Pharmaceutical Sciences & Analytical Research Journal
ISSN: 2640-6659

Review Article *Volume 2; Issue 2*

Function of Reactive Oxygen Species (ROS) Inside the Living Organisms and Sources of Oxidants

Loutfy H Madkour*

Chemistry Department, Al Baha University, Saudi Arabia

***Corresponding author**: Dr. Loutfy H Madkour, Chemistry Department, Al Baha University, Baljarashi 65635, Saudi Arabia, Tel: +966 533899075; Fax: +96677247272; E-mail: loutfy_madkour@yahoo.com

Received Date: June 24, 2019; **Published Date:** July 03, 2019

Abstract

Reactive oxygen species (ROS) is a generic name given to a variety of molecules and free radicals derived from molecular oxygen. The reduction of oxygen produces relatively stable intermediates. One electron-reduction produces superoxide anion, which is the precursor of most ROS. As most commonly used, ROS in this chapter refer to superoxide, hydrogen peroxide and their derivatives such as the hydroxyl radical. Reactive oxygen species (ROS) were initially recognized as toxic by-products of aerobic metabolism. In recent years, it has become apparent that ROS plays an important signaling role in plants, controlling processes such as growth, development and especially response to biotic and abiotic environmental stimuli. ROS include free radicals such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), as well as nonradical molecules like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and so forth. Stepwise reduction of molecular oxygen (O_2) by high-energy exposure or electron-transfer reactions leads to production of the highly reactive ROS. Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions.

Keywords: Reactive oxygen species (ROS); Highly reactive molecules; $O_2^{\bullet -}$; $^1O_2$; $^{\bullet}OH$; H_2O_2; Living organisms

Abbreviations: ROS: Reactive Oxygen Species; PMNs: Polymorphonuclear Neutrophils; SOD: superoxide dismutase; APX: Ascorbate Peroxidase; ETC: Electron Transport Chain; GPX: Glutathione Peroxidase; PTP: Permeability Transition Pore; GPX: Glutathione Peroxidase; PRX: peroxiredoxin; APX: Ascorbate Peroxidase; CAT: Catalase; H_2O_2: Hydrogen Peroxide; HOCl: Hypochlorous Acid; PTP: Permeability Transition Pore; DMPK: Dystrophy Protein Kinase

Introduction

At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, and DNA [1-6]. Reactive oxygen species (ROS) are key signaling molecules that play an important role in the progression of inflammatory disorders. An enhanced ROS generation by polymorphonuclear neutrophils (PMNs) at the site of inflammation causes endothelial dysfunction and tissue injury. The vascular endothelium plays an important role in passage of macromolecules and inflammatory cells from the blood to tissue. Under the inflammatory conditions, oxidative stress produced by PMNs leads to the opening of inter-endothelial junctions and promotes the migration of inflammatory cells across the endothelial barrier. The migrated inflammatory cells not only help in

Citation: Loutfy H Madkour. Function of Reactive Oxygen Species (ROS) Inside the Living Organisms and Sources of Oxidants. Pharma Sci Analytical Res J 2019, 2(2): 180023.

the clearance of pathogens and foreign particles but also lead to tissue injury. Reactive oxygen species (e.g. $^1O_2$, $O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$, OH^-) are partially reduced or activated forms of atmospheric oxygen (O_2). They are considered to be unavoidable byproducts of aerobic metabolism that have accompanied life on Earth ever since the appearance of oxygen-evolving photosynthetic organisms about 2.2–2.7 billion years ago [7]. Higher plants have thus evolved in the presence of ROS and have acquired dedicated pathways to protect themselves from ROS toxicity, as well as to use ROS as signaling molecules [8-12]. If kept unchecked, ROS concentrations will increase in cells and cause oxidative damage to membranes (lipid peroxidation), proteins, RNA and DNA molecules, and can even lead to the oxidative destruction of the cell in a process termed oxidative stress [13]. However, this process is mitigated in cells by a large number of ROS detoxifying proteins [e.g. superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PRX)], as well as by antioxidants such as ascorbic acid and glutathione (GSH) that are present in almost all subcellular compartments [14]. The active process of ROS detoxification in plant cells is also aided by different metabolic adaptations that reduce ROS production, and by maintaining the level of free transient metals such as Fe^{2+} under control, to prevent the formation of the highly toxic hydroxyl radical ($^{\bullet}OH$) via the Fenton reaction [15]. On the other hand, plants actively produce ROS that are used as signal transduction molecules.

ROS ($^1O_2$, $O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$, OH^-) are highly reactive, toxic molecules which are caused by various environmental stress conditions, increased oxidative stress and which cause damage on membrane, DNA, protein, photosynthetic pigments and lipids [16,17]. Reactive oxygen species (ROS) are deadly weapons used by phagocytes and other cell types, such as lung epithelial cells, against pathogens. ROS can kill pathogens directly by causing oxidative damage to biocompounds or indirectly by stimulating pathogen elimination by various nonoxidative mechanisms, including pattern recognition receptors signaling, autophagy, neutrophil extracellular trap formation, and T-lymphocyte responses. Thus, one should expect that the inhibition of ROS production promote infection. Increasing evidences support that in certain particular infections, antioxidants decrease and prooxidants increase pathogen burden. In this study, we review the classic infections that are controlled by ROS and the cases in which ROS appear as promoters of infection, challenging the paradigm. We discuss the possible mechanisms by which ROS could promote particular infections. These mechanisms are still not completely clear but include the metabolic effects of ROS on pathogen physiology, ROS-induced damage to the immune system, and ROS induced activation of immune defense mechanisms that are subsequently hijacked by particular pathogens to act against more effective microbicidal mechanisms of the immune system. Reactive oxygen species (ROS) are used by the immune system as weapons against pathogens; however, antioxidants have long been recognized as protectors of host organism against infections [18]. It is plausible that the paradox contained in these statements can be solved by the failure of antioxidants to neutralize the ROS involved in pathogen killing (e.g., by not reaching the appropriate location required to their action) and by the capacity of antioxidants to protect immune cells from the damage caused by ROS. The nature of the microbes and their susceptibility to ROS can also offer a clue to solve the paradox. ROS effectively combat certain microbes, whereas other microbes seem to thrive in oxidative environments. Recognizing the mechanisms by which ROS promote the clearance of microbes and the mechanisms by which particular microbes benefit from ROS generation will help to clarify the paradox.

Phagocytes reside within tissues or are recruited by inflammatory processes. Phagocytes recognize microbes through many molecular patterns displayed by them and try to engulf them. Once a microbe is phagocytosed, the nature of the molecules recognized on microbe's surface dictates the treatment enacted within the phagosome. Respiratory burst, a process by which NADPH oxidase (NADPH oxidase 2 [NOX2]) generates ROS in response to microbe recognition, is a possible outcome of this process and helps to get rid of many microbes. For instance, β-glucan on the surface of the fungi can engage dectin-1 on the surface of phagocyte [19]. Once fungi are phagocytosed, NOX2 is promptly assembled at the phagosome membrane and high amounts of superoxide ($O_2^{\bullet-}$) are discharged into the phagosome. The recognition of Escherichia coli generates comparatively smaller amounts of ROS than the recognition of Listeria monocytogenes, and these ROS are less promptly released into the phagosome [20]. However, the recognition and phagocytosis of Leishmania spp. is well studied, and, except when recognition is mediated by Fc receptor (FcR), virulent parasites do not usually trigger a severe respiratory burst [21]. Thus, microbes face different fates after phagocytosis dependent on the molecules presented at their surfaces and how they are targeted by innate immune receptors.

Once a pathogen is phagocytosed, it must subvert the respiratory burst, withstand its oxidative power, or escape the phagosome to survive. Microbe recognition sets the immune system in motion, and ROS are produced not only in the phagocyte respiratory burst but also in other cell compartments, such as mitochondria, as

intermediaries in many signal transduction pathways, such as leukocyte pattern recognition receptor (PRR) signaling. The generation of ROS is a prerequisite to the formation of neutrophil extracellular traps (NETs) [22] is actively involved in phagolysosomal formation and enzymatic degradation [23], autophagy [24-26] and ROS inhibition of mammalian target of rapamycin (mTOR) kinase [27,28]; chemoattraction and inflammation [29,30] cell death of infection reservoirs [31] antigenic presentation, T-helper (Th) polarization, and lymphocyte proliferation [32-37] iron redistribution among tissues [33] and cell compartment availability of iron [34-36] and foam cell formation [37,38]. Many of these mechanisms promote microbe clearance, whereas others can potentially contribute to microbe persistence.

Exogenous Source of Oxidants

Cigarette smoke

Cigarette smoke contains many oxidants and free radicals and organic compounds, such as superoxide and nitric oxide [39]. In addition, inhalation of cigarette smoke into the lung also activates some endogenous mechanisms, such as accumulation of neutrophils and macrophages, which further increase the oxidant injury.

Ozone exposure

Ozone exposure can cause lipid peroxidation and induce influx of neutrophils into the airway epithelium. Short-term exposure to ozone also causes the release of inflammatory mediators, such as MPO, eosinophil cationic proteins and also lactate dehydrogenase and albumin [40]. Even in healthy subjects, ozone exposure causes a reduction in pulmonary functions particulate matter (mixture of solid particles and liquid droplets suspended in the air) catalyzes the reduction of oxygen [41,42].

Hyperoxia

Hyperoxia refers to conditions of higher oxygen levels than normal partial pressure of oxygen in the lungs or other body tissues. It leads to greater production of reactive oxygen and nitrogen species [43,44].

Ionizing radiation

Ionizing radiation, in the presence of O_2, converts hydroxyl radical, superoxide, and organic radicals to hydrogen peroxide and organic hydroperoxides. These hydroperoxide species react with redox active metal ions, such as Fe and Cu, via Fenton reactions and thus induce oxidative stress [45,46]. Fibroblasts that were exposed to alpha particles had significant increases in intracellular $O_2^{\bullet-}$ and H_2O_2 production via plasma membrane-bound NADPH oxidase [47]. Signal transduction molecules, such as extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38, and transcription factors, such as activator protein-1(AP-1), nuclear factor-kB (NF-kB), and p53, are activated, which result in the expression of radiation response–related genes [48-53]. Ultraviolet A (UVA) photons trigger oxidative reactions by excitation of endogenous photosensitizers, such as porphyrins, NADPH oxidase, and riboflavins. 8-Oxo-7,8-dihydroguanine (8-oxoGua) is the main UVA-mediated DNA oxidation product formed by the oxidation of $^{\bullet}OH$ radical, 1-electron oxidants, and singlet oxygen that mainly reacts with guanine [54]. The formation of guanine radical cation in isolated DNA has been shown to efficiently occur through the direct effect of ionizing radiation [55,56]. After exposure to ionizing radiation, intracellular level of glutathione (GSH) decreases for a short term but then increases again [57].

Heavy metal ions

Heavy metal ions, such as iron, copper, cadmium, mercury, nickel, lead, and arsenic, can induce generation of reactive radicals and cause cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA [58]. One of the most important mechanisms of metalmediated free radical generation is via a Fenton-type reaction. Superoxide ion and hydrogen peroxide can interact with transition metals, such as iron and copper, via the metal catalyzed Haber–Weiss/Fenton reaction to form OH radicals.

$$Metal^{3+} + O_2^{\bullet-} \rightarrow Metal^{2+} + O_2 \quad \text{Haber-Weiss}$$

$$Metal^{2+} + H_2O_2 \rightarrow Metal^{3+} + OH^{-} + {}^{\bullet}OH \quad \text{Fenton reaction}$$

The role of oxidants in inducing inflammation has been vigorously investigated in all manner of experimental models. The consensus is they are fundamentally involved, but how they contribute to the response and whether antioxidant therapy is a valid means of arresting inflammation in patients remains largely unresolved. Among the commonly used inflammatory mediators used to simulate inflammation are included cytokines (e.g., TNF-α), the stress of hyperoxia, ischemia-reperfusion injury, bacterial toxins (LPS), and mediators that ligate cell surface receptors (PAF, thrombin, histamine, VEGF, and bradykinins). These and other mediators except LPS induce only a subset of changes that are associated with full-blown inflammation.

Endogenous Sources of ROS and Their Regulation in Inflammation

ROS are classically defined as partially reduced metabolites of oxygen that possess strong oxidizing capabilities. They are deleterious to cells at high concentrations but at low concentrations (exact concentrations still remaining to be defined), they serve complex signaling functions. They are injurious, because they oxidize protein and lipid cellular constituents and damage the DNA. At "physiological concentrations," ROS function as signaling molecules that regulate cell growth, the adhesion of cells toward other cells, differentiation, senescence, and apoptosis [59,60]. The concept of chronic or prolonged ROS production is considered central to the progression of inflammatory disease [61]. What are the biologically relevant ROS? The widely studied and understood family members are the superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl) [60]. Although others may be important in signaling and disease [60,61], their functions remain poorly understood. ROS are generated as byproducts of cellular metabolism through the electron transport chain (ETC) in mitochondria as well as via the cytochrome P450. The other major source, where ROS are not produced as by products, are the NADPH oxidases that are present in a variety of cells, especially the professional phagocytes and endothelial cells [62], which are central to the genesis of the inflammatory response [61].

$O_2^{\bullet -}$ is generated by one-electron reduction of O_2 through enzymatic catalysis by NADPH oxidase or xanthine oxidase (XO) or during electron transfer reactions in the ETC of mitochondria [60,63,64]. $O_2^{\bullet -}$ has a half-life of 10^6 ns [65], as it undergoes spontaneous dismutation to H_2O_2 (under physiological conditions k = 2 x 10^5 $M^{-1}s^{-1}$). This reaction can be accelerated to 10^4-fold by the enzyme SOD (K = 1.6 x 10^9 $M^{-1}s^{-1}$) [60,64]. In the presence of the transition metal iron, $O_2^{\bullet -}$ and H_2O_2, in turn, generate the highly reactive OH^- and $^{\bullet}OH$ (Haber-Weiss reaction). In the first step of this reaction, $O_2^{\bullet -}$ reacts with Fe^{3+} to form Fe^{2+} and O_2. However, this reaction is thermodynamically unfavorable under the physiological conditions [66]. The second step of this reaction is also known as Fenton's reaction and occurs under the biological conditions in which Fe^{2+} reacts with H_2O_2 to form both $^{\bullet}OH$ and OH^-. $^{\bullet}OH$ is defined as the most potent oxidizing species of biological membrane proteins and lipids and has an extremely short half-life [60,65,67]. At inflammatory sites where PMN are abundant, H_2O_2 and chloride generate HOCl by the enzyme myeloperoxidase, generally considered as being a PMN-specific enzyme [68]. The passage of $O_2^{\bullet -}$ across biological membranes is highly restricted because of its negative charge. However, certain transmembrane proteins, such as voltage-dependent anion channels (VDAC) found in mitochondria, allow transmembrane passage of $O_2^{\bullet -}$ produced in ETC [69]. H_2O_2, on the other hand, can cross biological membranes through aquaporin channels such as AQP3 and AQP8 which mediate membrane H_2O_2 uptake, raising the possibility that it can enter cells that are contacting one another [70-72]. ROS are produced from molecular oxygen as a result of normal cellular metabolism. ROS can be divided into 2 groups: free radicals and nonradicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When 2 free radicals share their unpaired electrons, nonradical forms are created. The 3 major ROS that are of physiological significance are superoxide anion ($O_2^{\bullet -}$,), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2). ROS are summarized in Table 1.

Oxidant	Formula	Reaction Equation
Superoxide anion	$O_2^{\bullet -}$	$NADPH + 2O_2 \leftrightarrow NADP^+ + 2\ O_2^{\bullet -} + H^+$ $2\ O_2^{\bullet -} + H^+ \rightarrow\ H_2O_2\ + O_2$
Hydrogen peroxide	H_2O_2	$Hypoxanthine + H_2O + O_2 \ \rightleftarrows Xanthine + H_2O_2$ $Xanthine + H_2O + O_2 \ \rightleftarrows$ uric acid $+ H_2O_2$
Hydroxyl radical	$^{\bullet}OH$	$Fe^{2+}\ + H_2O_2 \rightarrow\ Fe^{3+} + OH^- + {}^{\bullet}OH$
Hypochlorous acid	HOCl	$H_2O_2 + Cl^- \rightarrow HOCl\ +\ H_2O$
Peroxyl radicals	$ROO^{\bullet}$	$R^{\bullet}\ + O_2 \rightarrow\ ROO^{\bullet}$
Hydroperoxyl radical	$HOO^{\bullet}$	$O_2^-\ + H_2O \rightleftarrows\ HOO^{\bullet}\ + OH^-$

Table 1: Major Endogenous Oxidants.

Superoxide anion is formed by the addition of 1 electron to the molecular oxygen [73]. This process is mediated by nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase or xanthine oxidase or by mitochondrial electron transport system. The major site for producing superoxide anion is the mitochondria, the machinery of the cell to produce adenosine triphosphate. Normally, electrons are transferred through mitochondrial electron

transport chain for reduction of oxygen to water, but approximately 1 to 3% of all electrons leak from the system and produce superoxide. NAD(P)H oxidase is found in polymorphonuclear leukocytes, monocytes, and macrophages. Upon phagocytosis, these cells produce a burst of superoxide that lead to bactericidal activity. Superoxide is converted into hydrogen peroxide by the action of superoxide dismutases (SODs, EC 1.15.1.1). Hydrogen peroxide easily diffuses across the plasma membrane. Hydrogen peroxide is also produced by xanthine oxidase, amino acid oxidase, and NAD (P)H oxidase [74,75] and in peroxisomes by consumption of molecular oxygen in metabolic reactions. In a succession of reactions called Haber Weiss and Fenton reactions, H_2O_2 can breakdown to OH^- in the presence of transmission metals like Fe^{2+} or Cu^{2+}[76].

$O_2^{\bullet-} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$ Haber-Weiss

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$ Fenton reaction

$O_2^{\bullet-}$ itself can also react with H_2O_2 and generate OH^- [77,78]. Hydroxyl radical is the most reactive of ROS and can damage proteins, lipids, and carbohydrates and DNA. It can also start lipid peroxidation by taking an electron from polyunsaturated fatty acids.

Granulocytic enzymes further expand the reactivity of H_2O_2 via eosinophil peroxidase and myeloperoxidase (MPO). In activated neutrophils, H_2O_2 is consumed by MPO. In the presence of chloride ion, H_2O_2 is converted to hypochlorous acid (HOCl). HOCl is highly oxidative and plays an important role in killing of the pathogens in the airways [79]. However, HOCl can also react with DNA and induce DNA–protein interactions and produce pyrimidine oxidation products and add chloride to DNA bases [80,81]. Eosinophil peroxidase and MPO also contribute to the oxidative stress by modification of proteins by halogenations, nitration, and protein cross-links via tyrosyl radicals [82-84]. Other oxygen-derived free radicals are the peroxyl radicals ($ROO^{\bullet}$). Simplest form of these radicals is hydroperoxyl radical ($HOO^{\bullet}$) and has a role in fatty acid peroxidation. Free radicals can trigger lipid peroxidation chain reactions by abstracting a hydrogen atom from a sidechain methylene carbon. The lipid radical then reacts with oxygen to produce peroxyl radical. Peroxyl radical initiates a chain reaction and transforms polyunsaturated fatty acids into lipid hydroperoxides. Lipid hydroperoxides are very unstable and easily decompose to secondary products, such as aldehydes (such as 4-hydroxy-2,3-nonenal) and malondialdehydes (MDAs). Isoprostanes are another group of lipid peroxidation products that are generated via the peroxidation of arachidonic acid and have also been found to be elevated in plasma and breath condensates of asthmatics [85,86]. Peroxidation of lipids disturbs the integrity of cell membranes and leads to rearrangement of membrane structure. Hydrogen peroxide, superoxide radical, oxidized glutathione (GSSG), MDAs, isoprostanes, carbonyls, and nitrotyrosine can be easily measured from plasma, blood, or bronchoalveolar lavage samples as biomarkers of oxidation by standardized assays.

Mitochondria as Main Source of ROS in Autophagy Signalling

Although the question is still far from being solved, there are at least other two issues that deserve to be considered. The first is 'where ROS are so rapidly produced'. It would be actually more logical that a stimulus coming from the outside of the cell is transduced by a ROS-producing system located at, or nearby, the plasma membrane, such as the NADPH oxidase (NOX) complexes. Nevertheless, although attractive, this hypothesis has been verified only in macrophages upon bacterial infection, where ROS generated by NOX2 are indispensable for the recruitment of the microtubule-associated protein light chain 3 (LC3) on phagosomes that, thus modified, are degraded by autophagy to prevent pathogen escape [87]. A large amount of data, instead, converge to state that the mitochondria represent the principal source of ROS required for autophagy induction, [88,89] although they are not in close proximity to the plasma membrane. A possible explanation for this unexpected evidence is that nutrient deprivation suddenly results in energetic stress that, in turn, increases ATP demand and causes mitochondrial overburden to face up adverse conditions. As a consequence, electron leakage and ROS production also increase. Another hypothesis is that a still uncharacterized factor could act as transducer, linking the upstream autophagic signal with mitochondria. A good candidate could be HKII that, by sequestering mTORC1, could loosen its inhibition on permeability transition pore (PTP) and its ability to decrease ROS [90,91]. In support of this hypothesis, it has been reported that the two protein kinases positively regulating HKII activity, Akt and myotonic dystrophy protein kinase (DMPK), are negative modulators of autophagy [92,93].

ROS and Mitophagy

As principal sites of ROS production, mitochondria are the organelles that are able to turn on and tune autophagy. However, upon chronic impairment of mitochondrial function, ROS can be generated at high extent, thus shifting their role from bulk autophagy inducers into a self-removal signal for mitochondria through a selective process called mitophagy. This represents a fine mechanism of negative feedback regulation by which

autophagy eliminates the source of oxidative stress and protects the cell from oxidative damage. Although necessary, mitophagy represents an 'extreme decision' for a cell subjected to nutrient deprivation because of at least two main reasons. The first reason is that mitochondria underpin ATP production that is fundamental upon carbon source limitation. The second reason lies in the fact that mitochondria are relatively large organelles that require being beforehand fragmented in order to be properly recognized and engulfed within the autophagosomes [94]. Both these issues contribute to explain why mitochondria are in general refractory to undergo mitophagy, unless they are severely damaged. Recently, it has been proposed that under nutrient deprivation, mitochondria attempt to protect themselves from autophagic removal by promoting fusion and inhibiting fission events [95,96]. The combination of these two inputs results in mitochondrial elongation that further impedes organelle engulfment within the autophagosomes and, concomitantly, allows to maximize ATP production [96]. Only upon prolonged starvation, mitochondria depolarize and become fragmented in order to assist their removal by mitophagy.

So far, at least two different molecular mechanisms underlying mitophagy have been described and characterized. The first one is mediated by NIX/Bnip3L (Bcl-2/adenovirus E1B 19-kDa-interacting protein 3, long form), [97,98] an atypical BH3 protein that is able to directly recognize the autophagosomesited GABA receptor-associated protein (GABARAP), a functional homologue of LC3 and, in turn, allow mitochondria to be removed. This is a 'programmed' event, independent on any damage response that is required, for example, in mitochondrial elimination during erythrocyte differentiation [99,100]. The second mechanism is activated for the selective dismissal of impaired or dysfunctional mitochondria. It is responsive to mitochondrial depolarization and is regulated by the PTEN-induced putative kinase 1 (PINK1) and Parkin, [101] a ubiquitin E3 ligase whose mutations have been associated with familial form of Parkinson's disease. PINK1 is a Ser/Thr kinase that translocates on the outer mitochondrial membrane where it is stabilized by low mitochondrial transmembrane potential, thereby acting as real sensor of mitochondrial depolarization [102]. Here, PINK1 recruits Parkin [102] that ubiquitylates a number of outer mitochondrial membrane-located proteins, for example, the voltagedependent anion channel 1 (VDAC1) [103]. Once ubiquitylated, these proteins are recognized by p62/sequestosome 1 (p62/ SQSTM1, or simply p62), [103,104] a ubiquitin-binding protein acting as a scaffold for several protein aggregates and triggering their degradation through the proteasome, or the lysosome pathway via autophagy [105,106]. p62 contains an LC3 interacting region (LIR) [107] that has been indicated being fundamental for p62 to mediating mitophagy. Indeed, by means of this motif, p62 can bridge autophagy-targeted mitochondria to LC3 located on the autophagosomes surface, thereby driving their degradation. Recently a role for Ambra1 in mitophagy, driven by its selective interaction with LC3 and independent from Parkin and p62 is identified [108].

Production of ROS and Their Mechanisms of Biological Activities

ROS ($^1O_2$, $O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$, OH^-). However, other special ROS with biological effects exist. Nitric oxide, for example, is a short-lived molecule with a free electron that regulates many physiological functions by itself, some of which are associated with development [109,110]. Hydrogen peroxide is not as reactive as the hydroxyl radical yet the latter is readily generated when the former is in the presence of Fe^{3+} (Fenton reaction). Superoxide is also not very reactive but can react with nitric oxide to produce the very potent oxidant peroxynitrite [111]. Singlet oxygen, an electronically excited form of oxygen, is very reactive and produces clear effects on cells [112], but its biological relevance waits for more in vivo studies. Phagocytic cells, such as macrophages and neutrophils are also prominent sources of O_2^-. In the presence of invading pathogens like bacteria, phagocytic cells become activated and they generate O_2^- which attacks the invading pathogens as a part of the inflammatory response [113]. Superoxide anion is also produced from xanthine by the enzyme xanthine oxidase along with uric acid as waste products of purine metabolism [114]. Other intracellular sources of the generation of ROS include reactions involving cytochrome P450 enzymes [115], peroxisomal oxidases [116], and NAD(P)H oxidases [117]. Superoxide anion is important in the body because it generates other free radicals capable of causing cell injury [118,119]. Superoxide anion is impermeable to the cell membrane and mainly affects enzyme function [120]. Its mechanism of toxicity involves disassembly of iron-sulphur ([Fe–S]) clusters in proteins through the inactivation of iron regulatory protein-1 (IRP-1), causing release of iron and alterations of -SH residues [116]. Superoxide anion reacts with the potent vasodilator and cell-signaling molecule, nitric acid (NO), to form the toxic peroxynitrite ($ONOO^-$) product [119,120]. Superoxide anion can also undergo dismutationto form hydrogen peroxide (H_2O_2), either spontaneously or when catalyzed by the enzyme superoxide dismutase [118]. Although, H_2O_2 is not a free radical by definition, it is a biologically important oxidant because it selectively participates in hydroxyl radical generation, an extremely potent radical [120-122]. Hydrogen peroxide undergoes reactions with metal ions like ferrous (Fe^{2+}) or cuprous (Cu^{2+}) to form

ferric (Fe^{3+}) or cupric ions (Cu^{3+}) and hydroxyl ions, which is sometimes described as the Fenton reaction [123]. Also, because of its nonionized and low charged state, H_2O_2 has a long diffusion distance, since it readily diffuses through hydrophobic membranes as seen with the leakage of H_2O_2 from mitochondria [124]. H_2O_2 is broken down to O_2 and water by the antioxidant enzyme catalase [120,124,125]. In addition to catalase, glutathione peroxidase (GPx) can also break down H_2O_2 and other peroxides that are formed on lipids within the body to yieldwater and oxidized glutathione [120,124].

NO is synthesized through the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS), which exists in three known forms ofendothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS) which can be up-regulated under certain conditions to induce oxidetive stress [126]. At physiological concentrations, NO controls mitochondrial respiration, causing reversible inhibition of respiration by altering cytochrome C oxidase (Complex IV) activity, which is the terminal enzyme of the mitochondrial respiratory chain [127]. NO also binds to soluble guanylate cyclase in the vascular endothelium to control vascular tone [128]. Inducible NOS (iNOS) is up-regulated by oxidative stress, producing a burst of NO that far exceeds basal levels which can cause significant cellular injury via different mechanisms:

(1) NO may directly promote excessive peripheral vasodilation, resulting in vascular decomposition [128,129], and (2) NO may up-regulate the transcription of nuclear factor-κB (NF-κB), thus, initiating an inflammatory signaling pathway that, in turn, triggers numerous inflammatory cytokines [129]. Peroxynitrite, obtained from the reaction of O_2^- and NO, has been reported to modify proteins with thiol groups resulting in the generation of nitrosothiols, which can disrupt metal-protein interactions and lead to the generation of other metal-derived free radicals. peroxynitrite can react with CO_2 to form nitrogen dioxide ($^{\bullet}NO_2$), a radical of less activity than peroxynitrite but of longer diffusion distance [130].

Formation of superoxide anion

$$O_2 + e^- \xrightarrow{X} \text{Hypoxanthine} \xrightarrow{XO} \text{Uric acid} + O_2^{\cdot-} + H_2O_2$$

X: Xanthine; XO: Xanthine Oxidase

Formation of peroxynitrite and nitrogen dioxide

$$NO + O_2^{\cdot-} \longrightarrow ONOO^-$$

$$ONOO^- + CO_2 \longrightarrow NO_2^{\cdot-} + CO_3^{\cdot-}$$

Formation of hydroxyl ions via Fenton reactions

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot-} + OH^-$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{2+} + OOH^{\cdot} + H^+$$

Hydroxyl radical ($^{\bullet}OH$) is formed not only by the interaction between hydrogen peroxide and the reduced forms of metal ions, *i.e.*, Cu^{2+} and Fe^{2+}, but also by reduction of hydrogen peroxide as well as the interaction of superoxide with hydrogen peroxide [118,120]. The hydroxyl radical is particularly unstable and is the most reactive of the free radical molecules. In addition, it is capable of reacting rapidly and non-specifically with most biological molecules [120,131]. Despite having very short half-lives of nanoseconds, they can cause severe damage to cell and other intracellular structures because they can cause covalent crosslinking of a variety of biological molecules. They cause cell damage by initiating chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins [122,131-133]. Damage to DNA can cause mutations [133,134] and possibly lead to cancer [134], if not reversed by DNA repair mechanisms [134]. Also, damage to proteins causes enzyme inhibition, denaturation and protein degradation [131].

ROS are formed as unavoidable by-products of aerobic respiration and various other catabolic and anabolic processes [135] (Figure 1).

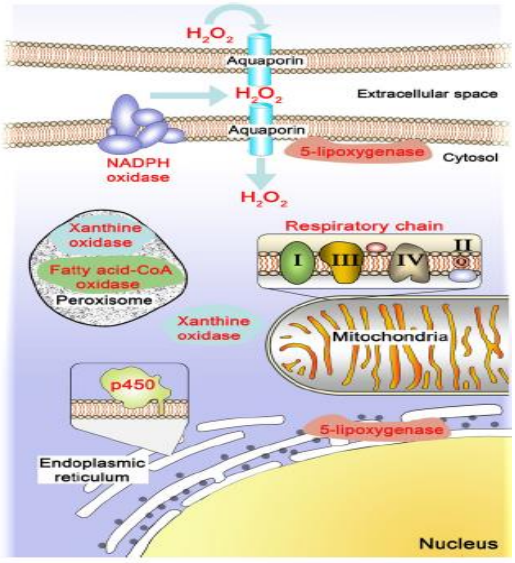

Figure 1: Places in the cell where ROS are produced. Major organelles that are known to be sources of ROS are depicted. The activity of the respiratory chain in the mitochondria is responsible of most ROS produced in aerobiosis. On the other hand, the metabolic pathway that drives the degradation of long chain fatty acids (i.e., β-oxidation) in the peroxisome is also an important ROS source, though the amount produced depends on the activity of this metabolic pathway, a property that is cell-type specific. The function of ROS produced by cytochrome p450 or NADPH oxidases may be restricted to the area where they are located. Specific cytochrome p450 are involved in the synthesis and degradation of steroid hormones and retinoic acid, relevant molecules in development. Peroxisomal or cytosolic xanthine oxidase is an enzyme that produces ROS from molecular oxygen, whose best-characterized function is in the final catabolism of purines. 5-lipoxygenase, an enzyme involved in the synthesis of leukotrienes, can be found associated with membranes or with the nuclear envelope. ROS can also function as paracrine signals, as hydrogen peroxide can cross from one cell to another through aquaporins.

For example, the respiratory chain produces essentially super-oxide that can be converted to peroxide by superoxide dismutases [136]. Among the enzymes that produce ROS by-products are (Figure 1) fatty acyl-CoA oxidase, xanthine oxidase, cytochrome p450 systems, cycloxygenases and lipoxygenases [137,138]. ROS are directly produced from oxygen by NADPH oxidases, a major family of enzymes whose catalytic subunits are encoded by Nox1–5 and Duox1–2 [139]. Although this activity was initially detected during phagocytosis, it is now known that these enzymes are broadly distributed among many tissues [139]. Interestingly, Nox enzymes are almost exclusive to multicellular organisms [140]. Due to the high reactivity of some ROS, the location where they are produced is critical for their biological effects (Figure 1). Nonetheless, although it is common to think that ROS are cell autonomous signals produced within the affected cell, there are examples in which ROS holding low reactivity, such as hydrogen peroxide, appear to mediate intercellular communication. Paracrine communication could result when ROS are released from normal (i.e., myofibroblasts) or apoptotic cells and affect neighboring cells [141,142]. It is frequently considered that hydrogen peroxide diffuses freely through biological membranes; however, its water-like electrostatic characteristics suggest a limited simple passive diffusion. Recently, it was found that specific aquaporins, initially described as water channels that are present in all living cells, facilitate hydrogen peroxide diffusion across cell membranes [143] (Figure 1). Diffusion of hydrogen peroxide also plays a role in the autocrine signaling mediated by NADPH oxidases (Figure 1). The diffusibility of ROS is a property that may contribute to determine the redox state of a community of cells or the propagation of ROS signals, mechanisms that could coordinate developmental events such as massive cell death.

Increased ROS Production in Photosynthesis during Drought

The commonly considered sources of ROS shown in Figure 2 potentially allow specificity because of their differences in chemical nature or location. Although drought may stimulate all three sources simultaneously,

and ROS are frequently associated with damage, at least some of these pathways may act rather as damage limitation (protective) processes.

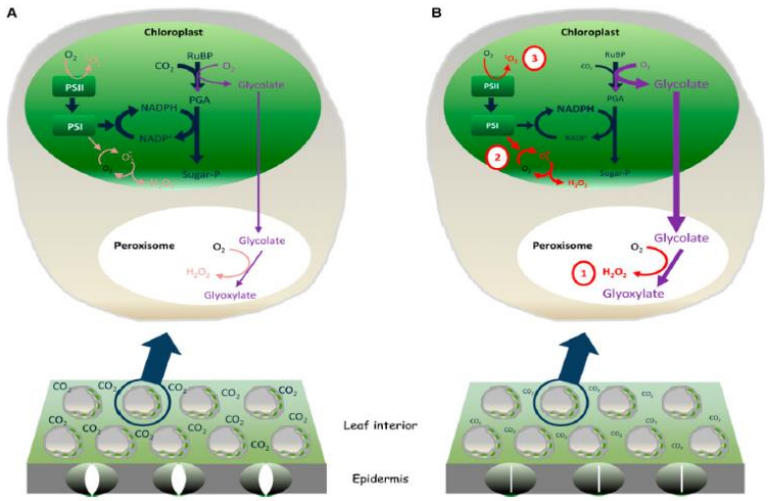

Figure 2: Current concepts of how drought increases the generation of ROS in photosynthesis. A Cartoon of leaf section in well-watered plants in which relatively high intercellular CO_2 concentrations (Ci) allow efficient regeneration of terminal oxidants and limit RuBP oxygenation. B, Drought-induced stomatal closure restricts CO_2 uptake, favoring photorespiratory production of H_2O_2 in the peroxisome (1) and possibly favoring production of superoxide and H_2O_2 (2) or singlet oxygen (3) by the photosynthetic electron transport chain. PGA, 3-Phosphoglyceric acid.

Much of the work on redox changes triggered by drought has focused on shoots. According to the dominant view of drought-induced oxidative stress in these organs, ROS production is increased by redox changes associated with photosynthesis. Even under optimal conditions, ROS can be produced at considerable rates inside the cell as part of metabolism [144]. In terms of production of H_2O_2, the most stable of the major ROS species, the chloroplast continues to receive the most attention; however, in many conditions in C_3 plants, the rate of H_2O_2 production may actually be higher in the peroxisomes [145]. Peroxisomal H_2O_2 production in the green tissues of C_3 plants is largely a result of the activity of glycolate oxidase. This enzyme is an essential part of the photorespiratory recycling pathway that is initiated by oxygenation of ribulose-1,5-bisphosphate (RuBP) in the chloroplast [146]. Glycolate production is considered to be accelerated during drought as intercellular CO_2 drops as a result of drought-induced stomatal closure. This favors RuBP oxygenation [147] and hence increased peroxisomal H_2O_2 production (Figure 2B, site 1). In the chloroplast, restrictions over reductant and ATP consumption during drought may also favor ROS production at two distinct sites within the electron transport chain. First, decreased availability of other oxidants for the chain may promote electron flow to O_2 in the Mehler reaction, thus stimulating superoxide and H_2O_2 production and accelerating the water-water cycle ([148], Figure 2B, site 2). Second, any overreduction of the electron transport chain is expected to enhance the probability of singlet oxygen generation in PSII ([149], Figure 2B, site 3). According to this view, production of superoxide and H_2O_2 at both sites 1 and 2 is associated with limitation of the accumulation of reduced intermediates and hence a decrease in the probability of singlet oxygen generation at site 3 (Figure 2). Several genes encoding expansins are among genes that are up-regulated at an early stage or in response to moderate drought [150]. Some of these adjustments in cell wall structure may involve ROS and thus one or more of several types of enzymes that are localized at the cell surface or apoplast and that use different reductants and cofactors to produce either superoxide or H_2O_2 (Figure 3).

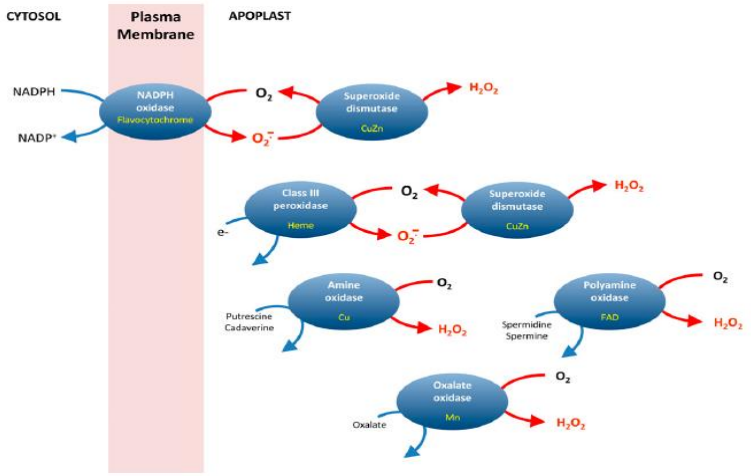

Figure 3: Multiple ROS-producing enzymes at the cell surface/exterior. Enzymes are shown in blue and their redox cofactors are indicated in yellow. Class III peroxidases may accept electrons from several types of compounds to generate superoxide, but in many cases their physiological reductant is not established [151].

These include NADPH oxidases, amine oxidases, polyamine oxidases, oxalate oxidases, and a large family of class III heme peroxidases [151-154]. Class III heme peroxidases may either use H_2O_2 to oxidize apoplastic substrates or reductants to produce superoxide from O_2 [151]. Although most of these ROS-producing enzymes have been implicated in pathogenesis responses [151,153,155,156], the roles of many of them in drought are less clear. However, enzymes such as oxalate oxidases may play important roles in the acclimation of root growth to drought [157]. In addition, the plasma membrane is rich in stress perception proteins such as receptor-like kinases that fulfill important roles in drought tolerance and cell wall function [158,159]. Many of these are regulated at the level of expression by changes in the cell redox state [160].

ROS Elimination

Levels of ROS are not only determined by production, but also by the rate of ROS degradation or inactivation. In general terms, the ultimate effect of antioxidants is to decrease the amount of active ROS. Cells have manyways to respond against ROS, including enzymatic and non-enzymatic antioxidants. It is the balance between the production and degradation of ROS that maintains the cellular homeostasis. Common non-enzymatic antioxidants are glutathione and thioredoxin [161,162]. Glutathione (γ-glutamyl-cysteinyl-glycine; GSH) synthesis is catalyzed by the sequential action of γ-glutamylcysteine synthetase and GSH synthetase. [163]. Once GSH is oxidized (GSSG), the reduced form can be regenerated by the GSH reductase (Gr). The balance between GSH and GSSG is a way to determine the redox state within the cell. On the other hand, thioredoxins (Trx), as well as glutaredoxins (Grx), are small proteins containing an active site with a redox-active disulfide [161]. These proteins maintain a reduced intracellular redox state in mammalian cells by the reduction of protein thiols. Two Trx and three Trx reductases (TrxR) are present in mammals, each with a distinct intracellular location [164]. Trx1 and TrxR1 are cytosolic or nuclear proteins, whereas Trx2 and TrxR2 are targeted to the mitochondria. Of the two Grx present in mammals, Grx2 appears to be in mitochondria and the nucleus [165].

Types of Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are produced from molecular oxygen as a result of normal cellular metabolism. ROS can be divided into 2 groups: free radicals and nonradicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When 2 free radicals share their unpaired electrons, nonradical forms are created. ROS in high concentrations have damaging actions, but in lower concentrations they can act as signaling molecules (Figure 4).

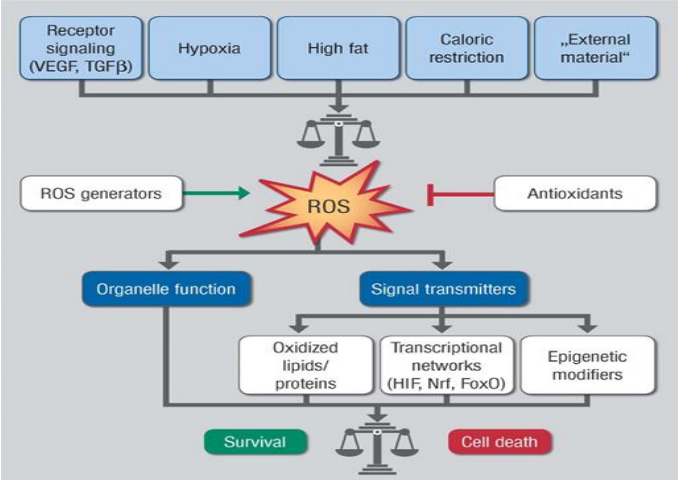

Figure 4: Summary scheme of ROS acting as signaling molecules in different disease settings but also in physiological processes.

ROS generated by the activation of enzymes such as NOX, xanthine oxidases, uncoupled NO synthases and other sources such as arachidonic acid metabolizing enzymes, lipoxygenases and cyclooxygenases, the cytochrome P450s, peroxidases and other hemoproteins, as well as ROS generated by mitochondria seem to play various roles in the cellular signaling network under different physiological and pathophysiological conditions. Various cellular antioxidant systems oppose ROS load thereby limiting not only cellular damage, but also contributing to ROS-dependent signaling. The delicate balance between ROS generation and ROS scavenging is disturbed by the different types of stress factors like salinity, drought, extreme temperatures, heavy metals, pollution, high irradiance, pathogen infection, etc. (Figure 5 (A)).

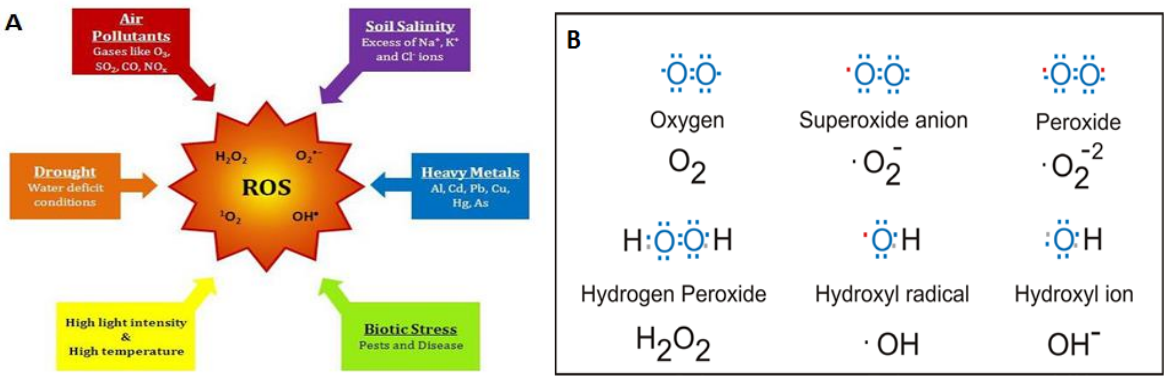

Figure: 5 (A) Various causes responsible for the generation of ROS.
(B) Electron structures of common reactive oxygen species. Each structure is provided with its name and chemical formula. The ● designates an unpaired electron.

Most reactive oxygen species are generated as by-products during mitochondrial electron transport. In addition ROS are formed as necessary intermediates of metal catalyzed oxidation reactions. Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation. The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including: superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and

nitric oxide. (Figure 5(B)). The major ROS include $^1O_2$, superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl anions (OH^-), hydroxyl radicals ($OH^{\bullet}$), and hypochlorous acid (HOCl). Superoxide is produced by NADPH oxidase/xanthine oxidase-derived reduction of molecular oxygen, uncoupled endothelial nitric oxide synthase (eNOS), or mitochondrial electron transport chain (ETC). Superoxide is rapidly dismutated to H_2O_2 by superoxide dismutase (SOD). However, in the presence of nitric oxide (NO), $O_2^{\bullet-}$rapidly reacts with NO, resulting in the formation of highly reactive peroxynitrite ($ONOO^-$), which is three to four times faster than dismutation of $O_2^{\bullet-}$ to H_2O_2. H_2O_2 can change to highly reactive HOCl at the inflammatory sites by an enzyme known as myeloperoxidase (MPO), which is abundantly expressed in neutrophils. H_2O_2 can also change to the highly toxic $OH^{\bullet}$ in presence of Fe^{2+} by Fenton's reaction. H_2O_2 is scavenged to H_2O and O_2 by catalase, glutathione peroxidase (GPX), or peroxiredoxins (Prx) antioxidant enzymes. Prx uses thioredoxin (Trx) to detoxify H_2O_2. These are very lethal and causes extensive damage to biomolecules such as lipids, proteins and DNA (Figure 6) and thereby affects normal cellular functioning [166].

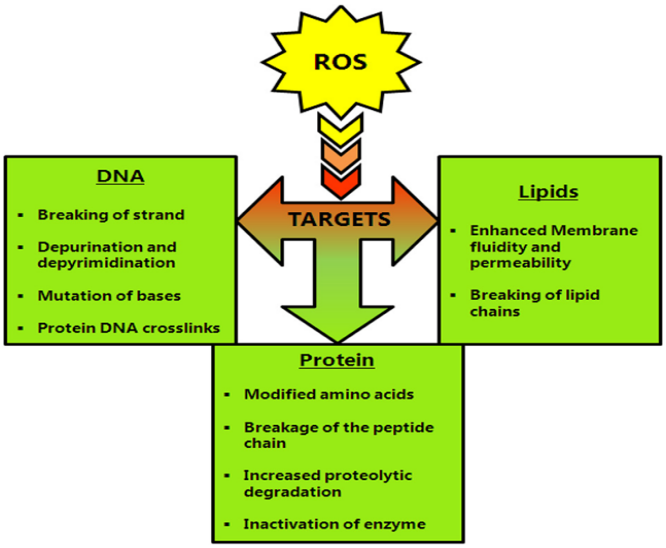

Figure 6: Reactive oxygen species (ROS) induced oxidative damage effects to tissues lipids, proteins, and DNA.

Bivalves are able to survive in a wide range of oxygen concentrations ranging from anoxic to high levels of dissolved oxygen. Variations in this ability have been proposed as an index of environmental stress. Oxyradicals ($O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$) can be highly toxic to aquatic organisms often resulting in lipid peroxidation in membranes, altered pyridine nucleotide redox status and DNA damage. Moreover, many xenobiotics are capable of modulating oxidative stress either by acting directly as redox cycling compounds (e.g. menadione) or as a consequence of biotransformation to quinones which are redox cycling (e.g. benzo (*a*) pyrene). The microsomes appear to be an important subcellular location for such activity since this is the site of cytochrome P-450 induction and catalysis. An attractive in vitro system for studying the production of oxyradicals in *M. edulis* lysosomes using the laser dye dihydrorhodamine 123 is available.

References

1. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1): 1-40.

2. Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine. 3rd Ed. New York: Oxford University Press.

3. Marnett LJ (1999) Lipid peroxidationdDNA damage by malondialdehyde. Mutat Res 424(1-2): 83-95.

4. Siems WG, Grune T, Esterbauer H (1995) 4-Hydroxynonenal formation during ischemia and reperfusion of rat small-intestine. Life Sci 57(8): 785-789.

5. Stadtman ER (2004) Role of oxidant species in aging. Curr Med Chem 11(9): 1105-1112.

6. Wang MY, Dhingra K, Hittelman WN, Liehr JG, deAndrade M, et al. (1996) Lipid peroxidation-induced putative malondialdehyde–DNA adducts in human breast tissues. Cancer Epidemiol Biomarkers Prev 5(9): 705-710.

7. Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, et al. (2011) ROS signaling: the new wave?. Trends Plant Sci 16(6): 300-309.

8. Foyer CH, Noctor G (2013) Redox signaling in plants. Antioxid Redox Signal 18(16): 2087-2090.

9. Vaahtera L, Brosche M, Wrzaczek M, Kangasjarvi J (2014) Specificity in ROS signaling and transcript signatures. Antioxid & Redox Signal 21(9): 1422-1441.

10. Considine MJ, Sandalio LM, Foyer CH (2015) Unravelling how plants benefit from ROS and NO reactions, while resisting oxidative stress. Ann Bot 116(4): 469-473.

11. Dietz KJ (2015)Efficient high light acclimation involves rapid processes at multiple mechanistic levels. J Exp Bot 66(9): 2401-2414.

12. Mignolet-Spruyt L, Xu E, Idanheimo N, Hoeberichts FA, Muhlenbock P, et al. (2016) Spreading the news: subcellular and organellar reactive oxygen species production and signalling. J Exp Bot 67(13): 383-3844.

13. Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7(9): 405-410.

14. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci. 9(10): 490-498.

15. Halliwell B, Gutteridge JM (2015) Free radicals in biology and medicine, New York, NY: Oxford University Press.

16. Apel K, Hirt H (2004) Ann Rev Plant Biol 55: 373-399.

17. Smirnoff N (1998) Plant resistance to environmental stress. Curr Opin Biotechnol 9(2): 214-219.

18. Puertollano MA, Puertollano E, de Cienfuegos GA, de Pablo MA (2011) Dietary antioxidants: immunity and host defense. Curr Top Med Chem 11(14): 1752-1766.

19. Drummond RA, Brown GD (2011) The role of Dectin-1 in the host defence against fungal infections. Curr Opin Microbiol 14(4): 392-39.

20. Wu W, Hsu Y-MS, Bi L, Songyang Z, Lin X (2009) CARD9 facilitates microbe-elicited production of reactive oxygen species by regulating the LyGDI-Rac1 complex. Nat Immunol 10(11): 1208-1214.

21. Ueno N, Wilson ME (2012) Receptor-mediated phagocytosis of Leishmania: implications for intracellular survival. Trends Parasitol 28(8): 335-344.

22. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, et al. (2004) Neutrophil extracellular traps kill bacteria. Science 303(5663): 1532-1535.

23. Rybicka JM, Balce DR, Khan MF, Krohn RM, Yates RM (2010) NADPH oxidase activity controls phagosomal proteolysis in macrophages through modulation of the luminal redox environment of phagosomes. Proc Natl Acad Sci U S A 107(23): 10496-10501.

24. Huang J, Canadien V, Lam GY, Steinberg BE, Dinauer MC, et al. (2009)Activation of antibacterial autophagy by NADPH oxidases. Proc Natl Acad Sci U S A 106(15): 6226-6231.

25. Huang J, Lam GY, Brumell JH (2011) Autophagy signaling through reactive oxygen species. Antioxid Redox Signal 14(11): 2215-2231.

26. Shin DM, Jeon BY, Lee HM, Jin HS, Yuk JM, et al. (2010) Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. PLoS Pathog 6(12): e1001230.

27. Li M, Zhao L, Liu J, Liu A, Jia C, et al. (2010) Multi-mechanisms are involved in reactive oxygen species regulation of mTORC1 signaling. Cell Signal 22(10): 1469-1476.

28. Tilton C, Clippinger AJ, Maguire T, Alwine JC (2011) Human cytomegalovirus induces multiple means to combat reactive oxygen species. J Virol 85(23): 12585-12593.

29. Niethammer P, Grabher C, Look AT, Mitchison TJ (2009) A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. Nature 459(7249): 996-999.

30. Yoo SK, Starnes TW, Deng Q, Huttenlocher A (2011) Lyn is a redox sensor that mediates leukocyte wound attraction in vivo. Nature 480(7375): 109-112.

31. Ashida H, Mimuro H, Ogawa M, Kobayashi T, Sanada T, et al. (2011) Cell death and infection: a double-edged sword for host and pathogen survival. J Cell Biol 195(6): 931-942.

32. Chan RC, Wang M, Li N, Yanagawa Y, Onoe K, et al. (2006) Pro-oxidative diesel exhaust particle chemicals inhibit LPS-induced dendritic cell responses involved in Thelper differentiation. J Allergy Clin Immunol 118(2): 455-465.

33. Gilmour MI (2012) Influence of air pollutants on allergic sensitization: the paradox of increased allergies and decreased resistance to infection. Toxicol Pathol 40(2): 312-314.

34. Mantegazza AR, Savina A, Vermeulen M, Perez L, Geffner J, et al. (2008) NADPH oxidase controls phagosomal pH and antigen cross-presentation in human dendritic cells. Blood 112(12): 4712-4722.

35. Moghaddam AE, Gartlan KH, Kong L, Sattentau QJ (2011) Reactive carbonyls are a major Th2-inducing damageassociated molecular pattern generated by oxidative stress. J Immunol 187(4): 1626-1633.

36. Tang H, Cao W, Kasturi SP, Ravindran R, Nakaya HI, et al. (2010) The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROSmediated signaling. Nat Immunol 11(7): 608-617.

37. Wang J, Ren Z, Xu Y, Xiao S, Meydani SN, et al. (2012) Epigallocatechin-3-gallate ameliorates experimental autoimmune encephalomyelitis by altering balance among CD4 + T-cell subsets. Am J Pathol 180(1): 221-234 2012.

38. Swirski FK, Nahrendorf M (2013) Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science 339(6116): 161-166.

39. Church DF, Pryor WA (1985) Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 64: 111-126.

40. Hiltermann JT, Lapperre TS, van Bree L, Steerenberg PA, Brahim JJ, et al. (1999) Ozone-induced inflammation assessed in sputum and bronchial lavage fluid from asthmatics: a new noninvasive tool in epidemiologic studies on air pollution and asthma. Free Radic Biol Med 27(11-12): 1448-1454.

41. Nightingale JA, Rogers DF, Barnes PJ (1999) Effect of inhaled ozone on exhaled nitric oxide, pulmonary function, and induced sputum in normal and asthmatic subjects. Thorax 54(12): 1061-1069.

42. Cho AK, Sioutas C, Miguel AH, Kumagai Y, Schmitz DA, et al. (2005) Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. Environ Res 99(1): 40-47.

43. Comhair SA, Thomassen MJ, Erzurum SC (2000) Differential induction of extracellular glutathione peroxidase and nitric oxide synthase 2 in airways of healthy individuals exposed to 100% O(2) or cigarette smoke. Am J Respir Cell Mol Biol 23(3): 350-354.

44. Matthay MA, Geiser T, Matalon S, Ischiropoulos H (1999) Oxidant-mediated lung injury in the acute respiratory distress syndrome. Crit Care Med 27(9): 2028-2030.

45. Biaglow JE, Mitchell JB, Held K (1992) The importance of peroxide and superoxide in the X-ray response. Int J Radiat Oncol Biol Phys 22(4): 665-669.

46. Chiu SM, Xue LY, Friedman LR, Oleinick NL (1993) Copper ion-mediated sensitization of nuclear matrix attachment sites to ionizing radiation. Biochemistry 32(24): 6214-6219.

47. Narayanan PK, Goodwin EH, Lehnert BE (1997) Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. Cancer Res 57(18): 3963-3971.

48. Tuttle SW, Varnes ME, Mitchell JB, Biaglow JE (1992) Sensitivity to chemical oxidants and radiation in CHO cell lines deficient in oxidative pentose cycle activity. Int J Radiat Oncol Biol Phys 22(4): 671-675.

49. Guo G, Yan-Sanders Y, Lyn-Cook BD, Wang T, Tamae D, et al. (2003) Manganese superoxide dismutase-mediated gene expression in radiationinduced adaptive responses. Mol Cell Biol 23(7): 2362-2378.

50. Azzam EI, de Toledo SM, Spitz DR, Little JB (2002) Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from a-particle irradiated normal human fibroblasts. Cancer Res 62(19): 5436-5442.

51. Leach JK, Van Tuyle G, Lin PS, Schmidt-Ullrich R, Mikkelsen RB (2001) Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res 61(10): 3894-3901.

52. Dent P, Yacoub A, Fisher PB, Hagan MP, Grant S (2003) MAPK pathways in radiation responses. Oncogene 22(37): 5885-5896.

53. Wei SJ, Botero A, Hirota K, Bradbury CM, Markovina S, et al. (2000) Thioredoxin nuclear translocation and interaction with redox factor-1 activates the AP-1 transcription factor in response to ionizing radiation. Cancer Res 60(23): 6688-6695.

54. Cadet J, Douki T, Gasparutto D, Ravanat JL (2003) Oxidative damage to DNA: formation, measurement and biochemical features. Mutat Res 531(1-2):5-23.

55. Yokoya A, Cunniffe SM, O'Neill P (2002) Effect of hydration on the induction of strand breaks and base lesions in plasmid DNA films by gammaradiation. J Am Chem Soc 124(30): 8859-8866.

56. Janssen YM, Van Houten B, Borm PJ, Mossman BT (1993) Cell and tissue responses to oxidative damage. Lab Invest 69(3): 261-274.

57. Iwanaga M, Mori K, Iida T, Urata Y, Matsuo T, et al. (1998) Nuclear factor kappa B dependent induction of gamma glutamylcysteine synthetase by ionizing radiation in T98G human glioblastoma cells. Free Radic Biol Med 24(7-8): 1256-1268.

58. Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 18(2): 321-336.

59. Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82(1): 47-95.

60. Thannickal VJ, Fanburg BL (2000) Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 279(6): L1005-L1028.

61. Griffith B, Pendyala S, Hecker L, Lee PJ, Natarajan V, et al. (2009) NOX enzymes and pulmonary disease. Antioxid Redox Signal 11(10): 2505-2516.

62. Pendyala S and Natarajan V (2010) Redox regulation of Nox proteins. Respir Physiol Neurobiol 174(3): 265-271.

63. Handy DE, Loscalzo J (2012) Redox regulation of mitochondrial function. Antioxid Redox Signal 16(11): 1323-1367.

64. Lambeth JD (2004) NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4(3): 181-189.

65. Saran M, Bors W (1989) Oxygen radicals acting as chemical messengers: a hypothesis. Free Radic Res Commun 7(3-6): 213-220.

66. Kehrer JP (2000) The Haber-Weiss reaction and mechanisms of toxicity. Toxicology 149(1): 43-50.

67. Graf E, Mahoney JR, Bryant RG, Eaton JW (1984) Ironcatalyzed hydroxyl radical formation. Stringent requirement for free iron coordination site. J Biol Chem 259(6): 3620-3624.

68. Hampton MB, Kettle AJ, Winterbourn CC (1998) Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. Blood 92(9): 3007-3017.

69. Han D, Antunes F, Canali R, Rettori D, Cadenas E (2003) Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. J Biol Chem 278(8): 5557-5563.

70. Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, et al. (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282(2): 1183-1192.

71. Hara-Chikuma M, Chikuma S, Sugiyama Y, Kabashima K, VerkmanAS, Inoue S, et al. (2012) Chemokine-dependent T cell migration requires aquaporin-3-mediated hydrogen peroxide uptake. J Exp Med 209(10): 1743-1752.

72. Miller EW, Dickinson BC, Chang CJ (2010) Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. Proc Natl Acad Sci U S A 107(36): 15681-15686.

73. Miller DM, Buettner GR, Aust SD (1990) Transition metals as catalysts of "autoxidation" reactions. Free Radic Biol Med 8(1): 95-108.

74. Dupuy C, Virion A, Ohayon R, Kaniewski J, Dème D, et al. (1991) Mechanism of hydrogen peroxide formation catalyzed by NADPH oxidase in thyroid plasma membrane. J Biol Chem 266(6): 3739-3743.

75. Granger DN (1988) Role of xanthine oxidase and granulocytes in ischemiareperfusion injury. Am J Physiol 255(62): 1269-1275.

76. Fenton HJH (1984) Oxidation of tartaric acid in the presence of iron. J Chem Soc 65: 899-910.

77. Haber F, Weiss JJ (1934) The catalytic decomposition of hydrogen peroxide by iron salts. Proc R Soc Lond Ser A 147(861): 332-351.

78. Liochev SI, Fridovich I (2002) The Haber–Weiss cycled 70 years later: an alternative view. Redox Rep 7(1): 55-57.

79. Klebanoff SJ (2005) Myeloperoxidase: friend and foe. J Leukoc Biol 77(5):598-625.

80. Whiteman M, Jenner A, Halliwell B (1997) Hypochlorous acid-induced base modifications in isolated calf thymus DNA. Chem Res Toxicol 10(11): 1240-1246.

81. Kulcharyk PA, Heinecke JW (2001) Hypochlorous acid produced by the myeloperoxidase system of human phagocytes induces covalent cross-links between DNA and protein. Biochemistry 40(12): 3648-3656.

82. Brennan ML, Wu W, Fu X, Shen Z, Song W, et al. (2002) A tale of two controversies: defining both the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidase and myeloperoxidasedeficient mice, and the nature of peroxidase-generated reactive nitrogen species. J Biol Chem 277(20): 17415-17427.

83. Denzler KL, Borchers MT, Crosby JR, Cieslewicz G, Hines EM, et al. (2001) Extensive eosinophil degranulation and peroxidase-mediated oxidation of airway proteins do not occur in a mouse ovalbumin-challenge model of pulmonary inflammation. J Immunol 167(3):1672-1682.

84. van Dalen CJ, Winterbourn CC, Senthilmohan R, Kettle AJ (2000) Nitrite as a substrate and inhibitor of myeloperoxidase. Implications for nitration and hypochlorous acid production at sites of inflammation. J Biol Chem 275: 11638-11644.

85. Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Garg ML (2000) Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. Lipids 35(9): 967-974.

86. Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, et al. (1999) Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. Am J Respir Crit Care Med 160(1): 216-220.

87. Huang J, Canadien V, Lam GY, Steinberg BE, Dinauer MC, et al. (2009) Activation of antibacterial autophagy by NADPH oxidases. Proc Natl Acad Sci U S A 106(15): 6226-6231.

88. Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417(1): 1-13.

89. Scherz-Shouval R, Elazar Z (2007) ROS, mitochondria and the regulation of autophagy. Trends Cell Biol 17(19): 422-427.

90. Roberts DJ, Tan-Sah VP, Ding EY, Smith JM, Miyamoto S (2014) Hexokinase-II positively regulates glucose starvation-induced autophagy through TORC1 inhibition. Mol Cell 53(4): 521-533.

91. da-Silva WS, Gomez-Puyou A, de Gomez-Puyou MT, Moreno-Sanchez R, De Felice FG, et al. (2004) Mitochondrial bound hexokinase activity as a preventive antioxidant defense: steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. J Biol Chem 279(38): 39846-39855.

92. Wang RC,Wei Y, An Z, Zou Z, Xiao G, Bhagat G et al. (2012) Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science 338(6109): 956-959.

93. Oude Ophuis RJ, Wijers M, Bennink MB, van de Loo FA, Fransen JA, et al. (2009) A tail-anchored myotonic dystrophy protein kinase isoform induces perinuclear clustering of mitochondria, autophagy, and apoptosis. PLoS One 4(11): e8024.

94. Campello S, Strappazzon F, Cecconi F (2014) Mitochondrial dismissal in mammals, from protein degradation to mitophagy. Biochim Biophys Acta 1837(4): 451-460.

95. Rambold AS, Kostelecky B, Elia N, Lippincott-Schwartz J (2011) Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. Proc Natl Acad Sci U S A 108(25): 10190-10195.

96. Gomes LC, Di Benedetto G, Scorrano L (2011) During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nat Cell Biol 13(5): 589-598.

97. Zhang J, Ney PA (2009) Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. Cell Death Differ 16(7): 939-946.

98. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, et al. (2010) Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep 11(1): 45-51.

99. Schweers RL, Zhang J, Randall MS, Loyd MR, Li W, et al. (2007) NIX is required for programmed mitochondrial clearance during reticulocyte maturation. Proc Natl Acad Sci U S A 104(49): 19500-19505.

100. Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, et al. (2008) Essential role for Nix in autophagic maturation of erythroid cells. Nature 454(7201): 232-235.

101. Youle RJ, Narendra DP (2011) Mechanisms of mitophagy. Nat Rev Mol Cell Biol 12(1): 9-14.

102. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, et al. (2010) PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol 189(2): 211-221.

103. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, et al. (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 12(2): 119-131.

104. Narendra D, Kane LA, Hauser DN, Fearnley IM, Youle RJ (2010) P62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. Autophagy 6(8): 1090-1106.

105. Vadlamudi RK, Joung I, Strominger JL, Shin J (1996) p62, a phosphotyrosine-independent ligand of the SH2 domain of p56lck, belongs to a new class of ubiquitin-binding proteins. J Biol Chem 271(34): 20235-20237.

106. Bjorkoy G, Lamark T, Johansen T (2006) p62/SQSTM1: a missing link between protein aggregates and the autophagy machinery. Autophagy 2(2): 138-139.

107. Ichimura Y, Kumanomidou T, Sou YS, Mizushima T, Ezaki J, et al. (2008) Structural basis for sorting mechanism of p62 in selective autophagy. J Biol Chem 283(33): 22847-22857.

108. Strappazzon F, Nazio F, Corrado M, Cianfanelli V, Romagnoli A, et al. (2015) AMBRA1 is able to induce mitophagy via LC3 binding, regardless of PARKIN and p62/SQSTM1. Cell Death Differ 22(3): 419-432.

109. Kuzin B, Roberts I, Peunova N, Enikolopov G (1996) Nitric oxide regulates cell proliferation during Drosophila development. Cell 87(4): 639-649.

110. Regulski M, Stasiv Y, Tully T, Enikolopov G (2004) Essential function of nitric oxide synthase in Drosophila. Curr Biol 14(20): R881-882.

111. Estévez AG, Spear N, Manuel SM, Radi R, Henderson CE et al. (1998) Nitric oxide and superoxide contribute to motor neuron apoptosis induced by trophic factor deprivation. J Neurosci 18(3): 923-931.

112. Klotz LO, Kröncke KD, Sies H (2003) Singlet oxygen-induced signaling effects in mammalian cells. Photochem. Photobiol Sci 2(2): 88-94.

113. Victor VM, Rocha M, De la Fuente M (2004) Immune cells: free radicals and antioxidants in sepsis. Int Immunopharmacol 4(3): 327-347.

114. Matesanz N, Lafuente N, Azcutia V, Martin D, Cuadrado A, et al. (2007) Xanthine oxidasederived extracellular superoxide anions stimulate activator protein 1 activity and hypertrophy in human vascular smooth muscle via c-Jun N-terminal kinase and p38 mitogen-activated protein kinases. J Hypertens 25(3): 609-618.

115. Zangar RC, Davydov DR, Verma S (2004) Mechanisms that regulate production of reactive oxygen species by cytochrome P450. Toxicol Appl Pharmacol 199(3): 316-331.

116. Schrader M, Fahimi HD (2004) Mammalian peroxisomes and reactive oxygen species. Histochem Cell Biol 122(4): 383-393.

117. Li WG, Miller FJ Jr, Zhang HJ, Spitz DR, Oberley LW, et al. (2001) H (2) O (2)-induced O (2) production by a non-phagocytic NAD(P)H oxidase causes oxidant injury. J Biol Chem 276(31): 29251-29256.

118. Cadenas E (2004) Mitochondrial free radical production and cell signaling. Mol Aspects Med 25(1-2): 17-26.

119. Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87(1): 315-424.

120. D'Autreaux B, Toledano MB (2007) ROS as signaling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 8(10): 813-824.

121. Pryor WA, Houk KN, Foote CS, Fukuto JM, Ignarro LJ, et al. (2006) Free radical biology and medicine: it's a gas, man!. Am J Physiol Regul Integr Comp Physiol 291(3): R491-511.

122. Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12(10): 1161-1208.

123. Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 18(2): 321-336.

124. Mates JM, Sanchez-Jimenez F (1999) Antioxidant enzymes and their implications in pathophysiologic processes. Front Biosci 4: D339-345.

125. Yu BP (1994) Cellular defenses against damage from reactive oxygen species. Physiol Rev 74(1): 139-62.

126. Ishikawa T, Kondo Y, Goda K, Fujisawa M (2005) Overexpression of endothelial nitric oxide synthase in transgenic mice accelerates testicular germ cell apoptosis induced by experimental cryptorchidism. J Androl 26(2): 281-288.

127. Zhang J, Jin B, Li L, Block ER, Patel JM (2005) Nitric oxide-induced persistent inhibition and nitrosylation of active site cysteine residues of mitochondrial cytochrome-c oxidase in lung endothelial cells. Am J Physiol Cell Physiol 288(4): C840-849.

128. Sandoo A, van Zanten JJ, Metsios GS, Carroll D, Kitas GD (2010) The endothelium and its role in regulating vascular tone. Open Cardiovasc Med J 4:302-312.

129. Szabo C (1998) Role of nitric oxide in endotoxic shock. An overview of recent advances. Ann N Y Acad Sci 851: 422-425.

130. Szabo C, Ischiropoulos H, Radi R (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov 6(8): 662-680.

131. Stadtman ER, Moskovitz J, Levine RL (2003) Oxidation of methionine residues of proteins: biological consequences. Antioxid Redox Signal 5(5): 577-582.

132. Slater TF (1984) Free-radical mechanisms in tissue injury. Biochem J 222(1): 1-15.

133. Aruoma OI, Halliwell B, Gajewski E, Dizdaroglu M (1991) Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. Biochem J 273(Pt 3): 601-604.

134. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004) Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 266(1-2): 37-56.

135. Halliwell B, (1991) Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 91(3C): 14S-22S.

136. Turrens JF (2003) Mitochondrial formation of reactive oxygen species. J Physiol 552(2): 335-344.

137. Wanders RJ, Waterham HR (2006) Biochemistry of mammalian peroxisomes revisited. Annu Rev Biochem 75: 295-332.

138. Soberman RJ, Christmas P (2003) The organization and consequences of eicosanoid signaling. J Clin Invest 111(8): 1107-1113.

139. Lambeth JD (2004) NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4(3): 181-189.

140. Lalucque H, Silar P (2003) NADPH oxidase: an enzyme for multicellularity? Trends Microbiol. 11(1): 9-12.

141. Pletjushkina OY, Fetisova EK, Lyamzaev KG, Ivanova OY, Domnina LV, et al. (2005) Long-distance apoptotic killing of cells is mediated by hydrogen peroxide in a mitochondrial ROS-dependent fashion. Cell Death Differ 12(11): 1442-1444.

142. Waghray M, Cui Z, Horowitz JC, Subramanian IM, Martinez FJ, et al. (2005) Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts. FASEB J 19(17): 854-856.

143. Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM, et al. (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282(2): 1183-1192.

144. Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119(3): 355-364.

145. Noctor G, Veljovic-Jovanovic SD, Driscoll S, Novitskaya L, Foyer CH (2002) Drought and oxidative load in the leaves of C_3 plants: a predominant role for photorespiration? Ann Bot (Lond) 89: 841-850.

146. Foyer CH, Bloom AJ, Queval G, Noctor G (2009) Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. Annu Rev Plant Biol 60: 455-484.

147. Cornic G, Briantais J-M (1991) Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (Phaseolus vulgaris L.) at different CO_2 concentrations and during drought stress. Planta 183: 178-184.

148. Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141(2): 391-396.

149. Fischer BB, Hideg E, Krieger-Liszkay A (2013) Production, detection, and signaling of singlet oxygen in photosynthetic organisms. Antioxid Redox Signal 18(16): 2145-2162.

150. Harb A, Krishnan A, Ambavaram MMR, Pereira A (2010) Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiol 154(3): 1254-1271.

151. O'Brien JA, Daudi A, Butt VS, Bolwell GP (2012) Reactive oxygen species and their role in plant defence and cell wall metabolism. Planta 236(3): 765-779.

152. Moschou PN, Paschalidis KA, Delis ID, Andriopoulou AH, Lagiotis GD, et al. (2008) Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H_2O_2 signatures that direct tolerance responses in tobacco. Plant Cell 20(6): 1708-1724.

153. Angelini R, Cona A, Federico R, Fincato P, Tavladoraki P, et al. (2010) Plant amine oxidases "on the move": an update. Plant Physiol Biochem 48(7): 560-564.

154. Marino D, Dunand C, Puppo A, Pauly N (2012) A burst of plant NADPH oxidases. Trends Plant Sci 17(1): 9-15.

155. Zhou F, Zhang Z, Gregersen PL, Mikkelsen JD, de Neergaard E, et al. (1998) Molecular characterization of the oxalate oxidase involved in the response of barley to the powdery mildew fungus. Plant Physiol 117(1): 33-41.

156. Torres MA, Jones JD, Dangl JL (2006) Reactive oxygen species signaling in response to pathogens. Plant Physiol 141(2): 373-378.

157. Voothuluru P, Yamaguchi M, Zhu J, Cho IJ, Oliver MJ, Simmonds J, Sharp RE (2011) Cell wall proteomics and apoplastic ROS: novel insights into root growth adaptation to water stress [abstract no. P13018]. Pp: 518.

158. Steinwand BJ, Kieber JJ (2010) The role of receptor-like kinases in regulating cell wall function. Plant Physiol 153(2): 479-484.

159. Marshall A, Aalen RB, Audenaert D, Beeckman T, Broadley, et al. (2012) Tackling drought stress: Receptor-like kinases present new approaches. Plant Cell 24(6): 2262-2278.

160. Munné-Bosch S, Queval G, Foyer CH (2013) The impact of global change factors on redox signaling underpinning stress tolerance. Plant Physiol 161(1): 5-19.

161. Holmgren A, Johansson C, Berndt C, Lonn ME, Hudemann C et al. (2005) Thiol redox control via thioredoxin and glutaredoxin systems. Biochem Soc Trans 33(6): 1375-1377.

162. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND (2004) Glutathione metabolism and its implications for health. J Nutr 134(3): 489-492.

163. Lu SC, (1999) Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J 13(10): 1169-1183.

164. Nakamura H (2005) Thioredoxin and its related molecules: update 2005. Antioxid Redox Signal 7(5-6): 823-828.

165. Lundberg M, Johansson C, Chandra J, Enoksson M, Jacobsson G, et al. (2001) Cloning and expression of a novel human glutaredoxin (Grx2) with mitochondrial and nuclear isoforms. J Biol Chem 276(28): 26269-26275.

166. Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17(7): 1866-1875.