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Review article

Reactive oxygen species: friend or foe?

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Abstract

Reactive oxygen species (ROS) are an inevitable by-product of cellular metabolism. ROS generation associated with interaction of ionizing radiation with biological molecule, and devoted enzymes in phagocytic cells (NADPH oxidase and myeloperoxidase) or may be the result of imbalanced radical generating and scavenging systems. Albeit the myth about ROS as Pandora's Box, it has several innovative physiological roles in body. ROS serve as signalling messengers for activation of transcription factors from cytokine-receptor interactions. It facilitates the evolution and membrane fusion of spermatozoa and oocyte during fertilization. NADPH oxidase enzyme and nitric oxide (NO) are function as potent vasodilators and immunity booster. ROS has been suggested as a prevalent regulator of several nuclear factors including erythroid 2-related factor 2 (Nrf2), nuclear factor kappa-B cells (NFκB), mitogen-activated protein kinase (MAPK) and p53 which further associated with plenty of signalling cascades. Under physiological conditions the amount of ROS generated in body can be counterbalance by natural anti-oxidant of body. However, the aberrant augmented level of ROS predominantly leads to various defined disorders comprising myocardial infarction, autoimmune diseases, atherosclerosis, alzheimer, parkinson and emphysema diseases. Ordinarily, it has been observed that physiological roles of ROS are knocked down in front of their pathological action. But here a need of glimpse about the explicit line of margin between patho-physiological function of ROS. Worthy of this note is to reveal the beneficial responsibility of ROS in different cellular pathways and metabolic functions, over its injurious consequence.

Keywords: Mitochondria, ROS, RNS, Physiology, Pathology, Signalling mechanism.

Abbreviations: ROS, Reactive oxygen species; NO, Nitric oxide; Nrf2, Nuclear factor erythroid 2-related factor 2; NFκB, Nuclear factor kappa-B cells; MAPK, Mitogen-activated protein kinase; TNFα, Tumor necrosis factor-α; PTP, Permeability transition pore; NADPH-oxidase, Nicotinamide adenine dinucleotide phosphate-oxidase; eNOS, Endothelial nitric oxide synthase; BH4, Tetrahydrobiopterin; RNS, Reactive nitrogen species; JNK, c-Jun N-terminal kinases; PKB/Akt, Protein kinase B; mTOR, Mammalian target of rapamycin; GPCR, G protein-coupled receptors; TLR, Toll-like receptors; ICAM-1, Intercellular adhesion molecule-1; cAMP, Cyclic adenosine monophosphate; Keap1, Kelch-like ECH-associated protein 1.

Introduction

Mitochondria are the primary site of ROS generation. During aerobic metabolism electron transport chain reaction leads to instantaneous production of ROS as an unavoidable by-product [1]. Mitochondrial ROS generation is composed of four multi protein complexes (complex I-IV). Complex I and III are widely identified as the core site of ROS generation [2]. Endogenous production of ROS may initiate numerous signalling cascades for maintaining homeostasis in physicochemical or patho-physiological states [3]. Recent extensive evidences suggested that conventionally mitochondrial ROS plays a pivotal role in managing several transcription factors and receptor interactions [4-5]. Despite of physiological role several evidences show debilitating proposition about ROS at higher level. Over production of ROS due to damaged mitochondria is considered as major predictor for the development of acute and chronic disorders. Increased level of ROS involved in the activation of wide range of gene expression and associated pathogenesis [6]. As the brain consumed 20% oxygen of the total body's oxygen consumption, the vulnerability of oxidative damage is most prominent in this area. The accumulation of cellular ROS destroys the normal redox state in neural cells and leads to neurodegenerative diseases [7]. Ma *et al.* (2012) concluded the overproduction of these ambiguity molecules by mitochondria of β-cell activate various insidious metabolic disorder (diabetes mellitus followed by β-cell failure) [8]. Moreover, evidences suggested that high glucose concentration increased oxidative stress and further contribute to diabetes and associated complications [9-10]. In addition, ROS interfere with nuclear factor kappa-B (NF-κB) and tumor necrosis factor-α (TNFα) pathway and may leads to rheumatoid arthritis [11]. Indeed, free radicals react with all biological macromolecules (lipids, proteins, nucleic acids and carbohydrates) which contribute to oxidative stress and activate

various apoptotic pathways. Further, intracellular ROS also affect the mitochondria by activation of caspases with the rapid release of cytochrome c which ultimately leads to apoptosis [12]. TNFα can induce ROS by interacting with TNF receptor (TNF-Rl) and leads to apoptosis/ necrosis. ROS induce apoptosis may affect 'Guardian of the genome' p53 which cause immense alteration in different signalling [13-14]. However, mitochondrial low oxidative stress or mitochondrial hormesis/mitohormesis prompts an adaptive reaction results including lower glucose metabolism, improve stress resistance, and influence mitochondrial signalling and metabolism [5]. In-addition, Perez-Matute *et al.* (2009) also supports that ROS act as a loyal second messenger at low level and regulates vital cellular functions [4]. NO is an imperative mediator of endothelial function and cardiovascular physiology because of its vaso-dilatory, anti-platelet, anti-proliferative, and anti-inflammatory potential. Increased bioavailability of NO in the blood vessels may improve the inflammation, atherosclerosis, hypertension, vascular endothelial dysfunction and associated cardiovascular diseases [15]. Moreover, recent evidence has shown that oxidative stress generated by reactive oxygen and nitrogen species monitor the array of physiological signalling in cardiovascular system [16]. The contentious facts on patho-physiological action of ROS can differentiate only upon its level in cells. Despite of the consciousness about these two parts of the coin, an overt line of boundary between physiological and pathological level of ROS is still unclear. Thus, there is a need of revisiting to understand the relation of oxidative stress level to precise health effect. The present review delineates positive liability of ROS in several cellular and metabolic pathways, more than its deleterious effects and put a requisite of measurable level of ROS, which may explain that how the physiological state get convert into pathological state.

Generation of reactive oxygen species

To begin with Richard Altmann, an elementary organism has been illustrated within the cells which conclusively recognized as 'bioblast' or powerhouse of the cell or mitochondria [17]. Primarily mitochondria was regarded as only an energy generator, but in last few decades' number of evidences revealed its extensive role in generating ROS and -associated fascinating mechanisms [18]. Mitochondria are the foremost site for *in vivo* production of free radical in continuous chain reaction mode, which extensively involved in various patho-physiological pathways. Superoxide is generated on the innermost membrane of mitochondria (matrix) [19]. This superoxide consecutively monitors mitochondrial functions including mitochondrial biogenesis, mitochondrial membrane permeability transition, lipid peroxidation, and mitochondrial DNA damage [20-21]. Mitochondrial ROS generation is organized by four multi-protein complexes (complex I-IV) [2]. Mitochondria comprise almost eight sites from where superoxide may generate [22]. Interestingly, out of them seven sites liberates generates superoxide within the matrix, but eighth (site IIIQo) on complex III and glycerol 3-phosphate dehydrogenase have an ability to discharge produced superoxide in inter membrane space. ROS moving in inter membrane space can easily cross the mitochondrial membrane in contrast to ROS present in the matrix [23]. Therefore, the ROS generated by site IIIQo on complex III and glycerol 3-phosphate dehydrogenase has more impact to participate in cellular signalling. In-addition, complex I (NADH CoQ reductase) has ability to stimulate the transfer of electron from NADH to co-enzyme Q which is responsible for production of significant amount of ROS. The translocation of proton from matrix to the inter membrane space revealed the potential of complex I to generate and release ROS as complex III. Moreover, complex II enzyme (succinate dehydrogenase) may reduce CoQ and

showing its involvement in production of superoxide [24-25]. Overall studies indicate that complex I, II and III have huge potential to generate ROS, but major contributors are complex I and complex III [2]. These contributions of producing ROS by each enzyme complex are variations in different tissue and pathologic conditions [26] [Figure 1].

Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase) is a multisubunit (membrane associated components, i.e., p22 phagocytes oxides, gp91phox and cytosolic component enzyme, i.e., p47phox, p40phox, p67phox, small GTPase Rac) enzyme which plays an integral role to catalyze the reduction of molecular oxygen to form superoxide. NADPH oxidase activation through translocation and accumulation of cytosolic proteins with the membrane turn out electron transference, which further combines with molecular oxygen and generate superoxide anion [27-28].

Endoplasmic reticulum (ER) is another major site of ROS production. It contains several proteins and enzymes including endoplasmic reticulum oxidoreductase, reduced glutathione and protein disulfide isomerase which participate in ER stress-induced ROS production. Protein folding is précised system which initiates the translaction of accurate folded protein to intended site. Any adversity in this system process leads to misfolding or immature protein aggregate and ER stress -associated ROS. Protein folding is carried out by the formation of disulfide bond which produce a specific oxidising environment. Alteration in the disulfide bond formation results to protein misfolding which leads to generate significant amount of ROS due to depletion of glutathione (GSH). Repetitive cycle of breakage and formation of disulfide bond degrade the level of GSH. Each cycle produce more and more ROS as

by-product. Moreover, accumulated unfolded proteins cause Ca^{+2} leakages in cytosol which further responsible for mitochondrial ROS production [29-30].

Redox signalling plays a vital role in physiological and patho-physiological regulation. Cellular redox reactions maintain the equilibrium between oxidized and reduced state of cysteines and methionines. Extracellular fluid infused protein may oxidized by reacting with thiol group present in cystein residue. This thiol reaction with protein can modify critical cellular signalling mechanism. Disufide bonding between thiol group of cystein and extracellular protein is the major source of ROS and radition induced cellular death. Regulation of protein redox state through thiol/disulfide bonding may maintain physiologic functions [29-31].

Endothelial nitric oxide synthase (eNOS), is an exceptional enzyme that has the ability to generate both free radicals (Peroxynitrite) and free radical scavenging (NO) agent. Coupling of eNOS with cofactor tetrahydrobiopterin (BH4) produces NO during the conversion of L-arginine to L-citrulline in the presence of NADPH-dependent enzyme. NO, is a key regulator of vascular and cardiac functions having anti-inflammatory, anti-platelet, anti-proliferative and anti-migratory properties [32]. However, uncoupled eNOS produces reactive nitrogen species (RNS). Superoxide reacts with NO and leads to versatile nitrogen species; Peroxynitrite may further convert in peroxynitrous acid by protonation and may yield nitrogen dioxide and a hydroxyl radical [33-34]. Peroxynitrite and peroxynitrous acid can cause lipid peroxidation and membrane damage in vessel wall [35].

Pathological role of ROS

Healthy mitochondria persistently generate low levels of superoxide during conventional respiration. On the other hand Injured and unsynchronized mitochondria

generate large amount of superoxide which further cause detriment to mitochondrial components including lipids, proteins, DNA and eventually cell death [1, 36]. Numerous studies have demonstrated the pathological role of ROS in different metabolic syndrome. Indeed, ROS regulate autophagy process by directly modifying major proteins including Atg4, Atg5 and Beclin. Higher level of ROS indirectly alters the vital signalling pathway including c-Jun N-terminal kinases (JNK), p38 which leads to autophagy. Intriguingly, ROS mediated autophagy process allied with numerous diseases, comprising neurodegenerative diseases, diabetes mellitus, myodegenerative diseases, crohn's disease, and different type of cancer. Elevated ROS also obstruct the key signalling of protein kinase B (PKB/Akt) and down regulate mammalian target of rapamycin (mTOR) [37-39]. Clinical evidences support the subclinical based concept of DNA damage associated activation of mutagenic and apparently carcinogenic factor [40]. Additionally, high oxidative stress has acknowledged as key mediator in the occurrence and development of diabetes mellitus and -associated complications. Furthermore, diabetes related oxidative alteration would affect cellular metabolism, functions, and gene expression [41-42]. Oxidative stress is the major pitfall of vascular endothelial dysfunction and associated cardiovascular pathology. eNOS-generated NO is an imperative endothelial factor which can obstruct pathological event for cardiovascular disorders, including platelet aggregation, inflammation, vascular proliferation, hypertension, and leukocyte adhesion [43-44]. Excessive extent of NADPH-oxidase derived superoxide reacts with NO and converts this into versatile nitrogen species "Peroxynitrite". Peroxynitrite further reduced the bioavailability of NO by initiating eNOS uncoupling by oxidizing BH4. Thus, a deficiency in production or bioavailability of NO leads to atherosclerotic events, vascular endothelial dysfunction and -associated

cardiovascular diseases [45-46]. Moreover, *in vitro* evidence revealed that ample amount of ROS triggered apoptosis in renal cells through activation of caspase-3 leads to nephrotoxicity [47-48]. About three decades before Baltimore invent one more ROS mediated cellular signalling (NFκB). It has been found NFκB signalling leads to atherosclerosis, schizophrenia and cancer generation [49-51]. NFκB has five distinct transcription factors (p65, p50, p52, c-Rel and RelB) which react with DNA through homology domain and may stimulate transcription of various genes. Furthermore, seven individual proteins of IκB family (inhibitory proteins) can modulate NFκB signalling cascade [52-54]. Schreck and his colleagues primarily revealed the important role of oxidative stress in stimulating NFκB and -associated transcription factors [55]. Oxidative stress mediated NFκB signalling activation may lead to cell inflammation, proliferation, and apoptosis [56-57]. ROS generated oxidative stress initiate glomerular, tubular injuries and may initiate NF-κB. NF-κB further alters the transcription of the intercellular adhesion molecule-1 and tumor necrosis factor (TNF-α) which cause renal injury [47-48, 58] [Figure 2]. ER stress associated production involved in wide range of pathologic condition such as cancer, neurodegenerative diseases, cardiac diseases, diabetes mellitus and muscle degeneration diseases [29-30]. Thus, mentioned all above evidences suggest that ROS generated oxidative stress may cause serious pathological condition. However, pathological role of ROS is only associated with its elevated level.

Physiological role of ROS

ROS including superoxide anions (O^{2-}) and hydrogen peroxide (H_2O_2), are highly reactive molecules which may leads to organ pathogenesis. However, it is recognized that low level of ROS act as vital signalling molecules for modulating the normal cellular processes [59]. ROS are salient mediators and signal modifiers during

numerous biological processes and cascades such as G protein-coupled receptors (GPCR), Notch [60], Wnt-$\beta\beta$-catenin [61], MAPK, JAK-STAT, NF-κB, and PI3K/AKT [62].

Physiological role of ROS associated with myocardium

Recent evidence suggests that ROS could serve as paracrine signalling mediators upon pathological stimulation [63]. Paracrine signalling in the epicardium and endocardium is associated with fibroblast growth factor and retinoic acid dependent signalling. These vital signalling pathways carry the proper growth and differentiation of the myocardium [64-65].

Physiological role of ROS associated with insulin sensitivity

ROS promotes tyrosine phosphorylation-dependent signaling (protein tyrosine phosphatises, PTP) which leads to phosphorylation of PTP1B and dephosphorylation of tyrosyl phosphorylated substrates and PTEN enzyme. PTP is regulated by cysteines which inactivate its nucleophilic properties and increase insulin sensitivity and glucose homeostasis. ROS also improve the insulin sensitivity by terminating PI3K signalling via dephosphorylation of phosphatidylinositol-3,4,5-triphosphate (PIP3) [66]. ROS play an important role in the regulation of insulin receptor kinase activity by auto phosphorylation of the insulin receptor kinase at Tyr-1158, Tyr-1162, and Tyr-1163 sites [67]. In general, insulin controls several physiological functions such as glucose metabolism, lipid metabolism and synthesis of protein. Loh et al. (2009) also revealed supportive evidence on the basis of *in vivo* study conducted by him suggesting the enhancement of insulin signalling by ROS [66].

Physiological role of ROS associated with autophagy

ROS regulate the process of autophagy which in turn implicated with various physiological processes and maintain the cell homeostasis. Autophagy is a process of engulfing or breaking of intracellular proteins and organelles of cells by lysosome and further repurposes the constituents for new biosynthesis. In-addition autophagy plays a central role in maintaining the homeostasis during starvation and prevention of pathogenic infection [37, 68]. Starvation activates PI3K signalling followed by mitochondrial ROS generation which inactivate the cysteine protease Atg4 and promote autophagy.

Physiological role of ROS associated with apoptosis

Mitochondrial ROS significantly leads to apoptosis which have physiologic functions during embryogenesis. Interestingly, mitochondrial cytochrome-C which leads to caspase signalling activation intensely arbitrated by direct or indirect ROS action and that ultimately causes cell death. In-spite of that, ROS have their own anti-apoptotic actions also [69]. Apoptosis has an imperative biological function in the improvement and homeostasis of cell masses [70]. Apoptosis also involved in certain physiologic action including involution of breast [71], endometrium detachment during menstruation [72], removal of T cells during thymus development [73], castration-induced prostate atrophy [74] and epidermal cell death [75]. Recent study revealed apoptosis-dependent cavitation is an essential step for the formation of embryogenesis and growth of mammary gland, lung, and kidney by epithelial lumen creation route [76].

Physiological role of ROS associated with immunity

The physiological role of ROS also includes their potential against pathogens in favour to immune response. Superoxide generation may kill the growth of bacteria

and resist the host from infection. Additionally, evidence supports that ROS derived NADPH oxidase also plays a crucial role in host protection [77-79]. ROS initiate the immunity of cells by causing oxidative burst via NADPH oxidase to kill the pathogens in cells. Uncoupling proteins 1–3 (UCPs1-3; mitochondrial anion carrier proteins) are supposed to play an important role in minimizing ROS discharge from the electron transport chain [80]. However, UCP2 in macrophages increase mitochondrial ROS for MAPK activation and oxidative burst agumentation in favour of pathogen elimination [81-82]. More recent studies have demonstrated that mitochondrial ROS are significantly initiated by Toll-like receptors (TLR) and associated pathways. The stimulation of cell-surface TLRs (TLR1, TLR2, and TLR4), but not endosomal TLRs (TLR3, TLR7, TLR8, and TLR9) leads to an increase in mitochondrial ROS production through ECSIT (evolutionarily conserved signalling intermediate in Toll pathways) and TRAF6 (tumour necrosis factor receptor-associated factor 6 signalling). Collectively, the study concluded that, TLRs which are prominently obtained from bacteria can produce immunity against bacterial infection by inducing ROS [83].

Physiological role of ROS associated with capacitation

ROS have another crucial liability in early events of capacitation. The capacitation is a term used to define the complex and not well-characterized process that allows spermatozoa to complete their preparations to fertilize oocytes. Some of the early events of capacitation include a calcium influx, rise in pH [84], increase in intracellular cyclic adenosine monophosphate (cAMP) [85] and the generation of low and organize level of superoxide anion [86]. Another important aspect of capacitation, reported more than ten years before is that the proteins from the fibrous sheath are subjected to tyrosine phosphorylation (P-Tyr) [87]. Moreover, post translational

modifications of these proteins become very important for sperm function, because spermatozoa are unable to synthesize proteins. Studies revealed that this potential role of protein P-Tyr is dependent on ROS [88-89]. Ford, (2004) publish a report which revealed the increment of capacitation by the addition of ROS and vice versa. This study also support ROS-induced cAMP and tyrosine kinase activation through protein tyrosine phosphorylation as signalling cascade for regulation of sperm function [90]. Conversely, excessive ROS generation may devastate its protective action in capacitation. The posivtive and negative influence of ROS generation on fertilizing ability but the threshold level of ROS-associated with sperm cell maturation is still unclear. Thus further revisit on this aspects is needed for the contemporary treatment of infertility [90-92].

Physiological role of ROS associated with signalling cascade

Nrf2 is another prominent regulator of cellular resistance to oxidants. Keap-1 (Kelch-like ECH-associated protein1) dependent and independent activation of Nrf2 plays a pivotal role in regulating anti-oxidant and anti-inflammatory defence mechanism through various physiological cellular signalling. It also revealed that low level of ROS can significantly initiate the activation of Nrf2 signalling [93-94].

Albeit, H_2O_2 is an inevitable by-product of cellular metabolism. The production of H_2O_2 is most commonly associated in neutrophils which play important defensive role in host. Moreover, some studies reveal that H_2O_2 act as novel signalling messenger at low level. Several prosurvival pathways regulated by H_2O_2 including kinase, oxidation of cysteines and transcriptional factors such as p53, NF-kappa B and AP-1 [95].

Thus, the diverse biological activities of oxygen radicals and related species are likely rooted in the oxygen-dependent evolution of complex life forms. These critical evidences collectively suggest the physiological role of ROS may offer a precious therapeutic option and that may be indispensable in cellular pathology.

Conclusion

For a long time, evidences pursuit the stochastic action of ROS but the devoted role of ROS has soared in both physiology and pathology. ROS is a double-edged sword, with protective and toxic capabilities. All pathological conditions instigate involvement of high level of ROS. However, low levels of ROS are not only suspicious against diseases, but also have the additional property to regulate physiological cellular signalling. Both these facts widely asserted and acknowledged, but the mystery regarding the level of ROS conversion from physiological action to pathological action is still unclear. Ample numbers of important cellular pathways, where ROS are involved. For instance, MAPK [96-97], 'Guardian of the genome' p53 [98-99], Hypoxia-inducible factor 1-α (HIF1-α) [100], SP-1 transcription factor [101-102], AP-1 transcription factor [101, 103], caspase regulation [104], cytokine [105], platelet derived growth factor [106-107] and fibroblast-derived growth factor [108], are well known remnant key signalling cascades which have been altered by ROS. Thus it is further important to clarify that what extant of ROS is protective and another one is aggressive. Concept becomes more worthy for the individuals getting anti-oxidant treatment in life-threatening diseases like cancer. Moreover, potent anti-oxidant may cause developmental deformity due to crucial reduction of ROS level as compared to its threshold level needed for therapeutic effect. In light of this view point we suggest there should be a precise border line between patho-phyisological actions with statistical level of ROS. This statistical approach may offer new

perspectives for better and in-time treatment of ROS -associated diseases with specific amount of anti-oxidant agents.

Conflict of interest

No conflict of interest.

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Legends for Figures:

Figure 1: Figure portrays the involvement of various sites in generation of ROS. NADPH-oxidase indicates nicotinamide adenine dinucleotide phosphate-oxidase; eNOS indicates endothelial nitric oxide synthase; NO indicates nitric oxide; RNS indicates reactive nitrogen species; ROS indicates reactive oxygen species.

Figure 2: Figure interpret the pre-diabetic-associated cardiovascular signalling alterations and their possible contribution to cardiovascular disease pathology. ROS indicates reactive oxygen species; TNFα indicates tumor necrosis factor-α; NFκB indicates nuclear factor kappa-B cells; mTOR indicates mammalian target of rapamycin; JNK indicates c-Jun N-terminal kinases; PI3K indicates phosphatidylinositide 3-kinase; PKB/Akt indicates protein kinase B; GPCR indicates G protein-coupled receptors; Nrf2 indicates nuclear factor erythroid 2-related factor 2; cAMP indicates cyclic adenosine monophosphate; MAPK indicates mitogen-activated protein kinase; TLR indicates toll-like receptors; HIF1-α indicates hypoxia-inducible factor 1-α.

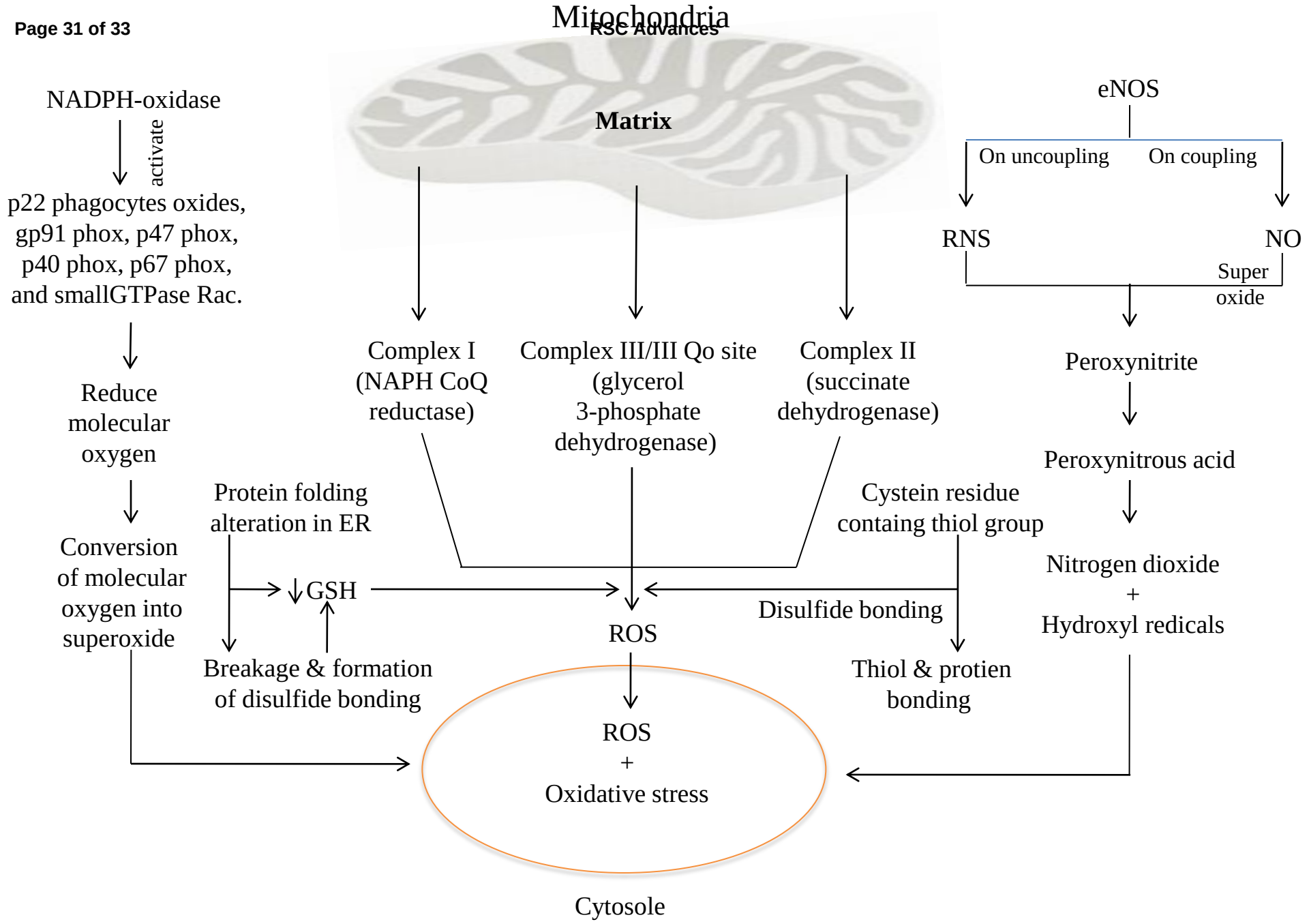

Mitochondria
Matrix
NADPH-oxidase
activate
p22 phagocytes oxides, gp91 phox, p47 phox, p40 phox, p67 phox, and smallGTPase Rac.
Reduce molecular oxygen
Conversion of molecular oxygen into superoxide
Complex I (NAPH CoQ reductase)
Complex III/III Qo site (glycerol 3-phosphate dehydrogenase)
Complex II (succinate dehydrogenase)
eNOS
On uncoupling
On coupling
RNS
NO
Super oxide
Peroxynitrite
Peroxynitrous acid
Nitrogen dioxide + Hydroxyl redicals
Protein folding alteration in ER
GSH
Breakage & formation of disulfide bonding
Cystein residue containg thiol group
Disulfide bonding
Thiol & protien bonding
ROS
ROS + Oxidative stress
Cytosole

Figure 1.

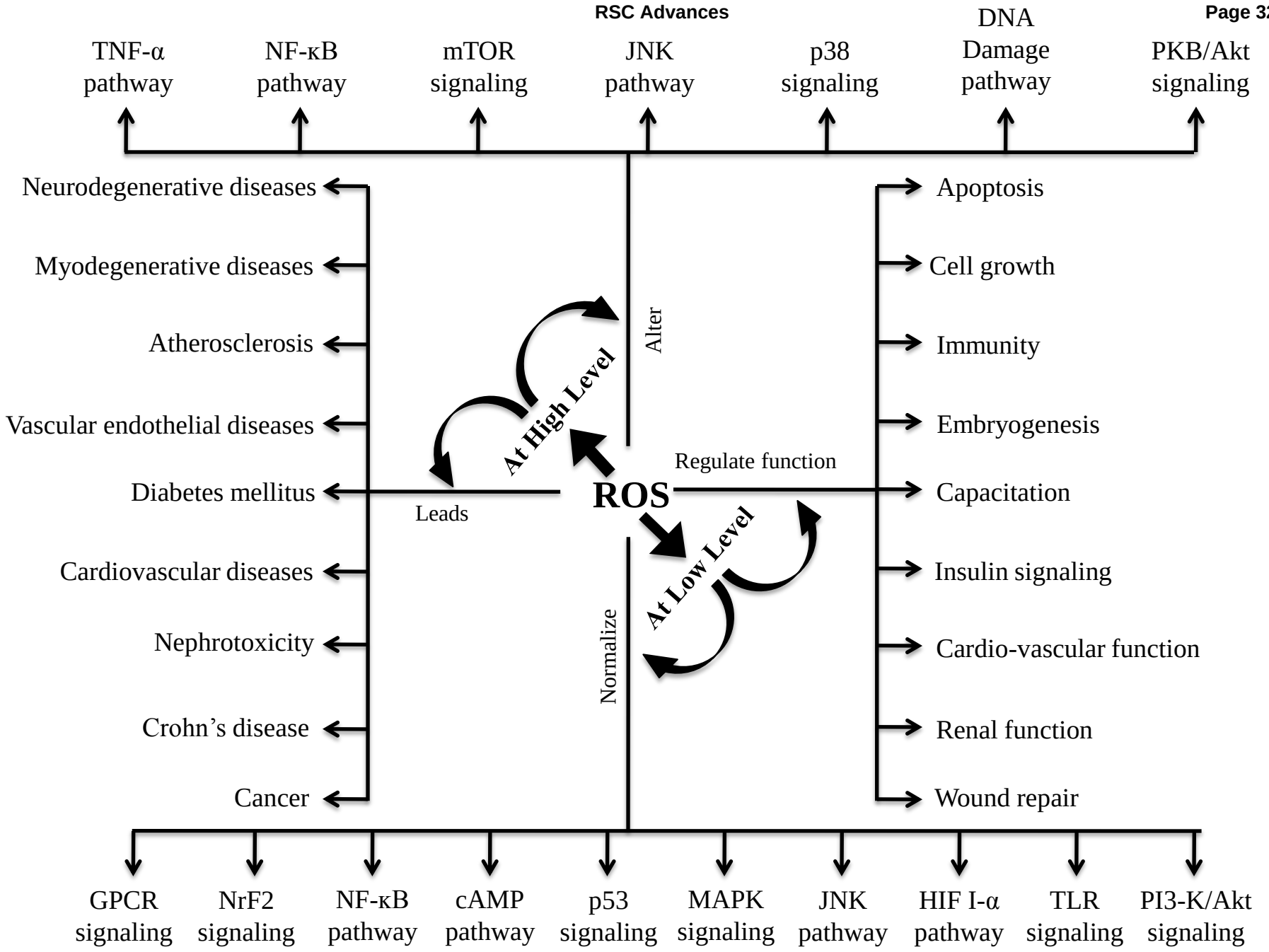
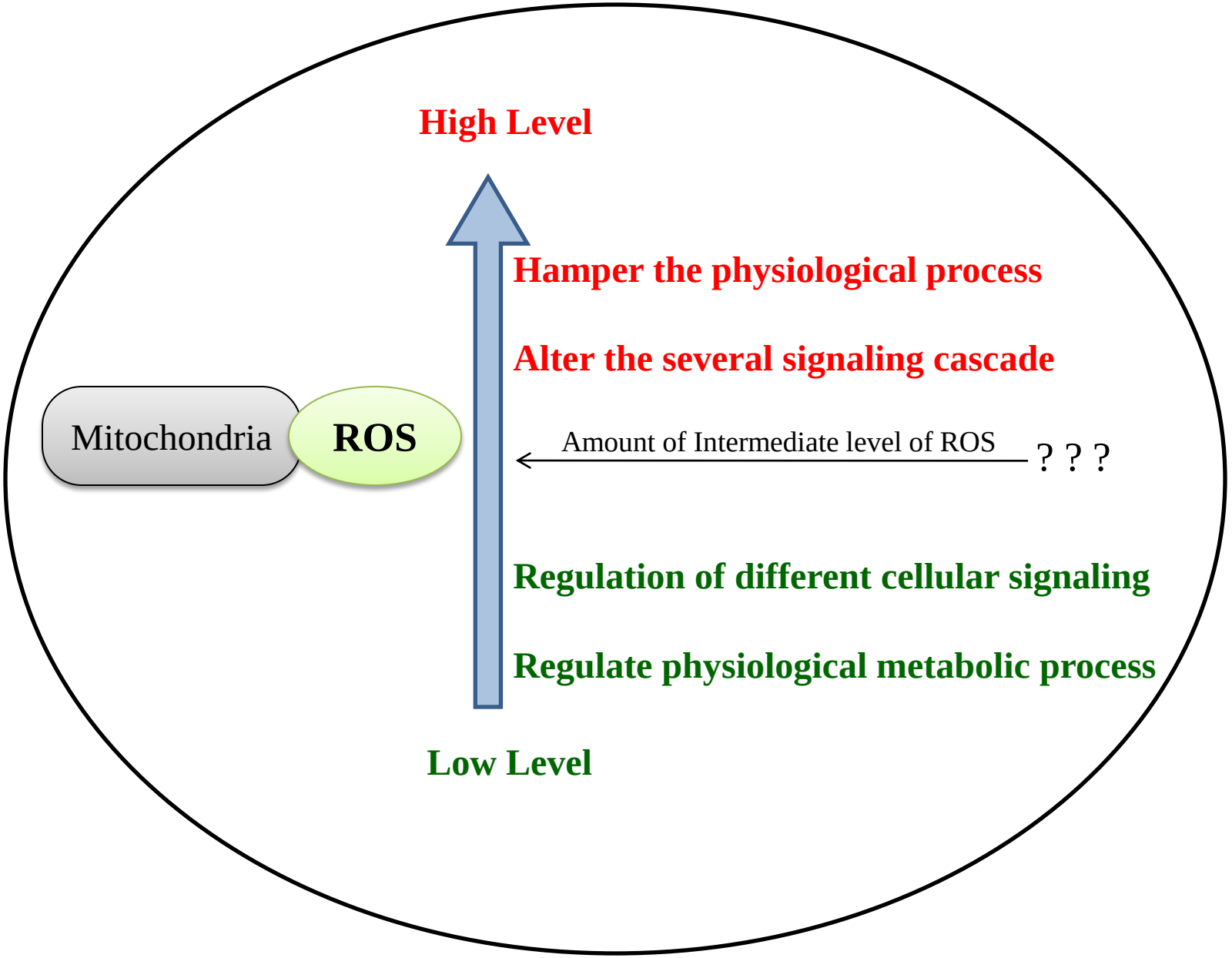

TNF-α pathway
NF-κB pathway
mTOR signaling
JNK pathway
p38 signaling
DNA Damage pathway
PKB/Akt signaling
Neurodegenerative diseases
Myodegenerative diseases
Atherosclerosis
Vascular endothelial diseases
Diabetes mellitus
Cardiovascular diseases
Nephrotoxicity
Crohn's disease
Cancer
Apoptosis
Cell growth
Immunity
Embryogenesis
Capacitation
Insulin signaling
Cardio-vascular function
Renal function
Wound repair
Alter
At High Level
Regulate function
ROS
Leads
At Low Level
Normalize
GPCR signaling
NrF2 signaling
NF-κB pathway
cAMP pathway
p53 signaling
MAPK signaling
JNK pathway
HIF I-α pathway
TLR signaling
PI3-K/Akt signaling

Figure 2.


High Level
Hamper the physiological process
Alter the several signaling cascade
Mitochondria
ROS
Amount of Intermediate level of ROS
? ? ?
Regulation of different cellular signaling
Regulate physiological metabolic process
Low Level