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Review Article

Mitochondrial Dysfunction and Mitophagy in Neurodegenerative Diseases

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Abstract

Mitochondria are critical in providing energy for neuronal development. They provide the majority of intracellular energy and perform important metabolic functions such as the Krebs cycle. Mitochondria contain their own mitochondrial DNA in a circular form, similar to bacterial genomes. Mitochondrial genomes encode several essential genes of the eukaryotic respiratory machinery, but most respiratory machinery components and factors controlling mitochondrial biogenesis are encoded in the nucleus. Mitochondria and the nucleus cooperate and communicate via retrograde signals, such as energy supply and redox signaling. This poorly understood communication is essential for balancing intracellular energy production and demand. Mitochondrial mutations could lead to dysfunctions in ATP production, calcium homeostasis, reactive oxygen species generation, and apoptotic signaling. Thus, mitochondrial dysfunction has been reported and discussed as part of neurodegenerative etiologies. There is no doubt that mitochondrial dysfunction, abnormal mitochondrial dynamics, and mitophagic degradation occur in neurodegenerative diseases.

Mitochondrial turnover maintains cellular homeostasis by eliminating defective mitochondria through a specific form of autophagy, an evolutionarily conserved eukaryotic response to stress conditions by which lysosome contents are used to breakdown cytoplasmic proteins and organelles. Both number of 'healthy' and 'mutated' mitochondria could be increased or decreased by fusion and fission. Selective uptake of mitochondria by autophagosomes is called mitophagy. Mitophagic events are highly selective processes controlled by oxidative stress and are accompanied by loss of membrane potential and ensuing mitochondrial degradation. This review discusses the role of mitochondria in neurodegenerative diseases. This review also explores the connection between neurodegeneration and mitophagy, a highly selective autophagic process of oxidative stress-induced mitochondrial degradation. It will further discuss the role of fusion and fission processes in maintaining homeostasis.

Abbreviations: $\Delta\psi$: Membrane Potential; $\rho°$: Rho Zero; Aβ: β Amyloid; AβPP: Aβ Precursor Protein; AD: Alzheimer's Disease; ADHD: Attention Deficit Hyperactivity Disorder; ARJP: Autosomal Recessive Juvenile- Onset Parkinsonism; ATP: Adenosine Triphosphate; CCCP: Cyanide *m*-Chlorophenyl Hydrazone; COX: Cytochrome c Oxidase; DRP1: Dynamin-Related Protein 1; *D-loop*: Displacement Loop; EB: Embryoid Body; ESC: Embryonic Stem Cell; ETC: Electron Transport Chain; FIS1: Fission 1 (mitochondrial outer membrane) Homologue; Gfer: Growth Factor erv1-like; GTP: Guanosine Triphosphate; HMG: High Mobility Group; HSP: Heavy Strand Promoter; LSP: Light Strand Promoter; NADH: Nicotinamide Adenine Dinucleotide; MFN1: Mitofusin 1; MFN2: Mitofusin 2; MPP+: 1-Methyl-4-Phenylpyridinium; mtDNA: Mitochondrial DNA; NFT: Neurofibrillary Tangles; OPA1: Optic Atrophy 1; OXPHOS: Oxidative Phosphorylation; PD: Parkinson's Disease; PINK1: Phosphatase and Tensin Homolog-Induced Putative Kinase 1; PMF: Proton Motive Force; ROS: Reactive Oxygen Species; rRNA: Ribosomal RNA; SNP: Single Nucleotide Polymorphisms; TFAM: Transcription Factor A; tRNA: Transfer RNA

Introduction

Neurodegenerative diseases are a heterogeneous group of neurological illnesses with degenerative or genetic etiologies. There are currently no effective treatments for diseases that cause progressive losses of memory and motor control. Alzheimer's disease (AD) and Parkinson's disease (PD) are common examples of neurodegenerative diseases (Table 1). Together, these results suggest that neurological disorders in general are associated with mitochondrial dysfunctions."

People with AD experience memory loss and other cognitive dysfunctions such as impaired movement, reasoning, and judgment. AD progressively damages various brain areas, leading to increasingly severe symptoms. It affects millions of people worldwide. Most cases of AD occur sporadically, although certain genes increase susceptibility. AD is characterized by cerebral β-amyloidosis in the form of β-amyloid (Aβ) plaques and tauopathy in the form of neurofibrillary tangles (NFTs), neuritic plaques, and neuropil threads. Familial AD represents the minority of AD cases and is caused by mutations in genes encoding the Aβ precursor protein (AβPP), presenilin 1 or presenilin 2. The pathological hallmarks observed in AD brains at autopsy are intracellular NFTs and extracellular senile plaques in the neocortex, hippocampus, and other subcortical regions essential for cognitive function. NFTs are formed from paired helical filaments composed of neurofilaments and hyperphosphorylated tau proteins. Senile plaques are formed mostly from the deposition of Aβ that results from AβPP cleavage. Elevated tau levels in the cerebrospinal fluid have long been recognized as a marker of dementia. Tau is typically found clumped in the hippocampus but can spread into other areas, especially the lateral temporal and parietal lobes. There is currently no early detection method for amyloid and tau buildup that could assist in preventing or curing AD. Delaying the onset of symptoms by 10-15 years would make a significant difference to AD patients, their families and caregivers, and the global economy. During the course of the disease, Aβ plaques and NFTs lead to the loss of nerve cell connections and eventually to the death of nerve cells and brain tissues. Moreover, studies have strongly indicated that mitochondrial dysfunction and oxidative stress occur in AD-affected brain regions [1,2].

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Neurodegenerative disease	Major associated protein	Impact
Alzheimer's disease	β-Amyloid	– Disrupts DNA repaired system, alters calcium homeostasis, and reduces cytochrome c oxidase activity.
Parkinson disease	Parkin	– Causes defective oxidative phosphorylation complex assembly, increased oxidative stress and disruption of mitochondrial morphology.
Amyotrophic lateral sclerosis	Superoxide dismutase 1	– Increases oxidative damage to mitochondrial DNA and decreases mitochondrial membrane potential
Huntington disease	Huntingtin	– Reduces respiratory chain complex function, generates reactive oxygen species and alters mitochondrial activity.

Table 1: Neurodegenerative diseases associated with mitochondrial dysfunction.

It has been shown that oxidative damage occurs before Aβ plaque formation, supporting a causative role of mitochondrial dysfunction and oxidative stress in AD [3,4]. For example, an increase in intracellular Aβ and oxidative stress was observed in neurons exposed to hydrogen peroxide [5,6]. Moreover, cytochrome oxidase impairment *in vitro* leads to increased expression of AβPP C-terminal fragments that contain the Aβ peptide [7] and decreased non-amyloid genic processing of AβPP [8]. This suggests that oxidative damage may lead to cross-linking and impaired solubility of Aβ [9]. Oxidative injury studies have also shown that oxidation of critical cysteine residues seems to be associated with the aggregation of tau proteins into paired helical filaments [10]. Additionally, reduced adenosine triphosphate (ATP) generation leads to activation of extracellular signal-regulated kinases 1 and 2, which phosphorylate tau proteins into a paired helical filament-like state similar to that seen in AD [11].

PD is another common neurodegenerative disorder characterized by the loss of dopaminergic neurons. The core symptoms of PD consist of the triad of resting tremor, bradykinesia, and rigidity. PD is caused by the loss of pigmented dopaminergic neurons in the substantia nigra and the presence of abnormal protein aggregates called Lewy bodies, which are cytoplasmic eosinophilic inclusions composed of the presynaptic protein α-synuclein. Studies have reported mitochondrial dysfunction in PD.

This review is mainly devoted to discussing the evidence that mitochondrial dysfunction and oxidative stress are heavily involved in AD pathogenesis. Furthermore, the interplay between mitochondrial abnormalities and autophagy in AD will be addressed. Finally, I will briefly discuss autophagy as a potential therapeutic target in AD and PD.

Mitochondria

Mitochondria are dual-membrane organelles found in all eukaryotic cells except erythrocytes and lens tissues. The mitochondrion is composed of the outer mitochondrial membrane, the intermembrane space, the inner mitochondrial membrane, the cristae space (formed by in folding of the inner membrane), and the matrix (the space within the inner membrane) (Figure 1).

The human mitochondrial genome consists of a 16.5 kb double-stranded circular DNA molecule that contains both non-coding and coding regions. The largest non-coding sequence in mammalian mtDNA is the displacement-loop (D-loop), which contains promoters and replication origins. The coding regions contain 37 genes, including 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and 13 genes that encode subunits of respiratory complexes I, III, IV and V (Figure 2) [12]. Alkaline gradient centrifugation separates mtDNA into a heavy strand and a light strand based on differential contents of guanosine and cytosine. The heavy strand encodes 2 rRNAs, 12 polypeptides and 14 tRNAs, while the light strand encodes 1 polypeptide and 8 tRNAs. The mitochondrial genetic code is similar to the universal genetic code, with 3 exceptions: (1) the TGA codon codes
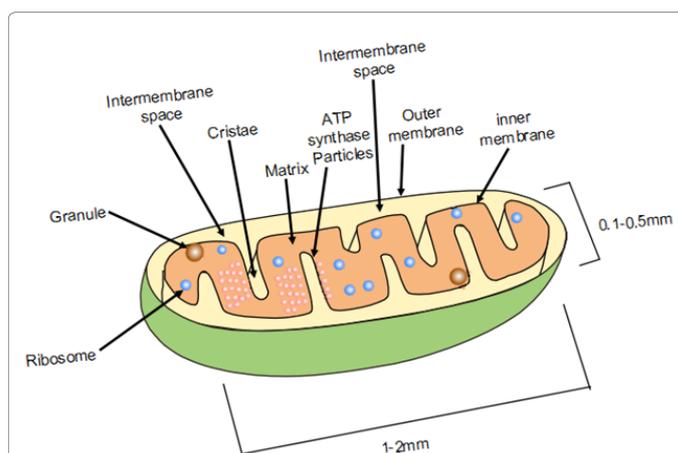
Figure 1: Structure of a mitochondrion.
This figure is modified from [111]
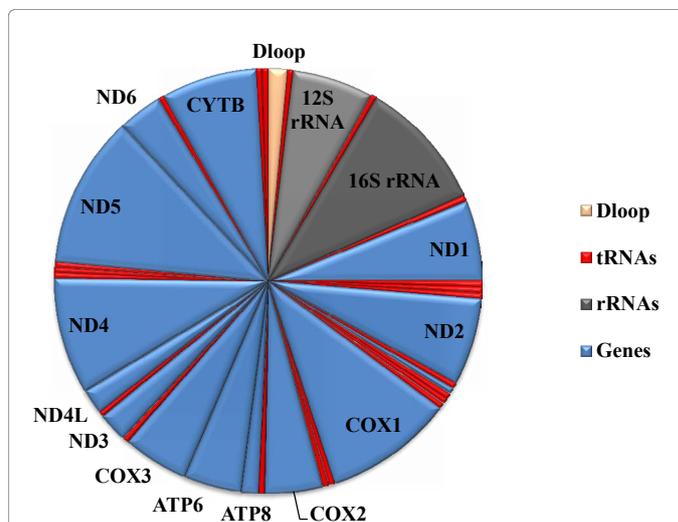
Figure 2: Schematic representation of the human mitochondrial genome.

mtDNA is a double-stranded circular molecule of approximately 16.5 kb, consisting of a heavy strand and a light strand. mtDNA consists of non-coding and coding regions. The non-coding *D-loop* region contains the origin of heavy strand replication and the transcription promoters for both strands. The origin of light strand replication is located approximately two-thirds of the way around the genome (at base pairs 16024-16569 and 1-576). The coding region encodes 13 protein subunits of the electron transport chain, 12 on the heavy strand and 1 on the light strand, along with 22 tRNAs and 2 rRNAs that are necessary for mtDNA transcription and protein synthesis. Proteins encoded by mtDNA, indicated in yellow, include ND1, 2, 3, 4, 4L, 5 and 6 (NADH dehydrogenase; Complex I); CYTB (cytochrome b; Complex III); COX1, 2, and 3 (cytochrome c oxidase; Complex IV); and ATP6 and 8 (ATP synthase; Complex V)

Abbreviations: mtDNA: Mitochondrial DNA; tRNA: Transfer RNA; rRNA: Ribosomal RNA; NADH: Nicotinamide Adenine Dinucleotide; ATP: Adenosine Triphosphate

for tryptophan instead of stop, (2) AGA and AGG code for stop instead of arginine, and (3) ATA codes for methionine instead of isoleucine [13]. It is thought that mitochondria originally derived from endosymbiotic prokaryotes because they share many features. For example, the circular mitochondrial genome is very similar to bacterial genomes [14].

Mitochondrial respiratory activity occurs via the electron transport chain (ETC). The ETC is composed of 5 complexes: Complex I (nicotinamide adenine dinucleotide dehydrogenase), Complex II (succinate dehydrogenase), Complex III (cytochrome reductase), Complex IV (cytochrome c oxidase, also called COX) and Complex V (ATP synthase). Energy obtained from electron transfer through these complexes is used to pump protons from the mitochondrial matrix into the intermembrane space. The net movement of positive charge across the inner membrane creates the membrane potential ($\Delta\psi$). The sum of $\Delta\psi$ and the pH gradient is the proton motive force (PMF), which accumulates across the mitochondrial inner membrane. The PMF allows Complex V to transfer protons back into the matrix to generate ATP from adenosine diphosphate and inorganic phosphate. As electrons flow through the complexes, they eventually reach Complex IV, where they are used to generate water from hydrogen ions and molecular oxygen (Figure 3) [15].

mtDNA Mutations

Mitochondrial diseases often result from mtDNA mutations and represent common inherited conditions. One out of every 7,634 newborns is affected by mitochondrial dysfunction (Table 2) [16]. Mitochondrial diseases are a heterogeneous group of disorders in which mitochondrial dysfunction can affect tissues with varying severity. Mitochondrial diseases most prominently affect cerebral neurons and skeletal muscles due to their high energy demands. The effects on cerebral neurons are especially severe during energy-intensive neurodevelopment.

Within mtDNA, several types of mutations have been identified, including point mutations, large-scale rearrangements, and reductions in mtDNA copy numbers, the last of which leads to mtDNA depletion [17], which is an autosomal recessive trait that can cause severe illnesses

Complex Name	Complex I NADH Dehydrogenase (NADH)	Complex II Succinate Dehydrogenase	Complex III Cytochrome Reductase (CYTB)	Complex IV Cytochrome C Oxidase (COX)	Complex V ATP synthase (ATPase)
mtDNA Subunits	7	0	1	3	2
nDNA Subunits	>18	4	8	10	10
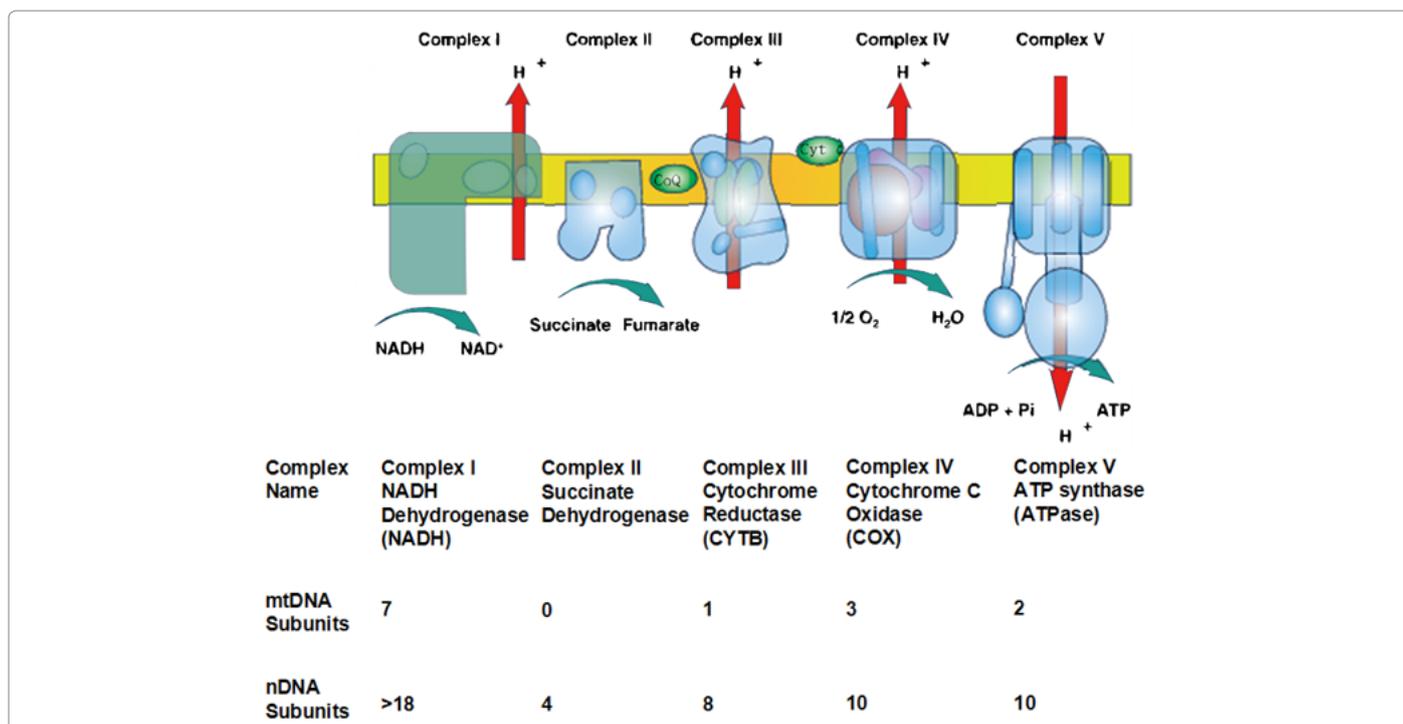
Figure 3: Stylized representation of the electron transport chain (ETC), the final stage of cellular respiration, where oxidative phosphorylation takes place.

The ETC consists of 5 protein complexes that contain polypeptides encoded by the nuclear and mitochondrial genomes (except for Complex II). The flow of electrons through the first 4 complexes releases protons (H+) from the mitochondrion and establishes the mitochondrial membrane potential. The transfer of these protons back into the mitochondrion through Complex V generates ATP. This figure is modified from [17]

Abbreviations: CoQ: Coenzyme Q; Cyt C: Cytochrome C; ANT: Adenosine Nucleoside Transporter; ADP: Adenosine Diphosphate; ATP: Adenosine Triphosphate; mtDNA: Mitochondrial DNA; nDNA: Nuclear DNA; P$_i$: Inorganic Phosphate

Syndrome and features	Mutations in mitochondrial genome	Genetics
Leigh syndrome: Neonatal subacute encephalopathy with bilateral symmetric midbrain and basal ganglia necrosis	tRNAs (Val, Trp), *MTATP6, MTCO3, MTND4, MTND5, MTND6*	Autosomal recessive, mitochondrial DNA (mtDNA), X-linked
Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)	tRNAs (Phe, Val, Leu, Gln, Trp, Ser, Ile), *MTND1, MTND4, MTND5, MTND6, MTCYB, MTCO3*	mtDNA
Myoclonic epilepsy with ragged-red fibres on muscle biopsy (MERRF)	tRNAs (Lys, His, Phe, Leu, Val)	mtDNA
Autism and attention deficit-hyperactivity disorder	tRNA (Leu, Lys), rearrangement in mtDNA	mtDNA
Alzheimer's and Parkinson's diseases	rRNA (16s), tRNAs (Gln, Trp) *MTND1, MTND2, MTCYB*	mtDNA

Source: MITOMAP

Table 2: Recognizable syndromes of mitochondrial dysfunction.

including neurodegenerative disorders [17,18]. Profound reductions in mtDNA are responsible for a series of syndromes that are collectively referred to as mtDNA-depletion syndromes [19]. Patients with these syndromes present with myopathies, including mitochondrial neurogastrointestinal encephalomyopathy syndromes. A patient suffering from mtDNA depletion exhibited altered TFAM levels, while mtDNA-deficient cultured cell lines exhibited greatly reduced TFAM levels [20]. Pathologies associated with these syndromes vary widely and can manifest pre- or post-natally. To study the consequences of mtDNA dysfunction, researchers have developed animal and cellular models with mitochondrial mutations, deletions, or depletion.

The mitochondrial dysfunction symptoms caused by mtDNA point mutations or deletions or by mitochondrial depletion can be observed in animal and cellular models. Several studies have described mice with abnormal mitochondria resulting from mtDNA mutations or deletions. To demonstrate the importance of mitochondrial morphology and function in cell-specific functions, my colleagues and I [21] established mitochondria-depleted cells called rho-zero cells (ρ°), which are mtDNA-depleted and generated *in vitro* through the application of different drugs, such as ethidium bromide, antibiotics, or nucleoside analogue reverse transcriptase inhibitors (a class of anti-HIV drugs). ρ° cells exhibit several common features: (1) they become autotrophic, relying on pyrimidine (uridine) and pyruvate supplementation for cell growth [22,23]; (2) they have low mtDNA copy numbers and reduced expression of mitochondrial-encoded genes but not nuclear-encoded genes; (3) they have low mitochondrial respiratory chain complex activity, with the exception of complex II; (4) they have low ATP concentrations and respiration rates; (5) they shift from aerobic to anaerobic metabolism if given supplemental pyruvate; and (6) they have an immature mitochondrial structure with a circular morphology, loss of tubular structure, and fewer cristae membranes.

Several studies have found that upon a reduction in oxygen consumption, antioxidants can reverse the increased ROS production in ρ° cells. In addition, ρ° cells have decreased levels of cell proliferation, mitotic cyclin gene expression, cyclin-dependent kinase inhibitors, retinoblastoma 1 phosphorylation and telomerase activity [24,25]. Researchers have observed that relative to control cells, ρ° cells exhibit

greater expression of mitochondrial biogenesis-related genes [26,27]. Moreover, they exhibit a normal distribution of cytochrome c within mitochondria during staurosporine-induced apoptosis (in spite of low mtDNA levels and respiratory function deficiencies). Consistently, caspase 3 activation and DNA fragmentation are unaffected in ρ° cells. The localization of nuclear factor kappa B is shifted towards the nucleus rather than the cytoplasm, which might be related to the observed resistance to apoptosis. Moreover, more of the mass in ρ° cells is associated with lysosome and peroxidation production [27-29]. Remarkably, the differentiation of SH-SY5Y neuroblastoma cells into neuron-like cells is not affected by defective mitochondria, as indicated by the presence of long neurites and secretory granules, which are typical of differentiating neuroblastoma cells [30]. These studies have suggested that ρ° cells have increased resistance to apoptosis.

Several transcription factors play important roles in mitochondrial biogenesis. One of them is mitochondrial transcription factor A (TFAM), which controls the mtDNA copy number [31] and stimulates mtDNA transcription [32]. TFAM (also known as mtTFA, mtTF1, TCF6 and TCF6L2) was initially characterized as a monomeric 25 kDa protein comprising a signal peptide (mitochondrial leading peptide) and two high-mobility group (HMG) motifs that are highly conserved across species (Figure 4) [33-35]. Abf2p, the yeast homologue, has features similar to the human variant but lacks the C-terminal tail, which is essential for transcription activity. If added to Abf2p, the tail converts the protein into a transcriptional activator [33]. Abf2p does not have a role in transcription but packages mtDNA. This has led many to speculate that TFAM might also have DNA-packaging activities [36].

The HMG motifs in TFAM recognize specific mtDNA regulatory sequences and bind upstream of the light- and heavy-strand promoters of the mitochondrial genome in a non-specific fashion [37,38] while facilitating the synthesis of RNA primers that are necessary to initiate mtDNA replication and transcription [39]. TFAM has a higher affinity for DNA near the light strand promoter (LSP) than for DNA near the heavy strand promoter (HSP) and stimulates transcription to a much greater extent from the LSP than from the HSP *in vitro* [40,41].

Early *in vitro* transcription studies dissecting the human

Figure 4: TFAM alignment across a wide range of species.

The image shows the N-terminal region, HMG boxes 1 and 2, linker region, and the C-terminal tail of TFAM in humans, mice, rats, and yeast. The amino acid residues conserved in all 4 species are indicated in red. The amino acid residues conserved in 3 species are indicated in blue. The dashes indicate sequence gaps due to different amino acid compositions

Abbreviations: Human: *Homo sapiens*; Mouse: *Mus musculus*; Rat: *Rattus norvegicus*; Yeast: *Saccharomyces cerevisiae*; TFAM: Transcription Factor A; HMG: High Mobility Group

mitochondrial transcription machinery showed that, in addition to mitochondrial RNA polymerase, several transcription factors were necessary for specific transcription initiation from mitochondrial promoters [34]. Low levels of TFAM are sufficient to recruit other mtDNA replication factors to increase the mtDNA copy number [19,42]. TFAM is necessary for mtDNA maintenance during development [43]. Its non-specific DNA binding activity shows that TFAM is involved in the transcriptional regulation of mtDNA.

Studies in mammals have reported that for every 1 TFAM molecule, there are between 15-20 bp and 35-50 bp [37] of mtDNA. Additionally, several studies have shown that mtDNA is organized in a chromatin-like higher-order structure, of which TFAM might be an integral part [42,44,45]. Studies have indicated that TFAM stabilizes mitochondrial chromatin and contributes to maintaining chromatin integrity.

TFAM's essential role in mtDNA maintenance during embryonic development is well documented [43]. Several studies have described mouse models of TFAM depletion and knockout. Larsson et al. [43] were the first to establish a TFAM knockout mouse model. The heterozygous knockout mice exhibited cardiac respiratory chain deficiency and reduced mtDNA copy numbers in all tissues, while homozygous knockout mouse embryos did not develop beyond day 10.5 (E10.5) because of severe mtDNA depletion and the absence of ETC function. Homozygous knockout mouse embryos exhibited numerous enlarged mitochondria with abnormal cristae and severe mtDNA depletion accompanied by the absence of OXPHOS. TFAM-deficient embryos were smaller than littermate controls on E8.5 and had delayed neural development, no optic discs, no recognizable cardiac structures, and indistinct somites [43]. These findings indicate that TFAM is critical for controlling mtDNA copy numbers during development.

TFAM has also been specifically knocked out in dopaminergic neurons, generating several transgenic mouse strains [46,47] including 1 termed "MitoPark" [48]. In early adulthood, MitoPark mice exhibit a PD phenotype with progressive impairment of motor function. They developed progressive symptoms that replicated many of the clinical features of PD, such as bradykinesia, rigidity and abnormal gait. The behavioral disturbances correlated with a slow degeneration of the nigrostriatal dopaminergic pathway. Dopaminergic neuron losses initially occurred primarily in the substantia nigra pars compacta and later extended to the ventral tegmental area. Many surviving dopaminergic neurons displayed abnormal morphology and contained α-synuclein and ubiquitin-immunoreactive inclusions, similar to the Lewy bodies seen in PD. This phenotype was accompanied by reduced mtDNA expression and respiratory chain defects within midbrain neurons. Intraneuronal inclusions also developed, and the cells eventually died [47,48]. These findings suggest that mitochondrial dysfunction plays a role in PD. Moreover, studies have shown a relationship between TFAM and AD. Clinical studies have shown that single nucleotide polymorphisms (SNPs) in TFAM (SNP rs1937, coding for S12T in the mitochondrial signal sequence) are associated with late-onset AD [49,50]. Collectively, these studies show that TFAM is important for mtDNA maintenance. Novel strategies to treat

mitochondrial disorders and neurological diseases will likely involve the manipulation of TFAM expression [51]. These studies also provide the rationale for experiments involving the manipulation of TFAM expression."

The multiple links between TFAM expression or activity and mitochondrial dysfunction suggest that TFAM is an important driving force in neurodegenerative disorders, including PD and AD, as well as in diabetes and mtDNA depletion disorders such as infantile mitochondrial myopathy, fatal childhood myopathy, skeletal muscle and mitochondrial encephalomyopathy, ocular myopathy, exercise intolerance and muscle wasting [20,52-56].

Dysfunction of Mitochondrial Dynamics

A mitochondrion is not a discrete, autonomous organelle, but rather part of a dynamic network akin to the endoplasmic reticulum. Mitochondria are highly dynamic organelles that are constantly engaged in intracellular fusion and fission, as shown, for instance, in murine embryonic fibroblasts [57]. The relative rates of fusion and fission determine the morphology of the mitochondrial network at any given time. In dividing cells, the mass of this mitochondrial network steadily increases throughout the cell cycle and normally divides approximately equally between daughter cells. Mitochondria prepare for cell divisions by increasing their numbers through mitochondrial fission. The mitochondria prepare for fission by synthesizing the vast majority of their components and importing proteins and lipids. Once sufficient mitochondria are available, the cell divides, and the mitochondria are distributed between the daughters.

Mitochondrial fission and fusion events are important processes not only in the normal functioning of the cell but also in pathophysiological situations in different cells and tissues (Table 3). Excessive fusion and fission activity result in abnormal mitochondrial ultrastructure and consequent neuronal death. For example, mitochondrial fission mediated by DRP1 is necessary for apoptotic cell death [58]. Studies have shown that dysfunction of mitochondrial fission proteins results in excessive mitochondrial fragmentation, damage to regions of nerve cell communication, synaptic injury, and eventual nerve cell death [59-63]. Furthermore, over-fragmentation or excessive mitochondrial fission from the oxidation of a dynamin-like transporter protein may cause synapse loss in AD [59]. The antihistamine dimebolin hydrochloride, a putative mitochondrial stimulant, has been suggested to improve cognition and behavior in patients with mild-to moderate AD [64].

Multiple proteins are involved in mitochondrial fusion and fission. Several important proteins are involved in the mitochondrial fusion mechanism in mammalian cells, such as mitofusin 1 (MFN1), mitofusin 2 (MFN2), and optic atrophy 1 (OPA1) [65]. These 3 proteins are large guanosine triphosphate (GTP) hydrolases that localize to different sites in mitochondria [66,67]. MFN1 and MFN2 insert into the outer membrane and form homo- and hetero-oligomers, while OPA1 localizes into the intermembrane space partially anchored to the inner membrane [65,67]. The presence of mitofusins on adjacent

Diseases	Mitochondrial function	Gene	Description
CMT2A	Fusion	MFN2	Autosomal dominant peripheral neuropathy
ADOA	Fusion	OPA1	Autosomal dominant optic atrophy
Unnamed	Fission	DRP1	Neonatal lethality

This table is modified from [66]
Abbreviations: CMT2A-Charcot-Marie-Tooth Type 2A; MFN2-Mitofusin-2; OPA1-Optic Atrophy-1; DRP1-Dynamin-Related Protein-1
Table 3: Diseases associated with perturbations in the mitochondrial fusion/fission machinery.

mitochondria is required during fusion because they form complexes that tether the mitochondria together [68]. Inhibition of mitofusins results in mitochondrial fragmentation and poor mitochondrial function [57,69]. Inhibition of OPA1 leads to mitochondrial fragmentation and severe aberrations in the structure of the cristae owing to a loss of mitochondrial fusion [70-72]. However, the specific functions of these proteins in membrane fusion remain undetermined [73]. They might contribute to membrane fusion by providing the necessary GTP hydrolysis and mitochondrial Δψ [68]."

In mammalian cells, mitochondrial fission is regulated by fission 1 (mitochondrial outer membrane) homologue (FIS1) and dynamin-related protein 1 (DRP1). FIS1 is localized uniformly on the mitochondrial outer membrane. The majority of DRP1 is located in the cytosol, from which it is recruited to the outer membrane during mitochondrial fission, but a sub-pool is localized to punctate spots on microtubules. A subset of these puncta mark future fission sites [73,74], and DRP1 contributes to membrane constriction during fission."

Inhibition of FIS1 and DRP1 inhibits fission, resulting in the elongation of mitochondrial tubules [17]. However, inhibition of FIS1 does not disrupt the mitochondrial localization of DRP1 [17]. DRP1 contributes to membrane constriction during mitochondrial fission, but the precise mechanism of fission remains undetermined.

Mitochondrial ROS and Apoptosis

Mitochondrial dysfunction may worsen with age due to accumulation of mtDNA mutations. Furthermore, a high number of neuronal Aβ aggregations inhibit key mitochondrial enzymes in the brain and in isolated mitochondria [75]. Aβ is a known potent generator of ROS and reactive nitrogen species in neural, microglial, and cerebrovascular cells [76-78]. Over-accumulation of Aβ in mitochondria is associated with diminished enzymatic activity of respiratory chain complexes III and IV [4]. Moreover, this leads to impairment of ATP production, oxygen consumption, and mitochondrial Δψ. It also leads to more superoxide radicals being formed and converted into hydrogen peroxide, causing oxidative stress, cytochrome c release and apoptosis. mtDNA mutations can expose mitochondria to high levels of oxidative damage, resulting in instability and irreparability of specific neurons [79].

ETC Impairment in Neurodegenerative Diseases

Little is known about the association between mitochondrial dysfunction and AD. Studies have shown that energy metabolism is impaired in AD by alterations in mitochondrial enzymes, such as Complexes I and IV [80,81]. The common AD biomarkers Aβ, tau and phosphorylated tau may be connected to mitochondrial dysfunctions such as decreased activity of ETC complexes and increased ROS production [6,82].

One possible connection between AD and mitochondrial dysfunction lies in the synaptosomes. Synaptosomal mitochondria are a subgroup of neuronal mitochondria specifically located at synapses. They play an essential role in fueling synaptic function by providing on-location energy. Their defects may lead to synaptic failure, which causes an early and pronounced pathology in AD. This has been investigated using the 5XFAD mouse model, which is a double transgenic AβPP/presenilin 1 model that co-expresses 5 AD mutations leading to accelerated plaque formation and increased Aβ production [83]." This model exhibits an early onset of plaque deposition (around 2 months of age) that spreads to most of the brain in parallel with astrocytosis, microgliosis and gender-based effects [84]. Female mice exhibit a significant increase in hippocampal plaque deposition that is not seen in male mice [85]. In addition to plaque pathology and gliosis, the 5XFAD mice also exhibit age-dependent synaptic degeneration indicated by reduction of synaptic markers [84]. Furthermore, there is another AD mouse model, the J20 line, which over-expresses human Aβ and exhibits early synaptosomal mitochondrial dysfunction. These mouse models display imbalanced mitochondrial dynamics favoring fission along with activated Parkin and light chain 3 recruitment correlating with progressive spatial learning and memory impairments. These results suggest that synaptosomal mitochondrial deficits are a primary pathology in Aβ-rich environments and further confirm the relevance of synaptosomal mitochondrial deficits in the development of AD [86]. Overall, it is suggested that the 5XFAD strain is a valid model for understanding both the detrimental and neuroprotective mechanisms of AD pathogenesis and for screening potential therapeutics [87].

Impairment of Mitophagy in Neurodegenerative Diseases

Mitophagy is an autophagic process that selectively degrades damaged mitochondria [88,89] and occurs in response to various conditions, such as nutrient depletion, mitochondrial dysfunction or red blood cell maturation. During nutrient depletion or caloric restriction, which can increase mitochondrial longevity, Uth1p (Atg1) activates autophagosome formation to break down unnecessary organelles for use as building blocks and nutrients and to increase the removal of oxidatively damaged mitochondria and mutated mtDNA [51].

Mitochondrial damage or dysfunction is 1 of the main inducers of mitophagy. Increased mitochondrial dysfunction might occur in post-mitotic cells of older organisms that exhibit abnormal mitochondrial morphology (e.g. swelling, low number of cristae and destruction of inner membranes) [90-92]. Autophagic events decrease during aging, which results in older animals having lower ATP production and respiration than younger ones [92].

There is frequent mitochondrial turnover in organs such as the brain, heart, liver and kidney [93]. Mitochondria are more vulnerable to oxidative damage and have less robust DNA repair systems than the nucleus [94]. Damage to mtDNA could cause the synthesis of abnormal mitochondrial proteins or disrupt synthesis altogether, which would exacerbate mitochondrial dysfunction. In this process, damaged mitochondria (e.g. those with a compromised Δψ or overproduction of ROS) release signals that promote uptake by autophagosomes, triggering degradation [95]. This provides a mechanism for mitochondrial quality control and a general, controlled cytoprotective response.

In neurodegenerative diseases, for example, the cell benefits from mitophagy through the selective removal of damaged and free radical-generating mitochondria. PD may be induced by an environmental toxin, 1-methyl-4-phenylpyridinium (MPP+), an inhibitor of ETC complex I. A precursor of MPP+, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, can also induce parkinsonism [96], as can PD-related genes (e.g. *PARK2* and *PINK1*) in autosomal-recessive juvenile-onset parkinsonism (ARJP) [97,98]. Studies of the mitochondrial proteins Parkin and phosphatase and tensin homolog-induced putative kinase 1 (PINK1) have established a direct link between defective mitochondria and mitophagy [99,100]. Studies have shown that loss of Parkin function results in vulnerability to oxidative damage, dopaminergic neuron loss, and abnormal mitochondrial structure (e.g. swollen, distorted mitochondrial morphology, and fragmented cristae). Loss-of-function mutations in Parkin decrease ATP production and enhance fission activity, resulting in cell death [101]. Loss of Parkin

activity also results in the loss of a cellular monitor of dysfunctional mitochondria, whereas overexpression of Parkin could help to protect against toxicity and mark damaged mitochondria for degradation [99,100].

PINK1 is a mitochondrial serine/threonine kinase that detects mitochondrial or cellular stress and is thought to prevent mitochondrial dysfunction [99]. Studies have shown that loss-of-function mutations in *PINK1* result in defects in mitochondrial morphology, dynamics, and function. This leads to an imbalance in mitochondrial fusion and fission in Drosophila [101]. Moreover, high levels of PINK1 activity are important for the translocation of Parkin to mitochondria, whereas overexpression of Parkin alone results in a cytosolic localization [102-105]. Studies have shown that Parkin targets dysfunctional mitochondria and recruits PINK1 to damaged mitochondria with low $\Delta\psi$ to promote autophagic degradation after treatment of cells with carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) [106]. The Parkin-PINK1 mechanism depends on voltage-dependent inhibition in the clearance process. Moreover, studies have shown that Parkin's cognate E2 coenzymes in this ubiquitin-dependent pathway either positively or negatively regulate the activation, translocation, and enzymatic functions of Parkin during mitochondrial quality control [104]. PINK1's proteolytic cleavage function allows the proteasome to clear cleaved fragments [106]. Overall, signals such as an increase in ROS production or a reduction in $\Delta\psi$ recruit the cytosolic forms of Parkin and PINK1 to fragmented mitochondria, which promote the engulfment of mitochondria by autophagosomes for degradation [99,100].

Mitophagy plays an essential role during differentiation when the elimination of mitochondria is required, such as during the differentiation of reticulocytes into erythrocytes. The autophagic gene *Nix* (also called *Bnip3L*) [95] is upregulated in erythroid cells during terminal differentiation [95,107]. Nix, a BH3-only member of the Bcl-2 family, induces mitophagy by disrupting $\Delta\psi$ [95]. Furthermore, mitophagy is also important for maintaining the number and function of mitochondria during stem cell differentiation and de-differentiation. Knock-down of growth factor erv1-like (Gfer) in embryonic stem cells (ESCs) upregulates DRP1, resulting in decreased pluripotency marker expression, smaller embryoid bodies (EBs) and loss of mitochondrial function (e.g. mitochondrial $\Delta\psi$, cytochrome c expression). Knockdown of Gfer in ESCs is also associated with fragmentation of mitochondria and an increase in apoptosis that reduces cells numbers but does not affect the ability to differentiate [108]. By contrast, overexpression of Gfer in ESCs reduces DRP1 expression and maintains pluripotency marker expression, the size of EBs, mitochondrial function (e.g. the mitochondrial $\Delta\psi$) and elongated mitochondrial morphology with well-defined cristae. These findings suggest that Gfer modulates mitochondrial fission activity to preserve mitochondrial function while maintaining pluripotency status in ESCs. Another study investigated the response of ESCs to CCCP-induced mitochondrial $\Delta\psi$ reduction and found that Parkin was upregulated in clustered perinuclear mitochondria [100,102,109]. These observations suggest that mitophagy controls mitochondrial quality within ESCs. Overall, this review suggests that mitochondrial dysfunction is highly associated with neurodegenerative disorders. Autophagy is important for maintaining homeostasis during development, differentiation and macromolecule synthesis and degradation [110]. Mitophagy is critical to maintaining a healthy population of mitochondria, and it will be a target of future drug discovery research.

Conclusion and Future Perspective

As discussed above, there is strong evidence implicating the role of mitochondrial dysfunction in neurodegenerative diseases (AD and PD). Mitochondrial mutation or depletion affects the neuro-health status, which may result from decrease in mitochondrial complexes activities, oxidative stress, and apoptosis. Furthermore, mitochondrial dynamics is critical for providing a mechanism to eliminate unhealthy mitochondria within neuronal cells and thus retain the healthy mitochondria. There are also other mechanisms, such as mitophagy, which are activated by signaling from unhealthy mitochondria (e.g. PINK1 or PARKIN). The development of transgenic mouse models of neurodegenerative diseases has significantly contributed to mitochondrial-specific therapeutics. Thus, studies of mitochondrial mutation, depletion, mitochondrial dis-ordinated dynamics and mitophagy-specific mechanisms can shed light in the field of mitochondrial-specific therapeutics and bring important advancements to clinical treatments for mitochondrial diseases.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflict of interest.

Author Contributions

Wrote or contributed to the writing of the manuscript: KLP and WEJ.

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