

Review Article

Mitochondria-Ros Crosstalk in the Control of Cell Death and Aging

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Reactive oxygen species (ROS) are highly reactive molecules, mainly generated inside mitochondria that can oxidize DNA, proteins, and lipids. At physiological levels, ROS function as “redox messengers” in intracellular signalling and regulation, whereas excess ROS induce cell death by promoting the intrinsic apoptotic pathway. Recent work has pointed to a further role of ROS in activation of autophagy and their importance in the regulation of aging. This review will focus on mitochondria as producers and targets of ROS and will summarize different proteins that modulate the redox state of the cell. Moreover, the involvement of ROS and mitochondria in different molecular pathways controlling lifespan will be reported, pointing out the role of ROS as a “balance of power,” directing the cell towards life or death.

1. Introduction

From the first observation of the real mitochondrial structure in 1950 [1], it was clear that this particular composition reflected the different functions of this organelle. Mitochondria are delimited by double-membrane architecture, an outer membrane of eukaryotic origin and an inner membrane characterized by the absence of cholesterol and the presence of cardiolipin, typical elements of bacterial membranes. The inner membrane is organized in characteristic folds, termed cristae, which protrude into the matrix and accommodate the respiratory chain complexes. In healthy cells, the inner membrane is impermeable to ions [2], which allows the electrons transport chain (ETC) to actively build up the proton gradient. The mitochondrial membrane potential ($\Delta\Psi_m$) results from the difference in electrical potential generated by the electrochemical gradient across the inner membrane. Through oxidative phosphorylation, mitochondria play their essential role to supply the cell with metabolic energy in the form of ATP. As a

consequence, they are also the primary source of cellular reactive oxygen species (ROS), especially at level of the respiratory chain complexes I and III, where electrons derived from NADH and ubiquinone can directly react with oxygen or other electron acceptors and generate free radicals [3, 4]. Therefore, ROS are a normal side product of the respiration process, and they react with lipids, protein, and DNA, generating oxidative damage. Indeed, mitochondria are the major site of ROS production, but also the major targets of their detrimental effects, representing the trigger for several mitochondrial dysfunctions. In this review, we will focus on this deadly liaison, with particular attention on ROS production, mitochondrial ROS targets, and their role in apoptosis, autophagy, and aging.

2. Mitochondrial ROS Production

Mitochondria are responsible for 90% of the energy that cells, and thus tissues, organs, and the body as a whole need

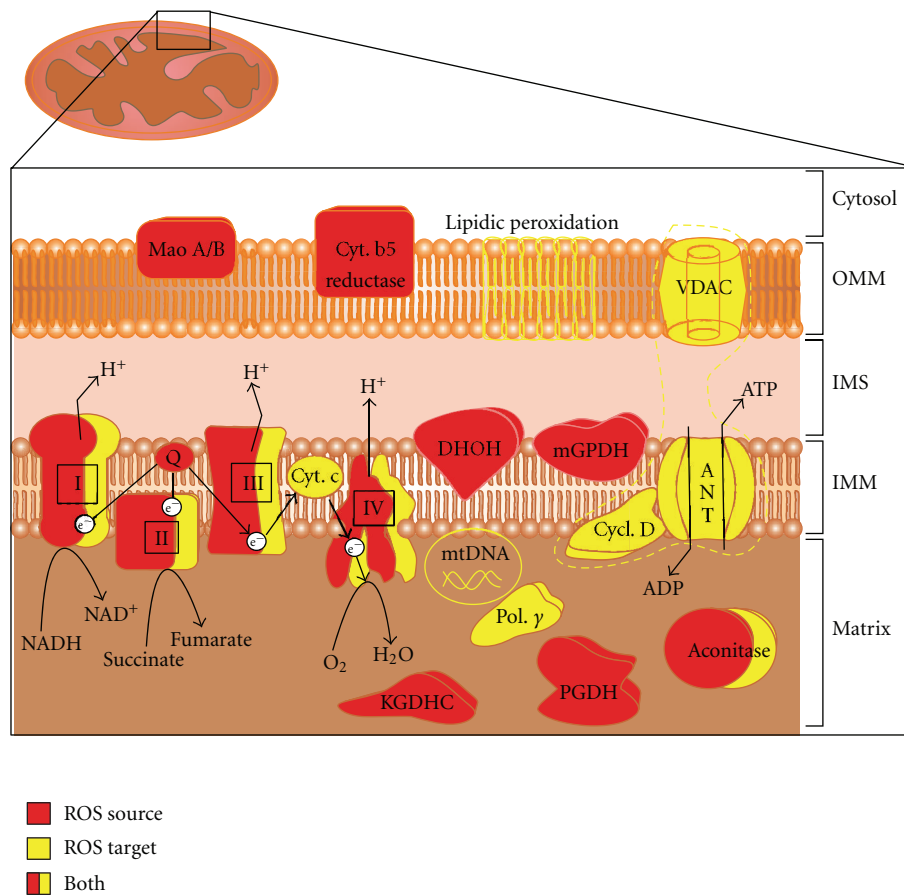


FIGURE 1: Mitochondrial sources of ROS and mitochondrial ROS targets. ROS generators (red) and ROS targets (yellow) are shown in their precise localizations inside mitochondria. Dotted yellow trace encloses the permeability transition pore components. See text, in particular sections 2 and 3, for further details. Abbreviations: OMM: outer mitochondrial membrane; IMS: intermembrane space; IMM: inner mitochondrial membrane; MAO A/B: monoamine oxidases A and B; Cyt. b5 reduct.: cytochrome b5 reductase; DHOH: dihydroorotate dehydrogenase; mGPDH: glycerol-3-phosphate dehydrogenase; I, II, III, and IV: Complex I to IV of the respiratory chain; Q: coenzyme Q; Cyt. c: cytochrome c; KGDHC: α -ketoglutarate dehydrogenase complex; PGDH: pyruvate dehydrogenase complex; e^- : electrons; VDAC, voltage-dependent anion channel, Cyc. D: cyclophilin D; ANT: adenine nucleotide translocase; Pol. γ : polymerase γ ; mtDNA: mitochondrial DNA.

to function. Hence, they are known as the “cells powerhouse,” the core of cellular energy metabolism, being the site of most ATP generation through mitochondrial oxidative phosphorylation (OXPHOS) [5].

In this process, electrons liberated from reducing substrates are delivered to O_2 establishing an electrochemical gradient used to drive ATP synthesis. During the OXPHOS, the reduction of oxygen by one electron at a time ($O_2 \rightarrow O_2^{\cdot-} \rightarrow H_2O_2 \rightarrow \cdot OH \rightarrow H_2O$) produces ROS, relatively stable intermediates with one unpaired electron [6–8]. Although Brown and Borutaite in their recent review have presented a number of examples supporting the point of view that mitochondria are not a major source of ROS in the cell [9], the fact that oxidative phosphorylation accounts for 90% to 95% of cellular oxygen consumption and that 3% from that pool can be converted to superoxide is a very strong argument in favour of mitochondria as a main source of this oxygen radical. Hence, it is a firm paradigm

that mitochondria are also the major source of ROS in mammalian cells (Figure 1).

The primary ROS generated by mitochondria, as a result of mono-electronic reduction of O_2 , is superoxide anion ($O_2^{\cdot-}$) that is the precursor of most ROS and a mediator in oxidative chain reactions. In vivo, $O_2^{\cdot-}$ is produced both enzymatically by NADPH oxidase, cytochrome P450-dependent oxygenases, and xanthine oxidase and non-enzymatically, when a single electron is directly transferred to O_2 [8]. In addition, $O_2^{\cdot-}$ may react with other radicals including nitric oxide ($NO\cdot$) producing reactive nitrogen species (RNS) [10]. Dismutation of $O_2^{\cdot-}$, either spontaneously [11], or through a reaction catalyzed by superoxide dismutases (SODs) [12], produces hydrogen peroxide (H_2O_2) [13]. H_2O_2 generated in mitochondria has many possible fates. Because H_2O_2 is relatively stable and membrane permeable, it can diffuse within the cell and be eliminated by cytosolic or mitochondrial antioxidant systems

such as catalase, glutathione peroxidase, and thioredoxin peroxidase [14]. Mitochondrially generated H_2O_2 can also act as a signaling molecule in the cytosol, affecting multiple networks that control, for example, cell cycle, stress response, energy metabolism, and redox balance [15–17].

When not metabolized, H_2O_2 can be further transformed to hydroxyl radical ($\bullet OH$), in the presence of metal ions, by Fenton reaction [18]. $\bullet OH$ is one of the strongest oxidants in nature, is highly reactive, and generally acts essentially as a damaging molecule. For this reason, mitochondria are believed to have developed efficient H_2O_2 removal systems, as well as metal-chelating mechanisms, such as chaperone proteins, preventing the formation of this radical.

At least, ten mammalian mitochondrial enzymes contribute to ROS production [17, 19] even if their capacity of ROS producing greatly differ in a tissues-specific manner [20]. *In vitro* experiments demonstrate that mitochondria isolated from mouse heart, brain, and kidney have selective substrate and inhibitor preferences for H_2O_2 generation and that the apparent sites of H_2O_2 generation are both substrate and organ specific [21].

The major bulk of mitochondrial ROS generation occurs at the electron transport chain, as a byproduct of respiration [7, 8, 22]. Cytochrome c, oxidase (Complex IV) is the terminal component of the ETC, receives four electrons from cytochrome c and reduces one O_2 molecule to two H_2O . It retains all partially reduced intermediates until full reduction is achieved and does not seem to release these intermediates in measurable quantities [6].

Historically, the first mitochondrial site producing ROS was identified at the Complex III located at the inner side of inner mitochondrial membrane (bc1 complex, ubiquinone: cytochrome c reductase). The primary ROS produced at this site is $O_2^{\bullet -}$, through the referred Q-cycle [23]. Despite the recent advances in understanding the structure of the bc1 complex, a mechanism of $O_2^{\bullet -}$ production is not yet known [24–26].

Succinate dehydrogenase (Complex II, SDH) is a flavo-protein located at the inner surface of inner mitochondrial membrane. Although oxidation of succinate can theoretically produce ROS with a high rate, significant $O_2^{\bullet -}$ formation from this enzyme has not been measured so it is still unclear whether SDH produces ROS *in situ*, in mitochondria. Despite the lack of ROS formation by Complex II itself, succinate is an important source of ROS in many tissues through a mechanism involving reverse electron transfer. This particular phenomenon may result from high mitochondrial membrane potential, which thermodynamically favours electron donor from Complex II to I. Because of this, succinate may promote ROS generation at Complex I level [6, 7].

Mitochondrial Complex I, “NADH dehydrogenase,” in the inner face of inner membrane provides a major entry point for electrons into respiratory chain. It is a significant source of ROS [27] in particular $O_2^{\bullet -}$ [28] and H_2O_2 although whether or not Complex I is the major site of ROS production in intact mitochondria *in vivo* is a complicated issue. Indeed all the evidence on ROS production by Complex I was obtained *in vitro* with isolated mitochondria [29].

There are other mitochondrial nonrespiratory chain enzymes that produce ROS although pinpointing their specific contribution to total mitochondrial ROS production remains unclear.

Mitochondrial cytochrome b5 reductase is located in the outer mitochondrial membrane [30]. The enzyme is widely distributed in mammalian tissues and may be involved in $O_2^{\bullet -}$ generation with a very high rate ~ 300 nmol per min per mg protein [31].

Monoamine oxidases (MAO-A and MAO-B) are also located in the outer mitochondrial membrane and ubiquitously expressed in various mammalian tissues. These enzymes catalyze the oxidation of biogenic amines accompanied by the release of H_2O_2 [32].

Dihydroorotate dehydrogenase (DHOH) is located at the outer surface of inner mitochondrial membrane. It catalyzes the conversion of dihydroorotate to the pyrimidine base, orotate, which is a step in the de novo synthesis of uridine monophosphate. The DHOH is ubiquitously distributed in mammalian tissues, and it is considered a mitochondrial source of $O_2^{\bullet -}$ and H_2O_2 although the capacity of DHOH to produce $O_2^{\bullet -}$ requires further clarification [33].

Mitochondrial dehydrogenase of α -glycerophosphate (Glycerol-3-Phosphate Dehydrogenase, mGPDH) is located at the outer surface of inner mitochondrial membrane. The mGPDH is involved in lipid metabolism and in the so-called glycerol phosphate shuttle capable of regenerating cytosolic NAD^+ from the $NADH$ formed in glycolysis. It is ubiquitously but unequally expressed in various mouse tissues and mediate the production of H_2O_2 [34, 35].

Mitochondrial aconitase is an enzyme localized to the matrix space of mitochondria; it participates in tricarboxylic acid cycle catalyzing a conversion of citrate to isocitrate. The enzyme contains an iron-sulfur cluster that can be oxidized by $O_2^{\bullet -}$ or H_2O_2 producing $\bullet OH$ [36].

Ketoglutarate dehydrogenase complex (KGDHC, 2-oxoglutarate dehydrogenase) is an integral mitochondrial enzyme tightly bound to the inner mitochondrial membrane on the matrix side [37]. It catalyzes the oxidation of α -ketoglutarate to succinyl-CoA in the tricarboxylic acid cycle. Structurally, KGDHC is composed of three enzymes: α -ketoglutarate dehydrogenase (E1 subunit), dihydrolipoamide succinyltransferase (E2 subunit), and lipoamide dehydrogenase (E3 subunit). The E3 component appears to be the principal ROS source generating $O_2^{\bullet -}$ and H_2O_2 [38, 39].

Pyruvate dehydrogenase complex (PGDH) localized into mitochondrial matrix contains flavoenzyme dihydrolipoamide dehydrogenase subunits like KGDHC, which are very important ROS sources [38].

3. Mitochondrial Targets of ROS

A wide range of mitochondrial ROS-induced damages has been described, including protein carbonylation, lipid peroxidation, or mtDNA damage. These damages, either individually or collectively can lead to a cellular energetic catastrophe. In this section, we are going to treat the most

important mitochondrial targets of ROS action, also summarized, with their mitochondrial localization, in Figure 1.

3.1. Oxidative Damage of mtDNA. The sequence and organization of the human mitochondrial genome has been presented for the first time by Anderson et al. in the landmark paper in 1981 [40]. Mitochondrial DNA (mtDNA) was identified as a 16.5 kb circular molecule encoding 13 polypeptides, 22 tRNAs, and 2 rRNAs. All of the above mentioned are components of mitochondrial respiratory machinery. MtDNA is attached to the IMM on the matrix side, rendering it particularly prone to oxidative damage, as the aforesaid localization is in immediate proximity to the cells' major source of ROS, that is to the mitochondrial respiratory chain. ROS accumulating within the matrix are in such a case explicitly deleterious. Additionally, the lack of introns, histones, and other DNA-protecting proteins as well as DNA repair systems inferior to those observed in nDNA allows increased deterioration [41, 42]. However, mitochondria possess a number of DNA repair enzymes such as MYH (2-OH-A/adenine DNA glycosylase) and OGG1 (8-oxoG DNA glycosylase) [43]. In nucleic acids, both the bases as well as the sugar are prone to ROS attacks. This may lead to modifications of base and abasic sites and result in strand breaks [44]. A good example that may be the addition of the hydroxyl radical to the double bonds may occur. In this case the reaction rate-limiting factor is diffusion of approximately 4.5×10^9 to $9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ [45]. The main outcome of the above-mentioned attacks is the hydroxylation of pyrimidines at C5 and C6 as well as purines at C4, C5, and C8. So far over 80 different oxidized bases in DNA exposed to oxidant factors, such as H_2O_2 and singlet oxygen, have been identified [45, 46]. Experiments from the mid 1980s led to the establishment of a dependable marker of these alterations—8-hydroxy-deoxyguanosine (8-OHDG) [47–51]. An increase in 8-OHDG is a sign of mtDNA fragmentation and is observed in events involving oxidative stress [52].

Alterations in the mitochondrial genome are reflected in a number of disorders, most of which involve impaired respiration and oxidative phosphorylation. As a matter of fact, over 150 pathogenic mutations have been identified in the mtDNA, which cause human diseases [53]. The scope of these disorders has been positively correlated with increasing age and tissues with high-energy demands [54–58]. Individuals may develop a number of symptoms including ataxia, dystonia, epilepsy, dementia, peripheral neuropathy, optic atrophy, and deafness [59]. A number of authors report associations between mtDNA point mutations and neurodegenerative diseases [60–63]. However, some of the mutations are yet to be confirmed and therefore are under dispute. On the other hand, in some cases, such as Parkinson's disease (PD), there seems to be a consistent PD-associated haplogroup [64–66]. Brains of aged individuals as well as those with neurodegenerative diseases exhibit increased levels of mutated mtDNA, particularly in substantia nigra neurons, as compared to hippocampal neurons [67]. It is not clear, whether these mutations are an effect of oxidative stress. Nevertheless, such explanation seems reasonable.

3.2. Oxidative Damage of Mitochondrial Proteins. Oxidative modification of proteins (oxidation of arginine, lysine, threonine, and proline residues) induces the formation of protein carbonyls, commonly detected under oxidative conditions, in aged cells and in aged animal tissues. On the other hand, peroxynitrite oxidizes tryptophan, cysteine, and tyrosine protein residues as well as unsaturated fatty acids and low molecular mass components of the antioxidant defense system like glutathione, α -tocopherol, and ascorbate. Additionally, oxidative damage can affect carbohydrates, which, when oxidized, may be used in glycation process causing the formation of glycation end products. It has been repeatedly shown that the accumulation rate of such oxidatively modified proteins (under oxidative stress or in aging) is tissue specific and depends on protein type [68].

3.2.1. Mitochondrial Respiratory Chain. The mitochondrial respiratory chain, due to its natural imperfections, can be considered a peculiar superoxide generator. In such a case, complexes of the respiratory chain are in the firsthand exposition to oxidative modification because they are present at the sites of ROS production. The high sensitivity of the respiratory chain complexes to oxidizing agents results from the fact that they contain iron-sulfur clusters (Complexes I, II, and III), heme groups (Complexes II, III, and IV) and copper centers in complex IV, all of which can be a site of direct ROS attack, and their oxidative modifications can manifest with a decrease of their enzymatic activity and dysfunction of the whole respiratory chain [69–71]. Interestingly, three mechanisms have been reported in the $\text{NO}\bullet$ inhibition of Complex I: S-nitrosation, tyrosine nitration and damage to FeS centres [72]. The inhibition of mitochondrial Complex I activity is consistently detected in Parkinson's disease patients as well as in mitochondrial toxin models of the disorder [73].

Cytochrome c (a component of OXPHOS) can be modified by nitration of its tyrosine residues, which also affects electron flow through the respiratory chain [74]. Nitration of a single tyrosine residue in cytochrome c by a relatively low dose of peroxynitrite resulted in the upregulation of its peroxidase activity for H_2O_2 and in the impairment of the membrane potential formation [75]. Oxidative damage of individual respiratory chain complexes results not only in a decreased efficiency of ATP production but can also increase ROS production by oxidatively affected OXPHOS subunits, contributing in this way to the intensification of oxidative stress.

3.2.2. Mitochondrial Carriers. The effect of oxidative stress (i.e., caused by site-specific metal-catalyzed oxidation) on mitochondrial carriers was studied intensively in a model exploiting components of the mitochondrial permeability pore: the adenine nucleotide translocase (ANT), porin (VDAC), and creatine kinase. ANT contains many proline, arginine, and lysine residues, which are very sensible for such type oxidation (systems: vanadyl/ H_2O_2 -generating hydroxyl free radicals or Cu^{2+} /tert-butyl hydroperoxide).

Moreover, ANT is localized in the IMM in cardiolipin-rich (containing unsaturated fatty acids) domains. This can explain its sensibility to oxidation also by lipid peroxidation products. In 1998, interesting studies of Sohal's group revealed that ANT in mitochondria of flight muscles is significantly carbonylated in senescent flies. Surprisingly, ANT was the only mitochondrial protein demonstrating age-related increase of carbonylation in this experimental model. In vitro experiments basing on the exposure of isolated mitochondria to the hydroxyl free radicals showed an increased level of carbonylated ANT exhibiting lower nucleotide exchange activity [76].

In addition to controlling the release of superoxide from mitochondria [77], VDAC, which accounts for about 10% of outer mitochondrial membrane (OMM) proteins, can also be a target for intracellular $O_2^- \bullet$. Madesh and Hajnoczky observed that VDAC-dependent permeabilization of the OMM and cytochrome c release can be caused by superoxide but not by H_2O_2 . Moreover, the observed effect of superoxide does not require the contribution of the proapoptotic proteins like Bak or Bax [78]. On the other hand, another component of the mitochondrial permeability transition pore (mPTP), mitochondrial creatine kinase, present in the intermembrane space (IMS) is affected by H_2O_2 and peroxynitrite. The drop in the creatine kinase activity is caused by oxidative modifications of its sulfhydryl groups and aromatic aminoacids [79, 80].

3.2.3. Permeability Transition Pore as a Ros Target. Based on the literature, permeability transition pore (mPTP) seems to be a multiprotein complex with variable composition. The main core of mPTP consists of the voltage-dependent anion channel (VDAC), ANT, and cyclophilin D localized in the outer-, inner mitochondrial membrane, and mitochondrial matrix, respectively [81–83]. Still there is no consensus concerning exact composition of mPTP. Recent studies with the use of transgenic VDAC1 or ANT1 knockout mice seem to contradict the stereotype model of PTP. To find more about the presence of VDAC, ANT, or cyclophilin D in the mPTP, refer to [84–86]. The other components, such as hexokinase, benzodiazepine receptor, and creatine kinase seem to play a regulatory role rather than being structural components of the mPTP. However, regardless of the composition of mPTP, it has been repeatedly described already in 90ties that oxidation of critical thiol residues of ANT induces opening of the mPTP and collapse of mitochondrial membrane potential, mitochondrial swelling, and cytochrome c release. When the thiol groups of ANT are in reduced state, probability of mPTP opening is much lower than when they are oxidised and cross-linkage of ANT thiol groups can occur. Especially oxidation of the Cys¹⁶⁰ and Cys²⁵⁷ of ANT promotes PTP opening [87]. Moreover, Cys⁵⁶ oxidation alters the conformation of ANT inducing it to become a nonspecific pore, thus mimicking the effect of Ca^{2+} which converts ANT into a large channel [88]. Sensitivity of ANT to the oxidizing agents makes mPTP one of the direct sites of ROS action in mitochondria.

3.2.4. Aconitase. In Section 2, we have argued m-aconitase as a source of ROS, but, at the same time, it can be considered a well-established example of oxidative damage targeting a mitochondrial enzyme. In fact, its susceptibility to oxidative damage mainly from superoxide is related to the iron-sulfur cluster present in the active site of the enzyme [89].

3.2.5. Polymerase γ . Nearly 10 years ago, the studies of Copeland's group confirmed that polymerase γ present in the mitochondrial matrix responsible for mtDNA replication can also be considered as an intrinsic target for ROS. The group found that the catalytic subunit of polymerase γ is one of the proteins most abundantly oxidized in the mitochondrial matrix and correlated oxidative modification of polymerase γ with the drop of its enzymatic activity. This in turn can result in the reduction of mtDNA repair and replication [90].

3.3. Peroxidation of Mitochondrial Lipids. In mitochondrial membranes, unsaturated fatty acids, being components of phospholipids, are very susceptible to oxidation by the hydroxyl radical. Lipid peroxidation products, lipid hydroperoxides, generate very reactive unsaturated aldehydes like 4-hydroxy 2-nonenal (4-HNE), malondialdehyde (MDA), and acrolein. Moreover, the generation of lipid radicals can induce a chain reaction leading to the generation of new radicals which intensifies lipid peroxidation. Lipid peroxides cause various effects in the cell. It has been reported that oxidatively modified lipids affect membrane fluidity; this is the case of lipid peroxidation at inner mitochondrial membrane level, leading to increased permeability to protons and uncoupling of oxidative phosphorylation [91]. Moreover, lipid radicals diffuse easily in the membranes and can covalently modify membrane proteins [92], as well as cause "lipoxidative" damage to the mtDNA. For example, 4-HNE inhibits ANT activity in isolated mitochondria due to the modification of critical sulfhydryl groups in ANT [93]. It has also been suggested that the protein carbonylation process is more often related to the lipid-protein oxidation than the direct protein oxidation by reactive oxygen species [94, 95].

4. Mitochondrial ROS Measurements

Direct imaging of ROS in biological samples has proven to be extremely challenging. A large amount of methods for ROS monitoring has been developed in the last years, including fluorimetric, spectrophotometric, and chemiluminescent assays [96].

Among different fluorescent assays, MitoSOX is largely used to visualize superoxide ions inside mitochondria [97]. This reagent is a cell permeant dye that once in mitochondria is oxidized by superoxide and trapped inside organelle, becoming weakly fluorescent. Subsequent binding with nucleic acid permit to the dye to show high fluorescence, with excitation at 510 nm and emission at 580 nm. It can be considered a superoxide-specific probe, because its reactivity for hydrogen peroxide or reactive nitrogen

species is relatively low. However, several caveats exist in using MitoSOX probe [98, 99]; one of the mostly frequent is, without doubts, the photochemical oxidation of the dye, an aspect which has to be taken into consideration for proper interpretation of results.

One of the widespread methods to detect H_2O_2 production by mitochondria is based on 2',7' dichlorofluorescein (DCF- H_2) oxidation. This dye is commercialized as a diacetate chemical probe, in order to be cell permeant, and, once deacetylated from cellular diesterases, it becomes impermeable and weakly fluorescent. DCF- H_2 , when oxidized by H_2O_2 , is converted into 2',7'-dichlorofluorescein (DCF), a high fluorescent component (λ_{ex} 500 nm $-\lambda_{em}$ 520 nm) [100]. DCF formation is directly proportional to H_2O_2 production; therefore, for data analysis is preferable to calculate variation in fluorophore accumulation speed. One of the most relevant limitations of DCF is that it cannot be used to measure H_2O_2 production exclusively inside mitochondria. Mitochondria peroxy yellow 1 (MitoPY1), a new type of fluorophore for imaging mitochondrial H_2O_2 in living cells with ROS and spatial specificity [101], may represent the answer to this problem, but further studies have to be performed to clarify its real efficacy.

Recently, a new highly specific fluorescent probe has been introduced in methods for ROS measurements. HyPer (distributed by Evrogen) is a genetically encoded fluorescent sensor specific for H_2O_2 and consists in a circularly permuted yellow fluorescent protein (cpYFP), inserted into the regulatory domain of OxyR, a hydrogen peroxide-sensitive transcription factor isolated from *E. coli*. HyPer is a ratiometric indicator; the excitation wavelength in normal condition is 420 nm, and, in the presence of H_2O_2 , a red-shift moving excitation to 500 nm occurs. Emission spectrum has a maximum at 516 nm, independently from oxidants activity. The most palatable feature is the possibility to direct HyPer to mitochondrial matrix using COX-targeting sequence, permitting directing fast measurements of mitochondrial ROS production [102]. HyPer is not the first ratiometric probe used for ROS assessment. In 2004, Tsien and coworkers developed ratiometric redox-sensitive versions of GFP, termed redox-sensitive green fluorescent proteins (roGFPs) [103, 104]. When a population of roGFP is oxidized, excitation increases at the 400 nm peak while diminishing at the 480 nm peak; the emission is measured at 510 nm. Because the indicator is genetically encoded, it can be targeted to specific proteins or organelles of interest and expressed in a wide variety of cells and organisms (for an exhaustive discussion of the biophysical properties and development of these probes, see [105]).

Fluorescent dyes show the high advantage to be easy to use, fast, and weakly invasive, leading to a quite accurate calibration using peroxide (or nitric oxide) donors. However, they are fast degradable and easily produce artefacts; in fact, as cited above, high light intensity could induce the activation of the dye, with consequent artefactual ROS generation, resulting in nonspecific signal amplification.

5. Deadly Liaisons: ROS and Mitochondria in the Control of Cell Death

As mentioned in the introduction, mitochondria are often targets of high ROS exposure with deleterious consequences, such as oxidative damage to mitochondrial DNA. This is certainly true, but recent evidence has suggested a deep involvement of ROS also in the extrinsic pathway of apoptosis.

The extrinsic receptor-mediated death pathway requires effective engagement between the death receptors found on the surface of the cell membranes and their respective ligands [106]. The receptor-mediated pathway involves death receptors from the tumor necrosis factor (TNF) superfamily such as TNF, CD95 (Fas), and TNF-related apoptosis-inducing ligand (TRAIL) receptors. Fas and TNFR1 activation is known to generate ROS in response to stimulation, which has been hypothesized to be due to the production of superoxide ($O_2^{\bullet-}$) because of the formation of lipid raft-derived NADPH oxidase platforms. This lipid raft-associated ROS downstream generation may be of high importance in induction of apoptosis or necrosis [107, 108]. Moreover, ROS are required for apoptosis induction by Fas ligand (FasL) in primary lung epithelial cells. ROS mediate the downregulation of FLIP (FLICE inhibitory protein, a strong inhibitor of apoptosis) by ubiquitination and subsequent degradation by proteasome or through nitric oxide (NO) scavenging that prevents FLIP S-nitrosation and cytoprotection [109].

Other evidences underline how ROS generation may influence the intracellular milieu, favouring the effective execution of the downstream events leading to extrinsic apoptosis. ROS has been shown to sensitize cancer cells to TRAIL-induced apoptosis [110], and massive upregulation of CD95 and TRAIL death receptors have also been observed in response to hydrogen peroxide, through a mechanism involving the activation of NF-kappaB [111]. Interestingly, the ability of H_2O_2 to promote apoptosis, through the activation of JNK, seems inhibited in lung fibroblasts from TNFR1-deficient mice [112]. ROS activation of JNK can induce extrinsic or intrinsic apoptotic signaling. $TNF\alpha$ is a potent activator of the MAPK cascade, and $TNF\alpha$ -induced ROS cause oxidation and inhibition of JNK-inactivating phosphatases, by converting their catalytic cysteine to sulfenic acid, with a consequent prolonged JNK activation, which is required for cytochrome c release and caspase 3 cleavage, as well as necrotic cell death [113].

Upstream of JNK is the redox-sensitive MAPK kinase kinase, ASK1. Under non-oxidizing conditions, reduced thioredoxin1 (Trx1) binds ASK1; the resultant Trx1/ASK1 complex, called "ASK1 signalosome," functions as a perfect redox switch. Robust and sustained cellular ROS induce the dissociation of oxidized Trx1 from the complex and let to complete the activation of ASK1, also through the recruitment of TRAF2/6 [114]. Moreover, ASK2, another member of the ASK family, binds and stabilizes ASK1 not only in the cytosol, but also in nucleus and mitochondria. Recently, Saxena et al. have shown how the thioredoxin-interacting protein TXNIP, a ubiquitously expressed redox protein that promotes apoptosis, could shuttle from nucleus to mitochondria under oxidative stress, forming a complex

with mitochondrial Trx2 and removing it from ASK1. This alleviation of Trx2-mediated inhibition results in phosphorylation of ASK1 and consequent induction of the mitochondrial pathway of apoptosis, with cytochrome c release and caspase-3 cleavage [115].

The major ROS target inside mitochondria is undoubtedly the permeability transition pore (see Section 3). Oxidative modifications of mPTP proteins will significantly impact mitochondrial anion fluxes [116]. In response to proapoptotic stimuli, including ROS and Ca^{2+} overload, the mPTP assumes a high-conductance state that allows the deregulated entry of small solutes into the mitochondrial matrix along their electrochemical gradient. This phenomenon, which is known as mitochondrial permeability transition (MPT), results in immediate dissipation of the mitochondrial membrane potential and osmotic swelling of the mitochondrial matrix [2]. Mitochondrial swelling is defined as an “increase in the volume of mitochondria due to an influx of fluid.” The mechanism concerns an early phase of mitochondrial swelling, which involves movement of water from the intercrystal spaces into the matrix; when this water movement continues, the pressure applied to the outer membrane from the increased matrix volume leads to the opening of mPTP and/or rupture of the mitochondrial outer membrane, allowing further matrix expansion [117]. This leads to the release of cytochrome c and the subsequent engagement of the Apaf-1-pro-caspase 9 apoptosome complex, which activates downstream effector caspases. Cytochrome c is present as loosely and tightly bound pools attached to the inner membrane by its association with cardiolipin; this interaction must first be disrupted to generate a soluble pool of this protein. In 2002, the group of Orrenius elegantly showed how oxidative modification of mitochondrial lipids, specifically cardiolipin, is fundamental for tightly bound pool of cytochrome c mobilization, that is so detached because of disturbances in membrane structure [118]. The cardiolipin-bound cytochrome c assumes the role of a membrane-bound peroxidase that can effectively catalyze oxidative stress and cause oxidation of cardiolipin if a source of oxidizing equivalents, such as H_2O_2 , is activated. This can lead to a putative “oxidative wave propagation:” membrane-bound cytochrome c may be seen as a mitochondrial death receptor transducing proapoptotic signals into executing oxidative cascades, with a consequent overload of oxidized cardiolipin species, detachment of cytochrome c from the membrane and formation of mPTP (reviewed in [119]). In this regard, it has been recently shown that overexpression of human telomerase reverse transcriptase (hTERT), the catalytic subunit of the telomerase holoenzyme, alleviates cellular ROS levels also through its mitochondrial localization, improving activity of cytochrome c oxidase, blocking cytochrome c release, mitochondrial membrane permeabilization, and inhibiting apoptosis in cancer cells [120].

The pathophysiological importance of intracellular redox balance and apoptosis regulation was also taken in to consideration during the study of cerebral cavernous malformations (CCMs) [121]. Ablation of Krit1, a protein whose loss of function has been associated to this particular pathogenesis, leads to a significant increase in intracellular

ROS levels, due to the modulation of the expression of the antioxidant protein SOD2 and to a drastic decrease of mitochondrial energy metabolism, with consequent increased susceptibility to oxidative damage [121]. In the same way, MUC1, an oncoprotein aberrantly expressed in acute myeloid leukemia (AML) cells, regulates ROS levels and the differentiation of hematopoietic cells [122]. MUC1 expression is associated with attenuation of endogenous and H_2O_2 -induced intracellular levels of reactive oxygen species [123], and, inhibition of MUC1 results in the disruption of redox balance and thereby AML cell death [122].

Redox regulation of proteins by moderate levels of ROS is observed in various signaling pathways, including autophagy, a catabolic pathway for degradation of intracellular proteins and organelles via the lysosome [124]. Autophagy is activated mainly by nutrient starvation, and it plays a dual role; it is primarily a surviving mechanism, but it also leads to cell death (called type III cell death) thus possibly acting as an alternative to apoptosis. It is generally accepted that ROS induce autophagy [125, 126], and that autophagy, in turn, serves to reduce oxidative damage [124]. Given that, antioxidants may represent natural inhibitors of this process. As a matter of fact, the p53-target gene TIGAR contributes to the regulation of intracellular ROS levels by modulation of the glycolytic pathway, increasing NADPH production (with a consequent decrease in intracellular ROS levels) and lowering the sensitivity of cells to p53-dependent apoptosis induced by oxidative stress [127]. In this manner, TIGAR inhibits autophagy induced by nutrient starvation and metabolic stress, in a p53-independent way [128]. Moreover, a mutant form of SOD1 ($\text{SOD1}^{\text{G93A}}$, associated with one-fifth of familial amyotrophic lateral sclerosis cases [129]) promotes ROS accumulation and autophagy [130, 131], despite its role in muscular atrophy induction has not been fully understood. In transgenic mice spinal cord, $\text{SOD1}^{\text{G93A}}$ interacts with p62, an LC3-binding partner known to target protein aggregates for autophagic degradation, suggesting that p62 may direct mutant SOD1 aggregates to autophagy [132].

Because mitochondria are, at the same time, primary source and target of ROS, they play a fundamental role in ROS-mediated autophagy regulation. Indeed, cells use a specialized form of autophagy, called mitophagy, to selectively eliminate defective mitochondria. The term, coined by Lemasters in 2007 [133], indicates a selective degrading process to maintain a healthy population of mitochondria. Increases in cellular ROS lead to loss of mitochondrial membrane potential ($\Delta\Psi_m$), which is considered a trigger for mitophagy [133]. Under serum deprivation, a typical decrease in mitochondrial membrane potential is observed in hepatocytes prior to engulfment by autophagosomes, whereas Cyclosporin A (CsA), a mPTP inhibitor, prevented this depolarization and the autophagosomal proliferation [134, 135]. However, in different setups represented by rat pituitary GH3 cells [136] and muscles [137], Cyclosporin A works as a strong autophagy-inducer: in particular, CsA treatment in skeletal muscles of collagen VI knockout mice ($\text{Col6a1}^{-/-}$), characterized by impaired autophagy, presence of abnormal mitochondria, and myofiber degeneration, promotes autophagy with a concomitant block of apoptotic

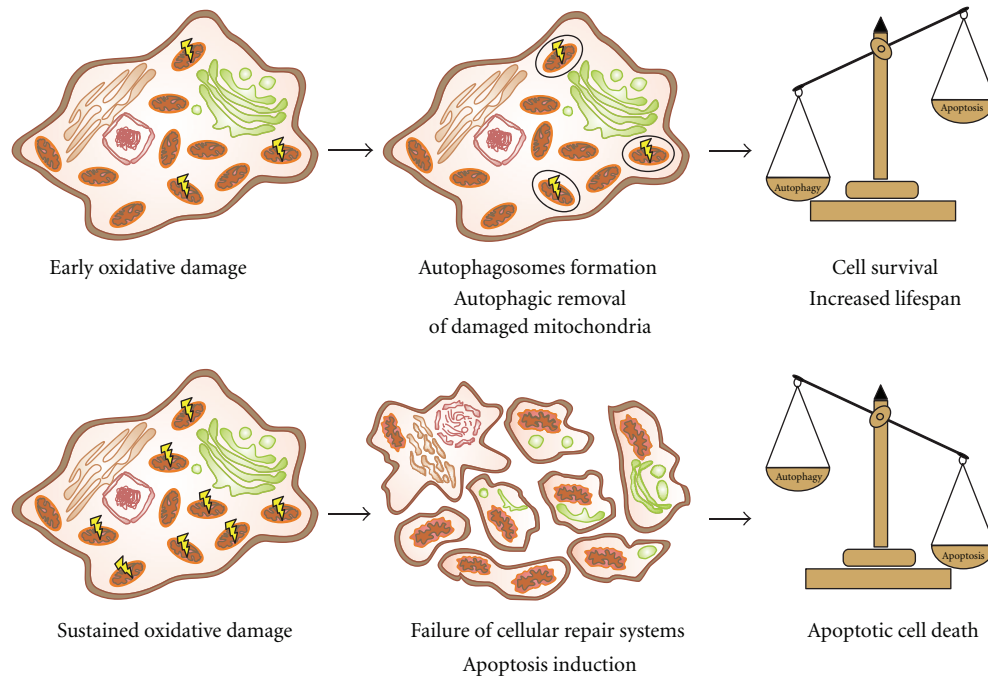


FIGURE 2: ROS levels control cell fate. Low production of ROS works as trigger of autophagic/mitophagic process, with consequent removal of damaged mitochondria and in turn cellular survival (upper panel). On the other hand, high levels of ROS lead to cell death promoting the apoptotic pathway when pro-survival attempt fails (lower panel).

degeneration and recovery of muscle strength [137]. This discrepancy suggests that mitochondrial depolarization is not general, leading event to rage the mitophagic process.

Abnormalities in mitochondrial respiration and increased oxidative stress are observed in cells and tissues from parkinsonian patients [138], which also exhibit increased mitochondrial autophagy [139]. Two genes, which encode the OMM kinase PINK1 and the E3 ubiquitin ligase parkin, are mutated in autosomal recessive Parkinson's disease [140]. Parkin is able to induce mitophagy through translocation from cytosol to mitochondria in many different cellular settings, after stress induction by mitochondrial uncouplers, which mimic damage by decreasing the mitochondrial membrane potential [141], or by the administration of oxidative stress inducers, such as Paraquat [142]. Moreover, Parkin may signal the selective removal of defective mitochondria within the cell. Overexpression of Parkin can eliminate mitochondria with deleterious COXI mutations in heteroplasmic cybrid cells, thereby enriching cells for wildtype mtDNA and restoring cytochrome c oxidase activity [143]. PINK1 (PTEN-induced kinase 1) mediates recruitment of parkin to the damaged mitochondria and activates parkin's ligase activity [144], thereby promoting autophagy. Recently, it has been shown that knockdown of PINK1 expression also leads to mitophagy in SH-SY 5Y cells, due to mitochondrial fragmentation and mitochondrial ROS production [145]. PINK1 knockdown led to decreased transmembrane potential, which was ameliorated by antioxidant MnTBAP treatment, suggesting that ROS production is upstream of mitochondrial depolarization [146].

Based on these observations, ROS act as signaling molecules influencing cell fate. Undoubtedly, redox regulation can promote both survival, during starvation for example, as well as cell death, during oxidative stress. ROS play the role of trigger in the early phase of autophagic process, inducing cytoprotection by eliminating potential sources of proapoptotic stimuli. On the other hand, if the pro-survival attempt fails, ROS cause cell death which involves either the autophagic or the apoptotic pathway, or both (Figure 2).

6. ROS as Key Regulators of Aging

The decline associated with aging is caused by the accumulation of ROS, as supported by cellular and biological data from different model systems and organisms [147]. Indeed defects in antioxidant defense mechanisms fail to protect against oxidative damage, reducing lifespan [148, 149] and causing cardiomyopathy, neurodegeneration [150], and cancer [151].

The relationship between mitochondria dysfunctions observed during aging and ROS production is still debated. However, it is clear that the decline of the integrity of mitochondria as a function of age is implicated in aging and age-related diseases [147].

Given this wide scientific evidence, many studies were aimed to identify the molecular mechanisms responsible for ROS deleterious effects on the aging process. Genes that extend lifespan partially included those involved in oxidative stress response, while partially were clustered in the IGF-1/insulin-like signaling pathway [152, 153].

A key mitochondrial effector is the adapter protein p66shc, which directly mediate, the production of ROS within the mitochondria [154], participating in intracellular pathways that control oxidative stress, apoptosis, and lifespan determination [155, 156]. Accordingly, p66shc knockout mice are one of the best characterized genetic model of longevity, is being more resistant to oxidative stress [157] and protected against age-dependent, ROS-mediated cardiovascular complications induced by diabetes [158–160]. On the contrary, overexpression of p66shc causes alterations of mitochondrial Ca^{2+} responses (which is an early event of mitochondrial damage) and fragmentation of mitochondrial network [161], leading to cytochrome c release and apoptosis [162]. Orsini and coworkers showed, for the first time, that oxidative stress promotes a translocation of part of the cytosolic pool of p66Shc to mitochondria, binding mtHsp70 in mitochondrial matrix [163]. The same group in 2005 claimed that p66Shc is rather present in the intermembrane space, where it interacts with cytochrome c. This interaction, which results in H_2O_2 production [162], can be supported by an earlier report showing that p66Shc has internally located mitochondrial targeting sequence [164]. In order to explain its activity, p66Shc has to be phosphorylated at serine 36 by $\text{PKC}\beta$, and this event leads to a consequent recognition by the prolyl isomerase Pin1, allowing p66Shc entrance into mitochondria [156]. Our recent data have indicated that p66Shc is also present in MAM (mitochondria-associate membranes) fraction, which consists in membranes interacting with mitochondria [165, 166]. Interestingly, the level of p66Shc in MAM fraction changes in an age-dependent manner (MAM fraction isolated from livers of old animals contained more p66Shc than MAM isolated from young individuals) [167]. In response to the oxidative stress, high p66Shc Ser36-phosphorylation status promotes both an additional intracellular ROS generation and reduction of the antioxidant defence system efficiency. The disturbance of the antioxidant enzymes level is connected with the recruitment of Akt to the p66Shc-FOXO3a complex, resulting in a direct inactivation of FOXO transcription factors by their phosphorylation [168, 169]. In this way, genes encoding antioxidant enzymes controlled by FOXO transcription factors are downregulated, with consequent increased ROS production and oxidative stress. Accordingly, decrease of phosphorylated p66Shc is accompanied by positive modulation of the antioxidant defence system [170].

Many alterations that extend lifespan affect not only stress response proteins, such as p66shc, but also nutrient sensors, such as insulin growth factor (IGF-1), target of rapamycin (TOR) protein kinase, AMP kinase (AMPK), sirtuins, and PGC-1 α [171]. Caloric restriction (CR) is the best example of signals that modulate the activity of nutrients sensors, and it is the most robust intervention to extend lifespan and ameliorate various diseases in mammals, reducing oxidative stress and damage [172, 173]. Despite CR was historically considered a nongenetic process regulating lifespan, many recent lines of evidence have suggested its role in several signaling pathways, causing ROS reduction and mitochondrial biogenesis [174].

Nutrient sensor IGF-1 signaling pathway was the first to be associated to CR-mediated regulation of lifespan in animals. Indeed, heterozygous knockout mice for the IGF-1 receptor (Igf1 +/-) live longer, show a delay in the onset of pathologies, and display greater resistance to oxidative stress [175, 176], suggesting that the effects of the IGF-1 pathway on longevity are closely related to mitochondrial protection from oxidative damage. Accordingly in *C. elegans*, mutations of downstream components of this pathway, such as age-1 (the homologue of PI3K) and akt-1 and akt-2 (the homologue of AKT1 and AKT2), result in extension of lifespan [153].

The most important targets regulated by IGF-1-signaling pathway are the transcription factors FoxO [177] and TOR protein kinase [178]. FoxO is required for the antiaging effects of the IGF-1 pathway [179, 180], once downregulated by CR, since it induces the expression of several antioxidative enzymes [181, 182], mediating the production of secondary signals that regulates lifespan.

TOR is highly conserved during evolution and functions as the major amino acid and nutrient sensor in the cell [183]. Much evidence points out the central role of this kinase in lifespan extension by CR. In yeast, *C. elegans*, and *Drosophila*, TOR is required for the effects of dietary restriction, and, importantly, its downregulation extends lifespan of all these models [184] and in mice too [185].

Recently, other signaling proteins have been shown to converge and regulate the FoxO transcription factors and thus oxidative stress and aging. Specifically, the proteins known as sirtuins, NAD-dependent histone deacetylases [186], are activated during CR, when the NAD/NADH ratio is elevated [187]. This group of proteins increases lifespan in yeast, *C. elegans*, and *Drosophila*, and it is thought to act similarly in mammals [188]. In this respect, aging is often associated with reduced sirtuins levels. Sirtuins are mainly antiaging genes via the promotion of mitochondrial function and autophagy and inhibition of apoptosis. They also have an inhibitory activity on ROS. Sirtuin function may be enhanced by restricting caloric intake or increasing physical activity, thereby, extending lifespan. Importantly, three of seven mammalian sirtuins (SIRT3, 4, and 5) are targeted to mitochondria, and SIRT1 is a regulator of mitochondrial biogenesis [189]. SIRT3 is required for the maintenance of mitochondrial integrity and metabolism during stress [190]. The protective effects of CR on oxidative stress and damage are diminished in mice lacking SIRT3. SIRT3 reduces cellular ROS levels dependent on SOD2. SIRT3 deacetylates two critical lysine residues on SOD2 (Lys^{53/89}) and promotes its antioxidative activity. Importantly, the ability of SOD2 to reduce cellular ROS and promote oxidative stress resistance is greatly enhanced by SIRT3 [191]. In a very recent paper, Chen et al. have reported that SOD2 is also acetylated at Lys⁶⁸ and that this acetylation decreases its activity. SIRT3 binds to, deacetylates, and activates SOD2. Increase of ROS levels stimulates SIRT3 transcription, leading to SOD2 deacetylation and activation [192].

Other studies confirmed that SIRT1 is part of the insulin/IGF-1 pathway, being able to deacetylate and, thus, activate FoxO transcription factors [193]. Moreover, SIRT1 can

activate PGC-1 α (peroxisome-proliferator-activated receptor γ coactivator-1 α). Several data linked PGC-1 α and aging. First, PGC-1 α is progressively downregulated during aging, and this event is prevented by CR [194]. Furthermore, oxidative stress is known to induce the expression of this gene [195]. In general, PGC-1 α improves mitochondrial dysfunction, the major phenotype of aging and age-related diseases, in a tissue-dependent fashion [196] and during CR promotes mitochondrial biogenesis [197], thereby lowering ROS production [198]. PGC-1 α is also directly activated by phosphorylation by AMPK that is the master nutrient sensor in the cell [199]. The same kinase is able to phosphorylate FoxO factors too [200].

Recently another protein, named Bmi1, has demonstrated to increase the expression of a collection of gene products involved in mitochondrial function and ROS production in aging. Cells defective in Bmi1 have significant mitochondrial dysfunction with a sustained increase in ROS that is sufficient to cause a marked increase in the intracellular levels of reactive oxygen species and subsequent engagement of the DNA damage response pathway. Also mice Bmi1 $-/-$ present numerous abnormalities including a severe defect in stem cell self-renewal, alterations in thymocyte maturation, and a shortened lifespan [201].

Aging is characterized also by the decline of the autophagic pathway [202]. As described in the previous section, autophagy is required in order to remove compromised mitochondria suffering from oxidative stress. Moreover, autophagy modulation in different model organisms has yielded very promising results suggesting that the maintenance of a proper autophagic activity contributes to extend longevity [203]. During aging, the number of mitochondria suffering from oxidative stress may increase, while their cleanup by the autophagic system may become limiting, leading to the accumulation of damaged mitochondria [204]. It is important to point out that a greater pool of functional mitochondria could ameliorate tissue damage by opposing cells against the gradual energetic decline occurring in cells as mitochondria become damaged during aging [171].

In this respect, the efficient and selective removal of damaged mitochondria by autophagy is a crucial element in the maintenance of cellular health since the accumulation of ROS from dysfunctional mitochondria and eventual cell death via apoptosis is avoided [204]. At the level of the organism, apoptosis will be the ultimate resort to remove seriously damaged cells. This will particularly affect the lifespan of nondividing cells, like neurons, thereby, affecting the lifespan of the whole organism. Indeed, the degenerative processes that arise in old organisms are partly caused by increased cell death of nondividing cells [205]. In any case, the accumulation of damaged mitochondria and their impaired removal is a hallmark of aging and will contribute to decreased cell viability. Interestingly, autophagy is regulated by the same signaling pathways that determine lifespan, acting as one of the downstream effectors of these signaling cascades. Autophagy is essential for the effects of the IGF-1/insulin pathway on longevity. The insulin/IGF-1 pathway regulates autophagy through TOR and FoxO, with opposite effects; TOR was shown to inhibit autophagy [206, 207],

while FoxO induces autophagy [208, 209]. Moreover, SIRT1 seems to be, at least in part, responsible for the direct induction of autophagy [210], as it forms complexes with several autophagy-related genes (ATG) proteins, causing their deacetylation and activation [211]. Autophagy is suggested to be crucial also for the onset of age-related diseases since knockout animals for ATG5 and ATG7 in the nervous system show the accumulation of abnormal proteins and massive neuronal loss leading to neurodegeneration with decreased lifespan [212, 213]. Altogether, these data suggest the presence in the cell of a redundant control of autophagy that allows to finely tune this process under a wide array of physiological and pathological conditions.

7. Concluding Remarks

Mitochondria and ROS signalling tightly control cellular homeostasis by regulating fundamental cell-death and cell-survival processes like apoptosis and autophagy. Therefore, ROS production may be considered a “balance of power,” directing the cell towards life or death. It is clear that many proteins that mediate apoptosis and autophagy directly affect ROS signalling, through translocation to the mitochondrial compartment and/or modulation of pro/antioxidant proteins. Although correlation between mitochondrial ROS and aging has been the subject of debate for over forty years, recent discoveries about the role of autophagy in cleaning up damaged mitochondria, prolonging lifespan, underline the importance of studying ROS dynamics and show that they continue to be extremely fashionable research targets.

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References

- [1] G. E. Palade, “An electron microscope study of the mitochondrial structure,” *Journal of Histochemistry and Cytochemistry*, vol. 1, no. 4, pp. 188–211, 1953.
- [2] S. Fulda, L. Galluzzi, and G. Kroemer, “Targeting mitochondria for cancer therapy,” *Nature Reviews Drug Discovery*, vol. 9, no. 6, pp. 447–464, 2010.

- [3] P. Jezek and L. Hlavata, "Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism," *International Journal of Biochemistry & Cell Biology*, vol. 37, no. 12, pp. 2478–2503, 2005.
- [4] F. L. Muller, Y. Liu, and H. van Remmen, "Complex III releases superoxide to both sides of the inner mitochondrial membrane," *Journal of Biological Chemistry*, vol. 279, no. 47, pp. 49064–49073, 2004.
- [5] G. C. Brown, "Control of respiration and ATP synthesis in mammalian mitochondria and cells," *Biochemical Journal*, vol. 284, no. 1, pp. 1–13, 1992.
- [6] A. J. Kowaltowski, N. C. de Souza-Pinto, R. F. Castilho, and A. E. Vercesi, "Mitochondria and reactive oxygen species," *Free Radical Biology and Medicine*, vol. 47, no. 4, pp. 333–343, 2009.
- [7] Y. Liu, G. Fiskum, and D. Schubert, "Generation of reactive oxygen species by the mitochondrial electron transport chain," *Journal of Neurochemistry*, vol. 80, no. 5, pp. 780–787, 2002.
- [8] J. F. Turrens, "Mitochondrial formation of reactive oxygen species," *Journal of Physiology*, vol. 552, no. 2, pp. 335–344, 2003.
- [9] G. C. Brown and V. Borutaite, "There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells," *Mitochondrion*. In press.
- [10] R. Radi, A. Cassina, and R. Hodara, "Nitric oxide and peroxynitrite interactions with mitochondria," *Biological Chemistry*, vol. 383, no. 3–4, pp. 401–409, 2002.
- [11] G. Loschen, A. Azzi, C. Richter, and L. Flohe, "Superoxide radicals as precursors of mitochondrial hydrogen peroxide," *FEBS Letters*, vol. 42, no. 1, pp. 68–72, 1974.
- [12] R. A. Weisiger and I. Fridovich, "Superoxide dismutase. Organelle specificity," *Journal of Biological Chemistry*, vol. 248, no. 10, pp. 3582–3592, 1973.
- [13] A. Boveris and B. Chance, "The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen," *Biochemical Journal*, vol. 134, no. 3, pp. 707–716, 1973.
- [14] J. Nordberg and E. S. Arnér, "Reactive oxygen species, antioxidants, and the mammalian thioredoxin system," *Free Radical Biology and Medicine*, vol. 31, no. 11, pp. 1287–1312, 2001.
- [15] W. Dröge, "Free radicals in the physiological control of cell function," *Physiological Reviews*, vol. 82, no. 1, pp. 47–95, 2002.
- [16] R. B. Hamanaka and N. S. Chandel, "Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes," *Trends in Biochemical Sciences*, vol. 35, no. 9, pp. 505–513, 2010.
- [17] A. A. Starkov, "The role of mitochondria in reactive oxygen species metabolism and signaling," *Annals of the New York Academy of Sciences*, vol. 1147, pp. 37–52, 2008.
- [18] B. Halliwell and J. M. Gutteridge, "Role of free radicals and catalytic metal ions in human disease: an overview," *Methods in Enzymology*, vol. 186, pp. 1–85, 1990.
- [19] V. Adam-Vizi and C. Chinopoulos, "Bioenergetics and the formation of mitochondrial reactive oxygen species," *Trends in Pharmacological Sciences*, vol. 27, no. 12, pp. 639–645, 2006.
- [20] G. Lenaz, "The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology," *IUBMB Life*, vol. 52, no. 3–5, pp. 159–164, 2002.
- [21] L. K. Kwong and R. S. Sohal, "Substrate and site specificity of hydrogen peroxide generation in mouse Mitochondria," *Archives of Biochemistry and Biophysics*, vol. 350, no. 1, pp. 118–126, 1998.
- [22] M. P. Murphy, "How mitochondria produce reactive oxygen species," *Biochemical Journal*, vol. 417, no. 1, pp. 1–13, 2009.
- [23] S. Dröse and U. Brandt, "The mechanism of mitochondrial superoxide production by the cytochrome bc₁ Complex," *Journal of Biological Chemistry*, vol. 283, no. 31, pp. 21649–21654, 2008.
- [24] A. Boveris, E. Cadenas, and A. O. Stoppani, "Role of ubiquinone in the mitochondrial generation of hydrogen peroxide," *Biochemical Journal*, vol. 156, no. 2, pp. 435–444, 1976.
- [25] E. Cadenas, A. Boveris, C. I. Ragan, and A. O. M. Stoppani, "Production of superoxide radicals and hydrogen peroxide by NADH ubiquinone reductase and ubiquinol cytochrome c reductase from beef heart mitochondria," *Archives of Biochemistry and Biophysics*, vol. 180, no. 2, pp. 248–257, 1977.
- [26] Q. Chen, E. J. Vazquez, S. Moghaddas, C. L. Hoppel, and E. J. Lesnfsky, "Production of reactive oxygen species by mitochondria: central role of complex III," *Journal of Biological Chemistry*, vol. 278, no. 38, pp. 36027–36031, 2003.
- [27] Y. Kushnareva, A. N. Murphy, and A. Andreyev, "Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)⁺ oxidation-reduction state," *Biochemical Journal*, vol. 368, no. 2, pp. 545–553, 2002.
- [28] J. F. Turrens and A. Boveris, "Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria," *Biochemical Journal*, vol. 191, no. 2, pp. 421–427, 1980.
- [29] M. L. Genova, B. Ventura, G. Giuliano et al., "The site of production of superoxide radical in mitochondrial Complex I is not a bound ubisemiquinone but presumably iron-sulfur cluster N₂," *FEBS Letters*, vol. 505, no. 3, pp. 364–368, 2001.
- [30] H. Nishino and A. Ito, "Subcellular distribution of OM cytochrome b-mediated NADH-semidehydroascorbate reductase activity in rat liver," *Journal of Biochemistry*, vol. 100, no. 6, pp. 1523–1531, 1986.
- [31] S. A. Whatley, D. Curti, F. Das Gupta et al., "Superoxide, neuroleptics and the ubiquinone and cytochrome b₅ reductases in brain and lymphocytes from normals and schizophrenic patients," *Molecular Psychiatry*, vol. 3, no. 3, pp. 227–237, 1998.
- [32] O. R. Kunduzova, P. Bianchi, A. Parini, and C. Cambon, "Hydrogen peroxide production by monoamine oxidase during ischemia/reperfusion," *European Journal of Pharmacology*, vol. 448, no. 2–3, pp. 225–230, 2002.
- [33] H. J. Forman and J. Kennedy, "Superoxide production and electron transport in mitochondrial oxidation of dihydrorotic acid," *Journal of Biological Chemistry*, vol. 250, no. 11, pp. 4322–4326, 1975.
- [34] T. Mráček, A. Pecinová, M. Vrbacký, Z. Drahota, and J. Houštěk, "High efficiency of ROS production by glycerophosphate dehydrogenase in mammalian mitochondria," *Archives of Biochemistry and Biophysics*, vol. 481, no. 1, pp. 30–36, 2009.
- [35] P. Jesina et al., "Glycerophosphate-dependent hydrogen peroxide production by rat liver mitochondria," *Physiological Research*, vol. 53, no. 3, pp. 305–310, 2004.
- [36] J. Vasquez-Vivar, B. Kalyanaram, and M. C. Kennedy, "Mitochondrial aconitase is a source of hydroxyl radical. An electron spin resonance investigation," *Journal of Biological Chemistry*, vol. 275, no. 19, pp. 14064–14069, 2000.

- [37] E. Maas and H. Bisswanger, "Localization of the α -oxoacid dehydrogenase multienzyme complexes within the mitochondrion," *FEBS Letters*, vol. 277, no. 1-2, pp. 189–190, 1990.
- [38] A. A. Starkov, G. Fiskum, C. Chinopoulos et al., "Mitochondrial α -ketoglutarate dehydrogenase complex generates reactive oxygen species," *Journal of Neuroscience*, vol. 24, no. 36, pp. 7779–7788, 2004.
- [39] L. Tretter and V. Adam-Vizi, "Generation of reactive oxygen species in the reaction catalyzed by α -ketoglutarate dehydrogenase," *Journal of Neuroscience*, vol. 24, no. 36, pp. 7771–7778, 2004.
- [40] S. Anderson, A. T. Bankier, and B. G. Barrell, "Sequence and organization of the human mitochondrial genome," *Nature*, vol. 290, no. 5806, pp. 457–465, 1981.
- [41] D. A. Clayton, "Transcription of the mammalian mitochondrial genome," *Annual Review of Biochemistry*, vol. 53, pp. 573–594, 1984.
- [42] D. L. Croteau and V. A. Bohr, "Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells," *Journal of Biological Chemistry*, vol. 272, no. 41, pp. 25409–25412, 1997.
- [43] Y. Nakabeppu, "Regulation of intracellular localization of human MTH1, OGG1, and MYH proteins for repair of oxidative DNA damage," *Progress in Nucleic Acid Research and Molecular Biology*, vol. 68, pp. 75–94, 2001.
- [44] M. S. Cooke, M. D. Evans, M. Dizdaroglu, and J. Lunec, "Oxidative DNA damage: mechanisms, mutation, and disease," *FASEB Journal*, vol. 17, no. 10, pp. 1195–1214, 2003.
- [45] M. D. Evans, M. Dizdaroglu, and M. S. Cooke, "Oxidative DNA damage and disease: induction, repair and significance," *Mutation Research*, vol. 567, no. 1, pp. 1–61, 2004.
- [46] R. A. Costa, C. D. Romagna, J. L. Pereira, and N. C. Souza-Pinto, "The role of mitochondrial DNA damage in the cytotoxicity of reactive oxygen species," *Journal of Bioenergetics and Biomembranes*, vol. 43, no. 1, pp. 25–29, 2011.
- [47] H. Kasai and S. Nishimura, "Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents," *Nucleic Acids Research*, vol. 12, no. 4, pp. 2137–2145, 1984.
- [48] R. A. Floyd, J. J. Watson, P. K. Wong, D. H. Altmiller, and R. C. Rickard, "Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation," *Free Radical Research Communications*, vol. 1, no. 3, pp. 163–172, 1986.
- [49] H. Kasai and S. Nishimura, "Hydroxylation of guanine in nucleosides and DNA at the C-8 position by heated glucose and oxygen radical-forming agents," *Environmental Health Perspectives*, vol. 67, pp. 111–116, 1986.
- [50] K. C. Cundy, R. Kohen, and B. N. Ames, "Determination of 8-hydroxydeoxyguanosine in human urine: a possible assay for in vivo oxidative DNA damage," *Basic life sciences*, vol. 49, pp. 479–482, 1988.
- [51] R. A. Floyd, M. S. West, K. L. Eneff, W. E. Hogsett, and D. T. Tingey, "Hydroxyl free radical mediated formation of 8-hydroxyguanine in isolated DNA," *Archives of Biochemistry and Biophysics*, vol. 262, no. 1, pp. 266–272, 1988.
- [52] F. M. Yakes and B. van Houten, "Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 2, pp. 514–519, 1997.
- [53] D. C. Wallace, "Mitochondrial DNA mutations in disease and aging," *Environmental and Molecular Mutagenesis*, vol. 51, no. 5, pp. 440–450, 2010.
- [54] M. Corral-Debrinski, T. Horton, M. T. Lott, J. M. Shoffner, M. F. Beal, and D. C. Wallace, "Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age," *Nature Genetics*, vol. 2, no. 4, pp. 324–329, 1992.
- [55] G. A. Cortopassi and N. Arnheim, "Detection of a specific mitochondrial DNA deletion in tissues of older humans," *Nucleic Acids Research*, vol. 18, no. 23, pp. 6927–6933, 1990.
- [56] A. Hiona, A. Sanz, G. C. Kujoth et al., "Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice," *PLoS ONE*, vol. 5, no. 7, Article ID e11468, 2010.
- [57] R. Pamplona, "Mitochondrial DNA damage and animal longevity: insights from comparative studies," *Journal of Aging Research*, vol. 2011, Article ID 807108, 9 pages, 2011.
- [58] H. J. Sung, W. Ma, P. Y. Wang et al., "Mitochondrial respiration protects against oxygen-associated DNA damage," *Nature Communications*, vol. 1, no. 1, 2010.
- [59] R. McFarland, R. W. Taylor, and D. M. Turnbull, "The neurology of mitochondrial DNA disease," *Lancet Neurology*, vol. 1, no. 6, pp. 343–351, 2002.
- [60] C. Vives-Bauza, A. L. Andreu, G. Manfredi et al., "Sequence analysis of the entire mitochondrial genome in Parkinson's disease," *Biochemical and Biophysical Research Communications*, vol. 290, no. 5, pp. 1593–1601, 2002.
- [61] M. T. Lin, D. K. Simon, C. H. Ahn, L. M. Kim, and M. Flint Beal, "High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain," *Human Molecular Genetics*, vol. 11, no. 2, pp. 133–145, 2002.
- [62] M. F. Beal, "Mitochondria take center stage in aging and neurodegeneration," *Annals of Neurology*, vol. 58, no. 4, pp. 495–505, 2005.
- [63] R. Smigrodzki, J. Parks, and W. D. Parker, "High frequency of mitochondrial complex I mutations in Parkinson's disease and aging," *Neurobiology of Aging*, vol. 25, no. 10, pp. 1273–1281, 2004.
- [64] D. G. Murdock, N. C. Christacos, and D. C. Wallace, "The age-related accumulation of a mitochondrial DNA control region mutation in muscle, but not brain, detected by a sensitive PNA-directed PCR clamping based method," *Nucleic Acids Research*, vol. 28, no. 21, pp. 4350–4355, 2000.
- [65] P. F. Chinnery, G. A. Taylor, N. Howell, D. T. Brown, T. J. Parsons, and D. M. Turnbull, "Point mutations of the mtDNA control region in normal and neurodegenerative human brains," *American Journal of Human Genetics*, vol. 68, no. 2, pp. 529–532, 2001.
- [66] A. Pyle, T. Foltynie, W. Tiangyou et al., "Mitochondrial DNA haplogroup cluster UKJT reduces the risk of PD," *Annals of Neurology*, vol. 57, no. 4, pp. 564–567, 2005.
- [67] A. Bender, K. J. Krishnan, C. M. Morris et al., "High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease," *Nature Genetics*, vol. 38, no. 5, pp. 515–517, 2006.
- [68] J. M. Carney, P. E. Starke-Reed, C. N. Oliver et al., "Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl- α -phenylnitron," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 9, pp. 3633–3636, 1991.

- [69] G. C. Brown, "Nitric oxide and mitochondrial respiration," *Biochimica et Biophysica Acta*, vol. 1411, no. 2-3, pp. 351-369, 1999.
- [70] A. Cassina and R. Radi, "Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport," *Archives of Biochemistry and Biophysics*, vol. 328, no. 2, pp. 309-316, 1996.
- [71] M. W. Cleeter, "Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide Implications for neurodegenerative diseases," *FEBS Letters*, vol. 345, no. 1, pp. 50-54, 1994.
- [72] G. C. Brown and V. Borutaite, "Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols," *Biochimica et Biophysica Acta*, vol. 1658, no. 1-2, pp. 44-49, 2004.
- [73] S. J. Chinta and J. K. Andersen, "Nitrosylation and nitration of mitochondrial complex i in Parkinson's disease," *Free Radical Research*, vol. 45, no. 1, pp. 53-58, 2011.
- [74] A. M. Cassina, R. Hodara, J. M. Souza et al., "Cytochrome c nitration by peroxynitrite," *Journal of Biological Chemistry*, vol. 275, no. 28, pp. 21409-21415, 2000.
- [75] H. Nakagawa, N. Komai, M. Takusagawa et al., "Nitration of specific tyrosine residues of cytochrome c is associated with caspase-cascade inactivation," *Biological and Pharmaceutical Bulletin*, vol. 30, no. 1, pp. 15-20, 2007.
- [76] L. J. Yan and R. S. Sohal, "Mitochondrial adenine nucleotide translocase is modified oxidatively during aging," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 22, pp. 12896-12901, 1998.
- [77] D. Han, F. Antunes, R. Canali, D. Rettori, and E. Cadenas, "Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol," *Journal of Biological Chemistry*, vol. 278, no. 8, pp. 5557-5563, 2003.
- [78] M. Madesh and G. Hajnóczky, "VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release," *Journal of Cell Biology*, vol. 155, no. 6, pp. 1003-1015, 2001.
- [79] O. Stachowiak, M. Dolder, T. Wallimann, and C. Richter, "Mitochondrial creatine kinase is a prime target of peroxynitrite-induced modification and inactivation," *Journal of Biological Chemistry*, vol. 273, no. 27, pp. 16694-16699, 1998.
- [80] M. Le Bras, M. V. Clément, S. Pervaiz, and C. Brenner, "Reactive oxygen species and the mitochondrial signaling pathway of cell death," *Histology and Histopathology*, vol. 20, no. 1, pp. 205-219, 2005.
- [81] M. W. McEnery, A. M. Snowman, R. R. Trifiletti, and S. H. Snyder, "Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 8, pp. 3170-3174, 1992.
- [82] A. Rasola and P. Bernardi, "The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis," *Apoptosis*, vol. 12, no. 5, pp. 815-833, 2007.
- [83] P. Bernardi and M. Forte, "The mitochondrial permeability transition pore," *Novartis Foundation Symposium*, vol. 287, pp. 157-164, 2007.
- [84] J. E. Kokoszka, K. G. Waymire, S. E. Levy et al., "The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore," *Nature*, vol. 427, no. 6973, pp. 461-465, 2004.
- [85] A. Krauskopf, O. Eriksson, W. J. Craigen, M. A. Forte, and P. Bernardi, "Properties of the permeability transition in *VDAC1*^{-/-} mitochondria," *Biochimica et Biophysica Acta*, vol. 1757, no. 5-6, pp. 590-595, 2006.
- [86] E. Basso, L. Fante, J. Fowlkes, V. Petronilli, M. A. Forte, and P. Bernardi, "Properties of the permeability transition pore in mitochondria devoid of cyclophilin D," *Journal of Biological Chemistry*, vol. 280, no. 19, pp. 18558-18561, 2005.
- [87] G. P. McStay, S. J. Clarke, and A. P. Halestrap, "Role of critical thiol groups on the matrix surface of the adenine nucleotide translocase in the mechanism of the mitochondrial permeability transition pore," *Biochemical Journal*, vol. 367, no. 2, pp. 541-548, 2002.
- [88] P. Costantini, A. S. Belzacq, H. L. A. Vieira et al., "Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis," *Oncogene*, vol. 19, no. 2, pp. 307-314, 2000.
- [89] L. J. Yan, R. L. Levine, and R. S. Sohal, "Oxidative damage during aging targets mitochondrial aconitase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 21, pp. 11168-11172, 1997.
- [90] M. A. Graziewicz, B. J. Day, and W. C. Copeland, "The mitochondrial DNA polymerase as a target of oxidative damage," *Nucleic Acids Research*, vol. 30, no. 13, pp. 2817-2824, 2002.
- [91] H. Huang and K. G. Manton, "The role of oxidative damage in mitochondria during aging: a review," *Frontiers in Bioscience*, vol. 9, pp. 1100-1117, 2004.
- [92] L. M. Sayre, D. Lin, Q. Yuan, X. Zhu, and X. Tang, "Protein adducts generated from products of lipid oxidation: focus on HNE and one," *Drug Metabolism Reviews*, vol. 38, no. 4, pp. 651-675, 2006.
- [93] H. L. Vieira, A. S. Belzacq, D. Haouzi et al., "The adenine nucleotide translocator: a target of nitric oxide, peroxynitrite, and 4-hydroxynonenal," *Oncogene*, vol. 20, no. 32, pp. 4305-4316, 2001.
- [94] Q. Yuan, X. Zhu, and L. M. Sayre, "Chemical nature of stochastic generation of protein-based carbonyls: metal-catalyzed oxidation versus modification by products of lipid oxidation," *Chemical Research in Toxicology*, vol. 20, no. 1, pp. 129-139, 2007.
- [95] P. A. Grimsrud, H. Xie, T. J. Griffin, and D. A. Bernlohr, "Oxidative stress and covalent modification of protein with bioactive aldehydes," *Journal of Biological Chemistry*, vol. 283, no. 32, pp. 21837-21841, 2008.
- [96] C. Batandier, E. Fontaine, C. Kériel, and X. M. Laverne, "Determination of mitochondrial reactive oxygen species: methodological aspects," *Journal of Cellular and Molecular Medicine*, vol. 6, no. 2, pp. 175-187, 2002.
- [97] K. M. Robinson, M. S. Janes, and J. S. Beckman, "The selective detection of mitochondrial superoxide by live cell imaging," *Nature Protocols*, vol. 3, no. 6, pp. 941-947, 2008.
- [98] J. Zielonka, M. Hardy, and B. Kalyanaram, "HPLC study of oxidation products of hydroethidine in chemical and biological systems: ramifications in superoxide measurements," *Free Radical Biology and Medicine*, vol. 46, no. 3, pp. 329-338, 2009.
- [99] J. Zielonka and B. Kalyanaram, "Hydroethidine- and MitoSOX-derived red fluorescence is not a reliable indicator of intracellular superoxide formation: another inconvenient truth," *Free Radical Biology and Medicine*, vol. 48, no. 8, pp. 983-1001, 2010.
- [100] M. Degli Esposti, "Measuring mitochondrial reactive oxygen species," *Methods*, vol. 26, no. 4, pp. 335-340, 2002.

- [101] B. C. Dickinson and C. J. Chang, "A targetable fluorescent probe for imaging hydrogen peroxide in the mitochondria of living cells," *Journal of the American Chemical Society*, vol. 130, no. 30, pp. 9638–9639, 2008.
- [102] V. V. Belousov, A. F. Fradkov, K. A. Lukyanov et al., "Genetically encoded fluorescent indicator for intracellular hydrogen peroxide," *Nature Methods*, vol. 3, no. 4, pp. 281–286, 2006.
- [103] G. T. Hanson, R. Aggeler, D. Oglesbee et al., "Investigating mitochondrial redox potential with redox-sensitive green fluorescent protein indicators," *Journal of Biological Chemistry*, vol. 279, no. 13, pp. 13044–13053, 2004.
- [104] C. T. Dooley, T. M. Dore, G. T. Hanson, W. C. Jackson, S. J. Remington, and R. Y. Tsien, "Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators," *Journal of Biological Chemistry*, vol. 279, no. 21, pp. 22284–22293, 2004.
- [105] M. B. Cannon and S. James Remington, "Redox-sensitive green fluorescent protein: probes for dynamic intracellular redox responses. A review," *Methods in Molecular Biology*, vol. 476, pp. 50–64, 2009.
- [106] C. Scaffidi, S. Fulda, A. Srinivasan et al., "Two CD95 (APO-1/Fas) signaling pathways," *EMBO Journal*, vol. 17, no. 6, pp. 1675–1687, 1998.
- [107] M. J. Morgan, Y. S. Kim, and Z. Liu, "Lipid rafts and oxidative stress-induced cell death," *Antioxidants and Redox Signaling*, vol. 9, no. 9, pp. 1471–1483, 2007.
- [108] P. L. Li and E. Gulbins, "Lipid rafts and redox signaling," *Antioxidants and Redox Signaling*, vol. 9, no. 9, pp. 1411–1415, 2007.
- [109] L. Wang, N. Azad, L. Kongkaneramt et al., "The Fas death signaling pathway connecting reactive oxygen species generation and FLICE inhibitory protein down-regulation," *Journal of Immunology*, vol. 180, no. 5, pp. 3072–3080, 2008.
- [110] K. Izeradjene, L. Douglas, D. M. Tillman, A. B. Delaney, and J. A. Houghton, "Reactive oxygen species regulate caspase activation in tumor necrosis factor-related apoptosis-inducing ligand-resistant human colon carcinoma cell lines," *Cancer Research*, vol. 65, no. 16, pp. 7436–7445, 2005.
- [111] S. H. Woo, I. C. Park, M. J. Park et al., "Arsenic trioxide sensitizes CD95/Fas-induced apoptosis through rosmidated upregulation of CD95/Fas by NF- κ B activation," *International Journal of Cancer*, vol. 112, no. 4, pp. 596–606, 2004.
- [112] C. Pantano, P. Shrivastava, B. McElhinney, and Y. Janssen-Heininger, "Hydrogen peroxide signaling through tumor necrosis factor receptor 1 leads to selective activation of c-jun n-terminal kinase," *Journal of Biological Chemistry*, vol. 278, no. 45, pp. 44091–44096, 2003.
- [113] H. Kamata, S. I. Honda, S. Maeda, L. Chang, H. Hirata, and M. Karin, "Reactive oxygen species promote TNF α -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases," *Cell*, vol. 120, no. 5, pp. 649–661, 2005.
- [114] G. Fujino, T. Noguchi, A. Matsuzawa et al., "Thioredoxin and TRAF family proteins regulate reactive oxygen species-dependent activation of ASK1 through reciprocal modulation of the N-terminal homophilic interaction of ASK1," *Molecular and Cellular Biology*, vol. 27, no. 23, pp. 8152–8163, 2007.
- [115] G. Saxena, J. Chen, and A. Shalev, "Intracellular shuttling and mitochondrial function of thioredoxin- interacting protein," *Journal of Biological Chemistry*, vol. 285, no. 6, pp. 3997–4005, 2010.
- [116] M. Zoratti and I. Szabo, "The mitochondrial permeability transition," *Biochimica et Biophysica Acta*, vol. 1241, no. 2, pp. 139–176, 1995.
- [117] A. Kaasik, D. Safiulina, A. Zharkovsky, and V. Veksler, "Regulation of mitochondrial matrix volume," *American Journal of Physiology*, vol. 292, no. 1, pp. C157–C163, 2007.
- [118] M. Ott, J. D. Robertson, V. Gogvadze, B. Zhivotovsky, and S. Orrenius, "Cytochrome c release from mitochondria proceeds by a two-step process," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 3, pp. 1259–1263, 2002.
- [119] V. E. Kagan, G. G. Borisenko, Y. Y. Tyurina et al., "Oxidative lipidomics of apoptosis: redox catalytic interactions of cytochrome c with cardiolipin and phosphatidylserine," *Free Radical Biology and Medicine*, vol. 37, no. 12, pp. 1963–1985, 2004.
- [120] I. R. Indran, M. P. Hande, and S. Pervaiz, "hTERT overexpression alleviates intracellular ROS production, improves mitochondrial function, and inhibits ROS-mediated apoptosis in cancer cells," *Cancer Research*, vol. 71, no. 1, pp. 266–276, 2011.
- [121] L. Goitre, F. Balzac, S. Degani et al., "KRIT1 regulates the homeostasis of intracellular reactive oxygen species," *PLoS ONE*, vol. 5, no. 7, Article ID e11786, 2010.
- [122] L. Yin, Z. Wu, D. Avigan et al., "MUC1-C oncoprotein suppresses reactive oxygen species-induced terminal differentiation of acute myelogenous leukemia cells," *Blood*, vol. 117, no. 18, pp. 4863–4870, 2011.
- [123] L. Yin, Y. Li, J. Ren, H. Kuwahara, and D. Kufe, "Human MUC1 carcinoma antigen regulates intracellular oxidant levels and the apoptotic response to oxidative stress," *Journal of Biological Chemistry*, vol. 278, no. 37, pp. 35458–35464, 2003.
- [124] R. Scherz-Shouval and Z. Elazar, "ROS, mitochondria and the regulation of autophagy," *Trends in Cell Biology*, vol. 17, no. 9, pp. 422–427, 2007.
- [125] R. Scherz-Shouval, E. Shvets, E. Fass, H. Shorer, L. Gil, and Z. Elazar, "Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4," *EMBO Journal*, vol. 26, no. 7, pp. 1749–1760, 2007.
- [126] M. B. Azad, Y. Chen, and S. B. Gibson, "Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment," *Antioxidants and Redox Signaling*, vol. 11, no. 4, pp. 777–790, 2009.
- [127] K. Bensaad, A. Tsuruta, M. A. Selak et al., "TIGAR, a p53-inducible regulator of glycolysis and apoptosis," *Cell*, vol. 126, no. 1, pp. 107–120, 2006.
- [128] K. Bensaad, E. C. Cheung, and K. H. Vousden, "Modulation of intracellular ROS levels by TIGAR controls autophagy," *EMBO Journal*, vol. 28, no. 19, pp. 3015–3026, 2009.
- [129] S. C. Barber, R. J. Mead, and P. J. Shaw, "Oxidative stress in ALS: a mechanism of neurodegeneration and a therapeutic target," *Biochimica et Biophysica Acta*, vol. 1762, no. 11–12, pp. 1051–1067, 2006.
- [130] G. Dobrowolny, M. Aucello, E. Rizzuto et al., "Skeletal muscle is a primary target of SOD1G93A-mediated toxicity," *Cell Metabolism*, vol. 8, no. 5, pp. 425–436, 2008.
- [131] N. Morimoto, M. Nagai, Y. Ohta et al., "Increased autophagy in transgenic mice with a G93A mutant SOD1 gene," *Brain Research*, vol. 1167, no. 1, pp. 112–117, 2007.
- [132] J. Gal, A. L. Ström, D. M. Kwinter et al., "Sequestosome 1/p62 links familial ALS mutant SOD1 to LC3 via an ubiquitin-independent mechanism," *Journal of Neurochemistry*, vol. 111, no. 4, pp. 1062–1073, 2009.

- [133] I. Kim, S. Rodriguez-Enriquez, and J. J. Lemasters, "Selective degradation of mitochondria by mitophagy," *Archives of Biochemistry and Biophysics*, vol. 462, no. 2, pp. 245–253, 2007.
- [134] S. P. Elmore, T. Qian, S. F. Grissom, and J. J. Lemasters, "The mitochondrial permeability transition initiates autophagy in rat hepatocytes," *The FASEB Journal*, vol. 15, no. 12, pp. 2286–2287, 2001.
- [135] S. Rodriguez-Enriquez, Y. Kai, E. Maldonado, R. T. Currin, and J. J. Lemasters, "Roles of mitophagy and the mitochondrial permeability transition in remodeling of cultured rat hepatocytes," *Autophagy*, vol. 5, no. 8, pp. 1099–1106, 2009.
- [136] Y. M. Yoo and E. B. Jeung, "Melatonin suppresses cyclosporine A-induced autophagy in rat pituitary GH3 cells," *Journal of Pineal Research*, vol. 48, no. 3, pp. 204–211, 2010.
- [137] P. Grumati, L. Coletto, P. Sabatelli et al., "Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration," *Nature Medicine*, vol. 16, no. 11, pp. 1313–1320, 2010.
- [138] N. Exner, B. Treske, D. Paquet et al., "Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin," *Journal of Neuroscience*, vol. 27, no. 45, pp. 12413–12418, 2007.
- [139] J. H. Zhu, F. Guo, J. Shelburne, S. Watkins, and C. T. Chu, "Localization of phosphorylated ERK/MAP kinases to mitochondria and autophagosomes in lewy body diseases," *Brain Pathology*, vol. 13, no. 4, pp. 473–481, 2003.
- [140] R. Scherz-Shouval and Z. Elazar, "Regulation of autophagy by ROS: physiology and pathology," *Trends in Biochemical Sciences*, vol. 36, no. 1, pp. 30–38, 2011.
- [141] R. J. Youle and D. P. Narendra, "Mechanisms of mitophagy," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 1, pp. 9–14, 2011.
- [142] D. Narendra, A. Tanaka, D. F. Suen, and R. J. Youle, "Parkin is recruited selectively to impaired mitochondria and promotes their autophagy," *Journal of Cell Biology*, vol. 183, no. 5, pp. 795–803, 2008.
- [143] D. F. Suen, D. P. Narendra, A. Tanaka, G. Manfredi, and R. J. Youle, "Parkin overexpression selects against a deleterious mtDNA mutation in heteroplasmic cybrid cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 26, pp. 11835–11840, 2010.
- [144] C. Vives-Bauza, C. Zhou, Y. Huang et al., "PINK1-dependent recruitment of Parkin to mitochondria in mitophagy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 1, pp. 378–383, 2010.
- [145] R. K. Dagda, S. J. Cherra, S. M. Kulich, A. Tandon, D. Park, and C. T. Chu, "Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission," *Journal of Biological Chemistry*, vol. 284, no. 20, pp. 13843–13855, 2009.
- [146] S. J. Cherra 3rd, R. K. Dagda, A. Tandon, and C. T. Chu, "Mitochondrial autophagy as a compensatory response to PINK1 deficiency," *Autophagy*, vol. 5, no. 8, pp. 1213–1214, 2009.
- [147] E. Verdin, M. D. Hirschey, L. W. S. Finley, and M. C. Haigis, "Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling," *Trends in Biochemical Sciences*, vol. 35, no. 12, pp. 669–675, 2010.
- [148] S. E. Schriener, N. J. Linford, G. M. Martin et al., "Extension of murine life span by overexpression of catalase targeted to mitochondria," *Science*, vol. 308, no. 5730, pp. 1909–1911, 2005.
- [149] V. I. Pérez, C. M. Lew, L. A. Cortez et al., "Thioredoxin 2 haploinsufficiency in mice results in impaired mitochondrial function and increased oxidative stress," *Free Radical Biology and Medicine*, vol. 44, no. 5, pp. 882–892, 2008.
- [150] R. M. Lebovitz, H. Zhang, H. Vogel et al., "Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 18, pp. 9782–9787, 1996.
- [151] D. C. Wallace, "A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine," *Annual Review of Genetics*, vol. 39, pp. 359–407, 2005.
- [152] M. Tatar, A. Bartke, and A. Antebi, "The endocrine regulation of aging by insulin-like signals," *Science*, vol. 299, no. 5611, pp. 1346–1351, 2003.
- [153] M. Barbieri, M. Bonafè, C. Franceschi, and G. Paolisso, "Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans," *American Journal of Physiology*, vol. 285, no. 5, pp. E1064–E1071, 2003.
- [154] M. Trinei, I. Berniakovich, E. Beltrami et al., "P66Shc signals to age," *Aging*, vol. 1, no. 6, pp. 503–510, 2009.
- [155] P. Pinton and R. Rizzuto, "P66Shc, oxidative stress and aging: importing a lifespan determinant into mitochondria," *Cell Cycle*, vol. 7, no. 3, pp. 304–308, 2008.
- [156] P. Pinton, A. Rimessi, S. Marchi et al., "Protein kinase C β and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc," *Science*, vol. 315, no. 5812, pp. 659–663, 2007.
- [157] E. Migliaccio, M. Giorgio, S. Mele et al., "The p66(shc) adaptor protein controls oxidative stress response and life span in mammals," *Nature*, vol. 402, no. 6759, pp. 309–313, 1999.
- [158] P. Francia, C. Delli Gatti, M. Bachschmid et al., "Deletion of p66shc gene protects against age-related endothelial dysfunction," *Circulation*, vol. 110, no. 18, pp. 2889–2895, 2004.
- [159] G. G. Camici, M. Schiavoni, P. Francia et al., "Genetic deletion of p66Shc adaptor protein prevents hyperglycemia-induced endothelial dysfunction and oxidative stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 12, pp. 5217–5222, 2007.
- [160] M. Rota, N. LeCapitaine, T. Hosoda et al., "Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66shc gene," *Circulation Research*, vol. 99, no. 1, pp. 42–52, 2006.
- [161] C. Giorgi, A. Romagnoli, P. Pinton, and R. Rizzuto, "Ca²⁺ signaling, mitochondria and cell death," *Current Molecular Medicine*, vol. 8, no. 2, pp. 119–130, 2008.
- [162] M. Giorgio, E. Migliaccio, F. Orsini et al., "Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis," *Cell*, vol. 122, no. 2, pp. 221–233, 2005.
- [163] F. Orsini, E. Migliaccio, M. Moroni et al., "The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential," *Journal of Biological Chemistry*, vol. 279, no. 24, pp. 25689–25695, 2004.
- [164] A. Ventura, M. Maccarana, V. A. Raker, and P. G. Pelicci, "A Cryptic Targeting Signal Induces Isoform-specific Localization of p46Shc to Mitochondria," *Journal of Biological Chemistry*, vol. 279, no. 3, pp. 2299–2306, 2004.
- [165] M. R. Wieckowski, C. Giorgi, M. Lebedzinska, J. Duszynski, and P. Pinton, "Isolation of mitochondria-associated

- membranes and mitochondria from animal tissues and cells," *Nature Protocols*, vol. 4, no. 11, pp. 1582–1590, 2009.
- [166] M. Lebedzinska, G. Szabadkai, A. W. E. Jones, J. Duszynski, and M. R. Wieckowski, "Interactions between the endoplasmic reticulum, mitochondria, plasma membrane and other subcellular organelles," *International Journal of Biochemistry and Cell Biology*, vol. 41, no. 10, pp. 1805–1816, 2009.
- [167] M. Lebedzinska, J. Duszynski, R. Rizzuto, P. Pinton, and M. R. Wieckowski, "Age-related changes in levels of p66Shc and serine 36-phosphorylated p66Shc in organs and mouse tissues," *Archives of Biochemistry and Biophysics*, vol. 486, no. 1, pp. 73–80, 2009.
- [168] S. Nemoto and T. Finkel, "Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway," *Science*, vol. 295, no. 5564, pp. 2450–2452, 2002.
- [169] S. Purdom and Q. M. Chen, "Linking oxidative stress and genetics of aging with p66Shc signaling and forkhead transcription factors," *Biogerontology*, vol. 4, no. 4, pp. 181–191, 2003.
- [170] M. Lebedzinska, A. Karkucinska-Wieckowska, C. Giorgi et al., "Oxidative stress-dependent p66Shc phosphorylation in skin fibroblasts of children with mitochondrial disorders," *Biochimica et Biophysica Acta*, vol. 1797, no. 6–7, pp. 952–960, 2010.
- [171] A. Raffaello and R. Rizzuto, "Mitochondrial longevity pathways," *Biochimica et Biophysica Acta*, vol. 1813, no. 1, pp. 260–268, 2011.
- [172] L. Fontana, L. Partridge, and V. D. Longo, "Extending healthy life span—from yeast to humans," *Science*, vol. 328, no. 5976, pp. 321–326, 2010.
- [173] S. D. Hursting, S. N. Perkins, J. M. Phang, and J. C. Barrett, "Diet and cancer prevention studies in p53-deficient mice," *Journal of Nutrition*, vol. 131, supplement 11, pp. 3092S–3094S, 2001.
- [174] G. López-Lluch, N. Hunt, B. Jones et al., "Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 6, pp. 1768–1773, 2006.
- [175] M. Holzenberger, J. Dupont, B. Ducos et al., "IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice," *Nature*, vol. 421, no. 6919, pp. 182–187, 2003.
- [176] M. Holzenberger, "The GH/IGF-I axis and longevity," *European Journal of Endocrinology*, vol. 151, supplement 1, pp. S23–S27, 2004.
- [177] A. Salminen and K. Kaarniranta, "Insulin/IGF-1 paradox of aging: regulation via AKT/IKK/NF- κ B signaling," *Cellular Signalling*, vol. 22, no. 4, pp. 573–577, 2010.
- [178] D. D. Sarbassov, S. M. Ali, and D. M. Sabatini, "Growing roles for the mTOR pathway," *Current Opinion in Cell Biology*, vol. 17, no. 6, pp. 596–603, 2005.
- [179] G. J. Kops, T. B. Dansen, P. E. Polderman et al., "Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress," *Nature*, vol. 419, no. 6904, pp. 316–321, 2002.
- [180] H. Daitoku and A. Fukamizu, "FOXO transcription factors in the regulatory networks of longevity," *Journal of Biochemistry*, vol. 141, no. 6, pp. 769–774, 2007.
- [181] J. Araujo, P. Breuer, S. Dieringer et al., "FOXO4-dependent upregulation of superoxide dismutase-2 in response to oxidative stress is impaired in spinocerebellar ataxia type 3," *Human Molecular Genetics*, vol. 20, no. 15, pp. 2928–2941, 2011.
- [182] D. Barsyte, D. A. Lovejoy, and G. J. Lithgow, "Longevity and heavy metal resistance in daf-2 and age-1 long-lived mutants of *Caenorhabditis elegans*," *FASEB Journal*, vol. 15, no. 3, pp. 627–634, 2001.
- [183] D. D. Sarbassov, D. A. Guertin, S. M. Ali, and D. M. Sabatini, "Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex," *Science*, vol. 307, no. 5712, pp. 1098–1101, 2005.
- [184] P. Kapahi, B. M. Zid, T. Harper, D. Koslover, V. Sapin, and S. Benzer, "Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway," *Current Biology*, vol. 14, no. 10, pp. 885–890, 2004.
- [185] D. E. Harrison, R. Strong, Z. D. Sharp et al., "Rapamycin fed late in life extends lifespan in genetically heterogeneous mice," *Nature*, vol. 460, no. 7253, pp. 392–395, 2009.
- [186] J. Landry, A. Sutton, S. T. Tafrov et al., "The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 11, pp. 5807–5811, 2000.
- [187] R. M. Anderson, M. Latorre-Esteves, A. R. Neves et al., "Yeast life-span extension by calorie restriction is independent of NAD fluctuation," *Science*, vol. 302, no. 5653, pp. 2124–2126, 2003.
- [188] S. Kyrilenko and A. Baniahmad, "Sirtuin family: a link to metabolic signaling and senescence," *Current Medicinal Chemistry*, vol. 17, no. 26, pp. 2921–2932, 2010.
- [189] M. Bernier et al., "Negative regulation of STAT3-mediated cellular respiration by SirT1," *The Journal of Biological Chemistry*, vol. 286, no. 22, pp. 1970–1979, 2011.
- [190] H. S. Kim, K. Patel, K. Muldoon-Jacobs et al., "SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress," *Cancer Cell*, vol. 17, no. 1, pp. 41–52, 2010.
- [191] X. Qiu, K. Brown, M. D. Hirschey, E. Verdin, and D. Chen, "Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation," *Cell Metabolism*, vol. 12, no. 6, pp. 662–667, 2010.
- [192] Y. Chen, J. Zhang, Y. Lin et al., "Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS," *EMBO Reports*, vol. 12, no. 6, pp. 534–541, 2011.
- [193] Y. Kobayashi, Y. Furukawa-Hibi, C. Chen et al., "SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress," *International Journal of Molecular Medicine*, vol. 16, no. 2, pp. 237–243, 2005.
- [194] M. M. Rahman, G. V. Halade, A. Bhattacharya, and G. Fernandes, "The fat-1 transgene in mice increases antioxidant potential, reduces pro-inflammatory cytokine levels, and enhances PPA Ry and SIRT-1 expression on a calorie restricted diet," *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 5, pp. 307–316, 2009.
- [195] R. M. Anderson, J. L. Barger, M. G. Edwards et al., "Dynamic regulation of PGC-1 α localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response," *Aging Cell*, vol. 7, no. 1, pp. 101–111, 2008.
- [196] R. Anderson and T. Prolla, "PGC-1 α in aging and anti-aging interventions," *Biochimica et Biophysica Acta*, vol. 1790, no. 10, pp. 1059–1066, 2009.
- [197] G. López-Lluch, P. M. Irusta, P. Navas, and R. de Cabo, "Mitochondrial biogenesis and healthy aging," *Experimental Gerontology*, vol. 43, no. 9, pp. 813–819, 2008.
- [198] J. T. Rodgers, C. Lerin, W. Haas, S. P. Gygi, B. M. Spiegelman, and P. Puigserver, "Nutrient control of glucose homeostasis through a complex of PGC-1 α and SIRT1," *Nature*, vol. 434, no. 7029, pp. 113–118, 2005.

- [199] S. Jäer, C. Handschin, J. St-Pierre, and B. M. Spiegelman, "AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 α ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 29, pp. 12017–12022, 2007.
- [200] E. L. Greer, D. Dowlatshahi, M. R. Banko et al., "An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*," *Current Biology*, vol. 17, no. 19, pp. 1646–1656, 2007.
- [201] J. Liu, L. Cao, J. Chen et al., "Bmi1 regulates mitochondrial function and the DNA damage response pathway," *Nature*, vol. 459, no. 7245, pp. 387–392, 2009.
- [202] D. J. Klionsky and S. D. Emr, "Autophagy as a regulated pathway of cellular degradation," *Science*, vol. 290, no. 5497, pp. 1717–1721, 2000.
- [203] G. Mariño, A. F. Fernández, and C. López-Otín, "Autophagy and aging: lessons from progeria models," *Advances in Experimental Medicine and Biology*, vol. 694, pp. 61–68, 2010.
- [204] J. P. Decuyper, "IP₃ Receptors, Mitochondria, and Ca²⁺ Signaling: implications for Aging," *Journal of Aging Research*, vol. 2011, Article ID 920178, 20 pages, 2011.
- [205] D. R. Green and G. Kroemer, "The pathophysiology of mitochondrial cell death," *Science*, vol. 305, no. 5684, pp. 626–629, 2004.
- [206] L. Fu, Y. A. Kim, X. Wang et al., "Perifosine inhibits mammalian target of rapamycin signaling through facilitating degradation of major components in the mTOR axis and induces autophagy," *Cancer Research*, vol. 69, no. 23, pp. 8967–8976, 2009.
- [207] Y. Liu and D. C. Bassham, "TOR is a negative regulator of autophagy in *Arabidopsis thaliana*," *PLoS ONE*, vol. 5, no. 7, Article ID e11883, 2010.
- [208] G. Juhász, L. G. Puskás, O. Komonyi et al., "Gene expression profiling identifies FKBP39 as an inhibitor of autophagy in larval *Drosophila* fat body," *Cell Death and Differentiation*, vol. 14, no. 6, pp. 1181–1190, 2007.
- [209] A. Sengupta, J. D. Molkenin, and K. E. Yutzey, "FoxO transcription factors promote autophagy in cardiomyocytes," *Journal of Biological Chemistry*, vol. 284, no. 41, pp. 28319–28331, 2009.
- [210] I. H. Lee et al., "A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 9, pp. 3374–3379, 2008.
- [211] E. Morselli, M. C. Maiuri, M. Markaki et al., "The life span-prolonging effect of sirtuin-1 is mediated by autophagy," *Autophagy*, vol. 6, no. 1, pp. 186–188, 2010.
- [212] T. Hara, K. Nakamura, M. Matsui et al., "Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice," *Nature*, vol. 441, no. 7095, pp. 885–889, 2006.
- [213] M. Komatsu, S. Waguri, T. Chiba et al., "Loss of autophagy in the central nervous system causes neurodegeneration in mice," *Nature*, vol. 441, no. 7095, pp. 880–884, 2006.