Int. J. Mol. Sci. **2012**, *13*, 10697-10721; doi:10.3390/ijms130910697

OPEN ACCESS
International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms

Review

Cell Signaling through Protein Kinase C Oxidation and Activation

Daniela Cosentino-Gomes [1,2,*], Nathália Rocco-Machado [1,2] and José Roberto Meyer-Fernandes [1,2]

[1] Institute of Medical Biochemistry, Federal University of Rio de Janeiro (UFRJ), CCS, Bloco H, Cidade Universitária, Ilha do Fundão, 21941-590, Rio de Janeiro, RJ, Brazil; E-Mails: rocco@bioqmed.ufrj.br (N.R.-M.); meyer@bioqmed.ufrj.br (J.R.M.-F.)

[2] Institute of National Science and Technology of Structural Biology and Bioimage (INCTBEB), CCS, Bloco H, Cidade Universitária, Ilha do Fundão, 21941-590, Rio de Janeiro, RJ, Brazil

* Author to whom correspondence should be addressed; E-Mail: danicosentino@yahoo.com.br; Tel.: +55-21-2562-6781; Fax: +55-21-2270-8647.

Received: 20 June 2012; in revised form: 2 August 2012 / Accepted: 13 August 2012 / Published: 24 August 2012

Abstract: Due to the growing importance of cellular signaling mediated by reactive oxygen species (ROS), proteins that are reversibly modulated by these reactant molecules are of high interest. In this context, protein kinases and phosphatases, which act coordinately in the regulation of signal transduction through the phosphorylation and dephosphorylation of target proteins, have been described to be key elements in ROS-mediated signaling events. The major mechanism by which these proteins may be modified by oxidation involves the presence of key redox-sensitive cysteine residues. Protein kinase C (PKC) is involved in a variety of cellular signaling pathways. These proteins have been shown to contain a unique structural feature that is susceptible to oxidative modification. A large number of scientific studies have highlighted the importance of ROS as a second messenger in numerous cellular processes, including cell proliferation, gene expression, adhesion, differentiation, senescence, and apoptosis. In this context, the goal of this review is to discuss the mechanisms by which PKCs are modulated by ROS and how these processes are involved in the cellular response.

Keywords: protein kinase C; reactive oxygen species; cell signaling

1. Introduction

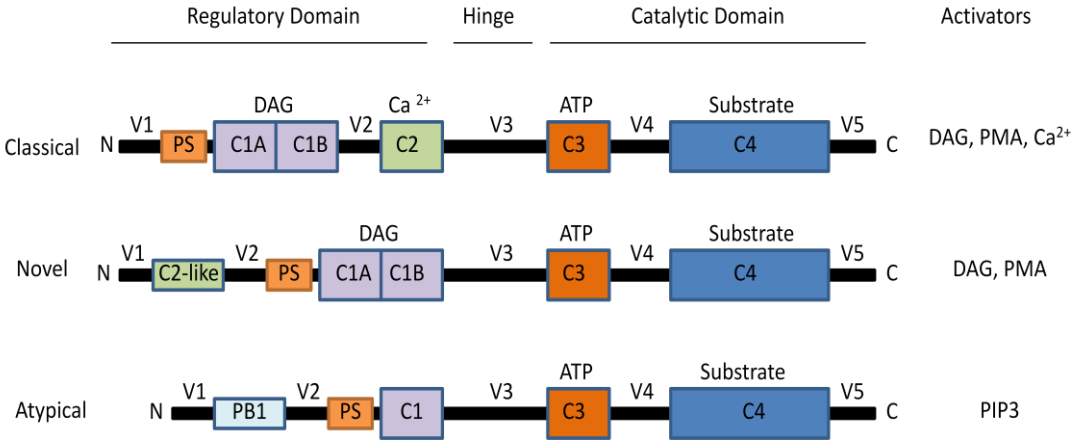
Phosphorylation, acetylation, ubiquitinylation, and glycosylation are among the most well-known post-translational modifications. The concept of protein oxidation as a post-translational modification has only recently gained acceptance. Protein oxidation occurs as an outcome of a chemical attack by reactive oxygen species (ROS) or reactive nitrogen species (RNS) on susceptible amino acids, such as tyrosine, tryptophan, histidine, lysine, methionine, and cysteine [1]. Indeed, it is important to note that signal transduction must occur in a coordinated manner in response to a stimulus. The key elements of a signaling response are reversibility and specificity [2]. In this way, an oxidation-dependent chain reaction may be short and employ only a low concentration of oxidants to avoid irreversible damage to cellular components. Thus, proteins that are reversibly modulated by these reactant molecules are of high interest. Kinases and phosphatases, which act coordinately in the regulation of signal transduction through the phosphorylation and dephosphorylation of target proteins, have been described to be key elements in ROS-mediated signaling events [3–6]. The major mechanism by which these proteins are modified by oxidation involves the presence of key redox-sensitive cysteine residues [7].

The goal of this review is to discuss the mechanisms by which protein kinase Cs (PKCs) can be modulated by ROS and how these processes are involved in the cellular response. The review will focus on the structural mechanism of action of ROS in the activity of these enzymes, how ROS interact with their target molecules, the regulation of these enzymes by oxidants, and finally the major consequences of this tightly controlled mechanism on cell signaling.

2. The Protein Kinase C Family

The PKC family is composed of serine/threonine protein kinases that are involved in a variety of pathways that regulate cell growth, differentiation, apoptosis, transformation and tumorigenicity. Most cells express more than one isoform, and each type of PKC mediates different cellular events [8,9]. The various PKC isoforms consist of NH_2-terminal regulatory domains and COOH-terminal catalytic domains [10]. They also have in common the pseudosubstrate site (PS), which keeps the protein in its inactive form [11]. However, they differ in their structure, cofactor requirement and substrate specificity [11]. Thus, the 10 members of the PKC family have been divided into three major groups: the classical PKCs (cPKCs), including the α, βI, βII, and γ isoforms; the novel PKCs (nPKCs), including the θ, η, ε, δ isoforms; and the atypical PKCs (aPKCs), including the ζ and ι/λ isoforms [12–14]. The cPKC subfamily members possess conserved (C1–C4) and variable (V1–V5) regions, which are presented in Figure 1 [11,12]. They require calcium, phosphatidylserine and diacylglycerol (DAG) or phorbol esters for activation. The nPKCs differ from cPKCs in that they lack the C2 homologous domain and do not require calcium for activation. Finally, aPKCs lack both the C2 and half of the C1 homologous domains, thus rendering them insensitive to DAG, phorbol esters, and calcium [10,15]. Figure 1 depicts the three classes of PKCs and their respective activators. PKCμ and PKCυ isorforms are now classified as members of the DAG receptor protein kinase D (PKD), which is a family of serine/threonine protein kinases classified as a subfamily of the Ca^{2+}/calmodulin-dependent kinase (CaMK) superfamily. For a complete review of PKD see [16,17].

Figure 1. Schematic sequence of protein kinase C (PKC) isozymes indicating the domain structure of the PKC subfamilies and their respective activators.



2.1. Activation of PKCs

PKC isoforms require serine/threonine phosphorylation for their activation. There are three distinct phosphorylation sites in the catalytic domain; the first and rate-limiting phosphorylation step occurs in the activation loop sequence [10,15]. Phosphoinositide-dependent kinase-1 (PDK-1) is the upstream kinase that directly phosphorylates the activation loop of many PKCs [10,15,18]. The activation cascade differs according to the PKC isoform. PDK-1 directs the activation of aPKCs, such as ζ, in a phosphoinositide 3-kinase (PI3K)-dependent manner. In this case, phosphorylation is regulated rather than constitutive. The PI3K pathway produces inositol phospholipids containing an additional phosphate at the third position, which activate PDK-1 and aPKC [19]. However, the phosphorylation at the activation loop of the cPKC and nPKC isozymes does not activate but rather promotes the autophosphorylation of two residues at the *C*-terminus. The first residue has been termed the "turn motif" because it is flanked by a proline residue, and the terminal site is called the "hydrophobic motif" because it is flanked by hydrophobic amino acid residues. The autophosphorylation produces a mature and fully phosphorylated enzyme that is able to respond to lipid second messengers [10,15,18,20].

Phospholipase C (PLC) is an enzyme that, when activated by growth factor receptors, hydrolyzes phosphatidylinositol 4, 5-bisphosphate (PIP_2) to generate DAG and inositol trisphosphate (IP_3), which subsequently mobilize intracellular calcium [21]. In the absence of activating cofactors, such as Ca^{2+} and DAG, PKCs are maintained in an inactive conformation by binding of the PS to the substrate-binding cavity [11,20]. The cPKCs are activated by both DAG, which binds to the C1 domain, and Ca^{2+}, which binds to the C2 domain. After Ca^{2+} signaling, the binding of DAG to the C1 domain increases the affinity of PKCs for membrane lipids, inducing conformational changes that lead to a catalytically competent form. Phorbol esters bind to the same site as DAG and mimic its activation [11,22,23]. Novel PKCs are independent of Ca^{2+} and activated directly by DAG [11]. Recent studies indicate the existence of another model in which cellular stimulation results in inducible phosphorylation at some of the three sites in PKCs [24–26]. Generally, activation is characterized by the translocation of the PKC from the cytosol to the plasma membrane [27]. However, the isoforms have different subcellular localizations, and this simple model cannot be applied for all of them.

2.2. PKC Receptors and Localization of Isoenzymes

PKC isoforms are differentially distributed not only with respect to tissue but also in terms of subcellular localization, suggesting that each isoform could have specific functions [10,11]. Intracellular receptors for inactivated kinases (RICKs) and intracellular receptors for activated kinases (RACKs) are involved in the specific translocation of PKCs from the cytosol to different compartments [28,29]. RICKs and RACKs are proteins that bind PKCs in an isozyme-specific and saturable manner, and any PKC activator should induce the release of PKC from RICK [29]. The interaction of PKCs with RACKs is mediated through the PKC regulatory domain. Moreover, RACKs have been shown to be responsible for the different cellular distribution of the same isoform in different cell types [11].

The α, ζ and δ PKC isoforms are expressed in all tissues [28], while other isoforms are expressed in a tissue specific-manner, such as βI in the spleen; βII in the spleen and brain; η in keratinocytes; and θ in skeletal muscle, T cells and epidermis [28,30]. Atypical PKCι/λ can be found in the testis and insulin-secreting cells [28]. Subcellular localization varies according to organism, tissue, and stimulation. Different diets can also affect the expression and localization of PKC isoforms [10,19,28,31–33].

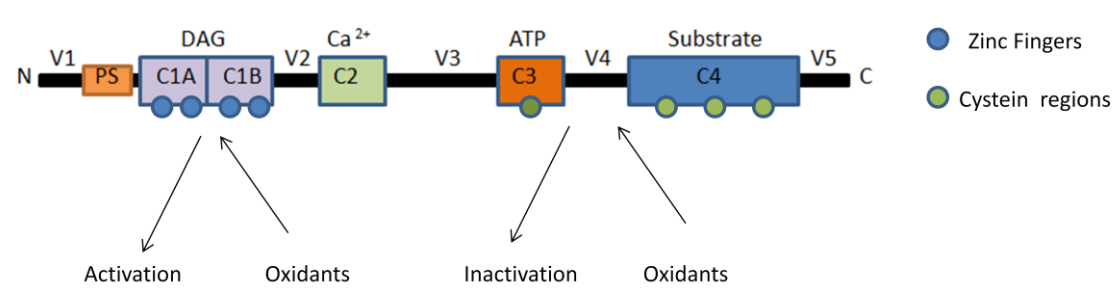
3. Regulation of Protein Kinase C by Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are produced in the natural course of metabolism. They can originate from different sources, including the mitochondrial electron transport chain, xanthine oxidase, myeloperoxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Nox enzymes), and lipoxygenase [3,25]. Nox enzymes and lipoxygenase are responsible for the production of ROS in response to hormones, growth factors and cytokines [34–36]. Among these oxygen metabolites are superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) [26]. These species are not all equally reactive with their prospective targets. Many of them have very short half-lives, leading to little relevance in terms of signaling. For example, the $^{\bullet}OH$ is the most unstable radical, reflecting its limited ability to transmit signals across any significant distance. In addition, O_2^- and H_2O_2 can be considered more stable species, and because of this feature, they may be the most favorable ROS to operate as signaling molecules [37]. ROS were once considered a dangerous product of cellular metabolism because, in high concentrations, they deleteriously affect DNA, lipids and proteins. Currently, these reactant molecules, in lower doses, are thought to act as important mediators of cell growth, adhesion, differentiation, senescence, and apoptosis by modifying key elements in protein regulatory sites [3,25].

In the PKC structure, two pairs of zinc fingers are found within the regulatory domain. They are sites of DAG and phorbol ester binding. Each zinc finger is formed by a structure that is composed of six cysteine residues and two zinc atoms, as presented in Figure 2. The high levels of cysteine residues render the regulatory domain susceptible to redox regulation [11,23]. The oxidants destroy the zinc finger conformation [38], and the autoinhibition is relieved, resulting in a PKC form that is catalytically active in the absence of Ca^{2+} or phospholipids. Cysteine residues are also found in the *C*-terminal catalytic domain, but they are uncoordinated in a different manner to those in the regulatory domain. Redox modifications at the *C*-terminal catalytic domain lead to the inactivation of the kinase

due to the loss of the free sulfhydryls required for its catalytic activity. Furthermore, the intracellular redox state has been shown to affect the distribution of the PKC isoforms [11,23]. Because all PKC isoforms possess zinc fingers and high concentration of cysteine residues located in the regulatory domain, as well as free sulfhydryls in the catalytic site, it seems that the regulation by oxidation is a common feature for this family. However, this signaling mechanism may vary according to the distinct isoform and different cell [11]. The aPKCs, for example, lack one of the two cysteine-rich zinc-finger regions in the regulatory domain [39]. This characteristic can render aPKCs to a distinct susceptibility of oxidative stress. In mouse embryonic fibroblasts, oxidative stress triggers translocation of PKCα, β, δ, and ε isoforms from the cytosol to the plasma membrane. Nevertheless, under the same conditions, PKCζ translocates to the nucleus [11]. PKCδ selectively regulates the activation of the inducible nuclear factor κB (NF-κB) in response to oxidative stress. The activation of the transcription factor NF-κB is often observed in cells exposed to oxidative stress [40]. In HeLa cells treated with exogenous H_2O_2 this signaling pathway was shown to be dependent of the activation of PKD by two coordinated signaling events: the phosphorylation of Tyr463 mediated by the Src-Abl signaling pathway, which then facilitates the second step, the phosphorylation of the PKD activation loop of Ser738/Ser742 by the Src-PKCδ pathway, leading to an increase in cell survival exposed to oxidative stress [41,42]. This coordinated mechanism seems to be specifically in response to oxidative stress, and relies on the activation of PKCδ and not of other PKC isoforms [41]. Later the same group showed that the resveratrol-dependent inhibition of PKD Ser738/Ser742 phosphorylation by PKCδ was able to block the activation of NF-κB in response to oxidative stress. Resveratrol inhibited NFκB activation by avoiding PKD association with IKK complex [40]. The specificity by which ROS may activate different PKC isoforms is not well established, but the localization and the intensity of ROS generation could contribute to the explanation of such different responses.

Figure 2. Schematic representation of susceptible sites to oxidation of PKCs.



4. Effect of Mitochondrial ROS Generation on PKC Activity

In addition to hormones and growth factors, ROS are currently recognized as initiating factors for signaling cascades in the cell, as they are involved in numerous physiological signaling pathways and cellular functions. Thus, sites of ROS production within the cell, such as mitochondria, or enzymes that are capable of one-electron transfer to O_2, such as Nox enzymes, xanthine oxidase and cytochrome P-450, play important roles in cellular metabolism and signaling [34–36].

The main superoxide producer in the cell is the respiratory chain, and the primary factor governing mitochondrial ROS generation is the redox state of the respiratory chain [43,44]. In fact, two of the

respiratory chain complexes (I and III) have long been recognized for their involvement in O_2^- production. With respect to complex III, two components were proposed to be auto-oxidizable electron donors to oxygen: semiubiquinone and reduced cytochrome b. The other site in the respiratory chain that is involved in O_2^- formation is complex I. In this large multi-subunit complex, electron transfer functions at near equilibrium, and thus, O_2^- production may be linked to both forward electron transport and reverse electron transport. For a complete review, see Rigoulet *et al.* 2011 [45].

PKCs exist in an inactive form and are activated depending on their subcellular redistribution and the availability of the appropriate substrates after a distinct stimulus [46,47]. Currently, much evidence supports the direct activation of different PKC isoforms by ROS generation [23,48]. The signaling pathway triggered upon PKC activation by ROS depends on the specific isoform, cell type, and the site of ROS generation. Thus, depending on the particular condition, this mechanism can be involved in either cell protection or death.

The presence of PKCs in the mitochondria has long been known, but their functions are not well defined [47,49]. The activation of PKCδ with phorbol myristate acetate (PMA) or H_2O_2 stimulates its accumulation in the mitochondria, and this fact can be related to apoptosis in different cell types [50–52]. On the other hand, accumulation of PKCε in the mitochondria was appointed a cardioprotective function [53].

In human melanoma cells, mitochondrial ROS were found to have a cytoprotective effect in cells treated with the chemotherapeutic silibinin. In a study performed by Jiang and colleagues [54], silibinin induced ROS generation in these cancer cells, with O_2^- being the major species responsible for the activation of PLC-dependent PKCγ, which is part of a pro-survival pathway to protect the cells from the cytotoxicity of silibinin [54].

PKC activation is also involved in cell damage induced by hyperglycemia. Hyperglycemia results in a loss of antioxidant reducing equivalents, which culminates in an overproduction of O_2^- and Sorbitol, the product resulting from the enzymatic conversion of glucose, can produce fructose by the action of sorbitol dehydrogenase. This reaction increases the ratio of $NADH/NAD^+$, enhancing the oxidized form of triose phosphates with *de novo* synthesis of DAG. DAG can then activate several PKC isoforms, which can lead to various clinical complications in diabetes [55,56]. In bovine vascular endothelial cells, the inhibition of mitochondrial metabolism or the overexpression of uncoupling protein-1 (UCP1) or superoxide dismutase (SOD), the enzyme responsible for the dismutation of O_2^- to H_2O_2, results in a decrease in mitochondrial ROS production, which prevents PKC activation and sorbitol accumulation [57].

Mitochondrial ROS also have an important role in pulmonary vasoconstriction induced by hypoxic conditions. In artery smooth muscle cells that were submitted to an acute hypoxic period, an increase in mitochondrial ROS production was shown, followed by the activation of total PKC and PKCε. The experiments performed by Rathore and colleagues [58] provided support for the specificity of mitochondrial ROS-induced signal transduction pathways in vasoconstriction induced by hypoxia. The activation of PKCs in response to hypoxic conditions in pulmonary artery smooth muscle cells is remarkable because different responses were seen in mesenteric arteries under the same conditions. Moreover, the external addition of H_2O_2 was able to mimic hypoxic responses in normoxic conditions, leading to an increase in PKCε activity in pulmonary arteries. Interestingly, H_2O_2 also significantly activated PKCε in mesenteric arteries although not under hypoxic conditions [58]. These experiments

highlight the importance of H_2O_2 as a second messenger, but the effectiveness of the signal triggered by this molecule may also be related to the site of its production, in this case, the mitochondria. In hepatocytes, mitochondrial ROS generation after cell exposure to sodium arsenite ($NaAsO_2$) was shown to induce PKCδ activation, which in turn activates c-Jun *N*-terminal kinases (JNK) leading to the progression of apoptosis. On the other hand, in the same study, the authors showed that treatment of hepatocytes with taurine was able to inhibit PKCδ and JNK activation, thus avoiding arsenic-induced ROS generation in the liver, preventing apoptosis [59]. In contrast, PKCδ is involved in γ-radiation activated signaling in splenic lymphocytes with a cytoprotective effect. In this case, radiotherapy treatment leaded to a decrease in the activities of antioxidant enzymes and an increase in cell oxidative damage. This effect was followed by an increase in PKCδ activation and degradation of IκBα during the earlier period of treatment. IκBα after degradation is known to release NF-κB, which acts in the nucleus as a transcription factor for many anti-apoptotic genes, thus indicating that the cell could be entering into a cytoprotective pathway. In this study, pretreatment of irradiated cells with curcumin-copper complex was shown to be effective in avoiding radiation-induced damage in these cells [60].

ROS-induced PKC activation also has an important function in the cardioprotective signaling cascade. Sevoflurane, an anesthetic with the ability to protect the myocardium from ischemia and reperfusion injury, triggers a protective signal transduction cascade [61]. The preconditioning elicited by sevoflurane involves the translocation of PKCα towards the mitochondria, which is induced by an increase in ROS production [62]. The fundamental role of PKCα in this cardioprotective effect is still unclear, but the authors suggest the participation of this enzyme in an anti-apoptotic signaling cascade [62]. On the other hand, PKCs can be involved in apoptosis induction through mitochondrial ROS generation. Treatment of head and neck squamous carcinoma cells (HNSCC) with *N*-(4-hydroxyphenyl)retinamide (4HPR), a synthetic retinoid effective in cancer chemoprevention and therapy was shown to increase mitochondrial ROS generation, which in turns activates PKC and MAPK kinases (MKK4,MKK3/6). Downstream of PKC activation is the activation of p38 and ERK, resulting in an enhancement of apoptosis via caspase 9 and caspase 3 pathway [63].

In addition to interactions between receptors and ligands, other events are involved in mitochondrial ROS-induced PKC activation. Several studies implicate that mechanical stretch increases ROS production in endothelial cells [64–66]. In bovine pulmonary artery endothelial cells, strain-induced perturbation of the plasma membrane is involved in the release of ROS to the cytosol, thus activating downstream effectors involved in the mechanotransduction signaling pathway. These signals involve the participation of the intact actin cytoskeleton and the phosphorylation of focal adhesion kinase (FAK) by PKCα. In this way, PKC activation is preceded by mitochondrial ROS production in stretch-induced endothelial cells [67].

5. PKC Phosphorylates Nox Subunits for ROS Generation

Nox enzyme is a complex multi-subunit enzyme that uses NADPH as a substrate to convert molecular oxygen into ROS (mainly O_2^- with secondary production of H_2O_2) in a regulated manner in response to different stimuli, including growth factors, cytokines and calcium signals [68,69]. The prototypical Nox enzyme from phagocytic cells is divided into two parts: a membrane bound

component, the flavocytochrome b_{558}, which is composed of two subunits, a large glycosylated gp91phox that forms the catalytic subunit and the smaller regulatory subunit p22phox, and a cytosolic component formed by four major proteins, the regulatory subunits p47phox, p67phox, and p40phox, and the small GTPase RAC [70–73]. To date, seven mammalian isoforms are known (Nox1-5, Duox1, 2) to be expressed in a wide variety of tissues, such as vascular smooth muscle, kidney, spleen, cerebrum, lungs, colon, ovary and others [74]. When cells are stimulated, the cytosolic components migrate almost instantly to the membrane, where they assemble with the flavocytochrome b_{558} to form the active enzyme, a process that is tightly regulated by protein-protein interactions and phosphorylation of the p47phox subunit [68,70–73,75].

PKC isoforms are upstream regulators of Nox. The activity of these enzymes is important for the assembly and activation of the Nox1-3 isoforms, which require the phosphorylation-dependent assembly of several cytosolic subunits to be catalytically active; this includes p47phox, which is the best studied of the phosphorylated Nox enzyme components [76]. The *C*-terminal sequence of p47phox has a basic charge, is rich in serine and arginine residues and has at least one proline-rich region (PRR) (amino acids 363–368) [71,77]. In resting cells, p47phox is not phosphorylated and possesses a highly basic charge (pI > 9). Upon phosphorylation, its pI shifts to the acidic range, giving rise to several phosphorylated isoforms, which correspond to different phosphorylated states [71,78]. Different PKC isoforms in various tissues have been described to be efficient in the phosphorylation of the p47phox subunit, as summarized in Table 1. On the other hand, both Nox4 and Nox5 do not require cytosolic subunits for their activation. Nox4 is a constitutively active enzyme and is bound to the integral membrane protein p22phox, whereas Nox5 is primarily regulated by calcium levels [79,80].

Table 1. Protein kinase C (PKC) isoforms involved in phosphorylation of p47phox subunit.

PKC group	PKC isoform	Cell/tissue type	References
Classical	PKCα	Kidney	[81]
	PKCβ	Leukemic cells; Neutrophils; and Monocytes.	[46,82–85]
Novel	PKCδ	Monocytes; Neuroblastoma; Neutrophils; and Fibroblast.	[75,86–88]
	PKCε	Pulmonary artery smooth muscle; and Myocytes.	[89,90]
Atypical	PKCζ	Leukocytes; Neurons; Hippocampus of mice; and Alveolus.	[91–94]

PKC is not the only protein kinase able to phosphorylate p47phox. PKA [95,96], MAPK ERK1/2 and p38MAPK [97,98], protein casein kinase 2 (CKII) [99], AKT [100,101], p21-activated kinase (PAK) [102], a phosphatidic acid-activated kinase [103] and src kinase [104] have also been shown to phosphorylate this subunit [71].

A growing body of research has shown that the activation of the PKC/Nox signaling complex regulates ROS levels and is involved in various pathophysiological conditions, including neurodegenerative disorders [105,106], human cardiovascular disease, such as atherosclerosis [95,107–111], hypertension [112,113], renal damage [91], diabetes [114–117], and cancer [118].

PKC has been shown to activate O_2^- generation by Nox enzymes in several types of cells, including phagocytes [46,71], cardiomyocytes [100], aortic endothelial cells [107], platelets [114], and renal mesangial cells [119].

The interaction between ligand and receptor is crucial to trigger the signaling pathways that involve the PKC and Nox enzymes. The response is different in each cell and involves distinct PKC and Nox isoforms. In vascular smooth muscle cells (VSMC), for example, three isoforms of Nox enzymes are present, Nox1, Nox2, and Nox4. They are thought to have different functions and subcellular localization. It seems that Nox1, when activated, is present on the cell surface and co-localizes with caveolins, while Nox2 can be located intracellularly or in the cell membrane. Nox4 is localized in focal adhesions and in nuclei [120–122]. In this model, Nox4 plays an important role in the constitutive production of ROS, while Nox1 would be necessary during pathological development [122–124]. Recently studies showed that Nox1 expression is involved in the hypertrophy of VSMCs induced by aldosterone and prostaglandin F2α. In both cases, the up-regulation of Nox1 may occur through the activation of PKCδ and the activating transcription factor 1 (ATF1) [122,125,126].

PKC can also activate Nox2. In human monocytes and murine macrophages PI3K and PKC pathways are involved in Nox2 stimulation in response to insulin. This mechanism triggers ERK1/2, p38MAPK and NFκB, which culminates in the activation of monocytes and proliferation of macrophages [127]. PKC-dependent Nox2 activation was also shown in coronary artery disease. Despite the concomitant expression of Nox2 and Nox4 in these arteries, Nox2 represents the main source of superoxide production in the disease, and this was related in part by an increase in monocyte/macrophage infiltration, which reflects the presence of inflammatory cells. On the other hand, Nox4 seems to be related to smooth muscles cells or myofibroblasts and is independent of the pathological condition [128].

Several studies demonstrate that Nox4 is inducible and can generate H_2O_2 in response to various hormone stimuli. Generation of H_2O_2 by Nox4 after insulin stimulation of differentiated adipocytes was shown to be important in both early and late events in insulin signal transduction, which includes the activation of insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation, and the activation of downstream serine kinases and glucose uptake [129]. Similar results were found in preadipocytes, where Nox4 acts indirectly to facilitate the tyrosine phosphorylation of IRS-1, stimuli required for the insulin-induced differentiation of these cells [130]. Nox4 was also stimulated by H_2O_2 and diacylglycerol, through a mechanism dependent of phospholipase A_2, but apparently independent of PKC activity. The arachidonic acid generated in this system by phospholipase A_2 is responsible for the oxidase activation and the increase of intracellular ROS production [131], similar results were found in mesangial cells [132].

It has been well established that angiotensin II exerts important intra–cellular signaling through ROS generation in many physiological and pathological conditions [133]. In many published works, angiotensin II has been shown to activate the Nox enzyme in a PKC-dependent manner [91,109,134–138]. Apoptosis of cardiac cells mediated by alcohol is dependent on the angiotensin II interaction with the angiotensin II type 1 (AT1) receptor, which subsequently activates PKCβI to phosphorylate and activate the Nox enzyme. Because high amounts of superoxide are generated in this process, this mechanism has also been related to alcoholic cardiomyopathy [134]. Angiotensin II also increased the basal and the NADPH stimulated superoxide production in coronary arteries, and this was found to be

modulated by PKC activity [128]. In addition, angiotensin II stimulates the epithelial Na^+-channel in the rat cortical collecting duct through the activation of Nox-dependent PKC phosphorylation [135]. In the renal system, this receptor agonist has a stimulatory effect in a cross talk mechanism between the connecting tubule in the renal cortex and the afferent arteriole, which is mediated by O_2^- that is generated primarily by PKC-dependent Nox activation [132]. In physiological conditions, angiotensin II also regulates O_2^- generation in the thick ascending limb of the renal medulla through the activation of PKCα and the stimulation of NADPH oxidase [81]. This mechanism is also associated with an increase in sodium absorption in this renal structure [136]. Similar results were obtained for skeletal muscle [137] and monocytes, in which increased ROS generation by angiotensin II caused an increase in the level of interaction between human monocytes and the extracellular matrix by favoring adhesion to laminin-1 [108]. In myocytes, White and collaborators showed that this angiotensin II-dependent activation of PKC/Nox inhibits the Na^+–K^+ pump [84]. Conversely, at the same time that angiotensin II triggers PKC-dependent Nox enzyme activation and ROS generation, it also stimulates the activity of antioxidant enzymes like catalase, SOD, and glutathione peroxidase (GPx) in the rat hypothalamus, a counteraction mechanism that exerts local control on ROS levels [138].

Angiotensin II can also act as key neuromodulator in central autonomic nuclei [139,140]. Vagal afferent neurons, in the nucleus of the solitary tract, play a major role in cardiovascular regulation and are a target of angiotensin II through activation of the AT1 receptor. In these cells intracellular Ca^{2+} and PKC activation are critical for AT1 receptor-induced Nox2 activation and ROS production. The authors suggest that this mechanism could play a role in the vascular dysregulation mediated by the central autonomic system [141].

Phorbol esters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or PMA, are also involved in PKC-dependent Nox activation. In human lung adenocarcinoma cells, the tumor-promoting TPA and the tumor necrosis factor-alpha (TNF-α) were implicated in the stimulation of the expression of the mitochondrial manganese-dependent isoform of SOD (MnSOD) mediated by the Nox enzyme pathway. This stimulation is in part regulated by PKCα, which phosphorylates a transcription factor of cAMP-responsive element-binding protein, or by the PKCε pathway, which culminates with the activation of forkhead transcription factor 3 (FOXO3) [142]. In a recent study, Kamiya and colleagues showed that the reduction of extracellular SOD and cytosolic copper/zinc SOD (Cu/ZnSOD) is dependent on the PKC/Nox complex during the TPA-induced monocytic differentiation of U937 cells, a leukemic cell line. Moreover, the nuclear factor kappa B (NF-κB) is involved only in the reduction of Cu/ZnSOD [143], suggesting differential regulation for both isoforms. In human umbilical vein endothelial cells, TNF-α was also shown to increase ROS generation via the PKCβII-dependent activation of vascular Nox enzyme. This mechanism has been suggested to be involved with apoptosis in these cells, but it occurs independently of ROS generation [144]. PMA influences the phosphorylation of Nox5 through the activation of the MAPK/ERK1/2 pathway, leading to an increase in ROS generation in fibroblasts [145] in a calcium-independent manner [146]. Although Nox5 requires calcium for its activation, the specific phosphorylation of serine/threonine residues in Nox5 affects the calcium sensitivity of Nox5, thus permitting its activation without a change in calcium levels in the cell [80,146,147]. In bovine coronary arteries, PKC is activated by phorbol 12,13-dibutyrate (PDBu). PDBu increases superoxide generation by Nox2 through both p47phox and

peroxide-dependent Src activation. The authors suggest that Nox2 contributes to vascular contractile mechanisms through PKC-dependent activation [148].

Several works also highlight the participation of formyl peptide formyl-methionyl-leucyl-phenylalanine (fMLP) in the activation of PKC and Nox enzyme. This participation includes superoxide generation, which in turn stimulates leukocytes, phagocytic cells, and neutrophils [46,72,84,91,149,150].

Although most biological functions of PKC have been recognized to occur at the plasma membrane or in the cytoplasm, several studies have pointed out the role of PKCs in nuclear functions [39]. Almost all the isoforms of PKC have been identified at nuclear level, except for the PKCμ isoform [39,151]. The distinct functions of PKCs in the nucleus may be a consequence of either, the activity of resident isoforms of the enzyme or the translocation of PKC from cytoplasm to the nucleus, which may occur in certain conditions and in a different cellular system, as a result of nuclear lipid signaling [39]. It seems that DAG is the driving force to attract cPKCs to the nucleus [39,152–155], while for nPKCs and aPKCs there has been no specific recognized lipid messenger for this function until now. However, Phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P3), one of the products of PI3K family members, has been proposed to act as the driving force for the nuclear translocation of the aPKC isoform PKC-ζ [39,156]. This PKC-ζ translocation through PtdIns(3,4,5)P3 signaling has been shown in nerve growth factor (NGF)-treated rat pheochromocytoma PC-12 cells [157,158], and in rat epatocytes treated with C2-ceramid [159].

The nuclear presence of PKC suggests an involvement of these enzymes in the regulation of DNA replication, RNA synthesis and processing, gene expression, transport between nucleus and cytoplasm, and chromatin structure [39]. Moreover, nuclear PKCs have been implicated in cell proliferation, differentiation, apoptosis, and cardiovascular diseases [39,160]. For a complete review of nuclear PKCs and their function in cells see [39,151,161].

Beside PKCs, Nox enzymes subunits have been described to be localized in subcellular compartments of different cells [162]. This includes the presence of Nox1 and Nox4 in endoplasmic reticulum [79,163], Nox2 in perinuclear compartments [164], and Nox4 at the nucleus of multiple kinds of cells, such as endothelial cells, VSMCs, and hepatic cells [121,165,166]. Nox4 is considered the predominant NADPH isozyme in the kidney with prevalent expression in the proximal tubule [167,168]. Regarding these concepts, recent data show that angiotensin II can induce a nuclear Nox4-dependent generation of ROS, through PI3K and PKC activation in isolated nuclei of cortical cells of kidney [169].

6. PKC-Induced ROS Generation

PKC also has been shown to promote the production of endogenous ROS to induce a positive feedback loop [48]. The β isoform of PKC induces ROS generation through mitochondrial damage [170]. The enzyme has two major splice variants, -βI and -βII, which result from alternative splicing and differ in their carboxyl-terminus and subcellular localization [171]. In melanoma, the reduced expression of the variant PKCβII decreases ROS generation and promotes the survival and growth of melanoma cells under oxidative stress conditions [172]. In chondrocytes that have lost their normal extracellular matrix and growth factor survival signals, the activation of PKCβI results in the production of mitochondrial ROS, which is required for a signal that mediates both apoptosis and

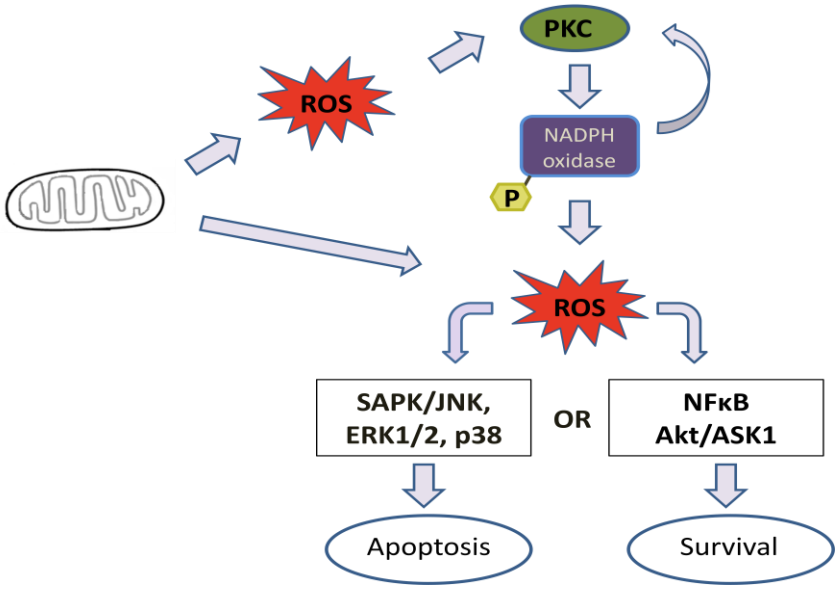
necrosis. Notwithstanding, in this same study, PKCδ activity was determined to be necessary for chondrocyte survival [48]. The PKCβ isoform is responsible for the activation/phosphorylation of the mitochondrial $p66^{shc}$ protein [173]. This protein is an alternatively spliced isoform of a growth factor adapter. When this isoform is activated in mitochondria, it can bind to cytochrome c, acting as an oxidoreductase and ROS-generating system and subsequently promoting organelle dysfunction and cell death [174]. In mouse embryonic fibroblasts, inhibition of PKCβ leads to the inhibition of mitochondrial $p66^{shc}$ phosphorylation, preserving mitochondrial morphology and reducing apoptosis in cells under oxidative stress [170].

In myeloid leukemia cells, ROS are not necessary for the translocation of PKCβII from the cytosol to the cell membrane. However, PKCβII was shown to be essential for ROS production induced by the PKC activator TPA. This ROS-dependent mechanism is associated with the stress-activated protein kinase pathway (SAPK). SAPK is associated with the molecular response of myeloid leukemia cells to TPA, through the phosphorylation and activation of c-jun, activating transcription factor 2 (ATF2) and Elk-1, contributing to the induction of the c-jun and Erg-1 early response genes involved in this signaling process [175]. The PKCε and PKCζ isoforms are also involved in mitochondrial ROS production. The neuroprotective effect of the sphingolipid C2-ceramide involves the participation of both isoforms, which promote an increase in the formation of mitochondrial ROS and a controlled opening of the mitochondrial permeability transition pore. In ischemia, these events prevent mitochondrial calcium overload, leading to a protective effect and the prevention of cell death during recovery of normal cell function [176]. In skeletal myocytes and glioma cells, PKCα is translocated to the mitochondria after activation by PMA, resulting in mitochondrial dysfunction through a decrease in complex I activity and an enhancement of ROS generation [47].

7. Conclusions

Because different PKC isoforms are present in a variety of cells and are involved in a number of signaling pathways, it is difficult to ascribe a common ROS-dependent mechanism for all PKC isotypes. However, it seems that under pathological conditions, a general signaling mechanism is triggered in mitochondrial dysfunction. In this context, an increase in ROS production by mitochondria activates local PKCs, which in turn activate Nox enzymes. Nox enzymes could also activate another group or the same group of PKCs in a feedback mechanism. Consequently, the redox state of the cell becomes imbalanced. Depending on the stimuli, proapoptotic signaling may occur through ROS activation of MAPK, such as SAPK/JNK, ERK1/2 and p38, or antiapoptotic signals ccould be triggered through activation of the NFκB or Akt/ASK1 pathway [177]. Figure 3 represents a general mechanism of cell signaling activation trough mitochondria/PKC/NADPH oxidase. This mechanism can be different depending on the cell and activation stimulus. More studies are necessary to elucidate the mechanisms that are involved in these signaling pathways.

Figure 3. General mechanism of cell signaling activation through mitochondria/ PKC/ nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. In pathological conditions, a mitochondrial dysfunction could lead to an increase in reactive oxygen species (ROS) generation. ROS can directly trigger cell signaling or activate different PKC isoforms, depending on cell type and stimulation. Activated PKC stimulates NADPH oxidase, which generates ROS. These ROS could activate another group or the same group of PKCs in a feedback mechanism or induce cell signaling. Depending on the stimulus, it could lead to cell survival or death.



Acknowledgements

This study was supported by grants from the Brazilian National Research Council (CNPq), the Carlos Chagas Filho Rio de Janeiro State Research Foundation (FAPERJ), the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES), and the National Institutes of Science and Technology, Brazil.

References

1. Spickett, C.M.; Pitt, A.R.; Morrice, N.; Kolch, W. Proteomic analysis of phosphorylation, oxidation and nitrosylation in signal transduction. *Biochim. Biophys. Acta* **2006**, *1764*, 1823–1841.
2. Tonks, N.K. Redox redux: Revisiting PTPs and the control of cell signaling. *Cell* **2005**, *121*, 667–670.
3. Leslie, N.R.; Lindsay, Y.; Ross, S.H.; Downes, C.P. Redox regulation of phosphatase function. *Biochem. Soc. Trans.* **2004**, *32*, 1018–1020.
4. Suzuki, Y.J.; Forman, H.J.; Sevanian, A. Oxidants as stimulators of signal transduction. *Free Radic. Biol. Med.* **1997**, *22*, 269–285.

5. Cosentino-Gomes, D.; Russo-Abrahão, T.; Fonseca-de-Souza, A.L.; Ferreira, C.R.; Galina, A.; Meyer-Fernandes, J.R. Modulation of *Trypanosoma rangeli* ecto-phosphatase activity by hydrogen peroxide. *Free Radic. Biol. Med.* **2009**, *47*, 152–158.
6. Cosentino-Gomes, D.; Meyer-Fernandes, J.R. Ecto-phosphatase in protozoan parasites: Possible roles in nutrition, growth and ROS sensing. *J. Bioenerg. Biomembr*. **2011**, *43*, 89–92.
7. Knock, G.A.; Ward, J.P.T. Redox regulation of protein kinases as a modulator of vascular function. *Antioxid. Redox Signal*. **2011**, *15*, 1531–1547.
8. Nishizuka, Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **1992**, *258*, 607–614.
9. Nishizuka, Y. Protein Kinase C and lipid signaling for sustained cellular responses. *FASEB J.* **1995**, *9*, 486–496.
10. Duquesnes, N.; Lezoualc'h, F.; Crozatier, B. PKC-delta and PKC-epsilon: Foes of the same family or strangers? *J. Mol. Cell. Cardiol.* **2011**, *51*, 665–673.
11. Giogi, C.; Agnoletto, C.; Baldini, C.; Bononi, A.; Bonora, M.; Marchi, S.; Missiroli, S.; Patergnani, S.; Poletti, F.; Rimessi, A.; *et al.* Redox control of Protein Kinase C: Cell and disease-specific aspects. *Antioxid. Redox Signal*. **2010**, *13*, 1051–1085.
12. Kikkawa, U.; Kishimoto, A.; Nishizuka, Y. The protein kinase C family: Heterogeneity and its implications. *Annu. Rev. Biochem*. **1989**, *58*, 31–44.
13. Svetek, J.; Schara, M.; Pecar, S.; Hergenhahn, M.; Hecker, E. Spectroscopic characterization of specific phorbol ester binding to PKC-receptor sites in membranes *in situ*. *Carcinogenises* **1995**, *10*, 2589–2592.
14. Rosse, C.; Linch, M.; Kermorgant, S.; Cameron, A.J.; Boeckeler, K.; Parker, P.J. PKC and the control of localized signal dynamics. *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 103–112.
15. Cenni, V.; Döppler, H.; Sonnenburg, E.D.; Maraldi, N.; Newton, A.C.; Toker, A. Regulation of novel protein kinase C epsilon by phosphorylation. *Biochem. J.* **2002**, *3*, 537–545.
16. Rozengurt, E.; Rey, O.; Waldron, R.T. Protein kinase D signaling. *J. Biol. Chem.* **2005**, *280*, 13205–13208.
17. Wang, Q.J. PKD at the crossroads of DAG and PKC signaling. *Trends Pharmacol. Sci.* **2006**, *27*, 317–323.
18. Toker, A.; Newton, A.C. Cellular signaling: Pivoting around PDK-1. *Cell* **2000**, *2*, 185–188.
19. Miyamae, M.; Rodriguez, M.M.; Camacho, S.A.; Diamond, I.; Mochly-Rosen, D.; Figueredo, V.M. Activation of epsilon protein kinase C correlates with a cardioprotective effect of regular ethanol consumption. *Proc. Natl. Acad. Sci. USA* **1998**, *4*, 8262–8267.
20. Newton, A.C. Regulation of the ABC kinases by phosphorylation: Protein kinase C as a paradigm. *Biochem. J.* **2003**, *2*, 361–371.
21. Steinberg, S.F. Structural basis of protein kinase C isoform function. *Physiol. Rev*. **2008**, *4*, 1341–1378.
22. Oancea, E.; Meyer, T. Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* **1998**, *3*, 307–318.
23. Gopalakrishna, R.; Jaken, S. Protein kinase C signaling and oxidative stress. *Free. Radic. Biol. Med.* **2000**, *9*, 1349–1361.

24. Saito, N.; Kikkawa, U.; Nishizuka, Y. The family of protein kinase C and membrane lipid mediators. *J. Diabetes Complic*. **2002**, *1*, 4–8.
25. Thannickal, V.J.; Fanburg, B.L. Reactive oxygen species in cell signaling. *Am. J. Physiol. Lung Cell. Mol. Physiol*. **2000**, *6*, 1005–1028.
26. Chiarugi, P.; Buricchi, F. Protein tyrosine phosphorylation and reversible oxidation: Two cross-talking posttranslation modifications. *Antioxid. Redox Signal.* **2007**, *1*, 1–24.
27. Almeida-Amaral, E.E.; Caruso-Neves, C.; Lara, L.S.; Pinheiro, C.M.; Meyer-Fernandes, J.R. *Leishmania amazonensis*: PKC-like protein kinase modulates the (Na^+ + K^+)ATPase activity. *Exp. Parasitol.* **2007**, *116*, 419–426.
28. Breitkreutz, D.; Braiman-Wiksman, L.; Daum, N.; Denning, M.F.; Tennenbaum, T. Protein kinase C family: On the crossroads of cell signaling in skin and tumor epithelium. *J. Cancer Res. Clin. Oncol.* **2007**, *11*, 793–808.
29. Mochly-Rosen, D.; Gordon, A.S. Anchoring proteins for protein kinase C: A means for isozyme selectivity. *FASEB J.* **1998**, *1*, 35–42.
30. Hug, H.; Sarre T.F. Protein kinase C isoenzymes: Divergence in signal transduction? *Biochem. J.* **1993**, *2*, 329–343.
31. Benoit, S.C.; Kemp, C.J.; Elias, C.F.; Abplanalp, W.; Herman, J.P.; Migrenne, S.; Lefevre, A.L.; Cruciani-Guglielmacci, C.; Magnan, C.; Yu, F.; *et al.* Palmitic acid mediates hypothalamic insulin resistance by altering PKC-theta subcellularlocalization in rodents. *J. Clin. Invest*. **2009**, *9*, 2577–2589.
32. Oyasu, M.; Fujimiya, M.; Kashiwagi, K.; Ohmori, S.; Imaeda, H.; Saito, N. Immunogold electron microscopic demonstration of distinct submembranous localization of theactivated γPKC depending on the stimulation. *J. Histochem. Cytochem.* **2008**, *56*, 253–265.
33. Judé, S.; Martel, E.; Vincent, F.; Besson, P.; Couet, C.; Ogilvie, G.K.; Pinault, M.; De Chalendar, C.; Bougnoux, P.; Richard, S.; *et al.* Dietary long-chain n-3 fatty acids modify blood and cardiac phospholipids and reduce protein kinase-C-delta and protein kinase-C-epsilon translocation. *Br. J. Nutr.* **2007**, *6*, 1143–1151.
34. Bae, Y.S.; Kang, S.W.; Seo, M.S.; Baines, I.C.; Tekle, E.; Chock, P.B.; Rhee, S.G. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J. Biol. Chem.* **1997**, *272*, 217–221.
35. Salmeen, A.; Barford, D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. *Antioxid. Redox Signal*. **2005**, *7*, 560–577.
36. Sundaresan, M.; Yu, Z.X.; Ferrans, V.J.; Irani, K.; Finkel, T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* **1995**, *270*, 296–299.
37. Chen, K.; Craige, S.E.; Keaney, J.F., Jr. Downstream targets and intracellular compartmentalization in Nox Signaling. *Antioxid. Redox Signal.* **2009**, *11*, 2467–2480.
38. Lin, Y.L.; Shivji, M.K.; Chen, C.; Kolodner, R.; Wood, R.D.; Dutta, A. The evolutionarily conserved zinc finger motif in the largest subunit of human replication protein A is required for DNA replication and mismatch repair but not for nucleotide excision repair. *J. Biol. Chem.* **1998**, *3*, 1453–1461.
39. Martelli, A.M.; Evangelisti, C.; Nyakern, M.; Manzoli, F.A. Nuclear protein kinase C. *Biochim. Biophys. Acta* **2006**, *1761*, 542–551.

40. Storz, P.; Döppler, H.; Toker, A. Activation loop phosphorylation controls protein kinase D-dependent activation of nuclear factor kappaB. *Mol. Pharmacol.* **2004**, *66*, 870–879.
41. Storz, P.; Döppler, H.; Toker, A. Protein kinase Cdelta selectively regulates protein kinase D-dependent activation of NF-kappaB in oxidative stress signaling. *Mol. Cell. Biol.* **2004**, *24*, 2614–2626.
42. Storz, P.; Toker, A. Protein kinase D mediates a stress-induced NF-kappaB activation and survival pathway. *EMBO J.* **2003**, *22*, 109–120.
43. Skulachev, V.P. Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants. *Q. Rev. Biophys.* **1996**, *29*, 169–202.
44. Lambert, A.J.; Brand, M.D. Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH: Ubiquinone oxidoreductase (complex I). *J. Biol. Chem.* **2004**, *17*, 39414–39420.
45. Rigoulet, M.; Yoboue, E.D.; Devin, A. Mitochondrial ROS generation and its regulation: Mechanisms involved in H_2O_2 signaling. *Antioxid. Redox Signal.* **2011**, *1*, 459–468.
46. Korchak, H.M.; Rossi, M.W.; Kilpatrick, L.E. Selective role for beta-protein kinase C in signaling for O-2 generation but not degranulation or adherence in differentiated HL60 cells. *J. Biol. Chem.* **1998**, *16*, 27292–27299.
47. Wang, Y.; Biswas, G.; Prabu, S.K.; Avadhani, N.G. Modulation of mitochondrial metabolic function by phorbol 12-myristate 13-acetate through increased mitochondrial translocation of protein kinase Calpha in C2C12 myocytes. *Biochem. Pharmacol.* **2006**, *28*, 881–892.
48. DelCarlo, M.; Loeser, R.F. Chondrocyte cell death mediated by reactive oxygen species-dependent activation of PKC-betaI. *Am. J. Physiol. Cell Physiol.* **2006**, *290*, 802–811.
49. Noland, T.A., Jr.; Dimino, M.J. Characterization and distribution of protein kinase C in ovarian tissue. *Biol. Reprod.* **1986**, *35*, 863–872.
50. Li, L.; Lorenzo, P.S.; Bogi, K.; Blumberg, P.M.; Yuspa, S.H. Protein kinase C delta targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector. *Mol. Cell. Biol.* **1999**, *12*, 8547–8558.
51. Majumder, P.K.; Pandey, P.; Sun, X.; Cheng, K.; Datta, R.; Saxena, S.; Kharbanda, S.; Kufe, D. Mitochondrial translocation of protein kinase C deltain phorbol ester-induced cytochrome c release and apoptosis. *J. Biol. Chem.* **2000**, *21*, 21793–21796.
52. Majumder, P.K.; Mishra, N.C.; Sun, X.; Bharti, A.; Kharbanda, S.; Saxena, S.; Kufe, D. Targeting of protein kinase C delta to mitochondria in the oxidative stress response. *Cell Growth Differ*. **2001**, *12*, 465–470.
53. Baines, C.P.; Zhang, J.; Wang, G.W.; Zheng, Y.T.; Xiu, J.X.; Cardwell, E.M.; Bolli, R.; Ping, P. Mitochondrial PKCepsilon and MAPK form signaling modules in the murine heart: Enhanced mitochondrial PKCepsilon-MAPK interactions and differential MAPK activation in PKCepsilon-induced cardioprotection. *Circ. Res.* **2002**, *8*, 390–397.
54. Jiang, Y.Y.; Huang, H.; Wang, H.J.; Wu, D.; Yang, R.; Tashiro, S.; Onodera, S.; Ikejima, T. Interruption of mitochondrial complex IV activity and cytochrome c expression activated O_2^--mediated cell survival in silibinin-treated human melanoma A375-S2 cells via IGF-1R-PI3K-Akt and IGF-1R-PLC γ-PKC pathways. *Eur. J. Pharmacol.* **2011**, *668*, 78–87.

55. Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature* **2001**, *13*, 813–820.
56. Rolo, A.P.; Palmeira, C.M. Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. *Toxicol. Appl. Pharmacol.* **2006**, *15*, 167–178.
57. Nishikawa, T.; Kukidome, D.; Sonoda, K.; Fujisawa, K.; Matsuhisa, T.; Motoshima, H.; Matsumura, T.; Araki, E. Impact of mitochondrial ROS production on diabetic vascular complications. *Diabetes Res. Clin. Pract.* **2007**, *77*, 41–45.
58. Rathore, R.; Zheng, Y.M.; Li, X.Q.; Wang, Q.S.; Liu, Q.H.; Ginnan, R.; Singer, H.A.; Ho, Y.S.; Wang, Y.X. Mitochondrial ROS-PKC epsilon signaling axis is uniquely involved in hypoxic increase in [Ca^{2+}] in pulmonary artery smooth muscle cells. *Biochem. Biophys. Res. Commun.* **2006**, *351*, 784–790.
59. Das, J.; Ghosh, J.; Manna, P.; Sil, P.C. Protective role of taurine against arsenic-induced mitochondria-dependent hepatic apoptosis via the inhibition of PKCdelta-JNK pathway. *PloS One* **2010**, *5*, e12602.
60. Kunwar, A.; Narang, H.; Priyadarsini, K.I.; Krishna, M.; Pandey, R.; Sainis, K.B. Delayed activation of PKCdelta and NFkappaB and higher radioprotection in splenic lymphocytes by copper (II)-Curcumin (1:1) complex as compared to curcumin. *J. Cell. Biochem*. **2007**, *102*, 1214–1224.
61. Weber, N.C.; Schlack, W. The concept of anaesthetic-induced cardioprotection: Mechanisms of action. *Best. Pract. Res. Clin. Anaesthesiol.* **2005**, *19*, 429–443.
62. Bouwman, R.A.; Musters, R.J.; van Beek-Harmsen, B.J.; de Lange, J.J.; Lamberts, R.R.; Loer, S.A.; Boer, C. Sevoflurane-induced cardioprotection depends on PKC-alpha activation via production of reactive oxygen species. *Br. J. Anaesth.* **2007**, *99*, 639–645.
63. Kim, H.J.; Chakravarti, N.; Oridate, N.; Choe, C.; Claret, F.X.; Lotan, R. *N*-(4-hydroxyphenyl) retinamide-induced apoptosis triggered by reactive oxygen species is mediated by activation of MAPKs in head and neck squamous carcinoma cells. *Oncogene* **2006**, *25*, 2785–2794.
64. Cheng, J.J.; Wung, B.S.; Chao, Y.J.; Wang, D.L. Cyclic strain-induced reactive oxygen species involved in ICAM-1 gene induction in endothelial cells. *Hypertension* **1998**, *31*, 125–130.
65. Wung, B.S.; Cheng, J.J.; Hsieh, H.J.; Shyy, Y.J.; Wang, D.L. Cyclic strain-induced monocyte chemotactic protein-1 gene expression in endothelial cells involves reactive oxygen species activation of activator protein 1. *Circ. Res.* **1997**, *81*, 1–7.
66. Ali, M.H.; Pearlstein, D.P.; Mathieu, C.E.; Schumacker, P.T. Mitochondrial requirement for endothelial responses to cyclic strain: Implications for mechanotransduction. *Am. J. Physiol. Lung Cell. Mol. Physiol*. **2004**, *287*, 486–496.
67. Ali, M.H.; Mungai, P.T.; Schumacker, P.T. Stretch-induced phosphorylation of focal adhesion kinase in endothelial cells: Role of mitochondrial oxidants. *Am. J. Physiol. Lung Cell. Mol. Physiol*. **2006**, *291*, 38–45.
68. Fontayne, A.; Dang, P.M.; Gougerot-Pocidalo, M.A.; El-Benna, J. Phosphorylation of p47phox sites by PKC alpha, betaII, delta, and zeta: Effect on binding to p22phox and on NADPH oxidase activation. *Biochemistry* **2002**, *18*, 7743–7750.
69. Lambeth, J.D. Nox enzymes and the biology of reactive oxygen. *Nat. Rev. Immunol*. **2004**, *4*, 181–189.

70. El Benna, J.; Faust, L.P.; Babior, B.M. The phosphorylation of the respiratory burst oxidase component p47phox during neutrophil activation. Phosphorylation of sites recognized by protein kinase C and by proline-directed kinases. *J. Biol. Chem.* **1994**, *23*, 23431–234316.
71. El-Benna, J.; Dang, P.M.; Gougerot-Pocidalo, M.A.; Marie, J.C.; Braut-Boucher, F. p47phox, the phagocyte NADPH oxidase/Nox2 organizer: Structure, phosphorylation and implication in diseases. *Exp. Mol. Med*. **2009**, *30*, 217–225.
72. Faust, L.R.; El Benna, J.; Babior, B.M.; Chanock, S.J. The phosphorylation targets of p47phox, a subunit of the respiratory burst oxidase. Functions of the individual target serines as evaluated by site-directed mutagenesis. *J. Clin. Invest.* **1995**, *96*, 1499–1505.
73. Quinn, M.T.; Gauss, K.A. Structure and regulation of the neutrophil respiratory burst oxidase: Comparison with nonphagocyte oxidases. *J. Leukoc. Biol.* **2004**, *76*, 76760–76781.
74. Kawahara, T.; Lambeth J.D. Molecular evolution of Phox-related regulatory subunits for NADPH oxidase enzymes. *BMC Evol. Biol.* **2007**, *7*, 178.
75. Bey, E.A.; Xu, B.; Bhattacharjee, A.; Oldfield, C.M.; Zhao, X.; Li, Q.; Subbulakshmi, V.; Feldman, G.M.; Wientjes, F.B.; Cathcart, M.K. Protein kinase C delta is required for p47phox phosphorylation and translocation in activated human monocytes. *J. Immunol.* **2004**, *1*, 5730–5738.
76. Harrigan, T.J.; Abdullaev, I.F.; Jourd'heuil, D.; Mongin, A.A. Activation of microglia with zymosan promotes excitatory amino acid release via volume-regulated anion channels: The role of NADPH oxidases. *J. Neurochem*. **2008**, *106*, 2449–2462.
77. Groemping, Y.; Rittinger, K. Activation and assembly of the NADPH oxidase: A structural perspective. *Biochem. J.* **2005**, *15*, 401–416.
78. Okamura, N.; Curnutte, J.T.; Roberts, R.L.; Babior, B.M. Relationship of protein phosphorylation to the activation of the respiratory burst in human neutrophils. Defects in the phosphorylation of a group of closely related 48-kDa proteins in two forms of chronic granulomatous disease. *J. Biol. Chem.* **1988**, *15*, 6777–6782.
79. Martyn, K.D.; Frederick, L.M.; von Loehneysen, K.; Dinauer, M.C.; Knaus, U.G. Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. *Cell. Signal.* **2006**, *18*, 69–82.
80. Fulton D.J. Nox5 and the regulation of cellular function. *Antioxid Redox Signal*. **2009**, *11*, 2443–2452.
81. Herrera, M.; Silva, G.B.; Garvin, J.L. Angiotensin II stimulates thick ascending limb superoxide production via protein kinase C(α)-dependent NADPH oxidase activation. *J. Biol. Chem.* **2010**, *285*, 21323–21328.
82. Dekker, L.V.; Leitges, M.; Altschuler, G.; Mistry, N.; McDermott, A; Roes, J.; Segal, A.W. Protein kinase C-beta contributes to NADPH oxidase activation in neutrophils. *Biochem. J.* **2000**, *1*, 285–289.
83. Korchak, H.M.; Corkey, B.E.; Yaney, G.C.; Kilpatrick, L.E. Negative regulation of ligand-initiated Ca^{2+} uptake by_PKC-beta II in differentiated HL60 cells. *Am. J. Physiol. Cell Physiol.* **2001**, *281*, 514–523.

84. Majumdar, S.; Kane, L.H.; Rossi, M.W.; Volpp, B.D.; Nauseef, W.M.; Korchak, H.M. Protein kinase C isotypes and signal-transduction in human neutrophils: Selective substrate specificity of calcium-dependent beta-PKC and novel calcium-independent nPKC. *Biochim. Biophys. Acta* **1993**, *16*, 276–286.
85. Siow, Y.L.; Au-Yeung, K.K.; Woo, C.W.; O, K. Homocysteine stimulates phosphorylation of NADPH oxidase p47phox and p67phox subunits in monocytes via protein kinase C beta activation. *Biochem. J.* **2006**, *15*, 73–82.
86. Brown, G.E.; Stewart, M.Q.; Liu, H.; Ha, V.L.; Yaffe, M.B. A novel assay system implicates PtdIns(3,4)P(2), PtdIns(3)P, and PKC delta in intracellular production of reactive oxygen species by the NADPH oxidase. *Mol. Cell* **2003**, *11*, 35–47.
87. Cheng, N.; He, R.; Tian, J.; Dinauer, M.C.; Ye, R.D. A critical role of protein kinase C delta activation loop phosphorylation in formyl-methionyl-leucyl-phenylalanine-induced phosphorylation of p47(phox) and rapid activation of nicotinamide adenine dinucleotide phosphate oxidase. *J. Immunol.* **2007**, *1179*, 7720–7728.
88. Nitti, M.; Furfaro, A.L.; Traverso, N.; Odetti, P.; Storace, D.; Cottalasso, D.; Pronzato, M.A.; Marinari, U.M.; Domenicotti, C. PKC delta and NADPH oxidase in AGE-induced neuronal death. *Neurosci. Lett.* **2007**, *18*, 261–265.
89. Rathore, R.; Zheng, Y.M.; Niu, C.F.; Liu, Q.H.; Korde, A.; Ho, Y.S.; Wang, Y.X. Hypoxia activates NADPH oxidase to increase $[ROS]_i$ and $[Ca^{2+}]_i$ through the mitochondrial ROS-PKCepsilon signaling axis in pulmonary artery smooth muscle cells. *Free Radic. Biol. Med.* **2008**, *1*, 1223–1231.
90. White, C.N.; Figtree, G.A.; Liu, C.C.; Garcia, A.; Hamilton, E.J.; Chia, K.K.; Rasmussen, H.H. Angiotensin II inhibits the Na^+–K^+ pump via PKC-dependent activation of NADPH oxidase. *Am. J. Physiol. Cell Physiol.* **2009**, *296*, 693–700.
91. Dang, P.M.; Fontayne, A.; Hakim, J.; El Benna, J.; Périanin, A. Protein kinase C zeta phosphorylates a subset of selective sites of the NADPH oxidase component p47phox and participates in formyl peptide-mediated neutrophil respiratory burst. *J. Immunol.* **2001**, *15*, 1206–1213.
92. Brennan, A.M.; Suh, S.W.; Won, S.J.; Narasimhan, P.; Kauppinen, T.M.; Lee, H.; Edling, Y.; Chan, P.H.; Swanson, R.A. NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. *Nat. Neurosci.* **2009**, *12*, 857–863.
93. Wu, D.M.; Lu, J.; Zheng, Y.L.; Zhang, Y.Q.; Hu, B.; Cheng, W.; Zhang, Z.F.; Li, M.Q. Small interfering RNA-mediated knockdown of protein kinase C zeta attenuates domoic acid-induced cognitive deficits in mice. *Toxicol. Sci.* **2012**, doi:10.1093/toxsci/kfs124.
94. Leverence, J.T.; Medhora, M.; Konduri, G.G.; Sampath, V. Lipopolysaccharide-induced cytokine expression in alveolar epithelial cells: Role of PKCζ-mediated p47phox phosphorylation. *Chem. Biol. Interact.* **2011**, *15*, 72–81.
95. El Benna, J.; Faust, R.P.; Johnson, J.L.; Babior, B.M. Phosphorylation of the respiratory burst oxidase subunit p47phox as determined by two-dimensional phosphopeptide mapping. Phosphorylation by protein kinase C, protein kinase A, and a mitogen-activated protein kinase. *J. Biol. Chem.* **1996**, *15*, 6374–6378.

96. Kramer, I.M.; van der Bend, R.L.; Verhoeven, A.J.; Roos, D. The 47-kDa protein involved in the NADPH:O_2 oxidoreductase activity of human neutrophils is phosphorylated by cyclic AMP-dependent protein kinase without induction of a respiratory burst. *Biochim. Biophys. Acta* **1988**, *16*, 189–196.
97. El Benna, J.; Han, J.; Park, J.W.; Schmid, E.; Ulevitch, R.J.; Babior, B.M. Activation of p38 in stimulated human neutrophils: Phosphorylation of the oxidase component p47phox by p38 and ERK but not by JNK. *Arch. Biochem. Biophys.* **1996**, *15*, 395–400.
98. Dewas, C.; Fay, M.; Gougerot-Pocidalo, M.A.; El-Benna, J. The mitogen-activated protein kinase extracellular signal-regulated kinase 1/2 pathway is involved in formyl-methionyl-leucyl-phenylalanine-induced p47phox phosphorylation in human neutrophils. *J. Immunol*. **2000**, *165*, 5238–5244.
99. Park, H.S.; Lee, S.M.; Lee, J.H.; Kim, Y.S.; Bae, Y.S.; Park, J.W. Phosphorylation of the leucocyte NADPH oxidase subunit p47(phox) by casein kinase 2: Conformation-dependent phosphorylation and modulation of oxidase activity. *Biochem. J.* **2001**, *15*, 783–790.
100. Chen, Q.; Powell, D.W.; Rane, M.J.; Singh, S.; Butt, W.; Klein, J.B.; McLeish, K.R. Akt phosphorylates p47phox and mediates respiratory burst activity in human neutrophils. *J. Immunol*. **2003**, *15*, 5302–5308.
101. Hoyal, C.R.; Gutierrez, A.; Young, B.M.; Catz, S.D.; Lin, J.H.; Tsichlis, P.N.; Babior, B.M. Modulation of p47PHOX activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. *Proc. Natl. Acad. Sci. USA* **2003**, *29*, 5130–5135.
102. Martyn, K.D.; Kim, M.J.; Quinn, M.T.; Dinauer, M.C.; Knaus, U.G. p21-activated kinase (Pak) regulates NADPH oxidase activation in human neutrophils. *Blood* **2005**, *1*, 3962–3969.
103. Waite, K.A.; Wallin, R.; Qualliotine-Mann, D.; McPhail, L.C. Phosphatidic acid-mediated phosphorylation of the NADPH oxidase component p47-phox. Evidence that phosphatidic acid may activate a novel protein kinase. *J. Biol. Chem.* **1997**, *13*, 15569–15578.
104. Chowdhury, A.K.; Watkins, T.; Parinandi, N.L.; Saatian, B.; Kleinberg, M.E.; Usatyuk, P.V.; Natarajan, V. Src-mediated tyrosine phosphorylation of p47phox in hyperoxia-induced activation of NADPH oxidase and generation of reactive oxygen species in lung endothelial cells. *J. Biol. Chem.* **2005**, *27*, 20700–20711.
105. Joglar, B.; Rodriguez-Pallares, J.; Rodriguez-Perez, A.I.; Rey, P.; Guerra, M.J.; Labandeira-Garcia, J.L. The inflammatory response in the MPTP model of Parkinson's disease is mediated by brain angiotensin: Relevance to progression of the disease. *J. Neurochem.* **2009**, *109*, 656–669.
106. Tsai, C.T.; Wang, D.L.; Chen, W.P.; Hwang, J.J.; Hsieh, C.S.; Hsu, K.L.; Tseng, C.D.; Lai, L.P.; Tseng, Y.Z.; Chiang, F.T.; *et al.* Angiotensin II increases expression of alpha1C subunit of L-type calcium channel through a reactive oxygen species and cAMP response element-binding protein-dependent pathway in HL-1 myocytes. *Circ. Res.* **2007**, *25*, 1476–1485.
107. Inoguchi, T.; Li, P.; Umeda, F.; Yu, H.Y.; Kakimoto, M.; Imamura, M.; Aoki, T.; Etoh, T.; Hashimoto, T.; Naruse, M.; *et al.* High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C—Dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* **2000**, *49*, 1939–1945.

108. Devaraj, S.; Dasu, M.R.; Singh, U.; Rao, L.V.; Jialal, I. C-reactive protein stimulates superoxide anion release and tissue factor activity in vivo. *Atherosclerosis* **2009**, *203*, 67–74.
109. Paletas, K.; Sailer, X.; Rizeq, L.; Dimitriadi, A.; Koliakos, G.; Kaloyianni, M. Angiotensin-II-dependent NHE1 activation in human monocytes. *J. Am. Soc. Hypertens.* **2008**, *2*, 173–181.
110. Romero, M.; Jiménez, R.; Sánchez, M.; López-Sepúlveda, R.; Zarzuelo, M.J.; O'Valle, F.; Zarzuelo, A.; Pérez-Vizcaíno, F.; Duarte, J. Quercetin inhibits vascular superoxide production induced by endothelin-1: Role of NADPH oxidase, uncoupled eNOS and PKC. *Atherosclerosis* **2009**, *202*, 58–67.
111. Koliakos, G.; Befani, C.; Paletas, K.; Kaloyianni, M. Effect of endothelin on sodium/hydrogen exchanger activity of human monocytes and atherosclerosis-related functions. *Ann. N. Y. Acad. Sci.* **2007**, *1095*, 274–291.
112. Dikalov, S.I.; Li, W.; Doughan, A.K.; Blanco, R.R.; Zafari, A.M. Mitochondrial reactive oxygen species and calcium uptake regulate activation of phagocytic NADPH oxidase. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2012**, *302*, 1134–1142.
113. Yang, J.; Lane, P.H.; Pollock, J.S.; Carmines, P.K. Protein kinase *C*-dependent NAD(P)H oxidase activation induced by type 1 diabetes in renal medullary thick ascending limb. *Hypertension* **2010**, *55*, 468–473.
114. Plumb, R.D.; El-Sherbeeny, N.A.; Dixon, L.J.; Hughes, S.M.; Devine, A.B.; Leahey, W.J.; McVeigh, G.E. NAD(P)H-dependent superoxide production in platelets: The role of angiotensin II and protein kinase C. *Clin. Biochem.* **2005**, *38*, 607–613.
115. Soetikno, V.; Watanabe, K.; Sari, F.R.; Harima, M.; Thandavarayan, R.A.; Veeraveedu, P.T.; Arozal, W.; Sukumaran, V.; Lakshmanan, A.P.; Arumugam, S.; *et al.* Curcumin attenuates diabetic nephropathy by inhibiting PKC-α and PKC-β1 activity in streptozotocin-induced type I diabetic rats. *Mol. Nutr. Food. Res*. **2011**, *55*, 1655–1665.
116. Ha, H.; Lee, H.B. Reactive oxygen species amplify glucose signalling in renal cells cultured under high glucose and in diabetic kidney. *Nephrology* (*Carlton*) **2005**, *10*, S7–S10.
117. Lin, R.Z.; Wang, T.P.; Hung, R.J.; Chuang, Y.J.; Chien, C.C.; Chang, H.Y. Tumor-induced endothelial cell apoptosis: Roles of NAD(P)H oxidase-derived reactive oxygen species. *J. Cell. Physiol*. **2011**, *226*, 1750–1762.
118. Wei, X.F.; Zhou, Q.G.; Hou, F.F.; Liu, B.Y.; Liang, M. Advanced oxidation protein products induce mesangial cell perturbation through PKC-dependent activation of NADPH oxidase. *Am. J. Physiol. Ren. Physiol.* **2009**, *296*, 427–437.
119. Griendling, K.K.; Ushio-Fukai, M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul. Pept.* **2000**, *28*, 21–27.
120. Touyz, R.M.; Chen, X.; Tabet, F.; Yao, G.; He, G.; Quinn, M.T.; Pagano, P.J.; Schiffrin, E.L. 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.* **2002**, *90*, 1205–1213.
121. Hilenski, L.L.; Clempus, R.E.; Quinn, M.T.; Lambeth, J.D.; Griendling, K.K. Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol*. **2004**, *24*, 677–683.

122. Shi, G.; Fu, Y.; Jiang, W.; Yin, A.; Feng, M.; Wu, Y.; Kawai, Y.; Miyamori, I.; Fan, C.; Activation of Src-ATF1 pathway is involved in upregulation of Nox1, a catalytic subunit of NADPH oxidase, by aldosterone. *Endocr. J.* **2011**, *58*, 491–499.
123. Lassègue, B.; Sorescu, D.; Szöcs, K.; Yin, Q.; Akers, M.; Zhang, Y.; Grant, S.L.; Lambeth, J.D.; Griendling, K.K. Novel gp91(phox) homologues in vascular smooth muscle cells: Nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. *Circ. Res.* **2001**, *88*, 888–894.
124. Wingler, K.; Wünsch, S.; Kreutz, R.; Rothermund, L.; Paul, M.; Schmidt, H.H. Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system *in vitro* and *in vivo*. *Free Radic. Biol. Med.* **2001**, *31*, 1456–1464.
125. Wei, H.; Mi, X.; Ji, L.; Yang, L.; Xia, Q.; Wei, Y.; Miyamori, I.; Fan, C. Protein kinase *C*-delta is involved in induction of Nox1 gene expression by aldosterone in rat vascular smooth muscle cells. *Biochemistry (Mosc)* **2010**, *75*, 304–309.
126. Fan, C.Y.; Katsuyama, M.; Yabe-Nishimura, C. PKCdelta mediates up-regulation of Nox1, a catalytic subunit of NADPH oxidase, via transactivation of the EGF receptor: Possible involvement of PKCdelta in vascular hypertrophy. *Biochem. J.* **2005**, *390*, 761–767.
127. San José, G.; Bidegain, J.; Robador, P.A.; Díez, J.; Fortuño, A.; Zalba, G. Insulin-induced NADPH oxidase activation promotes proliferation and matrix metalloproteinase activation in monocytes/macrophages. *Free Radic. Biol. Med.* **2009**, *46*, 1058–1067.
128. Guzik, T.J.; Sadowski, J.; Guzik, B.; Jopek, A.; Kapelak, B.; Przybylowski, P.; Wierzbicki, K.; Korbut, R.; Harrison, D.G.; Channon, K.M. Coronary artery superoxide production and Nox isoform expression in human coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **2006**, *26*, 333–339.
129. Mahadev, K.; Motoshima, H.; Wu, X.; Ruddy, J.M.; Arnold, R.S.; Cheng, G.; Lambeth, J.D.; Goldstein, B.J. The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H_2O_2 and plays an integral role in insulin signal transduction. *Mol. Cell. Biol.* **2004**, *24*, 1844–1854.
130. Schröder, K.; Wandzioch, K.; Helmcke, I.; Brandes, R.P. Nox4 acts as a switch between differentiation and proliferation in preadipocytes. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 239–245.
131. Colston, J.T.; de la Rosa, S.D.; Strader, J.R.; Anderson, M.A.; Freeman, G.L. H_2O_2 activates Nox4 through PLA2-dependent arachidonic acid production in adult cardiac fibroblasts. *FEBS Lett.* **2005**, *579*, 2533–2540.
132. Gorin, Y.; Ricono, J.M.; Wagner, B.; Kim, N.H.; Bhandari, B.; Choudhury, G.G.; Abboud, H.E. Angiotensin II-induced ERK1/ERK2 activation and protein synthesis are redox-dependent in glomerular mesangial cells. *Biochem. J.* **2004**, *381*, 231–239.
133. Ren, Y.; D'Ambrosio, M.A.; Wang, H.; Peterson, E.L.; Garvin, J.L.; Carretero, O.A. Mechanisms of angiotensin II-enhanced connecting tubule glomerular feedback. *Am. J. Physiol. Ren. Physiol.* **2012**, doi:10.1152/ajprenal.00689.2011.

134. Tan, Y.; Li, X.; Prabhu, S.D.; Brittian, K.R.; Chen, Q.; Yin, X.; McClain, C.J.; Zhou, Z.; Cai, L. Angiotensin II plays a critical role in alcohol-induced cardiac nitrative damage, cell death, remodeling, and cardiomyopathy in a protein kinase C/nicotinamide adenine dinucleotide phosphate oxidase-dependent manner. *J. Am. Coll. Cardiol.* **2012**, *59*, 1477–1486.
135. Sun, P.; Yue, P.; Wang, W.H. Angiotensin II stimulates epithelial sodium channels in the cortical collecting duct of the rat kidney. Am. *J. Physiol. Ren. Physiol.* **2012**, *302*, 679–687.
136. Silva, G.B.; Ortiz, P.A.; Hong, N.J.; Garvin, J.L. Superoxide stimulates NaCl absorption in the thick ascending limb via activation of protein kinase C. *Hypertension* **2006**, *48*, 467–472.
137. Cabello-Verrugio, C.; Acuña, M.J.; Morales, M.G.; Becerra, A.; Simon, F.; Brandan, E. Fibrotic response induced by angiotensin-II requires NAD(P)H oxidase-induced reactive oxygen species (ROS) in skeletal muscle cells. *Biochem. Biophys. Res. Commun.* **2011**, *8*, 665–670.
138. Silva, J.; Pastorello, M.; Arzola, J.; Zavala, L.E.; De Jesús, S.; Varela, M.; Matos, M.G.; del Rosario Garrido, M.; Israel, A. AT_1 receptor and NAD(P)H oxidase mediate angiotensin II-stimulated antioxidant enzymes and mitogen-activated protein kinase activity in the rat hypothalamus. *J. Renin-Angiotensin-Aldosterone Syst.* **2010**, *11*, 234–242.
139. Hogarty, D.C.; Speakman, E.A.; Puig, V.; Phillips, M.I. The role of angiotensin, AT1 and AT2 receptors in the pressor, drinking and vasopressin responses to central angiotensin. *Brain Res*. **1992**, *586*, 289–294.
140. Phillips, M.I.; Sumners, C. Angiotensin II in central nervous system physiology. *Regul. Pept.* **1998**, *78*, 1–11.
141. Wang, G.; Anrather, J.; Glass, M.J.; Tarsitano, M.J.; Zhou, P.; Frys, K.A.; Pickel, V.M.; Iadecola, C. Nox2, Ca^{2+}, and protein kinase C play a role in angiotensin II-induced free radical production in nucleus tractus solitarius. *Hypertension* **2006**, *48*, 482–489.
142. Chung, Y.W.; Kim, H.K.; Kim, I.Y.; Yim, M.B.; Chock, P.B. Dual function of protein kinase C (PKC) in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced manganese superoxide dismutase (MnSOD) expression: Activation of CREB and FOXO3a by PKC-alpha phosphorylation and by PKC-mediated inactivation of Akt, respectively. *J. Biol. Chem.* **2011**, *286*, 29681–29690.
143. Kamiya, T.; Makino, J.; Hara, H.; Inagaki, N.; Adachi, T. Extracellular-superoxide dismutase expression during monocytic differentiation of U937 cells. *J. Cell. Biochem.* **2011**, *112*, 244–255.
144. Deng, B.; Xie, S.; Wang, J.; Xia, Z.; Nie, R. Inhibition of protein kinase C β(2) prevents tumor necrosis factor-α-induced apoptosis and oxidative stress in endothelial cells: The role of NADPH oxidase subunits. *J. Vasc. Res*. **2012**, *49*, 144–159.
145. Pandey, D.; Fulton, D.J. Molecular regulation of NADPH oxidase 5 via the MAPK pathway. *Am. J. Physiol. Heart Circ. Physiol.* **2011**, *300*, 1336–1344.
146. Serrander, L.; Jaquet, V.; Bedard, K.; Plastre, O.; Hartley, O.; Arnaudeau, S.; Demaurex, N.; Schlegel, W.; Krause, K.H. Nox5 is expressed at the plasma membrane and generates superoxide in response to protein kinase C activation. *Biochimie* **2007**, *89*, 1159–1167.
147. Jagnandan, D.; Church, J.E.; Banfi, B.; Stuehr, D.J.; Marrero, M.B.; Fulton, D.J. Novel mechanism of activation of NADPH oxidase 5. Calcium sensitization via phosphorylation. *J. Biol. Chem.* **2007**, *282*, 6494–6507.

148. Gupte, S.A.; Kaminski, P.M.; George, S.; Kouznestova, L.; Olson, S.C.; Mathew, R.; Hintze, T.H.; Wolin, M.S. Peroxide generation by p47phox-Src activation of Nox2 has a key role in protein kinase C-induced arterial smooth muscle contraction. *Am. J. Physiol. Heart Circ. Physiol*. **2009**, *296*, 1048–1057.
149. Park, J.W.; Babior, B.M. Activation of the leukocyte NADPH oxidase subunit p47phox by protein kinase C. A phosphorylation-dependent change in the conformation of the *C*-terminal end of p47phox. *Biochemistry* **1997**, *36*, 7474–7480.
150. Baggiolini, M.; Boulay, F.; Badwey, J.A.; Curnutte, J.T. Activation of neutrophil leukocytes: Chemoattractant receptors and respiratory burst. *FASEB J.* **1993**, *7*, 1004–1010.
151. Martelli, A.M.; Sang, N.; Borgatti, P.; Capitani, S.; Neri, L.M. Multiple biological responses activated by nuclear protein kinase C. *J. Cell. Biochem*. **1999**, *74*, 499–521.
152. Divecha, N.; Banfić, H.; Irvine, R.F. The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. *EMBO J.* **1991**, *10*, 3207–3214.
153. Neri, L.M.; Borgatti, P.; Capitani, S.; Martelli, A.M. Nuclear diacylglycerol produced by phosphoinositide-specific phospholipase C is responsible for nuclear translocation of protein kinase C-alpha. *J. Biol. Chem.* **1998**, *273*, 29738–29744.
154. Sun, B.; Murray, N.R.; Fields, A.P. A role for nuclear phosphatidylinositol-specific phospholipase C in the G2/M phase transition. *J. Biol. Chem*. **1997**, *272*, 26313–26317.
155. Deacon, E.M.; Pettitt, T.R.; Webb, P.; Cross, T.; Chahal, H.; Wakelam, M.J.; Lord, J.M. Generation of diacylglycerol molecular species through the cell cycle: A role for 1-stearoyl, 2-arachidonyl glycerol in the activation of nuclear protein kinase C-betaII at G2/M. *J. Cell. Sci.* **2002**, *115*, 983–989.
156. Neri, L.M.; Borgatti, P.; Capitani, S.; Martelli, A.M. The nuclear phosphoinositide 3-kinase/AKT pathway: A new second messenger system. *Biochim. Biophys. Acta* **2002**, *1584*, 73–80.
157. Neri, L.M.; Martelli, A.M.; Borgatti, P.; Colamussi, M.L.; Marchisio, M.; Capitani, S. Increase in nuclear phosphatidylinositol 3-kinase activity and phosphatidylinositol (3,4,5) trisphosphate synthesis precede PKC-zeta translocation to the nucleus of NGF-treated PC12 cells. *FASEB J.* **1999**, *13*, 2299–2310.
158. Wooten, M.W.; Zhou, G.; Wooten, M.C.; Seibenhener, M.L. Transport of protein kinase C isoforms to the nucleus of PC12 cells by nerve growth factor: Association of atypical zeta-PKC with the nuclear matrix. *J. Neurosci. Res*. **1997**, *49*, 393–403.
159. Calcerrada, M.C.; Miguel, B.G.; Martín, L.; Catalán, R.E.; Martínez, A.M. Involvement of phosphatidylinositol 3-kinase in nuclear translocation of protein kinase C zeta induced by C2-ceramide in rat hepatocytes. *FEBS Lett*. **2002**, *514*, 361–365.
160. Borgatti, P.; Mazzoni, M.; Carini, C.; Neri, L.M.; Marchisio, M.; Bertolaso, L.; Previati, M.; Zauli, G.; Capitani, S. Changes of nuclear protein kinase C activity and isotype composition in PC12 cell proliferation and differentiation. *Exp. Cell Res.* **1996**, *224*, 72–78.
161. Buchner, K. The role of protein kinase C in the regulation of cell growth and in signalling to the cell nucleus. *J. Cancer Res. Clin. Oncol*. **2000**, *126*, 1–11.
162. Ushio-Fukai, M. Localizing NADPH oxidase-derived ROS. *Sci. STKE* **2006**, *2006*, re8.

163. Ambasta, R.K.; Kumar, P.; Griendling, K.K.; Schmidt, H.H.; Busse, R.; Brandes, R.P. Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. *J. Biol. Chem.* **2004**, *279*, 45935–45941.
164. Van Buul, J.D.; Fernandez-Borja, M.; Anthony, E.C.; Hordijk, P.L. Expression and localization of Nox2 and Nox4 in primary human endothelial cells. *Antioxid. Redox Signal.* **2005**, *7*, 308–317.
165. Kuroda, J.; Nakagawa, K.; Yamasaki, T.; Nakamura, K.; Takeya, R.; Kuribayashi, F.; Imajoh-Ohmi, S.; Igarashi, K.; Shibata, Y.; Sueishi, K.; *et al.* The superoxide-producing NAD(P)H oxidase Nox4 in the nucleus of human vascular endothelial cells. *Genes Cells* **2005**, *10*, 1139–1151.
166. Spencer, N.Y.; Yan, Z.; Boudreau, R.L.; Zhang, Y.; Luo, M.; Li, Q.; Tian, X.; Shah, A.M.; Davisson, R.L.; Davidson, B.; *et al.* Control of hepatic nuclear superoxide production by glucose 6-phosphate dehydrogenase and NADPH oxidase-4. *J. Biol. Chem.* **2011**, *286*, 8977–8987.
167. Geiszt, M.; Kopp, J.B.; Várnai, P.; Leto, T.L. Identification of renox, an NAD(P)H oxidase in kidney. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8010–8014.
168. Shiose, A.; Kuroda, J.; Tsuruya, K.; Hirai, M.; Hirakata, H.; Naito, S.; Hattori, M.; Sakaki, Y.; Sumimoto, H. A novel superoxide-producing NAD(P)H oxidase in kidney. *J. Biol. Chem.* **2001**, *276*, 1417–1423.
169. Pendergrass, K.D.; Gwathmey, T.M.; Michalek, R.D.; Grayson, J.M.; Chappell, M.C. The angiotensin II-AT1 receptor stimulates reactive oxygen species within the cell nucleus. *Biochem. Biophys. Res. Commun*. **2009**, *384*, 149–154.
170. Pinton, P.; Rimessi, A.; Marchi, S.; Orsini, F.; Migliaccio, E.; Giorgio, M.; Contursi, C.; Minucci, S.; Mantovani, F.; Wieckowski, M.R.; *et al.* Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science* **2007**, *315*, 659–663.
171. Becker, K.P.; Kitatani, K.; Idkowiak-Baldys, J.; Bielawski, J.; Hannun, Y.A. Selective inhibition of juxtanuclear translocation of protein kinase C betaII by a negative feedback mechanism involving ceramide formed from the salvage pathway. *J. Biol. Chem.* **2005**, *280*, 2606–2612.
172. Voris, J.P.; Sitailo, L.A.; Rahn, H.R.; Defnet, A.; Gerds, A.T.; Sprague, R.; Yadav, V.; Caroline Le Poole, I.; Denning, M.F. Functional alterations in protein kinase C beta II expression in melanoma. *Pigment. Cell Melanoma. Res.* **2010**, *23*, 216–224.
173. Migliaccio, E.; Giorgio, M.; Mele, S.; Pelicci, G.; Reboldi, P.; Pandolfi, P.P.; Lanfrancone, L.; Pelicci, P.G. The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* **1999**, *402*, 309–313.
174. Giorgio, M.; Migliaccio, E.; Orsini, F.; Paolucci, D.; Moroni, M.; Contursi, C.; Pelliccia, G.; Luzi, L.; Minucci, S.; Marcaccio, M.; *et al.* Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* **2005**, *122*, 221–233.
175. Datta, R.; Yoshinaga, K.; Kaneki, M.; Pandey, P.; Kufe, D. Phorbol ester-induced generation of reactive oxygen species is protein kinase cbeta -dependent and required for SAPK activation. *J. Biol. Chem.* **2000**, *275*, 41000–41003.

176. Agudo-López, A.; Miguel, B.G.; Fernández, I.; Martínez, A.M. Role of protein kinase C and mitochondrial permeability transition pore in the neuroprotective effect of ceramide in ischemia-induced cell death. *FEBS Lett*. **2011**, *585*, 99–103.
177. Frey, R.S.; Ushio-Fukai, M.; Malik, A.B. NADPH oxidase-dependent signaling in endothelial cells: Role in physiology and pathophysiology. *Antioxid. Redox Signal.* **2009**, *11*, 791–810.