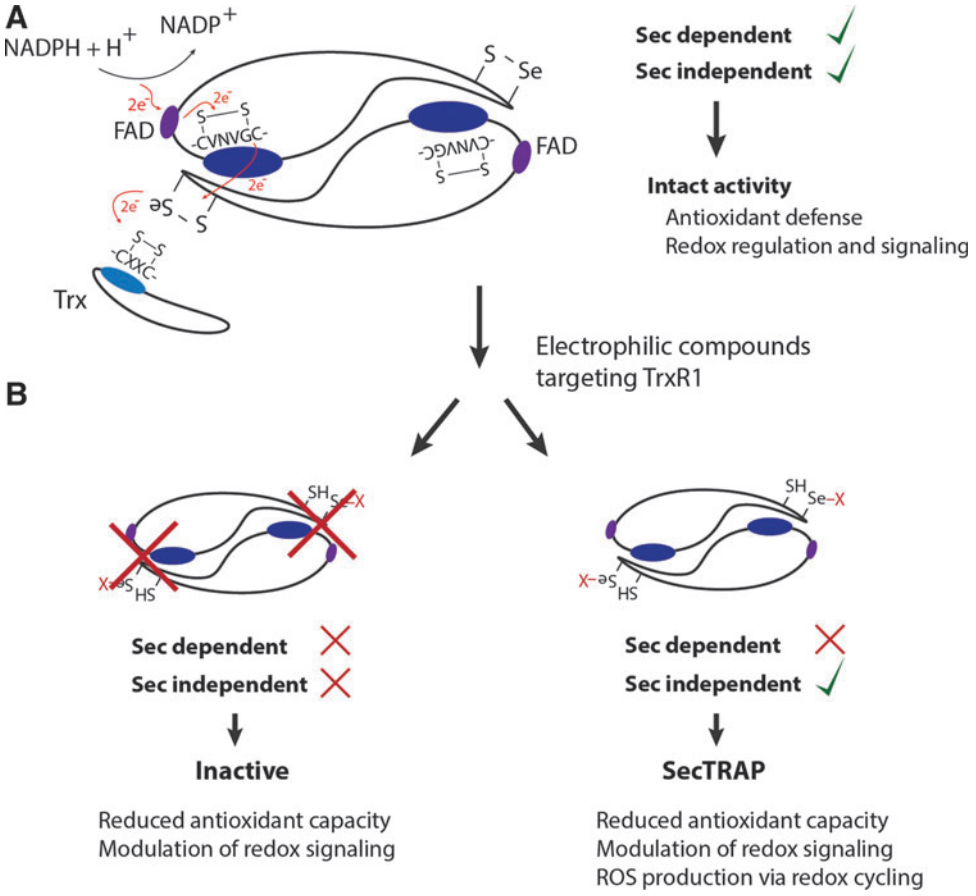

FIG. 5. Principal electron flow in normal catalysis and differential modes of inhibition. **(A)** Scheme of the head-to-tail homodimer confirmation of mammalian TrxR. The principal electron flow during normal catalysis is indicated. **(B)** Targeting *via* electrophilic compounds can either leave the enzyme completely inactive (*left*) or transform it into its pro-oxidant SecTRAP form (*right*), which was shown to promote ROS production *via* redox cycling. See section on SecTRAPs in the main article for more details. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

gene, that is, if diminished, TrxR1 activity leads to increased oxidative stress that does not kill the cells, and new synthesis will commence of native noninactivated TrxR1 (124). Finally, direct inhibition of TrxR1 can also have effects on Nrf2 activation not only as a result of diminished activity but also due to pro-oxidant gain of function in the forms of TrxR1 derivatized by electrophiles, as discussed next.

Formation of SecTRAPs—converting electrophilic challenges to oxidative signals

Some electrophilic compounds that target TrxR1 can yield a pro-oxidant gain of function in the protein, by transforming the enzyme into SecTRAPs (5, 11, 49). This peculiar effect is schematically shown in Figure 5B. Prooxidant properties of SecTRAPS were originally not only characterized in relation to induction of cell death on targeting of TrxR1 with electrophilic compounds in cancer cells but may also relate to the mechanisms of Nrf2 activation in cells that survive an electrophilic insult. SecTRAPs may be formed from the TrxR1 protein by compounds that derivatize the Sec residue of TrxR1, but leave the remaining redox-active moieties of the enzyme intact. Such modified TrxR1 species have lost their ability to catalyze their normal Sec dependent reactions but can still sustain a potent NADPH oxidase activity through redox cycling with certain substrates, such as quinone compounds. As listed in Table 1 and further discussed next, several strong Nrf2 activators target TrxR1 and also have the capacity to transform TrxR1 into SecTRAPs. Recently, it was proposed that an increased access to the N-terminal domain of TrxR1 can be promoted in SecTRAPs by conformational changes caused by modifications of the Sec residue (94, 195). In unmodified TrxR1, this access, and thus electron leakage and NADPH oxidase activity, was proposed to be prevented by efficient electron transfer to the Sec residue (94). When present at high concentrations, SecTRAPs were shown to be able to induce cell death *via* a combination of apoptosis and necrosis, which may thus contribute to the pronounced cytotoxicity of many TrxR inhibitors (5, 135, 202, 203). This may also explain why A549 cells having high TrxR1 levels are more susceptible toward the SecTRAP triggering compound cisplatin (4) than A549 cells having lower levels of the enzyme, as obtained using siRNA treatment (83). A similar phenomenon was illustrated in HCT116 and NIH 3T3 cells treated with thiophosphate and selenite. When given selenite supplementation, these cells increased their expression of TrxR1, which rendered them more sensitive toward cisplatin; whereas thiophosphate, on the other hand, promoted a more resistant phenotype due to expression of a less reactive Sec-to-Cys variants of the enzyme (245). Recently, the natural product shikonin was shown to promote SecTRAP formation from TrxR1 (77), which is also an Nrf2-activating compound (138). Another important TrxR1-targeting compound that induces SecTRAP properties is NAPQI (N-acetyl-p-benzoquinone imine), the hepatotoxic metabolite of acetaminophen, suggesting that the prooxidant properties of SecTRAPs formed in the liver on acetaminophen treatment may contribute to not only Nrf2 responses but also the liver damage seen on acetaminophen overdose (149). Several different compounds having the two combined properties of TrxR1

Table 1. Low-Molecular-Weight Compounds Affecting Both Nrf2 and TrxR1

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Curcuminoid	Curcumin, CAS: 458-37-7	Glutathione S-transferase induction in mice (283) NAD(P)H: quinone reductase (QR) induction in cell culture (9) HO-1 and phase II enzymes expression *via* Nrf2 (23, 223, 268) HO-1 induction *via* ROS, p38 activation, and phosphatase inhibition (212) Inhibits carcinogen-induced expression of phase I CYP450 enzymes and induction of phase II enzymes through Nrf2 activation in mice (liver and lung) (97) Induction of HO-1 *via* Nrf2 in rats (ischemic brain) (325)	Inhibition *in vitro* and in cell culture (38, 89, 154) Modified enzyme showed NADPH oxidase (SecTRAP) activity (38, 89)
Cinnamic acid ester	Caffeic acid phenethyl ester, CAS: 104594-70-9	HO-1 expression *via* Nrf2 (23, 161, 179) Phosphorylation of ERK, which activates Nrf2 independently of Keap1 and leads to HO-1 expression (163)	TrxR1 inhibition (noted as an unpublished observation) (154)
Cinnamic aldehyde	Cinnamaldehyde, CAS: 104-55-2 2-hydroxy-cinnamaldehyde CAS: 3541-42-2 5-Fluoro-2-hydroxycinnamaldehyde 2-benzoyloxycinnamaldehyde	Nrf2 protein induction and ARE activation (58, 186, 210, 318) TrxR1 upregulation (37, 58, 186) HO-1 upregulation (37, 186, 317, 318) NAD(P)H-quinone oxidoreductase upregulation (317) gamma-glutamyl-cysteine synthetase (GCS) upregulation (318) Cinnamaldehyde analogs with 2-Hydroxyl or 2-Benzoyloxy substitutions displayed enhanced Nrf2 activation (58)	Inhibition *in vitro* and in cell culture (58) No GR or Trx inhibition (58) Cinnamaldehyde analogs with a 2-Hydroxyl or 2-Benzoyloxy substitutions displayed enhanced TrxR1 inactivation (58)
Flavonol	Myricetin, CAS: 529-44-2	Nrf2 and HO-1 upregulation (111) Stimulated Nrf2 expression and Nrf2-ARE pathway activation (251) Expression of various phase I and II enzymes as well as antioxidant and stress response proteins (251)	Inhibition *in vitro* and in cell culture (199) Modified enzyme showed NADPH oxidase (SecTRAP) activity (199) Potential target site: Sec-residue (199)
Flavonol	Quercetin, CAS: 117-39-5	Nrf2 and HO-1 upregulation (111) Upregulation of GCS, GPx, GR, and GST—potentially in part *via* modulation of the p38-MAPK signaling pathway (101) Stimulated Nrf2 expression and Nrf2-ARE pathway activation (267) Induces nuclear translocation of Nrf2, gamma-glutamate-cysteine ligase catalytic subunit (GCLC) upregulation, and protection from H2O2 (20)	Inhibition *in vitro* and in cell culture (199) Modified enzyme showed NADPH oxidase (SecTRAP) activity (199) Potential target site: Sec-residue (199)
Flavan-3-ol	Catechin, CAS: 18829-70-4	Upregulation of GPx, GR, GSH, and HO-1 *via* Nrf2 in cell culture and rats (57)	Inhibition *in vitro* and in cell culture (199, 308)
Flavan-3-ol	Epicatechin, CAS: 490-46-0	Induction of phase II and stress response enzymes *via* Nrf2 activation in cell culture and mice (51, 180, 271)	Inhibition *in vitro* and in cell culture (308)

(*continued*)

Table 1. (Continued)

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Flavan-3-ol	Epicatechin gallate, CAS: 1257-08-5	Activation of the MAP kinase pathway and induction of ARE-mediated gene expression (54)	Inhibition *in vitro* and in cell culture (308)
Flavan-3-ol	Epigallocatechin gallate, CAS: 989-51-5	Nrf2 and HO-1 upregulation (111) Activation of the MAP kinase pathway and induction of ARE-mediated gene expression (54) ROS production and expression of oxidative stress-related genes (288) Elevated Nrf2, HO-1, and GSH levels with an increased activity of GPx, SOD, and Catalase, while NFkB and HNE levels were reduced in cisplatin-treated mice (263) HO-1 and bilirubin production *via* Nrf2 activation (335) Prevention of TNFa-induced NFkB activation *via* activation of Nrf2-Keap1 (156) Expression of GPx, HO-1, GCS, GST, and others *via* Nrf2 activation (225)	Inhibition *in vitro* and in cell culture (308) Potential target site: Cys- and Sec-residue (308, 330)
Flavanonol	Taxifolin, CAS:480-18-2	Nrf2 and HO-1 upregulation (111) Activation of ARE and upregulation of NQO1, GSTM1, and TXNRD1, among others (177) *Note*: One report found no Hmox1 upregulation or increase in HO-1 with Taxifolin, but with a derivative of taxifolin (302)	Inhibition *in vitro* and in cell culture (199) Modified enzyme showed NADPH oxidase (SecTRAP) activity (199) Potential target site: Sec-residue (199)
Hydroxyalkenal	4-Hydroxynonenal, CAS: 75899-68-2	Nuclear Nrf2 accumulation in murine macrophages and vascular smooth muscle cells; Nrf2 stimulated expression of A170, HO-1, and PrxI (143) Correlation of elevated 4-HNE levels and the expression of Nrf2 downstream targets in patients with McArdle (167), Alzheimer's (41), and Meniere's disease (40) Induction of TrxR1 and HO-1 *via* activation of the Nrf2 pathway in PC12 cells (55) Induction of HO-1 *via* Nrf2 activation in vascular endothelial cells (145) Induction of GSH and HO-1 in BAEC and rho0 cells (173) Induction of GSH in HUVECs (182)	Inhibition *in vitro* and in cell culture (46, 88, 219) Preferred target site: C-terminal active site (46, 88)

(*continued*)

Table 1. (Continued)

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Prostaglandin	15d-PGJ2, CAS: 87893-55-8	Induction of HO-1 and PrxI in mice (148) Activation of the Nrf2 pathway in HAECs (131) Induction of GSH and HO-1 *via* Nrf2 activation in BAECs (235) Induction of GSH in HUVECs (181, 182) Induced Nrf2 expression and nuclear translocation in Hepa-1c1c7 cells (62)	Inhibition *in vitro* and in cell culture (46, 219) Preferred target site: C-terminal active site (46)
Prostaglandin	Prostaglandin A1, CAS: 14152-28-4	Induction of HO-1 and PrxI in mice (148) Induction of GSH in HUVECs (181) Enhanced levels of HSF-1, HO-1, and HSP90alpha in an ischemic rat model (323) Modulation of HO-1, catalase, GPX1, Mn-SOD-2, and Cu/Zn-SOD-1 on mRNA and protein level in differentiated neuroblastoma (NB) cells (324)	Inhibition *in vitro* and in cell culture (46, 219) Preferred target site: C-terminal active site (46)
Quinone	Juglone, CAS:481-39-0	Induction of stress resistance in *Caenorhabditis elegans via* the activation of FOXO homologue DAF-16 and the Nrf2 homologue SKN-1 (68, 119, 140, 250)	Inhibition *in vitro* and in cell culture (49, 50, 83) Modified enzyme may function as NADPH oxidase (SecTRAP) (49)
Quinone	Naphthazarin, CAS: 475-38-7	Activation of the Nrf2/ARE pathway and induced expression of HO-1 and TrxR1 in primary neuronal and glial cultures (277) Nuclear translocation of Nrf2 in primary cultured astrocytes (60)	Inhibition *in vitro*, claimed in citation of a book chapter *(171)*
Quinone	PQQ Cofactor, CAS: 72909-34-3	Induces nuclear translocation of Nrf2 as well as expression of Nrf2, HO-1, and GCLC in glutamate-injured hippocampal neurons (334) and the cortex of glutamate-injected rats (333) Dietary PQQ modulates the transcription of genes that are important for cellular stress, mitochondriogenesis, cell signaling, and transport in rats (290)	Inhibited Trx1 reduction *in vitro* (321) Stimulated redox cycling with juglone (321)
Polyphenol	Ellagic acid, CAS: 476-66-4	Induced Nrf2 and HO-1 expression in aortas of mice, HAECs (70), as well as the livers of mice (109) Induced nuclear translocation and transcriptional activation of Nrf2 as well as increased expression of HO-1 and SOD in human keratinocyte (HaCaT) cells (133) Nrf2 activation as well as induced expression of Nrf2, HO-1, and NQO1 in human alveolar A549 cells (165)	Inhibition *in vitro* (Trx & DTNB) and in cell culture (278)

(*continued*)

Table 1. (Continued)

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Benzene derivative	Dinitrochlorobenzene (CDNB, DNCB), CAS: 97-00-7	Induced Nrf2 expression and nuclear translocation in Hepa-1c1c7 (59, 62) and THP-1 cells (213) Induction of Nrf2 and the downstream target genes HMOX-1, TXRND1, GCLM, and OSGIN-1 in primary human skin keratinocytes (3) and mtCCs (not OSGIN-1, but NQO1) (192)	Inhibition *in vitro* (Selenite) and in cell culture *(10, 12, 49, 50, 83, 192, 233)* Modified enzyme showed NADPH oxidase (SecTRAP) activity (10, 12, 89, 233) Target site: C-terminal Sec- and Cys-residues (10, 233)
Benzene derivative	Dinitrofluorobenzene (FDNB, DNFB), CAS: 70-34-8	Stabilizes Nrf2, which causes a strong induction of HMOX1 (200) Induced Nrf2 expression in THP-1 cells (213)	Inhibition *in vitro* (Selenite) *(233)* Modified enzyme showed NADPH oxidase (SecTRAP) activity (233) Potential target site: Sec-residue (233)
Isothiocyanate	Sulforaphane, CAS: 4478-93-7	Induction of TrxR1 on mRNA and protein level in MCF-7 cells (304) Induction of nuclear translocation of Nrf2 as well as TrxR1 mRNA in Caco-2 cells (151) Induced GSTM1, GSTP1, NQO1, and HO-1 mRNA expression in the upper airway of human subjects (258) Induction of NQO1 and HO-1 on mRNA and protein level in rat mammary and human breast epithelium (63) Is a potent Nrf2 inducer with a good bioavailability (87, 132) Upregulation of HO-1 and GCL expression, GSH content, ARE-binding, and protection from oxLDL-induced endothelial damage in HUVECs (137) Nuclear translocation and transactivation of Nrf2 as well as induction of γGCS, HO-1, and NQO1 on mRNA and protein level in NIH3T3 cells (84) and mtCCs (also TXNRD1 expression) (192) Induced Nrf2 protein expression, transactivation, and HO-1 expression in HepG2 cells (155)	Inhibition *in vitro* with Trx and DTNB as substrates, and in cell culture (136, 192)
Isothiocyanate	Benzyl isothiocyanate (BITC), CAS: 622-78-6	Induction of nuclear translocation of Nrf2 as well as TrxR1 mRNA in Caco-2 Cells (151) Upregulation of HO-1 and GCL expression, GSH content, ARE-binding, and protection from oxLDL-induced endothelial damage in HUVECs (137) Nuclear translocation and transactivation of Nrf2 as well as induction of γGCS, HO-1, and NQO1 on mRNA and protein level in NIH3T3 cells (84)	Inhibition *in vitro* with Trx and DTNB as substrates, and in cell culture (136)

(*continued*)

TABLE 1. (CONTINUED)

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Isothiocyanate	Phenethyl isothiocyanate (PEITC), CAS: 2257-09-2	Induction of nuclear translocation of Nrf2 as well as TrxR1 mRNA in Caco-2 Cells (151) Upregulation of HO-1 and GCL expression, GSH content, ARE-binding, and protection from oxLDL-induced endothelial damage in HUVECs (137) Nuclear translocation and transactivation of Nrf2 as well as induction of γGCS, HO-1, and NQO1 on mRNA and protein level in NIH3T3 cells (84)	Inhibition *in vitro* with Trx and DTNB as substrates, and in cell culture (136)
Isothiocyanate	Allyl isothiocyanate (AITC), CAS: 57-06-7	Nuclear translocation and transactivation of Nrf2 as well as induction of γGCS, HO-1, and NQO1 on mRNA and protein level in NIH3T3 cells (84) Induced Nrf2 protein expression, transactivation, and HO-1 expression in HepG2 cells (155)	Inhibition *in vitro* with Trx and DTNB as substrates, and in cell culture (136)
Arsenic compound	Arsenite (AsIII), CAS: 7784-46-5	Alterations of Nrf2-mediated gene expression among others (including upregulation of HO-1 and TrxR1) in HBE, 1T1, and HEK001 cells (74) Nrf2 stabilization and nuclear translocation as well as expression of A170, HO-1, and Prx1 in MC3T3-E1 cells (7) Nrf2 activation, nuclear translocation, and transactivation of NQO1 (mRNA and protein level) in Hepa1c1c7 cells and MEFs (117) Nrf2 activation and MRP1 expression in mouse kidneys (166) Nrf2 induction and transactivation as well as HO-1 induction in HEK193T and JAR cells (209)	Inhibition *in vitro* (with DTNB) (189), in cultured rat hepatocytes (190), and *in vivo* (rat liver (170) and rabbit liver (230))
Arsenic compound	Monomethylarsonous acid, CAS: 25400-23-1	Alterations of Nrf2-mediated gene expression among others (including upregulation of HO-1 and TrxR1) in HBE, 1T1, and HEK001 cells (74) Induction of the TrxR1 promoter and of TrxR1 on mRNA and protein level (216)	Inhibition in cultured rat hepatocytes (190) and Hela cells (310)
Arsenic compound	Arsenate (AsV), CAS: 12523-21-6	Nrf2 stabilization and nuclear translocation as well as expression of A170, HO-1, and Prx1 in MC3T3-E1 cells (7)	Low inhibition *in vitro* (DTNB) (189) but high *in vivo* (rat liver (170) and rabbit liver (230)), possibly due to metabolism to AsIII species.
Arsenic compound	Arsenic trioxide, CAS: 1327-53-3	Nrf2 stabilization and nuclear translocation as well as induced HO-1 and NQO1 expression in various multiple myeloma cell lines (220) Nrf2 stabilization and nuclear translocation as well as induced HO-1 expression in SVEC4-10 cells (303) Nrf2 activation and expression of various downstream target genes in a NCI-60 tumor cell line panel (191)	Inhibition *in vitro* (DTNB), in cell culture(196), and *in vivo* (rat liver) (170) Potential target sites: N-terminal and C-terminal active sites (196)

(*continued*)

Table 1. (Continued)

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Arsenic compound	Phenylarsine oxide, CAS: 637-03-6	Nrf2 activation in Hepa1c1c7 cells (118)	Binds the selenolthiol *in vitro* and thus inhibits the enzyme (157)
Gold compound	Auranofin, CAS: 34031-32-8	Nrf2 activation and induced HO-1, GCSh, and NQO1 expression in HepG2, Hela, U937, and Jurkat cells (160) Induced Nrf2 stabilization, nuclear translocation, and transactivation together with expression of HO-1 in THP-1 and MDA-MB 231 cells (164) as well as TXNRD1, NQO1, and GCLm in mtCCs (192) HO-1 induction in mice hepatocytes as well as in primary human and mouse hepatocytes (21)	Inhibition *in vitro* (DTNB) (238, 248, 259), in cell culture (75, 83, 142, 192, 248, 260), and *in vivo* (mice, renal tissue) *(332)*
Gold compound	Aurothioglucose, CAS: 12192-57-3	Nuclear translocation and transactivation; induction of HO-1, NQO1, TrxR1, and GCLM mRNA levels in murine-transformed Clara cells (mtCC) as well as NQO1 in adult murine lungs (192) Nrf2 activation and induced HO-1, GCSh, and NQO1 expression in HepG2 cells (160)	Inhibition *in vitro (123)*, in cell culture (cultured rat hepatocytes (190) and Hela cells (75, 310)), and *in vivo* (mice, all tissues (274) and lungs (192))
Gold compound	Aurothiomalate, CAS: 12244-57-4	Nrf2 activation and induced HO-1, GCSh and NQO1 expression in HepG2 cells (160)	Inhibition *in vitro* (DTNB) (238, 265, 300) and low inhibition in cell culture (260), possibly due to low uptake.
Gold compound	Gold (III) chloride, CAS: 13453-07-1	Nrf2 transactivation in HEK293T, HepG2, MCF7, and A172 cells (273)	Inhibition *in vitro* (DTNB) (238)
Platinum compound	Cisplatin, CAS: 15663-27-1	Nrf2 activation and AKR!C induction on mRNA and protein level in MCF7 cells (weak) (305) Induction of Nrf2 mRNA, protein, nuclear translocation, and transactivation as well as expression of NQO1, HO-1, GCLC, Mrp2, Mrp4, and Mdr1b in the kidneys of treated mice (2) Nrf2 activation in the human AREc32 mammary cell line (122) *Note*: Reduction of nuclear Nrf2 levels and HO-1 in kidneys of treated mice (263) (effect was reversed by EGCG treatment) *Note*: No effect of cisplatin on the Nrf2 system in Caco2 cells (306)	Inhibition *in vitro* (DTNB) and in cell culture (83, 120, 248) and cochlear organotypic cultures (67) Modified enzyme showed NADPH oxidase (SecTRAP) activity (248)
Platinum compound	Nedaplatin, CAS: 95734-82-0	Induced TrxR1, GST, SOD, GR, and GPx activities after 72h (H22 cells in a mouse model) (307) Induction of stress-response genes in the kidneys of mice (297)	Inhibition *in vivo* (H22 cells in a mouse model) (307)

(*continued*)

Table 1. (Continued)

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Platinum compound	Oxaliplatin, CAS: 63121-00-6	Nrf2 stabilization, nuclear translocation, and transactivation as well as HO-1 AK1C and NQO1 induction on protein and mRNA level in Caco2 cells (306) Nrf2- dependent induction of Nqo1 and Gsta1/2 in the small intestine of mice (306)	Inhibition in cell culture (83, 120) and cochlear organotypic cultures (67)
Gadolinium compound	Motexafin gadolinium, CAS: 156436-89-4	Increased HO-1 expression in some cell lines (HF1 & Wil-2), but not in others (Jurkat, DB, DHL4, etc.) (86) Transcriptional induction of stress-response genes in Ramos cells (174)	Inhibition *in vitro* (DTNB & Trx) (115) Modified enzyme may show NADPH oxidase (SecTRAP) activity (115)
Mercury compound	Mercuric chloride, CAS: 7487-94-7	Nrf2 induction on mRNA and protein level in THP-1 cells (309) Nrf2 activation and nuclear translocation as well as induced TrxR expression in mRNA and protein level (31) Nrf2 activation in the human AREc32 mammary cell line (122)	Inhibition *in vitro* (DTNB) and in cell culture (31, 45, 309)
Mercury compound	Monomethylmercury, CAS: 16056-34-1	Weak effects with late Nrf2 activation that results in induction of TrxR expression on mRNA level (31)	Inhibition *in vitro* (DTNB) and in cell culture (31, 45)
Sulfur mustard	2-chloroethyl ethyl sulfide, CAS: 693-07-2	Stimulated expression of antioxidant and stress-response genes (*e.g.*, TrxR, GST, catalase, SOD, GSTA1/2, GSTP1) (29) *Note*: Results unclear with CEES, as the compound may also reduce GSH levels, with strong Nrf2 inducers such as sulforaphane that are typically used to counter the effects of CEES (1).	Inhibition *in vitro* (DTNB) and in cell culture (153) Modified enzyme showed NADPH oxidase (SecTRAP) activity (153) Potential target site: Sec-residue (153)
Nitrosourea	Carmustine (BCNU), CAS: 154-93-8	Nrf2 activation and AKR1C induction on mRNA and protein level in MCF7 cells (weak) (305) Nrf2 activation in the human AREc32 mammary cell line (122)	Inhibition *in vitro* (DTNB) (107, 316) Potential target site: N- and C-terminal active sites (49) Modified enzyme has a reduced capacity to redox cycle with juglone (49)
Acetaminophen metabolite	NAPQI, CAS: 50700-49-7	Induced Nrf2 expression and nuclear translocation in Hepa-1c1c7 cells (59, 62)	Inhibition *in vitro* (DTNB) and *in vivo* (mouse liver) (149, 152, 242) Modified enzyme showed capacity to redox cycle (149, 152) Potential target site: Sec-residue (149, 152)
Schistosomicide	Oltipraz, CAS: 64224-21-1	Olipraz restores nuclear Nrf2 levels as well as HO-1 and SOD levels in mice on a high-fat diet (328) Increased expression of TrxR, Prx, NQO1, and GST μ on hepatic mRNA level in mice (81)	Inhibition *in vitro* (DTNB) (171)

(*continued*)

Table 1. (Continued)

Class of compound	*Compound, CAS nr.*	*Nrf2 related effects*	*TrxR1 related effects*
Statin	Simvastatin, CAS: 79902-63-9	Nrf2 activation and transactivation in MEFs (53)	Lowered TXNRD1 core promoter activity, reduced TrxR1 mRNA levels, and a reduction in cellular TrxR1 activity (HepG2 cells) (80)
		Nrf2 stabilization, translocation and transactivation, as well as expression of HO-1, NQO1, and GCLM on mRNA level in hCASMC cells (206)	*Note*: likely no direct inhibition of TrxR1 by simvastatin
Statin	Fluvastatin, CAS: 93957-54-1	Nrf2 stabilization, translocation and transactivation, as well as expression of HO-1, NQO1, and GCLM on mRNA and protein level in hCASMC cells (206)	Reduction in cellular TrxR1 activity (HepG2 cells) (80)
Unsaturated aldehyde	Acrolein, CAS: 107-02-8	Nrf2 activation and AKR1C induction on mRNA and protein level in MCF7 cells (305) Induction of TrxR mRNA and activity (241) Induction of HO-1 and NQO1 on protein level (253) Nrf2 activation in the human AREc32 mammary cell line (122)	Inhibition *in vitro* (DTNB) and in cell culture (241, 253) Potential target site: Sec-residue (241, 253) Modified enzyme showed enhanced NADPH oxidase (SecTRAP) activity (253)
Nitrogen mustard	Chlorambucil, CAS: 305-03-3	Nrf2 activation and AKR1C induction on mRNA and protein level in MCF7 cells (weak) (305) Nrf2 activation in the human AREc32 mammary cell line (122)	Inhibition *in vitro* (DTNB) (316) and cell culture (281)
Nitrogen mustard	Melphalan, CAS: 148-82-3	Nrf2 activation and AKR1C induction on mRNA and protein level in MCF7 cells (weak) (305)	Inhibition *in vitro* (DTNB) (316)

This table lists a selection of compound classes and specific compounds reported to affect Nrf2 signaling as well as TrxR1 activity, albeit not necessarily in the same study. The effects are summarized here together with citations to original articles reporting these effects. Except for a few uncertain findings as specifically noted in the table, all the reported effects agree with the notion of TrxR1 targeting and inhibition being functionally linked to Nrf2 activation.

targeting, with or without SecTRAP formation, and triggering of Nrf2 activation are listed in Table 1.

Additional effects of targeting Sec residues compared with Cys residues with electrophilic compounds include a greater flexibility of Sec toward substrates, efficient support for one-electron-transfer reactions, high nucleophilicity that leads to fast reaction rates with electrophiles, and increased resistance to inhibition of Sec *via* overoxidation (17, 105, 130, 228, 275). However, for an Sec residue in a selenoprotein to be derivatized by electrophiles, it also has to be solvent exposed and easily accessible, as in the case of TrxR1. The list of identified compounds that target TrxR1 is, indeed, extensive (39, 262) and includes naturally occurring substances such as flavonoids (35, 199), the lipid peroxidation product 4-hydroxy-2-nonenal (HNE) (88), curcumin (89), as well as many synthetic electrophilic compounds, of which some are in clinical use. Prominent examples of the latter include gold compounds such as auranofin (104) or aurothioglucose (274), platinum compounds, including cisplatin (15) and oxaliplatin (316), arsenic oxide (196), nitrosoureas (107), or dinitrohalobenzenes (12). Some TrxR1 inhibitors might react as reversible competitive inhibitors with regards to reduction of Trx, such as the green tea extracts epicatechin-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG) (308). However, such inhibition seems to be the exception. The majority of compounds targeting TrxR1 irreversibly inhibit the enzyme by covalent binding to the thiol/selenol groups of its active sites, as illustrated by the NADPH dependency of inhibition (104, 262, 336). A schematic illustration of the inhibitory pathways of Sec targeting in TrxR1 and the potential formation of SecTRAPs is shown in Figure 5B.

TrxR1 as a Gatekeeper of Nrf2 Activation

Having briefly discussed the functions and mechanisms of TrxR1 and Nrf2, as well as having noted the exceptionally reactive Sec residue of TrxR1, we shall here discuss the published results illustrating that TrxR1 may be viewed as a potent Nrf2 regulator and gatekeeper of Nrf2 activation.

TrxR1 attenuation or depletion leads to robust Nrf2 activation

A number of studies over the past few years have shown that an active Trx system, in particularly TrxR1, is important for counteracting Nrf2 activation (Fig. 6). Links between selenium and selenoprotein status, in general, and the activities of TrxR1 and GPx, in particular, to Nrf2 activation patterns have been discussed elsewhere (34, 224). Such functional links have been further demonstrated in a number of mouse knockout models. It was shown in several studies that a reduction of the total cellular selenoprotein pool either by nutritional selenium deficiency (36, 52, 61, 222, 237) or through a conditional knockout of the $tRNA^{Sec}$ gene (*Trsp*) that is required for Sec insertion into selenoproteins results in robust Nrf2 activation and induction of various phase II and antioxidant enzymes (43, 285). It was also shown that this

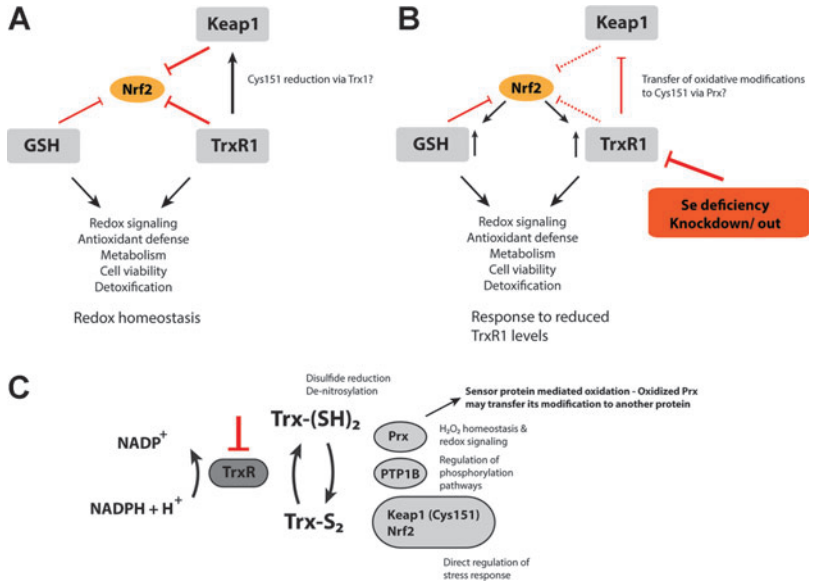

FIG. 6. TrxR1 as an essential negative regulator of Nrf2. (A) Normal, unstressed cells with the Trx- and GSH systems expressed at a basal level maintain redox homeostasis. Both systems act, together with Keap1, as negative regulators of Nrf2 transactivation counteracting oxidative and electrophilic insults. Furthermore, TrxR1 might directly prevent Keap1 inhibition by reducing the critical cysteine 151 *via* Trx1. **(B)** A reduction in the catalytic capacity of TrxR1 either by Se deficiency or due to knockdown or knockout leads to activation of Nrf2. This, in turn, promotes the expression of various enzymes of the Trx and GSH systems, boosting the antioxidant and detoxification capacity of the cell. **(C)** Loss of TrxR1 activity leads to direct interplay with Nrf2 signaling through several different mechanisms. The mechanisms of TrxR1 targeting leading to Nrf2 activation likely involve combinations of a reduced antioxidant capacity, changes in redox signaling-dependent pathways (particularly those mediated by Trx1), and direct regulatory effects on Keap1 and Nrf2. The lack of TrxR1 prevents Trx1 from its reductive functions, which leads to oxidation of Cys151 in Keap1, either directly or potentially *via* a transfer of oxidative equivalents from Prx (257). This latter mechanism would serve as an ''oxidative switch'' in the regulation of Keap1, as not only reduction is diminished but also oxidation is actively promoted. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Nrf2 induction phenotype is particularly dependent on lack of expression of housekeeping selenoproteins such as TrxR1 (42). Indeed, when the liver-expressed $tRNA^{Sec}$ gene (*Trsp*) was mutated in a manner so that only housekeeping, but not stress-related, selenoproteins were affected, the compensatory upregulation of phase II enzymes could be seen, thus indicating that TrxR1 might be the key selenoenzyme in regulation of Nrf2 (269). An interdependent relationship between TrxR1 and Nrf2 was also reported earlier by Trigona *et al.* and Mostert *et al.* (221, 294). A final validation of TrxR1 as a main selenoprotein that regulates Nrf2 was provided with a conditional knockout of only the *Txnrd1* gene in the mouse liver, encoding TrxR1, which gave a robust Nrf2 activation as a response (284).

Several additional cell- and animal-based studies have verified that diminished TrxR1 activity leads to Nrf2 activation. Fourquet *et al.*, for instance, suggested that shRNA-mediated TrxR1 knockdown could promote H_2O_2-mediated oxidation of Keap1 at Cys151 as well as Nrf2 stabilization (92), thus indicating that reduction of the intermolecular disulfide by the Trx system might be an important turn-off signal. Furthermore, Nrf2 stabilization and transactivation was observed in Clara cells on siRNA-mediated TrxR1 knockdown or inhibition (192), or in hepatocytes of liver-specific *Txnrd1* knockout mice as mentioned earlier. Such effects on Nrf2 on TrxR1 targeting have indeed been validated by several groups (44, 242, 284), as well as observed in *Txnrd1* knockout mouse embryonic fibroblasts (207, 284). Importantly, analyzed liver samples of *Txnrd1*-deficient mice did not appear to be oxidatively stressed as they lacked markers such as oxidized thioredoxin, oxidized glutathione, carbonylated proteins, or peroxidated lipids (284). The robust Nrf2 activation in *Txnrd1*-deficient conditions can, thus, not be directly explained by a general increase of overall oxidative stress. Immunostaining of tissue sections, Western blot analyses, as well as chromatin immunoprecipitation (ChIP) analyses were also employed to demonstrate that Nrf2 protein levels increased and that Nrf2 relocated to ARE sites in target genes as a result of *Txnrd1* knockout (284). Collectively, these observations are strongly suggestive of direct functional links between TrxR1 and Nrf2, which cannot be explained by increased oxidative stress on loss of cellular TrxR1 activity alone.

What makes TrxR1 a unique gatekeeper of Nrf2 activation?

The mechanisms behind Nrf2 activation when promoted by diminished TrxR1 activities are likely to be complex, with a combination of factors contributing differently depending on cellular context and redox state. One major consequence of lower TrxR1 activity is likely a lower capacity in Trx1-mediated processes, such as disulfide reduction and denitrosylation (198, 270). This will lead to a propagation of effects through various downstream events. One example would be effects on regulation of phosphorylation pathways, also potentially regulating Nrf2, by Trx1- or thioredoxin-related protein of 14 kDa (TRP14)-catalyzed reduction of key phosphatases such as protein-tyrosine phosphatase 1B (PTP1B) (65, 96). Trx1, furthermore, prevents Nrf2 activation directly *via* the reduction of Cys151 in Keap1 (92) or of Cys506 in the NLS region of Nrf2, which promotes the nuclear export (112), as discussed earlier. In addition, lower capacity in Trx1-mediated reduction of Prxs will affect H_2O_2 homeostasis and thus redox signaling pathways (255) (Fig. 3). However, it is also likely that additional specific links exist between the TrxR1-dependent reductive pathways and Nrf2 activation.

Peroxiredoxins are likely to be Trx-dependent sensors of oxidative stress

An interesting possibility would be direct oxidation of Keap1, Nrf2, or other relevant protein thiols in the Keap1/Nrf2 system, by Prxs in accordance with the sensor protein-mediated oxidation model. This model proposes that regulated oxidation of target proteins can occur *via* thiol exchange reactions using specific sensor proteins that are especially reactive with H_2O_2; in that context, peroxidases have been suggested as suitable candidates (257, 315). Recently, it was shown by Du *et al.* that Prx1 can also transmit oxidative equivalents to nonactive site cysteine residues of oxidized Trx1-S_2, thus generating overoxidized Trx1-S_4 forms that can be directly reduced by GSH or *via* Grx1, but not by TrxR1 (76). This mechanism was proposed to serve as a temporary shut-off signal to modulate Trx1-mediated redox signaling processes, such as activation of Nrf2, and will likely be promoted by diminished TrxR1 activity. Another example of a sensor protein-mediated oxidation in mammals involves transfer of the oxidation state of Prx4 to protein disulfide isomerase, which, in turn, promotes disulfide formation in target proteins (289). The best known case for this type of signaling is, however, seen in the yeast transcription factor Yap1 that is oxidized *via* the glutathione peroxidase-like protein GPx3 (69) and the thioredoxin peroxidase Tsa1 (236). Here, the sulfenic acid of the oxidized peroxidase forms an interdisulfide bond with Yap1, which on subsequent exchange with a second Cys residue in Yap1 generates an intramolecular disulfide and recycles GPx3 or Tsa1. Whether similar direct oxidative processes occur between a dedicated redox protein of mammals with Keap1 and Nrf2 is not yet clear. However, as discussed here, it is clear that TrxR1 targeting leads to robust Nrf2 activation. The molecular mechanism(s) leading to this activation have not yet been fully characterized. Importantly, TrxR1 is expressed at low submicromolar concentration in cells (16) but is, nonetheless, easily targeted at its Sec residue by electrophilic compounds or cellular stresses, such as nitrosylation or excessive oxidation, because of its unique reactivity as discussed earlier. Targeting of TrxR1, with its many downstream consequences, can thus easily translate into a robust Nrf2 response. There may also be differences in TrxR1 dependence for Nrf2 activation on oxidative stress as opposed to challenges with electrophiles. Indeed, almost every electrophilic compound that was found to activate Nrf2 also inhibits TrxR1, as discussed next in greater detail.

Electrophilic compounds typically modulate both TrxR1 and Nrf2

The same classes of compounds that inhibit TrxR1 (39) have been shown to also activate the Keap1-Nrf2 pathway (204), which may not be surprising as the mechanism for Keap1 inactivation involves modification of thiol groups, while TrxR1 is inhibited by similar targeting of its highly

reactive Sec residue. However, we propose that simultaneous targeting of TrxR1 with Nrf2 activation is not only circumstantial. Some prominent examples of compounds that both inhibit TrxR1 and activate Nrf2 include Michael acceptors such as curcumin (73), flavonoids (204) such as quercetin (267), polyphenols such as ellagic acid (161), Isothiocyanates (226) including sulforaphane (134), or metal compounds such as auranofin (164). It has been suggested that a main mechanism by which most of these compounds activate Nrf2 is *via* covalent modification of reactive Cys residues in Keap1 (169, 299), as also discussed earlier. However, based on the chemistry of known TrxR1 inhibitors and Nrf2-activating compounds, the highly reactive Sec residue in TrxR1, and the effects of TrxR1 depletion on Nrf2 activation, we here propose that TrxR1 may be a major target of most electrophilic Nrf2 activators. We also propose that inhibition of TrxR1 may be a major component of the mechanism(s) leading to Nrf2 activation (Fig. 7A). It stands to reason that Nrf2 activation *via* TrxR1 inhibition might be an event that could precede modification of Keap1, as a result of the high reactivity of the Sec residue in TrxR1. However, this has yet to be experimentally proven. Targeting events involving Keap1 and TrxR1 are likely to be fast and may also only be observed within an initial phase of exposure to electrophiles, as, in most cases, expression of novel nonmodified TrxR1 molecules will be induced by Nrf2 activation. It should also be noted that neither Trx1 nor TrxR1 has yet been identified as being major Nrf2 regulating proteins in systems-wide ''Nrf2 interactome'' studies (239, 296), although both Trx1 (gene *TXN*) and TrxR1 (*TXNRD1*) are found in the larger Nrf2 interactome if searched for in a database of Nrf2 network proteins, covering as much as 7,891 proteins in total (http://nrf2.elte.hu). It is, however, clear that a large number of compounds that inhibit TrxR1 also activate Nrf2. The combined effects of these electrophiles have not always been recognized in the same studies, but here we have compiled a list of original studies showing that electrophilic compounds that inhibit TrxR1 also activate Nrf2 (Table 1). We argue here that the dual effects of these compounds should not only be a coincidence but also a reflection of a causal relationship between TrxR1 targeting and Nrf2 activation.

The potential role of SecTRAP formation in Nrf2 activation by electrophiles

The combination of a diminished catalytic activity together with a gain of NADPH oxidase activity in the form of SecTRAPs enables TrxR1 to transform a minor electrophilic insult into an oxidative signal that might further promote activation of Nrf2 (Fig. 7B). Such events may precede random modification of Cys residues by electrophiles, thus enabling a faster stress response to boost the Nrf2-driven detoxification system. The prooxidant capacity of SecTRAPs also suggests that Nrf2 activation may be promoted before a loss of total TrxR1 activity in a cell, that is, it may be sufficient for a lower fraction of TrxR1 molecules to be converted to SecTRAPs for the signaling process to be initiated. An interesting mechanistic possibility is furthermore the additive effects of prooxidant SecTRAP formation with Prx1-mediated over-oxidation of Trx1, as discussed earlier. Such combined effects should be synergistic and could serve as potent mechanisms of signal transduction in the background of all cellular reductive pathways, thus being able to translate smaller electrophilic insults into efficient and appropriate patterns of Nrf2 activation.

Concluding Remarks

In this review, we have discussed many published results from cell and animal studies that collectively suggest that targeting of TrxR1 by oxidative stress, electrophiles, selenium deficiency, or genetic manipulation typically leads to robust Nrf2 activation. We propose that compelling evidence suggests that such targeting of TrxR1 should posit the enzyme to be a key regulator of Nrf2 activation, which is likely to play a central role in redox homeostasis, defense against oxidative stress, and regulation of redox signaling pathways. Furthermore, we have reasoned that this functional role of TrxR1 is linked to its central position in the Trx system, in

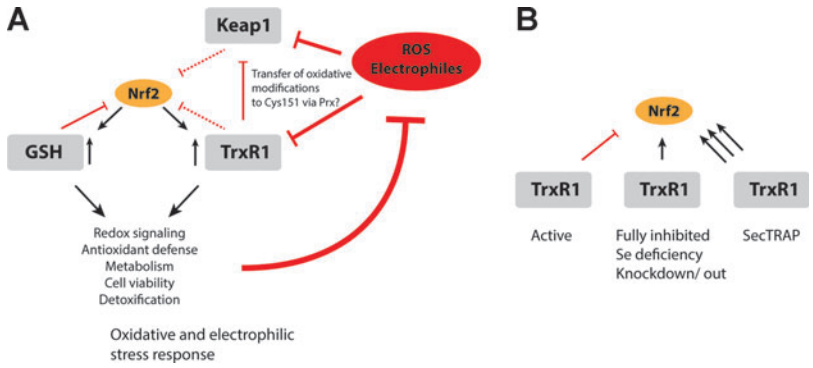

FIG. 7. Different modes of redox modulation and their effects on Nrf2 activation. (A) Both Keap1 and TrxR1 are subject to inhibition by electrophiles and ROS, which, in turn, leads to activation of Nrf2. The interplay between Keap1, TrxR1, and Nrf2 is constituted of a complex web of interactions. Here, it is proposed that TrxR1 targeting is part of the mechanisms regulating Nrf2 activation, as summarized in the figure and discussed further in the text. **(B)** Active TrxR1 is proposed to act as a gatekeeper to prevent Nrf2 activation. Direct inactivation of the enzyme promotes Nrf2 activation, as seen in *Txnrd1* knockout or knockdown models, or under selenium deprivation conditions. In addition, the formation of SecTRAPs can promote a very strong Nrf2 activation, by means of ROS production from converted TrxR1 protein having NADPH oxidase activity, in addition to a loss of reductive capacity. These mechanisms together identify TrxR1 as a potent regulator of Nrf2 activation. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

combination with the reliance of the enzyme on a solvent exposed, easily accessible, and highly reactive Sec residue. It is also likely that conversion of TrxR1 to prooxidant SecTRAPs on its targeting by certain classes of inhibitors should further contribute to the highly potent activation of Nrf2 seen with many electrophilic compounds. As discussed earlier and also indicated in Figure 7B, the activities of TrxR1 may regulate Nrf2 activation through three separate mechanisms:

1. An intact Trx system, with fully active TrxR1, is likely to counteract Nrf2 activation through several mechanisms. One mechanism would be through the antioxidant properties of the complete Trx system, including antioxidant properties of Trx1-dependent enzymes such as Prxs. Another possible mechanism could be through effects of keeping Keap1 in a reduced state, provided that Keap1 would be a substrate of Trx1 or another TrxR1-dependent oxidoreductase.
2. Loss of TrxR1 activity may activate Nrf2 solely through diminished antioxidant or reductive capacity of the Trx system. However, as discussed earlier, analyses of tissues or cells from knockout mouse models that lack TrxR1 have not displayed overt signs of oxidative stress or Trx1 oxidation. This suggests that loss of TrxR1 activity may also directly signal Nrf2 activation through some yet unrecognized mechanism of action. It is clear, however, that complete loss of TrxR1 activity triggers robust Nrf2 activation.
3. Inhibition of TrxR1 by electrophilic agents is a highly efficient event, for most compounds due to targeting of the reactive and accessible Sec residue in the enzyme. Such derivatization typically leads to Nrf2 activation, which may be partly be due to loss of TrxR1 capacity. However, the Nrf2 activation on treatment with electrophilic compounds can, in this case, also be due to formation of SecTRAPs, which due to a gain of function in the inhibited TrxR1 enzyme exaggerates such insults and converts the electrophilic challenge to an oxidative signal.

The evident links between TrxR1 targeting by electrophilic compounds to Nrf2 activation may also, possibly, be part of the longstanding question of the evolutionary pressure that resulted in an Sec-depending TrxR1 in mammals. Because *Drosophila melanogaster* is an animal that relies on a TrxR1 orthologue with Cys in place of Sec, this makes that enzyme much less susceptible to inhibition by electrophiles (105). In that context, it is interesting to note that the Nrf2/Keap1 orthologous system of *D. melanogaster* (286) seems to be even more important for xenobiotic responses than in mammals and, moreover, regulated by a smaller number of converging signals than found in mammals (218). It furthermore should be noted that although the fly relies on a TrxR1 orthologue without an excessively reactive Sec residue (105), the enzyme instead has the dual roles of keeping the Trx as well as the GSH system active, because the fly lacks GR (159). Thus, it may be possible that a biochemically less reactive TrxR1 orthologue in the fly might still signal to the corresponding Nrf2/Keap1 system, because its targeting would impair both of the two main reductive systems of the fly. In mammals, however, the Sec reactivity of TrxR1 may therefore have evolved to keep it sensitive to electrophiles, while GR in mammals is not and can therefore maintain the GSH pool in a reduced state even if TrxR1 becomes inhibited. However, these are only mere speculations as seed for thought for future research projects. The notion of TrxR1 targeting being intimately linked to Nrf2 activation in the fly has to our knowledge not yet been scrutinized, and in that particular case, it is not clear why the Cys-dependent TrxR1 would be more susceptible to electrophiles than the Cys residues in the Keap1 orthologue itself. We, therefore, do not currently know whether the tight functional links between TrxR1 targeting and Nrf2 activation is unique for animals that express a TrxR1-containing Sec, which would mainly include mammals (234), or whether the close functional links between TrxR1 and Nrf2 are more evolutionarily conserved and thus found also beyond the mammalian class of animals. If future studies would show that only Sec-containing TrxR1 is closely linked to Nrf2 activation, that aspect would indeed help explain why the enzyme has evolved to be a selenoprotein. The evolutionary pressure of retaining Sec-containing TrxR once it has been acquired seems to be high (47, 48), but the reason for this has remained a topic that is unanswered and still debated, although it has been suggested that the higher chemical reactivity of Sec in TrxR1 compared with Cys variants should be part of the answer (17, 105, 129, 195).

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Author Contributions

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Abbreviations Used

ARE/EpRE = antioxidant/electrophile responsive element
Crm1 = chromosome region maintenance 1; exportin
DNTB = 5,5′-dithiobis(2-nitrobenzoic) acid
ECG = epicatechin-gallate
EGCG (-) = -epigallocatechin-3-gallate
GPx = glutathione peroxidase
GR = glutathione reductase
Grx = glutaredoxin
GSH = glutathione
GSTs = glutathione-S-transferases
HNE = 4-hydroxy-2-nonenal
Keap1 = Kelch-like ECH-associated protein 1
NAPQI = N-acetyl-p-benzoquinone imine
NES = nuclear export signal
NLS = nuclear localization signal
Nrf2 = Nuclear factor (erythroid-derived 2)-like 2
PKC = protein kinase C
Prx = peroxiredoxin
PTP1B = protein-tyrosine phosphatase 1B
ROS = reactive oxygen species
Sec = Selenocysteine
SecTRAPs = Selenium-compromised thioredoxin reductase-derived apoptotic proteins
TRP14 = thioredoxin-related protein of 14 kDa
Trx = thioredoxin
TrxR = thioredoxin reductase