Chapter 2

Reactive Oxygen Species: The Good and the Bad

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Abstract

This chapter summarizes recent research on the biology of reactive oxygen species (ROS). The chapter is focused on the bimodal actions of ROS, which can be summarized as both beneficial and negative. The beneficial aspects of ROS are related to their effects on the redox state of cells and the important role that some ROS play in signaling cascade. The detrimental effects of ROS are related excess amounts of these chemical moieties, which are caused by excessive production and/or insufficient actions of endogenous antioxidants. The generation of these species is also discussed.

Keywords: reactive oxygen species, oxidative stress, superoxide

1. Introduction

Reactive oxygen species (ROS) are defined as chemically reactive oxygen radicals as well as non-radical derivatives of oxygen [1]. The varying range of reactivity each reactive oxygen species exhibits is crucial to its impact at the molecular level. Their significance in the development of many cardiovascular diseases is well known, but they also have beneficial roles in cells. Developing a balance between the overproduction of ROS and its utilization is important in maintaining healthy redox processes within the cells.

2. Generation of reactive oxygen species

The main types of reactive oxygen species discussed in this paper are superoxide and hydrogen peroxide, both of which play a large role in cardiovascular diseases. Additionally, the production of hydroxyl radicals and singlet oxygen will be mentioned, as these are the most

reactive, and subsequently dangerous, of the ROS. Lastly the generation of peroxynitrite will be considered. See **Table 1** for an overview of the mechanism of generation for each.

Superoxide is produced by the one-electron reduction of molecular oxygen. Superoxide is then converted to hydrogen peroxide via the mitochondrial enzyme superoxide dismutase (MnSOD), or into diatomic oxygen [2]. Hydrogen peroxide itself is fairly unreactive, but plays a role in the Fenton reaction to generate hydroxyl radicals that can be damaging to cellular structures and molecules. Haber and Weiss demonstrated in 1934 that a superoxide molecule and a hydrogen peroxide molecule could interact with each other to produce these reactive hydroxyl radicals in the following net reaction: $\bullet O_2^- + H_2O_2 \rightarrow \bullet OH + OH^- + O_2$ [3]. Hydroxyl radicals can also be formed from reactions with hypochlorous acid, which is produced by the enzyme myeloperoxidase [4]. The production of hydroxyl radicals in tissue is significant for its contribution to a variety of pathologies, but also for the fact that it cannot be removed enzymatically in the same way that superoxide is converted back to oxygen via SOD.

There are many enzymatic pathways by which ROS can be generated in the cell. Most pathways involve the initial production of superoxide, which, as previously indicated, lead to the production of even more reactive compounds. Although some enzymatic systems "intentionally" generate superoxide, e.g., NADPH oxidases, this ROS is also a consequence of metabolism. Specifically, in aerobic cellular respiration, superoxide is a byproduct of oxygen utilization. The electron transport chain (ETC) of the mitochondria is a major source its generation. The ETC is made up of three complexes and an ATP synthase enzyme; it functions by transferring electrons through a series of electron carriers. The transfer of electrons is coupled with the release of protons into the intermembrane space of the mitochondria, creating an electrochemical potential, Δp, across the inner membrane, which drives the production of ATP [5]. However, when electrons are leaked from the complexes, instead of being transferred, these leaked species are those that reduce oxygen to form superoxide.

The first complex in the ETC is composed of a flavin mononucleotide group and is a significant site of production of superoxides. The donation of electrons is initially provided to the chain by NADH to the FMN, which subsequently passes them along a chain of FeS to the reduction site CoQ [6]. Superoxide is produced when FMN is fully reduced; its degree of reduction has been shown to be dependent on the ratio of NADH/NAD+, with the proportion

Reactive oxygen species	Mechanism of generation
Superoxide (O^-_2)	Reduction of molecular oxygen in the electron transport chain of mitochondria [4, 6], and other enzymatic routes: monooxygenase, NADPH oxidase, xanthine oxidase [8, 10]
Hydrogen peroxide (H_2O_2)	Converted from O^-_2 by enzyme superoxide dismutase (SOD) [18]
Hydroxyl radical ($^\bullet OH$)	Produced in Haber-Weiss reaction from O^-_2 and H_2O_2 [2]
Singlet oxygen ($^1O_2$)	Produced in reaction of hypochlorous acid (HOCl) and H_2O_2 [3]
Peroxynitrite ($ONOO^-$)	Produced in reaction of nitric oxide (NO) and (O^-_2) [15]

Table 1. Production of reactive oxygen species.

of FMNs reduced correlating to a higher ratio [7]. This has been confirmed with the experimental addition of rotenone, a complex I inhibitor; its function is to limit the transfer of electrons away from complex I, creating a condition where they are "backed up" onto the NADH and are available for superoxide generation [7].

The third complex in the ETC is also a significant site of superoxide generation. Complex III is where electrons are transferred from CoQ to the cytochrome *c*. Changes in the Δp or in the reduction state of CoQ are contributors to the production of superoxide at Complex III, as well as the addition of the inhibitor antimycin [5]. Compared to complex I, the superoxide generation at this site is less significant.

Outside of the ETC, ROS can be generated in the mitochondria by different means. In the event that complex I is inhibited, and 2-oxoglutarate is added as a substrate, there is still a high level of superoxide generated by the enzyme *α*-ketoglutarate dehydrogenase, which also contains a flavin subunit and utilizes the reduced NADH pool of electrons that complex I is unable to use [8]. Cytochrome P450 is another enzyme within the mitochondria that has been implicated in ROS production. Its regular function involves complex reactions converting cholesterols and other steps in steroid biosynthesis; it catalyzes monooxygenase reactions that require electrons from NADPH, which can "leak" and interact with diatomic oxygen to produce superoxides [9]. These are just two examples of a variety of mitochondrial reactions involving the utilization of electrons that produces ROS at this organelle.

Mitochondrial respiration is not the only source of ROS generation. Other sources of ROS production include the processes of the enzymes nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), xanthine oxidoreductase, and myeloperoxidase [10].

NADPH oxidase functions as a multi-subunit enzyme which, via electrons donated by NADPH, can reduce oxygen to superoxide. NADPH oxidase is well known in leukocytes, but also exists in other tissues in different forms, such as in vascular smooth muscle. The leukocyte NADPH oxidase is primarily found in polymorph-nuclear neutrophils, or PMNs, and its function is the generation and subsequent release of superoxide as a mechanism for combating bacterial infection. The vascular NADPH oxidase is mainly activated by angiotensin II, but also thrombin, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), and other mechanical stimuli such as shear stress and strain [11]. Its function in these cells correlates to the development of cardiovascular disease, but also the dismuatation of superoxide results in the production of the vasodilator, H_2O_2 [12].

Xanthine oxidoreductase has two interconvertible forms as an enzyme, both of which are essential to purine catabolism by oxidizing hypoxanthine to xanthine to uric acid. Its dehydrogenase oxidizes NADH to NAD, while in its oxidase form, the enzyme is capable of producing both superoxide and hydrogen peroxide from diatomic oxygen [13].

Myeloperoxidase is highly significant not for the production of superoxides but because hypochlorous acid is its main product [14]. HOCl is a key intermediate for the generation of many different ROS and is, in itself, highly reactive—it can react with superoxides to produce hydroxyl radicals, or with hydrogen peroxide to produce a singlet oxygen [4]. Singlet

oxygens are extremely reactive species. Hypochlorous acid itself is implicated in various cellular reactions as well, such as initiating lipid peroxidation, or oxidizing protein sulfhydryl and thioether groups on proteins [4, 15, 16].

Nitric oxide, which is in the family reactive nitrogen species, plays a significant role in vascular tone in the circulatory system. While its generation is not discussed here, it is important to mention because it greatly increases in toxicity when it reacts with superoxide to generate peroxynitrite (ONOO—), a powerful oxidant [17]. Peroxynitrite itself reacts slowly, giving it selective reactivity within the cell, giving it wide implications for cellular pathology [18]. Despite its high reactivity, it is highly stable with a negative charge delocalized over the whole molecule, providing the ability to be a highly influential oxidant. **Table 1** summarizes the generation of reactive oxygen species.

3. The "good side" of ROS

Reactive oxygen species (ROS) have been given a considerable amount of scrutiny due to the disease states that they have been linked to, such as aging, cancer, and atherosclerosis [19]. However, ROS is imperative for redox homeostasis, as well as proper function in the cardiovascular system, and immune system. The body requires a balance in its ROS levels for homeostasis. If the level of ROS exceeds that which the body can handle, then oxidative stress occurs [20]. On the other hand, if the level is too low, reductive stresses occur and can also cause pathologies ranging from cancer to cardiomyopathy [21].

Redox regulation is imperative for the body to maintain proper signaling processes. These redox reactions usually entail ROS interacting with the amino acid cysteine on proteins. ROS modulates cell proliferation and apoptotic pathways to ensure proper regulation of the cell cycle and programmed cell death. There are multiple kinases in these pathways that interact with ROS. The mitogen- activated protein kinase (MAPK) has a MAPKKK upstream called apoptosis signal regulated kinase 1 (ASK1). ASK1 regulates transcription factors JNK and p38, which can trigger apoptosis by phosphorylating MAPKK4,3, and cGMP dependent protein kinase (PKG) and protein kinase A (PKA) are both activated by ROS as well and are involved in the MAPK signaling process. ROS can also inhibit protein phosphatases through cysteine oxidation that prevents the inhibitory actions of the phosphatase on MAPK signaling. Consequently, transcription factors such as p38 can be regulated this way as well. Protein tyrosine phosphatase (PTP) is oxidized and inhibited by ROS and helps maintain appropriate levels of growth factor signals. Tyrosine phosphatases are affected by ROS in a manner consistent with our concept of the redox window. Physiological levels of H_2O_2 will activate tyrosine kinases through cysteine oxidation to sulfenic acid; however, high levels of ROS oxidize cysteine into sulfinic and sulfonic acids which lead to complete inactivation of the phosphatase through irreversible modification of the catalytic cysteine [22]. Another major signaling pathway, phosphoinositide 3-kinase (PI3K), is regulated by ROS through oxidation reactions. The body maintains a homeostatic level of ROS because ROS products activate antioxidant genes through mechanisms such as PI3K-NFE2-like2 (Nrf2)-antioxidant response element

(ARE) [23]. Ref-1, also known as redox factor-1, is an endonuclease that is regulated through transcription factors such as activator protein 1 (AP-1), p53, nuclear factor kappa B (NFkB) and hypoxia inducible factor 1 (HIF-alpha). When cytoplasmic Ref-1 is subjected to oxidative stress, by exposure to ROS, it moves to the nucleus and helps the redox factor interact with transcription factors so an antioxidant defense system can be initiated [23].

Many of these redox regulatory pathways are evident when examining the impact of ROS on collateral blood vessel growth, which is a major area of interest in all forms of vascular disease, e.g., peripheral artery disease, ischemic heart disease. A conundrum about the role of ROS in coronary collateral growth pertains to observations that too much ROS, and the concurrent oxidative stress, inhibits collateral growth. On the opposite side of the spectrum, too little ROS and the consequential reductive stress, also inhibits coronary collateral growth. This optimal "level" of ROS has been dubbed "redox window" [24], which is the level of the redox state that is optimal for growth factor signaling. p53 is thought to be the connection between redox dependent and growth factor dependent signaling. Angiogenesis is mediated through a transcription factors, such as nuclear factor kappaB, and ROS such as H_2O_2, NO and other oxidants$_7$. H_2O_2 is a major mediator of HIF-1a [25], a major transcription factor required for vascularization in ischemic settings [26]. Vascular endothelial growth factor (VEGF) activates NADPH oxidase to produce ROS. ROS produced through this process works in conjunction with VEGF to trigger endothelial cell migration and proliferation. Vascular NADPH oxidase, a generator of ROS, is also triggered by angiotensin II (Ang II), which is a key component of angiogenesis. The necessity of ROS to appropriately activate angiogenesis is yet another exemplification of the beneficial use of ROS in the body [27]. The thyroid hormone can activate angiogenesis by triggering transcription factors such as VEGF and HIF-1a [28]. Both of which were described above as producers of ROS that further propagate the angiogenesis process.

Vascular smooth muscle cells require ROS for appropriate cell growth [29]. PDGF and thrombin are both agonists to help cell proliferation, and both agonists require ROS in their mechanisms to amplify and further their signal for greater cell growth [30]. ROS also plays an important role in the expression of transcription nuclear factor-kB, which helps the body's inflammatory process by activating the monocyte chemotactic protein-1 (MCP-1) and interleukin-6 [31]. Many reactive oxygen species, such as H_2O_2, play a big part in vasomotor tone such as vasorelaxation in the pulmonary, coronary and mesenteric systems [32].

Reactive oxygen species has an important role in the immune system. A lack of ROS in the immune system can cause disease states that impair an individual's ability to fight against foreign invasion. The innate immunity that utilizes macrophages, neutrophils, and dendritic cells are key. These cells use toll like receptors to determine a cell that is foreign to the body, such as a bacterium. As a part of the innate immune system, macrophages, neutrophils and dendritic cells can phagocytose foreign material and then express it to the acquired immune system. The phagocytosis process is made possible by the use of reactive oxygen species. As previously mentioned, the ROS used in this process is made on the endosomes of the phagocytosing cells using NADPH oxidase. The immune system ensures the production of ROS when a foreign substance is detected due to the toll like receptor-4 binding to NADPH

oxidase. Such makes certain that when a foreign substance is detected in the body and it binds to the toll like receptor-4, the NADPH oxidase is consequently triggered to make sure there is ROS production to breakdown the foreign entity [31].

Nitric oxide a reactive nitrogen species that easily diffuses across most tissues, but has a difficult time being carried through blood because oxyhemoglobin breaks it down. However, due to its rapid diffusion rates, it reacts with superoxide with diffusion limited kinetics, resulting in the formation of the potent oxidant, peroxynitrite. Although by convention peroxynitrite is viewed as deleterious [17], it is noteworthy to add that this species is used by the immune system destroy bacteria. Macrophages produce peroxynitrite, and this mechanism was found to kill amounts of *Escherichia coli* in proportion to the amount of peroxynitrite produced in the macrophage [33].

When ROS production is not appropriate, many disease states can occur. Chronic granulomatous disease (CGD) is a rare hereditary disease where there is a defect in the NADPH oxidase. As a result, infections such as pneumonia and osteomyelitis can occur. Since the body cannot fight the infection, it creates granulomas around the infections. The treatment for the disease is designed to help the immune system through antibacterial, antifungal and immunomodulatory therapy. Stem cell transplants and gene therapy are both definitive treatments used as the first clinical interventions [34].

All some reactive oxygen species such as superoxide and hydrogen peroxide have beneficial effects (at physiological levels), some reactive oxygen species, such as the hydroxyl radical, react and form bonds with almost all organic molecules in the body. As a result, this particular species is exclusively deleterious when produced within a cell. When the ROS levels deviate away from the "redox widow," imbalance in these systems occurs and detrimental consequences can be triggered [24].

However, while redox homeostasis of ROS is imperative for normal bodily function ranging from an effective immune system to angiogenesis, an imbalance in said homeostasis is not always terrible. When the body experiences acute trauma such as a hemorrhage, the renin-angiotensin system comes into play [35]. Angiotensin II helps constrict the blood vessels to increase the blood pressure that considerably drops due to the loss of blood [36]. Angiotensin II activates NADPH oxidase in smooth muscle cells causing a production of superoxides [37]. The superoxides partake in the angiotensin II mechanisms. However simultaneously there is a decrease in the nitric oxide present in the blood because NO scavengers, such as NOX, actively eliminate them [38]. By increasing the levels of superoxides and decreasing the levels of NO, vasodilation is minimized and platelet coagulation is much more effective [39]. A hemorrhage is a wonderful example of how the body naturally handles crises by tipping the redox homeostatic scale toward a greater production of superoxides.

4. The "bad side" of ROS

The production of mitochondrial reactive oxygen species (ROS) is found in both physiological and pathological conditions. When ROS production increases above basal level, however,

the excessive amounts of ROS can lead to pathologies ranging from autoimmune diseases to cardiomyopathies. As mentioned previously, there are numerous sources of ROS in a cell that may occur in cytosolic, extracellular, and mitochondrial domains. The relative amounts of mitochondrial ROS produced are indicative of the metabolic needs of the cell by acting as a mode of cell signaling [40]. At lower levels of production, the presence of ROS may be beneficially used as a metabolic response to hypoxia by regulating the stability of HIF-1a. Medium levels of ROS production are more indicative of an inflammatory response by activating mitogen-activated protein kinase (MAPK) and proinflammatory cytokines. Excessive levels of ROS production, however, become pathological, and may lead to mitochondrial and cell apoptosis through activation of the apoptosome protein complex. Interaction of apoptosis activating factor (APAF-1) with mitochondrial cytochrome c plays in integral role in activation of the apoptosome, which will then lead to the activation of a chain of apoptotic caspases. The decision of whether the cell enters a state of inflammation or apoptosis, dictated by relative amount of mitochondrial ROS found within the cell, highlights the importance of ROS in choosing which cell signaling pathway will proceed. Overproduction of ROS is observed to be the cause of inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, and atherosclerosis by over-activating MAPKs [40].

This state of overproducing ROS may be stimulated by a multitude of enzymes. An example includes myeloperoxidase (MPO), a subfamily of peroxidases, due to its role in producing hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2) during an immune response. MPO's, unique to neutrophils and monocytes, are active in respiratory burst, a cytotoxic mechanism to kill pathogens and bacteria. Excessive production of HOCl, however, may cause oxidative damage, apoptosis and inflammatory disease. The clinical significance of excessive ROS production through MPO can be seen in its role in the formation of nitrotyrosine in endothelial regions of inflammation, impairment of NO-dependent relaxation of blood vessels, and inactivation of select neutrophil granule contents during inflammation, which may then to a prolonged respiratory burst. These detriments are apparent in pathologies associated with MPO defects, such as atherosclerosis and plaque formation, multiple sclerosis and Alzheimer's disease [4].

When produced above basal levels necessary for cell signaling and transduction, the cell requires specific mechanism to eradicate ROS in order to return to physiological conditions. The toxic effects of excessive mitochondrial ROS production necessitate that the cell has developed antioxidant mechanisms to scavenge them after generation. These mechanisms to counteract ROS production include the use of the Superoxide Dismutase (SOD) family, which catalyze the initial reaction of O_2—to hydrogen peroxide (H_2O_2), a product that will eventually reduce to water through glutathione peroxidase and catalase. The SOD family, found in three isoforms, can be found within the cell cytoplasm, mitochondria and nucleus. The isoform SOD2 exemplifies the detrimental effects of overproduction of ROS: the removal of mitochondrial SOD2 in species such as yeast, flies and mice is associated with cardiomyopathy, aging and early death, and atherosclerosis. The SOD2 gene codes for the protein Manganese SOD (MnSOD), and is found within the inner mitochondrial membrane and dismutates superoxide anions, produced by the mitochondrial electron transport chain, into H_2O_2. Overproduction or incomplete metabolism of these superoxide anions can cause

oxidative damage. The importance of MnSOD was reinforced in a study where knockout mice for SOD2 displayed increased mitochondrial oxidative damage and cell apoptosis. The mice deficient of MnSOD died within 1 week due to either dilated cardiomyopathy or neurodegeneration [11]. Deficiencies in other members of the SOD family did not demonstrate the same severity in oxidative damage as SOD2. Additionally, MnSOD and SOD2 play imperative roles in the intrinsic pathway for apoptosis, through its involvement in mitochondrial permeability transition. Mitochondrial permeability transition utilizes mitochondrial cytochrome c, APAF-1 and caspase 9 to ensure cell death, an outcome possible from excessive production of ROS [41].

In physiological conditions, the presence of the ROS superoxide can be quickly eradicated by the presence of these SODs; however, when synthesized in close proximity to NO, the toxic radical peroxynitrite (ONOO-) may be spontaneously formed. The reaction, which does not require an enzyme, may outcompete the scavenging capabilities of SOD [18]. Peroxynitrite, once formed, can cross cell membranes through both anion channels or passive diffusion and reacts selectively throughout the cell by nitrating tyrosine residues on proteins [17]. The presence of nitrotyrosines will alter the conformation and function of proteins such as neurofilaments and actin, leading to pathologies such as atherosclerosis, myocardial ischemia and irritable bowel syndrome [17]. Additionally, peroxynitrites can oxidize the heme groups of various proteins, including hemoglobin, myoglobin and cytochrome c [18]. By reacting in the same manner with inducible NOS, peroxynitrite can alter negative feedback of itself in inflammatory conditions. Peroxynitrite may also damage DNA through oxidation of bases and the DNA backbone, and contribute to apoptosis. During reperfusion or states of inflammation, the mitochondrion produces higher levels of NO. NO has the effect of inhibiting complex IV of the electron transport chain, increasing electron leakage, and consequently, the formation of superoxide. Therefore, the subsequent increase in peroxynitrite from NO and superoxide, can cause mitochondrial oxidative damage and increase the amount of free radicals present [18]. These elevated concentrations of peroxynitrite and superoxide are found in endothelium due to the uncoupling of endothelial NOS and vascular NADPH oxidase. In pathological conditions, due to excessive ROS production, NO is altered to become ONOO—, preventing endothelial-dependent relaxation, and causing endothelial dysfunction. The initial adverse event due to the decrease in NO bioavailability is impaired endothelium-dependent vasodilation, which may spiral into long-term cardiovascular complications due to the decreased vasorelaxation. This dysfunction in vascular endothelium is then associated with pathologies ranging from hypertension, preeclampsia, and atherosclerosis to coronary artery disease [42].

The premise that inadequate scavenging of excessive ROS is detrimental to normal cellular function is reinforced by the existence of multiple antioxidant mechanisms, such as glutathione peroxidase (GPx). Decreased efficiency of GPx, which catalyzes the reduction of H_2O_2 to water using NADPH as a substrate, may lead to pathologies such as atherosclerosis and vascular inflammation [2]. Additionally, deficiencies in antioxidant enzymes such as peroxiredoxin and mitochondrial thioredoxin 2 (Trx2) lead to mitochondrial apoptosis and vascular pathologies and myocardial infarction. Knocking out these imperative enzymes within mice models demonstrate that insufficient removal of ROS due to inefficient antioxidant mechanisms will also lead to excessive ROS amounts and its damaging effects.

Other proteins involved with the cellular response to stress and in physiological conditions include the family of heat shock proteins. Heat shock proteins (HSP's), such as HSP70 and HSP27, are observed as stress-response proteins induced by ROS through the JAK-STAT pathway [43]. In normal, physiological conditions, HSP's serve as molecular chaperones required to prevent improper folding of proteins found within a cell. Their synthesis, however, is increased in response to environmental stresses in an attempt to prevent protein aggregation. Additionally, HSP's are observed to be directly involved in the signaling pathways that lead a cell to undergo apoptosis in response to stress. For example, the overproduction of the reactive oxygen species H_2O_2 activates the JAK-STAT pathway that leads to HSP70 production. The beneficial expression of HSP70 in response to ROS-induced stress was exemplified in the myocardium of transgenic mice [44]. Following a period of ischemia, the correction of metabolic acidosis and re-establishment of correct phosphate stores due to the presence of HSP70 exemplify its protective effects against cardiomyopathy. Likewise, the reduction of expression of heat shock protein 27 inhibits the regulation ROS-induced apoptosis in cardiomyocytes [45]. This observation is exemplified by the overproduction of Hsp27 in the rat cardiac cell line H92c in comparison to a control, and the consequent suppression of H_2O_2-induced injury and apoptosis and the protective increase in phosphatidylinositol 3-kinase (PI3K)—protein kinase B(Akt) pathway activation when plentiful amounts of Hsp27 are present [45]. Both Hsp70 and Hsp27 serve to demonstrate the integral role of heat shock proteins in both physiological conditions and stressful conditions such as ROS-induced oxidative stress.

The impact of the presence of excessive ROS may also be found in its role in protein post-translational modification, in both irreversible or reversible protein oxidative modifications. The interaction between ROS, reactive nitrogen species (RNS), and amino acid residues has been observed to lead to aging and protein dysfunction. Commonly, these post-translational modifications occur most readily on the thiol (—SH) functional group found on cysteine residues: the electron rich sulfur atom within the thiol group allows for the oxidization a cysteine to sulfenic, sulfinic or sulfonic acid in addition to other oxidative posttranslational modifications (Ox-PTM) such as nitrosylation, sulfhydration, glutathionylation, and sulfenylation [46]. When ROS/RNS react with the thiol through nitrosylation, studies have found the reaction serves a function for cardioprotection [47]. More specifically, nitrosylation of cysteine residues acts as a barrier during periods of oxidative stress against further modification and oxidative damage, and may therefore lead to a faster recovery time [47]. ROS/RNS reactions with the thiol through glutathionylation as a reversible Ox-PTM have been previously linked with neurodegenerative and cardiovascular disease. ROS also have the ability to form thiyl radicals (RS.) to react with thiolates and form disulfide bonds, causing static protein conformations. The formation of disulfide bonds can alter the geography of the protein, and change the conformation and therefore function of the protein itself. Sulfenylation, a highly reactive and unstable form of modification has been associated with irreversible oxidative damage and apoptosis of a cell. Other modifications can include carbonylation, and phosphorylation [48]. These modifications may alter the polarity of the amino acid, ultimately modulating cell signal transduction and its downstream effects. In addition, the modifications may alter metal cofactor interaction and may inadvertently affect inhibitor reactions and impact physiological and drug reactions. A common effect of protein carbonylation, specifically, may be protein inactivation; the inactivation of membrane

transporters such as glucose (GLUT) transporters and Na+-K+ ATPases may lead to a multitude of neurodegenerative disorders. Additionally, oxidation of residues such as methionine to a sulfoxide may occur as well, which may serve to decrease cell signaling, and cause phosphorylation [49]. The inactivation of important antioxidant mechanisms such as glutathione peroxidase and thioredoxin due to protein modification may occur and act to aggravate oxidative stress within a cell. Other modifications may induce the phosphorylation of HSP27 in an attempt to prevent the unfolding and degradation of imperative proteins necessary for cell metabolism [45]. The fluctuations in ROS production, which distinguishes physiological metabolism from pathological metabolism, is revealed through the relative amount of Ox-PTM of critical cysteine thiols due to its role in regulation of oxidative stimuli.

The amount of mitochondrial ROS ranging from lower, basal physiological levels to excessive pathological levels highlights the importance of ROS and its maintenance. Excessive ROS production or inadequate scavenging by the cell's antioxidant mechanisms may cause a multitude of complications within a cell, leading to mitochondrial oxidative damage or cell apoptosis. The overproduction of ROS by MPO demonstrates how excessive amounts of HOCl may lead to pathologies such as atherosclerosis, while deficiencies in SOD2/MnSOD, GPx, peroredoxin, and Trx2 demonstrate how inadequate scavenging may lead to vascular and inflammatory complications as well. The role of HSP's to prevent protein aggregation caused by ROS accumulation underscores the importance of cell signal transduction pathways in response to excessive ROS. The detrimental outcomes of high levels of ROS can be seen through the multitude of effects due to oxidative protein post-translational modifications. ROS, both good and bad, can widely affect the intricate network of a variety of distinct proteins found within a single cell.

5. Conclusions and gaps

We hope the readers understand the there are two sides to reactive oxygen species—a "good side" and a "bad side." We opine that if one reads the literature, ROS are equated with pathology, and adverse consequences. Our goal is this chapter was to reinforce the concept that these species have many important physiological actions. The largest gap we see in our understanding of ROS and their actions pertains to defining the boundaries of the redox window. This will be important to study and understanding since the boundary marks the transition of ROS from being beneficial to being detrimental.

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