Physiological role of reactive oxygen species as promoters of natural defenses

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ABSTRACT: It has been 60 yr since the discovery of reactive oxygen species (ROS) in biology and the beginning of the scientific community's attempt to understand the impact of the unpaired electron of ROS molecules in biological pathways, which was eventually noted to be toxic. Several studies have shown that the presence of ROS is essential in triggering or acting as a secondary factor for numerous pathologies, including metabolic and genetic diseases; however, it was demonstrated that chronic treatment with antioxidants failed to show efficacy and positive effects in the prevention of diseases or health complications that result from oxidative stress. On the contrary, such treatment has been shown to sometimes even worsen the disease. Because of the permanent presence of ROS in organisms, elaborate mechanisms to adapt with these reactive molecules and to use them without necessarily blocking or preventing their actions have been studied. There is now a large body of evidence that shows that living organisms have conformed to the presence of ROS and, in retrospect, have adapted to the bioactive molecules that are generated by ROS on proteins, lipids, and DNA. In addition, ROS have undergone a shift from being molecules that invoked oxidative damage in regulating signaling pathways that impinged on normal physiological and redox responses. Working in this direction, this review unlocks a new conception about the involvement of cellular oxidants in the maintenance of redox homeostasis in redox regulation of normal physiological functions, and an explanation for its essential role in numerous pathophysiological states is noted.—Roy, J., Galano, J.-M., Durand, T., Le Guennec, J.-Y., Lee, J. C.-Y. Physiological role of reactive oxygen species as promoters of natural defenses. FASEB J. 31, 3729–3745 (2017). www.fasebj.org

KEY WORDS: oxidative stress · redox pathologies · redox homeostasis · redox signaling

In redox biology, oxidative stress is defined as the increase of reduction potential or a large decrease in the reducing capacity of cellular redox couples (1). One infamous group of these molecules that is responsible for oxidative stress is the reactive oxygen species (ROS). As the name suggests, these are free radical species of oxygen that are in a more reactive state than molecular oxygen and can be reduced to varying degrees. Molecular oxygen is a diradical, containing 2 unpaired electrons with parallel spin configurations. Because electrons must have opposite spin to occupy the same orbit, electrons added to molecular oxygen must be transferred one at a time during its reduction (2), which results in several high-reactive intermediates (3). These ROS molecules are a relative new concept and this area of research in science was first described only 60 yr ago. Scientists have extended their insights into the complex effects of free radicals and ROS within the biological systems.

In 1954, Commoner *et al.* (4) used electron spin resonance spectroscopy to generate the first data that showed that skeletal muscle contains free radicals. For the first time, the presence of ROS in biological materials was discovered. Two years later, Harman (5, 6) hypothesized that endogenous oxygen radicals may be formed as byproducts of enzymatic reactions *in vivo*. He proposed that traces of iron would catalyze oxidative reactions *in vivo* and that peroxidative chain reactions were possible by analogy to the principle of *in vitro* polymer chemistry. Harman (5, 6) described free radicals that were produced during aerobic respiration as a molecule of evils that may account for

ABBREVIATIONS: ATII, angiotensin II; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; CaMKII, calmodulin kinase II; DDA, dendrogenin; HIF-1, hypoxia-inducible factor 1; Mϕ, macrophage; nNOS, neuronal NOS; NOX, NADP oxidase; PUFA, polyunsaturated fatty acid; RAR-α, retinoic acid receptor-α; ROS, reactive oxygen species; RyR2, ryanodine receptor 2; SOD, superoxide dismutase; T_{reg}, regulatory T; XO, xanthine oxidase

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doi: 10.1096/fj.201700170R

gross cellular damage, mutagenesis, cancer, or the degenerative process of biological aging.

Thereafter, the free radical theory of aging was born. At that time, ROS were considered by scientists as molecules that were uniformly injurious and damaging to tissues and organs. This idea has been entrenched in the minds of exercise physiologists for years. The theory gained credibility in 1969 when McCord and Fridovich (7) discovered the enzyme, superoxide dismutase (SOD), which brought the free radical theory in living organisms into a new era and that eventually convinced scientists that ROS are important for biology systems. This discovery provided the first compelling evidence of the *in vivo* generation of $O_2^{\cdot-}$, but through the subsequent elucidation of elaborate antioxidant defenses system (8). In addition, the localization of SOD was used as a tool to locate subcellular sites of $O_2^{\cdot-}$ generation, which led to the demonstration that mitochondria are the principal source of endogenous oxidants (9). In the 1970s, Chance discovered that intact cells (10) and mitochondria (11) were also a primary site of endogenous oxidant generator. These findings established the missing links of the rate of living theory. Furthermore, discoveries that showed ROS are produced in the body indirectly were fundamental. Thereafter, this became the starting point of a colossal number of research studies on the sources of $O_2^{\cdot-}$ production and its pathological and physiological roles; however, for a long time, there were doubts about the existence of ROS and their *in vivo* effects. At the beginning of the 1990s, the existence of ROS became indisputable with the use of the electron spin resonance technique that can trap electron spins from ROS molecules.

After the discovery of SOD, extensive research was conducted (12–14) and the scientific community impetuously developed multiple *in vitro* experiments to investigate, in priority, the negative effects of ROS and oxidative damage inflicted by radicals upon DNA (15), proteins (16), lipids (17), and other components of the cell. Therefore, the first part of this review focuses on the involvement of ROS as an essential trigger or secondary factor in the development of pathology highlighting inflammation and cardiovascular disease, and in the second part, we present the physiological implications of ROS as a trigger of molecular signaling.

INVOLVEMENT OF ROS IN THE DEVELOPMENT OF PATHOLOGY

ROS, such as $O_2^{\cdot-}$, $^1O_2$, H_2O_2, $^{\cdot}OH$, $^{\cdot}ONOO^-$, and hypochlorite, are known to be produced as byproducts of oxidative metabolism in which energy activation and electron reduction are involved. It is well understood that the production is enhanced during inflammation, aging, radiation exposure, endotoxic shock, and ischemia/reperfusion in the heart, intestine, liver, kidney, and brain. Mechanisms of ROS produced at the cellular level are not well understood; therefore, it is important to follow these mechanisms for the development of therapeutic strategies at cellular sites of dysfunction. Human tissues have a substantial ability to tolerate ROS under normal conditions. When production of ROS exceeds the capacity of antioxidant defenses, oxidative stress is inflicted, which leads to harmful effects on the function and structural integrity of biological tissues. These ROS free radicals are reactive intermediates and can trigger rapid chain reactions and cause damage to macromolecules, such as lipids, proteins, carbohydrates, and nucleic acids (18). As a consequence of the oxidative damage to these macromolecules, lipid peroxide, carbonyl, and glycated compounds are formed, as well as DNA base modification/strand breakage that subsequently leads to the loss of the functional and structural efficiency of proteins and DNA mutation (18). Considering the continuous generation of ROS, organisms have evolved complex enzymatic defenses against the attacks of free radicals, also termed antioxidant defense (catalase, SOD, glutathione). These enzymes and antioxidant molecules alone, however, are unable to control the oxidative damage and, instead, remove or repair with the aid of other enzymes, such as thioredoxin reductase and methionine sulfoxide reductase (19). Despite these antioxidant protections, it is impossible to escape the oxidative stress that results from an imbalance of oxidative and antioxidant molecules in favor of ROS that potentially leads to biological injuries (20), including the disruption of the redox signal (21). If not regulated properly, it becomes chronic and leads to aging and several age-related diseases and pathologies (22). However, these pathologies, in particular, inflammatory disorders and cardiac alterations presented below, are known to be dependent on ROS production as essential to, a trigger of, or a secondary factor in the genesis of disease.

Inflammatory disorders

An inflammatory phenomenon is linked to overproduction of ROS after stimulation of the expression of essential enzymes, such as eNOS (expressed in endothelial cell) and neuronal NOS (nNOS; expressed in cardiomyocytes), xanthine oxidase (XO), NADP oxidase (NOX), cyclooxygenase 1 and inducible enzymes NOX2, iNOS, and cyclooxygenase 2. The origin of the overproduced ROS is related to cytokines that are produced during inflammation as well as phagocytic cells, fibroblasts, and chondrocytes. As presented below, overproduction of ROS can be deleterious for acute and chronic inflammatory disease, which is known to be dependent on ROS production.

Acute inflammatory diseases

Zazzo (23) indicated the implications of ROS in the physiopathology of acute inflammatory diseases that include systemic inflammatory response syndrome, such as toxic shock, acute respiratory distress syndrome, vast burns, polytrauma, acute renal insufficiencies, and ischemia/reperfusion. For example, during acute respiratory distress syndrome, damage undergone by the endothelium of lung capillaries is a result of a massive activation of neutrophils that also induces excessive ROS within the inflammatory site (24).

This overproduction of ROS *via* activation of neutrophils by NOX is initiated by blood-borne chemotactic factors that are released after an inflammatory event, such as myocardial ischemia/reperfusion (25). In addition, during this inflammatory reaction, phagocytosis and overconsumption of oxygen take place. In 1994, Babior (26) described such reactions as a burst of respiration as a result of an increase in metabolic activity (50 times more than the normal condition). The burst is defined as a massive production of ROS in inflammation where neutrophils and macrophages (Mϕs) produce large quantities of ROS, especially $O_2^{\cdot -}$, to activate NOX.

Excess production of ROS is associated with the increment of procoagulative microparticles (*i.e.*, formation of microthrombosis), which triggers systemic inflammatory response syndrome (27) as described in Fig. 1. However, even if this burst of ROS plays a major role in inflammatory diseases and the oxidative damage may have the unwanted consequence of neutrophil activation; its importance in the physiological role of neutrophils to defend against infections, as in lung infection, cannot be ignored.

Chronic inflammatory diseases

Intestinal disease Chronic inflammatory disease of the intestine is often associated with an increase of ROS, mainly by $O_2^{\cdot -}$, produced by intestinal cells. It is suggested that the lipid peroxidation that follows is responsible for the change in intestinal epithelium function; however, we do not know whether ROS produced during this inflammation is the cause or the consequence of the inflammation (28).

Renal disease Molecular oxidation induced by ROS occurs in the tubules and renal glomerulus. Hypochlorite ions that are produced by enzymatic systems end in the formation of chlorinated proteins and lipids, which leads to tubule and renal glomerulus dysfunction. Through several observations, Klebanoff (29) suggested that myeloperoxidase takes part in renal dysfunction. Moreover, myeloperoxidase induces and aggravates the formation of autoantibody in necrotizing glomerulonephritis (30).

Osteoarthritis Maneesh *et al.* (31) showed oxidative stress to be associated with patients who present with degenerative osteoarthritis. Degenerative osteoarthritis is an articular inflammatory phenomenon in which ROS are formed by inflammatory cells by the activation of synoviocytes, endothelial cells, and chondrocytes. ROS are essentially produced by NOX, iNOS, and eNOS. Furthermore, ROS are a trigger factor that induces the degradation of collagen and proteoglycans to increase metalloproteases synthesis and the apoptosis of chondrocytes, which leads to cartilage annihilation.

Rheumatoid arthritis Rheumatoid arthritis is a systemic autoimmune disease that is characterized by chronic joint inflammation with infiltration of Mϕs and activated T cells (32). The pathogenesis of this disease is linked essentially with the formation of ROS at the site of inflammation. T cells that are isolated from the synovial fluid of patients with rheumatoid arthritis showed signs of decreased levels of intracellular glutathione and impaired phosphorylation of the adaptor protein linker for T cells (33). Altered subcellular localization of T cells has been shown to cause modification of intracellular glutathione levels (**Fig. 2**). Migration of monocytes and lymphocytes into the rheumatoid arthritis synovium is mediated by the abnormal expression of several adhesion molecules, including VCAM-1 (34). Although malignant tumors of the synovium are rare, it has been hypothesized that the presence of transformed cells (P53 mutation) in the synovium of patients with rheumatoid arthritis caused by ROS may lead to progressive joint destruction without malignant degeneration (35).

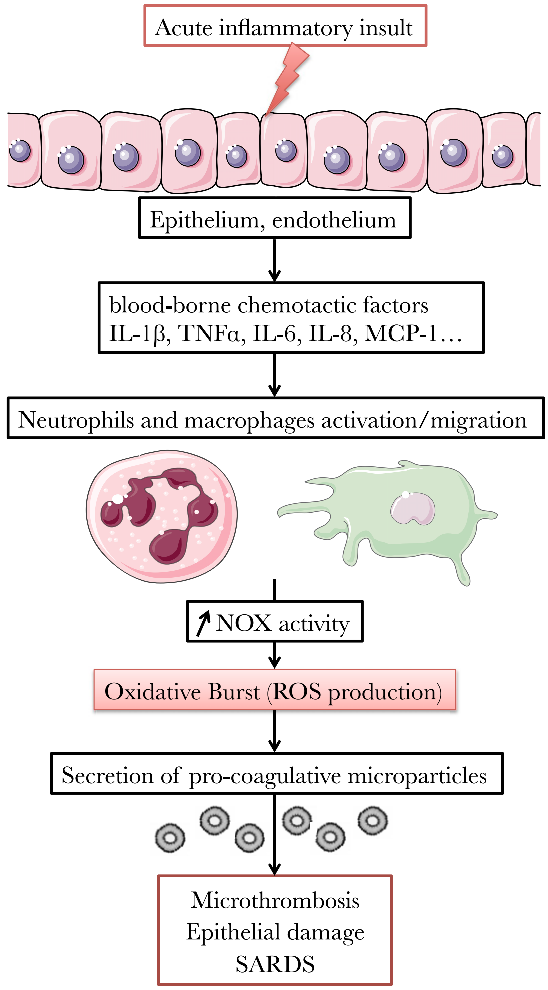

Figure 1. Impact of neutrophil activation and burst ROS production on severe acute respiratory distress syndrome (SARDS). An initial inflammation insult to the lung results in increased expression and release of blood-borne chemotactic factors, such as IL-1β, TNF-α, and IL-6, and of chemokines, such as IL-8 and monocyte chemoattractant protein 1 (MCP-1). This leads to the activation and recruitment of neutrophils and Mϕs into areas of inflamed sites. Activated cells are capable of increasing NOX2 activity to induce bursts of ROS production. These bursts secrete procoagulative microparticles (granule contents), cause bystander damage to host cells (endothelial and epithelial cells), and cause microthrombosis. Disruption of the endothelial-epithelial barrier allows protein-rich fluid to enter the alveolar space, which eventually results in alveolar flooding and respiratory failure. This burst of ROS is a trigger to exaggerate the inflammatory response and, of note, the occurrence of SARDS.

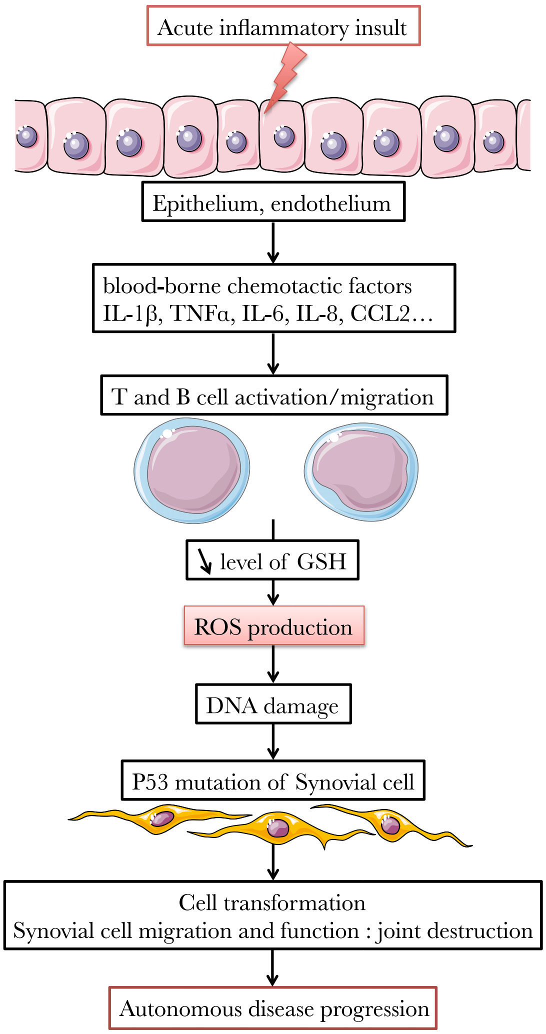

Figure 2. Impact of lymphocyte activation on autonomous disease progression. An initial inflammation insult results in increased expression and release of blood-borne chemotactic factors, such IL-1β, TNF-α, and IL-6, and of chemokines, such as IL-8 and monocyte chemoattractant protein 1. This leads to the activation and recruitment of T lymphocytes into the inflamed site. T and B cells show signs of decreased intracellular glutathione (GSH) levels, which induces ROS production that causes DNA damage to host cells (synovial cell of rheumatoid arthritis patients), eventually contributing to malignant tumors (P53 mutation). This action by ROS production may lead to progressive joint destruction without malignant degeneration, may exaggerate the inflammatory response, and may contribute to the pathogenesis of autonomous disease (rheumatoid arthritis).

Cardiac pathogenesis

ROS-induced oxidative stress in cardiac and vascular myocytes is associated with cardiovascular tissue injury (36). Chronic overproduction of ROS within the mitochondria of cardiac cells leads to mitochondrial DNA damage, and the accumulation of mutations causes cellular injuries and, consequently, crucial cardiac remodeling. Nonetheless, ROS play a role in the development of cardiovascular diseases, such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies (hypertrophic and dilated), cardiac hypertrophy, congestive heart failure, and blocks of conduction or still cardiac infarction (37). In addition, abnormalities in myocyte function as a result of increased oxidative stress are associated with the effects of ROS in subcellular organelles, which is considered the first step to the development of cardiac dysfunction.

Arrhythmias

Abnormalities of Ca^{2+} homeostasis are a fundamental feature of heart failure with contractile and energetic dysfunction, arrhythmia, transcriptional changes, and mitochondrial ROS production. Redox signaling has a significant impact on Ca^{2+} homeostasis of myocytes (38). In myocytes, ROS can target the protein involved in excitation-contraction coupling. Mechanisms that are implicated in these abnormalities include ryanodine receptor 2 (RyR2) protein hyperphosphorylation by PKA, and calmodulin kinase II (CaMKII). Oxidation-enhanced activation of PKA/CaMKII could potentially contribute to RyR2 dysfunction (39). In addition, nNOS colocalizes with RyR2, and it was reported that deficient nNOS-mediated RyR2 *S*-nitrosylation promotes thiol oxidation *via* XO-dependent ROS, which leads to increased diastolic Ca^{2+} and arrhythmia (40).

In the atrium, oxidative stress has been shown to take part in atrial fibrillation, and NOX2 has been implicated in this process. Indeed, NOX is a multimeric complex that is expressed in several tissues and cells—phagocytes, endothelial, epithelial, smooth muscle, and cardiac cells—and there are 7 enzymes: NOX 1–5 for NAD(P)H oxidase and DUOX 1 and 2 for dual oxidase. All multisubunit enzymes of NOX oxidize the soluble coenzyme NADPH, which results in the formation of $O_2^{\cdot -}$. In phagocytic cells, NOX plays a key role in the defense against pathogens. In other cells, NOX participates in cell signaling, during which it releases $O_2^{\cdot -}$ extracellularly for phagocytic cells or intracellularly for nonphagocytic cells. NOX2-derived ROS production was increased in the right atrial appendages of patients who underwent cardiac bypass surgery who developed postoperative atrial fibrillation (41). It is also interesting to observe that NOX2-dependent CaMKII oxidation promotes sinus node dysfunction *via* the apoptosis of sinoatrial cells in a mouse model of angiotensin II (ATII)–induced arrhythmias (42). It should be noted that other redox-sensitive mechanisms, such as the effects on L-type Ca^{2+} channels, plasmalemma Ca^{2+}-ATPase, Na^+/Ca^{2+} exchanger, K^+ channels, and Na^+ channels, can also contribute to the occurrence of arrhythmias (43).

Atherosclerosis

Animal experiments revealed significant amounts of iron pool in atherosclerotic lesions, which indicates that the iron-catalyzed formation of free radicals may act as the trigger factor in the process and development of atherosclerosis (44). In human endothelial cells, increased levels of $[Ca^{2+}]_i$ were observed that could potentially induce oxidative stress and can thus be an additional contributing factor in atherosclerosis progression. Furthermore, up-regulation of cholesterol and the enhanced uptake of oxidized LDL seems to be a key step in the development of atherosclerosis (45). Oxidized LDL accumulates in Mϕs and form foam cells, which establishes the initiation of atheroma formation (24). Subsequently, overproduction of ROS facilitates the activation of cells that are involved in atherosclerosis and the formation and progression of lesions (46).

Ischemia/reperfusion

During an ischemia/reperfusion period, plates of accumulated atheroma reduce the diameter of the vessel and increases rigidity, which leads to occlusion. In the coronary artery, a rupture, obstruction, or vascular occlusion, called stenosis, blocks the distributions of O_2 in the irrigation area by the vessel, which leads to an ischemic event. During ischemia/reperfusion, the cellular source of ROS within heart tissue includes cardiac myocytes, endothelial cells, and neutrophils. Within the cardiac myocytes, ROS can be produced by several sources, such as the mitochondrial respiratory chain, NOX (47), XO (48), and uncoupled NOS. Despite the low oxygen tension during ischemia, moderate ROS generation is thought to occur in the mitochondria (49–51). It has been recognized that mitochondrial complex I takes part in ischemia/reperfusion (52) and, currently, it is known that the major ROS generators in the mitochondria are the ubiquinone–ubiquinol mobile electron carriers, popularly known as coenzyme Q10. Ubiquinone normally accepts electrons from complexes I and III of the electron transport chain and transfers them to complex IV and cytochrome *c*. During an infarct, when oxygen is absent, there is no final electron acceptor for complex IV. Consequently, the ubiquinol pool is highly reduced and increases the level of ubisemiquinone radical (*i.e.*, the reduced form of ubiquinone). When oxygen returns to the mitochondria during reperfusion, ubisemiquinone donates electrons directly to the oxygen-generating $O_2^{\cdot -}$. This free radical reacts rapidly with the neighboring molecules, which leads to lipid peroxidation.

After ischemia, the massive burst of ROS during reperfusion originates from a different cellular source, but it has thus far not been identified. Moreover, the massive production of ROS during an ischemia/reperfusion event, in turn, leads to tissue injury that causes serious complications in organ transplantation, stroke, and myocardial infarction (51). The process of ischemia/reperfusion was intensively studied in human myocardial infarction and observed injuries were attributed to ROS released by neutrophil activation (53). In cardiomyocytes and inflammatory cells, NOX2 levels are increased early after acute myocardial infarction in both humans (54) and animal models (55). NOX activity might also be activated by NADPH, and it has been suggested that increased NADPH levels fuel $O_2^{\cdot -}$ production in heart failure (56). Numerous studies have investigated the deleterious effects of ischemia/reperfusion-induced ROS production by using various pharmacologic interventions (57). Of note, antioxidant treatment ameliorates both leukocyte adhesion and leukocyte-mediated heart injury in the postischemic period (58). To be complete, treatment with a synthetic SOD mimetic was shown to ameliorate tissue damage in a rat model of ischemia/reperfusion injury (59).

More recently, studies have demonstrated that NOX-derived ROS can promote autophagy (60), with NOX2 and NOX4 representing the isoforms of implicated NOX. NOX2- and NOX4-dependent autophagy plays an important role in the elimination of pathogens by phagocytes and in the regulation of vascular cell and cancer cell survival. Of interest, the authors found that the regulatory role for ROS from NOX2 complexes is also important in autophagy regulation in cardiomyocytes. NOX promotes the activation of autophagy and survival in cardiomyocytes in response to nutrient deprivation and ischemia *via* the protein kinase RNA-like endoplasmic reticulum kinase activation signaling pathway.

Cardiac hypertrophy

Mounting evidence has strongly implicated ROS signaling in the development of cardiac hypertrophy, which can either be compensatory and adaptive, or a maladaptive precursor to cardiac failure (61–64). Many extracellular factors can induce hypertrophy of cardiomyocytes, and several of the downstream signaling pathways that mediate the hypertrophic growth response to these factors can be activated directly or indirectly by ROS (65). For example, cardiomyocyte hypertrophy induced by GPCR agonists, such as ATII, α-adrenoceptor agonists, and endothelin-1, has been shown to involve endogenous ROS generation and activation of ERK1/2 and NF-κB (66). ATII induces cardiac hypertrophy *via* a G-protein–linked pathway that involves the generation of ROS and ROS-associated activation of several downstream signals, including MAPKs (67). Of interest, antioxidants were shown to inhibit ROS and block ATII-induced cardiac hypertrophy (68).

TNF-α–induced cardiomyocyte hypertrophy has also been reported to be associated with ROS-dependent activation of NF-κB. In addition, NOX2 has been confirmed to be involved in the cultured cardiomyocyte hypertrophy that is induced by endothelin-1 (69) and ATII (70), which further provides evidence that Akt activation may also be involved. In addition, redox signaling was also implicated in pressure overload–induced cardiac hypertrophy (62). Furthermore, mechanical strain may act as at least one prohypertrophic stimulus during pressure overload and, consistent with NOX2 reports, it has been shown that mechanical stress–induced cardiomyocyte hypertrophy may involve Rac1–ROS-dependent pathways that activate ERK1/2 (64) and p38 MAPK (71).

Vascular disease

Substantial evidence suggests the involvement of ROS generation in hypertension (72). ROS are generated within endothelial and vascular smooth muscle cells of the vascular wall, as well as by adventitia fibroblasts (73–75). The relationship between ROS and hypertension was suggested by Landmesser *et al.* (76), but it was some 40 yr later that this association was investigated in greater detail when it was demonstrated that ATII-mediated hypertension in rats increased vascular $O_2^{\cdot -}$ production *via* NOX activation (77).

In the vascular system, ROS production *via* NOX is triggered by stimulation of neuro-humoral vasoconstrictor agents, such as ATII, endothelin-1, and norepinephrine. The action of ATII *via* angiotensin type 1 receptors plays an important role in vasoconstriction (78). Furthermore, enzymatic reduction of molecular oxygen by eNOS no longer couples to L-arginine, which results in the generation of deleterious $O_2^{\cdot -}$ rather than protective NO (79). This eNOS uncoupling contributes to increase ROS production and endothelial dysfunction that have been observed in various vascular diseases (80), including hypertension (81). Muslin (82) has also shown that increased production of ROS is responsible for the activation of redox-sensitive p38 MAPK, which might be involved in the functional and structural changes that are associated with hypertension. To be complete, it is important to note that hypertension is associated with renal function. In hypertension, nNOS activity is impaired and the effect of NO donors is reduced, which leads to increased tubule-glomerular feedback and decreased renal blood flow and glomerular filtration (83). Overall, these functional changes cooperatively increase blood pressure acutely, whereas long-term hypertension is likely a result of tissue damage and remodeling.

As summarized in **Fig. 3**, the NOX complex is an important factor that is involved in heart dysfunction. In the short-term, ROS can increase blood pressure by stimulating the heart rate and blood pressure, which may also contribute to chronic hypertension by inducing myocardial hypertrophy. In extreme cases, ROS may be responsible for the emergence of stenotic lesions and fibrosis.

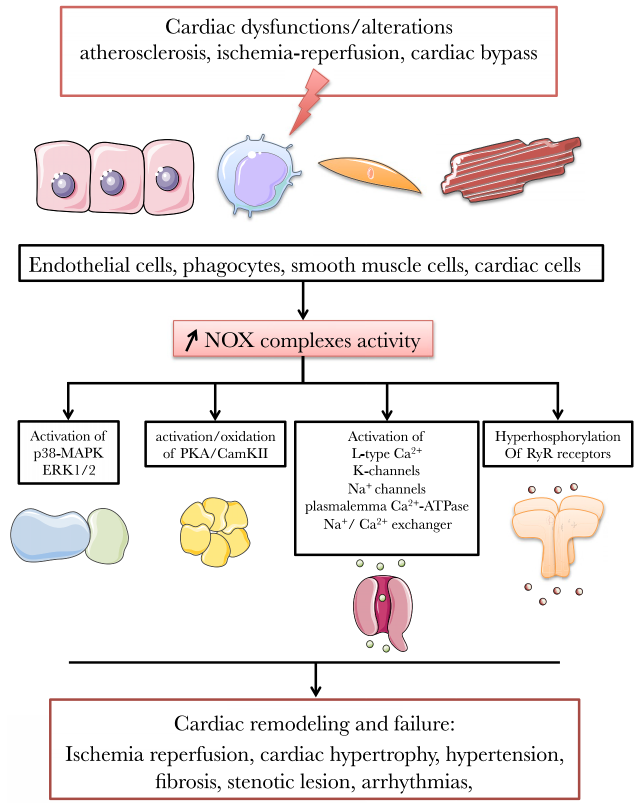

Figure 3. Involvement of NOX complex activities in disease progression. Heart dysfunction or an alteration event, such as atherosclerosis, ischemia/reperfusion, myocardial infarction, and cardiac bypass, results in the increased activity of the NOX complex to host cells (endothelial cells, phagocytes, smooth muscle cells, and cardiac cells). This activation induces oxidation, activation, modulation, or phosphorylation for complex proteins (p38-MAPK ERK1/2), channels (*i.e.*, L-type Ca^{2+}, K channels, Na^+ channels, plasmalemma Ca^{2+}-ATPase, and Na^+/Ca^{2+} exchanger), receptors (RyR2) and protein kinase (PKA and CaMKII). These chronic actions may lead to progressive cardiac remodeling and failure, such as cardiac hypertrophy, hypertension, fibrosis, stenotic lesion, and arrhythmias.

Other pathologies

Numerous pathologies are known to be dependent on ROS production and alterations and are cataloged in different reviews (22, 84, 85). Most chronic diseases are related to inflammatory rheumatism, inflammatory chronic diseases of the digestive system, bronchopulmonary diseases, skin infection, and chronic viral infections with oxidative stress. Diseases that are associated with ROS include cataract (86), cancer (84, 85), diabetes (87) and insulin resistance (88, 89), obstructive sleep apnea (90), HIV infection (91), asthma (92), psoriasis (93), and chronic granulomatous (94). Moderate levels of ROS have also been observed in neurologic disorders, such as Parkinson's disease (95), Alzheimer's disease, Huntington's disease, and in patients with either familial and sporadic amyotrophic lateral sclerosis (96) and schizophrenia (97). Aside from intrinsic causes in oxidative stress, extrinsic causes, such as smoking, alcohol, UVR, pollution, and intensive sport and psychosocial stress, are contributors to human diseases.

Nevertheless, scientific studies that demonstrate a role for oxidative stress in the aging process cannot be neglected. Indeed, multicellular organisms generally undergo qualitative changes with time (aging) that are associated with the progressive degeneration of biological functions, increased susceptibility to diseases, and increased probability of death within a period. This process, known as the theory of aging, may be defined as a progressive decline in the physiological functions of an organism after the reproductive phase of life. Harman (5), in 1956, proposed that free radicals play a role in the ageing process (*i.e.*, the accumulation of oxidized products in the body and the weakening of the antioxidant defense system contribute to aging of tissues, organs, and organisms). In addition, there are various indirect manifestations of oxidative stress in old age, including lipid peroxidation, DNA oxidation, protein oxidation, and a shift in the redox states of thiol/disulfide redox couples, such as glutathione, cysteine, and albumin. These manifestations suggest that the rate of ROS production per time unit increases with age; however, this conclusion must be tested experimentally.

PHYSIOLOGICAL IMPLICATIONS OF ROS

ROS are an essential part of many metabolic pathways. In fact, ROS are the spark of basic energy-producing processes. It is evident that oxidative stress takes part in several pathologies; however, numerous studies have highlighted the physiological role of ROS as promoters of natural defenses (22, 84). This may explain, in part, why many intervention studies with chronic antioxidants have failed to show efficacy and positive effects in the prevention of diseases or their complications. There is now a large body of evidence that shows that living organisms have not only adapted to an unfriendly coexistence with ROS but have developed advantageous mechanisms by which these molecules can be used.

With the goal of developing procedures to ameliorate undesirable ROS production for therapy in pathologies, instead, it would be important to identify the molecular effectors of redox biology that are involved in normal biological and physiological responses. By doing so, a new theory about the involvement of cellular oxidants in the maintenance of redox homeostasis to keep normal physiological function should be established. For 2 decades, ROS have undergone a shift from being considered molecules that invoke damage in oxidative stress to regulating signaling pathways that impinge on normal physiological and redox biological responses, as explained below.

Implication of ROS in skeletal muscle function

In the 1970s, researchers reported for the first time that lipid peroxidation is increased during exercise in humans and rats (98, 99). In the early 1980s, researchers began to understand the biological importance of this finding by identifying the first link between ROS and muscle function (100). Clearly, the idea that ROS are involved in normal muscle contraction dates to the 1990s (101). Reid *et al*. reported, for the first time in muscle, that low levels of ROS that are present in skeletal muscle under basal conditions are a requirement for normal movement and that antioxidant-mediated depletion of ROS from unfatigued skeletal muscle results in the inhibition of their contraction. After this finding, several researchers elucidated this hypothesis in studies on the relationship between ROS production and antioxidants, and proposed that exercise itself can be considered an antioxidant (102). Consequently, ROS that are produced in exercise have a physiological role, and it is conspicuous that they behave as signals to modulate adaptations of muscle to exercise.

Implication of ROS in excitation-contraction coupling

In cardiac muscle, studies suggest that ROS production has physiological effects in the excitation-contraction coupling protein process. Of note, Sánchez *et al.* (103) provided indirect evidence that during tachycardia, NOX2 contributes to RyR2 redox modifications, such as *S*-glutathionylation, that can sustain faster Ca^{2+} release during increased cardiac activity and in contractile force during exercise. Recently, Prosser *et al.* (104) confirmed these results and reported an important physiological role for acute stretch-induced activation of NOX2 in the mechanotransduction of Ca^{2+} release, hence the contractile force of the cardiomyocytes. The authors found that ROS produced by NOX2 are strategically localized to the sarcolemma and T-tubule membranes to permit rapid redox modification of RyR2 and the regulation of cardiac Ca^{2+} signaling, thereby tuning RyR2 Ca^{2+} signaling sensitivity. This may be an important physiological mechanism involved in the stretch-induced augmentation of contractile activity.

Impact of ROS in programmed cell death and cancer

Other than muscle exercise, the perception started to change with the assumption that ROS were inevitably deleterious to numerous tissues and the unquestionable beneficial effects of antioxidants. As direct exposure of cells to ROS, such as H_2O_2, caused multiple intracellular alterations, including the elevation of cytosolic Ca^{2+}, depletion of ATP, oxidation of NADH, and reduction of glutathione, it is evident that ROS contribute to cell death whenever they are generated in the apoptosis process. In addition, studies suggest that increases in cellular ROS production observed in apoptotic processes are triggered by various stimuli, including APO-1/Fas/CD95 ligands (105–107). Even so, the impact of ROS in programmed cell death suggests that they are deleterious and have no positive effects. Whether and how these ROS contribute to the induction of cell death depends on the signaling and execution pathways that are activated (108). The process that leads to proliferation or cell death depends on the condition of the ROS-producing cell. For example, in cancer, ROS production defends the organism by attacking the DNA of the cancer cell, even if it is limited compared with the proliferation of the normal cell (109), and, as mentioned previously, ROS are potential carcinogens, as they facilitate mutagenesis, tumor promotion, and progression (110).

Conversely, Cao *et al.* (111) recently observed that oridonin, a natural diterpenoid that is isolated from an herb (*Rabdosia rubescens*), regulated retinoic acid receptor-α (RAR-α) and contributed to the pathogenesis of various diseases, including cancer, especially acute promyelocytic leukemia. Of interest, oridonin stabilizes RAR-α protein by increasing cellular ROS levels *via* the activation of the NF-κB signaling pathway. Oridonin increased intracellular ROS levels, whereas pretreatment with the ROS scavenger, *N*-acetyl-L-cysteine, dramatically abrogated RAR-α stabilization, which indicates the positive role of ROS in oridonin-induced RAR-α stabilization (111). In addition, Ma *et al.* (112) observed that ROS production (H_2O_2) inhibits proliferation and induces apoptosis in MCF-7 breast cancer cells *via* the modulation of cell cycle and apoptosis-related genes, and inhibits migration by decreasing stress fibers *via* DLC1/RhoA signaling. The production and migration of the fibers, which are primarily composed of actin and myosin, were also suppressed by H_2O_2 in a more aggressive breast cancer cell line (MDA-MB-231). These results suggest that that proliferation of breast cancer cells is modulated directly by ROS *via* the modulation gene that encodes for the cellular cycle and apoptosis (112). These two recent findings changed the views of ROS as a deleterious molecule and even assigned it a potential essential role in the diminution of the progression in some cancers (**Fig. 4**).

ROS formation as a sensor for changes in oxygen concentration

Oxygen homeostasis is maintained in higher organisms by the tight regulation of red blood cells and *via* respiratory ventilation (113). Growing evidence indicates that an alteration in oxygen concentration is sensed independently by different ROS-producing proteins, including cytochrome *b*. Other studies suggest that a change in the rate of mitochondrial ROS may play a vital role in oxygen sensing by the carotids in the regulation of arterial blood oxygen (114).

In the event of hypoxia, the hormone erythropoietin, which is mainly produced by kidney and liver cells, regulates the total mass of erythrocytes—defined by red blood cell mass—in circulation. It is clear, that the modification in oxygen tension is sensed by changes in ROS production (115, 116). In addition, the expression of erythropoietin protein or mRNA was found to be strongly repressed by ROS when normoxic cells were treated with catalase *via* the stimulation of erythropoietin (117). This study strongly suggests that ROS are involved in the regulation of red blood cell mass and ventilation. Indeed, during an ischemia event, ROS repress erythropoietin protein, which is also known to increase red blood cell mass, and prevent hypertension and augmentation of blood viscosity (Fig. 4). The mechanism seems to be associated with the transcription factor, hypoxia-inducible factor 1 (HIF-1). When under normoxic conditions, it is rapidly mediated by the O_2-dependent degradation domain *via* the ubiquitin-proteasome pathway (118). The number of target genes that are activated by HIF-1 continues to increase and includes genes of protein products involved in angiogenesis, energy metabolism, erythropoiesis, cell proliferation and viability, vascular remodeling, and vasomotor responses, indicating the role of ROS in these contexts in response to hypoxia (119).

Urao *et al.* (120) used transgenic mice with endothelial cell-specific overexpression of human catalase and examined whether endogenous ROS in endothelial cells is required for neovascularization after hindlimb ischemia. In this study, they found a significant decrease in the expression of redox-sensitive VCAM-1 and monocyte chemotactic protein-1, which is required for inflammatory cell recruitment to ischemic tissues (120). Of interest, the researchers also observed a significant decrease in eNOS phosphorylation, which is known as a key regulator of angiogenesis and H_2O_2 to increase eNOS expression. They concluded that ROS, in particular H_2O_2, are positive effectors in postischemic reparative neovascularization, which aligns with previous reports that NOX2-derived ROS (121), NOX4-derived H_2O_2 (122, 123), or H_2O_2 derived from myeloid cells (124) are required for such a response. In addition, Kim *et al.* (125) showed that angiopoietin 1, which is known to play a role in angiogenesis after induction by NO (126), generates H_2O_2 and modulates the activation of p44/42 MAPK and p38 MAPK, thereby playing a critical role in tubule formation, cell migration, and angiogenesis (Fig. 4).

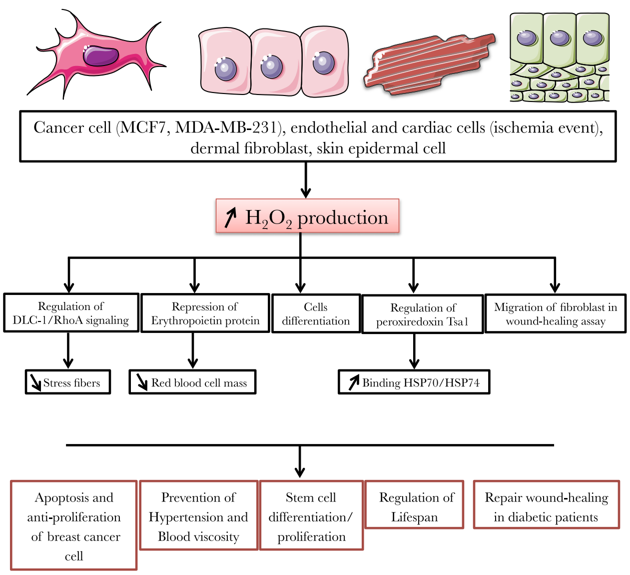

Figure 4. A new role for H_2O_2 signaling. In certain situations, such as an ischemia event or cancer, endothelial cells, cardiac cells, dermal fibroblasts, and skin epidermal cells can increase H_2O_2 production. This activity can induce activation, repression, differentiation, modulation, or migration for protein (erythropoietin and peroxiredoxin), cell (stem cell and fibroblast), and signaling pathways (DLC-1/RhoA). These actions may lead to the regulation of lifespan, a decrease in the proliferation of cancer cells (breast cancer), prevention of hypertension and blood viscosity, an increase in stem-cell differentiation/proliferation, and repair of wound healing in patients with diabetes, with H_2O_2 as the trigger messenger in physiological function.

ROS-mediated amplification of immune responses

For 20 yr, the immune response has been known as a redox-regulated process through the activation of T lymphocytes, which is significantly enhanced by ROS or by a shift in the intracellular glutathione redox state (127). Superoxide and/or physiologically relevant concentrations of H_2O_2 were shown to increase the production of IL-2 in various experimental studies (128). In addition, pharmacologic or genetic manipulation and dampening of the mitochondrial ROS generation can diminish T-cell activation *in vitro* and *in vivo*; however, NOX can be invoked in response to mitochondrial ROS to further sustain ROS levels to maintain T-cell activation (129). One study showed that uncoupling protein 2–knockout mice featured increased levels of mitochondrial ROS and increased immunity to bacterial pathogens (130). These findings suggest a moderate elevation of ROS in the immune system that might enhance normal immune function.

Recently, research has focused on the later stages of the immune response in diseases, which can involve not only the promotion of inflammation, but also its resolution. Among the regulators of inflammation that have garnered attention are regulatory T (T_{reg}) cells. T_{reg} cells are an important subset of T cells that lend themselves to immune tolerance, and data indicate that T_{reg} cells are relevant, notably, for atherosclerosis. A recent study has suggested a link between ROS and T_{reg} cells in which the commitment of the T_{reg} lineage is dependent on localized production of ROS and that scavenging ROS decreased the T_{reg}/T-cell effector balance. These studies have focused on ROS production that can determine T-cell fate, thereby potentiating T_{reg} production and decreasing arthritis (131, 132). In 2014, researchers investigated mice with elevated levels of ROS as a result of the deficiency of both GPx-1 and catalase on the T_{reg} function. The group found that dextran sodium sulfate–induced colitis was attenuated and T_{reg} cells were hyperfunctional in GPx-1- and catalase-knockout mice. This finding (**Fig. 5**) suggests that the function of regulatory lymphocytes is closely related to ROS levels and that inflammation may be attenuated appropriately in elevated ROS conditions (133). Taken together, as the endothelium is a major regulator of local inflammatory and immune responses and predominantly secretes ROS into the extracellular space, these new data suggest that endothelial-derived ROS could influence T-cell fate by potentiating T_{reg} differentiation (Fig. 5).

Moreover, there is evidence that the intracellular redox state also modulates the immunologic functions of MΦs. Hamuro *et al.* (134) reported that MΦs vary strongly in their release of prostaglandins, IL-6, and -12, depending on the intracellular content of glutathione. These data further display a new picture of ROS involvement in the regulation and limitation of inflammatory responses.

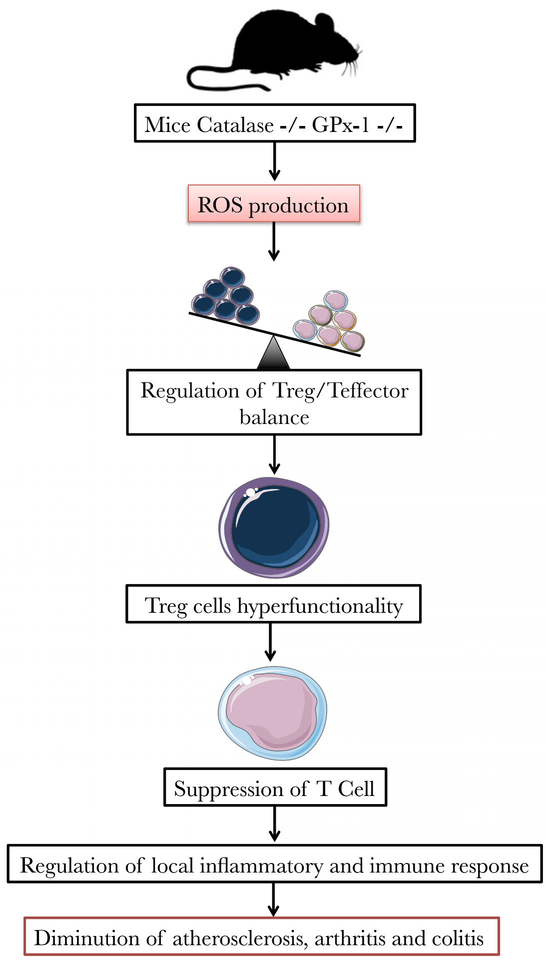

Figure 5. Regulation of inflammatory and immune response by production of ROS. ROS production can be induced genetically in mice without catalase and GPx protein (catalase$^{-/-}$ GPx$^{-/-}$). The production of ROS regulates T_{reg}/T effector cell balance, which induces hyperfunctionality of T_{reg} cells. T_{reg} cells suppress T cells, which induces the regulation of the local inflammatory and immune response. For this transgenic mouse model, ROS production is an important effector limiting inflammatory responses that can regulate the development of atherosclerosis, arthritis, and colitis.

Redox regulation of cell adhesion and migration

Controlled changes in the adhesive properties of cells and tissues play an important role in many biological processes. Cell adhesion plays a substantial role in embryogenesis, cell growth, differentiation, wound repair, and other processes; therefore, changes in the adhesive properties of cells and tissues are suggested to regulate redox tightly (135). Expression of cell adhesion molecules is stimulated by bacterial LPS and by various cytokines, such as TNF-α or IL-1 (136). ROS-treated endothelial cells induce the phosphorylation of the focal adhesion kinase, pp125FAK, a cytosolic tyrosine kinase that has been implicated in the oxidant-mediated adhesion process (137). Adhesion of leukocytes to endothelial cells is also enhanced by ROS (2.5-fold increase compared with control) (138). Of interest, this adherence was independent of the XO concentration and abolished by catalase but not by SOD, which suggests that ROS are the effective agents.

Recently, Falanga *et al.* (139) studied the influence of ROS production on cell migration by using a wound-healing assay, notably in patients with diabetes for whom the healing process is slow and impaired. The authors investigated the effect of the high-glucose environment and basic fibroblast growth factor (bFGF) on human dermal fibroblast migration. It is known that bFGFs multiply and play critical roles in the wound-healing process, and that a decrease in expression may disrupt the normal healing process in patients with diabetes (140). They found that bFGF significantly increased the migration of fibroblasts simultaneously with an increase in intracellular ROS (141). The study indicated ROS production (initiated by H_2O_2) as the major element to induce the migration of fibroblast *via* bFGFs in the presence of high-level glucose, which is essential for the would-healing process in patients with diabetes (Fig. 4).

More recently, Chandrasekaran *et al.* (142) hypothesized that ROS-mediated signaling is linked to bone morphogenetic protein (BMP) receptor activation to dendritic growth. In cultures of rat sympathetic neurons that were exposed to different antioxidants, BMP-induced dendritic growth was blocked in a concentration-dependent manner without altering axonal growth or neuronal cell survival (142). In addition, BMPs upregulated the expression of NOX2 in different cell types, and small interfering RNA knockdown of NOX2, but not NOX4, significantly decreased BMP7-induced dendritic growth. Collectively, these data support the hypothesis that ROS are involved in downstream signaling events that mediate BMP7-induced dendritic growth in sympathetic neurons, and suggest that ROS-mediated signaling positively modulates dendritic complexity in peripheral neurons.

Implication of ROS in stem-cell differentiation

ROS are also essential for stem-cell differentiation. Stem cells need to self-renew to maintain both the stem-cell pool and to differentiate to generate specialized tissues. The best-studied example of stem-cell characterization is the hematopoietic stem cell, which differentiates to provide myeloid and lymphoid progenitors throughout a lifespan. Juntilla *et al.* (143), observed that mouse hematopoietic stem cells that are deficient in proteins involved in signal transduction pathways (both AKT1 and AKT2) have reduced levels of ROS and impaired differentiation. Furthermore, numerous studies seem to indicate the role of ROS in differentiation processes. Owusu-Ansah and Banerjee (144) found that ROS triggered differentiation, whereas decreasing ROS impaired differentiation, in *Drosophila* hematopoietic progenitors. In 2012, Malinska *et al.* (145) observed that ROS synthesis in mitochondria by complex I can trigger muscle differentiation of human bone marrow mesenchymal stem cells. In epidermis, Hamanaka *et al.* (146) demonstrated that lowering mitochondrial ROS prevented differentiation in this cell process and, surprisingly, that it can be restored by supplementing with exogenous H_2O_2.

Similar observations were made of the regenerative capacity of spermatogonial and neural stem cells. The authors observed that spermatogonial stem cells that were depleted in ROS stopped proliferation but enhanced self-renewal when ROS levels were increased, and also induced the phosphorylation of stress kinases, p38 MAPK and JNK (147). As a follow-up to this report, the authors investigated ROS function in primary brain-derived neural progenitors. They discovered that pharmacologic and genetic manipulations that diminished cellular ROS levels interfered with normal neural stem cells and/or multipotent progenitor functions both *in vitro* and *in vivo*. This study identified a redox-mediated regulatory mechanism of neural stem cells function, which may have significant implications for brain injury, disease, and repair (148).

These data indicate that the generation of low levels of ROS are physiologically required to activate proliferative pathways, serving as a trigger signal to support stem-cell proliferation. In contrast, high levels of ROS impair stem-cell function by activating signaling pathways that limit self-renewal but do not necessarily cause cellular damage.

Implication of ROS in the regulation of aging

It is well understood that the production of ROS is enhanced during aging, but even if researchers attempted intervention studies for the reduction of ROS levels, the outcome is rather mixed and it is not clear whether ROS-induced damage is the underlying cause of aging (149). On the contrary, recent evidence suggests that ROS signaling is required for the maintenance of tissues, and that ROS elevation can activate cellular stress pathways to dampen tissue degeneration and promote healthy aging (150). The initial studies to support the theory of aging—the deleterious effects of ROS—comes from the observation that hypoxia increased the replication of human diploid fibroblasts with the lifespan (151). During hypoxia, ROS decrease, consequently leading to less accumulation of oxidative damage to increase the replication of the human fibroblast lifespan, but later studies have demonstrated that an augmentation of ROS production during hypoxia resulted in the activation of HIF to increase the lifespan (152). After this first contradiction of the theory of aging, Van Raamsdonk and Hekimi (153) observed that the deletion of SOD in mouse mitochondrial matrix elevated mitochondrial DNA damage, as well as cancer incidence, but did not accelerate aging and, instead, extended lifespan (154). Extended lifespan as a result of increased mitochondrial ROS seems to be dependent on glucose restriction (155), mitochondrial electron transport mutation (156) and diminished IGF signaling (157). At the protein level, the long-lived mitochondrial mutant in *Caenorhabditis elegans* seems to increase the replicative lifespan by ROS-dependent activation of HIF (156).

In other animal models (nematodes and mice), reduced activity of MCLK1 (heterozygous COQ7), a mitochondrial enzyme that is required for ubiquinone biosynthesis, was observed to have increased lifespan and mitochondrial ROS (158). Mice with long life were associated with less oxidative damage in the cytosolic proteins, which supports the idea that elevated ROS levels are paradoxically protective *via* the induction of stress pathways (159). The recent data in *C. elegans* and mouse models suggest the physiological role of ROS in the lifespan (**Fig. 6**). More recently, Hanzén *et al.* (160) reported a new concept in lifespan-protein quality control. The study revealed the role of peroxiredoxin,

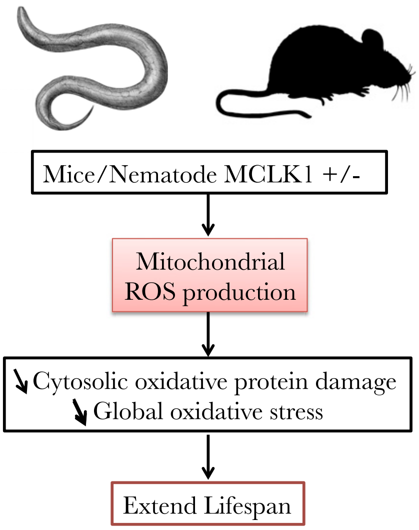

Figure 6. Regulation of lifespan by ROS production. ROS production can be induced genetically in mice and nematodes by reduction of MCLK1, a mitochondrial enzyme that is required for ubiquinone biosynthesis ($MCLK1^{+/-}$). This diminution can increase mitochondrial ROS production. Animal models show the lifespan to be associated with less oxidative damage to cytosolic proteins.

Tsa1 (the cytosolic peroxiredoxin in yeast), that facilitated the binding of Hsp70/104 chaperones to damaged proteins that were formed with aging *via* H_2O_2-specific redox switch in the Tsa1 peroxidatic cysteine (160). These data showed conceptually the new role for H_2O_2 signaling in proteostasis and lifespan control (Fig. 4).

Central role of ROS in lipid metabolism

Among the targets of ROS, unsaturated fatty acids, without doubt, are most attacked because of the abundance of double bonds in their structure. Unsaturated fatty acids are highly susceptible to abstract hydrogen atoms of the methylene (CH_2) group by $^{\cdot}OH$ and potentially initiate peroxidation. For several reasons, oxidation of lipids by ROS has been largely ignored by investigators, and the effects of the nonenzymatic products of these lipids remain largely unexplored. The reasons for this paucity of investigation could be a result of the rate of lipid oxidation *via* free radicals *in vivo*, which previously was thought to be negligible, or of the previously held idea that any form of lipid peroxidation is undesirable, as it is unconditionally toxic. Of the nonenzymatic oxygenated metabolites investigated, isoprostanes, namely 15-F_{2t}-isoprostanes (15-F_{2t}-IsoPs) from arachidonic acid [ω-6 polyunsaturated fatty acid (PUFA)], are commonly used as biomarkers of lipid peroxidation *in vivo* (161, 162). More recently, it has been shown that they are biologically active (163) as mediators of oxidant injury. They are vasoconstrictors in many species, and in various vascular beds (164) modulate platelet activity (165) and monocyte adhesion (166), as well as induce proliferation of endothelial and smooth muscle cells (167). In addition, oxidative stress is a feature of numerous pathologic conditions that occur in the perinatal period. For instance, newborns are subjected to oxidative stress that results from the rapid transition from a low-oxygen environment *in utero* to a relatively high-oxygen environment at birth. In 2012, Comporti *et al.* (168–170) showed that isoprostane levels are increased shortly after birth in response to increased oxygen tension mediated *via* activation of the thromboxane A_2 receptor and that isoprostane may serve as a novel physiological signal to stimulate postnatal ductus arteriosus closure.

As for ω-3 PUFA oxidation by ROS, Sethi's group (171) demonstrated, for the first time, that the metabolites generated could contribute to anti-inflammatory activities. The researchers showed that preincubation of endothelial cells with oxidized ω-3 PUFAs reduced the adhesion of monocytic cells to endothelial cells, but native ω-3 PUFAs had no effect. The researchers hypothesized that the reduced expression of adhesion molecules, such as VCAM-1, by endothelial cells decreased the interaction of phagocytes *via* the action of anti-inflammatory properties of the oxidized ω-3 PUFAs. In 2014, Jamil *et al.* (172) investigated the role of oxygenated metabolites of eicosapentaenoic acid from nonenzymatic oxidation, namely 5-F_{3t}-IsoPs, in the regulation of glutamatergic neurotransmission. In this study, the group revealed the beneficial role of this compound by reducing excitatory neurotransmitter release, thereby slowing the progression of ocular neuropathic disease by modulating K^+-induced glutamate release by 5-epi-5-F_{3t}-IsoP in isolated bovine retina (172).

Recently, Roy *et al.*, showed that the oxidation of ω-3 PUFAs by ROS releases 4(*RS*)-4-F_{4t}-NeuroP from docosahexaenoic acid, which is necessary to prevent isoproterenol-induced arrhythmias in mice with myocardial infarction (173), prevent early arrhythmias in rats after an ischemia/reperfusion period (174), or prevent breast cancer proliferation (175). As previously observed in different oxidative conditions (176), the researchers proposed that in oxidative stress conditions, such as ischemic events, 4(*RS*)-4-F_{4t}-NeuroP is responsible for the antiarrhythmic properties of ω-3 PUFAs by countering the cellular stress by ROS. They demonstrated that 4(*RS*)-4-F_{4t}-NeuroP could mediate the cardioprotective effect of ω-3 PUFAs by stabilizing the RyR2 complex (177). This discovery created a new perspective on products of nonenzymatic oxygenated metabolites of fatty acids as potent mediators in diseases that involve ROS production as a trigger factor. Overall, effects of nonenzymatic metabolites of ω-3 and ω-6 PUFAs by ROS oxidation are described in Roy *et al.* (178). These findings are relevant to the potential link between oxidative stress and the physiological role of peroxidation in lipids (**Fig. 7**).

In addition to unsaturated lipids of ω-3 and ω-6 PUFAs, antioxidant properties of cholesterol were noticed when the double-binding ligand became oxidized (179). In particular, the cholesterol-containing compounds, epoxide-bearing substances (produced by autoxidation *via* nonenzymatic mechanisms), are unstable because of the high

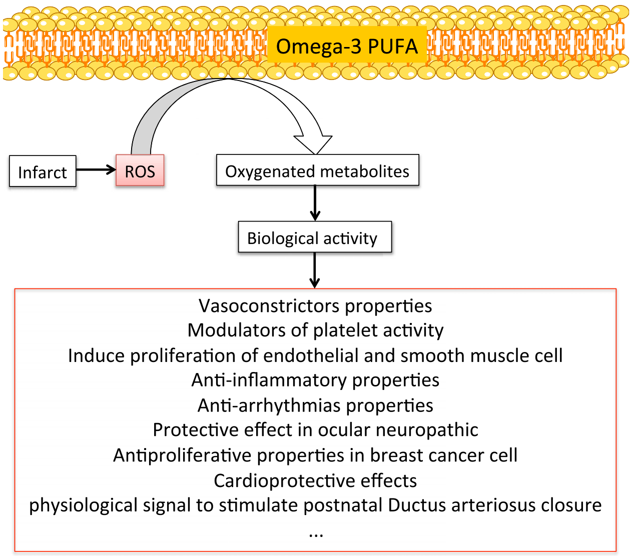

Figure 7. Physiological function of oxygenated metabolites of PUFAs. Under intense oxidative stress (an ischemia/reperfusion event), ROS can induce lipoperoxidation of PUFAs. This nonenzymatic oxidation induces the synthesis of oxygenated metabolites, which are biologically active. Oxygenated metabolites of PUFAs may exert vasoconstrictive properties, modulate platelet activity and the proliferation of endothelial and smooth muscle cells, and show that anti-inflammatory, antiarrhythmic, and antiproliferative properties have an effect on ocular neuropathic, cardioprotective, and physiological signals to stimulate postnatal ductus arteriosus closure.

reactivity of the epoxide ring toward nucleophiles, including amines, thiols, and the hydroxyl group, thus exhibiting antioxidant properties. Researchers showed that these oxidation products, such as in 5,6-epoxysterols, could be obtained *via* a nucleophilic substitution mechanism from cholesterol. Of interest, additional evidence points to the existence of active metabolites of cholesterol, namely, oxysterols 5,6a-EC. In 2013, researchers demonstrated an important role in carcinogenesis for dendrogenin (DDA), a natural metabolite in mammals that results from the enzymatic conjugation of 5,6α-EC with histamine (180). This oxysterol of DDA was not detected in cancer cell lines and was 5-fold lower in human breast tumors compared with normal tissues, which suggests dysregulation of DDA metabolism during carcinogenesis. De Medina *et al.* (181) established that DDA is a selective inhibitor of cholesterol epoxide hydrolase that can trigger tumor redifferentiation and growth control in mice as well as improved animal survival. The properties of DDA and its decreased level in tumors suggests important physiological functions in maintaining cell integrity, differentiation, and, possibly, immune system surveillance.

CONCLUSIONS

Although the discovery of free radicals raised several interests, it was not until 50–60 yr later that the existence of free radicals in living organisms was demonstrated and their responsibility for the theory of aging was suggested (4, 5, 7). The balance between the oxidative compounds that are derived from molecular oxygen and the antioxidant defenses in the body gives rise to a subtle harmony that allows ROS to exercise their physiological role without causing collateral damage to cells. This balance can be broken, for example, after an intense physical effort, which causes, then, cellular damage as a result of ROS, but the extent of damage depends on the nature of the ROS and its place of production. Even if it remains difficult to demonstrate the implication of direct or indirect oxidative stress in numerous pathologies, as described in this review, it is indisputable and collectively admitted that ROS play a fundamental role in numerous situations as ROS sensors (**Fig. 8**). However, the link between oxidative stress and pathologies is complex to determine. Of note, it is difficult to know whether oxidative stress is the origin or the consequence of the pathologies to which it is

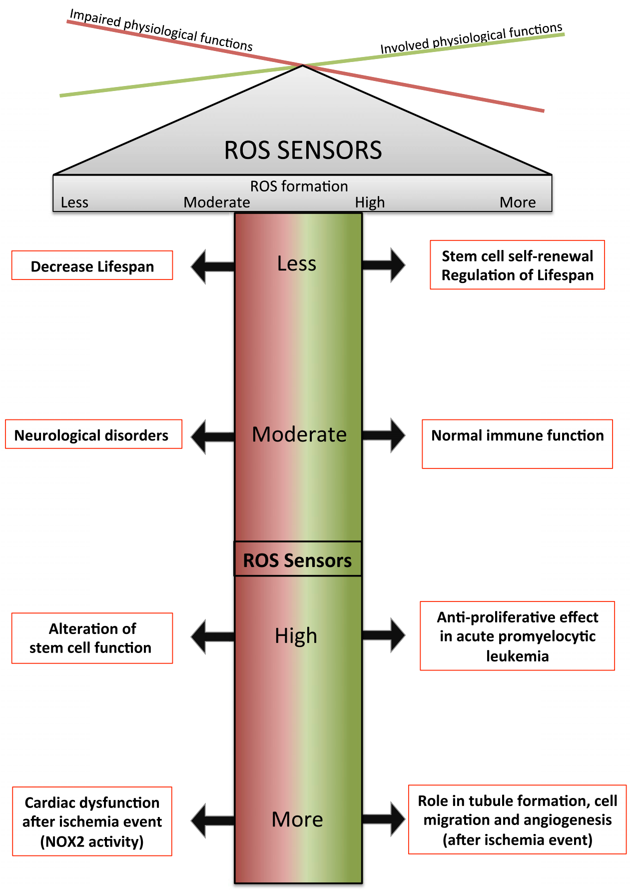

Figure 8. Involvement of ROS sensors. In numerous situations, it has been proposed that ROS formation at diverse levels (less, moderate, high, more) and to the same degree may take part in physiological functions but can also impair physiological functions. During ischemia/reperfusion, a burst of ROS can induce cardiac dysfunction (by NOX2 activity), but in other cases, is potentially responsible for tubule formation, cell migration, and angiogenesis. In the same physiological situation, during aging, ROS seem to regulate and decrease lifespan. Collectively, new data suggest that ROS can be considered to be ROS sensors.

bound. In addition, as explained in this review and depicted in Fig. 8, the level of ROS formation can be in different situations, in same (theory of aging) situation, or in an impaired physiological situation.

Addressing the regulatory role of ROS is certainly methodologically complex and not easily applicable to field studies because of the intrinsic properties of ROS and because the technical tools that have been developed to this point are neither standardized nor optimized for daily use, which would be of interest in preventive medicine. Yet research on chemicals with pro-oxidant activity increased dramatically, and, presently, the study of oxidative stress in physiology and redox status regulation has gained an important role in medicine, biochemistry, physiology, pharmacology, ecotoxicology, and, more recently, in the evolution of ecology. ROS work as redox messengers in regulatory processes in which the signal is delivered *via* redox chemistry (84, 182). The organism's response to a social or nonsocial environmental stimulus depends on a cascade of processes, starting from the perception of the stimulus to its translation into hormonal secretions, which, in turn, regulate the response itself. For example, research in the area of behavioral endocrinology has contributed to the identification of several mechanisms that regulate the extent and rate at which organisms respond to environmental influences (183). Because of these signaling properties in cell communication, ROS might also be important regulators of the way organisms respond to their environment. As such, the number of publications that involve ROS has increased over the last 10 yr (~10,000 articles published in 2005 compared with ~25,000 in 2015). FJ

ACKNOWLEDGMENTS

J.R. was supported by the Fondation pour la Recherche Médicale (FRM 40199). The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

J. Roy, J.-M. Galano, T. Durand, J. C.-Y. Lee, and J.-Y. Le Guennec designed the review (parts) and wrote the paper; and J. Roy created all figures.

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Received for publication February 24, 2017.
Accepted for publication May 22, 2017.