

# Thiol redox control via thioredoxin and glutaredoxin systems

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## Abstract

The Trx (thioredoxin) and Grx (glutaredoxin) systems control cellular redox potential, keeping a reducing thiol-rich intracellular state, which on generation of reactive oxygen species signals through thiol redox control mechanisms. Here, we give a brief overview of the human Trx and Grx systems. The main part focuses on our current knowledge about mitochondrial Grx2, which facilitates mitochondrial redox homeostasis during oxidative stress-induced apoptosis.

## Introduction

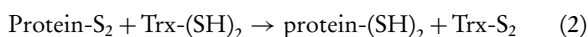
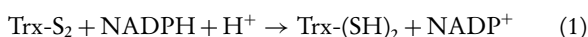
The cellular redox state is a crucial mediator of multiple metabolic, signalling and transcriptional processes in cells, and protein thiols in the form of cysteine residues are key players in redox sensing and regulation. Trxs (thioredoxins) and Grxs (glutaredoxins) are small (9–16 kDa) thiol-disulphide oxidoreductases, first identified as electron donors for ribonucleotide reductase in *Escherichia coli* [1,2]. They share a dicysteine active site motif (CXXC) in a common structure called the Trx-fold [3,4].

## The Trx system

Trxs have a remarkable number of functions in mammalian cells [5], which contain two distinct Trxs. Trx1 (gene *TXN1*) is a cytosolic or nuclear protein, which can be exported and can act as cytokine or chemokine [6], whereas Trx2 (*TXN2*) is targeted to mitochondria [7]. Trxs, which have a low redox potential, reduce protein disulphides utilizing two cysteine residues in their CGPC active site. The reduction of the resulting active site disulphide in Trx is catalysed by TrxR (Trx reductase) using electrons from NADPH. Three mammalian TrxR isoenzymes have been described: (i) cytosolic TrxR1 (*TXNRD1*) [8,9], (ii) mitochondrial TrxR2 (*TXNRD2*) [10,11] and (iii) the testis-specific TGR (Trx GSH reductase) [12]. Unlike their small bacterial counterparts, these enzymes are large selenoproteins. The homodimeric flavoenzymes contain a penultimate C-terminal selenocysteine in their GCSG active sites [13–15], which is also the reason for TrxRs' wide substrate specificity, including non-disulphide compounds [16].

The Trx system was originally described as a dithiol cofactor for *E. coli* ribonucleotide reductase. *E. coli* Trx and human platelet Trx were later shown to be general disulphide reductases by using fibrinogen [17], chorionic gonadotropin [18] and insulin [19,20]. The reaction can be followed by

NADPH according to eqns (1) and (2):

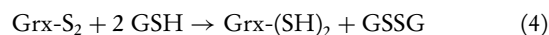
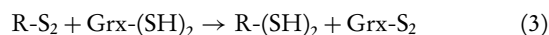


A large number of disulphide-containing proteins have been analysed using Trx and TrxR as sensitive probes for disulphide exposure and reactivity [21]. The term thiol redox control was introduced to describe the possible signalling function of Trx system-dependent control of protein function [22]. Today, we can see an explosive development of such applications (see e.g. [23]). Since the discovery of H<sub>2</sub>O<sub>2</sub> as a signalling molecule and of the peroxiredoxins, this is expanding even more into, for instance, control of phosphotyrosine phosphatase activity [24].

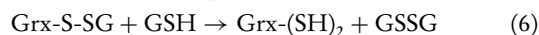
## The Grx system

The Grx system consists of Grx, GSH and NADPH-dependent GSH reductase. Grxs usually contain the active site motif CPYC and are able to catalyse reactions not only via a dithiol mechanism (as Trxs do), but also via a monothiol mechanism [25,26], which is required for the reduction of protein GSH-mixed disulphides (deglutathionylation).

Dithiol mechanism:



Monothiol mechanism:



where R-S-SG is a mixed disulphide with GSH.

Mammalian cells contain two Grxs. Cytosolic Grx1 (*GLRX1*) is involved in multiple cellular processes, e.g. protein disulphide reduction as in ribonucleotide reductase [27], dehydroascorbate reduction [28], regulation of transcription factors [29,30] and apoptosis [31,32]. The second mammalian Grx2 was identified more recently [33,34]. The human gene (*GLRX2*), located at chromosome 1q31.2-31.3,

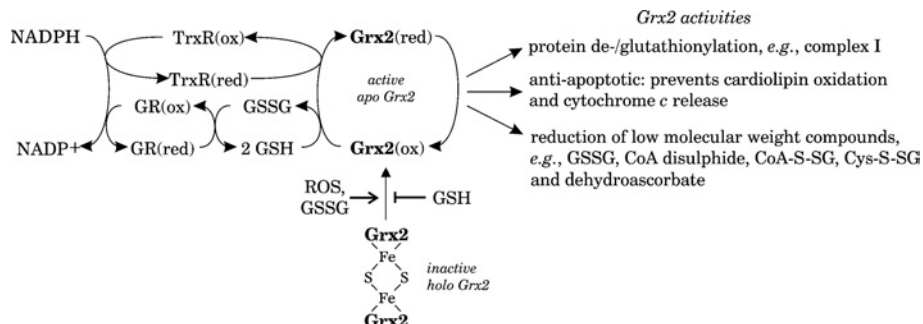
**Key words:** disulphide, glutaredoxin (Grx), GSH, thiol redox control, thioredoxin (Trx), thioredoxin reductase (TrxR).

**Abbreviations used:** Dox, doxorubicin; Grx, glutaredoxin; PAO, phenylarsine oxide; ROS, reactive oxygen species; Trx, thioredoxin; TrxR, thioredoxin reductase.

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**Scheme 1 | Summary of proposed activities and activation of Grx2 *in vivo***

A high GSH/GSSG ratio stabilizes holo-Grx2, whereas the [2Fe-2S] cluster is destroyed by oxidative stress leading to activation of Grx2 [47]. Grx2 can use electrons from both NADPH/GR (glutathione reductase)/GSH and NADPH/TrxR for the reversible deglutathionylation/glutathionylation of proteins [35,43] and from the reduction of low-molecular-mass disulphides and dehydroascorbate [35]. In addition, consequently, Grx2 protects the cells from apoptosis by preventing cytochrome *c* release [44,46].



gives rise to two alternatively transcribed Grx2 mRNA isoforms with alternative first exons. Both isoforms share the same Grx core, encoded by exons II to IV. The unusual active site CSYC is encoded by exon III and the GSH- and substrate-binding sites by exon III and exon IV respectively. Exon Ia encodes a mitochondrial translocation signal, while the second isoform (exon Ib and exon IV) was predicted to be localized in the nucleus. Corroboratively, Western-blot analysis demonstrated the presence of Grx2 in both nuclear and mitochondrial fractions from Jurkat cells [33].

The oxidized active site of Grx2, which in all other eukaryotic Grxs is exclusively reduced by GSH, is also a substrate for TrxR [35]. TrxR efficiently reduces both the active site disulphide and the Grx2-S-SG intermediate formed in the reduction of glutathionylated proteins, thus supporting both monothiol and dithiol reactions. At sufficiently oxidizing conditions the active site in Grx2 cannot be reduced by GSH. The direct reduction via TrxR enables Grx2 to reduce glutathionylated proteins and a series of low-molecular-mass substrates even during conditions of oxidative stress [35] (Scheme 1). The mitochondrial respiratory chain is the major source of ROS (reactive oxygen species) for most cells and increased production of ROS by complex I is involved in committing cells to apoptosis. ROS alter the ratio of GSH/GSSG, which can change the activity of many key proteins by formation of mixed disulphides of GSH with critical cysteine residues (glutathionylation) (see e.g. [36–41]). ROS formation by complex I is increased upon glutathionylation of two thiols in its NADH-binding pocket [42]. Grx2 is an efficient catalyst of monothiol reactions with high affinity for glutathionylated proteins [35], and Grx2 catalyses the reversible glutathionylation of complex I and other proteins of the inner mitochondrial membrane over a wide range of GSH/GSSG ratios [43].

HeLa cells with short interfering RNA-mediated silenced expression of Grx2 were dramatically sensitized to cell death, induced by the ROS-inducing agents Dox (doxorubicin)/adriamycin and PAO (phenylarsine oxide) [44]; the ED<sub>50</sub>

for Dox decreased 60-fold and that for PAO 40-fold. The cells did not show signs of a general increase in oxidative damage of proteins and were not sensitized to cadmium, a known inhibitor of Grx1 [31]. The protein levels of Grx2, determined by a sensitive ELISA, are at least 20-fold lower than those of Grx1 [45]. Together, these findings indicate the regulation of specific targets by Grx2 upon mitochondrial ROS production rather than a role as a general antioxidant. Overexpression of Grx2 in HeLa cells decreased the susceptibility of cells to apoptosis induced by 2-DG (2-deoxy-D-glucose) or Dox [46]. Grx2 prevented the loss of cardiolipin, and inhibited cytochrome *c* release and caspase activation. Overexpression of mitochondrial Grx2 provided better protection than overexpression of a cytosolic mutant lacking the mitochondrial translocation signal. Grx2 facilitates the maintenance of mitochondrial redox homeostasis upon treatment with apoptotic agents, thereby preventing cardiolipin oxidation and cytochrome *c* release, i.e. the induction of apoptosis [46].

Recently, we have characterized Grx2 as the first iron-sulphur cluster-containing member of the Trx-fold protein family [47]. In-depth spectroscopic analysis revealed the presence of a four cysteine-co-ordinated, non-oxidizable [2Fe-2S]<sup>2+</sup> cluster. Mutational analysis indicated that two cysteinyl groups outside the active site and unique to Grx2 served to complex the cluster and to dimerize Grx2. Co-immunoprecipitation of the  $\gamma$ -X-ray emitting <sup>55</sup>Fe isotope with Grx2 from two different human cell lines indicated the presence of the cluster *in vivo*. Iron-sulphur clusters are multipurpose structures that can undergo reversible redox reactions, influence protein structure, and act as catalytic centres and as sensors of iron and oxygen species [48,49]. Dimeric holo-Grx2 was enzymatically inactive, but cluster degradation and monomerization of Grx2 activated the oxidoreductase. Slow loss of the cofactor under aerobic conditions was prevented by the presence of GSH; GSSG as well as one-electron oxidants or reductants like ferricyanide and dithionite promoted monomerization and activation of Grx2. We

have proposed that the iron–sulphur cluster serves as a redox sensor for the activation of Grx2 during conditions of oxidative stress when free radicals are formed and the GSH pool becomes oxidized [47] (Scheme 1).

In summary, there is clear evidence that Grx2, whose biochemical properties are highly adapted to the mitochondrial environment dominated by variable redox conditions, attenuates apoptosis induced by ROS-producing agents. The questions to be answered next concern the underlying mechanisms and the identification of Grx2's molecular targets.

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