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DEVELOPING MASTER KEYS TO BRAIN PATHOLOGY, CANCER AND AGING FROM THE STRUCTURAL BIOLOGY OF PROTEINS CONTROLLING REACTIVE OXYGEN SPECIES AND DNA REPAIR

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Abstract—This review is focused on proteins with key roles in pathways controlling either reactive oxygen species or DNA damage responses, both of which are essential for preserving the nervous system. An imbalance of reactive oxygen species or inappropriate DNA damage response likely causes mutational or cytotoxic outcomes, which may lead to cancer and/or aging phenotypes. Moreover, individuals with hereditary disorders in proteins of these cellular pathways have significant neurological abnormalities. Mutations in a superoxide dismutase, which removes oxygen free radicals, may cause the neurodegenerative disease amyotrophic lateral sclerosis. Additionally, DNA repair disorders that affect the brain to various extents include ataxia–telangiectasia-like disorder, Cockayne syndrome or Werner syndrome. Here, we highlight recent advances gained through structural biochemistry studies on enzymes linked to these disorders and other related enzymes acting within the same cellular pathways. We describe the current understanding of how these vital proteins coordinate chemical steps and integrate cellular signaling and response events. Significantly, these structural studies may provide a set of master keys to developing a unified understanding of the survival mechanisms utilized after insults by reactive oxygen species and genotoxic agents, and also provide a basis for developing an informed intervention in brain tumor and neurodegenerative disease progression.

Key words: amyotrophic lateral sclerosis, ataxia–telangiectasia-like disorder, Werner syndrome, xeroderma pigmentosum, nitric oxide synthase, superoxide dismutase.

Proteins that control cellular levels of reactive oxygen species (ROS) or DNA damage responses are essential to the nervous system. This is because an imbalance of ROS may cause significant damage to the cell, which often produces a cytotoxic or mutational outcome. DNA is surprisingly susceptible to both exogenous and endogenous DNA damaging agents, considering that it is the carrier of genetic information. If the DNA damage is not corrected by repair mechanisms, deleterious mutations and genomic instability may arise, leading to aging and cancer. Additionally, since DNA repair mechanisms are imperfect accumulations of both nuclear and mitochondrial DNA (mtDNA) damage, post-mitotic cells may have a central role in neurodegeneration and aging pathologies. Therefore, the defenses against DNA damaging agents merit close attention for their significance to neuropathologies.

Responses to ROS and DNA damage have many critical links to neuroscience. The damage from stroke is likely linked to ROS, with resulting damage to membrane components, proteins, and DNA. The long-term stability of human embryonic stem cells, with their potential for repair of neuronal defects, depends upon their effective DNA repair responses. Damage within actively transcribed genes has to be repaired, otherwise defective proteins are likely to be produced, or the more bulky DNA lesions may block transcription, triggering cell death. DNA damage to regulatory regions can cause the stochastic deregulation of gene expression, and provide a mechanism for age-related degeneration and cell death. Mouse models involving the mutation of key DNA repair enzymes support a role for these proteins in the control of aging and cancer, as well as reveal the etiology of neurological abnormalities in the brain (Hoeijmakers, 2001). Defects in either ROS controlling enzymes or DNA double-strand break (DSB) repair genes are also the cause of several human hereditary diseases. These disorders present clinical phenotypes that

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*Abbreviations: Af*XPB, *Archaeoglobus fulgidus* XPB; AH, autoinhibitory helix; ALS, amyotrophic lateral sclerosis; ATLD, ataxia–telangiectasia-like disorder; BER, base excision repair; CaM, Ca^{2+}/calmodulin; CD, connecting domain; CS, Cockayne syndrome; CT, C-terminal tail; Cu,Zn SOD; copper,zinc superoxide dismutase; DRD, damage recognition domain; DSBs, double-strand breaks; dsDNA, double-stranded DNA; eNOS, endothelial nitric oxide synthase; FAD, flavin adenine dinucleotide; FALS, familial amyotrophic lateral sclerosis; FEN-1, flap endonuclease 1; FMN, flavin mononucleotide; HD1 and HD2, helicase domains 1 and 2; HR, homologous recombination; HRDC, helicase RNase D conserved domain; HRR, homologous recombination repair; iNOS, inducible nitric oxide synthase; MMR, mismatch repair; MnSOD, manganese superoxide dismutase; MnTBAP, manganese (III) tetrakis (4-benzoic acid) porphyrin; MR, Mre11-Rad50; MRN, Mre11/Rad50/Nbs1; mtDNA, mitochondrial DNA; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NOSox, N-terminal nitric oxide synthase catalytic oxygenase module; NOSred, C-terminal electron-supplying nitric oxide synthase reductase module; NTP, nucleotide triophosphate; PARP-1, poly(ADP-ribose)polymerase 1; ROS, reactive oxygen species; SOD, superoxide dismutase; SOD1, superoxide dismutase 1; SSBs, single-strand breaks; ThM, thumb domain; TTD, trichothiodystropy; WS, Werner syndrome; XP, xeroderma pigmentosum.

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are often linked to tumorigenesis and neurological abnormalities. Severe neurodegeneration is clearly apparent in some disorders, such as Cockayne syndrome (CS) (Lehmann, 2003), while more aging-related phenotypes are present in others, including Werner syndrome (WS) (Goto, 1997). Thus, the emerging knowledge of mutations and polymorphisms in key human ROS and DNA repair genes may provide an informed basis for improved strategies for interventions into brain tumors and neurodegenerative disorders. Yet, to date no unifying theory exists to explain the root causes of aging and neurodegenerative diseases.

Here, we focus on the important gains in our knowledge of the cellular mechanisms controlling ROS and DNA repair events, through insights provided by structural biochemistry studies. We describe the known roles, disease phenotypes and molecular mechanisms of key ROS and DNA repair proteins within the nervous system. We highlight how these proteins coordinate chemical steps within pathways, as well as integrate any signaling and response events, often through dynamic conformational changes in structure. The results of these studies may therefore provide a more unified understanding of molecular survival mechanisms from ROS and DNA damaging insults. Moreover, these findings may provide new templates for rationally based therapeutic design, for the treatment of brain tumors and neurodegenerative diseases.

ROS REMOVAL BY MANGANESE SUPEROXIDE DISMUTASE (SOD)

The high metabolic activity of post-mitotic neuronal cells typically produces significant quantities of ROS. However, neurons are also relatively sensitive to ROS and neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), Alzheimer disease and Parkinson disease have been linked to damage caused by ROS (Andersen, 2004). Ninety percent of the ROS in the cell are generated by mitochondria, as the toxic by-products of energy generation. Most of these free radicals are rapidly scavenged in the cell, by the SOD enzymes. SOD enzymes catalyze the disproportionation of superoxide anion radicals to molecular oxygen and hydrogen peroxide, and are the enzymes that provide the master controls for ROS levels in the cell (Silverman and Nick, 2002).

The important role of SOD in the brain was highlighted by genetic inactivation of the mitochondrial form of SOD, manganese superoxide dismutase (MnSOD), in mice. This resulted in dilated cardiomyopathy, hepatic lipid accumulation, and early neonatal death (Li et al., 1995). Treatment with a SOD mimetic, manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), rescued the $MnSOD^{-/-}$ mutant mice from this systemic pathology and dramatically prolonged their survival (Melov et al., 1998). However, surviving animals developed a pronounced movement disorder, which progressed to total debilitation by 3 weeks of age. Neuropathologic evaluation showed a striking spongiform degeneration of the cortex and specific brainstem nuclei. This was associated with gliosis and intramyelinic vacuolization, and was similar to that observed in cytotoxic edema and disorders associated with mitochondrial abnormalities, such as Leigh disease and Canavan disease. It was suggested that because of the failure of MnTBAP to cross the blood–brain barrier, progressive neuropathology is caused by excessive mitochondrial production of ROS (Melov et al., 1998), normally removed by MnSOD.

To define how MnSOD controls ROS levels in the cell, the molecular mechanism of MnSOD has been extensively characterized through combined structural and biochemical studies. The crystal structure of human MnSOD revealed that the enzyme forms a homotetramer (Borgstahl et al., 1992). Each subunit contains an N-terminal helical hairpin and a C-terminal α/β domain. The helical hairpins form two symmetrical four-helix bundles, which assemble the tetramer. The active site of each subunit is at the junction between the helical hairpin and the α/β domain. The active site contains four amino acid side chains, His26, His74, Asp159 and His163, and one solvent molecule. These coordinate a single manganese ion in a strained trigonal bipyramidal geometry. The active site of the enzyme also shows an apparent hydrogen-bonded network of side chains and water, which extends from the manganese bound solvent molecule at the active site, to solvent-exposed residues and the interface between subunits (Borgstahl et al., 1992; Silverman and Nick, 2002) (Fig. 1). This hydrogen-bonded network is suggested to support proton transfer in catalysis, providing a mechanism for delivering protons to the active site, and a means by which the pKa of the active-site water is regulated (Silverman and Nick, 2002).

Structure-based mutagenesis (Guan et al., 1998; Leveque et al., 2000; Hearn et al., 2003, 2004; Greenleaf et al., 2004) or chemical modification (Ayala et al., 2005a) of the hydrogen bonding partners has highlighted how these residues control the enzyme's activity. The network consists of the metal bound solvent forming a hydrogen bond with Gln143 Nε, which subsequently hydrogen bonds

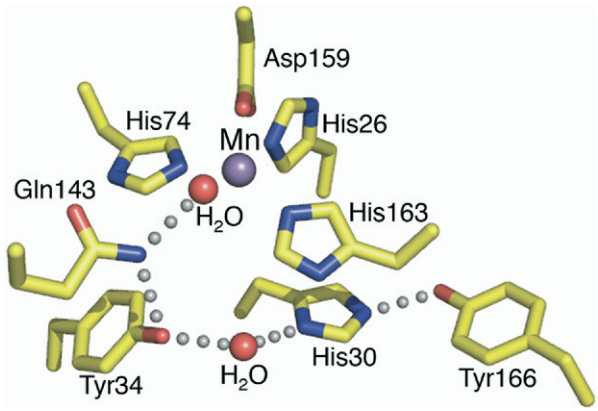

Fig. 1. The human MnSOD active site. In the wild type MnSOD structure (PDB code 1N0J) the His26, His74, His163, and Asp159 side chains and a water molecule chelate a single Mn^{2+} ion. The active site hydrogen bond network, depicted by gray spheres, starts at the metal bond solvent that forms a hydrogen bond to Gln143 and then follows with a bond to Tyr34. A conserved water molecule then mediates the hydrogen bond between Tyr34 and His30, and His30 also forms a hydrogen bond with Tyr166 from another subunit.