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p53: Redox Regulation

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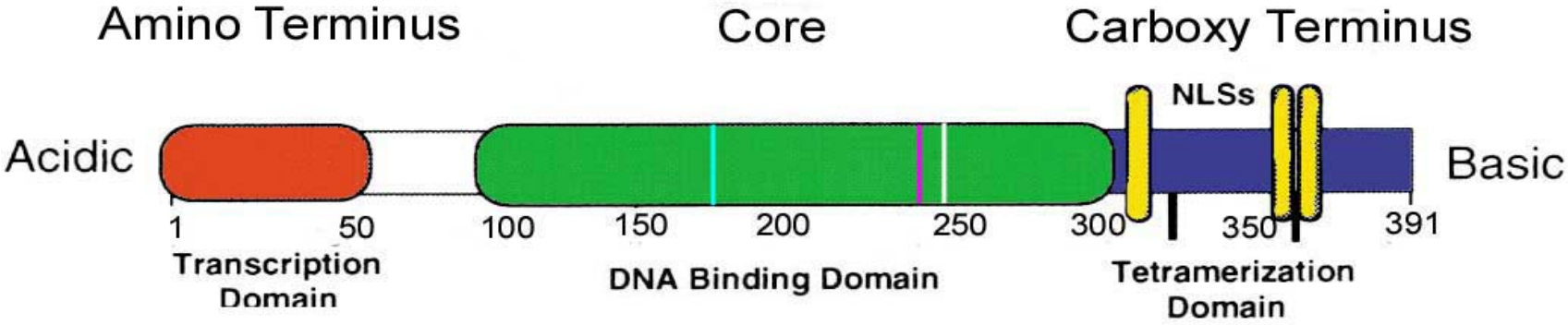
What is p53?

- A protein encoded by a TP53 gene that is located on human chromosome 17p 13.1; it regulates cell growth and is able to cause potentially cancerous cells to destroy themselves.
- In humans, a 393 residue phosphoprotein that is a tumor suppressor gene rather than an oncogene, because it is frequently inactivated or mutated in tumors (more than 50%) and transformed cells.
- Considered to be a guardian of the genome that maintains its genomic stability.
- First discovered almost 30 years ago as a 53 kDa cellular protein complexed with the Simian SV40 virus.
- Transcription factor (MW = 53 kDa), constitutively expressed in most cells and tissues.

Harris CC. Carcinogenesis. 1996; 17(6): 1187-98.

Structure of p53 is divided in three domains:



- Amino terminus: acidic domain containing a minimal transactivation domain, regulates transcriptive action of p53.
- Core domain: responsible for binding p53 to DNA.
- Carboxy terminus: contains multiple regulator signals and promotes the oligomerization of p53.

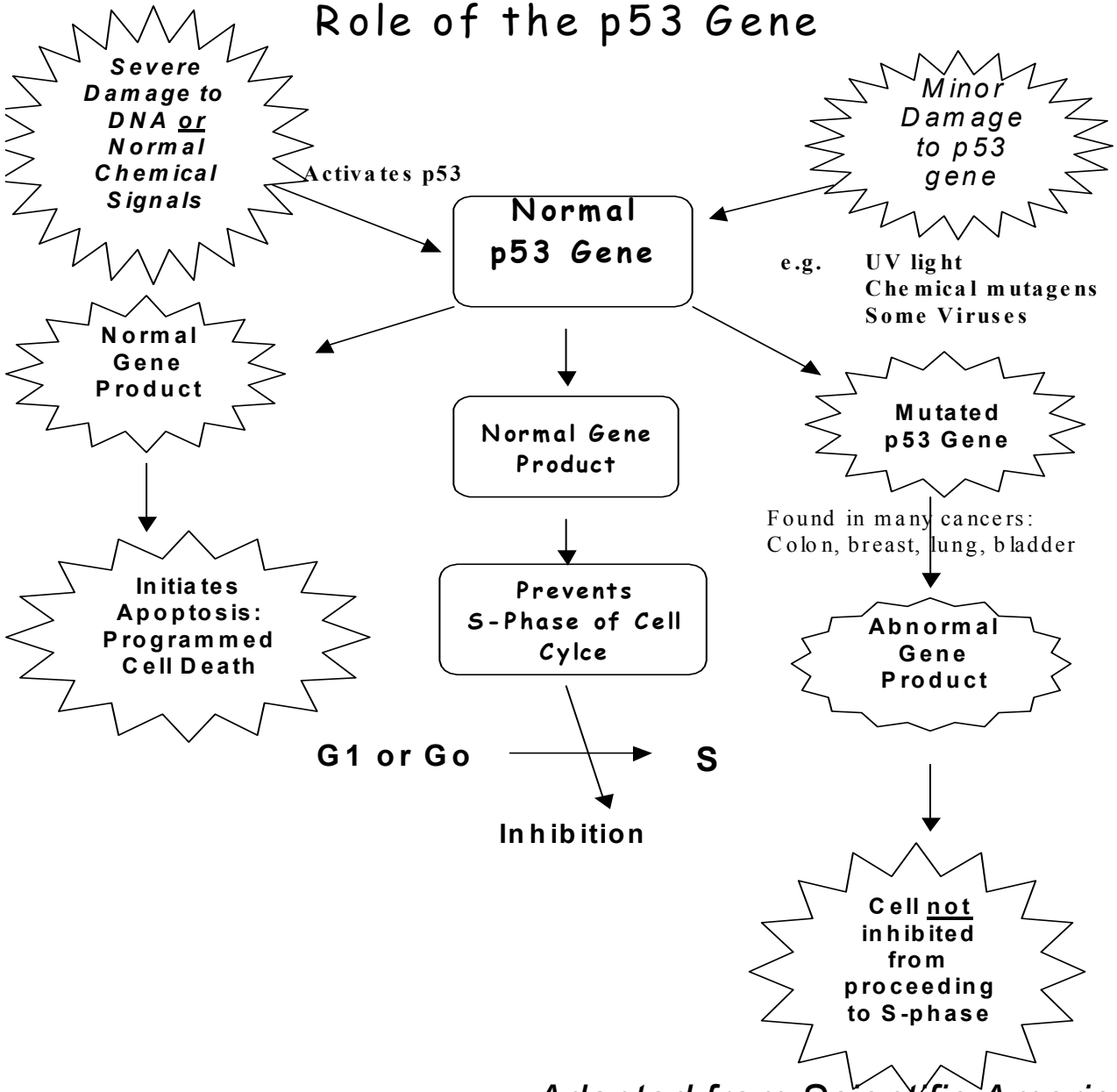
www.bimcore.emory.edu; *visited on 03/15/2005.*

Main functions of p53 in the cell:

- Involved in differentiation and development, DNA repair, DNA replication and transcription, senescence, and cell cycle checkpoints.
- Regulates cell cycle.
- Prevents genetic alterations.
- Induces apoptosis.

Bates S, Vousden KH. (1999) Mechanisms of p53-mediated apoptosis. Cell Mol Life Sci. ***55****: 28-37.*

Role of the p53 Gene



Adapted from Scientific American, 9/96.

p53 in normal versus abnormal cells:

- In normal cells, p53 has a rapid intracellular turnover and does not accumulate in cells.
- In abnormal cells, p53 is **induced** by stress-related signals:
  - *upstream signals* represent a form of cellular or genotoxic stress (DNA-damaging chemicals, irradiation, heat, depletion of ribonucleotides, hypoxia).
  - *downstream signals* are involved in overlapping, antiproliferative pathways modulating cell-cycle progression, apoptosis, DNA repair, differentiation, and senescence.

Agarwal ML et al. (1998) The p53 network. J Biol Chem. ***273****: 1-4.*

Activation of p53:

- Is thought to take place at both translational and post-translational level.
- Is thought to be prevented by p53 oxidation.

*(mentioned in Wu et al.(2000) p53 protein oxidation in cultured cells in response to pyrrolidine dithiocarbamate: a novel method for relating the amount of p53 oxidation in vivo to the regulation of p53-responsive genes. Biochem J. **351**: 87-93.)*

Stressors that perform redox modulation of p53:

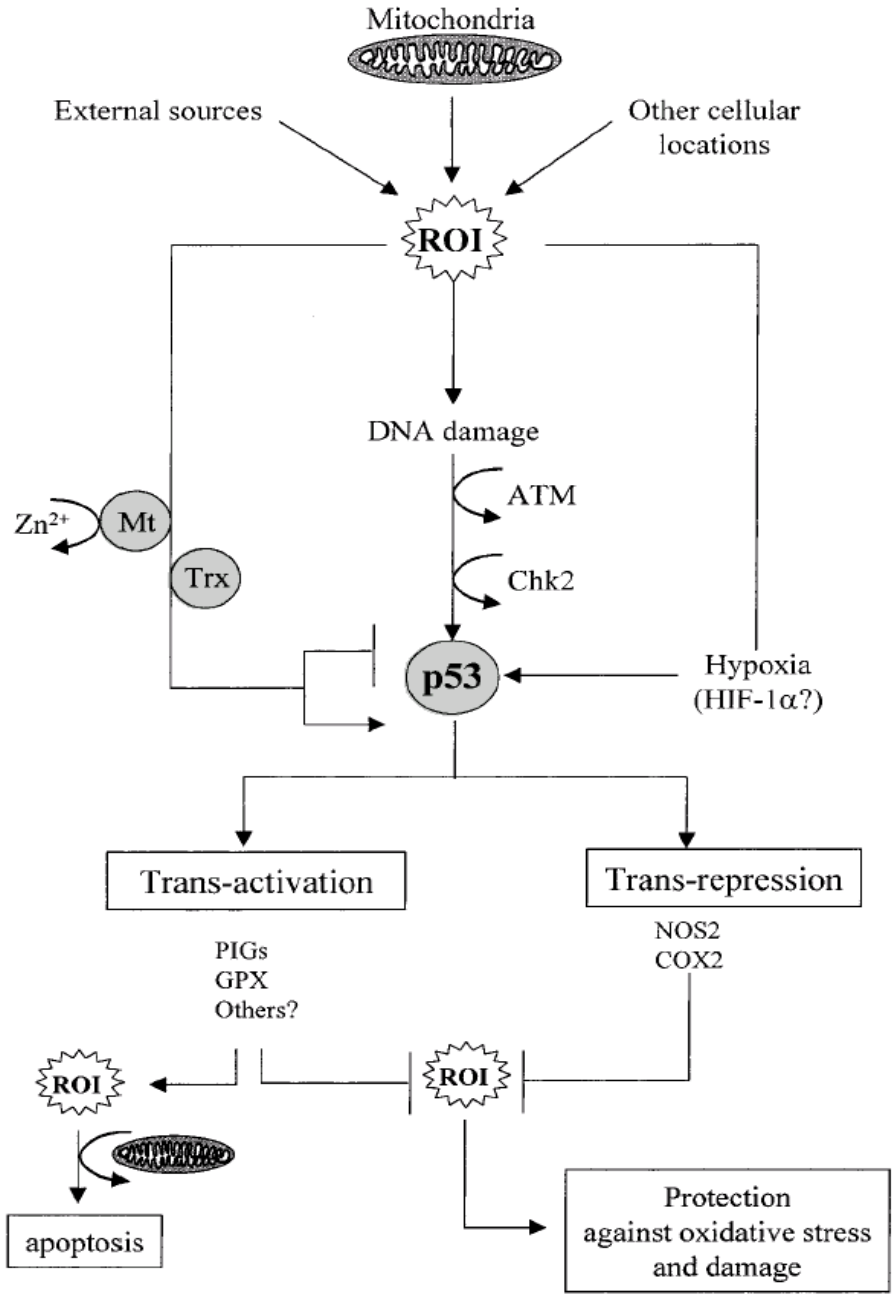
- Ultraviolet (UV) light.
- Ionizing radiation.
- Heat shock.
- Hypoxia.
- Hydrogen peroxide.
- **Reactive oxygen intermediates (ROI):**

ROI play distinct roles in the p53 pathway:

- Important activators of p53 due to their capacity to induce DNA strand breaks. *(Graeber et al. (1996) Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumors. Nature. **379**: 88-91.)*
- Regulate DNA-binding activity of p53 by modulating the redox status of a critical set of cysteines in the DNA-binding domain. *(Hainaut et al., (1993) Redox modulation of p53 conformation and sequence-specific DNA binding in vitro. Cancer Res. **53**: 4469-73.)*
- Play role in the **signaling pathways** regulated by p53 since p53 regulates several genes involved in ROI metabolism. *(e.g. Forrester et al., (1996) Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase expression by wt p53. Proc Natl Acad Sci USA. **93**: 2442-47.)*

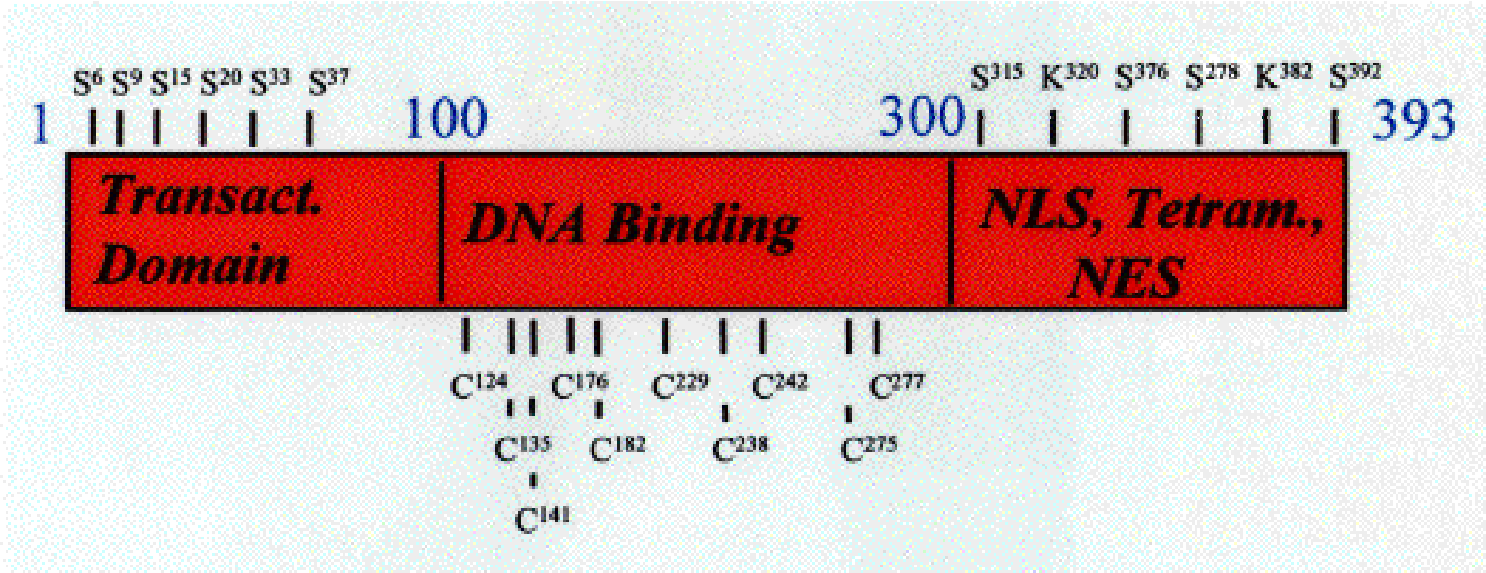
ROI and p53 signaling pathways:

Reactive Oxygen Intermediates are involved in a direct or indirect manner at several levels in the p53 signaling pathways, both as upstream regulators and downstream effectors.



Adapted from Hainaut et al. (2001) Zinc binding and redox control of p53. Andioxidants and redox signaling. ***3(4)****: 611-23.*

Mechanism of direct redox changes in p53 is regulated by direct alteration of its cysteine residues oxidation:



Primary protein sequence of p53. The numbered serine (S) and lysine (K) residues are sites of phosphorylation and acetylation respectively. All cysteine residue positions are shown.

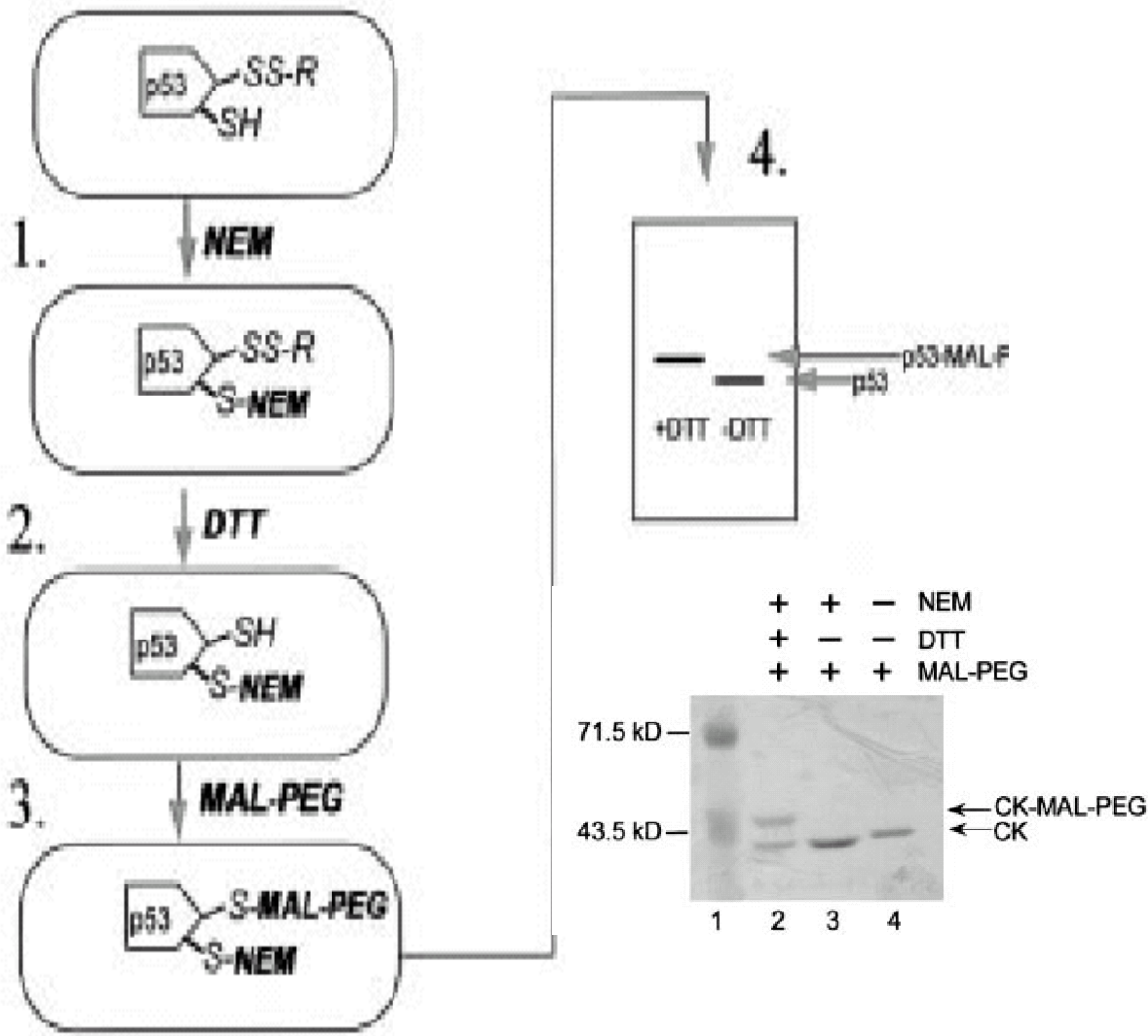
Adapted from Wu et al. (1999) Direct redox modulation of p53 protein. Gene Ther Mol Biol. ***4****: 119-32.*

Oxidation of cysteine residues in p53 can prevent its proper binding to DNA consensus sequence:

- Support: high concentrations of dithiothreitol (*DTT*, prevents oxidation) required for p53 to bind DNA.
- Support: inability of p53 to bind DNA consensus sequence when treated with the thiol alkylating agent N-ethyl maleimide (*NEM*, a sulfhydryl reagent). *(Rainwater et al. (1995) Role of cysteine residues in regulation of p53 function. Mol Cell Bol. **15**: 3892-3903.)*
- Therefore, it is important to maintain p53 cysteine residues in the reduced state for optimal p53 consensus sequence-dependent DNA binding.

Detecting p53 oxidation:

- Performed by a thiol-group tagging procedure
  - **NEM**: used to block all free thiol groups.
- **DTT**: used to reduce oxidized thiol groups that were resistant to NEM derivatization.
- **MAL-PEG** (methoxy-polyethylene glycol-maleimide): used to tag disulfide linked thiols that indicate p53 oxidation.



*Adapted from Wu et al. (2000) p53 protein oxidation in cultured cells in response to pyrrolidine dithiocarbamate. Biochem J. **351**: 87-93.*

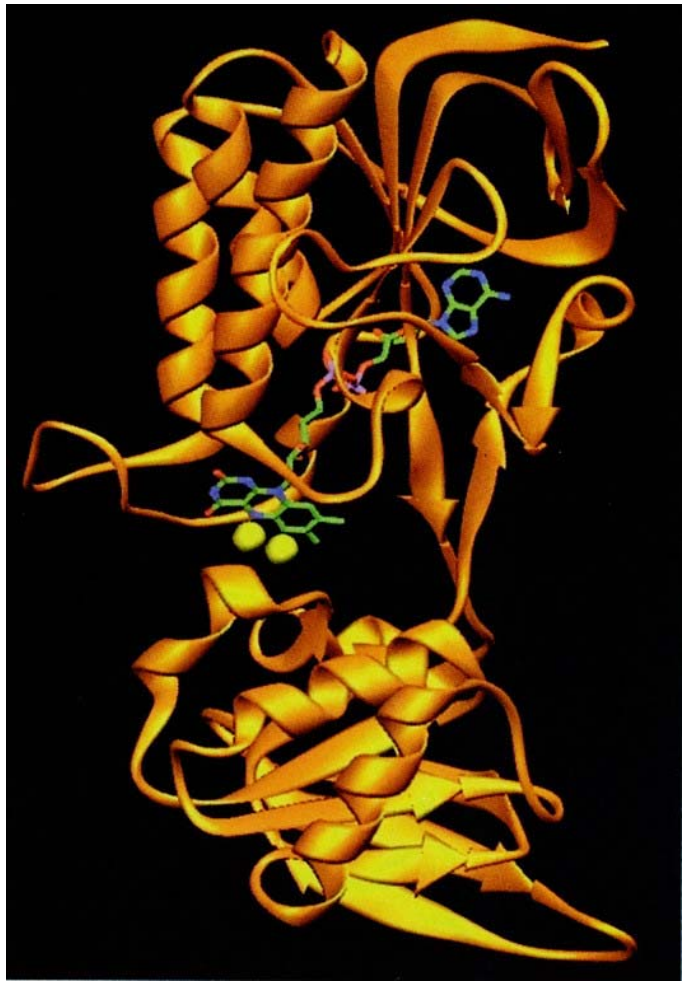
Enzymes that may be responsible for maintaining p53 in the reduced state:

- Redox factor-1(Ref-1):
  - a multifunctional protein that increases recombinant p53 binding to a p53 consensus sequence;
  - previously shown to increase the activity of Fos-Jun heterodimer binding to DNA;
  - in the presence of DTT, stimulates consensus DNA binding of full-length p53; this activity is inhibited when p53 lacks its C-terminal residue;
  - does not form a stable complex when reacting with p53
  - according to data, can stimulate p53 DNA binding activity through a non-redox and a redox mechanism.

Wu et al. (1999) Direct redox modulation of p53 protein. Gene Ther Mol Biol. ***4****: 119-32.*

Enzymes that may be responsible for maintaining p53 in the reduced state:

- Thioredoxin reductase:
  - a protein disulfide reductase that catalyzes NADPH-dependent reduction of the active site disulfide in oxidized thioredoxin;
  - was shown to rescue p53-dependent growth arrest by maintaining its transcriptional activity;
  - required to reduce the disulfide bond and restore p53 function.



*Wu et al. (1999) Direct redox modulation of p53 protein. Gene Ther Mol Biol. **4**: 119-132.*

Adapted from www.opbs.okstate.edu; visited on 03-18-2005.

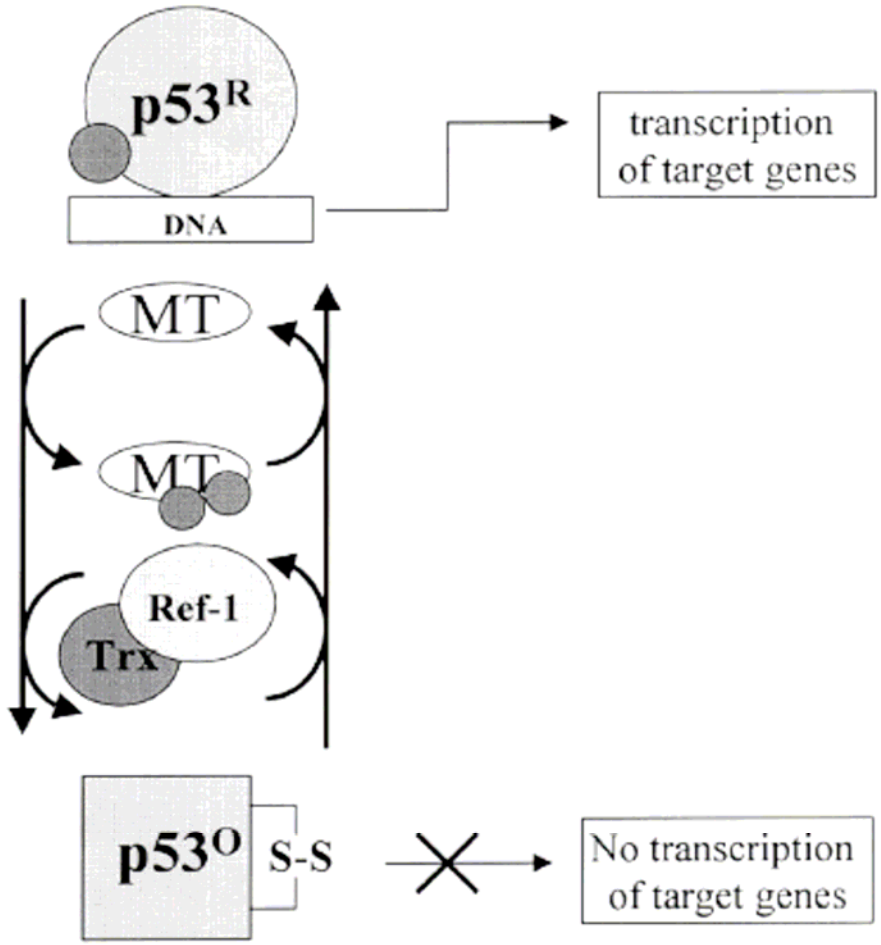
Enzymes that may be responsible for maintaining p53 in the reduced state:

- Metallothionein (MT):
  - an inducible protein that can bind up to seven zinc equivalents;
  - protects against toxic metal stress and controls physiological metal transfer reactions;
  - depending on the concentration, can help p53 to fold in the wild-type DNA binding conformation (low levels) or can act as a chelator and sequester metals (Zn^{2+}) which prevents the protein from folding into its active conformation (high levels).

*Wu et al. (1999) Direct redox modulation of p53 protein. Gene Ther Mol Biol. **4**: 119-132.*

A hypothetical scheme of control of p53 protein redox & activity:

The p53 protein is shown as being able to oscillate between two conformations, reduced active (circle) and oxidized inactive form (square). Metal ions (Zn^{2+}) are represented as small dark circles. The transition of p53 from oxidized to reduced form and back is regulated by Ref-1, Trx, and MT.



Adapted from Hainaut et al. (2001) Forum Review. Antiox Redox Signaling. ***3(4)****: 611-23.*

P53 can be redox regulated due to its cysteine residues:

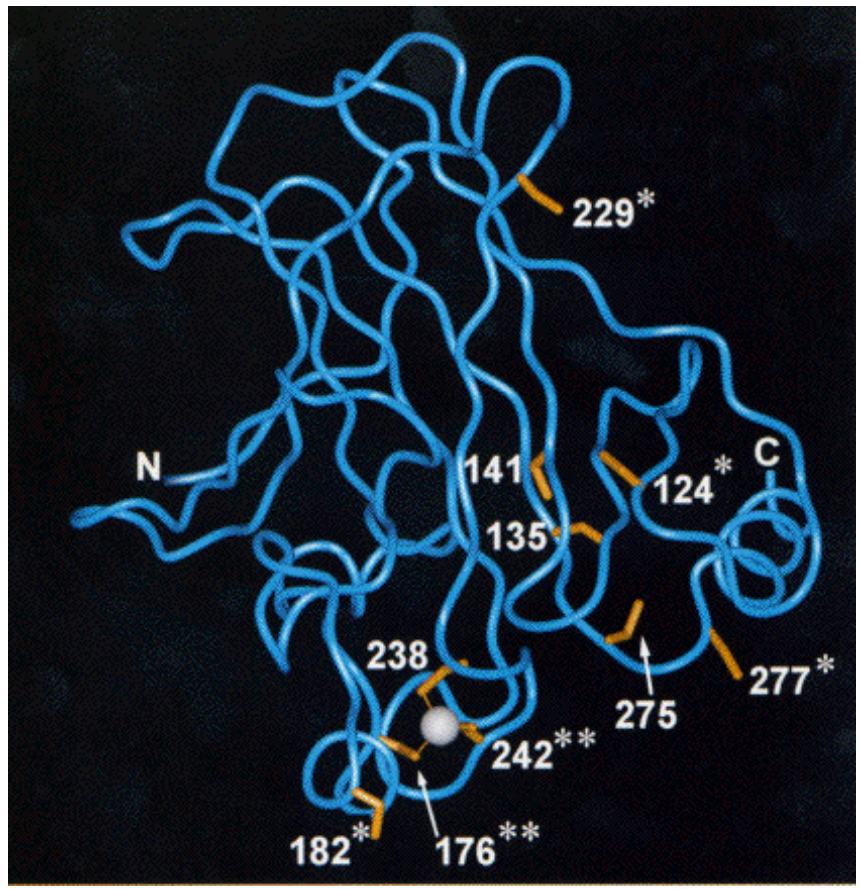
- Crystal structure of p53 reveals 10 cysteine residues.
- Based on the exposure to the solvent, cysteine residues are divided in three groups:

  1) a group that directly interacts with metals (Zn^{2+}) and is essential for DNA binding: sites 176, 238, 242;
  2) a group that is required for transactivation and suppression function: sites 124, 135, 141, 275;
  3) a group that does not exhibit any alterations when activities of p53 are measured: sites 182, 277.

Hainaut et al. (2001) Forum Review. Antiox Redox Signaling. ***3(4):*** *611-23.*

Potential sites of p53 cysteine residue oxidation:

- Oxidation of any of these residues may alter p53 activities.
- According to the crystal structure analysis, only two of these residues can theoretically form a disulfide bond: Cys 176, and Cys 242.



Adapted from Hainaut et al. (2001) Forum Review. Antiox Redox Signaling. ***3(4):*** *611-23.*

Summary:

- In normal cells, p53 is degraded whereas in abnormal cells it accumulates.
- P53 can be redox regulated, although this regulating is still poorly understood.
- ROI are important stressors in p53 redox regulation pathways.
- Redox regulation of p53 is possible due to its cysteine residues that can participate in forming sulfide bonds.
- More research needs to be done for better understanding of p53 redox regulation enzymes.

p53 can be regulated by:

- Phosphorylation
- Acetylation
- Ribosylation
- O-glycosylation
- Ubiquitination
- SUMOylation
- **Redox Regulation**