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Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis

Claire Henchcliffe* and M Flint Beal

SUMMARY

Parkinson disease (PD) is associated with progressive loss of dopaminergic neurons in the substantia nigra, as well as with more-widespread neuronal changes that cause complex and variable motor and nonmotor symptoms. Recent rapid advances in PD genetics have revealed a prominent role for mitochondrial dysfunction in the pathogenesis of the disease, and the products of several PD-associated genes, including *SNCA*, *Parkin*, *PINK1*, *DJ-1*, *LRRK2* and *HTR2A*, show a degree of localization to the mitochondria under certain conditions. Impaired mitochondrial function is likely to increase oxidative stress and might render cells more vulnerable to this and other related processes, including excitotoxicity. The mitochondria, therefore, represent a highly promising target for the development of disease biomarkers by use of genetic, biochemical and bioimaging approaches. Novel therapeutic interventions that modify mitochondrial function are currently under development, and a large phase III clinical trial is underway to examine whether high-dose oral coenzyme Q10 will slow disease progression. In this Review, we examine evidence for the roles of mitochondrial dysfunction and increased oxidative stress in the neuronal loss that leads to PD and discuss how this knowledge might further improve patient management and aid in the development of 'mitochondrial therapy' for PD.

KEYWORDS coenzyme Q10, genetics, mitochondrial dysfunction, oxidative stress, Parkinson disease

REVIEW CRITERIA

English language peer-reviewed published literature was searched in MEDLINE for reviews and original research using the keywords "Parkinson's disease", "mitochondria" and "oxidative stress"; along with the names of individual Parkinson disease-associated genes, either singly or in combination. Secondary references from these articles were then screened for inclusion.

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INTRODUCTION

Parkinson disease (PD) is a chronic, and often devastating, progressive neurodegenerative disease. Loss of dopaminergic neurons in the substantia nigra leads to the characteristic motor features of tremor, rigidity and bradykinesia, while more-widespread neuronal changes lead to complex and variable nonmotor symptoms. Strong evidence now exists to support a role for aberrant mitochondrial form and function, as well as increased oxidative stress, in the pathogenesis of PD.[1,2] A complex interplay occurs between mitochondria and other cellular machinery that affects cell survival, as mitochondria not only have a key role in electron transport and oxidative phosphorylation, but they are also the main cellular source of free radicals, and they are involved in calcium homeostasis and in the regulation and instigation of cell-death pathways (Figure 1). The products of a number of PD-associated genes are involved in these pathways and have recently been found to influence the balance of mitochondrial fission and fusion, thus affecting the maintenance of dynamic networks of mitochondrial tubular structures. In addition, studies of mitochondrial function in PD underscore a pathophysiological heterogeneity within this disorder; mitochondrial dysfunction is not detected in all individuals with PD, a factor that will be of critical importance in the development of individualized therapies in the years ahead. In this article, we highlight important recent advances in mitochondrial biology that have contributed to our understanding of PD pathogenesis and will aid in the development of future treatment strategies.

MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE DAMAGE

The most direct evidence for disrupted mitochondrial metabolism has come from studies of autopsy tissue and other tissue samples and *in vitro* cell cultures derived from patients with PD. One finding at autopsy is that the activity of complex I, a major component of the electron transport chain, is decreased in the substantia

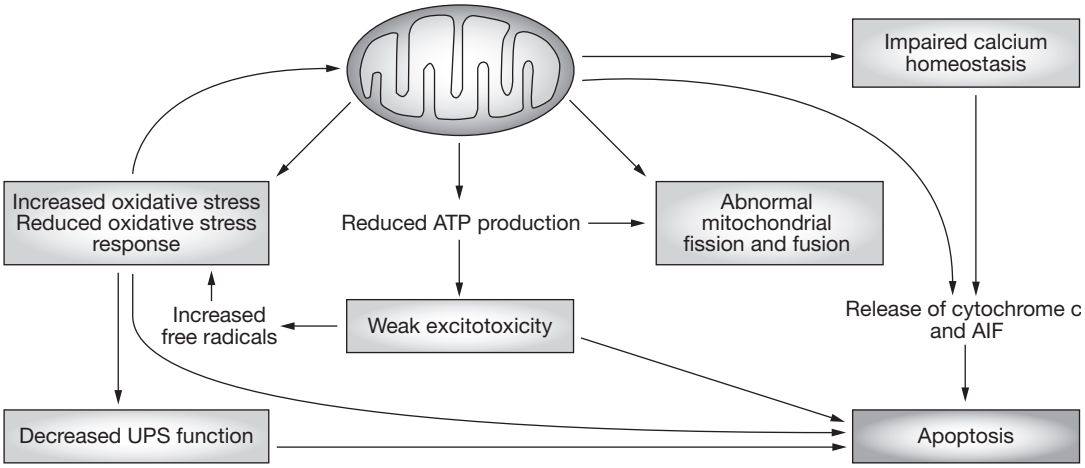

Figure 1 Mitochondrial dysfunction affects diverse cellular processes that can culminate in cell death. Mitochondrial dysfunction affects a number of cellular pathways, leading to damage of intracellular components and to cell death. Abnormal metabolic function, abnormal morphology, and impaired fission–fusion balance have all been observed in mitochondria in at least some forms of Parkinson disease. Mitochondria are a major source of free radicals in the cell, resulting in oxidative stress, but mitochondria are also integral to the oxidative stress response. Increased oxidative stress can lead to impaired function of the UPS, thereby further affecting cell survival. Mitochondria also sequester calcium when intracellular calcium levels rise during the excitotoxic process. The threshold for excitotoxicity might decrease if mitochondrial ATP production is impaired. Mitochondria also have a pivotal role in apoptotic cell death. Mitochondrial release of cytochrome c and other 'pro-apoptotic factors', such as AIF, into the cytoplasm triggers a cascade of events, culminating in cell death. Abbreviations: AIF, apoptosis-initiating factor; UPS, ubiquitin–proteasomal system.

nigra[3] and frontal cortex[4] in patients with PD, and sophisticated immunocapture techniques have demonstrated increased oxidative damage and reduced electron transfer rates through complex I subunits in these individuals.[5] This abnormality is predicted to render cells more vulnerable to Bax-induced apoptosis and conceivably contributes to the demise and dysfunction of cells during the PD disease process. Electron transport chain impairment might actually be systemic, as decreased complex I activity has been demonstrated in platelets,[6] and defective oxidative phosphorylation has been suggested to occur in skeletal muscle.[7] Moreover, fusion of platelets from individuals with PD and known complex I deficiency with mitochondria-deficient rho0 cell lines produces cell cybrids with complex I deficiency.[8,9] In addition to the *in vitro* findings, magnetic resonance spectroscopy studies, by examining high-energy phosphate levels in the temporoparietal region[10] and occipital lobe[11] and by measuring increased cerebral lactate levels in PD,[12,13] have demonstrated metabolic abnormalities consistent with mitochondrial dysfunction and a shift to anaerobic metabolism *in vivo* in humans with PD.

Mitochondrial dysfunction leads to increased oxidative stress. Oxidative damage to lipids, proteins and DNA,[14,15] as well as a decrease in the levels of the important antioxidant reduced glutathione,[16] has been detected in autopsy tissue from the brains of individuals with PD. These findings provide a plausible link between oxidative damage and formation of the Lewy body protein aggregates that are characteristic of PD, as oxidative damage induces α-synuclein aggregation and impairs proteasomal ubiquitination and degradation of proteins.[17] A recent study demonstrated higher mean plasma 8-hydroxydeoxyguanosine levels in patients with PD than in controls,[18] providing yet more evidence of systemic effects of the disease. Interestingly, increased serum levels of uric acid, a potent antioxidant, are associated with a lower risk of PD in men.[19] Recent advances in metabolomic, proteomic and transcriptomic approaches are anticipated to permit further identification of alterations at the molecular level that are relevant to mitochondrial metabolism in PD.

What are the consequences of abnormal mitochondrial function in PD? Mitochondria have an integral role in the apoptotic cell death

pathway; when the outer mitochondrial membrane is rendered permeable by the action of 'death agonists', such as Bax, cytochrome c is released into the cytosol, leading to caspase activation and apoptosis.[20] Similar pathways are also activated by opening of the mitochondrial permeability transition pore, an event that can occur under conditions of oxidative stress or electron transport chain inhibition, leading to collapse of the mitochondrial membrane potential. Mitochondrial dysfunction and oxidative stress might, therefore, 'reset' the threshold for activation of apoptotic pathways in response to Bax and other pro-apoptotic molecules. Impaired energy metabolism resulting from mitochondrial dysfunction has also been proposed to render cells vulnerable to 'weak excitotoxicity', which is proposed to result from changes in the energy-dependent cell membrane potential. This weak excitotoxic injury could potentially increase free radical generation and add to cellular injury.[21] Mitochondrial dysfunction and increased oxidative stress is also predicted to overload the ubiquitin–proteasomal system (UPS) of protein degradation, leading to accumulation of misfolded or damaged proteins.

ENVIRONMENTAL INFLUENCES ON MITOCHONDRIA AND COMPLEX I

Several complex I inhibitors replicate some of the key motor features of PD and cause death of dopaminergic neurons. For example, parkinsonism in humans has been reported to result from unintentional exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[22] Chronic infusion of the pesticide rotenone reproduces a parkinsonian syndrome in rats that is associated with selective loss of dopaminergic neurons and Lewy body-like fibrillar cytoplasmic inclusions containing ubiquitin and α-synuclein.[23] Administration of rotenone to *Drosophila melanogaster* results in levodopa-responsive locomotor deficits and loss of dopaminergic neurons.[24] Paraquat, a free radical generator, causes loss of dopaminergic neurons and motor deficits in rodents,[25] with a corresponding dramatic increase in free radical formation by mitochondria. The precise role of environmental complex I inhibitors in PD remains to be defined, but these results help to corroborate concerns over other environmental toxins. For example, a link has been suggested to exist between PD and workplace exposure to the industrial solvent trichloroethylene, which inhibits complex I and causes nigral cell loss in animals.[26]

GENETIC INFLUENCES ON MITOCHONDRIA AND COMPLEX I

Mitochondrial genetic alterations

Maternally inherited mutations in mitochondrial DNA (mtDNA) are rarely linked to PD, although one kindred with maternally inherited PD and complex I deficiency[27] and five families with a probable maternal PD inheritance pattern have been reported.[28] A missense mutation in the ND4 subunit of complex I has led to atypical parkinsonism and loss of nigral neurons in one kindred, although this mutation is more typically associated with Leber hereditary optic neuropathy.[29] Mutation in the gene encoding human mtDNA polymerase subunit γ (*POLG*) leads to clinical parkinsonism associated with multiple mtDNA deletions.[30] Parkinsonism is also seen as a feature of several well characterized mitochondrial disorders, including myoclonic epilepsy with ragged red fibers (MERRF) and mitochondrial encephalomyopathy with stroke-like episodes (MELAS). As is the case in individuals with *POLG* mutations, however, the diagnosis of MERRF and MELAS is almost always aided by other features not typically found in PD, such as progressive external ophthalmoplegia, seizures, neuropathy, and myopathy.

A clear mitochondrial genetic contribution is evident in cases of idiopathic PD, and this contribution is most probably attributable to acquired somatic mutations. Acquired mtDNA deletions have now been demonstrated in PD by long-range polymerase chain reaction studies of pooled laser microdissected neurons from brain tissue samples isolated at autopsy.[31] More-extensive mtDNA deletions, which are associated with cytochrome c oxidase deficiency, occur in patients with PD compared with age-matched controls, and oxidative damage leading to double-strand breaks in mtDNA might be instrumental in the acquisition of these somatic mutations.[32] In addition to these acquired mutations, studies have suggested that specific haplogroups, such as UJKT, might affect PD risk,[33] although the effects of these haplogroups are likely to vary between different populations.

Nuclear gene alterations affecting mitochondrial function and oxidative stress

A growing body of evidence indicates that the products of PD-associated genes have important effects on mitochondrial morphology, function and oxidative stress (Table 1, Figure 2). The effects of dysfunction of these genes are summarized in the sections that follow.

Table 1 Involvement of Parkinson disease-associated genes in mitochondrial dysfunction and oxidative stress.

Gene	Function of gene product	Observations	References
α-synuclein (*SNCA* or *PARK1*)	Not known	▪ Wild-type protein: reduces mitochondrial function, increases oxidative stress; overexpression plus MPTP administration leads to abnormal mitochondria; overexpression leads to association with mitochondrial membrane and cytochrome c release (in SHSY cells) or increased free radicals (in GT-17 cells) ▪ Knockout mutant: increased resistance to MPTP (in mice) ▪ Mutation (overexpression of the Ala53Thr form of α-synuclein): abnormal mitochondria, damage to mitochondrial DNA (in mice); increased cytochrome c release (in PC12 and SHSY cells); association with mitochondrial membrane (in SHSY cells); increased markers of oxidative stress (in NT-2/D1 and SK-N-MC cells)	Martin *et al.* (2006)[37] Parihar *et al.* (2008)[38] Song *et al*. (2004)[41] Ko *et al.* (2000)[84] Ostrerova-Golts *et al.* (2000)[85] Smith *et al.* (2005)[86] Paxinou *et al.* (2001)[87]
Parkin (*PARK2*)	Ubiquitin E3 ligase	▪ Partially associated with the mitochondrial outer membrane ▪ Localized to mitochondria in proliferating cells ▪ Interacts with PINK1 to promote mitochondrial fission ▪ Mutation: abnormal mitochondria, increased sensitivity to oxidative stress (in *Drosophila melanogaster*); decreased complex I and IV (in mice and humans); increased oxidative stress (in mice) ▪ Wild–type protein: involved in mitochondrial biogenesis and mitochondrial DNA replication; rescues *PINK1* mutant phenotype	Kuroda *et al.* (2006)[42] Greene *et al.* (2003)[43] Pesah *et al.* (2004)[44] Palacino *et al.* (2004)[45] Muftuoglu *et al.* (2004)[46] Poole *et al.* (2008)[47] Clark *et al.* (2006)[51] West *et al.* (2005)[65] Darios *et al.* (2003)[88] Park *et al.* (2006)[89]
PINK1 (*PARK6*)	Serine–threonine kinase	▪ Mitochondrial membrane localization ▪ Targeting by small interfering RNA: increased sensitivity to 1-methyl-4-phenylpyridinium and rotenone ▪ Mutation: abnormal mitochondria, increased sensitivity to oxidative stress (in *D. melanogaster*); reduced complex I activity and increased oxidative damage (in individuals with PD who carry the Gly309Asp substitution) ▪ Wild–type protein: reduces mitochondrial cytochrome c release, reduces apoptosis (in cell cultures); overexpression promotes mitochondrial fission	Silvestri *et al.* (2005)[48] Clark *et al.* (2006)[51] Yang *et al.* (2008)[52] Petit *et al.* (2005)[53] Park *et al.* (2006)[89] Gandhi *et al.* (2006)[90]
DJ-1 (*PARK7*)	Oxidative stress sensor, chaperone	▪ Oxidative stress causes relocalization to mitochondria (in the matrix and/or intermembrane space), is oxidized in the brains of patients with PD ▪ Protects against oxidative stress ▪ Targeting by small interfering RNA: increased sensitivity to oxidative stress (in *D. melanogaster*) ▪ Mutation: increased sensitivity to rotenone, paraquat, and hydrogen peroxide (in *D. melanogaster*); increased sensitivity to oxidative stress (in mice)	Bonifati *et al.* (2003)[56] Yokota *et al.* (2003)[57] Zhang *et al.* (2005)[58] Meulener *et al.* (2005)[60] Park *et al.* (2005)[61] Yang *et al.* (2005)[62] Shendelman *et al.* (2004)[63] Meulener *et al.* (2005)[64] Taira *et al.* (2004)[91] Choi *et al.* (2006)[92] Menzies *et al.* (2005)[93]
LRRK2 (*PARK8*)	Serine–threonine kinase	▪ Around 10% are located in outer mitochondrial membrane; kinase activity affects mitochondrial function	West *et al.* (2005)[65]
HTRA2 (*PARK13*)	Serine protease	▪ Localized to mitochondria, released during mitochondrial membrane permeabilization in programmed cell death ▪ Mutation: mitochondrial swelling, reduced membrane potential, reduced neuroprotection	Martins *et al.* (2004)[66] Strauss *et al.* (2005)[67]

Abbreviations: HTRA2, high temperature requirement protein A2; LRRK2, leucine-rich repeat kinase 2; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson disease; PINK1, PTEN-induced putative kinase 1.

α-*synuclein*

The α-synuclein protein is an abundant protein found particularly in axonal termini and is also a component of Lewy bodies. Certain mutations in the α-synuclein gene (*SNCA*; also known as *PARK1*), including those causing the Ala53Thr and Ala30Pro substitutions, as well as gene triplication (the triplicated locus is also known as PARK4), lead to autosomal dominant PD. The wild-type function of α-synuclein is not well

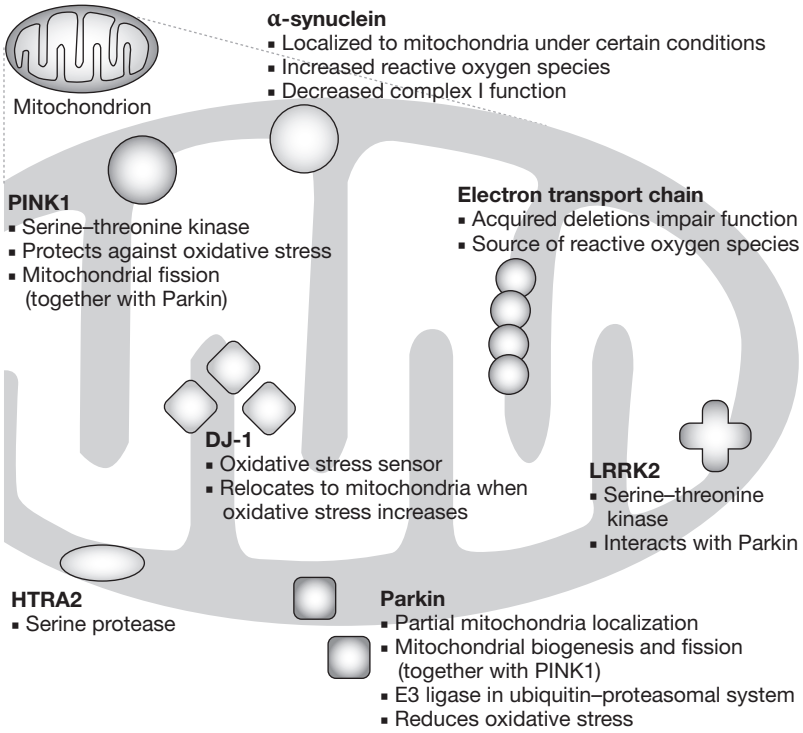

Figure 2 Products of PD-associated genes that affect mitochondrial function and oxidative stress. Acquired somatic mutations affect mitochondrial electron transport chain function, and such mutations are increased in the substantia nigra in patients with PD. Rare inherited mutations in genes encoding electron transport chain components have been associated with parkinsonism. Parkin, α-synuclein, PINK1, DJ-1, LRRK2 and HTRA2, are all encoded by nuclear genes, mutations in which can lead to PD, and all show a degree of localization to the mitochondria. Parkin is partially localized to the outer mitochondrial membrane, protects against oxidative stress, and has a hypothesized role in mitochondrial biogenesis. LRRK2 associates, at least in part, with the outer mitochondrial membrane; its precise function in that location is unclear, but it is thought to interact with Parkin. HTRA2 is a mitochondrial serine protease, the release of which might be involved in apoptotic cell death. PINK1 is a mitochondrial serine–threonine kinase that affords protection against oxidative stress and acts with Parkin to regulate the balance of mitochondrial fission and fusion. DJ-1 is relocated to mitochondria under conditions of oxidative stress and is thought to be neuroprotective under such conditions. The α-synuclein protein has an amino-terminal mitochondrial targeting sequence and, when overexpressed or under conditions of acidification, is at least partially associated with the inner mitochondrial membrane, where it might cause direct damage. Abbreviations: HTRA2, high temperature requirement protein A2; LRRK2, leucine-rich repeat kinase 2; PD, Parkinson disease; PINK1, PTEN-induced putative kinase 1.

understood, but there seems to be a reciprocal relationship between the activity of this protein and mitochondrial function.

Oxidative damage to α-synuclein affects the protein's aggregation—an effect that might partially explain the cellular toxicity of the protein. The α-synuclein protein contains an amino-terminal mitochondrial targeting sequence,[34] and acidification of the cytosol or overexpression of α-synuclein can cause the protein to become localized to mitochondria.[35,36] Moreover, when exposed to rotenone, human embryonic kidney cells that overexpress α-synuclein are more susceptible to cell death and have lower measurable levels of ATP than control cells.[36] Degenerating and dysmorphic mitochondria with evidence of DNA damage are present in brainstem neurons of mice that overexpress the human Ala53Thr form of α-synuclein.[37] The α-synuclein protein also seems to induce oxidative damage and mitochondrial release of cytochrome c.[38] In aging yeast, functional mitochondria are required for α-synuclein toxicity.[39] Mice with a knockout of the *Snca* gene demonstrate increased resistance to MPTP through modulation of complex I activity,[40] whereas administration of MPTP to mice that overexpress α-synuclein leads to swollen, morphologically abnormal mitochondria.[41]

Parkin

A number of mutations in the *Parkin* gene (also known as *PARK2*) lead to autosomal recessive PD. Parkin acts as a ubiquitin E3 ligase within the UPS, and this activity is vulnerable to oxidative damage. Parkin also seems to have a fundamental role in mitochondrial function, and the protein has been detected in mitochondria in replicating cells.[42] *Parkin*-null *D. melanogaster* mutants develop prominent apoptotic muscle degeneration with mitochondrial pathology and decreased resistance to oxidative stress.[43,44] Parkin-deficient mice have reduced striatal mitochondrial respiratory capacity, with decreased levels of the subunits of the mitochondrial electron transport complexes I and IV.[45] In addition, in humans with PD and in those who are homozygous for *Parkin* mutations, leukocyte mitochondrial complex I and IV activities are reduced.[46] A link between Parkin and oxidative stress is demonstrated by the finding that *Parkin*-null mice manifest increased protein and lipid peroxidation.[45]

A recent and intriguing finding is that Parkin is involved in the regulation of mitochondrial morphology.[47] Parkin affects mitochondrial biogenesis in conjunction with PTEN-induced putative kinase 1 (PINK1; see below), and overexpression of Parkin enhances transcription and replication of mitochondrial DNA.[42]

PTEN-induced putative kinase 1

Mutations in *PINK1* (also known as *PARK6*) lead to a rare autosomal form of PD. The PINK1 protein is a serine–threonine kinase localized to

the mitochondrial membrane via an 8 kDa amino-terminal mitochondrial targeting sequence.[48] The mitochondrial chaperone protein TRAP1 is a PINK1 substrate,[49] but PINK1 might not be exclusively mitochondrial and is probably dynamically distributed between various cellular compartments, including the cytosol. Cells isolated from individuals with a *PINK1* mutation that causes a Gly309Asp substitution have reduced complex I activity and evidence of increased oxidative damage compared with cells from control individuals.[50] In addition, a *Pink1* deficiency in *D. melanogaster* results in loss of dopaminergic cells, as well as enhanced susceptibility to oxidative stress and reduced ATP levels.[51] These *D. melanogaster* mutants also demonstrate reduced mitochondrial mass with disorganized morphology, similar to the effects of *Parkin* mutations. Such morphological changes could result simply from a dependency on oxidative phosphorylation for maintenance of intact mitochondrial networks, although there is evidence that PINK1 itself also regulates mitochondrial fission and fusion. Overexpression of PINK1 in mammalian or *D. melanogaster* cells results in increased mitochondrial fission. In *D. melanogaster*, altered function of Drp1, a component of the mitochondrial fission–fusion apparatus, modifies the *Pink1*-null phenotype.[52]

PINK1 also seems to possess neuroprotective properties. For example, wild-type, but not mutant, PINK1 attenuates staurosporine-induced apoptosis and reduces mitochondrial cytochrome c release when overexpressed in SH-SY5Y cells.[53] Expression of a small interfering RNA (siRNA) that targets *PINK1* increases susceptibility to 1-methyl-4-phenylpyridinium (MPP+) or rotenone,[53] and in human dopaminergic neurons expression of this siRNA leads to reduced long-term survival, along with mitochondrial dysfunction, abnormal mitochondrial morphology, and increased oxidative stress.[54]

Intriguingly, overexpression of Parkin in HeLa cells[55] can rescue some features of the PINK1-deficient phenotypes,[51] including altered mitochondrial membrane potential and morphology, indicating that PINK1 and Parkin function via a common pathway.

DJ-1

DJ-1 (also known as PARK7) has multiple functions, but its overall wild-type function seems to be to protect cells from oxidative-stress-related death.[56,57] Under conditions of oxidative stress, the DJ-1 protein relocalizes from the nucleus to the mitochondrial matrix and intermembrane space.[58] A mutation affecting the cysteine 106 residue prevents this process and attenuates the protection afforded by DJ-1 against oxidative stress and mitochondrial damage.[59]

Normal DJ-1 function protects cells against a variety of insults; *D. melanogaster* double knockout mutations of the *DJ-1* homologs *DJ-1*α and *DJ-1*β are exquisitely sensitive to rotenone and paraquat,[60] and DJ-1β-deficient flies display a locomotor deficit that is exacerbated by oxidative stress.[61] *DJ-1* knockout mice have enhanced sensitivity to MPTP, and their embryonic cortical neurons are more susceptible than wild-type neurons to oxidative stress.[61] Furthermore, introduction of an siRNA that targets *DJ-1*α into *D. melanogaster* increases levels of reactive oxygen species, increases sensitivity to oxidative stress, and results in degeneration of dopaminergic neurons.[62] DJ-1 might reduce α-synuclein aggregation,[63] and it might physically associate with α-synuclein.[64] In *D. melanogaster*, DJ-1 functions as a PTEN suppressor.[61]

Leucine-rich repeat kinase 2

Mutations in the leucine-rich repeat serine–threonine-protein kinase 2 gene (*LRRK2*; also known as *PARK8*) represent the most common known cause of familial PD; these mutations also account for cases of sporadic, late-onset PD. LRRK2 has a conserved serine–threonine kinase mitogen-activated protein kinase kinase kinase (MAPKKK) domain, and is a member of the Roc GTPase family. The commonly occurring Gly2019Ser substitution takes place in the MAPKKK domain, and the mutation augments kinase activity.[65] Although the majority of LRRK2 is present in the cytoplasm, approximately 10% of these proteins are associated with the outer mitochondrial membrane,[65] raising the question of whether mutant LRRK2 kinase hyperactivity might directly affect mitochondrial function.

HTRA2

High temperature requirement protein A2 (HTRA2; also known as OMI or PARK13) is a mitochondrial serine protease. Mutations in the gene encoding this protein are rare in individuals with PD, and mutations in *HTRA2* are suggested to be a susceptibility factor for PD. Homozygous *HtrA2* knockout mice develop striatal degeneration and parkinsonism[66] in the context of more-widespread neuronal loss. Expression of a

mutation that causes the Gly399Ser substitution or a polymorphism that produces an Ala141Ser substitution, both of which have been found in individuals with PD, leads to mitochondrial swelling, decreased mitochondrial membrane potential, and increased risk of staurosporine-induced cell death.[67] Permeabilization of the mitochondrial membrane by pro-apoptotic molecules might result in release of HTRA2 as part of the programmed cell death pathway.[68] The protease activity of HTRA2 is controlled by PINK1-dependent phosphorylation, and HTRA2 phosphorylation is decreased in brain tissue from individuals with PD who carry *PINK1* mutations.[69] A *PINK1* mutation that has been proposed to be pathogenic has also been found in individuals without PD, however, so its significance requires further clarification.[70]

Clearly, there is more to learn regarding the functions of the PD-associated genes and gene modifiers that have been identified to date. The data described above, however, support a direct or indirect role for several of these genes in both mitochondrial function and the cellular response to oxidative stress.

THERAPEUTIC IMPLICATIONS

The concept of 'mitochondrial therapy' is a new approach in PD, but it is being intensively tested. Coenzyme Q10 (CoQ10) is a naturally occurring antioxidant that affects mitochondrial depolarization and acts as an electron transporter for mitochondrial complexes I and II.[71] CoQ10 levels are low in mitochondria that have been isolated from individuals with PD, and the ratio of oxidized to reduced CoQ10 is greater in patients with PD than in controls, suggesting increased oxidative stress in the former. In mice and primates, CoQ10 protects against paraquat-induced oxidative stress, loss of dopaminergic neurons resulting from rotenone exposure, and both acute and chronic MPTP-induced dopaminergic cell death.[72–75]

The effects of CoQ10 in PD are not fully understood, but a recent magnetic resonance spectroscopy study in patients with progressive supranuclear palsy demonstrated a significant increase in the ratio of high-energy to low-energy phosphates (suggesting improved oxidative phosphorylation) in the occipital cortex after short-term administration of CoQ10.[76] In a small, randomized, double-blind, placebo-controlled study in untreated individuals with early PD, high-dose supplementation with CoQ10 at a dose of up to 1200 mg daily, in conjunction with α-tocopherol, was suggested to slow progression of disease in the absence of symptomatic benefit.[77] When administered at a dose of 2400 mg daily in early PD as part of the Neuroprotection Exploratory Trials in Parkinson Disease (NET-PD) program, the use of CoQ10 could not be rejected as futile on the basis of the prespecified criteria.[78] Much discussion has taken place, however, regarding the validity of these criteria. A large phase III trial comparing placebo, 1,200 mg, and 2,400 mg CoQ10 daily is now underway in an attempt to dispel this controversy.

Creatine, in the form of phosphocreatine, a high-energy phosphate, buffers cellular ATP and prevents opening of the mitochondrial permeability transition pore. Creatine attenuates MPP+-mediated toxicity in embryonic ventral mesencephalic neurons and is protective against MPTP. In individuals with PD who participated in the NET-PD investigation, creatine was well tolerated and its use could not be rejected as futile.[79] A large clinical trial is now planned to test the potential of CoQ10 as a disease-modifying agent.

Rasagiline is a monoamine oxidase type B inhibitor that produces a mild symptomatic benefit in patients with PD. The drug has multiple effects on mitochondrial function, including stabilization of the mitochondrial membrane potential,[80] and evidence from a delayed-start clinical trial of rasagiline suggests that the agent might have a disease-modifying effect.[81] Results from another delayed-start design phase III clinical trial are awaited.

Recent advances in the dissection of the complex cellular pathways that influence mitochondrial structure and function have exposed new potential therapeutic targets. The peroxisome-proliferator-activated receptor gamma coactivator (PGC)-1α is a transcriptional coactivator that regulates mitochondrial biogenesis and energy metabolism, thereby aiding in the maintenance of energy homeostasis. Resveratrol, a polyphenolic compound abundant in grape skins, induces expression of genes involved in mitochondrial biogenesis and oxidative phosphorylation by activating NAD-dependent deacetylase sirtuin-1 (SIRT1) and PGC-1α.[82] Although the effects of resveratrol in PD are unclear, the agent seems to protect against MPTP-induced dopaminergic neuron loss in mice.[83]

CONCLUSIONS AND FUTURE PROSPECTS

A growing body of evidence now indicates that mitochondrial dysfunction and oxidative stress have central roles in PD pathogenesis. Recent advances in understanding the genetics of PD in humans, as well as the use of animal models of PD, have enabled us to make important steps not only in identifying the proteins involved in the pathways of PD pathogenesis, but also in determining how these proteins interact, both of which have enabled us to link oxidative stress and mitochondrial dysfunction with abnormal UPS function.

Commercial genetic testing is now available for *SNCA*, *Parkin*, *PINK1* and *LRRK2* gene mutations, as well as for mutations in certain mitochondrial genes. Until the consequences of these mutations are better understood, the decision of whether to test for PD-associated gene mutations remains a matter of clinical judgment and should be made on the basis of a full and informed discussion between clinician and patient. In the future, however, a patient's genotype might conceivably have value in predicting the individual's response to treatments and disease-modifying interventions. We feel, therefore, that it is imperative for patients be encouraged to participate in research studies where appropriate, so that such questions can be addressed.

Strategies to block oxidative stress are effective in animal models of PD, and some encouraging data now exist to suggest that agents that affect mitochondrial function might slow down PD progression. To date, however, most of the promising data on neuroprotective agents in PD animal models have not adequately translated to patients in clinical trials. A lack of biomarkers that would be useful as surrogate end points and as measurements of intended therapeutic effects has markedly hampered trial design and the interpretation of results. We suggest that a better understanding of how mitochondrial function is altered *in vivo* in brain tissue in PD might be an important step in developing potential PD biomarkers. Rather than using these as diagnostic biomarkers in the conventional sense, they could provide a means of identifying endophenotypes. The susceptibility of an individual to developing PD probably involves a complex interplay of genetic factors, environmental factors, and aging, with the consequence that within a population of individuals with PD, considerable heterogeneity exists with regard to function of specific cellular pathways. A biomarker designed to detect mitochondrial dysfunction would enable an 'enriched' population—those with more-severe defects in mitochondrial metabolism—to be identified, and these individuals could be preferentially enrolled into neuroprotective trials of mitochondrial therapy. This would be a first step towards the rational individualization of neuroprotective regimens.

Importantly, the role of mitochondrial function and oxidative stress in disease pathogenesis is not specific to PD. There is evidence that these pathways are also involved in other neurodegenerative diseases such as Friedreich ataxia, Alzheimer disease, amyotrophic lateral sclerosis, and Huntington disease, among others. Potential exists, therefore, for considerable 'cross-talk' in the development of improved treatments for each of these conditions.

KEY POINTS

- Defective mitochondrial function and increased oxidative stress have been demonstrated in a subset of people with Parkinson disease (PD)
- The products of several nuclear genes associated with PD are linked to mitochondrial function
- Mitochondrial activity can also be affected by environmental factors that possibly contribute to PD pathogenesis
- Novel therapies that target mitochondrial function and oxidative stress, such as coenzyme Q10, are now in clinical trials to test whether they modify PD progression

References

1 Beal MF (2007) Mitochondria and neurodegeneration. *Novartis Found Symp* **287:** 183–192
2 Schapira AH (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet Neurol* **7:** 97–109
3 Schapira AH *et al.* (1990) Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* **54:** 823–827
4 Parker WD Jr *et al.* (2008) Complex I deficiency in Parkinson's disease frontal cortex. *Brain Res* **1189:** 215–218
5 Keeney PM *et al.* (2006) Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. *J Neurosci* **26:** 5256–5264
6 Haas RH *et al.* (1995) Low platelet mitochondrial complex I and complex II/III activity in early untreated Parkinson's disease. *Ann Neurol* **37:** 714–722
7 Penn AM *et al.* (1995) Generalized mitochondrial dysfunction in Parkinson's disease detected by magnetic resonance spectroscopy of muscle. *Neurology* **45:** 2097–2099

8 Swerdlow RH *et al.* (1996) Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann Neurol* **40:** 663–671
9 Gu M *et al.* (1998) Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease. *Ann Neurol* **44:** 177–186
10 Hu MT *et al.* (2000) Cortical dysfunction in non-demented Parkinson's disease patients: a combined ^{31}P-MRS and ^{18}FDG-PET study. *Brain* **123:** 340–352
11 Rango M *et al.* (2005) Parkinson's disease and brain mitochondrial dysfunction: a functional phosphorus magnetic resonance spectroscopy study. *J Cereb Blood Flow Metab* **26:** 283–290
12 Bowen BC *et al.* (1995) Proton MR spectroscopy of the brain in 14 patients with Parkinson disease. *AJNR Am J Neuroradiol* **16:** 61–68
13 Henchcliffe C *et al.*: Multinuclear magnetic resonance spectroscopy for *in vivo* assessment of mitochondrial function in Parkinson's disease. *Ann N Y Acad Sci,* in press
14 Dexter DT *et al.* (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J Neurochem* **52:** 381–389
15 Zhang J *et al.* (1999) Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. *Am J Pathol* **154:** 1423–1429
16 Perry TL *et al.* (1986) Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. *Neurosci Lett* **67:** 269–274
17 Jenner P (2003) Oxidative stress in Parkinson's disease. *Ann Neurol* **53 (Suppl 3):** S26–S36
18 Bogdanov M *et al.* (2008) Metabolomic profiling to develop blood biomarkers for Parkinson's disease. *Brain* **131:** 389–396
19 Weisskopf MG *et al.* (2007) Plasma urate and risk of Parkinson's disease. *Am J Epidemiol* **166:** 561–567
20 Green DR *et al.* (2004) The pathophysiology of mitochondrial cell death. *Science* **305:** 626–629
21 Beal MF (1998) Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. *Ann Neurol* **44 (Suppl 1):** S110–S114
22 Langston JW *et al.* (1983) Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219:** 979–980
23 Betarbet R *et al.* (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* **3:** 1301–1306
24 Coulom H *et al.* (2004) Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*. *J Neurosci* **24:** 10993–10998
25 Cicchetti F *et al.* (2005) Systemic exposure to paraquat and maneb models early Parkinson's disease in young adult rats. *Neurobiol Dis* **20:** 360–371
26 Greenamyre JT *et al.* (1994) Antiparkinsonian effects of remacemide hydrochloride, a glutamate antagonist, in rodent and primate models of Parkinson's disease. *Ann Neurol* **35:** 655–661
27 Swerdlow RH *et al.* (1998) Matrilineal inheritance of complex I dysfunction in a multigenerational Parkinson's disease family. *Ann Neurol* **44:** 873–881
28 Wooten GF *et al.* (1997) Maternal inheritance in Parkinson's disease. *Ann Neurol* **41:** 265–268
29 Simon DK *et al.* (1999) Familial multisystem degeneration with parkinsonism associated with the 11778 mitochondrial DNA mutation. *Neurology* **53:** 1787–1793
30 Luoma P *et al.* (2004) Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. *Lancet* **364:** 875–882
31 Bender A *et al.* (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet* **38:** 515–517
32 Srivastava S *et al.* (2005) Double-strand breaks of mouse muscle mtDNA promote large deletions similar to multiple mtDNA deletions in humans. *Hum Mol Genet* **14:** 893–902
33 Pyle A *et al.* (2005) Mitochondrial DNA haplogroup cluster UKJT reduces the risk of PD. *Ann Neurol* **57:** 564–567
34 Devi L *et al.* (2008) Mitochondrial import and accumulation of α-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem* **283:** 9089–9100
35 Cole NB *et al.* (2008) Mitochondrial translocation of alpha-synuclein is promoted by intracellular acidification. *Exp Cell Res* **314:** 2076–2089
36 Shavali S *et al.* (2008) Mitochondrial localization of alpha-synuclein protein in alpha-synuclein overexpressing cells. *Neurosci Lett* **439:** 125–128
37 Martin LJ *et al.* (2006) Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. *J Neurosci* **26:** 41–50
38 Parihar MS *et al.* (2008) Mitochondrial association of alpha-synuclein causes oxidative stress. *Cell Mol Life Sci* **65:** 1272–1284
39 Buttner S *et al.* (2008) Functional mitochondria are required for α-synuclein toxicity in aging yeast. *J Biol Chem* **283:** 7554–7560
40 Dauer W *et al.* (2003) Parkinson's disease: mechanisms and models. *Neuron* **39:** 889–909
41 Song DD *et al.* (2004) Enhanced substantia nigra mitochondrial pathology in human α-synuclein transgenic mice after treatment with MPTP. *Exp Neurol* **186:** 158–172
42 Kuroda Y *et al.* (2006) Parkin enhances mitochondrial biogenesis in proliferating cells. *Hum Mol Genet* **15:** 883–895
43 Greene JC *et al.* (2003) Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants. *Proc Natl Acad Sci USA* **100:** 4078–4083
44 Pesah Y *et al.* (2004) *Drosophila parkin* mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development* **131:** 2183–2194
45 Palacino JJ *et al.* (2004) Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J Biol Chem* **279:** 18614–18622
46 Muftuoglu M *et al.* (2004) Mitochondrial complex I and IV activities in leukocytes from patients with *parkin* mutations. *Mov Disord* **19:** 544–548
47 Poole AC *et al.* (2008) The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci USA* **105:** 1638–1643
48 Silvestri L *et al.* (2005) Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum Mol Genet* **14:** 3477–3492
49 Pridgeon JW *et al.* (2007) PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. *PLoS Biol* **5:** e172
50 Hoepken HH *et al.* (2007) Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. *Neurobiol Dis* **25:** 401–411
51 Clark IE *et al.* (2006) *Drosophila Pink1* is required for mitochondrial function and interacts genetically with *parkin*. *Nature* **441:** 1162–1166
52 Yang Y *et al.* (2008) Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci USA* **105:** 7070–7075
53 Petit A *et al.* (2005) Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J Biol Chem* **280:** 34025–34032
54 Wood-Kaczmar A *et al.* (2008) PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PLoS ONE* **3:** e2455

55 Exner N *et al.* (2007) Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. *J Neurosci* **27:** 12413–12418
56 Bonifati V *et al.* (2003) Mutations in the *DJ-1* gene associated with autosomal recessive early-onset parkinsonism. *Science* **299:** 256–259
57 Yokota T *et al.* (2003) Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. *Biochem Biophys Res Commun* **312:** 1342–1348
58 Zhang L *et al.* (2005) Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. *Hum Mol Genet* **14:** 2063–2073
59 Canet-Aviles RM *et al.* (2004) The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc Natl Acad Sci USA* **101:** 9103–9108
60 Meulener M *et al.* (2005) *Drosophila DJ-1* mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. *Curr Biol* **15:** 1572–1577
61 Park J *et al.* (2005) *Drosophila DJ-1* mutants show oxidative stress-sensitive locomotive dysfunction. *Gene* **361:** 133–139
62 Yang Y *et al.* (2005) Inactivation of *Drosophila* DJ-1 leads to impairments of oxidative stress response and phosphatidylinositol 3-kinase/Akt signaling. *Proc Natl Acad Sci USA* **102:** 13670–13675
63 Shendelman S *et al.* (2004) DJ-1 is a redox-dependent molecular chaperone that inhibits α-synuclein aggregate formation. *PLoS Biol* **2:** e362
64 Meulener MC *et al.* (2005) DJ-1 is present in a large molecular complex in human brain tissue and interacts with α-synuclein. *J Neurochem* **93:** 1524–1532
65 West AB *et al.* (2005) Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci USA* **102:** 16842–16847
66 Martins LM *et al.* (2004) Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol Cell Biol* **24:** 9848–9862
67 Strauss KM *et al.* (2005) Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum Mol Genet* **14:** 2099–2111
68 Ravagnan L *et al.* (2002) Mitochondria, the killer organelles and their weapons. *J Cell Physiol* **192:** 131–137
69 Plun-Favreau H *et al.* (2007) The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. *Nat Cell Biol* **9:** 1243–1252
70 Simon-Sanchez J *et al.* (2008) Sequencing analysis of OMI/HTRA2 shows previously reported pathogenic mutations in neurologically normal controls. *Hum Mol Genet* **17:** 1988–1993
71 Beal MF (2004) Mitochondrial dysfunction and oxidative damage in Alzheimer's and Parkinson's diseases and coenzyme Q10 as a potential treatment. *J Bioenerg Biomembr* **36:** 381–386
72 McCarthy S *et al.* (2004) Paraquat induces oxidative stress and neuronal cell death; neuroprotection by water-soluble coenzyme Q10. *Toxicol Appl Pharmacol* **201:** 21–31
73 Menke T *et al.* (2003) Coenzyme Q10 reduces the toxicity of rotenone in neuronal cultures by preserving the mitochondrial membrane potential. *Biofactors* **18:** 65–72
74 Beal MF *et al.* (1998) Coenzyme Q10 attenuates the 1-methyl-4-phenyl-1,2,3,tetrahydropyridine (MPTP) induced loss of striatal dopamine and dopaminergic axons in aged mice. *Brain Res* **783:** 109–114
75 Horvath TL *et al.* (2003) Coenzyme Q induces nigral mitochondrial uncoupling and prevents nigral cell loss in a primate model of Parkinson's disease. *Endocrinology* **144:** 2757–2760
76 Stamelou M *et al.* (2008) Short-term effects of coenzyme Q10 in progressive supranuclear palsy: a randomized, placebo-controlled trial. *Mov Disord* **23:** 942–949
77 Shults CW *et al.* (2002) Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. *Arch Neurol* **59:** 1541–1550
78 The NINDS NET-PD Investigators (2007) A randomized clinical trial of coenzyme Q10 and GPI-1485 in early Parkinson disease. *Neurology* **68:** 20–28
79 The NINDS NET-PD Investigators (2006) A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease. *Neurology* **66:** 664–671
80 Klivenyi P *et al.* (2006) Mice lacking alpha-synuclein are resistant to mitochondrial toxins. *Neurobiol Dis* **21:** 541–548
81 Parkinson Study Group (2004) A controlled, randomized, delayed-start study of rasagiline in early Parkinson disease. *Arch Neurol* **61:** 561–566
82 Lagouge M *et al.* (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell* **127:** 1109–1122
83 Lu KT *et al.* (2008) Neuroprotective effects of resveratrol on MPTP-induced neuron loss mediated by free radical scavenging. *J Agric Food Chem* **56:** 6910–6913
84 Ko L *et al.* (2000) Sensitization of neuronal cells to oxidative stress with mutated human α-synuclein. *J Neurochem* **75:** 2546–2554
85 Ostrerova-Golts N *et al.* (2000) The A53T α-synuclein mutation increases iron-dependent aggregation and toxicity. *J Neurosci* **20:** 6048–6054
86 Smith WW *et al.* (2005) Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc Natl Acad Sci USA* **102:** 18676–18681
87 Paxinou E *et al.* (2001) Induction of α-synuclein aggregation by intracellular nitrative insult. *J Neurosci* **21:** 8053–8061
88 Darios F *et al.* (2003) Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. *Hum Mol Genet* **12:** 517–526
89 Park J *et al.* (2006) Mitochondrial dysfunction in *Drosophila PINK1* mutants is complemented by parkin. *Nature* **441:** 1157–1161
90 Gandhi S *et al.* (2006) PINK1 protein in normal human brain and Parkinson's disease. *Brain* **129:** 1720–1731
91 Taira T *et al.* (2004) DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Rep* **5:** 213–218
92 Choi J *et al.* (2006) Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. *J Biol Chem* **281:** 10816–10824
93 Menzies FM *et al.* (2005) Roles of *Drosophila* DJ-1 in survival of dopaminergic neurons and oxidative stress. *Curr Biol* **15:** 1578–1582

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