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Forum Review

Reactive Species-Mediated Regulation of Cell Signaling and the Cell Cycle: The Role of MAPK

YAVUZ CAKIR and SCOTT W. BALLINGER

ABSTRACT

Cardiovascular disease development is significantly influenced by the effects of reactive species (RS). By virtue of their controlled production, regulation, and reactive nature, RS play important roles in the modulation of cellular signaling, growth, and death in the vasculature. Concentration gradients are important in determining the effects of RS. Low to moderate concentrations of RS act as mediators in signaling cascades and gene regulation, whereas high levels of RS cause cellular damage and death. Because a dual redox regulation state seems to exist in several signaling cascades, *e.g.*, RS often induce upstream initiating events, whereas downstream events are reliant on reductive processes, alterations in cellular redox states influence the activation/inactivation of signaling events and transcription factors. In this review, the relationships between RS, specific signal transduction pathways, and aspects of cell-cycle control are discussed. *Antioxid. Redox Signal.* 7, 726–740.

INTRODUCTION

Several disease states, including cardiovascular disease (CVD), are significantly influenced and perhaps initiated by altered regulation of reactive species (RS) (for review, see 30). The increasing importance of RS in the development of vascular diseases has become a focus of many recent articles, and RS play essential roles in modulation and mediation of cell function and damage (30, 43). For example, RS play a significant role in the development of atherosclerosis, a chronic inflammatory disease of the arterial intima, characterized by the formation of fibrous plaques composed of vascular smooth muscle cells (VSMCs), lipids, leukocytes, and extracellular matrix. Endothelial cell injury and dysfunction are thought to be the first step in the development of atherosclerosis, which ultimately leads to arterial wall thickening, plaque rupture, and occlusion that result in myocardial ischemia and infarction (90). Although there is a degree of controversy regarding the precise nature and sequence of *in vivo* events that mediate atherogenesis, it is clear that RS are components of the process (90). In this review, the relationships between RS, specific signal transduction pathways, and aspects of cell-cycle control that are believed to be important factors in the development of vascular disease are discussed.

REACTIVE OXYGEN AND NITROGEN SPECIES

RS or free radicals are atoms or groups of atoms with one or more unpaired electrons. They are often grouped into two categories: reactive oxygen species (ROS) and reactive nitrogen species (RNS). Both are produced during several physiological processes, including oxidative respiration and inflammation. Superoxide ($O_2^{\cdot -}$) and nitric oxide ($^{\cdot}NO$) represent the fundamental or "building block" species for ROS and RNS, respectively. Their generation and the resultant species related to their formation are important factors in vascular cell function and damage.

Superoxide ($O_2^{\cdot -}$) generation

$O_2^{\cdot -}$ is a negatively charged free radical that usually represents the first step in ROS production. It can be produced en-

Division of Molecular and Cellular Pathology, The University of Alabama at Birmingham, Birmingham, AL.

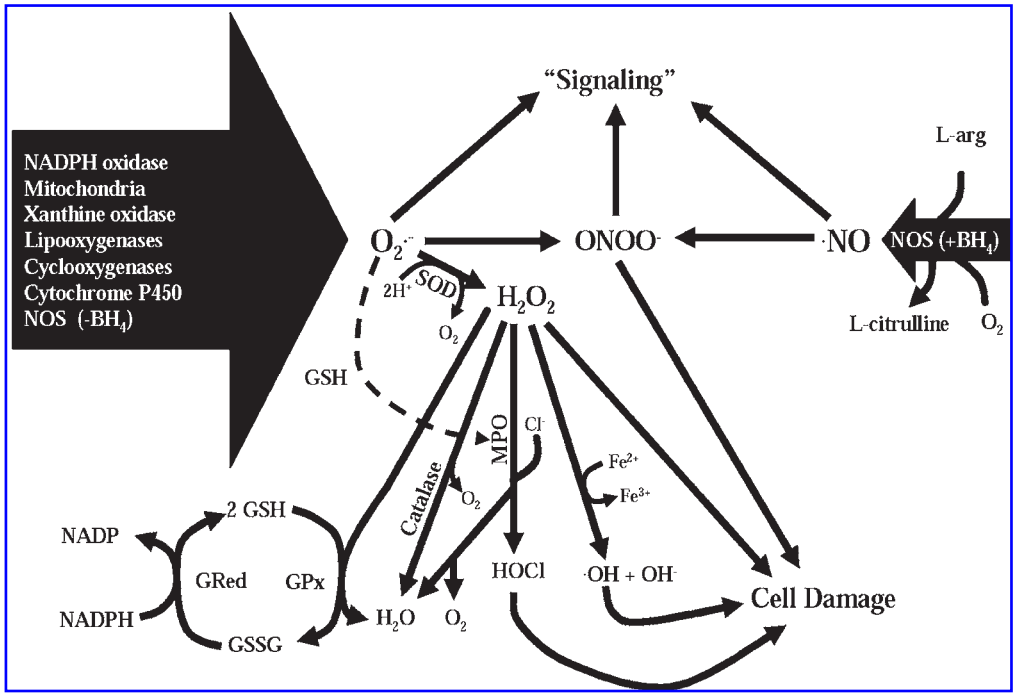

FIG. 1. Potential sources and actions of ROS and RNS. Superoxide ($O_2^{\bullet -}$) is generated from various sources and can enzymatically be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 can act as a signaling molecule, be dismutated by catalase, or be reduced by glutathione (GSH) to form water. Myeloperoxidase (MPO) degrades H_2O_2 to water and oxygen, producing hypochlorous acid (HOCl), a potent oxidant and reactant. MPO also reacts with $O_2^{\bullet -}$ in reduction reactions that increase HOCl production (dashed line). In the presence of transition metals, H_2O_2 can produce hydroxyl radicals ($^{\bullet}OH$) and hydroxide anion (OH^-). Nitric oxide ($^{\bullet}NO$) is produced by nitric oxide synthase (NOS), can act as a signaling molecule, or can react with $O_2^{\bullet -}$ to produce peroxynitrite ($ONOO^-$), which is capable of mediating a variety of biological effects, including cell damage and apoptosis.

zymatically or as a metabolic by-product (Fig. 1). However, the relative contribution of each $O_2^{\bullet -}$ production pathway toward the pathobiology of vascular disease has not been firmly established. The following discussion is not intended to be a comprehensive treatise on cellular sources of $O_2^{\bullet -}$, but provides examples of commonly investigated sources.

The NADPH oxidases are some of the more widely examined sources of enzymatically produced $O_2^{\bullet -}$. The NADPH oxidase found in phagocytic cells is a membrane-bound flavohemoprotein that produces low levels of $O_2^{\bullet -}$ until the cell becomes activated via signaling events that significantly increase NADPH oxidase activity (52). In this respect, the enzyme is probably an important source of $O_2^{\bullet -}$ in inflammation. Although both endothelial cells and VSMCs contain their own forms of NADPH oxidase (107), it is not yet apparent whether these isoforms are regulated and function in the same fashion as the phagocytic cell oxidase. Induction of NADPH oxidase and related $O_2^{\bullet -}$ generation have significant impact on the expression of the genes regulating signaling pathways in both vascular endothelium and smooth muscle cells (52, 78, 107). Increased ROS produced by NADPH oxidase may contribute to the oxidation of tetrahydrobiopterin (BH_4), a required cofactor for nitric oxide synthase (NOS) activity (Fig. 2). Loss or oxidation of BH_4 can result in production of $O_2^{\bullet -}$ by NOS via a NADPH oxidase-like activity (64). Studies in animal models lacking vascular endothelial NADPH oxidase activity show decreased atherosclerotic lesion formation (9). However, other studies examining the effects of decreased NADPH oxidase activity failed to show significant differences in atherogenesis, suggesting that other factors may play roles in influencing the onset of CVD (45, 58). Regardless, NADPH oxidase is a potentially important source of $O_2^{\bullet -}$ and, therefore, vascular oxidative stress.

Xanthine oxidase (XO) is derived from xanthine oxidoreductase, an enzyme that typically exists as xanthine dehydrogenase (XDH). Upon thiol oxidation, XDH is rapidly converted to XO (Fig. 2), by an enzyme involved in purine degradation that yields $O_2^{\bullet -}$ and hydrogen peroxide (H_2O_2), typically in a 1:2 ratio (85, 105, 114). Increased NADPH oxidase activity has been associated with increased XO activity, suggesting that NADPH oxidase may play a role in the conversion of XDH to XO (78, 120). Increased levels of circulating XO have been noted in a variety of diseases, including CVD (105). Circulating XO can specifically bind vascular endothelium, giving rise to the possibility that non-endothelial derived XO can contribute to increased vascular oxidant production (114).

Mitochondria are the primary cellular sources of $O_2^{\bullet -}$ (108) (Fig. 3). Whereas the majority of oxygen consumed by the mitochondrion is converted to water at complex IV, oxygen can pick up electrons directly from the flavin dehydrogenases and ubiquinol ($CoQH_2$) to generate $O_2^{\bullet -}$ (119). Deficiencies

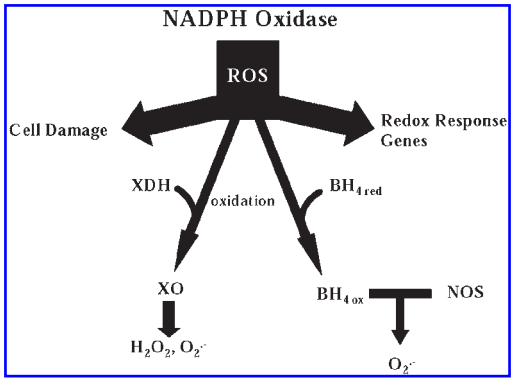

FIG. 2. NADPH oxidase in cell damage and regulation. Low to moderate ROS production mediated by NADPH oxidase can influence the expression of redox response genes, whereas high ROS production contributes to cell damage directly, or indirectly by converting xanthine dehydrogenase (XDH) to xanthine oxidase (XO) via thiol oxidation. Likewise NADPH oxidase-produced ROS are capable of oxidizing BH_4, which can increase $O_2^{\bullet -}$ production by NOS.

in mitochondrial antioxidants that scavenge $O_2^{\bullet -}$ can promote the onset of CVD *in vivo*, whereas their overexpression can be cardioprotective, suggesting that mitochondrial generated oxidants may play a role in atherogenesis (7, 21). Several factors, including $^{\bullet}NO$ concentration, cytokines, and electron transport efficiency, play important roles in modulating mitochondrial $O_2^{\bullet -}$ production (18, 84, 94).

$^{\bullet}NO$ generation

In the presence of molecular oxygen, NOS oxidizes one of the guanidino nitrogens of L-arginine to yield $^{\bullet}NO$ and L-citrulline (Fig. 1). There are three isoforms of NOS; all require L-arginine, O_2, NADPH, FAD, FMN, heme, and BH_4 for activity. Endothelial and neuronal NOS (eNOS and nNOS, respectively) are constitutively expressed and are regulated by cellular Ca^{2+} concentrations, whereas inducible NOS (iNOS) appears to be transcriptionally regulated (73).

The constitutive forms of NOS function as regulators of cell growth and function. Physiologically, $^{\bullet}NO$ diffused from the vascular endothelium is a strong endogenous vasodilator and plays important roles in cell proliferation, platelet aggre-

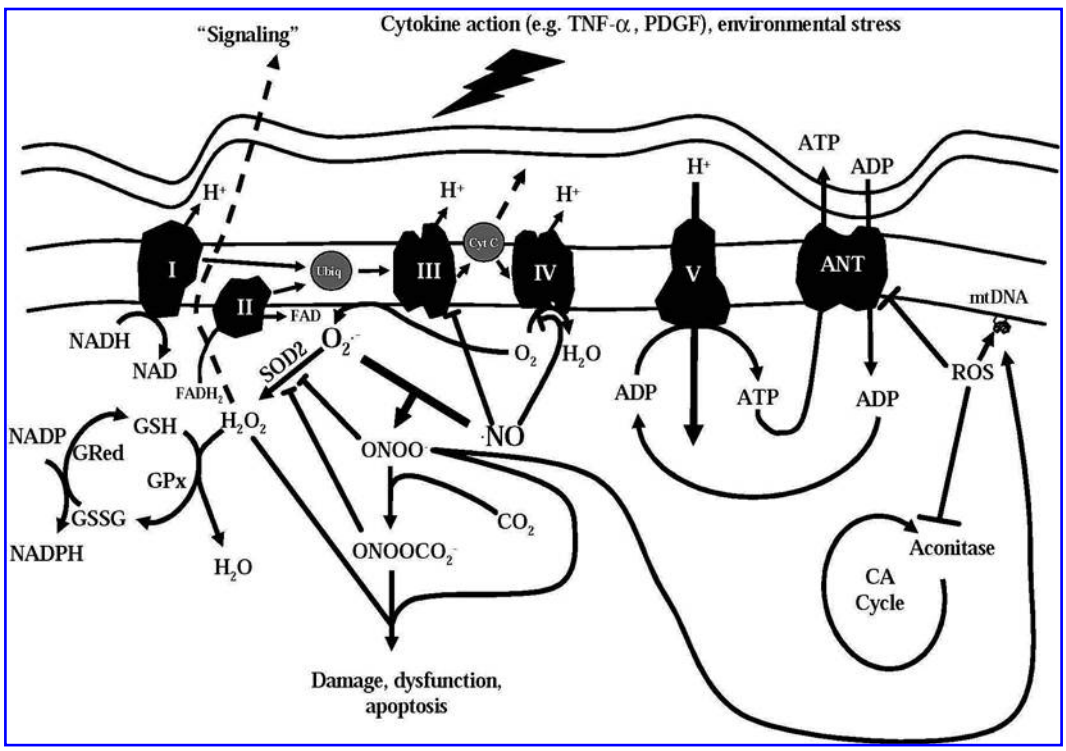

FIG. 3. Mitochondrial effects of ROS/RNS. $^{\bullet}NO$ can impair electron flow at cytochrome oxidase (complex IV) by oxidizing the heme group of cytochrome aa3, and is competitive with O_2. $^{\bullet}NO$ also inhibits the bc1 segment of complex III, resulting in increased autooxidation of ubisemiquinone and $O_2^{\bullet -}$ production. At low $^{\bullet}NO$ concentrations, the dismutation of $O_2^{\bullet -}$ and formation of H_2O_2 are favored, which can subsequently diffuse from the mitochondrion and act as a signaling molecule or be reduced by GSH to form H_2O. At higher $^{\bullet}NO$ concentrations, $O_2^{\bullet -}$ reacts with $^{\bullet}NO$ to form $ONOO^-$, which may promote cytochrome *c* release and apoptosis, inhibit complexes I and V, and inactivate aconitase. In the presence of CO_2, $ONOO^-$ yields nitrosoperoxycarbonate ($ONOOCO_2^-$), which has been proposed to diminish oxidation and increase nitration reactions. Increased levels of ROS cause mitochondrial DNA (mtDNA) damage and also reduce the transport and exchange of adenine nucleotides across the inner membrane by inactivating the adenine nucleotide translocase (ANT). Hence, modulation of ROS/RNS concentrations within the mitochondrion contributes to altered mitochondrial function, damage, and apoptosis.

gation, VSMC regulation, and leukocyte adhesion (16). The regulation of vascular blood flow and tone by endothelial derived •NO is related to its binding of heme groups of soluble guanylate cyclase, resulting in its activation and production of cyclic GMP, with subsequent VSMC relaxation (91). •NO has been shown to inhibit RNA and protein synthesis in VSMCs and inhibit VSMC proliferation (34, 91). In contrast, suppression of •NO synthesis is associated with increased endothelin-1 (ET-1) and platelet-derived growth factor (PDGF) expression, which are strong activators of VSMC growth and proliferation (103). Similarly, long-term suppression of •NO formation in rats induces VSMC proliferation (104). Loss of eNOS activity causes hypertension in mice and renders them more susceptible to CVD, potentially due to a noted increase in iNOS activity (46).

In contrast, in response to inflammatory stimuli, iNOS rapidly generates •NO at levels several times greater than its constitutive counterparts to affect cells in seconds (73). Although iNOS can be expressed at low levels in normal VSMCs, its expression can be significantly induced by cellular stress; activation of iNOS in atherosclerotic lesions can be stimulated in macrophages and VSMCs by several cytokines, including interleukin-1, tumor necrosis factor-α (TNF-α), and interferon (73). VSMC infiltration and iNOS overexpression have been observed in the intima of atherosclerotic vessels of long-term cholesterol-fed rabbits (10), and similar studies in human atherosclerotic tissues have also shown the overexpression of iNOS mRNA in macrophages and VSMCs (73). Animals lacking iNOS activity are more resistant to atherosclerotic lesion formation, indicating that iNOS activation and inflammatory response are important in atherogenesis (61, 74). Therefore, derangement in the release or production of •NO contributes to altered vasoregulation and the initiation and development of atherogenic events in the vasculature.

RS derived from $O_2^{\bullet-}$ and •NO

Peroxynitrite ($ONOO^-$). Production of $O_2^{\bullet-}$ and •NO results in the generation of additional RS that can contribute to cellular damage or modulate signaling molecules (Fig. 1). One of the more studied effects of $O_2^{\bullet-}$ is its diffusion-limited reaction with •NO to form $ONOO^-$, a molecule that has been implicated in the initiation of a number of deleterious biological effects, including protein modification, DNA damage, and lipid peroxidation (6, 66). The reactivity and biological effects of $O_2^{\bullet-}$, •NO, and $ONOO^-$ formation are influenced by several factors, including the relative concentrations of $O_2^{\bullet-}$ and •NO, antioxidant concentration gradients, and local cellular phasic (*e.g.*, hydrophobic versus hydrophilic environments) and compartmentalization (*e.g.*, organelles) characteristics (40). For example, in the mitochondrion, it is likely that $ONOO^-$ reacts with CO_2 to form a nitrosoperoxycarbonate intermediate ($ONOOCO_2^-$) (Fig. 3), which efficiently mediates nitration reactions ($ONOOCO_2^-$ leads to diminished oxidation yields with a concomitant increase in nitration reactions) (40, 49). The observations that 3-nitrotyrosine levels (a marker for RNS) are increased in atherogenic tissues and that CVD risk factor exposure increases their levels provide evidence that $ONOO^-$ can be an atherogenic factor (60, 66).

H_2O_2, hydroxyl radical (•OH), and hypochlorous acid (HOCl). Superoxide dismutase (SOD) converts $O_2^{\bullet-}$ into H_2O_2, which is a relatively stable species that is freely diffusible and, depending on its concentration and duration of production, can serve as a signaling molecule or damaging agent (Fig. 1). Studies have implicated H_2O_2 in the induction of a number of signaling events and expression of proteins, including aspects of vasoregulation and the stimulation of sGC (4, 20, 39, 56). High levels or chronic production of H_2O_2 has been shown to cause oxidation of protein and lipids, and induce DNA damage in the cell (6). In the presence of transition metals, H_2O_2 can yield highly reactive •OH (48). Oxidative stress mediated by H_2O_2 and •OH has been suggested as a primary effector in the age-related decrement of mitochondrial oxidative phosphorylation efficiency and adenine nucleotide translocase (ANT) activity (116). In the presence of myeloperoxidase (MPO), H_2O_2 is degraded to water and oxygen, also producing HOCl, which is a potent oxidant and at physiologic doses reacts rapidly with a myriad of biological molecules (41). HOCl inactivates enzymes, cross-links proteins, and depletes cells of glutathione, ATP, and ascorbate. Halogenated proteins are secondary products of the reaction of HOCl (or HOBr) with amines (41). MPO also reacts rapidly with $O_2^{\bullet-}$ in reduction reactions that increase the production of HOCl (86).

ROS and RNS effects on the mitochondrion

Mitochondria are important cellular organelles with critical functions related to energy production, cell signaling, growth, death, and thermogenesis. Somewhat paradoxically, mitochondria are both significant sources and targets of oxidative and nitrosative stress (Fig. 3).

Whereas ROS and RNS are capable of targeting a variety of subcellular components, the mitochondrial membranes, proteins, and mitochondrial DNA (mtDNA) are particularly sensitive to oxidative and nitrosative damage (108, 115). The mtDNA is sensitive to RS-mediated damage due to the lack of both protective histone and nonhistone proteins, a relatively limited DNA repair capability compared with the nucleus (7, 33), and its proximity to the inner membrane, making it especially susceptible to lipophilic species, and electrophiles generated within the membrane. Both $O_2^{\bullet-}$ and •NO are present in the mitochondrion, providing the fundamental species for further ROS and RNS generation within the mitochondrion (35).

In vitro studies have shown that RS induce a variety of effects, including preferential and sustained mtDNA damage, altered mitochondrial transcript levels and mitochondrial protein synthesis, and lowered mitochondrial redox potentials in vascular cells (6). Nitration of specific adducts on the SOD2 protein results in its inactivation (75), and decreased vascular SOD2-specific activities are associated with increased exposure to CVD risk factors (60). Several other important mitochondrial proteins are inhibited by RNS, including the mitochondrial electron transport complex proteins (18). Increased oxidative stress has been shown to inhibit ANT and aconitase activities (116); *ex vivo* studies in rat heart have shown that ischemia reduces myocardial ANT activity, and reperfusion further contributes to the loss of both ANT and oxidative phosphorylation capacities (31).

In vivo studies have shown that chronic ischemia increases both mtDNA deletions in human heart tissue (23) and cardiac mitochondrial sensitivity to inhibitors of cellular respiration (15). CVD patients have increased mtDNA damage compared with healthy controls in both the heart (23) and aorta (7). CVD risk factors increase aortic mitochondrial damage and, when combined, can act synergistically on mitochondrial damage and function (60). Cardiotoxic RS generators increase mtDNA deletions and lipid peroxidation in the myocardial mitochondria, and decreased SOD2 activity hastens atherosclerotic lesion development and increases aortic mtDNA damage in hypercholesterolemic mice (7, 31) Hence, it appears that mitochondrial damage and dysfunction can hasten CVD development, and that factors that make mitochondria more resistant to RS-mediated stress will be cardioprotective (7, 21). The demonstration that ·NO modulates mitochondrial electron transport through a reversible inhibition of complex IV, which influences ATP production, $O_2^{\bullet-}$ generation, and oxygen diffusion, suggests that the relative balance between ·NO, $O_2^{\bullet-}$, antioxidants, and additional factors (*e.g.*, mitochondrial uncoupling proteins, which can reduce mitochondrial ROS production) within the mitochondrion probably play an important role in regulating mitochondrial functions (18). Any factors that alter this balance can modify mitochondrial performance and impact cellular function.

SIGNALING PATHWAYS

Changes in the redox status of the cell

The interrelationships between the reductive and oxidative events that occur within the cell play an important role in the proper functioning of proteins and signaling molecules. In many cases, redox changes appear to be mediated through oxidation or reduction of protein sulfydryls, which can alter protein conformation and reduce or enhance DNA-binding activities and protein complex formations necessary for many signaling events (59, 102). In this regard, changes in the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) can influence the oxidation of protein cysteinyl thiols (59). Physiologically, SOD enzymes convert $O_2^{\bullet-}$ into H_2O_2, which can be metabolized to H_2O by GSH and glutathione peroxidase. This reaction increases the intracellular levels of GSSG, which may be converted back to GSH by NADPH-mediated GSH reductase (Figs. 1 and 3).

Increased oxidative stress can enhance the intracellular concentration of GSSG at the expense of GSH, potentially altering cellular redox status. For example, cellular redox signaling pathways may induce the oxidation of the sulfhydryl groups of cysteine residues on proteins to form chemical structures such as disulfide bonds (RSSR), sulfinic acid (RSO_2H) or sulfenic acid (RSOH) (106). The reduction of disulfides and the conversion to their previous conformation are enzymatically mediated by thiol reductants such as GSH, thioredoxin (TRX), and glutaredoxin (106). Restoration of GSSG and oxidized TRX to their reduced forms is reliant on NADPH-dependent GSH and TRX reductases (Fig. 4) (106). Consequently, alterations in cellular thiol redox status can influence the cellular redox state and the activation or inactivation of signaling pathways.

An extracellular stimulus causes a variety of responses by inducing several signaling molecules, including receptor tyrosine kinases (RTKs), serine/threonine kinases, phospholipases, and Ca^{2+} (53). By virtue of their diffusibility, controlled production, and regulation, ROS and RNS can serve as facile signaling molecules in the regulation of VSMCs by modulating several signaling cascades.

ROS generation by signaling pathways: ligand-activated stimulation

Several cytokines and growth factors induce the production and release of ROS that mediate signaling events in the vasculature (Fig. 4). The RTKs are directly linked to intracellular enzymes and phosphorylate their substrate proteins on tyrosine residues. Binding of growth factors to the extracellular domains of these receptors induces their cytosolic kinase domains, leading to phosphorylation of the receptor and intracellular target proteins. Growth factor or cytokine stimulation of membrane-bound RTKs, such as the epidermal growth factor (EGF) receptor (EGFR), causes rapid and significant elevation of ROS, which act as second messengers that alter signaling cascades (53, 102).

The production of ROS mediated by ligand-activated receptor signaling mechanisms and their stimulatory role as second messengers in VSMCs have been reported (37). Several growth factors and cytokines [*e.g.*, EGF, PDGF, vascular endothelial growth factor (VEGF), endothelium-derived constricting factors, endothelium-derived hyperpolarizing factors, phosphatidylinositol 3-kinase, protein kinase A, thrombin, G-protein Rac-1, ET-1, TNF-α, interleukin-1β, and angiotensin-II] can induce cellular ROS production via enzymatic and metabolic pathways resulting in hypertrophic response and induction of additional signaling pathways in VSMCs (1, 5, 30, 93). ET-1 induces growth factor release from VSMCs and ROS production by macrophages and causes the expression of adhesion molecule in endothelial cells (81). Angiotensin II promotes vascular growth and tone and may increase $O_2^{\bullet-}$ production by activating NADH and NADPH oxidases (37). $O_2^{\bullet-}$ and H_2O_2 produced by XO induce proliferation of rat aortic smooth muscle cells (88). PDGF activates flavoenzyme-dependent $O_2^{\bullet-}$ production in human aortic smooth muscle cells by protein kinase C (PKC)-sensitive cascades (77). Similarly, PDGF and VEGF increase the production of mitochondrial and/or NADPH oxidase generated ROS in VSMCs to induce endothelial cell growth and proliferation (1, 77).

ROS as signaling molecules: ligand-independent stimulation of receptor kinase activity

Reversible protein phosphorylation is the main feature of cellular signaling pathways, and ROS-mediated signal transduction pathways are also involved in phosphorylation events. ROS can activate protein tyrosine kinases and protein serine/threonine kinases via a process known as receptor "transactivation," which involves ligand-independent stimulation of receptor kinase activity (Fig. 4). For example, H_2O_2 transacti-

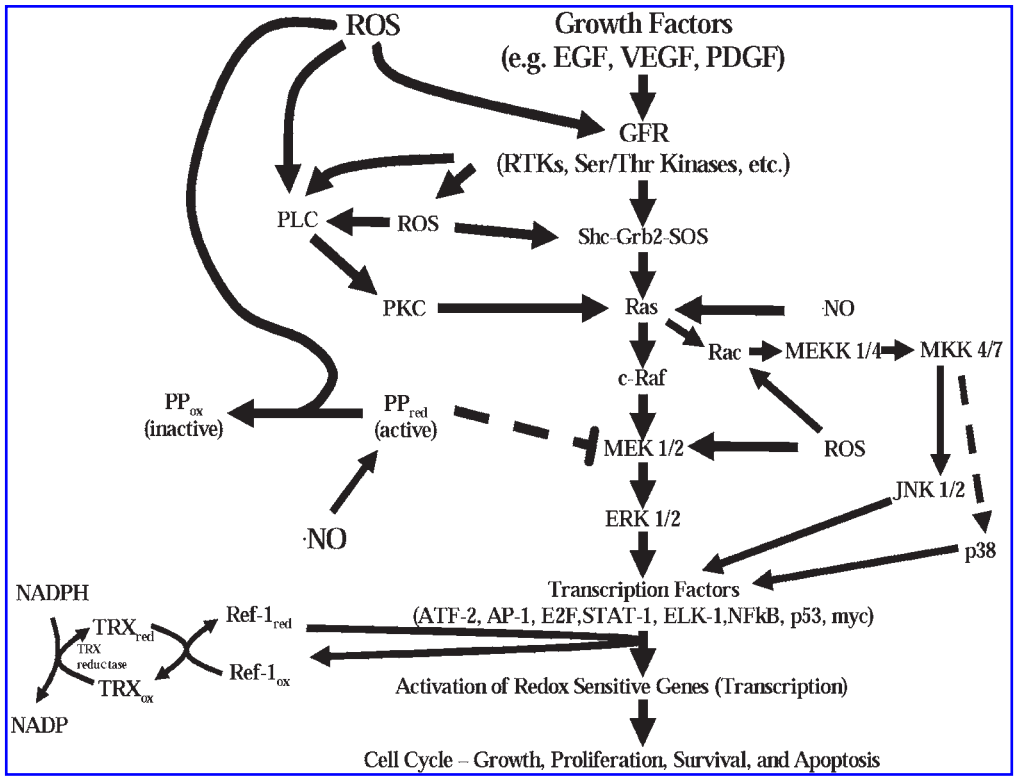

FIG. 4. Growth factor and ROS-mediated signal transduction pathways. Several growth factors induce the activation of redox-sensitive genes via MAPK pathways. MAPKs, a family of serine/threonine protein kinases, are involved in several cellular functions, including cell proliferation, cell differentiation, cell movement, and cell death. ROS transactivate protein tyrosine kinases and protein serine/threonine kinases to induce MAPK pathway. Autophosphorylation of the receptors results in their association with Ras guanine nucleotide exchange factors (GEF or SOS) as a result of SH2-mediated protein (Grb2) interactions. This interaction recruits SOS to the plasma membrane, where it activates Ras, which then binds to the Raf family protein kinases. c-Raf activates a dual specificity kinase called MAPK/ERK (MEK 1/2), and MEK 1/2 activation leads to the phosphorylation of both threonine and tyrosine residues of members of the ERK family. Stress-activated protein kinases (SAPK)/c-Jun N-terminal kinases (JNK) are members of the MAPK family and are induced by a variety of environmental stresses, including ROS. Stress signals are delivered to this cascade by members of small GTPases of the Rho family (Rac, Rho, cdc4). ROS or Ras-induced Rac activates MEKK1/4, which activates MKK4 (SEK) or MKK7, the SAPK/JNK kinases. The p38 MAPKs, another member of the MAPK family, may also be activated by MKK4 (dashed line). Activated MAPKs may phosphorylate a variety of target proteins and other protein kinases. Importantly, some fractions of activated MAPKs translocate to the nucleus to activate transcription factors and redox-sensitive genes for cell growth, proliferation, and apoptosis. ROS may also activate phospholipase C (PLC), inducing the PKC pathway, which then may activate MAPK pathway. ROS indirectly activate MAPK by oxidizing phosphatases (inactive PP_{ox}), whereas ·NO blocks MAPK pathway by activating phosphatases (active PP_{red}). Thioredoxin (TRX) and NADPH-mediated Ref-1 induces the activation of transcription factors that initiate cell growth, proliferation, survival, and apoptosis.

vates the EGFR with subsequent induction of both c-Jun N-terminal kinase (JNK) and eNOS activation (20, 65). H_2O_2 also stimulates the association of the Shc-Grb2-SOS complex with the EGFR that is required for induction of the downstream signaling pathways (87). Likewise, angiotensin II-mediated activation of the mitogen-activated protein kinase (MAPK) pathway involves H_2O_2–mediated EGFR transactivation (111). The transactivation of EGFR by ROS appears to be different from regular EGF-mediated receptor activation because tyrosine phosphorylation sites between these two types of receptor activation processes are not similar and may alter the recruitment of regulatory proteins (89). Oxidative stress can also activate distinct signaling cascades by altering the phosphorylation patterns of specific signaling proteins, as is the case of tyrosine versus serine phosphorylation of the p66 adaptor protein, a factor that is part of the signal transduction pathway that regulates stress-induced apoptosis (83). EGF induces tyrosine phosphorylation of p66, whereas ultraviolet light (UV) and H_2O_2 induce serine phosphorylation of p66, increasing susceptibility to oxidant-induced apoptosis (82). Hence, redox mechanisms are critical components of many signaling cascades involved in the enzymatic activation of cellular processes that can also be activated by growth factors or other stimuli.

MAPK

The MAPK pathway plays an important role in the modulation of several physiological events, including cell-cycle regulation, proliferation, and apoptosis. The MAPK pathway is involved in the phosphorylation of key enzymes and nuclear

transcription factors, and thus regulates their function. The association of the MAPK signaling pathway with oxidative stress has been demonstrated in numerous reports (Fig. 4) (39, 87, 102).

The MAPK pathway comprises three subgroups: the extracellular signal-regulated kinases 1 and 2 (ERK 1/2), the p38 MAPK, and the stress-activated protein kinase or c-Jun N-terminal kinase (SAPK/JNK) (95). The MAPK pathway can be activated by several growth factors, including EGF and PDGF, which bind to RTKs (Fig. 4). Many signals initiate the MAPK pathway through the activation of the small GTP-binding protein, Ras. In the ERK 1/2 pathway, EGFR activation by EGF leads to Ras activation, resulting in the phosphorylation of serine and threonine residues of Raf kinase, which activates a dual specificity kinase called MAPK/ERK (MEK 1/2). MEK 1/2 activation leads to the phosphorylation of both threonine and tyrosine residues of members of the ERK family (95). Once phosphorylated, activated ERK phosphorylates a variety of target proteins and other protein kinases. Simultaneously, ERK can translocate to the nucleus where it interacts with target genes or binds to serum response elements on the different promoters to induce immediate early response genes such as c-myc, c-jun, c-fos, and Elk-1 to stimulate cell growth and proliferation. Several growth factors induce the activation of ERK 1/2, which is typically associated with cell survival, and exposure of cells to UV and other environmental stressors induce the JNK and p38 subfamilies, which often mediate apoptotic events.

Various oxidative stressors, including $O_2^{\bullet -}$, H_2O_2, and $ONOO^-$, activate RTKs followed by the induction of downstream signaling pathways, including the MAPK pathway (Fig. 4) (39, 87). In VSMCs, the ERK 1/2 pathway is highly sensitive to $O_2^{\bullet -}$, whereas the JNK and p38 MAPKs are sensitive to high amounts of H_2O_2 (4, 110). ROS increases VSMC differentiation and maturation via a MAPK pathway in human aortic VSMCs (100). Antioxidants inhibit PDGF stimulation of MAPK in VSMCs, suggesting that ROS play a significant role in the MAPK signaling pathway (101). Similarly, heat-shock protein-mediated induction of MAPK signaling by ROS in VSMCs has also been reported (71). H_2O_2 stimulates PKC activity through oxidative modification at its amino regulatory domain, whereas oxidative modification of the carboxylic terminal domain results in complete inactivation of PKC. H_2O_2 activates phospholipase C, which produces inositol triphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. Inositol triphosphate stimulates Ca^{2+} release into the cytoplasm, and Ca^{2+} is bound by calmodulin, which can activate Ca^{2+}-dependent protein kinases, whereas diacylglycerol, along with phosphatidylserine, activates PKC, which phosphorylates serine and threonine residues (12).

MAPK pathways can also be influenced by RNS. For example, nitrosylation of the cysteine residue of Ras by RNS causes a conformational change and stimulates a GDP/GTP exchange leading to activation of MAPK pathways (Fig. 4) (63). Activated Ras protein can modulate several target proteins and signaling molecules involved in cell-cycle regulation, including PKC, protein kinase B, Raf, phosphatidylinositol 3-kinase, and MEK 1/2 (26). $^{\bullet}NO$-mediated activation of ERK 1/2 and the associated nuclear factor-κB (NFκB) response are Ras-dependent (63). $ONOO^-$ activates JNK in endothelial cells following shear stress (36), whereas $^{\bullet}NO$ inhibits activation of ERK 1/2, JNK, and p38 by angiotension II and PDGF in rat cardiac fibroblasts (113). Overall, the balance of MAPK pathway activation will dictate the impact of RS and resultant transcription factor induction, and thus promotion of cell survival or death.

Transcription factors

Activated signaling pathways lead to the induction of nuclear transcription factors, including c-fos and c-jun, both of which can associate as heterodimers (c-jun/c-fos) or a c-jun homodimer to constitute the activator protein-1 (AP-1) that binds DNA and activates cell-cycle regulatory genes (Fig. 4) (96). Growth factor-induced activation of the AP-1 complex involves the ERK subgroup of the MAPK pathway, whereas redox-induced activation involves the JNK and p38 subgroups of the MAPK pathway (19). In endothelial cells, H_2O_2 induces the JNK pathway, which activates c-jun, causing transactivation of the AP-1 complex, and concomitantly stimulates transcription factor ATF-2, which increases c-Jun expression (20, 38). The oxidative signaling cascades also activate the c-myc transcription factor that can induce VSMC proliferation, because suppression of c-myc expression with antisense oligonucleotides inhibited rat aortic VSMC proliferation, and led to cell-cycle arrest (11).

Recently, redox factor-1 (Ref-1, or AP endonuclease), a bifunctional enzyme involved in both DNA repair (base excision repair) and redox regulation of transcription factors (*e.g.*, AP-1), has been identified (Fig. 4) (54). The DNA repair and redox regulatory activities are in physically distinct domains of the Ref-1 protein, suggesting that repair of oxidative DNA damage and regulation of transcriptional response to oxidative stress may be related. Ref-1 regulates the DNA-binding activity of several nuclear transcription factors, including p53, AP-1, and NFκB through the modifications of cysteine residues of the target protein (44). For example, cysteine residues of Ref-1 interact with conserved cysteines within the DNA-binding domains of AP-1 and act as reductants, stimulating AP-1 activity. Oxidized Ref-1 can be regenerated via reaction with thiol reductants. Overexpression of Ref-1 increases VSMC proliferation by causing an increase in the cell-cycle S phase, whereas its suppression decreases PDGF-induced AP-1 activity and DNA synthesis (42), suggesting that Ref-1 may regulate the cell-cycle progression of VSMCs by modulating the redox activation of AP-1 and other transcription factors.

Phosphatases

The dephosphorylation of kinases by phosphatases is a common mechanism for protein activities. Phosphatases are crucial elements in most signaling cascades by virtue of their regulatory functions on the protein kinases, including MAPK. Failure to reverse phosphorylation events modulated by MAPK will result in impaired cellular function and cell-cycle progression (47).

Both phosphotyrosine phosphatases (PTPs) and serine/threonine phosphatases are redox-sensitive (Fig. 4) (25, 80). The active sites of PTPs include cysteine and arginine residues separated by five amino acids, which creates a low pK_a, making the cysteine residue more susceptible to oxidation that results in PTP inactivation (8). For example, H_2O_2 oxidizes the active site of redox-sensitive cysteine residues and, thus, is a potent inhibitor of PTPs. In this manner, ROS inhibit the dephosphorylation of MAPK (inactivation of PTPs), yet can also activate MAPK and, thus, influence the

expression of the transcription factors that regulate several signaling cascades. In contrast, the ·NO donor, SNAP, lowered the cytosolic concentration of Ca^{2+} and induced phosphotyrosine dephosphorylation of aortic smooth muscle cells (55). Similarly, SNAP induces protein tyrosine phosphatase activity and inhibits VSMC growth and proliferation (28).

CELL CYCLE

Cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CKIs)

Cell-cycle pathways are the end points of the signaling cascades and are defined as the program for cell growth and cell proliferation. The four broad phases of the cell cycle ($G_{0/1}$, S, G_2, and M) are regulated by extracellular stimuli, which in turn induce a number of proteins that regulate and control cell growth and proliferation (Fig. 5) (97). The proteins involved in controlling the cell cycle are the cyclins (cyclins A, D, and E), the CDKs (CDK2, 4, and 6), the CKIs (p16, p21, and p27), and other negative regulators, including the retinoblastoma protein (Rb) and p53, that regulate the effects of both CKI p21 and cyclin A protein (98). Cyclins D and E control S-phase entry, and many growth factor-mediated signaling pathways can activate their transcripts.

Certain events occur in early G_1 that influence cell-cycle progression (S phase) or entry into G_0 (Fig. 5). Growth factors can promote transcription of cyclin D protein, which associates with preexisting CDK4/6, forming active cyclin

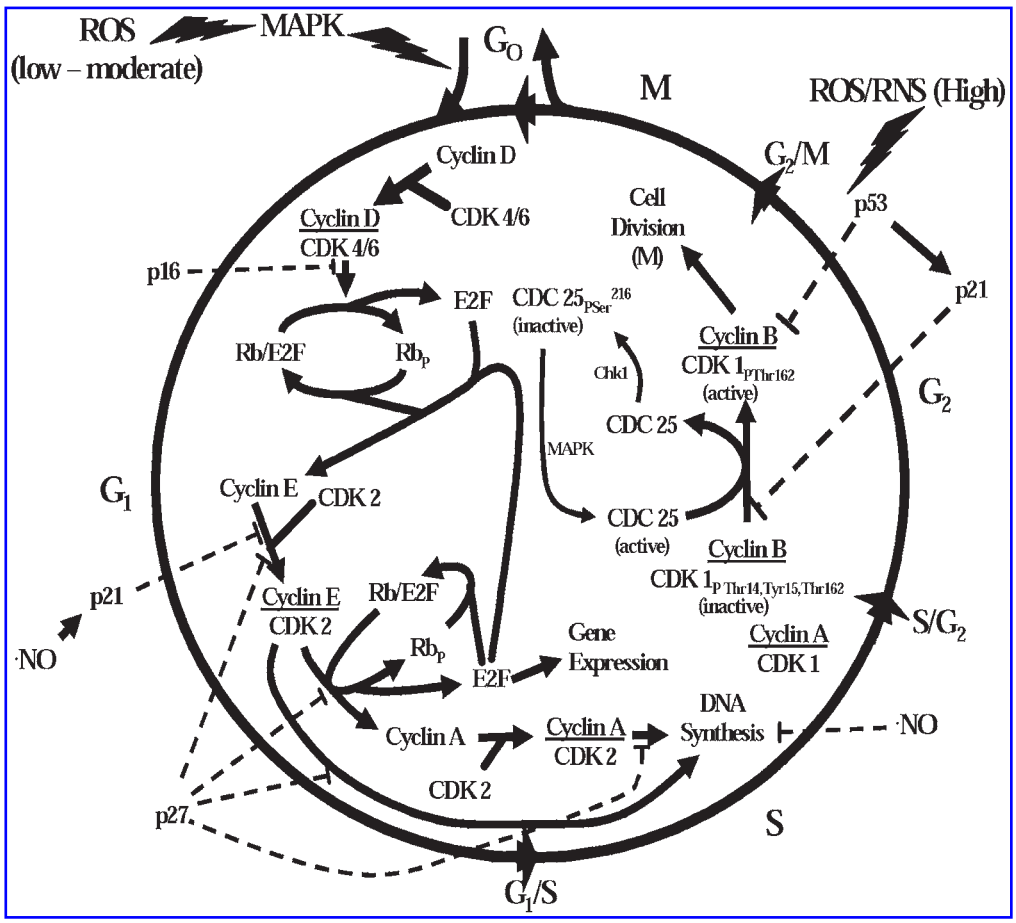

FIG. 5. Cell-cycle regulation. The MAPK pathway can induce cyclin D, which forms a cyclin D/CDK4/6 complex that phosphorylates the retinoblastoma (Rb), resulting in the release of E2F, which mediates gene expression and the induction and formation of cyclin E/CDK2 complex. The cyclin E complex phosphorylates a broad variety of proteins, including Rb, and promotes cell-cycle progression to late G_1, leading to the induction and formation of the cyclin A/CDK2 complex, which promotes cell-cycle progression through the G_1/S phase into S (DNA synthesis), and late S, S/G_2 interphase (cyclin A/CDK1). Cyclin B/CDK1 regulates G_2 to M phase progression in conjunction with the cyclin A/CDK1 complex. The cyclin B complex is inactive due to phosphorylation of Tyr^{15} residue, and is activated in its hypophosphorylated state (phosphorylated Thr^{160}), a process regulated by Cdc25 (dephosphorylation of Tyr^{15}). The active cyclin B complex promotes cell division (M phase). CDK inhibitor p16 induces early G_1 arrest by inhibiting cyclin D complex action, preventing phosphorylation of Rb. p21 can be induced by ·NO, leading to inhibition of cyclin E complex action, as can p27 (resulting in entry into G_0). In G_2, p53 can induce late G_2 arrest (inhibition of active cyclin B action) or mediate early G_2 arrest via p21 inhibition of Cdc25. In general, low to moderate ROS can induce MAPK pathways that lead to cell growth and proliferation, whereas high ROS induce DNA damage and/or MAPK pathways that activate p53, cell arrest, and apoptosis.

D/CDK4/6 complexes that phosphorylate Rb, which, when hypophosphorylated, sequesters several important factors required for cell-cycle progression (*e.g.*, transcription factor E2F) (29). Rb phosphorylation results in the release of these factors and promotes cell-cycle progression via activation of cyclin E transcription. Cyclin E associates with existing CDK2, forming an active complex that regulates several proteins, including E2F, to activate expression of cyclin A, which associates with CDK2 or 1 (Cdc2) to form active complexes that phosphorylate targets involved in the initiation of DNA synthesis, required for cell-cycle progression from G_1/S transition to S/G_2 (cyclin A/CDK2) and S/G_2 to M phase (cyclin A/CDK1) (68). Cyclin A/CDK2 may contribute to phosphorylation of Rb and histone H1, whereas cyclin A/CDK1 appears to phosphorylate histone H1 (29). Cdc7, a protein kinase unrelated to CDKs, is required for DNA synthesis during the S phase. Cyclin A accumulates during S phase, and its activation initiates transition to G_2. The synthesis of cyclin B begins during S phase, but the cyclin B/CDK1 complex is inactive due to phosphorylation of Tyr^{15}. The transition from G_2 to M is mediated via Cdc25 (a phosphatase) dephosphorylation (Tyr^{15}) of the cyclin B/CDK1 complex. Activated cyclin B complex catalyzes the phosphorylation of histone H1 and lamins, and is involved in the regulation of cellular events preceding cell division. Cyclin B/CDK1 regulates the progression of G_2 to M phase in conjunction with the cyclin A complex (97).

Cell-cycle progression is regulated by cyclin production and degradation, the phosphorylation of CDKs, cyclins, and other factors (*e.g.*, Rb), the association of cyclin/CDK complexes, and inhibition of the CDKs. Damage sustained during the cell cycle can induce a delay in the cell cycle, or a cell-cycle checkpoint. Cell-cycle checkpoints are actively induced pauses in progression through the cell cycle after exposure to damaging agents that allow for cellular repair of damage before reengaging the cell cycle. Severe damage results in apoptosis or permanent entry into G_0. In general, cells are very sensitive to DNA damage to induce cell-cycle arrest. In this regard, the timing of cell injury events and cell-cycle phases are important in determining which checkpoint is initiated. For example, if cell damage is incurred during the late phase of a cycle, the cell cycle will often progress into the next phase before arrest. Most cell checkpoints involve inhibition of cyclin/CDK complexes. Because CDK activities are regulated by CKIs, CKIs also influence cyclin activities and the cyclin/CDK-mediated (cyclin D/CDK4/6, cyclin E/CDK2) phosphorylation of the Rb protein that causes G_1 arrest (98). In proliferating cells, CKI p21 regulates the CDK complexes, whereas CKI p27 appears to be involved in CDK regulation during the G_0 phase and can be modulated by growth factors and mitogens (22).

ROS, RNS, and cell cycle

The regulation of the VSMC growth includes both apoptotic and proliferative pathways, and the balance between each determines the degree and level of cell growth. Several studies have shown that the cell cycle can be arrested in response to ROS and/or RNS (50, 92). Alteration of cellular redox state by depletion of GSH (increased ROS) results in delayed progression through G_1 and S phases, as well as G_2 arrest (Fig. 5). For example, peroxides inhibit cyclin E/CDK2 function and the related S phase entry in a dose-dependent manner (31) and can induce a G_1 or G_2 checkpoint through the inhibition of cyclin E/CDK2 and cyclin B/CDK1 activities, respectively (31). The G_2 checkpoint results in the delay in activation of cyclin B/CDK1 at the G_2/M border, potentially due to the checkpoint-activated kinase (Chk1)-mediated phosphorylation of Ser^{216} on Cdc25 phosphatase, leading to its inactivation (62).

At the nuclear level, p53 tumor suppressor gene regulates apoptosis and is a negative regulator of the cell cycle (Fig. 5). In response to environmental stress, oxidants, and UV irradiation, p53 induces CKI expression in pre-S-phase cells, inhibiting cyclin/CDK action and cell-cycle progression; or, in post-S-phase cells, p53 initiates apoptosis. p53 function is critical for G_1 checkpoint function, and acts as a transcriptional activator of numerous genes, including p21, which inhibits CDKs (118). Overexpression of p21 results in G_1 arrest, whereas inactivation of p53 results in the loss of the G_1 checkpoint. Suppression of p53 in VSMCs results in cellular proliferation in human coronary restenosis, whereas overexpression of p53 increases intracellular ROS/RNS in cultured cells (99) and blocks cellular growth of human VSMCs. These observations suggest that ROS/RNS-mediated apoptosis may protect the vascular wall by inducing an apoptotic cascade that is RS-dependent (51).

•NO protects the vessel wall from proliferating VSMCs by blocking the cell cycle at the S-phase entry and causing an arrest at the G_1 phase of the cell cycle (Fig. 5) (92). Studies have demonstrated that •NO induces CKI p21, which suggests that •NO blocks the G_1/S transition by suppressing cyclin/CDK-dependent cell-cycle progression (50). Similarly, •NO transiently activates the level of the CKI p21 after vascular injury, resulting in G_1 arrest in VSMCs to protect the vessel wall (117). •NO also inhibits the enzyme ribonucleotide reductase, which is essential for DNA synthesis, and its inhibition results in G_1 arrest (67). These observations suggest that •NO plays an important role in the regulation of VSMC cell cycle, potentially by modulating CKI expression.

APOPTOSIS

Apoptosis, or programmed cell death, is a highly regulated cell function that is essential for normal development of an organism (79). Specific signals can initiate apoptosis, upon which several biochemical and morphological changes occur within the cell.

Caspases

Caspases are cysteine-dependent proteases that are activated in the early stages of apoptosis and cleave major structural proteins of the cytoplasm and nucleus. In normal cells, caspases are present as inactive zymogens called procaspases that can be induced by other enzymes in response to apoptotic signals. The proapoptotic caspases play a crucial role in the apoptotic-signaling pathway and are divided into two groups: initiator caspases, which include procaspases-2, -8, -9, and -10; and executioner caspases, which include procaspases-3, -6, and

-7 (32). Caspases are activated by specific ligands that bind to cell-surface receptors called death receptors that relay apoptotic signals (14). Death receptors belonging to the TNF gene superfamily play a crucial role in both the induction of apoptosis and activation of caspase cascades within seconds of ligand binding. Caspases are induced either in response to ligand binding to death receptors (extrinsic apoptosis) or in response to signals originating from inside the cell (intrinsic apoptosis). In extrinsic apoptosis, a ligand binds to the death receptor, recruits adaptor molecules through its cytoplasmic death domains, and activates procaspase-8 by autoproteolytic cleavage, forming active caspase-8 that cleaves and activates additional caspases for the initiation of apoptosis (24).

Intrinsic apoptosis involves procaspase-9 and is induced downstream of mitochondrial proapoptotic events. During intrinsic apoptosis, cytochrome *c*, a key protein in the electron transport chain, forms a complex with apoptosis-activating factor-1 (APAF-1), a protease, which activates procaspase-9, and initiates the activation of downstream caspases (24). Once the initiator caspases have been induced, they can immediately activate the executioner procaspases-3, -6, and -7 that cleave protein substrates, leading to the stimulation of the apoptotic cascade.

Mitochondria in the regulation of intrinsic apoptosis

Besides amplifying and mediating intrinsic apoptotic pathways, mitochondria also play a crucial role in the initiation of apoptosis induced by DNA damage and oxidative stress (Fig. 3). Mitochondria regulate apoptosis by: (a) providing ATP required for the initiation of apoptosis; (b) releasing cytochrome *c* and apoptosis-inducing factors (AIF) that are involved in caspase activation; and (c) releasing proteins that neutralize endogenous inhibitors of apoptosis (72, 112). Cytochrome *c* and AIF initiate proteolytic activity that leads to nuclear damage and apoptosis. Because mitochondria play an important role in the regulation of cell death, a number of antiapoptotic and proapoptotic molecules and mechanisms associated with mitochondria have been identified.

The Bcl-2 family of proteins that are located in the outer mitochondrial membrane regulates cell survival and death. The sensitivity of cells to apoptotic stimuli can depend on the balance of pro- and antiapoptotic Bcl-2 proteins. Bcl-2 and Bcl-XL are antiapoptotic, whereas Bad and Bax are proapoptotic (13). The up-regulation of Bcl-2 and Bcl-XL proteins inhibits apoptosis by suppressing the generation of ROS, stabilizing mitochondrial membrane potential, preventing permeability transition pore formation, and blocking the release of cytochrome *c*, thereby suppressing the activation of the caspase cascade and apoptosis (3). On the other hand, the Bad and Bax proteins act as sensors of cellular damage or stress, and relocate to the surface of the mitochondrion following cellular stress, disrupting the normal function of the antiapoptotic proteins, leading to the formation of the "apoptosome" and the activation of the caspases to initiate apoptosis (2).

The membrane receptor-derived signaling pathways induced by low levels of ROS or the pathways inducing ROS production can stimulate cell proliferation and survival, whereas high levels of ROS levels are apoptotic and/or necrotic (17). Higher concentrations of ROS induce the oxidation of several lipids, proteins, and nucleic acids and the release of cytochrome *c*, enhancing apoptosis. Most apoptosis-inducing conditions and factors disrupt the mitochondrial membrane potential and permeability transition leading to cellular dysfunction: ATP synthesis is blocked, redox molecules such as NADH, NADPH, and glutathione are oxidized, and ROS are increasingly produced. In vascular cells, the activity of caspase-3 is regulated by ROS (69). TNF-α stimulates mitochondrial ROS production that is important in mediating cell death; mitochondrial complex I and II inhibitors reduce TNF-α-mediated mitochondrial ROS production and, thus, inhibit apoptosis (94). Likewise, up-regulation of SOD2 inhibits intrinsic apoptotic pathways, which underlines the importance of mitochondrial ROS generation in apoptosis (76).

•NO and apoptosis

Depending on the concentration and location, •NO can induce or inhibit apoptosis (Fig. 5). At low concentrations, •NO blocks apoptosis, whereas high levels are proapoptotic (109). The antiapoptotic effects of •NO are mediated via a number of mechanisms, including nitrosylation or inactivation of many of the caspases, including caspases-1, -3, and -8 (70). Other mechanisms include the activation of heat shock protein 70, which prevents the recruitment of procaspase-9 to the APAF-1 apoptosome, the activation of Bcl-2 and Bcl-XL, the activation of cyclic GMP signaling, which mediates the activation of cyclic GMP-dependent protein kinases, and the suppression of caspase activity and increased expression of antiapoptotic proteins (57, 70).

SUMMARY

The steady-state levels of oxidative and nitrosative species are important factors in the regulation of several signaling pathways and, thus, cell-cycle control. At low concentrations, RS can act as mediators in signaling cascades and gene regulation, whereas at high levels RS induce cell death and apoptosis. Signaling mechanisms regulated by RS are crucial in the control of vascular function, and this regulation is influenced by the relative concentrations of different RS within the vascular microenvironment. Under oxidative stress, cycling cells will show cell-cycle checkpoint responses using several different mechanisms to block or repair the damage caused by RS to maintain genomic stability. These mechanisms consist of oxidative response enzymes such as SOD, catalase, and glutathione peroxidase, antioxidants, DNA repair enzymes, and cell-cycle checkpoint systems. However, high levels of unregulated RS can severely impair cellular functions by inducing DNA damage and signaling cascades. Consequently, derangement of these steady-states potentially alters the regulation of these pathways and leads to chronic diseases, including CVD.

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ABBREVIATIONS

AIF, apoptosis-inducing factors; ANT, adenenine nucleotide translocase; AP-1, activator protein-1; APAF-1, apoptosis-activating factor-1; BH_4, tetrahydrobiopterin; CDK, cyclin-dependent kinase; CKI, CDK inhibitor; CVD, cardiovascular disease; EGF, epidermal growth factor; EGFR, EGF receptor; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK; MPO, myeloperoxidase; mtDNA, mitochondrial DNA; NFκB, nuclear factor-κB; •NO, nitric oxide; NOS, nitric oxide synthase; $O_2^{\bullet -}$, superoxide; •OH, hydroxyl radicals; $ONOO^-$, peroxynitrite; $ONOOCO_2^-$, nitrosoperoxycarbonate; PDGF, platelet-derived growth factor; PKC, protein kinase C; PTP, phosphotyrosine phosphatase; Rb, retinoblastoma protein; Ref-1, redox factor-1; RNS, reactive nitrogen species; ROS, reactive oxygen species; RS, reactive species; RTK, receptor tyrosine kinase; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; TRX, thioredoxin; UV, ultraviolet light; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell; XDH, xanthine dehydrogenase; XO, xanthine oxidase.

REFERENCES

1. Abid MR, Tsai JC, Spokes KC, Deshpande SS, Irani K, and Aird WC. Vascular endothelial growth factor induces manganese-superoxide dismutase expression in endothelial cells by a Rac1-regulated NADPH oxidase-dependent mechanism. *FASEB J* 15: 2548–2550, 2001.
2. Adams JM and Cory S. Apoptosomes: engines for caspase activation. *Curr Opin Cell Biol* 14: 715–720, 2002.
3. Antonsson B, Montessuit S, Lauper S, Eskes R, and Martino JC. Bax oligomerization is required for channel-forming activity in liposomes and to trigger cytochrome *c* release from mitochondria. *Biochem J* 345: 271–278, 2000.
4. Baas AS and Berk BC. Differential activation of mitogen-activated protein kinases by H_2O_2 and O_2^- in vascular smooth muscle cells. *Circ Res* 77: 29–36, 1995.
5. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, and Rhee SG. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem* 272: 217–221, 1997.
6. Ballinger SW, Patterson WC, Yan C-N, Doan R, Burow DL, Young CG, Yakes FM, Van Houten B, Ballinger CA, Freeman BA, and Runge MS. Hydrogen peroxide and peroxynitrite induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. *Circ Res* 86: 960–966, 2000.
7. Ballinger SW, Patterson WC, Knight-Lozano CA, Burow DL, Conklin CA, Hu Z, Reuf J, Horaist C, Lebovitz RM, Hunter G, McIntyre K, and Runge MS. Mitochondrial integrity and function in atherogenesis. *Circulation* 106: 544–549, 2002.
8. Barford D. Protein phosphatases. *Curr Opin Struct Biol* 5: 728–734, 1995.
9. Barry-Lane PA, Patterson C, van der Merwe M, Hu Z, Holland SM, Yeh ET, and Runge MS. p47phox is required for atherosclerotic lesion progression in ApoE(−/−) mice. *J Clin Invest* 108: 1513–1522, 2001.
10. Behr D, Rupin A, Fabiani JN, and Verbeuren TJ. Distribution and prevalence of inducible nitric oxide synthase in atherosclerotic vessels of long-term cholesterol-fed rabbits. *Atherosclerosis* 142: 335–344, 1999.
11. Bennett MR, Anglin S, McEwan JR, Jagoe R, Newby EC, and Evan GI. Inhibition of vascular smooth muscle cell proliferation in vitro and in vivo by c-myc antisense oligodeoxynucleotides. *J Clin Invest* 93: 820–828, 1994.
12. Blake RA, Walker TR, and Watson SP. Activation of human platelets by peroxovanadate is associated with tyrosine phosphorylation of phospholipase C gamma and formation of inositol phosphates. *Biochem J* 290: 471–475, 1993.
13. Borner C. The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. *Mol Immunol* 39: 615–647, 2003.
14. Bratton SB, MacFarlane M, Cain K, and Cohen GM. Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. *Exp Cell Res* 256: 27–33, 2000.
15. Brookes PS, Zhang J, Dai L, Zhou F, Parks DA, Darley-Usmar VM, and Anderson PG. Increased sensitivity of mitochondrial respiration to inhibition by nitric oxide in cardiac hypertrophy. *J Mol Cell Cardiol* 33: 69–82, 2001.
16. Buchwalow IB, Podzuweit T, Bocker W, Samoilova VE, Thomas S, Wellner M, Baba HA, Robenek H, Schnekenburger J, and Lerch MM. Vascular smooth muscle and nitric oxide synthase. *FASEB J* 16: 500–508, 2002.
17. Cai J and Jones DP. Superoxide in apoptosis. Mitochondrial generation triggered by cytochrome *c* loss. *J Biol Chem* 273: 11401–11404, 1998.
18. Cassina A and Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. *Arch Biochem Biophys* 328: 309–316, 1996.
19. Chang L and Karin M. Mammalian MAP kinase signalling cascades. *Nature* 410: 37–41, 2001.
20. Chen K, Vita JA, Berk BC, and Keaney JF Jr. c-Jun N-terminal kinase activation by hydrogen peroxide in endothelial cells involves Src-dependent epidermal growth factor receptor transactivation. *J Biol Chem* 276: 16045–16050, 2001.
21. Chen Z, Siu B, Ho Y-S, Vincent R, Chua CC, Hamdy RC, and Chua BH. Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. *J Mol Cell Cardiol* 30: 2281–2289, 1998.
22. Coats S, Flanagan WM, Nourse J, and Roberts JM. Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle. *Science* 272: 877–880, 1996.
23. Corral-Debrinski M, Stepien G, Shoffner JM, Lott MT, Kanter K, and Wallace DC. Hypoxemia is associated with mitochondrial DNA damage and gene induction: implications for cardiac disease. *JAMA* 266: 1812–1816, 1991.

24. Denault JB and Salvesen GS. Caspases: keys in the ignition of cell death. *Chem Rev* 102: 4489–4500, 2002.
25. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 5633–5642, 1998.
26. Deora AA, Win T, Vanhaesebroeck B, and Lander HM. A redox-triggered Ras-effector interaction. Recruitment of phosphatidylinositol 3′-kinase to Ras by redox stress. *J Biol Chem* 273: 29923–29928, 1998.
27. Deshpande NN, Sorescu D, Seshiah P, Ushio-Fukai M, Akers M, Yin Q, and Griendling KK. Mechanism of hydrogen peroxide-induced cell cycle arrest in vascular smooth muscle. *Antioxid Redox Signal* 4: 845–854, 2002.
28. Dhaunsi GS, Matthews C, Kaur K, and Hassid A. NO increases protein tyrosine phosphatase activity in smooth muscle cells: relationship to antimitogenesis. *Am J Physiol* 272: H1342–H1349, 1997.
29. Dowdy SF, Hinds PW, Louie K, Reed SI, Arnold A, and Weinberg RA. Physical interaction of the retinoblastoma protein with human D cyclins. *Cell* 73: 499–511, 1993.
30. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2002.
31. Duan J and Karmazyn M. Relationship between oxidative phosphorylation and adenine nucleotide translocase activity in two populations of cardiac mitochondria and mechanical recovery of ischemic hearts following reperfusion. *Can J Physiol Pharmacol* 67: 704–709, 1989.
32. Earnshaw WC, Martins LM, and Kaufmann SF. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 68: 383–424, 1999.
33. Forman HJ, Boveris A, and Prior WA (Eds). *Free radicals in Biology*. Orlando, FL: Academic Press, 1982, p. 65.
34. Garg UC and Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 83: 1774–1777, 1989.
35. Giulivi C, Poderoso JJ, and Boveris A. Production of nitric oxide by mitochondria. *J Biol Chem* 273: 11038–11043, 1998.
36. Go YM, Patel RP, Maland MC, Park H, Beckman JS, Darley-Usmar VM, and Jo H. Evidence for peroxynitrite as a signaling molecule in flow-dependent activation of c-Jun NH_2-terminal kinase. *Am J Physiol* 277 (4 Pt 2): H1647–H1653, 1999.
37. Griendling K, Minieri C, Ollerenshaw J, and Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 74: 1141–1148, 1994.
38. Gupta S, Campbell D, Derijard B, and Davis RJ. Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science* 267: 389–393, 1995.
39. Guyton KZ, Liu Y, Gorospe M, Xu Q, and Holbrook NJ. Activation of mitogen-activated protein kinase by H_2O_2. *J Biol Chem* 271: 4138–4142, 1996.
40. Guzik TJ, Nick EJ, West NE, Pillai R, Taggart DP, and Channon KM. Nitric oxide modulates superoxide release and peroxynitrite formation in human blood vessels. *Hypertension* 39: 1088–1094, 2002.
41. Hazen SL and Henecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J Clin Invest* 99: 2075–2081, 1997.
42. He T, Weintraub NL, Goswami PC, Chatterjee P, Flaherty DM, Domann FE, and Oberley LW. Redox factor-1 contributes to the regulation of progression from G0/G1 to S by PDGF in vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* 285: H804–H812, 2003.
43. Heitzer T, Schlinzig T, Krohn K, Meinertz T, and Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 104: 2673–2678, 2001.
44. Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, and Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc Natl Acad Sci U S A* 94: 3633–3638, 1997.
45. Hsich E, Segal BH, Pagano PJ, Rey FE, Paigen B, Deleonardis J, Hoyt RF, Holland SM, and Finkel T. Vascular effects following homozygous disruption of p47(phox): an essential component of NADP oxidase. *Circulation* 101: 1234–1236, 2000.
46. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, and Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 377: 239–242, 1995.
47. Hunter T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* 80: 225–236, 1995.
48. Ide T, Tsutsui H, Kinugawa S, Suematsu N, Hayashidani S, Ichikawa K, Utsumi H, Machida Y, Egashira K, and Takeshita A. Direct evidence for increased hydroxyl radicals orginating from superoxide in the failing myocardium. *Circ Res* 86: 152–157, 2000.
49. Ischiropoulos H. Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. *Arch Biochem Biophys* 356: 1–11, 1998.
50. Ishida A, Sasaguri T, Kosaka C, Nojima H, and Ogata J. Induction of the cyclin-dependent kinase inhibitor p21(Sdi1/Cip1/Waf1) by nitric oxide-generating vasodilator in vascular smooth muscle cells. *J Biol Chem* 272: 10050–10057, 1997.
51. Johnson TM, Yu ZX, Ferrans VJ, Lowenstein RA, and Finkel T. Reactive oxygen species are downstream mediators of p53-dependent apoptosis. *Proc Natl Acad Sci U S A* 93: 11848–11852, 1996.
52. Jones SA, O'Donnell VB, Wood JD, Broughton JP, Hughes EJ, and Jones OT. Expression of phagocyte NADPH oxidase components in human endothelial cells. *Am J Physiol* 271: H1626–H1634, 1996.
53. Jordan JD, Landau EM, and Iyengar R. Signaling networks: the origins of cellular multitasking. *Cell* 103: 193–200, 2000.
54. Kakolyris S, Kaklamanis L, Hickson ID, and Harris AL. Expression and subcellular localization of human AP endonuclease 1 (HAP1/Ref-1) protein: a basis for its role in human disease. *Histopathology* 33: 561–569, 1998.

55. Kaur K, Yao J, Pan X, Matthews C, and Hassid A. NO decreases phosphorylation of focal adhesion proteins via reduction of Ca in rat aortic smooth muscle cells. *Am J Physiol* 274: H1613–H1619, 1998.
56. Kim SM, Byun JS, Jung YD, Kang IC, Choi SY, and Lee KY. The effects of oxygen radicals on the activity of nitric oxide synthase and guanylate cyclase. *Exp Mol Med* 30: 221–226, 1998.
57. Kim YM, Chung HT, Kim SS, Han JA, Yoo YM, Kim KM, Lee GH, Yun HY, Green A, Li J, Simmons RL, and Billiar TR. Nitric oxide protects PC12 cells from serum deprivation-induced apoptosis by cGMP-dependent inhibition of caspase signaling. *J Neurosci* 19: 6740–6747, 1999.
58. Kirk EA, Dinauer MC, Rosen H, Chait A, Heinecke JW, and Le Boeuf RC. Impaired superoxide production due to a deficiency in phagocyte NADPH oxidase fails to inhibit atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 20: 1529–1535, 2000.
59. Kirlin WG, Cai J, Thompson SA, Daiaz D, Kavanagh TJ, and Jones DP. Glutathione redox potential in response to differentiation and enzyme inducers. *Free Radic Biol Med* 27: 1208–1218, 1999.
60. Knight-Lozano CA, Young CG, Burow DL, Hu Z, Uyeminami D, Pinkerton K, Ischiropoulos H, and Ballinger SW. Cigarette smoke exposure and hypercholesterolemia increase mitochondrial damage in cardiovascular tissues. *Circulation* 105: 849–854, 2002.
61. Koglin J, Glysing-Jensen T, Mudgett JS, and Russell ME. Exacerbated transplant arteriosclerosis in inducible nitric oxide-deficient mice. *Circulation* 97: 2059–2065, 1998.
62. Kumagai A and Dunphy WG. The Cdc25 protein controls tyrosine dephosphorylation of the Cdc2 protein in a cell free system. *Cell* 64: 903–914, 1991.
63. Lander HM, Ogiste JS, Pearce SF, Levi R, and Novogrodsky A. Nitric oxide-stimulated guanine nucleotide exchange on p21ras. *J Biol Chem* 270: 7017–7020, 1995.
64. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, and Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 111: 1201–1209, 2003.
65. Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, Yu ZX, Ferrans VJ, Howard BH, and Finkel T. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *J Biol Chem* 274: 7936–7940, 1999.
66. Leeuwenburgh C, Hardy MM, Hazen SL, Wagner P, Oh-ishi S, Steinbrecher UP, and Heinecke JW. Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. *J Biol Chem* 272: 1433–1436, 1997.
67. Lepoivre M, Fieschi F, Coves J, Thelander L, and Fontecave M. Inactivation of ribonucleotide reductase by nitric oxide. *Biochem Biophys Res Commun* 179: 442–448, 1991.
68. Lew DJ and Kornbluth S. Regulatory roles of cyclin dependent kinase phosphorylation in cell cycle control. *Curr Opin Cell Biol* 8: 795–804, 1996.
69. Li AE, Ito H, Rovira II, Kim KS, Takeda K, Yu ZY, Ferrans VJ, and Finklel T. A role for reactive oxygen species in endothelial cell anoikis. *Circ Res* 85: 304–310, 1999.
70. Li J, Billiar TR, Talanian RV, and Kim YM. Nitric oxide reversibly inhibits seven members of the caspase family via *S*-nitrosylation. *Biochem Biophys Res Commun* 240: 419–424, 1997.
71. Liao DF, Jin ZG, Baas AS, Daum G, Gygi SP, Aebersold R, and Berk BC. Purification and identification of secreted oxidative stress-induced factors from vascular smooth muscle cells. *J Biol Chem* 275: 189–196, 2000.
72. Liu X, Kim CN, Yang J, Jemmerson R, and Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. *Cell* 86: 147–157, 1996.
73. Luoma JS and Ylä-Herttuala S. Expression of inducible nitric oxide synthase in macrophages and smooth muscle cells in various types of human atherosclerotic lesions. *Virchows Arch* 434: 561–568, 1999.
74. MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, Trumbauer M, Stevens K, Xie QW, Sokol K, Hutchinson N, *et al*. Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell* 81: 641–650, 1995.
75. MacMillan-Crow LA, Crow JP, and Thompson JA. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. *Biochemistry* 37: 1613–1622, 1998.
76. Manna SK. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *J Biol Chem* 273: 13245–13254, 1998.
77. Marumo T, Schini-Kerth VB, Fisslthaler B, and Busse R. Platelet-derived growth factor-stimulated superoxide anion production modulates activation of transcription factor NF-kappaB and expression of monocyte chemoattractant protein 1 in human aortic smooth muscle cells. *Circulation* 96: 2361–2367, 1997.
78. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, and Harrison DG. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol Heart Circ Physiol* 285: H2290–H2297, 2003.
79. Meier P, Finch A, and Evan G. Apoptosis in development. *Nature* 407: 796–801, 2000.
80. Meng TC, Fukada T, and Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. *Mol Cell* 9: 387–399, 2002.
81. Michael JR, Markewitz BA, and Kohan DE. Oxidant stress regulates basal endothelin-1 production by cultured rat pulmonary endothelial cells. *Am J Physiol* 273: L768–L774, 1997.
82. Migliaccio E, Gorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, and Pelicci PG. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402: 309–313, 1999.
83. Nemoto S and Finkel T. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. *Science* 295: 2450–2452.
84. Packer MA, Porteous CM, and Murphy MP. Superoxide production by mitochondria in the presence of nitric

oxide forms peroxynitrite. *Biochem Mol Biol* 40: 527–534, 1996.
85. Parks DA, Williams TK, and Beckman JS. Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: a reevaluation. *Am J Physiol* 254: G768–G774, 1988.
86. Podrez EA, Abu-Soud HM, and Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic Biol Med* 28: 1717–1725, 2000.
87. Rao GN. Hydrogen peroxide induces complex formation of SHC-Grb2-SOS with receptor tyrosine kinase and activates Ras and extracellular signal-regulated protein kinases group of mitogen-activated protein kinases. *Oncogene* 13: 713–719, 1996.
88. Rao GN and Berk BC. Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. *Circ Res* 70: 593–599, 1992.
89. Ravid T, Sweeney C, Gee P, Carraway KL 3rd, and Goldkorn T. Epidermal growth factor receptor activation under oxidative stress fails to promote c-Cbl mediated down-regulation. *J Biol Chem* 277: 31214–31219, 2002.
90. Ross R. Mechanisms of disease: atherosclerosis—an inflammatory disease. *N Engl J Med* 340: 115–126, 1999.
91. Rudic RD, Shesely EG, Meada N, Smithies O, Segal SS, and Sessa WC. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *J Clin Invest* 101: 731–736, 1998.
92. Sarkar R, Gordon D, Stanley JC, and Webb RC. Cell cycle effects of nitric oxide on vascular smooth muscle cells. *Am J Physiol* 272: H1810–H1818, 1997.
93. Sattler M, Winkler T, Verma S, Byrne CH, Shrikhande G, Salgia R, and Griffin JD. Hematopoietic growth factors signal through the formation of reactive oxygen species. *Blood* 93: 2928–2935, 1999.
94. Schulze-Osthoff K, Bakker AC, Vanhaesebroeck B, Beyaert R, Jacob WA, and Fiers W. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J Biol Chem* 267: 5317–5323, 1992.
95. Seger R and Krebs EG. The MAPK signaling cascade. *FASEB J* 9: 726–735, 1995.
96. Shaulian E and Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 4: E131–E136, 2002.
97. Sherr CJ. Cancer cell cycles. *Science* 274: 1672–1677, 1996.
98. Sherr CJ and Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev* 9: 1149–1163, 1995.
99. Speir E, Modali R, Huang ES, Leon MB, Shawl F, Finkel T, and Epstein SE. Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. *Science* 265: 391–394, 1994.
100. Su B, Mitra S, Gregg H, Flavahan S, Chotani MA, Clark KR, Goldschmidt-Clermont PJ, and Flavahan NA. Redox regulation of vascular smooth muscle cell differentiation. *Circ Res* 89: 39–46, 2001.
101. Sundaresan M, Yu ZX, Fererans VJ, Irani K, and Finkel T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* 270: 296–299, 1995.
102. Suzuki HJF and Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 22: 269–285, 1997.
103. Sventek P, Turgeon A, and Schiffrin EL. Vascular endothelin-1 gene expression and effect on blood pressure of chronic ETA endothelin receptor antagonism after nitric oxide synthase inhibition with L-NAME in normal rats. *Circulation* 95: 240–244, 1997.
104. Takemoto M, Egashira K, Usui M, Numaguchi K, Tomita H, Tsutsui H, and Takeshita A. Important role of tissue angiotensin-converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *J Clin Invest* 99: 278–287, 1997.
105. Tan S, Gelman S, Wheat JK, and Parks DA. Circulating xanthine oxidase in human ischemia reperfusion. *South Med J* 88: 479–482, 1995.
106. Thomas JA, Poland B, and Honzatko R. Protein sulfhydryls and their role in the antioxidant function of protein *S*-thiolation. *Arch Biochem Biophys* 319: 1–9, 1995.
107. Touyz RM, Chen X, Tabet F, Yao G, He G, Quinn MT, Pagano PJ, and Schiffrin EL. Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. *Circ Res* 90: 1205–1213, 2002.
108. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* 552: 335–344, 2003.
109. Tzeng E, Kim YM, Pitt BR, Lizonova A, Kovesdi I, and Billiar TR. Adenoviral transfer of the inducible nitric oxide synthase gene blocks endothelial cell apoptosis. *Surgery* 122: 255–263, 1997.
110. Ushio-Fukai M, Alexander RW, Akers M, and Griendling KK. p38 mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy. *J Biol Chem* 273: 15022–15029, 1998.
111. Ushio-Fukai M, Griendling KK, Becker PL, Hilenski L, Halleran S, and Alexander RW. Epidermal growth factor receptor transactivation by angiotensin II requires reactive oxygen species in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 21: 489–495, 2001.
112. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, and Vaux DL. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* 102: 43–53, 2000.
113. Wang D, Yu X, and Brecher P. Nitric oxide and *N*-acetylcysteine inhibit the activation of mitogen-activated protein kinases by angiotensin II in rat cardiac fibroblasts. *J Biol Chem* 273: 33027–33034, 1998.
114. White CR, Darley-Usmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA, Parks DA, Tarpey MM, and Freeman BA. Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. *Proc Natl Acad Sci U S A* 93: 8745–8749, 1996.
115. Yakes FM and Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear

DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci U S A* 94: 514–519, 1997.

116. Yan L-J and Sohal RS. Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. *Proc Natl Acad Sci U S A* 95: 12896–12901, 1998.
117. Yang ZY, Simari RD, Perkins ND, San H, Gordon D, Nabel GJ, and Nabel EG. Role of the p21 cyclin-dependent kinase inhibitor in limiting intimal cell proliferation in response to arterial injury. *Proc Natl Acad Sci U S A* 93: 7905–7910, 1996.
118. Zhang H, Hannon GJ, and Beach D. p21-containing cyclin kinases exist in both active and inactive states. *Genes Dev* 8: 1750–1758, 1994.
119. Zhang Y, Marcillat O, Giulivi C, Ernster L, and Davies KJ. The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *J Biol Chem* 265: 16330–16336, 1990.
120. Zhang Z, Blake DR, Stevens CR, Kanczler JM, Winyard PG, and Symons MC. A reappraisal of xanthine dehydrogenase and oxidase in hypoxic reperfusion injury: the role of NADH as an electron donor. *Free Radic Res* 28: 151–164, 1998.

Address reprint requests to:
Scott W. Ballinger, Ph.D.
VH G019
1530 3rd Avenue South
Birmingham, AL 35294-0019

E-mail: sballing@path.uab.edu

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3. Gina M. DeNicola, Florian A. Karreth, Timothy J. Humpton, Aarthi Gopinathan, Cong Wei, Kristopher Frese, Dipti Mangal, Kenneth H. Yu, Charles J. Yeo, Eric S. Calhoun, Francesca Scrimieri, Jordan M. Winter, Ralph H. Hruban, Christine Iacobuzio-Donahue, Scott E. Kern, Ian A. Blair, David A. Tuveson. 2011. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* **475**:7354, 106-109. [CrossRef]
4. Irene L. Ibañez, Lucía L. Policastro, Ivanna Tropper, Candelaria Bracalente, Mónica A. Palmieri, Paola A. Rojas, Beatriz L. Molinari, Hebe Durán. 2011. H2O2 scavenging inhibits G1/S transition by increasing nuclear levels of p27KIP1. *Cancer Letters* **305**:1, 58-68. [CrossRef]
5. Feng-Yih Yu, Ting-Shuan Wu, Ting-Wei Chen, Biing-Hui Liu. 2011. Aristolochic acid I induced oxidative DNA damage associated with glutathione depletion and ERK1/2 activation in human cells. *Toxicology in Vitro* **25**:4, 810-816. [CrossRef]
6. Emiko Kasahara, Atsuo Sekiyama, Mika Hori, Kenjiro Hara, Nozomi Takahashi, Masami Konishi, Eisuke F Sato, Sohkichi Matsumoto, Haruki Okamura, Masayasu Inoue. 2011. Mitochondrial density contributes to the immune response of macrophages to lipopolysaccharide via the MAPK pathway. *FEBS Letters* . [CrossRef]
7. Eva Rath, Dirk Haller. 2011. Mitochondria at the interface between danger signaling and metabolism: Role of unfolded protein responses in chronic inflammation. *Inflammatory Bowel Diseases* n/a-n/a. [CrossRef]
8. Yalin Emre, Tobias Nübel. 2010. Uncoupling protein UCP2: When mitochondrial activity meets immunity. *FEBS Letters* **584**:8, 1437-1442. [CrossRef]
9. Mona Sedeek, Richard L Hébert, Chris R Kennedy, Kevin D Burns, Rhian M Touyz. 2009. Molecular mechanisms of hypertension: role of Nox family NADPH oxidases. *Current Opinion in Nephrology and Hypertension* **18**:2, 122-127. [CrossRef]
10. Jan Kajstura, Konrad Urbanek, Marcello Rota, Claudia Bearzi, Toru Hosoda, Roberto Bolli, Piero Anversa, Annarosa Leri. 2008. Cardiac stem cells and myocardial disease. *Journal of Molecular and Cellular Cardiology* **45**:4, 505-513. [CrossRef]
11. Jianfei Jiang, Natalia A. Belikova, Adam T. Hoye, Qing Zhao, Michael W. Epperly, Joel S. Greenberger, Peter Wipf, Valerian E. Kagan. 2008. A Mitochondria-Targeted Nitroxide/Hemigramicidin S Conjugate Protects Mouse Embryonic Cells Against Gamma Irradiation. *International Journal of Radiation Oncology*Biology*Physics* **70**:3, 816-825. [CrossRef]
12. F SHANG, L ZHAO, Q ZHENG, J WANG, Z XU, W LIANG, H LIU, S LIU, L ZHANG. 2006. Simvastatin inhibits lipopolysaccharide-induced tumor necrosis factor-# expression in neonatal rat cardiomyocytes: The role of reactive oxygen species. *Biochemical and Biophysical Research Communications* **351**:4, 947-952. [CrossRef]
13. Emily C. Rothstein , Pamela A. Lucchesi . 2005. Redox Control of the Cell Cycle: A Radical Encounter. *Antioxidants & Redox Signaling* **7**:5-6, 701-703. [Citation] [Full Text PDF] [Full Text PDF with Links]