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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Thioredoxin (Trx)

By

Tanja Hadzic

Department of Pathology
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

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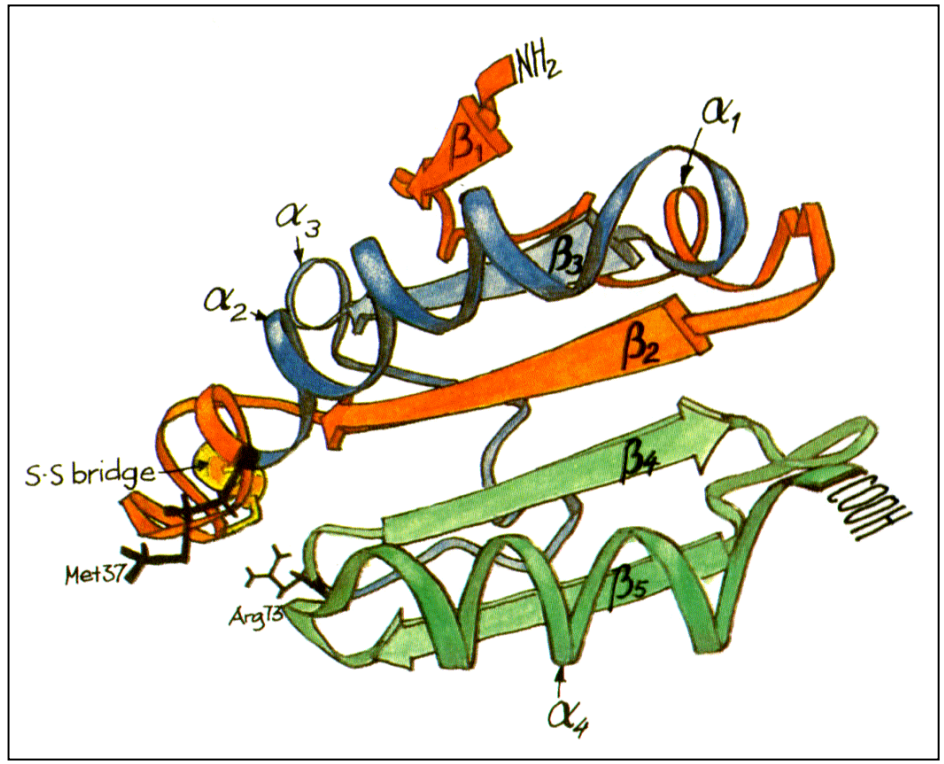
Background on Thioredoxin (Trx)

- Thioredoxin (Trx) is a 12-kDa protein with oxidoreductase activity.
- Trx is ubiquitous in both mammalian and prokaryotic cells.
- Trx was first identified in 1964 as a small redox protein from *Escherichia coli.*
- It was later re-discovered as adult T cell leukemia-derived factor (ADF) and an interleukin-2 receptor-inducing factor produced by human T-lymphotrophic virus type 1 (HTLV-1)-infected T cells.
- These proteins were found to be identical after the amino acid sequence of Trx was published (*Free Radic Biol Med*. **29**:312-322, 2000).

Structure of Thioredoxin (Trx)

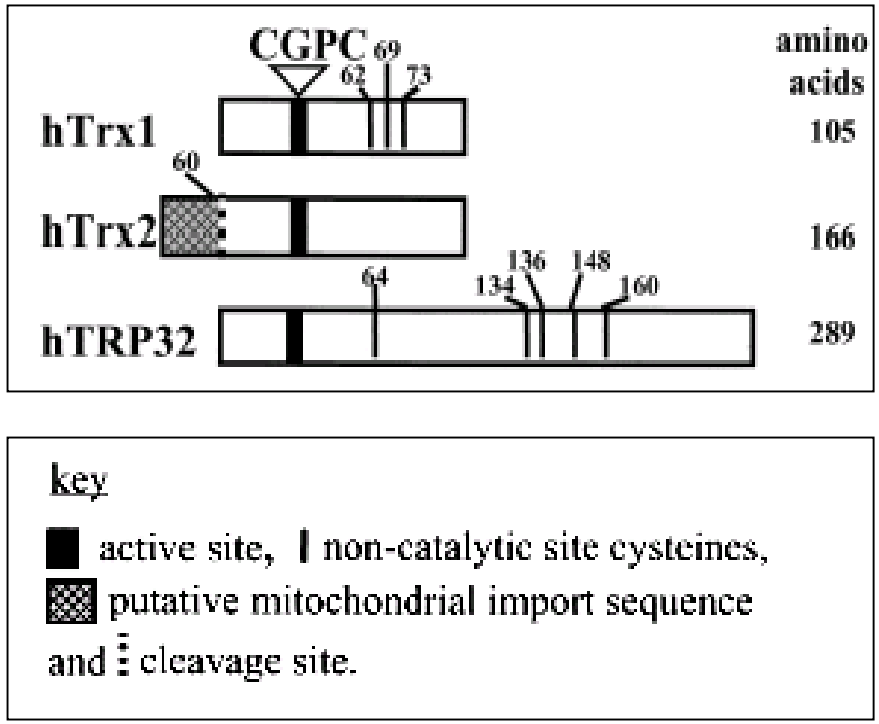
Thioredoxin has a stable tertiary structure known as the thioredoxin fold:

- Five-stranded β sheet that forms a hydrophobic core
- Four α helices on the external surface that surround the hydrophobic core
- The sulfhydryl bridge (S-S), located on the second β sheet to the second α helix, denotes the active site (*J Biol Chem*. **256**:6796-6803, 1981)



Mammalian Trx Family Members

- Thioredoxin-1 (Trx-1)
- Thioredoxin-2 (Trx-2)
- Thioredoxin-like protein TRP32
- Thioredoxins contain a conserved –Trp-Cys-Gly-Pro-Cys-Lys- redox catalytic site (*Free Radic Biol Med*. **29**:312-322, 2000)



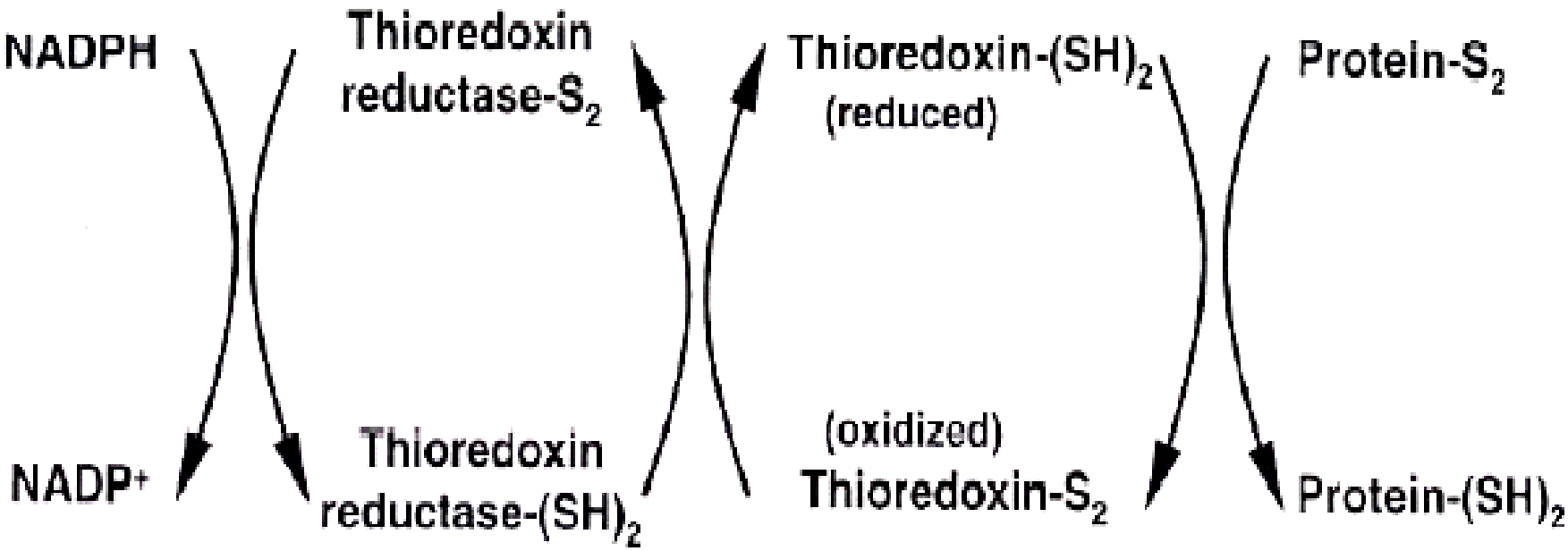
Trx Redox Biochemistry

- Trx conserved catalytic site –Trp-Cys-Gly-Pro-Cys-Lys- undergoes reversible oxidation through the transfer of reducing equivalents to a disulfide substrate ($X\text{-}S_2$) to form the cysteine-disulfide ($Trx\text{-}S_2$).
- The NADPH-dependent flavoprotein thioredoxin reductase (TR) then reduces the oxidized $Trx\text{-}S_2$ back to its free thiol form $Trx\text{-}(SH)_2$.
- This redox property allows Trx to act as an antioxidant, growth factor and regulator of transcription factors (*Free Radic Biol Med*. **29**:312-322, 2000):

$$Trx\text{-}(SH)_2 + X\text{-}S_2 \rightleftharpoons Trx\text{-}S_2 + X(SH)_2$$

$$Trx\text{-}S_2 + NADPH \xrightleftharpoons{TrxR} Trx\text{-}(SH)_2 + NADP^+$$

Trx, TrxR, and NADPH



This pathway illustrates the action of Trx:

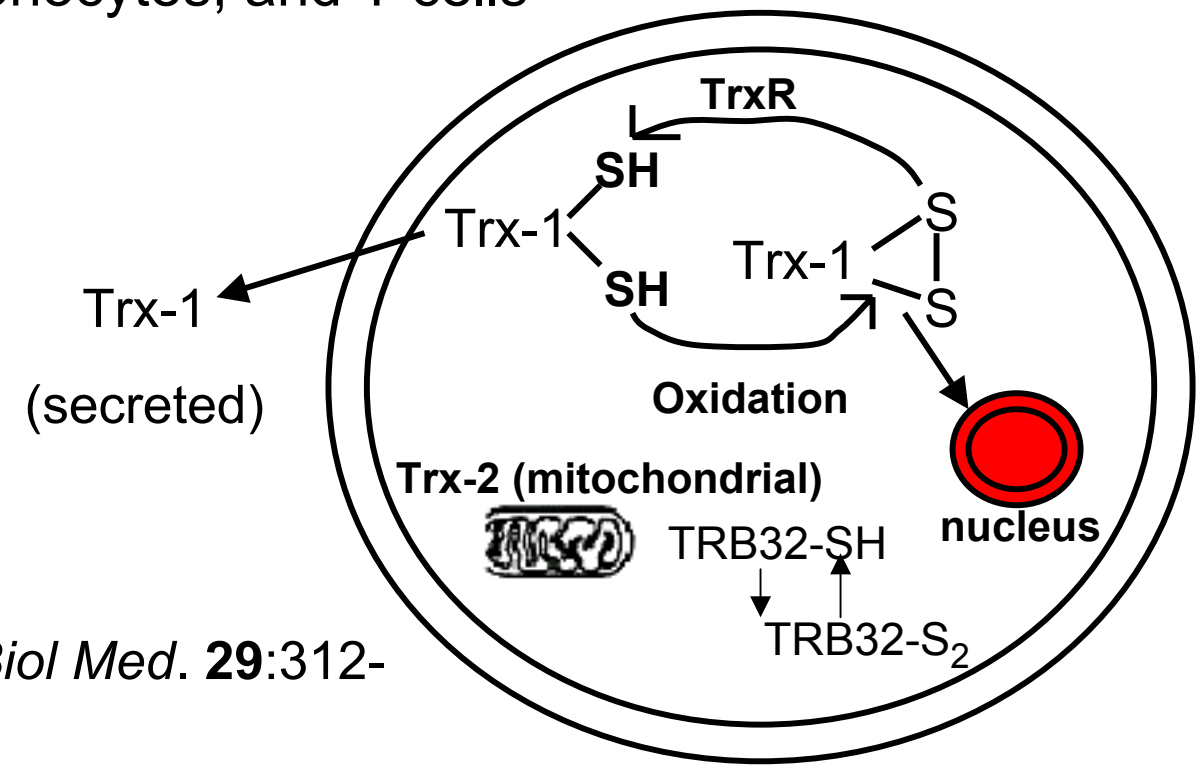
- NADPH and TrxR act to reduce Trx
- Trx then reduces cysteine residues on proteins

Trx Location-Intracellular

- Trx-1 is predominantly a cytosolic protein
  - Acts as a general protein disulfide oxidoreductase
  - Provides reducing equivalents to cytoplasmic thioredoxin peroxidases, which scavenge hydrogen peroxide and organic hydroperoxides (*Carcinog*. **10**:195-205, 2004)
- Trx-1 can also be found in the nucleus
  - Trx has no known nuclear localization sequence yet it has been detected in the nucleus
  - Trx has been suggested to bind to nuclear transport proteins, such as nuclear factor kappa B (NF-kB), in order to translocate to cell nucleus
- Trx-2 is found in the mitochondria
- TRB32 exists mainly in the cytosol (*Free Radic Biol Med*. **29**:312-322, 2000)

Trx Location-Extracellular

- The immune cells and various tumor cells secrete Trx-1 by a leaderless pathway.
- Extracellular Trx-1:
  - Functions as an autocrine growth factor
  - Induces chemotaxis of neutrophilic granulocytes, monocytes, and T cells



Free Radic Biol Med. **29**:312-322, 2000

Trx as an Antioxidant

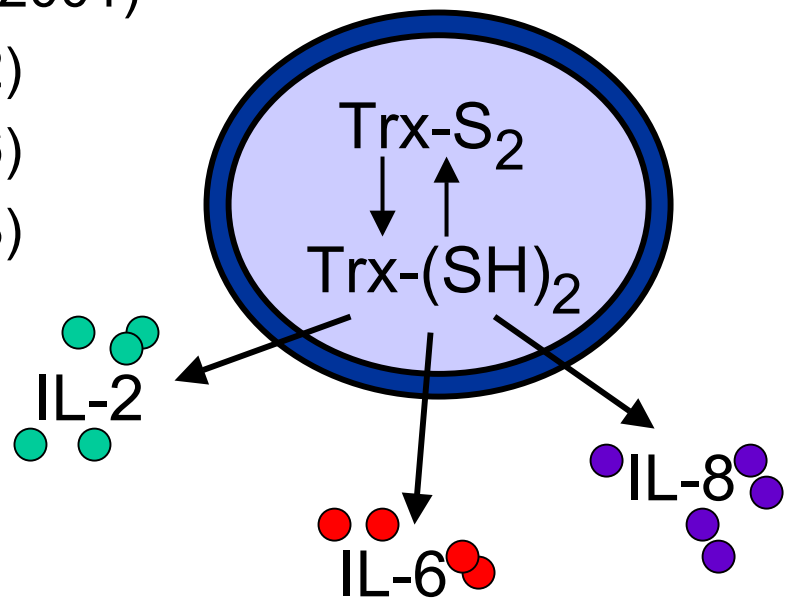
- Trx has been shown to reduce hydrogen peroxide (H_2O_2) (*J Biol Chem*. **263**:4984-4990, 1988):

  $$Trx\text{-}(SH)_2 + H_2O_2 \longrightarrow Trx\text{-}S_2 + 2H_2O$$

- Trx can function as an electron donor to human plasma glutathione peroxidase (*J Biol Chem*. **269**:29382-29384, 1994)
- Trx blocked nitric oxide (NO)-dependent inhibition of NO-synthase activity (*Am J Respir Cell Mol Biol.* **15**:410-419, 1996)
- Addition of Trx to A549 cells in vitro resulted in elevated mRNA levels and increased activity of manganese superoxide dismutase (MnSOD) (*Am J Respir Cell Mol Biol.* **17**:713-726, 1997).

Trx as a Growth Factor

- Trx stimulates growth of:
  - Lymphocytes
  - Normal fibroblasts
  - Hepatocytes
  - Cancer cells
- Trx has also been shown to enhance the secretion of cytokines associated with cell proliferation (*Free Radic Biol Med*. **31**:1287-1312, 2001)
  - Interleukin-2 (IL-2)
  - Interleukin-6 (IL-6)
  - Interleukin-8 (IL-8)



Trx as a Regulator of Transcription

- Nuclear Factor Kappa B (NF-kB)
  - Trx increases the DNA binding of NF-kB to DNA
  - DNA binding of NF-kB requires that cysteine 62 of p50 subunit be reduced by Trx
  - Trx is more potent activator of NF-kB than reduced glutathione and L-cysteine
  - Oxidized Trx inhibited the NF-kB binding to DNA (*Free Radic Biol Med*. **29**:312-322, 2000)
- Activating Protein-1 (AP-1)
  - Trx regulates activation of AP-1 (Fos and Jun homo- and heterodimers) by reducing a single conserved cysteine residue in the DNA binding domain
  - Trx does not reduce AP-1 directly but acts through nuclear redox protein Ref-1 (*Free Radic Biol Med*. **29**:312-322, 2000)

Trx as an Inhibitor of Apoptosis

- Stable transfection of mouse lymphoid cells with human Trx cDNA inhibited apoptosis induced by various agents (dexamethasone, thapsigargin, and etoposide).
- This inhibition was similar to the pattern of inhibition caused by transfection of the cells with the anti-apoptotic protein Bcl-2 (*Cancer Res.* **57**:5162-5167, 1997).
- Reduced Trx was shown to bind to and inhibit apoptosis signal-regulated kinase 1 (ASK-1).
- TRB32 was also found to bind to MST kinase, which is proteolytically activated by caspases during CD95/Fas-induced apoptosis (*Oncogene*. **16**:3029-3037, 1998).

Trx and Human Cancer

- Trx is overexpressed in human primary tumors compared to its levels in the corresponding normal tissue.

Table 1. Thioredoxin Overexpression by Human Primary Cancers

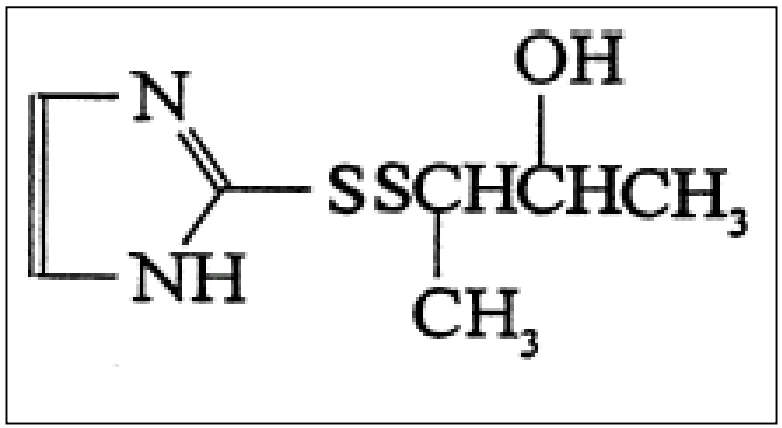
Tumor	Type	Number of subjects	Percent overexpressed	Reference
Lung	mRNA	10	50[a]	[6]
Colon	mRNA	10	60[a]	[54]
Cervix	Protein	79	78[b]	[110]
Hepatoma	mRNA, Protein	20	85	[104]
		25	52	[49]
Gastric	Protein	10		[41]
Squamous cell carcinoma	Protein	29	7 (differentiated)	[119]
		6 metastatic	6	
Myeloma	Protein	10	9	Unpublished
Non-Hodgkins lymphoma	Protein	20	7 (advanced)	Unpublished
Acute lymphocytic leukemia	Protein	33	15	Unpublished

[a] Compared to corresponding normal tissue from the same subject.
[b] Immunohistochemistry and comparison to normal tissue from the same subject.

Free Radic Biol Med. **29**:312-322, 2000

Trx and Human Cancer

- Trx overexpression in primary human tumors raise the possibility that Trx is involved in aggressive tumor growth and poor patient prognosis.
- Therefore, Trx could be a potential target for development of drugs to inhibit cancer growth.
- A few inhibitors of Trx have already been identified, including 1-methyl hydroxypropyl 2-imidazoloyl disulfide (PX-12).



PX-12

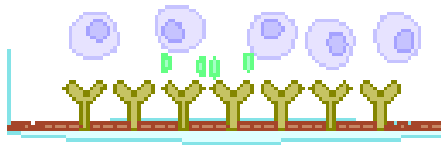
Free Radic Biol Med. **29**:312-322, 2000

Detection of Trx-ELISpot

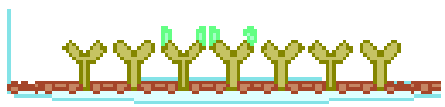
1 **Capture Antibody**

2 **Block unoccupied sites with protein**

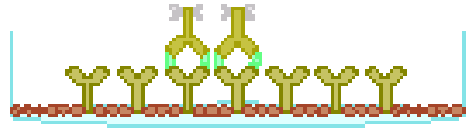
3 **Add cells**



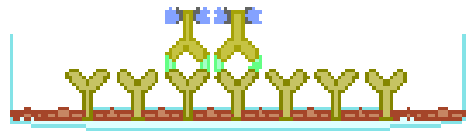
4 **Wash**



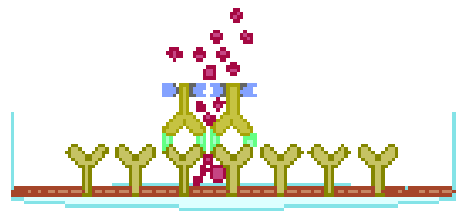
5 **Detection Antibody**



6 **Enzyme Avidin-HRP**



7 **Develop with substrate**



Methods Enzymol. **353**:22-35, 2002

Summary

- Trx is a protein with oxidoreductase activity.
- Three forms of Trx exist:
  - Trx-1 (cytosol)
  - Trx-2 (mitochondria)
  - TRB32 (cytosol)
- Trx has a conserved active site, -Trp-Cys-Gly-Pro-Cys-Lys-, where cysteine residues alternate between oxidized (S-S) and reduced (SH) states.
- The active site disulfide in Trx-S_2 is reduced to a dithiol in Trx-$(SH)_2$ by TrxR and NADPH.
- Trx uses its redox property to function as an antioxidant, growth factor and a regulator or transcription.
- Trx can work both inside and outside of the cell.