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*Review Article*

# REACTIVE OXYGEN SPECIES, ANTIOXIDANTS, AND THE MAMMALIAN THIOREDOXIN SYSTEM

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**Abstract**—Reactive oxygen species (ROS) are known mediators of intracellular signaling cascades. Excessive production of ROS may, however, lead to oxidative stress, loss of cell function, and ultimately apoptosis or necrosis. A balance between oxidant and antioxidant intracellular systems is hence vital for cell function, regulation, and adaptation to diverse growth conditions. Thioredoxin reductase (TrxR) in conjunction with thioredoxin (Trx) is a ubiquitous oxidoreductase system with antioxidant and redox regulatory roles. In mammals, extracellular forms of Trx also have cytokine-like effects. Mammalian TrxR has a highly reactive active site selenocysteine residue resulting in a profound reductive capacity, reducing several substrates in addition to Trx. Due to the reactivity of TrxR, the enzyme is inhibited by many clinically used electrophilic compounds including nitrosoureas, aurothioglucose, platinum compounds, and retinoic acid derivatives. The properties of TrxR in combination with the functions of Trx position this system at the core of cellular thiol redox control and antioxidant defense. In this review, we focus on the reactions of the Trx system with ROS molecules and different cellular antioxidant enzymes. We summarize the TrxR-catalyzed regeneration of several antioxidant compounds, including ascorbic acid (vitamin C), selenium-containing substances, lipoic acid, and ubiquinone (Q10). We also discuss the general cellular effects of TrxR inhibition. Dinitrohalobenzenes constitute a unique class of immunostimulatory TrxR inhibitors and we consider the immunomodulatory effects of dinitrohalobenzene compounds in view of their reactions with the Trx system. © 2001 Elsevier Science Inc.

**Keywords**—Thioredoxin, Thioredoxin reductase, Redox regulation, Inflammation, Oxidative stress, Antioxidant, Dinitrohalobenzene, Reactive oxygen species, Free radicals

## INTRODUCTION: OXIDANTS AND ANTIOXIDANTS

Reactive oxygen species (ROS) are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function, or excessive quantities, the state called oxidative stress. As the term ROS implies, intracellular production of those oxygen intermediates threatens the integrity of various biomolecules including proteins [1], lipids as well as lipoproteins involved in atherosclerosis [2], and DNA [3]. Oxidative stress is also proposed to be involved in the process of aging both by inducing damage to mitochondrial DNA and by other mechanisms [4,5].

Vital beneficial physiological cellular use of ROS is now being demonstrated in different areas including intracellular signaling and redox regulation. Nitric oxide (NO) was identified as a signaling molecule already in 1987 [6,7] and is now well known as a regulator of transcription factor activities and other determinants of gene expression [8]. Hydrogen peroxide and superoxide have similar intracellular effects [9–13]. Several cytokines, growth factors, hormones, and neurotransmitters use ROS as secondary messengers in the intracellular signal transduction [14]. The mechanism for altered transcription factor control could be either via decreased binding to promoter regions via oxidative damage to the DNA [15] or more direct by redox regulation of transcription factor activation [16–22] and/or altered DNA-

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Table 1. The Major ROS Molecules and Their Metabolism

| ROS molecule | Main sources | Enzymatic defense systems | Product(s) |
|---|---|---|---|
| Superoxide ($O_2^{\bullet -}$) | 'Leakage' of electrons from the electron transport chain<br>Activated phagocytes<br>Xanthine oxidase<br>Flavoenzymes | Superoxide dismutase (SOD)<br>Superoxide reductase (in some bacteria) | $H_2O_2 + O_2$<br>$H_2O_2$ |
| Hydrogen peroxide ($H_2O_2$) | From $O_2^{\bullet -}$ via superoxide dismutase (SOD)<br>NADPH-oxidase (neutrophils)<br>Glucose oxidase<br>Xanthine oxidase | Glutathione peroxidase<br>Catalases<br>Peroxiredoxins (Prx) | $H_2O + GSSG$<br>$H_2O + O_2$<br>$H_2O$ |
| Hydroxyl radical ($^{\bullet}OH$) | From $O_2^{\bullet -}$ and $H_2O_2$ via transition metals (Fe or Cu) | | |
| Nitric oxide (NO) | Nitric oxide synthases | Glutathione/TrxR | GSNO |

binding due to redox-induced modification of the transcription factor protein [22–25].

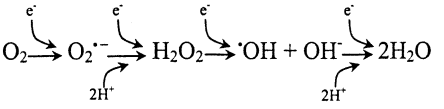
Here we shall briefly review the biochemical characteristics of the most common ROS molecules, their beneficial and adverse effects, and the different cellular systems that regulate their intracellular levels. We also provide references to other review articles describing these issues in further detail. We will then focus on the many oxidant and antioxidant reactions now known to involve, fully or in part, the mammalian thioredoxin system. This selenium-dependent enzymatic system has in recent years emerged at the very core of antioxidant regeneration and regulation of intracellular ROS levels.

## REACTIVE OXYGEN SPECIES (ROS)

ROS include a number of chemically reactive molecules derived from oxygen [26–29]. Some of those molecules are extremely reactive, such as the hydroxyl radical, while some are less reactive (superoxide and hydrogen peroxide). Intracellular free radicals, i.e., free, low molecular weight molecules with an unpaired electron, are often ROS and vice versa and the two terms are therefore commonly used as equivalents. Free radicals and ROS can readily react with most biomolecules, starting a chain reaction of free radical formation. In order to stop this chain reaction, a newly formed radical must either react with another free radical, eliminating the unpaired electrons, or react with a free radical scavenger—a chain-breaking or primary antioxidant.

In Table 1, the most common intracellular forms of ROS are listed together with their main cellular sources of production and the relevant enzymatic antioxidant systems scavenging these ROS molecules.

The step-wise reduction of molecular oxygen via 1-electron transfers, producing and also connecting the ROS molecules listed in Table 1, can be summarized as follows:




(Reaction 1)

On the intracellular level, the ROS formation and metabolism can be summarized as shown in Fig. 1. The three major forms of ROS shall now shortly be individually discussed in terms of pathways of formation and their cellular effects.

### *Superoxide ($O_2^{\bullet -}$)*

The superoxide anion created from molecular oxygen by the addition of an electron is, in spite of being a free radical, not highly reactive. It lacks the ability to penetrate lipid membranes and is therefore enclosed in the compartment where it was produced. The formation of superoxide takes place spontaneously, especially in the electron-rich aerobic environment in vicinity of the inner mitochondrial membrane with the respiratory chain (Fig. 1). Superoxide (as well as hydrogen peroxide) is also produced endogenously by flavoenzymes, e.g., xanthine oxidase [30] activated in ischemia-reperfusion [31]. Other superoxide-producing enzymes are lipoxygenase and cyclooxygenase [32,33]. The NADPH-dependent oxidase of phagocytic cells, a membrane-associated enzyme complex, constitutes an example of deliberate high-level $O_2^{\bullet -}$ production (see below). Enzymes similar to components of this complex are also present in nonphagocytic cells where their functions should be connected to signaling [14]. Two molecules of superoxide rapidly dismutate to hydrogen peroxide and molecular oxygen and this reaction is further accelerated by SOD (see below).

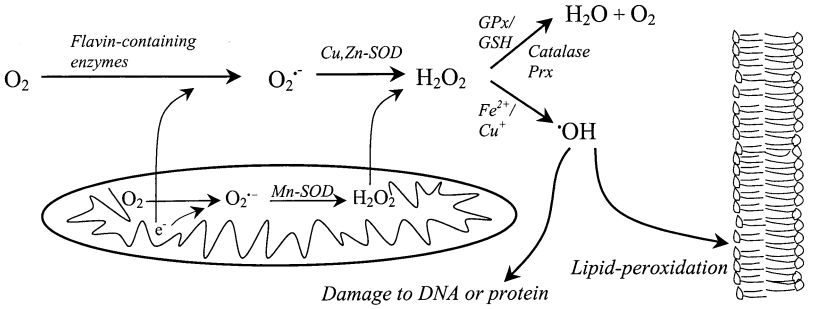



Fig. 1. Simplified nonstoichiometric scheme of oxidative and antioxidative systems in cells. Superoxide is produced in significant amounts intracellularly, both in the cytosol via flavin-containing enzymes, and in mitochondria, mainly due to escape of electrons from the respiratory chain. Two molecules of superoxide rapidly dismutate, either spontaneously or via superoxide dismutases to dioxygen and hydrogen peroxide, the latter permitting flux of ROS between cellular compartments. Hydrogen peroxide can be enzymatically metabolized to dioxygen and water by a number of different enzyme systems or converted to the hydroxyl radical, which is extremely reactive, via a chemical reaction catalyzed by transition metals.

*Hydrogen peroxide ($H_2O_2$)*

$H_2O_2$ is not a free radical but is nonetheless highly important much because of its ability to penetrate biological membranes. It plays a radical forming role as an intermediate in the production of more reactive ROS molecules including HOCl (hypochlorous acid) (Reaction 2) by the action of myeloperoxidase, an enzyme present in the phagosomes of neutrophils [34] and, most importantly, formation of $^{\bullet}OH$ (Reaction 3) via oxidation of transition metals.

$$H^+ + Cl^- + H_2O_2 \rightarrow HOCl + H_2O$$

(Reaction 2)

Another important function of $H_2O_2$ is carried out in its role as an intracellular signaling molecule [11,12] (see also below).

$H_2O_2$ once produced by the above mentioned mechanisms is removed by at least three antioxidant enzyme systems, namely catalases, glutathione peroxidases, and peroxiredoxins [35–37], as summarized in Fig. 1 and further discussed below.

*Hydroxyl radical ($^{\bullet}OH$)*

Due to its strong reactivity with biomolecules, $^{\bullet}OH$ is probably capable of doing more damage to biological systems than any other ROS [27,38]. The radical is formed from hydrogen peroxide in a reaction catalyzed by metal ions ($Fe^{2+}$ or $Cu^+$), often bound in complex with different proteins or other molecules. This is known as the Fenton reaction:

$$H_2O_2 + Cu^+/Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Cu^{2+}/Fe^{3+}$$

(Reaction 3)

Superoxide also plays an important role in connection with Reaction 3 by recycling the metal ions:

$$Cu^{2+}/Fe^{3+} + O_2^{\bullet -} \rightarrow Cu^+/Fe^{2+} + O_2$$

(Reaction 4)

The sum of Reactions 3 and 4 is the Haber-Weiss reaction; transition metals thus play an important role in the formation of hydroxyl radicals [28,38]. Transition metals may be released from proteins such as ferritin [39] and the [4Fe-4S] center of different dehydrases by reactions with $O_2^{\bullet -}$. This mechanism, specific for living cells, has been called the in vivo Haber-Weiss reaction [40].

*Nitric oxide (NO)*

Nitric oxide represents an odd member of the free radical family and is similar to $O_2^{\bullet -}$ in several aspects in that it does not readily react with most biomolecules despite its unpaired electron. On the other hand it easily reacts with other free radicals (e.g., peroxyl and alkyl radicals), generating mainly less reactive molecules, thus in fact functioning as a free radical scavenger; NO has, for example, been shown to inhibit lipid peroxidation in cell membranes [41,42]. Though, if $O_2^{\bullet -}$ is produced in large amounts in parallel with NO, the two react with each other to give $OONO^-$ (peroxynitrite), which is highly cytotoxic [43]. Peroxynitrite may react directly with diverse biomolecules in one- or two-electron reactions, readily react with $CO_2$ to form highly reactive nitroso peroxocarboxylate ($ONOOCO_2^-$), or protonated as peroxonitrous acid (ONOOH) undergo homolysis to form either $^{\bullet}OH$ and $^{\bullet}NO_2$ or rearrange to nitrate ($NO_3$). The individual rates of these different reactions of peroxynitrite will depend upon the pH, temperature, and type of compounds present in the surrounding milieu

[44]. Peroxynitrite, directly or via its reaction products, may oxidize LDL, release copper ions by destroying ceruloplasmin, and generally attack tyrosine residues in different proteins, as observed in many inflammatory diseases [45].

NO is synthesized enzymatically from L-arginine by NO synthase (NOS) [46–48] (Reaction 5).

$$\text{L-arginine} + O_2 + \text{NADPH} \rightarrow \text{L-citrulline} + \text{NO} + \text{NADP}^+ \quad \text{(Reaction 5)}$$

The complex enzymatic catalysis of NOS involves the transfer of electrons from NADPH, via the flavins FAD and FMN in the carboxy-terminal reductase domain, to the heme in the amino-terminal oxygenase domain, where the substrate L-arginine is oxidized to L-citrulline and NO. There are three main isoforms of the enzyme, i.e., neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS), which differ in their respective expression and activities [46–49].

In physiologic concentrations NO functions mainly as an intracellular messenger stimulating guanylate cyclase and protein kinases, thereby relaxing smooth muscle in blood vessels, among other effects. NO has the ability to cross cell membranes and can thereby also transmit signals to other cells [50]. When produced in larger amounts, as is the case when iNOS is induced by endotoxin and IFN-$\gamma$, NO becomes an important factor in redox control of cellular function [51]. Nitrosylation of proteins is known to regulate, for example, enzymatic activity [52,53]. NOS itself is regulated in this fashion and the inhibition is reversed by the thioredoxin system [54,55].

Excessive production of NO is counteracted by its conjugation with glutathione that results in the *S*-nitrosoglutathione adduct (GSNO). GSNO can in its turn be cleaved directly by mammalian TrxR or by the complete thioredoxin system, which again liberates GSH and NO under consumption of NADPH [56]. GSNO has also been shown to inhibit TrxR indicating a possible regulatory mechanism [56].

The total effect of NO on the redox status of cells is clearly multifaceted, though, in conclusion, in many respects it seems to function as an antioxidant and not an oxidant [28].

## PHYSIOLOGICAL FUNCTIONS OF ROS

### *Defense against infection*

When phagocytes are activated, they produce ROS in amounts enough to kill intruding bacteria [57]. ROS are in this system produced by the NADPH oxidase complex that converts $O_2$ to $O_2^{\bullet -}$ [58,59]. Superoxide is then reduced in the phagosome by SOD to $H_2O_2$ that can be further converted to HOCl by myeloperoxidase [60] (Reaction 2). Hypochlorous acid may then spontaneously form $^{\bullet}OH$ by Reactions 6 or 7. Note that Reaction 7 is analogous to the Fenton reaction (Reaction 3) but with HOCl taking the place of $H_2O_2$.

$$HOCl + O_2^{\bullet -} \rightarrow {}^{\bullet}OH + O_2 + Cl^- \quad \text{(Reaction 6)}$$

$$HOCl + Cu^+/Fe^{2+} \rightarrow {}^{\bullet}OH + Cl^- + Cu^{2+}/Fe^{3+} \quad \text{(Reaction 7)}$$

The two highly reactive ROS molecules thereby formed in phagosomes (HOCl and $^{\bullet}OH$) are highly toxic to bacteria ingested by the phagocyte and carry the direct antimicrobial effects of ROS. The hypochlorous acid produced in the myeloperoxidase reaction is also an important part of the antimicrobial defense by destruction of the DNA anchoring at the bacterial membrane, resulting in cessation of DNA replication [61].

### *Redox regulation of transcription factor activity*

ROS can directly affect the conformation and/or activities of all sulfhydryl-containing molecules, such as proteins or GSH, by oxidation of their thiol moiety. This type of redox regulation affects many proteins important in signal transduction and carcinogenesis such as protein kinase C, $Ca^{2+}$-ATPase, collagenase, and tyrosine kinases [17], among many other enzymes and membrane receptors; see [62] for further examples and references. For several transcription factors, ROS function as physiological mediators of transcription control [16–18,20, 21]. Well-known examples of redox-sensitive transcription factors are Nuclear Factor-$\kappa$B (NF-$\kappa$B) and Activator Protein-1 (AP-1) [9,17,18,22,63–68].

In the case of AP-1, a dimer of gene products from the *Jun* and *Fos* proto-oncogene families, expression is induced by several pro-oxidant conditions [69–72], including different types of irradiation [73]. However, the activity of AP-1 can also be regulated post-translationally. For example, DNA-binding of AP-1 increases upon reduction of critical cysteine residues and decreases when those residues are oxidized [23]. By addition of antioxidants or replacement of the critical Cys-residues with Ser, DNA-binding of AP-1 increases, whereas it conversely decreases after addition of oxidants. The increased activity in Cys to Ser mutant *Jun* or *Fos* [23] as well as after antioxidant treatment suggests a conformational change with increased activity in the reduced state. This resembles the association of *E. coli* Trx with T7 DNA Polymerase, which is dependent on the conformation of reduced Trx without dependence of oxidoreduc-

tase activity [74]. A similar mechanism is found in regulation of apoptosis signal-regulating kinase 1 (ASK1) by human Trx [75]; see further below. Regulation of AP-1 DNA-binding is further mediated by Ref-1, originally identified as an endonuclease. Ref-1 increases DNA-binding of AP-1 together with reduced thioredoxin [76]. AP-1 binding sites are present in the promoter regions of a large number of genes including collagenase, cytokines (including IL-3, IL-8, IL-9, TNF-$\alpha$, and IFN-$\gamma$), adhesion molecules related to atherosclerotic plaque formation, and factors involved in cell proliferation and tumor promotion [17–20,63, 77].

NF-$\kappa$B is regulated by redox mechanisms quite differently from AP-1, although ROS can trigger both of these transcription factors. Activation of NF-$\kappa$B occurs in the cytosol by degradation of the inhibitory protein I-$\kappa$B, a degradation which is induced by oxidative conditions and prevented by most antioxidant compounds [17,19]. Targets for NF-$\kappa$B include the genes corresponding to cytokines (including IL-1, IL-6, IL-8, and TNF$\alpha$), iNOS, adhesion molecules (E-selectin and VCAM-1), as well as MHC class I and II antigens, the IL-2 receptor, and different acute-phase proteins [64]. NF-$\kappa$B is also involved in the expression of HIV proteins by binding to the enhancer region of the long terminal repeat in HIV-1 [78]. Specific relations between the thioredoxin system and NF-$\kappa$B are considered below.

In *E. coli*, OxyR is a transcription factor specifically activated by $H_2O_2$ and responsible for initiating transcription of a number of antioxidant genes [79]. The structural basis of OxyR activation was recently revealed, showing how $H_2O_2$ induces a large structural remodeling of OxyR upon the rapid $H_2O_2$-catalyzed formation of an intramolecular disulfide in the protein [80]. In yeast, Yap1p is a transcription factor that in analogy to OxyR is required for the $H_2O_2$-dependent activation of many antioxidant genes, and the redox regulation of Yap1p is related to the yeast thioredoxin system [81]. It is possible that OxyR and/or YaP1p have yet undiscovered homologues in mammals, in addition to the many known mammalian redox-regulated transcription factors.

## ROS AS CAUSE OF OXIDATIVE DAMAGE

ROS are, due to their high reactivity, prone to cause damage, and are thereby also potentially toxic, mutagenic, or carcinogenic. The targets for ROS damage include all major groups of biomolecules, summarized as follows.

### *DNA*

ROS have been shown to be mutagenic [3,37], an effect that should be derived from chemical modification of DNA. A number of alterations (e.g., cleavage of DNA, DNA-protein cross links, oxidation of purines, etc.) are due to reactions with ROS, especially $^{\bullet}OH$. If the DNA-repair systems are not able to immediately regenerate intact DNA, a mutation will result from erroneous base pairing during replication. This mechanism may partly explain the high prevalence of cancer in individuals exposed to oxidative stress [3,37]. The fact that apoptosis in some cases is mediated by ROS [9] may in part be due to ROS-derived damage to DNA, but is also related to increased mitochondrial permeability, released cytochrome C, increased intracellular $Ca^{2+}$, and other effects [82].

The concept of ROS as an important factor in cellular and whole organism aging due to damage to mitochondrial DNA is an interesting theory [83–86]. This concept was recently challenged by a study based on extensive gene array analysis suggesting errors in the mitotic machinery and possibly in arachidonic acid metabolism as being important determinants for the aging process [87]. However, accumulating data do indicate that ROS contribute to aging [5] and, conversely, that superoxide dismutase and catalase mimetics may prolong the life span, at least that of *C. elegans* [88].

### *Lipids*

Lipid peroxidation is probably the most explored area of research when it comes to ROS; see [2,89] and references therein. Polyunsaturated fatty acids are, because of their multiple double bonds, excellent targets for free radical attacks. Such oxidation is also essential for the generation of atherosclerotic plaques [90,91]. The mechanism for plaque formation involves oxidation of low-density lipoproteins (LDL), uptake of those particles by phagocytes in the subendothelial space via their scavenger receptor, and finally, accumulation of these phagocytic cells in the subendothelial space, where they stimulate formation of atherosclerotic plaques.

Cardiovascular disease with plaque formation constitutes a large part of the total burden of disease, at least in western countries. Therefore, prevention or decrease of lipid peroxidation is of significant medical importance.

### *Proteins*

ROS have been shown to react with several amino acid residues in vitro, generating anything from modified and less active enzymes to denatured, nonfunctioning proteins [86,92]. Among the most susceptible amino

acids are sulfur- (or selenium)-containing residues. General antioxidant systems such as Trx, Grx, or GSH, or specific systems, such as methionine sulfoxide reductases to which Trx serves as electron donor [93], all uphold the protection of proteins from such modification.

## CELLULAR ANTIOXIDANT ENZYMES IN ADDITION TO THE THIOREDOXIN SYSTEM

Before discussing the thioredoxin system in further detail, being the true focus of this review, we shall briefly discuss the other cellular antioxidant enzyme systems serving to protect cells and organisms from the lethal effects of excessive ROS formation. The cellular antioxidant systems can be divided into two major groups, enzymatic and nonenzymatic. Low-molecular weight antioxidant compounds will be discussed later in relation to the thioredoxin system. Here, we will briefly review the antioxidant enzyme systems including SOD (E.C. 1.15.1.1), SOR, catalase (1.11.1.6), Prx, GPx (1.11.1.9), and other GSH-related systems; known reactions of these enzymes with the Trx system will also be pointed out.

### *Superoxide dismutases (SOD)*

Superoxide dismutases (SOD) were the first genuine ROS-metabolizing enzymes discovered [94]. In eukaryotic cells, $O_2^{\bullet -}$ can be metabolized to hydrogen peroxide by two metal-containing SOD isoenzymes, an 80-kDa tetrameric Mn-SOD present in mitochondria, and the cytosolic 32-kDa dimeric Cu/Zn-SOD (see Fig. 1). Bacteria contain both Mn-SOD and Fe-SOD [95] and in some cases also Cu,Zn-SOD [40]. In the reaction catalyzed by SOD, two molecules of superoxide form hydrogen peroxide and molecular oxygen and are thereby a source of cellular hydrogen peroxide (Reaction 8). The reaction catalyzed by SOD is extremely efficient, limited in essence only by diffusion.

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{(Reaction 8)}$$

In mitochondria, superoxide is formed in relatively high concentrations due to the leakage of electrons from the respiratory chain. The strictly mitochondrial Mn-SOD [96] is obviously essential or near essential since no inherited diseases have been found in which Mn-SOD is deficient, and knockout mice lacking Mn-SOD die soon after birth or suffer severe neurodegeneration [97]. Expression of Mn-SOD is, in contrast to Cu,Zn-SOD, induced by oxidative stress and, interestingly, also by Trx [98]. Cytosolic Cu,Zn-SOD [33,94] seems less important than Mn-SOD, and transgenic animals lacking this enzyme are able to adapt so that the phenotype appears normal [99]. Still, the ultimately lethal neurodegenerative disease amyotrophic lateral sclerosis (ALS) can be caused by mutations in the gene for cytosolic SOD [100,101], which increases the peroxidase activity of the enzyme [102]. A larger Cu,Zn-SOD distinct from the cytosolic form can also be found extracellularly [103].

### *Superoxide reductases (SOR)*

Recently, a novel type of superoxide scavenging enzyme was discovered, catalyzing the direct reduction of superoxide:

$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2 \quad \text{(Reaction 9)}$$

These enzymes, the superoxide reductases (SOR), contain iron and have so far only been found in anaerobic sulfate-reducing bacteria, here called desulfoferrodoxin [104], the anaerobic archaea *Pyrococcus furiosus*, [105] or the microaerophilic bacterium causing venereal syphilis, *Treponema pallidum* [106]. The latter organism lacks SOD and seems to rely solely on SOR for its elimination of superoxide. Yet there is no evidence for a mammalian SOR.

### *Catalases*

Catalases of many organisms are mainly heme-containing enzymes [107]. The predominant subcellular localization in mammalian cells is in peroxisomes, where catalase catalyze the dismutation of hydrogen peroxide to water and molecular oxygen:

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad \text{(Reaction 10)}$$

Catalase also has functions in detoxifying different substrates, e.g., phenols and alcohols, via coupled reduction of hydrogen peroxide:

$$H_2O_2 + R'H_2 \rightarrow R' + 2H_2O \quad \text{(Reaction 11)}$$

One antioxidative role of catalase is to lower the risk of hydroxyl radical formation from $H_2O_2$ via the Fenton-reaction catalyzed by Cu or Fe ions [26,28] (Reaction 3). Catalase binds NADPH, which protects the enzyme from inactivation and increases its efficiency [108,109]. In glucose 6-phosphate dehydrogenase deficiency, a disease in which NADPH is lowered in mature red blood cells, it is possible that the hemolysis is derived from inhibition of catalase rather than from less GR/GPx activity as previously believed (see [109] and references therein).

*Peroxiredoxins (Prx)*

Peroxiredoxins (Prx; thioredoxin peroxidases) are recently discovered enzymes capable of directly reducing peroxides, e.g., hydrogen peroxide and different alkyl hydroperoxides [35,36,110]. Thioredoxin (in mammalian cells), trypanothione (in trypanosomatids) or AhpF, a component of the alkyl hydroperoxide reductase system in *Salmonella typhimurium*, regenerate oxidized Prx formed in the catalytic cycle [111]. In the mitochondria of mammalian cells the mitochondrial thioredoxin system is probably a specific reductant of Prx [112]. Peroxiredoxins have been shown to inhibit apoptosis induced by p53 [113] and by hydrogen peroxide on a level upstream of bcl-2 [114]. As of today, at least 13 mammalian peroxiredoxins are known [35,36,115].

*Glutathione peroxidases (GPx)*

There are at least four different GPx in mammals (GPx1–4), all of them containing selenocysteine [116]. GPx1 and GPx4 (or phospholipid hydroperoxide GPx) are both cytosolic enzymes abundant in most tissues [37,117]. GPx4 has recently been found to have dual functions in sperm cells by being enzymatically active in spermatids but insoluble and working as a structural protein in mature spermatozoa [118]. Remarkably, by use of an alternative first exon, a GPx4 variant differing at the N-terminus is also specific for sperm nuclei where it participates in the spermatid chromatin condensation [119]. Thereby GPx4 has at least three completely different functions during sperm maturation. GPx2 (gastrointestinal GPx) and GPx3 (plasma GPx) are mainly expressed in the gastrointestinal tract and kidney, respectively [37,120]. Interestingly, GPx3 may be catalytically regenerated by the Trx system [121] and thioredoxin reductase seems to have an expression pattern in kidney highly reminiscent to that of GPx3 [122]. All glutathione peroxidases may catalyze the reduction of $H_2O_2$ using glutathione as substrate. They can also reduce other peroxides (e.g., lipid peroxides in cell membranes) to alcohols (Reaction 12).

$$ROOH + 2GSH \rightarrow ROH + GSSG + H_2O$$

(Reaction 12)

The catalytic mechanism proposed for reduction of hydroperoxides by GPx [123] involves oxidation of the active site selenolate ($Se^-$) to selenenic acid (SeOH). Upon addition of one molecule of GSH, the selenenic acid is transformed to a selenenylsulfide adduct with glutathione (Se-SG), which can be regenerated to the active selenolate and glutathione disulfide (GSSG) by addition of a second molecule of GSH. Thus, in the reaction, two molecules of GSH are oxidized to GSSG that subsequently can be reduced by GR, the major mammalian GSSG-reducing enzyme.

Some data has indicated that GPx should be of high antioxidant importance under physiological conditions [124] while others place the enzymes as important only at events of oxidative stress [125]. It is therefore interesting that knockout mice lacking GPx1 develop quite normally and even cope with exposure to oxidative stress in the form of hyperoxia [126]. Thus the function of GPx isoenzymes in antioxidant defense is still unclear, but the kinetic properties and widespread distribution still imply that they constitute major contributors to the total protection against oxidative damage.

*Other glutathione-related systems*

Glutathione (GSH) is the most abundant intracellular thiol-based antioxidant, prevalent in millimolar concentrations in all living aerobic cells. Its function is mainly as a sulfhydryl buffer, but GSH also serves to detoxify compounds either via conjugation reactions catalyzed by glutathione S-transferases (2.5.1.18) [127,128] or directly, as is the case with hydrogen peroxide in the GPx catalyzed reaction (Reaction 12). Oxidized glutathione (GSSG) is reduced by the NADPH-dependent flavoenzyme glutathione reductase (GR) (1.6.4.2) [129]. GSSG may also be efficiently reduced by thioredoxins of *E. coli*, *Plasmodium falciparum*, and human origin [130]. Moreover, *Drosophila melanogaster* lacks GR and solely uses a (selenium-independent) thioredoxin system for GSSG reduction [131].

Another class of proteins intimately related to GSH is the glutaredoxins (Grx), with functions overlapping those of thioredoxins. A major qualitative difference between Grx and Trx is that Grx can be reduced by GSH and is capable of reducing GSH mixed protein disulfides formed at oxidative stress, which should play an important role in the total cellular antioxidant defense [132]. Figure 2 summarizes the different GSH-related antioxidant systems.

*Low molecular weight antioxidant compounds*

A large number of low molecular weight compounds are considered to be antioxidants of biological importance, including vitamins C and E, different selenium compounds, lipoic acid, and ubiquinones. Those are all interacting with the mammalian thioredoxin system and will therefore be dealt with in conjunction with the review below of substrates of this enzyme system.

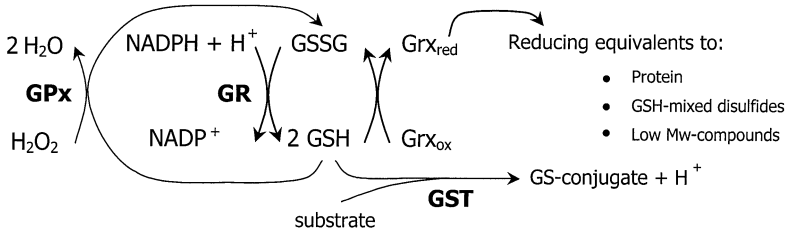



Fig. 2. Schematic summary of the major glutathione-associated antioxidant systems. Hydrogen peroxide is reduced by glutathione peroxidases (GPx) by oxidation of two molecules of glutathione (GSH) forming glutathione disulfide (GSSG) that subsequently can be reduced by glutathione reductase (GR) under consumption of NADPH. Glutathione also reduces glutaredoxins (Grx) that in their turn reduce various substrates. Specific for glutaredoxins is the reduction of glutathione mixed disulfides such as glutathionylated proteins. Glutathione S-transferases (GST) catalyze the conjugation of glutathione with other molecules, thereby functioning as an intermediate step in the detoxification of miscellaneous toxic substances.

## THE MAMMALIAN THIOREDOXIN SYSTEM

The thioredoxin system consists of the two antioxidant oxidoreductase enzymes thioredoxin (Trx) and thioredoxin reductase (TrxR) (1.6.4.5). The latter catalyzes the reduction of the active site disulfide in Trx using NADPH and, among other functions, reduced Trx is a general protein disulfide reductant (Fig. 3). Both TrxR and Trx have several antioxidant and other functions that will be described as follows.

### *Thioredoxin (Trx)*

Thioredoxins from both prokaryotic and mammalian organisms are reduced by mammalian TrxR, while *E. coli* TrxR has a narrow substrate specificity and only catalyzes the reduction of bacterial Trx [133–137]. Even though mammalian TrxR shows a wide substrate specificity, it should be emphasized that the physiological effects of cellular TrxR activity are closely linked to the different cellular functions of Trx although TrxR may also carry functions not related to those of Trx. The detailed and diverse functions and properties of Trx are not within the major scope of this review and will therefore only be presented in brief. For further information on activities of human Trx in relation to regulation of the immune system, see [19,138–145], whereas the Trx-dependent redox regulation of transcription factor activities is described in [17–20,24,76,77,146].

Thioredoxins are proteins with oxidoreductase activity and are ubiquitous in both mammalian and prokaryotic cells [135]. So far, three distinct variants of human Trx encoded by separate genes have been cloned and characterized in some detail. Most studied is the gene for the classical 12-kDa thioredoxin (Trx-1) [147]. The Trx-2 isoenzyme is located in mitochondria and includes a 60 amino acid N-terminal mitochondrial translocation signal [148]. The third, SpTrx, is a Trx variant highly expressed in spermatozoa [149]. Trx of all organisms including the human Trx-1, Trx-2, and SpTrx contain a conserved -Cys-Gly-Pro-Cys- active site, essential for the function as a general and potent protein disulfide oxidoreductase [133,136]. Specific protein disulfide targets for reduction by Trx are ribonucleotide reductase (RR) [150], PDI [151], and several transcription factors including p53, NF-$\kappa$B, and AP-1, the latter via Ref-1 (see below). As mentioned above, Trx is also a specific electron donor for many peroxiredoxins [35,36,110], highly important for the reduction of peroxides. Furthermore, reduced Trx prevents apoptosis via an inhibitory binding to apoptosis signal-regulating kinase 1 (ASK-1), whereas this binding is lost when Trx is oxidized [75].

A number of clinical conditions involve Trx [152, 153]. For example, Trx protects the lens from oxidative stress and cataract formation [154], inhibits reperfusion-induced arrhythmias in a rat heart model [155] indicating a protective effect in acute ischaemic heart disease and, extracellularly, increased plasma levels of Trx have been found in a number of diseases including hepatocellular carcinoma [156], AIDS [141,157], Sjögrens Syndrome [158], and rheumatoid arthritis [159].

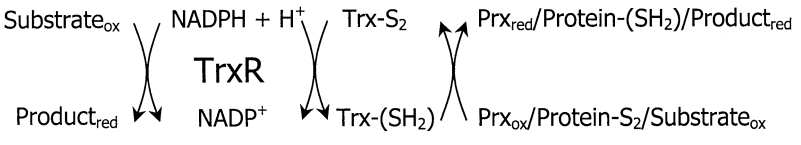



Fig. 3. Enzymatic reactions of the thioredoxin system. Thioredoxin reductase (TrxR) reduces the active site disulfide in thioredoxin (Trx) and several other substrates directly under consumption of NADPH. Reduced thioredoxin is highly efficient in reducing disulfides in proteins and peptides, including peroxiredoxins (Prx) and glutathione disulfide (GSSG).

*Trx and the immune system*

The immunologic functions of Trx were first investigated in conjunction with adult T-cell leukemia (ATL), a form of cancer now known to be caused by the retrovirus HTLV-I; see [19] and references therein. Initially, the name 'ATL-derived factor' (ADF) was coined for a protein found to induce the overexpression of CD25 (IL-2R$\alpha$), a protein overexpressed on the surface of all ATL leukemic cells. ADF was subsequently shown to be human Trx [140,160]. It is now established that other cells of the immune system [139,161] and various tumor cells [144,162] also secrete Trx, which occurs by a leaderless pathway [161].

Extracellular Trx exhibits autocrine growth factor-like effects [163] as well as co-cytokine activities stimulating the recruitment and proliferation of lymphocytes and the growth of tumor cells [140,162,164–166]. The synthesis of other cytokines (TNF, IL-1, IL-2, IL-6, and IL-8) is also regulated by Trx and, extracellularly, the protein induces chemotaxis of neutrophilic granulocytes, monocytes, and T-cells [166,167]. Peripheral blood cells secrete Trx upon conditions resembling bacterial infection such as stimulation with endotoxin or IFN [145]. Elevated levels of Trx inhibit the innate bacterial defense system that includes neutrophil migration [141], an effect that could possibly be beneficial in situations with excessive immune response. In patients with rheumatoid arthritis, Trx levels have been found to be increased both at the sites of inflammation and in serum [159].

Lately, a truncated form of Trx, Trx80, which is probably identical to eosinophilic cytotoxicity-enhancing factor [138], was found to be secreted from monocytes and T-cells [138,142,145]. This protein is not a substrate for TrxR and is not capable of reducing protein disulfides but has remarkable immunostimulatory capacities. Trx80 especially stimulates monocytes to proliferate and to express diverse surface antigens and these activated monocytes in turn stimulate T-cells to proliferate [142, 143]. The T-cell response to Trx80 is typical for the Th1-type and is mediated by IL-12 [143].

These findings briefly reviewed here show that extracellular Trx and Trx80 are important parts of the inflammatory response by acting as cytokines and co-cytokines. This should have relevance for understanding the effects in mammals upon inhibition of TrxR, as discussed last in this review.

*Trx and redox regulation of transcription factor activity*

Expression of Trx is induced by oxidative stress [147, 168] and this induction should most likely involve, among other factors, the antioxidant responsive element present in the Trx promoter [169]. An increased Trx expression should aim at an increased reduction of intracellular proteins and other biomolecules as part of the antioxidant defense. Antioxidant effects of Trx can also be mediated more indirectly. In general, Trx modulates the signal transduction properties of ROS; (see [17,19, 170] and references therein) by striving towards reduction of the intracellular disulfides induced by ROS and by lowering the levels of ROS directly. In the case of transcription factors, Trx and other antioxidants have been found to enhance AP-1 activation via their antioxidative potential [16,25]. Interestingly, oxidants may also activate AP-1 [17,20,72]. NF-$\kappa$B activation, on the other hand, is clearly inhibited by reduced Trx in the cytosol and by other antioxidants in a dose-dependent manner [77]. Trx in micromolar amounts, which is within the physiological range, is enough to inhibit NF-$\kappa$B activity [77], illustrating a high specificity rather than a general antioxidant effect of Trx.

When cells are exposed to oxidative stress in the form of radiation [24,171] or ferric nitrilotriacetate [172], Trx is translocated to the nucleus. Upon intranuclear translocation it could exert regulatory functions, e.g., regulate the DNA-binding of NF-$\kappa$B and AP-1 [76,171]. Upon nuclear translocation of NF-$\kappa$B, Trx in fact enhances DNA-binding of NF-$\kappa$B by reduction of an essential cysteine residue [146]. Thereby Trx stimulates the DNA-binding activity of NF-$\kappa$B once translocation to the nucleus has occurred. Trx also increases the DNA-binding activity of AP-1 via Ref-1 in the nucleus [76,171].

The interplay between pro- and antioxidants in the activation of AP-1 and NF-$\kappa$B and the cytosolic versus nuclear activities of Trx are intriguing and not fully understood. Trx certainly has several different effects in this transcription factor regulation. A model involving some of the possible mechanisms implicated will be presented in the last section of this review.

## MAMMALIAN THIOREDOXIN REDUCTASE

Thioredoxin reductase (TrxR) isoenzymes are NADPH-dependent homodimer oxidoreductases with one FAD per subunit that reduce the active site disulfide in oxidized Trx [133,134,152,173]. The molecular weight is 55-65 kDa per subunit in mammalian TrxR to be compared with 35 kDa in TrxR of prokaryotes, plants, or yeast [132–135,152,173]. With the first characterizations of TrxR purified from bovine tissue in 1977 [174] it became clear that mammalian TrxR also differs in catalytic properties from that of lower organisms. Many diverse and unique reactions have since been found to be catalyzed by mammalian TrxR, as reviewed below. In 1996, Thressa Stadtman and coworkers showed that TrxR purified from human lung adenocarcinoma cells or

T-cells were selenoproteins [175,176]. This finding certainly spurred further interest in the mammalian thioredoxin system and it is now clear that all mammalian TrxR isoenzymes contain selenium (see below). It had previously been known that several selenium compounds are metabolized by mammalian TrxR and with the TrxR protein in itself containing selenium, the enzyme is hence intimately linked with the functions of selenium in biological systems.

TrxR contains selenium in the form of selenocysteine (Sec), the naturally occuring selenium analogue of cysteine being recognized as the 21st amino acid [177,178]. In TrxR, this residue is located at the very C-terminus of the protein, within a tetrapeptide motif (-Gly-Cys-Sec-Gly-COOH) being conserved between species and present in all TrxR isoenzymes [175,179–182]. Sec is cotranslationally inserted into selenoproteins by an intricate synthesis machinery, which differs between prokaryotic and eukaryotic organisms. The interested reader is referred to other reviews on selenium incorporation [177,178,183], being out of the scope for this article. Here we shall merely conclude that the Sec in TrxR is essential for its enzymatic activities [179,184–186] and that TrxR activity is severely impaired in selenium-deficient animals [187] or human cell lines [188], a fact that may explain some of the effects of selenium deficiency. Addition of selenium to human cancer cells has been reported to increase the TrxR activity several-fold [189] as opposed to other selenium-containing enzymes including GPx. TrxR sems to be the only selenoprotein in *C. elegans* expressed at significant amounts [190], which implies that TrxR is particularly important in some higher organisms.

Mammalian TrxR reduces not only the disulfide in oxidized Trx, but also some other protein disulfides or a wide spectrum of oxidized low molecular weight compounds (see below) [137]. The catalytic mechanism of TrxR explaining the wide substrate specificity and involving the Sec residue has in recent years been unraveled in quite some detail. Mammalian TrxR is closely related to other mammalian flavoprotein disulfide oxidoreductases such as glutathione reductase or lipoamide dehydrogenase in both primary structure [179], crystal structure [191], and general features in the catalytic mechanism [192]. However, TrxR carries a C-terminal elongation containing the Sec residue, which is not present in lipoamide dehydrogenase or glutathione reductase [179] and which explains many of the uniques features of this enzyme. In the first part (the reductive half-reaction) of the reduction of mammalian TrxR by NADPH it is similar to that of GR, LipDH, and mercuric ion reductase [129,186,192]. This reduction involves transfer of electrons from NADPH via the enzyme-bound FAD to an active site disulfide formed by the cysteine residues in positions 59 and 64 in the N-terminal active site domain sequence being identical to that of GR (CVNVGC), thereby forming a dithiol. Electrons are then transferred from the dithiol of one subunit in the dimeric enzyme to a selenenylsulfide formed by the Cys-Sec pair in the C-terminal sequence of the other subunit [186]. This transfer of electrons to the other subunit in TrxR can be seen as taking the place of the reduction of GSSG by GR [186]. The reduced C-terminal selenolthiol motif can finally reduce any of the many substrates of mammalian TrxR1.

The active center of TrxR seems to be generally protected by the oxidation to a selenenylsulfide since when the enzyme is oxidized, it is resistant to carboxypeptidase or trypsin treatment [179,193], and to derivatization with electrophilic compounds [194]. This indicates a conformational change of the active site upon reduction/oxidation that could be related to the capacity of the enzyme to reduce such a large variety of substrates; in the reduced state, the active site is evidently easily accessible.

### *Mammalian TrxR isoforms*

Isoforms of TrxR have been found in mitochondria [195,196] and in the testis [180], with all isoenzymes having the same general domain organization and Sec-containing C-terminal active site as the previously characterized cytosolic enzyme. However, three different 5′ untranslated regions of cytosolic murine TrxR mRNA and an alternative upstream ATG codon have also been found [122,182], the importance of which is yet not clear. Interestingly, the testis isoenzyme of TrxR is capable of reducing both Trx and GSSG directly [181] and was recently named ‘thioredoxin and glutathione reductase’ (TGR). This TGR enzyme is elongated in its N-terminal end by an additional monothiol glutaredoxin domain that is proposed to receive electrons from the C-terminal Sec-containing center [181]. TGR is another illustration of the overlapping capacities between the Trx and GSH systems that are further pointed out by the fact that GR is absent in *Drosophila melanogaster*, where reduction of GSSG is instead carried out solely by the *Drosophila* Trx-system [131]. Most of the characteristics of TrxR described in this review are derived from studies on the mammalian cytosolic isozyme, TrxR1.

## WIDE SUBSTRATE SPECIFICITY OF TrxR

A striking characteristic of mammalian TrxR is the wide substrate specificity and direct reduction of different protein disulfides, many low molecular weight disulfide compounds, and nondisulfide compounds. This wide

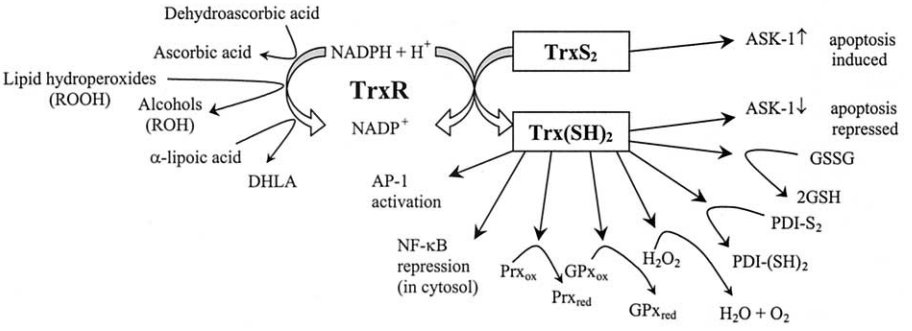



Fig. 4. Graphic summary of major physiological functions of the mammalian thioredoxin system. Reduction of hydrogen peroxide and regeneration of the active site in glutathione peroxidase are reactions that can also be directly catalyzed by TrxR. Thioredoxin-dependent reduction of hydrogen peroxide is highly efficient in conjunction with the activities of peroxiredoxins and to some extent glutathione peroxidases, although the latter also function without involvement of the thioredoxin system (via GSH and GR).

specificity is most likely explained by the unique and easily accessible Sec-containing redox active site, although this has not been directly experimentally proven for all of the substrates. It should be noted that GSSG and insulin are not substrates for TrxR1, though they can both be efficiently reduced by Trx [130,174].

Some of the different substrates in addition to Trx will be briefly described here, followed by a more extensive description of the important antioxidant compounds that can be recycled by mammalian TrxR.

Peroxides, including lipid hydroperoxides and hydrogen peroxide can directly be reduced by TrxR [185,197]. By this mechanism TrxR could function as an alternative enzymatic pathway for the detoxification of lipid hydroperoxides, otherwise mainly managed by GPx4. The high apparent $K_m$ of TrxR for hydrogen peroxide (2.5 mM), however, implies a role for TrxR only when levels are elevated [185]. Also, $H_2O_2$ has in cell experiments been shown to be able to transiently inactivate TrxR, possibly by induction of the selenenylsulfide motif or by further oxidation of the Sec residue [180]. This has been proposed to give TrxR the role of a cellular redox sensor [180]. The inactivation of TrxR by hydrogen peroxide cannot be seen in vitro [185] and these cellular reactions need to be studied further.

5,5′-dithio-bis(2-nitrobenzoic acid) (Ellman's reagent; DTNB) is an unspecific thiol reagent consisting of two nitrobenzene ring-structures connected by a disulfide. It is used for the general determination of free thiols, and after prior reduction also of disulfides, in proteins and peptides [198]. Mammalian TrxR specifically reduces the disulfide in DTNB by NADPH and the reaction is easily spectrophotometrically measurable due to the high absorbance of the $TNB^-$ anion being released [174]. This is the basis for the well-established DTNB-assay for mammalian TrxR activity [174,199,200].

Protein Disulfide-isomerases (PDI) constitute a family of proteins in the endoplasmic reticulum important for post-translational folding and processing of cellular proteins. Structurally, PDI proteins consist of one or several thioredoxin domains, with some domains also having redox active dithiol-disulfide motifs [201]. The Trx-like active site motifs present in PDI are reduced by mammalian TrxR, both directly and via reduced Trx [151,202].

Nitroblue Tetrazolium (NBT) has long been used to localize so called 'NADPH diaphorase activity' in cells and tissues and the enzyme proposed to catalyze the NADPH-dependent reduction of NBT is NO-synthase [203]. However, mammalian TrxR is also capable of reducing NBT under consumption of NADPH (Nordberg, Arnér, and Holmgren, unpublished observation) displaying Michaelis-Menten kinetics with $K_m$ of 2 mM and turnover number of around 16/min. The turnover number for NO-synthase with NBT is 39/min [204]. It therefore remains to be determined how much of the diaphorase activity detected in cells or tissues actually could be attributed to TrxR.

As additional substrates, TrxR also reduces various additional low molecular weight compounds including the antibacterial polypeptide NK-lysin, which is not active in its reduced form [205], L-cystine [199], alloxan [206], and Vit K [199]. Figure 4 summarizes some of the physiological substrates and functions of TrxR and of the Trx system as a whole.

## REGENERATION OF ANTIOXIDANTS BY MAMMALIAN THIOREDOXIN REDUCTASE

### *Selenium-containing enzymes and compounds*

The synthesis of selenoproteins in humans is Se-saturated at selenium intakes above 120 $\mu$g/d; still, a total significant reduction of cancer mortality is seen when individuals are Se supplemented with higher doses [207]. Se in the form of different low molecular compounds may excert some of those therapeutic effects. In vitro experiments have shown that certain Se compounds can induce apoptosis and cell cycle arrest in transformed

cells, and thereby inhibit the progression of cancer cell growth [208,209]. Se supplementation may indeed be preventive of the overall cancer incidence by as much as 50% [207]. It should be noted, however, that the therapeutic index is narrow and that selenium compounds may be severely toxic. The relationship between cancer and selenium is discussed in [210]. TrxR1 should play a central role for the pharmaceutical effects of selenium, not only by being one of the human selenoproteins but also through its role in metabolizing selenium-containing compounds.

TrxR reduces many selenium-containing compounds including selenite [211], selenocystine [197], and even the Sec-containing active site of plasma glutathione peroxidase [121]. For the active cotranslational incorporation of selenium into selenoproteins, conversion of inorganic selenium, e.g., in the form of selenite ($SeO_3^{2-}$), to hydrogen selenide ($HSe^-$) is necessary. This reduction can either be catalyzed directly via TrxR with or without Trx [211], or may involve the formation of selenodiglutathione (GS-Se-SG) adducts as an intermediate. The formation of GS-Se-SG requires presence of GSH that is formed by glutathione reductase (GR) and GR may also reduce GS-Se-SG directly [212]. However, TrxR is also highly efficient in reduction of GS-Se-SG [213]. Since selenium is an essential constituent of mammalian TrxR, the enzyme indeed plays a pivotal part in the metabolism of selenium compounds [214] and hence constitutes a link between selenium and the antioxidative cellular processes.

### *Ascorbic acid (vitamin C) and α-tocopherol (vitamin E)*

Ascorbate is water-soluble and has been shown to be a major antioxidant in human plasma as well as in and even across cell membranes [215,216]. It reduces α-tocopherol as well as peroxides and ROS such as superoxide [217]. The vitamin serves mainly to prevent lipid hydroperoxide formation in plasma lipoproteins, e.g., LDL, by reducing α-tocopherol radicals formed upon reaction with lipid peroxyl radicals [218]. This is, in turn, an important function in the prevention of atherosclerotic plaque formation [219]. Ascorbate also protects lipids in cell membranes by this mechanism. Intracellularly, in the aqueous phase, ascorbate and GSH act in concert to protect the cell from oxidative damage [220].

Ascorbate recycling in human cells is accomplished by several mechanisms and has been studied most thoroughly in red blood cells (RBC). Glutathione is believed to be the major reductant of dehydroascorbic acid (DHA) in erythrocytes [221] even though RBCs depleted of GSH are still able to recycle ascorbate [222]. The importance of alternative mechanisms for reduction of DHA becomes evident if cells are depleted of GSH, as is the case in oxidative stress. It has been shown that the thioredoxin system in such cases can function as a reductant of both DHA [223] and the ascorbyl free radical [224]. Other studies indicate the importance of the thioredoxin system in cells other than RBC [223]. The extent to which ascorbate is recycled by the thioredoxin system in vivo in different cells and conditions of growth is still unclear. Some cells are able to reduce DHA independent of both GSH and NADPH, suggesting still additional mechanisms for the recycling of ascorbate [225].

Vitamin E or α-tocopherol (α-TOH) is a lipid-soluble vitamin present in biological membranes. It contains a hydroxyl group by which it reacts with unpaired electrons and can reduce, e.g., peroxyl radicals. α-TOH is absorbed in the intestine and transported to the liver in chylomicrons. It is returned to the blood in VLDL particles that with lipoprotein lipase are degraded to LDL. LDL particles provide cells in peripheral tissues with cholesterol and are also important as a factor in atherogenesis [2,219]. Only 5–10 molecules of α-TOH exist in each LDL, in contrast to the abundance of oxidizable lipid molecules. This fact points out the need for compounds that can efficiently recycle α-TOH. In fact, since ascorbate recycles α-TOH and mammalian TrxR reduces dehydroascorbate, TrxR may play an important role for the total vitamin E antioxidant function. It has long been known that Se and vitamin E have mutual sparing effects, and TrxR could be the link explaining this fact, as has been proposed [226]. For a scheme summarizing the TrxR-mediated recycling of DHA and the interrelationship to vitamin E, see Fig. 5.

### *Lipoic acid*

R-α-Lipoic acid (LA; thioctic acid) is an essential prosthetic group in the dihydrolipoyl transacetylase ($E_2$) component of α-keto acid dehydrogenase complexes in mitochondria [227]. LA is covalently bound to a specific lysine side chain to form a lipoamide-like moiety. The dihydrolipoyl (lipoamide) dehydrogenase (LipDH) component ($E_3$) is finally responsible for the regeneration of lipoamide via $NAD^+$ and FAD. Thus, the in vivo function of LipDH in mitochondria is to oxidize dihydrolipoamide [227].

Free LA reduced to dihydrolipoic acid (DHLA) has powerful reductive capacity; the redox potential for the LA/DHLA-couple is in the range of −0.29 V [228] to −0.32 V [229]. The therapeutic use of free LA (or rather DHLA) has been explored in recent years. It is clear that LA/DHLA given as a drug has many antioxidant properties including metal-chelation [230], free radical quenching [230,231], and regeneration of other antioxi-

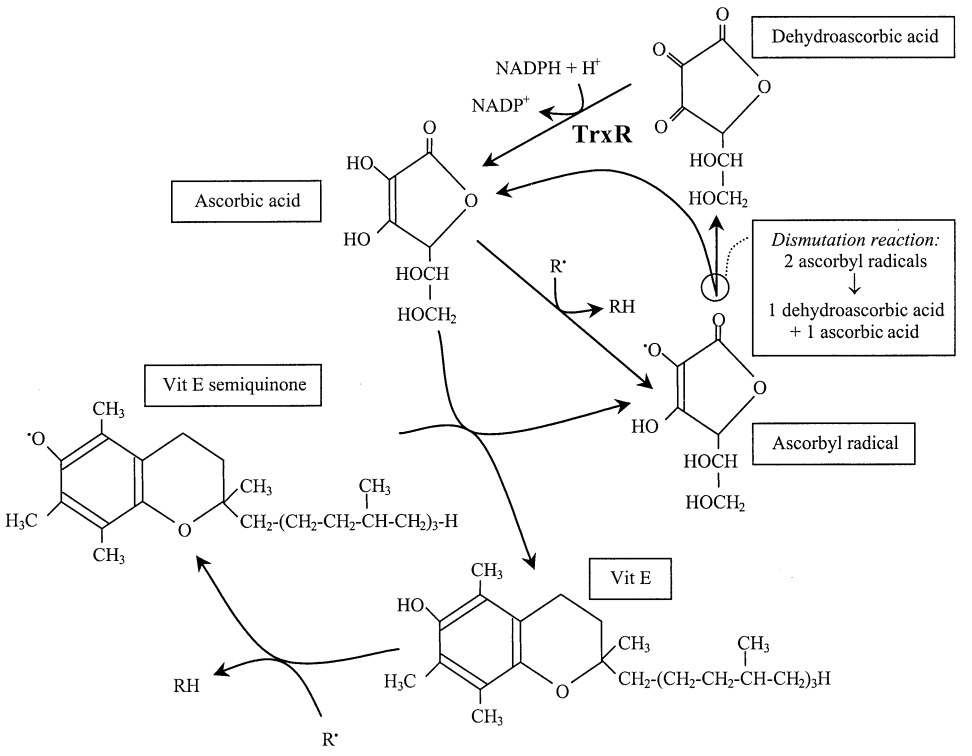



Fig. 5. The interplay between different species of vitamin C and vitamin E in relation to TrxR. Vitamin E is the major antioxidant in biological membranes and can react with free radicals to form vitamin E semiquinone, which in turn can be reduced back to vitamin E by ascorbic acid [62,297,298]. In this reaction, ascorbyl free radicals are formed, two of which can spontaneously dismutate, generating one molecule of ascorbic acid and one molecule of dehydroascorbic acid. TrxR can efficiently reduce the latter to ascorbic acid [223]. The direct reduction of ascorbyl free radical to ascorbic acid by TrxR has been shown indirectly by May and coworkers [224] but the catalytic mechanism is unclear and that pathway is left out in this scheme.

dants, including GSH, ascorbate, and vitamin E [232]. LA normally exists in micromolar amounts in blood [228] (majority being in the DHLA-form) but upon exogenous administration, LA is taken up via the intestine, distributed to the major organs and is to a large extent intracellularly reduced to DHLA [227]. Cells in culture are capable of taking up LA, reducing it to DHLA, and then releasing the reduced molecule [233]. Thus, LA and in particular its reduced form, DHLA, has properties that give the compound potentials as a pharmacological antioxidant. Clinical trials using LA in several different diseases have been conducted and are reviewed in [227, 232]. The major conclusions indicate that LA could be of therapeutic use in diabetes mellitus by preventing $\beta$-cell destruction, stimulating glucose uptake, protecting against atherosclerosis and cataracts, and by decreasing symptoms from diabetic neuropathy. In ischemia-reperfusion injury, LA has also been shown to prevent or decrease the damage that occurs due to ROS produced when the ischemic tissue is reoxygenated. LA has also been shown to have some effect in mushroom poisoning, liver disease, HIV-infection, heavy metal poisoning, and as an adjuvant in the treatment of Chagas disease. For further reviews on the subject see [227,230–232].

The enzymatic reduction of exogenously administered lipoic acid has been attributed to lipoamide dehydrogenase (LipDH) and glutathione reductase (GR) activities [234–236]. LipDH is a mitochondrial enzyme and is not prevalent in erythrocytes. GR was therefore postulated to be responsible for LA reduction in those cells, since the reduction was found to be dependent of NADPH and inhibited by mitomycin C, an inhibitor of FAD-dependent reductases [234]. In the liver, LipDH together with GR were proposed to reduce LA [227]. LA is also reduced efficiently in vitro by TrxR1 from calf thymus and liver, human placenta and rat liver, with $K_m$ values ranging from 0.49 to 1.00 mM [237]. In Table 2, the kinetic parameters of TrxR-catalyzed LA reduction are compared to those of LipDH and GR, showing that the $k_{cat}/K_m$ for TrxR exceeds that of GR but that LipDH has a higher capacity for LA reduction but with a higher $K_m$ than that found for TrxR. It should however be emphasized that TrxR catalyzes reduction of LA 2.49 times more efficiently than it catalyzes the reverse reaction,

Table 2. Comparison Between the Lipoic Acid-Reducing Activities of TrxR, GR, and LipDH

| Enzyme | $K_m$ (mM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ | Ref. |
|---|---|---|---|---|
| TrxR | 0, 71[a] | 368[a] | 518 | [237] |
| GR | 3.5 (S)[b] − 7.1 (R) | 112 (S) − 78 (R) | 32 (S); 11 (R) | [236] |
| LipDH | 5.5 (S) − 3.7(R) | 868 (S) − 21100 (R) | 158 (S); 5700 (R) | [286] |

Activities were determined using NADPH for TrxR and GR and NADH for LipDH.
[a] Substrate: Racemic $\alpha$-lipoic acid.
[b] (S) = S-enantiomer; (R) = R-enantiomer.

oxidation of DHLA, whereas LipDH catalyzes DHLA oxidation 20 times more efficiently than the LA reduction [237]. In absolute turnover when assayed at the same conditions (6–7 nM enzyme, 1 mM substrate, pH 8.0), TrxR was 15 times more effective in reducing LA (using 200 $\mu$M NADPH) than LipDH could reduce LA (using 200 $\mu$M NADH) [237]. These results strongly indicate that TrxR should serve an important part of the cytosolic reduction of LA, being necessary for its antioxidant properties as a drug.

The LA-reducing activity of the mitochondrial isoform of TrxR seems less than that of TrxR1 [238]; this isoenzyme should however contribute to the total LA reducing activity of mitochondria, previously believed to be solely due to LipDH.

### *Ubiquinone (coenzyme Q/Q10)*

Ubiquinone exerts its main natural function in mitochondria as a part of the electron transport chain, but is also present in low concentrations in plasma and in cell membranes where it functions as an antioxidant by preventing lipid peroxidation [239]. Regeneration of ubiquinone is managed by lipoamide dehydrogenase [240] and to some extent also by the other members of this family of enzymes, including TrxR [240].

The potential use of oral administration of ubiquinone is doubtful since the gastrointestinal absorption is saturable and only a few percent is redistributed to the blood. For further discussions of ubiquinone as an antioxidant see [241].

## INHIBITORS OF MAMMALIAN TrxR

The effects of inhibitors of TrxR should be profound in view of the multiple functions of the thioredoxin system in DNA synthesis and inflammatory processes as well as in the general defense against oxidative damage, as reviewed above. The striking species differences between the TrxR of mammals, prokaryotes and *P. falciparum* also makes the enzyme of lower organisms an appealing drug target in, e.g., bacterial infections and malaria [153,242].

### *Many electrophilic compounds are inhibitors of mammalian TrxR*

A number of low molecular weight compounds are known inhibitors of the selenocysteine-containing mammalian-type TrxR. Among others, these include arsenicals [243], 4-vinyl pyridine [194], iodo-acetic acid [194], and gold compounds used in the treatment of rheumatoid arthritis [244]. Because of the essential role of the Trx-system in DNA production by its participation in ribonucleotide reductase catalysis [150], TrxR should be an appealing target for anticancer drugs. Several chemotherapeutic anticancer compounds in clinical use do in fact inhibit TrxR, including quinones [245,246], retinoic acid [247], nitrosureas, e.g., carmustine (BCNU), fotemustine [248], and cisplatin [249]. The intracellular GSH adduct of cisplatin, GS-Pt, is an inhibitor of TrxR as well as of glutaredoxins [250]. The clinical relevance of TrxR and its inhibitors has recently been reviewed [153].

In view of retinoic acid as inhibitor [247] it should also be mentioned that cytosolic TrxR has been proposed to mediate cell death upon interferon and retinoic acid treatment, in the form of GRIM-12 (Gene associated with Retinoid-IFN-induced Mortality-12) [251,252]. However, the major part of those studies was based upon experiments with cultured cells transfected with a construct lacking the SECIS element necessary for selenocysteine incorporation, thereby resulting in an enzymatically inactive (or pro-oxidant?) truncated TrxR1 product. The mechanism of GRIM-12-associated cell death is therefore unclear and difficult to attribute to the physiological role of TrxR.

### *DNCB (1-chloro-2,4-dinitrobenzene) and the thioredoxin system*

Dinitrohalobenzenes such as 1-chloro-2,4-dinitrobenzene (DNCB; CDNB) are unique inhibitors of TrxR in that they induce a superoxide-producing NADPH oxidase activity in the enzyme [194,253], thereby exaggerating the pro-oxidant effects of TrxR inhibition. We shall therefore here discuss the effects of DNCB in relation to the thioredoxin system in some further detail.

DNCB is a small electrophilic molecule belonging to

the wider group of dinitrohalobenzenes, where fluoride, bromide, or iodide may take the place of chloride to form DNFB, DNBB, or DNIB, respectively. The different dinitrohalobenzenes have similar biological effects. In biomedicine, DNCB has long been used to probe cellular immunity and provoke delayed hypersensitivity responses by topical application to the skin. The sensitizing effects on the immune system that then occur have been explained by the delayed-type hypersensitivity (DTH) reaction in which DNCB is thought to function as a hapten [254]. Due to its immunostimulatory properties, i.e., provoking DTH as well as an immediate inflammatory reaction (see below), topical DNCB has been tested as an immunomodulatory treatment for a number of diseases characterized by varying degrees of immune suppression, including viral warts [255], HIV/AIDS [256–259], and alopecia areata [260,261]. The clinical value is, however, uncertain.

DNCB is also a well-characterized substrate for glutathione S-transferase [127]. In this reaction, GSH is conjugated with DNCB, rendering a less electrophilic, water-soluble product, which can be further metabolized. This is the in vivo metabolism of DNCB and the basis for its use as a laboratory agent for GSH-depletion in cell culture experiments [262].

More recently, DTH and GSH depletion have been questioned as the only molecular mechanisms for the inflammatory response to topical DNCB [263]. It is evident that there is a direct inflammatory effect of DNCB that cannot be explained by DTH or GSH-depletion, and it has even been shown that GSH levels in mouse skin increase upon epidermal application of DNCB [263]. In the same study, NADPH- and oxygen consumption was measured and found to be considerably higher in DNCB-treated skin cells than in controls [263]. Those findings indicate that oxidative stress is induced by DNCB, which may explain the immediate inflammatory response to DNCB upon its topical administration. Interactions with the thioredoxin system should provide a major part of the underlying biochemical mechanism for this effect.

In 1995 we found that DNCB induces an NADPH-oxidase activity in mammalian TrxR concomitant with an irreversible inhibition of the enzyme. Subsequently we showed that both the C-terminal selenocysteine and its neighboring cysteine were derivatized by DNCB and that the NADPH-oxidase activity resulted in production of superoxide [194]. The molecular mechanisms for this activity are clear, involving FAD-catalyzed generation of nitro anion radicals in the dnp moieties of the derivatized enzyme, and a model for a link to the inflammatory response to DNCB was proposed [264]. With recent results regarding the cellular functions of the thioredoxin system, this model has gained considerable credibility, which we will discuss here.

As mentioned above, inhibition of TrxR in cells should lead to a number of major potentially pro-oxidant effects. The major cellular consequences of TrxR inhibition by any inhibitor can be divided in the following probable events:

*Decreased TrxR activity*. The essence of TrxR inhibition is a decreased TrxR activity leading to decreased regeneration of the many TrxR substrates (see above) and less total antioxidant capacity. Many of the known TrxR inhibitors inactivate the enzyme at low concentrations and the TrxR activity should be decreased considerably upon exposure to inhibitor also in cells or whole tissues, even though many inhibitor studies were performed in vitro. Studies showing TrxR inhibition in living cells has been performed with organotellurium compounds [265], arsenicals [266], and aurothioglucose [267]. For DNCB and most of the other inhibitors, the inhibition is irreversible and should thereby remain until new enzyme has been synthesized and inhibitor is no longer present.

*Decreased Trx activity*. Regeneration of reduced Trx should naturally be impaired as a consequence of the decreased TrxR activity. Thereby the whole reductive capacity of the cell becomes heavily impaired.

*Increased production of ROS*. Inhibition of the thioredoxin system will, among the other effects, impair the function of peroxiredoxins [110,268] and lead to increased levels of ROS. DNCB-derivatized TrxR itself produces superoxide [194] and this will further increase the production of ROS and the subsequent oxidative stress. This may, moreover, mimic the intracellular signaling through $H_2O_2$ and other ROS [10,15], which in fact has been demonstrated, with DNCB treatment of cells mimicking the effects seen upon hydrogen peroxide production after treatment with epidermal growth factor [180].

*Decreased GSH-levels and increased GSSG*. As a general effect upon the increased oxidative stress after inhibition of the thioredoxin system, an increasingly oxidized intracellular milieu should induce GSSG formation and possibly glutathionylation of selected target protein thiols. Reversible protein glutathionylation, catalyzed by glutaredoxins, may in fact be an important part of physiological cellular redox signaling and should be increased at events of oxidative stress [269,270]. For DNCB, since the compound is metabolized through conjugation with GSH, cells may have decreased GSH levels upon treatment with DNCB and this should subsequently further impair the reductive capacity of the cells.

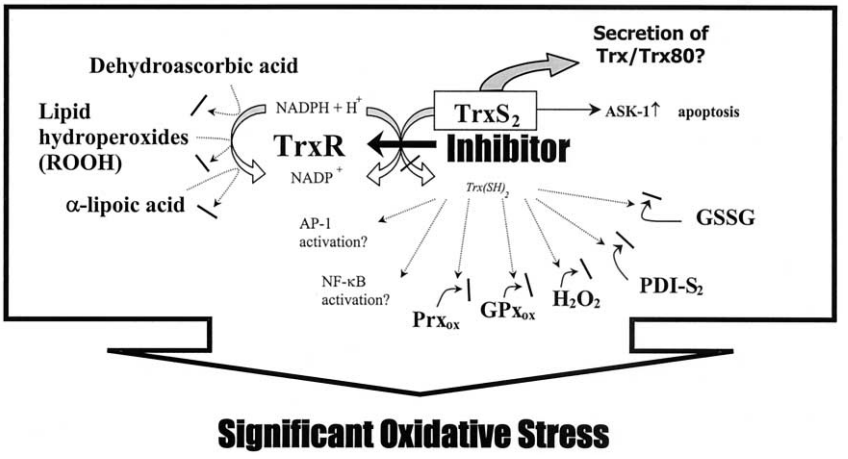



Fig. 6. Schematic model of the effects of TrxR-inhibition. Inhibition of thioredoxin reductase naturally leads to impaired function of thioredoxin system-dependent or –regulated reactions, as summarized in this figure (dashed arrows). The net result is a significant intracellular oxidative stress. Increased oxidation of thioredoxin may also lead to apoptosis via increased ASK-1 activity or to secretion of thioredoxin and its truncated Trx80 form. This scheme should be compared to the situation with a functional thioredoxin system as summarized in Fig. 4.

*Increased expression of Trx*. As a consequence of the decreased levels of reduced Trx and the total increased cellular oxidative stress, the expression of Trx will be induced [147,169,172,271–273].

*Increased secretion of Trx*. Secretion of Trx seems to be augmented when the intracellular concentrations of ROS are elevated and Trx is kept oxidized [264]. As reviewed above, extracellular Trx and Trx80 exert specific cytokine and chemokine activities that hence may give rise to an immunologic response upon TrxR inhibition [166]. Possibly with secretion of Trx, increased secretion of Trx80 may also be seen.

*Increased expression of NF-κB-dependent proteins*. Due to the elevated levels of ROS and decreased amount of reduced Trx in the cytosol, NF-κB should be activated with the gene activation by NF-κB being increased (see below).

*Increased expression of TrxR*. If the cells survive the significant oxidative stress, increased expression of newly synthesized TrxR, not being inhibited, should eventually restore the cells to a physiological redox balance. Increase of TrxR expression has indeed been confirmed upon treatment of cells with hydrogen peroxide, DNCB, calcium ionophore, PMA, IL-1β, lipopolysaccharide, and vitamin D3; see [274] and references therein. A fast response in TrxR expression is likely at least in part to be derived from a constitutively high transcription in combination with oxidant-induced stabilization of the TrxR mRNA [274].

The effects outlined here upon inhibition of TrxR (especially by dinitrohalobenzenes) should apply a massive oxidative stress to the cell. The response could either be recovery by overexpression of Trx and other antioxidative enzymes (including native, nonderivatized TrxR), or cell death via necrosis or apoptosis. Apoptosis is indeed directly prevented by reduced Trx via inhibition of ASK1 [75], and indirectly, via the peroxiredoxins [113,114]. Conversely, ASK1 is activated when Trx is oxidized [75] and interestingly, in the experiments showing this effect, DNCB was used as a means of increasing the intracellularly oxidized Trx species [75]. Figure 6 schematically summarizes the many cellular effects that should occur upon TrxR inhibition.

## IMMUNOMODULATORY EFFECTS OF DNCB DISCUSSED IN VIEW OF ITS INTERACTIONS WITH MAMMALIAN THIOREDOXIN REDUCTASE

When DNCB is applied to the skin it readily penetrates the stratum corneum to reach the cells underneath. Among those cells are the antigen-presenting Langerhans cells present, being key players in the immunologic defense in skin. According to the hapten theory, DNCB modifies proteins making them recognizable as antigens by Langerhans cells, thereby eliciting a DTH [254]. Recently, those effects have been studied extensively by measuring the cytokine response indicative of maturation of Langerhans cells upon treatment with DNCB and other allergens and irritants [275–279]. Exposure of antigen presenting cells to pathogens or allergens may lead to differentiation of naive T-helper cells ($CD4^+$) into either the Th1 or Th2 subtype [275,280]. Intracellular bacteria and *viridae* typically elicit a Th1 response (via, e.g., IL-12), while, e.g., parasitic worms cause differentiation into Th2-cells (via IL-6). One subset of T-helper

Table 3. Chemokine Profiles Induced by DNCB and the Correlation to Transcription Control by NF-κB

| Cytokine/receptor | Secreted by Th1/Th2 specifically | Induction (+) or inhibition (−) by DNCB | Inducing (+) or Inhibiting (−) NF-κB | Transcription of gene controlled by NF-κB | References |
|---|---|---|---|---|---|
| IL-1β | | + | + | | [278,279] |
| IL-2 | Th1 | | | + | [64] |
| IL-4 | Th2 | − | − | | [278,287–289] |
| IL-6 | | + | | + | [64,290] |
| IL-8 | | +* | | + | [64,291] |
| IL-10 | Th2 | − | − | | [278,287–289] |
| IL-12 | | + | | + | [292,293] |
| IL-18 | | + | | | [294] |
| TNF-α | Th1 | + | + | + | [64,279,295] |
| IFN-γ | Th1 | + | | + | [282,289] |
| MHC class II | | + | | + | [64,296] |
| CD40 | | + | + | | [278,290] |

* DNFB.

cells also produces cytokines to inhibit the proliferation of its counterpart [281]. This division is of critical importance since it generally directs the immune defense towards either cell-mediated immunity (Th1) or humoral immunity (Th2). In several studies, DNCB has been shown to cause a typical Th1 response, thereby strengthening the interpretation of its effects as being hapten mediated (see Table 3 and the references cited therein).

As observed by Aronica et al. [282], the profile of cytokines controlled by NF-κB is similar to the pattern of cytokines secreted by type 1 helper T-lymphocytes, and the Th1-dependent DTH response intimately relies on NF-κB for its induction. Those findings imply a function for NF-κB in the determination of T-cells towards either Th1 or Th2 effector cells. Could it be that DNCB is inducing NF-κB and thereby swings the immunologic response towards Th1 dominance? As seen in Table 3, there is a clear correlation between the cytokines shown to be induced by DNCB and their transcription control by NF-κB.

### *Redox regulation of NF-κB by Trx; implications for DNCB*

As described above, inactivation of TrxR should give increased levels of oxidized Trx and decreased levels of reduced Trx, and this should be especially pronounced in the inhibition or TrxR by DNCB, as the enzyme thereby is being transformed to a superoxide-generating enzyme. Low levels of ROS are modulators of cell signaling with their adverse effects counteracted by antioxidants (Trx/GSH, etc.). In the normal low-level ROS physiology Trx will, via its reductive properties, prevent NF-κB activation and its subsequent nuclear translocation [24]. Trx and activated NF-κB are further translocated into the nucleus upon oxidative stress, where Trx enhances the DNA-binding capacity of NF-κB [24]. In this manner Trx can exert both inhibitory and stimulatory effects on NF-κB, depending on its localization, which in turn depend on the level of cellular oxidative stress. The intracellular effects of DNCB-inactivated TrxR could very well lead to Trx-mediated activation of NF-κB, which would fit the results listed in Table 3 without the need for the hapten-related DTH reaction. The activation of NF-κB should be more direct and could be a mechanism for the immediate inflammatory response after topical DNCB treatment. An additional immunomodulatory effect of DNCB derivatization of TrxR could be an increased secretion of Trx and/or Trx80, as discussed in the next section.

### *Secretion of Trx and Trx80 and T helper cell differentiation as a response to DNCB*

When peripheral blood mononuclear cells (PBMC) are incubated with DNCB, they secrete Trx in full length and/or truncated form [264]. The truncated form of Trx, Trx80, stimulates PBMC and especially monocytes to produce IL-12 [143], thereby being a strong inducer of the Th1 response. By this mechanism, DNCB could also directly switch the T-cell response towards the Th1 side via subsequent extracellular effects derived from the intracellularly inhibited thioredoxin system, as depicted in Fig. 7.

### *Vitiligo; further support for an immunostimulatory interaction between TrxR and DNCB*

Patients with vitiligo, a disease in which the pigmentation of skin is defective, are known to show impaired DTH reactions and inflammation upon topical application of DNCB [283,284]. Sensitization with tuberculin [283] and *Candida albicans* antigen [284] were, however, in the same studies shown *not* to be suppressed. Vitiliginous skin was, most interestingly, recently reported to lack TrxR [285]. This finding could explain the

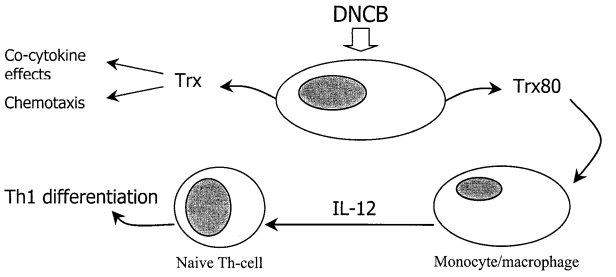



Fig. 7. Extracellular effects of DNCB via the thioredoxin system. Exposure of cells to DNCB may lead to secretion of Trx and/or Trx80 as a result of the inhibited TrxR and induced oxidative stress; (see Fig. 6 and the text). Extracellular Trx is known to exert co-cytokine functions, stimulate cytokine production, and to have mitogenic and chemokine effects (see text for details and references). Moreover, Trx80 stimulates monocytes to produce IL-12, a strong inducer of T-helper cell differentiation towards the Th1 type, as recently characterized by Pekkari et al. [143].

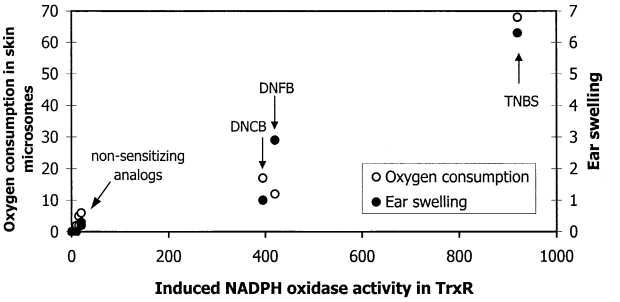



Fig. 8. Correlation between induction of NADPH oxidase activity in TrxR in vitro and oxygen consumption and mouse ear swelling provoked in vivo by dinitrohalobenzene compounds. The oxygen utilization in mouse skin microsomes (% $min^{-1}$) and the ear swelling response (× 0.01 mm) upon topical application of diverse dinitrohalobenzene compounds are shown on the Y-axes (from [263]). On the x-axis, the level of induced NADPH oxidase activity in TrxR ($\Delta mA_{340}$/min) by the same compounds is shown (from [194]). The effect of TNBS (2,4,6-trinitrobenzene sulfonic acid) on TrxR is an unpublished result of ours and is in the figure correlated to the effects on mice as reported for 2,4,6-trinitrobenzene chloride [263].

deficiency of DTH responses to DNCB in vitiligo and further corroborate the model proposed here, suggesting that in vivo interactions with TrxR indeed play an important role for the immunostimulatory effects of DNCB.

### *Inflammatory response to dinitrohalobenzenes in mice—correlation to interactions with TrxR in vitro*

An NADPH-dependent oxygen consumption in skin microsomes with clear correlation to the degree of mice ear swelling provoked upon topical administration of halobenzenes has been demonstrated [263]. We found that these parameters also correlated well with the NADPH oxidase activity of TrxR being induced in vitro by incubation with the same compounds (Fig. 8). Those findings are hence further experimental data supporting the view that dnp-derivatized TrxR may play a role in the molecular mechanism of the inflammatory response seen upon topical application of dinitrohalobenzene compounds.

## CONCLUDING REMARKS

As discussed, reactive oxygen species (ROS) have a number of effects in cells. In low amounts they modify and fine-tune intracellular signaling, and their potentially adverse effects are prevented by the different cellular antioxidant systems. When ROS are prevalent in higher amounts, if their production is excessive or the antioxidative systems are inadequate, oxidative stress is the result. Such condition can lead to necrosis or apoptosis. From a medical perspective, oxidative stress may lead to disease, e.g., atherosclerosis, inflammation, or cancer.

We have focused this review on the many interactions of the thioredoxin system with ROS molecules, with low molecular weight antioxidant compounds and with the other major cellular antioxidant enzymatic systems. We have discussed the cellular effects that may be the result of inhibition of TrxR. In more detail we have focused on the interactions between TrxR and DNCB, constituting a possible molecular mechanism for the immunostimulatory effects seen upon topical application of dinitrohalobenzene compounds. The central role played by the thioredoxin system for redox control of cell function and protection against oxidative damage is evident and more results will certainly be unraveled by future studies. It is also likely that novel pharmaceutical regimes will be developed based upon interactions with thioredoxin reductase and the thioredoxin system as a whole.

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## ABBREVIATIONS

$\alpha$-TOH—$\alpha$-tocopherol (Vitamin E)
AP-1—Activator Protein-1
ASK—Apoptosis signal-regulating kinase
Cys—cysteine
DHLA—Dihydrolipoic acid
DNCB—1-chloro 2,4-dinitrobenzene
Dnp—Dinitrophenyl
DTH—Delayed type hypersensitivity response
FAD—Flavin adenine dinucleotide (oxidized form)
GR—Glutathione reductase
GPx—Glutathione peroxidase
Grx—Glutaredoxin
GSH—Glutathione (reduced form)
GSSG—Glutathione disulfide
GST—Glutathione S-Transferase
LA—Lipoic acid
LipDH—Lipoamide dehydrogenase
NADH—Nicotinamide dinucleotide (reduced form)
NADPH—Nicotinamide dinucleotide phosphate (reduced form)
NF-$\kappa$B—Nuclear factor kappa B
PBMC—peripheral blood mononuclear cells
Prx—Peroxiredoxin
ROS—Reactive oxygen species
Sec—selenocysteine
-SH—thiol
SOD—Superoxide dismutase
SOR—Superoxide reductase
TrxR—Thioredoxin reductase
Trx—Thioredoxin