Virtual Free Radical School

MnSOD:
A Special Enzyme In A Special Place

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Superoxide Dismutase

- Superoxide dismutase (SOD) is one of the primary antioxidant enzymes.
- The first SOD was discovered by J. McCord and I. Fridovich in 1969. (McCord, & Fridovich, *JBC* 244:6049, 1969)
- Three members of SOD family have been identified in eukaryotes. They are copper-zinc containing SOD (CuZnSOD), manganese containing SOD (MnSOD), and extracellular SOD (EcSOD).
- Two isoforms of SOD in prokaryotes are FeSOD and MnSOD.
- SOD catalyzes the conversion of superoxide ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2).

$$2\ O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

How is MnSOD Made?

- MnSOD is encoded by nuclear chromatin in eukaryotic cells
- MnSOD mRNA migrates to the cytosol.
- MnSOD protein is made in ribosome as a precursor form, ~223 a.a.
- The MnSOD precursor is transported post-translationally into the mitochondrial matrix.
- The mitochondrial target sequence is clipped by a protease in an energy dependent manner; mature protein for human and most eukaryotes is then assembled into a tetramer.
- Eukaryotic MnSOD resides in the mitochondrial matrix.

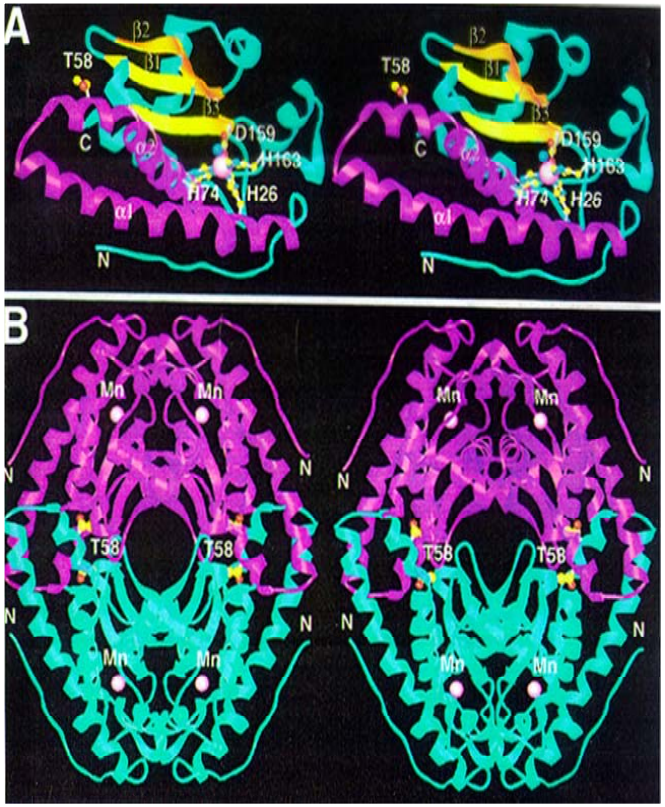
(Wispe, *BBA* 994:30036, 1989)

Facts About MnSOD Protein

- Most MnSODs from eukaryotes are homotetramers with four subunits and a total molecular weight of ~88 kDa.
- Most MnSODs are acidic, pI = 4-5.
- MnSOD can be inactivated by freeze/thawing processes; CuZnSOD is more resistant to this process.
- Human liver MnSOD was first sequenced in 1984, discovering 196 amino acids in each subunit.
- MnSOD is a highly conserved protein with >40% sequence homology among human, yeast, *E. coli,* and *B. stearothemophilus*, but no homology between MnSOD and CuZnSOD.
- Two cysteine residues were found in each subunite of human liver MnSOD with not disulfite bond within and between the subunites. Cysteine appears to be specific for the eukaryotic MnSOD.

(Barra, *JBC* 259:12595-12601, 1984)

The Structure of MnSOD



Each newly synthesized human MnSOD subunit has 223 amino acids. After transport into mitochondria, each mature MnSOD subunit has 198 amino acids.

One subunit has dimensions of about 40 X 47 X 49 Å and can be divided into two distinct domains: an N-terminal helical hairpin domain and a C-terminal a/b domain, containing a three-stranded antiparallel sheet and five helices.

Two subunits of MnSOD pair into a dimer with the active site manganese atoms near the dimer interface. Residues D159, H163, H26, H74, and a water molecule from each subunit contribute to the metal-binding site. For human MnSOD, two dimers further associate into a homotetramer with the dimensions of about 60 x 79 x 79 Å. (Borgstahl, *Cell* 71:107, 1992)

Polymorphism/mutation of MnSOD

Three forms of MnSOD polymorphism variant or mutation have been discovered.

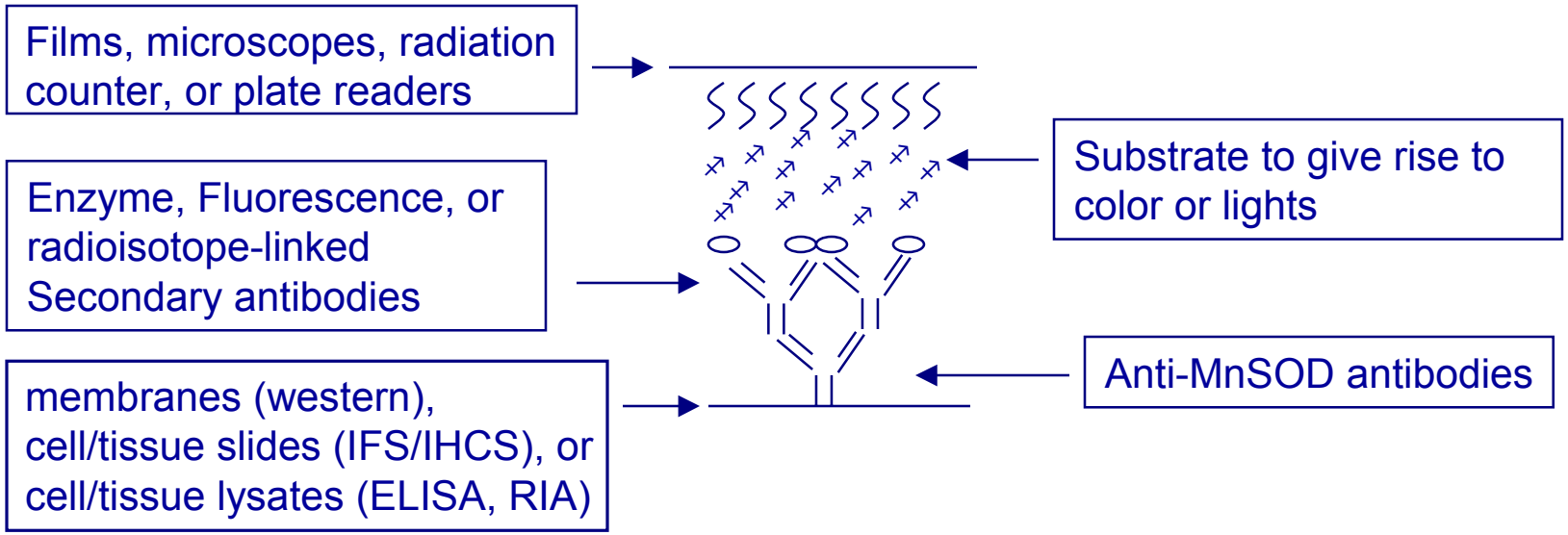
- In a genetic polymorphism variant of MnSOD, valine is changed to alanine at -9 position in the signal peptide. It was suggested that this change might affect the cellular location and mitochondrial transport of MnSOD. (Rosenblum, *PNAS* 93: 4471, 1996)

- A polymorphic variant/mutation of MnSOD at 58 position from isoleucine to threonine has decreased thermal stability and reduced enzymatic activity *in vivo and in vitro.* (Borgstahl, Biochemistry 35: 428, 1996; Zhang, *Cancer Res*. 59:6276, 1999)

- A mutation of MnSOD at 60 position from leucine to phenylalanine renders the MnSOD protein sensitive to redox regulation by intracellular thiols. (Hernandez-Saavedra and McCord, *Cancer Res.* 63:159, 2003)

Measurements of MnSOD
(Protein determination)

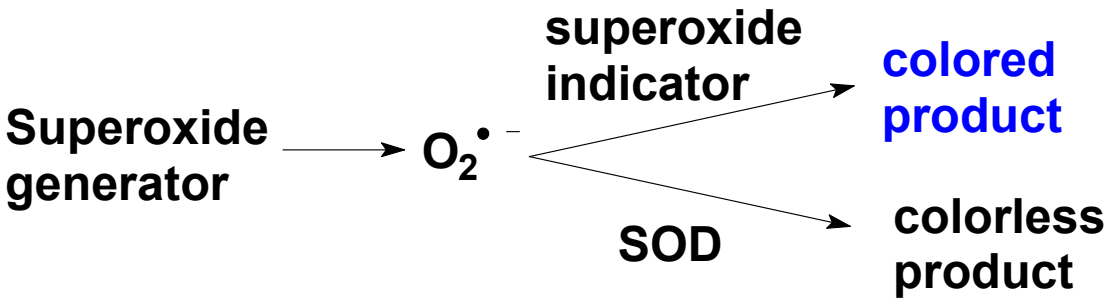
MnSOD protein can be detected by Western blotting analysis, immunofluorescent (immunohistochemistry) staining (IFS/IHCS), ELISA (enzyme-linked immunosorbent assay), or RIA (radioimmunoassay) using specific rabbit anti-human MnSOD serum.



A high quality rabbit anti-human MnSOD antibody is available from Upstate Group Inc., Waltham, MA and will cross-react with MnSOD of rat, mouse, dog, and hamster origin.

Measurements of MnSOD
(Activity assays)

Most assays are based on competition between SOD and $O_2^{\bullet -}$ indicators.



- Xanthine/xanthine oxidase is often used as superoxide generator.
- Cytochrome c is the oldest and most specific superoxide indicator.
- Nitroblue tetrazolium (NBT) is more sensitive but less specific than cytochrome c.
- Activity gel is the least sensitive but the easiest, and less labor intensive.

(Spitz, & Oberley, *Anal. Biochem*. 179:8, 1989;
Beauchamp, & Fridovich, *Anal. Biochem*. 44:276,1971)

Manipulation of Cellular MnSOD Levels

- Induction or suppression of endogenous MnSOD gene expression by chemicals, heating, radiation, or cytokines such as TNF-α.

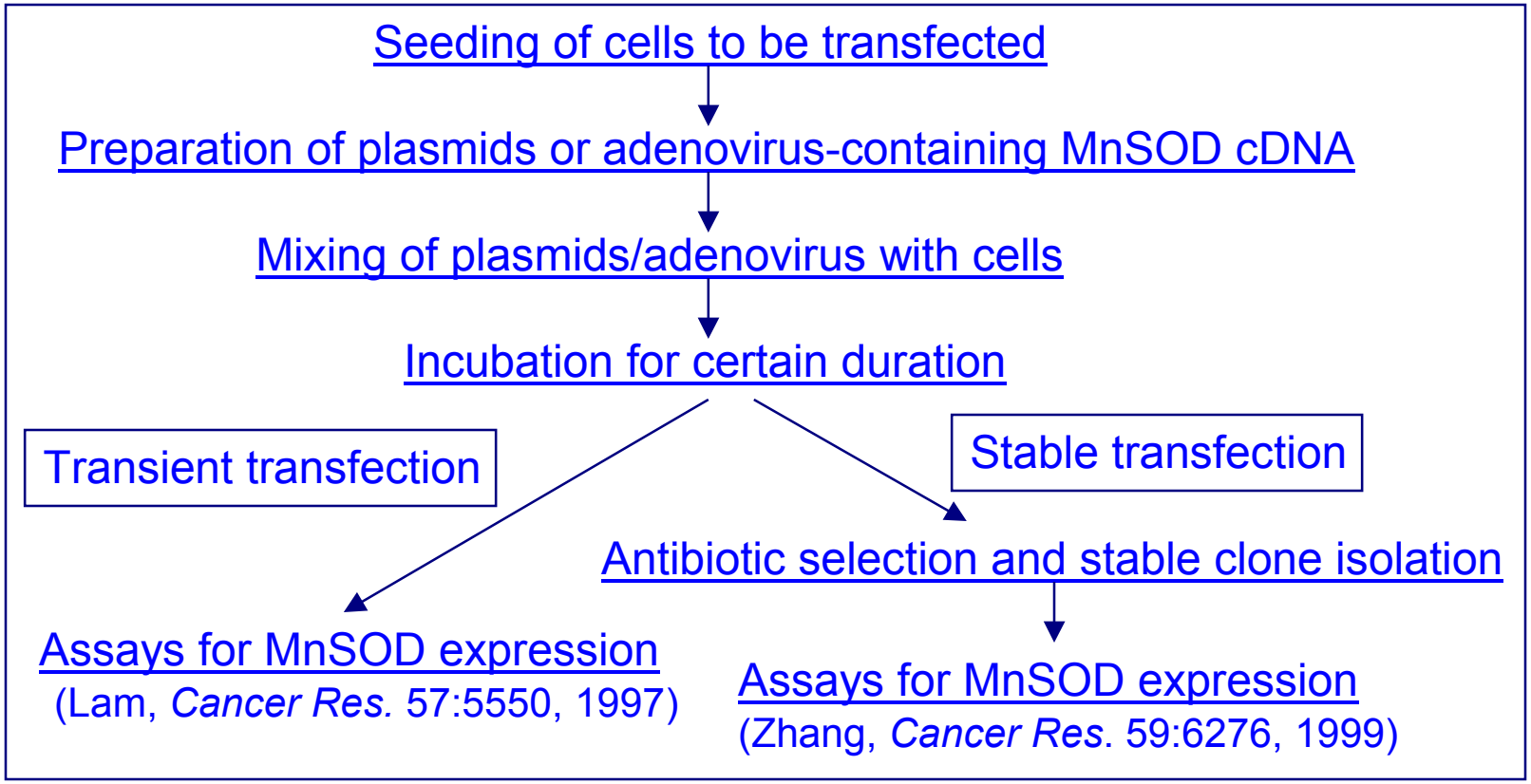
- Direct introduction of exogenous MnSOD protein by liposomal SOD or conjugated enzymes such as polyethylene glycol-SOD (Peg-SOD).

- Transfection/transduction of exogenous MnSOD cDNA by vector such as plasmids or adenovirus.

- Inhibition of endogenous MnSOD expression by antisense or RNAi techniques.

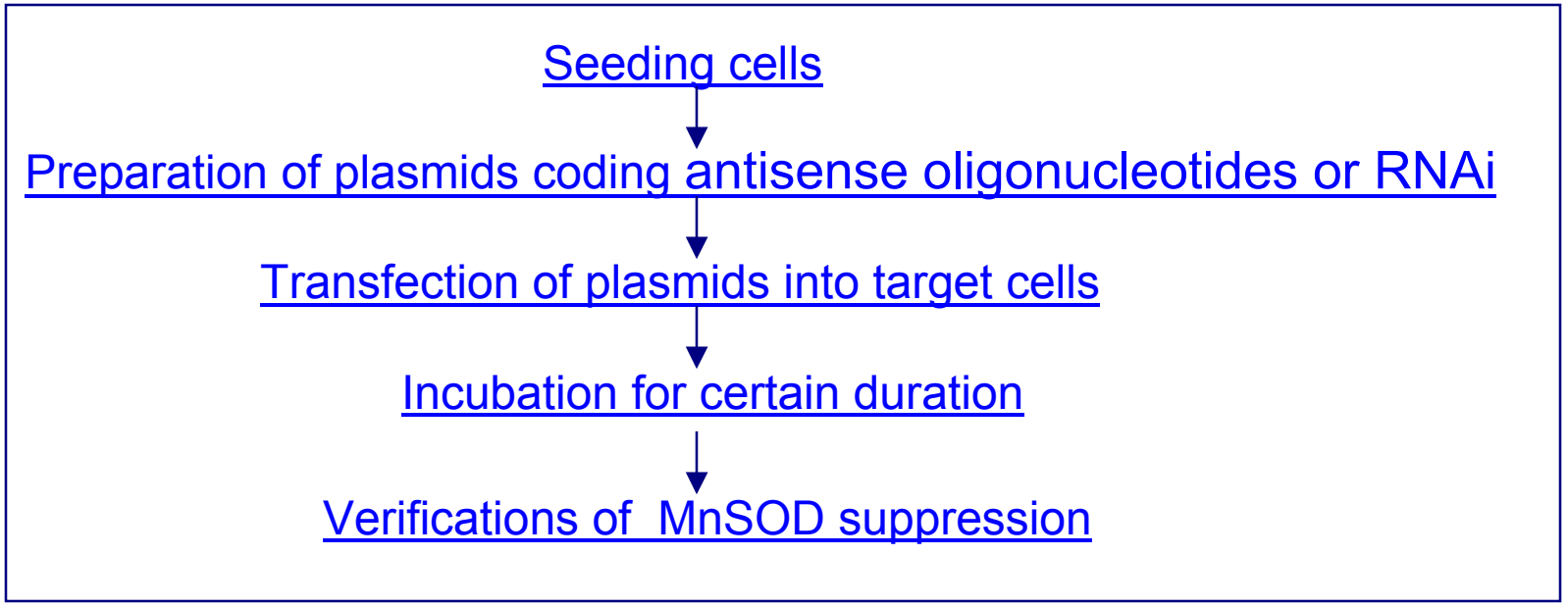
Transfection of MnSOD

Overexpression of MnSOD can be achieved by either stable transfection or transient transfection. The procedures for different forms of transfection are described in following diagram.



Suppression of MnSOD

Suppression of MnSOD can be achieved using antisense oligonucleotides or RNA interference techniques.



Biological Effects of MnSOD

MnSOD is necessary for aerobic life:

- MnSOD knock-out mice die within 1-18 days after birth, depending on their genetic background . (Li, *Nat. Genet*. 11:376, 1995)

MnSOD modulates intracellular signal pathways and gene expression

- MnSOD modulates the activation of several redox-sensitive transcriptional factors, such as NFκB and AP-1. (Zhang, *J. Virology* 76:355, 2002)

- MnSOD alters gene expression associated with apoptosis. (Manna, *JBC* 273:13245, 1998; Kiningham, *FASEB J.* 13:1601, 1999)

Biological Effects of MnSOD

MnSOD is important in maintaining intracellular ROS and redox balance:

- Increased MnSOD protects normal tissue against oxidative stress. (Epperly, *Military Medicine* 167:71, 2002)
- However, overexpression of MnSOD exceeding physiology conditions can lead to the accumulation of ROS and oxidative stress, which may contribute to tumor metastasis and angiogenesis. (Zhang, *JBC* 277:20919, 2002)
- On the other hand, the accumulation of ROS by overexpressing MnSOD and inhibition of H_2O_2 removal may beneficial to tumor therapy. (Oberley, *Antioxidants and Redox Signaling* 3:461, 2001)

Biological Effects of MnSOD

MnSOD is a tumor suppressor:

- Increasing MnSOD expression can suppress tumor cell growth *in vitro* and tumor formation in nude mice in a large variety of cancer types. (Oberley, *Antioxidants and Redox Signaling* 3:461, 2001)
- MnSOD transgenic mice showed resistance to chemical induced tumor formation and oxidative stress. (Zhao, *Cancer Res*. 61:6082, 2001)
- A 4-fold increase in breast cancer risk in premenopausal women who have homozygous for a MnSOD polymorphic variant (alanine at -9 position) was observed. (Ambrosone, *Cancer Res*. 59:603, 1999)

Summary

MnSOD is an critical antioxidant enzyme residing in mitochondria.

Overexpression of MnSOD can be achieved by stable or transient transfection.

MnSOD plays important and essential biological functions in aerobic life.