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Series: Current Trends in Aging and Age-Related Diseases

Opinion

Mitochondrial Dynamics: Coupling Mitochondrial Fitness with Healthy Aging

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Aging is associated with a decline in mitochondrial function and the accumulation of abnormal mitochondria. However, the precise mechanisms by which aging promotes these mitochondrial alterations and the role of the latter in aging are still not fully understood. Mitochondrial dynamics is a key process regulating mitochondrial function and quality. Altered expression of some mitochondrial dynamics proteins has been recently associated with aging and with age-related alterations in yeast, *Caenorhabditis elegans*, mice, and humans. Here, we review the link between alterations in mitochondrial dynamics, aging, and age-related impairment. We propose that the dysregulation of mitochondrial dynamics leads to age-induced accumulation of unhealthy mitochondria and contributes to alterations linked to aging, such as diabetes and neurodegeneration.

Mitochondria and Aging: A Connection of Uncertain Origin

Since Harman proposed the **mitochondrial free radical theory of aging** (MFRTA; see Glossary) [1] several decades ago, the connection between mitochondria and the aging process has been widely accepted. The function of mitochondria declines with age in several tissues and organisms, and is accompanied by the accumulation of morphological alterations and reduced respiration in these organelles [2]. However, less is known about the origin of these changes and whether they are a primary cause of aging or are secondary to aging itself. In addition, whether these changes are a consequence of mitochondrial damage or constitute an adaptive mechanism to mitigate age-dependent impairments remains controversial. It has been proposed that the accumulation of mtDNA mutations and reduced mitochondrial biogenesis may be responsible for the loss of mitochondrial function during aging in experimental animal models [3–5]. In addition, more recently, studies in yeast, *Caenorhabditis elegans*, and mice have demonstrated that alterations in the removal of damaged mitochondria also contribute to the accumulation of unhealthy mitochondria during aging [6–9]. Hence, down-regulation of proteins involved in **mitophagy** leads to mitochondrial damage and shortened lifespan in *C. elegans* and yeast [6,9], and activation of mitophagy increases mitochondrial health and lifespan in *C. elegans* and mice [7,8]. In this regard, mitochondrial dynamics is a key process in the regulation of both mitochondrial function and mitochondrial quality, and is implicated in mitophagy and mtDNA stability [10]. Importantly, alterations in mitochondrial dynamics have been described to alter lifespan in yeast, *Drosophila*, and *C. elegans* [9,11,12]. Based on these data, we hypothesize that the accumulation of mitochondrial damage precedes age-related mitochondrial alterations.

Trends

Mitochondrial abnormalities, characterized by a decline in mitochondrial function and the accumulation of damaged mitochondria, have been observed in various cell types and tissues from aged organisms.

Mitochondria are organized inside cells to form an interconnected and dynamic network, regulated by mitochondrial dynamics. Elimination of damaged mitochondria from this network is mediated by mitophagy. These two processes have a key role in mitochondrial function.

Both mitochondrial dynamics and mitophagy are altered in aging and age-related diseases. In addition, balanced mitochondrial dynamics are necessary for functional mitophagy.

Alteration of mitochondrial dynamics in aging could explain the accumulation of mitochondrial damage and be viewed as a mechanism linking a loss of mitochondrial fitness with unhealthy aging.

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Here, we summarize information regarding mitochondrial dynamics proteins and their regulatory role in mitochondrial fitness, as well as their association with age-related diseases. We postulate a new hypothesis to explain the origin of mitochondrial damage observed during aging; we propose that alterations in proteins involved in mitochondrial dynamics during aging can cause the accumulation of damaged and dysfunctional mitochondria, and this can contribute to age-related disorders.

Mitochondrial Dynamics

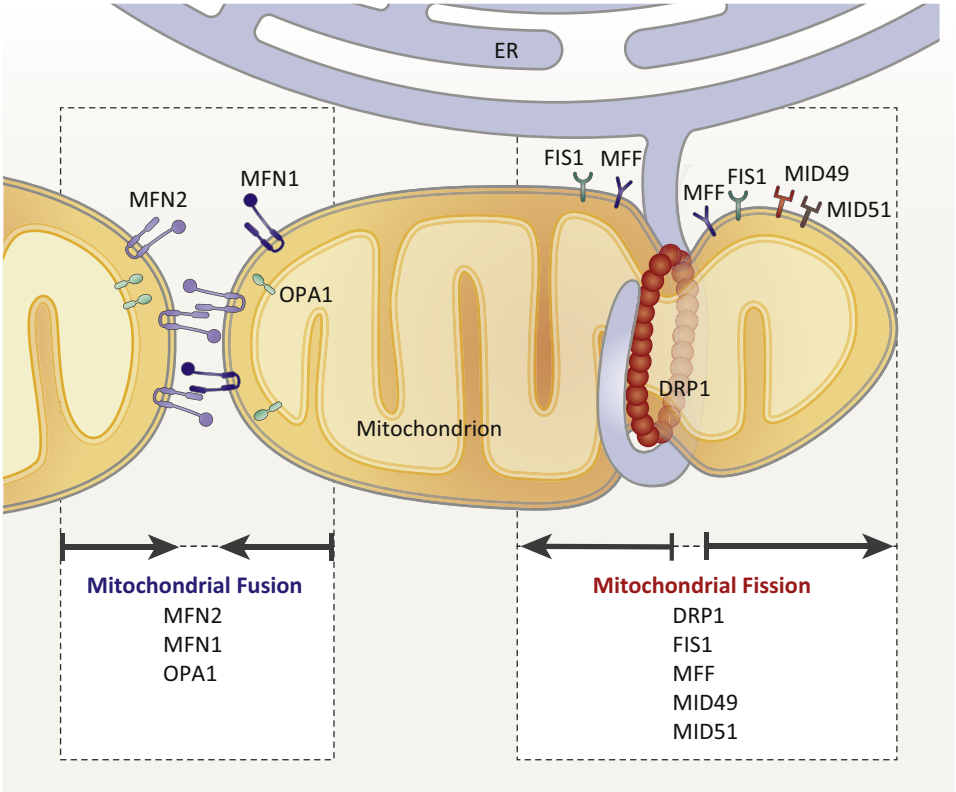
Previously, mitochondria were considered to be static and isolated organelles. However, it is now clear that they form a complex, interconnected, and highly dynamic network inside cells. This dynamism refers not only to changes in mitochondrial shape, but also to the movement of these organelles along the cytoskeleton. All these processes are referred to as 'mitochondrial dynamics', a process that is tightly regulated by **mitochondrial fusion and fission** events [10]. The physiological relevance of mitochondrial dynamics came to light with the discovery that mutations or alterations in mitochondrial dynamics proteins were associated with several human pathologies; these included neuropathies, such as **Charcot-Marie-Tooth Syndrome** and **Autosomal Dominant Hereditary Optic Atrophy** (ADOA) [13–15]; neurodegenerative diseases, such as Parkinson's disease (PD) [16]; atherosclerosis [17]; as well as metabolic syndromes, such as obesity and type 2 diabetes mellitus (T2DM) [18,19]. More recently, defects in mitochondrial dynamics proteins have also been linked to age-related **sarcopenia** in mice and humans [20,21]. To better understand these associations, we describe the components of the mitochondrial fusion and fission machinery and discuss their possible role in cellular homeostasis and aging.

Mitochondrial Fusion

Two adjacent mitochondria can fuse to form a more elongated mitochondrion by the action of mitochondrial fusion proteins (Box 1, Figure 1). Mitochondrial fusion is a two-step process. First, mitofusins catalyze outer mitochondrial membrane (OMM) fusion and then, optic atrophy gene 1 (OPA1) promotes inner mitochondrial membrane (IMM) fusion [22]. Mitofusins 1 and 2 (MFN1 and MFN2) form homo-oligomeric and hetero-oligomeric complexes that promote fusion, and this oligomerization is completely dependent on GTP hydrolysis and mediated by a heptad repeat region in the C-terminal region [23]. In addition, it was recently proposed that mitofusins can adopt either a constrained or permissive conformation for fusion, directed by intramolecular binding interactions [24]. Ablation of either *Mfn1* or *Mfn2* or abrogation of their GTPase activity in mammalian cells inhibited mitochondrial fusion, causing **mitochondrial network fragmentation** as a result of unopposed mitochondrial fission [25]. In addition, both the induction of apoptosis and the specific dissipation of **mitochondrial membrane potential** induced the cleavage of OPA1 to short isoforms and caused degradation of the long isoforms [26], leading to mitochondrial fragmentation. Overall, the coordinated action of mitofusins and OPA1 leads to the elongation of the mitochondrial network.

Box 1. Mitochondrial Fusion Proteins

Mitochondrial fusion is regulated in mammals by three large GTPases: mitofusin 1 (MFN1) and mitofusin 2 (MFN2), located in the outer mitochondrial membrane (OMM); and optic atrophy gene 1 (OPA1), located in the inner mitochondrial membrane (IMM) and in the intermembrane space [10] (Figure 1, main text). MFN2 is also present in the endoplasmic reticulum (ER) and enriched in the contact sites between the ER and mitochondria (MAMs) [111–113]. OPA1 is bound to the IMM by an N-terminal transmembrane domain, with most of the protein exposed to the intermembrane space. OPA1 is subject to complex post-transcriptional and post-translational regulation, yielding eight alternative spliced variants in humans and four in mice, which are also regulated by proteolytic cleavage [114]. Alternative splicing of OPA1 produces various long-forms (L-OPA1), which are cleaved to short-forms (S-OPA1) by specific mitochondrial proteases (the zinc metalloprotease OMA1, overlapping activity with the m-AAA protease, and the ATP-dependent zinc metalloprotease YME1-like protein 1 (YME1L)] [115].



Trends in Molecular Medicine

Figure 1. Proteins Involved in Mitochondrial Dynamics. Mitochondrial fusion is a two-step process in which outer mitochondrial membrane (OMM) fusion is mediated by homo- and hetero-oligomeric complexes between mitofusin 1 (MFN1) and mitofusin 2 (MFN2), and inner mitochondrial membrane (IMM) fusion is mediated by optic atrophy gene 1 (OPA1). Mitochondrial fission is mediated by the action of dynamin-related protein 1 (DRP1), which is recruited to mitochondria by different receptors: fission 1 homolog protein (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamics protein of 49 kDa (MID49), and mitochondrial dynamics protein of 51 kDa/mitochondrial elongation factor 1 (MID51/MIEF1). The endoplasmic reticulum (ER) interacts with mitochondria, marking the constriction sites in which mitochondrial fission will occur.

Mitochondrial Fission

Mitochondrial fission generates two distinct types of mitochondrion: one with a high mitochondrial membrane potential, and the other with a low mitochondrial membrane potential. The latter can recover its membrane potential and undergo fusion with another mitochondrion or remain depolarized, whereupon it is eliminated by mitophagy [27]. Mitochondrial fission is regulated by mitochondrial fission proteins (Box 2, Figure 1). Fission requires the recruitment of dynamin-related protein 1 (DRP1) to the OMM by fission 1 homolog protein (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamics protein of 49 kDa (MID49), and mitochondrial dynamics protein of 51 kDa/mitochondrial elongation factor 1 (MID51/MIEF1), forming dotted structures located on future mitochondrial scission sites [28]. Once recruited to the mitochondrial membrane, DRP1 constricts mitochondrial tubules to mediate membrane fission. It has been suggested that association of these constriction sites with the endoplasmic reticulum (ER) is an early step to mark the sites for DRP1 recruitment and mitochondrial fission [29].

Mitochondrial Dynamics in the Control of Mitochondrial Fitness

In addition to regulating mitochondrial shape, mitochondrial dynamics has important physiological relevance in many other cellular functions and processes. In this regard, the ablation of mitochondrial fusion and fission proteins in mice is embryonically lethal [25,30–32]. Indeed,

Glossary

Amyloid β (Aβ): peptides originating from the amyloid precursor protein (APP) by the action of beta and gamma secretases. The aggregation of Aβ molecules is involved in the formation of amyloid plaques in the brain, which are associated with Alzheimer's disease.
Anorexigenic POMC neurons: produce anorexigenic neuropeptides, which promote the loss of appetite and decreased food intake. The most important anorexigenic peptides are proopiomelanocortin (POMC) and its post-translational cleavage product alpha-melanocyte stimulating hormone (MSH), produced and released by POMC/CART neurons within the arcuate nucleus of the hypothalamus.
Autophagy: process by which the cell degrades its own cellular components through the lysosomal pathway. There are three types of autophagy: macroautophagy (termed 'autophagy'), microautophagy, and chaperone-mediated autophagy (CMA). Macroautophagy: engulfment of cytoplasmic components or organelles into the autophagosome, which fuses with the lysosome to form the autolysosome, where degradation occurs. Microautophagy: cytoplasmic material is trapped and engulfed directly by the lysosome. CMA: soluble cytosolic proteins are selected and targeted to the lysosome by chaperones.
Autophagic flux: rate of flow along the autophagy pathway. Its measurement is used as an indicator of autophagic degradation activity.
Autosomal dominant optic atrophy (ADOA): neuro-ophthalmic disease characterized by degeneration of the optic nerves, mainly affecting the retinal ganglion in the optic nerve, causing visual loss early in life.
Caloric restriction: condition characterized by a reduced energy intake through a dietary regimen, without incurring in malnutrition or reduction in essential nutrients.
Charcot-Marie-Tooth Syndrome (CMT): progressive neuropathy characterized by distal muscle weakness, predominantly of the lower extremities, leading to eventual disability. The disease is a clinically heterogeneous disorder classified as demyelinating forms (CMT1, CMT3, and CMT4) or axonal form (CMT2).

Box 2. Mitochondrial Fission Proteins

Mitochondrial fission is regulated by two classes of protein, namely a soluble cytosolic protein, dynamin-related protein 1 (DRP1), and mitochondria-bound proteins, including fission 1 homolog protein (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamics protein of 49 kDa (MiD49), and mitochondrial dynamics protein of 51 kDa/mitochondrial elongation factor 1 (MiD51/MIEF1) (Figure 1, main text) [10]. The central player in mitochondrial fission across species appears to be DRP1, a soluble GTPase that is ubiquitously expressed [10]. Ablation of *Drp1* leads to hypertubulated mitochondria in cells from yeast, worms, and mammals, highlighting the crucial role of this protein in mitochondrial fission. FIS1 is a tail-anchored protein of the OMM and was the first protein described to be involved in the recruitment of DRP1 to mitochondria [116]. However, there continues to be controversy regarding the exact role of FIS1 in mitochondrial division, probably due to differences in the cellular context [117]. Hence, in HeLa cells (a human cell line), FIS1 has been shown to be unnecessary for DRP1 recruitment and mitochondrial fission [117]. MFF is another protein found in the mitochondrial OMM, and it is the best-characterized DRP1 receptor. MFF physically interacts with DRP1, and its knockdown in mammalian cells reduces mitochondrial DRP1 recruitment and mitochondrial fission [117,118]. MID49 and MID51/MIEF1 are similar proteins located in the OMM and are also capable of interacting with DRP1; they also appear to be involved in DRP1 recruitment in mouse embryonic fibroblasts (MEF) and HeLa cells [119]. However, their exact role in mitochondrial fission is still unclear [120].

studies performed during the past decade have elucidated a role for mitochondrial dynamics in regulating mitochondrial function and metabolism, as well as apoptosis, calcium homeostasis, mtDNA stability, antiviral signaling, **autophagy**, and mitophagy. In the next section, we focus on two key aspects of mitochondrial dynamics associated with the maintenance of mitochondrial fitness.

Mitochondrial Dynamics Regulates Mitochondrial Function and Metabolism

Loss-of-function and overexpression approaches have revealed that mitochondrial dynamics regulates mitochondrial metabolism in a range of cells and tissues (Figure 2). The modulation of MFN2 expression has revealed a tight link between this mitofusin, cellular respiration, and metabolism. For instance, *Mfn2* depletion in mammalian cells induced mitochondrial dysfunction, characterized by a decrease in oxygen consumption coupled to ATP synthesis, increased proton leak, reduced mitochondrial membrane potential, increased production of reactive oxygen species (ROS), and reduced Coenzyme Q levels, thereby leading to reduced glucose, pyruvate, and fatty acid oxidation [18,33–35]. Conversely, overexpression of either the full-length or a C-terminal truncated version of MFN2 defective in mitochondrial fusion activity increased glucose oxidation and mitochondrial membrane potential in human HeLa cells, and mouse liver and muscle cells [36,37]. In addition, fibroblasts from patients with Charcot-Marie-Tooth Syndrome harboring *MFN2* mutations have also been shown to present abnormalities in mitochondrial metabolism, characterized by lower mitochondrial coupling and mitochondrial membrane potential [38]. These alterations appear to be specific to MFN2, because ablation of *Mfn1* in MEF cells or mouse liver has been reported to lead to the opposite phenotype, characterized by an increase in ATP-coupled and maximal respiration [39]. In addition to these effects, MFN2 is required for OPA1 cleavage, which is responsible for the modulation of **mitochondrial cristae** morphology occurring in mouse liver during the metabolic postabsorptive state (i.e., absorption of a meal) [40]. OPA1 has also been associated with alterations in mitochondrial metabolism. For instance, *Opa1* knockdown was found to reduce basal, ATP-coupled, and maximal respiration, together with a decrease in mitochondrial membrane potential and an increase in proton leak, in mouse embryonic fibroblasts (MEF), as well as in murine pancreatic β cells and T cells [34,41,42]. Moreover, Opa1 appears to be involved in regulating mitochondrial cristae shape, respiratory efficiency, and the assembly of respiratory chain supercomplexes in mice [43]. In addition to mitochondrial fusion proteins, DRP1 has also been associated with alterations in mitochondrial function. For example, repression of *Drp1* or the expression of a dominant negative mutant form of DRP1 was found to lead to a reduction in oxygen consumption and an increase in proton leak in mouse β cells and cardiomyocytes [27,44,45]. In another recent study, repression of *Drp1* in adult rat cardiomyocytes was documented to inhibit mitochondrial respiration, and this effect appeared to be independent

Foxo3 (FOXO3 in humans): transcription factor of the Forkhead family; also known as Forkhead box O3. Upon phosphorylation, FOXO3 translocates to the nucleus, where it regulates gene expression.
Hyperphagia: abnormally increased food intake, frequently associated with alteration of the function of specific hypothalamic areas involved in the control of feeding.
Leptin resistance: condition characterized by the lack of action of the anorectic hormone leptin, leading to a loss of suppression of food intake. This condition is associated with a loss of satiety and is linked to obesity.
Mitochondrial biogenesis: process by which cells increase their mitochondrial mass and number. It involves coordinated transcription and translation of both nuclear- and mitochondrial-encoded genes. A modulator of the process is the nuclear co-activator PGC-1α, which interacts with NRF2 and NRF1, which in turn activate the mitochondrial transcription factor TFAM, responsible for transcribing nuclear-encoded mitochondrial structural proteins and also those involved in mtDNA transcription and translation.
Mitochondrial cristae: organized folds of the inner mitochondrial membrane that increase the total surface area of that membrane, providing more space for the different components of the respiratory electron transport chain and allowing a faster production of ATP during oxidative phosphorylation.
Mitochondrial free radical theory of aging (MFRTA): initially postulated by Denham Harman in 1956, it states that organismal aging is a consequence of accumulation of free radicals over time, which induces oxidative damage to various proteins and cellular components, leading to their dysfunction. Although widely accepted, the theory remains unproven and has been challenged by several studies in several model organisms.
Mitochondrial fusion and fission: processes by which the mitochondrial network changes its morphology. Mitochondrial fusion leads to the formation of a more elongated mitochondrion through the fusion of two adjacent mitochondria, whereas mitochondrial fission

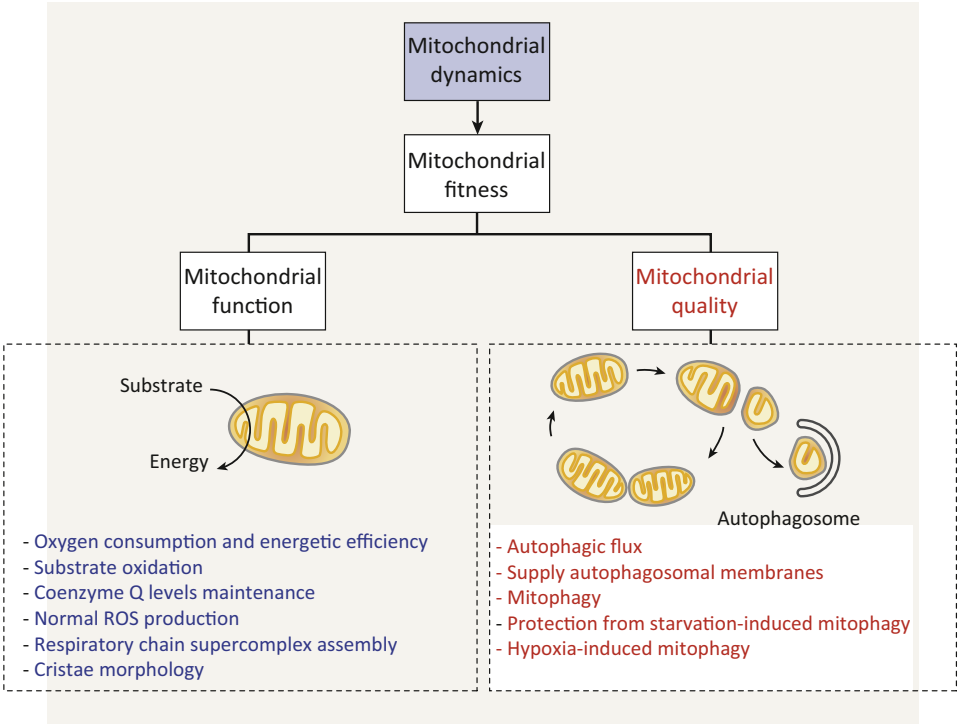


Figure 2. Mitochondrial Dynamics: A Key Process in the Preservation of Mitochondrial Fitness. Mitochondrial dynamics, by controlling several aspects of mitochondrial function and mitochondrial quality (summarized in the figure), is fundamental to the preservation of mitochondrial fitness. Mitofusins, optic atrophy gene 1 (OPA1), and dynamin-related protein 1 (DRP1) have been implicated in the control of mitochondrial metabolism, energetic efficiency, and reactive oxygen species (ROS) production; MFN2 is important for the maintenance of Coenzyme Q levels, and OPA1 is involved in respiratory chain supercomplexes assembly and cristae morphology. In addition, mitochondrial dynamics is also involved in some mitochondrial quality-control mechanisms, such as autophagy and mitophagy, by controlling autophagosomal membranes formation, autophagic flux, and mitophagy.

of mitochondrial fission, given that acute *Drp1* genetic manipulation yielded modest to no changes in mitochondrial morphology in this model [46]. Thus, the currently available data indicate that a correct balance between mitochondrial fusion and fission is crucial to maintain mitochondrial metabolism.

Mitochondrial Dynamics, Autophagy, and Mitochondrial Quality Control

Mitochondrial dynamics has been shown to participate in autophagy (Box 3, Figure 2). For instance, *MFN2* ablation in human HeLa cells, various mouse cultured cells (MEF, muscle, and cardiomyocytes), and mouse skeletal muscle has been associated with reduced autophagy [20,47,48]. *Mfn2* depletion in rat kidney cells also impaired starvation-induced autophagy by disrupting mitochondria–ER connections, which are necessary for supplying membranes during autophagosome formation [49]. The downregulation of *DRP1* has also been linked to a reduced **autophagic flux** in human senescent endothelial cells [50].

Importantly, mitochondrial fusion and fission appear to have key roles in mitophagy, thus contributing to mitochondrial quality control (Figure 2). For example, pioneering studies revealed that mitochondrial fission is needed for the segregation of damaged mitochondria from the mitochondrial network and posterior removal by mitophagy in mouse pancreatic β cells, in a process involving both Drp1 and Opa1 proteins [27]. Moreover, upon the induction of mitophagy in human dopaminergic SH-SY5Y cells and *Drosophila*, the ubiquitin ligase Parkin

generates two mitochondria by the division of an existing mitochondrion, generating a more fragmented mitochondrial network.
Mitochondrial membrane potential: difference in electric potential across the outer mitochondrial membrane, an important parameter of mitochondrial function.
Mitochondrial network fragmentation: remodeling of the mitochondrial network induced by mitochondrial fission and characterized by a punctuate or fragmented pattern of mitochondria inside the cell.
Mitophagy: selective degradation of mitochondria by autophagy. During mitophagy, mitochondria are tagged by specific mitophagic receptors, which interact with core autophagic proteins and are then directed to the autophagosome.
Muscle atrophy: wasting or loss of muscle tissue, characterized by a reduction in muscle mass and muscle size. Muscle atrophy is typical of situations associated with inactivity and temporary disabling circumstances. Other causes of muscle atrophy include: aging, burns, malnutrition, injuries such as broken bones, and stroke.
Orexigenic Agrp neurons: neurons producing orexigenic neuropeptides, such as AGRP neurons in in the arcuate nucleus of the hypothalamus. These neurons produce neuropeptide Y (NPY), which promotes appetite and food intake.
Purkinje cells: class of GABAergic neurons located in the cerebellum, which constitute the sole output of all motor coordination in the cerebellar cortex.
Rapamycin: natural macrocyclic lactone produced by *Streptomyces hygroscopicus* with immunosuppressant properties. Rapamycin inhibits mammalian target of rapamycin (mTOR) and, therefore, is also used as an activator of autophagy.
Sarcopenia: gradual loss of muscle mass, strength, and function during aging, which is associated with a loss of ability to perform everyday tasks. Muscle is lost at approximately 1–2% per year after the age of 50.
Stemness: characteristic of stem cells, which have the capacity to self-renew and the ability to generate differentiated cells.

Box 3. Autophagy

Autophagy is a crucial process in the degradation of long-lived proteins and organelles, thus having an important role in the maintenance of cellular homeostasis. It is regulated at the transcriptional, post-transcriptional, and post-translational levels. It can be nonselective (bulk autophagy) or selective, depending on the type of cargo that is sequestered to be degraded. Selective autophagy is determined by the presence of autophagy receptors, which tag the cargo, interact with the autophagosome marker LC3, and direct the cargo to the autophagosome. One type of selective autophagy involves the degradation of mitochondria, also called 'mitophagy'. Several mitophagy receptors have been described, such as ATG32 in yeast, and BNIP3, BNIP3L/NIX, FUNDC1, optineurin, and NDP52 in mammals [121,122]. In addition, in response to mitochondrial depolarization, the PINK1/Parkin pathway is activated, removing damaged mitochondria via mitophagy in mammalian cells [123]. Indeed, the kinase PINK1 is stabilized in the OMM and recruits the ubiquitin ligase Parkin in HeLa, human neuroblastoma, and MEF cells [124], which in turn ubiquitinates OMM proteins, such as voltage-dependent anion channel 1 (VDAC1), serving as mitophagic receptors [125]. In addition, PINK1 can also directly phosphorylate ubiquitin on mitochondria to induce the recruitment of optineurin and NDP52 and induce mitophagy in mammalian cells [122].

TAU: microtubule-associated protein abundant in neurons implicated in the stabilization of axonal microtubules. Hyperphosphorylation of TAU induces aggregation of the protein, which is involved in the pathogenesis of Alzheimer's disease.

(Box 3) ubiquitinated Mfn1 and Mfn2, leading to their proteasomal degradation and subsequent inhibition of mitochondrial fusion [51,52]. Mfn2 can also be phosphorylated by Pink1 (PINK1, humans), promoting its Parkin-mediated ubiquitination and acting as a Parkin receptor in mouse cardiomyocytes [53]. Genetic ablation of *Mfn2* in mouse skeletal muscle has also been found to lead to decreased autophagic degradation of mitochondria [20]. The link between mitochondrial dynamics and mitophagy has also been supported by the observation that, upon nutrient deprivation, elongation of mitochondria can protect the latter from autophagic degradation, thus maximizing energy production and supplying autophagosomal membranes during starvation in MEF cells. This effect appears to be mediated by the phosphorylation of DRP1, which is retained in the cytoplasm, where it can inhibit fission, leading to unopposed fusion [54,55]. In addition, downregulation of Drp1 has been reported to prevent mitochondrial autophagy in mouse heart [45,56]. DRP1 has also been described as necessary in mediating various forms of mitophagy, such as following hypoxic/ischemic injury in rat brain [57], and after hypoxia induction in HeLa cells; this might involve the interaction of DRP1 with the mitophagy receptor FUN14 domain-containing 1 Pan troglodytes (FUNDC1) [58]. Collectively, these results suggest that mitochondrial dynamics regulates mitochondrial quality control by modulation of autophagy and mitophagy.

Alterations of Mitochondrial Dynamics in Age-Related Diseases

Mitochondrial dynamics has emerged as a key process in the maintenance of cellular homeostasis. In this regard, mutations or alterations in mitochondrial fusion and fission proteins have been associated with several diseases [13–20]. Many of these, as described below, are age-related diseases. Consequently, we posit that the dysregulation of mitochondrial dynamics has a causative role in the pathogenesis of many of these conditions (Table 1).

Mitochondrial Dynamics and Metabolic Diseases

Mitochondrial dynamics operates under different metabolic requirements. Excess nutrients cause mitochondrial fragmentation, while low nutrient availability is linked to mitochondrial elongation [59]. Alterations in some mitochondrial dynamics proteins have been tightly associated with metabolic disease. For instance, *Mfn2* gene and protein expression in skeletal muscle were found to be reduced in obesity as well as in T2DM in both mice and humans [33,60]. Moreover, deficiency of Mfn2 in mouse skeletal muscle has been associated with glucose intolerance and insulin resistance in response to a high-fat diet (HFD) or upon healthy aging [18,20]. In addition, liver *Mfn2* ablation in mice has also been found to lead to impairments in glucose homeostasis, as evidenced by increased hepatic glucose production and insulin resistance [18]. Furthermore, *Mfn2* gene expression was reduced in mouse hypothalamic neurons in response to a HFD, and specific ablation of *Mfn2* (but not *Mfn1*) in **anorexigenic POMC neurons** has been documented to result in **leptin resistance**, **hyperphagia,** reduced energy expenditure, and obesity in mice [19]. However, ablation of either *Mfn2* or *Mfn1*

Table 1. Summary of Alterations in Mitochondrial Dynamics Proteins for Different Mammalian Age-Related Diseases

Mitochondrial Dynamics Protein	Alteration	Age-related disease/phenotype	Model/organism	Refs
Mfn2	Reduced expression	Obesity and T2DM: glucose intolerance and insulin resistance	Skeletal muscle of humans and rodents	[18,20,33,60]
		T2DM: increase hepatic glucose production and insulin resistance	Mouse liver	[18]
		Obesity: leptin resistance and energy imbalance	Hypothalamic neurons	[19,61]
		Cardiovascular disease: hyperproliferation of vascular smooth muscle cells	Hypertensive and atherosclerotic arteries from rats and mice	[17]
		Cardiovascular disease: cardiac hypertrophy and cardiomyopathy	Mouse cardiomyocytes	[53,67,68]
		Neurodegeneration	Mouse cerebellum (Purkinje cells and dopaminergic neurons)	[81–83]
		Sarcopenia: **muscle atrophy** and muscle dysfunction	Human, rat, and mouse muscle	[20,21,91, 94,96]
Mfn1	Reduced expression	T2DM: protection from diet-induced insulin resistance	Mouse liver	[39]
		Obesity: resistance to high-fat diet-induced weight gain	Agrp hypothalamic neurons	[61]
Opa1	Reduced expression	T2DM: impairment in insulin secretion and systemic glucose homeostasis	Mouse pancreatic β cells	[42]
		Cardiovascular disease: heart failure	Heart from humans, rats, and mice	[70–72]
	Increased expression	Cardiovascular disease: protection from ischemia-reperfusion (I/R) injury	Mouse heart	[73]
		Protection from denervation-induced muscle atrophy	Mouse muscle	[73]
		Protection from myopathy-associated muscle atrophy	Mouse muscle	[93]
Drp1	Reduced expression	T2DM and obesity: protection from diet-induced obesity and improvement of insulin resistance	Mouse muscle and liver	[62,63]
		Cardiovascular disease: development of cardiac dysfunction	Mouse heart	[45,78]
		Neurodegeneration: defects in synapsis, morphogenesis, plasticity, and axonal growth	Mouse brain	[31,84]
	Acute inhibition	Cardiovascular disease: protection from cardiac hypertrophy and function after I/R injury or myocardial infarction	Mouse heart	[76,77]
	Increased expression	Neurodegeneration: Alzheimer's and Huntington's diseases	Human brain biopsies	[88,89]
		Sarcopenia: muscle atrophy and dysfunction	Mouse skeletal muscle	[90,94]

in **orexigenic Agrp neurons** appeared to confer resistance to weight gain upon a HFD in mice [61]. This suggests that mitofusins have a distinct role in these two opposite (anorexigenic versus orexigenic) subpopulations of neurons in connection with the control of whole-body energy homeostasis. Interestingly, in contrast to the effects of *Mfn2* deficiency, specific ablation of *Mfn1* in mouse liver appears to protect against diet-induced insulin resistance, leading to enhanced mitochondrial respiration, probably due to an adaptive response [39]. Due to these differences, further studies are warranted to elucidate the precise contribution of these proteins in the control of energy homeostasis and metabolic disorders in different tissues.

From another angle, OPA1 alterations have also been associated with metabolic disturbances. In one study, silencing of OPA1 in mouse β cells was reported to impair insulin secretion and systemic glucose homeostasis [42].

In contrast to mitochondrial fusion proteins, the expression of mitochondrial fission proteins was found to be increased in skeletal muscle in mouse models of obesity [60]. Accordingly, pharmacological or genetic inhibition of Drp1 in skeletal muscle was shown to improve insulin signaling and systemic insulin resistance in mice with both genetic and diet-induced obesity [62]. Moreover, genetic ablation of *Drp1* in mouse liver was found to protect mice from diet-induced obesity and to increase energy expenditure [63]. Collectively, these data suggest that an imbalance in mitochondrial dynamics in the absence of counter-regulatory adaptations is linked to alterations in mitochondrial metabolism. In the studies relating to obesity and T2DM, it is possible that impaired mitochondrial dynamics contributes to disease pathogenesis and/or progression.

Mitochondrial Dynamics and Cardiovascular Disease

Recent studies have suggested that the impaired function of mitochondrial fusion and fission proteins is associated with cardiac dysfunction, cardiomyocyte development, and cardiovascular disease in rodents and *Drosophila* [53,64–66]. In particular, *Mfn2* gene and protein expression were found to be markedly reduced in hyperproliferative vascular smooth muscle cells (VSMCs) from hypertensive rat arteries and mouse atherosclerotic arteries [17]. Indeed, MFN2 was originally termed 'Hyperplasia Suppressor Gene' (HSG) because its dysregulation was initially discovered to trigger vascular proliferative disorders, such as atherosclerosis and restenosis [17]. Furthermore, *Mfn2* mRNA has been shown to be reduced in *in vitro* and *in vivo* in models of cardiac hypertrophy, such as neonatal rat ventricular myocytes, spontaneously hypertensive rats, mice with pressure-overload hypertrophy by transverse aortic constriction (TAC), and cardiomyopathy in mice due to cardiac-restricted overexpression of β2-adrenergic receptors [67]. In addition, *Mfn2* deletion in mouse cardiomyocytes induced progressive cardiomyopathy [53]. Combined *Mfn1* and *Mfn2* ablation in mouse adult heart also led to heart failure in the long-term [68]; interestingly, double *Mfn1/Mfn2* knockout (KO) mice were protected from acute ischemia-reperfusion (I/R) injury [69]. This suggests the existence of different possible roles for mitofusins in modulating heart physiology in response to short-term or long-term insults. OPA1 protein expression has also been reported to be decreased in human and rat models of heart failure (e.g., in an explanted failing human heart removed at transplant, and in a high coronary ligation rat model of heart failure) [70]. Furthermore, a mouse model carrying a heterozygous mutation of *Opa1* causing ADOA and leading to a 50% deletion of Opa1 protein was shown to develop late-onset cardiomyopathy [71]. Imbalanced Opa1 processing leading to accelerated Opa1 proteolysis has also been associated with dilated cardiomyopathy and heart failure in mice [72]. Conversely, increased Opa1 levels in mice have been shown to confer protection against I/R injury [73]. Therefore, inhibition of mitochondrial fusion concomitant with increased mitochondrial fission appears to be linked to heart disease, at least in rodents. In keeping with this, by inhibiting calcineurin, which dephosphorylates Drp1 and induces its

translocation to mitochondria [74], Drp1 translocation to mitochondria could be blocked in a mouse myocardial infarction (MI) model [75]. As a consequence, mitochondrial fission was inhibited, resulting in a protective effect against MI in the mice [75]. Moreover, acute pharmacological inhibition of Drp1 activity with mitochondrial division inhibitor-1 (Mdivi-1) has been shown to protect against heart hypertrophy, safeguarding cardiac function following I/R injury induction in adult murine cardiomyocytes [76], or following myocardial infarction in mice [77]. In contrast to acute Drp1 inhibition, chronic Drp1 dysfunction in both Drp1 mutant mice and cardiac-specific *Drp1* KO mice has been linked to the development of cardiac dysfunction [45]. These results suggest that Drp1 has a different role in acute or chronic heart dysfunction, possibly due to alterations in mitophagy; for example, impaired mitophagy might be induced by chronic Drp1 dysfunction, ultimately leading to heart damage [45,78]. In summary, the data clearly indicate that alterations in mitochondrial dynamics are linked to heart disease, although this connection is complex and differentially affected by acute or chronic cardiovascular insults.

Mitochondrial Dynamics and Neurodegenerative Diseases

Mutations in *MFN2* and *OPA1* are responsible for Charcot-Marie-Tooth 2A and ADOA neuropathies, respectively [13–15]. In addition, a mutation in *DRP1* has been associated with a severe type of infantile neurodegeneration in humans, characterized by microcephaly, abnormal brain development, optic atrophy, and hypoplasia [79]. KO models of these genes in various organisms, including *Drosophila*, zebrafish, and mice, have yielded similar phenotypes [80]. Thus, specific ablation of *Mfn2* in mouse brain has been shown to lead to degeneration of the cerebellum, as evidenced by alterations in dendritic outgrowth and spine formation of **Purkinje cells** as well as in the degeneration of dopaminergic neurons [81–83]. Furthermore, inhibition of mitochondrial fission has been reported to lead to defects in synaptic morphogenesis, plasticity, and axonal growth in rat hippocampal neurons and primary cultures of mouse forebrain [31,84]. Alterations in mitochondrial fusion and fission have also been linked to defects in neuronal development, plasticity, and function, and therefore, they are involved in several neurodegenerative diseases, such as PD, Alzheimer's disease (AD), and Huntington's disease (HD) [80]. Thus, genes mutated in HD and PD, such as leucine-rich repeat kinase 2 (*LRRK2*) and huntingtin (*HTT*), have been found to promote DRP1 activity both *in vitro* and *in vivo* in rat neurons, fibroblasts from patients with HD, postmortem HD brains [85,86], human dopaminergic SH-SY5Y neuroblastoma cells, as well as in differentiated rat primary cortical neurons overexpressing PD-associated mutants of *LRRK2* [87]. Importantly, mitochondrial fragmentation and an increased interaction between DRP1 and **amyloid β** (Aβ) or **TAU** have been observed in neurons from patients with AD [88]. In addition, the expression of *DRP1* is increased in the striatum and cortex of patients with HD [89]. Altogether, these data suggest that there are important functional connections between genes and/or proteins associated with neurodegenerative conditions and those associated with mitochondrial dynamics. Indeed, the correct balance between mitochondrial fusion and fission is clearly crucial for brain development and neuronal function.

Mitochondrial Dynamics, Muscle Atrophy, and Sarcopenia

Sarcopenia is the main cause of disability among older humans. Recent studies identified that alterations in mitochondrial dynamics could be involved in the degeneration of muscle mass and function with age. The first evidence came from the observation that *in vivo* overexpression of mitochondrial fission proteins Drp1 and Fis1 in muscle triggered skeletal muscle atrophy in mice, while inhibition of fission partially prevented muscle atrophy induced by muscle **Foxo3** overexpression [90]. In keeping with this observation, muscle-specific ablation of both *Mfn1* and *Mfn2* was shown to lead to muscle atrophy and abnormal muscle growth in mice [91]. A decrease in Mfn2 and an increase in Drp1 expression have also been implicated in disuse-induced muscle atrophy in mice [92]. Moreover, overexpression of Opa1 in mice has been

shown to lead to a protective effect against denervation-induced muscle atrophy [73] and primary mitochondrial myopathy [93]. Importantly, protein expression of mitochondrial fusion and fission proteins is reduced during aging in skeletal muscle in mice, rats, and humans [20,94–96]. In particular, studies in muscle-specific *Mfn2*-deficient mice demonstrated that a reduction in Mfn2 expression during aging drives muscle atrophy and sarcopenia [20]. It has been further proposed that MFN2 function is also linked to sarcopenia in humans, given that reduced MFN2 expression has been documented in muscle samples from sarcopenic older individuals relative to nonsarcopenic older controls [21].

Mitochondrial Dynamics As a Safeguard for Mitochondrial Health in Age-Related Disease

Maintenance of a healthy mitochondrial population is achieved by the efficient elimination of damaged mitochondria and appropriate **mitochondrial biogenesis**. In this regard, the proper coordination of these two processes has been demonstrated to be crucial for healthy aging in *C. elegans* [6]. Damaged mitochondria are eliminated by autophagy and/or mitophagy, and these processes are impaired during aging [97]. Alterations in several proteins involved in autophagy have been linked to age-related pathologies, premature aging, and a reduced lifespan in yeast, *C. elegans*, *Drosophila*, and mice [98]. Conversely, interventions designed to increase autophagy, such as **caloric restriction** and **rapamycin** treatment, can increase longevity in different model organisms [97–101], and promote beneficial effects on age-related diseases, such as cancer, metabolic diseases, muscle atrophy, cardiac dysfunction, and neurodegeneration, in mice, rhesus monkeys, and humans [102–106]. In addition, autophagy has been documented as a necessary process for the maintenance of cell **stemness,** and for preventing senescence in mouse muscle satellite cells [107]. Furthermore, defects in mitophagy have been linked to aging and age-related disease in various model organisms. For instance, in yeast, flies, and worms, a reduction in mitophagy led to a decreased lifespan, while increased mitophagy extended lifespan [7,9,108]. In addition, as previously mentioned, in mammals, defective mitophagy has been associated with several age-related diseases, including neurodegeneration, cardiovascular disease, and sarcopenia [20,66,90,109].

Mitochondrial dynamics is clearly crucial for mitochondrial metabolism and to ensure mitochondrial quality control. Therefore, we propose a model in which alterations in mitochondrial dynamics act as a double-edged sword, resulting in impaired mitochondrial health (Figure 3, Key Figure). On the one hand, alterations in mitochondrial fusion or fission proteins can promote intrinsic mitochondrial defects that lead to reduced mitochondrial activity and increased mitochondrial damage. On the other hand, unbalanced mitochondrial dynamics can impair autophagy and/or mitophagy and prevent the elimination of damaged mitochondria. Therefore, the final net effect of altered mitochondrial dynamics might be the combined induction and accumulation of damaged mitochondria. It is becoming clear that mitochondrial dysfunction and reduced autophagy and/or mitophagy are key features of aging. Moreover, mitochondrial dynamics can also be dysregulated during aging. As described, alterations in mitochondrial dynamics have been associated with aging and longevity in lower organisms (yeast, C. *elegans*, and *Drosophila)* as well as with age-related diseases and health span in mammals [9,11,12]. However, whether lifespan in mammals can be modulated by mitochondrial dynamics proteins remains to be determined.

Therefore, we propose that alterations in mitochondrial dynamics, which modulate mitochondrial function and quality, constitute a primary factor contributing to the underlying loss of mitochondrial health observed during aging. As such, we argue that defective mitochondrial dynamics can eventually contribute to cell and/or tissue dysfunction and to the pathogenesis of age-associated diseases and/or unhealthy aging.

Key Figure

Coupling Mitochondrial Dynamics and Mitochondrial Fitness with Healthy Aging

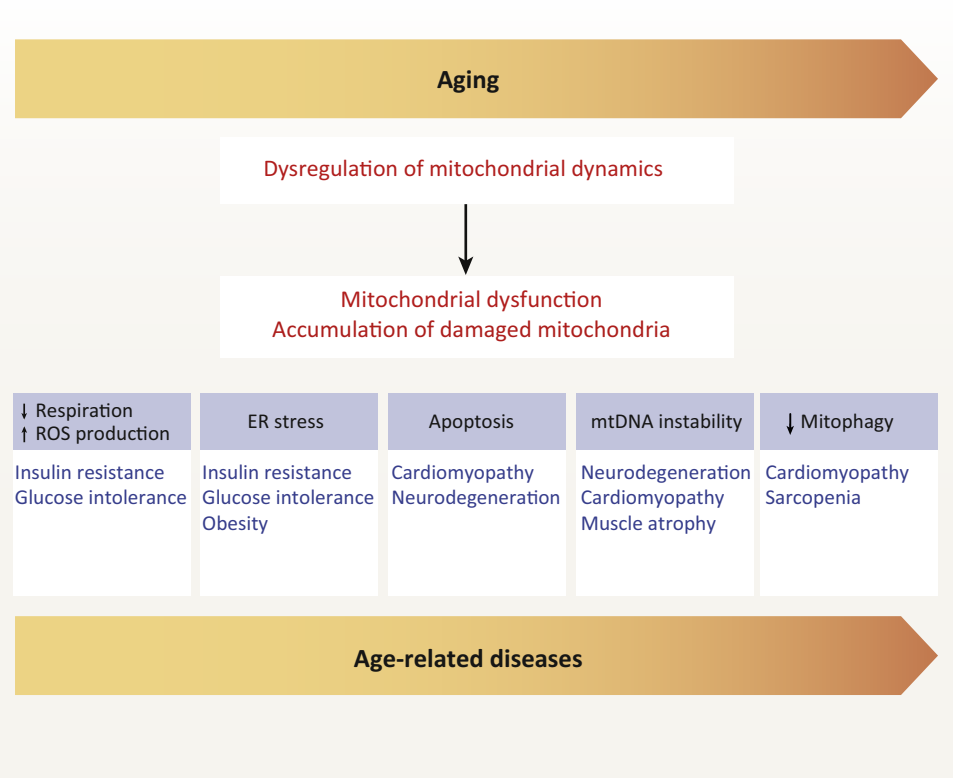


Figure 3. Aging is associated with dysregulated mitochondrial dynamics, which can lead to age-related diseases through different mechanisms and signaling pathways. For instance, increased reactive oxygen species (ROS) production has been associated with metabolic disease [18,62]. Induction of endoplasmic reticulum (ER) stress [cellular stress response or unfolded protein response (UPR) is activated upon loss of ER homeostasis and has been associated with pathogenic mechanisms of obesity and type 2 diabetes mellitus] can lead to metabolic disorders [18,19]. Induction of apoptosis can promote cardiomyopathy and neurodegeneration [70,76,89]. mtDNA instability has been associated with cardiomyopathy, neurodegeneration, and muscle atrophy [71,81,91]. Finally, reduction in mitophagy has been associated with cardiomyopathy and sarcopenia [20,110].

Concluding Remarks

Aging, or the loss of physiological integrity that leads to the progressive accumulation of damage and tissue dysfunction, has attracted scientific attention for many years. This interest has focused not only on the discovery of pathways oriented towards promoting longevity, but also on achieving healthy aging. In this regard, aging is associated with a decline in mitochondrial function and an accumulation of aberrant mitochondria [2]. Importantly, the expression of mitochondrial dynamics and autophagy and/or mitophagy proteins is altered with aging and in many age-related diseases [97]. This suggests that mitochondrial dynamics and autophagy are interconnected in the context of age-related diseases. From a novel perspective, we propose that dysregulation of mitochondrial dynamics constitutes a pivotal factor leading to the accumulation of damaged mitochondria observed during aging. Evidently, many questions

Outstanding Questions

Have all the components of mitochondrial dynamics and mitochondrial quality control systems been discovered? Although our knowledge of the proteins involved in mitochondrial dynamics and mitophagy is growing daily, it is possible that more, as yet unknown, proteins also participate in these processes.

What are the precise mechanisms behind the connection between mitochondrial dynamics and autophagy and/or mitophagy? Are they conserved in all cells and tissues or are they tissue specific? Recent data have shed light on this question, but more research in cells and model organisms is needed to gain a deeper mechanistic insight into this link.

The expression of fusion and fission proteins is altered in aged cells and tissues, but the factors leading to this alteration are still unknown. What are these factors? What is their nature? Are they genetic or are they affected by external stimuli? Can they be modulated and, if so, how? This information would help in designing interventions aimed at promoting healthy aging.

Could the alteration of mitochondrial dynamics during aging be the primary factor leading to mitochondrial abnormalities? Although some recent studies are pointing in this direction, more *in vivo* studies modulating the expression of the different proteins involved in mitochondrial dynamics and the analyses of aging-related phenotypes are required.

If mitochondrial dynamics during aging is confirmed to be the primary factor leading to mitochondrial abnormalities, can the proteins involved in mitochondrial dynamics be a promising therapeutic target for the treatment of age-related diseases and/or the promotion of healthy aging? In this regard, efforts should be oriented towards the discovery of new pharmacological compounds that are able to modulate the expression and/or activity of these proteins.

Box 4. Clinician's Corner

Concomitant with an increase in life expectancy, the development of age-associated diseases has also increased over the past century.

Alterations in mitochondrial function and quality are among the known factors postulated to be associated with aging and, for several decades, have been a focus of research. Mitochondrial dynamics is important for the maintenance of mitochondrial function and the regulation of mitochondrial quality. Mutations or altered expression of proteins regulating mitochondrial dynamics are associated with certain age-related diseases and neurodegenerative conditions (Parkinson's and Alzheimer's disease, and some neuropathies), cardiovascular disease, metabolic disease, and sarcopenia.

Scientific and economic efforts have been made previously and are still needed to decipher the molecular alterations driving aging and age-related diseases. This research will facilitate the identification of new putative pharmacological targets and the design of more efficient therapeutic approaches to treat various diseases.

The dysregulation of mitochondrial dynamics may underlie the loss of mitochondrial health during aging, thus representing a promising strategy to help combat aging and age-related diseases.

regarding the link between mitochondrial dynamics and aging remain (see Outstanding Questions, Box 4). In this regard, future studies should focus on the discovery of the putative factors responsible for the alteration of mitochondrial dynamics during aging, as well as on the molecular mechanisms underlying the connection between mitochondrial dynamics and mitophagy. Increased knowledge in this field might contribute to validating the mitochondrial dynamics network as a promising target for combating aging and, hopefully, certain age-related diseases.

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