Dummy Grant Application

For load and performance testing of the Sapphire system.

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It will contain some graphics as well as some text with a diagram or two…. Here is some text with some technical language…

Ursula Jakob and Dana Reichmann (eds.), Oxidative Stress and Redox Regulation, 2013, DOI: 10.1007/978-94-007-5787-5_14, © Springer Science+Business Media Dordrecht 2013 14. Role of Oxidative Stress in Aging D. Knoefler1, H. L. Tienson1, 2 and U. Jakob1, 3 (1) Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, USA (2) Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA (3) Department of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA U. Jakob Email: ujakob@umich.edu Abstract The question of why we age has given rise to many different theories over the last decades. One of the most popular and long-lasting hypothesis is the free radical theory of aging. It postulates that endogenously generated reactive oxygen species (ROS) accumulate over time, causing damage to cellular macromolecules and eventually leading to physiological decline, disease, aging, and death. Over the years, a multitude of correlative evidence has been collected in favor of this aging theory, including the discovery that aging and many age-related diseases are accompanied by substantial cellular oxidative damage. However, genetic manipulation of components of cellular antioxidant defense systems in model organisms, like Caenorhabditis elegans, Drosophila melanogaster or mice have generated conflicting results and suggested a more complex interplay between endogenous oxidants, antioxidants, and lifespan. The fact that ROS play important roles as second messengers in signaling processes, in hormesis, and during the oxidative burst in innate immune cells, likely contributes to the complexity of this issue. In this chapter, we present an overview of the most crucial experiments conducted to address the free radical theory of aging. Our conclusion is that ROS are major players involved in lifespan and aging but likely not (only) in their role as cytotoxic agents but as regulators of essential physiological processes in the cell. KeywordsRedox regulation – Lifespan – Reactive oxygen species – Caloric restriction – Insulin/IGF signaling – Free radical theory of aging 14.1 The Free Radical Theory of Aging Max Rubner was the first to suggest that aging might be connected to energy metabolism after he observed that organisms with different lifespans expend the same total amount of energy (Rubner 1908). The idea that organisms have a fixed amount of “vital substances”, which, when utilized faster, would shorten lifespan formed the basis of the ‘rate-of-living’ theory proposed by Raymond Pearl in 1921 (Pearl 1921). Although this theory was never proven to be valid, it drew attention to the concept that oxygen metabolism and lifespan might be connected. When Denham Harman realized that ionizing radiation, which induces the formation of oxygen radicals, causes biological effects that are very similar to the physiological changes that occur during aging, he postulated the ‘free radical theory of aging’ (Harman 1956). This hypothesis suggested that free radicals, which are generated by cells themselves, accumulate over time, leading to increased cell and tissue damage and eventually causing physiological decline and

death. The suggestion that harmful reactive oxygen species (ROS) are endogenously produced was initially received with skepticism but gained acceptance with the discovery of superoxide dismutase (SOD), an enzyme whose sole function is the specific removal of superoxide from cells and organisms (McCord and Fridovic 1969). The 'free radical theory of aging' was later modified to the 'mitochondrial free radical theory of aging' (Harman 1972) to take into account the fact that mitochondria are the major source and also the major target of ROS. To acknowledge the involvement of other non-radical oxygen species, like hydrogen peroxide, Harman's theory underwent a final re-definition and is now often referred to as the 'oxidative stress hypothesis of aging' (Yu and Yang 1996). Since the inception of the free radical theory of aging, numerous studies have been conducted providing convincing evidence that cells constantly produce ROS, not only during mitochondrial respiration but also during host defense, cell signaling and many other physiological and pathological events (Trachootham et al. 2008; Droge 2002). To counteract free oxygen radicals, aerobic organisms have evolved a number of highly efficient antioxidant defense systems, which include ROS detoxifying enzymes, small molecules ROS scavengers and oxidoreductases. These systems appear to work together to maintain a crucial balance of pro-oxidants and antioxidants within cells and sub-cellular compartments, a process commonly referred to as redox homeostasis (Finkel and Holbrook 2000). Shifting the equilibrium towards more oxidizing conditions (i.e., oxidative stress) either by increasing the levels of pro-oxidants or by decreasing the cell's antioxidant capacity, leads to the toxic accumulation of ROS, which damage cellular macromolecules, including nucleic acids, lipids and proteins. Oxidative damage has been associated with aging as well as many age-related conditions, including cancer, diabetes, artherosclerosis, cardiovascular diseases and a variety of neurodegenerative diseases (Barnham et al. 2004; Ceriello and Motz 2004; Victor et al. 2009; Reuter et al. 2010). Yet, despite the wealth of studies that have been conducted to test the free radical theory of aging, the jury is still out on whether radical formation is the primary cause of aging or represents a secondary effect of aging and age-related diseases. This is in part due to the recent realization that ROS are not toxic per se. In fact, it is now clear that cells need to maintain certain levels of oxidants to be able to differentiate, develop and to overall function properly (Finkel and Holbrook 2000). Many physiological processes, including cell signaling (Finkel 2011b; D'Autreaux and Toledano 2007; Ghezzi et al. 2005), protein folding (Kakihana et al. 2012; Margittai and Banhegyi 2010), development (Hernandez-Garcia et al. 2010), and immune response require the presence of certain levels of oxidants (Finkel 2011a). These findings imply that while shifting the redox balance towards pro-oxidants is clearly toxic to the cell, shifting the redox balance towards antioxidants might possible not be beneficial either, as it will interfere with the physiological role that low ROS levels play in cells and organisms. In the following chapter, we will summarize the current view on the role of ROS in aging and age-related diseases, attempting to provide a balanced assessment of the most popular aging theory postulated thus far. 14.2 Interplay of Oxidants and Antioxidants 14.2.1 Physiological Occurrence of Reactive Oxygen Species 14.2.1.1 Oxidant Generation in the Mitochondrial Electron Transport Chain (ETC) The electron transport chain (ETC) in mitochondria is generally considered to be the major source of ROS in the eukaryotic cell (Cadenas and Davies 2000). Mitochondria produce the energy to oxidatively phosphorylate ADP, utilizing an electrochemical proton gradient, which is generated by a series of redox reactions located in the inner membrane. In a stepwise reaction catalyzed by four enzyme complexes (I–IV), electrons are passed from NADH to the more electronegative electron acceptor oxygen. Three of the complexes (I, III, and IV) also function as proton pumps, which utilize the energy released from the electron transport chain to transfer protons from the matrix into the intermembrane space. The proton gradient is subsequently utilized by complex V (ATP synthase) to drive ATP production. Over 95% of inhaled oxygen is used in this process (Cadenas and Davies 2000). Although very efficient and tightly regulated, the electron transport chain can lead to mono- or

bivalent reduction of oxygen under physiological conditions, giving rise to superoxide anions and hydrogen peroxide, respectively (Cadenas and Davies 2000; Klotz and Sies 2009) (Fig. 14.1). It is estimated that up to 2% of the molecular oxygen used in mitochondria escapes in form of superoxide anion radicals (Chance and Williams 1956), with complex I and III considered to be the main superoxide producers (Turrens 1997). Not surprisingly, the generation of superoxide and hydrogen peroxide is thus dependent on the mitochondrial metabolic state. Excess of dietary substrates or decreased ATP production due to lack of ADP will stall the flow of electrons through the ETC, which increases electron leakage and hence ROS formation. In contrast, decreasing the metabolic rate by reducing the amount of substrate intake is thought to reduce electron leakage and thus to minimize superoxide and peroxide generation in mitochondria (Heilbronn and Ravussin 2003).

Oxidative Stress and Redox Regulation (pp. 388-392). Springer Netherlands. Kindle Edition.

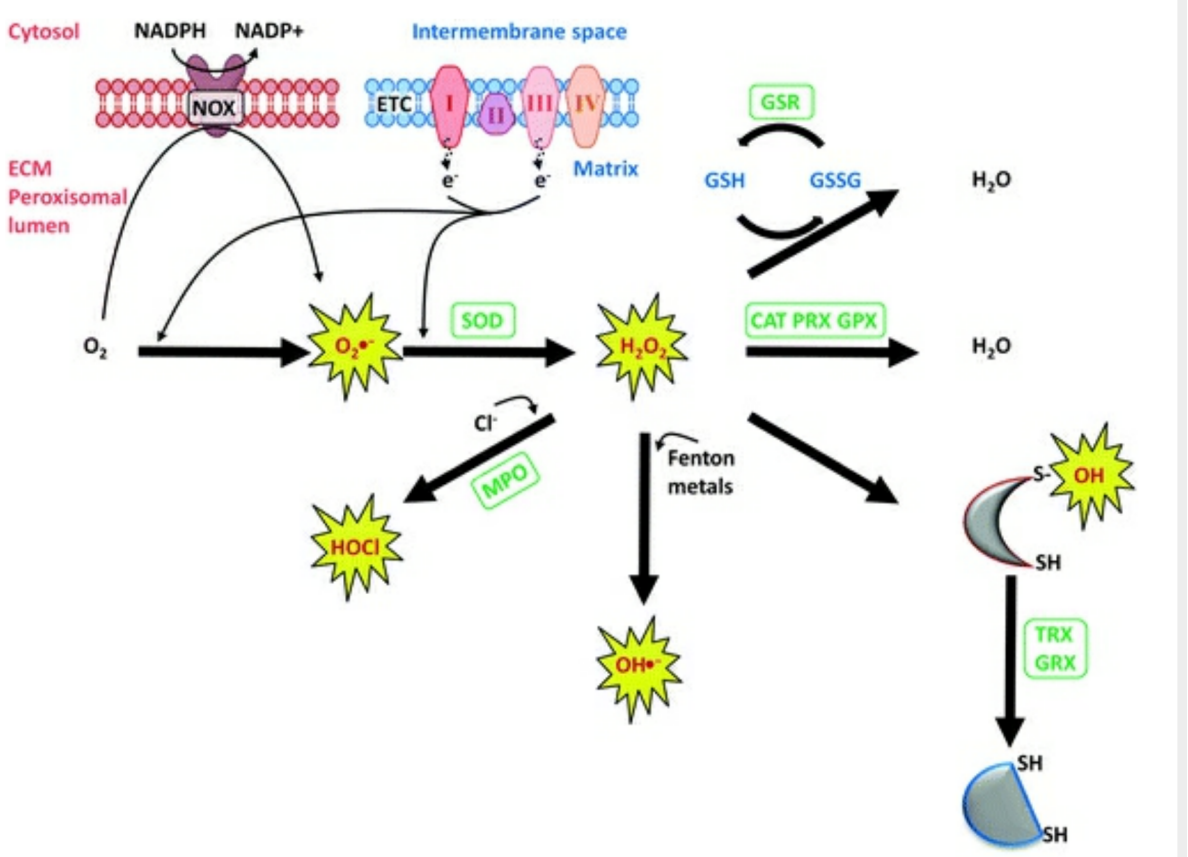

Fig. 14.1 Interplay between oxidants and antioxidants. Reactive oxygen species (ROS) are produced by members of the electron transport chain (ETC), located in the inner membrane of the mitochondria, and by transmembrane NADPH oxidases (NOXs), located in plasma and peroxisomal membranes. Electrons, which constantly leak during the electron transport chain, react with molecular oxygen to form superoxide or hydrogen peroxide (H_2O_2). NADPH oxidases utilize cytosolic NADPH to generate superoxide ($O_2^{\bullet -}$) either in peroxisomes or the extracellular matrix (ECM). Superoxide is rapidly dismutated to the slow-acting hydrogen peroxide (H_2O_2) in a process that is catalyzed by superoxide dismutase (SOD). H_2O_2 can react with chloride ions to generate the very potent oxidant hypochlorous acid (HOCl), in a process that is catalyzed by myeloperoxidases (MPO) within phagocytes. HOCl is a fast acting oxidant, targeting sulfur-containing amino acids and causing widespread protein aggregation *in vivo*. In the presence of Fenton metals (i.e., iron, copper), peroxide rapidly forms highly reactive hydroxyl radicals ($OH^{\bullet -}$), which react with and potentially destroy all cellular macromolecules in their vicinity. To detoxify H_2O_2, cells utilize a combination of enzymatic clearance systems, consisting of catalase (CAT), peroxiredoxin (PRX), and glutathione peroxidase (GPX), as well as non-enzymatic small molecule scavengers. One of these scavengers is the small tripeptide glutathione (GSH), which becomes oxidized to GSSG in the process. Regeneration of GSH is achieved by glutathione reductase (GSR). Other peroxide scavengers are surface thiols in proteins, which undergo sulfenic acids (SOH) formation (Hansen et al. 2009; Murphy 2012). Sulfenates are either directly reduced by the thioredoxin (TRX) system or undergo S-glutathionylation, which is reversed by the glutaredoxin (GRX) system

14.2.1.2 Oxidant Production by NADPH Oxidases and Dual Oxidases