

# Mitochondrial dynamics, cell death and the pathogenesis of Parkinson's disease

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**Abstract** The structure and function of the mitochondrial network is regulated by mitochondrial biogenesis, fission, fusion, transport and degradation. A well-maintained balance of these processes (mitochondrial dynamics) is essential for neuronal signaling, plasticity and transmitter release. Core proteins of the mitochondrial dynamics machinery play important roles in the regulation of apoptosis, and mutations or abnormal expression of these factors are associated with inherited and age-dependent neurodegenerative disorders. In Parkinson's disease (PD), oxidative stress and mitochondrial dysfunction underlie the development of neuropathology. The recessive Parkinsonism-linked genes *PTEN-induced kinase 1* (*PINK1*) and *Parkin* maintain mitochondrial integrity by regulating diverse aspects of mitochondrial function, including membrane potential, calcium homeostasis, cristae structure, respiratory activity, and mtDNA integrity. In addition, *Parkin* is crucial for autophagy-dependent clearance of dysfunctional mitochondria. In the absence of *PINK1* or *Parkin*, cells often develop fragmented mitochondria. Whereas excessive fission may cause apoptosis, coordinated induction of fission and autophagy is believed to facilitate the removal of damaged mitochondria through mitophagy, and has been observed in some types of cells. Compensatory mechanisms may also occur in mice lacking *PINK1* that, in contrast to cells and *Drosophila*, have only mild mitochondrial dysfunction and lack dopaminergic neuron loss. A better understanding of the relationship between the specific changes in mitochondrial dynamics/turnover and cell death will be instrumental to identify

potentially neuroprotective pathways steering *PINK1*-deficient cells towards survival. Such pathways may be manipulated in the future by specific drugs to treat PD and perhaps other neurodegenerative disorders characterized by abnormal mitochondrial function and dynamics.

**Keywords** *PINK1* · *Parkin* · Mitophagy · Calcium · Cristae · Mitochondria

## Mitochondrial structure and function

Among the most important functions of mitochondria are the synthesis of adenosine triphosphate (ATP) through oxidative phosphorylation, fatty acid oxidation, regulation of redox and calcium signaling, and the control of apoptosis in response to a variety of adverse extracellular and intracellular signals [1]. As descendants of ancient prokaryotes, mitochondria have inner and outer membranes that separate the matrix from the intermembrane space (IMS) and the IMS from the cytosol. The inner mitochondrial membrane (IMM) is highly folded into the cristae and harbors the respiratory chain complexes of the electron transport chain and ATP synthase. Cells contain many mitochondria, each carrying several copies of a small circular mitochondrial genome (16,569 bp in humans) in the matrix. The mammalian mitochondrial genome contains 37 genes, which encode 13 protein subunits of the respiratory complexes I, III, IV and V (ATP synthase) as well as 22 tRNAs and 2 rRNAs that are required for the translation of mitochondrial transcripts. The remainder of the 900–1000 estimated mitochondrial proteins [2], as well as additional tRNAs required for translation, are nucleus-encoded and must be imported into mitochondria via an organelle-specific import machinery [3].

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## Mitochondrial fission and fusion

Mitochondria can change their shape, size and inner membrane structure in a dynamic fashion. In addition, the mitochondria of a single cell do not function in isolation, but form a complex reticulum whose morphology undergoes continuous changes in response to metabolic stimuli and signaling pathways. These changes are regulated by fission and fusion events that remodel mitochondrial membranes. Several excellent reviews have been published recently on the machinery and regulation of mitochondrial dynamics [4–11]. Therefore, I will limit the discussion on the core proteins and most important regulatory mechanisms involved in these processes.

The molecular machinery of mitochondrial fission and fusion is best characterized in yeast [4]. In mammalian cells, mitochondrial fission depends on the GTPase dynamin-related protein (Drp1) [12–14]. To promote fission, Drp1 must translocate from the cytosol to the outer mitochondrial membrane (OMM), where it oligomerizes into ring-like structures and constricts the mitochondria through GTP hydrolysis. In addition, fission requires OMM proteins including hFis1 and others [5, 11, 15–21]. Control of mitochondrial fission occurs primarily through post-translational modifications of Drp1, as well as direct Drp1-interacting proteins that increase the mitochondrial pool of Drp1 [10, 22]. In proliferating cells mitochondria must undergo fission during mitosis, which is promoted by Cdk1/cyclin B-dependent phosphorylation of Drp1 on Ser-585 (rat Drp1; Ser-618 in human Drp1) [23]. In addition, the conserved residue Ser-637 (human Drp1; Ser-656 in rat Drp1) is phosphorylated by cyclic AMP-dependent protein kinase (PKA) and dephosphorylated by calcineurin [24]. Expression of the phosphomimetic Ser656Asp Drp1 mutant resulted in a marked elongation and perinuclear clustering of mitochondria, while non-phosphorylatable Ser656Ala Drp1 caused mitochondrial fragmentation [24, 25]. In agreement with this, dephosphorylation of Drp1 by calcineurin enhanced the translocation of Drp1 to mitochondria and promoted fission [26]. Thus, the phosphorylation status of Ser-637 (Ser-656 in rats) determines Drp1 localization and activity [24, 26–28]. In addition, both sumoylation [29–32] and ubiquitination [33] affect fission. Drp1 interacts with Ubc9 and Small ubiquitin-like modifier 1 (Sumo1), and transient transfection of YFP-tagged Sumo1 into cells dramatically increased mitochondrial fission, with a portion of YFP-Sumo1 colocalizing with endogenous Drp1 at mitochondrial fission sites [29]. In contrast, overexpression of SUMO1/sentrin-specific peptidase 5 (SEN5) rescued Sumo1-induced mitochondrial fission, partly through destabilization of Drp1, while silencing of SEN5 expression increased mitochondrial fission [31]. Recently, mitochondrial-anchored protein ligase (MAPL) has been

identified as the first mitochondrial SUMO E3 ligase [32]. Overexpression of MAPL stimulates fission [34], and MAPL binds to Drp1 in vivo and sumoylates Drp1 in vitro [32]. Conversely, silencing of MAPL leads to a decrease in the levels of Drp1 and SUMO conjugates [32]. These experiments show that MAPL-dependent sumoylation of Drp1 is associated with increased fission [29, 31, 32]. The ubiquitin E3 ligase MITOL/MARCH-V is an integral protein of the OMM with its RING finger domain exposed to the cytosol. MITOL has been shown to ubiquitinate both hFis1 and Drp1, increasing their turnover [35]. Conversely, depletion of MITOL by RNAi resulted in increased mitochondrial fragmentation, and MITOL overexpression could rescue mitochondrial fragmentation induced by overexpression of hFis1, suggesting that MITOL1 is an inhibitor of fission [35]. However, the results of another group published more recently pointed in the opposite direction, because cells with silenced MARCH-V expression showed abnormally elongated mitochondria with increased interconnectivity, indicative of reduced fission and/or increased fusion. The latter group proposed that wildtype MARCH-V facilitates the assembly and/or disassembly of functional fission complexes on the OMM through ubiquitination of Drp1, thereby promoting fission [36]. Finally, MARCH-V was also shown to bind to Mfn2 and to promote mitochondrial fusion in an Mfn2-dependent manner [37]. Thus, while MITOL/MARCH-V clearly affects mitochondrial morphology, further studies are necessary to clarify its exact mechanism.

Mitochondrial fusion relies on the coordinated action of three GTPases. Mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) are integral outer membrane proteins that tether organelles for OMM fusion by mediating homotypic and heterotypic interactions between adjacent mitochondria [38–40]. Inner membrane fusion is mediated by optical atrophy 1 (Opa1), which is an IMM protein with a soluble IMS form generated through proteolytic processing [40–42]. In addition, Opa1 plays an important role in the maintenance and regulation of IMM structure [41, 43, 44]. In yeast, several proteins with regulatory functions in mitochondrial fusion have been identified [10]. For example, the IMS protein Ups1 regulates the alternative topogenesis and sorting of Mgm1 (the yeast homologue of Opa1) in the IMM under certain metabolic conditions [45]. PRELI (protein of relevant evolutionary and lymphoid interest) is the human orthologue of Ups1 [46], but whether it fulfills a similar function in mammals is unknown.

## Importance of mitochondrial transport and subcellular distribution for neuronal function

In neurons, mitochondria are particularly abundant at synapses and in unmyelinated axon segments of the

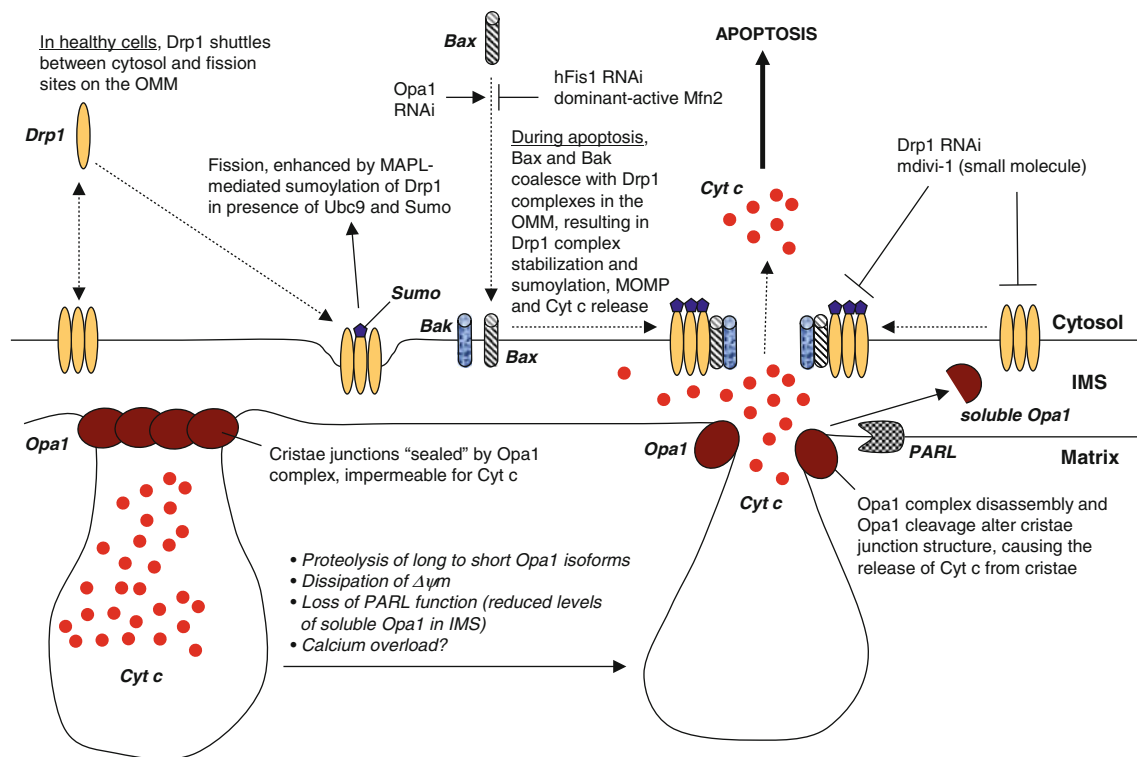
peripheral nervous system [47–50]. Mitochondria concentrate and are recruited to subcellular regions with high metabolic requirements, for example in the vicinity of active growth cones in neurons [51]. Pharmacological and genetic studies have demonstrated that mitochondria regulate neuronal activity, synaptic plasticity and neurotransmitter release by controlling synaptic  $\text{Ca}^{2+}$  concentrations and ATP-dependent vesicle movements [47, 52–59]. In addition, manipulation of Drp1 and Opa1 function that reduced dendritic mitochondrial content in hippocampal neurons caused the loss of synapses and dendritic spines, demonstrating the importance of subcellular mitochondrial positioning for the development and morphological plasticity of neuronal processes [51]. Trafficking of mitochondria in neurons relies on the molecular motor proteins kinesin-1 (KIF1B) and kinesin-3 (KIF5B) as well as cytosolic dynein [10, 49, 60]. In *Drosophila*, the two proteins Miro and Milton function as an adaptor complex that anchors mitochondria to kinesin-1 [53, 58, 61]. Miro is a GTPase anchored to the outer mitochondrial membrane, whereas Milton is a cytosolic protein that binds to Miro and kinesin heavy chain [61]. Mammalian Miro recruits Grif-1 (the mammalian homologue of Milton) to mitochondria in a GTPase-dependent way, resulting in enhanced transport of mitochondria towards the distal ends of hippocampal processes [62]. Miro also plays an important role as a calcium sensor and the regulation of calcium-dependent mitochondrial transport [63, 64]. The interaction of Miro with kinesin is calcium-sensitive, and increased  $\text{Ca}^{2+}$  concentrations in the physiological range disrupt the interaction of Miro with KIF5, resulting in mitochondrial arrest [64]. In addition, upon activation of *N*-methyl-*D*-aspartate (NMDA) receptors Miro positioned mitochondria at the postsynaptic site, where calcium concentrations and energy demand were high [64]. *Drosophila dMiro* mutants retained their mitochondria in the neuron soma and failed to distribute the organelles to axons, whereas *dMiro* overexpression resulted in the accumulation of mitochondria in terminal boutons of the fly neuromuscular junction [53]. In mammalian cells, overexpression of Miro increased mitochondrial trafficking in both anterograde and retrograde direction by increasing the overall proportion of mobile mitochondria [65]. In contrast, RNAi-mediated Miro gene silencing decreased the number of mobile mitochondria [62]. Collectively, these experiments show that Miro and Milton regulate mitochondrial positioning to sites of increased energy usage in a calcium-dependent manner [66, 67]. Other recently identified proteins affecting mitochondrial transport are syntabulin, which connects mitochondria to KIF5B and is involved in activity-dependent anterograde transport of mitochondria to presynaptic sites [68, 69] and syntaphilin, which serves as a docking receptor for mitochondria in axons and may allow an

increased concentration of mitochondria to be maintained in the synapse [50]. Finally, both ectopic expression and knockdown of the E3 ubiquitin ligase MULAN interfered with mitochondrial trafficking and morphology, suggesting that mitochondrial transport is also regulated by ubiquitin-dependent processes [70].

### Regulation of apoptosis by mitochondrial fission and fusion proteins

Apoptosis is accompanied by changes in mitochondrial morphology. Often, mitochondrial fission is an early event during apoptosis and it has been shown that silencing of hFis results in increased net fusion and resistance to staurosporine-, actinomycin D- or anti-Fas-induced apoptosis [71]. Likewise, inhibiting mitochondrial fission by expression of dominant-negative Drp1<sup>K38A</sup> decreased apoptosis, albeit to a lesser degree [71]. Silencing of hFis1 expression blocked apoptosis by inhibiting the translocation of cytosolic Bax to mitochondria [71], while Drp1<sup>K38A</sup> reduced cytochrome *c* release and apoptosis without significantly blocking Bax translocation [71, 72]. However, although mitochondrial fission often coincides with apoptosis, fission per se is dispensable for apoptosis [73, 74]. In addition, fission and the release of cytochrome *c* and other pro-apoptotic molecules can occur independently from each other. First, cells depleted of both hFis1 and Opa1 were resistant to apoptosis despite showing extensive mitochondrial fission [71]. Second, a recently developed chemical Drp1 inhibitor (mdivi-1) was shown to block mitochondrial outer membrane permeabilization (MOMP) induced by cleaved Bid in a cell-free in vitro assay with purified mitochondria, in which mitochondrial fission was unlikely to occur [75]. Mdivi-1 prevents the assembly of Drp1 complexes on the outer membrane of mitochondria, suggesting that Drp1 may be important for the induction of MOMP independent of mitochondrial fission [75, 76]. Third, although the anti-apoptotic proteins Bcl-x<sub>L</sub> and Mcl-1 could inhibit the release of cytochrome *c* in cells overexpressing Bax/Bak, they did not reverse the mitochondrial fragmentation, indicating that MOMP and mitochondrial fission are separable events [77, 78]. It has been shown that Bax and Bak colocalize with mitochondrial fission sites, Drp1 and Mfn2 during apoptosis [79]. These Bax/Bak-dependent apoptotic fission complexes are very stable and coincide with Drp1 sumoylation [80]. Taken together, these data implicate Drp1 in the regulation of apoptosis through influencing MOMP. MOMP may be accompanied or enhanced by mitochondrial fission, but does not strictly depend on it [74] (Fig. 1).

In contrast, mitochondrial fusion proteins conferred protection against apoptosis [71, 72, 81, 82]. For example,



**Fig. 1** Mitochondrial fission and fusion proteins in apoptosis. Mitochondrial outer membrane permeabilization (MOMP) is stimulated by Bax/Bak-dependent formation of Sumo-modified Drp1 complexes at the OMM. In healthy cells Drp1 shuttles between mitochondrial fission sites and the cytosol. At the beginning of apoptosis, Drp1 gets locked to the OMM and forms stable, Bax/Bak-dependent complexes that promote MOMP. In addition, Bax and Bak become colocalized with Drp1 complexes as well as Mfn2 punctae on the OMM. Bax-dependent sumoylation of Drp1 coincides with apoptosis and may be required for Drp1 complex stabilization, suggesting that Sumo modification of Drp1 may enhance apoptosis. However, Drp1 sumoylation also stimulates fission, which can occur during apoptosis but is not a prerequisite for cell death. Genetic manipulations (Drp1 RNAi) or chemical inhibitors (mdivi-1) that reduce Drp1 complex formation or stability decrease MOMP. Silencing of hFis1 expression and expression of a dominant-active form of the fusion protein Mfn2 inhibit translocation of Bax to

mitochondria and Bax activation, while Opa1 RNAi has the opposite effect. Therefore, manipulations that increase net fusion reduce the release of cytochrome c and apoptosis, while fission proteins can increase apoptosis. In normal cells, an Opa1 complex at the IMM maintains the cristae junctions impermeable for cytochrome c, thereby preventing most of the cytochrome c molecules from reaching the OMM, where they can be released into the cytosol after MOMP. During apoptosis, the Opa1 complex becomes disassembled in a Bax/Bak-dependent manner, which results in a structural change of the cristae junctions and the release of the cristae cytochrome c pool into the remainder of the IMS, with access to OMM "pores". Opa1 complex disassembly may also occur upon dissipation of  $\Delta\psi_m$  and proteolytic processing, which correlates with conversion of longer to shorter Opa1 isoforms. In addition, excess mitochondrial calcium may promote Opa1 complex disassembly. For details and references, please see main text

knocking down Opa1 expression enhanced sensitivity to apoptosis, characterized by Bax translocation to mitochondria, release of cytochrome c, nuclear fragmentation and caspase activation [71]. Furthermore, a dominant-active mutant of Mfn2 with reduced GTP hydrolysis activity promoted increased fusion and interfered with the translocation and activation of Bax, thereby protecting mitochondria against free radical-induced permeability transition [72]. Finally, several studies showed that Opa1, through its role in maintaining cristae structure and junctions, attenuates the release of cytochrome c from mitochondria [81, 83, 84]. Cristae are tubular structures that trap about 85% of the cytochrome c molecules within the folds of the IMS, with their "neck" or junction stabilized

by a complex of Opa1 oligomers (Fig. 1). Frezza et al. showed that induction of apoptosis by Bid correlated with the disruption of Opa1 oligomers that keep the cristae junctions impermeable for cytochrome c, indicating remodeling of an Opa1 complex at the cristae junction during apoptosis [85]. Yamaguchi et al. confirmed this result, although in this study a narrowing of the cristae junctions was observed after Opa1 complex disassembly [86]. In addition, MOMP occurred under these conditions, as expected [86]. Opa1 complex disassembly and MOMP resulted in the release of cytochrome c, Smac/DIABLO and Opa1, which could be inhibited by small molecule drugs against serine proteases of the OMM [86]. Interestingly, two of these drugs were able to block MOMP but failed to

inhibit the disassembly of Opa1 complexes [86]. In addition, overexpression of Opa1<sup>Q297V</sup>, a mutant that is resistant to disassembly, inhibited cristae junction remodeling, cytochrome *c* release and apoptosis in transiently transfected 293T cells, without blocking activation of Bax and induction of MOMP [86]. Collectively, these results show that Bax/Bak-induced disassembly of Opa1 complexes and MOMP can occur independently from each other, and that both events are necessary for the release of the complete mitochondrial pool of cytochrome *c* and Opa1 [84, 86] (Fig. 1). Although cristae junction remodeling and MOMP can occur independently, it has been hypothesized that activated Bax/Bak act through some OMM proteins that transmit the signal to Opa1 at the IMM, either directly or indirectly via a protein in IMS [86]. In an earlier study it was shown that Bik-induced cristae remodeling could be inhibited by expression of dominant-negative Drp1<sup>K38E</sup> [87], indicating that Drp1 may relay the death signal to Opa1 in the IMM. In addition, inhibition of mitochondrial calcium uptake also interfered with Bik-induced opening of cristae [87]. In thapsigargin-treated cells undergoing ER stress, Opa1 and cytochrome *c* release required mitochondrial permeability transition (MPT) and Bax, as well as the matrix peptidyl-prolyl-isomerase cyclophilin-D [88]. However, because calcium-induced MPT also occurred with purified mitochondria stripped of their outer membrane and the voltage-dependent anion channel, it was proposed to be an inner membrane phenomenon directly affecting Opa1 release [88].

Additional regulation of Opa1 function can occur through post-transcriptional and post-translational mechanisms. The Opa1 primary transcript undergoes alternative splicing [89] and at least five Opa1 protein isoforms have been detected in mammalian cells [90]. Based on exon-specific RNAi experiments, it has been suggested that Opa1 transcripts containing exon 4 are important for mitochondrial fusion, while mRNAs containing exons 4b or 5b encode Opa1 isoforms that regulate cytochrome *c* release [91]. The IMM protease presenelin-associated rhomboid-like (PARL) is involved in the generation of a soluble Opa1 isoform present in the IMS [92]. Mitochondria from PARL-deficient mice display faster cristae remodeling and cytochrome *c* release during apoptosis, showing that Opa1 processing influences apoptosis [92, 93]. Consistent with this idea, it has recently been shown that deletion of the *prohibitin-2* (*Phb2*) gene in mice, which leads to the selective loss of long isoforms of Opa1 and aberrant cristae morphogenesis, renders cells more vulnerable to apoptosis [94, 95]. However, prohibitins most likely act as scaffolding proteins at the IMM and are not proteases themselves, and their effect on Opa1 processing is thus indirect. Finally, the expression of the mitochondrial AAA protease paraplegin and dissipation of  $\Delta\psi_m$  resulted

in the proteolytic conversion of long to short Opa1 isoforms and mitochondrial fragmentation [96, 97]. However, RNAi experiments in HeLa cells showed that only Yme1, another m-AAA protease, is required for the constitutive processing of a subset of longer Opa1 isoforms and that Yme1 depletion results in increased fusion and mitochondrial interconnectivity [98].

In summary, Opa1 plays a critical role in the regulation of the structure and tightness of cristae junctions, where Opa1 complex disassembly at the IMM causes the release of cytochrome *c* from cristae. Cristae junction remodeling may be further regulated through constitutive and induced processing of Opa1 by mitochondrial proteases and  $\Delta\psi_m$ . Several other proteins affecting mitochondrial dynamics have been implicated in apoptosis regulation. These factors, and a more detailed overview of the relationship of mitochondrial structure and apoptosis have been described recently in reviews specifically dedicated to this topic [11, 99].

### Mitochondrial function and dynamics in Parkinson's disease

Rapidly growing evidence implicates impaired mitochondrial function and dynamics in the pathogenesis of PD. Data supporting a causal relationship between abnormal mitochondrial function and dynamics, cell death and dopaminergic neuron degeneration has come from a host of studies that addressed the functions of Parkin, PINK1 and other proteins linked to recessive familial Parkinsonism in cultured cells, flies and mice [100–102]. Collectively, these experiments have shown that Parkin and PINK1 not only influence the function and morphology of mitochondria, but also promote the degradation of dysfunctional organelles and possibly regulate the transport of mitochondria in axons. In the following sections, I will discuss the activities of Parkin and PINK1 relevant to these processes, and describe metabolic and anatomic features of the dopaminergic system that may explain its exquisite vulnerability to perturbations in mitochondrial function and dynamics.

Parkin maintains mitochondrial integrity and protects against oxidative and unfolded protein stress

Mutations in the *Parkin* (*PARK2*) gene are associated with autosomal recessive juvenile Parkinsonism (AR-JP) [103, 104]. The E3 ubiquitin protein ligase Parkin plays a role in the proteasome-mediated turnover of several proteins in vitro [105–108], some of which accumulate in the brains of AR-JP patients and/or mice carrying a targeted deletion of the *Parkin* gene, showing that they are authentic Parkin substrates in vivo [109–111]. *Drosophila* lacking Parkin

display increased sensitivity to oxidative stress, dopaminergic neuron loss, and severe structural mitochondrial abnormalities in muscle and germline tissues associated with apoptotic muscle degeneration and male sterility [112–114]. Ablation of Parkin in mice resulted in respiratory defects, reduced expression of subunits of respiratory complexes I and IV, decreased levels of anti-oxidant proteins, increased protein and lipid peroxidation [115], and abnormalities in dopamine neurotransmission, but no loss of dopaminergic neurons [116, 117]. Parkin-deficient zebrafish showed selective dopamine neuron loss associated with complex I impairment [118]. Fibroblasts from patients with *Parkin* mutations showed reduced mitochondrial connectivity, complex I activity and ATP synthesis, and were more sensitive to rotenone-induced death [119]. In agreement with these findings, Parkin overexpression increased mitochondrial membrane potential, selectively enhanced the expression of mitochondrial complex I subunits and reduced accumulation of reactive oxygen species (ROS) in mitochondria of transfected cells [120]. Interestingly, transgenic flies overexpressing familial PD-linked mutants of Parkin (R275 W and Q311X) also exhibited dopaminergic neurodegeneration, vacuolization of mitochondria, concentric membranous structures within mitochondria and disruption of cristae [121, 122]. Likewise, age-dependent loss of nigral dopaminergic neurons and striatal dopamine fibers were observed in BAC-transgenic mice expressing Parkin<sup>Q311X</sup>, along with accumulation of proteinase K-resistant  $\alpha$ -synuclein and progressive hypokinesia [123]. These studies suggest that certain *Parkin* mutations may cause dopamine neurodegeneration by a toxic gain-of-function.

Recently, Parkin has been linked to DNA repair [124, 125]. Parkin-deficient fibroblasts exhibited reduced DNA excision repair in assays where a luciferase reporter construct, which had been mutagenized in vitro by UV light or incubation with H<sub>2</sub>O<sub>2</sub>, was transfected into wildtype or Parkin<sup>-/-</sup> fibroblasts [124]. The defect in DNA excision repair could be restored by cotransfection of wildtype, but not pathogenic mutant Parkin, into Parkin<sup>-/-</sup> cells [124]. As a possible mechanism, it was shown that Parkin interacts with the proliferating cell nuclear antigen (PCNA), which coordinates DNA excision repair, and that inhibiting this interaction with a dominant-negative Parkin fragment also reduced the ability of Parkin to stimulate DNA repair [124]. A recent study with SH-SY5Y neuroblastoma cells showed that overexpression of Parkin enhanced replication and transcription of mitochondrial DNA, and protected mitochondrial DNA against oxidative damage by stimulating mitochondrial DNA repair [125].

Taken together, these data implicate Parkin in several diverse neuroprotective pathways, including ubiquitin-dependent degradation of toxic proteins, inhibition of

oxidative stress, maintenance of mitochondrial structure, function and DNA integrity, as well as the stimulation of presynaptic dopamine neurotransmission.

PINK1 acts as a kinase and protects against oxidative stress

Homozygous mutations in the *PARK6* gene, that encodes the protein PTEN-induced kinase 1 (PINK1), are the second most frequent cause for autosomal recessive early-onset Parkinsonism (EOPD) [126–131]. Recently, certain compound heterozygous *PINK1* mutations have been found in patients with late-onset sporadic PD [132]. PINK1 is a ubiquitously expressed serine/threonine kinase with a short N-terminal mitochondrial targeting sequence that directs import of PINK1 into mitochondria [126, 133–137]. However, PINK1 has also been detected in the cytosol [133, 134, 138, 139], and one group showed substantial amounts of endogenous PINK1 in the microsomal fraction co-localized with calnexin, suggesting an association with the endoplasmic reticulum [139]. The formation of the cytosolic PINK1 isoforms depends on import and processing of full-length PINK1 within mitochondria [140]. Within mitochondria, PINK1 has been localized both to the IMM facing the IMS [133, 140], and the OMM with the kinase domain facing the cytosol [141]. It has been shown that EOPD-associated mutations at least partially reduce PINK1 kinase activity, either by directly affecting the kinase domain or destabilizing PINK1 [134, 142, 143]. However, familial PD mutations do not interfere with mitochondrial import of PINK1 [133, 134]. Three PINK1 substrates have been identified to date. These are the mitochondrial protease HtrA2 [144], the mitochondrial chaperone TRAP1 (Hsp75) [142] and Parkin [145]. Both HtrA2 and TRAP1 are present in the IMS, while most of Parkin is located in the cytosol. HtrA2 is phosphorylated by PINK1 on serine 142 in response to the activation of the p38 stress-signaling pathway, and phospho-mimetic HtrA2 mutants suggest that this phosphorylation increases HtrA2 protease activity [144]. Increased HtrA2 protease activity may mediate neuronal survival under stress, as suggested by the phenotype of *HtrA2* knockout mice, which develop neurodegeneration in the striatum, leading to a disorder with a Parkinsonian phenotype and death at 1 month of age [146]. Additionally, the observation that phosphorylation of serine 142 of HtrA2 is significantly reduced in the brains of PD patients with *PINK1* mutations shows that HtrA2 is a true PINK1 substrate in vivo [144]. Phosphorylation of TRAP1 by PINK1 is necessary to protect cultured cells against oxidative stress-induced cytochrome c release and apoptosis [142], and overexpression of TRAP1 in the rat brain reduced oxidative stress and infarct size after focal ischemia and improved mitochondrial function under these

conditions [147]. Finally, in cells PINK1 directly phosphorylates Parkin on threonine 175, which causes a striking redistribution of Parkin from the cytosol to mitochondria [145]. As discussed later, PINK1 and Parkin function in a common pathway to maintain the integrity of the mitochondrial network, by preventing mitochondrial dysfunction (PINK1, Parkin) and promoting degradation of defective mitochondria through autophagy (Parkin).

#### Effects of PINK1 ablation on mitochondrial function

Several groups have generated mammalian cells with stable or transient PINK1 gene silencing by RNA interference (RNAi). Characterization of these cells revealed reduced mitochondrial membrane potential [148–151], increased oxidative stress [149, 151–153], increased sensitivity to Parkinsonian toxins and complex I inhibitors [150, 154–156], reduced respiration and ATP synthesis [153, 157, 158], decreased mitochondrial DNA levels and respiration [149] and proteasomal deficits associated with  $\alpha$ -synuclein aggregation [158]. Mitochondrial defects were also detected in fibroblasts and immortalized lymphoblasts from PD patients with G309D and W437Stop PINK1 mutations, which displayed lower respiratory activity, decreased activity of cytochrome c oxidase and elevated levels of lipid peroxidation [159, 160]. Recently, it has been shown that mitochondria of PINK1-deficient neurons accumulate higher basal levels of  $\text{Ca}^{2+}$  in the matrix (due to reduced calcium efflux capacity) and display reduced mitochondrial  $\text{Ca}^{2+}$  storage capacity associated with  $\text{Ca}^{2+}$  overload [151]. As a consequence, PINK1-deficient neurons were significantly more vulnerable to  $\text{Ca}^{2+}$ -induced loss of  $\Delta\psi_m$  and subsequent permeability transition pore opening [151]. In addition, increased production of ROS was observed secondary to mitochondrial  $\text{Ca}^{2+}$  overload [151].

#### Effects of PINK1 ablation on mitochondrial morphology

The consequences of *PINK1* gene silencing on mitochondrial morphology and dynamics have been controversial and apparently contradictory. However, several groups have observed inner membrane abnormalities, such as decreased cristae density and disorganized or swollen cristae [148–150, 152]. Enlarged and swollen mitochondria were detected in human neurons after PINK1 gene silencing by RNAi [149]. In COS7 cells, which possess dynamic mitochondrial networks overexpression of PINK1 increased the number of cells with fragmented mitochondria [161], whereas simultaneous overexpression of PINK1 and dominant-negative Drp1<sup>K38A</sup> resulted in more cells with long tubular mitochondria [161]. Likewise, several

lines of PINK1-deficient *Drosophila* developed prominent mitochondrial abnormalities, such as fragmented cristae and hollow-appearing mitochondria, swollen and enlarged mitochondria, as well as dopamine neuron death and apoptotic muscle degeneration [162–164]. Overexpression of the mitochondrial fission protein Drp1 rescued mitochondrial abnormalities and other defects in flies, while heterozygous loss-of-function mutations in the *drp1* gene were mostly lethal in the absence of PINK1 or Parkin. In contrast, reduced expression of the mitochondrial fusion proteins Opa1 and Mfn2 significantly suppressed muscle degeneration and abnormal mitochondrial morphology in the absence of PINK1 and Parkin [161, 164, 165]. These results show that, in *Drosophila* and certain mammalian cells, shifting the net mitochondrial dynamics towards fission is protective, while decreased fission has the opposite effect. This has led to the suggestion that PINK1 promotes fission and/or inhibits fusion [165].

However, inconsistent with this interpretation, several groups have reported increased mitochondrial fission in cells lacking PINK1 [148, 150, 152]. For example, human M17 neuroblastoma cells with a lentiviral vector-mediated stable PINK1 knockdown exhibited mitochondrial fragmentation, which was exaggerated by expression of Drp1 and rescued by Drp1 RNAi, suggesting that PINK1 antagonizes mitochondrial fission and/or promotes fusion [150]. The same authors also showed that the amount of Ser-637 phosphorylated Drp1 is reduced by about 30% in PINK1-deficient cells due to the activation of the calcium-dependent phosphatase calcineurin [150]. Moreover, the calcineurin inhibitor FK506 blocked both Drp1 dephosphorylation and mitochondrial fragmentation [150]. Dephosphorylation of Drp1 by calcineurin was shown by others to enhance the translocation of Drp1 to mitochondria and promote fission [26]. However, despite increased calcineurin activity and reduced Drp1 phosphorylation, the mitochondrial levels of Drp1 were not higher in M17 cells with silenced PINK1 expression compared to control cells [150]. This suggests that mitochondrial fragmentation in these cells is not a consequence of increased mitochondrial Drp1 recruitment. However, silencing of PINK1 was associated with increased GTPase activity of Drp1 in vitro [150], which may increase fission in vivo. Another group recently showed that silencing of PINK1 by siRNAs resulted in a higher percentage of cells containing truncated and fragmented mitochondria [157]. These alterations in mitochondrial morphology were associated with reduced ATP synthesis and could be reversed by overexpression of Mfn2, Opa1 or dominant-negative Drp1<sup>K38E</sup> [157]. Collectively, these experiments show that lack of PINK1 in cultured cells results in mitochondrial fragmentation that depends on the fission protein Drp1 and can be ameliorated by overexpression of fusion proteins Mfn2 and Opa1 [157].

In mice, deletion of the PINK1 gene resulted in reduced evoked dopamine release and impaired corticostriatal long-term potentiation and long-term depression, in the absence of dopaminergic neuron degeneration [166]. Furthermore, mice devoid of PINK1 exhibit selective mitochondrial defects in the striatum, with an increased number of large mitochondria, impaired activities of respiratory complexes I and II, and reduced aconitase activity [167]. In addition, an age-dependent functional decay of cortical mitochondria was observed [167]. Respiration of partially purified cortical mitochondria was reduced more significantly by oxidative stress and heat shock in the absence of PINK1 [167], suggesting an increased sensitivity of PINK1-deficient mitochondria to exogenous stressors. Mitochondria from mice expressing low levels of abnormal PINK1 transcripts as a result of the attempt to target the G309D mutation to the endogenous *PINK1* gene exhibited progressive reduction in mitochondrial preprotein import and reduced respiration, as well as increased aggregation of mitochondria under proteasomal stress [168]. Primary neurons from PINK1<sup>-/-</sup> mice, and human dopaminergic neurons with silenced PINK1 expression develop widespread mitochondrial dysfunction, such as reduced  $\Delta\psi_m$  and morphological abnormalities, increased oxygen radical production and heightened vulnerability to apoptosis [149]. Interestingly, human neurons with silenced PINK1 expression showed compensatory adaptations, including increased numbers of mitochondria, age-dependent elevated expression of specific respiratory complexes, and increased total citrate synthase activity [149].

#### Relationship of mitochondrial function and dynamics and pathways affected in PINK1-deficient cells

Because mitochondrial function and dynamics influence each other, it is difficult to study whether mitochondrial dysfunction in PINK1-deficient cells is a cause or consequence of abnormal mitochondrial morphology. However, the discrepant findings with respect to mitochondrial morphology in cells and animals lacking PINK1, as discussed above, suggest that alterations in mitochondrial morphology may occur secondary to mitochondrial dysfunction.

Several groups observed a reduced  $\Delta\psi_m$  in cells devoid of PINK1 (see above). Changes in  $\Delta\psi_m$  play important roles in regulating size and axonal transport of mitochondria [169], and chemical uncouplers that disrupt the  $\Delta\psi_m$  block mitochondrial transport [170]. In cultured embryonic chicken dorsal root ganglion neurons, 90% of mitochondria with high  $\Delta\psi_m$  were transported towards the growth cone, whereas 80% of mitochondria with low  $\Delta\psi_m$  were transported towards the cell body, demonstrating that the  $\Delta\psi_m$  determines the direction of mitochondrial transport in these

neurons [170]. While FK506 rescued mitochondrial fragmentation in M17 human neuroblastoma with silenced PINK1 expression (presumably by inhibition of calcineurin-mediated dephosphorylation of Drp1), it only partially ameliorated the deficit in  $\Delta\psi_m$ , supporting the view that the alterations in mitochondrial connectivity occurred secondary to the impaired  $\Delta\psi_m$  [150].

Abnormal cristae structure is another defect frequently observed in PINK1-deficient cells (see above). PINK1 was recently shown to interact with mitofilin [171], a mitochondrial IMS protein that acts as an organizer of cristae morphology and junctions [172]. This suggests the possibility that PINK1 controls cristae structure via mitofilin. In the absence of PINK1, abnormal cristae morphology and junctions may enhance the vulnerability of cells to apoptosis.

Dysregulated mitochondrial calcium homeostasis may also contribute to changes in mitochondrial dynamics. Calcium efflux from mitochondria is compromised in PINK1-deficient mammalian neurons due to a defect of the mitochondrial Na<sup>+</sup>/Ca<sup>2+</sup> exchanger [151]. This results in mitochondrial calcium overload and in turn increased ROS production via activation of NADPH oxidase, inhibition of the glucose transporter and impaired respiration [151]. Moreover, mitochondria from PINK1-deficient neurons were more susceptible to calcium-induced opening of the mitochondrial permeability transition pore [151]. Interestingly, increased mitochondrial fission caused by overexpression of Drp1 protected against calcium-induced mitochondrial abnormalities and subsequent apoptosis [173]. This raises the possibility that mitochondrial fragmentation in PINK1<sup>-/-</sup> cells may be a protective, adaptive response to mitochondrial calcium overload.

Lastly, oxidative stress could affect mitochondrial dynamics in PINK1-deficient cells. Mitochondrial fragmentation and upregulation of autophagy in PINK1<sup>-/-</sup> cells were mediated by mitochondrial oxidant production [152]. Mitochondria from the cerebral cortex of PINK1<sup>-/-</sup> mice were more susceptible to H<sub>2</sub>O<sub>2</sub>-induced respiratory defects, suggesting reduced ROS resistance of PINK1-deficient mitochondria [167]. It has recently been proposed that PINK1 may have a critical function in the oxidative stress defense downstream of PI3 kinase/Akt signaling and activation of FOXO transcription factors [174], which play important roles in protecting cells against oxidative stress via upregulation of several antioxidant enzymes [175]. FOXO3a binds to the *PINK1* promoter and activates transcription of the *PINK1* gene [174]. Transcriptional activation of *PINK1* by FOXO3a attenuates the loss of reduced glutathione (GSH) in cytokine-deprived lymphocytes and increases lymphocyte survival [174]. Consistent with these results, GSH levels and mitochondrial respiration were reduced in cells after PINK1 gene silencing [153], and the



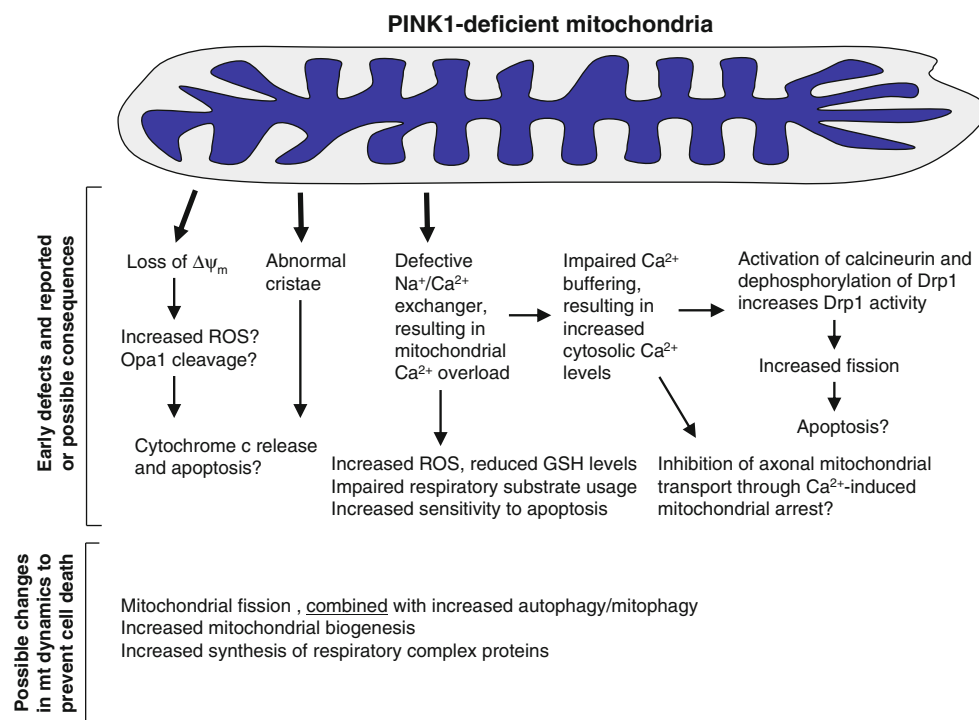
amount of oxidized glutathione (GSSG) was significantly elevated in fibroblasts from PD patients harboring the familial G309D PINK1 mutation [159].

In summary, a major role of PINK1 is to maintain mitochondrial integrity by preventing calcium- and oxidative stress-induced mitochondrial dysfunction, loss of  $\Delta\psi_m$ , and abnormal cristae structure (Fig. 2). In the absence of PINK1, impaired mitochondrial function may lead to secondary changes in mitochondrial dynamics, such as mitochondrial fragmentation, which may require increased Drp1 activity. While excessive fission likely exaggerates mitochondrial defects [150], limited fission can have protective effects as seen in *Drosophila* [161, 164, 165]. This may be attributed to the stimulatory effects of fission on mitophagy [176], respiration and ATP synthesis [177, 178], maintenance of mitochondrial DNA [178] and the transport of mitochondria to synapses during synaptogenesis [179]. It is not clear whether fusion may have been activated in those PINK1-deficient cell lines with swollen and enlarged mitochondria [149] and in

*Drosophila* lacking PINK1. Fusion inhibits mtDNA abnormalities [180, 181] and is neuroprotective [182]. However, PINK1-deficient flies developed dopaminergic neuron loss and muscle degeneration, suggesting that the enlarged mitochondrial phenotype may have been caused by a mechanism other than enhanced fusion.

PINK1 and Parkin cooperate in the elimination of dysfunctional mitochondria and unfolded proteins

Flies lacking Parkin and PINK1 showed strikingly similar phenotypes, including dopaminergic neuron loss, apoptotic muscle degeneration and severe morphological defects of the mitochondria. While Parkin was able to rescue all phenotypes of PINK1-deficient flies, PINK1 was ineffective in complementing the defects in Parkin<sup>-/-</sup> flies [162, 163, 183]. Overexpression of Parkin also rescues mitochondrial fragmentation in PINK1 knockdown cells [148, 152, 157], but not vice versa [157]. These results establish that PINK1 and Parkin function in a common pathway to maintain



**Fig. 2** Early mitochondrial defects and postulated downstream effects and compensatory mechanisms in cells devoid of PINK1. Early defects likely include impaired  $\Delta\psi_m$ , abnormal cristae morphology and calcium overload, resulting in increased mitochondrial ROS production, decreased respiration and enhanced sensitivity to apoptosis due to facilitated membrane permeability transition. Increased activity of the calcium-dependent phosphatase calcineurin has been detected in cells with PINK1 RNAi, resulting in Drp1 dephosphorylation and increased GTPase activity, which may lead to enhanced fission. In addition, the disrupted mitochondrial calcium homeostasis in PINK1-deficient neurons may block mitochondrial

transport in neurons through calcium-dependent inhibition of Miro. Upregulation of autophagy as well as increased mitochondrial biogenesis and respiratory complex synthesis have been observed in neurons lacking PINK1, and proposed to be protective compensatory mechanisms. Fragmented mitochondria have also been found frequently, though not always, in PINK1<sup>-/-</sup> cells. Mitochondrial fragmentation may serve a protective function by facilitating the degradation of dysfunctional mitochondria, especially in conjunction with increased autophagy. However, excessive fission may cause apoptosis. For details and references, please see main text

mitochondrial integrity and dynamics, with Parkin acting downstream of PINK1.

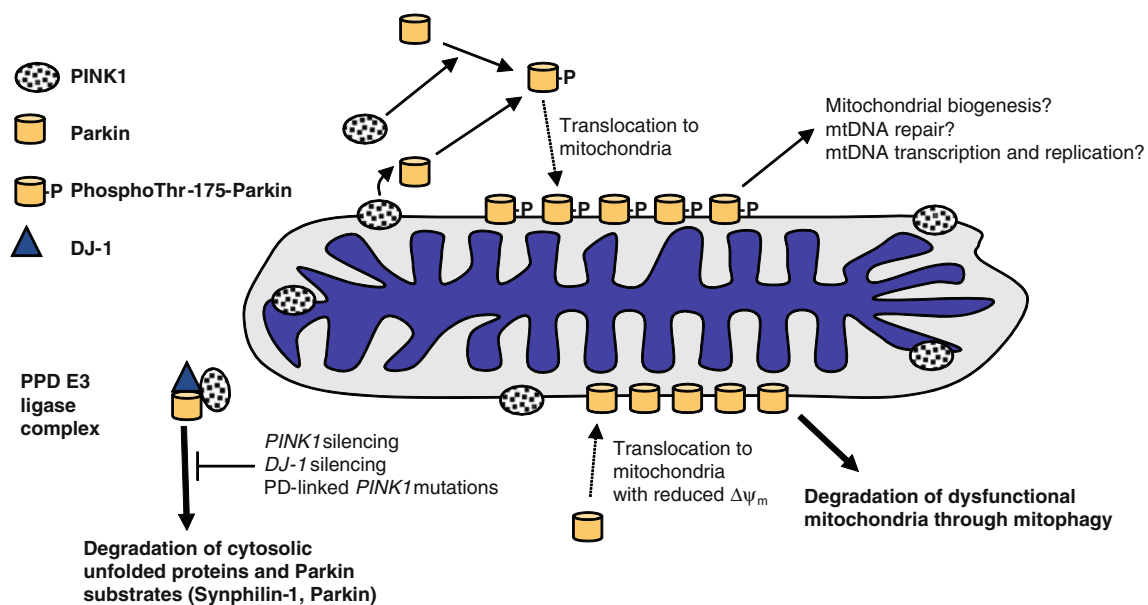
Parkin may rescue the mitochondrial defects in  $PINK1^{-/-}$  flies and cells by various ways, and the stimulation of autophagocytic degradation of functionally impaired mitochondria is likely one of them. Autophagy is an intracellular, lysosome-mediated degradation pathway for damaged proteins, organelles and membranes [184]. Autophagy is essential for neuronal survival, as shown by the fact that mice with neuron-specific deletions of the core autophagy genes *Atg5* and *Atg7* develop neurodegeneration, progressive deficits in motor function, accumulation of ubiquitin-positive inclusions and premature death [185, 186]. It has been demonstrated that Parkin is selectively recruited to dysfunctional mitochondria with low  $\Delta\psi_m$ , promoting their engulfment and destruction in autophagosomes [187, 188]. This suggests that impaired removal of defective mitochondria may underlie the pathogenesis of AR-JP and possibly sporadic PD. Accordingly, a major function of Parkin is to stimulate mitophagy, and it has been proposed that Parkin may ubiquitinate as yet unknown mitochondrial proteins [189], thereby targeting mitochondria to autophagosomes in a manner similar to ubiquitin-tagged peroxisomes [190]. However, further studies with cells expressing ligase-deficient Parkin only, and identification of mitochondrial Parkin substrates will be necessary to confirm that ubiquitination plays a role in this process.

A recent overexpression study in human neuroblastoma cells revealed that PINK1 controls the translocation of Parkin from the cytosol to mitochondria through direct phosphorylation of Parkin on threonine 175 [145]. Recruitment of Parkin to the mitochondrial surface depended on the PINK1 kinase activity, implicating phosphorylation as an obligatory step [145]. In contrast, PINK1 gene silencing led to significantly lower amounts of Parkin in the mitochondria, and endogenous Parkin levels were also dramatically reduced in mitochondria from PINK1-deficient *Drosophila* compared to normal flies [145]. Phosphorylation of Parkin by PINK1 is conserved in flies (threonine 187 in *Drosophila* Parkin) and necessary for full rescue of the mitochondrial deficit in Parkin-deficient flies [145], suggesting that PINK1-mediated recruitment of Parkin to mitochondria serves a protective function. However, it is unlikely that PINK1-dependent redistribution of Parkin to mitochondria also enhances mitophagy, because overexpression of PINK1 suppressed injury-induced autophagy [152]. Therefore, phosphorylated Parkin in mitochondria likely has other roles. Possible functions of phosphorylated Parkin include replacing the degraded mitochondria by enhancing mitochondrial biogenesis [120] and/or limiting mitochondrial damage through the stimulation of mitochondrial DNA repair,

replication and transcription [125] (Fig. 3). One possibility is that the PINK1 kinase activity towards Parkin increases upon activation of certain stress signaling pathways, resulting in enhanced recruitment of Parkin to mitochondria under conditions that might harm these organelles. This in turn could allow Parkin to repair damaged mitochondria and replace discarded organelles by new ones. Consistent with this idea, it has been shown that the activation of p38 kinase enhances PINK1-mediated phosphorylation of HtrA2 [144]. In the absence of PINK1, no phosphorylated Parkin would reach the mitochondria in stressed cells, potentially causing the propagation of faulty organelles. However, the reduced  $\Delta\psi_m$  in  $PINK1^{-/-}$  cells may trigger enough (unphosphorylated) Parkin to associate with defective mitochondria [187], resulting in their autophagocytic destruction. Nonetheless, the autophagy system would ultimately be overwhelmed by the mass of defective mitochondria, and neuron death would ensue.

Impaired autophagy may indeed be central to PD pathogenesis [191], because several other PD genes interact with autophagy. Alpha-synuclein, which aggregates in Lewy bodies in sporadic and dominant familial PD [192–194], is degraded by both macroautophagy and chaperone-mediated autophagy (CMA) [195]. Familial PD mutants of  $\alpha$ -synuclein bind to a lysosomal cargo receptor, thereby inhibiting their own degradation as well as the degradation of other CMA substrates in lysosomes [196]. In addition, DJ-1, linked to recessive EOPD [197], has been implicated in paraquat-induced autophagy [198].

CMA is particularly important for the degradation of cytosolic proteins [199] and, in conjunction with ubiquitin-dependent protein degradation by the proteasome, serves to prevent the accumulation and aggregation of misfolded and toxic proteins in cells. Recently, an ubiquitin E3 ligase complex comprised of Parkin, PINK1 and DJ-1 (termed PPD complex) has been identified in cells and human brain lysates, and shown to promote the degradation of unfolded proteins and Parkin substrates, such as Synphilin-1 [200]. Overexpression of PINK1 in neuroblastoma cells significantly enhanced Parkin-mediated degradation of heat shock-induced misfolded proteins, while genetic ablation of either *PINK1* or *DJ-1* resulted in increased accumulation of Parkin substrates in heat-shocked, but not normal, neurons [200]. In addition, both PINK1 and Parkin suppressed the toxicity of  $\alpha$ -synuclein in cell and animal models of PD [201–204]. Collectively, these results suggest yet another mechanism by which PINK1 and Parkin cooperate to confer neuroprotection, namely the degradation of aggregation-prone, potentially toxic cytosolic proteins (Fig. 3). Finally, some of the Parkin substrates are known to aggregate and, at least one, the Pael receptor induces autophagy when overexpressed [205]. Therefore, inhibiting the accumulation of aggregation-prone proteins could



**Fig. 3** Model for the cooperation of PINK1 and Parkin in the regulation of mitochondrial integrity and turnover, and the degradation of cytosolic aggregation-prone proteins. Cytosolic Parkin is effectively recruited to the surface of mitochondria with reduced  $\Delta\psi_m$ , promoting the destruction of functionally impaired mitochondria in autophagosomes. PINK1 phosphorylates Parkin on Thr175, which results in redistribution of Parkin from the cytosol to mitochondria. PINK1-deficient cells and *Drosophila* have lower amounts of mitochondria-associated Parkin, suggesting that PINK1 physiologically increases the mitochondrial Parkin pool. The function of phosphorylated Parkin in mitochondria is not known. It is unlikely to be involved in mitophagy but, based on recent findings, may serve

to stimulate the repair, replication and transcription of mtDNA, or to replace degraded organelles through mitochondrial biogenesis. A cytosolic E3 ubiquitin ligase complex containing PINK1, Parkin and DJ-1 has been implicated in the ubiquitination and degradation of Parkin substrates and unfolded/misfolded proteins that accumulate during heat shock, including Synphilin-1 and Parkin. Ablation of either *PINK1* or *DJ-1* reduced the proteasome-mediated degradation of these substrates, while overexpression of wildtype PINK1 but not PD-mutant PINK1 enhanced it. It has been suggested that the function of the PPD complex is to degrade potentially toxic proteins that accumulate and aggregate during oxidative stress. For details and references, please see main text

prevent saturation of autophagy under conditions that enhance protein aggregation, such as oxidative stress in PD. Although a small amount of the PPD complex was found in the mitochondrial fraction in cells overexpressing the three recessive PD proteins, it is unclear whether the PPD complex also ubiquitinates mitochondrial proteins. Ubiquitination plays an important role in the regulation of mitochondrial dynamics [33, 206]. However, none of the currently known Parkin substrates are mitochondrial proteins, but additional studies addressing this possibility may be warranted.

Is there a link between PINK1, mitochondrial transport and dopamine neurotransmission?

As described earlier, Miro is an important calcium-sensitive regulator of axonal mitochondrial transport. In addition, overexpression of Miro affects mitochondrial morphology, at least in part by suppressing Drp1-mediated fission, resulting in mitochondrial thread formation and elongated mitochondria that more readily reached the dendrites in primary cortical neurons [65]. In transfected cells, PINK1 forms a multiprotein complex with Miro and

Milton, suggesting that PINK1 may control mitochondrial transport and morphology via regulation of Miro and/or Milton [171]. Moreover, dysfunctional  $Ca^{2+}$  homeostasis in PINK1-deficient neurons and impaired calcium storage capacity of PINK1-deficient mitochondria [151] may cause the disruption of the Miro-KIF5 interaction, thereby blocking mitochondrial transport and energy supply in axons and synapses [63, 64]. Further experiments are necessary to study whether a defect of axonal mitochondrial transport does in fact exist in neurons lacking PINK1. If so, such a defect might explain the abnormal dopamine neurotransmission in mice lacking PINK1 [166], because mitochondrial dynamics and axonal trafficking of mitochondria are essential for synaptic function and neurotransmission [47–50].

Characteristics that may render the dopaminergic system particularly vulnerable to abnormal mitochondrial function and dynamics

Several features of the nigrostriatal system and the physiology of dopaminergic neurons may help to explain why dopaminergic neurons are particularly sensitive to

perturbations in mitochondrial function and dynamics. For example, it has been shown that the mitochondrial density and mass are lower in the substantia nigra dopaminergic neurons, which preferentially degenerate in PD, compared to non-dopaminergic neurons or dopaminergic neurons of the ventral tegmental area that are spared from death in PD [207]. In addition, cytoplasmic dopamine is prone to auto-oxidation and enzymatic oxidation, which can lead to impaired mitochondrial respiration and membrane permeability [208, 209]. A proteomic study recently showed that several chaperones playing important roles in mitochondrial protein quality control and cristae organization, as well as proteins of the electron transport chain and enzymes of the matrix were covalently modified by dopamine, after exposure of rat brain mitochondria to dopamine quinone (DAQ) in vitro [210]. In addition, dopamine was shown to enhance mtNOS activity [211] and increase the susceptibility of PC12 cells to nitric oxide-dependent inhibition of respiration [212]. Dopamine also inhibited mitochondrial complex I-driven respiration when added short-term or long-term to the medium of cultured SH-SY5Y cells [213]. Moreover, administration of exogenous dopamine to hippocampal neurons blocked the trafficking of mitochondria [214]. Finally, dopamine has been shown to interfere with CMA [215] and to promote autophagy-dependent cell death via upregulation of  $\alpha$ -synuclein expression [216]. Therefore, if not sequestered efficiently into synaptic vesicles, dopamine may affect mitochondrial respiration and transport. In addition, through its effects on autophagy, dopamine may interfere with autophagy-dependent clearance of aggregated proteins and dysfunctional mitochondria. Importantly, dopamine has been shown to covalently modify Parkin in living dopaminergic cells, which led to reduced Parkin solubility and inactivation of the Parkin E3 ligase activity [217]. Catechol-modified Parkin was specifically detected in the substantia nigra but not in other brain regions of PD patients, suggesting that dopamine-dependent inactivation of Parkin contributes to dopaminergic neuron loss [217]. Because Parkin is important for mitochondrial function and stimulates the clearance of dysfunctional organelles through mitophagy, dopamine-dependent Parkin inhibition may lead to mitochondrial dysfunction and impaired mitophagy. Collectively, these results show that dopamine and its metabolites can negatively affect mitochondrial homeostasis, rendering dopaminergic neurons more susceptible to further impairments of mitochondrial function, dynamics and clearance.

In addition, high levels of clonally expanded mtDNA deletions were shown to be present in individual pigmented neurons of the substantia nigra in aged people and PD patients [218, 219]. Because mitochondrial fission and fusion inhibit the propagation of mutant mtDNA genomes

[178, 180, 220], abnormalities in mitochondrial dynamics could result in an expansion of mitochondria harboring mutant genomes in dopaminergic neurons, causing energy depletion and cell death.

Finally, a common feature of the neuronal populations that degenerate in PD is that they all have disproportionately long and thin axons in relation to their cell body [221]. This might pose a challenge for neurons, because they have to transport mitochondria over long distances in the axons, and the narrow diameter of these processes may be particularly sensitive to alterations in mitochondrial shape and size that could interfere with mitochondrial trafficking. It is also believed that the neuronal degeneration in PD starts at the axon terminals [221], which would be affected first by impaired mitochondrial dynamics due to their increased energy requirement and the strict dependence of synaptic function on mitochondria [47].

## Conclusions

Significant progress has been made over the past decade in our understanding of the mechanisms and pathways that govern mitochondrial function and dynamics, and how pathogenic alterations of these mechanisms may contribute to the development of several neurodegenerative disorders, including autosomal dominant optic atrophy and Charcot-Marie-Tooth disease [222, 223], PD [101, 102] and Alzheimer's disease [224, 225]. With relevance to PD, PINK1 maintains mitochondrial integrity through the regulation of mitochondrial membrane potential, cristae structure and calcium homeostasis. In addition, via direct phosphorylation of its mitochondrial substrates TRAP1 and HtrA2, PINK1 likely regulates mitochondrial protein quality control and proteolysis under conditions of stress.

Parkin is important for the repair of damaged and mutated mtDNA, the replication and transcription of the mitochondrial genome, as well as mitochondrial biogenesis. In addition, Parkin specifically eliminates dysfunctional mitochondria by promoting their degradation in autophagosomes. This may be particularly important in cells lacking PINK1, which accumulate mitochondria with impaired  $\Delta\psi_m$ , and may explain how Parkin overexpression rescued the mitochondrial defects in PINK1-deficient flies and cells. In addition, the coordinated induction of fission and autophagy observed in PINK1-deficient neurons likely represents a compensatory response, which serves to fragment morphologically abnormal mitochondria and enhance their degradation in lysosomes (mitophagy). Finally, PINK1, Parkin and DJ-1 act in a complex to stimulate ubiquitin-dependent proteasomal degradation of aggregation-prone proteins, thus preventing the accumulation of potentially neurotoxic proteins. Understanding these

mechanisms of injury and compensation in PINK1-deficient neurons is critical for the development of future therapies for PD and other neurodegenerative disorders caused by abnormal mitochondrial function and dynamics.

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