Production of Reactive Oxygen Species and Its Implication in Human Diseases

Shalini Mani

Abstract

Reactive oxygen species (ROS) are chemical molecules with one unpaired electron and mostly derived from molecular oxygen. It is produced in all the mammalian system by various exogenous and endogenous sources. Mitochondria are major sources of ROS production and they are produced as a respiratory by-product. The main sites of superoxide radical production in the respiratory chain are complexes III and I; however, other mitochondrial enzymes are also involved in the production of ROS. Because of the presence of one unpaired electron, ROS is highly reactive, and it may cause oxidative damage to the biomolecules and cell organelles and hence may affect the cellular physiology and their survivability.

A variety of diseases have been associated with excessive ROS production leading to mitochondrial damage, apoptosis, and necrosis. The interrelationship between ROS and mitochondria suggests shared pathogenic mechanisms in mitochondrial and ROS-related diseases. Some common diseases, known to be caused by ROS and mitochondrial damages, are several mitochondrial diseases, neurodegenerative diseases, and aging. In the present chapter, we have summarized the molecular mechanisms of ROS production, its damaging effect on cellular physiology, as well as the existing evidence of mitochondrial ROS involvement in human diseases.

Keywords

Reactive oxygen species • Superoxides • Mitochondria • Electron transport chain • Mitochondrial DNA • Permeability transition pore

S. Mani (✉)
Department of Biotechnology, Jaypee Institute of Information Technology,
A-10, Sector-62, Noida 201307, Uttar Pradesh, India
e-mail: shalini.mani@jiit.ac.in;
mani.shalini@gmail.com

V. Rani and U.C.S. Yadav (eds.), *Free Radicals in Human Health and Disease*,
DOI 10.1007/978-81-322-2035-0_1,

1 Introduction

During the course of evolution, organisms developed the capacity to utilize molecular oxygen as the terminal oxidant in respiration in order to gain energy as an advantage over the anaerobic pathway by a factor of 18. By utilizing the molecular oxygen as a terminal electron acceptor, these aerobic organisms were now enabling metabolism of their organic carbon for energy production. In this respiratory process, oxygen is consumed along with metabolic substrates, while ATP, water, and carbon dioxide are generated. However, the presence of intracellular oxygen also allowed inadvertent redox reactions by oxygen radicals to damage critical biomolecules. Based upon the work done by Priestley, Scheele, and Lavoisier, the dual role of oxygen as sustainer and destroyer of life was realized [1], but the exact mechanism was not known. During 1959, for the first time, Gerschman showed that oxygen toxicity is due to the generation of reactive oxygen species (ROS) or free radicals [1].

2 Production of ROS

ROS is a phase used to describe a variety of molecules and free radicals (chemical species with one unpaired electron) derived from molecular oxygen. The ground state oxygen may be converted to the much more reactive forms either by energy transfer or by electron transfer reactions. The former leads to the formation of singlet oxygen, whereas the latter results in the sequential reduction to superoxide, hydrogen peroxide, hydroxyl radical, etc. ROS is a collective term that includes not only oxygen radicals (superoxide and hydroxyl) but also some non-radical derivatives of molecular oxygen (O_2) such as hydrogen peroxide (H_2O_2). Thus, it is a broader expression and includes hydrogen peroxide and lipid peroxide with no unpaired electron, superoxide ($^{\bullet}O_2^-$), hydroxyl ($^{\bullet}OH^-$), peroxyl ($ROO^{\bullet-}$), alkoxy ($RO^{\bullet}$) radicals, radicals of nitric oxide ($^{\bullet}NO$), nitrogen dioxide ($^{\bullet}NO_2$), peroxynitrite ($^{\bullet}ONOO^-$), ozone (O_3), and possibly singlet oxygen. Though hydrogen peroxide and lipid peroxide are not free radicals, they act as reservoirs for the highly reactive $^{\bullet}OH^-$, $ROO^{\bullet}$, and $RO^{\bullet}$ radicals. The production of these reactive species occurs continuously in the organism, and depending upon the sources, this production may be endogenous or exogenous.

2.1 Exogenous Sources of ROS

Environmental agents including non-genotoxic carcinogens can directly generate or indirectly induce ROS in cells. Exposure of the cell to gamma irradiation results in the production of a whole range of radical and non-radical species from ionization of intracellular water (e.g., aqueous electron, $^{\bullet}OH^-$, H_2O_2). Even exposure to nonionizing irradiation such as UV-C (<290 nm), UV-B (290–320 nm), and UV-A (320–400 nm) can indirectly produce a variety of ROS including O_2, H_2O_2, and $^{\bullet}O_2^-$ radicals [2]. Air pollutants such as car exhaust, cigarette smoke, and industrial contaminants encompassing many types of NO derivatives constitute major sources of ROS that attack and damage the organism either by direct interaction with the skin or following inhalation into the lung. Many of the drugs, such as bleomycin and Adriamycin, whose mechanism of action is mediated via ROS production, are also a major source of ROS. Narcotic drugs and anesthetizing gases are further considered major contributors to the production of ROS [3]. A large variety of xenobiotics (e.g., toxins, pesticides, and herbicides such as paraquat) and chemicals (e.g., mustard gas, alcohol) produce ROS as a by-product of their metabolism in vivo [3]. One of the major exogenous sources of oxidants is food. A large portion of the food consumed is oxidized to a higher degree and contains different kinds of oxidants such as peroxides, aldehydes, oxidized fatty acids, and transition metals. Food debris that reaches the intestinal tract places an enormous oxidative pressure on the intestinal tract mucosa [3].

2.2 Endogenous ROS Production in Cell Organelles

Although the exposure of the organism to ROS is extremely high from exogenous sources, the exposure to endogenous sources is much more important and extensive, because it is a continuous process during the life-span of every cell in the organism. Among the very varied endogenous sources, mitochondria, endoplasmic reticulum (ER), and peroxisomes are important cellular organelles which are involved in the ROS production.

2.2.1 ROS Production in Mitochondria

The reduction of oxygen to water in the mitochondria for ATP production occurs through the donation of four electrons to oxygen to produce water. Mitochondrial electron transport chain (ETC) reduces 95 % of O_2 by tetravalent reduction to H_2O without any free radical intermediates [4, 5]. However, the remaining 5 % of oxygen is reduced via the univalent pathway in which free radicals are produced. Mitochondria from different tissues may vary conspicuously in their capacity to produce ROS using different substrates, and this capacity may be related to membrane composition, animal species, and age.

During the process of ROS production, several major oxygen derivatives are formed, and considerable quantities of superoxide and hydrogen peroxide (H_2O_2) are formed. The mitochondrial electron transport chain is a multicomponent system involved in a series of oxidation-reduction reactions between redox couples and pairs, transfer of electrons from a suitable donor (reductant) to a suitable electron acceptor (oxidant) (Fig. 1) [6]. These oxidation-reduction reactions involve either the transport

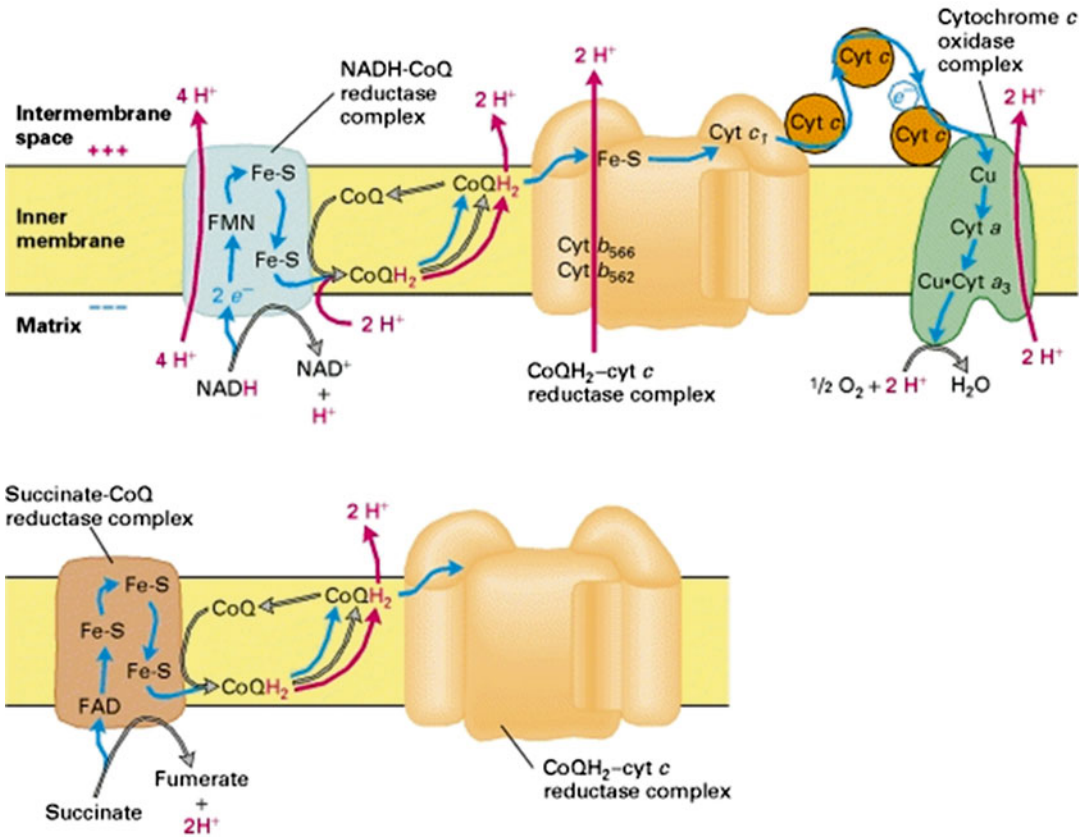

Fig. 1 Stepwise flow of electrons through the electron transport chain from NADH, succinate, and $FADH_2$ to O_2 [6]

of electrons only, as in the case of the cytochromes, or electrons and protons together, as occurs between NADH and FAD. The part of the ETC that actually uses O_2 is the terminal oxidase enzyme, cytochrome oxidase. Cytochrome oxidase releases no detectable oxygen radicals into free solution. However, during the transfer of electrons through earlier components of the transport chain, a few electrons do leak out directly on to O_2, resulting in the generation of $^{\bullet}O_2^-$ [6–8].

The major sites of ROS formation in the respiratory chain lay within respiratory complexes I and III, with a general consensus that production at complex I is about half of that at complex III. The roles of the various complexes that constitute the ETC along with their contribution to the generation of ROS are discussed as follows (Fig. 2) [9]:

2.2.1.1 Complex I

Complex I also known as the NADH-coenzyme Q reductase or NADH dehydrogenase. It is huge, 850,000 kD, and is composed of more than 30 subunits. It contains an FMN prosthetic group and seven or more Fe-S clusters. This complex binds NADH and transfers two electrons in the form of a hydride to FMN to produce NAD^+ and FMNH2, which involves the transfer of electrons one at a time to a series of iron-sulfur complexes. It is the major site for ROS production. It has been proposed that the participation of the reduced flavin mononucleotide in the active site for NADH oxidation and this mechanism are supported in mitochondria by correlations between the NAD(P) + potential and O_2 reduction. Two possible sites of oxygen reduction in complex I are either the flavin moiety or the quinone-binding site [10].

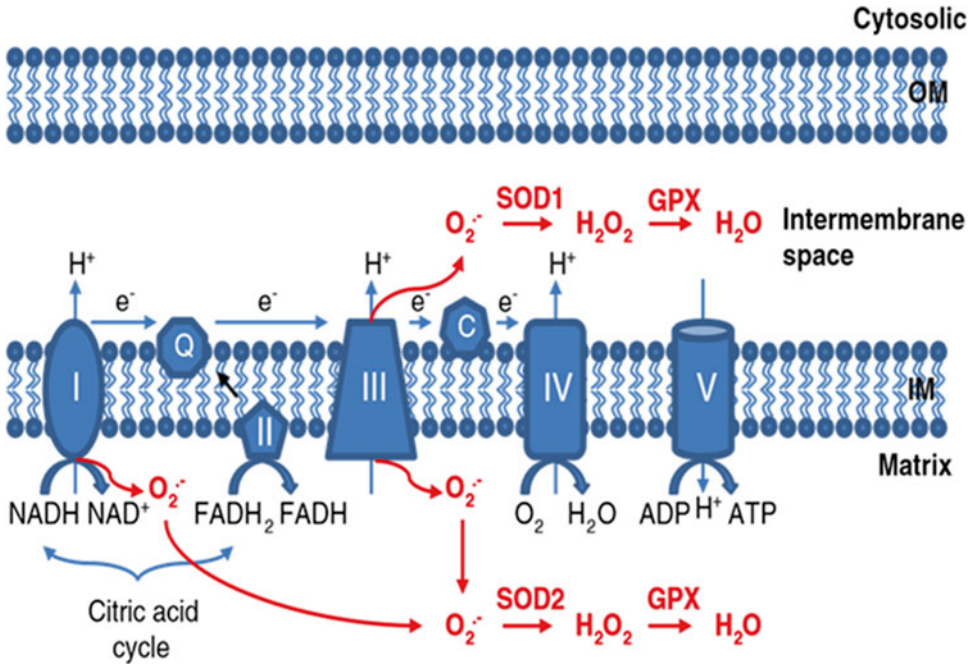

Fig. 2 Production and disposal of mtROS. Electrons (e^-) donated from NADH and $FADH_2$ pass through the electron transport chain and ultimately reduce O_2 to form H_2O at complex IV. MtROS are produced from the leakage of e^- to form superoxide (O_2^-) at complex I and complex III. O_2^- is produced within matrix at complex I, whereas at complex III O_2^- is released toward both the matrix and the intermembrane space. Once generated O_2^- is dismutated to H_2O_2 by superoxide dismutase 1 (SOD1) in the intermembrane space and by SOD2 in the matrix. Afterward H_2O_2 is fully reduced to water by glutathione peroxidase (GPX). Both O_2^- and H_2O_2 produced in the process are considered as empty ROS. *OM* outer membrane, *IM* inner membrane (Reproduced with permission from [9])

Fast rates of H_2O_2 generation have been observed in succinate-supported respiration, the mechanism involving reverse electron flow from succinate to NAD^+, providing reducing equivalents to a redox carrier of complex I that serves as a site of ROS formation [11–15]. The process is inhibited by rotenone which blocks the electron flow between the ubiquinone and the iron-sulfur center N-2 [4, 16, 17].

In addition, studies of intact mitochondria or submitochondrial particles have suggested the role of quinone-binding site in ROS production [10]. This site reduces several quinones to unstable semiquinone forms, which further reduced the oxygen to superoxide, whereas water-soluble CoQ homologues used as electron acceptors from isolated complex I stimulate H_2O_2 generation [18]. The evidence that inhibitors binding to the three quinone-binding sites of the complex stimulate superoxide production proposes that the oxygen reduction site lies upstream of the quinone-binding sites [18, 19].

2.2.1.2 Complex II

It is also known as succinate dehydrogenase. It is composed of four subunits. Two of which are iron-sulfur proteins, and the other two subunits together bind FAD through a covalent link to a histidine residue. These two subunits are called flavoprotein 2 or FP2. Complex II contains three Fe-S centers. In the first step of this complex, succinate is bound and a hydride is transferred to FAD to generate FADH2 and fumarate. FADH2 then transfers its electrons one at a time to the Fe-S centers. FAD functions as a two-electron acceptor and a one-electron donor. The final step of this complex is the transfer of two electrons one at a time to coenzyme Q to produce CoQH2.

ROS is generated in the reverse reaction, with electrons being supplied from the reduced ubiquinone pool. The contribution of complex II in generation of ROS is relatively lower than complex I [20].

Complex II as a source of ROS was demonstrated when electrons are channeled through complex II rather than complex I, while in both cases, reaching complex III yielded higher ROS levels [21]. Carboxin inhibits ROS production stimulated by antimycin in complex II and cause destabilization of stable semiquinone SQs of complex II [18]. A related inhibition of ROS generation was observed by carboxin in COS-7 cells respiring on glucose [22] showing that complex II may be a primary source of ROS in intact cells [18]. Auto-oxidation of flavin is the source of superoxide generation stimulated by one-electron acceptor cytochrome c, and ferricyanide [23] was shown to exhibit the direct role of complex II in ROS generation in purified succinate dehydrogenase (SDH) [18].

2.2.1.3 Complex III

This complex is also known as coenzyme Q-cytochrome c reductase because it passes the electrons from CoQH2 to cyt c through a very unique electron transport pathway called the Q cycle. In complex III, there are two b-type cytochromes and one c-type cytochrome, and along with complex I, it is also a major site of ROS generation [24].

The first evidence that complex III generates superoxide was shown by antimycin insensitive reduction of cytochrome c mediated by superoxide radicals [25]. The source of superoxide in the enzyme has been either assumed to be cytochrome b566 or ubisemiquinone (SQ) or Rieske iron-sulfur center [26]. The coenzyme Q is fully reduced in the inner side of the mitochondrial membrane (ubiquinol, QH2) and then migrates to the outer side of the inner membrane carrying 2 H^+ that become part of the pool needed to sustain ADP phosphorylation. Once on the outer side of the membrane, one electron is transferred to cytochrome c1 (via the Rieske Fe-S protein), resulting in the formation of $Q^{\bullet}$. The second electron is needed to reduce cytochrome b, but eventually some electrons leak to oxygen, producing $O_2^{-\bullet}$ [27]. SQ at the center o is assumed to be a major candidate in univalent oxygen reduction: CoQ may be transformed by a safe electron carrier to a superoxide generator when protons are allowed to penetrate the inner membrane of mitochondria [11].

2.2.2 ROS Production in Endoplasmic Reticulum

Similar to mitochondria, ER is another membrane-bound intracellular organelle, but unlike mitochondria, it is primarily involved in lipid and protein biosynthesis. ER when under stress produces ROS mainly by two mechanisms during disulfide bond formation [28]. First, ROS are produced as a by-product during transfer of electrons from protein thiol to molecular oxygen by endoplasmic reticulum oxidoreductin-1 (ERO-1) and protein disulfide-isomerase (PDI) [28]. Alternatively, ROS can be created during misfolding of protein due to depletion of GSH [29, 30], since after GSH is consumed, thiols are repaired enabling them to interact with ERO-1/PDI and to be re-oxidized [28]. These steps generate consecutive cycles of disulfide bond formation and breakage, with each cycle producing more ROS as a by-product [31]. The second mechanism presumes ROS are generated by unfolded proteins, independent of the formation of disulfide bonds [28]. Accordingly, accumulation of unfolded proteins in the ER elicits Ca^{2+} leakage into the cytosol, increasing ROS production in the mitochondria [32]. The mechanism of ROS production in ER is explained in Fig. 3 [28].

2.2.3 ROS Production in Peroxisomes

Peroxisomes participate in fatty acid oxidation and contain peroxide-producing enzymes. Peroxisomes are an important source of total cellular H_2O_2 production. Peroxisomes in mammals play an important role in a variety of metabolic pathways such as fatty acid α- and β-oxidation, ether phospholipid biosynthesis, glyoxylate metabolism, amino acid catabolism, polyamine oxidation, and oxidative part of the pentose phosphate pathway [33]. Peroxisomes contain a variety of enzymes that generate H_2O_2 as part of their normal catalytic cycle. These enzymes, which are essentially flavoproteins, include acyl-CoA oxidases, urate oxidase, D-amino acid oxidase, D-aspartate oxidase, L-pipecolic acid oxidase,

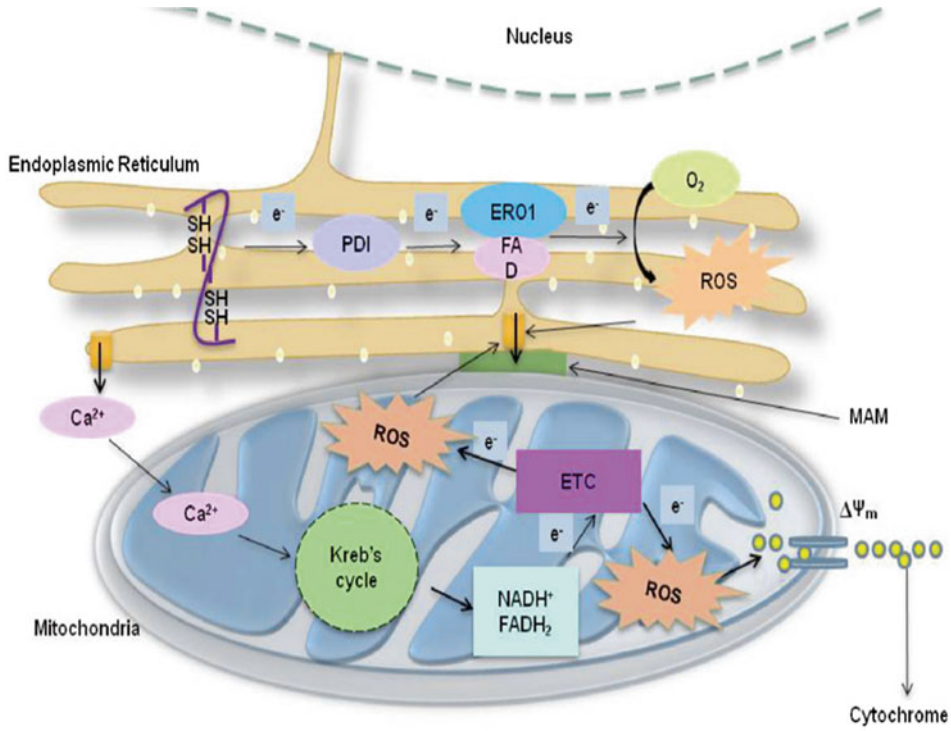

Fig. 3 ER and mitochondrial associated reactive oxygen species (ROS) production under ER stress (Reproduced with permission from [28])

L-α-hydroxy acid oxidase, polyamine oxidase, and xanthine oxidase [34]. As peroxisomes contain a large number of ROS-producing enzymes, hence using all the abovementioned metabolic pathways, different types of ROS such as hydrogen peroxide, superoxide, nitric oxide radicals, hydroxyl radical, and peroxynitrites are produced. Catalase is also a peroxisomal enzyme which metabolizes the hydrogen peroxide formed in these organelles. Peroxisomal catalase utilizes H_2O_2 produced by these oxidases to oxidize a variety of other substrates by "peroxidative" reactions. These types of oxidative reactions are particularly important in liver and kidney cells in which peroxisomes detoxify a variety of toxic molecules (including ethanol) that enter the circulation.

3 ROS-Induced Cellular Damage

These free radicals and other activated oxygen species are continuously formed in our body and important for cellular physiology at its low concentration (will be discussed in the next chapter). However, on top of their physiological function, they may also be damaging to the cellular integrity due to its high reactivity, at its high concentration. Due to the presence of an unpaired electron ($^{\bullet}O_2^-$ and $^{\bullet}OH^-$) or the ability to extract electrons from other molecules (H_2O_2, $HOCl^-$), ROS can readily react with all types of biomolecules and damage the cellular structure. Among the biological targets, most vulnerable to oxidative damage, are proteinaceous enzymes, lipidic membranes, and DNA. By targeting these biomolecules, ROS may directly harm its main site of production (mitochondria) and influence the cellular viability, via mitochondria dependent or independent pathways.

3.1 Effect on Mitochondria

The mitochondrion is the major site for ROS generation. Thus, the first site of damage by the ROS is the various biomolecules (like proteins, lipid, and nucleic acids) present within the mitochondrion itself. It has been demonstrated that after exposing cells to oxidative stress, mitochondrial DNA (mtDNA) damage is more extensive and persists longer than damage in nuclear DNA (nDNA). Several reasons may contribute to this selective vulnerability: (i) mtDNA lacks histones which are protective against free radical damage; (ii) mtDNA lacks an adequate repair system, rendering it unable to cope with the extensive damage, especially strand breaks; (iii) mtDNA has very few non-coding sequences, therefore increasing the likelihood of a DNA alteration to affect a gene; and (iv) mtDNA is located near the inner mitochondrial membrane, a major site of oxygen radical production [35]. The highly reactive ROS induces damage to mtDNA which includes single- and double-strand breaks, abasic sites, and purine and pyrimidine base damage. The mtDNA codes primarily for the proteins that form the complexes of the electron transport chain. Accumulation of mutations in the mtDNA as a result of oxidative damage results in the production of proteins that are less efficient. This dysfunction of the OXPHOS system may eventually lead to a hindrance in the ATP generation capacity and decreased ATP synthesis of the cell as well as increased leakage of ROS [36].

Excessive ROS production in the mitochondria also results in the damage to the lipids. ROS formation triggers lipid peroxidation which adversely affects the OXPHOS system as well as the mitochondrial membrane potential. The products of lipid peroxidation will interact with the lipids present in the mitochondrial bilayer, impairing its function and resulting in the opening of the mitochondrial permeability transition pore (MPTP). The opening of the MPTP leads to dysregulation of calcium homeostasis and may affect the entire cellular metabolism [37]. The susceptibility of mitochondrial function to oxidative damage may further lead to the induction of various reactions affecting the cellular viability in the affected cell, as explained in the next sections.

3.2 Effect on Cellular Viability

When a cell is exposed to oxidative stress, it may result in the activation of various signaling pathways that lead to the elimination of the damaged cell. These mechanisms include apoptosis, necrosis, and autophagy among others [38]. The entire mechanism is summarized in Fig. 4.

3.2.1 Apoptosis

Apoptosis is a form of programmed cell death characterized by several morphological changes in the dying cell like nuclear condensation and fragmentation, blebbing of the cell membrane, and swelling. It is controlled by extrinsic (receptor-mediated) as well as intrinsic (mitochondria-mediated) signaling pathways. Mitochondrion, which is the site of ROS generation, is an important regulator of cell survival as well as cell death. ROS plays an important role in the activation of the signaling pathways that ultimately lead to apoptosis [39]. The MPTP is a major player in apoptosis as well as necrosis as its induction under various conditions triggers cell death via either of the mechanisms. Increase in the intracellular levels of ROS triggers the opening of the MPTP and a subsequent decrease in the mitochondrial membrane potential. The opening of the MPTP leads to the release of mitochondrial intermembrane proteins like cytochrome c from the mitochondria and into the cytosol [39]. The release of the cytochrome c into the cytosol results in the

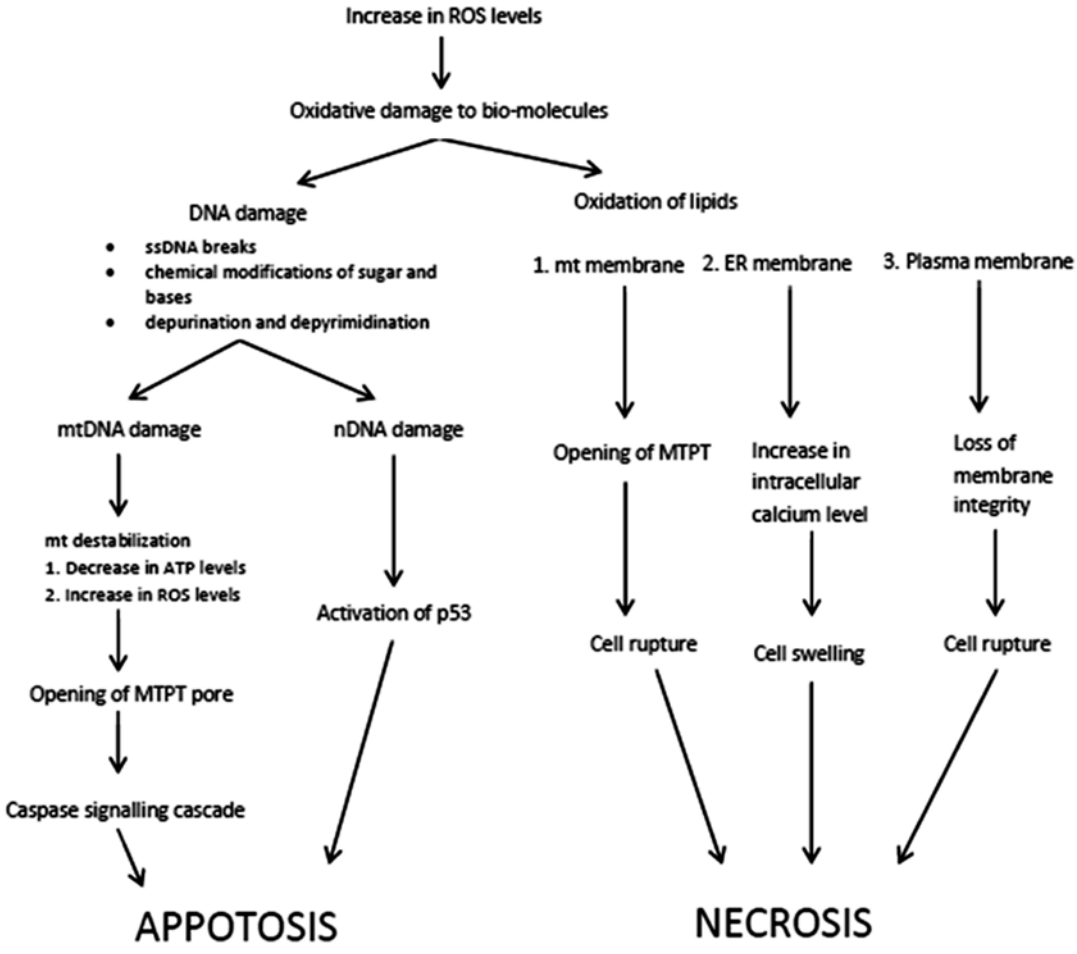

Fig. 4 Detailed mechanism of ROS-mediated apoptosis and necrosis

formation of the apoptosome complex by its interaction with apoptotic protease-activating factor 1 (Apaf-1). In this caspase-dependent signaling pathway, the apoptosome complex recruits procaspase 9 which induces the activation of the downstream effectors, caspase 3 and caspase 7, leading to apoptosis [40]. Oxidative stress also affects the mitochondrial regulation of calcium homeostasis. Rise in calcium levels also results in the opening of the MPTP, thus triggering apoptosis [32]. The intrinsic pathway is mediated by death receptors that stimulate the activation of initiator caspases which lead to apoptosis (41). The majority of the death receptors belong to the tumor necrosis factor-α (TNF-α) family of death receptors (like Fas, TRAIL, TNFR1, etc.). ROS serve as an important signaling molecule in the caspase-signaling pathway initiated by TNF-α. The ligation of TNF-α to its receptor, tumor necrosis factor receptor 1(TNF-α-TNFR1 pathway), is of particular interest as it activates the c-Jun N-terminal kinase (JNK) pathway. The JNK pathway has diverse functions including cellular differentiation, proliferation, and apoptosis [41]. The levels of the ROS in the cell as well as the duration of JNK activation will determine whether the pathway would lead to cell survival or cell apoptosis. The sustained JNK activation by high ROS levels leads to receptor-mediated cell death [40, 41]. Increased in the level of mitochondrial ROS may also cause damage to nuclear DNA, and p53 is capable of sensing the DNA damage and may further direct the cell toward apoptosis [42].

3.2.2 Necrosis

Necrosis, unlike apoptosis, is an unregulated process of cell death and is morphologically characterized by a gain in cell volume, swelling of organelles, and plasma membrane rupture, which results in the loss of intracellular contents [38].

Necrosis can occur in response to varied physiological conditions like ischemia, hypoxia, irradiation, pathogen attack, oxidative stress, etc. ROS is an important mediator of cellular necrosis. Excessive production of ROS can contribute to necrotic cell death by causing degradation of biomolecules and thus cause damage to intracellular organelles [43]. ROS initiates damage to lipids by targeting the double bonds present in the polyunsaturated fatty acids which have increased propensity for oxidative damage. The lipid oxidation of these fatty acids which make up the plasma membrane as well as organelle membranes results in the loss of the integrity of these membranes. Plasma membrane degradation can lead to the loss and failure of ion channels (such as ATP-dependent sodium-potassium pumps, calcium pumps, etc.) which are important for maintaining critical ion balance of the cell. The loss of the membrane potential across the mitochondrial inner membrane leads to the rupture of plasma membrane and causes the opening of the MPTP. It further causes the loss of the proton gradient and a shutdown of the OXPHOS [44]. Damage to the membranes of ER, which is a store of calcium ions, leads to an increase in intracellular calcium concentration as a result of ER leakage which acts as a signal for cell necrosis. Calcium ions may activate various calcium-dependent proteases like calpains and cathepsin thus triggering necrosis because of damage to intracellular proteins [44].

3.2.3 Autophagic Cell Death

Autophagy (self-eating) is a multistep process that is characterized by the vesicular sequestration and degradation of long-lived cytoplasmic proteins and organelles, for example, mitochondria [45]. The resulting double-membrane vesicle is termed an autophagosome. Autophagy is typically observed in cells that are exposed to a variety of metabolic and therapeutic stresses, including growth factor deprivation, inhibition of the receptor tyrosine kinase/Akt/mammalian target of rapamycin (mTOR) signaling, shortage of nutrients, ischemia/reperfusion, inhibition of proteasomal degradation, accumulation of intracellular calcium, and ER stress [46, 47]. ROS may provide a common link between cellular stress signals and the initiation of autophagy, as ROS accumulation has been reported to result in inactivation of the cysteine protease ATG4, which in turn causes accumulation of the ATG8-phosphoethanolamine precursor that is required for the initiation of autophagosome formation [48].

4 Mitochondrial Dysfunction and ROS in Human Diseases

Mitochondrial diseases are the diseases which are caused by inherited or spontaneous mutations in mtDNA or nDNA which lead to altered functions of the proteins or RNA molecules of mitochondria. As mitochondria perform so many different functions in different tissues, there are different mitochondrial diseases due to dysfunction of the mitochondria, which can be broadly categorized into mitochondrial diseases, neurodegenerative disorders, and aging. The detail of ROS and human diseases will be discussed in the later section of this book. However, a brief overview is mentioned here.

4.1 Mitochondrial Diseases

Several clinical syndromes are associated with mtDNA mutations, the most common being NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa), MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), MERRF (myoclonic epilepsy and ragged-red fibers), LHON (Leber hereditary optic neuropathy), KSS (Kearns-Sayre syndrome), ophthalmoplegia, ataxia, retinitis pigmentosa, cardiac conduction defect, and elevated cerebrospinal fluid protein. mtDNA encodes few proteins which are involved in the electron transport chain, the main source of ROS in cells. This special situation highlights the theory of the "vicious cycle," a theory attractive within the realm of degenerative processes [49]. In which, random primary mitochondrial mutations initially induce a defect in the respiratory chain that leads to the leakage of ROS from the ETC. Later, ROS may trigger accumulation of secondary mtDNA mutations which intensify the mitochondrial respiratory defects and increasing production of ROS from mitochondria [49].

Several studies have demonstrated that mtDNA mutations associated with human disease lead to defects like ETC complex dysfunction, increased ROS production, and oxidative damage, as is the situation in MELAS, where hydroxyl radical damage to mtDNA can be accelerated by a specific mitochondrial genotype associated with the disease [50]. Many clinical syndromes including fatal infantile lactic acidosis, adult onset exercise intolerance, focal dystonia, LHON, cardiomyopathy with cataracts, hepatopathy with tubulopathy, Leigh's disease, cataracts and developmental delay, and lactic academia have been associated with isolated complex I deficiencies [51].

4.2 Mitochondrial Dysfunction in Neurodegenerative Diseases

Increased level of ROS in neurodegenerative processes may affect normal mitochondrial parameters like ATP production, mitochondrial membrane potential, MPTP activation, and calcium uptake. Overproduction of free radicals leads to neurodegeneration. Neural cells suffer functional or sensory loss in neurodegenerative diseases. Apart from several other environmental or genetic factors, oxidative stress leading to free radical attack on neural cells contributes grievous role to neurodegeneration. These changes can lead to neuronal death, mainly through excitotoxic pathways, involving oxidation of macromolecules and apoptosis, thus affecting pathogenesis of common neurodegenerative diseases such as Parkinson disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Friedreich's ataxia (FA) [52]. Subsequently, enzymatic deficiencies in the electron transport chain were identified in additional neurodegenerative diseases: complex IV deficiency in AD and ALS [53–55] and complex II and III in HD and FA. In HD and FA, the genetic defects appear to be in nuclear genes that encode nonrespiratory proteins (huntingtin for HD and frataxin for FA) [52]. This fact suggests that the observed respiratory deficiencies are secondary to these primary pathogenic factors.

4.3 Mitochondrial Dysfunction in Aging

Mitochondrial ROS involvement in aging is strongly suggested by experimental data [56]. As mt DNA is naked and hence it is constantly exposed to ROS generated by mitochondrial electron transport chain. As a result, large number of mutations in mtDNA may exponentially get accumulated with age. The simultaneous increase in lipid peroxidation and oxidation of mitochondrial proteins adds to the oxidative stress effects, initiating the vicious cycle of molecular degeneration. This putative vicious cycle can operate at different rates in various tissues, leading to differential accumulation of oxidative damage, which could explain the differences in functional impairment and deterioration of different tissues in the aging process. There is a substantial evidence that damage to mtDNA accumulates with age. Finally, several mtDNA point mutations also increase with normal aging [57]. The point mutation of mitochondrial DNA characteristic for MERRF disease is found also in healthy people of different ages [58]. But still it is not clear whether such mutations are generated by ROS-mediated damage. Apart from these common clinical features, ROS is also known to participate in many pathological conditions including cardiovascular diseases, malignancies, autoimmune diseases, and neurological degenerative diseases.

5 Conclusion

ROS is produced in all the cells as default process, and mitochondria plays a central role in ROS generation. The excess amounts of ROS are known to trigger the oxidative damage to various biomolecules of the cell like proteins, lipids, and nucleic acids that result in the degradation and damage of cellular organelles. The excessive accumulation of ROS may trigger the process of cell death via apoptosis, necrosis, and autophagy. The side effect of ROS on cellular physiology, morphology, and viability may further get affected in the form of several pathological conditions.

More than 200 disorders have been described in literature in which ROS were important for the initiation state of a disease or produced during its course.

Our understanding of the intricate relationship between mitochondrial function, ROS production, ROS damage, and the development of a clinical phenotype is still very limited. Mitochondria are cellular organelles that perform vital functions essential for ATP production, homeostasis, and metabolism. They are integral to different cell death and survival pathways. These roles identify mitochondria as a potential target for drugs to treat metabolic and hyperproliferative diseases.

Therapies that target the generation of ROS in the mitochondria can help in the treatment of various diseases associated with oxidative damage. Hence, there is a need to do extensive research in this area, in such a way that we can decrease the ROS level in the mitochondria and thus improving or managing the health of the diseased person.

Acknowledgment Author wishes to acknowledge Nancy Taneja, Arshpreet Kalsi, and Alok Tandon for their contribution in editing the manuscript.

References

1. Gerschman R (1959) Oxygen effects in biological systems. Sym Spec Lect XXI Int Congr Physiol Soc 21:222–226
2. Zhang X, Rosenstein BS, Wang Y et al (1997) Identification of possible reactive oxygen species involved in ultraviolet radiation-induced oxidative DNA damage. Free Radic Biol Med 23:980–985
3. Halliwell B (1991) Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 91:14S–22S
4. Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527–605
5. Shigenaga MK, Hagen TM, Ames BN (1994) Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci U S A 91:10771–10778
6. Lodish H, Berk A, Zipursky SL et al (2000) Molecular cell biology, 4th edn. W. H. Freeman, New York, Section 16.2, Electron transport and oxidative phosphorylation
7. Nohl H, Hegner D (1978) Do mitochondria produce oxygen radicals in vivo? Eur J Biochem 82:563–567

8. Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421–427
9. Li X, Fang P, Mai J et al (2013) Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. J Hematol Oncol 6:1–19
10. Hirst J, King M, Pryde K (2008) The production of reactive oxygen species by complex I. Biochem Soc Trans 36:976–980
11. Cino M, Del Maestro RF (1989) Generation of hydrogen peroxide by brain mitochondria: the effect of reoxygenation following post decapitative ischemia. Arch Biochem Biophys 269:623–638
12. Hansford RG, Hogue BA, Mildaziene V (1997) Dependence of H_2O_2 formation by rat heart mitochondria on substrate availability and donor age. J Bioenerg Biomembr 29:89–95
13. Korshunov SS, Skulachev VP, Starkov AA (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett 416:15–18
14. Korshunov SS, Korkina OV, Ruuge EK et al (1998) Fatty acids as natural uncouplers preventing generation of O2d− and H_2O_2 by mitochondria in the resting state. FEBS Lett 435:215–218
15. Kwong LK, Sohal RS (1998) Substrate and site specificity of hydrogen peroxide generation in mouse mitochondria. Arch Biochem Biophys 350:118–126
16. Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 19:421–427
17. Liu Y, Fiskum G, Schubert D (2002) Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem 80:780–787
18. Lenaz G (2001) The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. IUBMB Life 52:159–164
19. Hinkle P, Butow RA, Racker E et al (1967) Partial resolution of the enzymes catalyzing oxidative phosphorylation. XV. Reverse electron transfer in the flavin-cytochrome beta region of the respiratory chain of beef heart submitochondrial particles. J Biol Chem 242:5169–5173
20. Quinlan C, Orr A, Perevoshchikova I (2012) Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. J Biol Chem 287:27255–27264
21. Cortopassi G, Wang E (1995) Modelling the effects of age-related mtDNA mutation accumulation: complex I deficiency, superoxide and cell death. Biochim Biophys Acta 1271:171–176
22. McLennan HR, Degli Esposti M (2000) The contribution of mitochondrial respiratory complexes to the production of reactive oxygen species. J Bioenerg Biomembr 32:153–162
23. Zhang L, Yu L, Yu CA (1998) Generation of superoxide anion by succinate cytochrome c reductase from bovine heart mitochondria. J Biol Chem 273:33972–33976
24. Chen Q, Vazquez E, Moghaddas S et al (2003) The reverse reaction, with electrons supplied from the reduced ubiquinone pool. J Biol Chem 278:36027–36031
25. Loschen G, Azzi A, Flohe L (1973) Mitochondrial H_2O_2 formation: relationship with energy conservation. FEBS Lett 33:84–87
26. Nohl H, Gille L, Scho¨nheit K et al (1996) Conditions allowing redox-cycling ubisemiquinone in mitochondria to establish a direct redox couple with molecular oxygen. Free Radic Biol Med 20:207–213
27. Turrens JF (2003) Mitochondrial production of reactive oxygen species. J Physiol 2:335–344
28. Bhandary B, Marahatta A, Kim HR et al (2003) An involvement of oxidative stress in endoplasmic reticulum stress and its associated disease. Int J Mol Sci 14:434–456
29. Santos CX, Tanaka LY, Wosniak J et al (2009) Mechanisms and implication of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductase, mitochondrial electron transport, and NADPH oxidase. Antioxid Redox Signal 11:2409–2427
30. Tu BP, Weismann JS (2002) The FAD and O(2) dependent reaction cycle of Erol-mediated oxidative protein folding in the endoplasmic reticulum. Mol Cell 10:983–994
31. Higa A, Chevet E (2012) Redox signalling loops in the unfolded protein response. Cell Signal 24:1548–1555
32. Malhotra JD, Kaufman RJ (2007) Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double edged sword? Antioxid Redox Signal 9:2277–2293
33. Angermüller S, Bruder G, Völkl A et al (1987) Localization of xanthine oxidase in crystalline cores of peroxisomes. A cytochemical and biochemical study. Eur J Cell Biol 45:137–144
34. Fransen M, Nordgren M, Wang B et al (2012) Role of peroxisomes in ROS/RNS- metabolism: implications for human disease. Biochim Biophys Acta 1822:1363–1373
35. Zeviani M, Antozzi C (1997) Mitochondrial disorders. Mol Hum Reprod 3:133–148
36. Gutierrez J, Ballinger S, Darley-Usmar V et al (2006) Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells. Circ Res 99:924–932
37. Ott M, Gogvadze V, Orrenius S et al (2007) Mitochondria, oxidative stress and cell death. Apoptosis 12:913–922
38. Fulda S, Gorman A, Hori O et al (2010) Cellular stress responses: cell survival and cell death. Int J Cell Biol 2010:214074
39. Datta K, Sinha S, Chattopadhyay P (2000) Reactive oxygen species in health and disease. Natl Med J India 13:304–310
40. Circu M, Yee A (2010) Reactive oxygen species, cellular redox systems and apoptosis. Free Radic Biol Med 48:749–762

41. Han-Ming S, Pervaiz S (2006) TNF receptor superfamily-induced cell death: redox dependent execution. FASEB J 20:1589–1598
42. Liu Y, Kulesz-Martin M (2001) p53 protein at the hub of cellular DNA damage response pathways through sequence-specific and non-sequence-specific DNA binding. Carcinogenesis 22:851–860
43. Nele Vanlangenakker N, Berghe T, Krysko D et al (2008) Molecular mechanisms and pathophysiology of necrotic cell death. Curr Mol Med 8:207–220
44. Zong XW, Thompson C (2006) Necrotic death as a cell fate. Genes Dev 20:1–15
45. Eskelinen EL (2008) New insights into the mechanisms of macroautophagy in mammalian cells. Int Rev Cell Mol Biol 266:207–247
46. Lum JJ, Bauer DE, Kong M et al (2005) Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 120:237–248
47. Ogata M, Hino SI, Saito A et al (2006) Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 26:9220–9231
48. Scherz-Shouval R, Shvets E, Fass E et al (2007) Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J 26:1749–1760
49. Kirkinezosa IG, Moraesa CT (2001) Reactive oxygen species and mitochondrial diseases. Cell Dev Biol 12:449–457
50. Yakes FM, Van Houten B (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci U S A 94:514–519
51. Tanaka M, Kovalenko SA, Gong JS et al (1996) Accumulation of deletions and point mutations in mitochondrial genome in degenerative diseases. Ann N Y Acad Sci 786:102–111
52. Parker WD, Boyson SJ, Parks JK (1989) Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann Neurol 26:719–723
53. Mutisya EM, Bowling AC, Beal MF (1994) Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. J Neurochem 63:2179–2184
54. Borthwick GM, Johnson MA, Ince PG et al (1999) Mitochondrial enzyme activity in amyotrophic lateral sclerosis: implications for the role of mitochondria in neuronal cell death. Ann Neurol 46:787–790
55. Xu GP, Dave KR, Moraes CT et al (2001) Dysfunctional mitochondrial respiration in the wobbler mouse brain. Neurosci Lett 300:141–144
56. Brookes PS, Land JM, Clark JB et al (1998) Peroxynitrite and brain mitochondria: evidence for increased proton leak. J Neurochem 70:2195–2202
57. Munscher C, Rieger T, Muller-Hocker J et al (1993) The point mutation of mitochondrial DNA characteristic for MERRF disease is found also in healthy people of different ages. FEBS Lett 317:27–30
58. Zhang C, Linnane AW, Nagley P (1993) Occurrence of a particular base substitution (3243 A to G) in mitochondrial DNA of tissues of ageing humans. Biochem Biophys Res Commun 195:1104–1110