Themed Issue: Mitochondrial Pharmacology: Energy, Injury & Beyond

# REVIEW

# Unravelling mitochondrial pathways to Parkinson's disease

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Mitochondria are essential for cellular function due to their role in ATP production, calcium homeostasis and apoptotic signalling. Neurons are heavily reliant on mitochondrial integrity for their complex signalling, plasticity and excitability properties, and to ensure cell survival over decades. The maintenance of a pool of healthy mitochondria that can meet the bioenergetic demands of a neuron, is therefore of critical importance; this is achieved by maintaining a careful balance between mitochondrial biogenesis, mitochondrial trafficking, mitochondrial dynamics and mitophagy. The molecular mechanisms that underlie these processes are gradually being elucidated. It is widely recognized that mitochondrial dysfunction occurs in many neurodegenerative diseases, including Parkinson's disease. Mitochondrial dysfunction in the form of reduced bioenergetic capacity, increased oxidative stress and reduced resistance to stress, is observed in several Parkinson's disease models. However, identification of the recessive genes implicated in Parkinson's disease has revealed a common pathway involving mitochondrial dynamics, transport, turnover and mitophagy. This body of work has led to the hypothesis that the homeostatic mechanisms that ensure a healthy mitochondrial pool are key to neuronal function and integrity. In this paradigm, impaired mitochondrial dynamics and clearance result in the accumulation of damaged and dysfunctional mitochondria, which may directly induce neuronal dysfunction and death. In this review, we consider the mechanisms by which mitochondrial dysfunction may lead to neurodegeneration. In particular, we focus on the mechanisms that underlie mitochondrial homeostasis, and discuss their importance in neuronal integrity and neurodegeneration in Parkinson's disease.

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## Abbreviations

α-syn, α-synuclein; ER, endoplasmic reticulum; DRP1, dynamin-related protein 1; HtrA2, serine protease high-temperature-regulated A2; MFN, mitofusin; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mTOR, mammalian protein target of rapamycin; OPA1, optic atrophy 1; PINK1, PTEN-induced putative kinase; QC, quality control; TRAP1, mitochondrial chaperone receptor-associated protein 1; UPR, unfolded protein response

## Introduction

In humans, the brain uses more energy than any other organ, accounting for approximately 20% of the body's total demand (Sokoloff, 1960; Clarke and Sokoloff, 1999). Mitochondria are tiny powerhouses that play a critical role in supplying the brain with energy. They provide energy by coupling the pumping of protons through the inner mitochondrial membrane (IMM) to the generation of ATP by ATP synthase, a highly complex molecular machine. It is accepted that acquisition of mitochondria by ancestral eukaryotic cells represents a crucial event in evolution (Gray et al., 1999); this

allowed the appearance of highly evolved multicellular life forms on our planet, eventually including humans with complex brains. Mitochondria do not only play a key role in supplying energy to cells, but they are also capable of causing cell death through the activation of cellular suicide programmes (reviewed in Youle and Strasser, 2008). Critically, uncontrolled cell proliferation or cell death can result in diseases such as cancer or neurodegeneration. Human cells contain several mitochondria, and each organelle contains multiple copies of a small genome encoded by the mitochondrial DNA (mtDNA; Legros et al., 2004). Human mitochondria are the result of uniparental inheritance from the maternal line. Mitochondrial mass and function differ significantly across different cell types, and are dynamically regulated by a variety of physiological cues, including physical activity and nutrient availability. An increase in mitochondrial numbers in individual cells is mediated by mitochondria biogenesis, a process involving the synthesis and import of macromolecules to existing mitochondria and the replication of the mtDNA. This is followed by the fission of one mitochondrion into two daughter mitochondria. Mitochondria accumulate damage during cell life as a consequence of their metabolic functions. Cells have, therefore, evolved multiple redundant quality control (QC) mechanisms to cope with mitochondrial damage. These mechanisms include the removal of damaged components from the organelles through the molecular QC pathways or, in extreme cases, the total removal of damaged mitochondria through autophagic degradation of the organelles (mitophagy); this is known as the organellar QC pathway (reviewed in de Castro et al., 2011; Rugarli and Langer, 2012).

Mitochondrial bioenergetics, homeostasis and QC are particularly important in human neurons, which are postmitotic cells that perform complex functions over many decades. Mitochondrial dysfunction has been implicated in many neurodegenerative diseases.

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting 1–2% of people over 65 years of age. It is characterized by the progressive loss of dopaminergic and non-dopaminergic neurons, and the development of intracellular aggregates of the protein α-synuclein (α-syn). The majority of PD is sporadic (90–95% of the cases), but approximately, 5–10% of cases are inherited through PD-related genes (Bonifati, 2007; Gasser, 2009). Decades of investigation of toxin-based models of PD, genetic models of PD and sporadic PD, have unveiled a number of potential molecular biomarkers of pathology that include protein aggregation, proteasomal stress, oxidative stress, mitochondrial dysfunction, lysosomal dysfunction and aberrant autophagy (reviewed in Dexter and Jenner, 2013). A robust molecular biomarker should be related to the fundamental features of neuropathology and its mechanism, and should also correlate with disease progression. Perhaps, unsurprisingly, there is no identified biomarker that can be found uniformly in all the genetic models and also in sporadic PD. One of the most ubiquitous markers is the presence of α-syn aggregation, and this is seen in all sporadic disease, but may be absent in certain genetic forms of PD such as PARKIN-associated PD. Oxidative stress is described in many studies of sporadic PD, toxin models and certain genetic models (in particular, models based on pink1, parkin, DJ-1 and α-syn),

although it remains controversial whether this is a bystander effect of neurotoxicity or a primary pathogenic process. Interpreting this data is complicated by the fact that any biomarker of sporadic PD can only be identified from post-mortem tissue or CSF, where dissection of primary and secondary mechanisms is not possible. Lysosomal dysfunction and aberrant chaperone-mediated autophagy is prominent in certain genetic models of PD (based on mutations in ATP13A2, GBA and α-syn), and altered expression of markers such as LAMP2A and hsp70 in the substantia nigra (SN) of sporadic PD (Alvarez-Erviti et al., 2010). Models based on adenoviral delivery of wild-type (Lundblad et al., 2012) or A53T mutant α-syn (Chung et al., 2009) to rat nigral neurons have demonstrated altered vesicle exocytosis and impaired dopaminergic neurotransmission. These events are followed by axonal damage, with dystrophic swollen axons and aberrant axonal transport. Interestingly, the axonopathy occurs as an early feature and precedes neuronal death, and has thus been proposed to be a possible early biomarker in these models. In addition, an α-syn-induced increase in activated microglia was reported in this model, with a concomitant increase in striatal cytokines, suggesting that the inflammatory response may also be an early biomarker for disease. It is evident that the cellular processes of protein degradation, protein aggregation, oxidative stress and mitochondrial function interact with each other, and so their coexistence as possible biomarkers of disease is frequently seen in models.

The role of this review is to address the importance and relevance of mitochondrial dysfunction as a biomarker for the pathogenesis of PD. Interestingly, the autosomal recessive forms of the disease are caused by loss of function mutations in genes encoding proteins that intrinsically localize to mitochondria (phosphatase and tensin homologue-induced putative kinase 1, DJ-1), or that relocate to this organelle upon mitochondrial damage (PARKIN), where they appear to regulate mitochondrial function. Mitochondrial dysfunction has also been reported in models based on the autosomal dominant forms of PD (α-syn, LRRK2), and these proteins have been shown to localize to mitochondria (Devi et al., 2008) (Papkovskaia et al., 2012). Mitochondrial toxins have been used for many years to generate animal models of PD that recapitulate varying degrees of the sporadic disease. Taken together, these data strongly suggest a potential role for mitochondrial dysfunction in the pathogenesis of PD. However, several important questions remain. It is not known if the observed mitochondrial pathology is the primary cause of pathogenesis in PD, or if mitochondrial damage is a consequence of damaged neurons. In addition, the specific mitochondrial processes involved in neurotoxicity in PD models and in the disease itself remain to be elucidated. Finally, it is unclear what the physiological roles of the PD proteins are in mitochondrial health. Here, we provide an overview of some of the recent advances in our understanding of the role of mitochondrial dysfunction in PD. We address the evidence for all aspects of mitochondrial pathology observed in different disease models, and we place particular emphasis on the emerging data regarding the mitochondrial QC pathways. We reflect on the relative importance of these pathways in PD, and how recent advances in our understanding of such pathways may direct better therapeutic approaches in the future.

## Mitochondrial bioenergetics and PD

The major mitochondrial function is generation of ATP, the main energy source for cells. Neurons are heavily reliant on mitochondrial ATP production for a range of high energy-demanding processes, including the maintenance of ion gradients through ATP-dependent pumps, axonal transport and synaptic neurotransmission. The respiratory chain is situated in the IMM and consists of four multiprotein redox complexes and two mobile electron carriers. The transfer of electrons from NADH and flavin adenine dinucleotide to oxygen, occurs through a series of redox reactions. The energy released by oxidation generates a proton gradient across the IMM that is used by complex V for the synthesis of ATP (oxidative phosphorylation). As the electrons flow through the respiratory chain, there is a small constitutive leak of high-energy electrons that are able to react with oxygen, and generate a free radical superoxide anion. Complexes I and III are the major sites of superoxide production. The brain consumes a disproportionate amount of oxygen compared with the rest of the body, and an estimated 2% of this oxygen will be converted to superoxide. Partially reduced forms of oxygen are highly active; they chemically interact with biological molecules, resulting in oxidation of protein, DNA and RNA, and peroxidation of lipids. Thus, cells also possess a range of mechanisms to neutralize the free radicals, including small-molecule antioxidants, such as glutathione and vitamin E, and antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. When the balance of reactive oxygen species (ROS) production and antioxidant defence is perturbed, accumulation of excessive ROS occurs in a state termed 'oxidative stress' (reviewed in Moran *et al.*, 2012). Of note, there is an age-related increase in mitochondrial ROS production, with a concomitant reduction in antioxidant defences, particularly in post-mitotic cells such as neurons. In the mitochondrial theory of aging, an age-related decline in the function of the respiratory chain causes increasing ROS, with subsequent accumulation of mtDNA mutations (reviewed in Wallace, 2005). These alterations result in progressively deficient respiratory chain components, which lead to impaired respiration and further oxidative stress. This may render aged neurons more susceptible to cell death in late onset neurodegenerative diseases.

Studies of *post-mortem* brains have demonstrated that there is significant evidence for oxidative stress in PD; there are increased levels of lipid peroxidation (in the form of malondialdehyde and 4-hydroxynonenal), increased levels of protein carbonyls and increased levels of 8-hydroxydeoxyguanosine in the PD brain, compared with controls (Dalfo *et al.*, 2005; Seet *et al.*, 2010). The major source of the oxidative stress is postulated to be an increase in ROS production induced by inhibition of respiration. Inhibition of respiration leads to a block in the electron transfer along the respiratory chain, increases the leak of high-energy electrons that form free radicals and concomitantly reduces ATP production. Indeed, complex I deficiency and reduction in glutathione are observed in the SN of PD patients (reviewed in Smeyne and Smeyne, 2013), and has been variably reported in other tissues, including platelets, lymphocytes and skeletal muscle (reviewed in (Schapira, 2008).

Models of PD that are based on the administration of toxins have demonstrated repeatedly that complex I inhibition is sufficient to cause dopaminergic neuronal toxicity by inducing a mitochondrial bioenergetic deficit (reviewed in Martinez and Greenamyre, 2012). Intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces an acute and irreversible version of parkinsonism in humans (Langston and Ballard, 1983). MPTP-treated primates develop nigrostriatal degeneration and clinical symptoms of PD (Burns *et al.*, 1983). MPTP-treated mice develop nigral dopaminergic (DA) neuron loss, neuronal inclusions of α-syn and progressive behavioural deficits (Fornai *et al.*, 2005). The active toxic species, MPP+, binds complex I and disrupts electron flow along the respiratory chain, resulting in lowered ATP production and increased ROS production. Mice overexpressing Cu/Zn SOD (SOD2), have reduced MPTP-induced neuronal death (Przedborski *et al.*, 1992).

Rotenone is a specific mitochondrial complex I inhibitor that results in increased leakage of electrons and, thus, increased superoxide generation. Chronic infusion of rotenone in rats (Betarbet *et al.*, 2000) or chronic oral administration in mice (Inden *et al.*, 2011), results in selective loss of nigral DA neurons and the presence of neuronal α-synpositive inclusions, as well as behavioural and motor symptoms akin to PD. Although rotenone is lipophilic, it crosses the blood brain barrier, and results in a systemic complex I deficit, that is selectively toxic to DA neurons.

Models based on the genes known to cause autosomal recessive and dominant forms of PD also point to the importance of mitochondrial bioenergetics in disease pathogenesis. PINK1 is a mitochondrial kinase, and there is strong evidence for its role in mitochondrial bioenergetics. *pink1*-deficient *Drosophila melanogaster* (Clark *et al.*, 2006) and *pink1* knockout mouse models (Gautier *et al.*, 2008) all share common features of mitochondrial abnormalities (Gispert *et al.*, 2009; Heeman *et al.*, 2011), increased ROS production and increased sensitivity to oxidative stress, albeit in the absence of neurodegeneration. Of note, vitamin K2, which acts as an electron transporter in *Drosophila*, is able to improve electron transfer in *pink1*-deficient *Drosophila* models and in complex I inhibition models, and thus restores ATP production and rescues the mitochondrial pathology (Vos *et al.*, 2012). Overexpression of a yeast complex I NADH dehydrogenase in *pink1* mutant flies is also able to rescue the mitochondrial pathology (Vilain *et al.*, 2012). Reversal of the bioenergetic deficit in both of these models is sufficient to reverse the observed pathologies, suggesting that impaired respiration and its consequences on ATP production are fundamental to *PINK1*-associated PD models, and may even precede aberrations observed in other mitochondrial processes. Fibroblasts from patients with *PINK1* mutations also exhibit respiratory chain impairment (Abramov *et al.*, 2010; Rakovic *et al.*, 2010). *PINK1*-deficient human neuronal models display impaired respiration, increased mitochondrial and cytosolic ROS production, reduced ATP production and mitochondrial membrane depolarization (Gandhi *et al.*, 2009). Interestingly, the inhibited respiration in *PINK1*-associated PD is secondary to the reduced supply of respiratory chain substrates, and can be reversed by improving the supply of complex I substrates,

again supporting a central role for impaired bioenergetics in PD.

PARKIN (PARK2) functions as an E3 ubiquitin protein ligase that is mainly cytosolic but can translocate to damaged mitochondria. *parkin*-deficient *Drosophila* models develop a mitochondrial phenotype and neuronal degeneration that is similar to *pink1*-deficient flies (Greene *et al.*, 2003; Pesah *et al.*, 2004), and furthermore demonstrate a genetic interaction between *parkin* and *pink1* (Yang *et al.*, 2006; Deng *et al.*, 2008). Additionally, parkin-deficient mitochondria have decreased activity of complexes I and IV, increased ROS species and increased sensitivity to complex I toxins (Whitworth *et al.*, 2005). *Parkin*-null mice display impaired respiration in isolated striatal mitochondria and evidence of oxidative damage to proteins and lipids (Palacino *et al.*, 2004), although in the absence of neurodegeneration (Itier *et al.*, 2003). Fibroblasts from PD patients with *PARKIN* mutations show inhibited respiration and reduced ATP production (Mortiboys *et al.*, 2008).

DJ-1 is a small highly conserved protein that can be oxidized at its cysteine residues during oxidative stress, and therefore acts as an ROS scavenger (Taira *et al.*, 2004). Oxidative stress induces relocalization of DJ-1 from the cytosol to the mitochondria (Canet-Aviles *et al.*, 2004). Certain models of *DJ-1*-deficient *Drosophila* demonstrate fragmented mitochondria, impaired bioenergetics, increased ROS production and sensitivity to complex I toxins (Yang *et al.*, 2005). *DJ-1*-null mice do not develop overt neurodegeneration, although they are more susceptible to MPTP-induced neurotoxicity (Kim *et al.*, 2005). Isolated mitochondria from *DJ-1*-null mice also exhibit increased ROS levels (Andres-Mateos *et al.*, 2007), suggesting an antioxidant role for DJ-1. Several other roles have also been proposed for DJ-1: DJ-1 binds RNA in an oxidation- dependent manner, and its RNA targets include mitochondrial genes and genes involved in glutathione metabolism (van der Brug *et al.*, 2008). DJ-1 stabilizes Nrf2, which is a major regulator of the transcription of the antioxidant response genes (Clements *et al.*, 2006). DJ-1 acts as a redox-sensitive molecular chaperone that can inhibit α-syn aggregation (Shendelman *et al.*, 2004). DJ-1 has also been reported to act as a metal binding protein and, thus, protect cells from copper and mercury-mediated cytotoxicity (Bjorkblom *et al.*, 2013).

α-syn is predominantly a presynaptic protein, but a subpopulation of α-syn localizes to mitochondrial membranes under conditions of cellular stress or cytosolic acidification (Cole *et al.*, 2008), and it is also found in the mitochondria of DA neurons in *post-mortem* human PD brains (Devi *et al.*, 2008). In cell models, overexpression of α-syn results in inhibition of complex I and mitochondrial membrane depolarization (Hsu *et al.*, 2000). Transgenic mice overexpressing A53T α-syn mutant under the mouse *PrP* promoter, show neurodegeneration characterized by intraneuronal inclusions, cell death and mitochondrial pathology, including altered morphology, mitochondrial DNA damage and oxidative-nitrative stress (Martin *et al.*, 2006). α-syn transgenic mice also show defects in complex I activity in the SN in the absence of neurodegeneration (Stichel *et al.*, 2007). Interestingly, α-syn knockout mice are resistant to the effects of MPTP, suggesting that α-syn is required downstream of complex I (Dauer *et al.*, 2002). Importantly, the mitochondrial dysfunction and oxidative stress seen in PD promote α-syn aggregation from its monomeric state to oligomers and fibrils (Conway *et al.*, 1998; Giasson *et al.*, 2000). Hence, there may be a bidirectional effect in which α-syn causes mitochondrial dysfunction, and this in turn exacerbates the misfolding of α-syn. However, it is not clear whether mitochondrial respiratory chain impairment and oxidative stress or α-syn aggregation is the initiating event in the pathogenesis of PD.

## Mitochondrial calcium homeostasis and PD

Calcium homeostasis is critical to normal neuronal function. Depolarization of the neuronal membrane induces opening of voltage-gated calcium channels, resulting in an increase in cytosolic calcium. The location, shape and amplitude of the calcium signal are able to regulate diverse processes, such as neurotransmitter release, vesicle recycling, axonal transport and neuronal plasticity. It is therefore vital to be able to modulate the peak and recovery of the calcium signal in different ways at different cellular locations. This is achieved in part by the ability of mitochondria to sequester and release calcium ions (reviewed in Vos *et al.*, 2010), thus maintaining the large calcium gradient across the cell's plasma membrane. Mitochondrial calcium uptake is mediated by the negative mitochondrial membrane potential and by a low-affinity calcium uniporter. Once in the matrix, calcium is buffered by forming reversible calcium phosphate complexes. Calcium can then be extruded from mitochondria via the $Na^+/Ca^{2+}$ exchanger (reviewed in Rizzuto *et al.*, 2012). The calcium-buffering capacity of mitochondria is important in all neurons, but it is particularly important in DA neurons; these neurons utilize an autonomous pacemaker mechanism based on L-type calcium channels, and are therefore exposed to frequent influxes of calcium (Surmeier *et al.*, 2011). This places such neurons under increased demand for ATP to maintain the cytosolic calcium concentration via ATP-dependent pumps. Importantly, deregulated calcium uptake or efflux leads to a state of mitochondrial calcium overload. This results in opening of the mitochondrial permeability transition pore, resulting in an influx of solutes into the matrix, osmotic swelling and rupture of the outer mitochondrial membrane. Release of apoptotic mediators from the intermembrane space into the cytosol is then able to trigger cell death. Mitochondrial calcium overload is reported to occur in rotenone models of PD, rendering rotenone-treated cells vulnerable to glutamate excitotoxicity (Yadava and Nicholls, 2007). Impaired mitochondrial efflux and mitochondrial calcium overload, with subsequent opening of the permeability transition pore and apoptotic cell death, are also reported in *PINK1*-deficient neurons (Gandhi *et al.*, 2009; Akundi *et al.*, 2011). Mitochondria and the endoplasmic reticulum (ER) are functionally and physically interconnected at contact sites that enable high concentrations of calcium to be generated (microdomains) and transferred to mitochondria. This function is vital for calcium signalling. There is evidence that α-syn, PARKIN and DJ-1 are necessary for the ER-mitochondrial transfer of calcium, and that the dysregulation of this process leads to abnormal mitochondrial calcium accumulation via an alteration in ER-mitochondrial contact sites (Cali *et al.*, 2012a; 2012b).

## Defective mitochondrial QC and PD

Mitochondrial QC mechanisms operate in a stepwise manner. Firstly, molecular QC involves molecular chaperones and proteases that assist the proper folding and assembly of mitochondrial proteins, and ensure the disposal of terminally damaged proteins. Secondly, organellar QC ensures the orderly disposal and recycling of terminally damaged mitochondria through mitophagy.

PINK1 and PARKIN are responsible for mitochondrial QC maintenance. Loss-of-function mutations in these proteins are associated with autosomal recessive parkinsonism (Valente *et al.*, 2004; Bonifati, 2007). Genetic studies in *Drosophila* show that mitochondrial dysfunction due to loss of function mutations in either *pink1* or *parkin*, can result in DA neuron degeneration (Whitworth *et al.*, 2005; Park *et al.*, 2006). It has been shown that *parkin* overexpression improves the mitochondrial phenotype in *pink1*-deficient flies, supporting the concept that *parkin* works genetically downstream of *pink1*. Accumulation of dysfunctional mitochondria is found in the brains of PD patients, highlighting the pivotal role of this pathway in PD pathology.

The PINK1-PARKIN axis acts to guarantee mitochondrial integrity at various levels. Upon mild mitochondrial dysfunction, PINK1 activates a molecular QC process, the mitochondrial unfolded-protein response (UPR$^{mt}$). In the UPR$^{mt}$, molecular chaperones and proteases are modulated to monitor the folding and assembly of mitochondrial proteins. In this scenario, PINK1 induces activation of the serine protease high-temperature-regulated A2 (HtrA2) (Plun-Favreau *et al.*, 2007) and phosphorylation of mitochondrial chaperone receptor-associated protein 1 (TRAP1; Pridgeon *et al.*, 2007), to eliminate misfolded proteins and to restore damaged mitochondria. It is reported that *Htra2* knockout mice show an accumulation of misfolded proteins in brain mitochondria (Moisoi *et al.*, 2009). In addition, the brains of PD patients carrying mutations in PINK1 show a reduction in HtrA2 phosphorylation (Plun-Favreau *et al.*, 2007). Furthermore, loss of *Trap1* in a *Drosophila* model causes a decline in mitochondrial function, and restoration of its expression in neurons rescues the phenotype of *pink1* mutant flies (Costa *et al.*, 2013).

Valuable insights into the mechanisms regulating UPR$^{mt}$ have been obtained from studies in *Caenorhabditis elegans*. In this nematode, the mitochondrial matrix protease CLPP-1 acts as a stress sensor, and through its proteolytic activity, transduces a signal to the cell nucleus that causes the transcriptional activation of stress-related genes. This signalling cascade involves the accumulation of the ubiquitin-like protein UBL-5 (Benedetti *et al.*, 2006) and the activation of the transcription factor DVE-1 (Haynes *et al.*, 2007). In addition, the transcription factor ATFS-1 is also capable of activating the UPR$^{mt}$ upon mitochondrial stress. Once in the nucleus, ATFS-1 accumulates and activates the expression of mitochondrial chaperones and other genes required to compensate for metabolic stress (Nargund *et al.*, 2012). The UPR$^{mt}$ can also be activated by 'mitonuclear imbalance', an imbalance between the expression of nuclear versus mitochondrial encoded mitochondrial proteins (Houtkooper *et al.*, 2013; Mouchiroud *et al.*, 2013). In this context, the central metabolic intermediate, NAD$^+$ positively regulates ageing by enhancing the UPR$^{mt}$. This occurs via NAD$^+$-dependent sirtuins such as SIRT1 (Mouchiroud *et al.*, 2013), which controls mitochondrial biogenesis through the acetylation of targets including the PPAR coactivator-1α (PGC-1α). It is therefore attractive to consider whether targeting NAD$^+$ metabolism is a viable strategy to prevent age-associated neurodegenerative diseases associated with mitochondrial dysfunction, such as some forms of PD.

## Mitophagy and PD

Once the molecular QC system is overwhelmed, organellar QC processes form a second line of defence. Organellar QC combines fusion, fission and mitophagy to promote the sequestration, sorting and elimination of functionally impaired mitochondria (Figure 1). PINK1 seems to have a pivotal function in organellar QC; it recruits PARKIN to impaired mitochondria, which targets them for mitophagy, and thereby promotes the removal of defective mitochondria (Figure 1B). The importance of the PINK1-PARKIN axis in promoting organellar QC through mitophagy has been demonstrated *in vivo* in *Drosophila*. Impairment of mitophagy in *pink1* and *parkin* mutant flies was shown to affect the turnover of defective mitochondria, as well as the selective nonmitophagic turnover of mitochondrial respiratory complexes (Vincow *et al.*, 2013), that accumulate in *pink1* and *parkin* mutant flies (de Castro *et al.*, 2011). The PINK1-dependent-recruitment of PARKIN to damaged mitochondria is also reported to involve the F-box domain-containing protein (Fbxo7; Burchell *et al.*, 2013) and interestingly, mutations in *FBXO7* have been identified in families with autosomal recessive early-onset PD, similar to that caused by mutations in *PINK1* or *PARKIN* (Shojaee *et al.*, 2008; Di Fonzo *et al.*, 2009; Paisan-Ruiz *et al.*, 2010). Additionally, PINK1 has been shown to interact with components of the autophagy machinery such as beclin1 (Michiorri *et al.*, 2010) and LC3 (Kawajiri *et al.*, 2010).

If both molecular and organellar QC mechanisms fail to rescue the damaged mitochondria, mitochondrial components and pro-apoptotic proteins are released into the cytosol, including cytochrome c (CytC); once released in the cytosol, CytC activates the apoptotic pathway, causing the irreversible demise of the cell (reviewed in Youle and Strasser, 2008). In the light of these events, the importance of the PINK1-PARKIN axis in maintaining both molecular and organellar QC is clear. It is conceivable that mutations in these genes in PD patients result in loss of function of these proteins and thus failure of one of the QC systems. Failure of one of these QC mechanisms ultimately results in loss of DA neurons, and might have a causative role in PD.

Selective autophagy of mitochondria that are damaged beyond the aid of molecular QC allows their basic macromolecule components to be recycled. This selective mitochondrial autophagy (mitophagy) begins with the isolation of the defective organelle in the autophagosome, which later fuses with a lysosome to generate an autolysosome. Here, the hydrolytic degradation of mitochondrial components takes place. When failure of mitophagy occurs, damaged mitochondria may accumulate in the cell and may be detrimental to cell function.
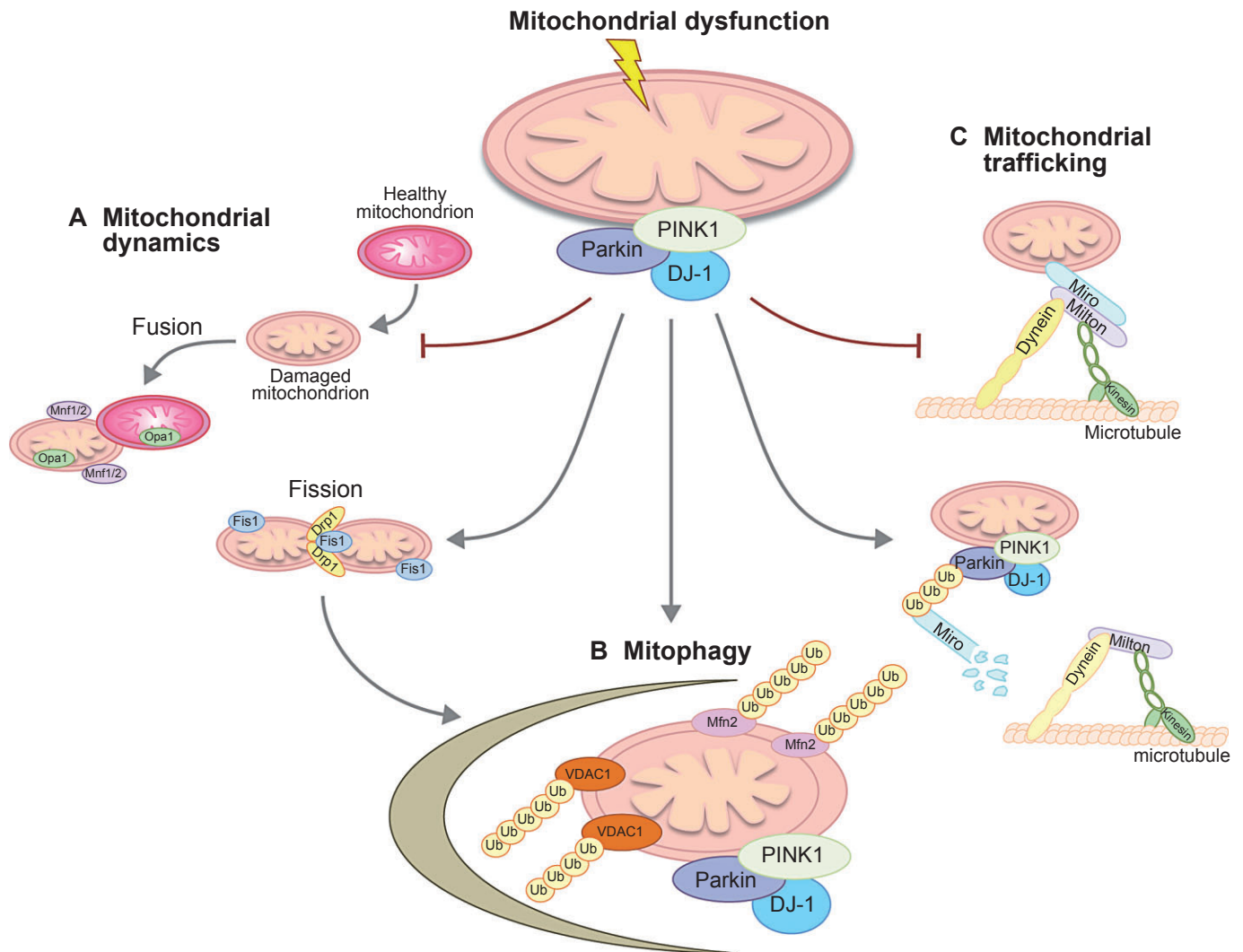
**Figure 1**

Modulation of mitochondrial dysfunction by the PINK1/PARKIN pathway. (A) Mitochondrial dynamics contribute to the maintenance of mitochondrial integrity. Upon mitochondrial dysfunction, mtDNA mutations or misfolded proteins accumulate. The PINK1/PARKIN axis inhibits the fusion process to avoid the spreading of damaged mitochondrial components. However, this axis also enhances the fission process to induce the physical separation of damaged mitochondria. Healthy components are rescued and damaged ones are eliminated by mitophagy. (B) Mitophagy is required to eliminate damaged mitochondria. When the organelle is deeply impaired, PINK1 recruits PARKIN for ubiquitination of outer membrane proteins, which labels irreversibly damaged mitochondria for mitophagy. (C) Mitochondrial trafficking is arrested upon mitochondrial dysfunction. PINK1 inhibits mitochondrial movement along microtubules, recruits PARKIN for ubiquitination and induces ubiquitin-dependent degradation of Miro. The above information is summarized from references discussed in the text.

Dysregulation of mitophagy is associated with neurode-generative diseases such as PD (reviewed in Youle and Narendra, 2011). The PD-related proteins PARKIN and PINK1 are potential regulators of mitophagy, ensuring the maintenance of mitochondrial QC. Following mitochondrial damage, PINK1 is required for the recruitment of PARKIN to dysfunctional mitochondria to promote their degradation (Matsuda et al., 2010; Narendra et al., 2010; Vives-Bauza et al., 2010).

Furthermore, mitochondrial dynamics such as the fission and fusion processes are important to maintain mitochondrial integrity. Fission is believed to be important for mitophagy because this process is delayed by inhibition of

division. During PARKIN recruitment, mitochondrial dynamics are shifted towards fission rather than fusion, partly due to the degradation of mitofusins (MFNs; Gegg et al., 2010; Tanaka et al., 2010; Ziviani et al., 2010) Moreover, many mutations in mitochondrial fission/fusion genes, such as MFN2, OPA1 and DRP1, are associated with neurodegenerative diseases (Alexander et al., 2000; Zuchner et al., 2004).

### Possible role of autophagy-inducing agents in the modulation of mitophagy

A multitude of different agents can induce autophagy, but their ability to induce mitophagy is debated. Rapamycin is a metabolite isolated from the bacterial strain *Streptomyces*

*hygroscopicus*; the study of its action in yeast led to the discovery of the protein target of rapamycin (TOR). The mammalian TOR (mTOR) is encoded by *FRAP1* and acts as a sensor of energy, nutrients, growth factors, redox and stress to increase protein synthesis and inhibit autophagy.

Rapamycin is a well-known inducer of autophagy. Rapamycin antagonizes mTOR, resulting in the activation of the translation inhibitor 4E-BP. Moreover, it has been shown that rapamycin treatment can improve mitochondrial defects in cell lines generated from PD patients with *PARKIN* mutations (Tain *et al.*, 2009). Rapamycin-induced autophagy was shown to suppress PD symptoms in a mouse model; inhibition of mTOR by rapamycin also increased the lifespan of mice by inducing a combination of increased autophagy and decreased mRNA translation (Rieker *et al.*, 2011). Growing evidence indicates that autophagy can be regulated by the UPR in response to ER stress (UPR[ER]; reviewed in Wouters and Koritzinsky, 2008). In this context, salubrinal, a compound that protects cells from ER stress by inhibiting the dephosphorylation of the eukaryotic translation initiation factor 2 subunit α (Boyce *et al.*, 2005), was shown to enhance paraquat-induced autophagy (Niso-Santano *et al.*, 2011), as well as to mitigate DA neurodegeneration (Colla *et al.*, 2012).

Metformin is a drug used for the treatment of type 2 diabetes; it reduces blood glucose, thus decreasing hepatic gluconeogenesis and stimulating glucose uptake in muscle (Stumvoll *et al.*, 1995; Hundal *et al.*, 2000). The mechanism of action of metformin is based on the activation of AMP-activated PK (AMPK), a protein that regulates energy metabolism, and is activated by an imbalance in the AMP/ATP ratio. A reduction in ATP levels promotes the phosphorylation of multiple proteins by AMPK to activate ATP-generating pathways such as glycolysis. Therefore, AMPK can modulate a variety of pathways, inhibiting the ones that are very energy expensive. mRNA translation requires up to 45% of the total cellular energy (Hardie, 2004); AMPK inhibits mRNA translation and promotes the activation of autophagy by inhibiting mTOR. The ability of metformin to affect mitophagy has been debated; the drug was found to affect erythrocyte metabolism, a cell type that lacks mitochondria (Rapin *et al.*, 1991; Muller *et al.*, 1997). In contrast, it has been recently reported that metformin inhibits the mitochondrial respiratory chain complex I and prevents oxidative mitochondrial phosphorylation, suggesting a role in the modulation of mitochondrial metabolism (El-Mir *et al.*, 2000). Furthermore, it has been showed that metformin can reduce the risk of PD in patients with type 2 diabetes (Wahlqvist *et al.*, 2012). As the modulation of autophagy is now possible using a variety of therapeutic strategies (reviewed in Rubinsztein *et al.*, 2012), decreasing the activity of the mTOR pathway by autophagy-inducing drugs could represent a promising target for the treatment of neurodegenerative diseases.

## Molecular scenario of mitochondrial dynamics in the mitophagic pathway

Mitochondrial dynamics contribute to the maintenance of mitochondrial integrity. Repetitive cycles of fusion and fission occur to guarantee the presence of healthy mitochondria in the cell and efficient distribution of their components (mtDNA, proteins and respiratory chain components) during mitochondrial turnover. These processes are regulated by GTPases that operate on mitochondrial membranes. Mitochondrial fission ensures the separation of daughter mitochondria through GTPases acting on the outer membrane. During fusion, different proteins act on the outer and inner mitochondrial membranes.

Fission is mediated by dynamin-related protein 1 (DRP1) through the formation of a multimeric complex; this complex wraps around the outer mitochondrial membrane (OMM), and causes physical scission of the membrane by exerting a mechanical force. In contrast, fusion is mediated by optic atrophy 1 (OPA1), MFN1 and MFN2 that act on the inner and outer membranes. Depolarization of the mitochondrial membrane can influence the fusion process by causing loss of OPA1 (Head *et al.*, 2009) and the MFNs, resulting in mitochondrial fragmentation. Fragmented mitochondria are often targeted for degradation through mitophagy (Figure 1B).

Excessive fusion, due to overexpression of *OPA1*, prevents mitophagy (Twig and Shirihai, 2011). Additionally, knockdown of *MFN2*, precludes mitophagy by impairing the transfer of lipids from mitochondria to autophagosomes (Hailey *et al.*, 2010). Conversely, fission is considered the prelude to mitophagy. This notion is supported by genetic manipulation in mammalian cells, where *DRP1* silencing decreases mitophagy (Twig *et al.*, 2008).

Another important player in the machinery of mitochondrial dynamics is α-syn, a cytoplasmic protein discovered to be the major component of intracellular aggregates (Lewy bodies) that are common to most familial and sporadic PD cases. α-syn can modulate the balance of mitochondrial dynamics by inhibiting membrane fusion and causing fragmentation through its interaction with the membrane (Nakamura *et al.* 2011). α-syn down-regulation results in elongated mitochondria in cell culture. In contrast, its overexpression induces mitochondrial fragmentation, which is rescued by co-expression of *PINK1*, *PARKIN* or *DJ-1* (Kamp *et al.*, 2010). Overexpression of α-syn in neurons results in fragmentation of mitochondria prior to cell death in a DRP1-independent manner. In fact, the mitochondrial fragmentation is induced by a direct interaction of α-syn oligomers with the mitochondrial membrane (Nakamura *et al.*, 2011).

Ultimately, mitophagy requires proper functioning of mitochondrial dynamics to avoid accumulation of damaged mitochondria. An imbalance in the molecular machinery towards fission, rather than fusion, promotes the clearance of damaged mitochondria by mitophagy. In neurons, there is an intimate link between mitochondrial dynamics and mitophagy (reviewed in Van Laar and Berman, 2013). It was reported that the Parkin-dependent mitophagy of depolarized mitochondria in neurites required a block in mitochondrial transport, showing that these two processes are closely linked (Cai *et al.*, 2012). In support of this, it has been showed that MFN2 is also involved in Parkin recruitment to damaged mitochondria; moreover, PINK1 phosphorylates MFN2 and promotes its degradation through PARKIN ubiquitination (Chen and Dorn, 2013). Even though opposite effects on mitochondrial dynamics have been proposed to be promoted by the PINK1-PARKIN axis (reviewed in Van Laar and Berman, 2013), the observation that the fusion proteins MFN1 and MFN2 are targets for PARKIN-dependent ubiquitination and

degradation, supports a mechanism where the primary role of the PINK1-PARKIN axis is to inhibit mitochondrial fusion.

## Aberrant mitochondrial trafficking in PD

Mitochondrial trafficking is essential for all cells, but it is particularly important for neurons due to their unique morphology. Axons and dendrites extend for millimetres, even up to centimetres in length. Synapses are often located at the extremities of the cell, and require a considerable energy supply; thus, the demand on mitochondrial ATP production is very high. ATP cannot diffuse from the soma to distant synapses; therefore, mitochondria are transported along axons to provide ATP to meet local demands. The transport of mitochondria in neurons from the cell soma along the axon (anterograde axonal transport), occurs through the association with the kinesin family motor proteins KIF1Bα and KIF5, while the transport back to the cell soma (retrograde transport), is mediated by cytosolic dynein (Hollenbeck and Saxton, 2005; Hirokawa et al., 2010). The efficient transport of mitochondria is vital in neurons as it is critical for synaptic formation and function, via proper distribution of mitochondria throughout neurites. In the soma, neurons have a highly interconnected mitochondrial network together with well-separated, individual mitochondria that travel up and down an extensive network of axons and dendrites. In neurites mitochondria provide energy in the form of ATP for synaptic function, and act as a calcium-buffering system, ensuring efficient synaptic transmission (Ly and Verstreken, 2006). Consequently, the aberrant regulation of mitochondrial transport, fission or fusion greatly alters synapse formation and function (Li et al., 2004; 2008; Verstreken et al., 2005). As a consequence of their highly specialized nature, the distribution of mitochondria in neurons plays a key role in maintaining neuronal energy reserves in multiple locations within the same cell that, in turn, requires a delicate balance of mitochondrial dynamics.

Recent studies have highlighted the presence of a motor/adaptor complex on the mitochondrial surface that is responsible for mitochondrial transport. This complex includes four proteins: Miro (RhoT1/2), the heavy chain of kinesin-1, Milton (TRAK1/2) and dynein. Milton mediates the binding between kinesin-1 and Miro, and dynein interacts with Milton and Miro (Stowers et al., 2002; Fransson et al., 2003; 2006; Guo et al., 2005; Glater et al., 2006).

Miro is a Rho GTPase protein that works as an anchor; it is attached to the surface of the OMM, where it binds milton (Glater et al., 2006). GTPase-defective mutants of Miro show aggregation of mitochondria, suggesting that Miro has a role in regulating mitochondrial trafficking, fusion and fission (Fransson et al., 2006).

PINK1 and PARKIN are associated with the Miro/Milton complex, suggesting the involvement of the PINK1/PARKIN pathway in mitochondrial trafficking (Weihofen et al., 2009; Wang et al., 2011). PINK1 phosphorylates Miro on several sites, which induces ubiquitination by PARKIN and results in proteasomal degradation of Miro. This results in the irreversible removal of the complex from the mitochondrial surface and block of mitochondrial movement. pink1 knowdown in Drosophila axons results in enhanced anterograde transport (Liu et al., 2012), whereas overexpression of PINK1 or PARKIN

in both mammalian and Drosophila neurons results in inhibition of mitochondrial trafficking (Figure 1C) (Chan et al., 2011; Wang et al., 2011). In neurons, Parkin-dependent mitophagy was reported to occur primarily in somatodendritic compartments and not in axons (Cai et al., 2012).

During trafficking, mitochondria are more inclined to fuse compared with when they are stationary (Liu and Hajnoczky, 2009). The degradation of Miro by the PINK1/PARKIN pathway is considered to be important to prevent the fusion and the exchange of mitochondrial components between damaged and healthy mitochondria. Indeed, mitochondrial transport defects caused by pink1 inactivation represent one of the key pathogenic events that contribute to PD pathogenesis in the Drosophila model (Liu et al., 2012). Because mutations in PINK1 and PARKIN are found in PD patients, it is reasonable to propose that they can affect mitochondrial trafficking in PD patients.

## Mitochondria, inflammation and PD

Failure of the organellar QC pathway, which ensures the removal of damaged mitochondria through mitophagy, can lead to the release of compartmentalized mitochondrial molecules. Once in the cytosol, some of these molecules, such as CytC, Smac/DIABLO and HtrA2/OMI, are capable of activating apoptotic routines that lead to cell death. It is reported that the failure of mitophagy results in the release of mtDNA into the cell cytosol. In a manner similar to bacterial DNA, mtDNA is capable of activating a Toll-like receptor (TLR) 9-mediated inflammatory response in cardiomyocytes, inducing myocarditis and dilated cardiomyopathy (Oka et al., 2012). Accordingly, defects in autophagy in LC3B knockout mice result in the release of mtDNA into the cytosol, activation of caspase-1, and elevated levels of the pro-inflammatory cytokine IL-β (Nakahira et al., 2011).

Data obtained from both post-mortem and in vivo studies in PD patients suggest that neuroinflammatory mechanisms could contribute to the non-cell autonomous death of dopaminergic neurons (Hirsch and Hunot, 2009). Accordingly, it is conceivable that failure of mitochondrial clearance through mitophagy in PD patients could be linked to inflammation by promoting the activation of the TLR9 pathway. The TLR9-mediated inflammatory response can increase the level of pro-inflammatory cytokines, including IL-1β (Miura et al., 2010). This cytokine plays a key role in the neuroinflammatory processes in PD through at least two separate mechanisms of neurotoxicity: direct binding to surface receptors on dopaminergic neurons or activation of glial cells. Through these mechanisms, IL-1β can activate cell death pathways in dopaminergic neurons. Thus, the DNA released from defective mitochondria that bypass mitophagy could be a contributing factor in triggering the non-cell autonomous dopaminergic cell death in PD.

## Mitochondrial DNA deletions and misrepair mechanisms in PD

Typically, human cells have between 50 and several hundred mitochondria. Each mitochondrion has 5 to 10 copies of mtDNA (Legros et al., 2004), contained in structures known as nucleoids. As the mitochondrial genome is present in multiple copies in each organelle, mutant mitochondrial

genomes can coexist with normal genomes in a situation known as heteroplasmy. This implies that once a critical threshold of mutant DNA copies is reached, normal mitochondrial function may be altered. This may have important consequences for cellular and organismal viability. The mutation rate of mtDNA is known to be higher than nuclear DNA (nDNA); in contrast to nDNA, mtDNA lacks protective coating with histones. Furthermore, mtDNA is in close proximity to the inner membrane, where DNA-damaging agents such as ROS are continually produced. To counteract this damage, mitochondria contain ROS-detoxifying enzymes such as SOD2, and a group of repair enzymes that counteract oxidative DNA damage. Among these, the orthologue of the *Escherichia coli* MutT (MTH1) hydrolyses oxidized purine nucleoside triphosphates, protecting mtDNA from the consequences of mis-incorporation during replication or repair. Increased levels of oxidized nucleosides are detected in patients with neurological diseases such as PD (Nakabeppu, 2001). For example, increases in 8-oxoguanine, a purine by-product of oxidative damage, can result in mismatched pairing with adenine and cause G to T and A to C substitutions in the genome. mtDNA mismatches can lead to mitochondrial impairment and cellular dysfunction, particularly in post-mitotic cells with high energy demands such as skeletal muscle and neurons.

High levels of mtDNA deletions have been described in the SN neurons in PD (Bender *et al.*, 2006). One major question is whether the accumulation of mtDNA mutations contributes to PD pathogenesis by the gradual replacement of wild-type DNA, failure to synthesize mitochondrial proteins, causing respiratory chain deficiency, neuronal dysfunction and ultimately, neuronal death. *Post-mortem* studies of brains harbouring high levels of mtDNA deletions (due to point mutations in the nuclear encoded mitochondrial DNA polymerase, POLG) demonstrate that such deletions are indeed associated with complex I deficiency, but that this is not necessarily correlated with parkinsonism (Palin *et al.*, 2013). mtDNA deletions and complex I deficiency may occur with α-syn pathology but also exist without any α-syn aggregation (Reeve *et al.*, 2013), raising the issue of whether a primary mitochondrial defect is required for α-syn aggregation. Interestingly, transgenic mice with a proofreading defective form of POLG (D257A) show high levels of mtDNA deletions without significant functional or morphological mitochondrial impairment, and with no increased susceptibility to mitochondrial toxins. Compensatory mechanisms, such as an increase in mtDNA copy number and OPA1-related enhancement of mitochondrial cristae, appeared to be responsible for protection of mitochondrial function and neuronal survival in this study (Perier *et al.*, 2013). Taken together, this work suggests that primary mitochondrial defects cause an array of phenotypes, some of which include PD-related neurodegeneration, although mitochondrial bioenergetic failure is clearly possible in the absence of SN neuronal death, protein aggregation and PD. However, it is possible that the compensatory mechanisms for the initial defect may be responsible for modulating which phenotype is seen, and this highlights the importance of the balance between mitochondrial adaptation and QC mechanisms, and mitochondrial bioenergetic dysfunction.

## Conclusions

The landscape of mitochondrial pathology in neurodegeneration has radically transformed in the past 5 years. There is an increasing body of evidence that mitochondrial pathology has a primary role in the pathogenesis of certain genetic forms of PD, although the evidence for its primary role over other pathogenic processes in sporadic PD remains controversial. Due to the inherent difficulty in identifying pathogenesis in sporadic disease (that can only be currently studied many years after the primary events have occurred), this controversy is likely to remain for some time. There has been a major shift in the view of mitochondria as ATP- and ROS-producing organelles, to recognizing a much more diverse role of mitochondria in trafficking, transport, QC and turnover. For the past 20 years, the bioenergetic deficit has been the most frequently recognized and well-characterized aspect of mitochondrial pathology in most PD models. However, the more recently discovered pathways may hold the key to understanding how mitochondrial dysfunction leads to cell death. The autosomal recessive models have uncovered the importance of mitochondrial QC and transport for maintaining neuronal function and integrity. The new key questions are no longer simply whether a bioenergetic defect in mitochondria is sufficient to cause PD, but whether a failure in the mitochondrial compensatory mechanisms (transport, dynamics and clearance) is sufficient, or necessary, to cause PD. How a failure of mitochondrial transport and clearance leads to gradual neuronal loss still remains to be explored, but it is possible that it induces a modest but persistent disruption to the cellular energy capacity, and this affects specific cellular processes such as synaptic transmission and axonal transport that would eventually lead to neuronal dysfunction and death.

Mitochondrial QC pathways such as mitophagy can be studied in isolation, for example, in cell cultures with different mutations. However, it is clear that the *in vivo* models, based on genetic forms of PD, show mitochondrial pathology affecting several different pathways concomitantly: respiratory chain abnormalities, calcium dysregulation, mitochondrial morphology changes, altered dynamics and changes in mitophagy. The processes responsible for counteracting mitochondrial dysfunction (Figure 2) are clearly interdependent and interact with other mitochondrial processes in ways that are not yet well understood. Unravelling the primary events in mitochondrial dysfunction and the sequence of molecular events that leads to cell death is a major challenge.

Future work should explore how these isolated processes work in concert to result in neuronal death. There is some evidence that the redox state and ROS levels of mitochondria affect their dynamics. For example, inhibition of complexes I and III can induce mitochondrial fission (Pletjushkina *et al.*, 2006). Conversely, mitochondrial fusion appears to enhance ATP production (Mitra *et al.*, 2009). In response to ROS production, mitochondrial dynamics may be used to alter their numbers or spatial distribution to sequester or limit local ROS release. Mitophagy is now a well-characterized process that occurs in response to damaged or depolarized mitochondria. However, the upstream physiological processes that induce such events *in vivo* are not well understood. The relationship between mtDNA mutations, impaired energy production,
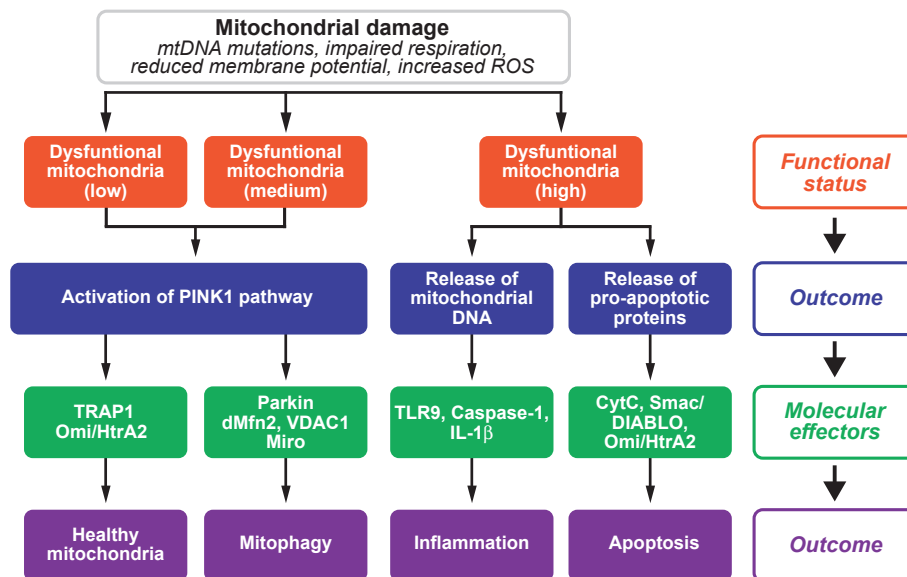
**Figure 2**

Cellular processes acting downstream of mitochondrial dysfunction. Increasing levels of mitochondrial damage lead to correspondingly higher levels of mitochondrial dysfunction (here represented by the categorical classifiers 'low', 'medium' and 'high'). Low levels of mitochondrial dysfunction are counteracted at the molecular level by the PINK1 pathway. This acts on intra-organellar proteases and chaperones, such as HtrA2/OMI and TRAP1, and suppresses the Miro-dependent transport of dysfunctional mitochondria. The combined output of these processes is the restoration of mitochondrial health. Higher levels of mitochondrial dysfunction might overwhelm the molecular QC. In this case, PINK1 promotes the degradation of mitochondria with 'medium' levels of dysfunction through mitophagy. This is the organellar QC pathway. Finally, if both molecular and organellar QC pathways downstream of PINK1 fail, the mitochondria will ultimately release pro-apoptotic proteins and mtDNA, resulting in either apoptosis or inflammation. The above information is summarized from references discussed in the text.

excessive ROS production, fission–fusion processes and mitophagy is unclear, but these processes are likely to be interrelated. Elucidating the complexity of these interactions will help to understand whether different PD proteins are involved in distinct but overlapping functions within mitochondria, or whether they are all implicated in the same pathway.

The relevance of such interactions is particularly important when considering therapeutic options for PD. Based on the hypothesis that respiratory chain impairment is sufficient to induce neurodegeneration in PD models, there have been extensive attempts to improve electron transport pharmacologically using Co-enzyme Q10 (Co-Q10) and to scavenge free radicals using vitamin E. Co-Q10 transfers electrons from complexes I and II to complex III. In MPTP-treated mice and primates, the administration of Co-Q10 exerts neuroprotective effects, reduces dopaminergic neuron death and attenuates α-syn aggregation (Beal *et al*., 1998; Cleren *et al*., 2008). Based on the recent reports in *pink1*-deficient flies, improving respiratory chain function (using improved electron transfer) should be sufficient to reverse mitochondrial deficits. However, a large meta-analysis of vitamin E, Co-Q10 and glutathione trials in PD patients, has not confirmed significant benefits from any of these approaches (Weber and Ernst, 2006). The inability to translate the findings from the animal models of PD to clinical benefit may be attributed to several factors, such as the bioavailability of the reducing molecules in the human brain and the effective targeting of mitochondria. Finally, oxidative stress may need to be targeted in

the very early presymptomatic stages of the disease to be effective.

The work highlighted in this review suggests further reasons for the ineffectiveness of current antioxidant therapy to date. Excessive ROS production may mediate toxicity by affecting other processes, such as mitochondrial dynamics and mitophagy, and these may not be effectively targeted by current pharmacological methods. Up-regulation of 4E-BP by the drug rapamycin activates autophagy and improves the phenotype of *pink1* and *parkin* mutant *Drosophila* (Tain *et al*., 2009). This provides a rational basis for developing therapies to target impaired mitophagy in PD. Therapeutic approaches designed to enhance the activity of PINK1 have the potential to suppress mitochondrial defects associated with PD-related loss-of-function mutations in this gene. In this context, it was demonstrated that the administration of the ATP analogue kinetin triphosphate, a PINK1 neo-substrate, increases its kinase activity and results in improved activation of the PINK1-dependent mitochondrial QC (Hertz *et al*., 2013). This indicates that employing pharmacological agents capable of increasing PINK1 kinase function could be used for treating diseases associated with defective mitochondrial QC such as some forms of PD.

A final possibility remains that the mitochondrial dysfunction seen in PD is truly heterogeneous. There may be multiple entry points in parallel processes that induce mitochondrial and neuronal dysfunction, and that ultimately converge to induce cell death. In this scenario, it will be necessary to design treatments that affect a range of targets

within the mitochondria to be effective. The complexity of the mitochondrial pathology observed in neurodegenerative diseases may thus require a complex therapeutic approach.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

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