THIOL REDOX SYSTEMS

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THIOL REDOX SYSTEMS

• Thioredoxin system
• Glutaredoxin system
• Redundant, but not identical
THIOREDOXIN AND ITS FUNCTIONS

• Disulfide reductase
• Two major antioxidant system; Trx and GSH
• First sequence 1968 by Arne Holmgren
• Trx system activity is mainly shown by transferring electrons to peroxiredoxins (Prxs), Methionine sulfoxide reductases (MSR), and some redox-sensitive transcription factors (Nrf2, NFkB, p53)
• Thioredoxin reduced by TrxR, GSH can serve as back up system.
ISOFORMS OF THIOREDOXIN

- Two Trx systems
  - Cytosolic Trx1 (2+3 Cys in active site)
  - Mitochondrial Trx2 (2 Cys in active site)

Fig. 3. Thioredoxin and glutathione antioxidant systems in mammalian cells. Thioredoxin and glutathione systems are the two major thiol-dependent antioxidant systems in mammalian cells. (A) Mammalian thiol-dependent redox system in cytosol, nucleus. Thioredoxin system provide the electron to thioredoxin-dependent peroxidases (Prx1&2), which can efficiently remove reactive oxygen species as glutathione peroxidase (Gpx1). Moreover, thioredoxin reduces methionine sulfoxide reductases and is involved in the repair of oxidized proteins. Trx regulated the activities of many oxidative-sensitive transcription factors such as NF-κB, NF-κ, and P53 and thus is involved in the redox signaling. The GSH system can serve as a backup system to reduce thioredoxin when the electron transfer pathway from TrxR1 is blocked. (B) Mammalian mitochondrial thiol-dependent redox systems. Mitochondrial Gox2 can be reduced by mitochondrial TrxR2 and GSH. Mitochondrial Prx3 can be reduced by both mitochondrial Trx2 and Gox2. The thick black lines represent the direct reaction between the protein and reactive oxygen species in a fast reaction rate, which is also shown in the other figures.
STRUCTURE OF THIOREDOXIN

- 12 kDa disulfide reductase
- Conserved Cys-Gly-Pro-Cys active site
- Trx is ubiquitously distributed from archaea, bacteria to human
- Five β-strands - internal core
- Four α-helices and a short stretch of helix surround the β-sheets (like a sandwich)
- Active site disulfide is located after the β2-sheet forming the N-terminal portion of α2
- Thioredoxin fold structure
  - α/β protein fold
  - Common for enzymes catalyzing disulfide bond formation
    - E.g. glutaredoxin, peroxiredoxin, and glutathione peroxidase)
Schematic representation of Trx system. Disulfide form of Trx1 (-S-S-, oxidized) is reduced to dithiol form (-SH, reduced) by Trx reductase (TrxR) and NADPH. Reduced Trx catalyzes the reduction of disulfides (-S-S-) within multiple oxidized cellular proteins. Proteins that are known to be directly reduced by Trx include RNR (Holmgren, 1985), Prxs (Rhee, Kang, Chang, Jeong, & Kim, 2001), ASK1 (Nadeau, Charette, Toledano, & Landry, 2007), PTEN (Lee et al., 2002), HDAC4 (Ago et al., 2008), NF-kB (Matthews, Wakasugi, Virelizier, Yodoi, & Hay, 1992), and Ref-1 (Silber et al., 2002). Transcription factors AP-1 and HIF-1α are indirectly activated by Trx through intermediate Ref-1.
THIOREDOXIN REDUCTASE

- Selenium-containing flavoprotein
- Oxidoreductase
- Forms homodimers
- Basic function: reduce sulfide bonds

Figure 1. Electrons are transferred from NADPH to FAD, to the N-terminal redox active disulfide in one subunit of TrxR and then to the C-terminal active site of the other subunit.
THIOREDOXIN REDUCTASE

- **Mammals**
  - Cytosol
  - Mitochondria
  - Testis-specific
  - Human: 3 isoforms TrxR1, TrxR2, TRXR3

- **Plants**
  - Cytosol
  - Mitochondria
  - Chloroplast

Saccoccia et al., 2014
GLUTAREDOXIN

- Also known as thioltransferase
- Work together with thioredoxin as a component of GSH system
- Catalyze reduction of protein disulfides, proteins thionylated by GSH
- Target proteins: ribonucleotide reductase, complex I, other mitochondrial inner membrane proteins, dehydroascorbate (oxidized vitamin C) in human placenta, the lens, and RBCs, PTP-1B, Ras, actin
In *E. coli*, Grx exists in 4 isoforms: Grx1-4

In human, Grx exists in 2 isoforms: Grx1 in cytosol and Grx2 in mitochondrion and nucleus

Grx has 3 main structural regions: active site (Cys22-Cys25), the GSH binding site, and a hydrophobic site
GSH

- The main thiol based redox buffer of the cell and part of the Grx system
- Non-protein, consists only of three amino acids (Glu-Cys-Gly)
- Biosynthesis occurs in the cytosol, catalysed by glutamate cystein ligase and glutathione synthetase

Musgrave et al
GSH REGULATION

• Co-regulated by Met4p, Yap1p, GSH feedback inhibition.
• Both expression of GSH1 and 2 as well enzyme activity are inhibited by GSH feedback loop
• Oxidative stress induces expression of GSH1 and GSH2
• Yap1p is a stress response induced transcription factor which in conjunction with intercellular GSH and oxidative stress regulate induced expression of GSH1 and GSH2
GSH CONCENTRATION

• GSH levels and cellular redox status is controlled by GSH synthesis, as well as export from the cells

• Multidrug resistance-associated proteins (Mrp/Abcc) appear to mediate GSH export and homeostasis, also export oxidized glutathione derivatives (GSSG, GS-NO, and glutathione-metal complexes)

• Glutathione is imported into the cell via glutathione transporters

• Several papers show the existence of glutathione transporters in the membrane of intracellular membranes to move glutathione around the cell and into organelles.

• GSH concentrations in the total cell are estimated to be around 7mM, with an range from 0.5mM-10mM. Lower concentrations in the cytosol and nucleus, but higher concentrations in the mitochondria and ER (around 15mM)

• Significant difference in GSH:GSSG ratio between organelles. In the cytosol and mitochondria 30-100:1, in the ER 1-3:1.
GSH CYCLING

- GSH gets oxidized by multiple enzymes, in the process of reducing their respective substrate protein.
- Oxidized GSH forms a dimer GSSG.
- GSSG is reduced back to 2GSH by Glutathione Reductase.

GLUTATHIONE REDUCTASE

- Dimeric disulfide oxidoreductase
- Forms homodimers
- Catalyzes the reduction of glutathione disulfide (GSSG) to glutathione (sulphydryl; GSH)

**Figure 2. Two parts of cycle:**

**Reduction:**
NADPH reduces FAD $\rightarrow$ FAD$^-$
FAD$^-$ breaks disulfide Cys$_{58}$-Cys$_{63}$
NADP$^+$ released, new NADPH

**Oxidation:**
Cys$_{63}$ attacks GSSG sulfide creating disulfide bond + GS$^-$ anion
His$_{467}$ of GSR protonates GS$^-$ ion and releases first GHS
Repeat with Cys$_{58}$ attach to eventually form second GSH
GLUTATHIONE REDUCTASE

Mammals
- Cytosol
- Mitochondria
- Nucleus

Plants
- Cytosol
- Mitochondria
- Chloroplast