Free Rad. Res., Vol. 34, pp. 325-336
Reprints available directly from the publisher
Photocopying permitted by license only

Invited Review
Manganese Superoxide Dismutase in Disease

LEE ANN MACMILLAN-CROW[a,b,c,*] and DANIELLE L. CRUTHIRDS[c]

[a]*Departments of Surgery,* [b]*Pathology and* [c]*Pharmacology, University of Alabama at Birmingham 1900 8th Avenue South Birmingham AL 35294 USA*

Accepted for publication by Prof. V. Darley-Usmar

(Received 13 August 2000)

Manganese superoxide dismutase (MnSOD) is essential for life as dramatically illustrated by the neonatal lethality of mice that are deficient in MnSOD. In addition, mice expressing only 50% of the normal compliment of MnSOD demonstrate increased susceptibility to oxidative stress and severe mitochondrial dysfunction resulting from elevation of reactive oxygen species. Thus, it is important to know the status of both MnSOD protein levels and activity in order to assess its role as an important regulator of cell biology.

Numerous studies have shown that MnSOD can be induced to protect against pro-oxidant insults resulting from cytokine treatment, ultraviolet light, irradiation, certain tumors, amyotrophic lateral sclerosis, and ischemia/reperfusion. In addition, overexpression of MnSOD has been shown to protect against pro-apoptotic stimuli as well as ischemic damage. Conversely, several studies have reported declines in MnSOD activity during diseases including cancer, aging, progeria, asthma, and transplant rejection. The precise biochemical/molecular mechanisms involved with this loss in activity are not well understood. Certainly, MnSOD gene expression or other defects could play a role in such inactivation. However, based on recent findings regarding the susceptibility of MnSOD to oxidative inactivation, it is equally likely that post-translational modification of MnSOD may account for the loss of activity. Our laboratory has recently demonstrated that MnSOD is tyrosine nitrated and inactivated during human kidney allograft rejection and human pancreatic ductal adenocarcinoma. We have determined that peroxynitrite ($ONOO^-$) is the only known biological oxidant competent to inactivate enzymatic activity, to nitrate critical tyrosine residues, and to induce dityrosine formation in MnSOD. Tyrosine nitration and inactivation of MnSOD would lead to increased levels of superoxide and concomitant increases in $ONOO^-$ within the mitochondria which, could lead to tyrosine nitration/oxidation of key mitochondrial proteins and ultimately mitochondrial dysfunction and cell death. This article assesses the important role of MnSOD activity in various pathological states in light of this potentially lethal positive feedback cycle involving oxidative inactivation.

Keywords: MnSOD, tyrosine nitration, mitochondria, peroxynitrite, disease, activity, nitric oxide, transplant, ischemia/reperfusion, apoptosis, and cancer

Abbreviations:(MnSOD), Manganese superoxide dismutase; ($ONOO^-$), peroxynitrite; (NO), nitric oxide; ($O_2^{\cdot-}$), superoxide; (ROS), reactive oxygen species; (I/R), ischemia/reperfusion

BIOLOGICAL IMPORTANCE OF MNSOD

MnSOD (also referred to as SOD2) is the major antioxidant in the mitochondria. Its primary function is to catalyze the dismutation of super-

* Corresponding Author.

oxide ($O_2^{\cdot-}$) which is continually generated via misfires in the electron transport chain as well as other sources. MnSOD is a nuclear encoded protein that is transported into the mitochondria via an amino-terminal targeting sequence and subsequently cleaved to form its native homotetrameric structure of 96 kDa. Recent studies with MnSOD knockout mice provide unequivocal evidence that MnSOD is essential for life--a finding that presumably extends to humans as well (see "KnockOut" section below). This is not the case for the other two superoxide dismutase family members--the cytosolic Cu, Zn SOD (SOD1) or the extracellular (EC) SOD (SOD3)- in which no lethality is observed following gene deletion [1,2].

MNSOD KNOCKOUT STUDIES

The essential nature of MnSOD was demonstrated by the seminal discovery by Li et al. [3] and Lebovitz et al. [4] that deletion mutation of the MnSOD gene in mice resulted in death within 5–21 days of birth. The neonatal mice exhibit several pathological features including: myocardial injury, neurodegeneration, lipid peroxidation, fatty liver, anemia, and severe mitochondrial damage depending on what strain background the mutant gene was expressed. Specifically, the individual respiratory chain enzymes of these animals were significantly affected. Complex I of heart mitochondria appeared to be deficient and histochemical staining indicates a significant decrease in succinate dehydrogenase activity. There was also a marked deficiency in complex II activity as seen in frozen heart and skeletal muscle [3]. These deficiencies were attributed to the increased reactive oxygen species (ROS) produced within the mitochondria, which have been shown to lead to inactivation of such Fe-S cluster containing enzymes [5]. It is well-established that mitochondria are a major source of ROS and that MnSOD functions to preserve mitochondrial integrity by scavenging $O_2^{\cdot-}$ and preventing amplification of ROS production [6,7,8].

MnSOD (-/+) heterozygous knockout mice (expressing 50% of the normal compliment of MnSOD) appear to develop normally and fail to show any overt phenotypic change compared to wild-type animals [3,4]. However, recent studies have demonstrated that mitochondria from MnSOD (-/+) knockout mice do show oxidative damage and alterations in mitochondrial function [9]. Interestingly, studies utilizing the MnSOD (-/+) mice have demonstrated decreases in activities of other antioxidants including glutathione, as well as NADH oxidoreductase, and aconitase. In many studies, these deficiencies have been shown to correlate with an increase in oxidative damage within the mitochondria. Consistent with this, these animals have significantly lower respiratory control ratios. Thus, it became clear that any condition that led to loss of MnSOD activity (even only a 50% reduction) in mitochondria would have serious, and potentially lethal consequences.

DISEASES DEMONSTRATING ALTERED MNSOD PROTEIN AND/OR ACTIVITY

The purpose of this review is to better understand the role that MnSOD activity has in disease situations with specific emphasis on transplantation, ischemia/reperfusion (I/R), and cancer. In addition, the review will focus on changes to MnSOD protein levels and/or activity, and not to altered MnSOD gene expression [10,11,12]. Clearly, cytokines including tumor necrosis factor alpha (TNFα), interferon gamma (INFγ), lipopolysachride (LPS), and interlukin-1 (IL-1) have been shown to induce MnSOD expression [13,14,15,16,17]. The molecular mechanisms involved with MnSOD induction by these agents are complex, cell type specific, and largely unknown [16,18]. One should exercise caution when interpreting experiments that only demonstrate MnSOD mRNA induction: every

attempt should be made to correlate the observed effects with actual MnSOD protein and activity levels. We will offer specific examples that illustrate the need for protein and activity determinations in numerous disease situations along with speculations regarding the consequence of such changes.

Organ Transplantation

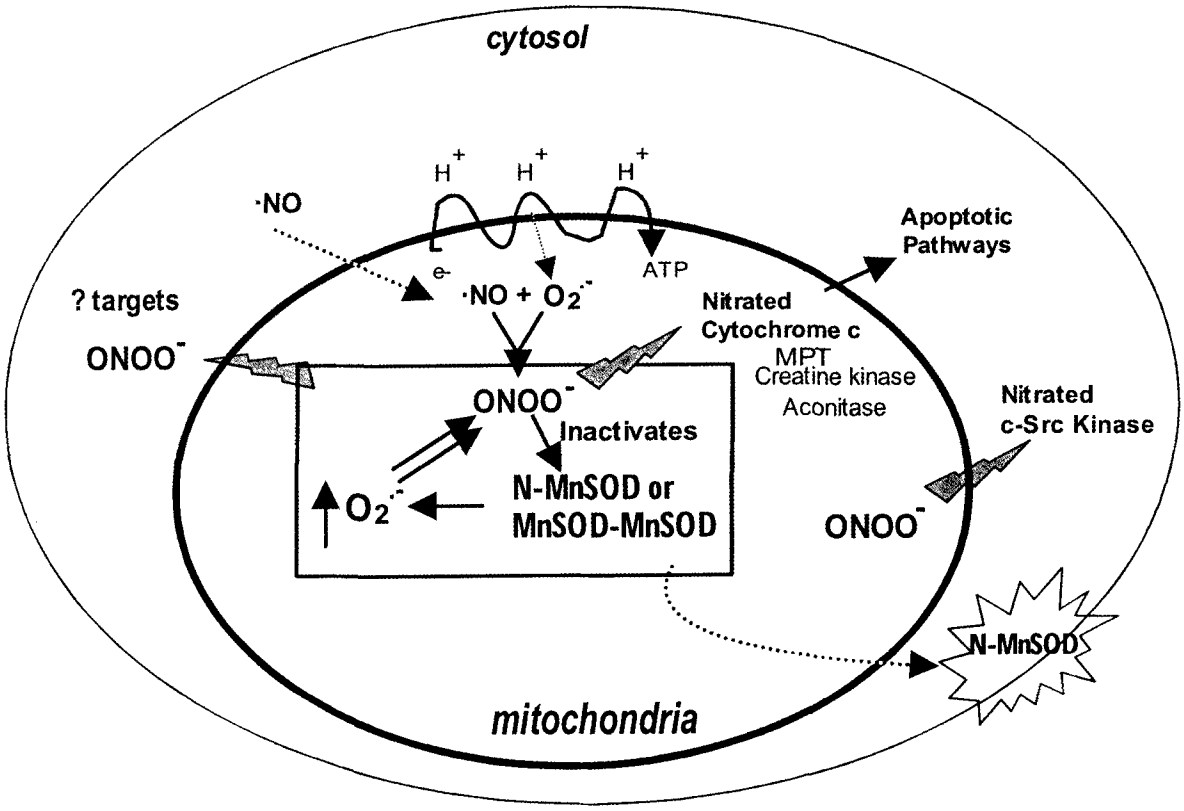
Shortly after the reports involving the MnSOD knockout animals, our laboratory demonstrated that MnSOD was tyrosine nitrated and inactivated during human chronic renal allograft rejection [19]. MnSOD was the first endogenously nitrated protein to be unambiguously identified [19]. Tyrosine nitrated MnSOD was identified by immunoprecipitating nitrated proteins in a rejected human kidney homogenate followed by microsequencing [19]. We had no preconceived notions that MnSOD would be a primary nitration target and were as surprised as anyone at its discovery. Despite an elevation in overall MnSOD protein levels in rejecting renal extracts, specific activity of MnSOD was dramatically reduced in rejecting renal extracts compared to non-rejecting renal extracts. Tyrosine nitration and inactivation of MnSOD would lead to increased levels of $O_2^{\cdot-}$ and concomitant increases in $ONOO^-$ within the mitochondria which, could lead to tyrosine nitration/oxidation of key mitochondrial proteins and ultimately mitochondrial dysfunction and cell death (Schematic 1). In any regard, this study demonstrated that MnSOD protein was susceptible to endogenous post-translational tyrosyl modification under pathologic conditions.

A more recent study evaluated heart transplant recipients up to six years following transplant for alterations in the antioxidant enzymes MnSOD, Cu,Zn SOD, and glutathione peroxidase (GPx) [20]. During the first year, there were no significant changes in antioxidant enzyme activity. However, between 1 and 6 years post-transplant, the activities of GPx and Cu,Zn SOD were increased significantly, while MnSOD activity showed a small but significant decrease. The authors concluded that early (less than one year) post-transplant recipients do not incur "oxidative stress". However, after one year, there appears to be abundant oxidative stress, which appears to remain years after transplantation.

Ischemia/Reperfusion Injury

Ischemia/reperfusion (I/R) is one of the earliest forms of injury following most surgical procedures including organ transplantation. Excessive ROS production occurs during I/R [21,22,23,24] and overwhelms endogenous antioxidants leading to increased cellular injury. Mitochondria are extremely sensitive to I/R injury leading to changes in oxidative phosphorylation, ATP depletion, a rise in intracellular calcium, mitochondrial swelling, and loss of respiratory complex activity [25,26,27,7].

Recently, Dobashi et al. examined the role of several antioxidant enzymes (catalase, GPX, Cu,Zn SOD, and MnSOD) following I/R injury using an *in vivo* rat kidney model [28]. I/R injury was induced by clamping the left renal vessels for 30, 60 or 90 minutes, followed by reperfusion for either 2 or 24 hours. During the I/R time course, 60 or 90 minutes of ischemia alone decreased the activities of all the antioxidants--an effect that was amplified following 2 or 24 hours of reperfusion. Interestingly, 60 or 90 minutes of ischemia followed by 24 hours of reperfusion helped to normalize MnSOD activity to control levels. These corresponding functional effects were correlated with both mRNA and protein levels of the antioxidants, such that the observed recovery of MnSOD activity following 24 hours of reperfusion was found to be due to elevation of MnSOD mRNA and protein levels. Preliminary studies in our laboratory using a similar *in vivo* rat kidney I/R model suggest that MnSOD is tyrosine nitrated during I/R injury. More specifically, tyrosine nitrated MnSOD is



SCHEMATIC 1 Model illustrating cytotoxicity following $ONOO^-$-mediated inactivation of MnSOD. Inactivation of MnSOD initiates a viscous positive feedback loop generating excess $ONOO^-$ within the mitochondria, leading to further nitration/oxidation of other mitochondrial/cellular proteins including cytochrome c, c-Src kinase, creatine kinase, aconitase, and induction of the mitochondrial permeability transition (MPT)

observed following 30 minutes of ischemia alone, as well as ischemia combined with 1, 3, or 6 hours reperfusion, but not after 16 hours of reperfusion (MacMillan-Crow, unpublished observations, 2000). These data are consistent with increased ROS-mediated injury during I/R.

Because MnSOD activity has been shown to decline during I/R injury [27,28], numerous studies have been designed to up-regulate MnSOD activity and determine the extent of protection from I/R injury. One such study examined the effects of I/R damage on hearts (*in vitro* using a Langendorff preparation and *in vivo* using a left coronary artery ligation model) of transgenic mice overexpressing MnSOD [24]. *In vitro*, hearts from MnSOD overexpressing transgenic animals had a significantly better functional recovery than non-transgenic hearts. Furthermore, *in vivo*, the infarct size in hearts from transgenic animals was 35% less compared to non-transgenic animals. In addition to protecting against I/R, MnSOD overexpression serves to decrease steady-state $O_2^{\cdot-}$ levels thereby preventing the formation of other ROS such as $ONOO^-$. Another elegant study using transgenic mice overexpressing MnSOD demonstrated that elevated MnSOD reduced membrane lipid peroxidation and tyrosine nitration following cerebral ischemia [21].

Role of MnSOD in Apoptosis

MnSOD can protect against pro-apoptotic events following either addition of agents leading to an induction of MnSOD protein or creating cell lines that overexpress MnSOD [21,29,30,31,32,33]. Overexpression of MnSOD in neuronal cells prevented apoptosis induced by excess iron, amyloid β-peptide, and nitric oxide donors [21]. All of these agents resulted in increased nitration of proteins, membrane lipid peroxidation and mitochondrial damage, which were largely prevented by overexpression of MnSOD. This study supports the notion that elevation of $O_2^{\cdot -}$ and subsequent $ONOO^-$ was involved with neuronal apoptosis in this system. Bruce-Keller et al., utilized neurons (with and without pretreatment with TNFα) or genetically engineered pheochromocytoma cells (PC6) that overexpressed MnSOD and exposed the cells to the fungal toxin 3-nitropropinic acid (3-NP). 3-NP has been shown to inhibit mitochondrial complex II activity resulting in decreased mitochondrial function, ATP synthesis, and cell death and is thought to mimic the deficits observed in Huntington's disease [30,34]. This study showed that either TNF-mediated induction of MnSOD activity in neurons or overexpression of MnSOD in a neuronal cell line was able to prevent 3-NP-induced neuronal apoptosis. Moreover, increased MnSOD activity prevented: 1) the decrease in mitochondrial membrane potential 2) the decline in ATP levels 3) the increase in calcium release, and 4) caspase-3 activation.

TNFα signaling pathways have been shown to result in activation of caspase-3 and apoptosis as well as activation of c-jun kinase (JNK), MAP kinase kinase (MEK), activated protein 1 (AP-1), and nuclear transcription factor (NF-κβ) [35]. Overexpression of MnSOD has been shown to prevent the TNFα mediated activation of the above processes as well as TNFα-independent mechanisms leading to apoptosis [36]. Another study demonstrated that overexpression of MnSOD in cells could protect against apoptosis elicited by ROS produced from *within* the mitochondria [37]. ROS were generated by treating cells with respiratory chain inhibitors (antimycin or rotenone), an event that caused cellular apoptosis via a caspase-3 and poly (ADP-ribose) polymerase mediated pathway.

MnSOD in Cancer

The role of MnSOD in cancer remains controversial. Many investigators have reported that tumor cells/tissues contain decreased MnSOD activity [38] whereas recent reports describe an increase of MnSOD protein in tumor cell lines [41,42] and tissue [43]. However, there are few published reports that actually measured MnSOD protein and activity in tumor tissue [39,40]. The failure to measure activity could account for some of these discrepancies. Studies that measure only MnSOD protein via immunohistochemical or ELISA techniques provide little insight into the status of MnSOD activity, and assessment of tumor tissue versus tumor cell lines are likely to produce variable results. One suggestion when comparing MnSOD protein/activity levels within tumor cell lines, would be to obtain a true normal cell line or normal tissue for accurate determination. Others suggest that defects in MnSOD gene expression [44], specifically mutations in the DNA sequence of the promoter region, could account for the lowered MnSOD protein levels in tumor cells [45].

In any case, tumors that possess very low MnSOD activity are particularly intriguing in light of recent studies that indicate that MnSOD is essential for survival of normal cells. Numerous studies have shown that restoration of MnSOD activity in transformed cancer cells (via transfection of MnSOD cDNA) results in a slowing of tumor growth in nude mice as well as alteration of the transformed phenotype of cancer cells [46,47,48,49,50,51,52,53]. Again, the biochemical mechanism for this alteration is

unclear. Thus, the paradox exists such that in normal cells a deficiency in MnSOD causes cell death, while tumor cells thrive in the face of a similar deficiency.

We have recently shown that nitrotyrosine levels are dramatically elevated during human pancreatic cancer [54] and that MnSOD is tyrosine nitrated and inactivated in these tissues [55]. When compared to control human pancreas (donor tissue deemed not suitable for transplantation), the protein level was not altered, but the activity of MnSOD was decreased. This could provide one explanation for the lowered activity of MnSOD in tumor tissue. Another hypothesis for this paradox could be that tyrosine nitration of MnSOD could either induce the protein to be translocated out of the mitochondria during neoplasia or alter its ability to be imported into the mitochondria (Schematic 1). This phenomenon may explain previous reports demonstrating elevated plasma MnSOD levels from cancer patients compared to non-cancerous patients [56]. How this mitochondrial matrix protein finds its way into the serum is unclear and it would be quite interesting to determine whether the serum localized MnSOD were active and/or tyrosine nitrated.

Irradiation of tumors is thought to kill tumor cells by increasing ROS. Overexpression of MnSOD but not Cu,Zn SOD or EC SOD protects cells against radiation-induced cytotoxicity and conversely, overexpression of antisense MnSOD RNA increases cytotoxicity. Transfection of cells with MnSOD lacking the mitochondrial targeting sequence does not provide protection against radiation, indicating that localization of MnSOD into the mitochondria is essential. Consistent with this interpretation, insertion of the mitochondrial signal sequence into Cu,Zn SOD or EC SOD results in significant protection. These results strongly suggest that mitochondrial production of ROS results in radiation induced cell death and that increased dismutase activity within the mitochondria can protect against radiation induced cytotoxicity [57].

Other Diseases

Many other diseases have been reported to possess decreased MnSOD activity. For example, MnSOD activity appears to decline with increased age in certain areas of the mouse brain [58]. In addition, MnSOD protein levels are decreased in progeria skin fibroblasts [59]. Both of these reports suggest that the aging process may be related to loss of antioxidant protection including MnSOD function. It has also been shown that overexpression of the human immunodeficiency virus type 1 regulatory protein Tat, leads to a reduction in MnSOD expression and activity [60]. Furthermore, 3′-Azido-2′,3′-dideoxythymidine (AZT, zidovudine) treatment of Tat transgenic mice induces a decline in MnSOD activity, which could account for some of the toxicity observed following AZT treatment [61]. MnSOD activity from neutrophils isolated from human asthmatics was reduced when compared to normal neutrophils [62].

On the other hand, numerous diseases have been reported to show an increase in MnSOD mRNA and protein levels (many don't report on enzyme activity). Influenza virus infection of human airway epithelial cells has been shown to induce MnSOD expression [14], while treatment of mice infected with influenza virus with recombinant MnSOD alleviated pulmonary toxicity following infection [63]. Again these apparent contradictions in outcome could be reconciled related to discrepant measurements of MnSOD mRNA, protein, and activity in cell culture versus animals.

The fatal neurodegenerative disease, familial amyotrophic lateral sclerosis (ALS), has been associated with several, single amino acid mutations in Cu,Zn SOD. Despite the obvious role of Cu,Zn SOD, reports have shown increased MnSOD protein [64,65] and activity [66] in ALS patient samples. Another report indicated potential gene defects of MnSOD in sporadic ALS patients [67]. Clearly, the disease is associated with oxidative damage as well as mitochondrial

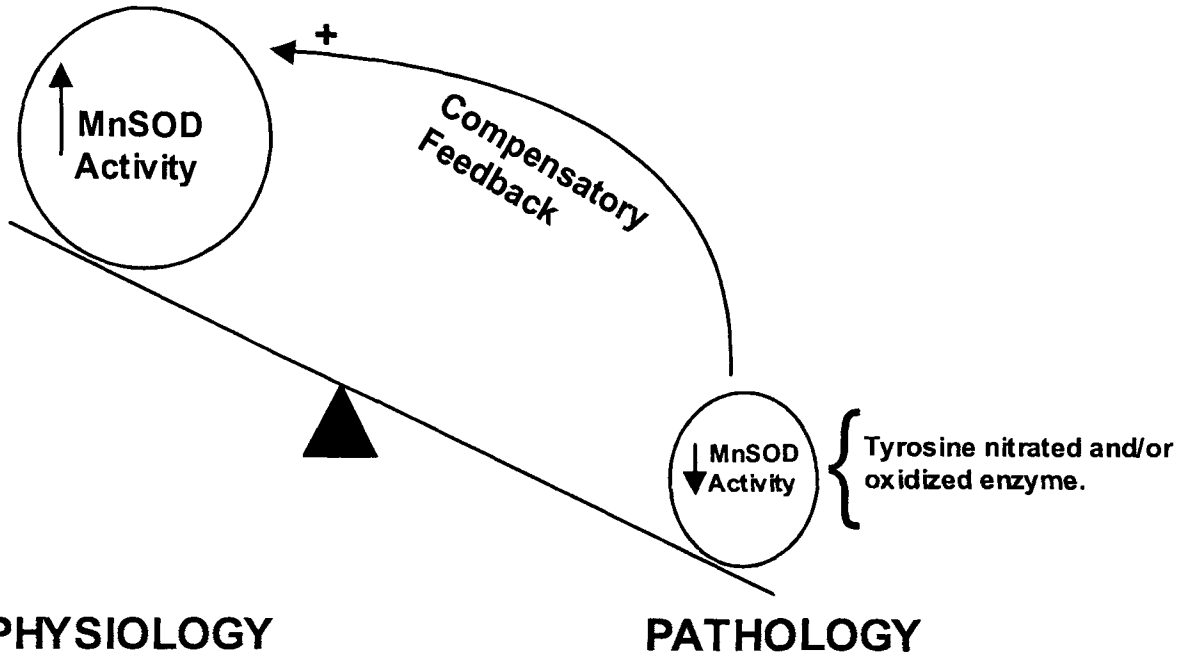
dysfunction and exciting new reports using ALS transgenic mice indicate that lowered MnSOD activity greatly exacerbates the disease process [68]. Likewise, MnSOD activity appears to play an important role in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity (a model of Parkinson's Disease). Specifically, MPTP treatment of mice resulted in severe neurotoxicity including elevation of tyrosine nitration; however, MPTP treatment in MnSOD overexpressing mice produced significantly less neurotoxicity and tyrosine nitration [69].

MECHANISM OF $ONOO^-$-MEDIATED INACTIVATION OF MNSOD

Virtually any protein containing one or more tyrosyl residues can be nitrated *in vitro* upon addition of a nitrating agent. However, the mere ability for a protein to be nitrated *in vitro* does not indicate that the protein is a likely target of endogenous nitration. Furthermore, it is important to determine whether tyrosine nitration of a protein alters its biologic function. Much attention and debate has been devoted to determining the precise reactive nitrogen species responsible for protein tyrosine nitration (not the focus of this review). Further identification of the specific targets of endogenous nitration are essential to provide clues to the pathological mechanisms.

Our earlier studies suggested that not only was MnSOD inactivated and tyrosine nitrated but also appeared to form non-reducible, covalently bound higher molecular mass structures during chronic rejection of human renal allografts [19]. Therefore, further studies were designed to determine the biochemical mechanism involved with MnSOD nitration and inactivation. Initially, treatment of recombinant human MnSOD with different reactive oxygen/nitrogen species including ($ONOO^-$, NO, hypochlorous acid (HOCl) plus nitrite, and hydrogen peroxide) followed by activity measurements revealed that only $ONOO^-$ and HOCl resulted in inhibition of dismutase activity [70]. Both of these strong oxidants have been shown to modify tyrosine residues through formation of dityrosine (3,3'-dityrosine), a stable covalent modification resulting from reaction of two tyrosyl radicals, leaving a product with a fluorescent emission distinct from tyrosine itself. $ONOO^-$ totally inhibited MnSOD activity and produced both dityrosine and nitrotyrosine, while HOCI only produced dityrosine and resulted in a ~50% reduction in activity [70]. Thus, partial activity loss can be attributed to nitration of tyrosine residues and to dityrosine formation with the two modifications being additive. In addition, dityrosine formation in MnSOD could account for the higher molecular weight structures observed *in vivo* and *in vitro*. These studies provided additional evidence that $ONOO^-$ was the likely culprit leading to nitration and inactivation of MnSOD *in vivo* [70].

We also identified the specific tyrosine residues that were nitrated *in vitro* following $ONOO^-$ exposure as residues 34, 45, and 193, with the active site tyrosine (Y34) being the most susceptible to nitration [71]. However, a recent study with a site-directed mutant of MnSOD revealed that nitration of the active site tyrosyl residue was not required for inactivation. We found that replacement of Y34 with phenylalanine (Y34F) yielded an enzymatically active protein that could still be nitrated and inactivated by $ONOO^-$, suggesting that nitration and/or oxidation of other tyrosines was equally important [72]. Collectively, these results suggest that complete inactivation of MnSOD by $ONOO^-$ can occur independent of the active site tyrosine residue and includes not only nitration of critical tyrosine residues but also tyrosine oxidation and subsequent formation of dityrosine.



SCHEMATIC 2 Model depicting the balance between MnSOD activity and pathology. Oxidatively modified MnSOD including tyrosine nitration and/or oxidation remain as plausible mechanisms leading to lowered MnSOD activity during pathology. Lowered MnSOD activity may signal a compensatory positive feedback mechanism leading to elevated expression of MnSOD and possible reversion to a physiologic situation

CONCLUSION: IMPORTANCE OF ACTIVE MNSOD IN MITOCHONDRIA

MnSOD is structurally very different from Cu,Zn SOD and, unlike Cu,Zn SOD, appears to be essential for life. MnSOD (-/+) heterozygous knockout mice develop normally and fail to show any overt phenotypic change [4,3], however mitochondria from these mice do show oxidative damage and alterations in mitochondrial function [9,73,74]. By extension, our findings that MnSOD is tyrosine nitrated and inactivated during chronic renal allograft rejection and pancreatic cancer suggest that loss of MnSOD activity (even a 50% loss) could be fundamentally related to the cellular pathology seen in these diseases. Therefore, a delicate balance exists in that physiologic situations appear to maintain a normal or elevated MnSOD activity, while during many pathologic situations a decrease in MnSOD activity prevails (Schematic 2). An event resulting in decreased MnSOD activity could signal a compensatory feedback mechanism leading to elevated MnSOD activity and possibly reversion to a physiologic state. In any regard, oxidatively modified MnSOD including tyrosine nitration and/or oxidation remain as plausible mechanisms leading to lowered MnSOD activity during pathology.

From an evolutionary standpoint, comparison of human MnSOD to human Cu,Zn SOD is intriguing. Human Cu,Zn SOD contains no tyrosine residues and to date appears to be very resistant to reasonable levels of reactive oxygen species, while human MnSOD contains 9 tyrosine residues, some of which can be nitrated and oxidized by low micromolar concentrations of $ONOO^-$. It is intriguing to question why the dis-

mutase shown to be more critical for cell survival is more susceptible to inactivation? It is reasonable to speculate that oxidatively modified MnSOD plays an additional role, possibly involving apoptotic pathways. Irrespective of any additional roles, it must be emphasized that MnSOD is needed to ensure survival of normal cells and is simultaneously susceptible to oxidative inactivation. Thus, changes in MnSOD activity need not depend on rates of *de novo* protein synthesis or degradation and can occur very quickly under pathological conditions. Even though the precise series of subsequent biochemical events remain to be elucidated, it is clear that loss of MnSOD activity could have catastrophic consequences for the mitochondria, the cell, and ultimately, the entire organism.

Acknowledgements

Lee Ann MacMillan-Crow, Ph. D. is supported by a Scientist Development Grant from the American Heart Association. We thank Dr. John P. Crow for helpful comments.

References

1. A. G. Reaume, J. L. Elliott, E. K. Hoffman, N. W. Kowall, R. J. Ferrante, D. F. Siwek, H. M. Wilcox, D. G. Flood, M. F. Beal, R. H. Brown, Jr., R. W. Scott, and W. D. Snider (1996) Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nature Genetics*, 13, 43–47.
2. L. M. Carlsson, J. Jonsson, T. Edlund, and S. L. Marklund (1995) Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. *Proceedings of the National Academy of Sciences of the United States of America*, 92, 6264–6268.
3. Y. Li, T. T. Huang, E. J. Carlson, S. Melov, P. C. Ursell, J. L. Olson, L. J. Noble, M. P. Yoshimura, C. Berger, P. H. Chan, and et al (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nature Genetics*, 11, 376–381.
4. R. M. Lebovitz, H. Zhang, H. Vogel, J. Cartwright, L. Dionne, N. Lu, S. Huang, and M. M. Matzuk (1996) Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 9782–9787.
5. C. L. Keen, M. S. Golub, M. E. Gershwin, B. Lonnerdal, and L. S. Hurley (1988) Studies of marginal zinc deprivation in rhesus monkeys. III. Use of liver biopsy in the assessment of zinc status. *American Journal of Clinical Nutrition*, 47, 1041–1045.
6. L. L. Dugan, S. L. Sensi, L. M. Canzoniero, S. D. Handran, S. M. Rothman, T. S. Lin, M. P. Goldberg, and D. W. Choi (1995) Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate. *Journal of Neuroscience*, 15, 6377–6388.
7. C. A. Piantadosi and J. Zhang (1996) Mitochondrial generation of reactive oxygen species after brain ischemia in the rat. *Stroke*, 27, 327–331.
8. G. C. Brown (1999) Nitric oxide and mitochondrial respiration. [Review] [155 refs]. *Biochimica et Biophysica Acta*, 1411, 351–369.
9. M. D. Williams, H. Van Remmen, C. C. Conrad, T. T. Huang, C. J. Epstein, and A. Richardson (1998) Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *Journal of Biological Chemistry*, 273, 28510–28515.
10. E. M. Grasbon-Frodl, S. Kosel, O. Riess, U. Muller, P. Mehraein, and M. B. Graeber (1999) Analysis of mitochondrial targeting sequence and coding region polymorphisms of the manganese superoxide dismutase gene in German Parkinson disease patients. *Biochemical & Biophysical Research Communications*, 255, 749–752.
11. Y. Xu, A. Krishnan, X. S. Wan, H. Majima, C. C. Yeh, G. Ludewig, E. J. Kasarskis, and D. K. St. Clair (1999) Mutations in the promoter reveal a cause for the reduced expression of the human manganese superoxide dismutase gene in cancer cells. *Oncogene*, 18, 93–102.
12. S. Borrello, M. E. De Leo, and T. Galeotti (1993) Defective gene expression of MnSOD in cancer cells. *Molecular Aspects of Medicine*, 14, 253–258.
13. R. G. Liu, G. R. Buettner, and L. W. Oberley (2000) Oxygen free radicals mediate the induction of manganese superoxide dismutase gene expression by TNF-alpha. *Free Radical Biology & Medicine, 28(8)*, 1197–1205.
14. D. B. Jacoby and A. M. Choi (1994) Influenza virus induces expression of antioxidant genes in human epithelial cells. *Free Radical Biology & Medicine*, 16, 821–824.
15. L. S. Gibbs, P. J. Del Vecchio, and J. B. Shaffer (1992) Mn and Cu/Zn SOD expression in cells from LPS-sensitive and LPS- resistant mice. *Free Radical Biology & Medicine*, 12, 107–111.
16. W. Gwinner, C. C. Tisher, and H. S. Nick (1995) Regulation of manganese superoxide dismutase in glomerular epithelial cells: mechanisms for interleukin 1 induction. *Kidney International*, 48, 354–362.
17. Y. Kifle, J. Monnier, S. E. Chesrown, M. K. Raizada, and H. S. Nick (1996) Regulation of the manganese superoxide dismutase and inducible nitric oxide synthase gene in rat neuronal and glial cells. *Journal of Neurochemistry*, 66, 2128–2135.
18. J. E. White, H. Y. Lin, F. B. Davis, P. J. Davis, and M. F. Tsan (2000) Differential induction of tumor necrosis factor alpha and manganese superoxide dismutase by endotoxin in human monocytes: role of protein tyrosine kinase, mitogen-activated protein kinase, and nuclear factor kappaB. *Journal of Cellular Physiology*, 182, 381–389.
19. L. A. MacMillan-Crow, J. P. Crow, J. D. Kerby, J. S. Beckman, and J. A. Thompson (1996) Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. *Proceedings*

of the National Academy of Sciences of the United States of America, 93, 11853–11858.

20. I. Schimke, M. Schikora, R. Meyer, H. P. Dubel, D. Modersohn, F. X. Kleber, and G. Baumann (2000) Oxidative stress in the human heart is associated with changes in the antioxidative defense as shown after heart transplantation. *Molecular & Cellular Biochemistry*, 204, 89–96.
21. J. N. Keller, M. S. Kindy, F. W. Holtsberg, D. K. St Clair, H. C. Yen, A. Germeyer, S. M. Steiner, A. J. Bruce-Keller, J. B. Hutchins, and M. P. Mattson (1998) Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. *Journal of Neuroscience*, 18, 687–697.
22. M. Fujimura, Y. Morita-Fujimura, M. Kawase, J. C. Copin, B. Calagui, C. J. Epstein, and P. H. Chan (1999) Manganese superoxide dismutase mediates the early release of mitochondrial cytochrome C and subsequent DNA fragmentation after permanent focal cerebral ischemia in mice. *Journal of Neuroscience*, 19, 3414–3422.
23. P.H. Chan (1996) Role of oxidants in ischemic brain damage. [Review]. *Stroke*, 27, 1124–1129.
24. Z. Chen, B. Siu, Y. S. Ho, R. Vincent, C. C. Chua, R. C. Hamdy, and B. H. Chua (1998) Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. *Journal of Molecular & Cellular Cardiology*, 30, 2281–2289.
25. I. A. Sammut, K. Burton, E. Balogun, P. Sarathchandra, K. J. Brooks, T. E. Bates, and C. J. Green (2000) Time-dependent impairment of mitochondrial function after storage and transplantation of rabbit kidneys. *Transplantation*, 69, 1265–1275.
26. J. M. Weinberg, M. A. Venkatachalam, N. F. Roeser, and I. Nissim (2000) Mitochondrial dysfunction during hypoxia/reoxygenation and its correction by anaerobic metabolism of citric acid cycle intermediates. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 2826–2831.
27. M. Gupta, K. Dobashi, E. L. Greene, J. K. Orak, and I. Singh (1997) Studies on hepatic injury and antioxidant enzyme activities in rat subcellular organelles following in vivo ischemia and reperfusion. *Molecular & Cellular Biochemistry*, 176, 337–347.
28. K. Dobashi, B. Ghosh, J. K. Orak, I. Singh, and A. K. Singh (2000) Kidney ischemia-reperfusion: Modulation of antioxidant defenses. *Molecular & Cellular Biochemistry*, 205, 1–11.
29. R. Kinscherf, H. P. Deigner, C. Usinger, J. Pill, M. Wagner, H. Kamencic, D. Hou, M. Chen, W. Schmiedt, M. Schrader, G. Kovacs, K. Kato, and J. Metz (1997) Induction of mitochondrial manganese superoxide dismutase in macrophages by oxidized LDL: its relevance in atherosclerosis of humans and heritable hyperlipidemic rabbits. *FASEB Journal*, 11, 1317–1328.
30. A. J. Bruce-Keller, J. W. Geddes, P. E. Knapp, R. W. McFall, J. N. Keller, F. W. Holtsberg, S. Parthasarathy, S. M. Steiner, and M. P. Mattson (1999) Anti-death properties of TNF against metabolic poisoning: mitochondrial stabilization by MnSOD. *Journal of Neuroimmunology*, 93, 53–71.
31. M. W. Epperly, J. A. Bray, P. Esocobar, W. L. Bigbee, S. Watkins, and J. S. Greenberger (1999) Overexpression of the human manganese superoxide dismutase (MnSOD) transgene in subclones of murine hematopoietic progenitor cell line 32D cl 3 decreases irradiation-induced apoptosis but does not alter G2/M or G1/S phase cell cycle arrest. *Radiation Oncology Investigations*, 7, 331–342.
32. P. Mantymaa, T. Siitonen, T. Guttorm, M. Saily, V. Kinnula, E. R. Savolainen, and P. Koistinen (2000) Induction of mitochondrial manganese superoxide dismutase confers resistance to apoptosis in acute myeloblastic leukaemia cells exposed to etoposide. *British Journal of Haematology*, 108, 574–581.
33. B. Lindau-Shepard, J. B. Shaffer, and P. J. Del Vecchio (1994) Overexpression of manganous superoxide dismutase (MnSOD) in pulmonary endothelial cells confers resistance to hyperoxia. *Journal of Cellular Physiology*, 161, 237–242.
34. M. F. Beal, E. Brouillet, B. G. Jenkins, R. J. Ferrante, N. W. Kowall, J. M. Miller, E. Storey, R. Srivastava, B. R. Rosen, and B. T. Hyman (1993) Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *Journal of Neuroscience*, 13, 4181–4192.
35. B. G. Darnay and B. B. Aggarwal (1997) Early events in TNF signaling: a story of associations and dissociations. *J. Leukoc. Biol.*, 61, 559–566.
36. S. K. Manna, H. J. Zhang, T. Yan, L. W. Oberley, and B. B. Aggarwal (1998) Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *Journal of Biological Chemistry*, 273, 13245–13254.
37. K. K. Kiningham, T. D. Oberley, S. Lin, C. A. Mattingly, and D. K. St Clair (1999) Overexpression of manganese superoxide dismutase protects against mitochondrial-initiated poly(ADP-ribose) polymerase-mediated cell death. *FASEB J.*, 13, 1601–1610.
38. L. W. Oberley and G. R. Buettner (1979) Role of superoxide dismutase in cancer: a review. [Review] [104 refs]. Cancer Research, 39, 1141–1149.
39. L. W. Oberley, I. B. Bize, S. K. Sahu, S. W. Leuthauser, and H. E. Gruber (1978) Superoxide dismutase activity of normal murine liver, regenerating liver, and H6 hepatoma. *Journal of the National Cancer Institute*, 61, 375–379.
40. M. L. McCormick, T. D. Oberley, J. H. Elwell, L. W. Oberley, Y. Sun, and J. J. Li (1991) Superoxide dismutase and catalase levels during estrogen-induced renal tumorigenesis, in renal tumors and their autonomous variants in the Syrian hamster. *Carcinogenesis*, 12, 977–983.
41. W. Zhong, T. Yan, R. Lim, and L. W. Oberley (1999) Expression of superoxide dismutases, catalase, and glutathione peroxidase in glioma cells. Free *Radical Biology & Medicine*, 27, 1334–1345.
42. C. S. Cobbs, D. S. Levi, K. Aldape, and M. A. Israel (1996) Manganese superoxide dismutase expression in human central nervous system tumors. *Cancer Research*, 56, 3192–3195.
43. R. Izutani, S. Asano, M. Imano, D. Kuroda, M. Kato, and H. Ohyanagi (1998) Expression of manganese superoxide dismutase in esophageal and gastric cancers. *Journal of Gastroenterology*, 33, 816–822.

44. S. Borrello, M. E. De Leo, and T. Galeotti (1993) Defective gene expression of MnSOD in cancer cells. *Molecular Aspects of Medicine*, 14, 253–258.
45. X. S. Wan, M. N. Devalaraja, and D. K. St Clair (1994) Molecular structure and organization of the human manganese superoxide dismutase gene. *DNA & Cell Biology*, 13, 1127–1136.
46. S. L. Church, J. W. Grant, L. A. Ridnour, L. W. Oberley, P. E. Swanson, P.S. Meltzer, and J. M. Trent (1993) Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proceedings of the National Academy of Sciences of the United States of America*, 90, 3113–3117.
47. J. J. Li, L. W. Oberley, D. K. St Clair, L. A. Ridnour, and T. D. Oberley (1995) Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase. *Oncogene*, 10, 1989–2000.
48. E. W. Lam, R. Zwacka, J. F. Engelhardt, B. L. Davidson, F. E. Domann, Jr., T. Yan, and L. W. Oberley (1997) Adenovirus-mediated manganese superoxide dismutase gene transfer to hamster cheek pouch carcinoma cells. *Cancer Research*, 57, 5550–5556.
49. N. Li, T. D. Oberley, L. W. Oberley, and W. Zhong (1998) Overexpression of manganese superoxide dismutase in DU145 human prostate carcinoma cells has multiple effects on cell phenotype. *Prostate*, 35, 221–233.
50. T. Yan, L. W. Oberley, W. Zhong, and D. K. St Clair (1996) Manganese-containing superoxide dismutase overexpression causes phenotypic reversion in SV40-transformed human lung fibroblasts. *Cancer Research*, 56, 2864–2871.
51. R. Liu, T. D. Oberley, and L. W. Oberley (1997) Transfection and expression of MnSOD cDNA decreases tumor malignancy of human oral squamous carcinoma SCC-25 cells. *Human Gene Therapy*, 20, 585–595.
52. S. L. Church, J. W. Grant, L. A. Ridnour, L. W. Oberley, P. E. Swanson, P. S. Meltzer, and J. M. Trent (1993) Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proceedings of the National Academy of Sciences of the United States of America*, 90, 3113–3117.
53. W. Zhong, L. W. Oberley, T. D. Oberley, and D. K. St Clair (1997) Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. *Oncogene*, 14, 481–490.
54. S. M. Vickers, L. A. MacMillan-Crow, M. Green, C. Ellis, and J. A. Thompson (1999) Association of increased immunostaining for inducible nitric oxide synthase and nitrotyrosine with fibroblast growth factor transformation in pancreatic cancer. *Archives of Surgery*, 134, 245–251.
55. L. A. MacMillan-Crow and J. A. Thompson (1999) Immunoprecipitation of nitrotyrosine-containing proteins. *Methods Enzymology*, 301, 135–145.
56. N. Taniguchi (1992) Clinical significances of superoxide dismutases: changes in aging, diabetes, ischemia, and cancer. [Review] [232 refs]. *Advances in Clinical Chemistry*, 29, 1–59.
57. G. H. Wong (1995) Protective roles of cytokines against radiation: induction of mitochondrial MnSOD. [Review]. *Biochimica et Biophysica Acta*, 1271, 205–209.
58. F. Cardozo-Pelaez, S. Song, A. Parthasarathy, C. Hazzi, K. Naidu, and J. Sanchez-Ramos (1999) Oxidative DNA damage in the aging mouse brain. *Movement Disorders*, 14, 972–980.
59. T. Yan, S. Li, X. Jiang, and L. W. Oberley (1999) Altered levels of primary antioxidant enzymes in progeria skin fibroblasts. *Biochemical & Biophysical Research Communications*, 257, 163–167.
60. S. C. Flores, J. C. Marecki, K. P. Harper, S. K. Bose, S. K. Nelson, and J. M. McCord (1993) Tat protein of human immunodeficiency virus type 1 represses expression of manganese superoxide dismutase in HeLa cells. *Proceedings of the National Academy of Sciences of the United States of America*, 90, 7632–7636.
61. O. Prakash, S. Teng, M. Ali, X. Zhu, R. Coleman, R. A. Dabdoub, R. Chambers, T. Y. Aw, S. C. Flores, and B. H. Joshi (1997) The human immunodeficiency virus type 1 Tat protein potentiates zidovudine-induced cellular toxicity in transgenic mice. *Archives of Biochemistry & Biophysics*, 343, 173–180.
62. B. Z. Joseph, J. M. Routes, and L. Borish (1993) Activities of superoxide dismutases and NADPH oxidase in neutrophils obtained from asthmatic and normal donors. *Inflammation*, 17, 361–370.
63. R. W. Sidwell, J. H. Huffman, K. W. Bailey, M. H. Wong, A. Nimrod, and A. Panet (1996) Inhibitory effects of recombinant manganese superoxide dismutase on influenza virus infections in mice. *Antimicrobial Agents & Chemotherapy*, 40, 2626–2631.
64. G. McEachern, S. Kassovska-Bratinova, S. Raha, M. A. Tarnopolsky, J. Turnbull, J. Bourgeois, and B. Robinson (2000) Manganese superoxide dismutase levels are elevated in a proportion of amyotrophic lateral sclerosis patient cell lines. *Biochemical & Biophysical Research Communications*, 273, 359–363.
65. Y. Liu, B. R. Brooks, N. Taniguchi, and H. A. Hartmann (1998) CuZnSOD and MnSOD immunoreactivity in brain stem motor neurons from amyotrophic lateral sclerosis patients. *Acta Neuropathologica*, 95, 63–70.
66. I. C. Shaw, P. S. Fitzmaurice, J. D. Mitchell, and P. G. Lynch (1995) Studies on cellular free radical protection mechanisms in the anterior horn from patients with amyotrophic lateral sclerosis. *Neurodegeneration*, 4, 391–396.
67. M. Tomblyn, E. J. Kasarskis, Y. Xu, and D. K. St Clair (1998) Distribution of MnSOD polymorphisms in sporadic ALS patients. *Journal of Molecular Neuroscience*, 10, 65–66.
68. O. A. Andreassen, R. J. Ferrante, P. Klivenyi, A. M. Klein, L. A. Shinobu, C. J. Epstein, and M. F. Beal (2000) Partial deficiency of manganese superoxide dismutase exacerbates a transgenic mouse model of amyotrophic lateral sclerosis. *Annals of Neurology*, 47, 447–455.
69. P. Klivenyi, D. St. Clair, M. Wermer, H. C. Yen, T. Oberley, L. Yang, and M. F. Beal (1998) Manganese superoxide dismutase overexpression attenuates MPTP toxicity. *Neurobiology of Disease*, 5, 253–258.
70. L. A. MacMillan-Crow, J. P. Crow, and J. A. Thompson (1998) Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. *Biochemistry*, 37, 1613–1622.
71. F. Yamakura, H. Taka, T. Fujimura, and K. Murayama (1998) Inactivation of Human Manganese-Superoxide Dismutase by Peroxynitrite is Caused by Exclusive

Nitration of Tyrosine 34 to 3-Nitrotyrosine. *Journal of Biological Chemistry*, 273, 14085–14089.
72. L. A. MacMillan-Crow and J. A. Thompson (1999) Tyrosine modifications and inactivation of active site manganese superoxide dismutase mutant (Y34F) by peroxynitrite. *Archives of Biochemistry & Biophysics*, 366, 82–88.
73. H. Van Remmen, C. Salvador, H. Yang, T. T. Huang, C. J. Epstein, and A. Richardson (1999) Characterization of the antioxidant status of the heterozygous manganese superoxide dismutase knockout mouse. *Archives of Biochemistry & Biophysics*, 363, 91–97.
74. S. Melov. P. Coskun, M. Patel, R. Tuinstra, B. Cottrell, A. S. Jun, T. H. Zastawny, M. Dizdaroglu, S.I. Goodman, T. T. Huang, H. Miziorko, C. J. Epstein, and D. C. Wallace (1999) Mitochondrial disease in superoxide dismutase 2 mutant mice. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 846–851.