

# Balance is the challenge – The impact of mitochondrial dynamics in Parkinson's disease

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## ABSTRACT

Impaired mitochondrial function has been implicated in neurodegeneration in Parkinson's disease (PD) based on biochemical and pathoanatomical studies in brains of PD patients. This observation was further substantiated by the identification of exogenic toxins, i.e. complex I inhibitors that directly affect mitochondrial energy metabolism and cause Parkinsonism in humans and various animal models. Recently, insights into the underlying molecular signalling pathways leading to alterations in mitochondrial homeostasis were gained based on the functional characterization of mitoprotective genes identified in rare forms of inherited PD. Using *in vitro* and *in vivo* loss of function models of the Parkin, PINK1, DJ-1 and Omi/HtrA2 gene, the emerging field of mitochondrial dynamics in PD was established as being critical for the maintenance of mitochondrial function in neurons. This underscored the concept that mitochondria are highly dynamic organelles, which are tightly regulated to continuously adapt shape to functional and anatomical requirements during axonal transport, synaptic signalling, organelle degradation and cellular energy supply. The dissection of pathways involved in mitochondrial quality control clearly established the PINK1/Parkin-pathway in the clearance of dysfunctional mitochondria by autophagy and hints to a complex interplay between PD-associated proteins acting at the mitochondrial interface. The elucidation of this mitoprotective signalling network may help to define novel therapeutic targets for PD via molecular modelling of mitochondria and/or pharmacological modulation of mitochondrial dynamics.

**Keywords** Autophagy, fission, fusion, mitochondria, neurodegeneration, Parkinson's disease.

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## Parkinson-associated genes and mitochondrial homeostasis

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. Although for the majority of patients the underlying cause of the disease is still unknown, current concepts agree that genetic susceptibility factors acting together with environmental risk factors contribute to the most frequent sporadic form of the disease. Presently, 16 genetic disease loci have been identified, based on linkage studies in rare familial forms of PD and association studies in large samples of sporadic PD patients. These genes and the respective proteins provided the first insight into molecular pathways leading to neurodegeneration [1,2]. The identification of PD patients with variants in nuclear genes encoding mitochondrial proteins was the first genetic support for a number of biochemical findings that implicated impaired mitochondrial function in PD pathogenesis [3–5].

Mutations in four nuclear genes have been identified, and the proteins they encode are actively transported to (Parkin) or into (PINK1, Omi/HtrA2, DJ-1) the mitochondria [6–9]. These pro-

teins play an important role in the maintenance of mitochondrial homeostasis as loss of function mutations that were observed in PD patients, lead to mitochondrial dysfunction *in vivo* and *in vitro*. Therefore these genes provide the first molecular link between mitochondrial maintenance and neurodegeneration in PD.

Loss of function mutations in the PARK2 gene encoding Parkin, a multifunctional E3 ubiquitin protein ligase, and the PARK6 gene encoding the 581 amino acid protein PTEN-induced kinase 1 (PINK1) together represent the most frequent cause for early onset PD [10–12]. Homozygous deletions as well as multiplications, insertions, frameshifts and missense mutations in the *Parkin* gene are known to cause a loss of protein function leading to impaired mitochondrial integrity [13]. Interestingly, promoter polymorphisms putatively influence the transcription level of the *Parkin* gene. Heterozygous mutations have also been identified as risk factors for the common

sporadic form of PD because of reduced penetrance and therefore may contribute to the typical late-onset of the disease [14–16].

The Parkin protein was subsequently linked to the ubiquitin-proteasome protein degradation system because of its ubiquitin E3 ligase activity and suggested potential implications for the degradation of PD-associated interacting proteins, i.e. alpha-synuclein (PARK1), synphilin-1 or LRRK2 (PARK8) [17–21]. However, no accumulation of the implicated substrates of Parkin was identified under conditions of compromised ligase activity *in vivo* to date [22]. Recent studies rather hint to a critical role of Parkin in mitochondrial quality control [23]. Parkin was found to be selectively recruited from the cytosol onto dysfunctional mitochondria defined by reduced membrane potential to promote their autophagic clearance from the cell [24]. This was mediated by an accumulation of the PD-associated protein PINK1 in damaged mitochondria and supported the concept of PINK1 mediating signals of mitochondrial dysfunction to Parkin, which subsequently promotes the elimination of dysfunctional mitochondria [25].

PINK1 is a putative serine/threonine kinase, which is predominantly imported to mitochondria via its N-terminal mitochondrial targeting sequence; however, a minor fraction also localizes to the cytosol [26–29]. After transport into the mitochondria, PINK1 is suggested to reside at the inner mitochondrial membrane. It spans the intermembrane space [30] reaching the outer mitochondria membrane with the kinase domain facing the cytosol [31]. The proposed physiological role of PINK1 in mitochondria includes the maintenance of mitochondrial integrity via regulation of mitochondrial membrane potential, cristae structure and calcium homeostasis [32,33]. In addition, the PINK-1-dependent phosphorylation of mitochondrial proteins, i.e. the PD-associated serine protease Omi/HtrA2, the molecular chaperone TNF-receptor associated protein-1 (TRAP1) and cytoplasmic proteins, i.e. Parkin, in response to cellular stress provided a first mechanistic link to how PINK1 could activate mitochondrial protein quality control [34–36].

Mutations in the PINK1 gene affecting the kinase domain or C-terminal regions of the protein contributed to the disease because of the loss of kinase function and/or effects on protein stability that lead to morphological abnormalities of mitochondria and impaired energy metabolism [32]. These functional observations provided the first evidence for a common signalling pathway formed by different mitochondrially targeted proteins in the pathogenesis of PD [37]. Similar to Parkin, heterozygous PINK1 mutations have been discovered in sporadic late-onset PD patients, suggesting a potential role of single PINK1 mutations as susceptibility factors for typical PD caused by impaired mitochondrial homeostasis [14,38].

Loss of function mutations in the DJ-1 gene (PARK7) were identified in rare forms of autosomal-recessive PD, including

point mutations and deletions that both lead to impaired gene expression or protein instability [39–41]. DJ-1 is a 189 amino acid protein, which is highly conserved among different species and exerts multiple functions including modulation of transcription, chaperone function, regulation of mitochondrial homeostasis and modulation of basal autophagy [42–46]. Major implications for its pathophysiological function were defined by its role as an oxidative stress sensor and its cytoprotective function upon oxidation at a cysteine residue in position 106 of the protein via *in vitro* and *in vivo* experiments [42,47,48]. This cytoprotective effect was mediated by the mitochondrial translocation of DJ-1 leading to increased DJ-1 dimer formation at the outer mitochondrial membrane [48]. Loss of DJ-1 function leads to mitochondrial damage and causes increased vulnerability to complex I inhibition as shown *in vivo* [47,49,50].

The mitochondrial serine-protease Omi/HtrA2 (PARK13) was first linked to neurodegeneration based on observations that loss of function mutations or complete knockout of the protein caused neurodegeneration with a parkinsonian phenotype in mice [8,51]. To date, three point mutations leading to amino acid changes (A141S, G399S and R404W) were identified in German and Belgian cohorts of PD patients as potential risk factors for the disease [4,52]. Moreover, nine heterozygous mutations in the 5' and 3' regulatory region of the gene were exclusively found in patients and suggested a role of differential transcriptional regulation of Omi/HtrA2 in neurodegeneration [52]. The G399S mutation has been subsequently found in healthy controls, giving rise to reduced penetrance of the respective variant [53]. *In vitro* functional characterization of two point mutations in the Omi/HtrA2 gene revealed loss of protease function [4]. Omi/HtrA2 is a nuclear encoded protein that is actively transported into mitochondria and resides in the intermembrane space. A proapoptotic function of Omi/HtrA2 by caspase activation was shown upon release of Omi/HtrA2 from damaged mitochondria into the cytosol [54]. However, the loss of its protective role within mitochondria was subsequently identified as the primary cause of neurodegeneration in different *in vivo* and *in vitro* models of loss of Omi/HtrA2 serine protease function [8,51]. Most interestingly, activation of the serine protease activity of Omi/HtrA2 was found to be modulated by the PD-associated kinase PINK1, as PINK1 mediates phosphorylation of Omi/HtrA2 *in vitro* and *in vivo* [34].

Therefore, these genetic links provide strong evidence for a role of mitochondrial dysfunction in neurodegeneration in PD and were the starting point for the dissection of molecular signalling pathways that control mitochondrial homeostasis.

## Genetic causes of mitochondrial dysfunction

Mitochondria are particularly important to maintain proper neuronal function and plasticity. There is increasing evidence

that disruption of mitochondrial function and dynamics are responsible for different neurodegenerative diseases [55]. Next to their main function to serve as a cellular energy supply providing the majority of the cellular ATP, mitochondria also act as a calcium buffer, regulators of lipid metabolism and regulators of apoptosis [56]. As neurons in the brain are major consumers of energy, it is not surprising that impaired mitochondrial function results in loss of neuronal integrity [55]. In support, defects in mitochondrial energy supply as reflected by reduced complex I activity in the substantia nigra pars compacta (SNpc) of PD patients was an early finding from pathoanatomical and biochemical studies [57]. Moreover, the relevance of mitochondrial complex I activity was underscored by observation in drug addicts, who displayed a progressive parkinsonian syndrome attributed to MPTP-contaminated drugs [58]. MPP<sup>+</sup>, the active metabolite of MPTP, is a potent complex I inhibitor and enters neurons via the dopamine receptor, causing mitochondrial dysfunction associated with oxidative stress [59]. This concept of toxin-mediated mitochondrial damage leading to neurodegeneration was subsequently confirmed in different animal models. However, besides all epidemiological efforts to date no consistent environmental factor causing PD has been identified [60,61].

By contrast, during the last 10 years PD-associated genes that directly influence mitochondrial function caused by modulation of intramitochondrial reactive oxygen species (ROS), mitochondrial membrane potential (MMP), and mitochondrial oxygen consumption have been identified. Wood-Kaczmar et al reported increased ROS levels and reduced MMP in primary neurons derived from PINK1 knockout mice as well as in human PINK1 knock-down dopaminergic neurons differentiated from mesencephalic stem cells [62]. The increased ROS levels were found to be related to a dysregulation of the mitochondrial Na<sup>+</sup>/Ca<sup>2+</sup> exchanger followed by an intramitochondrial Ca<sup>2+</sup> overload, which subsequently causes a stimulation of ROS production via NADPH oxidase [63]. Moreover, Parkin is known to influence mitochondrial homeostasis. Overexpression of Parkin prevented the formation of ROS in the mitochondria and increased the MMP [64]. This protective effect in mitochondria of Parkin-overexpressing cells was resulting from an improved complex I activity [64]. *Vice versa* an increased protein oxidation, lipid-peroxidation and reduced amounts of complex I and complex IV subunits of the respiratory chain were described in parkin deficient mice [65].

Similar defects in mitochondrial integrity were found in a model of loss of DJ-1 function based on embryonal fibroblasts from DJ-1 knockout mice (MEF), where impaired energy metabolism was reflected by reduced complex I activity [43]. Of note colocalization of DJ-1 with complex I was described under physiological conditions *in vitro*. These findings suggested a direct role of DJ-1 in maintaining the integrity of the mitochon-

drial complex I and thereby support studies that report an intramitochondrial localization of activated DJ-1 [9,66]. For Omi/HtrA2, another PD-associated protein, the localization in the intermembrane space of mitochondria is generally accepted. The *in vitro* characterization of loss of function mutations identified in PD patients revealed similar alterations of mitochondrial function as described in cells devoid of Omi/HtrA2 protein derived from different models of the disease, including reduced mitochondrial membrane potential and characteristic morphological changes, i.e. swelling of these organelles [4,67,68].

Therefore impaired mitochondrial function was a consistent finding in all studies characterizing effects of loss of Parkin, PINK1, DJ-1 and Omi/HtrA2 *in vitro* and *in vivo*. The fact that not only in mammalian but also in invertebrate models, i.e. *Drosophila melanogaster*, a similar effect was observed, argues in favour of the involvement of these genes in highly conserved signalling pathways. Indeed an intact mitochondrial network is required to maintain important functions such as the distribution of mitochondrial DNA, which although encoding only a few proteins, is essential for key functions of mitochondria, as oxidative phosphorylation [69]. Therefore, the preservation of mitochondrial integrity is a fundamental cell survival process for non-dividing cells like neurons.

## Regulation of mitochondrial homeostasis

Mitochondria are highly dynamic organelles that are engaged in repeated cycles of fusion and fission events allowing to migrate throughout the cell and to participate in quality control of the mitochondrial population by selective degradation of dysfunctional organelles [70]. The fusion process of two mitochondria results in the exchange of soluble intermembrane space and matrix proteins. Moreover, it allows mitochondria to interact with each other, likely serving as a protecting mechanism by forming networks and diluting toxic intermediates. On the other hand, fission is responsible for the division and sequestration of damaged parts of the organelle and therefore – together with mitochondrial biogenesis – allows for mitochondrial renewal, redistribution and proliferation into synapses [71]. Therefore a well-balanced machinery that controls both events is essential for maintaining the metabolic function of mitochondria and allowing to rapidly adapt to changes in cellular requirements. Once this sensible system is disequibrated because of toxic insult or genetic defects, fission processes may overrule and lead to fragmentation and subsequent autophagic clearance of damaged and inactive mitochondria. By contrast, pathological fusion events leading to increased ROS production within affected mitochondria have been reported [72]. If more severe, imbalance towards fusion can cause cell death and induce neurodegeneration. These elongation phenotypes of

mitochondria have been described in terms of cellular senescence and recently loss of MARCH5, an ubiquitin E3 ligase responsible for Mfn1 degradation, has been described to contribute to cellular senescence defined by elongated mitochondria and increased senescent-associated beta-galactosidase activity [72–74].

Mitochondria appear as discrete tubules or interconnected networks in living cells [69]. Mitochondrial outer- and inner-membrane fusion events are coupled *in vivo*, but dissection of this process *in vitro*, allowed for the identification of the fusion machinery. Mitofusins 1 and 2 (Mfn1, Mfn2) are integral proteins, located in the outer mitochondrial membrane and required for outer membrane fusion to control mitochondrial network architecture [75] (Fig. 1). Mfn2 shows additional functions concerning mitochondrial metabolism [76] or tethering the endoplasmic reticulum to mitochondria [77]. The fusion of the inner mitochondrial membrane requires a second dynamin-related GTPase (OPA1) (Fig. 1). OPA1 is present as a membrane-bound as well as soluble form, whereas the membrane-bound proteins are responsible for the fusion of the inner membranes [78]. It has been shown that mammalian mitofusins and OPA1 mediate distinct sequential fusion steps indicating

that fusion of the outer and inner membranes is not tightly coupled [78].

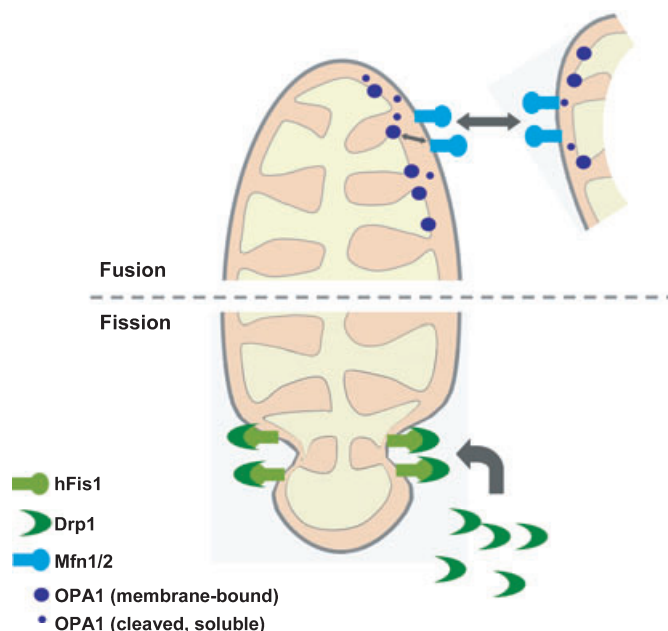
Similarly, two key proteins that control mitochondrial fission events were identified. The dynamin-related protein 1 (Drp1) is recruited from the cytosol to mitochondria during fission [79] and acts in concert with the mitochondrial outer membrane protein Fis1 [80]. From experiments on yeast it has been suggested that Fis1 may function as a receptor for the yeast homologue of Drp-1 [81] (Fig. 1). However, the role of the human homologue, hFis1, in recruiting Drp1 to mitochondria is less clear, as Drp1 recruitment is not affected by knockdown of hFis1 [82]. Nevertheless, hFis1 silencing effectively inhibited mitochondrial fission. This fact implicates that hFis1 is a downstream partner of Drp1 [83].

The critical role of regulation of mitochondrial fusion-fission events for the maintenance of neuronal function is reflected by the identification of mutations in known key regulators as OPA1 and Mfn2. Loss of function mutations in the gene encoding fusion factor OPA1 are the most frequent cause of inherited optic neuropathy, a condition that is characterized by gradual loss of vision, colour vision defects and temporal optic atrophy [84]. Recently mutations in the fusion protein Mfn2 were identified as a cause of a peripheral neuropathy (Hereditary motor and sensory neuropathy type 2A and type 6; HMSN2A2, HMSN6), that manifests with muscular weakness, loss of reflexes and sensory defects, i.e. decreased sensation of pain or vibration [85]. These findings indicate that the tight regulation of mitochondrial homeostasis is critical for neuronal function and that impairments caused by mutations in proteins controlling these events are related to neurodegenerative diseases [85,86]. Only recently, a direct involvement of PD-associated genes in the regulation of mitochondrial dynamics was defined.

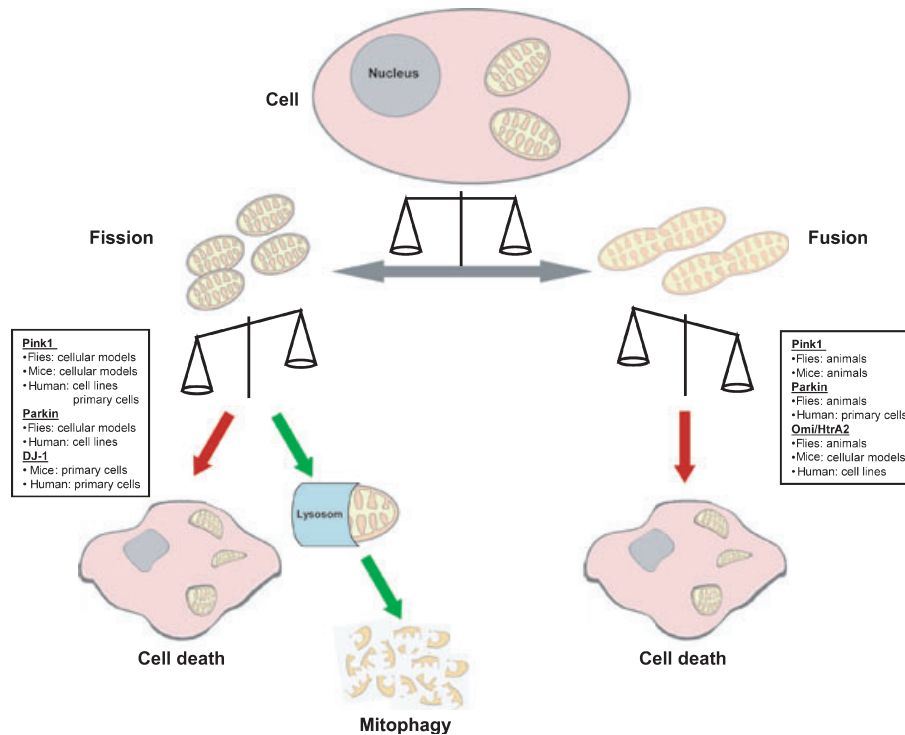
## Role of PD-associated proteins in mitochondrial dynamics

Based on the functional characterization of different loss of function models *in vitro* and *in vivo*, new signalling pathways were defined that show direct modulation of PD-associated proteins of the above mentioned fusion–fission machinery (Fig. 2).

Currently most data exist on the PINK1/Parkin pathway and defined a role of these proteins in the modulation of mitochondrial function and dynamics to ensure mitochondrial quality. First evidence for a common pathway with PINK1 acting upstream of parkin came from *in vivo* studies in *Drosophila* [87]. Loss of either *Drosophila* PINK1 or parkin function resulted in a mitochondrial phenotype including male sterility, apoptotic muscle degeneration, defects in mitochondrial morphology and increased sensitivity to oxidative stress. At that time this was unexpected for loss of parkin function as it was rather



**Figure 1** Schematic view on the role of key regulators in mitochondrial fusion and fission events. While Mitofusins 1 and 2 together with OPA1 are responsible for the fusion of mitochondrial outer and inner membrane, respectively, hFis1 and Drp1 play an important role in mitochondrial fission. 187 × 169 mm (300 × 300 DPI).



**Figure 2** Schematic view of the fusion–fission balance and the consequences of imbalance. Under physiological conditions, mitochondrial fusion and fission events are balanced to maintain the organelles homeostasis. Once this sensitive system is impaired and a shift towards increased fusion or fission occurs, cellular function is critically altered. Loss of PINK1, Parkin or DJ-1 function in experimental models (specified in the left box) may lead to an imbalance towards fission and causes either lysosomal degradation of impaired mitochondria maintaining cellular integrity (green arrows) or may directly drive the cell into apoptosis, if mitochondrial damage is severe and causes release of pro-apoptotic proteins (red arrow). By contrast, an imbalance towards fusion can be observed under conditions of loss of PINK1, Parkin and Omi/HtrA2 function in experimental models (specified in the right box). Because of the inability of lysosomes to degrade entire mitochondrial network structures, but only single mitochondria, the shift towards fusion events may result in loss of cellular integrity as a result of ineffective clearance of dysfunctional mitochondria (red arrow). 244 × 197 mm (300 × 300 DPI).

implicated in protein degradation pathways [21]. Interestingly, rescue of the defects caused by loss of PINK1 was observed upon overexpression of parkin [13,87]. The complementary experiment with overexpression of PINK1 in the *parkin* knockout background was not successful, showing that parkin acts downstream of PINK1. The related mitochondrial phenotypes included elongated and swollen mitochondria, abnormalities of the cristae such as fragmentation and were subsequently confirmed by independent studies [88–91]. Flight muscle degeneration and mitochondrial morphological alterations in *pink1* or *parkin* knockout flies were not only restored by transgenic overexpression of *parkin*, but also strongly suppressed by increased *drp1* gene dosage and heterozygous loss of function mutations affecting the mitochondrial fusion-promoting factors OPA1 and Mfn2 [88]. Therefore it was suggested that the PINK1/Parkin pathway promotes mitochondrial fission in flies under physio-

logical conditions and that loss of mitochondrial integrity was resulting from an increase of fusion events.

Based on studies suggesting genetic epistasis between PINK1 and Parkin, subsequent *in vitro* experiments in different vertebrate and mammalian disease models were initiated to define the precise molecular mechanisms involved in the regulation of mitochondrial morphology and function [32]. The role of PINK1 as an upstream interactor of Parkin was further supported by the identification of Parkin as a substrate for phosphorylation by PINK1 kinase activity [92].

Downregulation of PINK1 or Parkin in human HeLa or SH-SY5Y cells resulted in abnormal mitochondrial morphology, however, in mammalian models fragmented mitochondria, with decreased cristae density were observed, when compared with control cells expressing physiological PINK1 or Parkin [32,90,93]. Rescue of the morphological phenotype resulting

from loss of PINK1 or Parkin was shown by overexpression of Mfn2, OPA1 or a dominant negative variant of Drp1, suggesting that a decrease in mitochondrial fusion or an increase in fission was associated with a loss of Parkin or PINK1 function [90]. Indeed Drp1-dependent fragmentation of mitochondria caused by loss of either PINK1 or Parkin function has been subsequently confirmed by independent studies [93,94]. Similar to this observation, but in contrast to the results from the *in vivo* studies in flies, an *in vitro* model of *pink1* or *parkin* silencing in *Drosophila* S2 cells also resulted in an increase of the fragmentation of the mitochondrial network meaning a shift towards fission events [90].

The first *ex vivo* experiments in primary human fibroblasts derived from patients with different PINK1 mutations reveal similar defects in mitochondrial morphology as observed in knock down models in human cell lines [32,95]. In contrast to loss of function of PINK1, for Parkin mutant fibroblasts from patients, a higher degree of mitochondrial branching was found. The same result has been shown by knockdown of *Parkin* in control fibroblasts confirming that a Parkin deficiency in humans is sufficient to explain these mitochondrial effects [96]. Rescue experiments concerning the morphological phenotype of loss of function of Parkin in human fibroblasts have not been performed.

The apparent discrepancies between the different models of loss of Parkin or PINK1 function may be species-specific; however, another explanation may be the use of different paradigms. Whereas RNAi-mediated gene knockdown causes acute treatment effects, measurements in cells derived from knockout animals or patients carrying disease-causing loss of function mutations represent a chronic model in which cellular compensatory mechanisms could be active. This concept would attribute a mitochondrial fission to an acute response to mitochondrial damage induced by massive reduction of endogenous PINK1 or Parkin protein, whereas the observed increased fusion of mitochondria in knockout animals or *ex vivo* models from patients may represent a compensatory stress response caused by chronic loss of protein function.

Indeed, the quantitative ultrastructural analysis of the striatum of PINK1 knockout (KO) mice or Parkin KO mice revealed no gross changes in the structure, average size or total number of mitochondria, although an increased tendency for large mitochondria was detected in the PINK1 KO model [97,98]. Thus the mitochondrial defects observed in PINK1 KO mice were not as striking as in KO flies, probably because of better compensatory mechanisms. In support of acute effects on mitochondrial dynamics, in primary mouse neurons, a decrease in mitochondrial length and connectivity as an early response to acute PINK1 down-regulation has been observed [90]. Thus mitochondrial fragmentation obviously appears as an early phenomenon upon acute PINK1/Parkin silencing in primary

mouse neurons and *Drosophila* S2 cells, whereas PINK1/Parkin-deficient flies or KO mice show imbalance towards fusion processes and therefore elongated mitochondrial structures.

Characteristics of the mitochondrial morphology in loss of function models of DJ-1 still have to be examined in more detail. Recently in embryonic fibroblasts from DJ-1 KO mice first evidence for impaired mitochondrial morphology was reported [43,46]. Both of these studies are based on experiments with different KO models. Their results show consistency in terms of a reduction in mitochondrial connectivity accompanied by an increase in fragmentation of mitochondria in models devoid of DJ-1. Mitochondrial interconnectivity was rescued by stable expression of human wild-type DJ-1 showing that loss of DJ-1 is sufficient to cause increased fission in these cells [46]. However, no alterations of the key regulators of fusion/fission processes were observed [43]. First insight into human DJ-1 pathology derived from an *ex vivo* model of fibroblasts from patients carrying a homozygous E64D mutation in the *DJ-1* gene [40]. Analyses of mitochondrial morphology revealed similar characteristic alterations including a decreased mitochondrial network [43]. Interestingly, the levels of DJ-1 protein were largely reduced in homozygous mutation carriers compared with unaffected heterozygous siblings [43]. This strongly supports the loss of function phenotype in patients carrying the E64D mutation in the *DJ-1* gene.

Loss of function models of Omi/HtrA2 consistently revealed changes of mitochondrial ultrastructure [8,67]. Our group identified a novel direct involvement of the mitochondrial serine protease in the regulation of the fusion protein OPA1 based on an Omi/HtrA2 KO model using MEF [99]. The loss of Omi/HtrA2 resulted in increased mitochondrial fusion with significantly increased numbers of elongated mitochondria. Mechanistically this was linked to increased levels of easily extractable OPA1 protein in the mitochondria. Complementation of KO cells with wild-type Omi/HtrA2 reversed not only the mitochondrial phenotype but also restored OPA1 levels to normal in murine and human disease models [67]. In contrast to the divergent findings in acute as well as chronic models of loss of PINK1 and Parkin function, for Omi/HtrA2 KO or silencing models, either invertebrates or mammalian, a consistent elongation phenotype was observed.

Together these findings provide first insight into direct modulation of the mitochondrial fusion–fission machinery by proteins related to PD pathogenesis and give rise to a potential role of these proteins in mitochondrial dynamics to assure mitochondrial quality control (summarized in Table 1).

## Macro-autophagy and mitochondrial clearance

Macro-autophagy is a general process to degrade proteins and cellular components and organelles. Products determined



Table 1 Continued

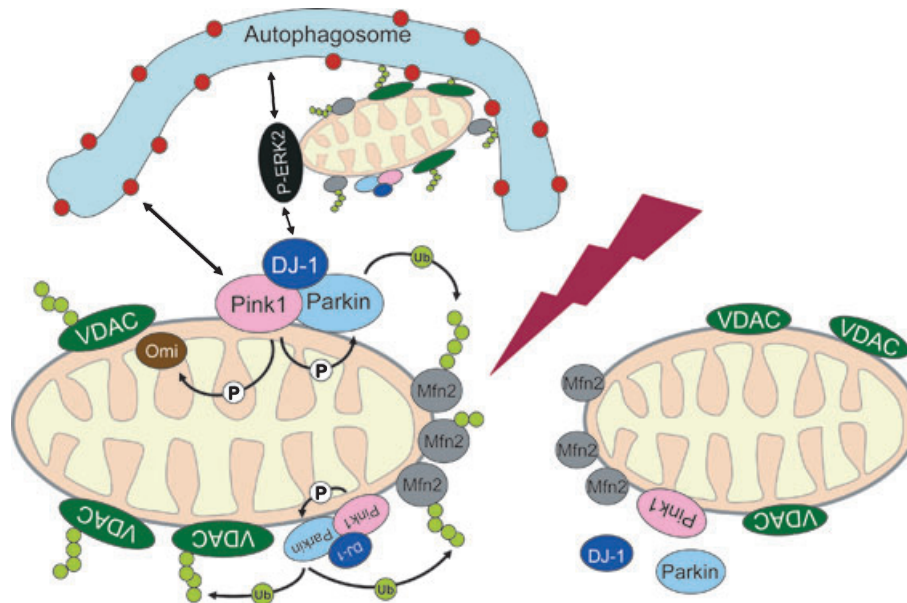
Loss of function of...		Parkin	DJ-1	Omi/HtrA2
Human	Cell lines	<b>PINK1</b> Truncated, fragmented or swollen mitochondria (SH-SY5Y) [90,93], (HeLa) [32, 90] Decreased cristae density (SH-SY5Y) [90, 93], (HeLa) [32, 90] Impaired mitophagy (HeLa) [100]	Increased mitochondrial fragmentation resulting from increased mitochondrial fission (SH-SY5Y) [90] Impaired mitophagy (HeLa) [99]	Less resistance to oxidative stress (M17 neuroblastoma) [42] (SK-N-BE) [48]  Abnormal cristae structure (HeLa) [67] Altered accessibility and extractability of the mitochondrial fusion protein OPA1 (HeLa) [67]
Primary cells		Truncated, fragmented or swollen mitochondria [32, 90] Decreased cristae density [32] Increased ROS and reduced MMP [62]	Higher degree of mitochondrial branching [96]	Reduced mitochondrial branching [43] increased fragmentation of mitochondria [43] impaired mitophagy [43]

for degradation are engulfed by the autophagosome. Autophagosomes fuse with lysosomes and the included proteins or cellular organelles are subsequently degraded by hydrolytic enzymes. In this context, mitophagy describes the specific degradation of mitochondria by the above mentioned mechanisms (Fig. 3). To maintain mitochondrial homeostasis, these organelles have to undergo biogenesis of functional mitochondria and in parallel effective degradation of impaired mitochondria from the cell. Recent studies showed that proper turnover of dysfunctional mitochondria by macroautophagy plays an important role in neurodegenerative diseases such as Parkinson's disease.

Most experimental evidence was provided for a novel role of PINK1 and Parkin as a sensing system for the detection and subsequent degradation of impaired mitochondria by macroautophagy. In this concept, PINK1 mediates the selective recruitment of Parkin to dysfunctional mitochondria and causes Parkin activation by phosphorylation in its RING finger domain [92,99]. PINK1 seems to be localized to mitochondria irrespective of their functional status, but undergoes voltage-dependent cleavage only on functional organelles, whereas – under conditions of mitochondrial impairment – this cleavage is prevented and full-length PINK1 accumulates [25]. Increased levels of PINK1 on dysfunctional mitochondria then promote the recruitment of Parkin to initiate selective degradation of mitochondria [24]. Indeed functional Parkin was necessary to initiate mitophagy after toxic insult in an overexpression model in human cell lines [99]. In mammalian cells, a specific E3 ligase activity of Parkin was required to (i) autoubiquitinate Parkin via lysine in position 63 (K63) and (ii) ubiquitinate the outer mitochondrial membrane protein VDAC1 via lysine at position 27 (K27; [99]). These specific ubiquitination steps were critical for the recruitment of p62 as an adaptor protein to mediate mitochondrial degradation by autophagy [25,99–101] (Fig. 3).

Using a *Drosophila* model, recently the mitochondrial fusion factor Mfn was identified as another substrate of parkin-mediated ubiquitination [100]. Specific ubiquitination of the outer membrane protein Mfn marked dysfunctional mitochondria for lysosomal turnover. The increased degradation of the fusion factor Mfn then causes fission of mitochondria, which is essential for subsequent mitophagic degradation [100]. This was consistent with the observed elongated phenotype of mitochondria in *Drosophila* models of loss of Parkin function [87]. Whether Mfn also serves as a substrate of Parkin in mammalian cells remains to be determined, however, first results from *ex vivo* experiments in fibroblasts of patients carrying loss of function mutations in the Parkin gene show consistent findings with increased mitochondrial elongation and connectivity [96].

Besides its indirect modulation of mitophagy via Parkin activation, PINK1 was also shown to directly modulate key proteins of the lysosomal degradation pathway. Indeed direct



**Figure 3** Model of mitochondrial clearance mechanisms controlled by PD-associated genes PINK1, Parkin, DJ-1 and Omi/HtrA2. The PD-associated genes PINK1 (pink), Parkin (light blue), DJ-1 (dark blue), and Omi/HtrA2 (brown) are known to influence mitophagy. Under stress condition, PINK-1 is thought to be stabilized at the outer membrane of dysfunctional mitochondria (left). By phosphorylation, it could modulate the activity of the mitochondrial serine protease Omi/HtrA2 and/or Parkin, the latter in response being recruited to mitochondria. Moreover, PINK1 has been reported to directly interact with the autophagic marker protein LC-3 (red). Parkin was shown to regulate the abundance of the mitochondrial fusion protein mitofusin 2 (Mfn2; grey) at the outer membrane by ubiquitination (light green), thereby inhibiting mitochondrial fusion events in *Drosophila*. In a mammalian model, Parkin was shown to ubiquitinate the outer mitochondrial membrane protein VDAC (dark green) to mark dysfunctional mitochondria for subsequent p62-mediated recruitment to the autophagosome. DJ-1 may be involved in the regulation of mitochondrial p-ERK2 (black), thereby promoting the clearance of dysfunctional mitochondria by the autophagosome. 250 × 165 mm (300 × 300 DPI).

interaction of full-length PINK1 with the beclin1 was recently shown to be necessary to promote autophagy independently of its kinase function [102]. In addition, molecular binding between PINK1 and the autophagic marker LC3-II confirmed a direct link between this mitochondrial protein and mitophagy [101].

The significance of autophagy as a cellular degradation pathway for dysfunctional mitochondria in PD was further substantiated by the role of DJ-1 in the regulation of basal autophagy [43]. Loss of function in DJ-1 results in reduced lysosomal activity and impaired mitophagy with accumulation of dysfunctional mitochondria [43]. The way DJ-1 regulates autophagic function remains to be determined. Recent studies suggest a role of ERK1/2 signalling in the regulation of autophagy [103], as ERK2 kinase activity and/or mitochondrial ERK2 localization induced autophagy including mitochondrial clearance [103] (Fig. 3). In this context, loss of DJ-1 function caused decreased mitochondrial ERK2 activation in MEF [43].

A potential unifying concept for the interaction of the above mentioned PD-associated proteins was provided by a recent study defining a complex formed by Parkin, PINK1 and DJ-1

that mediates ubiquitination and subsequent degradation of misfolded proteins, including the Parkin substrate synphilin-1 [104]. Whether this ligase complex also mediates specific ubiquitination of target proteins at the outer mitochondrial membrane to promote the degradation of dysfunctional organelles remains to be determined.

## Conclusion

The dissection of molecular signalling pathways leading to neurodegeneration in inherited forms of PD revealed defects at different steps of mitochondrial quality control including stress sensing, morphological alterations and mitochondrial clearance in case of severe damage of the organelle. Together with findings from other neurodegenerative disorders these results corroborate a central role of impaired mitochondrial quality control in neuronal cell death in PD and provide a major conceptual advance that unifies the previous concepts of genetic and environmental causes of mitochondrial dysfunction in PD.

Why forms of PD associated with mitochondrial dysfunction typically do not display such alterations observed in patients with classical mitochondrial disorders (i.e. morphological changes of white matter in brain imaging or clinical signs as myopathy and diabetes mellitus) remains to be determined. It can be speculated that PD-associated mutations in mitochondrial proteins result in a milder phenotype than 'classical' mitochondrial disorders. This would comply with the more severe clinical features in mitochondrial pathologies and with the later age at disease onset in PD patients with mitochondria-associated gene mutations. Nevertheless, typical mitochondria-associated pathologies like myopathy may be present in PD patients, however, may remain clinically unapparent, e.g. an asymptomatic myopathy was described as a feature of Parkin-related parkinsonism [105]. Another feature that is also observed in complex I and complex IV disorders, i.e. diabetes mellitus, is found to be associated with neurodegeneration in PD (reviewed in [106]).

Whether genes identified in autosomal dominant PD also converge to pathways related to mitochondrial dysfunction remains to be determined. Interestingly, also for the most common cause of inherited PD, mutations in the LRRK2 gene (PARK8), subcellular localization to mitochondria was described [107]. Recently a potential role of LRRK2 in the regulation of mitochondrial function was defined by the investigation of the invertebrate homologue *lrrk1* in *C. elegans* [108]. It was shown that *lrrk1* provides a protective effect against mitochondrial damage in *C. elegans* that was independent of its kinase function. Even the prototypic aggregation prone protein alpha-synuclein was recently functionally and physically linked to mitochondrial dysfunction based on partial translocation into mitochondria [109]. Future studies need to define the role of LRRK2 and alpha-synuclein on mitochondrial dynamics using *in vivo* and patient-based *ex vivo* models.

The emerging field of mitochondrial quality control indicates that targeting mitochondrial dynamics as a therapeutic approach might provide a novel tool to interfere with neurodegeneration in this common disorder.

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