acta medica

REVIEW ARTICLE

An overview of the recent knowledge about mitochondrial fusion and fission and related neurodegenerative diseases

Evrim AKSU[1], [BSc.],
Burcu BALCI-HAYTA[1]*, [PhD],
Assist. Prof. Dr.

1 Hacettepe University Faculty of Medicine, Department of Medical Biology

* *Corresponding Author: Burcu Balci-Hayta. Hacettepe University Faculty of Medicine, Department of Medical Biology, 06100, Sıhhiye, Ankara – Turkey*

e-mail: burcub@hacettepe.edu.tr

This review article has not been presented previously in a congress or symposium.

Received 28 June 2016; accepted 17 August 2016; published online 8 September 2016

ABSTRACT

Mitochondria undergo fusion and fission events and are called as dynamic organelles. The combined effect of these opposing events gives rise to a mitochondrial network that is necessary for cells to maintain effective and dynamic subcellular organization. The counterbalance of these two mechanisms determines morphology and size of the organelle. Moreover, fusion and fission regulate and maintain function and distribution of organelle, as well as cellular homeostasis. They are recognized to be an important constituent of cellular quality control mechanisms at the organelle level. The underlying mechanisms of mitochondrial fusion and fission machinery appear to be conserved in different species. Recently, identification of key molecular components and their extensive post-translational regulation has opened new directions for exploring mitochondrial biology and novel therapeutic targets for various diseases related to mitochondrial dysmorphology and dysfunction. This review summarizes recent advances in the detailed understanding of mitochondrial dynamics and the role of fusion and fission events in specific diseases.

Key words: Mitochondrial dynamics, neurodegenerative diseases, mitochondrial fusion, mitochondrial fission

Introduction

Mitochondria are vital organelles involved in various functions within the cell, including cellular respiration through the production of ATP, amino acid and iron/sulfur cluster biosynthesis, fatty acid β-oxidation, cell death, as well as Ca^{2+} buffering and signaling [1-3]. They are considered as semi-autonomous organelles that contain specialized and complete genetic systems that must be coordinated with cellular demands. Mitochondrial DNA is a double-stranded, circular molecule consisting of 16.569 base pairs (bp.) that encodes 37 genes, 13 of which encode structural subunits of the oxidative phosphorylation system. The majority (~98%) of mitochondrial proteins is encoded by the nuclear DNA and is imported from the cytosol. It is noteworthy to mention that mitochondrial function is important for cell life and death, and is controlled by several factors [4-6]. In the cell, mitochondria form highly dynamic, interconnected networks that are sustained by continuous opposing events of mitochondrial fission and fusion [7-8]. Mitochondrial movement and fission were first observed in live chick embryonic cells with light microscopy in the 1910s [9]. During 1950s, mitochondria have been observed by electron microscopy as bean-shaped organelles. This idea was accepted until the 1990s, when mitochondria were recognized to be mobile organelles that continuously move, fuse, and divide as observed by phase contrast microscopy [10-11].

Mitochondrial fusion occurs by joining both inner and outer membranes of two mitochondria synchronically in order to produce single mitochondrion. Conversely, mitochondrial fission is the process of separation of a single mitochondrion into two or more morphologically distinct structures [12]. These two mechanisms, called as mitochondrial dynamics, create a connected tubular mitochondrial network throughout the cell [4]. The morphology and number of mitochondria differ according to cell type and cellular metabolic needs [13]. Mitochondrial fusion and fission are the primary processes controlling mitochondrial size, length and number [14], and the balance between these

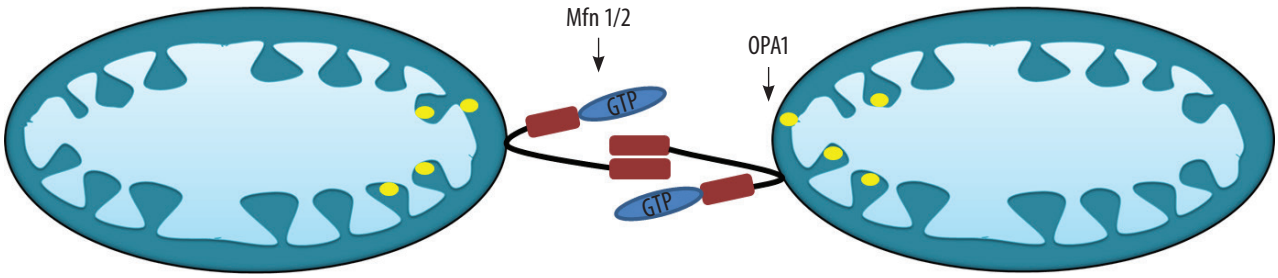

Figure 1. Schematic representation of the mammalian mitochondrial fusion machinery, mediated by dynamin family of GTPases; Mfn1, Mfn2, and OPA1 proteins. Mfn1 and Mfn2 have been shown to facilitate tethering of two mitochondria and fusion of outer membranes while OPA1 plays a role in fusion of inner membranes.

two events affect mitochondrial morphology [15]. Increased rate of fusion gives rise to elongated, interconnected mitochondrial networks, and unbalanced fission causes fragmented, separate mitochondria [16]. In addition, mitochondrial fission mediates the movement of intermembrane space proteins (IMS) to the cytosol, thereby aiding apoptosis [14]. Therefore, mitochondrial dynamics has an important role in maintenance of organelle function, distribution and development, and these antagonistic events participate in mitochondrial quality control at the organelle level. They have also appeared as important regulators of mitophagy and apoptosis pathways [15]. In the past decade, altered mitochondrial dynamics has also been linked to the pathomechanism of various diseases that are not considered to involve mitochondria, such as cancer, cardiovascular, neurodegenerative, and neuromuscular diseases. Impaired mitochondrial dynamics have negative effects on bioenergetic supply and associated with the pathogenesis of several different diseases. Although some disorders related to altered mitochondrial dynamics [Parkinson's Disease (PD), Charcot- Marie-Tooth Disease Type 2A (CMT2A), Autosomal Dominant Optic Atrophy (ADOA), and Alzheimer's Disease (AD)] result from a defect in any of the genes encoding molecular mediators of mitochondrial dynamics or their post-translational regulators, most reflect changes in mitochondrial dynamics triggered by changes in the cellular milieu. Extensive studies have been conducted to clarify the exact mechanisms underlying mitochondrial dynamics and the regulation of the mitochondrial network. In this review, we summarize the most recent studies about mitochondrial dynamics and the role of impaired mitochondrial dynamics in neurological disorders.

Mitochondrial Fusion

Mitochondrial fusion is a multistep process in which the inner and outer mitochondrial membranes fuse in a coordinated manner. This is assisted by several dynamin family of proteins, which includes various large GTPases having different cellular functions. The fusion mechanism is conserved in evolution across multiple species. As shown in Figure 1, the first step in mammalian mitochondrial fusion is the tethering of two fusing mitochondria and subsequent fusion of the outer membranes mediated by Mitofusin 1 and 2 (Mfn1 and Mfn2, respectively). Two isoforms of mitofusin, Mfn1 and Mfn2, were the first key components identified in mitochondrial fusion machinery [17] and are 81% identical. They are localized to the mitochondrial outer membrane and can form homo- and hetero-oligomeric antiparallel coiled-coil structures between adjacent fusing mitochondria in *trans* [18-19]. However, the fusion of the inner membranes requires the interaction of an IMS protein, Optic atrophy protein 1 (OPA1) in the second step. OPA1, unlike mitofusins, is not required on both mitochondria to promote membrane fusion [20]. These two steps in the fusion process depend on energy released by the hydrolysis of GTP (Figure 1) [21]. In addition, mitochondrial membrane lipids, such as cardiolipin, phosphatidic acid and phosphatidylethanolamine also play an important role in mitochondrial fusion by mixing and structural rearrangement of mitochondrial membrane lipid bilayers and matrix contents which are essential for organelle function [20-22]. Fusion of the mitochondrial outer membrane is usually synchronized with inner membrane fusion. However, in some instances, when the mitochondrial membrane potential is disrupted, outer mitochondrial membrane fusion can take place without inner membrane fusion [23].

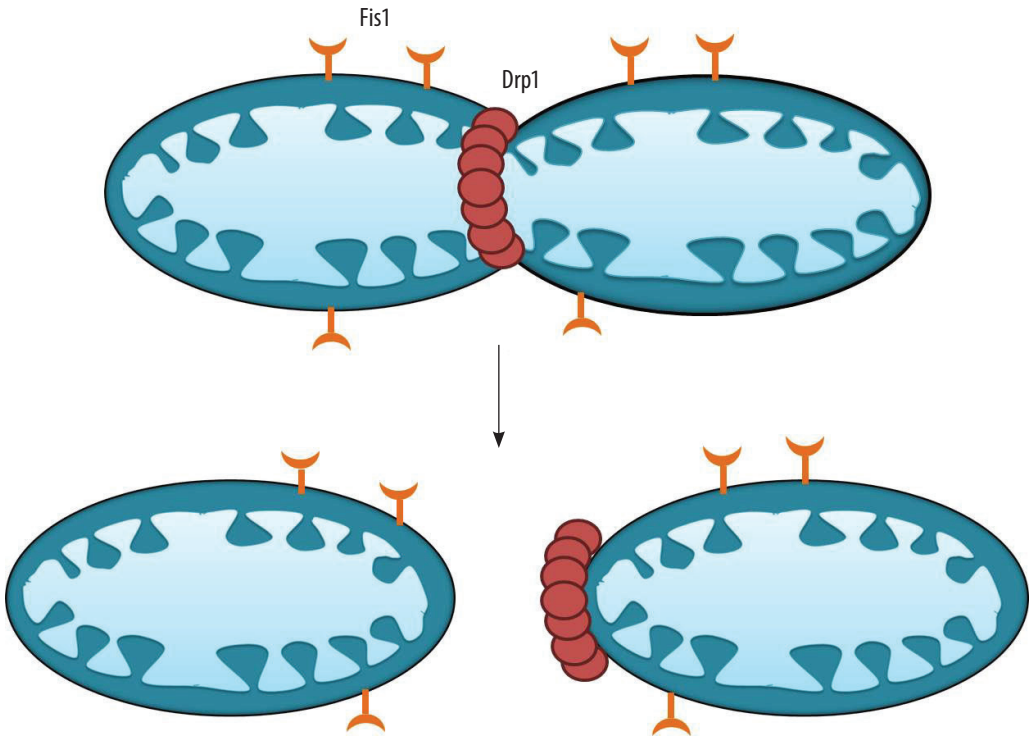

Figure 1. Schematic representation of the mammalian mitochondrial fission machinery, mediated by Fis 1 and Drp 1. Drp1 is shown to be recruited to fission site by Fis1. After recruitment, Drp1 forms spirals around mitochondrion and helps split of the mitochondrion by squeezing inner and outer membranes.

Mitochondrial fusion rate is increased by low levels of cellular stress. In order to reduce the accumulation of mitochondrial DNA mutations and oxidized proteins, healthy mitochondria fuse with partially damaged ones and override the damaged mitochondrion's function [24]. Also, components that are missing in defective mitochondria can be restored by adjacent mitochondria as a consequence of fusion [3]. Impaired mitochondrial fusion can lead to the cellular accumulation of damaged mitochondria, which may result in mitophagy. Since mitochondrial fusion is vital for mitochondrial DNA stability and cellular respiration, cells that lack mitochondrial fusion activity have reduced respiratory capacity and cell growth. It is also suggested that in fusion-deficient cells, mitochondrial DNA is absent in many mitochondria [16].

Mitochondrial fusion is also essential for embryonic development and survival, even if it was demonstrated to be unnecessary for cell survival *in vitro*. It was shown that Mfn1 or Mfn2 single knockout mice die during midgestation owing to mitochondrial fusion deficiency in the placenta. However, Mfn1 and Mfn2 double knockout embryos die even earlier, although embryonic fibroblasts obtained from double knockout mice can survive in cell culture [25-26].

Mitochondrial Fission

The key components of mitochondrial fission machinery in mammals are Dynamin-related protein 1 (Drp1) and Mitochondrial fission 1 protein (Fis1) [17]. Drp1 is a dynamin family member GTPase that is mostly found in the cytosol. It is also found in association with spotted vesicles attached to endoplasmic reticulum and microtubules, the sites where fission starts [27]. As shown in Figure 2, Drp1 accumulates on outer mitochondrial membranes and squeezes both outer and inner membranes simultaneously by building up spirals around mitochondria through GTPase activity. Fis1, anchored in outer mitochondrial membrane, is accepted as the main receptor protein for Drp1. Previous studies showed that decreased level of human Fis1 (hFis1) results in elongation of the organelle while overexpression leads to mitochondrial fragmentation [28-29]. In addition to Fis1, there are other proteins localized in the outer membrane involved in Drp1 recruitment, such as Mitochondrial fission factor (Mff) and Mitochondrial dynamics proteins MiD49 and MiD51 [15]. Downregulation of Mff prevents the recruitment of Drp1 from the cytosol, while Mff overexpression results in the association of Drp1 with the outer mitochondrial membrane [30]. MiD49 and MiD51 are known to recruit

Table 1. Key regulatory components of mitochondrial dynamics [21-66]

Function	Protein	Regulator	Activity
Fusion	Mfn2	Bcl-2-associated X protein (Bax) & Bcl-2 homologous antagonist killer (Bak)	Assembly of Mfn2- containing complexes
		B-cell lymphoma-extra large (Bcl-xL)	Unknown
		Mfn 1	Complementation of mutant forms of Mfn2 in CMT2A
		Membrane-associated RING-CH protein 5 (MARCH-V / MITOL)	Possible ubiquitination and degradation
		Mitofusin-binding protein (MIB)	Negative regulator of mitofusins
		Stomatin-like protein 2 (STOML2)	Unknown
	OPA1	Presenilins-associated rhomboid-like protein (PARL)	Proteolytic processing
		Paraplegin	Proteolytic processing
		High Temperature Requirement Protein A2 (HTRA2)	Proteolytic processing
Fission	Drp1	Cyclin B- Cyclin-dependent kinase (Cyclin B-CDK)	Phosphorylation during cell cycle
		Cyclic adenosine monophosphate dependent kinase (cAMP-dependent kinase)	Inhibition of assembly and GTPase activity by phosphorylation
		Small ubiquitin-like modifier-1 (SUMO-1)	SUMOylation
		SUMO1/Sentrin Specific Protease 5 (SENP5)	Removal of SUMO
		Bax and Bak	Mitochondrial association of Drp1
		Fis1	Mitochondrial association of Drp1
		MARCH-V / MITOL	Ubiquitination and degradation
	Fis1	MARCH-V / MITOL	Ubiquitination and degradation

Drp1 to mitochondria. However, it is controversial whether these proteins affect fission event positively or negatively [31-32]. One study indicated that knockdown of *MiD51* gene gives rise to an increase in mitochondrial fission, suggesting that MiD51 inhibits Drp1 activity [32]. However, a more recent study demonstrated that single or double knockdown of *MiD49 and MiD51* genes cause a reduction in mitochondrial fission [33]. Clearly, further studies are required to elucidate the exact function of MiD49 and MiD51 proteins. In addition to these proteins, cardiolipin, one of the mitochondrial membrane lipids, has been shown to facilitate Drp1 activity by recruiting it to outer membrane [34]. The exact mechanism of Drp1 recruitment to mitochondrial fission sites is still under investigation. Recent studies suggest an endoplasmic reticulum (ER)-associated mechanism in which the ER surrounds mitochondrial tubules at the site where fission will start. Then, elongation of actin filaments and recruitment of Myosin 2, regulated by phosphatidic acid metabolism, facilitate mitochondrial constriction at the ER contact site prior to Drp1 recruitment [22]. After the accumulation of Drp1 on the outer membrane, it builds up spirals around mitochondrial tubules and constricts outer and inner mitochondrial membranes [3-35-36].

Regulation of Mitochondrial Fusion and Fission

Fusion and fission events should be tightly regulated to retain a healthy mitochondrial population in the cell. Although complex regulatory mechanisms were not understood yet, several cellular signaling pathways are suggested to play vital roles in modulating the activity of fusion and fission mechanisms [4]. These mechanisms include control of key proteins involved in mitochondrial dynamics at either transcriptional or post-translational level [17]. Known post-translational regulatory components are summarized in Table 1.

Diseases Associated with Defects in Mitochondrial Dynamics

Maintaining healthy mitochondrial dynamics is vital for cells since defects in any step of mitochondrial fusion and fission leads to human diseases. Disorders associated with mitochondrial dynamics can be divided into two groups. The first group consists of diseases primarily associated with impaired mitochondrial fusion and fission. Monogenic mutations in nuclear or mitochondrial genes encoding proteins involved in mitochondrial dynamics or their regulatory elements result in neurological diseases such as Charcot-Marie-Tooth Disease Type 2A (CMT2A) and Autosomal Dominant Optic Atrophy (DOA). Additionally, recent studies have revealed that impaired mitochondrial dynamics can also affect complex diseases that are not assumed to be associated with mitochondria such as cancer, cardiovascular and neurodegenerative diseases like Alzheimer's and Parkinson's diseases [16].

Charcot-Marie-Tooth Disease Type 2A (CMT2A)

CMT2A is characterized by degeneration of peripheral nerves, particularly the neurons with the longest axons, that is why the distal extremities are the first affected parts of the body [37]. Neuronal degeneration leads to the deterioration of distal sensory and motor neurons [38]. Patients suffer from distal muscle weakness [39] and exhibit atypical gait and foot deformation [40]. The age of onset differs among patients and the disease tends to progress slowly [17]. Defects in the intracellular transportation of mitochondria to synapses are observed in CMT2A, which negatively affects bioenergetics of neurons [40-41]. However, the exact pathomechanism has not yet been clarified [42]. Heterogeneous mutations in *Mfn2* gene cause a neurogenic disease termed Charcot-Marie-Tooth Disease Type 2A [43]. More than 40 different mutations have been observed in *Mfn2*, most of which accumulate in the GTPase domain [14]. Since Mfn2 can form heterodimers with Mfn1, wildtype Mfn1 can compensate for the damage caused by mutations in Mfn2. This has led to speculation that differences in Mfn1 expression level determine disease severity, so CMT2A may be treated by regulating Mfn1 expression level in neurons [44]. Since most Mfn2 mutations occur in the GTPase domain that is necessary for Mfn2 function, it has been hypothesized that Mfn2 haploinsufficiency causes CMT2A [43]. Another possibility is that normal Mfn2 alleles are negatively affected by mutant Mfn2 alleles due to a dominant negative effect [45].

Autosomal Dominant Optic Atrophy (ADOA)

ADOA, the first disorder of mitochondrial dynamics, is the most common inherited childhood optic neuropathy [42-46]. The disease generally presents in childhood or early adolescence and patients slowly undergo progressive vision loss due to death of ganglion cells which receive visual information from photoreceptors and transmit it to the related part of the brain [47]. At the cellular level, defective mitochondrial fusion is observed in ADOA, which consequently makes neurons vulnerable to apoptosis [40]. The responsible gene in ADOA is *OPA1*. Nearly half of *OPA1* mutations give rise to severe immature truncated proteins and therefore, haploinsufficiency may be the cause of optic atrophy [48-49]. In addition, minor truncations or single amino acid changes may lead to the formation of a dominant-negative form of OPA1 which can have a negative effect on wild-type OPA1 [42].

It was thought that ADOA and CMT2A affect specific tissues; however, it is now known that clinical features of these diseases can coincide with each other. That is, the optic nerve may be affected by mutations in Mfn2, likewise, peripheral nerves can be affected by OPA1 mutations [50]. It has been suggested that defective mitochondrial replication and increased deletion of mitochondrial DNA are also associated with pathogenic OPA1 and Mfn2 mutations in post-mitotic tissues [51-52].

Parkinson's Disease (PD)

PD is an age-related progressive neurodegenerative disease in which the death of dopaminergic neurons in the substantia nigra results in resting tremors, muscle rigidity, and bradykinesia [40]. Several studies have led to the identification of monogenic forms of the PD which are caused by mutations in different genes, including *α-synuclein (SNCA), Parkin RBR E3 Ubiquitin Protein Ligase (PARK2), PTEN-induced putative kinase 1 (PINK1), Parkinsonism Associated Deglycase (DJ1)* and *Leucine-rich repeat kinase 2 (LRRK2)* and *High Temperature Requirement Protein A2 (HTRA2)* associated with mitochondrial dysfunction and oxidative stress [53].

Mutations in *PINK1* and *PARK2* genes, encoding PINK1 and Parkin proteins respectively, are the

major causes of the autosomal-recessive early-onset form of PD. These mutations cause upregulation of Drp1-mediated mitochondrial fission, and therefore neuronal cell death [3-4].

PINK1 is a serine-threonine kinase that prevents stress-induced mitochondrial depolarization and apoptosis and decreases oxidative stress, thereby protecting neurons [54-55]. In addition, it is essentially repressed in healthy mitochondria via translocation to the inner mitochondrial membrane and degradation by the Presenilins-associated rhomboid-like protein (PARL) [3]. However, in damaged mitochondria translocation of PINK1 is inhibited; therefore, PINK1 cannot be degraded by PARL protease, and is concentrated on the outer mitochondrial membrane. PINK1 accumulation, a sign of an unhealthy mitochondrion, mediates Parkin recruitment from the cytosol to damaged mitochondrion via its kinase activity. Parkin is a ubiquitin E3 ligase that ubiquitinates abnormal proteins in neurons and flags them for degradation, thereby protecting neural cells [56]. After PINK1 phosphorylates Parkin, Parkin ubiquitinates proteins on the outer mitochondrial membrane, leading to elimination of the damaged mitochondrion, a process called mitophagy [57]. Although mutations in *PINK1* and *PARK2* genes give rise to defects in the mitochondrial dynamics and so leads to PD, the exact relationship between mitochondrial dynamics and PINK1/Parkin is far from clear. Understanding the detailed pathway of mitochondrial dynamics in neurons may lead to further therapeutic applications for PD.

Alzheimer's Disease (AD)

AD, the most frequently diagnosed form of dementia, is characterized by the accumulation of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles in the hippocampus and other subcortical regions vital for cognitive function, resulting in selective neuronal death [40-58-59]. Patients undergo progressive memory loss due to synaptic dysfunction [60]. Even though the exact molecular pathway is not clear, it is suggested that Aβ plaques lead to functional disruption of synapses by binding dendritic spines [61]. While most AD cases are sporadic, a minority of them are familial resulted from mutations in the genes encoding amyloid β precursor protein (AβPP), presenilin 1 (PS1), or presenilin 2 (PS2) [59]. In one study, elevated expression of *Drp1, OPA1, Mfn1*, and *Mfn2* and decreased expression of *Fis1* was observed in hippocampi of AD patients compared to age-matched control individuals [62]. Another study showed that amyloid β accumulation gives rise to decrease in Drp1 expression. Therefore, it was concluded that an imbalance in mitochondrial fusion and fission could play a role in the pathomechanism of AD by causing dysfunction of mitochondria in neurons [63].

Conclusion

Mitochondrial fusion and fission, quality control mechanisms at the organelle level, are vital processes for mitochondrial morphology and function. Although the exact mechanisms of fusion and fission are yet to be clarified, it is known that maintaining proper mitochondrial dynamics is crucial for cellular health. In addition to the neurological diseases directly associated with mitochondrial dynamics, organelle dysfunction and structural abnormalities have also been linked to the pathomechanism of various diseases that are not classically considered to involve mitochondria [64]. Cells with high and changing energy demand, such as neurons and skeletal muscle cells, contain a large number of mitochondria to maximize ATP production by aerobic respiration. It is therefore not surprising that mitochondrial dysfunction and ultrastructural alterations also play a relevant role in the pathogenesis of several other neurodegenerative/ neuromuscular disorders [65]. However, the relationship between mitochondrial dysfunction, structural abnormalities and mitochondrial dynamics has not been fully revealed. Therefore, further studies should be conducted regarding the role of mitochondrial dynamics in the pathogenesis of these neurological diseases.

REFERENCES

[1] **Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS.** Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am J Physiol Cell Physiol 2004; 287: C817-833.

[2] **Lill R, Diekert K, Kaut A, Lange H, Pelzer W, Prohl C,** *et al.* The essential role of mitochondria in the biogenesis of cellular iron-sulfur proteins. Biol Chem 1999; 380: 1157-1166.

[3] **Youle RJ, van der Bliek AM.** Mitochondrial fission, fusion, and stress. Science 2012; 337: 1062-1065.

[4] **Westermann B.** Mitochondrial fusion and fission in cell life and death. Nat Rev Mol Cell Biol 2010; 11: 872-884.

[5] **Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J,** *et al.* Sequence and organization of the human mitochondrial genome. Nature 1981; 290: 457-465.

[6] **Bibb MJ, Van Etten RA, Wright CT, Walberg MW, Clayton DA.** Sequence and gene organization of mouse mitochondrial DNA. Cell 1981; 26: 167-180.

[7] **Parra V, Verdejo H, del Campo A, Pennanen C, Kuzmicic J, Iglewski M,** *et al.* The complex interplay between mitochondrial dynamics and cardiac metabolism. J Bioenerg Biomembr 2011; 43: 47-51.

[8] **Westermann B.** Merging mitochondria matters: cellular role and molecular machinery of mitochondrial fusion. EMBO Rep 2002; 3: 527-531.

[9] **Lewis MR, Lewis WH.** Mitochondria in Tissue Culture. Science 1914; 39: 330-333.

[10] **Rizzuto R, Brini M, De Giorgi F, Rossi R, Heim R, Tsien RY,** *et al.* Double labelling of subcellular structures with organelle-targeted GFP mutants in vivo. Curr Biol 1996; 6: 183-188.

[11] **Bereiter-Hahn J, Voth M.** Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc Res Tech 1994; 27: 198-219.

[12] **Cagalinec M, Safiulina D, Liiv M, Liiv J, Choubey V, Wareski P,** *et al.* Principles of the mitochondrial fusion and fission cycle in neurons. J Cell Sci 2013; 126: 2187-2197.

[13] **Benard G, Karbowski M.** Mitochondrial fusion and division: Regulation and role in cell viability. Sem Cell Dev Biol 2009; 20: 365-374.

[14] **Detmer SA, Chan DC.** Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol 2007; 8: 870-879.

[15] **van der Bliek AM, Shen Q, Kawajiri S.** Mechanisms of Mitochondrial Fission and Fusion. Cold Spring Harb Perspect Biol 2013; 5: a011072-a011072.

[16] **Chan DC.** Mitochondrial Fusion and Fission in Mammals. Annu Rev Cell Dev Biol 2006; 22: 79-99.

[17] **Chan DC.** Fusion and Fission: Interlinked Processes Critical for Mitochondrial Health. Annu Rev Genet 2012; 46: 265-287.

[18] **Detmer SA, Chan DC.** Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol 2007; 8: 870-879.

[19] **Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC.** Structural basis of mitochondrial tethering by mitofusin complexes. Science 2004; 305: 858-862.

[20] **Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC.** Mitofusins and OPA1 Mediate Sequential Steps in Mitochondrial Membrane Fusion. Mol Biol Cell 2009; 20: 3525-3532.

[21] **Benard G, Karbowski M.** Mitochondrial fusion and division: Regulation and role in cell viability. Semin Cell Dev Biol 2009; 20: 365-374.

[22] **Frohman MA.** Role of mitochondrial lipids in guiding fission and fusion. J Mol Med (Berl) 2015; 93: 263-269.

[23] **Malka F, Guillery O, Cifuentes-Diaz C, Guillou E, Belenguer P, Lombès A,** *et al.* Separate fusion of outer and inner mitochondrial membranes. EMBO reports 2005; 6: 853-859.

[24] **Santel A, Frank S, Gaume B, Herrler M, Youle RJ, Fuller MT.** Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. J Cell Sci 2003; 116: 2763-2774.

[25] **Chen H, Chan DC.** Physiological functions of mitochondrial fusion. Ann N Y Acad Sci 2010; 1201: 21-25.

[26] **Chen H, Chomyn A, Chan DC.** Disruption of fusion results in mitochondrial heterogeneity and dysfunction. J Biol Chem 2005; 280: 26185-26192.

[27] **Pitts KR, Yoon Y, Krueger EW, McNiven MA.** The dynamin-like protein DLP1 is essential for normal distribution and morphology of the endoplasmic reticulum and mitochondria in mammalian cells. Mol Biol Cell 1999; 10: 4403-4417.

[28] **Yoon Y, Krueger EW, Oswald BJ, McNiven MA.** The Mitochondrial Protein hFis1 Regulates Mitochondrial Fission in Mammalian Cells through an Interaction with the Dynamin-Like Protein DLP1. Mol Cell Biol 2003; 23: 5409-5420.

[29] **Stojanovski D, Koutsopoulos OS, Okamoto K, Ryan MT.** Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. J Cell Sci 2004; 117: 1201-1210.

[30] **Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, Youle RJ,** *et al.* Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. J Cell Biol 2010; 191: 1141-1158.

[31] **Palmer CS, Osellame LD, Laine D, Koutsopoulos OS, Frazier AE, Ryan MT.** MiD49 and MiD51, new components of the mitochondrial fission machinery. EMBO reports 2011; 12: 565-573.

[32] **Zhao J, Liu T, Jin S, Wang X, Qu M, Uhlen P,** *et al.* Human MIEF1 recruits Drp1 to mitochondrial outer membranes and promotes mitochondrial fusion rather than fission. EMBO J 2011; 30: 2762-2778.

[33] **Loson OC, Song Z, Chen H, Chan DC.** Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. Mol Biol Cell 2013; 24: 659-667.

[34] **Joshi AS, Thompson MN, Fei N, Huttemann M, Greenberg ML.** Cardiolipin and mitochondrial phosphatidylethanolamine have overlapping functions in mitochondrial fusion in Saccharomyces cerevisiae. J Biol Chem 2012; 287: 17589-17597.

[35] **Hatch AL, Gurel PS, Higgs HN.** Novel roles for actin in mitochondrial fission. J Cell Sci 2014; 127: 4549-4560.

[36] **Klecker T, Bockler S, Westermann B.** Making connections: interorganelle contacts orchestrate mitochondrial behavior. Trends Cell Biol 2014; 24: 537-545.

[37] **Chen H, Chan DC.** Physiological functions of mitochondrial fusion. Ann NY Acad Sci 2010; 1201: 21-25.

[38] **Zuchner S, Vance JM.** Emerging pathways for hereditary axonopathies. J Mol Med (Berl) 2005; 83: 935-943.

[39] **Yu-Wai-Man P, Carelli V, Chinnery PF.** 197th ENMC international workshop: Neuromuscular disorders of mitochondrial fusion and fission – OPA1 and MFN2 molecular mechanisms and therapeutic strategies. Neuromuscular Disord 2014; 24: 736-742.

[40] **Archer SL.** Mitochondrial dynamics—mitochondrial fission and fusion in human diseases. N Engl J Med 2013; 369: 2236-2251.

[41] **Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC.** Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J Cell Biol 2003; 160: 189-200.

[42] **Okamoto K, Shaw JM.** Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. Annu Rev Genet 2005; 39: 503-536.

[43] **Züchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL,** *et al.* Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. Nat Genet 2004; 36: 449-451.

[44] **Detmer SA, Chan DC.** Complementation between mouse Mfn1 and Mfn2 protects mitochondrial fusion defects caused by CMT2A disease mutations. J Cell Biol 2007; 176: 405-414.

[45] **Chan DC.** Mitochondria: Dynamic Organelles in Disease, Aging, and Development. Cell 2006; 125: 1241-1252.

[46] **Alexander C, Votruba M, Pesch UE, Thiselton DL, Mayer S, Moore A,** *et al.* OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. Nat Genet 2000; 26: 211-215.

[47] **Delettre C, Lenaers G, Pelloquin L, Belenguer P, Hamel CP.** OPA1 (Kjer Type) Dominant Optic Atrophy: A Novel Mitochondrial Disease. Mol Gen Metab 2002; 75: 97-107.

[48] **Liesa M, Palacin M, Zorzano A.** Mitochondrial Dynamics in Mammalian Health and Disease. Physiol Rev 2009; 89: 799-845.

[49] **Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P,** *et al.* Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nat Genet 2000; 26: 207-210.

[50] **Chen H, Chan DC.** Mitochondrial dynamics-fusion, fission, movement, and mitophagy-in neurodegenerative diseases. Hum Mol Gen 2009; 18: R169-R176.

[51] **Amati-Bonneau P, Valentino ML, Reynier P, Gallardo ME, Bornstein B, Boissiere A,** *et al.* OPA1 mutations induce mitochondrial DNA instability and optic atrophy 'plus' phenotypes. Brain 2008; 131: 338-351.

[52] **Hudson G, Amati-Bonneau P, Blakely EL, Stewart JD, He L, Schaefer AM,** *et al.* Mutation of OPA1 causes dominant optic atrophy with external ophthalmoplegia, ataxia, deafness and multiple mitochondrial DNA deletions: a novel disorder of mtDNA maintenance. Brain 2008; 131: 329-337.

[53] **Henchcliffe C, Beal MF.** Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol 2008; 4: 600-609.

[54] **Dagda RK, Cherra SJ, Kulich SM, Tandon A, Park D, Chu CT.** Loss of PINK1 Function Promotes Mitophagy through Effects on Oxidative Stress and Mitochondrial Fission. J Biol Chem 2009; 284: 13843-13855.

[55] **Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S,** *et al.* Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004; 304: 1158-1160.

[56] **Cherra Iii SJ, Dagda RK, Tandon A, Chu CT.** Mitochondrial autophagy as a compensatory response to PINK1 deficiency. Autophagy 2014; 5: 1213-1214.

[57] **Narendra D, Tanaka A, Suen DF, Youle RJ.** Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 2008; 183: 795-803.

[58] **Smith MA.** Alzheimer disease. Int Rev Neurobiol 1998; 42: 1-54.

[59] **Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G.** Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. BBA Mol Basis Dis 2010; 1802: 2-10.

[60] **DeKosky ST, Scheff SW.** Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 1990; 27: 457-464.

[61] **Lacor PN.** Synaptic Targeting by Alzheimer's-Related Amyloid Oligomers. J Neurosci 2004; 24: 10191-10200.

[62] **Wang X, Su B, Lee Hg, Li X, Perry G, Smith MA,** *et al.* Impaired Balance of Mitochondrial Fission and Fusion in Alzheimer's Disease. J Neurosci 2009; 29: 9090-9103.

[63] **Wang X, Su B, Fujioka H, Zhu X.** Dynamin-like protein 1 reduction underlies mitochondrial morphology and distribution abnormalities in fibroblasts from sporadic Alzheimer's disease patients. Am J Pathol 2008; 173: 470-482.

[64] **DiMauro S, Bonilla E, Davidson M, Hirano M, Schon EA.** Mitochondria in neuromuscular disorders. Biochim Biophys Acta 1998; 1366: 199-210.

[65] **Katsetos CD, Koutzaki S, Melvin JJ.** Mitochondrial dysfunction in neuromuscular disorders. Semin Pediatr Neurol 2013; 20: 202-215.

[66] **Cerveny KL, Tamura Y, Zhang Z, Jensen RE, Sesaki H.** Regulation of mitochondrial fusion and division. Trends Cell Biol 2007; 17: 563-569.