

Chapter 14

Role of Oxidative Stress in Aging

D. Knoefler, H.L. Tienson, and U. Jakob

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

D. Knoefler

Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, USA

H.L. Tienson

Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, USA

Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA

U. Jakob (✉)

Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI, USA

Department of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA

e-mail: ujakob@umich.edu

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.

Keywords Redox 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, atherosclerosis, 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

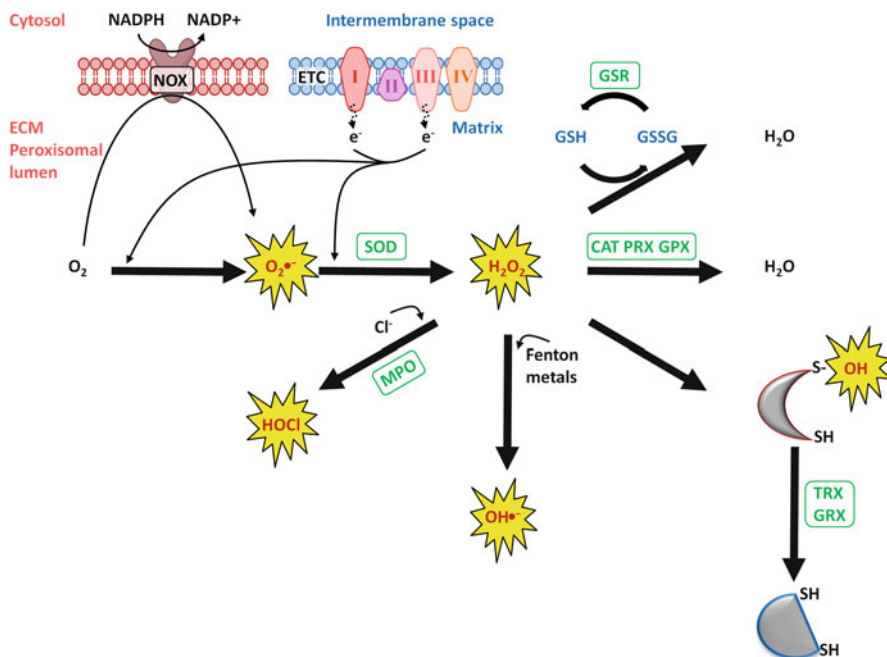


Fig. 14.1 Interplay between oxidants and antioxidants. Reactive oxygen species (ROS) are produced by members of the electron transport chain (ETC), located in the inner membrane of the mitochondria, and by transmembrane NADPH oxidases (NOXs), located in plasma and peroxisomal membranes. Electrons, which 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 ($\text{O}_2^{\bullet-}$) 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

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).

14.2.1.2 Oxidant Production by NADPH Oxidases and Dual Oxidases

NADPH oxidases (NOX) and dual oxidases (DUOX), which are universally distributed in cells and organisms, generate ROS upon exposure to a variety of stimuli, including growth factors, cytokines or bacterial invasion (Lambeth 2004) (Fig. 14.1). During the innate immune response, for instance, invading microorganisms are engulfed by phagocytes. This process triggers the activation of phagocytic NADPH oxidases, which produce large quantities of superoxide anions by transferring electrons from NADPH to oxygen (i.e., respiratory burst). Superoxide radicals are then converted to hydrogen peroxide, which is either directly released into the phagosome of phagocytes or used by myeloperoxidases in neutrophils (a subgroup of phagocytes) to form the potent antimicrobial hypochlorous acid (the active ingredient of household bleach). Dysfunction of phagocytic NADPH oxidase has been implicated in a number of inheritable immunodeficiency's, such as chronic granulomatous disease (Bedard and Krause 2007), which is characterized by the inability of the innate immune system to kill invading pathogens due to a failure to produce sufficient amounts of ROS (Lambeth 2004).

In addition to NADPH oxidases in phagocytic cells, isoforms of NADPH oxidases are involved in a host of other physiological processes. Growth factors, such as angiotensin II, platelet-derived growth factor (PDGF), or vascular endothelial growth factor (VEGF) utilize NOX-mediated ROS signaling to regulate angiogenesis and blood pressure, among other processes (Lambeth 2004; Ushio-Fukai and Nakamura 2008). Thyroidal NADPH dual oxidases, in contrast, provide hydrogen peroxide for thyroid hormone synthesis (Nauseef 2008; Dupuy et al. 1991). Typically membrane-bound, NADPH oxidases utilize cytosolic NADPH to generate superoxide either in the extracellular matrix or the lumen of intracellular organelles (Fig. 14.1). While superoxide itself is not membrane permeable, it is either transported to other cell compartments by ion channels or converted into the highly diffusible hydrogen peroxide. Many NADPH oxidases are ubiquitously expressed and are thought of being capable of generating higher ROS levels in a regulated manner than those continuously produced during respiration (Krause 2007). It is thus not surprising that increased expression and/or activity of several NOX family members has been implicated to play a key role in a number of age-related diseases, including cancer, cardiovascular diseases and neurodegenerative disorders (Bedard and Krause 2007; Krause 2007).

14.2.1.3 Oxidants as By-Products of Biochemical Reactions

In addition to reactions catalyzed by proteins of the ETC and by NADPH oxidases, many other cellular reactions have been shown to produce ROS. In peroxisomes, for example, electrons generated during the β -oxidation of long fatty acids are transferred onto molecular oxygen instead of components of the ETC, thereby producing hydrogen peroxide. Oxidative deamination of aromatic (dietary) amines and monoamine neurotransmitters, such as serotonin and dopamine, is catalyzed by monoamine oxidases (MAO) in a process that leads to the production of potentially neurotoxic by-products, including ammonia and hydrogen peroxide (Bortolato et al. 2008). Other major endogenous ROS producers belong to the heme-containing cytochrome P450 protein superfamily. Members of this family are involved in oxidizing endogenous substrates as well as a broad range of exogenous compounds, including drugs, carcinogens and other xenobiotics. Since the monooxygenation of these substrates is inefficiently coupled to the electron transfer from NADPH to cytochrome P450, it causes a continuous leakage of electrons, resulting in ROS formation even in the absence of substrates (Zangar et al. 2004). Some xenobiotic compounds such as alcohol or drugs can further increase the P450-uncoupling reaction, thereby increasing ROS generation even more. The need to maintain low intracellular ROS level has apparently resulted in the development of feedback mechanisms as the presence of high ROS levels was recently found to decrease cytochrome P450 levels (Zangar et al. 2004).

14.2.2 Antioxidants – Maintaining the Balance

14.2.2.1 Detoxification Systems and ROS Scavengers

Superoxide anions are known to spontaneously dismutate to hydrogen peroxide (H_2O_2) at a slow rate. *In vivo*, this process is massively accelerated by the presence of superoxide dismutases (SODs), which are located in the cytosol, the mitochondrial intermembrane space and matrix, as well as in the extracellular space (Fridovic 1972; Zelko et al. 2002). While H_2O_2 is less reactive and more stable than other ROS (Giorgio et al. 2007), it reacts rapidly with free ferrous (Fe^{2+}) iron in the Fenton reaction, which generates hydroxyl radicals, one of the most reactive oxygen species known. Hence, removal of peroxide is of utmost importance to avoid widespread oxidative damage. Enzymatic clearance of hydrogen peroxide is performed by catalases, glutathione (GSH) peroxidases, and peroxiredoxins (Fig. 14.1). Catalases are mainly found in the cytosol and peroxisomes, as well as in the mitochondrial matrix of some highly metabolically active tissues, such as heart and liver (Radi et al. 1991; Salvi et al. 2007). They catalyze the decomposition of two hydrogen peroxide molecules to oxygen and two water molecules, using one of the fastest turnover rates known for any enzyme (Nicholls et al. 2001). While catalases contribute to hydrogen peroxide decomposition at high hydrogen peroxide concentrations, selenocysteine-

containing GSH peroxidases work efficiently at low peroxide levels, suggesting that they serve as the predominant peroxide scavengers under physiological H_2O_2 concentrations (Makino et al. 1994). GSH peroxidases catalyze the reduction of hydrogen peroxide to water by utilizing reduced glutathione (GSH) as electron donor. GSH, a highly abundant tripeptide is oxidized in this process to disulfide-bonded GSSG, and subsequently regenerated by GSH reductase, a NADPH-dependent oxidoreductase. The third group of peroxide-detoxifying enzymes is constituted by peroxiredoxins, which compensate for their slow reaction rates with extremely high cellular concentrations, making them one of the most abundant enzymes in many organisms. The structure, function and role of peroxiredoxins in response to oxidative stress is expertly reviewed in Chap. 4 of this book.

In addition to the various antioxidant enzymes that clear reactive oxygen species, organisms have evolved various small molecules such as glutathione, metallothioneins and vitamins, which are capable of scavenging oxygen radicals. While the non-protein thiol γ -L-glutamyl-L-cysteinyl-glycine (glutathione) can act as reductant for peroxide (Fig. 14.1) and free radicals (Orrenius and Moldeus 1984), metallothioneins, which are low molecular weight metal-containing proteins, are capable of scavenging hydroxyl radicals and superoxide (Thornalley and Vasak 1985). The water-soluble ascorbate (vitamin C) scavenges oxygen radicals in aqueous solution whereas α -tocopherol (vitamin E) protects membranes from radical formation (Niki 1987).

14.2.2.2 Maintaining and Restoring Redox Homeostasis

One of the most ROS-sensitive and reactive target in the cell is the sulfur-containing amino acid cysteine. Many cysteine thiols in proteins have been shown to rapidly react with peroxide, HOCl and/or NO, thereby forming a variety of different oxidative modifications, including sulfenic acid and disulfide bond formation, mixed disulfides with glutathione (S-glutathionylation) or S-nitrosylation (Winterbourn and Hampton 2008). Because of their high sensitivity towards oxidation, cysteine thiols are also the amino acids of choice for those proteins, whose function is regulated by the redox conditions of the environment. Redox sensitive proteins are found to play roles in the majority of cellular functions, ranging from signal transduction (e.g., phosphatases and kinases) and gene expression (e.g., p53) to metabolism (e.g., GapDH) and proteostasis (e.g., Cdc-48) (Brandes et al. 2009; Kumsta et al. 2011). Oxidative modification of redox sensitive cysteines leads to the transient activation (e.g., OxyR, Hsp33, Nrf2) or inactivation (e.g., phosphatases) of a protein's function, making thiol-modifications uniquely able to fine-tune cellular pathways and response systems to the cellular redox environment.

Two highly conserved enzymatic systems, the thioredoxin system and the glutaredoxin system are responsible for maintaining redox homeostasis and reducing most forms of oxidative thiol modifications in proteins (expertly reviewed in Chap. 3). The thioredoxin system consists of the small oxidoreductase thioredoxin, which uses direct thiol-disulfide exchange reactions to reduce sulfenic

acids, disulfide bonds or S-nitrosylated cysteines in proteins (Collet and Messens 2010). Thioredoxins are then reduced by thioredoxin reductase, a selenocysteine-containing enzyme in eukaryotes, which utilizes NADPH as the ultimate electron donor. The second redox system consists of the small redox enzymes glutaredoxins, which directly interact with oxidized protein thiols. In contrast to thioredoxins, which are reduced by thioredoxin reductase, glutaredoxins are non-enzymatically reduced by reduced glutathione. Oxidized glutathione is subsequently regenerated by glutathione reductase, which, like thioredoxin reductase, uses NADPH as an electron donor (Holmgren et al. 2005).

As outlined above, both thioredoxin and glutaredoxin systems depend on reduced NADPH as electron source, making both systems and hence the cellular redox status ultimately dependent on the cellular NADPH/NADP⁺ ratio (Schafer and Buettner 2001). The major source of NADPH within the cell is the pentose phosphate pathway, which generates two molecules of NADPH for every oxidized glucose-6-phosphate molecule. The strict dependence of the cellular redox status on NADPH explains the need for efficient re-routing of glucose from glycolysis to the pentose phosphate pathway under conditions of oxidative stress (Godon et al. 1998; Grant 2008). Oxidative modification and inactivation of key enzymes of glycolysis seem to contribute to these changes in glucose flux, illustrating how redox-sensitive metabolic enzymes play an active part in the oxidative stress defense of organisms (Brandes et al. 2011). Deficiency in glucose-6-phosphate dehydrogenase, which catalyzes the first step of the pentose phosphate pathway, results in lower intracellular NADPH/NADP⁺ ratios and increased oxidative stress and has been associated with premature cell senescence and a number of different disease conditions (Ho et al. 2007).

14.3 Evidence in Support of the ‘Free Radical Theory of Aging’

14.3.1 *Oxidative Damage Increases with Age*

As outlined above, the free radical theory of aging states that progressive accumulation of ROS during the lifespan of an organism contributes and potentially causes the decline in fitness and might even determine longevity (Finkel and Holbrook 2000). A large amount of correlative evidence has been collected demonstrating a significant increase in oxidative damage to nucleic acids, lipids and proteins as organisms age, using a variety of different model systems ranging from bacteria to human (Golden et al. 2002; Sohal et al. 2002; Muller et al. 2007). By far the most commonly used assay to assess oxidative damage in cells and organisms is the analysis of protein carbonylation. This irreversible oxidative modification occurs primarily on the side chains of prolines, arginines, lysines and threonines in response to oxidative stress (Dalle-Donne et al. 2003). It is commonly assayed by a variety of semi-quantitative techniques, which rely on the detection of carbonylated

side chains upon *ex vivo* derivatization (Moller et al. 2011). Unfortunately, none of the available assays are able to determine what proportion of a protein population is affected to what extent, making the effects of protein carbonylation on the physiology of an organism difficult to assess. No distinction can be drawn whether an increased signal results from an increase in the number of carbonylation sites within a few protein molecules, or from carbonylating an increasing proportion of a protein population at a single, physiologically important site. Nevertheless, it is a convenient method to monitor trends in protein oxidation and has been extensively used in aging research. Tissues of mice fed *ad libitum* showed increasing levels of protein carbonylation with age (Sohal et al. 1994) and tissues derived from individuals with premature aging diseases (e.g., progeria) exhibited higher level of protein carbonyls compared to age-matched healthy humans (Stadtman 1992). The extent of protein carbonylation has also been shown to increase during the last third of the lifespan in tissues from different organisms, including house flies, *C. elegans*, rats and humans, summarized in (Levine 2002).

14.3.2 (Some) Oxidants Accumulate with Age

In addition to evaluating the effects of oxidative stress on proteins, lipids and DNA as a read-out for intracellular oxidative damage, assays have been developed that directly measure the concentration of ROS, such as superoxide or peroxide in aging organisms. The obtained results were not always consistent with the free radical theory of aging as it was shown, for instance, that the concentration of hydrogen peroxide in *Drosophila* homogenates increases during the first trimester of their life but remains stable during the remainder of the lifespan (Sohal et al. 1990). In contrast, mitochondrial matrix hydrogen peroxide was observed to increase during aging in *Drosophila* (Cocheme et al. 2011). Peroxide levels also increased in aging *C. elegans* population as was recently demonstrated by using chromosomally encoded peroxide-specific sensor proteins (Back et al. 2012) whereas microsomal superoxide anion production actually declined from reproductive to senescent age, with long-lived mutant animals (i.e., age-1) exhibiting higher superoxide anion levels than the age-matched control animals (Vanfleteren 1993). In mice, both mitochondrial superoxide and hydrogen peroxide release from heart, brain and kidney tissues increased with the age of the animals (Sohal et al. 1994).

14.3.3 (Some) Antioxidant Levels Decrease with Age

Age-induced oxidative damage can either be caused by increased ROS production, decreased detoxification, or a combination thereof. Many studies have been conducted to assess the activity of ROS-detoxifying enzymes in young and old organisms, with the goal to either confirm or rule out the model that older animals

have lower levels of ROS-detoxifying activity than young animals, hence the accumulation of oxidative damage. As exemplified below, while such correlation does seem to exist for certain antioxidant systems in some tissues and model systems, it does not generally apply to all ROS or model systems, making a more differentiated discussion necessary.

14.3.3.1 Superoxide Dismutases

Comparative analysis of superoxide dismutase activity in kidney, brain and heart tissue of young and old mice did not reveal any significant alteration in total SOD activity (Sohal et al. 1994). Similarly, activity of Cu/ZnSOD in liver homogenates of mice between 4, 12, or 18 months of age appeared unchanged while analysis of MnSOD activity revealed even an increase of SOD activity with age (Andziak et al. 2005). These results suggested that SOD activity levels in mice do not change with age. Cu/ZnSOD activity in brain tissues of aging rats, however, showed a gradual decline in activity, which appeared to be caused by a decrease in SOD expression levels (Semsei et al. 1991).

Studies in other model systems were consistent with the results obtained in mice and showed that SOD activity levels either remained unaltered during the lifespan (i.e., *C. elegans*) or linearly increased with age (i.e., *Drosophila lysates*) (Vanfleteren 1993; Sohal et al. 1990). Expression levels of Cu/ZnSOD, as determined by mRNA and steady state SOD analysis, remained relatively constant in aged flies (Radyuk et al. 2004). These results ruled out the possibility that a significant decrease in SOD activity and/or level was directly responsible for the oxidative damage observed in aging organisms.

14.3.3.2 Catalases, Glutathione Peroxidases and Peroxiredoxins

Studies assessing the activity of peroxide-detoxifying enzymes during the lifespan revealed a relatively consistent trend, indicating that the peroxide detoxifying capacity of organisms does indeed decrease with age. Analysis of the catalase activity in liver samples of mice, for example, showed a decline with the age of the animals (Perichon and Bourre 1995). A significantly decreased level of catalase and glutathione peroxidase activity was also observed in liver homogenates of 18 months old mice in comparison to 12 months old animals (Andziak et al. 2005), a finding that was independently confirmed for catalase in heart tissue and for glutathione peroxidase in kidney (Sohal et al. 1994). Brain tissue of aged mice, however, exhibited increased catalase and glutathione peroxidase activity (Sohal et al. 1994). This result was in contrast to brain samples of rats, which exhibited a gradual decline in catalase activity with age that coincided with a decrease in catalase mRNA (Semsei et al. 1991).

Analysis of non-rodent model systems were overall also more consistent, revealing kinetics of catalase activity in *Drosophila* that seem to follow a bell-shaped curve with higher levels of catalase in young animals as compared to older animals

(Sohal et al. 1990; Durusoy et al. 1995). In a subsequent more thorough analysis, catalase expression was shown to be both time- and tissue-specific, coinciding with pulses of ecdysteroid synthesis during development followed by a small decline as flies aged (Klichko et al. 2004). Studies in young *C. elegans* adults revealed a similar initial increase in the catalase activity and a decline as the worms aged (Vanfleteren 1993). Dramatic changes in expression level were also observed for peroxiredoxin 5 in *Drosophila*, which showed the highest expression level during embryogenesis, followed by a decline during aging (Radyuk et al. 2009). These results are largely consistent with the idea that the activity levels of peroxide-detoxifying enzymes decrease as animals age, potentially leading to the accumulation of peroxide in aging tissue.

14.3.3.3 Glutaredoxin, Thioredoxin and NADPH

To assess whether changes in the activity of the cellular redox systems contribute to the oxidative damage observed in aging organisms, expression analyses were conducted to monitor the activity of the thioredoxin and glutaredoxin system. An early study focusing on the thioredoxin system in rat kidneys reported decreasing levels of both thioredoxin and thioredoxin reductase with the age of the animals (Cho et al. 2003). The same study also found decreased levels of reduced glutathione and glutathione reductase activity in older rat kidneys as compared to young animals. These results were independently confirmed in aged rat muscles, where expression levels of both mitochondrial thioredoxin reductase and cytosolic thioredoxin were significantly reduced (Rohrbach et al. 2006). In contrast, however, levels of mitochondrial thioredoxin appeared to increase with age (Rohrbach et al. 2006). Moreover, comparative analysis of the glutathione and thioredoxin system in the heart muscle of young and old rats did not reveal any significant changes but did reveal an increase in oxidized GSSG levels, indicative of a pro-oxidant shift in the glutathione reduction potential (Jacob et al. 2010). A pro-oxidant shift in the glutathione pool has also been reported to occur in multiple other tissues of aging mice and rats, generally caused by an increase in oxidized glutathione and sometimes accompanied by a decrease in reduced glutathione. These changes tend to be most significant in liver tissues (Rebrin and Sohal 2008). Given that both glutaredoxin and thioredoxin systems rely heavily on reduced NADPH to maintain redox homeostasis, this shift in redox potential might be partially explained by a decrease in cellular NADPH levels, which has been observed to occur in the neurons of aging rats (Parihar et al. 2008). Studies in invertebrates confirmed some of the results obtained in rodents. It was shown, for instance, that the reduced glutathione pool sharply declines in older flies (Sohal et al. 1990). Moreover, caloric restriction, one of the few near-universal life prolonging measures (see Sect. 14.5.2) has been shown to partially reverse the detected changes in redox potential (Someya et al. 2010; Cho et al. 2003; Rohrbach et al. 2006). In summary, these studies provide convincing evidence that a combined decline in the cellular antioxidant capacity occurs with the age of the animal, which likely contributes to the accumulation of oxidative damage.

14.4 Do Manipulations of the Cell's Antioxidant Capacity Affect Lifespan?

After years of correlative studies, big hopes were spawned with the development of methods that enable genetic manipulations of model organisms, as they should allow direct and unambiguous testing of the validity of the free radical theory of aging. If correct, modulating endogenous ROS levels either by deleting or overexpressing specific antioxidant systems should have clear effects on lifespan. Below is a summary of the current state of affairs, based on genetic manipulation studies conducted in mice, *Drosophila* and *C. elegans*. An overview of the published results can be found in Tables 14.1 and 14.2.

14.4.1 Effects of Manipulating Superoxide Levels on Lifespan of Model Organisms

14.4.1.1 Effects of SOD Deletion

Deletion of either cytosolic Cu/ZnSOD1 or mitochondrial MnSOD2 in mice was found to significantly reduce their lifespan, illustrating the importance of preventing toxic ROS accumulation in mammals (Elchuri et al. 2005; Li et al. 1995). Deletion of cytosolic Cu/ZnSOD1 was also associated with increased oxidative damage and led to age-related loss of skeletal muscle mass and an increased incidence of liver cancer (Muller et al. 2006; Elchuri et al. 2005). Deletion of mitochondrial MnSOD2 had even more severe effects and led to early neonatal death with many animals dying within the first 16 days after birth (Li et al. 1995; Huang et al. 2001). This mortality rate increased further with higher oxygen concentrations (i.e., hyperoxia) (Asikainen et al. 2002). Yet the severity of the phenotype suggested that the decreased lifespan might be not purely due to accelerated aging. Partially depleting the levels of mitochondrial MnSOD2 by making *sod2*^{+/-} heterozygous mice did not affect their resistance to hyperoxia (Asikainen et al. 2002; Tsan et al. 1998) but did increase their sensitivity towards oxidative stressors (Van Remmen et al. 2003, 2004). These animals showed increased oxidative damage of mitochondrial and nuclear DNA and a significant increase in tumor incidence and age-associated biomarkers, like cataracts and immune response. Yet, their lifespan was unaffected by the mutation (Van Remmen et al. 2003). Homozygous deletion of extracellular SOD (EcSOD) in mice was found to increase their sensitivity to hyperoxia but the lifespan of the animals appeared to not be affected (Carlsson et al. 1995; Sentman et al. 2006). These results provided experimental evidence that deletion of either cytosolic or mitochondrial SOD causes increased oxidative damage and early death in mammals, although it is still inconclusive whether these two phenotypes are directly related.

Table 14.1 Effects of deletion of antioxidant genes on stress resistance and lifespan in model organisms

Antioxidant system	Species	Stress resistance	Lifespan	Refs
Cu/Zn Superoxide dismutase				
Δ sod1	Mm		↓	1,2
Δ sod1	Dm	↓	↓	3
Δ sod1	Ce	↓	↓↔ ^a	4–7
Δ sod1 + Δ sod2; Δ sod3; Δ sod4; Δ sod5	Ce	↓	↓↔	6, 7
Δ ECsod	Mm	↓	↔	8, 9
Mn Superoxide dismutase				
Δ sod2 ^{Het,b}	Mm	↓	↔	10, 11
Δ sod2	Dm	↓ ^{Het}	↓ ^b	12
Δ sod2	Ce	↓↔	↑↔	4, 6, 7
Δ sod2 + Δ sod1; Δ sod3; Δ sod4; Δ sod5	Ce	↓	↑↔	6, 7
Δ sod1 Δ sod2 Δ sod3 Δ sod4 Δ sod5	Ce	↓	↔ ^c	13
Catalase				
Δ cat	Mm	↔	↔ ^d	14
Δ cat	Dm	↓	↓	15, 16
Δ ctl1	Ce		↔	17
Δ ctl2	Ce		↓	17
Peroxiredoxin				
Δ prdx1	Mm		↓	18
Δ prdx2 ^e	Dm	↓	↓	19
Δ prdx2	Ce	↓	↓	20, 21
Δ prdx5	Dm	↓	↓ ^f	22
Glutathione peroxidase				
Δ gpx1	Mm	↓↔	↔	23–25
Δ gpx4 ^{Het,g}	Mm		↑	26
Thioredoxin				
Δ trx1	Ce	↓	↓	27, 28
Δ trx2 ^{Het,g}	Mm		↔	2
Δ trx2	Dm	↓	↓	29, 30

1 Elchuri et al. 2005, 2 Perez et al. 2009a, 3 Phillips et al. 1989, 4 Yen et al. 2009, 5 Yanase et al. 2009, 6 Doonan et al. 2008, 7 Van Raamsdonk and Hekimi 2009, 8 Carlsson et al. 1995, 9 Sentman et al. 2006, 10 Van Remmen et al. 2003, 11 Van Remmen et al. 2004, 12 Duttaroy et al. 2003, 13 Van Raamsdonk and Hekimi 2012, 14 Ho et al. 2004, 15 Mackay and Bewley 1989, 16 Griswold et al. 1993, 17 Petriv and Rachubinski 2004 18 Neumann et al. 2003, 19 Lee et al. 2009a, 20 Kumsta et al. 2011, 21 Olahova et al. 2008, 22 Radyuk et al. 2009, 23 Ho et al. 1997, 24 Fu et al. 1999, 25 Zhang et al. 2009, 26 Ran et al. 2007, 27 Miranda-Vizuete et al. 2006, 28 Jee et al. 2005, 29 Tsuda et al. 2010, 30 Svensson and Larsson 2007

Mm *M. musculus*, Dm *D. melanogaster*, Ce *C. elegans*, Het heterozygous

^aSod1 deletion reduces hypothermia-induced lifespan extension

^bHomozygous deletion has severe health effects (Li et al. 1995; Huang et al. 2001)

^cMaximum lifespan extended

^dObservation up to 1 year of age

^eNull mutation or RNAi knockdown, stress resistance assessed in RNAi knock-down

^fAccelerated death during development, maximum lifespan normal

^gHomozygous deletion embryonic lethal (Yant et al. 2003; Nonn et al. 2003)

Table 14.2 Effects of overexpression of antioxidant genes on stress resistance and lifespan in model organisms

Antioxidant system	Species	Stress resistance	Lifespan	Refs
Cu/Zn SOD overexpression				
<i>sod1</i> +/- <i>sod2</i>	Mm		↔	1–3
<i>sod1</i> (motorneurons)	Dm	↑	↑	4
<i>sod1</i> (ubiquitous)	Dm	↑	↑	5
<i>sod1</i> +/- <i>sod2</i>	Dm	↔	↔↑ ^a	6–8
<i>sod1</i>	Ce	↓	↑	9, 10
Mn SOD overexpression				
<i>sod2</i> +/- <i>cat</i>	Dm	↑	↑↔↓ ^{b,c}	7, 11–14
<i>sod2</i>	Ce		↑	10
Catalase overexpression				
<i>cat</i> (mitochondria)	Mm		↑	15
<i>cat</i> (peroxisomes)	Mm		↔	2, 15
<i>cat</i>	Dm	↑	↓↔ ^d	6, 11, 16
<i>ctl1</i> + <i>ctl2</i> + <i>ctl3</i>	Ce		↓ ^e	9
Peroxioredoxin overexpression				
<i>prx2</i> (neurons)	Dm	↑	↑	17
<i>prx5</i>	Dm	↑	↑	18
Thioredoxin overexpression				
<i>trx1</i>	Mm	↑	↑↔ ^f	19, 20
<i>trx1</i> (neurons)	Ce		↑	21
<i>trx2</i>	Dm	↑	↑	22, 23

1 Huang et al. 2000, 2 Perez et al. 2009b, 3 Rando et al. 1998, 4 Parkes et al. 1998, 5 Reveillaud et al. 1991, 6 Sun and Tower 1999, 7 Sun et al. 2004, 8 Seto et al. 1990, 9 Doonan et al. 2008, 10 Cabreiro et al. 2011, 11 Mockett et al. 2010, 12 Mockett et al. 1999, 13 Bayne et al. 2005, 14 Sun et al. 2002, 15 Schriener et al. 2005, 16 Griswold et al. 1993, 17 Lee et al. 2009a, 18 Radyuk et al. 2009, 19 Mitsui et al. 2002, 20 Perez et al. 2011, 21 Miranda-Vizuete et al. 2006, 22 Svensson and Larsson 2007, 23 Seong et al. 2001

^aSimultaneous overexpression of *sod1* and *sod2* increased the lifespan additively

^bMnSOD has slightly decreased lifespan (4–5%)

^cSimultaneous overexpression of *sod2* and catalase in mitochondria decreased lifespan by 43%

^dTwo *cat+* strains were tested; while one strain did not show a change in lifespan, the other strain had a significantly decreased lifespan

^eDeaths by internal hatching

^fWhile overexpression of *trx1* in male mice significantly extended earlier part of life (maximum lifespan was unaffected), female mice showed no significant change in lifespan

Very similar results were obtained in *Drosophila*, where deletion of either cytosolic *sod1* or mitochondrial *sod2* significantly decreased lifespan (Phillips et al. 1989; Duttaroy et al. 2003). Again deletion of the mitochondrial isoform caused more severe defects, as demonstrated by the finding that a large fraction of flies died

within the first 24 h after eclosion (Duttaroy et al. 2003). Genetic manipulation of SOD levels in *C. elegans*, however, yielded a significantly more ambiguous picture, contributing to a substantial amount of controversy in the field (Doonan et al. 2008). At this point, it is unclear to what extent the presence of additional cytoplasmic and mitochondrial SOD homologues (SOD-5; SOD-3) might have affected the outcome of some of these studies. Initially it was reported that deletion of the major cytosolic Cu/ZnSOD-1 increases the sensitivity of *C. elegans* to superoxide-inducing paraquat and shortens *C. elegans*' lifespan while deletion of the mitochondrial MnSOD-2 also increased sensitivity towards oxidative stressors but did not affect lifespan (Yen et al. 2009; Yanase et al. 2009; Doonan et al. 2008). An independent study in which all five SOD isoforms of *C. elegans* were manipulated either individually or in combination also reported increased sensitivity towards oxidative stressors like paraquat and juglone for *sod-1*, *sod-2*, and *sod-3* mutants but did not confirm reduced lifespan for any of the tested mutants (Van Raamsdonk and Hekimi 2009). In fact, deletion of mitochondrial MnSOD-2 (*sod-2*) alone or in combination with other SOD isoforms was found to even extend *C. elegans*' maximum lifespan by 5–19 days (Van Raamsdonk and Hekimi 2009). This result, however, requires further confirmation as an independent study observed an increase in oxidative stress sensitivity for *sod-2* deletion mutants but did not observe any beneficial effects on lifespan (Doonan et al. 2008). In an attempt to ultimately address the question regarding the significance of SOD in the lifespan of *C. elegans*, Van Raamsdonk and Hekimi went through the effort to construct a quintuple mutant strain, which lacked all five SOD-homologues. Importantly, this mutant strain showed the same mean lifespan as wild type worms but a significantly increased maximum lifespan (Van Raamsdonk and Hekimi 2012). The authors found that the quintuple mutant strain was highly sensitive to superoxide-inducing paraquat, heat shock and osmotic stress. Yet, the strain was more resistant to exogenous peroxide treatment than wild type strains. This increased peroxide resistance was apparently due to the massive upregulation of catalases, PRDX-2 and thioredoxin reductase 1, which seem to serve as compensatory mechanisms to deal with ROS. In summary, these results suggest that deletion of certain superoxide dismutase isoforms affects the capacity of organisms to deal with oxidative stressors, and causes a decline in fitness. It remains to be determined, however, to what extent superoxide-toxicity truly constitutes an aging factor.

14.4.1.2 Effects of SOD Overexpression and SOD Mimetics

Many gene deletions have been shown to affect the health and/or cause early death in organisms without being directly involved in regulating aging. Thus, a by far more desirable and interpretable phenotype is that of lifespan extension, which, if the free radical theory is indeed correct, should be achieved by increasing the ability of organisms to detoxify otherwise harmful ROS. This should reduce oxidative damage and hence extend lifespan. Much has been done to address this question and stimulation of superoxide detoxification can now either

be achieved genetically by overexpressing select SOD isoforms, or chemically by application of SOD mimetics, such as EUK-8 or EUK-134, which detoxify superoxide *in vivo*.

Studies performed in mice showed that the lifespan of mice was unaffected even when both mitochondrial MnSOD and cytosolic Cu/ZnSOD were simultaneously overexpressed (Huang et al. 2000; Perez et al. 2009b). It is of note, however, that Cu/ZnSOD overexpressing mice showed increased lipid peroxidation, suggesting that SOD overexpression might lead to the accumulation of peroxide in cells and tissues, hence potentially off-setting the beneficial effects of enhanced superoxide detoxification (Rando et al. 1998).

Studies in *Drosophila* were highly conflicting with results ranging from (i) 40% lifespan extension upon overexpression of human Cu/ZnSOD in fly motoneurons (Parkes et al. 1998), to (ii) mild lifespan-enhancing effect in flies overexpressing bovine Cu/Zn SOD under the control of the ubiquitous actin 5C gene promoter (Reveillaud et al. 1991), to (iii) no changes in lifespan upon overexpressing of additional copies of either *Drosophila* Cu/ZnSOD or MnSOD (Seto et al. 1990; Mockett et al. 2010), to even (iv) a reduction in lifespan in strains overexpressing mitochondrial MnSOD (Mockett et al. 1999), even when co-overexpressed with catalase (Bayne et al. 2005). It is conceivable that the different effects of SOD overexpression on *Drosophila* lifespan are due to differences in cultivation conditions. Indeed, it has been shown that administration of antioxidants does not cause an extension of mean or maximum lifespan if the control group has an optimal lifespan, while the survival could be extended by antioxidant treatment when the lifespan of the control group was sub-optimal (Kohn 1971). A beneficial effect of Cu/ZnSOD overexpression seems to occur in strains which are rather short-lived, implying that optimal SOD level already exist in wild type strains exhibiting a normal lifespan (Orr and Sohal 2003). It is also possible that expression of antioxidant enzymes affects lifespan in a tissue and/or stage-dependent fashion (Mockett et al. 1999). For instance, overexpression of either cytosolic Cu/ZnSOD or mitochondrial MnSOD initiated during adulthood using a heat shock-inducible expression system extended both mean and maximum lifespan (Sun et al. 2002; Sun and Tower 1999). However, while the inducible expression system enables researchers to work in identical genetic strain backgrounds (Sun and Tower 1999), initiation of transgene expression is based on a heat pulse, which might itself have some negative effects on those strains not overexpressing SOD (Mockett et al. 1999).

Studies in *C. elegans* revealed that despite the fact that overexpression of cytosolic Cu/ZnSOD-1 increases the levels of cellular protein oxidation and enhances the sensitivity of animals towards paraquat-induced oxidative stress, it does increase *C. elegans* lifespan (Doonan et al. 2008; Cabreiro et al. 2011). Overexpression of mitochondrial MnSOD-2 was found to also extend the lifespan of worms by approximately 25% (Cabreiro et al. 2011). A set of independent studies agreed with these results by showing that treatment of worms with the SOD/catalase mimetics EUK-8, EUK-134 or platinum nanoparticles resulted in a significantly increased mean and maximum lifespan of wild type worms, and restored wild type-like lifespan in a short-lived *mev-1* mutant (Kim et al. 2008; Melov et al. 2000). Yet these

studies were questioned by reports that showed that treatment with the same SOD mimetic (i.e., EUK-8) causes a dose-dependent shortening of lifespan, particularly at higher doses, suggesting that the effects of antioxidant interventions might be dependent on culture conditions (Keaney and Gems 2003). Moreover, while EUK-8 treatment protected worms against paraquat-toxicity, it did not extend lifespan in non-paraquat treated worms (Keaney et al. 2004). Note that treatment of worms with lower concentrations of the superoxide-generating compounds paraquat or juglone has recently been found to cause lifespan extension in worms (Heidler et al. 2010; Lee et al. 2010), suggesting that superoxide levels need to be carefully monitored in worms before final conclusions can be drawn about the efficiency of SOD mimetics in worms.

In summary, changing superoxide dismutase activity in mice, *Drosophila* or *C. elegans* either by deletion or overexpression of the respective enzymes did not reveal a clear picture about the effects of superoxide on lifespan. The results reflect a much more complex correlation between the amount of superoxide produced at distinct stages in life, the levels of antioxidants present, and the lifespan. It remains to be determined to what extent superoxide-toxicity might have contributed to the observed lifespan reduction and early deaths observed in mitochondrial MnSOD null mutants in fly and mice, and if this truly constitutes superoxide-mediated aging. Another intriguing question is whether overexpression of superoxide dismutases actually adds to cellular oxidative stress since higher SOD activity inevitably increases the hydrogen peroxide pool in the cells.

14.4.2 Effects of Altering the Hydrogen Peroxide Clearance System on Lifespan of Model Organism

The hydrogen peroxide clearance system typically consists of catalases, peroxiredoxins and glutathione peroxidases. In the following section we will summarize the effects that genetic manipulation of the individual branches of the hydrogen peroxide clearance system has on the lifespan of mice, *Drosophila* and *C. elegans*.

14.4.2.1 Effects of Catalase Deletion and Overexpression

Catalase-deficient mice develop normally and are healthy at least up to 1 year of age. They do not reveal any increased sensitivity towards hyperoxia or photochemically induced oxidative stress even though tissue slices from catalase knockout mice show slower rates in decomposing extracellular H₂O₂ (Ho et al. 2004). Mice expressing human catalase under the control of the endogenous promoter and regulatory elements, which results in peroxisome-specific overexpression of catalase similar to endogenous gene expression (Chen et al. 2003), did not show any lifespan extension even when Cu/ZnSOD was co-overexpressed (Perez et al. 2009b). In contrast, mice overexpressing the human catalase gene targeted to the mitochondria showed an

improved rate of peroxide clearance as well as reduced H₂O₂ production, decreased oxidative damage, and delayed development of cardiac pathology. Most importantly, these mice showed an extension of both median and maximum lifespan by 5 and 5.5 months, respectively (Schriner et al. 2005). These results provide first evidence that increased peroxide clearance from mitochondria might have beneficial effects on mammalian lifespan.

Drosophila mutants with catalase activities of less than 2% showed severe viability effects (Mackay and Bewley 1989). An independent study confirmed these findings, and demonstrated that the lack of catalase activity in *Drosophila* results in decreased viability and lifespan (Griswold et al. 1993). Increasing the levels of catalase in *Drosophila*, however, had either no effect on lifespan (Griswold et al. 1993; Mockett et al. 2010; Sun and Tower 1999) or caused a moderately reduced lifespan, depending in part on the insertion site of the transgene and the magnitude of catalase overexpression (Mockett et al. 2010; Sun and Tower 1999). Only when combined with the simultaneous overexpression of Cu/ZnSOD, was overexpression of catalase found to be beneficial for lifespan (Orr and Sohal 1994). This study was, however, contradicted by another group, who reported that the combined overexpression of catalase and Cu/ZnSOD did not result in a benefit beyond the lifespan expanding effect observed for Cu/ZnSOD overexpressing flies (Sun and Tower 1999).

Similar to the results in *Drosophila* where deletion of catalase significantly reduced lifespan, deletion of peroxisomal catalase 2 (*ctl-2*) in *C. elegans* was found to cause progeric phenotypes and a shortened lifespan. In contrast, deletion of the cytosolic *ctl-1* isoform had no effect on the lifespan of worms (Petriv and Rachubinski 2004). To test whether overexpression of catalase increased *C. elegans* lifespan, the *ctl-1*, *ctl-2*, *ctl-3* gene cluster was overexpressed, generating tenfold higher catalase activity (Doonan et al. 2008). Lifespan measurements did not reveal any significant effect of catalase overexpression in *C. elegans*. In fact, it increased mortality rate by internal hatching, an event that could be suppressed by the simultaneous overexpression of Cu/ZnSOD-1.

These studies suggest that animals lacking catalase accumulate high levels of hydrogen peroxide, which might contribute to a decline in fitness and causes early death at least in some model systems. Reducing peroxide levels by overexpressing catalase in mitochondria appears to have beneficial effects on mammalian lifespan but does not seem to enhance the lifespan of invertebrates.

14.4.2.2 Effects of Peroxiredoxin Deletion and Overexpression

Mice lacking cytosolic peroxiredoxin 1 (*prdx1*) exhibit increased DNA damage and a higher incidence of hemolytic anemia and cancer starting at 9 months of age, which effectively reduces their lifespan (Neumann et al. 2003). Deletion of peroxiredoxin 2 (*prdx2*) also results in hemolytic anemia and increased levels of

oxidative stress but the mutant mice seem otherwise healthy and fertile, and their lifespan has not been determined (Lee et al. 2003b). Deletion of the 1-Cys peroxiredoxin 6 (*prdx6*) results in elevated ROS level and increased oxidative damage and heightens the animal's susceptibility towards oxidative stress-mediated damage (Wang et al. 2003). These results confirm the important role that peroxiredoxins play on peroxide detoxification. To our knowledge, only one study presented lifespan data of peroxiredoxin deletion mice so far (Neumann et al. 2003). Adenovirus-mediated overexpression of PRDX6 in the lungs of mice was found to promote resistance to hyperoxia-induced oxidative stress. So far, no lifespan experiments have been reported with these mice.

RNAi knock down of *prx2* in flies resulted in an increased sensitivity to paraquat-mediated oxidative stress. Additionally, flies with a reduction in PRX2 activity, either through RNAi knockdown or a loss of function mutation, exhibited a shortened lifespan (Lee et al. 2009a). The deletion of peroxiredoxin 5 (*Prx5*) in *Drosophila* also leads to a slightly shortened mean lifespan, which appears to be mainly caused by accelerated dying during day 5–10 after eclosion. These results suggest that PRX5 might be important in proliferating tissues and maybe required for embryonic development (Radyuk et al. 2009). After development, the mortality rate of *prx5* null mutants was found to be similar to the mortality rate of wild type flies, resulting in the same maximum lifespan for both wild type and *prx5* mutants (Radyuk et al. 2009). The modest lifespan defect of *prx5* null flies was, however, severely enhanced under oxidative stress conditions (Radyuk et al. 2009). Neuronal expression of either human PRX2 or the *Drosophila* PRX2 homolog as well as ubiquitous expression of PRX5 was found to enhance the resistance of flies to oxidative stress and to extend their lifespan, providing excellent evidence that in flies, detoxification of peroxide through peroxiredoxins prolongs life (Radyuk et al. 2009; Lee et al. 2009a).

Peroxiredoxin 2 (PRDX-2), which is one of the most abundant proteins in *C. elegans*, plays a crucial role for hydrogen peroxide clearance in worms (Kumsta et al. 2011; Olahova et al. 2008). Animals lacking PRDX-2 are much more sensitive to sublethal hydrogen peroxide treatments than wild type *C. elegans*, fail to recover from exogenous peroxide treatment, and exert progeric phenotypes as well as symptoms of chronic oxidative stress (Kumsta et al. 2011; Olahova et al. 2008). Most importantly, *prdx-2* deletion worms show a severe lifespan defect, which seems most pronounced at lower temperatures (Kumsta et al. 2011; Olahova et al. 2008).

These studies show that deletion of peroxiredoxin has some of the most severe effects on the lifespan of model organisms, illustrating its importance in hydrogen peroxide clearance. Importantly, overexpression of peroxiredoxin in *Drosophila* significantly extends lifespan, which is very similar to the effects that overexpression of mitochondrial catalase has in mice. These results suggest that enhanced detoxification of peroxide might indeed be a lifespan-extending factor.

14.4.2.3 Effects of Glutathione Peroxidase Deletion and Overexpression in Mice

In addition to catalases and peroxidases, mice also contain several different glutathione peroxidases, which appear to play a prominent role in detoxifying low levels of peroxide. Mice lacking glutathione peroxidase 1 (*gpx1*) display a normal and healthy development, an unaltered capacity to decompose hydrogen peroxide, a wild type-like protein and lipid oxidation pattern and the same sensitivity to hyperoxia (Ho et al. 1997). While the mutant mice do show an increased sensitivity towards diaquat-induced oxidative stress (Fu et al. 1999) and increased cataract occurrence (Wolf et al. 2005), their lifespan is not affected by the absence of *gpx1* even in combination with a *sod2*^{+/-} heterozygous mutation (Zhang et al. 2009). In contrast, glutathione peroxidase 4 (*gpx4*) null mutants were found to be embryonic lethal (Yant et al. 2003). Expression of human GPX-4 was able (i) to rescue the embryonic lethality of *gpx4*^{-/-} mice, (ii) to reduce apoptosis and (iii) to increase cell survival after oxidative insult (Ran et al. 2004). Heterozygous *gpx4*^{+/-} mice showed an increased median lifespan but neither a change in maximum lifespan nor in age-related mortality rate, indicating that aging itself is not slowed in these animals (Ran et al. 2007).

14.4.3 Manipulation of the Thioredoxin/Glutaredoxin System

Genetic manipulations of the thioredoxin system are limited, yet the studies conducted so far all seem to point to the fact that maintenance of cellular redox homeostasis is crucial for development and lifespan. Deletion of either cytosolic thioredoxin 1 or mitochondrial thioredoxin 2 in mice is embryonic lethal (Nonn et al. 2003; Matsui et al. 1996). Heterozygous *trx2*^{+/-} mice have increased ROS generation and oxidative damage compared to wild type mice but their lifespan is unchanged (Perez et al. 2008, 2009a). Overexpression of TRX-1 was found to extend lifespan of mice (Mitsui et al. 2002). This result was, however, put into question as the lifespan of the control mice was significantly shorter than expected. A more recent study by Perez et al. reported that overexpression of mouse *trx1* causes an increase in the mean lifespan of male mice without affecting their maximal lifespan. These results suggest that the observed decrease in oxidative damage is beneficial to male mice early in life but might not aid in extending lifespan later in life (Perez et al. 2011). Studies in *Drosophila* showed that flies lacking *trx-2* are short-lived while flies overexpressing *trx-2* show enhanced survival upon exposure to hydrogen peroxide or paraquat (Tsuda et al. 2010; Svensson and Larsson 2007). Moreover, a gain-of-function-mutation of *trx* conferred longevity to *Drosophila* (Seong et al. 2001). Very similar results were also obtained in *C. elegans*, where overexpression of *trx-1* was found to extend the mean lifespan of *C. elegans* (Miranda-Vizuete et al. 2006) while deletion of *trx-1* resulted in a reduced resistance to oxidative stress as well as in a shortened mean and maximum lifespan (Miranda-Vizuete et al. 2006; Jee et al. 2005).

14.4.4 Where Are We Now, What Should We Think?

The many conflicting results obtained with genetic manipulations of antioxidant enzymes in a variety of different organisms over the last few years clearly illustrate the complexity of redox homeostasis and its role in lifespan. In general, deletion of antioxidant enzymes appears to have one of two outcomes. One being so serious that the organism is severely affected – thus decreased lifespan may not be a direct result of premature aging. The other one having little to no effect at all, suggesting either significant redundancy with other antioxidant enzymes or implying that their function is so highly specialized as to not affect longevity under “normal” conditions. Hence, it is probably unwise to draw significant conclusions from deletion studies. Overexpression studies could be viewed as a more direct approach to analyze the influence of antioxidant systems on aging. However, confusion arose from studies where the same genetic manipulations revealed different effects in different labs. This suggests that specific growth conditions and/or differences in the strain background might be additional sources that influence lifespan and need to be carefully controlled and monitored.

14.5 Lifespan Extension and the Free Radical Theory of Aging – What Works and Why

14.5.1 Lifespan Extension by Manipulating the Electron Transport Chain

Manipulation of endogenous ROS production has been used as an alternative approach to assess the role of ROS in aging and lifespan. Screens for mutants with increased sensitivity towards the superoxide generator paraquat in *C. elegans* resulted initially in the identification of the mitochondrial *mev-1* mutant (Ishii et al. 1990). *Mev-1* encodes a subunit of succinate dehydrogenase cytochrome b, a component of mitochondrial complex II (Ishii et al. 1998). Mutants in *mev-1* were found to exert elevated levels of superoxide anions (Senoo-Matsuda et al. 2001), reduced Cu/ZnSOD activity levels and a significantly shortened lifespan (Ishii et al. 1990), which could be reversed by treatment with superoxide dismutase/catalase mimetics (Melov et al. 2000). Similarly, *Drosophila* mutants of succinate dehydrogenase subunit b (complex II) also exhibited increased levels of mitochondrial hydrogen peroxide production, enhanced sensitivity towards hyperoxia and a shorter lifespan (Walker et al. 2006). Furthermore, *C. elegans* mutants of complex I (i.e., *gas-1*) were found to have elevated levels of superoxide anion and also displayed a shortened lifespan (Kondo et al. 2005; Hartman et al. 2001). These findings seemed to support the free radical theory of aging as they suggested that the decrease in lifespan, which is observed in mitochondrial mutants is caused by increased oxidant levels (Senoo-Matsuda et al. 2001; Sedensky and Morgan 2006). More recent reports indicated, however, that the model that a compromised mitochondrial ETC

causes increased oxidant production and hence shorter lifespan is far more complex than previously anticipated. In fact, worms carrying a mutation in *isp-1*, an iron-sulfur protein of complex III, showed increased resistance towards oxidative stress, reduced oxygen consumption, and an extended lifespan, indicating that a reduction in ETC function might also positively affect the lifespan of *C. elegans* (Feng et al. 2001). RNAi-mediated screens for longevity genes further confirmed these results by finding a tenfold overrepresentation of genes involved in mitochondrial function, whose knock-down improved H₂O₂ tolerance (although paraquat tolerance was reduced) and extended lifespan (Lee et al. 2003a). Selective targeting of individual components of the ETC, including proteins of complex I, III, IV and V causes a significant extension in lifespan in *Drosophila* and *C. elegans* (Dillin et al. 2002; Copeland et al. 2009). It is of note that reducing ETC function had to be initiated during *C. elegans* development to achieve lifespan extension whereas reduction of ETC components during adulthood of *C. elegans* resulted in lowered ATP level but no changes in lifespan (Dillin et al. 2002). These results imply that the rate of mitochondrial respiration during development is at least partly responsible for adjusting *C. elegans*' growth rate, development, and adult lifespan. The developmental window during which the intervention seems to be successful ends by the third or early fourth larval stage of *C. elegans*, indicating that an event occurring during larval development might set the clock for lifespan (Rea et al. 2007). One mechanism, which seems to play a role in the lifespan extension mediated by ETC reduction is the mitochondria-specific unfolded protein response (UPR), which can be induced in a cell-non-autonomous way, meaning that signals from one tissue can trigger or control processes in other tissues (Durieux et al. 2011) (Fig. 14.2).

It is of note that many long-lived strains with mutations in the ETC, such as *nuo-6*, *isp-1* and *clk-1* showed elevated ROS level, which speaks clearly against the original idea that accumulation of ROS is toxic *per se* (Yang and Hekimi 2010; Lee et al. 2010). In fact, treatment of *nuo-6* and *isp-1* mutants with the antioxidant N-acetylcysteine (NAC) abolished the observed lifespan extension, suggesting that (transient) accumulation of oxidants might actually be required for the lifespan-prolonging phenotype of those mutants (Yang and Hekimi 2010). In a similar way, Yang and co-workers showed that treatment with low concentrations of the superoxide generator paraquat caused a transient superoxide boost, which significantly increased the lifespan in *C. elegans* despite an apparent increase in oxidative damage as assessed by protein carbonylation (Yang and Hekimi 2010). These results are in good agreement with the recently postulated mitohormesis theory, which will be discussed in detail later in this chapter.

Deletion of *clk-1* has been shown to increase ROS levels (Lee et al. 2010), UV-resistance (Murakami and Johnson 1996) and lifespan in worms (Wong et al. 1995). A heterozygous deletion mutant of the *clk-1* homologue in mice (*mclk1*) was found to also accumulate higher mitochondrial oxidative protein damage and, most importantly, to extend lifespan in mice (Liu et al. 2005; Lapointe and Hekimi 2008). What appears to contribute to this lifespan extending effect is the mitochondrial ROS-mediated activation of the transcription factor HIF-1 α , which in

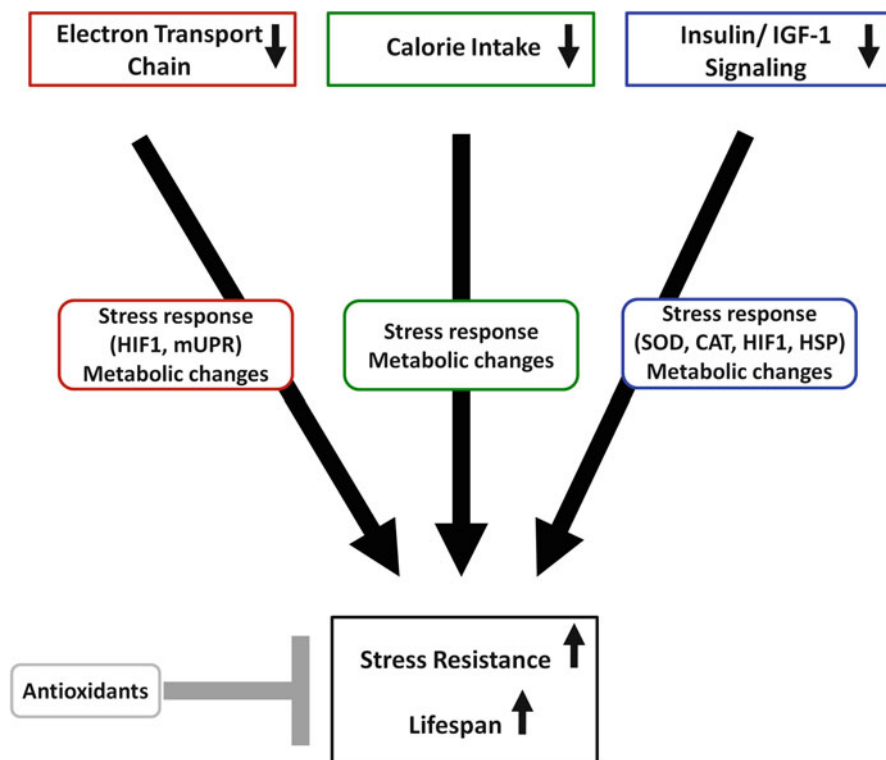


Fig. 14.2 Interventions that extend lifespan. Reduction of components of the electron transport chain (ETC), a decrease in the caloric intake, or a decline in Insulin/IGF-1 signaling extends lifespan in a variety of different organisms. One factor that might contribute to the observed lifespan extension is an increased stress resistance. The role that oxidants play in these lifespan-extending interventions is not fully elucidated yet. However, it has been demonstrated that antioxidants typically interfere with the observed lifespan extensions. Note: Some ETC mutants can also be short-lived

mice is involved in regulating the inflammatory immune response (Wang et al. 2010) (Fig. 14.2). This result led to speculations that the increased lifespan of *mclk1*^{+/-} mice results from an elevated resistance to pathogenic infection especially in aging mice (Wang et al. 2010). Observations in *C. elegans* suggested that compromised respiration could be involved in the ROS-mediated activation of HIF-1, which is indeed responsible for the lifespan extension observed in *C. elegans clk-1* and *isp-1* mutants (Lee et al. 2010). Similarly, the lifespan enhancing effect observed in *C. elegans* upon administering low levels of the superoxide-generator paraquat might also work through the ROS-induced activation of HIF-1, suggesting that the HIF-1 mediated lifespan extension might be evolutionarily conserved (Lee et al. 2010).

Taken together, these studies suggest that lifespan extension achieved by reducing mitochondrial respiration is not simply caused by minimizing the output of

harmful reactive oxygen species and decreasing oxidative damage. It rather seems to involve the activation of complex pathways, including the unfolded protein response (UPR) (Durieux et al. 2011), cell-cycle checkpoint control (Rea et al. 2007), changes in HIF-1-mediated gene expression (Lee et al. 2010) and possibly a switch in energy metabolism (Rea and Johnson 2003). That respiratory mutants do not mediate their longevity by one unifying feature was also suggested by the finding that reduction of HIF-1 (either by mutation or RNAi) significantly reduced the extended lifespan of *isp-1* and *clk-1* mutants but only partially affected the long-lived phenotype of other mitochondrial mutants, such as *cyc-1* or *cco-1* (subunits of complex III and IV) (Lee et al. 2010). This finding might also explain the apparently controversial results concerning the role of ROS in lifespan extension of mitochondrial mutants. While antioxidant treatment was found to not affect lifespan extension of some mitochondrial RNAi mutants (Durieux et al. 2011), other studies reported that superoxide is in fact required to mediate lifespan extension (Yang and Hekimi 2010).

14.5.2 *Lifespan Extension by Caloric Restriction*

Reduction of the daily caloric intake by 30% (dietary or caloric restriction) routinely extends lifespan up to 50% in a variety of different model organisms, including yeast, flies, *C. elegans*, mice and primates (McCay et al. 1935; Fontana et al. 2010). Calorically restricted mice have been found to have lower mitochondrial generation of superoxide and hydrogen peroxide than animals fed *ad libitum* (Sohal et al. 1994; Lambert and Merry 2004; Bevilacqua et al. 2004). Similarly, animals with reduced caloric intake show lower levels of age-accompanying oxidative DNA damage and oxidative protein damage as measured by protein carbonylation, and reveal higher levels of NADPH, specifically in the mitochondria of brain, liver, heart, kidney, ear and eyes of mice (Dubey et al. 1996; Sohal et al. 1994; Someya et al. 2010). Hence, caloric restriction appears to decrease oxidative damage by shifting the glutathione pool to a more reducing redox potential relative to age-matched controls. Whereas earlier findings seemed to indicate that caloric restriction resulted in a lowered metabolic rate, hence the decrease in oxidative damage, more recent studies that were corrected for body mass suggested quite the opposite (Houthoofd et al. 2002). In fact, caloric restriction appears to enhance mitochondria biogenesis (Lopez-Lluch et al. 2006) and to increase the rate of respiration (Lin et al. 2002; Nisoli et al. 2005) (Fig. 14.2). These results, although initially counterintuitive, are fully consistent with recent studies in *C. elegans*, which showed that 2-deoxyglucose (DOG)-mediated glucose restriction during adulthood increased mitochondrial respiration and ROS production, and significantly extended the lifespan (Schulz et al. 2007). Interestingly, pre-treatment of these animals with antioxidants, such as NAC or vitamin E abolished the beneficial effect of glucose restriction on lifespan (Schulz et al. 2007). These findings led to the model of mitohormesis, in which generation of slightly elevated levels of oxidants through increased respiration during a

defined time in life enhances expression of antioxidant genes and with that the capacity of organisms to detoxify ROS. Contrary to glucose-restriction, glucose-supplementation of *C. elegans*' diet prevented longevity of *daf-2* worms, a mutant of the Insulin signaling pathway (described in 14.5.3), and shortened the lifespan of wild type animals by inhibiting the transcription factor DAF-16 (Lee et al. 2009b; Schlotterer et al. 2009). Interestingly, increased ROS generation was found at day 15 in worms fed on high-glucose-diet, suggesting that the duration of increased ROS level (or the magnitude) might make a difference in lifespan determination (Schlotterer et al. 2009).

Although it is now generally accepted that caloric restriction increases respiratory rates, the jury is still out whether ROS levels increase, stay unchanged or decrease. Several studies in calorically restricted yeast, flies or rodents observed either no change or even a decrease in ROS production accompanying increased cellular respiration (Barros et al. 2004; Bevilacqua et al. 2004; Miwa et al. 2004; Cocheme et al. 2011; Ash and Merry 2011). So while caloric restriction clearly increases respiratory rates, it might also lead to more efficient respiration possibly through mild uncoupling between electron transport and oxidative phosphorylation (Barros et al. 2004; Bevilacqua et al. 2004; Lopez-Lluch et al. 2006). A mild uncoupling of the two processes could result in an increase of ETC function, which could lead to less opportunity for electron leakage, and hence more efficient mitochondria function and less ROS formation. This conclusion of course argues against a hormetic effect in lifespan extension. However, since CR can be considered as a mild stress condition, it could lead to the observed increase in antioxidant defenses by other means.

14.5.3 Manipulation of the Insulin/IGF-1 Signaling (ILS) Pathway

Genetic manipulation of the ILS pathway has been shown to reproducibly modulate lifespan in *Drosophila*, *C. elegans* and mice (Longo and Finch 2003). As outlined below, interference with the insulin signaling pathway appears to affect levels of both oxidants and antioxidants in organisms, providing additional support for a role of ROS in lifespan determination. Insulin/IGF-1 signaling is a highly conserved pathway, which has been implicated in a multitude of physiological processes, including stress response, diapause, reproduction, metabolism, growth, and aging (Tatar et al. 2003). Signaling through the Insulin/IGF-1 receptor occurs via a phosphorylation cascade, which ultimately causes phosphorylation of the forkhead transcription factor FOXO, and prevents FOXO from its translocation into the nucleus. Conversely, lack of the IGF-1 receptor or disruption of the kinase cascade promotes FOXO's translocation into the nucleus and allows the transcriptional regulator to induce the expression of a variety of stress-related genes (Kenyon 2005). FOXO-controlled genes encode for heat shock proteins, for proteins involved in pathogen resistance, metabolism (e.g. β -oxidation of fatty acids and

gluconeogenesis), transcriptional repression and protein degradation as well as for antioxidant enzymes, such as superoxide dismutase, catalase and glutathione S-transferases (reviewed by Murphy 2006) (Fig. 14.2). These findings suggested that the lifespan extension observed in mutants with compromised insulin signaling might be, at least in part, due to increased oxidative stress protection.

Studies in *C. elegans* also revealed that mutants lacking the insulin/IGF-1 receptor *daf-2* or the phosphoinositide kinase PI3K (*age-1*) show increased level of SOD and catalase activity, significantly increased oxidative stress resistance and exhibit very extended lifespans (Honda and Honda 1999; Johnson 1990; Kenyon et al. 1993; Brys et al. 2007). Similar results were observed in *Drosophila*, where deletion of either the insulin receptor or CHICO, the fly insulin receptor substrate, increases both oxidative stress resistance and lifespan (reviewed by (Giannakou and Partridge 2007)). The lifespan-extending features were found to strictly depend on the presence of the FOXO transcription factor *daf-16* as *C. elegans* mutants lacking DAF-16 show increased sensitivity towards paraquat-mediated oxidative stress and are severely short-lived (Yanase et al. 2002; Lin et al. 2001). *Daf-16* mutant worms also show significantly increased protein damage as measured by protein carbonylation, providing further confirmation that these animals experience increased levels of oxidative stress (Yanase et al. 2002).

That the insulin signaling pathway not only influences the lifespan of invertebrates but also of vertebrates became clear when female mice carrying a heterozygous mutation of the insulin-like growth factor type 1 receptor gene *igf-1r^{+/-}* (homozygous null mutation of IGF-1R has been found to be embryonic lethal) were found to be more resistant to oxidative stress and were long-lived (Holzenberger et al. 2003). These results were consistent with earlier studies on the IGF-1R substrate p66^{Shc}, whose mutation was found to increase oxidative stress resistance and extend lifespan (Migliaccio et al. 1999). Subsequent studies with tissue-specific knock-outs of the IGF-1R in the adipose tissue of mice further confirmed these results and showed that these mice have an extended lifespan (Bluhner et al. 2003). At this point it is still unclear which of the many FOXO-regulated genes are ultimately responsible for the observed lifespan extension in worms and other organisms, and what exact role(s) ROS play in the ILS-mediated lifespan regulation. A RNAi-mediated knock-down of FOXO-target genes, including glutathione transferase, cytosolic *ctl-1*, peroxisomal *ctl-2*, or mitochondrial superoxide dismutase *sod-3* was found to individually reduce the long lifespan of *daf-2* mutants while deletion of the cytosolic superoxide dismutase *sod-1* had no effect on the lifespan of *daf-2* mutants. Moreover, loss of the extracellular superoxide dismutase *sod-4* further extended *daf-2*-mediated lifespan (Murphy et al. 2003; Ayyadevara et al. 2005; Doonan et al. 2008). That lifespan-extension by compromised Insulin/IGF-1-signaling is not due to a reduction of oxidants and decreased oxidative damage became obvious when long-lived *daf-2* mutants were found to have higher respiratory rates, and exhibit increased levels of mitochondrial ROS level (Houthoofd et al. 2005; Zarse et al. 2012). Moreover, their lifespan was slightly reduced upon treatment with antioxidants (Yang and Hekimi 2010; Brys et al. 2007; Zarse et al. 2012). These results were highly reminiscent of studies conducted with some long-lived

ETC mutants, which also showed increased ROS level that seemed necessary for lifespan extension (see Sect. 14.5.1). When Insulin/IGF-1 signaling was reduced during adulthood of worms, an increase in respiration was observed that caused a transient increase in ROS, which eventually led to increased activity of catalase and SOD (Zarse et al. 2012). The observed lifespan extension was diminished when antioxidants were supplemented (Zarse et al. 2012). The observation that treatment of worms with the superoxide generator juglone caused nuclear translocation (i.e., activation) of DAF-16 while exposure to hydrogen peroxide lead to phosphorylation (i.e., inactivation) of DAF-16 (Weinkove et al. 2006; Nemoto and Finkel 2002) suggested that the type of oxidant and possibly its sub-cellular accumulation might affect signaling processes, oxidative stress resistance and ultimately the lifespan of organisms.

14.6 Is the Free Radical Theory of Aging Still Valid?

Taken all the published studies together, it is now clear that both the expression levels of antioxidants and the production of oxidants are optimized for individual organisms and finely balanced. Any drastic change in oxidant or antioxidant level brings about the possibility to negatively affect tightly regulated processes, such as development, differentiation, signaling, host defense, metabolism, and ultimately lifespan (Fig. 14.3). Future studies aimed to further manipulate the interplay between endogenous oxidants and antioxidants will provide, at most, more correlative evidence. However, to ultimately prove or disprove the free radical theory of aging, we need to temporally and spatially dissect both positive and negative effects of distinct ROS-levels on individual processes during the lifetime of an organism to define which levels, timings and kinetics are required for proper functioning of the organism. Only when we understand the effects of ROS *in vivo* can we begin to manipulate their levels with a predictable outcome. The development of quantitative *in vivo* ROS sensors and read-outs of cellular targets of distinct ROS levels provides us now with the opportunity to make clear distinctions between onset, duration and magnitude of oxidant formation, and to assign the role of ROS in development, proliferation, host defense, aging and disease.

Over the past decades, our view on reactive oxygen species has changed from branding them as undesired, potentially harmful by-products of aerobic life to acknowledging their importance in many physiological processes. The necessity of certain levels of oxidants became even more obvious by the observations that antioxidants were capable of reversing beneficial effects on physiology and lifespan, and by the discovery that many transcription factors and signaling pathways can be influenced by reactive oxygen species (Hamanaka and Chandel 2010; Trachootham et al. 2008). Hence, we need to now move beyond the free radical theory of aging to acknowledge the fact that ROS are major players in lifespan and aging but likely not (only) in their function as cytotoxic agents but as regulators of essential physiological processes in the cell.

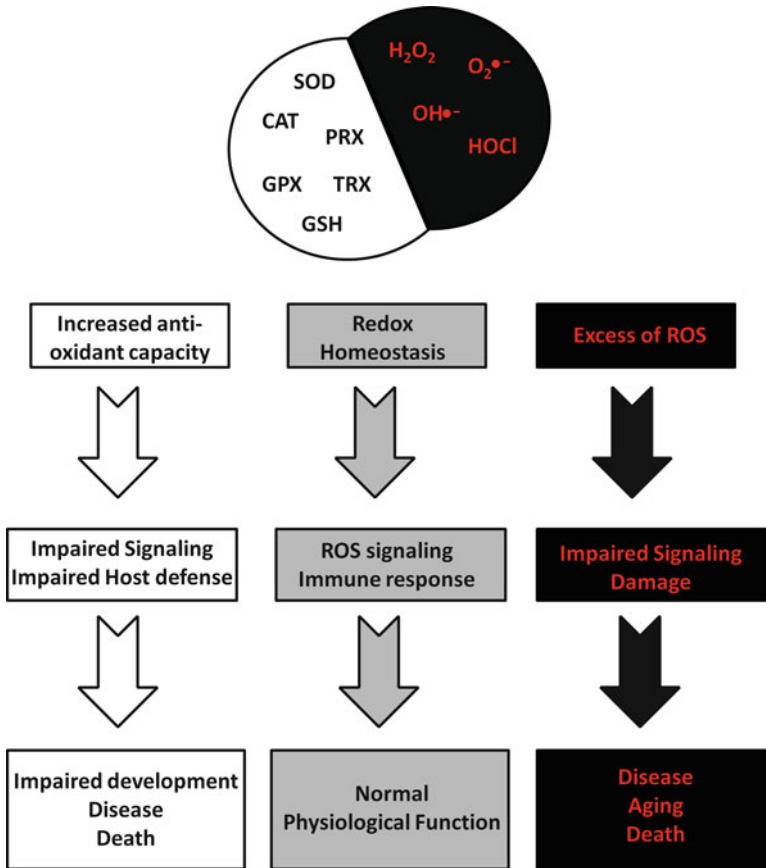


Fig. 14.3 Maintaining redox homeostasis – Essential for the physiology of organisms. Maintaining the proper redox balance between oxidants, such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet-}$), hypochlorous acid (HOCl) and antioxidants, like catalase (CAT), peroxiredoxin (PRX), glutathione peroxidase (GPX), superoxide dismutase (SOD), thioredoxin (TRX), glutathione (GSH) is essential for correct physiological processes. Shift towards more oxidizing conditions (i.e., oxidative stress) or increase in the antioxidant capacity of the organism will result in pathophysiological conditions

References

- Andziak B, O'Connor TP, Buffenstein R (2005) Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat. *Mech Ageing Dev* 126:1206–1212
- Ash CE, Merry BJ (2011) The molecular basis by which dietary restricted feeding reduces mitochondrial reactive oxygen species generation. *Mech Ageing Dev* 132:43–54
- Asikainen TM, Huang TT, Taskinen E, Levenon AL, Carlson E, Lapatto R, Epstein CJ, Raivio KO (2002) Increased sensitivity of homozygous Sod2 mutant mice to oxygen toxicity. *Free Radic Biol Med* 32:175–186

- Ayyadevara S, Dandapat A, Singh SP, Benes H, Zimniak L, Reis RJS, Zimniak P (2005) Lifespan extension in hypomorphic *daf-2* mutants of *Caenorhabditis elegans* is partially mediated by glutathione transferase *CeGSTP2-2*. *Aging Cell* 4:299–307
- Back P, de Vos WH, Depuydt GG, Matthijssens F, Vanfleteren JR, Braeckman BP (2012) Exploring real-time in vivo redox biology of developing and aging *Caenorhabditis elegans*. *Free Radic Biol Med* 52:850–859
- Barnham KJ, Masters CL, Bush AI (2004) Neurodegenerative diseases and oxidative stress. *Nat Rev Drug Discov* 3:205–214
- Barros MH, Bandy B, Tahara EB, Kowaltowski AJ (2004) Higher respiratory activity decreases mitochondrial reactive oxygen release and increases life span in *Saccharomyces cerevisiae*. *J Biol Chem* 279:49883–49888
- Bayne ACV, Mockett RJ, Orr WC, Sohal RS (2005) Enhanced catabolism of mitochondrial superoxide/hydrogen peroxide and aging in transgenic *Drosophila*. *Biochem J* 391:277–284
- Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 87:245–313
- Bevilacqua L, Ramsey JJ, Hagopian K, Weindruch R, Harper ME (2004) Effects of short- and medium-term calorie restriction on muscle mitochondrial proton leak and reactive oxygen species production. *Am J Physiol Endocrinol Metab* 286:E852–E861
- Blüher M, Kahn BB, Kahn CR (2003) Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–574
- Bortolato M, Chen K, Shih JC (2008) Monoamine oxidase inactivation: from pathophysiology to therapeutics. *Adv Drug Deliv Rev* 60:1527–1533
- Brandes N, Schmitt S, Jakob U (2009) Thiol-based redox switches in eukaryotic proteins. *Antioxid Redox Signal* 11:997–1014
- Brandes N, Reichmann D, Tienson H, Leichere LI, Jakob U (2011) Using quantitative redox proteomics to dissect the yeast redoxome. *J Biol Chem* 286:41893–41903
- Brys K, Vanfleteren JR, Braeckman BP (2007) Testing the rate-of-living/oxidative damage theory of aging in the nematode model *Caenorhabditis elegans*. *Exp Gerontol* 42:845–851
- Cabreiro F, Ackerman D, Doonan R, Araiz C, Back P, Papp D, Braeckman BP, Gems D (2011) Increased life span from overexpression of superoxide dismutase in *Caenorhabditis elegans* is not caused by decreased oxidative damage. *Free Radic Biol Med* 51:1575–1582
- Cadenas E, Davies KJA (2000) Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* 29:222–230
- Carlsson LM, Jonsson J, Edlund T, Marklund SL (1995) Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. *Proc Natl Acad Sci USA* 92:6264–6268
- Ceriello A, Motz E (2004) Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 24:816–823
- Chance B, Williams GR (1956) The respiratory chain and oxidative phosphorylation. *Adv Enzymol Relat Subj Biochem* 17:65–134
- Chen XL, Mele J, Giese H, van Remmen H, Dolle MET, Steinhilber M, Richardson A, Vijg J (2003) A strategy for the ubiquitous overexpression of human catalase and CuZn superoxide dismutase genes in transgenic mice. *Mech Ageing Dev* 124:219–227
- Cho CG, Kim HJ, Chung SW, Jung KJ, Shim KH, Yu BP, Yodoi J, Chung HY (2003) Modulation of glutathione and thioredoxin systems by calorie restriction during the aging process. *Exp Gerontol* 38:539–548
- Cocheme HM, Quin C, McQuaker SJ, Cabreiro F, Logan A, Prime TA, Abakumova I, Patel JV, Fearnley IM, James AM, Porteous CM, Smith RAJ, Saeed S, Carre JE, Singer M, Gems D, Hartley RC, Partridge L, Murphy MP (2011) Measurement of H₂O₂ within living *Drosophila* during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix. *Cell Metab* 13:340–350
- Collet JF, Messens J (2010) Structure, function, and mechanism of thioredoxin proteins. *Antioxid Redox Signal* 13:1205–1216

- Copeland JM, Cho J, Lo T Jr, Hur JH, Bahadorani S, Arabyan T, Rabie J, Soh J, Walker DW (2009) Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain. *Curr Biol* 19:1591–1598
- D'Autreaux B, Toledano MB (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 8:813–824
- Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R (2003) Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta* 329:23–38
- Dillin A, Hsu AL, Arantes-Oliveira NA, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C (2002) Rates of behavior and aging specified by mitochondrial function during development. *Science* 298:2398–2401
- Doonan R, McElwee JJ, Matthijssens F, Walker GA, Houthoofd K, Back P, Matscheski A, Vanfleteren JR, Gems D (2008) Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in *Caenorhabditis elegans*. *Genes Dev* 22:3236–3241
- Droge W (2002) Free radicals in the physiological control of cell function. *Physiol Rev* 82:47–95
- Dubey A, Forster MJ, Lal H, Sohail RS (1996) Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. *Arch Biochem Biophys* 333:189–197
- Dupuy C, Virion A, Ohayon R, Kaniewski J, Deme D, Pommier J (1991) Mechanism of hydrogen-peroxide formation catalyzed by nadph oxidase in thyroid plasma-membrane. *J Biol Chem* 266:3739–3743
- Durieux J, Wolff S, Dillin A (2011) The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 144:79–91
- Durusoy M, Diril N, Bozcuk AN (1995) Age-related activity of catalase in different genotypes of *Drosophila-melanogaster*. *Exp Gerontol* 30:77–86
- Duttaroy A, Paul A, Kundu M, Belton A (2003) A Sod2 null mutation confers severely reduced adult life span in *Drosophila*. *Genetics* 165:2295–2299
- Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, Epstein CJ, Huang TT (2005) CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* 24:367–380
- Feng JL, Bussiere F, Hekimi S (2001) Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. *Dev Cell* 1:633–644
- Finkel T (2011a) Signal transduction by mitochondrial oxidants. *J Biol Chem* 287(7):4434–4440
- Finkel T (2011b) Signal transduction by reactive oxygen species. *J Cell Biol* 194:7–15
- Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. *Nature* 408:239–247
- Fontana L, Partridge L, Longo VD (2010) Extending healthy life span—from yeast to humans. *Science* 328:321–326
- Fridovic I (1972) Superoxide radical and superoxide dismutase. *Acc Chem Res* 5:321–326
- Fu YX, Cheng WH, Ross DA, Lei XG (1999) Cellular glutathione peroxidase protects mice against lethal oxidative stress induced by various doses of diquat. *Proc Soc Exp Biol Med* 222:164–169
- Ghezzi P, Bonetto V, Fratelli M (2005) Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation. *Antioxid Redox Signal* 7:964–972
- Giannakou ME, Partridge L (2007) Role of insulin-like signalling in *Drosophila* lifespan. *Trends Biochem Sci* 32:180–188
- Giorgio M, Trinei M, Migliaccio E, Pelicci PG (2007) Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals? *Nat Rev Mol Cell Biol* 8:722–728
- Godon C, Lagniel G, Lee J, Buhler JM, Kieffer S, Perrot M, Boucherie H, Toledano MB, Labarre J (1998) The H₂O₂ stimulon in *Saccharomyces cerevisiae*. *J Biol Chem* 273:22480–22489
- Golden TR, Hinerfeld DA, Melov S (2002) Oxidative stress and aging: beyond correlation. *Aging Cell* 1:117–123
- Grant CM (2008) Metabolic reconfiguration is a regulated response to oxidative stress. *J Biol* 7:1
- Griswold CM, Matthews AL, Bewley KE, Mahaffey JW (1993) Molecular characterization and rescue of acatalasemic mutants of *Drosophila-melanogaster*. *Genetics* 134:781–788

- Hamanaka RB, Chandel NS (2010) Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. *Trends Biochem Sci* 35:505–513
- Hansen RE, Roth D, Winther JR (2009) Quantifying the global cellular thiol-disulfide status. *Proc Natl Acad Sci USA* 106:422–427
- Harman D (1956) Aging – a theory based on free-radical and radiation-chemistry. *J Gerontol* 11:298–300
- Harman D (1972) Biologic clock – mitochondria. *J Am Geriatr Soc* 20:145–147
- Hartman PS, Ishii N, Kayser EB, Morgan PG, Sedensky MM (2001) Mitochondrial mutations differentially affect aging, mutability and anesthetic sensitivity in *Caenorhabditis elegans*. *Mech Ageing Dev* 122:1187–1201
- Heidler T, Hartwig K, Daniel H, Wenzel U (2010) *Caenorhabditis elegans* lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. *Biogerontology* 11:183–195
- Heilbronn LK, Ravussin E (2003) Calorie restriction and aging: review of the literature and implications for studies in humans. *Am J Clin Nutr* 78:361–369
- Hernandez-Garcia D, Wood CD, Castro-Obregon S, Covarrubias L (2010) Reactive oxygen species: a radical role in development? *Free Radic Biol Med* 49:130–143
- Ho YS, Magnenat JL, Bronson RT, Cao J, Gargano M, Sugawara M, Funk CD (1997) Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. *J Biol Chem* 272:16644–16651
- Ho YS, Xiong Y, Ma WC, Spector A, Ho DS (2004) Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. *J Biol Chem* 279:32804–32812
- Ho HY, Cheng ML, Chiu DT (2007) Glucose-6-phosphate dehydrogenase—from oxidative stress to cellular functions and degenerative diseases. *Redox Rep* 12:109–118
- Holmgren A, Johansson C, Berndt C, Lonn ME, Hudemann C, Lillig CH (2005) Thiol redox control via thioredoxin and glutaredoxin systems. *Biochem Soc Trans* 33:1375–1377
- Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloen A, Even PC, Cervera P, le Bouc Y (2003) IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421:182–187
- Honda Y, Honda S (1999) The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J* 13:1385–1393
- Houthoofd K, Braeckman BP, Lenaerts I, Brys K, de Vreese A, van Eygen S, Vanfleteren JR (2002) Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in *Caenorhabditis elegans*. *Exp Gerontol* 37:1371–1378
- Houthoofd K, Fidalgo MA, Hoogewijs D, Braeckman BP, Lenaerts I, Brys K, Matthijssens F, de Vreese A, van Eygen S, Munoz MJ, Vanfleteren JR (2005) Metabolism, physiology and stress defense in three aging *Ins/IGF-1* mutants of the nematode *Caenorhabditis elegans*. *Aging Cell* 4:87–95
- Huang TT, Carlson EJ, Gillespie AM, Shi Y, Epstein CJ (2000) Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. *J Gerontol A Biol Sci Med Sci* 55:B5–B9
- Huang TT, Carlson EJ, Kozy HM, Mantha S, Goodman SI, Ursell PC, Epstein CJ (2001) Genetic modification of prenatal lethality and dilated cardiomyopathy in Mn superoxide dismutase mutant mice. *Free Radic Biol Med* 31:1101–1110
- Ishii N, Takahashi K, Tomita S, Keino T, Honda S, Yoshino K, Suzuki K (1990) A methyl viologen-sensitive mutant of the nematode *Caenorhabditis elegans*. *Mutat Res* 237:165–171
- Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. *Nature* 394:694–697
- Jacob MH, Janner Dda R, Araujo AS, Jahn MP, Kucharski LC, Moraes TB, Dutra Filho CS, Ribeiro MF, Bello-Klein A (2010) Redox imbalance influence in the myocardial Akt activation in aged rats treated with DHEA. *Exp Gerontol* 45:957–963

- Lee C, Vanoaica L, Lee J, Park BJ, Ahnn J (2005) Thioredoxin is related to life span regulation and oxidative stress response in *Caenorhabditis elegans*. *Genes Cells* 10:1203–1210
- Johnson TE (1990) Increased life-span of age-1 mutants in *Caenorhabditis-elegans* and lower gompertz rate of aging. *Science* 249:908–912
- Kakihana T, Nagata K, Sitia R (2012) Peroxides and peroxidases in the endoplasmic reticulum: integrating redox homeostasis and oxidative folding. *Antioxid Redox Signal* 16:763–771
- Keaney M, Gems D (2003) No increase in lifespan in *Caenorhabditis elegans* upon treatment with the superoxide dismutase mimetic EUK-8. *Free Radic Biol Med* 34:277–282
- Keaney M, Matthijssens F, Sharpe M, Vanfleteren J, Gems D (2004) Superoxide dismutase mimetics elevate superoxide dismutase activity *in vivo* but do not retard aging in the nematode *Caenorhabditis elegans*. *Free Radic Biol Med* 37:239–250
- Kenyon C (2005) The plasticity of aging: insights from long-lived mutants. *Cell* 120:449–460
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C-Elegans* mutant that lives twice as long as wild-type. *Nature* 366:461–464
- Kim J, Takahashi M, Shimizu T, Shirasawa T, Kajita M, Kanayama A, Miyamoto Y (2008) Effects of a potent antioxidant, platinum nanoparticle, on the lifespan of *Caenorhabditis elegans*. *Mech Ageing Dev* 129:322–331
- Klichko VI, Radyuk SN, Orr WC (2004) Profiling catalase gene expression in *Drosophila melanogaster* during development and aging. *Arch Insect Biochem Physiol* 56:34–50
- Klotz LO, Sies H (2009) Cellular generation of oxidants: relation to oxidative stress. In: *Redox signaling and regulation in biology and medicine*. Wiley-VCH, Weinheim, pp 45–61
- Kohn RR (1971) Effect of antioxidants on life-span of C57bl mice. *J Gerontol* 26:378–380
- Kondo M, Senoo-Matsuda N, Yanase S, Ishii T, Hartman PS, Ishii N (2005) Effect of oxidative stress on translocation of DAF-16 in oxygen-sensitive mutants, *mev-1* and *gas-1* of *Caenorhabditis elegans*. *Mech Ageing Dev* 126:637–641
- Krause KH (2007) Aging: a revisited theory based on free radicals generated by NOX family NADPH oxidases. *Exp Gerontol* 42:256–262
- Kumsta C, Thamsen M, Jakob U (2011) Effects of oxidative stress on behavior, physiology, and the redox thiol proteome of *Caenorhabditis elegans*. *Antioxid Redox Signal* 14:1023–1037
- Lambert AJ, Merry BJ (2004) Effect of caloric restriction on mitochondrial reactive oxygen species production and bioenergetics: reversal by insulin. *Am J Physiol Regul Integr Comp Physiol* 286:R71–R79
- Lambeth JD (2004) NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4:181–189
- Lapointe J, Hekimi S (2008) Early mitochondrial dysfunction in long-lived *Mcl1*(+/-) mice. *J Biol Chem* 283:26217–26227
- Lee SS, Lee RYN, Fraser AG, Kamath RS, Ahringer J, Ruvkun G (2003a) A systematic RNAi screen identifies a critical role for mitochondria in *C-elegans* longevity. *Nat Genet* 33:40–48
- Lee TH, Kim SU, Yu SL, Kim SH, Park DS, Moon HB, Dho SH, Kwon KS, Kwon HJ, Han YH, Jeong S, Kang SW, Shin HS, Lee KK, Rhee SG, Yu DY (2003b) Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. *Blood* 101:5033–5038
- Lee KS, Iijima-Ando K, Iijima K, Lee WJ, Lee JH, Yu K, Lee DS (2009a) JNK/FOXO-mediated neuronal expression of fly homologue of peroxiredoxin II reduces oxidative stress and extends life span. *J Biol Chem* 284:29454–29461
- Lee SJ, Murphy CT, Kenyon C (2009b) Glucose shortens the life span of *C. elegans* by downregulating DAF-16/FOXO activity and aquaporin gene expression. *Cell Metab* 10:379–391
- Lee SJ, Hwang AB, Kenyon C (2010) Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. *Curr Biol* 20:2131–2136
- Levine RL (2002) Carbonyl modified proteins in cellular regulation, aging, and disease. *Free Radic Biol Med* 32:790–796
- Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, Epstein CJ (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 11:376–381

- Lin K, Hsin H, Libina N, Kenyon C (2001) Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet* 28:139–145
- Lin SJ, Kaerberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC, Fink GR, Guarente L (2002) Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 418:344–348
- Liu XX, Jiang N, Hughes B, Bigras E, Shoubridge E, Hekimi S (2005) Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of *mclk1* increases cellular fitness and lifespan in mice. *Genes Dev* 19:2424–2434
- Longo VD, Finch CE (2003) Evolutionary medicine: from dwarf model systems to healthy centenarians? *Science* 299:1342–1346
- Lopez-Lluch G, Hunt N, Jones B, Zhu M, Jamieson H, Hilmer S, Cascajo MV, Allard J, Ingram DK, Navas P, de Cabo R (2006) Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc Natl Acad Sci USA* 103:1768–1773
- Mackay WJ, Bewley GC (1989) The genetics of catalase in *Drosophila-melanogaster* – isolation and characterization of acatalasemic mutants. *Genetics* 122:643–652
- Makino N, Mochizuki Y, Bannai S, Sugita Y (1994) Kinetic studies on the removal of extracellular hydrogen peroxide by cultured fibroblasts. *J Biol Chem* 269:1020–1025
- Margittai E, Banhegyi G (2010) Oxidative folding in the endoplasmic reticulum: towards a multiple oxidant hypothesis? *FEBS Lett* 584:2995–2998
- Matsui M, Oshima M, Oshima H, Takaku K, Maruyama T, Yodoi J, Taketo MM (1996) Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. *Dev Biol* 178:179–185
- McCay CM, Crowell MF, Maynard LA (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. *J Nutr* 10:63–79
- McCord JM, Fridovic I (1969) Superoxide dismutase an enzymic function for erythrocyte (Hemocuprein). *J Biol Chem* 244:6049–6055
- Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ (2000) Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 289:1567–1569
- Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG (1999) The p66(shc) adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402:309–313
- Miranda-Vizuete A, Gonzalez JCF, Gahmon G, Burghoorn J, Navas P, Swoboda P (2006) Lifespan decrease in a *Caenorhabditis elegans* mutant lacking TRX-1, a thioredoxin expressed in ASJ sensory neurons. *FEBS Lett* 580:484–490
- Mitsui A, Hamuro J, Nakamura H, Kondo N, Hirabayashi Y, Ishizaki-Koizumi S, Hirakawa T, Inoue T, Yodoi J (2002) Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. *Antioxid Redox Signal* 4:693–696
- Miwa S, Riyahi K, Partridge L, Brand MD (2004) Lack of correlation between mitochondrial reactive oxygen species production and life span in *Drosophila*. *Stratag Eng Neglig Senescence Why Genuin Control Aging May Be Foreseeable* 1019:388–391
- Mockett RJ, Orr WC, Rahmandar JJ, Benes JJ, Radyuk SN, Klichko VI, Sohal RS (1999) Overexpression of Mn-containing superoxide dismutase in transgenic *Drosophila melanogaster*. *Arch Biochem Biophys* 371:260–269
- Mockett RJ, Sohal BH, Sohal RS (2010) Expression of multiple copies of mitochondrially targeted catalase or genomic Mn superoxide dismutase transgenes does not extend the life span of *Drosophila melanogaster*. *Free Radic Biol Med* 49:2028–2031
- Moller IM, Rogowska-Wrzesinska A, Rao RSP (2011) Protein carbonylation and metal-catalyzed protein oxidation in a cellular perspective. *J Proteomics* 74:2228–2242
- Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R, Huang TT, Epstein CJ, Roberts LJ 2nd, Csete M, Faulkner JA, Van Remmen H (2006) Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. *Free Radic Biol Med* 40:1993–2004

- Muller FL, Lustgarten MS, Jang Y, Richardson A, van Remmen H (2007) Trends in oxidative aging theories. *Free Radic Biol Med* 43:477–503
- Murakami S, Johnson TE (1996) A genetic pathway conferring life extension and resistance to UV stress in *Caenorhabditis elegans*. *Genetics* 143:1207–1218
- Murphy CT (2006) The search for DAF-16/FOXO transcriptional targets: approaches and discoveries. *Exp Gerontol* 41:910–921
- Murphy MP (2012) Mitochondrial thiols in antioxidant protection and redox signaling: distinct roles for glutathionylation and other thiol modifications. *Antioxid Redox Signal* 16:476–495
- Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424:277–284
- Nauseef WM (2008) Biological roles for the NOX family NADPH oxidases. *J Biol Chem* 283:16961–16965
- Nemoto S, Finkel T (2002) Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. *Science* 295:2450–2452
- Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, van Etten RA (2003) Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 424:561–565
- Nicholls P, Fita I, Loewen PC (2001) Enzymology and structure of catalases. *Adv Inorg Chem* 51(51):51–106
- Niki E (1987) Interaction of ascorbate and alpha-tocopherol. *Ann N Y Acad Sci* 498:186–199
- Nisoli E, Tonetto C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO (2005) Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310:314–317
- Nonn L, Williams RR, Erickson RP, Powis G (2003) The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. *Mol Cell Biol* 23:916–922
- Olahova M, Taylor SR, Khazaipoul S, Wang JL, Morgan BA, Matsumoto K, Blackwell TK, Veal EA (2008) A redox-sensitive peroxiredoxin that is important for longevity has tissue- and stress-specific roles in stress resistance. *Proc Natl Acad Sci USA* 105:19839–19844
- Orr WC, Sohal RS (1994) Extension of life-span by overexpression of superoxide-dismutase and catalase in *Drosophila-melanogaster*. *Science* 263:1128–1130
- Orr WC, Sohal RS (2003) Does overexpression of Cu, Zn-SOD extend life span in *Drosophila melanogaster*? *Exp Gerontol* 38:227–230
- Orrenius S, Moldeus P (1984) The multiple roles of glutathione in drug-metabolism. *Trends Pharmacol Sci* 5:432–435
- Parihar MS, Kunz EA, Brewer GJ (2008) Age-related decreases in NAD(P)H and glutathione cause redox declines before ATP loss during glutamate treatment of hippocampal neurons. *J Neurosci Res* 86:2339–2352
- Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL (1998) Extension of *Drosophila* lifespan by overexpression of human SOD1 in motoneurons. *Nat Genet* 19:171–174
- Pearl R (1921) *The biology of death*. Utica, N.Y.: Science Press
- Perez VI, Lew CM, Cortez LA, Webb CR, Rodriguez M, Liu Y, Qi W, Li Y, Chaudhuri A, van Remmen H, Richardson A, Ikeno Y (2008) Thioredoxin 2 haploinsufficiency in mice results in impaired mitochondrial function and increased oxidative stress. *Free Radic Biol Med* 44:882–892
- Perez VI, Bokov A, van Remmen H, Mele J, Ran QT, Ikeno Y, Richardson A (2009a) Is the oxidative stress theory of aging dead? *Biochim Biophys Acta Gen Subj* 1790:1005–1014
- Perez VI, van Remmen H, Bokov A, Epstein CJ, Vijg J, Richardson A (2009b) The overexpression of major antioxidant enzymes does not extend the lifespan of mice. *Aging Cell* 8:73–75
- Perez VI, Cortez LA, Lew CM, Rodriguez M, Webb CR, van Remmen H, Chaudhuri A, Qi WB, Lee S, Bokov A, Fok W, Jones D, Richardson A, Yodoi J, Zhang Y, Tominaga K, Hubbard GB,

- Ikeno Y (2011) Thioredoxin 1 overexpression extends mainly the earlier part of life span in mice. *J Gerontol A Biol Sci Med Sci* 66:1286–1299
- Perichon R, Bourre JM (1995) Peroxisomal beta-oxidation activity and catalase activity during development and aging in mouse-liver. *Biochimie* 77:288–293
- Petriv OI, Rachubinski RA (2004) Lack of peroxisomal catalase causes a progeric phenotype in *Caenorhabditis elegans*. *J Biol Chem* 279:19996–20001
- Phillips JP, Campbell SD, Michaud D, Charbonneau M, Hilliker AJ (1989) Null mutation of copper-zinc superoxide-dismutase in *Drosophila* confers hypersensitivity to paraquat and reduced longevity. *Proc Natl Acad Sci USA* 86:2761–2765
- Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA (1991) Detection of catalase in rat heart mitochondria. *J Biol Chem* 266:22028–22034
- Radyuk SN, Klichko VI, Orr WC (2004) Profiling Cu, Zn-superoxide dismutase expression in *Drosophila melanogaster*—a critical regulatory role for intron/exon sequence within the coding domain. *Gene* 328:37–48
- Radyuk SN, Michalak K, Klichko VI, Benes J, Rebrin I, Sohal RS, Orr WC (2009) Peroxiredoxin 5 confers protection against oxidative stress and apoptosis and also promotes longevity in *Drosophila*. *Biochem J* 419:437–445
- Ran QT, Liang HY, Gu MJ, Qi WB, Walter CA, Roberts LJ, Herman B, Richardson A, van Remmen H (2004) Transgenic mice overexpressing glutathione peroxidase 4 are protected against oxidative stress-induced apoptosis. *J Biol Chem* 279:55137–55146
- Ran Q, Liang HY, Ikeno Y, Qi WB, Prolla TA, Roberts LJ, Wolf N, Vanremmen H, Richardson A (2007) Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. *J Gerontol A Biol Sci Med Sci* 62:932–942
- Rando TA, Crowley RS, Carlson EJ, Epstein CJ, Mohapatra PK (1998) Overexpression of copper/zinc superoxide dismutase: a novel cause of murine muscular dystrophy. *Ann Neurol* 44:381–386
- Rea S, Johnson TE (2003) A metabolic model for life span determination in *Caenorhabditis elegans*. *Dev Cell* 5:197–203
- Rea SL, Ventura N, Johnson TE (2007) Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*. *PLOS Biol* 5:2312–2329
- Rebrin I, Sohal RS (2008) Pro-oxidant shift in glutathione redox state during aging. *Adv Drug Deliv Rev* 60:1545–1552
- Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010) Oxidative stress, inflammation, and cancer how are they linked? *Free Radic Biol Med* 49:1603–1616
- Reveillaud I, Niedzwiecki A, Bensch KG, Fleming JE (1991) Expression of bovine superoxide-dismutase in *Drosophila-melanogaster* augments resistance to oxidative stress. *Mol Cell Biol* 11:632–640
- Rohrbach S, Gruenler S, Teschner M, Holtz J (2006) The thioredoxin system in aging muscle: key role of mitochondrial thioredoxin reductase in the protective effects of caloric restriction? *Am J Physiol Regul Integr Comp Physiol* 291:R927–R935
- Rubner M (1908) *Das problem der lebensdauer und seine beziehungen zu ernahrung*. Oldenbourg, München
- Salvi M, Battaglia V, Brunati AM, la Rocca N, Tibaldi E, Pietrangeli P, Marcocci L, Mondovi B, Rossi CA, Toninello A (2007) Catalase takes part in rat liver mitochondria oxidative stress defense. *J Biol Chem* 282:24407–24415
- Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 30:1191–1212
- Schlotterer A, Kukudov G, Bozorgmehr F, Hutter H, Du XL, Oikonomou D, Ibrahim Y, Pfisterer F, Rabbani N, Thornalley P, Sayed A, Fleming T, Humpert P, Schwenger V, Zeier M, Hamann A, Stern D, Brownlee M, Bierhaus A, Nawroth P, Morcos M (2009) *C-elegans* as model for the study of high glucose-mediated life span reduction. *Diabetes* 58:2450–2456

- Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, van Remmen H, Wallace DC, Rabinovitch PS (2005) Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308:1909–1911
- Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M (2007) Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* 6:280–293
- Sedensky MM, Morgan PG (2006) Mitochondrial respiration and reactive oxygen species in mitochondrial aging mutants. *Exp Gerontol* 41:237–245
- Semsei I, Rao G, Richardson A (1991) Expression of superoxide-dismutase and catalase in rat-brain as a function of age. *Mech Ageing Dev* 58:13–19
- Senoo-Matsuda N, Yasuda K, Tsuda M, Ohkubo T, Yoshimura S, Nakazawa H, Hartman PS, Ishii N (2001) A defect in the cytochrome b large subunit in complex II causes both superoxide anion overproduction and abnormal energy metabolism in *Caenorhabditis elegans*. *J Biol Chem* 276:41553–41558
- Sentman ML, Granstrom M, Jakobson H, Reaume A, Basu S, Marklund SL (2006) Phenotypes of mice lacking extracellular superoxide dismutase and copper- and zinc-containing superoxide dismutase. *J Biol Chem* 281:6904–6909
- Seong KH, Ogashiwa T, Matsuo T, Fuyama Y, Aigaki T (2001) Application of the gene search system to screen for longevity genes in *Drosophila*. *Biogerontology* 2:209–217
- Seto NOL, Hayashi S, Tener GM (1990) Overexpression of Cu-Zn superoxide-dismutase in *Drosophila* does not affect life-span. *Proc Natl Acad Sci USA* 87:4270–4274
- Sohal RS, Arnold L, Orr WC (1990) Effect of age on superoxide-dismutase, catalase, glutathione-reductase, inorganic peroxides, Tba-reactive material, Gsh Gssg, Nadph Nadp+ and Nadh Nad+ in *Drosophila-melanogaster*. *Mech Ageing Dev* 56:223–235
- Sohal RS, Ku HH, Agarwal S, Forster MJ, Lal H (1994) Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech Ageing Dev* 74:121–133
- Sohal RS, Mockett RJ, Orr WC (2002) Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radic Biol Med* 33:575–586
- Someya S, Yu W, Hallows WC, Xu JZ, Vann JM, Leeuwenburgh C, Tanokura M, Denu JM, Prolla TA (2010) Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell* 143:802–812
- Stadtman ER (1992) Protein oxidation and aging. *Science* 257:1220–1224
- Sun JT, Tower J (1999) FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Mol Cell Biol* 19:216–228
- Sun JT, Folk D, Bradley TJ, Tower J (2002) Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult *Drosophila melanogaster*. *Genetics* 161:661–672
- Sun JT, Molitor J, Tower J (2004) Effects of simultaneous over-expression of Cu/ZnSOD and MnSOD on *Drosophila melanogaster* life span. *Mech Ageing Dev* 125:341–349
- Svensson MJ, Larsson J (2007) Thioredoxin-2 affects lifespan and oxidative stress in *Drosophila*. *Hereditas* 144:25–32
- Tatar M, Bartke A, Antebi A (2003) The endocrine regulation of aging by insulin-like signals. *Science* 299:1346–1351
- Thornalley PJ, Vasak M (1985) Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochim Biophys Acta* 827:36–44
- Trachootham D, Lu WQ, Ogasawara MA, Valle NRD, Huang P (2008) Redox regulation of cell survival. *Antioxid Redox Signal* 10:1343–1374
- Tsan MF, White JE, Caska B, Epstein CJ, Lee CY (1998) Susceptibility of heterozygous MnSOD gene-knockout mice to oxygen toxicity. *Am J Respir Cell Mol Biol* 19:114–120
- Tsuda M, Ootaka R, Ohkura C, Kishita Y, Seong KH, Matsuo T, Aigaki T (2010) Loss of Trx-2 enhances oxidative stress-dependent phenotypes in *Drosophila*. *FEBS Lett* 584:3398–3401

- Turrens JF (1997) Superoxide production by the mitochondrial respiratory chain. *Biosci Rep* 17:3–8
- Ushio-Fukai M, Nakamura Y (2008) Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. *Cancer Lett* 266:37–52
- van Raamsdonk JM, Hekimi S (2009) Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. *PLOS Genet* 5:e1000361
- van Raamsdonk JM, Hekimi S (2012) Superoxide dismutase is dispensable for normal animal lifespan. *Proc Natl Acad Sci* 109:5785–5790
- van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, Alderson NL, Baynes JW, Epstein CJ, Huang TT, Nelson J, Strong R, Richardson A (2003) Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Phys Genomics* 16:29–37
- van Remmen H, Qi WB, Sabia M, Freeman G, Estlack L, Yang H, Guo ZM, Huang TT, Strong R, Lee S, Epstein CJ, Richardson A (2004) Multiple deficiencies in antioxidant enzymes in mice result in a compound increase in sensitivity to oxidative stress. *Free Radic Biol Med* 36:1625–1634
- Vanfleteren JR (1993) Oxidative stress and aging in *Caenorhabditis elegans*. *Biochem J* 292:605–608
- Victor VM, Rocha M, Sola E, Banuls C, Garcia-Malpartida K, Hernandez-Mijares A (2009) Oxidative stress, endothelial dysfunction and atherosclerosis. *Curr Pharm Des* 15:2988–3002
- Walker DW, Hajek P, Muffat J, Knoepfle D, Cornelison S, Attardi G, Benzer S (2006) Hypersensitivity to oxygen and shortened lifespan in a *Drosophila* mitochondrial complex II mutant. *Proc Natl Acad Sci USA* 103:16382–16387
- Wang XS, Phelan SA, Forsman-Semb K, Taylor EF, Petros C, Brown A, Lerner CP, Paigen B (2003) Mice with targeted mutation of peroxiredoxin 6 develop normally but are susceptible to oxidative stress. *J Biol Chem* 278:25179–25190
- Wang DT, Malo D, Hekimi S (2010) Elevated mitochondrial reactive oxygen species generation affects the immune response via hypoxia-inducible factor-1 alpha in long-lived *Mcl1(+/-)* mouse mutants. *J Immunol* 184:582–590
- Weinkove D, Halstead JR, Gems D, Divecha N (2006) Long-term starvation and ageing induce AGE-1/PI 3-kinase-dependent translocation of DAF-16/FOXO to the cytoplasm. *BMC Biol* 4:e1741–e7007
- Winterbourn CC, Hampton MB (2008) Thiol chemistry and specificity in redox signaling. *Free Radic Biol Med* 45:549–561
- Wolf N, Penn P, Pendergrass W, van Remmen H, Bartke A, Rabinovitch P, Martin GM (2005) Age-related cataract progression in five mouse models for anti-oxidant protection or hormonal influence. *Exp Eye Res* 81:276–285
- Wong A, Boutis P, Hekimi S (1995) Mutations in the *Clk-1* gene of *Caenorhabditis elegans* affect developmental and behavioral timing. *Genetics* 139:1247–1259
- Yanase S, Yasuda K, Ishii N (2002) Adaptive responses to oxidative, damage in three mutants of *Caenorhabditis elegans* (*age-1*, *mev-1* and *daf-16*) that affect life span. *Mech Age Dev* 123:1579–1587
- Yanase S, Onodera A, Tedesco P, Johnson TE, Ishii N (2009) SOD-1 deletions in *Caenorhabditis elegans* alter the localization of intracellular reactive oxygen species and show molecular compensation. *J Gerontol A Biol Sci Med Sci* 64:530–539
- Yang W, Hekimi S (2010) A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLOS Biol* 8:e1000556
- Yant LJ, Ran QT, Rao L, van Remmen H, Shibata T, Belter JG, Motta L, Richardson A, Prolla TA (2003) The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic Biol Med* 34:496–502
- Yen K, Patel HB, Lublin AL, Mobbs CV (2009) SOD isoforms play no role in lifespan in ad lib or dietary restricted conditions, but mutational inactivation of SOD-1 reduces life extension by cold. *Mech Ageing Dev* 130:173–178

- Yu BP, Yang R (1996) Critical evaluation of the free radical theory of aging – a proposal for the oxidative stress hypothesis. *Ann N Y Acad Sci* 786:1–11
- Zangar RC, Davydov DR, Verma S (2004) Mechanisms that regulate production of reactive oxygen species by cytochrome P450. *Toxicol Appl Pharmacol* 199:316–331
- Zarse K, Schmeisser S, Groth M, Priebe S, Beuster G, Kuhlow D, Guthke R, Platzer M, Kahn CR, Ristow M (2012) Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. *Cell Metab* 15:451–465
- Zelko IN, Mariani TJ, Folz RJ (2002) Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic Biol Med* 33:337–349
- Zhang YQ, Ikeno Y, Qi WB, Chaudhuri A, Li Y, Bokov A, Thorpe SR, Baynes JW, Epstein C, Richardson A, van Remmen H (2009) Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. *J Gerontol A Biol Sci Med Sci* 64:1212–1220