Rise and Fall of Reactive Oxygen Species (ROS): Implications in Aging and Neurodegenerative Disorders

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Introduction

Around 3.4 billion years ago, when photosynthetic bacteria first evolved on earth, there was a gradual but gigantic increase in molecular oxygen concentration in the earth's atmosphere. This grand event marked the beginning of a shift from anaerobic to aerobic life forms on earth. These aerobic organisms evolved a method to metabolize molecular oxygen to water and carbon dioxide in the inner membrane of their mitochondria for generation of energy through a process called oxidative phosphorylation. In spite of being extremely beneficial for cellular energetics, the process of oxidative phosphorylation also results in generation of harmful Reactive Oxygen Species (ROS) in the cells due to partial reduction of oxygen in the mitochondrial compartments. Approximately 5% of the total oxygen taken in by the cells is converted to ROS.

Within the mitochondria, ROS is produced at several sites along the Electron Transport Chain (ETC), coupled with a process called oxidative phosphorylation via complex V/ATP synthase. ETC involves four major complexes, namely: Complex I/ NADH dehydrogenase, Complex II/ Succinate dehydrogenase, Complex III/ Coenzyme Q and Complex IV/ Cytochrome c oxidase. The process of energy generation involves restricted oxidation of electron donors – NADH or FADH, which generates a potential difference across the inner membrane of mitochondria. Thereafter, the potential energy of protons is utilized by ATP synthase to phosphorylate ADP to ATP (energy molecule). Direct and imprecise interactions of NADH/FADH derived electrons with molecular oxygen or any other electron acceptors lead to generation of ROS [1]. Major complexes of ETC indulged in generation of ROS include complex I and complex III [2]. Figure 1 shows a schematic diagram of the flow of electrons across the ETC and the major sites involved in ROS production. Interestingly, Uncoupling Proteins (UCP) present in mitochondrial membranes have been found to minimize the generation of excessive ROS by leaking the protons inside mitochondria from the cytoplasm, thereby reducing the overall membrane potential [3].

Though mitochondria have been found to be the major contributors (~90%) of cellular ROS, several other organelles and enzymes are also involved in the production of ROS [1]. The amount of ROS present inside a cell has phenomenal physiological significance. Increased levels of ROS lead to cellular damage and reduced levels result in impairment of various signaling pathways essential for cellular proliferation and operation of host defense mechanisms. Therefore, a delicate balance between the levels of oxidants and antioxidants is essential to maintain the optimum levels of ROS. An efficient antioxidant defense system which includes enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GRd) etc., ensures the optimal level of cellular ROS and any imbalance or impairment in this system results in oxidative stress and its subsequent consequences [4].

ROS and Aging

It was in the mid-1950s when Denham Harman proposed the "Free radical theory of aging" and supported the hypothesis which postulated a major involvement of ROS in aging process [5]. It was proposed that generated ROS confers oxidative damage to all cellular macromolecules such as proteins, lipids, nucleic acids etc. Progressively, accumulation of unrepaired damaged molecules within the cellular compartments results in disturbed homeostasis and aging [6]. Thus, the process of aging has been found to be inversely related with the rate of ROS generation and oxidative damages.

Interestingly, in spite of being a major production site of intracellular ROS, mitochondria are one of the key targets of ROS attacks and therefore, mitochondrial dysfunctioning has been found to be deeply associated with progression of aging and manifestation of several neurodegenerative disorders [7-9]. Mitochondrial elements such as lipids, oxidative phosphorylation enzymes and mitochondrial DNA are particularly predisposed to attack by ROS due to their close proximity with the ROS production site. Absence of any structural histone protection and lack of repair mechanism in mitochondrial genome makes it a prime target of ROS attacks. Damage to mitochondrial proteins also results in reduced affinity with substrates and gradual loss in their functioning [10]. All these changes collectively form a "vicious cycle" which was initially triggered by mitochondrial impairments, caused by enhanced level of ROS. Gradual accumulation of such losses increase the demand of energy to drive the repair processes and such conditions further aggravate the mitochondrial impairments.

Over the past years, several direct and indirect evidences have demonstrated a direct link between ROS and aging *in-vivo*. In agreement, several theories attempted to correlate ROS with impaired mitochondrial function, activation of cellular senescence and molecular inflammation and suggested them as the basic mechanisms regulating aging process [11]. Recent studies have reported that major organs such as liver, heart, brain, skeletal muscles etc. exhibit enhanced level of ROS with aging. Aging mediated progressive shift in cellular redox status and accumulation of oxidative damages have also been reported in several model systems [12]. Therefore, gradual rise in level of ROS and changes in cellular redox status appears to be one of the major factors which drive the aging phenomenon and other associated features.
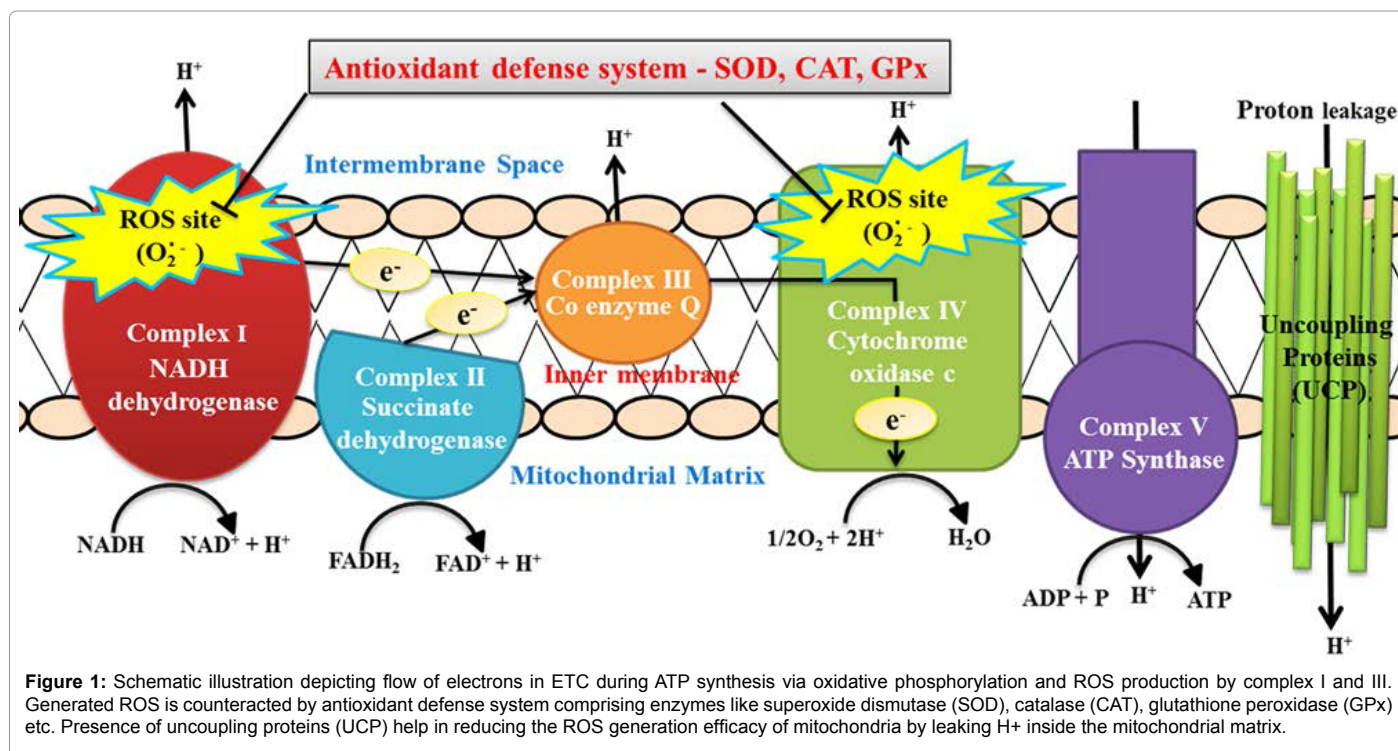
Decline in antioxidant defence system is yet another significant aspect contributing to aging process. Several studies have reported a decline in antioxidant defence system with aging [13]. Interestingly, dietary supplementation of antioxidants or overexpression of genes encoding antioxidant enzymes (SOD, CAT) have been demonstrated to

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Figure 1: Schematic illustration depicting flow of electrons in ETC during ATP synthesis via oxidative phosphorylation and ROS production by complex I and III. Generated ROS is counteracted by antioxidant defense system comprising enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) etc. Presence of uncoupling proteins (UCP) help in reducing the ROS generation efficacy of mitochondria by leaking H+ inside the mitochondrial matrix.

delay aging in various animal models. Dietary supplement of SOD and/ or over expression of Cu/ZnSOD and MnSOD antioxidant enzymes have been shown to increase the lifespan in *C. elegans*, *Drosophila* and mouse models [14-16]. Thus, exogenous supplementation of antioxidants or overexpression of gene(s) encoding antioxidant enzymes could be a fascinating approach to delay the aging process and its deleterious effects.

Despite much of these evidences which support oxidative theory of aging, a definite cause and mechanistic relationship between ROS imbalance and aging phenomenon has not been strongly established yet. Thus, it is essential to gain detailed insights of the fundamental mechanisms of oxidative stress mediated physiological changes which accumulate during aging. This would facilitate the development of a better and integrative understanding of aging process and pathogenesis of related disorders.

ROS and Neurodegeneration

An aging brain is one of the major depositories of free radicals. This is primarily because brain is the most metabolically active organ of the human body which consumes almost one-fifth of the total oxygen inspired by the individual. To further add to the risk factors, anti-oxidant enzymes which are required for metabolizing free radical species are relatively deficient in the Central Nervous System (CNS) [17]. In addition, the blood brain barrier also prevents the access of antioxidants such as vitamin E to the brain. All these factors coalesce to increase the susceptibility of the neurons and glial cells to the damaging effects of ROS and thus, render the brain more vulnerable to oxidative stress. Since the neurons are post-mitotic in nature, any damage to brain tissues by agents like ROS tends to be cumulative with age. Such a progressive loss of structure or function of neurons causing neuronal death is termed as neurodegeneration. Although dispute persists regarding oxidative stress being the cause or consequence of the neurodegeneration, growing body of evidences provides links

for it being involved in the propagation of cellular injury that finally culminates in cell death [18,19]. Some of the leading examples of such cases include Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (HD).

ALS provides a good example to establish a role of oxidative stress in neurodegeneration since about 3-20% cases of familial ALS were found to be associated with mutations in the gene encoding anti-oxidant enzyme SOD1 [20]. Dysfunctional SOD1 leads to an increase in cellular oxidative stress. A mouse model of PD expressing α-synuclein shows enhanced oxidative stress which is primarily driven by mitochondrial dysfunction brought about by administration of MPTP (1-methyl 4-phenyl-1, 2, 3, 6-tetrahydropyridine) [21]. Accelerated dopamine metabolism may also produce oxidative by-products which may in turn, oxidise α-synuclein [22]. Several other PD-related mutations in genes like PTEN-Induced Putative Kinase 1 (PINK1) and Parkin have also been shown to result in mitochondrial impairment and increased oxidative stress in cell cultures as well as in mouse models [23,24]. Similarly, loss-of-function of DJ-1, an oxidative stress sensor, has been found to be associated with increased mitochondrial ROS in mouse embryonic fibroblasts [25]. In case of AD, anti-oxidant enzymes like CAT, GPx and GRd exhibit reduced activities in affected regions of brain [26]. Furthermore, Amyloid-β (Aβ) plaques, the characteristic facet of AD, have been implicated in intracellular ROS generation by inducing a series of events upon interaction with nerve cell membranes [27]. Intriguingly, Aβ has also been demonstrated to participate in mitochondrial fragmentation leading to increased ROS production [28]. As found in other cases or neurodegenerative disorders, HD models also exhibit reduced CAT activity and enhanced glutamate-mediated excitatory receptor activity which may lead to enhanced production of ROS [29,30].

Oxidative stress induced damage to cellular macromolecules is a characteristic feature of all age-onset neurodegenerative disorders. Widespread DNA damage, owing to the strong local oxidative

milieu, is quite distinctly apparent as increased levels of 8-OHdG, a marker of ROS induced DNA damage, are often found in ALS, PD, AD and HD samples. Similarly, markers for lipid peroxidation like 4-Hydroxynonenal (HNE), Malondialdehyde (MDA), lipid hydroperoxides, Thiobarbituric Acid Reactive Substances (TBARS) and isoprostanes have also been found at elevated levels in all of these disease conditions. Protein oxidation is yet another prominent feature of neurodegenerative disorders. An increased presence of 3-nitrotyrosine and protein carboxyl moieties have been reported in all the four neurodegenerative disorders as noted above [31]. Oxidative stress causes proteins to mis-fold and form aggregates inside the cells. Such mis-folded protein aggregates manage to escape proteasomal machinery mediated degradation pathways, and therefore, eventually leads to toxicity and neurodegeneration. For instance, oxidation of SOD1 *in-vitro* leads to enhanced protein aggregation in ALS [32]. ROS induced plaque formation in the affected areas of the CNS has been reported in AD [26]. Moreover, β-amyloid has been demonstrated as neurotoxic and its toxicity was found to be mediated by free radicals [33].

An age-dependent elevation in the concentration of tissue metal ions has also been considered as a major catalyst for aggregate formation during progression of neurodegenerative disorders [34]. For example, hyper-metalation of Aβ by redox-active copper and iron metal ions results in increased rate of oxidation and subsequent production of soluble oxidised form of the peptide which are resistant to the endogenous cellular defense machinery. As a result, the oxidised peptides accumulate within the synapse wherein the high concentrations of Zn^{2+} causes copper/iron-metallated Aβ to precipitate, which could be potentially toxic for the nerve cells [35]. Mutated dopamine has also been demonstrated to interact with iron and accumulate in the cytoplasm which in turn leads to enhanced ROS production and worsening of the Parkinson's disease conditions [35].

Concluding Remarks and Future Perspectives

Over the years, extensive research has been carried out with a focus on deciphering the role of oxidative stress in progression of aging and manifestation of age-onset neurodegenerative disorders. Biomarkers of oxidative stress are continuously being examined to develop potential approaches to regulate biological aging and development of associated disorders. Although, it is increasingly clear now that aging is regulated by explicit signaling pathways and ROS imbalance functions as a catalyst in this phenomenon; several aspects of this association are still enigmatic. Model system based comprehensive research programs are expected to provide further details which would help in designing novel therapeutic approaches and preventive measures.

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