Regulation of Signal Transduction by Reactive Oxygen Species in the Cardiovascular System

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Abstract: Oxidative stress has long been implicated in cardiovascular disease, but more recently, the role of reactive oxygen species (ROS) in normal physiological signaling has been elucidated. Signaling pathways modulated by ROS are complex and compartmentalized, and we are only beginning to identify the molecular modifications of specific targets. Here, we review the current literature on ROS signaling in the cardiovascular system, focusing on the role of ROS in normal physiology and how dysregulation of signaling circuits contributes to cardiovascular diseases, including atherosclerosis, ischemia–reperfusion injury, cardiomyopathy, and heart failure. In particular, we consider how ROS modulate signaling pathways related to phenotypic modulation, migration and adhesion, contractility, proliferation and hypertrophy, angiogenesis, endoplasmic reticulum stress, apoptosis, and senescence. Understanding the specific targets of ROS may guide the development of the next generation of ROS-modifying therapies to reduce morbidity and mortality associated with oxidative stress. (Circ Res. 2015;116:531-549. DOI: 10.1161/CIRCRESAHA.116.303584.)

Key Words: cardiovascular diseases ■ oxidative stress ■ reactive oxygen species ■ signal transduction

For decades, oxidative stress was defined as a cellular imbalance between oxidants and reductants. Now it is clear that differences in subcellular and tissue compartmentalization of reactive oxygen species (ROS) contribute to stress responses.1 Recent research has shown that ROS signaling pathways are complex, compartmentalized, and in many cases essential for normal cardiovascular physiology. In addition, ROS signaling and oxidative stress have been implicated systemically or acutely in a variety of cardiovascular diseases and conditions, including atherosclerosis, ischemia–reperfusion injury, diabetic vascular disease, arrhythmia, myocardial infarction (MI), hypertrophy, cardiomyopathy, and heart failure. The differences between normal redox signaling required for cell survival and function and excess or inappropriate activation of redox circuits during oxidative stress are important to understand. Attempts at treating diseases with antioxidants prophylactically have been largely ineffective and in some cases harmful2–4; there is a definite need for improvement in timing, targeting, and a reduction in off-target effects. In this review, we will summarize the current literature on ROS signaling in the cardiovascular system focusing on the role of ROS in normal physiology and how dysregulation of signaling circuits contributes to cardiovascular disease. A detailed understanding of these pathways allows the development of more targeted therapies to reduce morbidity and mortality associated with oxidative stress.

Key ROS in Signaling and Oxidative Stress

ROS can be loosely defined as reactive molecules containing oxygen. Several ROS including superoxide (O$_2^*$), hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOO$^{-}$), and the hydroxyl radical (HO$^*$) are all produced in biological systems. O$_2^*$ and H$_2$O$_2$ are produced enzymatically and are involved in both reversible, physiological signaling processes and the pathologies associated with oxidative stress. Other ROS, such as OONO$^{-}$ and HO$^*$, are not considered as signaling molecules because of their highly reactive nature and irreversible modifications but can nonetheless contribute to oxidative stress and tissue damage pathologically. In addition, the reactive nitrogen species nitric oxide (NO) is sometimes considered to be a ROS. For the purpose of this review, we will only discuss NO in the greater context of O$_2^*$ and H$_2$O$_2$-mediated signaling.

Superoxide

O$_2^*$ is produced biologically by many enzymes, including NADPH oxidases (Nox), xanthine oxidase (XO), lipooxygenase, myeloperoxidase, uncoupled endothelial NO synthase (eNOS), and the mitochondrial respiratory chain, via a 1-electron reduction of molecular oxygen. O$_2^*$ can spontaneously dismutate to H$_2$O$_2$ (rate constant=8×10$^4$ M$^{-1}$ sec$^{-1}$) or be converted to H$_2$O$_2$ by the enzyme superoxide dismutase (SOD) (rate constant=2×10$^5$ M$^{-1}$ sec$^{-1}$; Figure 1). Although much of the O$_2^*$ produced is rapidly converted to H$_2$O$_2$,
which is thought to mediate downstream signaling, some direct modifications such as oxidation of (FeS)_4 clusters and heme groups are directly attributable to O_2•− (Figure 1).

Hydrogen Peroxide

H_2O_2 is primarily formed by the dismutation of O_2•− by SOD (Figure 1). In the case of Nox4, H_2O_2 may be produced directly before O_2•− leaves the enzyme. H_2O_2 is of particular interest in signaling because of its higher stability compared with oxygen radicals and the ability to cross biological membranes. H_2O_2 reacts with low pKa protein thiols, such as those on cysteine and methionine to form disulfide bonds (-SSR) or sulfenic acid (-SOH), which can influence protein function (Figure 1). These modifications are reversible by antioxidative mechanisms, such as glutathione peroxidase (Gpx) and peroxiredoxin. Further oxidation by H_2O_2 can form sulfinic acid (-SO_2H), which has been demonstrated to be reversible in peroxiredoxin by the enzyme sulfiredoxin (Srx), and sulfonic acid (-SO_3H), which is not readily reversible and is not thought to be involved in signaling. It is important to note that not only does H_2O_2 act on adjacent targets, but because it is relatively stable, it can also serve paracrine functions, as between endothelial and smooth muscle cells to regulate vasomotor tone.

Hydroxyl Radical

HO• is highly reactive, with a biological half-life of \(10^{-9}\) s. It is formed by the decomposition of H_2O_2, which is catalyzed by free metals in Fenton chemistry or by radiation-excited molecular oxygen reacting with H_2O. Because of its nonselective and often irreversible reactivity, HO• can be damaging to many biological molecules, including amino acids/proteins, carbohydrates, lipids, and DNA. Certain biological scavengers, such as glutathione, can react with HO• to prevent damage. In general, HO• is not considered a signaling molecule except as an intermediate because of its nonselective and reactive nature and will not be thoroughly discussed in this review. It should be noted, however, that HO• plays a role in the oxidative damage associated with oxidative stress.

Biological Sources of ROS

NADPH Oxidases

Nox proteins constitute a family of membrane-associated, multunit enzymes that catalyze the reduction of molecular oxygen using NADPH as an electron donor. The Nox family is composed of 7 members, Nox1-5 and Duox1/2, with varying...
binding partners and activities. Only Nox1, Nox2, Nox4, and Nox5 are present in cardiovascular tissues. Excessive or off-target myeloperoxidase activity is thought to mediate the antimicrobial phagocyte respiratory burst.

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ROS that contribute to lipid oxidation in atherosclerosis. Increased expression of 5-lipoxygenase (5-LO) in atherosclerotic plaques and abdominal aortic aneurisms. Reduced expression of 5-LO is effective at attenuating the contribution of $O_2^−$ to this injury.

Lipoxygenase Enzymes of the lipoxygenase family catalyze the oxidation of polyunsaturated fatty acids, including those involved in the biosynthesis of inflammatory leukotriene molecules. Lipoxygenases have been implicated in lipid oxidation in atherosclerosis. Increased expression of 5-lipoxygenase (5-LO) is found in atherosclerotic plaques and abdominal aortic aneurisms. Reduced expression of 5-LO or knockout of 12/15-LO reduces atherosclerosis in a LDL-R null model.

Myeloperoxidase Myeloperoxidase is a heme-containing peroxidase expressed in neutrophils and monocytes that forms hypohalous acids from $H_2O_2$ and halides (Cl−, Br− and I−) or pseudohalide (SCN−). The hypohalous acids are strong oxidants thought to mediate the antimicrobial phagocyte respiratory burst. Excessive or off-target myeloperoxidase activity is thought to produce ROS that contribute to lipid oxidation in atherosclerosis. In addition, myeloperoxidase may be transferred from neutrophils to endothelial cells via β2-integrin–mediated cell–cell contact.

Myeloperoxidase-derived oxidants have also been implicated in left ventricular remodeling after MI.

NO Synthase Uncoupling of eNOS by reduced availability of substrates and cofactors, such as BH4 and L-arginine, results in increased $O_2^−$ production and reduced NO. Peroxynitrite (ONOO−), formed by the reaction of NO with $O_2^−$ at a rate thought to be higher than $O_2^−$ dismutation by SOD, is a strong oxidant that can pass through membrane anion channels, but it is not expected to diffuse freely in an outward direction, thus limiting its potential paracrine effects. Peroxynitrite is, however, thought to contribute to eNOS uncoupling and oxidation of BH4 increases ROS production. Several factors have been linked to reduced bioavailability of NOS cofactors, including smoking, hypertension, diabetes mellitus, ischemia–reperfusion injury, coronary artery disease, and oxidative stress. The endothelial localization makes uncoupled eNOS both a contributor to endothelial dysfunction and a prime target of therapeutics to limit its detrimental effects.

Mitochondria Another source of $O_2^−$ in cardiovascular cells is from the mitochondria as a byproduct of respiration. In particular, complexes II and III of the mitochondrial respiratory chain exhibit electron leak during respiration that produces $O_2^−$. In the case of complex I, $O_2^−$ is only released into the matrix, whereas $O_2^−$ is released on both sides of the inner mitochondrial membrane from complex III. Antioxidants in the mitochondria, such as SOD2, rapidly degrade or sequester $O_2^−$ to reduce reactivity. Perhaps because of high concentrations of mitochondria in cardiac tissue, reduced mitochondrial antioxidant capacity results in cardiac dysfunction.

Antioxidants A large network of proteins and signaling pathways regulates the breakdown of ROS. From a systems perspective, overproduction of ROS in the cell can misregulate thiol redox circuits by changing the redox state of thioredoxins, glutathione, and other cysteine pools. Ultimately, overproduction or high levels of exposure of a cell to exogenous ROS leads to a state of oxidative stress, which can result in DNA damage, reduced growth, metabolic problems, and cell death. Thus, antioxidant systems are critically important to regulate ROS-mediated signaling temporally and spatially.

In addition to the control of redox potential by glutathione- and thioredoxin-based redox circuits, antioxidant enzymes, such as SOD, catalase, and Gpx, rapidly break down ROS to less reactive or nonreactive products. These proteins are key in preventing damage from oxidative stress. In response to oxidative stress, cells activate transcription of protective antioxidant genes via redox-sensitive transcription factors, such as nuclear factor (erythroid 2–related) factor 2 (Nrf2).

Nrf2 binds to the promoters of genes containing the cis-acting antioxidant response element transcriptional element. Many antioxidant response element genes are involved in detoxification, such as glutathione-S-transferases and NAD(P)H dehydrogenase. Numerous antioxidant genes contain antioxidant response elements, including catalase, Gpx, peroxiredoxin, and thioredoxin (see Ma for more detail). Nrf2 is retained in the cytoplasm by binding to Keap1 (INrf2), which scaffolds to actin filaments. Keap1 also acts as an adaptor for the E3 ubiquitin ligase Cul3 and targets Nrf2 for proteosomal degradation by ubiquitination. Oxidation of Keap1 results in disruption of the complex and translocation of Nrf2 to the nucleus.

Superoxide Dismutases SODs rapidly convert $O_2^−$ to $H_2O_2$. Most mammalian cells contain 3 forms of SOD (SOD1, SOD2, and SOD3) with differing localizations. SOD1, a Cu/Zn SOD, is expressed in the cytoplasm and regulates angiogenesis and vasomotor tone. Genetic deletion of SOD1 leads to increased $O_2^−$ and ONOO−, resulting in increased vasoconstrictor responses and impaired endothelium-dependent relaxation, but no
major change in blood pressure. Ischemia-induced vascular permeability is increased in these mice, but neovascularization is impaired. However, transgenic overexpression of SOD1 reduces angiotensin II (AngII)-induced hypertension. SOD2 is also known as mitochondrial manganese SOD because of its localization. Deletion of SOD2 induces perinatal lethality because of cardiomyopathy, whereas SOD2 mice develop age-related hypertension and accelerated atherosclerosis on an ApoE background. SOD3 (also Cu/Zn containing), which is secreted and then tethered to the outer plasma membrane, is particularly important in the cardiovascular system because of high expression in blood vessels, lung, and heart. Manipulation of SOD3 has no effect on basal blood pressure, but SOD3 deficiency enhances blood pressure in response to AngII infusion. These mice also show defective neovascularization, but the role of SOD3 in atherosclerosis remains unclear.

**Catalase**

Catalase catalyzes the decomposition of $H_2O_2$ to water and oxygen. Catalase is a tetramer containing 4 heme groups. The heme-iron is initially oxidized by $H_2O_2$ to form a high-valence intermediate known as compound I. Further reaction with $H_2O_2$ reduces compound I, forming molecular oxygen and water. Although catalase is highly abundant in many tissues, catalase null mice develop normally. Peroxiredoxins likely provide the necessary redundancy in $H_2O_2$ breakdown to allow for normal development. Overexpression of catalase protects against aneurysm formation but inhibits collateralization.

**Glutathione Peroxidase**

Gpx catalyzes the decomposition of $H_2O_2$ and lipid hydroperoxides to water or corresponding alcohols using reduced glutathione (GSH). Gpx exhibits higher expression in cardiovascular tissues than catalase, and because of its ability to reduce $H_2O_2$ and lipid peroxides, Gpx is thought to play a more critical protective antioxidant role in the cardiovascular system than catalase. Indeed, studies of Gpx knockout mice exhibit impaired angiogenesis, endothelial dysfunction, increased susceptibility to ischemia–reperfusion injury, and various other cardiovascular phenotypes that are thought to be mediated by increased ROS. A recent review discusses Gpx in more detail.

**Peroxiredoxins**

Peroxiredoxins are a class of 6 antioxidant enzymes that are typically classified by characteristics of their $H_2O_2$-sensitive catalytic cysteines as 2-Cys (Prx1-4), atypical 2-Cys (Prx5) and 1-Cys (Prx6). Peroxiredoxin enzymes are highly expressed in cardiovascular tissues and in circulating erythrocytes, presumably to protect hemoglobin from oxidation leading to anemia. Peroxiredoxin isoforms 3 to 6 are downregulated in failing myocardium. Peroxiredoxin 4 has been proposed to be a biomarker of oxidative stress based on evidence that patients with increased peroxiredoxin 4 have elevated CVD risk and mortality. Peroxiredoxin 6 exhibits both Gpx and phospholipase A activities and is necessary for the activation of Nox2 in endothelial cells and neutrophils.

**Thioredoxin**

The thioredoxins are ubiquitously expressed antioxidants with a highly conserved CGPC catalytic motif that reduce substrate proteins by cysteine thiol-disulfide exchange. In mammals, 2 thioredoxins are present, thioredoxin-1 in the cytosol and thioredoxin 2 in the mitochondria. Mice with transgenic thioredoxin overexpression exhibit reduced ROS-related cardiovascular toxicity. Altered thioredoxin levels have been identified in atherosclerosis.

**Compartmentalization of ROS Signaling**

As with any regulated signaling process, compartmentalization of redox signaling plays a key role in defining the cellular response generated by the initial ROS-based signal. The subcellular localization of ROS-producing enzymes and antioxidants is tightly regulated. ROS detection in real-time by novel methods, such as the $H_2O_2$ sensor HyPer, confirms spatially regulated subcellular localization of ROS production in response to certain stimuli. For example, by targeting HyPer to the endoplasmic reticulum (ER), Wu et al demonstrated localized $H_2O_2$ production by Nox4 in response to tunicamycin and HIV-1 Tat. Datla et al showed dynamic ROS production localized to focal adhesions in a model of nocodazole-induced focal adhesion turnover. Finally, Nox4-derived ROS have been detected in the perinuclear space.

Both ROS-producing enzymes (Nox1/Nox2) and antioxidant enzymes (SOD1) are associated with a pool of redox endosomes in nonphagocytic cells, including vascular smooth muscle cells (VSMCs), fibroblasts, and potentially cardiomyocytes. These redoxosomes are formed in response to cytokine signaling or other external stimuli, mediating downstream signaling by acting on spatially restricted molecular targets, and often require anion channels to help neutralize charge and facilitate transport of the signal across membranes. In addition, endosomes can mediate cross talk between spatially distinct signaling circuits. Because the localization of ROS production likely plays a large role in which signaling pathways are activated, targeting antioxidants to particular compartments to disrupt pathological signaling would likely be more effective than the ubiquitous prophylactic antioxidant treatments that have been attempted in the past.

**Role of ROS in Fundamental Cellular Responses**

ROS contribute to the cardiovascular diseases by several mechanisms (see ROS and Oxidative Stress in Cardiovascular Disease section of this article). Many of these processes are also important during development, in minute-to-minute regulation of blood flow, and in physiological adaptations to environmental stresses. In the following sections, we will discuss signaling pathways that are modified by ROS organized by functional response: cell migration/adhesion, cell proliferation/hypertrophy, ER stress/autophagy, and apoptosis/senescence. Depending on the source and severity of ROS production, as well as the cardiovascular cell type, ROS can contribute to some or all of these pathways simultaneously.
Phenotypic Modulation

One of the most fundamental roles of ROS in the vasculature is in phenotypic regulation of VSMCs. These cells are normally differentiated and contractile, but in response to certain environmental cues can undergo phenotypic modulation to become synthetic, proliferative, and migratory. Nox4 expression and activity are important for maintaining the differentiated phenotype. Regulation of smooth muscle–specific gene expression in VSMCs by Nox4 involves the activation of p38 mitogen-activated protein kinase (MAPK), which regulates the activity of the SMC transcription factors serum response factor and myocardin-related transcription factor A. Actin itself is also directly oxidized by the flavoprotein oxidoreductase MICAL-2 in the nucleus, which promotes G-actin disassembly and increases nuclear translocation of the myocardin-related transcription factor A. In contrast, Nox1 expression and activity are associated with a reduction in differentiation markers and increased migration and growth.

ROS produced by Nox proteins have also been implicated in cardiac cell differentiation. Nadworny et al induced the differentiation of c-kit+ cardiac precursor cells into mature cells and observed upregulation of Nox2 and Nox4 during differentiation. Furthermore, silencing of Nox2 and Nox4 increased the expression of c-kit+ cardiac precursor cell genes, such as c-kit and Flk-1, and decreased the expression of differentiation genes. Nox4 activation of c-jun upregulates the cardiac differentiation transcription factor GATA-4 in pluripotent embryonal carcinoma cells.

Cell Migration and Adhesion

VSMC, endothelial cell, and fibroblast migration play important roles in vessel development and repair. ROS regulate multiple steps in the migratory process, including tyrosine kinase signaling, integrin engagement, focal adhesion formation, and PI3K signaling. The amount and location of ROS production are critical; for example, although ROS are necessary for collagen formation, excess ROS can actually inhibit collagen formation.

Overactivation of migratory signaling in prodisease conditions, either by excessive extracellular oxidative stress or by hyperactivation of the normal healing response, can lead to inappropriate vascular cell migration, macrophage infiltration, and lesion formation.

Cell migration is a multistep process involving the creation and dissolution of focal adhesions coordinated with cytoskeleton contraction to mediate movement (Figure 2). The cell first establishes polarity by sensing a gradient of a migratory stimulus. For example, after vascular injury, platelet-derived growth factor (PDGF) and PDGF receptor expression increase, providing a stimulus for VSMC migration. The PDGF receptor dimerizes and autophosphorylates, providing binding sites for phospholipase C, Src, and phosphoinoside 3-kinase (PI3K). PI3K promotes the formation of PIP3, which activates Rho guanine nucleotide exchange factors to stimulate Rho-GTPase family members, such as Rho, Rac, and cdc42. Rac activates several Nox family members, notably Nox1 and Nox2, which can further increase ROS signaling, depending on the cell type. Activation of Rho by direct oxidation of a redox-sensitive motif has also been identified by Aghajanian et al. Rac and Rho are critical mediators of actin polymerization and the formation of focal adhesions, which are essential for protrusion and attachment and reattachment as the cell moves forward. Activation of the PDGF receptor is limited by the low molecular weight protein tyrosine phosphatase (LMW-PTP), but in the presence of ROS, LMW-PTP is inactivated by direct oxidation on Cys12 and Cys17 to form an inactivating disulfide bond, thus further amplifying the signal.

After sensing the migratory stimulus, the leading edge of the migrating cell forms as lamellipodia extend in the direction of movement. Nox1-dependent activation of SSH1L and oxidation of 14-3-3 in PDGF-stimulated VSMCs results in the dephosphorylation and subsequent activation of the actin filament disassembly protein coflin (Figure 2). This activation of coflin results in actin depolymerization, a necessary step in the formation of new actin filaments that result in protrusion of lamellipodia and increased cell migration.

Integrin binding and engagement in newly formed lamellipodia is essential for cell adhesion and migration. Recently, integrins have been demonstrated to be directly modified by ROS. de Rezende et al identified redox-sensitive cysteines in the integrin α7 subunit that influence integrin binding to laminin. Integrin engagement has been reported to induce an oxidative burst, which may play a role in cell adhesion and spreading; however, the mechanism is not well understood. Integrins sense and bind extracellular matrix proteins and in turn phosphorylate focal adhesion kinase (FAK), which recruits SH2- and SH3-domain–containing proteins, such as Src, PI3K, Grb7, PLCγ, and other focal adhesion proteins such as paxillin and vinculin into focal complexes that mature into stronger focal adhesions via a Rho-dependent pathway. Continuous focal adhesion turnover is essential for effective migration because both too much turnover and too little turnover seem to inhibit cell migration.

Phosphatases seem to be major redox targets during migration. Chiarugi et al demonstrated that increased ROS inhibit the activity of a phosphatase for FAK, which is necessary for subsequent focal adhesion formation and spreading. They showed that oxidation of the LMW-PTP mentioned above during cell adhesion inhibits its ability to bind and dephosphorylate FAK. Others have implicated the redox-sensitive phosphatase Shp2 in FAK activation. Shp2 is inactivated by direct oxidation, which ultimately increases FAK activity (Figure 2). In addition to FAK, LMW-PTP targets the PDGF receptor and p190RhoGAP, a guanine nucleotide exchange factor for Rho. The tyrosine phosphatase PTP-PEST regulates paxillin and the Rho guanine nucleotide exchange factor Vav2. PTP1B, which binds to integrins and the p130cas scaffold, is also sensitive to oxidation.

The focal adhesion complex is tethered to the cell’s actin cytoskeleton. Actin itself can be modified by ROS, either enhancing or inhibiting polymerization, depending on the type and amount of oxidant. In actively migrating endothelial cells, elevated levels of ROS are detected, and treatment with the SOD mimetic MnTMPyP abolishes actin monomer incorporation at the barbed end of growing actin filaments. However, the same concentration of H2O2 that causes fragmentation of F-actin in fibroblasts leads to reorganization of F-actin into stress fibers in endothelial cells, suggesting that oxidation can be environment or cell type dependent.
The force that is necessary to move the cell forward toward the leading edge is generated by actin myosin interactions. The myosin light chain is regulated primarily by calcium-calmodulin, but can be influenced by the activity of the redox-sensitive GTPase Rho and the potentially redox-sensitive cdc42. In pulmonary smooth muscle, ROS activation of Rho results in the deactivation of myosin light chain phosphatase via ROCK, which promotes contraction.

In addition to redox regulation of migratory signaling, matrix degradation, a necessary step in migration, is also regulated by ROS. Matrix metalloproteinases that degrade extracellular matrix proteins are regulated by ROS transcriptionally by activation of Akt/nuclear factor (NF)-κB and extracellular signal-regulated kinase 1/2 (ERK1/2), and directly by a cysteine switch mechanism. Although activation of matrix metalloproteinases may be protective in the case of mild atherosclerosis, in more severe plaques matrix metalloproteinase activation can result in plaque instability and rupture by breaking down extracellular matrix.

Integrin-associated matricellular protein receptors, such as CD47 and CD36, have recently been shown to be involved in pathological ROS production. The activation of CD47 and CD36 by thrombospondin-1 inhibits NO signaling in VSMCs and may contribute to eNOS uncoupling. The activation of matrix metalloproteinases may be protective in the case of mild atherosclerosis, in more severe plaques matrix metalloproteinase activation can result in plaque instability and rupture by breaking down extracellular matrix.

**Vascular Tone and Cardiac Contraction**

As with most other vascular functions, ROS not only contribute to homeostatic regulation of vascular tone but also are associated with hypertension when produced in excess. Similarly, ROS have been implicated in inotropic dysfunction in cardiomyopathy/heart failure.

A key regulator of vascular tone is NO produced in the endothelium by eNOS (Figure 3A). NO activates guanylate cyclase by binding to its associated heme, which increases cGMP production resulting in protein kinase G (PKG) activation and ultimately smooth muscle cell relaxation. Oxidative modification of the eNOS substrate BH4 by peroxynitrite, uncouples the enzyme, switching production from NO to O₂⁻ and contributing to deoxycorticosterone acetate salt hypertension in mice. eNOS itself is redox sensitive by reversible S-glutathionylation on several reactive cysteine residues, including C382, C689, and C908, in an elevated GSSG:GSH environment. Crabtree et al demonstrated that both of these mechanisms of eNOS uncoupling are additive but functionally independent. Although these 2 mechanisms result in decreased NO production in response to increased ROS, ROS can also paradoxically increase eNOS function. Cai et al demonstrated that H₂O₂ produced by Nox in response to AngII stimulation increases NO production by eNOS, at least in part by increasing eNOS expression. However, this induction of eNOS is unlikely to be sufficient to compensate for oxidative stress. Tong et al demonstrated that H₂O₂ produces NOx in arterial smooth muscle oxidizes sarcoplasmic reticulum calcium transport ATPase (SERCA), ultimately reducing cellular response to NO. Stimulation of murine endothelial cells with AngII results in increased O₂⁻ production by Nox2 and the mitochondria, resulting in AngII-induced hypertension. In fact, several transgenic animal models with altered vascular Nox expression have altered blood pressure responses.

H₂O₂ can act as a vasodilator, as first identified by the group of Wolin et al in rat cremasteric arterioles. It was soon demonstrated that H₂O₂ acts through the cGMP pathway, but it has only been recently appreciated that PKG itself can be activated by oxidation via disulfide bond formation, explaining in part why H₂O₂ causes direct vasodilation in some conditions.
vascular beds. cGMP depletion sensitizes PKG to oxidation, especially in resistance arteries, suggesting a compensatory mechanism of vasodilation when NO is depleted. In human coronary arteries, H$_2$O$_2$ causes dimerization and activation of PKG, leading to subsequent opening of smooth muscle large-conductance Ca$^{2+}$-activated K$^+$ channels (BK(Ca)) and hyperpolarization. Similarly, chronic hypoxia is accompanied by increased H$_2$O$_2$ and PKG activation, which may in turn partially counteract pulmonary hypertension.

Elevated H$_2$O$_2$ also plays a role in constriction by multiple pathways. Approximately 30 years ago, Heinle demonstrated that direct application of H$_2$O$_2$ induces vasoconstriction of the carotid artery. Since then, it has been shown that AngII-induced contraction produces H$_2$O$_2$ by Nox activation, which mediates vascular constriction by activating ERK1/2 and p38 MAPK in smooth muscle cells (Figure 3A). In addition, ROS mediate activation of RhoA by reversible oxidation of reactive cysteines C16/C19. Rho activation increases Rho kinase activity, leading to constriction by inhibiting myosin light chain phosphatase. Although the exact mechanisms have yet to be fully defined, differences in ROS production by Nox proteins and altered extracellular SOD levels in response to hypoxia in pulmonary versus coronary cells may help to explain the difference.

Alternative pathways, such as elevation of intracellular Ca$^{2+}$ caused by redox modification of L-type Ca$^{2+}$ channels (LTCC) in vascular smooth muscle, may play a role in chronic constriction and hypertension (Figure 3A), but additional work is necessary to understand the role of these channels better. Regulation of BK(Ca) channels has also been implicated in redox control of vascular tone. Ultimately, the regulation of vessel tone by ROS is likely dependent on concentration and localization. Moreover, the acute effect of a short-term elevation of ROS may activate different signaling pathways related to contractility when compared with chronic elevation of ROS, which can activate antioxidant response element genes and other compensatory antioxidant mechanisms.

ROS regulation of cardiac contractility occurs at multiple levels (Figure 3B). Excessive ROS production by overactive Nox, XO, or mitochondria results in cardiomyopathy because of direct modification of ion channels and transporters, as well as altered intracellular contractility signaling. Cardiac contraction begins with the propagation of an action potential by the rapid activation of voltage-gated sodium channels, such as Na$_{v}$.1,5 and voltage-dependent LTCCs. Oxidation of redox-sensitive methionine residues on Na$_{v}$.1,5 impairs the inactivation of the channel resulting in elevated intracellular Na$^+$. LTCC activation increases intracellular Ca$^{2+}$ resulting in Ca$^{2+}$-induced Ca$^{2+}$ release by the ryanodine receptor. Although the α1c pore-forming subunit of LTCC has been demonstrated to be sensitive to oxidation that reduces the peak Ca$^{2+}$ current carried by the channel, ROS-mediated activation of CaMKII, PKA, and PKC seem to result in a net increase in Ca$^{2+}$

Figure 3. Redox signaling pathways in contraction. A, Vascular smooth muscle cell (VSMC) contraction proteins and their relationship to endothelial cell (EC)–derived nitric oxide (NO). B, Cardiac contraction proteins. Dark gray, Redox modified proteins. CamKII indicates calcium/calmodulin-dependent protein kinase II; eNOS, endothelial NO synthase; GC, guanylate cyclase; GSH, glutathione; GSSG, glutathione disulfide; H$_2$O$_2$, hydrogen peroxide; LTCC, L-type Ca$^{2+}$ channels; MAPK, mitogen-activated protein kinase; MLCP, myosin light chain phosphatase; NCX, sodium/calcium exchanger; O$_2^-$, superoxide; ONOO$^-$, peroxynitrite; PKG, protein kinase G; ROK, Rho-associated protein kinase; ROS, reactive oxygen species; RyR2, ryanodine receptor; SERCA, sarcoendoplasmic reticulum calcium transport ATPase; SMC, smooth muscle cell; and XO, xanthine oxidase.
current by LTCC ultimately. Moreover, the activation of CaMKII itself by Ca\(^{2+}\)/calmodulin is augmented by the oxidation of M281/282.\(^{135}\) Ultimately, the sarcoplasmic reticulum calcium channel ryanodine receptor is activated by the influx in Ca\(^{2+}\) by LTCC to release sarcoplasmic reticulum Ca\(^{2+}\) into the cytosol. Key cysteine residues in the RyR are oxidized to form activating disulfide bonds, resulting in increased cytosolic Ca\(^{2+}\) and ultimately heightened cardiac contractility.

During diastole, Ca\(^{2+}\) efflux and reuptake by the sarcoplasmic reticulum occurs (Figure 3B). The sodium/calciuexchanger mediates Ca\(^{2+}\) efflux by removing 1 Ca\(^{2+}\) for 3 imported Na\(^{+}\) ions. There have been conflicting reports about ROS activating or inhibiting ion flux via sodium/calcium exchanger.\(^{139,140}\) Additional work is necessary to identify ROS-sensitive coactivators or related post-translational modifications. An analysis of potentially redox-sensitive cysteines failed to identify directly modified residues.\(^{141}\) The reuptake of Ca\(^{2+}\) by the sarcoplasmic reticulum after contraction is regulated by the Ca\(^{2+}\)-ATPase SERCA. SERCA contains the highly reactive cysteine C674, which reduces SERCA activity when H\(_2\)O\(_2\) induces its S-glutathionylation.\(^{142,143}\) However, S-glutathionylation of C674 also occurs in response to NO, which increases SERCA activity to promote relaxation.\(^{144}\) Additional work is necessary to clarify the role of S-glutathionylation of C674 and whether other still-to-be-identified modifications may contribute to SERCA activity.

### Proliferation and Hypertrophy

Like migration, cell proliferation is an important process in development, as well as wound healing and repair. In cases of oxidative stress in the vasculature, excessive cell proliferation can contribute to a growing atherosclerotic plaque or a narrowing artery because of restenosis after percutaneous coronary intervention.\(^{145}\) In VSMCs, many of the same factors that induce a migratory phenotype, such as PDGF, also activate proliferation pathways, and in the case of AngII, hypertrophy (Figure 4). In mice with VSMC-specific overexpression of the p22phox subunit of Nox1, increased H\(_2\)O\(_2\) and increased aortic hypertrophy are observed in response to AngII.\(^{146,147}\) In the heart, cardiac remodeling and hypertrophy associated with oxidative stress contribute to reduced output and progression to heart failure.\(^{147}\)

ROS play a regulatory role in many proliferation pathways, and ROS production is tightly regulated during the cell cycle.\(^{148}\) Low levels of ROS regulate the activation of tyrosine kinase receptors by inactivating inhibitory protein tyrosine phosphatases.\(^{96}\) High levels of ROS inactivate growth factor signaling mediators and activate cell cycle arrest proteins. However, redox regulation of phosphatases may have different consequences in migration and proliferation. Similar to their role in migration, ROS-mediated inactivation of active site cysteines in PTP1B and Shp1/2 results in increased proliferation.\(^{96,148,149}\) Redox regulation of LMW-PTP, in contrast, mediates growth inhibition.\(^{150}\)

Growth factor stimulation also activates the redox-sensitive PI3K cascade (Figure 4). Src is activated, in part, by ROS-mediated disulfide bond formation between C245 and C487.\(^{151}\) Src activates PI3K, which phosphorylates the phospholipid PIP\(_2\) to its active form PIP\(_3\). This is reversed by the PIP\(_3\) phosphatase PTEN, which is inactivated by oxidation in cases of growth factor stimulation and oxidative stress.\(^{152}\) PI3K itself was identified as a target of oxidation by a high throughput method, but the functional consequence of such a modification is unknown. Because PIP\(_3\) is an activator of PH domain proteins such as PDK1 and Akt, oxidation of PTEN ultimately activates Akt. Akt is also oxidized on Cys310, which may regulate Akt binding to PDK1.\(^{154}\) The redox-sensitive p38 MAPK and MAPK-activated protein kinase-2 additionally complex with and activate Akt in response to AngII-stimulated ROS in VSMCs.\(^{156}\) Ultimately, Akt activates the transcription factors AP1 and NF-κB to promote cell cycle progression.

Several proliferation-related transcription factors are directly modified by oxidation. c-Jun and STAT3 are both negatively regulated by S-glutathionylation.\(^{157,158}\) S-glutathionylation was also observed in p53; however, it is not known how this modification may affect cardiovascular signaling.\(^{159}\)

Cardiac hypertrophy is usually considered as physiological (adaptive) or pathophysiological (progressing to heart failure). ROS play a role in both types of hypertrophy and can be either protective, as is the case for Nox4-induced preservation of capillary density during chronic pressure overload,\(^{160}\) or injurious, as with Nox2-mediated activation of ERK, ASK1, and NF-κB signaling pathways during AngII-induced hypertrophy. It is likely that redox signaling, as opposed to oxidative stress, plays a role in compensatory hypertrophy, whereas chronic stress conditions lead to a more generalized activation of ROS sensitive pathways. For example, treatment with antioxidants in neonatal rat cardiomyocytes reduces tumor necrosis factor-α- and AngII-induced hypertrophy.\(^{161}\) ROS have also been implicated in α-adrenergic receptor-mediated hypertrophy in rat ventricular myocytes.\(^{162}\) Oxidative stress is reported to oxidize cysteine residues on Ras directly,\(^{163}\) activating downstream signaling to PI3K, Raf, MAPK/ERK, and ERK1/2.\(^{167}\) This increased Ras activity in response to α-adrenergic receptor stimulation is blocked by the overexpression of thioredoxin in-1.\(^{165}\) ASK1, which is a redox-sensitive kinase because of thioredoxin binding in its reduced form,\(^{168}\) and Akt169 activate NF-κB, which transcribes inflammatory genes involved in the hypertrophic response. Oxidative stress can also mediate JNK activation and PKC-mediated activation of ETS-1, which contributes to the increase in matrix metalloproteinase activity and reduction in collagen synthesis that is observed in myocardial remodeling. The exact molecular target of ROS in these signaling pathways is often unclear and awaits further redox proteomic analysis.

ROS can also contribute to hypertrophy by regulating cardiac calcium channels and transporters, similar to their role in cardiac contraction.\(^{142}\) Sustained calcium release caused by oxidative stress contributes to cardiac remodeling.\(^{171}\) Hypoxia has been implicated in both mitochondrial-dependent and Nox-dependent cytosolic calcium release. Ca\(^{2+}\) channel blockers, such as amiodipine and bendipidine,\(^{174}\) and intracellular calcium chelators, such as BAPTA-AM,\(^{176}\) reduce oxidative stress, potentially by preventing the cross talk between mitochondrial ROS production and subsequent activation of Nox enzymes.\(^{177}\) In addition, the redox-sensitive deactivation and degradation of SERCA reduces calcium reuptake into the ER resulting in sustained calcium elevation in the cytosol.\(^{178}\)
Sustained elevation of intracellular calcium in cardiac muscle results in myocardial dysfunction and hypertrophy. One mechanism is via calmodulin-dependent activation of the serine/threonine phosphatase calcineurin (protein phosphatase 3). Protein phosphatase 3 dephosphorylates the transcription factor NFAT, which translocates to the nucleus to transcribe hypertrophy-promoting genes.\textsuperscript{179} NFAT activity and localization were demonstrated to be redox sensitive by Kalivendi et al.\textsuperscript{180} in a model of doxorubicin-mediated oxidative stress. The authors showed that the treatment with antioxidants inhibits Dox-mediated nuclear translocation of NFAT in cardiac cells. Similar results were found after intermittent hypoxia, where the SOD mimetic tempol prevents the activation of NFAT.\textsuperscript{181}

**Angiogenesis**

Neovascularization and angiogenesis are ROS-sensitive processes that combine many of the mechanisms described above, including proliferation, migration, and adhesion. Angiogenesis is induced by the activation of proangiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietin-1 in endothelial cells. Angiogenesis is particularly relevant in cancer biology because vascularization is required for tumors to grow greater than a few millimeters in diameter.

In tumors, hypoxia induces VEGF expression by activating hypoxia-inducible factor-1α in a ROS-dependent manner. Hypoxia-inducible factor-1α stability and subsequent activity can be regulated by redox-regulation of prolyl hydroxylase 2.\textsuperscript{182} Hypoxia-inducible factor-1α activation results in the production of angiopoietin,\textsuperscript{183} which activates Tie2 and increases VEGF expression, in turn activating the redox-sensitive VEGF receptor 2 (VEGFR2). Oxidation of VEGFR2 by disulfide linkage between C1199 and C1206 is inactivating and is reversed by peroxiredoxin II.\textsuperscript{184} VEGFR2 is also negatively regulated by dephosphorylation by PTP1B,\textsuperscript{185} which is inactivated by ROS.\textsuperscript{186} In this way, ROS can both activate and inhibit the activity of VEGFR2 in certain contexts. The activation of VEGFR2 results in increased ROS production by Nox via Rac1 activation\textsuperscript{187} and IQGAP1.\textsuperscript{188}

Osteopontin was recently identified by Lyle et al.\textsuperscript{189} as an important player in the redox regulation of angiogenesis. The authors found that increased translation of osteopontin occurs in response to the redox-regulated phosphorylation of 4E-BP1 at S65. Concomitantly, prolonged exposure to H$_2$O$_2$ increases osteopontin transcription. When ROS levels are reduced in vivo, osteopontin is not upregulated and neovascularization is impaired, supporting osteopontin’s role in ischemic neovascularization.\textsuperscript{190}

Elevated ROS production in angiogenesis also results in the activation of the transcription factors Sp1, Ref-1, NF-κB, p53, AP-1, and ETS-1 although the precise redox modifications remain unclear.\textsuperscript{191} These transcription factors upregulate gene expression of proteins involved in migration, adhesion,
survival, and proliferation, all of which promote neovascularization and angiogenesis.

Nox4 has also been implicated in angiogenesis because of eNOS activation in endothelial cells. Transgenic mice with an endothelial-specific overexpression of Nox4 recovered from hindlimb ischemia more rapidly and exhibited enhanced capillary formation. When the Nox4 transgenic mice were crossed with eNOS null mice, increased angiogenesis was not observed, suggesting that the H$_2$O$_2$ produced by Nox4 may be responsible for regulated eNOS expression, as proposed by Cai et al. Conversely, in global Nox4 knockout mice, angiogenesis is impaired. Although the mechanisms by which Nox4 induces angiogenesis have not been fully worked out, overexpression of Nox4 in vitro was found to increase receptor tyrosine kinase activation and subsequently ERK phosphorylation.

**ER Stress and Autophagy**

The ER is involved in multiple cellular processes, including calcium homeostasis, protein transport/secretion, and protein folding/post-translational modification. There is a certain amount of error that occurs in this process, which results in the production of unfolded and misfolded proteins that can contribute to ER stress. To counteract this, the unfolded protein response (UPR) can regulate the rate of secretion of proteins, as well as recognize and degrade misfolded proteins and protein aggregates via the proteasome and autophagy. ER stress generally increases cellular ROS generation, in part by increasing calcium release, which increases ROS production by the mitochondria. This completes a positive feedback loop as oxidative stress contributes to ER stress and is directly involved in protein secretion, folding, and degradation. In this section, we will discuss the influence of ROS on ER stress and proteasome- and autophagy-related pathways.

The ER lumen is an oxidative environment compared with the cytosol, with a high ratio of GSSG/GSH. A recent study by Wu et al. however, did not find increased free H$_2$O$_2$ in the ER compared with the cytosol using the redox probe HyPer, suggesting that much of the observed redox potential is protein bound. The oxidative ER environment is conducive to the formation of disulfide bonds. A key class of thiol oxidoreductase chaperone proteins in the ER, protein disulfide isomerases (PDIs), catalyze the formation and breakage of disulfide bonds between cysteine residues of proteins in a process known as oxidative folding. PDI has emerged as a key redox-sensitive player in protein folding and ER stress. PDI is composed of 4 thioredoxin domains and contains redox-sensitive cysteines whose oxidation state can influence the protein binding and activity of PDI. In its reduced form, PDI acts as an isomerase, whereas oxidation of PDI enables it to form disulfide bridges. Reduced PDI can bind another ER oxidoreductase, ER oxidoreductin 1 (Ero1), which is capable of oxidizing PDI and produces H$_2$O$_2$ in the ER as a consequence (Figure 5). Ero1 is thought to be essential for PDI activity by generating internal disulfide bonds and transferring them via PDI to target proteins. Recent evidence suggests that the inhibition of PDI in cells can contribute to ER stress and apoptosis. Toldo et al. found that overexpression of PDI protects against myocardial damage in an acute MI mouse model. The authors suggest that PDI activity is antiapoptotic, in part, by increasing the activity of SOD1.

Oxidative stress in the ER can result in the misfolding of proteins, which are recognized by the chaperone binding immunoglobulin protein (Figure 5). Binding to misfolded proteins causes binding immunoglobulin protein to dissociate from UPR transmembrane proteins, including protein kinase-like ERK, activating transcription factor 6, and inositol requiring protein-1, leading to their activation. Notably, in human aortic smooth muscle cells, activation of inositol requiring protein-1 by 7-ketocholesterol increases Nox4 expression.

Additional information about ROS and the UPR can be found in a recent review by Santos et al.

Nox proteins have also been implicated in ROS production during ER stress. Overexpression of PDI in VSMCs results in increased ROS, Nox1 mRNA, and Nox4 expression in response to AngII. In addition, PDI was found to associate with Nox subunits p22phox, Nox1, and Nox4, potentially regulating trafficking and activity of the enzymes. Moreover, Nox4 has been shown to localize to the ER and to activate PTP1B, which regulates epidermal growth factor receptor trafficking.

In addition to mediating the physiological activation of PTP1B, ER-resident Nox4 also plays a role in ER stress. In endothelial cells, ER stress induced by HIV-1 Tat and tumicin activates Nox4-derived ROS production in the ER. This results in Ras and ERK1/2 activation, which induces autophagy. Autophagy involves the breakdown of excess or dysfunctional cellular components by lysosomes. Autophagy can be activated by ROS-mediated protein misfolding, as above, or via the absence of growth factors to maintain cellular energy levels, which results in the inactivation of mammalian target of rapamycin (mTor). mTor activity is redox sensitive via the Tsc1/2-Rheb GTPase pathway (Figure 5). Yoshida et al. demonstrated that the thiol cross linking agent PAO activates mTor1. The authors propose that inactivating cross linking of cysteine residues in Tsc1/2 could be responsible for the observed regulation of mTor, but mutational analysis has not been performed yet to verify this assumption. Thus, ROS signaling pathways are implicated in both activation and inhibition of autophagy, depending on the ER stress stimulus.

There is compelling evidence that autophagy plays a role in cardiovascular disease, and in many cases this leads to cell death. In the case of MI and ischemia/reperfusion injury, cardiomyocytes undergo oxidative stress, which results in autophagy-mediated cell death. Although autophagy-induced cell death in cardiomyocytes in this case is detrimental, there are scenarios in which this cell death can be beneficial. The drug everolimus, for example, induces macrophages to undergo autophagy-mediated cell death in atherosclerotic plaques. Autophagy can also reduce cardiac hypertrophy in the cases of heart failure.

**Apoptosis and Senescence**

Apoptosis, or programmed cell death, is a process whereby cells shrink and self-phagocytize with minimal inflammation occurring in the surrounding tissue. This occurs naturally during development, but pathologically occurs in response to DNA damage and certain types of cell stress. In some contexts, apoptosis can be protective, such as preventing cells that...
may become malignant from taking hold. However, in the cardiovascular system, apoptosis can result in tissue damage and reduced cardiac function. Much of the damage to the heart that occurs after MI is because of apoptosis of tissue that was oxygen deprived. During reperfusion of these oxygen-deprived tissues, large amounts of damaging ROS are produced enzymatically by many sources, including XO, the electron transport chain, and Nox. Apoptosis also contributes to cardiomyopathy because of remodeling in chronic hypertension. Apoptosis signaling is closely related to senescence, with the expression and activity of key regulators, such as p53 determining cell fate in cases of cellular stress.

Senescence is an irreversible halt in replication that occurs caused by damage or aging. Apoptosis and senescence are both consequences of oxidative stress in cardiovascular cells, including cardiomyocytes, endothelial cells, and VSMCs. Although the 2 endpoints are different, many of the signaling pathways that contribute to senescence or death are related. The ultimate outcome of oxidative damage is controlled by the level of damage and whether the cell is able to repair itself.

Cultured cardiomyocytes undergo apoptosis after exposure to as little as 10 μmol/L of H₂O₂, which induces the tumor suppressor transcription factor p53 and Bad expression (Figure 6), as well as cytochrome C release. Recent evidence by Del Re et al implicates oxidative stress–mediated oxidation and activation of K-Ras in this response, which activates Bax via Mst1 and BCL-xL to promote apoptosis. In addition, p53 is a redox-sensitive component of the DNA damage response. Briefly, DNA breaks and certain types of oxidative modifications are sensed by repair proteins and damage sensors. If the amount of damage passes a threshold, ATM kinase is activated, which phosphorylates downstream tumor suppressors such as CHK2, H2AX, and p53. p53 is a key determinant of whether cells enter apoptosis or senescence. p53 activity promotes the expression of p21, a master regulator of senescence in oxidative stress. p53 also modulates the expression of caspase proteases that play an essential role in apoptosis.

Although DNA damage is a major cause of apoptosis, other cellular signals can likewise result in apoptosis. As noted above, increased oxidative stress can result in heightened protein misfolding and aggregation leading to ER stress. In ER stress, the serine/threonine protein kinase inositol requiring protein-1 is activated, which activates the UPR and, in cases of severe stress, initiates apoptosis by degrading prosurvival miRNAs, as well as activating Jnk and Bax, thus initiating apoptosis by promoting cytochrome C release from the mitochondria via the mitochondrial apoptosis-induced channel pore formation. Activation of the UPR also activates CEBP-homologous protein, which inactivates Bcl-2 and promotes apoptosis by multiple mechanisms. Treatment with the antioxidant N-acetyl cysteine prevents MAPK activation upstream of Jnk, indicating that ROS are involved. One potential mechanism is via ROS-mediated activation of thioredoxin/Ask1 (Figure 6). Activation of thioredoxin/Ask1 promotes Jnk activation via MKK4/7. In addition, ROS control the expression and activity of Bax/Bad regulator Bcl-2 and Bad itself by regulating ubiquitination and phosphorylation. Mice lacking proapoptosis genes, such as Bax, exhibit less myocardial cell death after ROS-producing reperfusion injury. However, simply eliminating apoptosis genes does not always prevent cell death. In Bax/Bak double-knockout cells, DNA damage induces a ROS-mediated necrosis process induced by p53 and cathepsin.

Cytokine signaling by tumor necrosis factor-α has a demonstrated role in apoptosis via the ROS-dependent activation of Jnk. Kamata et al showed that treatment with antioxidants prevents tumor necrosis factor-α–mediated cell death by reducing oxidation of the MAPK phosphatases. ROS activation of NF-κB conversely acts to inhibit Jnk activity and reduces ROS production to promote cell survival.
To protect against apoptosis caused by ROS, protective genes, such as PTEN and Nrf2, are activated. Nrf2 induces the expression of the cell survival gene Bcl-2 to inhibit p53 and prevent apoptosis. Nrf2 knockdown during hypoxia was demonstrated to reduce cell survival, further supporting Nrf2’s protective role. Nrf2 also regulates mitochondria function, promotes the expression of antioxidant enzymes, and reduces the GSSG:GSH ratio to reduce additional oxidative damage.

**ROS and Oxidative Stress in Cardiovascular Disease**

As noted above, ROS are key players in normal cardiovascular physiology and signaling, but many redox-sensitive pathways are also activated during the development of disease. Moreover, certain catastrophic events, such as mechanical injury or the disruption of blood flow, result in increased local ROS production and reduced capacity of the cells to degrade or disperse these reactive molecules. Oxidative stress in turn influences signaling pathways that contribute to altered cell migration, proliferation/senescence, apoptosis, ER stress, and autophagy, which ultimately contribute to cardiovascular disease.

Exposure of endothelial and smooth muscle cells to circulating hormones and growth factors stimulates localized production of ROS that modify specific signaling molecules or alter cellular GSH:GSSG ratios that regulate entire circuits. AngII, for instance, is well known to induce hypertrophy in part because of localized production of ROS. The activation of the AT1 receptor by AngII rapidly induces ROS production by the activation of Nox and because of positive feedback, additional ROS is produced in the mitochondria. PDGF is a potent migratory stimulus for VSMCs that activates ROS production by activating Rac.

When normal signaling goes awry, or when ROS are produced in excess, the result is cardiovascular pathology. For example, high blood pressure is associated with impaired vasorelaxation and increased ROS production by Noxes. One key mechanism by which ROS contributes to vessel tone is via the inactivation of NO by $O_2^{•−}$. $O_2^{•−}$ produced by Nox can react with NO to form OONO$^{−}$, preventing the vasodilatory effect of NO. ROS also contribute to hypertension by regulating signaling in the renal and central nervous systems.

In atherosclerosis, lesions form in areas of oscillatory or disturbed blood flow. This may be, in part, because of sensing of stretch or disturbed flow by baroreceptors and subsequent CNS feedback, but cells in culture also exhibit increased ROS production in response to mechanical stimuli, suggesting that a redox-coupled mechanosensor is present in the cells themselves. Cyclic stretch and shear stress from disturbed flow is associated with Nox1 activation and vascular remodeling.

One of the most rapid and severe inducers of oxidative stress in the cardiovascular system is reperfusion after a period of ischemia. Ischemia can result from microvascular injury, embolism, occlusion caused by atherosclerosis, MI/stroke or during surgical procedures, such as transplants. On the restoration of blood flow, vascular cells produce more ROS and less NO. In cases of prolonged ischemia, xanthine dehydrogenase begins to function in reverse as an oxidase, producing peroxynitrite.

![Redox signaling in apoptosis and senescence](image)

**Figure 6. Redox signaling in apoptosis and senescence.** Dark gray, Redox modified proteins. ATMK indicates ataxia telangiectasia mutated kinase; Bad, Bcl2-associated agonist of cell death; Bak, Bcl-2 homologous antagonist killer; Bax, Bcl-2-associated X protein; Bcl-2, B-cell CLL/lymphoma 2; CHK2, checkpoint kinase 2; CHOP, CEBP-homologous protein; ER, endoplasmic reticulum; ETC, electron transport chain; Jnk, c-Jun N-terminal kinase; MKK4/7, mitogen-activated protein kinase kinase 4/7; TNF, tumor necrosis factor; TNFR, TNF receptor; UPR, unfolded protein response; and XO, xanthine oxidase.
excess ROS. Because of the rapid and severe influx of ROS and inflammation that occurs in ischemia/reperfusion injury, much of the surrounding tissue undergoes apoptosis if left untreated. Reperfusion injury is thought to be a major contributor to death associated with MI. Experimental efforts targeting antioxidants and scavengers to affected areas during reperfusion to reduce oxidative damage show promise; however, with the exception of the scavenger edaravone, which has limited use in the treatment of ischemic stroke, there is little translation of this research to the clinic.

Diabetic vascular disease is another area in which strong support exists for a role of ROS (for details, see a recent review by Giacco and Brownlee). ROS production by elevated glucose occurs by multiple mechanisms. Glucose can react with proteins to form an Amadori product, which is oxidized to form an advanced glycation end product. Advanced glycation end product activates the RAGE receptor to stimulate intracellular ROS production via Nox1. Mitochondrial ROS also play a major role in diabetic vascular disease. Diabetic oxidative stress results in cardiac and vascular dysfunction including altered vascular tone, inflammation, and proliferation. Diabetics exhibit increased rates of atherosclerosis and cardiomyopathies, likely in part because of hyperglycemia-induced oxidative stress.

Oxidative stress is also thought to be both a cause and a consequence of aging. ROS produced in the mitochondria as a consequence of respiration can damage mitochondria and cellular components over time, which ultimately results in more ROS production and oxidative stress. A study of rat cardiac mitochondria found increased oxidative stress markers and antioxidant enzyme activity in aged (24 months) rat cardiac interfibrillar mitochondria compared with tissue isolated from younger (6 months) rats. In addition, mice with a mitochondrial targeted overexpression of catalase exhibit prolonged lifespan and reduced cardiac aging.

Conclusions

Given the fact that ROS act as signaling molecules in so many cellular processes, it can be difficult to separate pathological oxidative stress from normal physiological signaling. Whereas once oxidative stress was defined as an oxidative overload in an entire cell or tissue, recent work supports the idea of localized redox imbalance within a cell having pathological signaling consequences. Such is the case in growth factor induction of ROS signaling in fibrosis and atherosclerosis, which causes pathological cell migration and extracellular matrix deposition. The development of therapeutics to target oxidative stress pathways must therefore focus on subcellular ROS or downstream molecular signals to avoid undesirable off-target effects. Before such therapies can be developed, we must have a better understanding of the ROS-sensitive signaling pathways in the cardiovascular system. Additional work should focus on the identification of specific molecular targets that regulate redox circuits which modify cellular functions; dissecting the role of compartmentalization of ROS-generating and ROS-catabolizing enzymes; and understanding the whole body consequences of inhibiting specific sources of ROS to predict side effects of targeted therapies.

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References


20. Jerke U, Rolle S, Purfürst B, Luft FC, Nauseef WM, Kettritz R. β2 integrin-mediated cell-cell contact transfers active myeloperoxidase from...


Circulation Research January 30, 2015


120. Liu G, Abramson JJ, Zable AC, Pessah IN. Direct evidence for the existence and functional role of hyperreactive sulfhydryls on the ryanodine receptor-triadin complex selectively labeled by the coumarin maleimide


Mistry Y, Poolman T, Williams B, Herbert KE. A role for mitochondrial oxidants in stress-induced premature senescence of...


Regulation of Signal Transduction by Reactive Oxygen Species in the Cardiovascular System

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