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Review

The Balance of MFN2 and OPA1 in Mitochondrial Dynamics, Cellular Homeostasis, and Disease

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Abstract: Mitochondrial dynamics, governed by fusion and fission, are crucial for maintaining cellular homeostasis, energy production, and stress adaptation. MFN2 and OPA1, key regulators of mitochondrial fusion, play essential roles beyond their structural functions, influencing bioenergetics, intracellular signalling, and quality control mechanisms such as mitophagy. Disruptions in these processes, often caused by MFN2 or OPA1 mutations, are linked to neurodegenerative diseases like Charcot-Marie-Tooth disease type 2A (CMT2A) and Autosomal Dominant Optic Atrophy (ADOA). This review explores the molecular mechanisms underlying mitochondrial fusion, the impact of MFN2 and OPA1 dysfunction on oxidative phosphorylation and autophagy, and their role in disease progression. Additionally, we discuss the divergent cellular responses to MFN2 and OPA1 mutations, particularly in terms of proliferation, senescence, and metabolic signalling. Finally, we highlight emerging therapeutic strategies to restore mitochondrial integrity, including mTOR modulation and autophagy-targeted approaches, with potential implications for neurodegenerative and metabolic disorders.

Keywords: mitochondria; mitochondrial dynamics; neurodegenerative diseases; autophagy; mitophagy; oxidative phosphorylation; proliferation; senescence; mTOR signalling

1. Introduction

Mitochondria are dynamic organelles constantly undergoing fusion and fission to maintain cellular balance and adapt to metabolic needs. These processes, known as mitochondrial dynamics, are essential for preserving the mitochondrial structure, optimizing energy production, and supporting intracellular signalling. By responding to environmental and physiological signals, mitochondria adjust their shape to help cells cope with stress and energy fluctuations [1,2]. Fusion is mediated by GTPase proteins, including mitofusins (MFN1 and MFN2) in the outer mitochondrial membrane (OMM) and optic atrophy 1 (OPA1) in the inner mitochondrial membrane (IMM) [3,4]. These proteins maintain mitochondrial elongation, preserve membrane potential, and regulate oxidative phosphorylation (OxPhos) and cellular quality control mechanisms like mitophagy. These processes are particularly crucial in high-energy tissues such as the brain, where neuronal health and function heavily depend on mitochondrial dynamics [5,6]. Conversely, fission is primarily driven by dynamin-related protein 1 (Drp1) [7,8]. Beyond their well-characterized roles in mitochondrial fusion, MFN2 and OPA1 influence broader cellular processes [9–11]. Disruptions in mitochondrial dynamics, often caused by mutations in MFN2 (OMIM #608507) or OPA1 (OMIM #605290), are associated with several neurodegenerative diseases and hereditary neuropathies, such as Charcot-Marie-Tooth disease type 2A (CMT2A, OMIM #609260) and Autosomal Dominant Optic Atrophy (ADOA, OMIM #165500). This review explores the structural and functional complexities of MFN2 and OPA1, focusing on their roles in mitochondrial quality control and bioenergetics and their involvement in cellular differentiation and metabolic signalling. By highlighting emerging therapeutic strategies targeting these proteins, we aim to enlighten their potential to restore mitochondrial integrity and cellular homeostasis in the context of neurodegenerative diseases.

2. Structural insights into MFN2 and OPA1

MFN2 and OPA1 possess sophisticated structures and undergo precise regulation, enabling their diverse functions.

MFN2: Structure and Function. MFN2 is a nuclear-encoded protein localized to the outer mitochondrial membrane (OMM) [12]. The MFN2 gene (OMIM *603560) resides on chromosome 1 at position 36.22, encompassing 19 exons and producing two transcript variants. These variants encode two protein isoforms: Isoform 1 (757aa; 86,402Da) and Isoform 2 (436aa; 50,041Da). MFN2 contains several conserved functional domains arranged sequentially from the N-terminal to the C-terminal: a GTPase domain, two coiled-coil domains (HR1 and HR2), a proline-rich domain, and two transmembrane domains [13]. The GTPase domain is critical for mediating the fusion process, with motifs G1 to G5 forming the catalytic centre and coordinating GTP binding and hydrolysis [14,15]. The HR2 domain enables tethering of adjacent mitochondria through an antiparallel coiled-coil dimeric structure, forming both homotypic (MFN1–MFN1 or MFN2–MFN2) and heterotypic dimers (MFN1–MFN2) [16,17]. Additionally, MFN2 possesses a unique N-terminal P21 Ras-binding domain, distinguishing it functionally from its homolog MFN1 [18]. While MFN1 is predominantly expressed in the heart and testis, MFN2 is abundant in the brain, underscoring its specialized role in neural tissue [19].

OPA1: Isoforms and Functional Domains. OPA1, a nuclear-encoded protein located in the inner mitochondrial membrane (IMM), is encoded by the OPA1 gene (OMIM #605290) on chromosome 3q29. This gene spans over 60 kb and produces eight isoforms through alternative splicing, primarily involving exons 4, 4b, and 5b [20]. Key isoforms include Isoform 1: 960aa (111,631Da); Isoform 2: 997aa (115,884Da); Isoform 3: 961aa (111,822Da). OPA1 contains several functional regions, including an N-terminal mitochondrial targeting sequence (MTS), a GTPase domain, two coiled-coil domains, and a GTPase effector domain (GED). These regions are critical for anchoring OPA1 to the IMM, mediating protein-protein interactions, and enabling GTP-dependent fusion activity [21]. After mitochondrial import, OPA1 undergoes proteolytic cleavage at specific sites: under stress conditions, OPA1 cleaves at site S1 (corresponding to exon 5). YME1L cleaves at site S2 (corresponding to exon 5b) during normal conditions [22]. This processing generates two OPA1 forms: long OPA1 (l-OPA1) and short OPA1 (s-OPA1). While l-OPA1 anchors to the IMM, s-OPA1 resides in the intermembrane space (IMS). Both forms are essential for mitochondrial fusion, with l-OPA1 facilitating homotypic interactions and s-OPA1 enhancing the fusion process [23].

3. MFN2 and OPA1 in the Mitochondrial Fusion Process

Mitochondrial fusion involves several key proteins and interactions across the outer and inner mitochondrial membranes (OMM and IMM) [6,24]. The fusion of the OMM is initiated by the mitofusins, MFN1 and MFN2, which tether two mitochondria through interactions between their HR2 domains. This tethering is followed by GTP hydrolysis-induced conformational changes, driving membrane fusion. While MFN1 is primarily responsible for completing OMM fusion, MFN2 plays a supportive role, including mediating OMM fusion and regulating mitochondria–endoplasmic reticulum (mito-ER) contact sites, also known as the ER mitochondria-associated membranes (MAMs). Several processes known to be regulated or directly occurring at MAM have been reported to be influenced by the presence of MFN2 (see paragraphs 4 and 6). Recent structural studies of MFN1 and MFN2 have provided mechanistic insights into their roles in mitochondrial fusion [25,26]. However, the physiological relevance remains under investigation due to the absence of the transmembrane domain in these structures and uncertainties surrounding their topology [27]. Once OMM fusion is complete, IMM undergoes fusion, a process mediated by OPA1. OPA1 exists in two forms: long OPA1 (L-OPA1) and short OPA1 (S-OPA1), generated through proteolytic cleavage. L-OPA1 facilitates homotypic interactions between membranes but requires S-OPA1 to complete the fusion process. Studies have demonstrated that L-OPA1 interacts with *cardiolipin*, a mitochondrial-specific phospholipid, to form trans complexes capable of driving membrane fusion. S-OPA1

enhances this process, indicating that both isoforms are essential for IMM fusion [23,28]. The coordination of OMM and IMM fusion remains an unresolved aspect of mitochondrial dynamics. One model suggests that MFN1-mediated GTP hydrolysis stimulates the oligomerization of OMM fusion pores, subsequently facilitating IMM fusion. OPA1 may then form right-turned helical assemblies, potentially activated by MFN1, to drive IMM fusion [29]. This coordinated interaction ensures that mitochondrial fusion occurs seamlessly, maintaining organelle integrity and function. Mitochondrial fusion is crucial in maintaining mitochondrial quality by selectively preventing the reintegration of defective mitochondria with lower membrane potential [30]. Additionally, it facilitates the exchange of mitochondrial DNA (mtDNA) and proteins, diluting damage and supporting mitochondrial health [31]. In the presence of mtDNA mutations, fusion helps mitigate defects in respiratory chain proteins, highlighting its importance in sustaining bioenergetic efficiency [32]. Disruption of this process due to the loss of key fusion regulators, such as MFN1, MFN2, or OPA1, leads to a decrease in mtDNA copy numbers and an increase in mitochondrial dysfunction [33].

4. Mitochondrial Fusion Proteins in the Regulation of Oxidative Phosphorylation

The efficiency of OxPhos—the process of producing ATP via the electron transport chain—is tightly regulated by mitochondrial dynamics. MFN2 and OPA1, key proteins governing mitochondrial fusion and *cristae* structure—invaginations of the inner mitochondrial membrane where respiratory chain complexes are located—directly influence bioenergetic efficiency and cellular metabolic states. Elongated mitochondria, formed through fusion mediated by MFN2 and OPA1, enhance OxPhos efficiency and ATP production [34,35]. In contrast, fragmented mitochondria often exhibit impaired OxPhos, reduced mitochondrial DNA (mtDNA) content, and elevated reactive oxygen species (ROS) levels, contributing to cellular dysfunction [36–38]. Recent research highlights the adaptability of mitochondria under metabolic stress. During nutrient deficiency, mitochondria dynamically segregate into specialized subpopulations: ATP-producing units and biosynthetic hubs. One subpopulation, devoid of *cristae* but enriched in pyrroline-5-carboxylate synthase (P5CS), supports proline and ornithine biosynthesis, optimizing metabolic flexibility through fusion-fission cycles [39]. This segregation exemplifies how mitochondrial dynamics align with cellular metabolic demands.

MFN2: A Key Regulator of Fusion, Bioenergetics and Beyond. MFN2 is a critical regulator of mitochondrial fusion and bioenergetics, and its role extends beyond mere mitochondrial dynamics. Alterations in mitochondrial fusion, often caused by impaired MFN2-GTPase activity, lead to severe metabolic dysfunctions, including suppressed OxPhos, diminished ATP production, and mtDNA instability. Such dysfunctions are implicated in pathological conditions such as neurodegeneration and metabolic syndromes [37]. As a tethering factor, MFN2 facilitates direct communication between the mitochondria and the ER [40,41]. This interaction is crucial in maintaining cellular bioenergetics by limiting ROS production, alleviating ER stress, and facilitating calcium transfer from the ER to mitochondria. These processes sustain NADH levels, which are critical for OxPhos [42,43]. Furthermore, MFN2-mediated fusion enhances OxPhos during periods of high metabolic demand, such as cell proliferation, though proliferative cells often shift to glycolysis to meet biosynthetic needs [34,44]. Despite the central role of MFN2 in tethering, conflicting findings exist regarding the effects of MFN2 loss on mito-ER contacts. Some studies suggest that the loss of MFN2 reduces these contacts, impairing mitochondrial function and increasing cellular stress [45]. In contrast, other studies suggest that the absence of MFN2 may increase these contacts, complicating the interpretation of its precise role in metabolic regulation [46]. A detailed discussion of this controversy is beyond the scope of this review and can be found in other literature [47,48]. In addition to its functions in mitochondrial dynamics, MFN2 is involved in mitochondrial transport along axons, a process essential for ensuring neurons meet their energetic and metabolic demands [49]. MFN2 facilitates the attachment of mitochondria to microtubules via interactions with motor proteins like Miro and Milton, ensuring

efficient mitochondrial distribution [50]. Interestingly, while MFN2 knockout impairs mitochondrial transport along axons, similar defects are not observed in OPA1-deficient neurons, despite both conditions causing comparable mitochondrial fragmentation [10,51]. This distinction highlights MFN2's multifaceted role, extending beyond mitochondrial fusion to influence a broader spectrum of cellular processes that support cellular metabolism and energy production [9].

OPA1: Master Regulator of Cristae Structure and Supercomplexes Assembly. OPA1 plays a central role in maintaining mitochondrial *cristae* structure organizing respiratory chain complexes (RCCs) into supercomplexes (RCS). These supercomplexes optimize electron transfer and maximize OxPhos' efficiency, highlighting the importance of *cristae* integrity in bioenergetics [52,53]. Overexpression of OPA1 in mice enhances *cristae* integrity, improving OxPhos and ATP production, while its knockdown disrupts mitochondrial membrane potential and respiration, underscoring its essential role in mitochondrial function [53]. OPA1 acts as a metabolic sensor, interacting with solute transporters in the inner mitochondrial membrane (IMM) to regulate *cristae* remodelling in response to cellular energy demands. This remodelling is independent of OPA1's fusion activity and is crucial for maintaining respiration and cell survival during metabolic stress, such as nutrient deprivation [53]. Studies suggest OPA1 interacts with mitochondrial solute carriers (*i.e.*, SLC25A), sensing substrate availability and adjusting mitochondrial function, accordingly, thus bridging bioenergetics and metabolic signalling [54]. Modulating these proteins could enhance OxPhos efficiency, reduce ROS production, and improve cellular resilience under metabolic stress. Additionally, understanding the role of mitochondrial solute carriers in OPA1-mediated *cristae* remodelling offers new avenues for designing interventions tailored to specific metabolic conditions.

5. Dynamic Changes in Mitochondrial Structure and Quality Control

Dynamic changes in mitochondrial structure are central to quality control mechanisms that maintain cellular and mitochondrial health [55]. These mechanisms enable the mitochondrial network to adapt to challenges such as protein misfolding, oxidative stress, and organelle damage [56,57]. Mitochondrial quality control encompasses transcriptional responses mediated by factors that shuttle between mitochondria and the nucleus—such as those involved in retrograde signalling and the mitochondrial unfolded protein response (mtUPR)—as well as stress-induced mitochondrial hyperfusion (SIMH), which enhances mitochondrial resilience under acute stress. These mechanisms also involve the degradation of misfolded proteins, regulating mitochondrial proteostasis, and the selective removal of damaged mitochondria through processes like mitophagy and autophagy [58,59].

Mitochondrial Retrograde Signaling. Mitochondria contain multiple copies of a small genome, the mitochondrial DNA (mtDNA), with most of the mitochondrial proteome encoded by nuclear genes regulated by transcription factors (TFs) and their associated cofactors [60–63]. Thus, maintaining mitochondrial homeostasis critically depends on nuclear transcription and signalling pathways that inform the nucleus of mitochondrial dysfunctions and changes in cellular metabolism [64,65]. This regulation occurs partly through nuclear TFs and cofactors modulated by cytosolic mediators such as ROS and the NAD/NADH ratio [66,67]. When mitochondrial dysfunction occurs, retrograde signalling pathways convey mitochondrial distress to the nucleus, triggering adaptive changes in nuclear gene expression and cellular physiology [68,69].

Stress-Induced Mitochondrial Hyperfusion (SIMH). Acute stressors, such as oxidative damage or starvation, trigger SIMH, a protective response characterized by elongated mitochondrial networks. SIMH enhances bioenergetic efficiency, ATP production, and cellular resilience under stress conditions [70,71]. This response is regulated by the inhibition of Drp1, suppressing mitochondrial fission, and the activation of OPA1 and MFN1, promoting fusion [35,71]. Notably, SIMH prevents mitophagy during nutrient deprivation, preserving mitochondrial function and sustaining energy production [35,72]. Additionally, it enhances calcium buffering, reduces ROS, and supports oxidative metabolism [70]. In contrast, nutrient excess induces mitochondrial fragmentation, shifting metabolism from oxidative phosphorylation to glycolysis, leading to lipid accumulation and

metabolic dysfunction [1]. Fragmented mitochondria produce excessive ROS, contributing to oxidative stress and cellular damage [73,74]. One proposed mechanism is that excessive fragmentation disrupts IMM and cristae structure, impairing electron transport chain (ETC) complexes and hindering electron transfer and proton transport [75–77]. This electron and proton leakage decreases ATP production while increasing ROS levels, and activates Drp1, further promoting mitochondrial fission and perpetuating oxidative stress in a positive feedback loop [78,79].

Integrated Stress Response (ISR) as a Key Regulator of Stress Adaptation. Mitochondria play a pivotal role in the Integrated Stress Response (ISR), a conserved mechanism that adjusts metabolism and protein synthesis under stress conditions. Triggered by mitochondrial dysfunction, nutrient deprivation, or oxidative stress, the ISR downregulates general protein synthesis while selectively upregulating stress-adaptive genes to conserve energy and enhance cellular resilience [80,81]. For instance, OPA1 deficiency in muscle cells activates the ISR, leading to mitochondrial fragmentation, suppression of mTORC1 signalling, and stimulation of AMP-activated protein kinase (AMPK) activity [72]. This dual regulation reduces anabolic processes like protein synthesis while promoting catabolic pathways such as fatty acid oxidation to restore energy balance [82]. Moreover, genetic inhibition of mitochondrial function or treatment with mitochondria-targeted antioxidants can activate AMPK signalling, triggering mitochondrial fragmentation and inducing catabolic pathways as a survival mechanism [83]. In response to mitochondrial injury, AMPK activation can also increase the expression of fusion proteins such as MFN1 and OPA1, promoting a hyper-tubular mitochondrial state that enhances OxPhos and ATP production under stress conditions [37,84,85]. The role of AMPK in mitochondrial dynamics varies depending on the cellular context, the type of stress, the metabolic state, and specific cellular demands. Notably, chronic ISR activation and impaired mitochondrial fusion are linked to neurodegenerative diseases such as Alzheimer's and Parkinson's [86–90]. These mechanisms highlight the intricate relationship between mitochondrial dynamics and stress adaptation, underscoring their critical role in maintaining cellular function and homeostasis.

The mitochondrial unfolded protein response (mtUPR) exemplifies a crucial pathway for maintaining mitochondrial proteostasis [91,92]. Protein homeostasis depends on a balance between unfolded proteins and the folding capacity of cellular compartments. Mitochondria possess their chaperones and proteases for quality control; however, when misfolded proteins exceed this capacity, mitochondrial dysfunction ensues, triggering mtUPR to enhance protein folding and degradation [93,94]. This retrograde signalling activates the transcription of nuclear-encoded mitochondrial chaperones and proteases, sustaining mitochondrial proteostasis. While not strictly a quality control mechanism, *retrograde communication* mitigates mitochondrial dysfunction, preventing further cellular damage [92,95].

Proteolytic Systems and Mitochondrial Proteostasis. The ubiquitin-proteasome system (UPS) plays a crucial role in maintaining mitochondrial proteostasis by clearing defective proteins and facilitating the replacement of non-functional components [92,96]. In addition to its degradative function, the UPS supports protein transport across the mitochondrial outer membrane by targeting import-deficient polypeptides for ubiquitination, marking them for proteasomal degradation [92,97]. Recently, it has been observed that disruption of mitochondrial protein import triggers a quality control mechanism in which YME1L1, the ATP-dependent protease involved in OPA1 cleavage, degrades unassembled translocase components, preventing the precursor stalling in the translocase of the outer membrane complex. This process is a protective response to maintain mitochondrial quality control and cellular viability. Import plugging leads to a cell growth defect and the loss of YME1L1 yeast exacerbates this inhibition, underscoring the protective role of YME1L1 [98].

6. Autophagy/Mitophagy and Mitochondrial Dynamics

Beyond the coordinated action of chaperones and proteases, mitochondrial quality control relies on autophagy, a fundamental mechanism for degrading dysfunctional organelles and protein aggregates. Autophagy can be non-selective, ensuring survival during nutrient deprivation, or selective, targeting specific cellular components such as mitochondria—a process known as *mitophagy*

[99–102]. This process begins with phagophore formation, likely originating from the ER, trans-Golgi, or endosomes, which expands to engulf damaged components, forming an autophagosome that subsequently fuses with a lysosome to create an autolysosome, where cargo degradation occurs through hydrolases [103,104]. Mitophagy plays a crucial role in mitochondrial turnover and proteostasis, preventing the accumulation of dysfunctional organelles that could otherwise compromise cellular homeostasis [105,106]. A key regulator of this pathway is the PINK1/Parkin system, where PINK1 accumulates on OMM of damaged mitochondria, recruiting Parkin, an E3 ubiquitin ligase, which ubiquitinates MFNs, leading to mitochondrial fragmentation and clearance [107–113]. Unfolded mitochondrial proteins can trigger mtUPR, and activate PINK1/Parkin-dependent mitophagy, reinforcing mitochondrial quality control [99]. Additionally, PINK1-phosphorylated MFN2 serves as a Parkin receptor, marking mitochondria for degradation [10], while Parkin-mediated MFN2 inhibition regulates ER-mitochondria crosstalk, influencing autophagic signalling [110,114]. This integrated network of mtUPR, UPS, autophagy, and mitophagy ensures mitochondrial function is preserved, preventing cellular damage and dynamically adapting mitochondrial activity to metabolic demands under physiological and stress conditions [105,106]. Mitochondrial dynamics dictate whether mitochondria undergo fusion, preserving their function, or fragmentation, facilitating mitophagy [115]. During mitophagy, fragmentation exposes “eat-me” signals, promoting autophagosome formation and lysosomal degradation [116]. In contrast, fusion prevents degradation during periods of nutrient scarcity, forming interconnected mitochondrial networks that enhance ATP production [117]. OPA1 is a crucial regulator of this balance, interacting with FUNDC1 and BNIP3 to mediate autophagy and mitophagy [118,119]. Moreover, mitochondrial-derived vesicles (MDVs) selectively remove damaged mitochondrial components, predominantly occurring at MAMs, where MFN2 localises and orchestrates mitochondrial fusion and autophagosome formation [103,120,121]. MFN2 depletion disrupts MAM integrity, impairing starvation-induced autophagy and hindering autophagosome formation [121,122]. Furthermore, MFN2 deletion in cardiomyocytes and mouse embryonic fibroblasts leads to autophagosome accumulation, as it blocks autophagosome-lysosome fusion, a crucial step in autophagic degradation, ultimately resulting in metabolic dysfunction, defective lipid metabolism, and reduced mitochondrial oxidative phosphorylation [123–125]. These findings highlight the critical role of MFN2-Rab7 interactions, which regulate autophagosome-lysosome fusion, further linking mitochondrial dynamics to autophagic regulation [123]. More recently, MFN2 has been identified as an interactor with novel autophagy-related partners, including RAB5C, a key endosomal regulator of mitochondrial homeostasis, and SLC27A2 [126].

7. Metabolic Cues Driving Mitochondrial Fusion

Mitochondrial bioenergetics is essential for cellular function, linking metabolic signals to regulate mitochondrial fusion and fission [24,127]. This dynamic nature allows mitochondria to adapt to fluctuating cellular demands by integrating their activity with physiological processes and shaping metabolism. Under nutrient scarcity, mitochondria undergo significant remodelling, characterized by reduced *cristae* density, respiratory capacity, and size, alongside increased contact with the ER. These changes facilitate calcium and lipid flux, enabling the cell to balance energy under metabolic stress [35,128]. Proteolytic inactivation of OPA1, mediated by MFN2, further promotes mito-ER contact formation, underscoring the importance of mitochondrial dynamics in stress adaptation [128,129].

Regulation by Metabolic Sensor Kinases and Post-Translational Modifications. Metabolic sensor kinases, including mTOR, AMPK, and PKA, dynamically regulate mitochondrial fusion and fission by integrating extracellular signals such as nutrients, oxygen, and growth factors [130,131]. For example, mTOR signalling enhances mitochondrial fusion by modulating OPA1 expression and activity, while AMPK activation during nutrient deprivation suppresses mTORC1 and promotes fusion, increasing energy efficiency [35,131–133]. Post-translational modifications (PTMs), such as phosphorylation, ubiquitylation, and sumoylation, provide an additional layer of regulation.

Phosphorylation of Drp1 at Ser637 by PKA inhibits its fission activity, promoting mitochondrial hyperfusion [134]. Meanwhile, ubiquitylation of MFN2 by E3 ligases modulates its fusion activity in response to specific *stimuli*, enabling precise adaptation of mitochondrial dynamics to cellular needs [135].

8. Mitochondrial Dynamics in Neuronal Differentiation and Bioenergetics

Mitochondrial dynamics are essential for neuronal differentiation, given the high energy demands of neurons. These processes support critical functions such as synaptic transmission, axonal transport, signal propagation, calcium flux, and glutamate cycling [136]. Unlike astrocytes, which predominantly rely on glycolysis, neurons depend on oxidative phosphorylation, highlighting the metabolic diversity within the nervous system [137,138]. Proteomic analyses further confirm this distinction, showing that neuronal mitochondria possess specialized respiratory chain complexes adapted for oxidative metabolism, whereas astrocytic mitochondria exhibit a reduced OxPhos capacity. During neuronal differentiation, mitochondria undergo significant remodelling, which includes alterations in their abundance, localization, and metabolic activity. This process is tightly regulated by MFN2 and OPA1, which facilitate the metabolic transition from glycolysis to oxidative phosphorylation (OxPhos), a crucial step for differentiation [139–141]. MFN2 plays a particularly critical role by optimizing bioenergetics, maintaining mitochondrial integrity, and regulating key signalling pathways, such as PI3K-Akt. Silencing MFN2 disrupts mitochondrial function and differentiation, while its overexpression enhances mitochondrial efficiency and promotes differentiation in neural progenitor cells [142,143]. Interestingly, silencing MFN2 paradoxically enhances neural differentiation by increasing Akt phosphorylation in embryonic stem cells, suggesting a context-dependent regulatory role [144]. A similar phenomenon occurs during cardiomyocyte differentiation, where elevated MFN2 and reduced OPA1 levels promote mitochondrial elongation, enhancing OxPhos to meet the increased energy demands of mature cells [145]. This highlights the direct influence of mitochondrial morphology on cellular bioenergetics during differentiation. The structure of mitochondrial *cristae*, regulated by OPA1, plays a pivotal role in neuronal differentiation. *Cristae* support respiratory chain complex (RCC) organization, facilitating efficient electron transfer and ATP production. OPA1 remodelling is crucial for meeting the increased OxPhos demands of differentiating neurons, while OPA1 dysfunction impairs respiration and reduces differentiation efficiency, underscoring its significance in neuronal function and pathology [146,147].

9. Pathological Implications of MFN2 and OPA1 Mutations

Neurons are particularly vulnerable to mitochondrial dynamics defects due to their reliance on properly positioned mitochondria for ATP production and calcium buffering [9]. MFN2 is essential for mitochondrial transport along axons, while OPA1 is critical for respiratory efficiency and mitochondrial genome stability. Although both proteins regulate mitochondrial fusion, their mutations lead to distinct pathologies due to their unique roles and the specific vulnerabilities of neuronal subtypes [13].

MFN2 Mutations and Charcot-Marie-Tooth Disease Type 2A (CMT2A). MFN2 mutations are primarily associated with CMT2A, an autosomal dominant axonal neuropathy characterized by distal limb weakness, muscle atrophy, and sensory loss [148,149]. Over 60 pathogenic variants have been identified, often affecting the GTPase or coiled-coil domains (Figure 1). These mutations disrupt mitochondrial fusion, trafficking, and bioenergetics, leading to neuronal energy deficits and impaired calcium homeostasis [148,150]. Dysfunctional MFN2 impairs axonal transport, causing mitochondrial aggregation in the soma and reduced distribution to distal axons [151]. Both retrograde and anterograde transport are affected, leading to mislocalized mitochondria, and compromised neuronal survival [125,152]. Loss-of-function MFN2 mutations result in fragmented mitochondrial networks with reduced OxPhos activity, while gain-of-function mutations may lead to abnormal

mitochondrial morphology or toxic effects on neurons [153]. A dominant-negative mechanism often exacerbates dysfunction by interfering with wild-type MFN2 activity [154]. Additionally, mutant MFN2 proteins disrupt interactions with motor proteins, impairing mito-ER connectivity, calcium signalling, and lipid synthesis [155,156]. Interestingly, motor neurons expressing mutant MFN2 show apoptosis resistance due to p53 suppression, which enhances mitochondrial turnover as a compensatory mechanism [157,158] (Table 1). Potential therapeutic approaches for CMT2A focus on improving mitochondrial function and axonal transport. Strategies include modulating tubulin and Miro acetylation to facilitate mitochondrial motility and reducing stress-induced transcriptional changes affecting axonal organelle transport pathways [144].

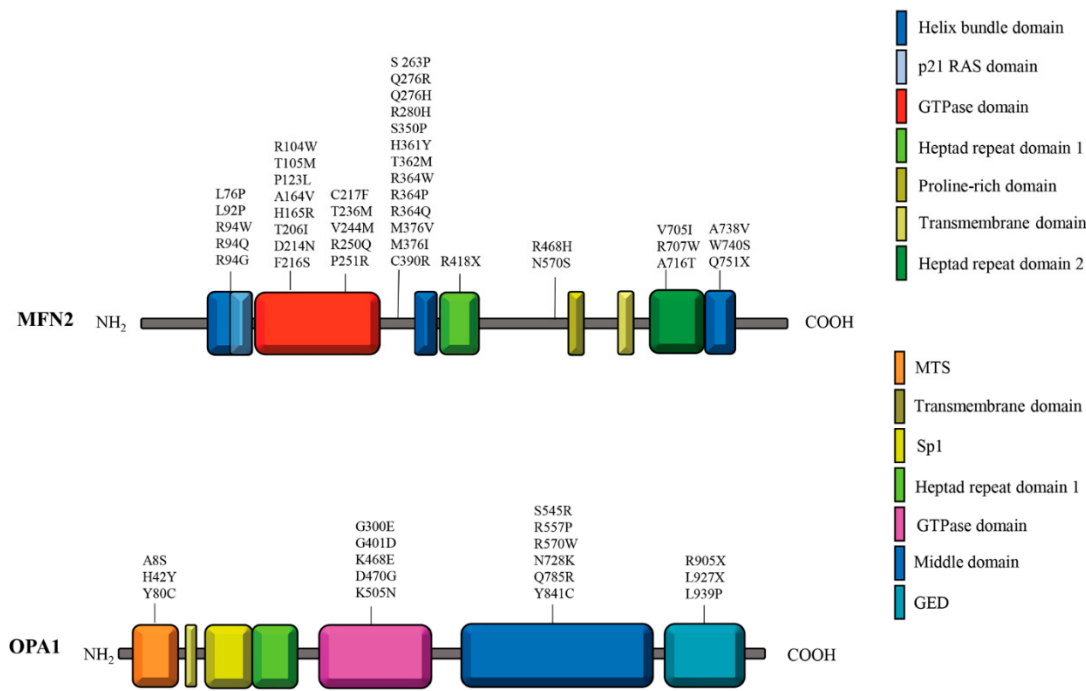


Figure 1. Secondary structure and commonly reported variants of MFN2 and OPA1 proteins. The schematic representation of MFN2 (above) highlights its structural domains: Helic bundle domain, p21 RAS domain, GTPase domain, heptad repeat domain 1 (HR1), a proline-rich domain, transmembrane domain, and heptad repeat domain 2 (HR2), each depicted in distinct colours. Below, OPA1 is illustrated with its key structural features: MTS (Mitochondrial Targeting Sequence), a transmembrane domain, Sp1, heptad repeat domain 1, GTPase domain, middle domain, and GED (GTPase Effector Domain), also represented with corresponding colours. The most reported variants for both proteins are indicated in their respective domains.

OPA1 Mutations and Autosomal Dominant Optic Atrophy (ADOA). Mutations in OPA1 are associated with autosomal dominant optic atrophy (ADOA), the most common mitochondrial optic neuropathy, characterized by retinal ganglion cell degeneration and progressive vision loss [159]. Over 370 pathogenic variants have been identified, including splicing mutations, frameshift deletions, and missense variants in the GTPase domain (Figure 1). These mutations disrupt inner membrane fusion and *cristae* organization, leading to respiratory dysfunction and mitochondrial fragmentation [160,161]. OPA1 haploinsufficiency reduces protein expression, sometimes triggering enhanced mitophagy and autophagy as compensatory responses to respiratory dysfunction [160,162–168]. Paradoxically, inhibiting autophagy in these cases has been shown to protect certain neuronal populations in mouse models [167,168]. However, no major alterations in the mitochondrial network

were detected in other cases, correlating with low mitophagic/autophagic activity [161,169]. Some missense mutations operate through a dominant-negative mechanism, leading to excessive mitochondrial fragmentation despite normal OPA1 expression levels [161] (Table 2). Additionally, OPA1-deficient neurons exhibit decreased BNIP3 expression, a key regulator of mitophagy. Notably, restoring BNIP3 levels reactivates autophagic and mitophagic activity, highlighting its therapeutic potential [169,170]. Several studies have investigated therapeutic methods to restore mitochondrial fusion, regulate autophagy and mitophagy, and target compensatory mechanisms such as the downregulation of BNIP3. One potential strategy involves small molecules that enhance mitochondrial fusion and bioenergetics. For instance, Wang et al. (2012) identified M1, a compound that increased mitochondrial fusion and respiratory complex expression, offering a potential treatment avenue [171]. Additionally, Mdivi-1, a well-known DRP1 inhibitor, has been shown to increase OPA1 expression in cells carrying OPA1 variants, reducing excessive mitophagy and significantly improving cellular function [172]. Another approach focuses on direct OPA1 GTPase activation, which can promote mitochondrial fusion as a protective mechanism in various diseases [173]. Altogether, these therapeutic strategies hold promise for mitigating neurodegenerative disease progression and improving neuronal survival and function in disorders such as CMT2A and ADOA [10,156]. For further insights into therapeutic developments, readers are referred to recent comprehensive reviews [174–176].

Table 1. Mitochondrial alterations in CMT2A2 cellular models.

Cell Type	MFN2 mutation	Remarks	References
Skin fibroblasts from patients with CMT2A	1) P123L 2) L92P	1) Mitochondrial fragmentation and abnormal mitochondrial accumulation around the nucleus 2) Mitochondrial fragmentation	Verhoeven K. et al., 2006
Skin fibroblasts from 4 patients with CMT2A2	M21V R364Q A166T	Reduced cellular respiration: uncoupling causing decreased ATP/O Reduced $\Delta\Psi_m$	Loiseau D. et al., 2007
Skin fibroblasts from patients with CMT2A	T105M I213T V273G	Mitochondrial morphology, mtDNA integrity and and respiratory enzyme activities are unchanged Extensive mitochondrial fusion	Amiott E. A. et al., 2008
Skin fibroblasts from patients with CMT2A	p.D210V	Respiratory chain defects Multiple mtDNA deletions Defect in mtDNA damage repair system Fragmentation of the mitochondrial network	Rouzier C. et al., 2012
Skin fibroblasts derived from 4 patients with CMT2A	M376V R707P V226_S229del Q74R	Mitochondrial respiratory chain dysfunction Decreased mtDNA copy number High levels of mtDNA depletion	Vielhaber S. et al., 2013
Motor neurons derived from iPSCs of patients with CMT2A	R364W	Reduced mitochondrial trafficking with slower anterograde and retrograde velocities along axons. Electrophysiological impairments including increased excitability, higher sodium current density, and reduced inactivation of voltage-dependent sodium and calcium channel	Saporta et al., 2015
Motor neurons derived from iPSCs of patients with CMT2A	A383V	Decreased respiratory chain activity: complexes II and III Reduced mitochondrial mass and mtDNA content No mtDNA alterations	Rizzo F. et al., 2016

		Decrease of mitochondrial trafficking leading to perinuclear aggregation No survival and morphometric defects Increased resistance to apoptosis Increased autophagic and mitophagic flux	
Motor neurons derived from iPSCs of patients with CMT2A	R94Q	Abnormal mitochondrial morphology: shorter mitochondrial length within neurites, presence of abnormal mitochondria with loss of crista Reduction in the percentage of moving mitochondria Decrease in ATP levels in neurites Increased toxicity sensitivity to vincristine and paclitaxel	Ohara et al., 2017
Motor neurons derived from iPSCs of patients with CMT2A	R94Q	Hyper-connectivity: increase in burst rate Alterations in mitochondrial morphology: reduced mitochondrial elongation and increase in circularity Impairment in axonal transport: decrease in the speed of mitochondria and lysosomes and the proportion of active mitochondria and lysosomes moving within the cells Defects in OxPhos: decrease in mitochondrial basal respiration. Transcriptomic analysis: enrichment in “PI3K-AKT signalling” and respiratory chain pathway	Van Lent et 2021
Skin fibroblasts derived from CMT2A patients	R364W M376V W740S	Mitochondrial mass and mtDNA levels are unchanged Moderate disturbances in Ca ²⁺ homeostasis Reduced ER-mitochondria contacts	Larrea D. et al., 2019
Skin fibroblasts from CMT2A patient	C217F	Mitochondrial mass and mtDNA levels and integrity unchanged Mitochondrial fragmentation Reduced ΔΨ _m Reduction of respiratory chain complexes activity Transcriptomic analysis: enrichment in “PI3K-AKT signalling” Reduced autophagy and increased cellular proliferation (mTORC2/AKT activation)	Zanfardino P. et al., 2022

Table 2. Mitochondrial Alterations in ADOA/ADOA Plus Cellular Models.

Cell Type	OPA1 mutation	Remarks	References
Fibroblasts from 3 patients with ADOA plus	V903Gfs3, E221K QT86Sfs15, H957Y QT86Sfs*15, H957Y	Increased mitochondrial fragmentation Depletion of mtDNA Altered mitochondrial localization Increased mitophagy flux	Liao C. et al., 2017

Fibroblasts from 7 ADOA patients	1) S545R 2) R445H 3) I382M 4) R824* (truncated, low levels) 5) R557* (truncated, low levels) 6) I382M	1) and 2) Fragmented and punctiform mitochondria 3) Mild mitochondrial fragmentation 1), 2), and 3) Loss of mitochondrial volume 1) and 2) Mild uncoupling of oxidative phosphorylation 4) and 6) Severe defects in oxidative phosphorylation Altered autophagy 4) and 5) Mild mtDNA depletion	Kane M. S. et al., 2017
Lymphoblastoid cells derived from ADOA patients	P400A	Decreased mtDNA copy number Reduced levels of 4 mtDNA-encoded polypeptides Respiratory capacity defects ATP synthesis defects Altered mitochondrial membrane potential Increased ROS production Increased apoptosis Mitochondrial morphological defects (fragmentation and swelling)	Zhang et al., 2017
Lymphoblastoid cell lines from ADOA patient	c.1444-2A>C (splicing variants, deletion of the 15th exon in mRNA transcript, low protein levels)	Respiratory chain activity defects More punctate mitochondria clustered in the perinuclear region, No marked depletion of mtDNA or mitochondrial mass Reduced ATP synthesis Reduced $\Delta\Psi_m$ Increased ROS production Increased mitophagy	Sun C. et al., 2021
Skin fibroblasts from ADOA plus patient	H42Y	Mitochondrial mass unchanged mtDNA levels slightly increased mtDNA integrity unchanged Mitochondrial fragmentation Reduced $\Delta\Psi_m$ Reduction of respiratory chain complexes activity Increased ROS production Transcriptomic analysis: enrichment in “p21WAF1/CIP1 and p53 pathways” along with downregulation of mitotic cell cycle genes Reduced autophagy and increased expression of senescence markers (SA- β galactosidase; p53 and p21) associated with lower mTORC2 activity	Zanfardino P. et al., 2024

10. Distinct Cellular Outcomes of MFN2 and OPA1 Mutations

Recent studies (Zanfardino et al., 2022; 2024) have revealed distinct cellular effects of MFN2 and OPA1 mutations, particularly concerning mitochondrial dynamics, autophagy, and cellular phenotypes. Researchers analysing primary fibroblasts carrying the MFN2^{C217F} and OPA1^{H42Y} mutations found significant mitochondrial fragmentation, depolarization, and impaired respiration, primarily due to decreased activity of respiratory chain complexes. These findings suggest a dominant-negative effect of the mutations, highlighting their detrimental impact on mitochondrial quality control [177–179]. Both MFN2 and OPA1 mutations reduced the formation of autophagosomes, indicating a shared defect in autophagy regulation. Interestingly, despite the

accumulation of damaged and fragmented mitochondria, autophagy was not activated in either mutant fibroblast line. The mitophagy machinery remained intact, suggesting that the observed defects arise from impaired signalling or autophagosome biogenesis, rather than a failure of the mitophagy process itself [177,180]. Transcriptomic profiling uncovered differential cellular responses between MFN2 and OPA1 mutations. In MFN2-mutated cells, genes associated with the PI3K/AKT signalling pathway and cell proliferation were upregulated, correlating with increased mTORC2/AKT activation, accelerated cell division, and reduced autophagy—consistent with the anabolic effects of PI3K/AKT/mTOR signalling [181,182] (Figure 2). These findings align with previous research demonstrating that MFN2 suppresses mTORC2 activity, influencing AKT phosphorylation at Ser473, a mechanism implicated in cancer growth and metastasis [183]. Additionally, in pancreatic cancer cells, MFN2 enhances autophagy by inhibiting PI3K/AKT/mTOR signalling, underscoring its role in maintaining MAM integrity and energy balance [178,184]. Conversely, OPA1-mutated fibroblasts exhibited a distinct profile, characterized by p21WAF1/CIP1 and p53 pathway activation, and downregulation of mitotic cell cycle genes. This expression pattern suggests an early senescent phenotype, confirmed by elevated SA- β -Gal activity. Additionally, these cells showed increased ROS levels, indicating oxidative stress and mitochondrial dysfunction, which may drive senescence [185–188]. In OPA1 mutants, impaired mTORC2 activation, cellular senescence, and weakened autophagic responses were observed. Researchers suggested that mitochondrial dysfunction in OPA1-deficient cells contributes to premature ageing by suppressing AMPK responsiveness, a key regulator of autophagy and metabolic balance and preventing mTORC1 inhibition [180,189] (Figure 2). In support of this, previous studies in mice demonstrated that OPA1 deletion in muscle cells induces premature senescence and early death, further linking OPA1 mutations, mitochondrial dysfunction, and senescence [72,190]. Moreover, OPA1 overexpression in Chang cells has been linked to mitochondrial fusion and senescence markers, reinforcing its role in ageing [191]. Similarly, aged mesenchymal stromal/stem cells (MSCs) and diseased postmitotic cells accumulate dysfunctional mitochondria with altered OPA1 levels, further emphasizing its role in aging and cellular decline [190,192,193].

Therapeutic Implications. The PI3K/AKT/mTOR pathway has emerged as a key regulator of the divergent outcomes observed in MFN2 and OPA1 mutants. Pharmacological modulation of this pathway effectively normalized cellular phenotypes in mutant cell models. In MFN2-mutated cells, mTOR inhibition with *torin1* restored normal cell proliferation, while AKT inhibition with *miransertib* improved both autophagy and cell proliferation [177,179]. In OPA1-mutated cells, mTORC1 inhibition with *everolimus* counteracted senescence and restored autophagy [180] (Figure 2). These findings underscore the crucial link between mitochondrial dysfunction, organelle morphology, and cellular signalling. The PI3K/AKT/mTOR pathway serves as a key sensor of mitochondrial distress, orchestrating the subsequent cellular adaptations [144]. Targeting this pathway presents a promising therapeutic strategy to counteract the pathological consequences of MFN2 and OPA1 mutations and restore mitochondrial function [177–180].

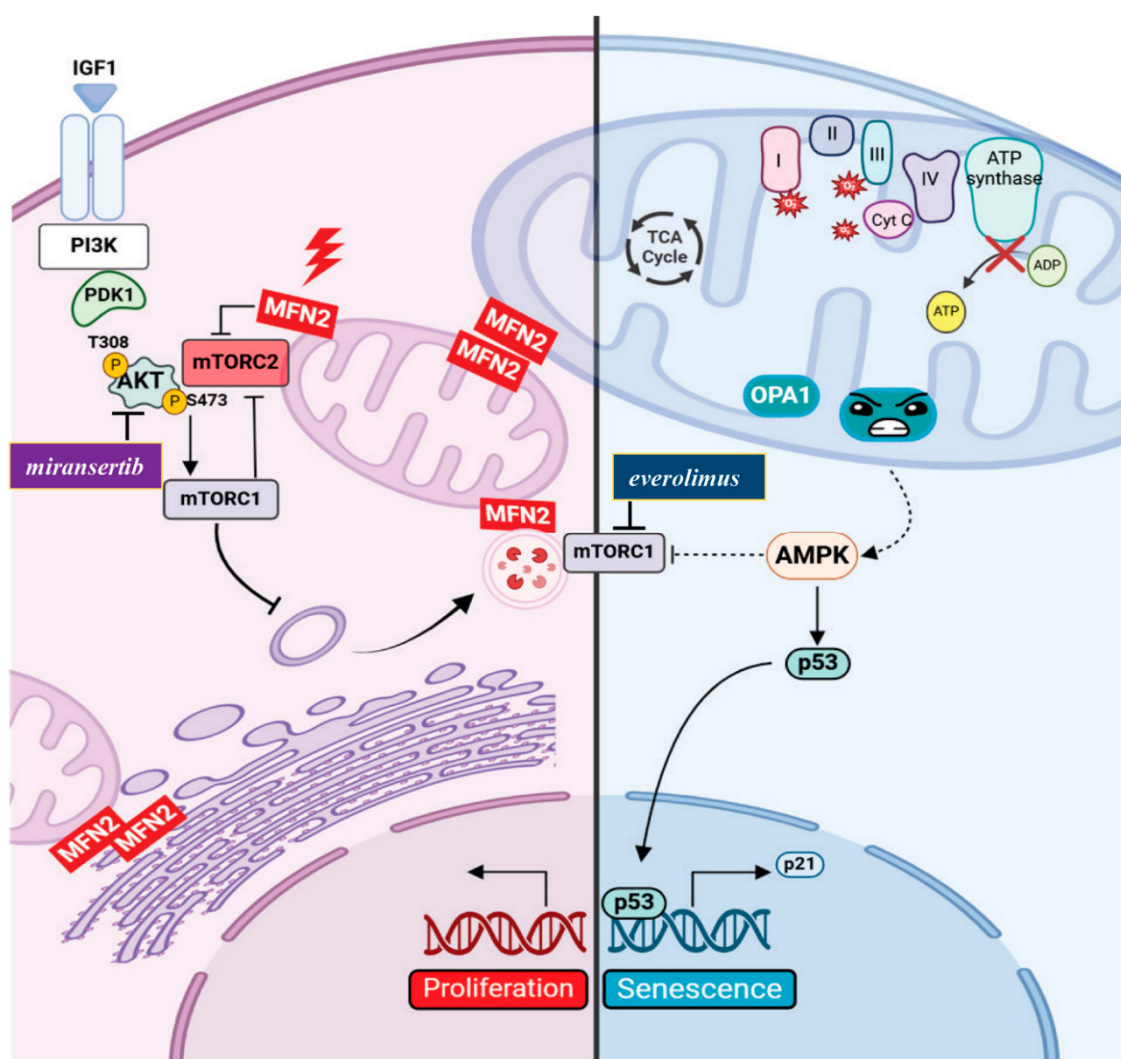


Figure 2. Schematic representation of altered processes in CMT2A and ADOA fibroblasts.

Phosphatidylinositol 3-kinase (PtdIns3K) catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PtdIns2P) to phosphatidylinositol-3,4,5-trisphosphate (PtdIns3P), recruiting proteins such as 3-phosphoinositide-dependent kinase (PDK1). PDK1 phosphorylates AKT at Thr(308), while second phosphorylation at Ser(473) by mTORC2 fully activates AKT, promoting cellular proliferation. MFN2, localized at the outer mitochondrial membrane (OMM) or mitochondria-associated ER membranes (MAMs), suppresses mTORC2 kinase activity, thereby inhibiting AKT.

Mutant MFN2 (*red lightning*) loses this inhibitory function, leading to hyperactivated AKT, reduced autophagy, and increased proliferation. AKT promotes proliferation directly or via mTORC1 and inhibits autophagy through both mTORC1-dependent and independent mechanisms. *Miransertib* (*violet*), an AKT inhibitor, restores autophagy and normalizes proliferation (*left panel*).

AMPK, a serine-threonine kinase, acts as an energy sensor, maintaining cellular homeostasis by promoting survival and autophagy through mTORC1 suppression (*right panel*). OPA1, located in the inner mitochondrial membrane (IMM), regulates mitochondrial function. Mutant OPA1 (*blue emoticon*) induces mitochondrial depolarization (green mitochondrion), increases ROS production, and impairs the electron transfer chain (ETC). These dysfunctions lead to premature senescence and a decline in AMPK responsiveness (*dotted line*), reducing autophagy initiation and cell survival.

OPA1-driven mitochondrial dysfunction contributes to p53 activation, which regulates stress response genes, including the cell cycle inhibitor p21. Increased p53 and p21 expression in OPA1-

mutated cells promotes senescence. However, senescence and autophagy defects can be reversed by inhibiting mTORC1 using *everolimus* (blue) (right panel).

11. Concluding Remarks

MFN2 and OPA1 are crucial regulators of mitochondrial dynamics, playing key roles in balancing fusion and fission to maintain mitochondrial structure, quality control, and energy production in response to cellular conditions. MFN2 primarily functions at the outer mitochondrial membrane, while OPA1 operates at the inner membrane mitochondrial fusion plays a vital role in the exchange of mitochondrial components, which helps to prevent the buildup of defective proteins and mutated mtDNA and allows for functional complementation. During periods of metabolic stress, such as starvation or cellular senescence, mitochondrial fusion is enhanced and forms elongated networks. These networks support oxidative phosphorylation (OxPhos) and help cells evade mitophagy. In contrast, severe mitochondrial damage, excessive nutrient levels, or increased cell proliferation trigger mitochondrial fission, resulting in smaller mitochondria. This fission process facilitates the redistribution of mitochondria during mitosis and helps isolate damaged mitochondria for degradation through mitophagy (Figure 3). Although mitophagy is a specialized form of autophagy, it is independently regulated in response to mitochondrial damage. Despite this, both processes can interact to maintain cellular homeostasis. For instance, autophagy complements mitophagy by responding to broader metabolic cues, ensuring the health of the cell. Central to the coordination of these processes is the mTOR/AKT pathway, which integrates signals related to nutrient availability, energy status, mitochondrial integrity, and even cellular differentiation. This coordination between mitochondrial dynamics and cellular function is also reflected in the dynamic regulation of mitochondrial morphology. The balance between fusion and fission is critical for metabolic adaptation, as elongated mitochondria, regulated by OPA1 and MFN2, enhance OxPhos, whereas fragmented mitochondria favor glycolysis. These shifts are particularly evident during cellular reprogramming. For instance, the generation of induced pluripotent stem cells (iPSCs) is linked to a fragmented mitochondrial network and increased glycolysis. In contrast, differentiation restores mitochondrial elongation and enhances OxPhos. Disruptions in the balance of mitochondrial function, such as those caused by mutations in the MFN2 and OPA1 genes, can negatively impact cellular health, especially in neurons. The initiation of mitophagy, the process where damaged mitochondria are removed, is determined by the extent of mitochondrial damage or dysfunction and the associated energy status of the cell [194]. When mitochondria are not significantly harmed, with respect to the high-energy costs, the cell tends to avoid their removal through mitophagy. The cell can recognize a decline in energy production resulting from mitochondrial changes. In response, it may initiate nonselective autophagy to acquire the nutrients needed for vital biosynthetic processes [195]. Research has indicated that mitochondrial dysfunction is associated with increased autophagic markers across different cellular disease models. For instance, in fibroblasts deficient in OxPhos, mitophagy is not increased but there is an accumulation of autophagosomes, lysosomes and late autophagic vacuoles [196]. This accumulation is thought to result from a trigger of nonselective autophagy, which occurs in response to a pseudo-starvation state. When mitochondria are significantly impaired, cells can trigger mitophagy and activate a signalling pathway that encourages nonselective autophagy. Many studies highlight the role of specific mitophagy factors in regulating the overall autophagic response to mitochondrial damage [197]. Investigating how the absence of these factors affects autophagy initiation, rate, extent, and overall cell survival would provide valuable insights. Research conducted in yeast demonstrates that mitophagy is consistently accompanied by nonselective autophagy, indicating a strong connection between mitochondrial *status* and autophagic regulation [197]. However, it remains unclear whether mitochondrial fission is universally required for mitophagy or if only necessary under specific conditions. General autophagy-inducing situations, such as starvation, also trigger mitophagy, but the mechanisms governing selective mitochondrial degradation in these contexts remain uncertain. Furthermore, it is possible to identify conditions that allow the induction of mitophagy while non-selective autophagy

is not triggered simultaneously. For example, in mammalian cells experiencing mild oxidative stress, DRP1-dependent mitophagy occurs without the activation of nonselective autophagy, suggesting that mitophagy can be regulated independently [198]. Additionally, it's important to note that mitochondrial fragmentation due to MFN2 and OPA1 mutations does not always lead to autophagy, as certain factors, such as cell proliferation (as seen in CMT2A) or premature senescence (as in ADOA), can suppress the autophagic process (Figure 3). MFN2 and OPA1 have differing effects on these cellular processes through their influence on mTOR/AKT signalling, a key regulator in either cancer or neurodegeneration. In CMT2A, dysfunction of MFN2 disrupts mitochondrial transport and bioenergetics, leading to a suppression of autophagy and an increase in cellular proliferation, potentially promoting excessive survival of neuronal stem cells. Conversely, mutations in OPA1 impair mitochondrial quality control, driving senescence and worsening neurodegeneration in ADOA by failing to efficiently clear damaged mitochondria. Although MFN2 and OPA1 have overlapping functions, their distinct roles contribute to the specific disease phenotypes observed in CMT2A and ADOA. Both conditions involve mitochondrial fragmentation and dysfunction; however, their impact on the regulation of cellular function differs, which in turn shapes disease progression in unique ways.

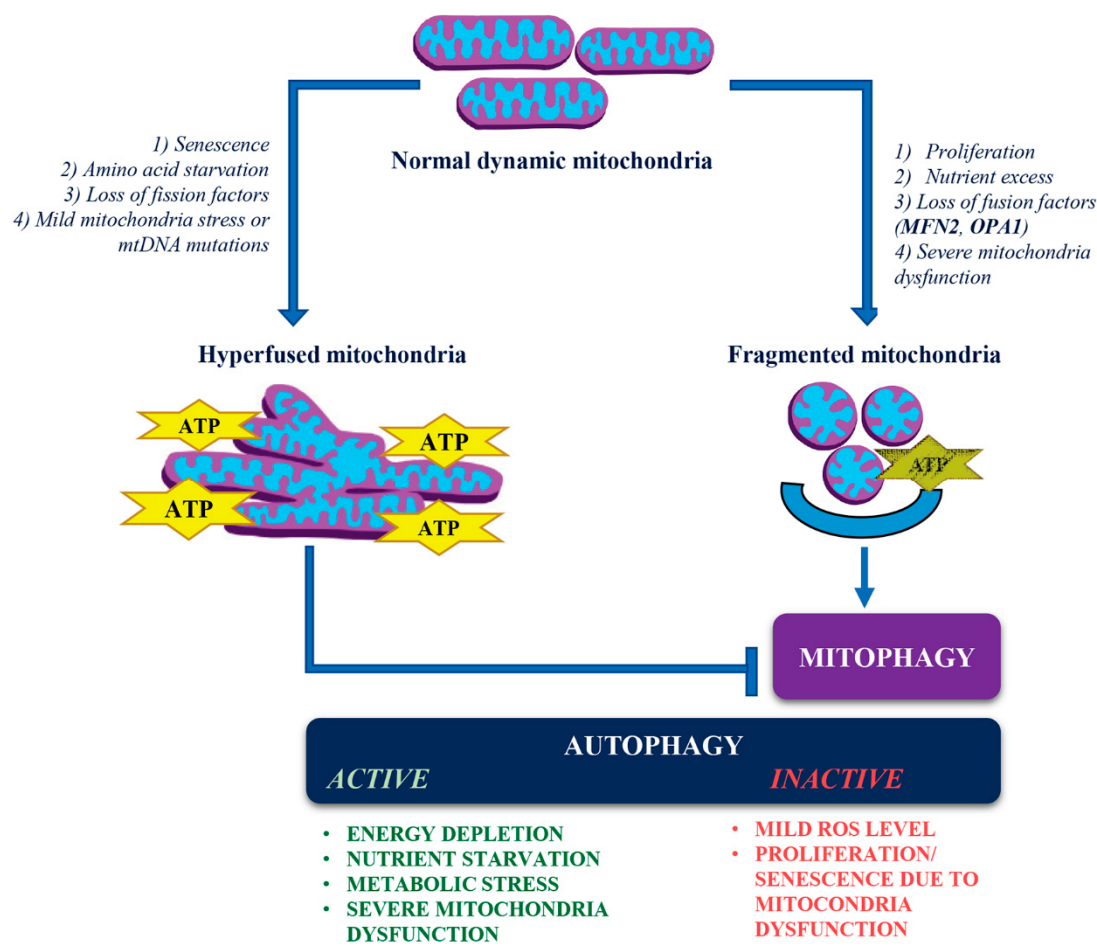


Figure 3. The Balance Between Mitochondrial Dynamics and Cellular Homeostasis.

This illustration depicts the contrasting effects of different cellular conditions on mitochondrial dynamics and homeostasis.

1. Mitochondrial Hyperfusion:
 - Triggered by cellular senescence, amino acid starvation, loss of fission factors, mild mitochondrial stress, or mitochondrial DNA (mtDNA) mutations.
 - Supports oxidative phosphorylation (OxPhos) and ATP production.

- Prevents autophagic clearance of damaged mitochondria by mitophagy despite cellular stressors.

2. Mitochondrial Fragmentation:

- Induced by cellular proliferation, nutrient excess, loss of fusion factors (like MFN2 and OPA1), and severe mitochondrial dysfunction.
- Promotes selective autophagic clearance (mitophagy) of dysfunctional mitochondria.

Mitophagy typically occurs due to mitochondrial dysfunction, autophagy can also respond to severe cellular stress, driven by factors like energy depletion, nutrient starvation, and metabolic stress.

3. Autophagy Regulation:

- There are instances when, despite mitochondrial fragmentation, autophagy may not be activated, particularly in contexts such as cell proliferation (as seen in Charcot-Marie-Tooth disease type 2A) or premature senescence (observed in Autosomal Dominant Optic Atrophy).
- Under mild oxidative stress, mitophagy is activated; however, the induction of non-selective autophagy may not occur simultaneously.

This graphical abstract illustrates the delicate interplay between mitochondrial dynamics and cellular homeostasis and the implications that disruptions in this balance can have on cellular health and disease.

12. Future Directions

The divergent effects of MFN2 and OPA1 mutations raise critical questions about their shared and distinct roles in disease progression:

- Could the proliferative changes observed in CMT2A2 fibroblasts and cancer models of MFN2 dysfunction also occur in neuronal stem cells affected by CMT2A?
- Does senescence caused by OPA1 deficiency directly contribute to neurodegeneration in ADOA, and is this related to impaired autophagy?
- How does metabolic reprogramming influence the progression of mitochondrial diseases, and can interventions be implemented to restore the balance of autophagy and mitophagy?

To address these questions, future research should concentrate on advanced neuronal stem cell models, co-cultures, and 3D organoids that better replicate *in vivo* environments. Investigating the interactions between mitochondrial dysfunction, metabolic shifts, and mTOR/AKT signalling within these systems will provide essential insights into disease mechanisms. Furthermore, targeting autophagy and mitophagy pathways therapeutically presents promising opportunities for intervention. The potential of mTOR inhibitors (such as everolimus and torin1) and modulators of MAM integrity should be examined for their ability to restore autophagic flux, enhance mitochondrial quality control, and prevent neurodegeneration. Understanding the relationship between mitochondrial dysfunction, autophagy, metabolism, and signalling pathways is crucial for developing novel therapies. These advancements could benefit not only those with mitochondrial and neurodegenerative diseases but also have wider implications in the fields of ageing and cancer.

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