# ALZHEIMER'S DISEASE
## AND TREATMENT

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# Mitochondrial dysfunction and the role of Mitophagy in Alzheimer's Disease

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## Abstract

Mitochondrial dysfunction and alteration in energy regulation occurs prior to the disease-defining amyloid beta peptide (Aβ) and Tau pathologies in the pathogenesis of Alzheimer's disease (AD). Dysfunctional Mitophagy, the process of removal of defective mitochondria through a complex and integrated cellular network is dysfunctional in AD. Mitophagy complements to synaptic dysfunction by prompting Aβ and Tau accumulation due to increasing oxidative stress and cellular energy insufficiencies leading to cognitive deficits. This chapter describes the role of mitochondria and its alterations in AD pathology. Furthermore, this chapter also deals with the quality control mechanisms in mitochondria with specific focus on mitophagy pathways, role of altered mitophagy in AD and the regulation of mitophagy as a novel therapeutic avenue for prevention and treatment of AD.

**Key words:** Mitochondrial dysfunction; Mitophagy; autophagy; PINK1; Parkin; Alzheimer's disease

## Introduction

Mitochondrion is a key organelle sub-serving various biological functions in the cells of virtually all eukaryotic organisms. Mitochondria play a key role in maintaining cellular homeostasis, which include Adenosine triphosphate (ATP) production by oxidative phosphorylation, intracellular calcium signaling and finally initiation of apoptotic cell death and control of inflammatory response [1-3]. In terms of energy regulation in mitochondria, electrons pass through the electron transport chain from high-energy substrates to oxygen to produce ATP by oxidative phosphorylation. Reactive oxygen species (ROS) for example superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (-OH) are toxic byproducts of oxidative phosphorylation [4]. ROS causes oxidative damage to mitochondrial lipids, DNA, and proteins, making mitochondria more prone to ROS production. These damaged mitochondria release high levels of $Ca^{2+}$ and cytochrome c to the cytosol and trigger apoptosis [5]. Accordingly, ensuring proper elimination of dysfunctional mitochondria is imperative to cellular survival, and mitochondrial damage have been implicated in aging, diabetes, and neurodegenerative diseases.

In the brain, mitochondria are responsible for synapse formation in developing neuronal circuits and in the maintenance of synapses in the adult hippocampus. Synaptic plasticity, which is the cellular model and hallmark of learning and memory, involves increase in the size and functional strength of the synapses, which in turn relates to the functional mitochondria [6]. Neurons possess numerous mitochondria to produce ATP, establish membrane excitability and thereby responsible for effective neurotransmission. For example, dendritic spines of excitatory glutamatergic synapses (N-methyl-D-aspartate-NMDA receptors) contain high proportion of metabolically active mitochondria which causes large amounts of $Ca^{2+}$ influx and perturbation in neuronal $Ca^{2+}$ levels promote neuronal death [7]. Notably, impaired mitochondria activate caspase-3-dependent apoptosis by releasing cytochrome-C. Hence, the maintenance of healthy mitochondrial pool is essential for appropriate neuronal function. Various quality control pathways at the mitochondria are a) the degradation of misfolded mitochondrial proteins, b) mitochondrial fission and fusion, c) engulfment and mitophagy [8,9]. Recent studies throw light on the molecular signaling mechanisms that manage mitophagy and regulate the removal

of damaged mitochondria during developmental processes and aging. Moreover, compromised mitophagy has been involved in various neurodegenerative diseases such as Parkinson's disease, AD and aging [10-12]. Therefore, mitophagy serves as a pivotal role in the development, function, and survival of neurons.

### Mitochondrial dyfunction in Alzheimer's disease

Alzheimer disease (AD) is the most common form of dementia characterized by progressive and irreversible cognitive deficits affecting millions of people worldwide. The social and economic burden is such that, by the year 2050, one new case of AD is expected to develop every 33 seconds or nearly a million new cases per year. The total estimated prevalence is expected to be 13.8 million making this disease, a major public health concern globally [13]. Pathologically, there is accumulation of a) extracellular β-amyloid plaques in which Aβ peptides polymerize into insoluble fibrils and b) intracellular/intraneuronal neurofibrillary tangles (NFT) which consists of abnormally phosphorylated tau protein polymerizing into paired helical filaments (PHFs) leading to neuronal death [14].

Mitochondrial dysfunction resulting in energy deficiency is an essential mechanism in the initial stages of AD. Various studies have investigated the role of mitochondrial dysfunction and oxidative damage in the pathogenesis of AD [15-17]. Swerdlow and Khan proposed the mitochondrial cascade hypothesis to explain late-onset, sporadic AD in which mitochondrial dysfunction is the primary event that causes Aβ deposition, NFTs formation and synaptic degeneration [18,19]. Neurons require constant supply of energy for its normal functioning. Neurons have a limited glycolytic capacity and they depend on mitochondrial aerobic oxidative phosphorylation for energy needs. Interestingly, oxidative phosphorylation is a major source of endogenous toxic free radicals, including hydrogen peroxide ($H_2O_2$), hydroxyl ($OH^-$) and superoxide ($O_2^-$) radicals that are products of normal cellular respiration. These reactive oxygen species generated are constantly neutralized by several efficient enzymatic processes like superoxide dismutase (SOD), glutathione peroxidase (GPx), superoxide reductase (SRed), catalase (CAT), peroxiredoxin (Prx) and thioredoxin/thioredoxin reductase (Trx/TrxRed) [20]. If there is an excess of reactive oxygen species generation, which overcomes the neuronal capacity to neutralize them, can lead to oxidative stress followed by mitochondrial dysfunction and neuronal damage ensue. Reactive oxygen species produced by mitochondria target mitochondrial components such as lipids, proteins, and DNA. The deficiency of histones and weakened capacity of DNA repair in mitochondrial DNA (mtDNA) render the mitochondria vulnerable to oxidative stress events. The alterations in energy metabolism is validated by PET imaging of the brain which show reduced radiolabeled glucose uptake into neurons and biochemical assaysdisplaying reduced activity of mitochondrial enzymes involved in oxidative phosphorylation and the TCA cycle [21]. Hence, mitochondrial damage leads to aberrant processing of amyloid precursor protein (APP) to form amyloid plaques and pTau [22].

### Amyloid beta and mitochondrial dysfunction

Several lines of evidence suggest that mitochondrial dysfunction initiate aberrant APP processing to form Aβ generation. Aβ pathology is exacerbated upon administration of toxins that diminish mitochondrial function and by genetic deletion of proteins necessary for the suppression of reactive oxygen species [23,24]. For instance, Complex-I dysfunction induced by rotenone lead to reduced mitochondrial membrane potential, ATP levels and ultimately increased ROS formation. This Complex-I derived reactive oxygen species enhanced the amyloidogenic APP processing and increased Aβ levels in an *in vitro* neuronal model [25]. Similarly, mitochondrial dysfunction precedes the Aβ pathology in brains of AD mouse models [26,27]. Gamma-secretase activity is increased by oxidative stress that causes covalent modification of nicastrin (γ-secretase complex protein) by the membrane lipid peroxidation product, 4-hydroxynonenal-HNE [28]. Increase in beta-secretase (BACE1) protein and activity are induced by HNE and this can be due to positive feedback loop wherein enhanced gamma secretase activity, increases BACE1 activity and expression thereby triggering Aβ generation [29]. Arachidonic acid is the chief source for HNE and it is present in the mitochondrial membranes. Together, accumulating evidence suggests that amyloidogenic APP processing in late-onset AD occurs due to increased generation of ROS by dysfunctional mitochondria in neurons. Hypoxia also increases Aβ production *in vitro* and *in vivo* by increasing ROS via reduced complex III activity [30-33]. To substantiate this finding, AD prevalence is increased in patients with a stroke history [34,35]. Finally, antimycin, a selective complex III inhibitor, also increases Aβ levels in HEK293 cells [25]. Overall, ROS generated by mitochondrial dysfunction, activate amyloidogenic APP processing contributing to initiation and progression of AD.

Mitochondrial function and its integrity is unfavorably affected by toxicity of Aβ and pTau in this manner stimulating a vicious cycle [36]. Decreased mitochondrial ATP production, decreased activity of mitochondrial enzymes and functions, and increased levels of mitochondrial ROS are characteristic of neurons exposed to aggregated Aβ. Similarly, cells with mutant APP producing high levels of Aβ exhibit increased superoxide production and decreased ATP levels [25]. Of note, several studies have stated that there is a robust link between Aβ deposition and Complex-IV activity of the respiratory chain. The data suggest that the mitochondrial dysfunction induced by Aβ is perhaps mediated by significant nitric oxide production that diminishes Complex-IV activity [37]. One possible neurotoxic mechanism was that Aβ is taken up and transported by the mitochondrial translocase of outer membrane in turn inhibiting protein import of nuclear encoded Complex-IV subunits [38]. Additional studies proposed that the sequestration of heme or the collaboration between Aβ and the Aβ-binding alcohol dehydrogenase influence the Complex-IV deficits. It was also proposed that Aβ over-production in mitochondria is inhibited by a γ-secretase inhibitor, reestablishing nitric oxide and ATP levels, indicating a direct involvement of Aβ in these neurotoxic mechanisms [39]. Surprisingly, the activity of the human presequence protease PreP and Complex-IV activity was reduced in AD brains compared to age-matched controls [40,41]. Mitochondria at glutamatergic synapse are prone to injury by aggregating Aβ due to high $Ca^{2+}$ influx and energy demand during synaptic activation [42]. Reduced mitochondrial membrane potential, impaired glutamatergic neurotransmission, glucose transport and oxidative stress caused by exposure of synapses to Aβ results in excitotoxic degeneration [43]. Aβ damages mitochondrial dynamics by compromised balance of fission and fusion, in addition Aβ causes formation of mitochondrial transition pores (mPTP) via interaction with cyclophilin D (CypD). Consequently, inhibition of mPTP formation by blocking CypD is a coherent target for prospective therapeutic AD strategies.

### Tau and mitochondrial dysfunction

Neurofibrillary tangles and microtubule-associated protein

Tau (MAPT) are also characteristic of AD. Mitochondria, lysosomes, and cell organelles are transported within the neurons through microtubules. Tau is a microtubule-associated protein that stabilizes neuronal microtubules under normal physiological conditions. The most common post-translational modifications of tau proteins are phosphorylation and O-glycosylation [44]. Hyperphosphorylation of Tau leads to detachment and destabilization of microtubules resulting in their depolymerization. Cognitive impairment seen in AD has been related to the accumulation of pTau aggregates and fibrils in the soma and neurites of degenerating neurons [45]. Like Aβ pathology, mitochondrial dysfunction also leads to pTau and NFT pathology. Mitochondrial dysfunction induced ROS initiates lipid peroxidation of the cell membranes thereby promoting pTau and its aggregation [46]. To support this, rotenone-a Complex-I inhibitor infused into the rat brain resulted in increased pTau levels [47]. Mice with genetic mitochondrial SOD2 deficiency have shown exhibit increased oxidative stress and pTau expression in the brain and this was neutralized by antioxidants. These experimental data provide strong evidence for the role of dysfunctional mitochondria in Tau pathology in AD.

Hyperphosphorylated Tau has shown to impede mitochondrial transport, causing energy deprivation and oxidative stress at the synapse [48]. Mice expressing P301L-mutant human Tau reveals decreased levels of mitochondrial Complex-V decreased mitochondrial respiration and increased levels of ROS in affected brain regions [49]. Tau over expression alters the distribution of various organelles identified to be transported by microtubule-dependent motor proteins. Accordingly, mitochondria are highly enriched in synapses and play an important role in neurotransmission. Transgenic pR5 mice, a mouse that overexpressed the mutant P301 of tau protein, showed a decrease of mitochondrial complexes activities, mitochondrial depolarization, impaired respiration, and high ROS levels [50]. Mitochondrial dysfunction was also observed in 3xTg-AD mice prior to the development of amyloid plaque. Brain samples from 3xTg-AD exhibited mitochondrial impairment, with a decrease in mitochondrial respiration, and pyruvate dehydrogenase (PDH) activity as early as three months of age. These mice also displayed increased oxidative stress by an increase in hydrogen peroxide production and lipid peroxidation [26]. Alterations of mitochondrial dynamics are tributary to microtubule depolymerization and a subsequent inability of the cell to shuttle healthy mitochondria into axons, dendrites and remove dysfunctional mitochondria by mitophagy. Furthermore, pTau encloses into the mitochondrial membrane and hinder Parkin-mediated mitophagy [51]. An N-terminal Tau present in the mitochondria of human AD brains is associated with hampered mitochondrial metabolism established by reduced expression of Cytochrome-C oxidase and cyclooxgenase (COX IV) [52]. A vicious cycle becomes mounted which potentiates tau hyperphosphorylation, along with Aβ overproduction and deposition. Variations in mitochondrial distribution occur secondary to pathological changes in tau approving the consequence of tau to mitochondrial trafficking observed in animal models [53]. Overall, targeting microtubule network constitutes a promising strategy for pharmacological therapy in AD.

## QUALITY CONTROL IN MITOCHONDRIA: ROLE OF MITOPHAGY

### Different quality control mechanisms in the mitochondria:

Structurally, mitochondria consist of the outer mitochondrial membrane, the intermembrane space, inner mitochondrial membrane, and mitochondrial matrix (stroma). Several quality control mechanisms play a significant role in maintaining homeostasis in the mitochondria. Mitochondria possess proteolytic system termed AAA protease complexes, present in the inner membrane to degrade unfolded membrane proteins [54]. The second mechanism is the removal of selective mitochondrial proteins especially the oxidized mitochondrial proteins by the

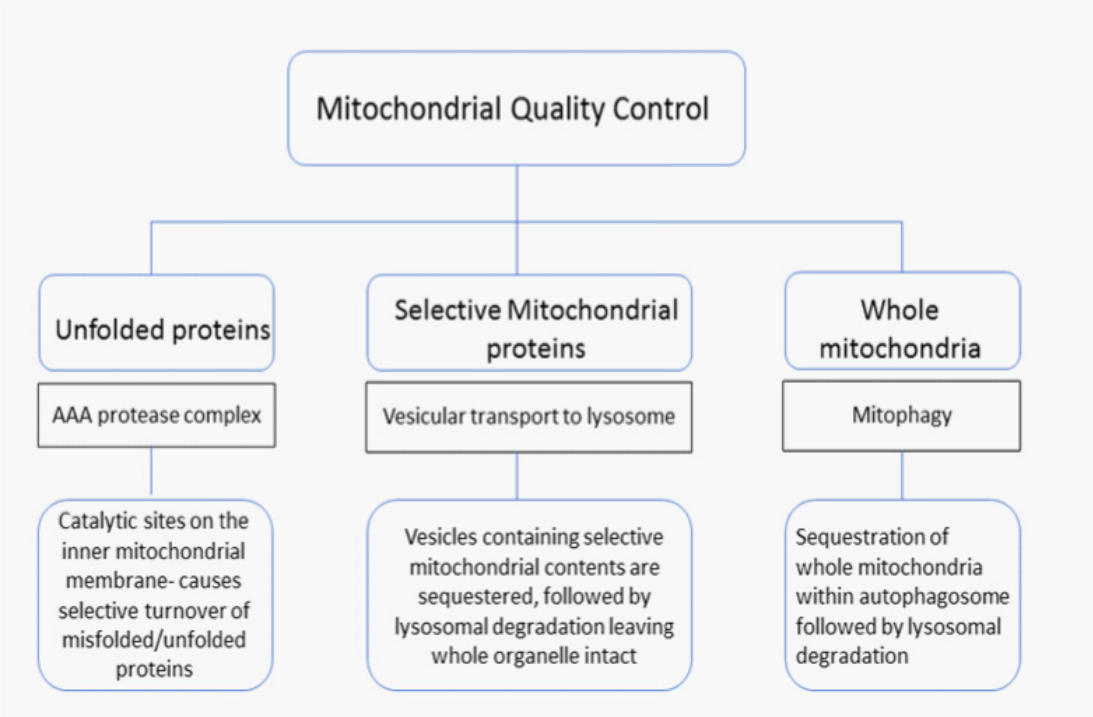


**Figure 1:** Pathways of mitochondrial quality control include unfolded/misfolded proteins degraded by AAA protease complex, Vesicular formation of mitochondrial proteins followed by lysosomal degration and finally through mitophagy involving autophagosome-lysosome formation

lysosomal pathway. This involves formation of vesicle bud from the mitochondrial tubules, which sequester selected mitochondrial components and later deliver to the lysosomes for degradation thereby leaving the whole organelle intact [55]. The above two mentioned mechanisms participate for removal of only a subset of damaged mitochondrial proteins. For the bulk degradation (entire mitochondria), a highly regulated process called mitophagy is involved which is described as follows.

### Mitophagy and its mechanisms

The word autophagy is derived from Greek word "auto" meaning self and "phagy" meaning eating. It is a physiologically conserved process wherein the homeostasis is maintained through protein degradation and turnover of damaged organelle for new cell formation. "Mitophagy" was first described by Dr. John Lemasters in 2005, who proposed the selective form of autophagy which occurs only in the mitochondria [56]. Briefly, the process involves engulfment of cytoplasmic substrates in autophagic vesicles which are then fused to lysosomes for degradation. Proper execution of mitophagy requires the mitochondria to possess or display a specific tag that is activated or modified. This process is crucial so that mitochondria destined for mitophagy are distinguished from those that are not. Several tag proteins facilitate the process of mitophagy, including phosphatase and tensin homolog (PTEN) induced putative kinase 1 (PINK1), Parkin, BCL2 interacting protein 3 (BNIP3), NIX [also known as BNIP3 like (BNIP3L)], Bcl2-like protein 13 (Bcl2-L-13), and FUN14 domain containing 1 (FUNDC1) which are described in the following sections.

The PINK1/Parkin pathway is one of the most characteristic pathway, which helps in clearing the damaged mitochondria. PINK1 is a serine/thereonine kinase that contains a mitochondrial targeting sequence allowing for its mitochondrial localization [57]. In healthy mitochondria, PINK1 enters the inner membrane through TIM/TOM complex and then cleaved by various proteases including the mitochondrial-processing protease (MPP) and the inner membrane presenilin-associated rhomboid-like protease (PARL) which results in ultimate proteolytic degradation of the mitochondria[58,59]. However, in the damaged mitochondria, there is decrease in mitochondrial membrane potential leading to stabilization of PINK1 on the outer mitochondrial membrane [60]. PINK1 phosphorylates mitofusin 2 (mfn2) and ubiquitin leading to recruitment of Parkin (E3 Ubiquitin ligase) to the outer mitochondrial membrane. Parkin polyubiquitinates several mitochondrial proteins that are then recognized by the ubiquitin-binding proteins optineurin (OPTN), p62, NDP52, and NBR1, leading to autophagosome formation which fuses with the lysosome and causes degradation of the mitochondria [61,62]. Thus, PINK1 and Parkin form the minimal machinery for recruitment of the canonical autophagy players to target organelles. Parkin-mediated mitophagy requires ubiquitin-binding adaptor protein p62/SQSTRM1 which accumulates on depolarized mitochondria and facilitate recruitment of damaged mitochondria to autophagosomes. PINK1 and Parkin has been shown to directly interact with the Beclin-1 PI3K complex [63,64]. Beclin-1 is involved in various cellular process including positive regulator of autophagy, neuronal homeostasis, apoptosis and in clearance of mutant proteins. Parkin dependent recruitment of Ambra1 (Activating Molecule in Beclin 1-Regulated Autophagy) leads to binding to LC3, formation of pre-autophagosomal membranes around damaged mitochondria and potentiate mitophagy. In contrast, AMBRA1 can induce mitophagy through Parkin independent manner [65]. Therefore, Parkin can cause either ubiquitination of the mitochondrial outer membrane proteins or recruitment of Ambra1, which contribute to mitophagy of damaged mitochondria. Parkin dependent mitophagy also involves cytoplasmic E3 ubiquitn ligase SMURF1 which play a role in delivering mitochondria to the autophagosome through its membrane targeting domain [66]. Other function of Parkin is to promote mitochondrial biogenesis through degradation of a transcriptional repressor PARIS (Parkin Interacting Substrate). PARIS represses the expression of the transcriptional coactivator, PGC-1α (master regulator of mitochondrial biogenesis) and NRF-1 by binding to insulin response sequences in the PGC-1α promoter. Loss of PARIS releases the mitochondrial biogenesis transcription factor PGC1α to activate its target genes, thereby promoting mitochondrial homeostasis [67].

Conversely, PINK1 can lead to low-level mitophagy in a Parkin-independent manner in *in vitro* mammalian systems [68]. Several other PINK1/Parkin-independent mitophagy pathways have been identified. A mitochondrial outer membrane protein, the BCL-2 homology 3 (BH3)-containing protein NIP3-like X (NIX, also known as BNIP3L), was shown to play an important role in the elimination of mitochondria in erythrocytes [69] and in the neurons [70]. NIX acts as a selective mitophagy receptor and binds to LC3 on the membranes [71]. Similarly, another mitochondrial outer membrane protein called FUN14 domain containing 1 (FUNDC1), regulates autophagic degradation of the mitochondria in response to hypoxia. FUNDC1 has a LIR (LC3 interacting region) required for recruitment of LC3 [72]. Under hypoxic conditions, this LIR is dephosphorylated by the mitochondrial phosphatase phosphoglycerate mutase family member 5 (PGAM5), thus increasing its physical association with LC3 and promoting mitophagy [73]. Cardiolipin-an inner mitochondrial membrane phospholipid externalizes to the outer membrane upon mitochondrial damage in neuronal cells with LC3 containing cardiolipin-binding sites thereby indicating neuronal mitophagy [74]. Altogether, these observations indicate that specific mitophagy receptors on the mitochondrial outer membrane play an essential role in mitochondrial degradation by recruiting autophagy machinery to mitochondria. Finally, these mitophagy receptors bind to proteins associated with nascent autophagosomes (LC3 and GABARAP family proteins covalently bound to the phagophore membrane lipid phosphatidylethanolamine) via LC3-interacting region (LIR) motifs. The formation of the protein bridges between the outer mitochondrial

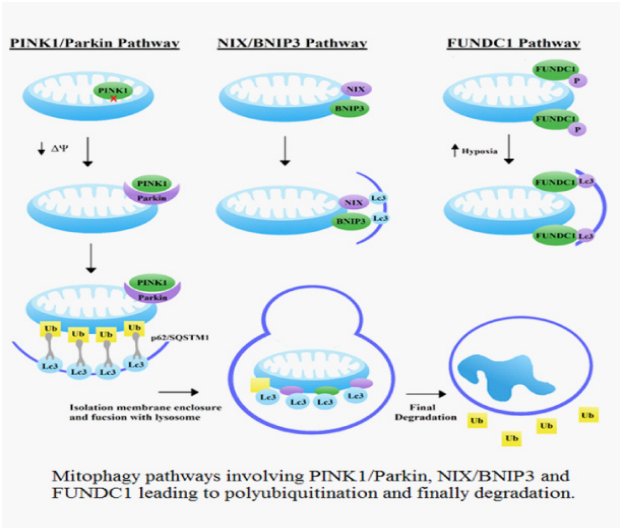


**Figure 2:** Different Mitophagy pathways

membrane and the phagophore membrane result in elongation (mediated by LC3 proteins) and closure (mediated by GABARAP proteins) of the phagophore membrane thereby completely engulfing the mitochondrion. The final stage of mitophagy is the fusion of the autophagosome with a lysosome, mediated by the phagophore LC3-binding proteins PLEKHM1, HOPS and the lysosome membrane-associated protein Rab7. Lastly, lysosomal hydrolases degrade the mitochondrion. The molecular mechanisms involved in mitophagy is depicted in Figure 2.

### Mitophagy in Neurons

Most of the signaling pathways of mitophagy described have been characterized in non-neuronal cells whereas neurons possess a distinct structural and functional activity. Mitochondrial damage and dysfunctional mitophagy have been implicated in AD [75], however it is not clear whether it is the upregulation or downregulation of mitophagy that contributes to the pathology. In addition, various studies have indicated contrasting results about the role of Pink1/Parkin pathway in neurons. Few studies have shown that depolarization of neuronal mitochondria does not recruit Parkin [76], whereas other studies indicate the role of Parkin mediated mitophagy in neurons[77,78]. Clearly, further experimentation is required to define the role of the PINK1/Parkin-mediated pathway of mitophagy in neurons. Mitochondrial biogenesis and clearance of damaged mitochondria by lysosomes occurs in the soma of neurons; this poses a unique problem for mitochondrial turnover because most mitochondria are located within the dendritic process and terminal axons [79]. The scarcity of lysosomes in axons further supports this idea that mitochondrial degradation occurs in the soma. As a result, organelles must be delivered by axonal transport to the soma for degradation. Mitochondrial turnover and elimination is an essential process in the protection of neurons against oxidative damage and degeneration. The life cycle of dysfunctional axonal mitochondria is largely unknown. For instance, it is unclear if these damaged mitochondria are transported to the soma for autophagic degradation. As well, the concept of retrograde movement of depolarized mitochondria remains controversial [80]. Mitochondrial damage that results in the destruction of Miro (Mitochondrial Rho GTPase) and arrests mitochondrial movement causes dysfunction in the PINK1/Parkin pathway [77]. If retrograde transport is necessary for the completion of mitophagy, then it is unknown at which stage the damaged mitochondria are transported from the axons. Sequestering the damaged organelle within an autophagosome before translocation to the soma may be beneficial to the neuron; the retrograde transport of the autophagosome would be independent of Miro [81]. Evidence of autophagic markers within the axons further suggests that autophagosomes can form in neuronal processes [82]. Further support of this concept is based on distal formation and retrograde transport of autophagosomes in primary neuronal axons, with mitochondrial markers also being observed [83]. Conversely, lysosomal markers have been identified as colocalizing with autophagosomes in distal axons, suggesting that lysosomal degradation is possible outside the soma. It is relevant to note that the previously mentioned studies focus on the total population of autophagosomes, instead of the mitophagosomes created in response to damage. The contribution of mitochondrial dysfunction to neurodegenerative diseases like AD will be better understood once the dynamics of damaged neuronal mitochondria and the molecular players of neuronal mitophagy have been determined.

### Mitophagic alterations in AD

Recent studies propose that neurons affected in AD experience mitochondrial dysfunction and a bioenergetic deficit that occurs early and promotes the disease-defining amyloid beta peptide (Aβ) and Tau pathologies [84]. For the process of mitophagy to occur, the mitochondrion within the autophagosome undergoes fusion with a lysosome to form an autolysosome in which proteases degrade the mitochondrion. A characteristic finding noted in neurons of AD is the abnormal accumulation of autophagosomal vacuoles due to lysosomal dysfunction possibly secondary to dysregulation of neuronal $Ca^{2+}$ homeostasis [85,86]. Hippocampal CA1 neurons of AD patients showed upregulation of autophagy related genes, increased lysosomal biogenesis (activation of TFE3 transcription factor and several other target genes), accumulation of LC3-II and p62 in autolysosomes indicating impaired autophagic flux [87]. In addition, levels of cathepsin D (primary protease in lysosomes), LAMP-1 (component of lysosomal membranes) and ubiquitylated proteins increase in exosomes of AD and pre-AD patients indicating compromised lysosomal function and accumulation of undegraded substances in the neurons [88]. Hence, mitophagy is stimulated secondary to mitochondrial dysfunction while lysosome function is impaired thereby contributing to the prominent accumulation of autophagosomes in neurons in AD.

Dysfunctional fusion between autophagosomes and lysosomes contribute to compromised mitophagy in AD. For instance, oxidative stress which is associated with AD, leads to autophagosomes accumulation in mouse corticalneurons [89]. Studies in the brain of the AD triple transgenic mice model (3xTg) showed significant increase in LC3-II levels and LC3II:LC3-I ratios indicating accumulation of autophagosomes in the neurons due to dysfunctional clearance through lysosomal pathway [90,91]. Impaired mitophagy is characteristic of mutations in PS1 gene. Remarkably, wild-typePS1 is required for lysosomal acidification and familial AD possessing PS1mutations result in lysosomal alkalization and reduced lysosomal hydrolase activity [92]. Neurons of AD patients and *in vitro* cell lines overexpressing APP mutation show increase in parkin translocation to mitochondria, autophagosome accumulation and lysosomes with undigested mitochondria indicating that autophagosome accumulation might represent deficient lysosomal activity [93]. These findings indicate that defective mitophagy might predispose to pathogenesis of AD.

Alterations in proteins involved in mitochondrial fission and fusion (Drp1 and mitofusin), mitochondrial biogenesis (PGC-1α) and mitochondrial stress responses (SIRT3 and SIRT1) are noted in AD [94,95]. Mitochondrial fission and fusion events are important processes in pathophysiological situations in different cells and tissues where they remove damaged and dysfunctional molecules from the mitochondria. Some of the specialized proteins controlling these events include Fis1, Drp1 [96,97]and Opa1 [98]. The amount of each protein present determines the amount of fission and fusion occurring. Before fission, damaged DNA and proteins are segregated to one side of the mitochondrion such that only one of the 'daughter' mitochondria contain damaged molecules and is targeted for mitophagy while the other daughter mitochondrion is normal [99]. Studies have shown that dysfunction of mitochondrial fission proteins results in excessive mitochondrial fragmentation, damage to regions of neuronal communication, synaptic injury, and eventual neuronal death [100-102]. Mitochondrial biogenesis refers to formation of new mitochondria from the existing ones. Genes regulating mitochondrial biogenesis (PGC-1α, TFAM, NRF2, and TFEB) have been shown to be downregulated in postmortem

brains of AD patients when compared with age-matched control subjects indicating that these genes have inverse relationship to AD pathogenesis [103]. Consistent with the result, APP23 transgenic mice injected with lentiviral vector (LV)-hPGC-1α into the hippocampus and cortical areas showed decreased Aβ plaques and improved spatial and recognition memory, probably due to downregulation of β-secretase [104].

Sirtuins are a group of $NAD^+$-dependent deacetylases that regulate energy metabolism, stress response and hence implicated in wide variety of metabolic and age-related diseases including AD. Out of the seven sirtuins identified, SIRT1 located in the nucleus and SIRT3 in the mitochondria are associated with neuroprotection. Decreased SIRT1 protein expression in the brain of AD patients has been associated with Aβ and pTau accumulation [105]. By up regulating PGC-1α, SIRT1 induces autophagy/mitophagy by activating various autophagy proteins (ATG5, ATG7, ATG8/LC3), stabilizing PINK1 and increasing mitophagy proteins Nix/BNIP3L and LC3 [106, 107].SIRT3 deacetylates FOXO3-a subclass of the fork head family of transcription factors important in regulating cellular stress and longevity. This SIRT3 mediated effect on FOXO3 induces clustering of p62 (a major autophagy protein) on ubiquitinated mitochondrial substrates and the formation of autolysosomes [108]. SIRT3 also protect mitochondria and neurons against excitotoxic and metabolic stress and apoptosis by a SOD2 and cyclophilin D deacetylation-dependent mechanism [95]. Neurons of APP/PS1 double mutant AD mice show reduced SIRT3 levels [106,107]. Diminished SIRT1 and SIRT3 activity can cause mitochondrial dysfunction and mitophagy inhibition, leading to accumulation of damaged mitochondria. Hence reduced levels of these sirtuins might contribute to pathogenesis of neurodegenerative diseases like AD.

$NAD^+$ is a cellular metabolite, which is important for biochemical pathways including glycolysis, TCA cycle, oxidative phosphorylation and ATP production. Additionally, it plays a vital role in maintaining mitochondrial health and biogenesis, stem cell turnover, neuroplasticity and neuronal stress resistance [111]. Proper functioning of the neurons depends on homeostasis between mitochondrial biogenesis and mitophagy through the NAD+/SIRT1–PGC-1α pathway and the DAF-16/FOXO3 pathway as described earlier. When $NAD^+$ levels are diminished, mitophagy is compromised, resulting in the accumulation of misfolded proteins and subsequent neuronal death [112]. $NAD^+$-dependent enzymes like poly (ADP-ribose) polymerase-1 (PARP1), cADP-ribose hydrolase (CD38) and CD157 are also involved in neuronal stress response [113]. PARP-1 is an enzyme which play a vital role in repair of DNA breaks using NAD+ as a cofactor. Increase in the level of PARP1 activity along with proteins that are PARylated (PolyADPribosylated) for example Electron Transport Chain (ETC) accumulate in brain of AD patients which might be due to the effect of oxidative stress [114-116]. CD38 is an enzyme that catalyzes the synthesis and hydrolysis of cADP-ribose (cADPR) from $NAD^+$ to ADP-ribose and facilitates $Ca^{2+}$ release from the endoplasmic reticulum. Transgenic mice with APP/PS1 mutation and lacking CD38 show reduced Aβ levels and improved learning and memory [117]. PARP1 activation due to oxidative stress causes $NAD^+$ depletion, which contribute to decreased sirtuin activity, decreased mitophagy and mitochondrial dysfunction. This suggests that $NAD^+$ up-regulation can reverse the impaired brain-energy metabolism and possibly oxidative stress that are implicated in cognitive decline.

Evidence from human and animal models of AD has shown that unrepaired oxidative damaged nuclear and mitochondrial DNA occurs early in the disease process. The two major DNA repair mechanisms in the neurons i.e. base excision repair (BER) and DNA double-strand break repair are compromised in AD [118]. Chronic elevation of PARP1 seen in AD might reflect defective DNA repair pathways described above [119]. Neuronal BER requires a key enzyme-DNA polymerase β (Polβ) and its haplo-insufficiency leads to increased hippocampal neuronal death in a triple transgenic AD mice model [116]. Furthermore, Down's syndrome which predispose to AD is also associated with reduced Polβ levels. Hence, these DNA damage induced neurodegeneration may be due to impaired mitochondrial function caused by inhibition of $NAD^+$/sirtuin–PGC-1α pathway. Investigation into the connection between DNA repair deficiency and mitophagy/mitochondrial dysfunction in AD is a promising frontier for future research.

### Therapeutics to enhance mitophagy for AD

As the initiation and progression of various neurodegenerative diseases involve mitochondrial dysfunction and dysfunctionalautophagy/mitophagy, therapeutic avenues targeting mitophagy may have broad translational applications. Non-pharmacological approaches like caloric restriction, intermitting fasting, and exercise have been shown to reduce mitochondrial oxidative stress, stimulate mitochondrial biogenesis resulting in the enhancement of autophagy/mitophagy [120,121]. For example, more autophagosomes were noted in cerebral cortical neurons after GFP-LC3 transgenic mice could fast for 24-48 hours [122]. Similarly, neuroprotective effects of exercise by increased SIRT3 expression in hippocampal and cortical cells were noted in mice, which were made to run on wheels [95]. One of the proposed mechanisms by which exercise and fasting stimulate mitochondrial biogenesis in neurons is through BDNF signaling and upregulation of PGC-1α [94]. Pharmacological methods to induce mitophagy include bioactive natural and synthetic compounds, such as antibiotics and plant metabolites. Actinonin, a naturally occurring antibiotic increases mitophagy in neuronal stem cells derived from the mt-Keima mouse, through inducing a specific mitochondrial ribosomal and RNA decay pathway in cells. However, the connection between this pathway and mitochondrial quality control remains to be completely elucidated [123,124]. Similarly, Urolithin A, a derivative from pomegranate fruit has been shown to induce mitophagy both *in vitro* and *in vivo*. For example, Urolithin A extends the lifespan of C. elegans and improves muscle function in mice through mitochondrial maintenance via upregulation of mitophagy though the molecular mechanisms have not been investigated [125]. A different set of compounds called sirtuin-activating compounds (resveratrol and metformin) have been shown to increase mitophagy [126, 127]. Improving sirtuin activities (SIRT1 and SIRT3) can also be through elevating cellular NAD+ levels. Treatment with nicotinamide, a precursor of NAD+ ameliorated Aβ, Tau pathologies, learning and memory deficits in triple transgenic AD mice [90]. Additionally, nicotinamide increased mitochondrial resistance to oxidative stress, upregulated autophagy, increased the activities of PI3K–Akt, MAPK/ERK1/2, SIRT1 and the transcription factor CREB. Reduction in PARP-1 levels along with markers of oxidative stress and increased endogenous antioxidant enzyme activity were noted with nicotinamide treatment in a rat model of Aβ neurotoxicity [128]. Moreover, treatment of APP-mutant transgenic mice with nicotinamide riboside, an $NAD^+$ precursor, elevated levels of $NAD^+$ and PGC-1α in the cerebral cortex and ameliorated cognitive deficits[107, 129]. Hence, interventions that increase neuronal $NAD^+$ levels may be a potential thera-

peutic target for AD patients. Mitochondrial uncoupling agents such as DNP stimulate autophagy and preserve neuronal function in animal models of AD [130].

Treatment of mice with 2-deoxyglucose increased mitochondrial function, stimulated autophagy and reduced the pathologies in a mice model of AD through inducing mild bioenergetic stress and stimulation of ketogenesis[131]. Agents that induce mild bioenergetics stress or inhibit the mTOR pathway may induce mitophagy. Finally, the mTOR inhibitor rapamycin with autophagy/mitophagy-inducing activity ameliorated cognitive deficits and reduced Aβ pathology in an APP-mutant mouse AD model[132]. Jointly these outcomes make accessible a justification for upcoming challenging of compounds that induce mitophagy, in preclinical AD models.

## Summary

Mitochondrial dysfunction is an early feature in the pathogenesis of AD much before the discernible cognitive deficits are noted. Impaired mitophagy can promote Aβ and pTau pathology, which in turn can cause mitochondrial dysfunction and defective mitophagy leading to synaptic dysfunction and neuronal death in AD. Various interventions that stimulate mitophagy can preserve neuronal homeostasis, thereby preventing the cognitive deficits. Further research into the molecular mechanisms of compromised mitophagy in various *in vitro* and *in vivo* AD models are necessary and may provide novel therapeutic strategies for this widespread global disease.

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