Reactive Oxygen Species in Metabolic and Inflammatory Signaling

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Abstract

Reactive oxygen species (ROS) are well known for their role in mediating both physiological and pathophysiological signal transduction. Enzymes and subcellular compartments that typically produce ROS are associated with metabolic regulation, and diseases associated with metabolic dysfunction may be influenced by changes in redox balance. In this review, we summarize the current literature surrounding ROS and their role in metabolic and inflammatory regulation, focusing on ROS signal transduction and its relationship to disease progression. In particular, we examine ROS production in compartments such as the cytoplasm, mitochondria, peroxisome and endoplasmic reticulum, and discuss how ROS influence metabolic processes such as proteasome function, autophagy and general inflammatory signaling. We also summarize and highlight the role of ROS in the regulation metabolic/inflammatory diseases including atherosclerosis, diabetes and stroke. To successfully develop improved therapies that target oxidative signaling, it is vital to understand the balance ROS signaling plays in both physiology and pathophysiology, and how manipulation of this balance and the identity of the ROS may influence cellular and tissue homeostasis. An increased understanding of specific sources of ROS production and an appreciation for how ROS influence cellular metabolism may help guide us in this effort to treat cardiovascular diseases.

Keywords

ROS; Metabolism; Inflammation; Cardiovascular

Introduction

Reactive oxygen species (ROS) regulate cellular homeostasis and act as prime modulators of cellular dysfunction contributing to disease pathophysiology. ROS are byproducts of numerous enzymatic reactions in various cell compartments, including the cytoplasm, cell membrane, endoplasmic reticulum (ER), mitochondria and peroxisome, as part of basal metabolic function. They are also generated specifically by enzymes such as NADPH oxidases and serve a signaling function in the cell. Depending upon the source of ROS, cell

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type and tissue environment, ROS signaling may participate in normal physiological processes or contribute to a maladaptive response that leads to metabolic dysfunction and inflammatory signaling. Diseases associated with elevated inflammatory signaling and metabolic dysfunction such as atherosclerosis, diabetes and stroke are associated with an altered redox balance.[1–3] Understanding the role of ROS signaling in the regulation of metabolic activity, inflammatory activation and diseases associated with metabolic dysfunction is important in our pursuit of novel therapies to treat these diseases. This review highlights the role of ROS signaling in basic metabolic processes and inflammatory signaling, and focuses on how this regulation contributes to disease development.

1. Sources of ROS and their role in metabolic function

I. Cytoplasmic

Cytoplasmic ROS (cytoROS) production is a cornerstone of cellular signaling and disease pathophysiology. One of the most well-known sources of cytoROS is the NADPH oxidase (NOX) family of enzymes. NOX2 (or gp91^{phox}) is well-characterized for its role in phagocytic function;[4] however, it and three homologs, NOX1, NOX4, and NOX5, are expressed throughout the cardiovascular system.[5] NOX proteins produce O_2^- through NADPH electron exchange, and NOX-dependent ROS production influence many metabolic processes and disease states.[6] Briefly, endothelial cell (EC) NOX-dependent ROS production drives hypoxia inducible factor 1α (HIF1α)-mediated glucose transporter 1 (GLUT1) expression, hexokinase activity and resultant glycolysis in response to low oxygen tension as part of the angiogenic response.[7] In response to inflammatory stimuli, neutrophil phosphofructokinase 2 colocalizes with NOX2, inducing its activation. $NADP^+$ produced as a byproduct of NOX2 O_2^- generation is used to facilitate an elevated glycolytic rate. A locally increasing NADPH concentration as a by-product of increased glycolysis is hypothesized to further enhance NOX2 activity; however, this has yet to be proven.[8]

In addition to NOX-dependent ROS production, the nitric oxide synthases (endothelial, neuronal and inducible) are sources of cytoplasmic ROS. Endothelial nitric oxide synthase (eNOS) produces O_2^- through its oxygenase domain in a Ca^{2+}/calmodulin-dependent reaction in the absence of tetrahydrobiopterin (BH_4).[9] Dysregulated or uncoupled eNOS is a hallmark of cardiovascular and metabolic diseases.[10] The oxygenase domain is vital for O_2^- production from neuronal and inducible NOS (nNOS and iNOS, respectively), and O_2^- generation is dependent upon reduced L-arginine.[11, 12] NOS uncoupling in various disease states results in a dysregulated NO response whereby synthesized NO combines with O_2^- to produce peroxynitrite. Peroxynitrite enhances pentose phosphate pathway (PPP) activity through stimulation of glucose-6-phosphate dehydrogenase (G6PD), leading to elevated NADPH.[13] A similar mechanism has been noted in response to H_2O_2, a downstream metabolite of O_2^-.[14] Thus, in the transient setting, peroxynitrite, and even H_2O_2, may promote a protective response through PPP-dependent increases in reducing equivalents. However, chronic stimulation of the PPP by ROS may instigate a toxic feedback loop whereby increased NADPH production results in excessive O_2^- through NOX.[15] In support of both hypotheses, G6PD deficiency and overexpression are protective against oxidative

stress[16,17] However, further investigation into how ROS and metabolic diseases affect NADPH shuttling is needed.

CytoROS also induce AMP-activated protein kinase (AMPK) activity.[18] AMPK is a central regulator of cellular metabolism implicated in multiple metabolic functions including glycolysis, lipid metabolism, mitochondrial function, cell growth and autophagy (discussed below).[19] Hypoxia can regulate AMPK activity through an ROS-dependent ER Ca^{2+}/ Stromal interaction molecule $1/Ca^{2+}$/calmodulin-dependent protein kinase kinase beta pathway in addition to indirect regulation via a change in the AMP/ATP ratio.[20] H_2O_2 can directly modulate AMPK activity and downstream metabolic function through oxidation and S-glutathionylation of the α- and β-subunits of AMPK by targeting cysteines 299 and 304.[21] ROS-mediated impairment of the mitochondrial respiratory chain can also increase the AMP/ATP ratio which can activate AMPK,[22] and peroxynitrite induces AMPK activation in bovine aortic ECs through a c-SRC(Tyr[416])/phosphoinositide 3-kinase (PI-3K)/ phosphoinositide-dependent kinase-1 (Ser[241]) pathway. Peroxynitrite-dependent AMPK phosphorylation at Thr[172] leads to phosphorylation of acetyl CoA carboxylase at Ser[79], resulting in inactivation and increased fatty acid oxidation.[23]

Low oxygen tension in ECs increases glycolysis as part of the angiogenic response through NOX-mediated, ROS-induced HIF1α signaling.[7] However, increased ROS signaling in some disease states inactivates the glycolysis rate-limiting enzyme pyruvate kinase M2, and diverts glycolytic substrates into the PPP pathway to generate reducing equivalents needed for ROS detoxification, thereby acting as a protective mechanism.[24] In total, cytoROS signaling plays a critical role in the PPP and glycolytic pathways (Figure 1). Below we will outline the contributions of various cytoROS-producing entities in the development of metabolic disorders.

II. Mitochondria

Mitochondria are central regulators of aerobic energy production. Proper respiratory chain function requires a delicate balance between pro-oxidant and anti-oxidant systems. Importantly, mitochondrial respiration relies on electron transfer and a proton gradient to drive ATP production. ROS are a natural byproduct of this process; however, inflammatory and metabolic diseases are associated with perturbed mitoROS production.[25, 26] MitoROS are generated by numerous mechanisms including Complexes I-III (Figure 2). Complex I serves as an entry point for electrons from NADH into the respiratory chain. O_2^- is produced from the interaction of O_2 with reduced FMN when the matrix NADH/NAD+ ratio is high, resulting in O_2^- release into the matrix.[27] In addition, mitoROS is produced through complex I via reverse electron transfer (RET). RET occurs in a 2-step process involving (1) reduced coenzyme Q (CoQ), and (2) a change in proton motive force that drives electrons back into complex I. Complex III is also a source of mitoROS. Under normal conditions, electrons flow from the CoQ pool to cytochrome C. Although complex III produces very low levels of O_2^-, in the presence of Antimycin A, the Q_i site is inhibited, which promotes O_2^- production from the Q_o site due to the interaction between O_2 and a ubisemiquinone bound to the Q_o site.[28] O_2^- generated from complex III is mainly released into the intermembrane space, but upon dismutation, H_2O_2 may diffuse into the matrix.[29]

While complex I and III are well known for their contribution to mitoROS production, complex II may also serve as an ROS producing complex.[30] Complex II oxidizes succinate to fumarate as part of the Krebs cycle and acts as the site of ubiquinone reduction in the electron transport chain (ETC). ROS are produced from complex II when both complex III and complex I are inhibited. Complex II ROS production is believed to proceed through a forward mechanism involving electrons from succinate, or in a reverse mechanism where electrons are provided through a reduced ubiquinone pool. Both mechanisms result in ROS production from the complex II flavin site.[30]

Other than complex-derived mitoROS, enzymes involved in metabolic reactions produce mitoROS (reviewed in [31, 32]). Of importance, the α-ketoglutarate dehydrogenase (KGDHC) and pyruvate dehydrogenase (PDC) complexes produce ROS as a result of both forward electron transfer and RET.[33, 34] KGDHC, PDC and branched-chain α-keto acid dehydrogenase complex, a dehydrogenase complex that catalyzes the oxidative decarboxylation of α-ketoacids, are thought to produce significantly more ROS than complex I.[35] Recent evidence indicates PDC and KGDHC-derived ROS may be regulated through protein-S-glutathionylation. Interestingly, S-glutathionylation during forward electron transfer may attenuate ROS production, whereas S-glutathionylation during RET may increase ROS from PDC and KGDHC[36] In addition to generating ROS, KGDHC is also an early target of oxidative stress. Similarly, aconitase, an enzyme responsible for the isomerization of citrate to isocitrate, is responsive to ROS.[37, 38] In conditions of low oxidative stress, when aconitase activity and Krebs cycle substrate production is diminished, α-ketoglutarate levels and NADH production can be maintained through glutamate via glutaminolysis; however, in the presence of high oxidative stress, KGDHC is inhibited, reducing NADH production and mitochondrial respiratory capacity.[38] RET-induced mitoROS can inactivate aconitase and pyruvate dehydrogenase kinase 2 (negative regulator of PDC) through reversible oxidation of cysteine 45 and 392 when the $NADH/NAD^+$ ratio is elevated. This regulation is hypothesized to promote acetyl-CoA production via PDC from carbohydrates while simultaneously inhibiting β-oxidation, resulting in cytoplasmic export of citrate and stimulation of fatty acid synthesis.[39] Reduced aconitase activity is associated with aging and diseases associated with metabolic dysfunction.[40]

Recent evidence indicates that a subset of NOX4 is localized to the mitochondria and is a regulator of mitoROS generation. NOX4 expression is increased in kidney cortex from diabetic rats and facilitates glucose-induced mitoROS production. Likewise, NOX4 induces cysteine oxidation, and resultant decreased activity, of aconitase and citrate synthase in cardiac myocytes,[41] and influences mitochondrial morphology and complex I activity.[42] Additionally, ATP production from normal mitochondrial respiration negatively regulates mitochondrial NOX4-induced ROS production through direct interaction between ATP and the NOX4 Walker A binding motif,[43] which suggests mitochondrial NOX4 may play a dynamic role in metabolic function.

Apart from ROS producing pathways, impairment of mitoROS scavenging pathways results in ROS accumulation leading to organelle and cell dysfunction. Within the mitochondria, the predominant ROS buffering systems include the glutaredoxin (Grx), glutathione and thioredoxin (Trx) systems.[44] Dismutation of O_2^- into H_2O_2 occurs through the superoxide

dismutase (SOD) family of proteins.[45] In the matrix, dismutation primarily proceeds through SOD2 (MnSOD), whereas in the intermembrane space, dismutation is carried out by SOD1 (Cu, Zn-SOD). H_2O_2 decomposition into O_2 and H_2O then occurs via the GSH redox system, which includes glutathione reductases, peroxidases (GPX) and peroxiredoxins (Prdx).[46]

The Trx and Grx systems also play a prominent role in mitochondrial ROS buffering. The mitochondrial Trx system involves thioredoxin-2 (Trx2), thioredoxin reductase-2 (TrxR2) and members of the Prdx family of proteins. Importantly, the antioxidant effect of Trx2 activity results from reduction of other oxidized proteins, mainly the Prdxs. Oxidization of Trx2 is reversible and is remedied through TrxR2 using NADPH as an electron donor. [47] Homozygous knockout (KO) of Trx2 is embryonically lethal, and heterozygous mice, while viable, show decreased mitochondrial respiratory function and increased mitoROS production,.[48] Likewise, cardiac-specific TrxR2 KO mice exhibit cardiac structural changes, dysregulation of autophagy, decreased oxygen consumption and a change in their metabolic profile.[49]

Mitochondrial Grx family members include Grx2 and Grx5. Grx2 regulates O_2^- production from complex 1 by catalyzing glutathionylation of two thiol groups, and Grx5 regulates iron/sulfur enzymes.[50–52] However, information regarding how the GRX and Trx systems regulate cardiovascular function is limited. Cumulatively, mitochondria maintain a delicate balance between oxidant and anti-oxidant systems, and dysregulation can result in organelle dysfunction resulting in metabolic stress.

MitoROS can also influence HIF1α stabilization and cell proliferation.[53, 54] Hypoxia-induced HIF1α promotes a metabolic shift favoring anaerobic glycolysis and reduced mitochondrial respiration by upregulating glucose transporters and glycolytic enzymes while also inhibiting PDC through activation of pyruvate dehydrogenase kinase 1.[55, 56] Reduced PDC activity causes decreased Krebs cycle and ETC flux, which may in turn attenuate mitoROS production in hypoxic conditions. Likewise, HIF1α induces mitophagy resulting in decreased mitochondrial mass, O_2 consumption and resultant ROS generation. Thus, in hypoxic conditions, mitoROS favor the stabilization of HIF1α, which induces a metabolic shift towards glycolysis, leading to reduced mitochondrial activity and mitoROS production. [57, 58] In normoxic conditions, inhibition of ETC function using rotenone (complex I inhibitor) or TTFA (complex II inhibitor) induces cell death through induction of mitoROS-mediated autophagy.[59] AMPK, a regulator of uncoordinated 51-like kinase 1 (ULK1) activity and downstream autophagy, can be regulated by mitoROS, and mitoROS contribute to autophagy through AMPK/ULK1-dependent signaling (discussed below).[60, 61] Similarly, AMPK is responsible for the mitoROS/HIF1α-dependent extension of life span observed in *Caenorhabditis elegans* (*C. elegans*).[62] Slightly elevated levels of mitoROS in mice are also associated with extension of lifespan.[63] Interestingly, mice with heterozygote deletion of mouse clock-1, a protein involved in CoQ biosynthesis, exhibit increased longevity, increased inflammatory cytokine production and macrophage activation that is dependent upon elevated mitoROS. These mice also show enhanced resistance to infection, which may be due to the observed increase in macrophage phagocytic activity.[64]

Mitochondrial fission and fusion are closely associated with mitochondrial function and can influence, and be influenced by, mitoROS production. (For a comprehensive review of mitochondrial fission and fusion, please refer to references.[65, 66] With regard to mitochondrial fission, the small cytoplasmic GTPase dynamin-related protein-1 (Drp1) is implicated in ROS signaling. Drp1 activity regulates mitoROS production and downstream mitochondrial functional changes in a variety of environments,[67–69] and oxidative stress influences Drp1 mitochondrial translocation and resultant fission.[70] Mitochondrial fission also precedes mitophagy, which can be regulated by oxidative stress and is thought to be a negative regulator of mitoROS signaling through selective degradation of dysfunctional mitochondria.[71, 72] Drp1 undergoes numerous post-translational modifications including S-nitrosylation and phosphorylation, and data suggest that ROS signaling contributes to serine 616 phosphorylation and activation of Drp1 GTPase activity.[73–75] However, the contribution of ROS signaling to the regulation of other fission proteins is currently unknown and is an area ripe for future investigation.

Similar to Drp1 and mitochondrial fission, ROS signaling may regulate mitochondrial fusion. The inner mitochondrial membrane GTPase optic atrophy protein-1 (OPA1) is regulated by reactive oxygen species modulator 1 (ROMO1). In response to ROS, ROMO1 is inactivated leading to OPA1 cleavage, cristae remodeling and mitochondrial fission.[76] Likewise, deletion of OPA1 induces morphological irregularities, respiratory defects and ROS generation.[77] In addition to OPA1, mitofusin 1 and 2 activity can regulate and be regulated by ROS.[78–80] The precise mechanism and contribution of fission/fusion-regulated ROS signaling to metabolic function remains to be explored; however, the contribution of ROS and mitochondrial morphology regulation in the setting of metabolic diseases will be explored below.

In summary, the mitochondria are dynamic players in metabolic regulation and signaling. MitoROS are produced as part of normal mitochondrial function, but various cellular stresses augment ROS levels either through increased oxidant production or decreased antioxidant activity. As discussed, mitoROS can regulate cellular metabolic function (illustrated in Figure 2), and in **section 2**, we will discuss the contribution of mitoROS to metabolic and cardiovascular diseases.

III. Peroxisome

Peroxisomes, like mitochondria, are vital organelles in aerobic metabolism that regulate key processes such as α- and β-oxidation, glyoxylate metabolism, amino acid catabolism, the pentose phosphate pathway, ketogenesis, polyamine oxidation and isoprenoid and cholesterol metabolism.[81] Peroxisomes are also a significant source of ROS. In particular, peroxisomes produce H_2O_2 due to an abundance of O_2-consuming oxidases, which include acyl-CoA oxidases (ACOX), D-amino acid oxidase, D-aspartate oxidase, polyamine oxidase, xanthine oxidase (also produces O_2^-), L-α-hydroxyacid oxidase and L-pipecolic oxidase.[82, 83] Unlike the mitochondria, peroxisomal electron transfer does not lead to ATP generation. Instead, free electrons are transferred to H_2O to form H_2O_2.[83] In addition, peroxisomes can produce nitric oxide through NOS.[84] However, similar to mitochondria, peroxisomes contain numerous oxidant scavenging enzymes including GPX, catalase, Prdx1

and 5, peroxisomal membrane associated protein 20, SOD1 and SOD2.[82] For an in-depth review of peroxisomal enzymes, please refer to[85].

Recently, a new role for peroxROS in mTOR complex (mTORC) 1 activity was defined. In response to elevated peroxisomal β-oxidation, peroxROS activate tuberous sclerosis proteins 1 and 2 (TSC1 and TSC2), which are bound to the peroxisomal assembly proteins peroxin 19 and 5 (Pex19 and Pex5), respectively. ROS induce TSC2-mediated GTPase activity of Rheb, leading to mTORC1 inhibition and autophagy induction.[86] Pex5 binds ataxia-telangiectasia mutated (ATM) and localizes it to the peroxisomal membrane. In response to peroxROS, ATM activation (Ser[1981]) induces AMPK and TSC2 activity and downstream mTORC1 inhibition, ULK1 activation and pexophagy. As part of this mechanism, ATM activation also induces the phosphorylation of Pex5 (Ser[141]), triggering ubiquination at lysine 209 and subsequent binding of p62, which is required for peroxisomal targeting to autophagosomes (Figure 3).[87] In addition to pexophagy regulation, peroxROS can also disrupt the mitochondrial redox balance and promote mitochondrial fission/fragmentation[88] and mitochondrial-mediated cell death.[89]

IV. ROS and the Endoplasmic Reticulum

The ER has a well-established role in metabolic and cardiovascular diseases[90, 91] due to its roles in Ca^{2+} handling, protein synthesis/folding and regulation of the secretory pathway. Protein-folding is highly sensitive to ER redox status and dysregulation of disulfide bond formation in response to ER stress increases luminal oxidative stress leading to a decline in ER function.[92] One of the most well understood routes for disfulfide bond introduction into folded proteins is the protein disulfide-isomerase (PDI) and ER oxidoreductin 1 (ERO1) pathway. PDI introduces disulfide bonds through thiol oxidation in folding substrates leaving PDI in a reduced state. Reduced PDI is re-oxidized through ERO1, which transfers acquired electrons through a flavin adenine dinucleotide cofactor to molecular oxygen, forming H_2O_2. ER H_2O_2 can further be used by Prdx4 to re-oxidize PDI, thereby increasing the efficiency of ERO1-mediated disulfide bond transfer.[93, 94] Overexpression of a human hyperactive mutant of ERO1 induces severe oxidative stress and induction of the unfolded protein response (UPR), an ER stress response involved in the pathogenesis of metabolic and cardiovascular diseases, highlighting the sensitivity of the ER to changes in redox balance.[95] Furthermore, UPR activation can induce ERO1 activation leading to increased oxidative stress and sustained UPR signaling, and administration of antioxidants can attenuate the UPR and improve downstream protein secretion.[96] In addition to the PDI/ERO1 pathway, ROS is produced through the membrane associated monooxygenase system via cytochrome p450, cytochrome b_5 reductase and through ER-localized NOX4 (Figure 4).[97–99]

As part of this redox balance, the ER contains an antioxidant system that is vital to proper ER function: peroxiredoxins (Prdx4, mentioned above) and glutathione peroxidases (GPX7 and GPX8). GPX7/8 contain a KDEL ER localization sequence, making them ER-specific ROS scavengers. GPX7/8 both interact with ERO1α, one of two isoforms of ERO1, at cysteine[208]/cysteine[41]. Reduced GPX7/8 scavenges ERO1-derived H_2O_2, leaving GPX7/8 in an oxidized form.[100] Oxidized GPX7, through its cysteine 86 residue, can bind to the cysteine[41/420] residue of glucose-regulated protein, 78 kDa (GRP78). This binding promotes

GRP78 oxidation and enhanced protein refolding chaperone activity. Silencing of GPX7 induces oxidative stress, accumulation of misfolded proteins and induction of the UPR.[101] PDI oxidation is regulated by GPX7/8 as well.[100]

The ER and mitochondria sit in close proximity to one another, and changes in the ER redox balance influence mitochondrial function. In response to ER stress, activating transcription factor 4 and C/EBP homologous protein (CHOP) induce ERO1α. In mitochondrial-associated ER membranes (MAMs), ERO1α oxidizes the type 1 inositol 1,4,5-trisphosphate receptor (IP$_3$R) inducing mitochondrial Ca^{2+} uptake and mitoROS production through mitochondrial respiration.[102, 103] ERO1α-mediated cytoplasmic Ca^{2+} efflux through the ER IP$_3$R is also hypothesized to contribute to NOX2-dependent ROS stimulation via CAMKII. [100] SHC-transforming protein 1 isoform p66 (p66[shc]) is a regulator of cellular oxidative stress[104] that translocates to MAMs in response to cellular stress and produces mitoROS through cytochrome C oxidation,[105] and is capable of inducing ER stress[106] and inhibiting mTOR-dependent anabolic metabolism (Figure 4).[107]

In summary, maintenance of the ER redox balance is critical to proper ER function, and alterations in ER ROS producing and scavenging pathways provoke ER stress and contribute to metabolic dysfunction.

V. ROS and Inflammation

Inflammation and metabolism are intricately intertwined, considering that numerous metabolic and cardiovascular disorders exhibit chronic low-grade inflammation.[108] Canonical NF-κB signaling is associated with insulin resistance, obesity and atherosclerosis, [108–110] and circulating dietary factors such as fatty acids and glucose can trigger inflammatory signaling. It has also been suggested that NF-κB may regulate metabolic reprogramming favoring aerobic glycolysis.[111] The influence of ROS on NF-κB signaling may depend upon the cellular location of oxidation (cytoplasmic vs nuclear).[112] In general, ROS are known to activate NF-κB in response to inflammatory agonists.[113] NF-κB nuclear translocation occurs in response to H_2O_2[114] through a mechanism involving IκBα tyrosine phosphorylation (Tyr[42]), phosphorylation of the serine/threonine PEST domain with subsequent degradation via calpain, and p65 phosphorylation (ser[529]).[115, 116] ROS-induced NF-κB is inhibited by SOD2 overexpression,[117] and the NOX family of proteins also influence,[118] and are influenced by, NF-κB activity.[119, 120]

Specific inflammatory agonists utilize ROS as part of their signaling cascades. IL-1β induces active endosomal IL-1R complex assembly that involves MyD88 and NOX2 ROS-induced tumor necrosis factor (TNF) receptor associated factor (TRAF) 6 endosomal recruitment.[121] NOX4 activity is required for lipopolysaccharide (LPS)-induced NF-κB activation,[122] and TNFα-induced NF-κB activation increases antioxidant expression leading to decreased TNFα-induced apoptotic signaling through a ROS/JNK pathway.[123]

Flow-induced activation of NF-κB is regulated by ROS signaling as well. Flow-mediated EC dysfunction and monocyte adhesion is dependent upon NOX-derived O_2^- regulation of NF-κB inducing kinase (NIK) and IKK signaling leading to NF-κB activation.[124–126] As part of this response, bone morphogenic protein 4 and p21-activated kinase act as upstream

regulators of flow-induced ROS generation and downstream NF-κB signaling, contributing to oscillatory shear stress-induced vascular dysfunction and atherosclerotic lesion development.[126, 127]

An abundance of evidence points to a role for mitoROS in regulating inflammatory signaling. In response to inflammatory stimuli, proinflammatory cytokines are synthesized and released from cells. Importantly, mitoROS contribute to LPS-induced cytokine release[25], thrombin induced NF-κB activation via IP_3R Ca^{2+} signaling,[128] and lysophosphatidylcholine-induced AP-1 activity and downstream endothelial activation.[129] Likewise, mitochondrial H_2O_2 production contributes to endothelial NF-κB activation in aged rat arteries,[130] and inhibition of mitoROS through ETC inhibition abrogates hypoxia-induced endothelial NF-κB activation and IL-6 secretion.[131] Evidence also indicates that mitoROS may be a downstream result of NF-κB activation as well.[132]

RET-induced mitoROS are involved in metabolic changes associated with macrophage activation during inflammation. Macrophage proinflammatory signaling is supported in part by metabolic repurposing that favors glycolysis-derived ATP production and RET-induced mitoROS generation through mitochondrial hyperpolarization and succinate oxidation.[133] RET-derived mitoROS promote HIF1α stabilization,[134] leading to regulation of glycolytic capacity and IL-1β mRNA and protein expression.[135,133] In response to LPS, immune cells secrete IL-1β through the inflammasome. MitoROS signaling is a primary regulator of NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome activation. NLRP3 inflammasome activation consists of two phases: (1) a priming phase where agonists such as LPS stimulate NF-κB-mediated NLRP3, IL-1β and interleukin-18 (IL-18) transcription, and (2) an activation phase where a multi-protein complex consisting of NLRP3, Apoptosis-associated speck-like protein containing a CARD, and caspase 1 is assembled.[136] The fully assembled NLRP3 inflammasome, along with activation via potassium efflux,[137] lysosomal destabilization and mitoROS,[138, 139] regulates the maturation of IL-1β and IL-18.[140] Thus, in LPS-stimulated macrophages, RET-induced mitoROS production, as a result of a metabolic shift towards glycolysis, regulates 1L-1β transcription (inflammasome priming), but may also regulate the maturation and secretion of IL-1β (inflammasome activation). However, alternative pathways of IL-1β processing independent of inflammasome activity also exist.[141]

Thioredoxin-interacting protein (TxNIP), a negative regulator of Trx, is an ROS-regulated proapoptotic factor that mediates mitoROS and NOX4 activity and influences glucose-induced inflammasome activation in two ways.[142, 143] First, TxNIP-dependent inhibition of Trx induces ROS which further exacerbate inflammasome activity and inflammatory cytokine signaling. Second, during inflammasome activation, TxNIP dissociates from Trx and interacts with NLRP3, which is required for proper inflammasome activity in response to glucose stimulation.[142] In addition, IRE1α, an ER stress protein, increases inflammasome activity by regulating TxNIP mRNA stability.[144, 145]

Apart from ROS-dependent inflammasome activation, ROS are critical to macrophage phagocytic activity. Importantly, metabolic and cardiovascular diseases associated with chronic inflammation display impairments in macrophage phagocytic/efferocytic activity

that are correlated with changes in ROS signaling. NOX2-dependent oxidative signaling is required for sufficient phagocytic function and pathogen/apoptotic cell degradation,[146] as shown by increased lung inflammation and abdominal aortic aneurysm progression in myeloid NOX2 KO mice.[147, 148]

Macrophage NOX-derived ROS induce microtubule-associated protein 1A/1B-light chain 3 (LC3) translocation to the phagosome, which is required for lysosomal fusion and phagosomal clearance in LC3-associated phagocytosis (LAP).[149] Toll-like receptor signaling promotes mitochondrial recruitment to phagosomes where augmented mitoROS kill phagocytosed bacteria and enhance NOX-dependent ROS production.[150] As part of LAP, Drp1-mediated mitochondrial fission increases cytosolic Ca^{2+} through inhibition of the mitochondrial Ca^{2+} uniporter and mitoROS production, which are required for efficient phagosomal sealing and LAP-mediated apoptotic cell degradation.[151] Drp1-dependent mitochondrial fission and resultant ROS production also contribute to NF-κB activation in T cells,[152] and changes in mitochondrial dynamics regulate T cell metabolic reprogramming. [153]

Mer tyrosine kinase (MerTK) is an essential membrane protein in macrophages that participates in efferocytosis. In response to inflammatory stimuli, proteolytic cleavage and inhibition of MerTK activity impair efferocytosis and downstream resolution of inflammation.[154] In response to LPS, TLR4-TIR-domain-containing adapter-inducing interferon-β signaling induces MerTK shedding through a NOX2/PKCα/ p38 MAPK and a Disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) pathway, and macrophage-specific ADAM17 deletion protects against MerTK shedding *in vivo*.[155] MitoROS and AMPK also play a significant role in macrophage efferocytosis. Apoptotic cell-released lysophosphatidylcholine diminishes mitochondrial membrane potential and ATP production coupled with concomitant mitoROS generation and downstream AMPK activation. AMPK facilitates both metabolic reprograming towards glycolysis and tubulin synthesis that are needed for macrophage chemokinesis and efficient efferocytosis.[156] Macrophages experience defective efferocytosis in response to TNFα as well, potentially through phospholipase A2/arachidonic acid-dependent ROS and Rho activity.[157]

Overall, inflammation is an underlying component to many diseases including those that exhibit metabolic distress. ROS act as central regulators of inflammatory signaling, particularly with respect to NF-κB activation and inflammasome signaling (Figure 5). The following sections will highlight the contribution of ROS to specific cellular functions and their role in regulating metabolic dysfunction in various diseases.

VI. Secondary Products of ROS

Secondary products of ROS also mediate inflammatory signaling in a variety of cellular environments. For example, lipid peroxidation products, such as 4-hydroxynonenal, and oxidized phospholipids (OxPLs) regulate NF-κB activation and inflammatory signaling through numerous pathways,[158–160] and can propagate ROS signaling; however, whether secondary ROS product signaling is protective or instigates a pathophysiological response may depend on ROS concentration, cell-type and cellular stress.[159] In regards to pathophysiology, lipid peroxidation and OxPLs have been implicated in atherogenesis.

[161,162] Mechanistically, both products can regulate NF-κB activity and chemokine release resulting in arterial wall inflammation and immune cell recruitment.[163–165] However, incubation of LPS-treated mice or macrophages with OxPLs suppresses NF-κB activation and TNFα secretion, suggesting that OxPLs may be protective in some conditions.[166] It should also be noted that in a single agonist condition, OxPLs may influence macrophage polarization and contribute to the macrophage pro-inflammatory response.[167]

Secondary products of ROS can also regulate metabolic function in various tissues and cellular environments. In response to OxPLs, EC glycolytic and proliferative capacity are increased and are dependent upon NRF2 signaling.[168] Similarly, oxidation of cholesterol may promote a protective response in macrophages by increasing autophagy and promoting effective efferocytosis.[169] Lipid peroxidation can increase autophagy in rat VSMCs through an ER stress/JNK-dependent mechanism.[170] In the mitochondria, lipid peroxidation affects membrane fluidity and is capable of modulating ETC complex activity, Krebs cycle enzymes, proteostasis and mitochondrial membrane potential (Figure 2).[171] Overall, while secondary products are known to influence various enzymes and contribute to diseased states such as atherosclerosis and cardiac diseases, specific signal transduction pathways have yet to be fully elucidated. Nevertheless, secondary products of ROS signaling have a clear role in metabolic and inflammatory signaling.

VII. ROS and Autophagy

Autophagy is a highly conserved catabolic process in which cytoplasmic macromolecules and organelles are delivered to lysosomes for degradation.[172] Constitutive basal levels of autophagy support metabolic homeostasis by promoting a balance between protein synthesis and degradation, as well as organelle biogenesis and degradation; however, dysregulated autophagic flux contributes to metabolic and inflammatory diseases.[172,173]. For an extensive review on the regulation of autophagic machinery, please refer to[174].

Oxidative stress regulates autophagic flux through its influence on autophagic gene transcription, protein activity and organellular degradation.[175] ROS induce macroautophagy (commonly referred to as autophagy), and selective degradation of oxidized proteins through chaperone-mediated autophagy (CMA) is important for cellular viability during periods of oxidative stress.[176]. Oxidative modification of CMA substrates increases their susceptibility to degradation, and CMA is enhanced through LAMP2a upregulation in cells challenged with ROS.[176] Little is known about the effect of ROS on microautophagy and further work is needed to distinguish the roles of macroautophagy, microautophagy, and CMA in response to oxidative stress.

In addition to the role of ROS in autophagic flux, ROS are required for autophagy induced by starvation,[177] dopamine,[178] sodium selenite,[179] mitochondrial electron transport chain inhibitors TTFA and rotenone,[180] TNFα[181] and LPS.[182] The source and identity of ROS that mediate these effects remain unclear. Sources of ROS located at or near the plasma membrane, such as NOXs, are prime candidates to transduce signals from external stimuli into the cell. However, this hypothesis has only been verified in macrophages through LAP.[149]

As mentioned, ROS can influence AMPK activation. In response to ROS, cytoplasmic ATM promotes liver kinase B1 (LKB1)-dependent AMPK activation and downstream autophagy through the regulation of TSC2 activity and mTOR inhibition.[183,184] Furthermore, overexpression of catalase blocks H_2O_2-induced autophagy,[177,182] and starvation-induced H_2O_2 production regulates cysteine oxidation of Atg4, a negative regulator of autophagy that delipidates LC3-II to LC3-1 and inhibits autophagosome formation.[177] Starvation methods including the removal of glucose, L-glutamine, pyruvate and selenium or amino acids can also increase O_2^- levels.[185] Interestingly, amino acid starvation alone increases intracellular H_2O_2 levels, and overexpression of SOD2 in serum-starved, amino acid-starved and H_2O_2-treated cells attenuates ROS production and inhibits autophagy.[185] Mitochondrial antioxidants, such as SS-31 and SOD2, can block autophagy induced by stressors including mitochondrial inhibitors,[180] sodium selenite,[179] and immobilization.[186] Given the critical role of ROS in the regulation of autophagy, further work is needed to clarify the specific mechanisms by which ROS influence autophagic machinery.

ROS are also implicated in regulating the selective degradation of mitochondria through mitophagy. MitoROS can trigger mitochondrial permeability transition pore opening and a burst of mitoROS, which has been described as ROS-induced ROS release.[187, 188] Thus, ROS produced by damaged mitochondria may act as a self-removal signal,[175] and while this hypothesis needs further verification, the benefits of selectively recycling mitochondria with oxidized proteins and damaged DNA as a secondary defense against oxidative stress are clear and have been demonstrated in yeast.[72,189] This is supported by recent work suggesting that ROS are required for Phosphatase and tensin homolog-induced kinase 1/ Parkin-dependent mitophagy.[190]

VIII. ROS and the Proteasome

The proteasome is a highly organized multimeric complex responsible for the selective hydrolysis of cytoplasmic, nuclear and ER proteins[191] which supports metabolic homeostasis by maintaining a stable pool of free amino acids for protein synthesis.[192] The proteasome also promotes cellular viability in response to oxidative stress.[175, 193] Amino acids are continuously subjected to oxidative modification as a consequence of aerobic respiration, and most of these modifications are irreversible and irreparable. Studies using antisense oligonucleotides against critical proteasome subunits [194] or proteasome inhibitors [195] have implicated the proteasome as the primary pathway for degrading oxidized proteins. Thus, the proteasome serves as an important secondary defense against oxidants.

The central catalytic component of the proteasome is the barrel-shaped 20S core particle (CP) composed of four heptameric rings consisting of two outer "gate-keeping" α rings and two identical inner β rings that contain catalytically active/proteolytic β subunits.[191,196] The 20S CP may be flanked by regulatory subunits through α subunit interaction[193] which modulate 20S CP activity and substrate specificity.[197] The most common of these subunits is the 19S regulatory particle (RP),[197] which along with the 20S CP, comprises the 26S proteasome. The 26S proteasome is a key component of the ubiquitin proteasome pathway (UPP) and regulates the degradation of ubiquitinated proteins.[197,198] The 19S RP recognizes polyubiquitinated substrates and hydrolyzes ATP to unfold and translocate target proteins

into the 20S CP.[197] The 20S CP may also associate with other regulatory proteins, such as PA28αβ, PA28γ, and PA200, for ubiquitin/ATP-independent protein degradation.[198,199]

Mildly oxidized proteins selectively and rapidly undergo proteolysis, and strong evidence suggests the 20S proteasome is largely responsible for this degradation.[193,195,200] H_2O_2 impairs ubiquitin conjugation and reduces ubiquitin/ATP-dependent proteolysis;[201] however, disruption of the ubiquitin system does not affect oxidized protein degradation,[195] suggesting that the 20S ubiquitin/ATP-*independent* proteasome is responsible for protein degradation during oxidative stress. Concordant with these findings, in vitro studies using purified 26S show that the 26S proteasome, even in the presence of ATP and a functional ubiquitination system, does not degrade oxidized proteins.[193] However, both the 26S and 20S proteasome are sensitive to oxidative stress.[202] In fact, in the presence of the activator PA28αβ, exogenous ROS enhances 20S protease activity[202] while H_2O_2 disrupts 26S proteasome complex integrity and reversibly dislocates the 20S CP from the 19S RP.[200]. This dissociation is accompanied by a loss of 26S proteasome activity and enhances cell survival following H_2O_2-induced oxidative stress.[200] Interestingly, dissociation of the 26S proteasome increases the fraction of 20S proteasome, which may account for an increase in cell survival.[200] Consistent with this notion is the observation that yeast deficient in 26S assembly are more resistant to oxidative stress than their wild type counterparts.[203]

Some evidence, however, supports a role for the 26S proteasome in degrading oxidized proteins. Ubiquitin carboxyl-terminal hydrolase 14 (USP14), a 26S proteasome-associated deubiquitinating enzyme, decreases ubiquitin-protein conjugate degradation by disassembling polyubiquitin chains.[204] Inhibition of USP14 enhances cell survival and reduces the accumulation of oxidized proteins in cells challenged by menadione,[204] suggesting that ubiquitination and the 26S proteasome are important for degrading oxidized proteins. Moreover, the expression of Ubiquitin conjugating enzyme (UBC) 4, an E2 enzyme, promotes the degradation of glutathionylated proteins in lens fiber cells, and this degradation is blocked by a dominant negative form of ubiquitin and proteasome inhibitors. [205] Several studies also suggest that the 26S proteasome is critical for cellular viability during recovery from oxidative stress. Cells treated with H_2O_2 exhibit a transient increase in proteolytic activity and in ubiquitin conjugation post-treatment.[201, 206] While oxidative stress negatively regulates 26S activity, 26S proteasome activity is almost completely restored 24 hours post-treatment.[202] Finally, it has been observed that certain oxidized proteins are preferentially ubiquitinated.[207] Thus, there is evidence to support a role for both the 20S and 26S proteasome in degrading oxidized proteins. However, further work may help to distinguish the different roles of the 20S and 26S proteasome in dealing with specific oxidized substrates.

The proteasome plays an important role in mitigating the effects of ROS, and oxidative modification of proteasome subunits modulates proteasome activity. Carboxylation of Regulatory Particle Triphosphate (RPT) 3, an ATPase subunit in the 19S RP, impairs ATPase activity and decreases 26S proteasome activity.[208]. Additionally, both carbonylation and 4-hydroxy-2-nonenal (HNE) modification of two α-subunits in the 20S proteasome impair ubiquitin/ATP-independent proteolysis.[209] Interestingly, S-glutathionylation directly modulates specific proteolytic activity of the 20S proteasome.[210] Low doses of GSH or

GSSG enhance the chymotrypsin-like activity of purified proteasome, while higher doses inhibit this activity.[210] The effect of S-glutathionylation seems specific to the chymotrypsin-like activity, as GSH and GSSG do not affect the trypsin-like activity of purified proteasomes.[210]

Other post-translational modifications also modulate proteasome activity in response to oxidative stress. Poly-ADP ribosylation of the 20S proteasome by the redox-sensitive enzyme poly-ADP ribose polymerase enhances the chymotrypsin-like activity of the 20S proteasome in K562 leukemia cells.[211] In addition, apoptosis signal-regulating kinase 1 (Ask1) is activated by oxidative stress,[212] and Ask1 phosphorylation of RPT5, a 19S subunit, negatively regulates 26S proteasome activity.[213] Significantly, Ask1 is required for ROS-induced inhibition of 26S proteasome activity.[214] Thus, Ask1-dependent proteasome phosphorylation may act as a critical regulatory mechanism for proteasome activity during oxidative stress.

Following oxidative stress, E1 activating enzyme expression and ubiquitin conjugation are increased in bovine lens epithelial cells.[206] Increased expression appears to be due to increased translation of E1 mRNA.[206] Consistent with the 20S proteasome playing a critical role in degrading oxidized proteins, H_2O_2 treatment increases $\alpha3$, $\alpha4$, $\beta1$, and $\beta2$ 20S proteasome subunit expression without affecting the 19S RPS subunit S4.[215] Interestingly, upregulation of immunoproteasome subunits has also been reported.[215, 216] Low-molecular-mass protein (LMP) 2, LMP7, LMP10 are β subunits that may replace constitutive β subunits in the 20S proteasome upon interferon-γ stimulation,[217] and increased expression of these subunits has been reported in neural cells in response to exogenous ROS.[216] The transcription factors that regulate immunoproteasome and standard proteasome subunit expression following oxidative stress require further study.

2. Cardiovascular and Metabolic Diseases

I. Atherosclerosis

NOX—Atherosclerosis is mediated in large part through subendothelial lipoprotein retention, endothelial dysfunction, vascular remodeling and a heightened inflammatory response, and is an underlying cause of heart disease and stroke.[218,219] Of importance, metabolic disease risk factors typically observed in patients with obesity and diabetes (changes in cholesterol, elevated triglycerides, insulin resistance) increase the risk for atherosclerosis.[220,221] Although the link between oxidized LDL and atherosclerosis was discovered nearly 30 years ago,[222] the NOX enzyme family was only defined in the mid-1990s. Some of the first studies performed exploring NOX-induced ROS concluded that NOX-derived ROS have little influence on lesion development in ApoE^{−/−} mice on normal chow diets. Neither p47[phox−/−] (NOX1 and NOX2 activator) nor NOX2 (gp91[phox−/y]) mice crossed with ApoE^{−/−} mice show changes in aortic sinus lesion area, although NOX2[−/y]ApoE^{−/−} mice exhibit reduced plasma triglyceride and cholesterol levels.[223,224] However, these findings appear to be aortic sinus-specific, as further analysis of p47[phox−/−] ApoE^{−/−} and NOX2[−/y] ApoE^{−/−} mice (on chow and high-fat/western-type diets) revealed a reduction in descending aortic lesion development.[225–227]

NOX1$^{-/y}$ ApoE$^{-/-}$ mice are protected from both atherogenic diet-induced and diabetes-induced (atherogenic diet + streptozatocin, STZ) atherosclerosis,[228–230] associated with decreased ROS production, inflammatory signaling and aortic macrophage infiltration. NOX1 likely exerts its effects in part by reducing VSMC proliferation and migration,[231] and its phosphorylation on threonine 429 (Th4^{429}) may be a contributing factor.[232] However, the role of NOX1 in the initiation and progression of atherosclerosis remains controversial because recent findings in NOX1-deficient ApoE$^{-/-}$ mice indicate NOX1 may be protective against hyperlipidemia and plaque instability in response to a western-type diet.[233]

Similar to NOX1, the role of NOX2 in lesion etiology remains controversial. As mentioned, NOX2$^{-/y}$ ApoE$^{-/-}$ mice fed an atherogenic diet show reductions in the development and progression of atherosclerosis.[226] Likewise, treatment of high fat diet fed ApoE$^{-/-}$ mice with a NOX2-specific inhibitor reduces aortic lesion area,[234] and while there are compelling data regarding a role for global NOX2-induced ROS in plaque development, the tissue-specific role of NOX2 remains ill-defined. In ApoE$^{-/-}$ mice, EC-specific overexpression of NOX2 increases inflammatory signaling and macrophage infiltration in early lesions, but does not influence diet-induced lesion progression.[235] Bone marrow transplantation between p47$^{phox -/-}$ ApoE$^{-/-}$ mice and p47$^{+/+}$ ApoE$^{-/-}$ mice shows that suppression of either bone marrow cell or vascular wall O_2^- production attenuates western-type diet induced atherosclerosis.[236] Moreover, suppression of NOX2 in bone marrow cells is associated with reduced plasma oxLDL, suggesting NOX2-derived ROS may regulate oxLDL production. oxLDL is scavenged and internalized by the lectin-like oxidized low density lipoprotein receptor 1 (LOX-1) and contributes to atherogenesis through ROS-dependent mechanisms involving NF-κB activation,[237] inhibition of AKT/eNOS signaling,[238] macrophage proinflammatory cytokine production and cell death,[239,240] and VSMC apoptosis.[241] The importance of NOX2-derived ROS within macrophages is also seen in its role in pathogen degradation after phagocytosis through a process called macropinocytosis. [242, 243]

Given the diverse roles of both NOX1 and NOX2 in vascular disease, it is not surprising that NOX4 can also be both a harbinger of lesion development and a protector. As a protector, NOX4-derived ROS appear to be critical in maintaining vessel homeostasis in mouse models of atherosclerosis, because NOX4$^{-/-}$ Ldlr$^{-/-}$ and NOX4$^{-/-}$ ApoE$^{-/-}$ mice experience endothelial dysfunction and increased plaque burden as well as increased plaque formation in partial ligation plus high fat diet models.[244,245] However, in diabetes-induced atherosclerosis, global NOX4 deletion may be protective or augment diabetes-induced lesion development, depending on the time frame.[227, 229] NOX4 deletion in a 10-week STZ model of diabetes reduces plaque burden associated with T cell activation and infiltration,[227] but in a 20-week model of STZ-induced diabetes, NOX4 deletion augments VSMC collagen deposition and proliferation, which may exacerbate lesion progression.[246] The contribution of NOX4 in diabetic lesion development may also differ between early and advanced lesions, as NOX4 deletion has a minimal effect on early diabetes-induced plaque progression in the aortic arch,[229] but is critical to suppressing the inflammatory response in advanced lesions.[230]

In tissue-specific NOX4 KO models, a more specific role for NOX4 signaling in atherosclerosis emerges. Expression of an EC-specific NOX4 mutation (human P437H

dominant negative mutation) in ApoE$^{-/-}$ mice exacerbates lesion progression in response to STZ;[247] whereas EC overexpression of wild-type NOX4 attenuates high-fat diet-induced lesion development.[248] As mentioned, NOX4 is upregulated in advanced lesions, as opposed to the upregulation of NOX1 and NOX2 in the early phases of plaque development. [225,249,250] While these studies indicate that this upregulation may be protective, other studies have shown NOX4 upregulation may promote lesion progression. Importantly, upregulated NOX4 in advanced plaques correlates with VSMC dysfunction and plaque instability,[249] and smooth muscle-specific NOX4 deletion protects against western-type diet-induced atherosclerosis.[251] Aged ApoE$^{-/-}$ mice fed a western-type diet and aged human carotid VSMCs exhibit increased mitochondrial NOX4 induction with parallel increases in mitoROS production, and inhibition of NOX4 increases aged VSMC mitochondrial complex I and II activity, decreases mitoROS and attenuates VCAM-1 induction.[250] ER-derived NOX4 signaling is also implicated in VSMC apoptosis in response to 7-ketocholesterol, a major cholesterol oxidation product found in human plaques. 7-ketocholesterol upregulates IRE1 activity leading to JNK/AP-1-dependent NOX4 gene induction. Increased ROS production through NOX4 induces ER stress proteins GRP78 and CHOP and the apoptosis regulator Bcl-2-associated X protein (Bax).[252] A role for NOX2 in CHOP induction has also been observed,[253] and *in vivo* silencing of CHOP attenuates lesion area and plaque necrosis in hyperlipidemic mice.[254,255]

The PPP pathway can serve as both a biosynthetic pathway for nucleotides and a factory for NADPH production resulting in increased reducing capacity or NOX activity and O_2^- production. G6PD catalyzes the first step of the PPP and generates NADPH as a by-product. With regard to atherosclerosis, deficiency in G6PD abrogates O_2^- production and resultant inflammatory signaling and lesion growth in ApoE$^{-/-}$ mice fed a western-type diet.[256] However, G6PD overexpression in ECs reduces TNFα-induced ROS production and increases eNOS activity, suggesting a protective role for this protein.[257] Future studies should aim to clarify the circumstances by which G6PD contributes to pro- and anti-oxidant signaling, as well as the role of other PPP enzymes, in NOX activity and atherogenesis.[257]

As noted above, a plethora of evidence indicates a role for NOX-induced ROS in atherogenesis, although these molecules play both a positive and negative role in lesion progression. It is becoming increasingly clear that no model of atherosclerosis is the same. Changes in diet (composition and timing), genetic background, and age all influence NOX signaling. Tissue-specific knockouts have helped to create a clearer picture of the role of NOX in various tissues as it pertains to atherosclerosis, and continued investigation will determine the suitability of NOX enzymes as potential therapeutic targets.

Mitochondrial ROS—MitoROS signaling plays a major role in atherosclerosis and associated vascular complications, and induction of mitoROS is correlated with human plaque development.[53] In general, scavenging of mitoROS attenuates atherosclerosis development and complications associated with atherosclerosis.[250,258] Genetic inhibition of mitoROS may also have beneficial effects on lesion development. Suppression of mitoROS signaling in macrophages through the overexpression of mito-targeted catalase reduces lesion area, inflammatory signaling and immune cell infiltration into the aortic root of Ldlr $^{-/-}$ mice fed a western-type diet. In cultured mitoCatalase-overexpressing macrophages, LPS

and oxLDL-induced mitoROS promote IKKβ phosphorylation (serine 177) and downstream p65 phosphorylation (serine 536), and inhibition of this pathway blocks macrophage MCP-1 induction.[259] Furthermore, bone marrow transfer from mitoCatalase-expressing mice to aged WT Ldlr^{−/−} mice diminishes neutrophil extracellular traps and lesion area, but is unable to perturb increased plasma cholesterol levels.[260]

As discussed previously, a dynamic interplay exists between mitoROS production and mitochondrial morphology. With regard to atherosclerosis and metabolic dysfunction, high glucose can induce mitoROS through Drp1-dependent mitochondrial fission in human ECs, and inhibition of fission with the pharmacological inhibitor mitochondrial division inhibitor-1 (Mdivi-1) attenuates diabetes-induced aortic oxidative stress, cell adhesion molecule expression and aortic root lesion development.[261] However, there appears to be a cell type-specific role for mitochondrial fission in disease progression, as myeloid Drp1 silencing in Ldlr^{−/−} mice fed a western-type augments plaque necrosis due to impaired efferocytosis.[151]

Uncoupling protein 2 (UCP2) is a mitochondrial inner membrane protein and is reported to regulate ROS generation through feedback inhibition involving ROS-induced uncoupling and proton leak.[262] UCP2 expression is increased in C57BL6 mice in response to an atherogenic diet, and deletion of UCP2 increases oxidative stress, endothelial dysfunction, VCAM-1 expression, macrophage infiltration and atherogenesis.[263,264] Likewise, xanthine oxidase is found in various cellular compartments including the mitochondria, and pharmacological inhibition via febuxostat or tungsten reduces chemokine expression, endothelial dysfunction and lesion development.[265, 266]

Altogether, mitoROS play a significant role is lesion etiology. Given their unique position in regulating cellular energy metabolism, it is no surprise that alteration in mitoROS production influences mitochondrial function and cellular homeostasis and is a possible cause of lesion progression. There are numerous ROS producing complexes within the mitochondria, and teasing out their role in atherosclerosis is of paramount importance.

ROS Scavenging—Similar to inhibition of ROS producing enzymes, increasing ROS scavenging appears to have a beneficial effect on lesion development. Mice overexpressing catalase and catalase + SOD1,[267] Prdx4,[268] and Trx2 (EC-specific)[269] all exhibit decreased lesion development. In contrast, deletion of ROS scavenging systems exacerbates lesion progression, as seen in mice with deletion of NF-E2 related factor 2,[270] GPX1,[271,272], SOD2,[273] and Prdx1 and 2.[274,275]

The paraoxanase (PON) family of proteins may also serve an anti-oxidant role in atherosclerosis through their ability to hydrolyze lipid peroxides.[276] Overexpression of the PON transgenic cluster (PONs 1,2 and 3 together) promotes plaque stability via increased collagen synthesis, decreased necrotic core area and reduced oxLDL and inflammatory markers. PON1 overexpression may also reduce monocyte-to-macrophage differentiation and promote macrophage resistance against oxLDL-induced foam cell formation[277] and induction of the LPS + IFNγ inflammatory phenotype.[278,279] In contrast, deletion of PON1 increases vascular oxidative stress and leukocyte adhesion,[280] and deletion of PON2

exacerbates lesion development.[281,282] Recent evidence suggests that PON2 may localize to the mitochondrial inner membrane where it is bound to CoQ and contributes to ROS scavenging and maintenance of complex I and III activity.[281]

Cumulatively, ROS signaling has been shown to be an underlying causative factor that contributes to the complex etiology of atherosclerosis in animal models (Supplementary Table I). The studies presented highlight a unique role for ROS in the regulation of cellular responses to various atherogenic stimuli, and tissue-specific studies have begun to elucidate the role ROS signaling might play in various cell types and their contribution to lesion progression. However, even in the presence of an abundance of data indicating a causative role for ROS in atherosclerosis, mechanistic details regarding metabolic regulation and its role in ROS signaling are lacking.

II. Diabetes/Obesity

Obesity creates an increased risk for the development of cardiovascular and metabolic diseases including atherosclerosis, diabetes mellitus and hypertension.[283] Obesity is associated with insulin resistance and hyperglycemia, which contribute to the development of type II diabetes mellitus, and clinical investigation has yielded novel insight into how diabetes-related complications contribute to systemic vascular and metabolic dysfunction. Clinical and animal research consistently point to oxidative stress as an underlying factor that mediates obesity- and diabetes-related health ailments.[284,285] Importantly, glucose metabolism can produce ROS via sorbitol metabolism, hexosamine metabolism, α-ketoaldehyde production, PKC activation, glycation and oxidative phosphorylation.[286]

Vascular System—Hyperglycemia is a consequence of diabetes and insulin resistance and plays a significant role in the accompanying vascular complications (atherogenesis, endothelial dysfunction, inflammation, altered vascular tone). Early studies highlighted the ability of glucose and free fatty acids to stimulate ROS production in vascular cells including VSMCs, ECs and human leukocytes, and showed that high glucose can impair PPP activity and generation of reducing equivalents.[287–290] Since then, investigation has focused on unique pathways by which ROS may regulate vascular complications in diabetes and obesity.

ROS can regulate vascular tone in response to hyperglycemia via regulation of PKCβ and inhibition of eNOS activity.[291] Hyperglycemia-induced ROS production activates the 26S proteasome leading to ubiquitination and degradation of the BH_4 synthesis rate limiting enzyme guanosine 5′-triphosphate cyclohydrolase I (GTPCH). BH_4 deficiency dysregulates eNOS activity which impairs endothelial-dependent relaxation, and treatment of STZ-injected mice with TEMPOL restores GTPCH, BH_4 and attenuates endothelial dysfunction.[292] Furthermore, PKCβII induces endothelial dysfunction through p66[shc]-mediated mitoROS production.[291,293] Hyperglycemia increases p66[shc] acetylation (Lys[81]), which precedes PKCβII-induced phosphorylation (Ser[36]) of p66[shc], and inhibition of p66[shc] reduces mitoROS, aortic lipid peroxidation and restores eNOS activity and endothelial-dependent relaxation in diabetic mice.[291, 293] PKCβ also regulates vascular tone through impairment in the large conductance Ca^{2+}-activated K^+ channel. In response to high glucose,

the β1 subunit of the Ca^{2+}-activated K^+ channel is degraded through PKCβ-stimulated NOX1 and NOX4 activation, inhibition of AKT and activation of the forkhead box protein O3a/F-box only degradation pathway.[294]

As with atherosclerotic lesion development, NOX-derived ROS play an important role in vascular homeostasis in diabetes and obesity. Inhibition of NOX activity attenuates diabetes-induced impairment of endothelial-dependent relaxation,[295] and aged $NOX2^{-/y}$ mice fed a high fat diet are protected from obesity, dyslipidemia, insulin resistance and endothelial dysfunction.[296] Similar results have been observed in arteries from diabetic $NOX1^{-/y}$ mice, [297] and mice treated with siRNA targeting $p22^{phox}$,[298] as well as $NOX2^{-/y}$ mice with EC-specific overexpression of a human dominant negative (DN) variant of the insulin receptor that causes EC-specific insulin resistance and reduced vascular relaxation.[299] Proinflammatory stimuli such as TNFα can induce NOX activity in diabetic mice resulting in impaired vasorelaxation,[300] and NOX-derived ROS downregulate cGMP-dependent protein kinase I, which normally promotes vascular relaxation.[301] Furthermore, high glucose induces NOX4 upregulation via a PKCζ and NF-κB-dependent pathway.[302] Insulin like growth factor 1 (IGF-1) enhances high glucose-induced $NOX4/p22^{phox}$ complex formation and activation of VSMCs, and induces NOX4 Tyr491 phosphorylation leading to NOX4 binding to the SH2 domain of growth factor receptor-bound protein 2 (Grb2). NOX4/Grb2 association is needed for NOX4 localization to the SHPS-1 plasma membrane scaffold in VSMCs and STZ-injected mouse vasculature. Disruption of the NOX4/Grb2 association inhibits Src oxidation *in vivo* as well as VSMC proliferation.[303]

ROS produced by NOX also reduce vascular endothelial growth factor (VEGF) expression and post-ischemic neovascularization in diabetic mice. $NOX2^{-/y}$ mice show increased bone marrow mononuclear cell (BM-MNC) to EC differentiation and injection of BM-MNCs from $NOX2^{-/y}$ mice into diabetic mice enhances neovascularization.[304] Furthermore, endothelial progenitor cells from diabetic patients show a reduced reendothelialization capacity that can be normalized through inhibition of $p47^{phox}$.[305] Together, these data suggest a major role for ROS-mediated signaling in both facilitating dysregulated vascular tone and impairment of angiogenesis/neovascularization in diabetic ischemic tissue.

One consequence of hyperglycemia is the generation of advanced glycation end products (AGEs), covalent adducts formed between glucose and plasma proteins. AGEs contribute to diabetic-related maladies including neuropathy, retinopathy and cardiomyopathy.[306] Given its direct contact with blood, the endothelium is a prime target for AGE-mediated signaling. Importantly, aortic ECs express the receptor for AGEs (RAGE), which is increased in response to high glucose via mitoROS production,[307] and incubation of ECs with diabetic red blood cells expressing AGEs increases VCAM-1 induction and tissue factor production through RAGE-induced NOX activation.[308] AGE stimulation may also dysregulate eNOS activity via increased NOX and mitoROS within the coronary endothelium of diabetic patients.[309] Silencing of NOX2 in macrophages prevents AGE-induced tissue factor expression as well.[308] NOX silencing is also beneficial in reducing AGE-induced apoptosis and NF-κB signaling in VSMCs.[308, 310] Further, high glucose-induced mitoROS production increases PKC activation, hexosamine pathway activation and AGE formation through DNA

strand break-mediated poly(ADP-ribose) polymerase activation and GAPDH poly(ADP-ribosyl)ation.[311]

As mentioned, mitoROS play a significant role in modulating vascular function in diabetes and obesity through both AGE signaling and modulating vascular tone, and potentially through changes in mitochondrial morphology. Isolated coronary ECs from diabetic mice exhibit increased mitochondrial fission that can be attenuated with *in vivo* TEMPOL delivery.[312] High glucose-induced mitochondrial fission induces mitoROS production,[312,313] which may be a result of increased pyruvate uptake following increased fission,[314] as well as an instigating step in apoptosis.[313] Silencing of Drp1 or Fis1 inhibits high glucose-induced mitoROS and restores eNOS activity and cGMP production, suggesting a role for mitochondrial fission-induced ROS in regulating vascular tone.[315] Likewise, high glucose-induced VSMC proliferation can be inhibited by attenuating mitochondrial fission-dependent NOX activity.[316] Low blood glucose also commonly occurs in diabetic patients who lack tight glycemic control, and low glucose enhances mitoROS production through Drp1-dependent mitochondrial fission. Suppression of mitochondrial fission in response to low glucose inhibits mitoROS production, increases NO bioavailability and restores endothelial-dependent vascular relaxation.[317] Moreover, NOX-induced Drp1-driven mitochondrial fission contributes to inflammasome activation by palmitate and elevated free fatty acids, which are often observed in diabetes.[318]

Much evidence supports an ROS contribution to the regulation of vascular tone and inflammatory signaling in diabetes and obesity (Supplementary Table II). The interaction of ROS and the metabolic changes that occur in obesity and diabetes is less clear, and is a subject for further investigation.

III. Stroke

Ischemic stroke is a leading cause of death and long term disability in the United States,[319] and patients who exhibit metabolic risk factors including diabetes mellitus, obesity and dyslipidemia are at a greater risk of experiencing stroke-related events.[320] Within minutes of hypoxia and glucose deprivation, a complex cascade of molecular events ensues, involving depolarization of neurons, increased Ca^{2+} influx, ATP depletion and release of the excitatory neurotransmitter glutamate.[321] Activation of glutamate receptors leads to a further increase in intracellular Ca^{2+}, activation of NOS and NOX signaling, mitochondrial dysfunction and neuronal death. Although hypoxia and glucose deprivation play a major role in the neurodegeneration induced by stroke, a role for ROS is clear. Indeed, several clinical studies have shown a correlation between elevated oxidative stress and brain ischemia,[322,323] and decreasing oxidative stress may be protective against stroke-induced complications.[324,325].

MitoROS are involved in the pathophysiology of cerebral ischemia as well as reperfusion injury.[326] Importantly, depletion of SOD2 increases mortality, stroke volume, brain edema and cytochrome C-mediated neuronal apoptosis following transient and permanent middle cerebral artery occlusion (MCAO).[327,328] In response to stroke, mitoROS arise from various sources including, complex I and IV, monoamine oxidase, p66[shc], mitochondrial BKCa and mK$_{ATP}$ channels, cytochrome b5 reductase and dihydroorotate dehydrogenase.[329–333] In patients who experience acute ischemic stroke, p66[shc] gene expression is increased in

peripheral blood monocytes, and post-ischemic knockdown of p66[shc] in mice undergoing transient MCAO improves survival and functional outcomes and reduces stroke lesion volume.[334] p66[shc] knockdown attenuates transient MCAO (tMCAO)-induced blood-brain barrier (BBB) permeability, which is associated with a reduction in NOX ROS-induced claudin-5 degradation.[335] NOX signaling represents an important source of ROS in the pathophysiology of stroke. Several studies have correlated NOX2 and NOX4 depletion with decreased infarct volume, oxidative stress, BBB permeability and neutrophil infiltration after MCAO.[324,336–339] Similar results have been reported with non-specific Nox2 inhibitors, apocynin and diphenylene iodonium,[337,340] and injection of glutathione into rats subjected to tMCAO reduces infarct volume and increases cell survival signaling.[341] Further, NOX2 deletion prevents tMCAO-induced IL-1β, TNFα, and CC-chemokine ligand 2/3 upregulation,[342] as well as glutamate toxicity through suppression of complexin II/soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) interaction.[343] However, a recent study hypothesizes that deletion of NOX2 only delays infarct progression while simultaneously increasing angiogenesis, but is unable to prevent neuronal loss.[344] Similarly, NOX2 deletion may have a protective effect only in models of transient occlusion, as NOX2 deletion has no effect on permanent MCAO-induced neurological dysfunction.[345]

A role for tissue-specific NOX4 signaling in cerebral ischemia has also recently been elucidated. Overexpression of NOX4 in pericytes increases BBB permeability following permanent MCAO in mice, which coincides with increased MMP-9 activity and phosphorylation of NFκB.[346] Similarly, in response to tMCAO, EC NOX4^{−/−} mice have increased BBB stability associated with less autophagy-related stress, but show no reduction in neuronal cell death. In contrast, neuronal NOX4^{−/−} mice display reduced neuronal cell death but no change in BBB stability.[339] Separate from NOX2 and NOX4, there have been conflicting reports regarding the role of NOX1 in stroke pathophysiology. NOX1 depletion decreases infarct volume, improves neurological outcome and reduces cerebral edema in mice subjected to tMCAO; however, these effects may be independent of NOX1-ROS signaling.[347] Adenoviral-mediated knockdown of NOX1 in rats undergoing tMCAO promotes increased functional recovery, which is associated with reduced infarct size and neuronal cell death.[348] However, two different groups have shown that NOX1 depletion does not affect infarct volume, brain edema or neurological score following tMCAO.[324,349]

As mentioned, diabetes is a risk factor for stroke,[350] and elevated blood glucose at stroke onset predisposes individuals to more severe functional outcomes and increased mortality. [351] Mechanistically, glucose injection into mice at the time of reperfusion after tMCAO increases NOX-dependent ROS production and neuronal cell death compared to normoglycemic controls, and p47[phox−/−] mice are protected from this response.[352] Furthermore, systemic glucose is regulated by the ventromedial nucleus of the hypothalamus (VMH), and glucose sensing in the VMH is partly regulated by Drp1-dependent mitoROS signaling.[353] Glucose load induces Drp1 activity through UCP2 in VMH, and UCP2-mediated mitochondrial fission reduces ROS, a mechanism that seemingly argues against the hypothesis that mitochondrial fission induces ROS, although increased Drp1-dependent mitophagy in response to permanent brain ischemia may play a role in mitigating ROS production.[354] Depletion of UCP2 increases infarct volume in mice after tMCAO[355] and alters whole body glucose utilization and insulin sensitivity. Re-expression of UCP2 in the

VMH rectifies changes in glucose utilization and insulin sensitivity,[356] and UCP2 overexpression in mice subjected to tMCAO reduces brain damage and neurological dysfunction.[357]

Oxygen sensing and glucose metabolism are central to regulating brain homeostasis and contribute to ischemic pathophysiology. Prolyl hydroxylase domain proteins (PHDs) regulate hypoxic signaling, most notably through regulation of HIF1α activity. PHD1$^{-/-}$ mice are protected against the deleterious consequences of permanent brain ischemia.[358] PHD1 deficiency reduces neuronal glycolysis and glucose consumption while concomitantly increasing glutamine oxidation, thereby maintaining mitochondrial respiration and energy homeostasis. This is vital for protection against ischemic-related injury as glucose is diverted towards the PPP pathway, thus increasing oxidative PPP flux and reducing equivalents needed for ROS scavenging in response to an ischemic insult.[358] A role for oxidant scavenging has also been noted in ischemic pathophysiology. Prdx2 overexpression reduces infarct size and neurological deficits in mice through attenuation of DNA damage and PARP1/p53 pro-death signaling.[359] GPX3 deficiency promotes vascular dysfunction and platelet-dependent arterial thrombosis and increases cerebral infarct size in mice with permanent MCAO.[360] Conversely, overexpression of GPX1 attenuates tMCAO-induced edema, microglial activation, neutrophil infiltration and neuronal cell death compared to WT mice.[361] Similar results have been observed in SOD1 mice.[362]

Cumulatively, although evidence supports a major role for ROS signaling in stroke pathophysiology, few studies have delved into cell-type specific roles of ROS. Given the dynamic interplay between ECs, pericytes, VSMCs, astrocytes and immune cells in the development and progression of stroke, additional experiments are warranted. In particular, with the unique metabolic alteration that occurs during stroke, more emphasis should be placed on how ROS signaling affects nutrient shuttling and metabolism in the ischemic tissue.

3. Antioxidant Trials

Animal studies have unequivocally suggested that ROS are a viable target for therapeutic intervention in the treatment of cardiovascular disease. However, clinical trials have yielded less than favorable results,[363,364] and although these results are negative, they have helped to identify why current approaches fail and how to target future interventions. Most antioxidant trials were conducted with various vitamins (A, C and E), and although vitamins have antioxidant properties, their effects may be too broad and concentrations may never reach the levels needed to attenuate ROS production, nor be as effective in ROS scavenging by tissue antioxidant defenses. Concerns have arisen regarding efficacy of synthetic compared to natural antioxidant formulations, as well as mode of delivery (e.g., pill vs diet). Furthermore, disease progression takes time, especially in the case of atherosclerosis and heart failure. To date, most studies have only looked at a small window of time (~5 years) during more advanced stages of disease, which may not be long enough to uncover positive effects. It has also become increasingly clear that ROS are vital to maintaining physiological function, and thus general scavenging of ROS may compromise cellular homeostasis and augment disease progression in some cases. This is especially important as we consider the

location and source of ROS, the specific roles of various types of ROS and how changes in concentration may affect cellular signaling. For instance, H_2O_2 has been found to induce both vasoconstriction and vasorelaxation in blood vessels treated with KCL and phenylephrine, respectively.[365] Likewise, suppression of endothelial H_2O_2 in mice results in hypotension and prevents the upregulation of eNOS in response to exercise.[366] On a similar note, O_2^-, while largely known for its role in arterial vasoconstriction and mediating EC and VSMC dysfunction, has also been found to regulate both vasoconstriction and vasodilation in a concentration-dependent manner in the cerebral vasculature,[367] and is critical to platelet activation and immune cell phagocytic function/recruitment.[368,369] ROS signaling in the setting of hypoxia has also been speculated to both instigate and inhibit angiogenesis, which may be dependent upon the local tissue environment and disease setting.[305,369] Finally, in many instances, antioxidants scavenge free radicals but do not affect H_2O_2, which as described throughout this review, is responsible for many of the pathophysiological effects on the vasculature.

While numerous studies highlight the physiological and pathophysiological differences in ROS signal transduction and their effect on cellular function, the takeaway remains the same. Instead of broad-spectrum ROS scavenging, it may be more effective to consider therapies that target specific sources of ROS, or limiting the production of specific ROS so as not to disrupt basic physiological function. We also need to consider how specific ROS influence different diseases. For example, in some cases, inhibition of O_2^- may not be as effective as scavenging H_2O_2 and vice-versa. However, inhibiting a specific source of ROS such as NOX may be beneficial. To this end, GKT137831, a promising NOX1/4 inhibitor, is currently in phase II clinical trials for diabetic kidney disease.[370] Human studies will be needed to test if new therapies targeted to specific sources of ROS, such as mito-tempol, will be beneficial in the treatment of cardiovascular disease.

4. Future Investigation/Concluding Remarks

One of the challenges in dissecting the role of ROS in cardiovascular pathology is that ROS are produced not only as natural by-products of metabolic reactions in various cellular compartments, but they also serve as signaling molecules that regulate specific biochemical pathways in normal cell function and survival. Dysregulation of ROS signaling, or excess non-specific production of ROS, can influence disease pathophysiology. As highlighted in this review, ROS are particularly important in cellular metabolism and inflammatory signaling. Thus, it is not surprising that ROS play a significant role in diseases associated with metabolic dysregulation and inflammation.

There are myriad areas where further investigation is warranted. Importantly, we have seen much emphasis on cytoplasmic and mitochondrial ROS signaling; however, there is a dearth of information regarding the contribution of peroxisomal and endoplasmic reticular ROS to cellular homeostasis. New organelle-targeted probes and antioxidants will help to tease out the contribution of organellular/compartmental-specific ROS and their influence on critical cellular processes including aerobic/anaerobic respiration, β-oxidation, fatty acid synthesis, protein translation and post-translational modifications. Such tools will also provide insight into how ROS-induced inflammatory signaling may affect and be affected by this regulation,

given the relationship between inflammatory and metabolic signaling. In addition, with the advent of cre-lox technology and the development of tissue-specific and inducible knockout mouse models, research should continue to delineate the tissue-specific role of ROS signaling in metabolic and cardiovascular diseases. Cardiovascular and metabolic disease etiology is complex, and understanding tissue-specific redox signaling will be important in our effort to develop new and novel therapies to treat disease. Metabolic dysregulation is a primary driver of cellular dysfunction and disease progression, and understanding the contribution and influence of ROS on metabolic processes poses an exciting area for scientific discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviation

ACOX
Acyl-CoA oxidase

ADAM17
A disintegrin and metalloproteinase domain-containing protein 17

AGE
Advanced glycation end product

AMPK
AMP-activated protein kinase

AP-1
Activator protein 1

ASC
Apoptosis-associated speck-like protein containing a CARD

ASK1
Apoptosis signal-regulating kinase 1

ATM
ataxia-telangiectasia mutated

BBB
Blood-brain barrier

BH4
tetrahydrobiopterin

BM-MNC
Bone marrow mononuclear cell

BMP4
Bone morphogenic protein 4

CaMKKβ
Ca^{2+}/calmodulin-dependent protein kinase kinase Beta

Cat
Catalase

CCCP
Carbonyl cyanide 3-chlorophenylhydrazone

CHOP
C/EBP homologous protein

CMA
Chaperone-mediated autophagy

CoQ
Coenzyme Q

CP
Core particle

CytoROS
Cytoplasmic ROS

DN
Dominant negative

Drp1
Dynamin-related protein 1

EC
Endothelial cell

eNOS
Endothelial nitric oxide synthase

ER
Endoplasmic Reticulum

ERO1
ER oxidoreductin 1

ETC

Electron transport chain

G6PD

Glucose-6-phosphate dehydrogenase

GlUT1

Glucose transporter 1

GPX

Glutathione peroxidase

Grb2

Growth factor receptor-bound protein 2

GRP78

Glucose-regulate protien 78

Grx

glutaredoxin

GTPCH

Guanosine 5′-triphosphate cyclohydrolase I

HIF1α

Hypoxia inducible factor 1α

IGF-1

Insulin like growth factor 1

IKK

IκB kinase

IL-18

Interleukin 18

IL-1β

Interleukin 1 Beta

iNOS

Inducible NOS

IP3R

Inositol 1,4,5-trisphosphate receptor

IκBα

Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha

JNK

c-Jun N-terminal kinase

KGDHC
α-ketoglutarate dehydrogenase

KO
Knockout

LAP
LC3-associated phagocytosis

LC3
Microtubule-associated protein 1A/1B-light chain 3

LKB1
Liver kinase B1

LMP
Low-molecular-mass protein

LOX-1
Lectin-type oxidized LDL receptor 1

LPP
Lipid peroxidation product

LPS
Lipopolysaccharide

MCU
Mitochondrial calcium uniporter

MerTK
Mer Tyrosine Kinase

MICU1
mitochondrial calcium uptake 1

Mitochondrial-associated ER membrane
MAM

MitoROS
Mitochondrial ROS

mTORC
Mammalian target of rapamycin complex

NAC
N-acetyl cysteine (CC

NF-κB
Nuclear factor kappa-light-chain-enhancer of activated B cells

NIK
NF-κB inducing kinase

NLRP3
NLR family pyrin domain containing 3

nNOS
Neuronal NOS

NOX
NADPH oxidase

ONOO
Peroxynitrite

OPA1
Optic atrophy protein 1

OxPL
Oxidized Phospholipid

P66SHC
SHC-transforming protein 1 isoform p66

PDC
pyruvate dehydrogenase

PDHK
Pyruvate dehydrogenase kinase

PDI
Protein disulfide-isomerase

Pex
Peroxin

PHD
Prolyl hydroxylase domain protein

PINK1
(PTEN)-induced kinase 1

PON
Paraoxanase

PPP
Pentose phosphate pathway

Prdx
peroxiredoxin

RAGE
Receptor for AGE

RET
Reverse electron transfer

ROMO1
ROS modulator 1

ROS
Reactive oxygen species

RP
Regulatory particle

RPT
Regulatory particle triphosphate

SNARE
Soluble *N*-ethylmaleimide-sensitive factor attachment protein

SOD
Superoxide dismutase

STZ
Streptozatocin

tMCAO
Transient middle cerebral artery occlusion

TNFα
Tumor necrosis factor alpha

TRAF
TNF Receptor associated factor

Trx
Thioredoxin

TrxR
Thioredoxin reductase

TSC
Tuberous sclerosis protein

TxNIP
Thioredoxin-interacting protein

UBC
Ubiquitin conjugating enzyme

UCP2

Uncoupling protein 2

ULK1

uncoordinated 51-like kinase 1

UPP

Ubiquitin proteasome pathway

UPR

Unfolded protein response

USP14

Ubiquitin carboxyl-terminal hydrolase 14

VCAM-1

Vascular cell adhesion molecule 1

VDAC1

Voltage dependent anion channel 1

VEGF

Vascular endothelial growth factor

VMH

Ventromedial nucleus of the hypothalamus

VSMC

Vascular smooth muscle cell

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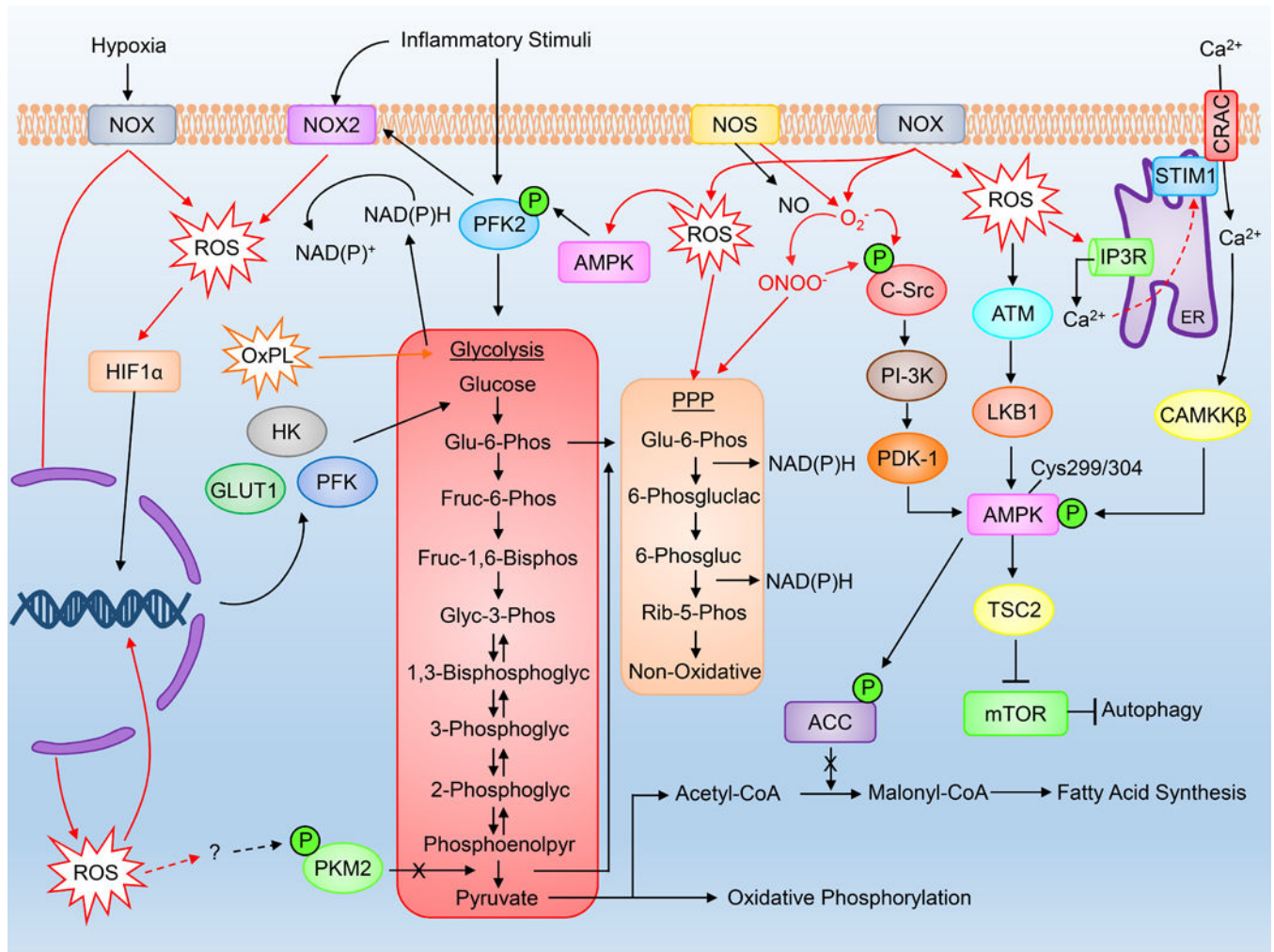
Figure 1. Cytosolic ROS production and regulation of cytosolic metabolic pathways
Cytosolic ROS are formed most notably through NOX activity and influence metabolic processes including glycolysis and downstream oxidative phosphorylation, pentose phosphate pathway activity and autophagy. Please refer to the abbreviation table for full names of listed proteins.
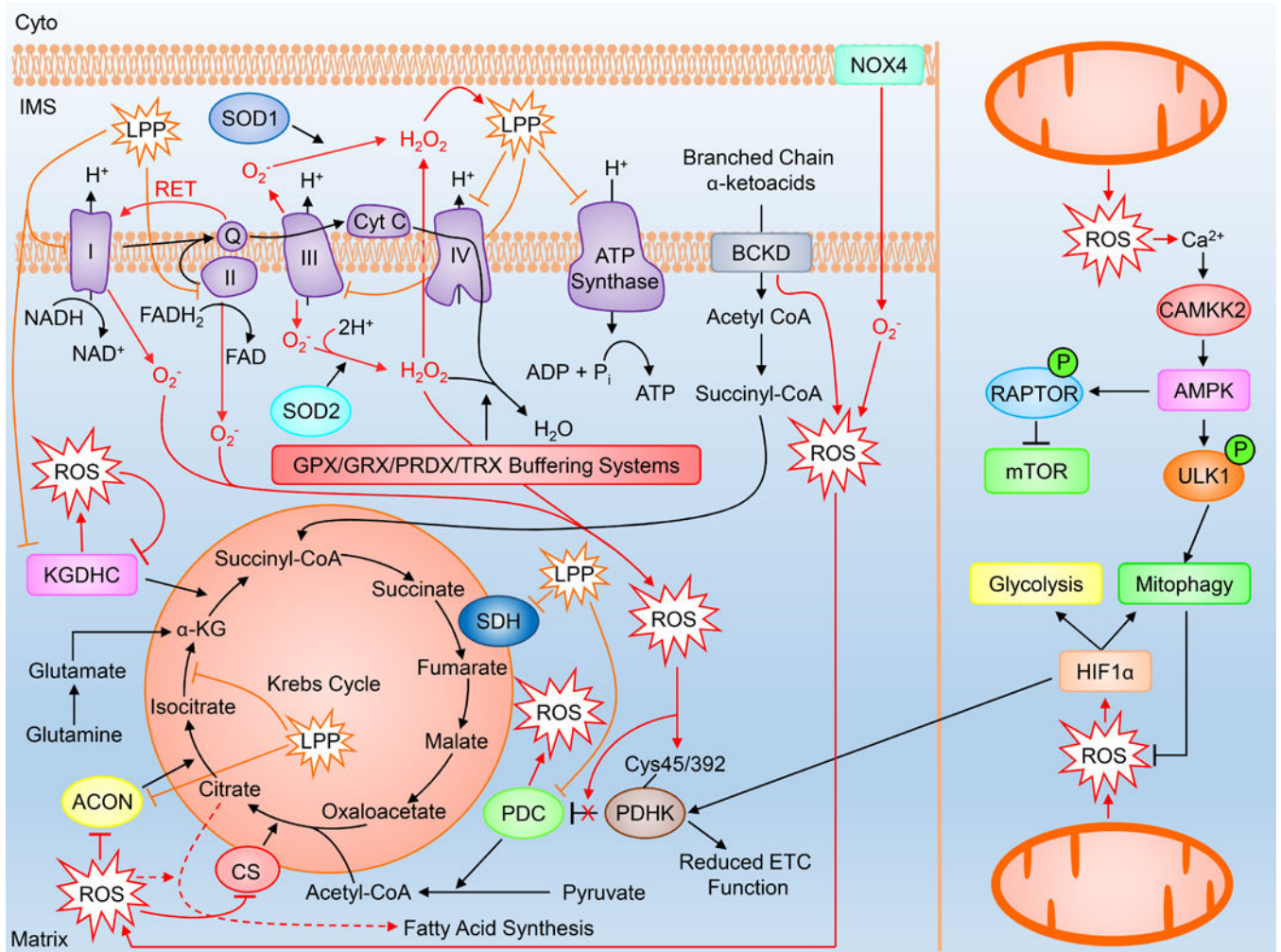
Figure 2. Mitochondrial ROS production
MitoROS are produced as a normal byproduct of mitochondrial respiration and metabolic enzymatic activity. Under settings of increased ROS generation as a result of dysregulated enzymatic activity and cellular stress, mitoROS can influence metabolic pathways including the Krebs cycle, fatty acid synthesis, ATP generation, glycolysis and mitophagy. Cyto=cytoplasm, IMS=intermembrane space, LPP=lipid peroxidation product. Please refer to the abbreviation table for full names of listed proteins.
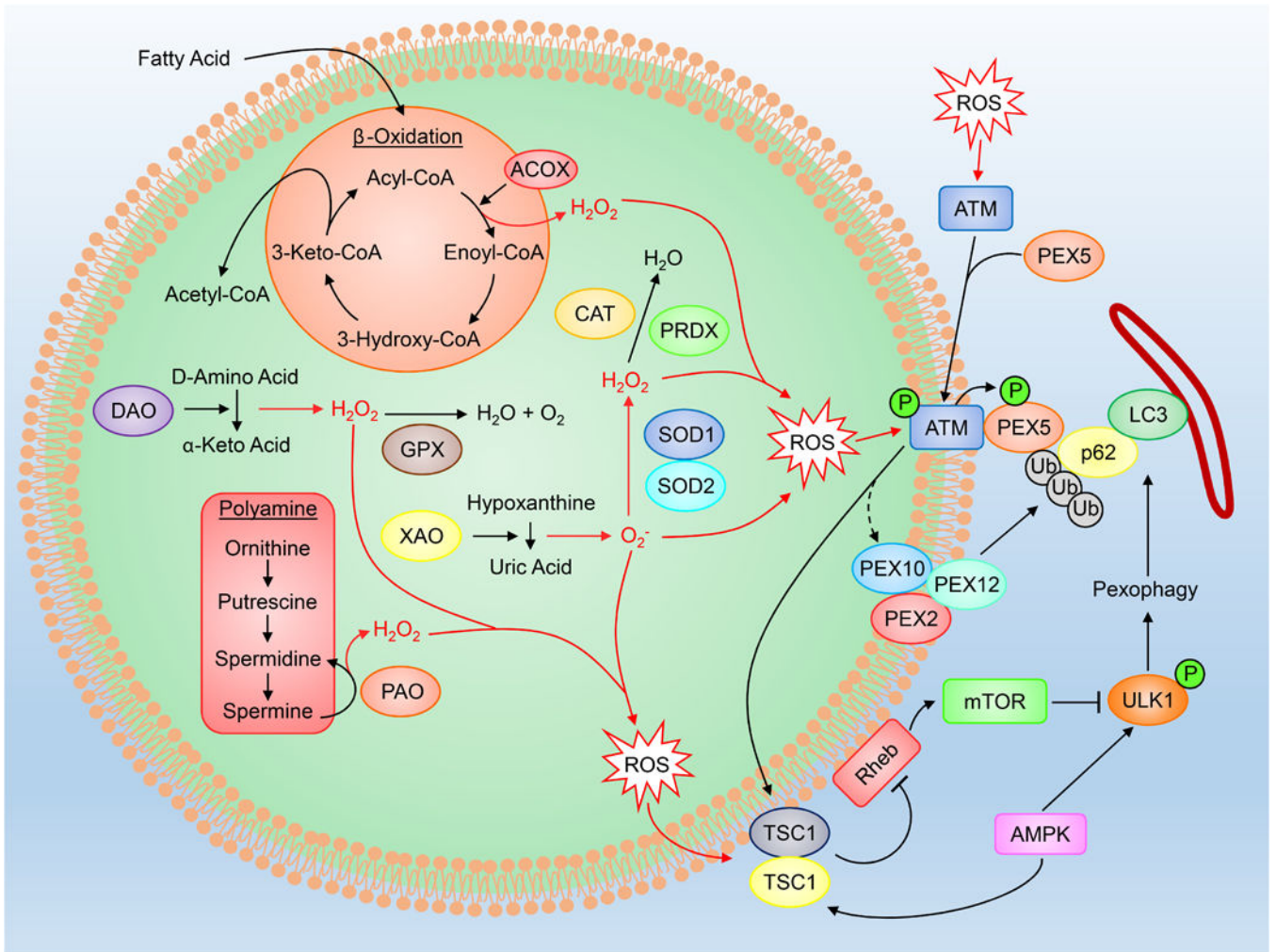
Figure 3. Peroxisomal ROS and metabolism
PeroxROS are produced as byproducts of enzymatic reactions within β-oxidation, polyamine synthesis, D-amino acid deamination and hypoxanthine oxidation, and have been found to be key regulators of pexophagy. Full names of abbreviations are listed in the accompanying table.
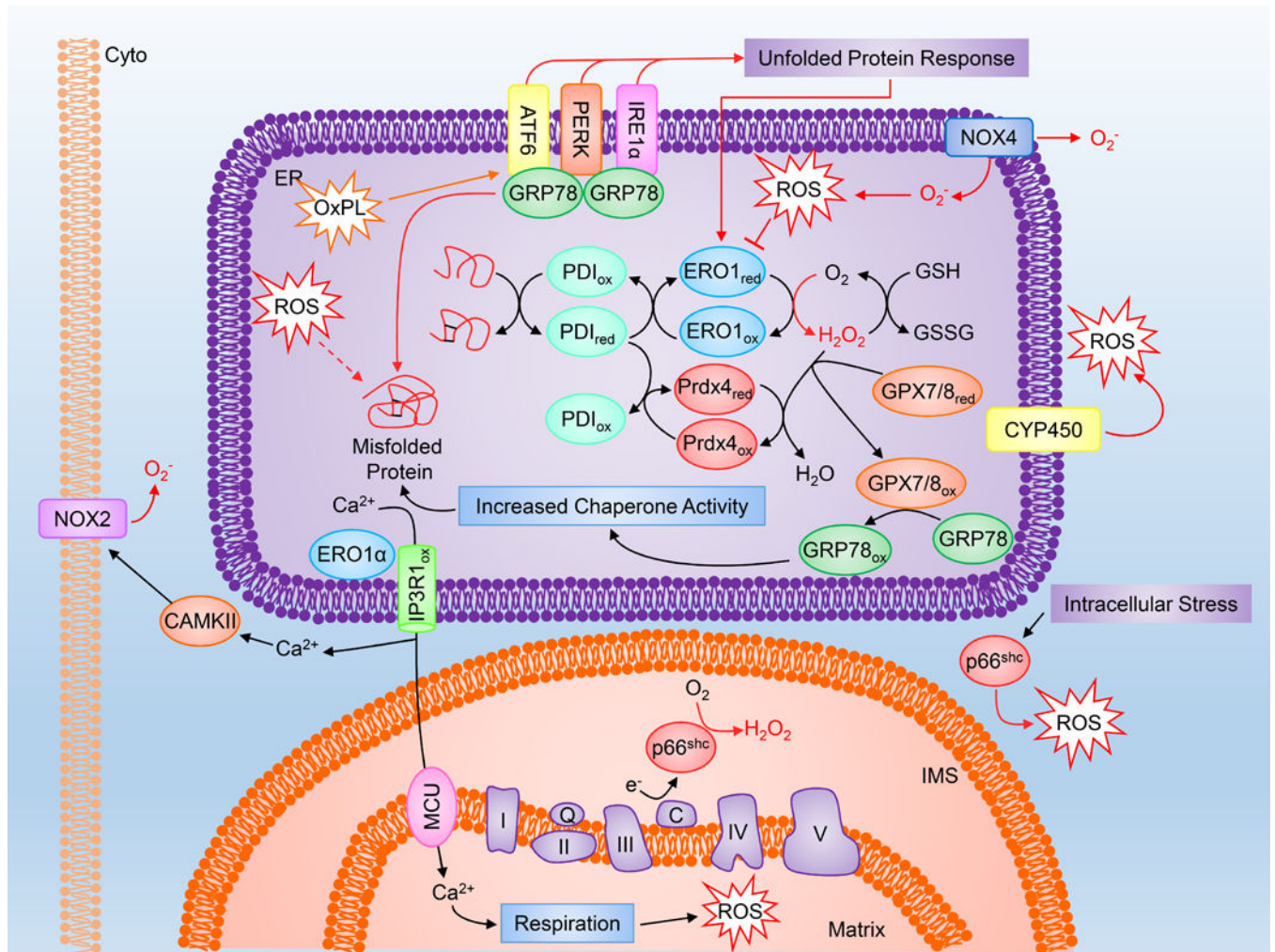
Figure 4. Endoplasmic reticulum and ROS

The ER is highly sensitive to redox status, and altered ROS signaling can influence protein folding, Ca^{2+} release and mitochondrial respiration. Please refer to the abbreviation table for full names of depicted proteins.
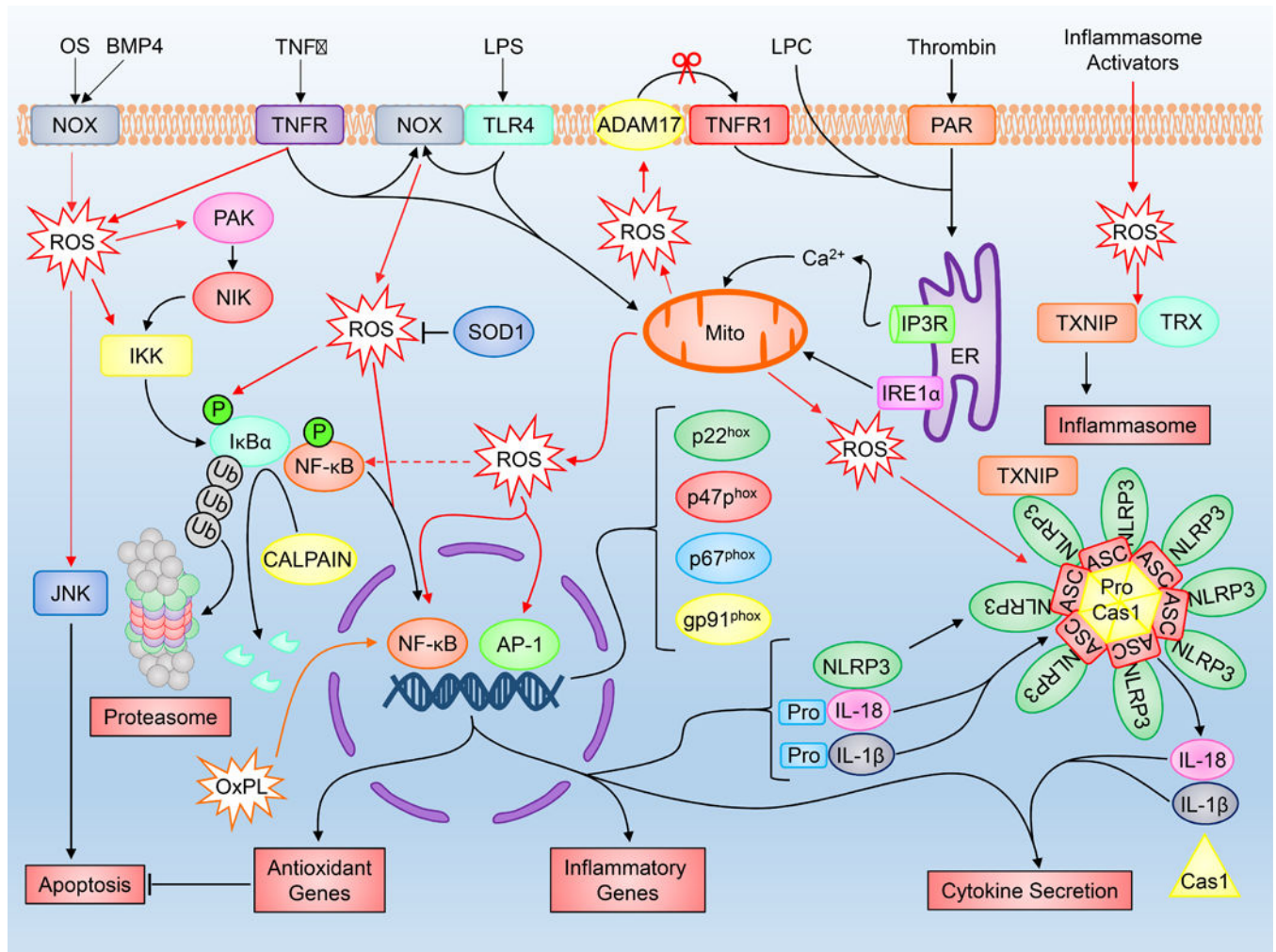
Figure 5. Inflammation and ROS

Various inflammation-inducing stimuli including TNFα, LPS, thrombin and oscillatory shear stress influence ROS production through sources including NOX and the mitochondria. Elevated ROS production as a result of inflammatory signaling can mediate canonical NF-κB activation and downstream inflammatory gene induction, proteasome activity, antioxidant gene transcription, inflammasome activation and cytokine secretion. Full names for abbreviations are listed in the accompanying table.