5 Importance of Mitochondrial Quality Control in Parkinson's Disease: The Potential Interplay of Mitochondrial Unfolded Protein Response and Mitophagy

Yew Mun Lee, Dongxue Hu, and Yih-Cherng Liou

Abstract

A substantial body of empirical studies has highlighted that the maintenance of a "healthy" mitochondrial network is imperative for the mitigation of neurodegenerative diseases. This maintenance is especially important in the nervous system made up of many neurons that act as important communicators. Neurons are energy-hungry cells that require high amounts of energy to function. A large part of the energy is obtained from the mitochondria, viewed as power plants of mammalian cells. Indeed, mitochondrial dysfunction plays a key role in the pathogenesis of many neurodegenerative diseases such as Parkinson's disease (PD). To prevent this, mitochondria have evolved mitochondrial quality control mechanisms such as the mitochondrial unfolded protein response (UPR^{mt}) and mitophagy to prevent or remove "unhealthy" populations of mitochondria in neurons. Interestingly, numerous studies in the last few decades have shed light on the implications of these two pathways in PD, of which both processes seem to share a close relationship with each other. Henceforth, in this book chapter, we aim to accentuate the mechanisms of both UPR^{mt} and mitophagy in mammalian cells and identify the potential interplay which these two machineries might have in the prevention and potential therapeutic targets of PD.

Keywords

Parkinson's disease · Mitochondrial dynamics · Mitochondrial unfolded protein response (UPR^{mt}) · Mitophagy · Oxidative phosphorylation (OXPHOS) · Reactive oxygen species (ROS) · Parkin · PINK1 · Mitochondrial DNA (mtDNA) · Ubiquitin

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5.1 Introduction

A mammalian cell is made up of numerous organelles that work hand in hand to sustain its viability. Although the nucleus holds the most important genetic information that is crucial to the survival of the cell, another organelle is responsible for providing important energy in the form of adenosine triphosphate (ATP) through the process of oxidative phosphorylation (OXPHOS) to sustain its function. This "power-packed" organelle has the most peculiar origin in the mammalian cell, dating back more than 1.5 billion years ago. Then, the prokaryotic cells entered into the eukaryotic cells to become what is commonly known as the mitochondrion or, in plural, the mitochondria [1]. Despite being the power plant of a mammalian cell, its importance cannot be highlighted greater enough than in the brain. The brain is one of the highest energy-consuming organs of the human body, requiring as much as 20% of the basal oxygen consumption to fuel its function [2], of which neurons are estimated to consume as much as 80% of the total energy generated from the brain [3]. The energy produced from the mitochondria fuels crucial neuronal functions, such as the ATP-dependent neurotransmission [4–6]. Therefore, the role of mitochondria is critical for the neurological system to work.

Despite the importance of the mitochondria in sustaining the physiological function of the brain, they bring along an unwanted baggage that can be detrimental to the cell. The main driver of ATP production as highlighted earlier comes from the process of OXPHOS. This exact process would also lead to the generation of reactive oxygen species (ROS). Negatively, an increasing level of accumulated ROS would become a source of cellular toxicity. This observation has led to scientists coining the "free radical theory of aging" [7]. In physiology, besides being a by-product of OXPHOS during ATP production, ROS also have a role as signalling molecules in the brain. As such, ROS are involved in processes such as mounting an immune response, eliciting inflammatory processes, expressing synaptic plasticity, and also playing a role in learning and memory of the brain function [8, 9]. On the flip side, the excessive accumulation of ROS would lead to a buildup of oxidative stress that can damage the proteins and DNA. The accumulative damages incurred would eventually lead to the onset of cellular death as observed in the pathogenesis of many neurodegenerative diseases like Parkinson's disease (PD) [10, 11].

5.1.1 Reactive Oxygen Species

Antioxidative mechanisms are present to alleviate the deleterious accumulation of ROS in the mitochondria. However, there is a tipping point when even such mechanisms are being overwhelmed. This would lead to the onset of mitochondrial dysfunction that is observed in many diseases, including neurodegenerative diseases as well as aging [12]. Furthermore, even with numerous studies demonstrating the prominent role of ROS in the pathogenesis of PD, the use of antioxidants as a treatment for PD remains modest [13]. Therefore, there is a need to improve our

understanding of the safeguarding of mitochondrial health beyond that of the ROS in order to better identify new therapeutic interventions for PD. In this chapter, we will emphasize on the importance of mitochondrial quality control that helps to prevent mitochondrial dysfunction in the brain. In particular, we intend to discuss how mitochondrial unfolded protein response (UPR^{mt}) and mitophagy play a responsible role in neurons, as well as the interplay between these two crucial factors. In addition, the content will also cover how the potential loss of mitochondrial quality control will lead to the pathogenesis of PD.

5.2 The Functional Importance of Mitochondria in the Neurons

The brain is a highly complex organ that consists of highly differentiated cells forming different regions of the brain to create a network of intricate signalling processes which governs the functions of the entire body. The major workhorses of the brain are neurons. They are specialized cells which differ in morphology based on their location and function in the brain and the rest of the body. As highlighted previously, neurons are energy-hungry cells, and they consume the majority of the energy produced in the brain to drive their functions. These include propagating electrical signals, maintaining the ionic balance in the cell to generate action potential, and releasing and capturing of neurotransmitters such as dopamine between neurons [14]. As glucose oxidation—which generates less ATP than lipids oxidation—is the major source of energy in the brain to reduce excessive production of ROS, the process of OXPHOS in the mitochondria is crucial for the energy needs of the neurons [15].

Even though mitochondria are found in most of the mammalian cells, a previous research by Reifschneider et al. [16] has shown that cells derived from different organs of the rat exhibit different mitochondrial protein composition, specifically the proteins involved in the process of OXPHOS. Of note, neurons are terminally differentiated cells; their life spans are almost parallel with that of the whole organism. Therefore, damaged neurons are rarely or never regenerated, and they seem to be more susceptible to the accumulation of defective mitochondria during aging [17, 18]. Thus, the mitochondrial quality control in neurons is paramount to their function and survival. In addition, the morphological and structural complexities of neurons such as the presence of synapses require the mitochondria to be distributed to these locations and produce energy required for neuronal function [19, 20]. Indeed, there are functional differences between the synaptic mitochondria and the non-synaptic mitochondria as observed in rats by Borras et al. [21, 22]. Furthermore, the effects of aging have a greater impact on synaptic mitochondria in terms of reduced respiratory rate and increased sensitivity to calcium insult as compared to non-synaptic mitochondria [23, 24]. Collectively, these negative effects highlight the maintenance of a healthy pool of mitochondria as the utmost crucial factor for synaptic neurons' function. The buildup of "unhealthy" mitochondria otherwise would lead to the onset of mitochondrial dysfunction,

potentially causing age-related diseases such as PD. Indeed, mitochondrial dysfunction has been observed by Reeve et al. [25] within the synapses of *substantia nigra* (SN) neurons from PD patients. In the next section, we will discuss the role of mitochondrial dysfunction in the manifestation of PD.

5.3 Mitochondrial Dysfunction in Parkinson's Disease

PD is the second most common neurodegenerative disease after Alzheimer's disease (AD) [26]. The established hallmarks of PD include the loss of dopaminergic neurons in the midbrain *SN pars compacta* (SNpc) and the accumulation of α-synuclein (α-syn) containing Lewy bodies. After almost four decades of research, there is a substantial amount of evidence to support the notion that mitochondrial dysfunction is an important trigger for the development of PD (reviewed in [27]). The establishment of mitochondrial dysfunction not only contributes to the loss of OXPHOS and its calcium regulation, but more importantly, the disruption of the mitochondrial quality control processes such as the UPR^{mt} and mitophagy has been implicated to play a pivotal role in the manifestation of PD (Fig. 5.1).

Ever since the application of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that led to the inhibition of complex I (NADH/ubiquinone oxidoreductase) of the mitochondrial electron transport chain (ETC) and the induction of parkinsonism in humans [28], there are ever-increasing evidences that tie mitochondrial dysfunction to PD. More studies have identified the reduction of complex I activity in other areas of the brain, on top of the SNpc in sporadic PD patients [29–35]. These evidences clearly suggest that a perturbation to the normal function of the mitochondria could trigger parkinsonism, highlighting further the relationship between mitochondrial dysfunction and PD.

On top of the perturbation to the ETC, genetic alterations of the mitochondrial DNA (mtDNA) have been identified to contribute to the pathogenesis of PD. Multiple deletions of mtDNA have been observed from postmortem analyses of human brains from both aged people and idiopathic PD patients [36, 37]. The first empirical evidence that altered mtDNA could lead to the pathogenesis of PD came from genetic analysis on the mutation of mtDNA polymerase gamma gene (*POLG*). Mutations of *POLG* lead to the accumulation of deletions to multiple regions of the mtDNA in muscle cells, developing levodopa-responsive parkinsonism with severe SNpc dopaminergic neuronal loss [38, 39]. Interestingly, from the studies on *POLG* mutant mice yielding high levels of mtDNA deletions, the authors did not observe any increase of mitochondrial dysfunction nor degeneration to the SNpc dopaminergic neurons, which are the hallmarks of PD [40, 41]. However, perturbation to the mitochondrial transcription factor A (TFAM), which causes partial depletion of the mtDNA, can disrupt complex VI (cytochrome C oxidase) activity. This decrease in complex VI activity is reminiscence of a progressive parkinsonism phenotype [42]. Collectively, these observations further suggest that mitochondrial dysfunction from the loss of OXPHOS activity contributes greatly to the pathogenesis of PD.

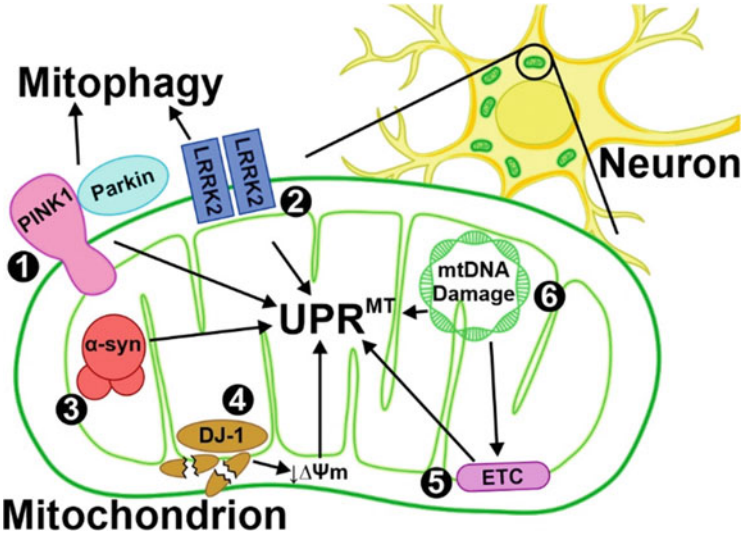

Fig. 5.1 Implications of mitochondrial dysfunction in Parkinson's disease (PD). (1) Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)/parkin RBR E3 ubiquitin protein ligase (Parkin) pathway has been well documented in PD phenotype and has been demonstrated to be involved in both the mitochondrial unfolded protein response (UPR^{mt}) and mitophagy. (2) Leucine-rich repeat kinase 2 (LRRK2) perturbation has also been identified in PD patients and has been known to be involved in mitochondrial dynamics and mitophagy. (3) The accumulation of alpha-synuclein (α-syn) is one of the major hallmarks of PD, and its accumulation in the mitochondria has been suggested to cause mitochondrial dysfunction. (4) The reduced expression of DJ-1 could lead to mitochondrial dysfunction in the form of reduced mitochondrial potential (ΔΨm). (5) Perturbation to the electron transport chain (ETC), particularly complex I, has been demonstrated to induce parkinsonism in humans. (6) The damage and subsequent deletions or mutations to the mitochondrial DNA (mtDNA) have been observed to contribute to mitochondrial dysfunction and lead to the potential pathogenesis of PD. All listed processes have been known to trigger the UPR^{mt} process

Indeed, efforts for identifying important genes have significantly contributed to the understanding of the familial forms of PD. Proteins encoded by these genes include α-syn, leucine-rich repeat kinase 2 (LRRK2), PTEN-induced putative kinase 1 (PINK1), parkin RBR E3 ubiquitin protein ligase (Parkin), DJ-1, and vacuolar protein sorting 35 (VPS35). They play important roles in the function of mitochondria (Fig. 5.1). In addition to the well-documented functions of α-syn and the Pink1/Parkin pathway in PD, mutant LRRK2 has also been identified as a potential pathogenic target of PD as it can disrupt mitochondrial mobility and affect the initiation of mitophagy [43]. Furthermore, the downregulation of DJ-1 was also identified to increase mitochondrial fragmentation and reduce the mitochondrial membrane potential (ΔΨm). These detrimental effects could be mitigated by the overexpression of PINK1 and Parkin [44–46]. The mutation of VPS35 was also found to induce mitochondrial dysfunction by the impairment to the mitochondrial dynamic process [47]. The vital physiological functions of these proteins highly

associate with the mitochondrial dysfunction and pathogenesis of PD. They could contribute to important mitochondrial quality control processes such as the UPR^{mt}, mitophagy, and mitochondrial dynamics (reviewed in [27]). These mechanisms are involved in maintaining a pool of "healthy" mitochondria. In the next few sections, we will discuss how the loss of these mechanisms through the age of time could lead to the onset of PD.

5.4 The Mitochondrial Unfolded Protein Response (UPR^{mt})

The process of UPR^{mt}, controlled by the communication between the mitochondria and the nucleus, is a transcriptionally regulated mechanism activated by many types of mitochondrial dysfunction [48–56]. The importance of UPR^{mt} is signified during the decline in mitochondrial function, where it is activated to promote repair and recovery of mitochondrial function from disruptions caused by various insults such as toxins, pathogens [51, 52, 57], and mutations to the respiratory genes [49, 54] in order to maintain cellular functions [49, 51, 54, 58].

The existence of UPR^{mt} was discovered almost 20 years ago by Zhao et al. [56]. The authors overexpressed the mutant form of the mitochondrial matrix protein, ornithine transcarbamylase (OCT), in fibroblasts named COS-7 cells derived from monkey kidney tissue. The mutant OCT was observed to irreversibly misfold and aggregate in the mitochondria, leading to an increased accumulation of nuclear transcripts that encode for several mitochondrial chaperones and proteases. These changes were suggested by the authors for the cell to balance out the amount of protein aggregation (proteostasis) in the mitochondria. Due to its similarity to the UPR mechanism in the endoplasmic reticulum (ER), it was thus coined as UPR^{mt}. During this decade or so, the progress of understanding UPR^{mt} continued mainly in the *Caenorhabditis elegans* model [59]. In recent times, scientists working on UPR^{mt} shifted to the use of mammalian model systems, gaining new knowledge that positioned UPR^{mt} as more than just regulating the proteostasis of the mitochondria [59]. In short, a growing number of evidences have suggested that UPR^{mt} is central in detecting mitochondrial dysfunction and communicates to the nucleus for initiating potential mitochondrial recovery mechanisms.

5.4.1 The UPR^{mt} During Mitochondrial Dysfunction

As highlighted earlier, the onset of mitochondrial dysfunction is triggered by a broad array of mitochondrial stress responses that include the accumulation of misfolded and aggregated proteins in the mitochondrial matrix and the perturbation of mitochondrial OXPHOS activity. These mitochondrial stresses were found to be able to activate UPR^{mt} (Fig. 5.2). Majority of the studies in elucidating the mechanisms of UPR^{mt} were conducted in *C. elegans* (reviewed in [59]). In the later sections of this chapter, we will focus our attention on the current knowledge of UPR^{mt} in the mammalian system.

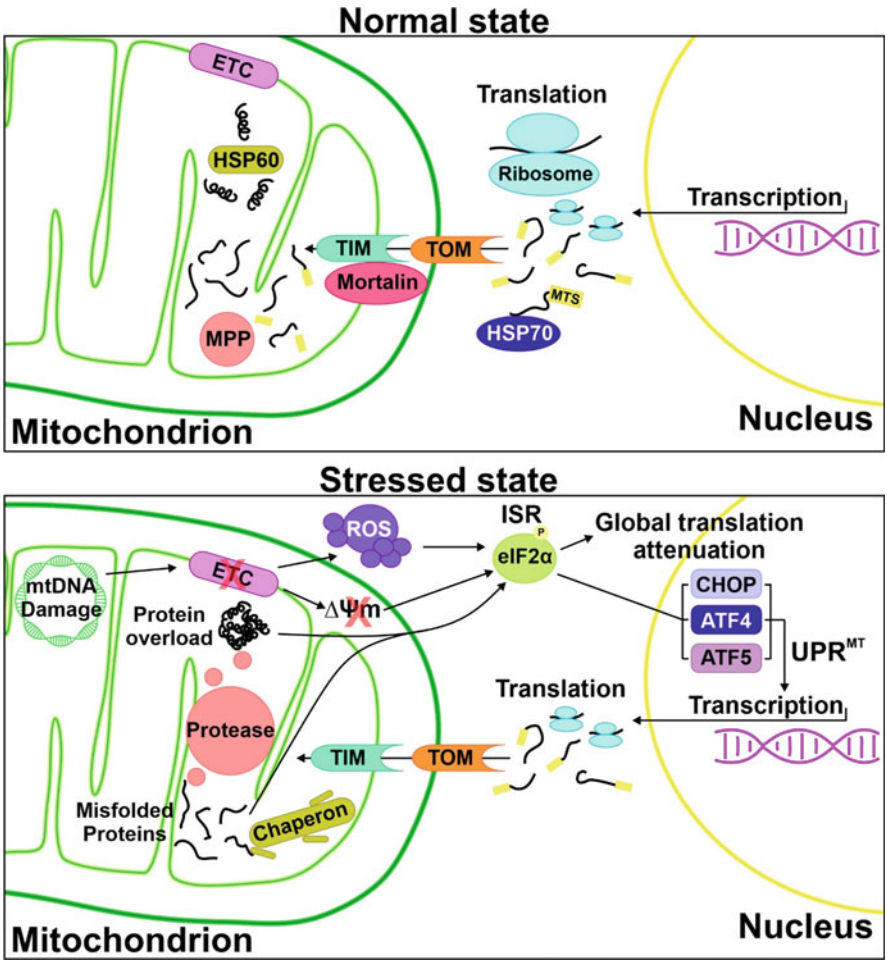

Fig. 5.2 The process of mitochondrial unfolded protein response (UPR^{mt}) and its function during mitochondrial stress. During the normal state of the mitochondria, important protein precursors are synthesized from the nucleolar DNA and imported into the mitochondrial matrix via the translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM) complexes. Each of the protein precursors has a mitochondria-targeting sequence (MTS) that is required for their import. The import process is aided by chaperone proteins such as heat shock protein 70 (HSP70). Mitochondrial chaperone proteins such as mortalin located in the TIM complex are also on hand to help the import of the protein precursors. Once inside the mitochondrial matrix, the protein precursors are processed by first removing the MTS via the protease mitochondrial processing peptidase (MPP) to become mature polypeptides. Subsequently, the mature polypeptides are folded into their proper structure by protein chaperons such as HSP60. Conversely, at the stressed state, mitochondria can be stressed by the presence of mitochondrial DNA (mtDNA) damage, perturbation of the electron transport chain (ETC) leading to reduced membrane potential ($\Delta\Psi m$) and the increase in reactive oxygen species (ROS) production, and the accumulation of excessive mitochondrial proteins or misfolded proteins. The damage to the mtDNA could lead to translation of non-functional proteins of the ETC and cause the ETC to malfunction. The subsequent generation of ROS would lead to damaged proteins and misfolded proteins that would accumulate. In addition, the loss of the $\Delta\Psi m$ will also block the import of the protein precursors. This stressed state would lead to the activation of the integrated stress response (ISR) pathway by the phosphorylation of the

5.4.1.1 The Relationship Between the Mitochondrial Protein Import and UPR

As mentioned previously, many preceding studies of UPR^{mt} have been done using the *C. elegans* model. The most important finding from these studies is that the mitochondrial protein import is a key UPR^{mt} regulatory event [54] and most of the triggers of UPR^{mt} are able to influence the mitochondrial protein import [59]. Of note, the mammalian mitochondria each consists of a mitochondrial genome that encodes for a total of 13 proteins. They are made up of subunits of the respiratory chain and the ATP synthase and the components of the mitochondrial translation system: the ribosomal RNAs and transfer RNAs [60]. As the number of proteins encoded in the mitochondria is small, most of the proteins required for a functional and healthy mitochondrion are transcribed in the nucleus and synthesized in the cytosol, before they are imported into the mitochondrion (Fig. 5.2). However, instead of the fully folded protein, protein precursors that remain unfolded are imported. The association of these protein precursors with ATP-dependent molecular chaperones, heat shock protein 70 (HSP70) and 90 (HSP90), prevents their degradation and aggregation in the cytosol [61]. The protein precursors are subsequently directed and imported into the mitochondria with their N-terminal mitochondria-targeting sequence (MTS) [62]. Once imported, the MTS on the protein precursors is cleaved in the mitochondrial matrix by the mitochondrial processing peptidase (MPP) to allow the precursors to become mature polypeptides.

The import of these protein precursors into the mitochondrial matrix, where most of the mitochondrial proteins are located, involves passing through the two main mitochondrial translocase complexes. First is the translocase of the outer membrane (TOM) complex consists of seven components: Tom5, Tom6, Tom7, Tom20, Tom22, Tom40, and Tom70 [63, 64]. Second is the translocase of the inner membrane (TIM23) complex consists of Tim17, Tim21, Tim23, Tim44, Tim50, Tim14/Pam18, Tim16/Pam16, Pam17, mitochondrial HSP70 (mtHSP70), and Mgel1 [65]. In addition, the import through the TIM23 complex requires an intact mitochondrial $\Delta\Psi m$ along with the hydrolysis of ATP.

Improper mitochondrial protein import, misfolding, and mutations of the mtDNA respiratory genes can affect the proteostasis in the mitochondria, resulting in mitochondrial dysfunction [51]. Henceforth, protein chaperones that avoid protein misfolding or aggregation are crucial for the mitochondrial protein import of the protein precursors (Fig. 5.2) [66]. The importing process is governed by a few important chaperons. They include HSP70 and its interactor HSP60, mitochondrial HSP70 (mtHSP70; also known as mortalin), HSP10, and tumor necrosis factor receptor-associated protein 1 (TRAP1) [67–70]. On top of ensuring proper protein

Fig. 5.2 (continued) eukaryotic translation initiation factor 2 subunit 1 (eIF2α). Moreover, the stress response pathway could trigger the inhibition of global protein translation and activate three transcription factors: C/EBP homologous protein (CHOP), activating transcription factor 4 (ATF4), and ATF5. These transcription factors will increase the transcription of important protein chaperons or proteases to help mitigate the mitochondrial stress and prevent mitochondrial dysfunction

folding, the system of removing unfolded or irreversibly misfolded proteins is equally important in maintaining mitochondrial proteostasis. Players in maintaining mitochondrial proteostasis include ATP-dependent proteases iAAA (intermembrane space facing) and mAAA (matrix facing), Lon protease (LONP1), Clp protease proteolytic subunit (CLPP), high-temperature requirement protein A2 (HTRA2), and ATP23 [71–75].

The system of protein chaperons and proteases ensures that the accumulation of misfolded proteins in the mitochondrial matrix and mutations in the mtDNA does not lead to mitochondrial dysfunction. Therefore, the triggering of the UPR^{mt} would lead to the transcriptional activation to produce these chaperons and proteases to mitigate potential mitochondrial dysfunction. Despite the similarity in the transcriptional response of the activation of UPR^{mt} in both *C. elegans* and mammalian models, the regulation of UPR^{mt} is more complicated in the mammalian cells as the research continues to understand this process better.

5.4.1.2 The Regulation of UPR^{mt} in Mammalian Cells

The UPR^{mt} regulator, the activating transcription factor associated with stress-1 (ATFS-1), found in *C. elegans*, belongs to the family of bZIP transcription factor [50, 54]. Similarly, research over the last few years in mammalian models of UPR^{mt} has yielded the identification of three basic leucine zipper (bZIP) transcription factors in C/EBP homologous protein (CHOP), activating transcription factor (ATF4), and ATF5. These transcription factors are associated with the integrated stress response (Fig. 5.2; ISR) [76–79]. The expression of the three transcription factors is governed by the phosphorylation of the eukaryotic translation initiation factor 2 subunit 1 (eIF2α). The phosphorylation of eIF2α is initiated by four kinases as part of the integrated stress response (ISR) pathway originating from a diverse range of cellular stresses such as the ER stress [78].

Studies over the years have revealed that mitochondrial stress of various types is able to induce the expression of CHOP, ATF4, and ATF5, including the genes that are involved in the UPR^{mt} [80–86]. Particularly, studies have demonstrated that CHOP, ATF4, and ATF5 are all required to regulate and induce the expression of UPR^{mt} genes during mitochondrial dysfunction [80, 81, 84, 86]. Although CHOP, ATF4, and ATF5 are involved in mitigating mitochondrial dysfunction, it remains unclear how the three transcription factors together with the ISR pathway are regulated during the whole process of this mitochondrial recovery.

Of interest, among the three transcription factors, ATF5 has been suggested by Florese et al. [84] to be an ortholog to the *C. elegans* ATFS-1. The authors demonstrated that ATF5 could rescue the activity of UPR^{mt} in ATFS-1-deficient *C. elegans*. From the same group, they further highlighted that ATF5 itself is able to respond directly to mitochondrial stress under the mechanism of the mitochondrial import regulation, much like the *C. elegans* ATFS-1. They further demonstrated that ATF5 is required to activate the transcription of several mitochondrial chaperone and protease genes in times of mitochondrial stress. In addition, studies have also demonstrated that ATF4 is able to protect mammalian cells against mitochondrial stress [86, 87] via the expression of ISR metabolic genes [86, 88]. Despite the

findings, the coordination among CHOP, ATF4, and ATF5 in the regulation of UPR^{mt} during mitochondrial dysfunction remains unknown. Interestingly, only ATF5 contains the MTS like ATFS-1, suggesting that ATF5 could be the main transcription factor regulating the transcriptional activation of the UPR^{mt} genes. As hypothesized by Shpilka and Haynes [59], CHOP, ATF4, and a phosphorylated eIF2α might induce the expression of ATF5 since the transcription of ATF5 was found to be dependent on the activity of CHOP and ATF4 [77, 89–91]. Much remains to be studied to fully understand the regulation of the UPR^{mt} in mammalian cells.

5.5 Mitophagy

Mitophagy is a mitochondria-specific autophagic process that was first observed under the electron microscope in the 1960s, showing the mitochondria with various extents of degradation in lysosomes [92, 93]. However, it was not until 2005 that Lemasters [94] proposed the term "mitophagy" to describe the selective elimination of mitochondria. Mitophagy mediates the removal of non-functional mitochondria to maintain a pool of "healthy" mitochondria and has been associated with physiological and pathological processes [95]. Mitophagy is involved in various cellular events, such as hypoxic stress, reticulocyte maturation, overt mitochondrial damage, and post-fertilization removal of paternal mtDNA [96]. Furthermore, mitophagy defects have been known to impede mitochondrial function and could lead to the accumulation of abnormal mitochondria, causing impairments to cells and tissues [97].

Upon initiation of mitophagy, isolation membranes (phagophores) are recruited to mitochondria through interactions between receptor proteins and microtubule-associated protein 1 light chain 3 (LC3) anchored on phagophores. The recruited phagophores then expand and encapsulate the targeted mitochondrion, leading to the formation of mitophagosome. The mitophagosome is subsequently fused with a lysosome for the degradation of mitochondria [98]. Multiple mechanisms are involved in the regulation of targeted elimination of mitochondria, and they are largely categorized into two major types: ubiquitin-dependent and ubiquitin-independent mitophagy (Fig. 5.3) [99].

5.5.1 The Mechanism of Ubiquitin-Dependent Mitophagy

PINK1/Parkin-mediated mitophagy is the most well-investigated and the best-described mitophagy pathway [98, 100]. PINK1 contains an MTS, a putative transmembrane (TM) helix, an N-terminal linker region (NT linker), a Ser/Thr kinase domain, and a conserved C-terminal extension (CTE) [101, 102]. Under a healthy physiology state, PINK1 is transported into the mitochondria via its MTS [103] and then cleaved by four proteases: MPP, ClpXP, presenilin-associated rhomboid-like (PARL) protease, and m-AAA [104, 105]. The cleavage yields a 52 kDa

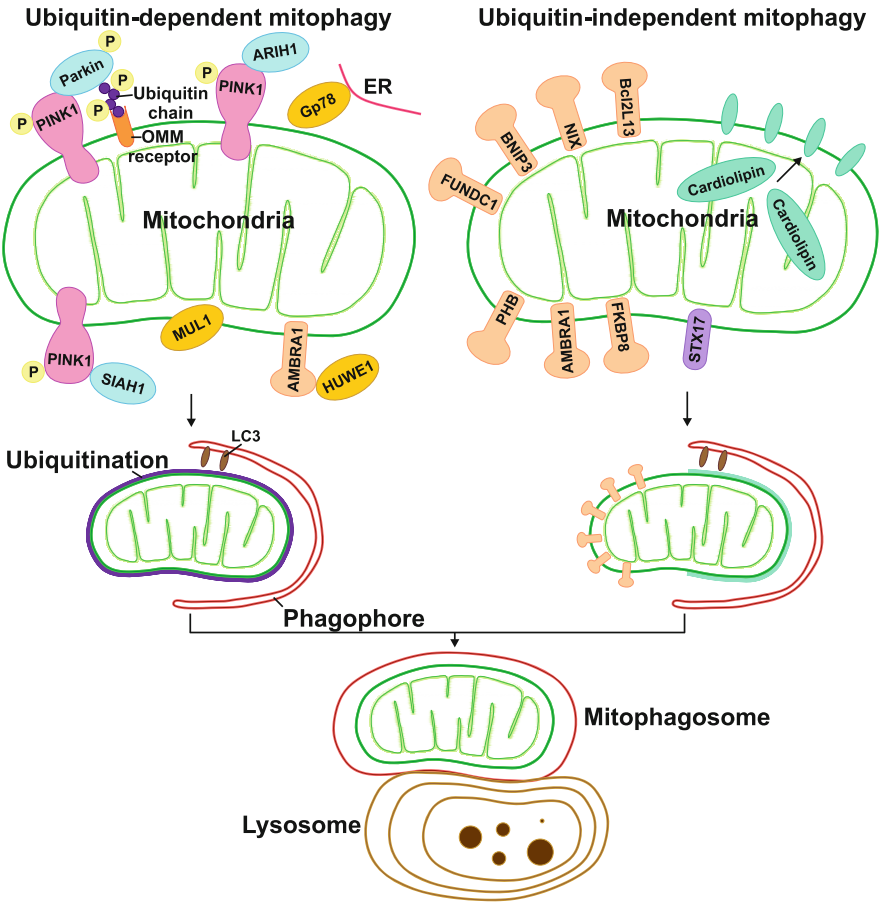

Fig. 5.3 The process of mitophagy. The two main classes of mitophagy lie in how they recruit the phagophore to trigger the process of mitophagy (digestion of the mitochondria). These two main classes are either ubiquitin-dependent or ubiquitin-independent. For the ubiquitin-dependent mitophagy, the main feature is that the outer mitochondrial membrane (OMM) is covered with phospho-ubiquitin chains for phagophore recruitment. The most prominent of this process is initiated by the PTEN-induced putative kinase 1 (PINK1)/parkin RBR E3 ubiquitin protein ligase (Parkin) pathway. The stabilization of PINK1 to the OMM allows it to be autophosphorylated and then phosphorylates ubiquitin. This process allows Parkin to bind to the phospho-ubiquitin and to PINK1 for its phosphorylation and activation. The phosphorylated Parkin will then recruit more phospho-ubiquitin to the OMM proteins to form polyubiquitin chains. Other E3 ubiquitin ligases, such as ariadne RBR E3 Ub protein ligase 1 (ARIH1) and synphilin-1 recruited seven in absentia homolog 1 (SIAH1), have also been identified for this process. Moreover, other E3 ubiquitin ligases that do not require PINK1 to ubiquitinate the OMM have been identified, including mitochondrial ubiquitin ligase 1 (MUL1), WWE domain-containing protein ligase 1 (HUWE1), and glycoprotein 78 (Gp78). HUWE1 works with activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1), an OMM receptor, while Gp78 is localized in the endoplasmic reticulum (ER) membrane. For ubiquitin-independent mitophagy, it mainly functions with OMM receptors that can directly recruit the phagophore, such as Bcl2-like protein 13 (Bcl2L13), Nip3-like protein X (NIX), BCL2-interacting protein 3 (BNIP3), FUN14 domain-containing 1 (FUNDC1), prohibitins (PHB), AMBRA1, and FK506-binding protein 8 (FKBP8). Recently, syntaxin 17 (STX17), not an OMM receptor, has also been identified to be able to initiate mitophagy via a

product, which is retro-translocated into the cytoplasm and further degraded by the proteasome via the N-end rule pathway after being ubiquitinated [106, 107]. These processes account for the low levels of PINK1 under basal conditions.

Upon ΔΨm dissipation, PINK1 is stabilized on the outer mitochondrial membrane (OMM) [108, 109], whereby it is activated by autophosphorylation [110]. Subsequently, PINK1 phosphorylates the E3 ligase Parkin and the neighboring ubiquitin chains located on the OMM. The phosphorylated ubiquitin chains recruit and activate the phosphorylated Parkin. The phosphorylation of both Parkin and ubiquitin chains contributes to the activation of Parkin [111–117]. On the OMM, Parkin next ubiquitinates itself along with other OMM proteins, including mitofusin (Mfn), voltage-dependent anion-selective channel protein 1 (VDAC1), and mitochondrial Rho GTPase (Miro) [112, 118–120]. The increased level of ubiquitin on the OMM would generate a feedforward loop for Parkin recruitment, thereby amplifying the mitophagy signals (Fig. 5.3) [121].

Recently, our group examined the role of ROS as a promoting factor for the process of mitophagy in PD [122]. The idea of ROS as a trigger remains controversial as previous studies have shown that the application of ROS scavengers did not suppress the carbonyl cyanide m-chlorophenylhydrazone (CCCP)-induced Parkin recruitment using HeLa cells [123–125]. We hypothesized that this failure is due to the inadequacy of ROS scavenging, causing the levels of ROS to remain high and initiate mitophagy. Indeed, using a combination of N-acetyl-L-cysteine (NAC) and catalase, we inhibited the increase of ROS levels after CCCP treatment and eventually block the PINK1-dependent Parkin translocation to the mitochondria needed to initiate mitophagy. Moreover, the overexpression of VDAC1 also led to an increase in Parkin translocation to the mitochondria, suggesting that the increased ROS production might have contributed to this phenomenon. Indeed, treatment with NAC and catalase are able to block the Parkin translocation induced by VDAC1 overexpression. Moreover, our recent study also showed that the loss of a mitochondrial fission protein, mitochondrial elongation factor 1 (MIEF1), also known as mitochondrial dynamic protein of 51 kDa (MiD51), can increase mitochondrial ROS levels [126]. The increase in ROS levels can induce PINK1/Parkin-mediated mitophagy, which can be blocked by adding NAC to MIEF1 knockdown (KD) HeLa cells. Taken together, we highlighted that ROS may contribute as a trigger for PINK1/Parkin-dependent mitophagy. Since ROS are also potential triggers for UPR^{mt}, it will be of interest in the future to investigate if this process of ROS-induced mitophagy might be downstream of UPR^{mt} when the ROS level becomes too much for UPR^{mt} to protect the mitochondria

Fig. 5.3 (continued) microtubule-associated protein 1 light chain 3 (LC3)-independent pathway. In addition, cardiolipin, a type of diphosphatidylglycerol lipid in the mitochondria, is also able to recruit the phagophore. Once the phagophore is recruited via interactions with LC3 and surrounds the mitochondrion, a mitophagosome is formed. The mitophagosome will then fuse to a lysosome to degrade the mitochondria and complete the mitophagy process

In addition to Parkin, several alternative ubiquitin E3 ligases, such as mitochondrial ubiquitin ligase 1 (MUL1) [127], synphilin-1 recruited seven in absentia homolog 1 (SIAH1) [128], ariadne RBR E3 Ub protein ligase 1 (ARIH1) [129], glycoprotein 78 (Gp78) [130], and WWE domain-containing protein ligase 1 (HUWE1) [131], have been identified. HUWE1 is recruited to the mitochondria from the cytosol by its cofactor activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1) and works collaboratively to mediate mitophagy [131]. Unique among these E3 ligases, Gp78 is localized on the ER membrane, where it initiates mitophagy at the ER-mitochondria-associated area [130]. Similar with Parkin, these E3 ligases generate polyubiquitin chains on OMM (Fig. 5.3). The polyubiquitin chains can recruit autophagic receptors onto the OMM, which would then connect with the phagophores to damaged mitochondria for cargo engulfment, followed by autophagic degradation. Optineurin (OPTN) [132, 133], calcium-binding and coiled-coil domain-containing protein 2 (NDP52) [133], p62 [118], Tax1-binding protein 1 (TAX1BP1) [133], and next to BRCA1 gene 1 protein (NBR1) [96, 134, 135] are currently known receptors that have been linked to ubiquitin-dependent mitophagy in mammalian cells.

5.5.2 The Mechanism of Ubiquitin-Independent Mitophagy

In the other type of mitophagy, certain OMM receptors or lipid could directly bridge the mitochondria and autophagy machinery to mediate mitophagy, thus activating what is known as the ubiquitin-independent mitophagy [97]. These receptors or lipid includes FUN14 domain-containing 1 (FUNDC1), prohibitins (PHB), BCL2-interacting protein 3 (BNIP3), Nip3-like protein X (NIX), Bcl2-like protein 13 (Bcl2L13), FK506−binding protein 8 (FKBP8), AMBRA1, and the OMM phospholipid cardiolipin (Fig. 5.3) [136, 137]. These proteins mediate mitophagy in response to various cellular stimuli, such as mitochondrial depolarization (e.g., cardiolipin, FKBP8), hypoxia (e.g., NIX, FUNDC1, BNIP3), energetic stress (e.g., PHB), and developmental signals during differentiation (e.g., NIX) [96, 136, 137]. Interestingly, we recently found that the depletion of mitochondrial fission 1 protein (Fis1) leads to aberrant syntaxin 17 (STX17) accumulation on the mitochondria. The translocation of STX17 leads to the interaction with autophagy-related 14 (ATG14) and recruitment of downstream autophagic proteins to induce mitophagy in a PINK1/Parkin-independent manner [138]. These results suggest that STX17 might be a new receptor for mitophagy. Unlike the other receptors mentioned above, STX17 initiates mitophagy by recruiting autophagic proteins other than LC3.

5.5.3 Mitochondrial Dynamics and Mitophagy

Mitochondria are highly dynamic organelles, which continuously undergo the process of fusion and fission. Fusion leads to the formation of interconnected networks,

which enables content exchange between mitochondria to maintain the mitochondrial genome and proteome integrity. On the other hand, fission results in the fragmentation of mitochondria and can facilitate the elimination of dysfunctional components [139, 140]. Therefore, mitochondrial dynamics are closely associated with mitophagy. In mammals, several dynamin-related GTPases mediate mitochondrial fusion and fission: mitofusin 1/2 (Mfn1/2), optic atrophy 1 (OPA1), dynamin-related protein 1 (Drp1), fission 1 protein (Fis1), MiD49/51 (MIEF1), and mitochondrial fusion factor (Mff) [141].

Interestingly, we previously found that the loss of death-associated protein 3 (DAP3) can significantly reduce the phosphorylation of Drp1 at Ser637 to promote mitochondrial fission [142]. Physiologically, DAP3 is a mitochondrial ribosomal protein located in the mitochondrial matrix and was initially thought to be solely involved in apoptosis. Depletion of DAP3 leads to defects in the mitochondria-encoded protein synthesis along with the abnormal imbalance of the mitochondrial fission and fusion processes due to the increased fission rate as mentioned above. Furthermore, we observed the inhibition of autophagy by the loss of DAP3. Collectively, our findings provided new insights into the potential interplay between mitochondria-encoded protein synthesis, mitochondrial dynamics, and autophagy. Since the perturbation of mitochondria-encoded protein synthesis could affect mitochondrial proteostasis as mentioned previously, depletion of DAP3 could have triggered UPR^{mt}, further suggesting the link between UPR^{mt} and mitophagy (selective autophagy) via mitochondrial dynamics. In addition, manipulation of the mitochondrial dynamic proteins can also be an indirect method to regulate the mitophagic process. Several lines of evidence have already demonstrated such possibility [143–147]. For example, D'Amico et al. [147] enhanced the process of mitophagy via the upregulation of Mff in both old nematodes and mice. And intriguingly, this intervention brought significant benefits to maintain the pool of "healthy" mitochondria during aging.

5.6 The Relationship of UPR^{mt} and Mitophagy

The processes of UPR^{mt} and mitophagy share a close relationship in that they are both activated by similar mitochondrial stress mechanisms like misfolded proteins [148], mtDNA mutations [53, 149, 150], perturbation to the OXPHOS activity [51], and the decreased levels of the mitochondrial chaperon mortalin (mtHSP70) [151]. Both the activation of UPR^{mt} and mitophagy are dependent on the perturbation to the mitochondrial import. This dependence of the mitochondrial import in both mitochondrial quality control processes suggests that a mitochondrion senses its ability to import proteins as a way to gauge its "health" status. As highlighted in a review by Pickles et al. [96], the authors suggest that if one considers the mitochondria as a large network in a mammalian cell, the global operation of UPR^{mt} would drive the import of chaperons and proteases to improve the general health of the mitochondria. In parallel, those import-compromised mitochondria would be degraded via mitophagy. As for mitochondria with diminished import

function, they could potentially be rescued by the activation of UPR^{mt} to avoid mitophagy. Nevertheless, it remains to be further investigated how these two mechanisms are co-regulated. In this section, we will highlight key events that could be considered important triggers of PD in association with both UPR^{mt} and mitophagy.

5.6.1 The Role of UPR^{mt} in Parkinson's Disease

The association of UPR^{mt} and PD was identified in a link between the accumulation of α-syn in the mitochondria and the reduction in mitochondrial protein import. A study by Devi et al. [152] identified a possible MTS within the human α-syn protein sequence, suggesting the potential import of α-syn into the mitochondrial matrix. Furthermore, the authors also observed the increased amount of α-syn in the mitochondrial fractions obtained from the postmortem SNpc region of PD patients. The accumulation of α-syn in the mitochondria could lead to its interaction and inhibition of the function of complex I, resulting in the induction of UPR^{mt} (Fig. 5.4) [51, 55]. Moreover, Ludtmann et al. [153] also demonstrated that α-syn could interact with complex V (ATP synthase) of the mitochondria, leading to changes in the mitochondrial morphology. The interaction between α-syn and complex V will eventually cause cell death by the opening of the mitochondrial permeability transition pore. In addition, a study by Di Maio et al. [154] revealed that posttranslationally modified species of α-syn are able to impair the mitochondrial protein import system, subsequently causing mitochondrial dysfunction. The impairment of the mitochondrial protein import system is mainly caused by the disruption to Tom20-Tom22 interaction due to the binding of α-syn to the MTS receptor site in Tom20 (Fig. 5.4). The interaction of α-syn to the MTS receptor site in Tom20 is further strengthened by the identification of the MTS in α-syn protein sequence highlighted previously. However, more research would need to further understand how α-syn could influence UPR^{mt} and lead to the onset of PD.

Further association of PD to UPR^{mt} lies in the substantial decrease in levels of mortalin observed from the analysis of mitochondria derived from the SN region of PD patients as compared to age-matched healthy patients [155]. A number of studies have identified genetic variations to the gene encoding mortalin in a Spanish and German cohort, although it is only a handful of patients harboring these mortalin variants with pathogenesis to PD [156, 157]. In the German cohort study, Burbulla et al. [157] went on to identify a A476T variant of mortalin as a potential risk factor for PD, where its overexpression would lead to increased ROS levels caused by the induction of proteolytic stress. Moreover, the authors demonstrated that the mitochondrial dysfunction induced by the KD of mortalin could only be rescued by wild-type mortalin, but not PD-associated variants of the mortalin, in human embryonic kidney 293 (HEK293) cells. Furthermore, it was shown that these mortalin variants in yeast could also cause mitochondrial dysfunction, particularly perturbations to mitochondrial proteostasis [158]. This association of mutant mortalin and mitochondrial dysfunction further suggests that mitochondrial dysfunction is a risk factor for

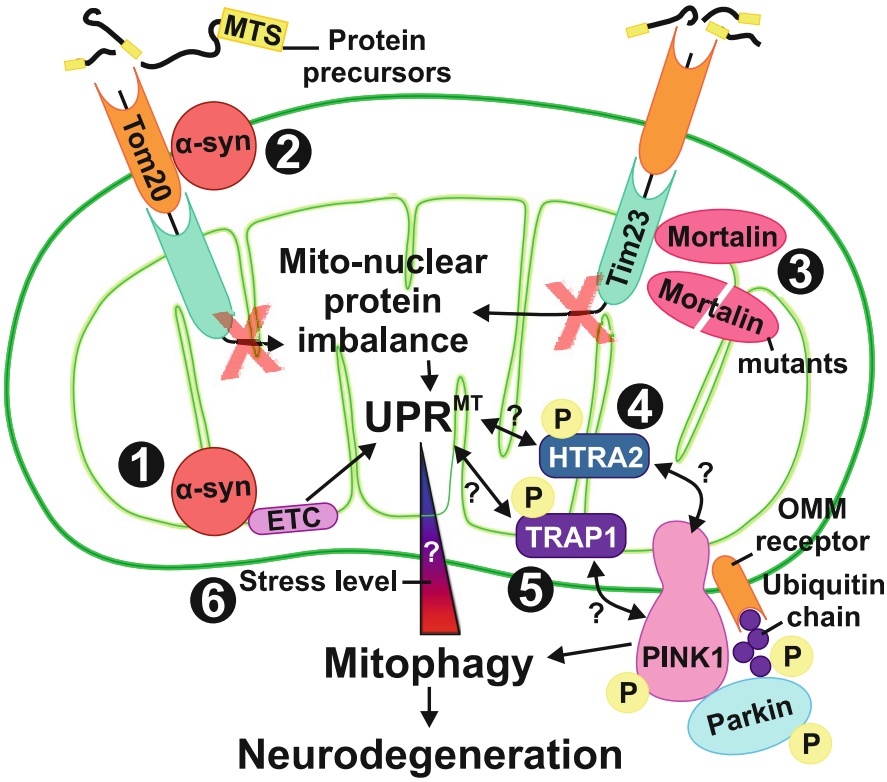

Fig. 5.4 The implications of mitochondrial unfolded protein response (UPR^{mt}) and mitophagy in Parkinson's disease (PD). (1) Alpha-synuclein (α-syn), one of the hallmarks of PD, can bind to components of the electron transport chain (ETC). The binding will perturb the ETC function and induce UPR^{mt}. (2) α-Syn can interact with the translocase of the outer membrane 20 (Tom20) and affect the mitochondrial protein import. The perturbation of the mitochondrial protein import can induce a mito-nuclear protein imbalance and trigger the UPR^{mt}. (3) The mutant mortalin, identified from PD patients, is not able to bind to the translocase of the inner membrane 23 (Tim23) as a chaperon to aid in the import of protein precursors. The absence of mortalin binding to Tim23 can affect mitochondrial import and imbalance on the mito-nuclear protein to trigger UPR^{mt}. (4) High-temperature requirement protein A2 (HTRA2), a protease in the mitochondria, can associate with the protein PINK1. The mutation to HTRA2 can lead to similar mitochondrial dysfunction as PINK1/Parkin deficiency. (5) Tumor necrosis factor receptor-associated protein 1 (TRAP1), another chaperon, can also associate similarly with PINK1/Parkin as with HTRA2. However, the exact mechanism of both TRAP1 and HTRA2 association with PINK1/Parkin and their roles in UPR^{mt} in PD patients remain unknown. (6) It has been suggested that UPR^{mt} is an initial stress response mechanism to protect the function of the mitochondria. However, if the perturbation to the mitochondria reaches a critical point beyond repair, mitophagy will be triggered to remove the "unhealthy" mitochondria and prevent widespread mitochondrial dysfunction. Subsequently, if there are extensive mitochondrial dysfunction throughout the neuron, neuronal death might be triggered, leading to neurodegeneration. MTS: mitochondrial target sequence; OMM: outer mitochondrial membrane

PD (Fig. 5.4). Mortalin has also been found to interact with DJ-1 [157, 159], a protein associated with PD, α-syn [159], and Parkin [160]. Interestingly, studies have shown that mitochondrial dysfunction induced from the depletion of mortalin can be rescued by the overexpression of PINK1 and Parkin [151, 161]. Despite all these findings, the number of PD patients harboring the genetic variants of mortalin remains low [162, 163], suggesting that mortalin might not be a main contributor to the pathogenesis of PD.

The chaperon TRAP1, another member of UPR^{mt}, was found to associate with PINK1 in vivo, potentially linking UPR^{mt} to PD. To this end, Costa et al. [164] generated a TRAP1-deficient *Drosophila* model and identified similar features of mitochondrial dysfunction in PINK1- and Parkin-deficient *Drosophila*. The increased expression of PINK1 and Parkin in the neurons was able to rescue the mitochondrial dysfunction by TRAP1 deficiency. However, TRAP1 expression is only able to partially rescue mitochondrial dysfunction in Parkin mutant flies. Conversely, Zhang et al. [165] showed that TRAP1 is able to rescue the mitochondrial dysfunction in SH-SY5Y neuroblastoma cells after depletion of PINK1, but did not show any effect on the mitochondrial dysfunction induced by depletion of Parkin. Data from these studies seem to point out that TRAP1 has a role in the PINK1-Parkin pathway that seeks to maintain the population of "healthy" mitochondria, and PINK1 alterations affect TRAP1 function [166].

Similar to TRAP1, the phosphorylation of UPR^{mt} serine protease HTRA2 was found to increase in a PINK1-dependent manner [167]. In addition, Tain et al. [168] used a *Drosophila* model and demonstrated that HTRA2 mutant flies have similar locomotor phenotypes that correlated with mitochondrial dysfunction as compared to that in both the PINK1 and Parkin mutant flies. Moreover, HTRA2 expression is able to rescue locomotor deficits induced by PINK1 deficiency. In contrast, Whitworth et al. [169] failed to repeat the rescue effect of HTRA2 in Parkin mutant flies. Collectively, these studies identified the potential association of TRAP1-, HTRA2-, PINK1-, and Parkin-associated stress pathways (Fig. 5.4). It would be interesting to investigate how exactly the UPR^{mt} process could be affected in PD. Moreover, since the PINK1/Parkin pathway is also implicated in the process of mitophagy, the research summarized above also suggests the need to further understand the potential reciprocal regulation of UPR^{mt} and mitophagy in maintaining the health of the mitochondria.

5.6.2 The Role of Mitophagy in Parkinson's Disease

Defects in mitophagy have been identified in many neurodegenerative diseases, including AD, Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and also PD, making the aberrant accumulation of damaged mitochondria a hallmark of major neurological pathologies [98, 136]. Since the accumulation of damaged mitochondria is associated with the development of neuronal health, understanding the pathological relationship

between mitophagy defects and neurodegenerative disease is of great importance. Unfortunately, the mechanism of how mitophagy plays a role in PD remains elusive.

As with UPR^{mt}, two PD-associated proteins, PINK1 and Parkin, have been strongly linked to damaged mitochondria tagged for degradation via mitophagy. There have been increasing evidences of a decline in mitophagy during aging, leading to a decline in the clearance of dysfunctional mitochondria and the accumulation of dysfunctional mitochondria during the development of neurodegenerative diseases [170–173]. As highlighted previously, the accumulation of α-syn in PD can make SN neurons vulnerable with the increase in mitochondrial dysfunction. Therefore, the decline in mitophagy in PD would reduce the clearance of "unhealthy" mitochondria, eventually reaching a critical point that would trigger neuronal cell death [174]. Very recently, Moskal et al. designed a high-throughput method to identify small molecules that increase Parkin recruitment to damaged mitochondria upon mitophagy induction. The approach led to the discovery of several rho-associated, coiled-coil-containing protein kinase (ROCK) inhibitors, which promote Parkin-mediated mitophagy via recruiting hexokinase 2 (HK2) to mitochondria. Among them, the molecule SR3677 has been demonstrated to improve the longevity and climbing ability of PD flies challenged with parkinsonian toxin paraquat, suggesting its neuroprotective effects [175].

As highlighted previously, VPS35 deficiency in dopamine (DA) neurons could lead to impaired mitochondrial dynamics. In physiology, VPS35 promotes the degradation of MUL1 which leads to the stabilization of MFN2. The deficiency of VPS35 affects the regulation of the mitochondrial dynamics, leading to mitochondrial dysfunction, potentially underlying the pathogenesis of PD [47]. MUL1 was also reported to be involved in the induction of mitophagy, but its importance in the mitophagy in PD remains to be investigated.

Indeed, the importance of mitophagy in neurons has been highlighted in several studies of mitophagy inducers which have been shown to have promising effects in prolonging health span and protecting neurons in animal models [137, 176–182]. For example, urolithin A (UA) and antibiotic actinonin (AC) are two natural compounds that have been reported to induce mitophagy in *C. elegans* and mouse models [176–178]. Treating either UA or AC on AD mouse or *C. elegans* model showed restored neuronal mitophagy and ameliorated cognitive decline. Of note, UA and AC can elevate the level of mitophagy-related proteins, including PINK1, Parkin, beclin-1, Bcl2L13, AMBRA1, and phospho-unc-51 like autophagy activating kinase 1 (ULK1), indicating a robust induction of mitophagy [178]. UA is a metabolite derived from ellagitannins. Interestingly, in a rotenone-induced PD rat model, the treatment of ellagitannin-rich pomegranate extract was reported to prevent PD-like features and enhance the activity of mitochondrial aldehyde dehydrogenase 2 (ALDH2) in the midbrain homogenate, which can protect cells against oxidative stress. UA was also detected in the brain and plasma after the intake of pomegranate juice [179]. Of note, a recent phase I clinical trial showed UA have a favorable safety profile and induced a molecular signature of improved mitochondrial health [180]. The above results suggest UA is a promising compound as a potential PD therapy. In addition to UA and AC, other mitophagy inducers such as

NAD^+ precursors, including nicotinamide, nicotinamide mononucleotide, and nicotinamide riboside, have also been reported to bring benefits to ameliorate PD phenotypes [137, 178, 181, 182]. The underling mechanism has been discussed by Fang [183].

Collectively, mitophagy has been shown to be an important process in the protection against PD. However, the underlying processes that lead to the loss of mitophagy in PD remain elusive; thus, more studies may help better understand the role of mitophagy in the onset of PD. In addition, further exploration into more mitophagy inducers could lead to potential therapies for PD patients.

5.7 Conclusion and Future Perspectives

Mitochondria are important organelles in mammalian cells. The presence of mitochondrial dysfunction as highlighted in this chapter has been implicated in many diseases including neurodegenerative diseases like PD. Mitochondria have also been associated with the mitochondrial free radical theory of aging. Thus, ensuring that the population of mitochondria remains healthy is an important aspect for mammalian cells to function and survive. Indeed, as summarized in this chapter, we have discussed about the intricate and complex mito-protective pathways that have been evolutionarily placed in the mitochondria for the safekeeping of its function. Of which the UPR^{mt} and mitophagy are present to response to the varying degrees of stress the mitochondria are undergoing. These mitochondrial quality control processes allow the mammalian cells to take precautionary measures to ensure that the accumulation of "unhealthy" mitochondria does not occur. A perturbation to any of these mitochondrial quality control processes would have grave implications such as the triggering of apoptotic death for the cell.

Therefore, it is not a surprise that the loss of these mitochondrial quality control processes would lead to undesirable consequences, such as the onset of neurodegenerative diseases. However, we are still far from understanding how the mitochondrial quality control processes, such as UPR^{mt} and mitophagy, could have contributed to PD. Herein, we have encapsulated that UPR^{mt} and mitophagy do share similar processes such as the PINK/Parkin pathway. However, whether both UPR^{mt} and mitophagy have any crosstalk with each other or how they could co-regulate still requires further investigations. Indeed, despite the decades of research being conducted in understanding the pathogenesis of PD, our increased understanding of PD did not translate to any viable therapeutic approaches. Even when understanding that the end point of PD is the induction of neuronal death, the use of anti-apoptotic drugs is unsuccessful in clinical trials against neurodegenerative diseases [184]. Henceforth, it seems that the use of neuroprotective therapy to slow down or prevent PD is a better alternative. Nevertheless, we are still far from understanding how the process of mitochondrial quality control could be administrated as a neuroprotective treatment.

In this chapter, we have highlighted that the processes of mitophagy and UPR^{mt} are perceived to be closely linked. It has been suggested that the UPR^{mt} is the

immediate repair mechanism for the cell to attempt to mitigate the low stress-induced perturbations of the mitochondria, while mitophagy is considered as the later stage of "unhealthy" mitochondrial removal to prevent the onset of cellular death (Fig. 5.4; reviewed in [185]). Therefore, elucidating the co-regulation of these two pathways could improve our knowledge on the mechanism of mitochondrial stress sensors. It could also determine how the neurons track the health status of the mitochondria to determine its fate and to prevent the potential triggering of cell death. With the improved details in the early developments of PD, potential therapeutic targets can be identified.

References

1. Sagan L. On the origin of mitosing cells. J Theor Biol. 1967;14(3):255–74. https://doi.org/10.1016/0022-5193(67)90079-3.
2. Aiello LC, Wheeler P. The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. Curr Anthropol. 1995;36(2):199–221.
3. Hyder F, Rothman DL, Bennett MR. Cortical energy demands of signaling and nonsignaling components in brain are conserved across mammalian species and activity levels. Proc Natl Acad Sci. 2013;110(9):3549. https://doi.org/10.1073/pnas.1214912110.
4. Sheng ZH, Cai Q. Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. Nat Rev Neurosci. 2012;13(2):77–93. https://doi.org/10.1038/nrn3156.
5. Cai Q, Tammineni P. Mitochondrial aspects of synaptic dysfunction in Alzheimer's disease. J Alzheimers Dis. 2017;57(4):1087–103. https://doi.org/10.3233/jad-160726.
6. Devine MJ, Kittler JT. Mitochondria at the neuronal presynapse in health and disease. Nat Rev Neurosci. 2018;19(2):63–80. https://doi.org/10.1038/nrn.2017.170.
7. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol. 1956;11(3):298–300. https://doi.org/10.1093/geronj/11.3.298.
8. Kishida KT, Klann E. Sources and targets of reactive oxygen species in synaptic plasticity and memory. Antioxid Redox Signal. 2007;9(2):233–44. https://doi.org/10.1089/ars.2007.9.ft-8.
9. Veal EA, Day AM, Morgan BA. Hydrogen peroxide sensing and signaling. Mol Cell. 2007;26(1):1–14. https://doi.org/10.1016/j.molcel.2007.03.016.
10. Casetta I, Govoni V, Granieri E. Oxidative stress, antioxidants and neurodegenerative diseases. Curr Pharm Des. 2005;11(16):2033–52.
11. Weng M, Xie X, Liu C, Lim KL, Zhang CW, Li L. The sources of reactive oxygen species and its possible role in the pathogenesis of Parkinson's disease. Parkinsons Dis. 2018;2018:9163040. https://doi.org/10.1155/2018/9163040.
12. Wu Y, Chen M, Jiang J. Mitochondrial dysfunction in neurodegenerative diseases and drug targets via apoptotic signaling. Mitochondrion. 2019;49:35–45. https://doi.org/10.1016/j.mito.2019.07.003.
13. Filograna R, Beltramini M, Bubacco L, Bisaglia M. Anti-oxidants in Parkinson's disease therapy: a critical point of view. Curr Neuropharmacol. 2016;14(3):260–71. https://doi.org/10.2174/1570159x13666151030102718.
14. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Overview of neuron structure and function. Molecular cell biology. 4th ed. New York: W. H. Freeman; 2000.
15. Schönfeld P, Reiser G. Why does brain metabolism not favor burning of fatty acids to provide energy? Reflections on disadvantages of the use of free fatty acids as fuel for brain. J Cereb Blood Flow Metab. 2013;33(10):1493–9. https://doi.org/10.1038/jcbfm.2013.128.
16. Reifschneider NH, Goto S, Nakamoto H, Takahashi R, Sugawa M, Dencher NA, et al. Defining the mitochondrial proteomes from five rat organs in a physiologically significant

context using 2D blue-native/SDS-PAGE. J Proteome Res. 2006;5(5):1117–32. https://doi.org/10.1021/pr0504440.
17. Kowald A, Kirkwood TB. Accumulation of defective mitochondria through delayed degradation of damaged organelles and its possible role in the ageing of post-mitotic and dividing cells. J Theor Biol. 2000;202(2):145–60. https://doi.org/10.1006/jtbi.1999.1046.
18. Terman A, Kurz T, Navratil M, Arriaga EA, Brunk UT. Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis theory of aging. Antioxid Redox Signal. 2010;12(4):503–35. https://doi.org/10.1089/ars.2009.2598.
19. Obashi K, Okabe S. Regulation of mitochondrial dynamics and distribution by synapse position and neuronal activity in the axon. Eur J Neurosci. 2013;38(3):2350–63. https://doi.org/10.1111/ejn.12263.
20. Lin MY, Sheng ZH. Regulation of mitochondrial transport in neurons. Exp Cell Res. 2015;334(1):35–44. https://doi.org/10.1016/j.yexcr.2015.01.004.
21. Borrás C, Sastre J, García-Sala D, Lloret A, Pallardó FV, Viña J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic Biol Med. 2003;34(5):546–52. https://doi.org/10.1016/s0891-5849(02)01356-4.
22. Borrás C, Gambini J, López-Grueso R, Pallardó FV, Viña J. Direct antioxidant and protective effect of estradiol on isolated mitochondria. Biochim Biophys Acta. 2010;1802(1):205–11. https://doi.org/10.1016/j.bbadis.2009.09.007.
23. Lores-Arnaiz S, Bustamante J. Age-related alterations in mitochondrial physiological parameters and nitric oxide production in synaptic and non-synaptic brain cortex mitochondria. Neuroscience. 2011;188:117–24. https://doi.org/10.1016/j.neuroscience.2011.04.060.
24. Lores-Arnaiz S, Lombardi P, Karadayian AG, Orgambide F, Cicerchia D, Bustamante J. Brain cortex mitochondrial bioenergetics in synaptosomes and non-synaptic mitochondria during aging. Neurochem Res. 2016;41(1-2):353–63. https://doi.org/10.1007/s11064-015-1817-5.
25. Reeve AK, Grady JP, Cosgrave EM, Bennison E, Chen C, Hepplewhite PD, et al. Mitochondrial dysfunction within the synapses of substantia nigra neurons in Parkinson's disease. Parkinson's Dis. 2018;4(1):9. https://doi.org/10.1038/s41531-018-0044-6.
26. Lebouvier T, Chaumette T, Paillusson S, Duyckaerts C, Bruley d, Varannes S, Neunlist M, et al. The second brain and Parkinson's disease. Eur J Neurosci. 2009;30(5):735–41. https://doi.org/10.1111/j.1460-9568.2009.06873.x.
27. Chen C, Turnbull DM, Reeve AK. Mitochondrial dysfunction in Parkinson's disease-cause or consequence? Biology. 2019;8(2):38. https://doi.org/10.3390/biology8020038.
28. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science. 1983;219(4587):979–80.
29. Schapira A, Cooper J, Dexter D, Clark J, Jenner P, Marsden C. Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem. 1990;54(3):823–7.
30. Parker WD Jr, Boyson SJ, Parks JK. Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann Neurol. 1989;26(6):719–23.
31. Parker WD Jr, Parks JK, Swerdlow RH. Complex I deficiency in Parkinson's disease frontal cortex. Brain Res. 2008;1189:215–8.
32. Krige D, Carroll MT, Cooper JM, Marsden CD, Schapira AH. RKQPDR. Platelet mitochondria function in Parkinson's disease. Ann Neurol. 1992;32(6):782–8.
33. Blin O, Desnuelle C, Rascol O, Borg M, Saint Paul HP, Azulay J, et al. Mitochondrial respiratory failure in skeletal muscle from patients with Parkinson's disease and multiple system atrophy. J Neurol Sci. 1994;125(1):95–101.
34. Haas RH, Nasirian F, Nakano K, Ward D, Pay M, Hill R, et al. Low platelet mitochondrial complex I and complex II/III activity in early untreated Parkinson's disease. Ann Neurol. 1995;37(6):714–22.
35. Swerdlow RH, Parks JK, Miller SW, Davis RE, Tuttle JB, Trimmer PA, et al. Origin and functional consequences of the complex I defect in Parkinson's disease. Ann Neurol. 1996;40(4):663–71.

36. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet. 2006;38(5):515–7.
37. Kraytsberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW, Khrapko K. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. Nat Genet. 2006;38(5):518–20.
38. Luoma P, Melberg A, Rinne JO, Kaukonen JA, Nupponen NN, Chalmers RM, et al. Parkinsonism, premature menopause, and mitochondrial DNA polymerase γ mutations: clinical and molecular genetic study. Lancet. 2004;364(9437):875–82.
39. Davidzon G, Greene P, Mancuso M, Klos KJ, Ahlskog JE, Hirano M, et al. Early-onset familial parkinsonism due to POLG mutations. Ann Neurol. 2006;59(5):859–62.
40. Perier C, Bender A, García-Arumí E, Melia MJ, Bove J, Laub C, et al. Accumulation of mitochondrial DNA deletions within dopaminergic neurons triggers neuroprotective mechanisms. Brain. 2013;136(8):2369–78.
41. Dai Y, Clark J, Zheng K, Kujoth GC, Prolla TA, Simon DK. Somatic mitochondrial DNA mutations do not increase neuronal vulnerability to MPTP in young POLG mutator mice. Neurotoxicol Teratol. 2014;46:62–7.
42. Ekstrand MI, Terzioglu M, Galter D, Zhu S, Hofstetter C, Lindqvist E, et al. Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Proc Natl Acad Sci. 2007;104(4):1325–30.
43. Hsieh CH, Shaltouki A, Gonzalez AE, Bettencourt da Cruz A, Burbulla LF, St Lawrence E, et al. Functional impairment in miro degradation and mitophagy is a shared feature in familial and sporadic Parkinson's disease. Cell Stem Cell. 2016;19(6):709–24. https://doi.org/10.1016/j.stem.2016.08.002.
44. Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum Mol Genet. 2011;20(1):40–50. https://doi.org/10.1093/hmg/ddq430.
45. Wang X, Petrie TG, Liu Y, Liu J, Fujioka H, Zhu X. Parkinson's disease-associated DJ-1 mutations impair mitochondrial dynamics and cause mitochondrial dysfunction. J Neurochem. 2012;121(5):830–9. https://doi.org/10.1111/j.1471-4159.2012.07734.x.
46. McCoy MK, Cookson MR. DJ-1 regulation of mitochondrial function and autophagy through oxidative stress. Autophagy. 2011;7(5):531–2. https://doi.org/10.4161/auto.7.5.14684.
47. Tang FL, Liu W, Hu JX, Erion JR, Ye J, Mei L, et al. VPS35 deficiency or mutation causes dopaminergic neuronal loss by impairing mitochondrial fusion and function. Cell Rep. 2015;12(10):1631–43. https://doi.org/10.1016/j.celrep.2015.08.001.
48. Schulz AM, Haynes CM. UPRmt-mediated cytoprotection and organismal aging. Biochim Biophys Acta. 2015;1847(11):1448–56.
49. Durieux J, Wolff S, Dillin A. The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell. 2011;144(1):79–91.
50. Haynes CM, Yang Y, Blais SP, Neubert TA, Ron D. The matrix peptide exporter HAF-1 signals a mitochondrial UPR by activating the transcription factor ZC376. 7 in C. elegans. Mol Cell. 2010;37(4):529–40.
51. Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsyuba E, Knott G, et al. Mitonuclear protein imbalance as a conserved longevity mechanism. Nature. 2013;497(7450):451–7.
52. Liu Y, Samuel BS, Breen PC, Ruvkun G. Caenorhabditis elegans pathways that surveil and defend mitochondria. Nature. 2014;508(7496):406–10.
53. Martinus RD, Garth GP, Webster TL, Cartwright P, Naylor DJ, Høj PB, et al. Selective induction of mitochondrial chaperones in response to loss of the mitochondrial genome. Eur J Biochem. 1996;240(1):98–103.
54. Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science. 2012;337(6094):587–90.

55. Yoneda T, Benedetti C, Urano F, Clark SG, Harding HP, Ron D. Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. J Cell Sci. 2004;117(18):4055–66.
56. Zhao Q, Wang J, Levichkin IV, Stasinopoulos S, Ryan MT, Hoogenraad NJ. A mitochondrial specific stress response in mammalian cells. EMBO J. 2002;21(17):4411–9.
57. Pellegrino MW, Nargund AM, Kirienko NV, Gillis R, Fiorese CJ, Haynes CM. Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. Nature. 2014;516 (7531):414–7.
58. Lin Y-F, Haynes CM. Metabolism and the UPRmt. Mol Cell. 2016;61(5):677–82.
59. Shpilka T, Haynes CM. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. Nat Rev Mol Cell Biol. 2018;19(2):109–20. https://doi.org/10.1038/nrm.2017.110.
60. 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(5806):457–65. https://doi.org/10.1038/290457a0.
61. Young JC, Hoogenraad NJ, Hartl FU. Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70. Cell. 2003;112(1):41–50.
62. Pfanner N. Protein sorting: recognizing mitochondrial presequences. Curr Biol. 2000;10(11): R412–5.
63. Model K, Meisinger C, Kühlbrandt W. Cryo-electron microscopy structure of a yeast mitochondrial preprotein translocase. J Mol Biol. 2008;383(5):1049–57.
64. Bausewein T, Mills DJ, Langer JD, Nitschke B, Nussberger S, Kühlbrandt W. Cryo-EM structure of the TOM core complex from Neurospora crassa. Cell. 2017;170(4):693–700.
65. Mokranjac D, Neupert W. The many faces of the mitochondrial TIM23 complex. Biochim Biophys Acta. 2010;1797(6-7):1045–54.
66. Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Ulrich Hartl F. Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem. 2013;82:323–55.
67. Deocaris CC, Kaul SC, Wadhwa R. On the brotherhood of the mitochondrial chaperones mortalin and heat shock protein 60. Cell Stress Chaperones. 2006;11(2):116.
68. Walter S. Structure and function of the GroE chaperone. Cell Mol Life Sci. 2002;59 (10):1589–97.
69. Okamoto T, Yamamoto H, Kudo I, Matsumoto K, Odaka M, Grave E, et al. HSP60 possesses a GTPase activity and mediates protein folding with HSP10. Sci Rep. 2017;7(1):1–11.
70. Song HY, Dunbar JD, Zhang YX, Guo D, Donner DB. Identification of a protein with homology to hsp90 that binds the type 1 tumor necrosis factor receptor. J Biol Chem. 1995;270(8):3574–81.
71. Gerdes F, Tatsuta T, Langer T. Mitochondrial AAA proteases—towards a molecular understanding of membrane-bound proteolytic machines. Cell Res. 2012;1823(1):49–55.
72. Haynes CM, Petrova K, Benedetti C, Yang Y, Ron D. ClpP mediates activation of a mitochondrial unfolded protein response in C. elegans. Dev Cell. 2007;13(4):467–80.
73. Quirós PM, Español Y, Acín-Pérez R, Rodríguez F, Bárcena C, Watanabe K, et al. ATP-dependent Lon protease controls tumor bioenergetics by reprogramming mitochondrial activity. Cell Rep. 2014;8(2):542–56.
74. Clausen T, Kaiser M, Huber R, Ehrmann M. HTRA proteases: regulated proteolysis in protein quality control. Nat Rev Mol Cell Biol. 2011;12(3):152–62.
75. Quirós PM, Langer T, Lopez-Otin C. New roles for mitochondrial proteases in health, ageing and disease. Nat Rev. 2015;16(6):345–59.
76. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell. 2003;11 (3):619–33.
77. Teske BF, Fusakio ME, Zhou D, Shan J, McClintick JN, Kilberg MS, et al. CHOP induces activating transcription factor 5 (ATF5) to trigger apoptosis in response to perturbations in protein homeostasis. Mol Biol Cell. 2013;24(15):2477–90.

78. Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. The integrated stress response. EMBO Rep. 2016;17(10):1374–95.
79. Dey S, Baird TD, Zhou D, Palam LR, Spandau DF, Wek RC. Both transcriptional regulation and translational control of ATF4 are central to the integrated stress response. J Biol Chem. 2010;285(43):33165–74.
80. Kim KH, Jeong YT, Oh H, Kim SH, Cho JM, Kim Y-N, et al. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. Nat Med. 2013;19(1):83.
81. Chung HK, Ryu D, Kim KS, Chang JY, Kim YK, Yi H-S, et al. Growth differentiation factor 15 is a myomitokine governing systemic energy homeostasis. J Cell Biol. 2017;216 (1):149–65.
82. Münch C, Harper JW. Mitochondrial unfolded protein response controls matrix pre-RNA processing and translation. Nature. 2016;534(7609):710–3.
83. Aldridge JE, Horibe T, Hoogenraad NJ. Discovery of genes activated by the mitochondrial unfolded protein response (mtUPR) and cognate promoter elements. PLoS One. 2007;2:9.
84. Fiorese CJ, Schulz AM, Lin Y-F, Rosin N, Pellegrino MW, Haynes CM. The transcription factor ATF5 mediates a mammalian mitochondrial UPR. Curr Biol. 2016;26(15):2037–43.
85. Horibe T, Hoogenraad NJ. The chop gene contains an element for the positive regulation of the mitochondrial unfolded protein response. PLoS One. 2007;2:9.
86. Quirós PM, Prado MA, Zamboni N, D'Amico D, Williams RW, Finley D, et al. Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. J Cell Biol. 2017;216(7):2027–45.
87. Bao XR, Ong S-E, Goldberger O, Peng J, Sharma R, Thompson DA, et al. Mitochondrial dysfunction remodels one-carbon metabolism in human cells. elife. 2016;5:e10575.
88. Mishra P, Chan DC. Mitochondrial dynamics and inheritance during cell division, development and disease. Nat Rev. 2014;15(10):634–46.
89. Zhou D, Palam LR, Jiang L, Narasimhan J, Staschke KA, Wek RC. Phosphorylation of eIF2 directs ATF5 translational control in response to diverse stress conditions. J Biol Chem. 2008;283(11):7064–73.
90. Fusakio ME, Willy JA, Wang Y, Mirek ET, Al Baghdadi RJ, Adams CM, et al. Transcription factor ATF4 directs basal and stress-induced gene expression in the unfolded protein response and cholesterol metabolism in the liver. Mol Biol Cell. 2016;27(9):1536–51.
91. Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, et al. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev. 2004;18(24):3066–77.
92. Ashford TP, Porter KR. Cytoplasmic components in hepatic cell lysosomes. J Cell Biol. 1962;12(1):198–202. https://doi.org/10.1083/jcb.12.1.198.
93. De Duve C, Wattiaux R. Functions of lysosomes. Annu Rev Physiol. 1966;28:435–92.
94. Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Rejuvenation Res. 2005;8(1):3–5.
95. Babbar M, Basu S, Yang B, Croteau DL, Bohr VA. Mitophagy and DNA damage signaling in human aging. Mech Ageing Dev. 2020;186:111207. https://doi.org/10.1016/j.mad.2020.111207.
96. Pickles S, Vigié P, Youle RJ. Mitophagy and quality control mechanisms in mitochondrial maintenance. Curr Biol. 2018;28(4):R170–R85. https://doi.org/10.1016/j.cub.2018.01.004.
97. Palikaras K, Lionaki E, Tavernarakis N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat Cell Biol. 2018;20(9):1013–22. https://doi.org/10.1038/s41556-018-0176-2.
98. Cai Q, Jeong YY. Mitophagy in Alzheimer's disease and other age-related neurodegenerative diseases. Cell. 2020;9(1):150. https://doi.org/10.3390/cells9010150.
99. Dombi E, Mortiboys H, Poulton J. Modulating mitophagy in mitochondrial disease. Curr Med Chem. 2018;25(40):5597–612. https://doi.org/10.2174/0929867324666170616101741.

100. Evans CS, Holzbaur ELF. Quality control in neurons: mitophagy and other selective autophagy mechanisms. J Mol Biol. 2020;432(1):240–60. https://doi.org/10.1016/j.jmb.2019.06.031.
101. Rasool S, Trempe J-F. New insights into the structure of PINK1 and the mechanism of ubiquitin phosphorylation. Crit Rev Biochem Mol Biol. 2018;53(5):515–34. https://doi.org/10.1080/10409238.2018.1491525.
102. Schubert AF, Gladkova C, Pardon E, Wagstaff JL, Freund SMV, Steyaert J, et al. Structure of PINK1 in complex with its substrate ubiquitin. Nature. 2017;552(7683):51–6. https://doi.org/10.1038/nature24645.
103. Sekine S. PINK1 import regulation at a crossroad of mitochondrial fate: the molecular mechanisms of PINK1 import. J Biochem. 2019; https://doi.org/10.1093/jb/mvz069.
104. Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J Cell Biol. 2010;191(5):933–42. https://doi.org/10.1083/jcb.201008084.
105. Greene AW, Grenier K, Aguileta MA, Muise S, Farazifard R, Haque ME, et al. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. EMBO Rep. 2012;13(4):378–85. https://doi.org/10.1038/embor.2012.14.
106. Sekine S, Youle RJ. PINK1 import regulation; a fine system to convey mitochondrial stress to the cytosol. BMC Biol. 2018;16(1):2. https://doi.org/10.1186/s12915-017-0470-7.
107. Yamano K, Youle RJ. PINK1 is degraded through the N-end rule pathway. Autophagy. 2013;9(11):1758–69. https://doi.org/10.4161/auto.24633.
108. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol. 2010;189(2):211–21. https://doi.org/10.1083/jcb.200910140.
109. Narendra DP, Jin SM, Tanaka A, Suen D-F, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. 2010;8(1):e1000298. https://doi.org/10.1371/journal.pbio.1000298.
110. Rasool S, Soya N, Truong L, Croteau N, Lukacs GL, Trempe J-F. PINK1 autophosphorylation is required for ubiquitin recognition. EMBO Rep. 2018;19:4. https://doi.org/10.15252/embr.201744981.
111. Shiba-Fukushima K, Imai Y, Yoshida S, Ishihama Y, Kanao T, Sato S, et al. PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. Sci Rep. 2012;2:1002. https://doi.org/10.1038/srep01002.
112. Durcan TM, Fon EA. The three P's of mitophagy: PARKIN, PINK1, and post-translational modifications. Genes Dev. 2015;29(10):989–99. https://doi.org/10.1101/gad.262758.115.
113. Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, et al. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. J Cell Biol. 2014;205(2):143–53. https://doi.org/10.1083/jcb.201402104.
114. Kazlauskaite A, Kondapalli C, Gourlay R, Campbell David G, Ritorto Maria S, Hofmann K, et al. Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. Biochem J. 2014;460(1):127–41. https://doi.org/10.1042/bj20140334.
115. Kazlauskaite A, Kelly V, Johnson C, Baillie C, Hastie CJ, Peggie M, et al. Phosphorylation of Parkin at Serine65 is essential for activation: elaboration of a Miro1 substrate-based assay of Parkin E3 ligase activity. Open Biol. 2014;4:130213. https://doi.org/10.1098/rsob.130213.
116. Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, et al. Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature. 2014;510(7503):162–6. https://doi.org/10.1038/nature13392.
117. Okatsu K, Koyano F, Kimura M, Kosako H, Saeki Y, Tanaka K, et al. Phosphorylated ubiquitin chain is the genuine Parkin receptor. J Cell Biol. 2015;209(1):111–28. https://doi.org/10.1083/jcb.201410050.

118. Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol. 2010;12(2):119–31. https://doi.org/10.1038/ncb2012.
119. Ziviani E, Tao RN, Whitworth AJ. Drosophila Parkin requires PINK1 for mitochondrial translocation and ubiquitinates. Mitofusin. 2010;107(11):5018–23. https://doi.org/10.1073/pnas.0913485107.
120. Wang X, Winter D, Ashrafi G, Schlehe J, Selkoe D, et al. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. Cell. 2011;147(4):893–906. https://doi.org/10.1016/j.cell.2011.10.018.
121. Ordureau A, Sarraf Shireen A, Duda David M, Heo J-M, Jedrychowski Mark P, Sviderskiy Vladislav O, et al. Quantitative proteomics reveal a feedforward mechanism for mitochondrial parkin translocation and ubiquitin chain synthesis. Mol Cell. 2014;56(3):360–75. https://doi.org/10.1016/j.molcel.2014.09.007.
122. Xiao B, Goh JY, Xiao L, Xian H, Lim KL, Liou YC. Reactive oxygen species trigger Parkin/PINK1 pathway-dependent mitophagy by inducing mitochondrial recruitment of Parkin. J Biol Chem. 2017;292(40):16697–708. https://doi.org/10.1074/jbc.M117.787739.
123. Narendra D, Tanaka A, Suen D-F, Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol. 2008;183(5):795–803. https://doi.org/10.1083/jcb.200809125.
124. Ding WX, Ni HM, Li M, Liao Y, Chen X, Stolz DB, et al. Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming. J Biol Chem. 2010;285(36):27879–90. https://doi.org/10.1074/jbc.M110.119537.
125. Joselin AP, Hewitt SJ, Callaghan SM, Kim RH, Chung Y-H, Mak TW, et al. ROS-dependent regulation of Parkin and DJ-1 localization during oxidative stress in neurons. Hum Mol Genet. 2012;21(22):4888–903.
126. Xian H, Liou Y-C. Loss of MIEF1/MiD51 confers susceptibility to BAX-mediated cell death and PINK1-PRKN-dependent mitophagy. Autophagy. 2019;15(12):2107–25. https://doi.org/10.1080/15548627.2019.1596494.
127. Rojansky R, Cha M-Y, Chan DC. Elimination of paternal mitochondria in mouse embryos occurs through autophagic degradation dependent on PARKIN and MUL1. elife. 2016;5:e17896. https://doi.org/10.7554/eLife.17896.
128. Szargel R, Shani V, Abd Elghani F, Mekies LN, Liani E, Rott R, et al. The PINK1, synphilin-1 and SIAH-1 complex constitutes a novel mitophagy pathway. Hum Mol Genet. 2016;25(16):3476–90. https://doi.org/10.1093/hmg/ddw189.
129. Villa E, Proïcs E, Rubio-Patiño C, Obba S, Zunino B, Bossowski JP, et al. Parkin-independent mitophagy controls chemotherapeutic response in cancer cells. Cell Rep. 2017;20(12):2846–59. https://doi.org/10.1016/j.celrep.2017.08.087.
130. Fu M, St-Pierre P, Shankar J, Wang PT, Joshi B, Nabi IR. Regulation of mitophagy by the Gp78 E3 ubiquitin ligase. Mol Biol Cell. 2013;24(8):1153–62. https://doi.org/10.1091/mbc.E12-08-0607.
131. Di Rita A, Peschiaroli A, Acunzo D, Strobbe D, Hu Z, Gruber J, et al. HUWE1 E3 ligase promotes PINK1/PARKIN-independent mitophagy by regulating AMBRA1 activation via IKKα. Nat Commun. 2018;9(1):3755. https://doi.org/10.1038/s41467-018-05722-3.
132. Richter B, Sliter DA, Herhaus L, Stolz A, Wang C, Beli P, et al. Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. Proc Natl Acad Sci. 2016;113(15):4039. https://doi.org/10.1073/pnas.1523926113.
133. Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature. 2015;524(7565):309–14. https://doi.org/10.1038/nature14893.

134. Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RLJ, et al. Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. Hum Mol Genet. 2011;20(9):1726–37. https://doi.org/10.1093/hmg/ddr048.
135. Kirkin V, Lamark T, Sou Y-S, Bjørkøy G, Nunn JL, Bruun J-A, et al. A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol Cell. 2009;33(4):505–16. https://doi.org/10.1016/j.molcel.2009.01.020.
136. Wang Y, Liu N, Lu B. Mechanisms and roles of mitophagy in neurodegenerative diseases. CNS Neurosci Ther. 2019;25(7):859–75. https://doi.org/10.1111/cns.13140.
137. Chakravorty A, Jetto CT, Manjithaya R. Dysfunctional mitochondria and mitophagy as drivers of Alzheimer's disease pathogenesis. Front Aging Neurosci. 2019;11:311. https://doi.org/10.3389/fnagi.2019.00311.
138. Xian H, Yang Q, Xiao L, Shen H-M, Liou Y-C. STX17 dynamically regulated by Fis1 induces mitophagy via hierarchical macroautophagic mechanism. Nat Commun. 2019;10(1):2059. https://doi.org/10.1038/s41467-019-10096-1.
139. Sabouny R, Shutt TE. Reciprocal regulation of mitochondrial fission and fusion. Trends Biochem Sci. 2020; https://doi.org/10.1016/j.tibs.2020.03.009.
140. Held NM, Houtkooper RH. Mitochondrial quality control pathways as determinants of metabolic health. Bioessays. 2015;37(8):867–76. https://doi.org/10.1002/bies.201500013.
141. Wu NN, Zhang Y, Ren J. Mitophagy, mitochondrial dynamics, and homeostasis in cardiovascular aging. Oxidative Med Cell Longev. 2019;2019:9825061. https://doi.org/10.1155/2019/9825061.
142. Xiao L, Xian H, Lee KY, Xiao B, Wang H, Yu F, et al. Death-associated protein 3 regulates mitochondrial-encoded protein synthesis and mitochondrial dynamics. J Biol Chem. 2015;290(41):24961–74. https://doi.org/10.1074/jbc.M115.673343.
143. Burman JL, Pickles S, Wang C, Sekine S, Vargas JNS, Zhang Z, et al. Mitochondrial fission facilitates the selective mitophagy of protein aggregates. J Cell Biol. 2017;216(10):3231–47. https://doi.org/10.1083/jcb.201612106.
144. Lang A, Anand R, Altinoluk-Hambüchen S, Ezzahoini H, Stefanski A, Iram A, et al. SIRT4 interacts with OPA1 and regulates mitochondrial quality control and mitophagy. Aging. 2017;9(10):2163–89. https://doi.org/10.18632/aging.101307.
145. Rana A, Oliveira MP, Khamoui AV, Aparicio R, Rera M, Rossiter HB, et al. Promoting Drp1-mediated mitochondrial fission in midlife prolongs healthy lifespan of Drosophila melanogaster. Nat Commun. 2017;8(1):448. https://doi.org/10.1038/s41467-017-00525-4.
146. Niu Y-J, Nie Z-W, Shin K-T, Zhou W, Cui X-S. PINK1 regulates mitochondrial morphology promoting mitochondrial fission in porcine preimplantation embryos. FASEB J. 2019;33(7):7882–95. https://doi.org/10.1096/fj.201802473R.
147. D'Amico D, Mottis A, Potenza F, Sorrentino V, Li H, Romani M, et al. The RNA-binding protein PUM2 impairs mitochondrial dynamics and mitophagy during aging. Mol Cell. 2019;73(4):775–87. https://doi.org/10.1016/j.molcel.2018.11.034.
148. Jin SM, Youle RJ. The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria. Autophagy. 2013;9(11):1750–7.
149. Hämäläinen RH, Manninen T, Koivumäki H, Kislin M, Otonkoski T, Suomalainen A. Tissue- and cell-type–specific manifestations of heteroplasmic mtDNA 3243A> G mutation in human induced pluripotent stem cell-derived disease model. Proc Natl Acad Sci. 2013;110(38):E3622–E30.
150. Suen D-F, Narendra DP, Tanaka A, Manfredi G, Youle RJ. Parkin overexpression selects against a deleterious mtDNA mutation in heteroplasmic cybrid cells. Proc Natl Acad Sci. 2010;107(26):11835–40.
151. Burbulla LF, Fitzgerald J, Stegen K, Westermeier J, Thost A, Kato H, et al. Mitochondrial proteolytic stress induced by loss of mortalin function is rescued by Parkin and PINK1. Cell Death Dis. 2014;5(4):e1180.

152. Devi L, Raghavendran V, Prabhu BM, Avadhani NG, Anandatheerthavarada HK. Mitochondrial import and accumulation of α-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. J Biol Chem. 2008;283 (14):9089–100.
153. Ludtmann MHR, Angelova PR, Horrocks MH, Choi ML, Rodrigues M, Baev AY, et al. α-synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. Nat Commun. 2018;9(1):2293. https://doi.org/10.1038/s41467-018-04422-2.
154. Di Maio R, Barrett PJ, Hoffman EK, Barrett CW, Zharikov A, Borah A, et al. α-Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. Sci Transl Med. 2016;8(342):342. https://doi.org/10.1126/scitranslmed.aaf3634.
155. Jin J, Hulette C, Wang Y, Zhang T, Pan C, Wadhwa R, et al. Proteomic identification of a stress protein, mortalin/mthsp70/GRP75: relevance to Parkinson disease. Mol Cell Proteomics. 2006;5(7):1193–204.
156. De Mena L, Coto E, Sánchez-Ferrero E, Ribacoba R, Guisasola LM, Salvador C, et al. Mutational screening of the mortalin gene (HSPA9) in Parkinson's disease. J Neural Transm. 2009;116(10):1289–93.
157. Burbulla LF, Schelling C, Kato H, Rapaport D, Woitalla D, Schiesling C, et al. Dissecting the role of the mitochondrial chaperone mortalin in Parkinson's disease: functional impact of disease-related variants on mitochondrial homeostasis. Hum Mol Genet. 2010;19 (22):4437–52.
158. Goswami AV, Samaddar M, Sinha D, Purushotham J, D'Silva P. Enhanced J-protein interaction and compromised protein stability of mtHsp70 variants lead to mitochondrial dysfunction in Parkinson's disease. Hum Mol Genet. 2012;21(15):3317–32.
159. Jin J, Li GJ, Davis J, Zhu D, Wang Y, Pan C, et al. Identification of novel proteins associated with both α-synuclein and DJ-1. Mol Cell Proteomics. 2007;6(5):845–59.
160. Davison EJ, Pennington K, Hung CC, Peng J, Rafiq R, Ostareck-Lederer A, et al. Proteomic analysis of increased Parkin expression and its interactants provides evidence for a role in modulation of mitochondrial function. Proteomics. 2009;9(18):4284–97.
161. Yang H, Zhou X, Liu X, Yang L, Chen Q, Zhao D, et al. Mitochondrial dysfunction induced by knockdown of mortalin is rescued by Parkin. Biochem Biophys Res Commun. 2011;410 (1):114–20.
162. Freimann K, Zschiedrich K, Brüggemann N, Grünewald A, Pawlack H, Hagenah J, et al. Mortalin mutations are not a frequent cause of early-onset Parkinson disease. Neurobiol Aging. 2013;34(11):2694.
163. Chung SJ, Kim M-J, Ryu H-S, Kim J, Kim YJ, Kim K, et al. Lack of association of mortalin (HSPA9) and other mitochondria-related genes with risk of Parkinson's and Alzheimer's diseases. Neurobiol Aging. 2017;49:215.
164. Costa A, Loh S, Martins LM. Drosophila Trap1 protects against mitochondrial dysfunction in a PINK1/parkin model of Parkinson's disease. Cell Death Dis. 2013;4(1):e467.
165. Zhang L, Karsten P, Hamm S, Pogson JH, Müller-Rischart AK, Exner N, et al. TRAP1 rescues PINK1 loss-of-function phenotypes. Hum Mol Genet. 2013;22(14):2829–41.
166. Pridgeon JW, Olzmann JA, Chin L-S, Li L. PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. PLoS Biol. 2007;5(7):e172.
167. Plun-Favreau H, Klupsch K, Moisoi N, Gandhi S, Kjaer S, Frith D, et al. The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. Nat Cell Biol. 2007;9(11):1243–52.
168. Tain LS, Chowdhury RB, Tao RN, Plun-Favreau H, Moisoi N, Martins LM, et al. Drosophila HtrA2 is dispensable for apoptosis but acts downstream of PINK1 independently from Parkin. Cell Death Differ. 2009;16(8):1118–25.
169. Whitworth AJ, Lee JR, Ho VM-W, Flick R, Chowdhury R, McQuibban GA. Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin. Mechanisms. 2008;1(2-3):168–74. https://doi.org/10.1242/dmm.000109.

170. Diot A, Morten K, Poulton J. Mitophagy plays a central role in mitochondrial ageing. Mamm Genome. 2016;27(7-8):381–95. https://doi.org/10.1007/s00335-016-9651-x.
171. Fivenson EM, Lautrup S, Sun N, Scheibye-Knudsen M, Stevnsner T, Nilsen H, et al. Mitophagy in neurodegeneration and aging. Neurochem Int. 2017;109:202–9. https://doi.org/10.1016/j.neuint.2017.02.007.
172. Shi R, Guberman M, Kirshenbaum LA. Mitochondrial quality control: the role of mitophagy in aging. Trends Cardiovasc Med. 2018;28(4):246–60. https://doi.org/10.1016/j.tcm.2017.11.008.
173. Moreira OC, Estébanez B, Martínez-Florez S, Paz JA, Cuevas MJ, González-Gallego J. Mitochondrial function and mitophagy in the elderly: effects of exercise. Oxidative Med Cell Longev. 2017;2017:2012798. https://doi.org/10.1155/2017/2012798.
174. Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing Res Rev. 2014;14(100):19–30. https://doi.org/10.1016/j.arr.2014.01.004.
175. Moskal N, Riccio V, Bashkurov M, Taddese R, Datti A, Lewis PN, et al. ROCK inhibitors upregulate the neuroprotective Parkin-mediated mitophagy pathway. Nat Commun. 2020;11(1):88. https://doi.org/10.1038/s41467-019-13781-3.
176. Ryu D, Mouchiroud L, Andreux PA, Katsyuba E, Moullan N, Nicolet-Dit-Félix AA, et al. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. Nat Med. 2016;22(8):879–88. https://doi.org/10.1038/nm.4132.
177. Sun N, Yun J, Liu J, Malide D, Liu C, Rovira II, et al. Measuring in vivo mitophagy. Mol Cell. 2015;60(4):685–96. https://doi.org/10.1016/j.molcel.2015.10.009.
178. Fang EF, Hou Y, Palikaras K, Adriaanse BA, Kerr JS, Yang B, et al. Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. Nat Neurosci. 2019;22(3):401–12. https://doi.org/10.1038/s41593-018-0332-9.
179. Kujawska M, Jourdes M, Kurpik M, Szulc M, Szaefer H, Chmielarz P, et al. Neuroprotective effects of pomegranate juice against Parkinson's disease and presence of ellagitannins-derived metabolite-urolithin A-in the brain. Int J Mol Sci. 2019;21(1):202. https://doi.org/10.3390/ijms21010202.
180. Andreux PA, Blanco-Bose W, Ryu D, Burdet F, Ibberson M, Aebischer P, et al. The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial and cellular health in humans. Nat Metab. 2019;1(6):595–603. https://doi.org/10.1038/s42255-019-0073-4.
181. Lu L, Tang LE, Wei W, Hong Y, Chen H, Ying W, et al. Nicotinamide mononucleotide improves energy activity and survival rate in an model of Parkinson's disease. Exp Ther Med. 2014;8(3):943–50.
182. Lehmann S, Loh SHY, Martins LM. Enhancing NAD salvage metabolism is neuroprotective in a PINK1 model of Parkinson's disease. Biol Open. 2017;6(2):141–7. https://doi.org/10.1242/bio.022186.
183. Fang EF. Mitophagy and NAD inhibit Alzheimer disease. Autophagy. 2019;15(6):1112–4. https://doi.org/10.1080/15548627.2019.1596497.
184. Investigators TPSGP. Mixed lineage kinase inhibitor CEP-1347 fails to delay disability in early Parkinson disease. Neurology. 2007;69(15):1480–90. https://doi.org/10.1212/01.wnl.0000277648.63931.c0.
185. Jang JY, Blum A, Liu J, Finkel T. The role of mitochondria in aging. J Clin Invest. 2018;128(9):3662–70. https://doi.org/10.1172/jci120842.