A cell biological perspective on mitochondrial dysfunction in Parkinson disease and other neurodegenerative diseases

Wim Mandemakers, Vanessa A. Morais and Bart De Strooper*

Center for Human Genetics, K.U.Leuven and Department of Molecular and Developmental Genetics, VIB, Herestraat 49, 3000 Leuven, Belgium

*Author for correspondence (e-mail: Bart.destrooper@med.kuleuven.be)

Accepted 13 March 2007
Journal of Cell Science 120, 1707-1716 Published by The Company of Biologists 2007
doi:10.1242/jcs.03443

Summary

Dysfunction of mitochondria is frequently proposed to be involved in neurodegenerative disease. Deficiencies in energy supply, free radical generation, Ca^{2+} buffering or control of apoptosis, could all theoretically contribute to progressive decline of the central nervous system. Parkinson disease illustrates how mutations in very different genes finally impinge directly or indirectly on mitochondrial function, causing subtle but finally fatal dysfunction of dopaminergic neurons. Neurons in general appear more sensitive than other cells to mutations in genes encoding mitochondrial proteins. Particularly interesting are mutations in genes such as *Opa1*, *Mfn1* and *Dnm1l*, whose products are involved in the dynamic morphological alterations and subcellular trafficking of mitochondria. These indicate that mitochondrial dynamics are especially important for the long-term maintenance of the nervous system. The emerging evidence clearly demonstrates the crucial role of specific mitochondrial functions in maintaining neuronal circuit integrity.

Key words: Neurodegenerative, Mitochondria, Oxidative stress, Parkinson disease, OXPHOS, Apoptosis, Mitochondrial dynamics

Introduction

Neurodegenerative diseases are characterized by the progressive death of neurons and result in memory loss, movement problems, cognitive deficits, emotional alterations and behavioral problems. Environmental factors (toxins), genetic mutations in a variety of genes, and finally, and most importantly, old age, are the major risk factors. Disease-linked mutations in genes provide key clues to the molecular mechanisms involved in the pathology and have therefore been studied intensively over the last decades (Table 1). Here, we focus on mutations causing Parkinson disease (PD). The study of these mutations and of mutations causing other diseases indicates that mitochondrial dysfunction is an important contributor to neurodegenerative processes.

PD is a chronically progressive, age-related neuro-degenerative disease characterized by progressive resting tremor, rigidity, bradykinesia, gait disturbance, postural instability and dementia. A major neuro-pathological feature is the degeneration of dopamine neurons in the substantia nigra pars compacta (SNc) and in other brainstem regions. The loss of inhibitory dopaminergic innervation causes over-activity of the subthalamic nucleus and the globus pallidus, which causes the movement symptoms (Obeso et al., 2000). Lewy bodies are a second neuropathologic feature of PD. These are eosinophilic cellular inclusions comprising a dense core of filamentous material surrounded by a halo of fibrils, which mainly consists of α-synuclein (see below) (Cookson, 2005).

Several hypotheses for the progressive and selective neurodegeneration in PD have been proposed. Mitochondrial dysfunction, however, has been linked with PD for a long time. In the early 1980s, a group of young designer-drug abusers showed PD-like symptoms upon exposure to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a synthetic by-product of heroin production (Langston et al., 1983). The active metabolite of MPTP, MPP^+, selectively enters dopaminergic neurons via the dopamine transporter and potently inhibits mitochondrial complex I (Vila and Przedborski, 2003). This results in increased oxidative stress, intracellular Ca^{2+} levels and excitotoxicity, and decreased energy production, eventually culminating in neuronal damage and death. Exposure to rotenone, another complex I inhibitor, produces a similar phenotype in rodents (Betarbet et al., 2000; Manning-Bog et al., 2002; Sherer et al., 2003). Furthermore, particular mitochondrial DNA (mtDNA) polymorphisms and haplotypes are associated with risk of PD (Autere et al., 2004; Pyle et al., 2005; van der Walt et al., 2003), and mutations in mtDNA or in the nuclear-encoded mtDNA polymerase-G (POLG) cause PD-like symptoms (Luoma et al., 2004). Finally, dopaminergic neurons in the SNc are particularly sensitive to somatic mtDNA mutations, and accumulation of these is greater in PD patients compared with age-matched controls (Bender et al., 2006; Kraytsberg et al., 2006). A critical threshold of approximately 60% mutated mtDNA molecules must be reached to cause dopaminergic neuronal cell death (Rossignol et al., 2003). The remaining unaffected mtDNA molecules cannot produce sufficient quantities of proteins such as cytochrome *c* oxidase (COX), and the respiratory chain becomes deficient.

Recently, several rare forms of inherited PD have been recognized (Polymeropoulos et al., 1997). The identification of the genes mutated has provided a major boost in our understanding of the molecular mechanism(s) causing PD

Journal of Cell Science

disease. Below, we briefly summarize the salient features of the proteins encoded by these genes [*SNCA* (encoding α-synuclein), *PARK2* (encoding parkin) *PARK7* (encoding PARK7; also known and hereafter referred to as DJ-1), *PINK1* (encoding the PTEN induced putative kinase 1 PINK1) and *LRRK2* (encoding the leucine-rich repeat kinase 2 LRRK2)] and then focus on the evidence that mutations in these genes impinge on mitochondrial function.

α-Synuclein

α-Synuclein (Fig. 1A) is a natively unfolded protein that upon binding to lipid can form oligomeric or amyloidogenic filaments. Fibrillar forms of α-synuclein are the major structural component of Lewy bodies, providing compelling evidence that it plays a major role in the pathogenesis of PD (Spillantini et al., 1998). Missense mutations in α-synuclein (A30P, E46K, A53T), and gene duplications and triplications have been identified in familial cases of PD (reviewed in Savitt et al., 2006). α-Synuclein is localized predominantly in synaptic terminals and in the cytosol. Although its normal function is not quite clear, studies in zebra finch indicate a potential role in synaptic plasticity (George et al., 1995). More, but inconsistent, information on the role of α-synuclein at synapses has come from studies in mice lacking α-synuclein or both α-synuclein and β-synuclein. Although one study demonstrated dramatic loss of synaptic reserve vesicles and an increase in synaptic depression in α-synuclein-null mice ($Snca^{tm1Nbm}$) (Cabin et al., 2002), two other studies only show mild ($Snca^{tm1Ros1}$) (Abeliovich et al., 2000) or no effects on these parameters $Snca^{tm1Sud}$ (Schluter et al., 2003). Also, mice lacking both α-synuclein and β-synuclein ($Snca^{tm1Sud}$-$Sncb^{tm1Sud}$) show no defects in synaptic plasticity and synaptic vesicle cycling. These mice, however, do show a 20% reduction in brain dopamine levels, which is not observed in single-knockout mice and points towards functional redundancy between these proteins (Chandra et al., 2004). Because of these controversial data, the normal function of α-synuclein remains debated, although a role in synaptic integrity seems likely. Indeed, loss of α-synuclein accelerates degeneration of presynaptic terminals in mice lacking *Dnajc5* ($Dnajc5^{tm1Sud}$), encoding the chaperone protein cystein-string protein α (CSPα). Thus, *Snca* and *Dnajc5* are in a genetic pathway important for the integrity of synaptic nerve terminals (Chandra et al., 2005).

Transgenic mouse models overexpressing wild-type or mutant forms of α-synuclein display disappointingly few symptoms relevant to PD (reviewed in Fleming et al., 2005). However, these mice do show an increased vulnerability of SNc neurons to MPTP (Fleming et al., 2005). Overexpression of α-synuclein in *Drosophila melanogaster* results in adult-onset loss of dopaminergic neurons, the appearance of filamentous intraneuronal inclusions containing α-synuclein and locomotory dysfunction. This model closely recapitulates the essential features of the human disorder (Bilen and Bonini, 2005; Feany and Bender, 2000). It is still unclear how mutant or overexpressed α-synuclein can lead to neuronal cell death. *SNCA* overexpression might block ER-Golgi trafficking (Cooper et al., 2006) by sequestering vesicle-trafficking proteins, such as Ypt1p, and interfering with its function. Conversely, overexpression of these, in particular Ypt1p and its ortholog Rab1, can attenuate α-synuclein-induced toxicity. Similar pathological mechanisms might be involved in synaptic vesicle transport (Cooper et al., 2006). For instance the scaffolding protein septin 4 (Sept4) frequently co-aggregates with α-synuclein in Lewy bodies in PD and is often deficient in dopaminergic nerve terminals in the striatum (Ihara et al., 2003; Ihara et al., 2007). α-Synuclein appears to interact with Sept4, and dopaminergic neurons deficient in Sept4 become more susceptible to further dysfunction and degeneration in PD (Ihara et al., 2007). *Sept4*-knockout mice ($Sept4^{tm1Ksh}$) display decreased dopamine release (Ihara et al., 2007). Ihara et al., therefore propose that Sept4 and α-synuclein are components of presynaptic complexes at the dopaminergic terminals, and that lack of the physiological association with Sept4 facilitates self-aggregation of α-synuclein.

Specific sensitivity of dopaminergic neurons to mitochondrial stress

Why are dopaminergic neurons so sensitive to α-synuclein toxicity? Dopamine is inherently unstable and can generate reactive oxygen species (ROS) and, via monoamine oxidase (MAO), H_2O_2 (Lotharius et al., 2002). Dopamine is synthesized in the cytosol and rapidly pumped by vesicular monoamine transporter 2 (VMAT2, also known as SLC18A2) into synaptic vesicles, where the low vesicular pH and the absence of MAO limit its breakdown. Defects in the early secretory pathway could cause a shortage of synaptic vesicles and reduce delivery of VMAT2 to the synapse. This would impede dopamine loading and increase cytosolic dopamine levels and consequently ROS production. Although dopamine itself may not be toxic, dopamine metabolites play a role in α-synuclein aggregation (Galvin, 2006) and dopamine-α-synuclein adducts stabilize α-synuclein protofibrils (Conway et al., 2001).

Although there is no evidence for mitochondrial localization of α-synuclein, overexpression of mutant α-synuclein sensitizes neurons to oxidative stress and damage by dopamine metabolites and mitochondrial toxins such as MPP^+ and 6-hydroxydopamine, resulting in increased protein carbonylation and lipid peroxidation in vitro and in vivo (Orth et al., 2003). Interestingly, α-synuclein-knockout mice ($Snca^{tm1Wtd}$, $Snca^{tm1Sud}$, ($Snca^{tm1Ros1}$) mice have marked resistance to MPTP and other mitochondrial toxins, such as malonate and 3-nitropropionic acid (Dauer et al., 2002; Fornai et al., 2005; Klivenyi et al., 2006). α-Synuclein deficiency seems to result in a reduction of oxidative stress and, in addition, it has been proposed that α-synuclein oligomers generate pore-like structures (Lashuel et al., 2002), which might disrupt the membranes of organelles such as mitochondria.

Parkin

Homozygous mutations in parkin (Fig. 1B) (Kitada et al., 1998) account for about half of all cases of autosomal recessive PD (ARPD) (Abbas et al., 1999; Lucking et al., 2000). A few dominant mutations have also been described (West and Maidment, 2004). Parkin functions as an E3 ubiquitin ligase (Shimura et al., 2000) catalyzing the ubiquitylation of damaged proteins, which leads to their degradation by the 26S proteasome (Ciechanover, 1998). Thus, mutations in parkin should impact on the unfolded protein stress response, resulting in accumulation of one or more of its presumed

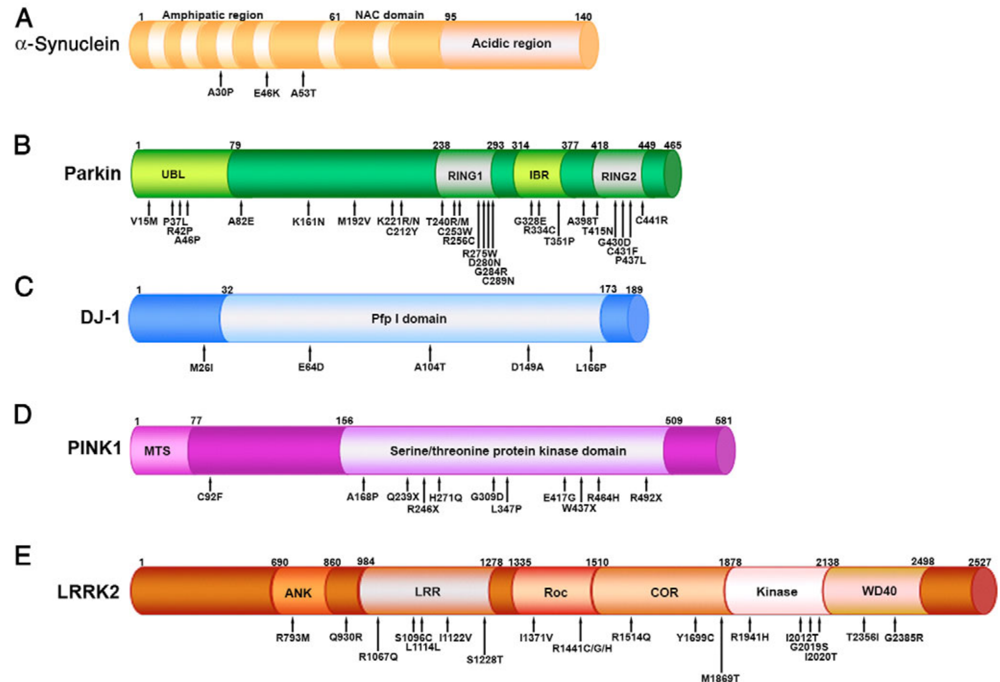

Fig. 1. Models of human PD-associated proteins. (A) α-Synuclein is a 140 amino acid protein belonging to a family of related synucleins that includes β- and γ-synuclein. It has an N-terminal amphipathic region containing six imperfect repeats with a KTKEGV consensus sequence, a hydrophobic central region that contains the non-amyloid-β component (NAC) domain, and a highly acidic C-terminal tail containing several phosphorylation sites. (B) Parkin is a 465 amino acid protein that functions as an E3 ubiquitin ligase. It contains an N-terminal ubiquitin-like (UBL) domain that binds to RPN10 subunit of the 26S proteasome system, a central linker region, and a C-terminal RING domain comprising two RING finger motifs (RING1 and RING2) separated by an in-between-RING (IBR) domain. (C) DJ-1 is a highly conserved 189 amino acid protein that is ubiquitously and abundantly expressed in most mammalian tissues and belongs to the DJ-1/ThiJ/PfpI superfamily. (D) PINK1 is a highly conserved 581 amino acid protein that is ubiquitously expressed. It localizes to the mitochondria via an N-terminal mitochondrion-targeting motif (MTS). Furthermore, it shares sequence similarity with Ca^{2+}/calmodulin-dependent protein kinase I and contains a catalytic serine/threonine kinase domain. (E) LRRK2 is a 2537 amino acid complex multi-domain protein that consists of a ankyrin-repeat region (ANK), an N-terminal leucine-rich repeat domain (LRR), a GTPase Roc domain (Roc) followed by associated C terminal of Roc (COR), a mitogen-activated kinase kinase kinase domain, and C-terminal WD40 repeat (approximately 40 amino acid repeats that form a β-propeller structure that might serve as a rigid scaffold for protein interactions). Approximate positions of missense mutations causing PD are indicated with arrows.

substrates and consequent death of neurons in the SNc (reviewed in Cookson, 2005). In general, no Lewy bodies are observed (Farrer et al., 2001; Mori et al., 1998; Sasaki et al., 2004).

Parkin also impacts on mitochondrial function. The *Drosophila* parkin-null (*park*^{25}) mutant displays reduced lifespan, excessive apoptosis, flight muscle degeneration and male sterility. Microscopy shows swollen mitochondria and severe disruption and disintegration of the cristae (Greene et al., 2003). Microarray analysis revealed upregulation of genes involved in oxidative stress and electron transport, including an orthologue of the mammalian peripheral benzodiazepine receptor (PBR), which is localized at mitochondria (both mitochondrial inner and outer membrane localization have been reported) and protects against ROS (Casellas et al., 2002; Greene et al., 2005; Mukherjee and Das, 1989). A genomic screen in the *park*^{25} flies identified loss-of-function of glutathione S-transferase (GstS1) as one of the strongest enhancers of fly death (Greene et al., 2005). GSTS1 is an important antioxidant and flies lacking the genes encoding both parkin and GSTS1 display progressive degeneration of dopaminergic neurons and increased oxidative damage (Whitworth et al., 2005). Loss of parkin in mice (*Park2*^{tm1shm}) results in increased dopamine levels and reduced synaptic excitability in the striatum, and behavioral defects (Goldberg et al., 2003). Remarkably, reduced expression of several proteins involved in mitochondrial function and oxidative stress, including subunits of complexes I and IV, is observed as well (Palacino et al., 2004). Mitochondria isolated from the striatum of *Park2*^{tm1shm} mice have decreased mitochondrial respiratory capacity and show evidence of increased formation of lipid by-products of ROS, such as protein carbonyls and lipid peroxides (Palacino et al., 2004). Intriguingly, parkin deficiency alone is not sufficient to cause neurodegeneration, which suggests additional unknown triggers are important for the disease process (Goldberg et al., 2003; Itier et al., 2003; Perez and Palmiter, 2005).

The mechanism by which parkin might regulate mitochondrial function remains unclear. Loss-of-function of this E3 ligase might lead to accumulation of oxidatively damaged proteins. Various putative parkin substrates, such as CDCrel-1/Sept5, CDCrel-2/Sept4, Pael-R, cyclin E, synphilin-1, p38/JTV1 and FBP1, accumulate in parkin-associated PD, whereas only p38/JTV1 and FBP1 also accumulate in sporadic PD and parkin knockout (*Park2*^{tm1Tmd}) mice, which suggests these might be genuine parkin substrates (reviewed in Moore et al., 2005a). None of these is localized to mitochondria, and their accumulation might only indirectly affect mitochondrial function. However, recently parkin has been shown to ubiquitylate the PD-associated protein LRRK2 (see below), which in turn activates auto-ubiquitylation of parkin (Smith et al., 2005). Interestingly, West and co-workers have shown that approximately 10% is localized to the mitochondrial outer membrane (West et al., 2005). Parkin itself is sensitive to oxidative stress: dopamine-quinone and s-nitrosylation covalently modify it and inactivate its E3 ligase activity

Journal of Cell Science

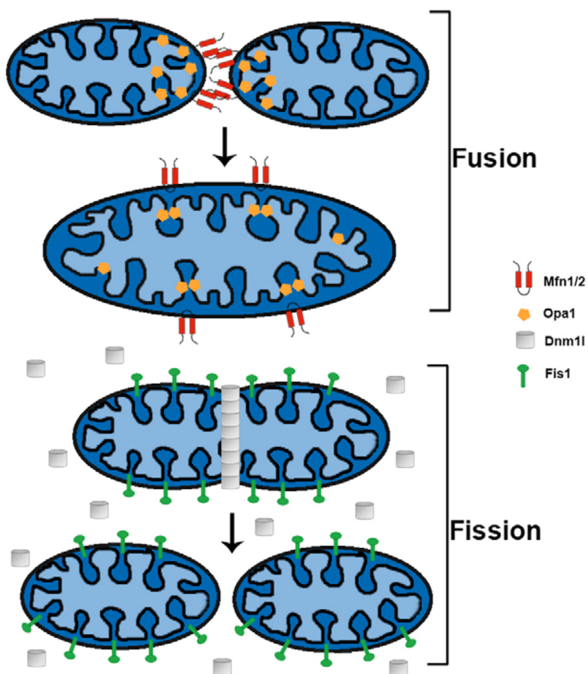

Fig. 2. Schematic representation of mitochondrial fusion and fission events. During fusion mitofusin 1 or 2 (Mfn1/2) proteins link two juxtaposed mitochondria through their coiled-coil domains. This is followed by outer, and subsequently inner, membrane fusion, which is GTP dependent and regulated by Opa1. During fission, Dnm1l is recruited from the cytosol to the outer mitochondrial membrane, where it interacts directly or indirectly with Fis1, leading to constriction of mitochondria and sequential separation of the inner and outer membrane.

(Chung et al., 2004; LaVoie et al., 2005). This suggests the possibility of a feedback loop and a more general mechanism for parkin dysfunction in the pathogenesis of sporadic PD. A genetic interaction between *parkin* and the mitochondrial kinase *Pink1* in *D. melanogaster* (see below) provides further evidence for parkin deficiency playing a general role in PD via mitochondria (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Recent data also show that parkin and Pink1 might physically interact (Moore, 2006). Although the mitochondrial localization of Pink1 is clear (see below), it is still debated whether parkin is associated with mitochondria.

DJ-1

Mutations in the gene encoding DJ-1, which encodes a putative sensor of oxidative stress, are rare causes of PD. DJ-1 (Fig. 1C) is ubiquitously and abundantly expressed in most mammalian tissues (Bandopadhyay et al., 2004; Olzmann et al., 2004). The crystal structure shows that it exists as a dimer in solution (Miller et al., 2003; Moore et al., 2003). An L166P PD-causing mutation destabilizes the dimerization of DJ-1, which indicates that this is functionally important. Cysteine residues, particularly Cys106, in DJ-1 become converted to cysteine sulfinic acid (Cys-SO_2H) upon oxidative stress (Canet-Aviles et al., 2004; Mitsumoto and Nakagawa, 2001). DJ-1 can also eliminate H_2O_2 in vitro by becoming oxidized itself and could thus function as a scavenger of ROS (Taira et al., 2004). Overexpression of the gene encoding DJ-1 protects against oxidative injury whereas knocking it down by RNAi enhances susceptibility to oxidative stress (Taira et al., 2004; Yokota et al., 2003).

Some of the protective actions of DJ-1 might occur at the transcriptional level (Bonifati et al., 2003). DJ-1 binds to PIAS proteins, a family of SUMO-1 ligases that modulate the activity of various transcription factors (Takahashi et al., 2001). Junn et al. show that wild-type DJ-1 sequesters Daxx in the nucleus, preventing it from binding and activating its effector kinase apoptosis signal-regulating kinase 1 (Ask1) in the cytoplasm (Junn et al., 2005). Others show that DJ-1 is a transcriptional co-activator that interacts with the nuclear proteins p54nrb and PSF (Xu et al., 2005) again to protect against apoptosis. DJ-1 also stabilizes the antioxidant transcriptional master regulator Nrf2 (Clements et al., 2006) by preventing association with its inhibitor protein Keap1. Interestingly, Nrf2 is located in the nucleus in PD nigral neurons (Ramsey et al., 2007). DJ-1 might thus act as a transcriptional co-factor that regulates the response to oxidative stress. In addition, the cysteine sulfinic acid modification of DJ-1 might enhance its association with mitochondria (Canet-Aviles et al., 2004), and oxidative stress promotes its interaction with parkin, linking DJ-1 and parkin in a common pathway (Moore et al., 2005b). Thus, DJ-1 may play a crucial role both sensing and conferring protection against a range of oxidative stressors, using different mechanisms. Flies and mice deficient in the gene encoding DJ-1 are indeed more susceptible to oxidative toxins (Kim et al., 2005; Menzies et al., 2005; Meulener et al., 2005; Park et al., 2005; Yang et al., 2005). Circumstantial evidence thus strongly indicates that DJ-1 directly or indirectly protects against mitochondrial dysfunction.

PINK1

Homozygous or compound heterozygous mutations in the *PINK1* gene (Fig. 1D) cause forms of PD that display a wide phenotypic spectrum, from early onset with atypical features to late-onset PD with symptoms similar to those in sporadic cases (Hatano et al., 2004; Valente et al., 2004a; Valente et al., 2004b). PINK1 is an ubiquitously expressed kinase (Silvestri et al., 2005; Valente et al., 2004a). It tends to be poorly soluble and has a propensity to aggregate, which might explain its presence in 10% of Lewy bodies in sporadic PD (Gandhi et al., 2006). Overexpression of *PINK1* protects cells from apoptosis affected by the mitochondrial pathway, whereas pathogenic mutations of *PINK1* in the kinase domain (E240K, L489P and K219M) disrupt this property (Petit et al., 2005). Increased lipid peroxidation and increased expression of mitochondrial superoxide dismutase, glutathione reductase and glutathione-S-transferase occur in primary fibroblasts and immortalized lymphoblasts from three patients homozygous for the *G309D-PINK1* mutation (Hoepken et al., 2007). To date, no in vivo studies of *Pink1* loss-of-function in mammalian models have been reported. However, loss-of-function mutants in *D. melanogaster* display a spectacular phenotype: male sterility, muscle and dopaminergic neuronal degeneration and increased sensitivity to stressors (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). The similarities to *D. melanogaster* parkin mutants are striking, and overexpression of parkin rescues the mitochondrial dysfunction caused by *Pink1* deficiency, which

Journal of Cell Science

further argues for a common mitochondrial pathway in PD (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Parkin can restore normal mitochondrial morphology, DNA and protein content, but does not rescue the sensitivity of *Pink1* mutant flies to stress-induced apoptosis. This suggests that *Pink1* acts upstream of *parkin* to protect mitochondrial integrity (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). PINK1 is the only PD-associated protein that clearly localizes to mitochondria and might be crucial for our understanding of the contribution of mitochondrial dysfunction to PD.

LRRK2

Mutations in the *LRRK2* gene, especially *LRRK2* G2019S (Fig. 1E), are very common in familial forms of PD but also in sporadic PD. In the latter, the absence of a family history suggests reduced penetrance or a de novo occurrence of the mutation (Di Fonzo et al., 2005; Kachergus et al., 2005; Nichols et al., 2005). The G2019S mutation has indeed variable penetrance, reaching 17% at 50 years and 85% at 70 years, a profile that mimics the occurrence of idiopathic sporadic PD. Little is known about LRRK2 function. It is a multidomain protein that has both a kinase and GTPase domain (Fig. 1E) (Gloeckner et al., 2006; West et al., 2005). Significantly, three PD-associated mutations, two in the kinase domain (G2019S and I2020T) and one in the Ras of complex/C-terminal of Roc (ROC/COR) GTPase domain (Bosgraaf and Van Haastert, 2003) (R1441C), increase LRRK2 autophosphorylation, hinting at a dominant gain-of-function mechanism in PD (Gloeckner et al., 2006; West et al., 2005; West et al., 2007). Indeed, over-expression of R1441C, Y1699C or G2019S LRRK2 can induce neuronal degeneration in mouse primary cortical neurons (Greggio et al., 2006; Smith et al., 2006; Smith et al., 2005). Interestingly, PD-associated *LRRK2* mutations that enhance kinase activity induce a progressive reduction in neurite length and branching in neurons in vitro and in vivo, which is reversed by loss of *LRRK2* (Macleod et al., 2006).

Neurons that express PD-associated *LRRK2* mutations also display prominent phospho-tau-positive inclusions that colocalize with the lysosomal marker LAMP1. The neurons ultimately undergo apoptosis (Macleod et al., 2006). It is known that hyperphosphorylation of tau leads to aggregate formation and axonal/neuronal degeneration in many neurodegenerative diseases (reviewed in Lee et al., 2001). Tau might be a molecular target for LRRK2 kinase activity, because the two proteins colocalize in inclusions. However, Macleod et al. have failed to detect evidence of direct phosphorylation of tau by LRRK2 in vitro and favor a model in which LRRK2 indirectly modifies tau through modulation of the activity of other kinases or phosphatases previously implicated in tau phosphorylation, such as GSK3β (Lee et al., 2001). Additional studies will be necessary to pinpoint the action of LRRK2 in these signaling cascades.

Confocal studies in primary cortical neurons and ultrastructural analysis in rodent basal ganglia detect LRRK2 in association with membranous and vesicular structures such as lysosomes, endosomes, transport vesicles and mitochondria (Biskup et al., 2006; West et al., 2005). Subcellular fractionation confirms that approximately 10% of total LRRK2 is associated with the mitochondrial outer membrane in rodent brain (Biskup et al., 2006; West et al., 2005). LRRK2 might therefore have a role in mitochondrial physiology, although this remains speculation.

Mitochondrial dynamics and neuronal dysfunction

As discussed above, the inherent stress imposed upon dopaminergic neurons by dopamine metabolism could explain the particular sensitivity of these particular neurons to mitochondrial dysfunction. Other neurons, however, are also sensitive to mitochondrial dysfunction (see Table 1). Mitochondria are remarkably dynamic organelles that undergo continual cycles of fusion and fission (Fig. 2). Strikingly, several proteins that are encoded by genes mutated in neurodegenerative diseases are involved in mitochondrial dynamics, which suggests that this is particularly important for the integrity of the nervous system. Fusion and fission events mix membranes and contents, but are also likely to be involved in control of the subcellular localization of mitochondria. Dramatic changes in morphology are also observed during apoptosis. The balance between fusion and fission thus determines not only the overall morphology of mitochondria in cells but has also important consequences for mitochondrial function (reviewed in Chan, 2006). Given the complex topology and energy needs of the nervous system, it is not so surprising that defects in mitochondrial dynamics lead to neurological disease.

Mutations in human mitofusin-2 (*MFN2*) cause Charcot-Marie-Tooth disease (Zuchner et al., 2004). Mitofusins Mfn1 and Mfn2 are essential GTPases localized to the mitochondrial outer membrane. Both proteins form complexes in trans that tether mitochondria together, promoting mitochondrial fusion (Chen et al., 2003; Koshiba et al., 2004). Deletion of *Mfn1* or *Mfn2* results in mitochondrial fragmentation due to unbalanced fission (Chen et al., 2005; Koshiba et al., 2004). Optic atrophy 1 (OPA1) is another example of a protein involved in mitochondrial dynamics. Mutations in its gene are the major cause of genetic forms of blindness (Delettre et al., 2000). OPA1 is a dynamin-related GTPase and localizes to the intermembrane space of mitochondria, where it is tightly associated with the inner membrane (Cereghetti and Scorrano, 2006; Chan, 2006; Olichon et al., 2002). Interestingly, *Opa1* interacts genetically with *Mfn1*, which suggests it coordinates the fusion reactions of outer and inner membranes (Cipolat et al., 2004).

These proteins also affect the subcellular localization of mitochondria. Overexpression of Mfn1 in primary cultures of neurons results in decreased localization of mitochondria to dendritic spines, whereas overexpression of dynamin-1-like (Dnm1l, also known as Drp1), a protein involved in mitochondrial fission (see below), results in re-localization of mitochondria to the spines (Li et al., 2004). Remarkably, mitochondrial and synaptic activities influence each other: repetitive depolarization results in fusion of mitochondria and their relocation to the spines.

Mitochondria are particularly abundant at the other sites of synapses: axons and synaptic terminals. They are probably needed there to provide the enormous amounts of ATP associated with the transmission of axon potentials. Loss-of-function mutations in the *D. melanogaster* genes encoding the coiled-coil protein Milton (Stowers et al., 2002) or the Rho-related GTPase Miro (Guo et al., 2005), which are both involved in transport of mitochondria along microtubules, lead to severe phenotypes characterized by partial or complete loss

Table 1. Mutated proteins that lead to neurodegenerative disorders

Protein	Disease	Function	References
A Mutant proteins with primary mitochondrial localization			
PANK2	Brain iron accumulation (NBIA)	Mitochondrial kinase involved in the biosynthesis of coenzyme A, an essential enzyme for energy metabolism, fatty acid synthesis and degradation, neurotransmitter and glutathione metabolism	reviewed in Hayflick, 2003
MFN2	Charcot-Marie-Tooth type 2A	Mitochondrial outer membrane GTPase that is involved in mitochondria dynamics/fusion	Kijima et al., 2005
GDAP1	Charcot-Marie-Tooth type 4A	Mitochondrial outer membrane protein probably involved in fusion and cristae dynamics	Niemann et al., 2005
Frataxin (FXN)	Friedreich ataxia	Involved in the heme biosynthesis, formation of iron-sulfur clusters and iron detoxification in mitochondria	reviewed in Bencze et al., 2006
Paraplegin (SPG7)	Hereditary spastic paraplegia	Mitochondrial inner-membrane metalloprotease involved in assembly of the respiratory chain complexes	reviewed in Rugarli and Langer, 2006
HSP60 (SPG13)	Hereditary spastic paraplegia	Mitochondrial chaperone involved in protein folding	reviewed in Rugarli and Langer, 2006
OPA1	Dominant optic atrophy	Mitochondrial inner membrane dynamin-related GTPase involved in maintaining *cristae* integrity and mediating mitochondrial fusion	reviewed in Olichon et al., 2006
OPA3	Recessive optic atrophy	Mitochondrial protein with unknown function	Votruba, 2004
ATPase6	Neuropathy ataxia and retinitis pigmentosa	Component of the ATP synthase of the mitochondrial respiratory chain involved in the assembly of the F_1F_0 complex	Cortes-Hernandez et al., 2007
OMI/HTRA2	Associated with Parkinson disease	Mitochondrial intermembrane space serine protease which is released into the cytosol upon pro-apoptotic stimuli	Strauss et al., 2005
PINK1	Parkinson disease	Mitochondrial serine/treonine kinase that appears to be critical to prevent oxidative stress	Petit et al., 2005; Valente et al., 2004a
B Mutant proteins potentially affecting mitochondrial function			
SOD1	Amyothrophic lateral sclerosis	Superoxide dismutase that partially localizes to the mitochondrial intermembrane space and matrix, where the mutant form possibly interacts with mitochondrial chaperone proteins affecting mitochondrial protein import	Liu et al., 2004
Huntingtin via PGC-1α and p53	Huntington disease	Cytosolic protein whose function remains unclear. However, mutant Huntingtin influences transcriptional activity of p53 and PGC-1α	Bae et al., 2005; Cui et al., 2006
DJ-1	Parkinson disease	Partially localizes to the mitochondrial intermembrane space and matrix, and acts as a redox sensor protecting against cell death especially induced by oxidative stress	Canet-Aviles et al., 2004
LRRK2	Parkinson disease	Kinase that partially associates with the outer mitochondrial membrane	reviewed in Mata et al., 2006
Parkin	Parkinson disease	Ubiquitin E3 ligase that partially associates with the outer mitochondrial membrane where it has been shown to protect against mitochondrial swelling and cytochrome *c* release	Darios et al., 2003

of neurotransmission. In a genetic screen for modifiers of synaptic neurotransmission, Verstreken et al. (Verstreken et al., 2005) identified the *D. melanogaster* Dnm1l orthologue *Drpl* as essential for sustained neurotransmission during rapid stimulation. *Drpl* deficiency results in synaptic depletion of mitochondria, and although basic neurotransmission activity appears to be sustained, the lack of mitochondria makes it impossible to mobilize a reserve pool of neurotransmitter vesicles that is essential for rapid transmission. DNM1L is a cytoplasmic protein and needs FIS1, a mitochondrial outer membrane protein, to induce fission of mitochondria (Yoon et al., 2003; Yu et al., 2005). The process of fission and fusion thus depends on a complex molecular machinery, providing ample opportunities for speculation on potential roles of kinases such as LRRK2 and PINK1 in its regulation. Interestingly, the LRRK2 GTPase domain shares significant sequence similarity with the Rho GTPase domain of Miro (Guo et al., 2005). Miro and Milton directly interact and this interaction is essential for anterograde transport of mitochondria (Glater et al., 2006). Recently, mammalian orthologues of Milton have been identified, OIP106 and GRIF1 (also known as Trak1 and Trak2, respectively) (Brickley et al., 2005; Fransson et al., 2006). Interestingly, OIP106 is mutated in hypertonic mice, which show motor pathway defects in the central nervous system (Gilbert et al., 2006).

Apoptosis and disturbances in energy metabolism in neurodegenerative disease

The proteins involved in mitochondrial dynamics not only are important for the subcellular distribution of these organelles in neurons but also contribute directly or indirectly to other mitochondrial functions. OPA1 illustrates this very well. Overexpression of OPA1 induces mitochondrial elongation and affects transport but also protects against apoptosis. Recently it has become clear that the two functions are part of two genetically distinct pathways (Cipolat et al., 2004; Cipolat et al., 2006). Mitochondrial elongation requires membrane-bound OPA1 and depends on MFN1, but not MFN2, whereas the anti-apoptotic function of OPA1 requires a soluble form of OPA1 that is generated by the mitochondrial rhomboid protease PARL. This soluble form of OPA1 is loosely bound to the inner membrane, apparently partially in complex with membrane-bound forms of OPA1, forming a multimeric complex that regulates the tightness of mitochondrial cristae junctions and therefore cytochrome *c* release during apoptosis (Cipolat et al., 2006; Frezza et al., 2006).

Journal of Cell Science

Mitochondrial fission is similarly closely associated with the apoptosis pathway: mitochondrial fragmentation occurs early in apoptosis, and inhibition of DNM1L or FIS1 can protect against cell death (Frank et al., 2001; Lee et al., 2004). Increased rates of apoptosis could theoretically contribute to neurodegeneration, although the evidence for apoptosis in human brain material remains controversial (Dauer and Przedborski, 2003).

It is not clear to what extent fusion and fission contribute to the maintenance of the oxidative phosphorylation (OXPHOS) pathway. The mixing of DNA and protein content during fusion probably reduces the effect of accumulating mutations in mtDNA, possibly stabilizing mitochondrial performance. Again, this must be confirmed in animal model studies. Recently, Ekstrand et al. showed that the specific inactivation of mitochondrial transcription factor A (Tfam) in dopaminergic neurons leads to defects in the OXPHOS pathway that result in PD-like symptoms in mice (Ekstrand et al., 2007). Evidence from studies of other genetic diseases corroborates the concept that disturbances of the OXPHOS system contribute to neurodegeneration. Mutations in frataxin (*FXN*) (Table 1) cause Friedreich ataxia (Wilson, 2006). Deletion of the yeast orthologue of frataxin causes accumulation of iron in the mitochondria, loss of mtDNA and impaired OXPHOS activity (Koutnikova et al., 1997; Wilson and Roof, 1997). Additional studies have demonstrated severe deficiencies in the activities of Complexes I, II and III and aconitase, and iron accumulation in tissue from Friedreich ataxia patients (Rotig et al., 1997).

Mutations in the human genes encoding HSP60 (*HSPD1*) and paraplegin (*SPG7*) (Table 1) cause hereditary spastic paraplegias. HSP60 is a mitochondrial matrix protein chaperone and plays a role in the folding and import of mitochondrial proteins (Casari et al., 1998). Paraplegin is a subunit of a mitochondrial AAA protease and is localized to the inner mitochondrial membrane (Rugarli and Langer, 2006). Patients with *SPG7* mutations have compromised complex I activity and their cells display increased sensitivity to oxidative stress (Elleuch et al., 2006). Apart from clearing misfolded protein, the protease is also involved in the activation of a mitochondrial ribosomal subunit (Nolden et al., 2005).

Finally, OXPHOS impairment leading to neurodegeneration can also be caused by mutations in proteins that are not or only partially localized to mitochondria and therefore probably indirectly affect mitochondrial homeostasis (Table 1). For instance, reduced activity of several key components of the OXPHOS system, including complexes II, III and IV, occurs in Huntington disease at an advanced stage of the disorder (Browne and Beal, 2004). Although the exact function of huntingtin protein remains unknown (Truant et al., 2006), mutant huntingtin binds to several key transcription factors and co-activators, such as p53 (Bae et al., 2005) and the peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) (Cui et al., 2006). Repression of this transcription co-activator leads to impaired mitochondrial function and enhances the of pathogenesis Huntington disease (St-Pierre et al., 2006).

Conclusions and Perspectives

The evidence linking mitochondrial dysfunction to neurodegenerative disorders and, in particular, PD is impressive. It remains, however, speculation whether the mutations in the different PD genes all finally converge on a common mitochondrial pathway and whether such a pathway is also relevant for understanding of sporadic PD. The genes shown to be involved in mitochondrial dysfunction in PD and other neurodegenerative diseases provide us with a handle to address some important questions. Hopefully we will be able to lay out progressively a map of proteins and functional interactions that describes a molecular network linking the different genes and proteins to a common final pathway. An important goal, for instance, is the identification of the physiological substrates for the kinases PINK1 and LRRK2. If they are proteins relevant to mitochondrial relevant proteins we could obtain clues to whether the OXPHOS, cell death or fusion/fission pathways are part of such a final pathway. In neurodegenerative diseases other than PD, deregulation of mitochondrial dynamics seems to be a recurrent theme. Whether this is also true for PD is an intriguing possibility but has thus far remained little explored.

In vivo model systems for various neurodegenerative diseases provide a powerful tool to investigate genetic interactions and unravel a hypothetical genetic pathway involved in the (dys)regulation of mitochondrial function in neurodegenerative disease. In this respect, knockout and transgenic mutant mouse models of PINK1 and LRRK2 are eagerly awaited. Furthermore, it will be interesting to investigate whether proteins, such as PGC-1α, that are apparently involved in other neurodegenerative diseases are also of relevance in PD.

Finally, further work will significantly contribute to our knowledge of mitochondrial function. Challenging questions are whether mitochondria have tissue-specific features and whether one can therapeutically exploit mitochondrial function to treat neurodegenerative disease. The new genetic and cell biological studies in human and animals provide ample starting points for us to tackle these important challenges.

Research in the group of B.D.S. is supported by a Pioneer award from the Alzheimer's Association, the Fund for Scientific Research, Flanders; K. U. Leuven (GOA); and Federal Office for Scientific Affairs, Belgium (IUAP). Wim Mandemakers is a visiting postdoctoral researcher of the FWO. V.M. is a Marie Curie Fellow of the EU.

References

Abbas, N., Lucking, C. B., Ricard, S., Durr, A., Bonifati, V., De Michele, G., Bouley, S., Vaughan, J. R., Gasser, T., Marconi, R. et al. (1999). A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. *Hum. Mol. Genet.* **8**, 567-574.

Abeliovich, A., Schmitz, Y., Farinas, I., Choi-Lundberg, D., Ho, W. H., Castillo, P. E., Shinsky, N., Verdugo, J. M., Armanini, M., Ryan, A. et al. (2000). Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* **25**, 239-252.

Autere, J., Moilanen, J. S., Finnila, S., Soininen, H., Mannermaa, A., Hartikainen, P., Hallikainen, M. and Majamaa, K. (2004). Mitochondrial DNA polymorphisms as risk factors for Parkinson's disease and Parkinson's disease dementia. *Hum. Genet.* **115**, 29-35.

Bae, B. I., Xu, H., Igarashi, S., Fujimuro, M., Agrawal, N., Taya, Y., Hayward, S. D., Moran, T. H., Montell, C., Ross, C. A. et al. (2005). p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. *Neuron* **47**, 29-41.

Bandopadhyay, R., Kingsbury, A. E., Cookson, M. R., Reid, A. R., Evans, I. M., Hope, A. D., Pittman, A. M., Lashley, T., Canet-Aviles, R., Miller, D. W. et al. (2004). The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. *Brain* **127**, 420-430.

Bencze, K. Z., Kondapalli, K. C., Cook, J. D., McMahon, S., Millan-Pacheco, C., Pastor, N. and Stemmler, T. L. (2006). The structure and function of frataxin. *Crit. Rev. Biochem. Mol. Biol.* **41**, 269-291.

Bender, A., Krishnan, K. J., Morris, C. M., Taylor, G. A., Reeve, A. K., Perry, R. H.,

Jaros, E., Hersheson, J. S., Betts, J., Klopstock, T. et al. (2006). High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat. Genet.* **38**, 515-517.

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V. and Greenamyre, J. T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* **3**, 1301-1306.

Bilen, J. and Bonini, N. M. (2005). Drosophila as a model for human neurodegenerative disease. *Annu. Rev. Genet.* **39**, 153-171.

Biskup, S., Moore, D. J., Celsi, F., Higashi, S., West, A. B., Andrabi, S. A., Kurkinen, K., Yu, S. W., Savitt, J. M., Waldvogel, H. J. et al. (2006). Localization of LRRK2 to membranous and vesicular structures in mammalian brain. *Ann. Neurol.* **60**, 557-569.

Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M. et al. (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* **299**, 256-259.

Bosgraaf, L. and Van Haastert, P. J. (2003). Roc, a Ras/GTPase domain in complex proteins. *Biochim. Biophys. Acta* **1643**, 5-10.

Brickley, K., Smith, M. J., Beck, M. and Stephenson, F. A. (2005). GRIF-1 and OIP106, members of a novel gene family of coiled-coil domain proteins: association in vivo and in vitro with kinesin. *J. Biol. Chem.* **280**, 14723-14732.

Browne, S. E. and Beal, M. F. (2004). The energetics of Huntington's disease. *Neurochem. Res.* **29**, 531-546.

Cabin, D. E., Shimazu, K., Murphy, D., Cole, N. B., Gottschalk, W., McIlwain, K. L., Orrison, B., Chen, A., Ellis, C. E., Paylor, R. et al. (2002). Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. *J. Neurosci.* **22**, 8797-8807.

Canet-Aviles, R. M., Wilson, M. A., Miller, D. W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M. J., Ringe, D., Petsko, G. A. and Cookson, M. R. (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.

Casari, G., De Fusco, M., Ciarmatori, S., Zeviani, M., Mora, M., Fernandez, P., De Michele, G., Filla, A., Cocozza, S., Marconi, R. et al. (1998). Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell* **93**, 973-983.

Casellas, P., Galiegue, S. and Basile, A. S. (2002). Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem. Int.* **40**, 475-486.

Cereghetti, G. M. and Scorrano, L. (2006). The many shapes of mitochondrial death. *Oncogene* **25**, 4717-4724.

Chan, D. C. (2006). Mitochondrial fusion and fission in mammals. *Annu. Rev. Cell Dev. Biol.* **22**, 79-99.

Chandra, S., Fornai, F., Kwon, H. B., Yazdani, U., Atasoy, D., Liu, X., Hammer, R. E., Battaglia, G., German, D. C., Castillo, P. E. et al. (2004). Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. *Proc. Natl. Acad. Sci. USA* **101**, 14966-14971.

Chandra, S., Gallardo, G., Fernandez-Chacon, R., Schluter, O. M. and Sudhof, T. C. (2005). Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell* **123**, 383-396.

Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E. and Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J. Cell Biol.* **160**, 189-200.

Chen, H., Chomyn, A. and Chan, D. C. (2005). Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J. Biol. Chem.* **280**, 26185-26192.

Chung, K. K., Thomas, B., Li, X., Pletnikova, O., Troncoso, J. C., Marsh, L., Dawson, V. L. and Dawson, T. M. (2004). S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science* **304**, 1328-1331.

Ciechanover, A. (1998). The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J.* **17**, 7151-7160.

Cipolat, S., Martins de Brito, O., Dal Zilio, B. and Scorrano, L. (2004). OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc. Natl. Acad. Sci. USA* **101**, 15927-15932.

Cipolat, S., Rudka, T., Hartmann, D., Costa, V., Serneels, L., Craessaerts, K., Metzger, K., Frezza, C., Annaert, W., D'Adamio, L. et al. (2006). Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* **126**, 163-175.

Clark, I. E., Dodson, M. W., Jiang, C., Cao, J. H., Huh, J. R., Seol, J. H., Yoo, S. J., Hay, B. A. and Guo, M. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* **441**, 1162-1166.

Clements, C. M., McNally, R. S., Conti, B. J., Mak, T. W. and Ting, J. P. (2006). DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. *Proc. Natl. Acad. Sci. USA* **103**, 15091-15096.

Conway, K. A., Rochet, J. C., Bieganski, R. M. and Lansbury, P. T., Jr (2001). Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science* **294**, 1346-1349.

Cookson, M. R. (2005). The biochemistry of Parkinson's disease. *Annu. Rev. Biochem.* **74**, 29-52.

Cooper, A. A., Gitler, A. D., Cashikar, A., Haynes, C. M., Hill, K. J., Bhullar, B., Liu, K., Xu, K., Strathearn, K. E., Liu, F. et al. (2006). Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* **313**, 324-328.

Cortes-Hernandez, P., Vazquez-Memije, M. E. and Garcia, J. J. (2007). ATP6 homoplasmic mutations inhibit and destabilize the human F1F0-ATP synthase without preventing enzyme assembly and oligomerization. *J. Biol. Chem.* **282**, 1051-1058.

Cui, L., Jeong, H., Borovecki, F., Parkhurst, C. N., Tanese, N. and Krainc, D. (2006). Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* **127**, 59-69.

Darios, F., Corti, O., Lucking, C. B., Hampe, C., Muriel, M. P., Abbas, N., Gu, W. J., Hirsch, E. C., Rooney, T., Ruberg, M. et al. (2003). Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. *Hum. Mol. Genet.* **12**, 517-526.

Dauer, W. and Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron* **39**, 889-909.

Dauer, W., Kholodilov, N., Vila, M., Trillat, A. C., Goodchild, R., Larsen, K. E., Staal, R., Tieu, K., Schmitz, Y., Yuan, C. A. et al. (2002). Resistance of alpha-synuclein null mice to the parkinsonian neurotoxin MPTP. *Proc. Natl. Acad. Sci. USA* **99**, 14524-14529.

Delettre, C., Lenaers, G., Griffoin, J. M., Gigarel, N., Lorenzo, C., Belenguer, P., Pelloquin, L., Grosgeorge, J., Turc-Carel, C., Perret, E. et al. (2000). Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat. Genet.* **26**, 207-210.

Di Fonzo, A., Rohe, C. F., Ferreira, J., Chien, H. F., Vacca, L., Stocchi, F., Guedes, L., Fabrizio, E., Manfredi, M., Vanacore, N. et al. (2005). A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet* **365**, 412-415.

Ekstrand, M. I., Terzioglu, M., Galter, D., Zhu, S., Hofstetter, C., Lindqvist, E., Thams, S., Bergstrand, A., Hansson, F. S., Trifunovic, A. et al. (2007). Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. *Proc. Natl. Acad. Sci. USA* **104**, 1325-1330.

Elleuch, N., Depienne, C., Benomar, A., Hernandez, A. M., Ferrer, X., Fontaine, B., Grid, D., Tallaksen, C. M., Zemmouri, R., Stevanin, G. et al. (2006). Mutation analysis of the paraplegin gene (SPG7) in patients with hereditary spastic paraplegia. *Neurology* **66**, 654-659.

Farrer, M., Chan, P., Chen, R., Tan, L., Lincoln, S., Hernandez, D., Forno, L., Gwinn-Hardy, K., Petrucelli, L., Hussey, J. et al. (2001). Lewy bodies and parkinsonism in families with parkin mutations. *Ann. Neurol.* **50**, 293-300.

Feany, M. B. and Bender, W. W. (2000). A Drosophila model of Parkinson's disease. *Nature* **404**, 394-398.

Fleming, S. M., Fernagut, P. O. and Chesselet, M. F. (2005). Genetic mouse models of parkinsonism: strengths and limitations. *NeuroRx* **2**, 495-503.

Fornai, F., Schluter, O. M., Lenzi, P., Gesi, M., Ruffoli, R., Ferrucci, M., Lazzeri, G., Busceti, C. L., Pontarelli, F., Battaglia, G. et al. (2005). Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proc. Natl. Acad. Sci. USA* **102**, 3413-3418.

Frank, S., Gaume, B., Bergmann-Leitner, E. S., Leitner, W. W., Robert, E. G., Catez, F., Smith, C. L. and Youle, R. J. (2001). The role of dynamin-related protein **1**, a mediator of mitochondrial fission, in apoptosis. *Dev. Cell* **1**, 515-525.

Fransson, S., Ruusala, A. and Aspenstrom, P. (2006). The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochem. Biophys. Res. Commun.* **344**, 500-510.

Frezza, C., Cipolat, S., Martins de Brito, O., Micaroni, M., Beznoussenko, G. V., Rudka, T., Bartoli, D., Polishuck, R. S., Danial, N. N., De Strooper, B. et al. (2006). OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* **126**, 177-189.

Galvin, J. E. (2006). Interaction of alpha-synuclein and dopamine metabolites in the pathogenesis of Parkinson's disease: a case for the selective vulnerability of the substantia nigra. *Acta Neuropathol.* **112**, 115-126.

Gandhi, S., Muqit, M. M., Stanyer, L., Healy, D. G., Abou-Sleiman, P. M., Hargreaves, I., Heales, S., Ganguly, M., Parsons, L., Lees, A. J. et al. (2006). PINK1 protein in normal human brain and Parkinson's disease. *Brain* **129**, 1720-1731.

George, J. M., Jin, H., Woods, W. S. and Clayton, D. F. (1995). Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron* **15**, 361-372.

Gilbert, S. L., Zhang, L., Forster, M. L., Anderson, J. R., Iwase, T., Soliven, B., Donahue, L. R., Sweet, H. O., Bronson, R. T., Davisson, M. T. et al. (2006). Trak1 mutation disrupts GABA(A) receptor homeostasis in hypertonic mice. *Nat. Genet.* **38**, 245-250.

Glater, E. E., Megeath, L. J., Stowers, R. S. and Schwarz, T. L. (2006). Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. *J. Cell Biol.* **173**, 545-557.

Gloeckner, C. J., Kinkl, N., Schumacher, A., Braun, R. J., O'Neill, E., Meitinger, T., Kolch, W., Prokisch, H. and Ueffing, M. (2006). The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Hum. Mol. Genet.* **15**, 223-232.

Goldberg, M. S., Fleming, S. M., Palacino, J. J., Cepeda, C., Lam, H. A., Bhatnagar, A., Meloni, E. G., Wu, N., Ackerson, L. C., Klapstein, G. J. et al. (2003). Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. *J. Biol. Chem.* **278**, 43628-43635.

Greene, J. C., Whitworth, A. J., Kuo, I., Andrews, L. A., Feany, M. B. and Pallanck, L. J. (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc. Natl. Acad. Sci. USA* **100**, 4078-4083.

Greene, J. C., Whitworth, A. J., Andrews, L. A., Parker, T. J. and Pallanck, L. J. (2005). Genetic and genomic studies of Drosophila parkin mutants implicate oxidative stress and innate immune responses in pathogenesis. *Hum. Mol. Genet.* **14**, 799-811.

Greggio, E., Jain, S., Kingsbury, A., Bandopadhyay, R., Lewis, P., Kaganovich, A., van der Brug, M. P., Beilina, A., Blackinton, J., Thomas, K. J. et al. (2006). Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol. Dis.* **23**, 329-341.

Guo, X., Macleod, G. T., Wellington, A., Hu, F., Panchumarthi, S., Schoenfield, M., Marin, L., Charlton, M. P., Atwood, H. L. and Zinsmaier, K. E. (2005). The GTPase

Journal of Cell Science

Journal of Cell Science

dMiro is required for axonal transport of mitochondria to Drosophila synapses. *Neuron* **47**, 379-393.

Hatano, Y., Li, Y., Sato, K., Asakawa, S., Yamamura, Y., Tomiyama, H., Yoshino, H., Asahina, M., Kobayashi, S., Hassin-Baer, S. et al. (2004). Novel PINK1 mutations in early-onset parkinsonism. *Ann. Neurol.* **56**, 424-427.

Hayflick, S. J. (2003). Unraveling the Hallervorden-Spatz syndrome: pantothenate kinase-associated neurodegeneration is the name. *Curr. Opin. Pediatr.* **15**, 572-577.

Hoepken, H. H., Gispert, S., Morales, B., Wingerter, O., Del Turco, D., Mulsch, A., Nussbaum, R. L., Muller, K., Drose, S., Brandt, U. et al. (2007). Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. *Neurobiol. Dis.* **25**, 401-411.

Ihara, M., Tomimoto, H., Kitayama, H., Morioka, Y., Akiguchi, I., Shibasaki, H., Noda, M. and Kinoshita, M. (2003). Association of the cytoskeletal GTP-binding protein Sept4/H5 with cytoplasmic inclusions found in Parkinson's disease and other synucleinopathies. *J. Biol. Chem.* **278**, 24095-24102.

Ihara, M., Yamasaki, N., Hagiwara, A., Tanigaki, A., Kitano, A., Hikawa, R., Tomimoto, H., Noda, M., Takanashi, M., Mori, H. et al. (2007). Sept4, a component of presynaptic scaffold and lewy bodies, is required for the suppression of alpha-Synuclein neurotoxicity. *Neuron* **53**, 519-533.

Itier, J. M., Ibanez, P., Mena, M. A., Abbas, N., Cohen-Salmon, C., Bohme, G. A., Laville, M., Pratt, J., Corti, O., Pradier, L. et al. (2003). Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. *Hum. Mol. Genet.* **12**, 2277-2291.

Junn, E., Taniguchi, H., Jeong, B. S., Zhao, X., Ichijo, H. and Mouradian, M. M. (2005). Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. *Proc. Natl. Acad. Sci. USA* **102**, 9691-9696.

Kachergus, J., Mata, I. F., Hulihan, M., Taylor, J. P., Lincoln, S., Aasly, J., Gibson, J. M., Ross, O. A., Lynch, T., Wiley, J. et al. (2005). Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. *Am. J. Hum. Genet.* **76**, 672-680.

Kijima, K., Numakura, C., Izumino, H., Umetsu, K., Nezu, A., Shiiki, T., Ogawa, M., Ishizaki, Y., Kitamura, T., Shozawa, Y. et al. (2005). Mitochondrial GTPase mitofusin 2 mutation in Charcot-Marie-Tooth neuropathy type 2A. *Hum. Genet.* **116**, 23-27.

Kim, R. H., Smith, P. D., Aleyasin, H., Hayley, S., Mount, M. P., Pownall, S., Wakeham, A., You-Ten, A. J., Kalia, S. K., Horne, P. et al. (2005). Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. *Proc. Natl. Acad. Sci. USA* **102**, 5215-5220.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605-608.

Klivenyi, P., Siwek, D., Gardian, G., Yang, L., Starkov, A., Cleren, C., Ferrante, R. J., Kowall, N. W., Abeliovich, A. and Beal, M. F. (2006). Mice lacking alpha-synuclein are resistant to mitochondrial toxins. *Neurobiol. Dis.* **21**, 541-548.

Koshiba, T., Detmer, S. A., Kaiser, J. T., Chen, H., McCaffery, J. M. and Chan, D. C. (2004). Structural basis of mitochondrial tethering by mitofusin complexes. *Science* **305**, 858-862.

Koutnikova, H., Campuzano, V., Foury, F., Dolle, P., Cazzalini, O. and Koenig, M. (1997). Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nat. Genet.* **16**, 345-351.

Kraytsberg, Y., Kudryavtseva, E., McKee, A. C., Geula, C., Kowall, N. W. and Khrapko, K. (2006). Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat. Genet.* **38**, 518-520.

Langston, J. W., Ballard, P., Tetrud, J. W. and Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**, 979-980.

Lashuel, H. A., Hartley, D., Petre, B. M., Walz, T. and Lansbury, P. T., Jr (2002). Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature* **418**, 291.

LaVoie, M. J., Ostaszewski, B. L., Weihofen, A., Schlossmacher, M. G. and Selkoe, D. J. (2005). Dopamine covalently modifies and functionally inactivates parkin. *Nat. Med.* **11**, 1214-1221.

Lee, V. M., Goedert, M. and Trojanowski, J. Q. (2001). Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* **24**, 1121-1159.

Lee, Y. J., Jeong, S. Y., Karbowski, M., Smith, C. L. and Youle, R. J. (2004). Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol. Biol. Cell* **15**, 5001-5011.

Li, Z., Okamoto, K., Hayashi, Y. and Sheng, M. (2004). The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* **119**, 873-887.

Liu, J., Lillo, C., Jonsson, P. A., Vande Velde, C., Ward, C. M., Miller, T. M., Subramaniam, J. R., Rothstein, J. D., Marklund, S., Andersen, P. M. et al. (2004). Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. *Neuron* **43**, 5-17.

Lotharius, J., Barg, S., Wiekop, P., Lundberg, C., Raymon, H. K. and Brundin, P. (2002). Effect of mutant alpha-synuclein on dopamine homeostasis in a new human mesencephalic cell line. *J. Biol. Chem.* **277**, 38884-38894.

Lucking, C. B., Durr, A., Bonifati, V., Vaughan, J., De Michele, G., Gasser, T., Harhangi, B. S., Meco, G., Denefle, P., Wood, N. W. et al. (2000). Association between early-onset Parkinson's disease and mutations in the parkin gene. *N. Engl. J. Med.* **342**, 1560-1567.

Luoma, P., Melberg, A., Rinne, J. O., Kaukonen, J. A., Nupponen, N. N., Chalmers, R. M., Oldfors, A., Rautakorpi, I., Peltonen, L., Majamaa, K. et al. (2004). Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: clinical and molecular genetic study. *Lancet* **364**, 875-882.

Macleod, D., Dowman, J., Hammond, R., Leete, T., Inoue, K. and Abeliovich, A. (2006). The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron* **52**, 587-593.

Manning-Bog, A. B., McCormack, A. L., Li, J., Uversky, V. N., Fink, A. L. and Di Monte, D. A. (2002). The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J. Biol. Chem.* **277**, 1641-1644.

Mata, I. F., Wedemeyer, W. J., Farrer, M. J., Taylor, J. P. and Gallo, K. A. (2006). LRRK2 in Parkinson's disease: protein domains and functional insights. *Trends Neurosci.* **29**, 286-293.

Menzies, F. M., Yenisetti, S. C. and Min, K. T. (2005). Roles of Drosophila DJ-1 in survival of dopaminergic neurons and oxidative stress. *Curr. Biol.* **15**, 1578-1582.

Meulener, M., Whitworth, A. J., Armstrong-Gold, C. E., Rizzu, P., Heutink, P., Wes, P. D., Pallanck, L. J. and Bonini, N. M. (2005). Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. *Curr. Biol.* **15**, 1572-1577.

Miller, D. W., Ahmad, R., Hague, S., Baptista, M. J., Canet-Aviles, R., McLendon, C., Carter, D. M., Zhu, P. P., Stadler, J., Chandran, J. et al. (2003). L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin-proteasome system. *J. Biol. Chem.* **278**, 36588-36595.

Mitsumoto, A. and Nakagawa, Y. (2001). DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. *Free Radic. Res.* **35**, 885-893.

Moore, D. J. (2006). Parkin: a multifaceted ubiquitin ligase. *Biochem. Soc. Trans.* **34**, 749-753.

Moore, D. J., Zhang, L., Dawson, T. M. and Dawson, V. L. (2003). A missense mutation (L166P) in DJ-1, linked to familial Parkinson's disease, confers reduced protein stability and impairs homo-oligomerization. *J. Neurochem.* **87**, 1558-1567.

Moore, D. J., West, A. B., Dawson, V. L. and Dawson, T. M. (2005a). Molecular pathophysiology of Parkinson's disease. *Annu. Rev. Neurosci.* **28**, 57-87.

Moore, D. J., Zhang, L., Troncoso, J., Lee, M. K., Hattori, N., Mizuno, Y., Dawson, T. M. and Dawson, V. L. (2005b). Association of DJ-1 and parkin mediated by pathogenic DJ-1 mutations and oxidative stress. *Hum. Mol. Genet.* **14**, 71-84.

Mori, H., Kondo, T., Yokochi, M., Matsumine, H., Nakagawa-Hattori, Y., Miyake, T., Suda, K. and Mizuno, Y. (1998). Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. *Neurology* **51**, 890-892.

Mukherjee, S. and Das, S. K. (1989). Subcellular distribution of "peripheral type" binding sites for [3H]Ro5-4864 in guinea pig lung. Localization to the mitochondrial inner membrane. *J. Biol. Chem.* **264**, 16713-16718.

Nichols, W. C., Pankratz, N., Hernandez, D., Paisan-Ruiz, C., Jain, S., Halter, C. A., Michaels, V. E., Reed, T., Rudolph, A., Shults, C. W. et al. (2005). Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. *Lancet* **365**, 410-412.

Niemann, A., Ruegg, M., La Padula, V., Schenone, A. and Suter, U. (2005). Ganglioside-induced differentiation associated protein 1 is a regulator of the mitochondrial network: new implications for Charcot-Marie-Tooth disease. *J. Cell Biol.* **170**, 1067-1078.

Nolden, M., Ehses, S., Koppen, M., Bernacchia, A., Rugarli, E. I. and Langer, T. (2005). The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. *Cell* **123**, 277-289.

Obeso, J. A., Rodriguez-Oroz, M. C., Rodriguez, M., Lanciego, J. L., Artieda, J., Gonzalo, N. and Olanow, C. W. (2000). Pathophysiology of the basal ganglia in Parkinson's disease. *Trends Neurosci.* **23**, S8-S19.

Olichon, A., Emorine, L. J., Descoins, E., Pelloquin, L., Brichese, L., Gas, N., Guillou, E., Delettre, C., Valette, A., Hamel, C. P. et al. (2002). The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. *FEBS Lett.* **523**, 171-176.

Olichon, A., Guillou, E., Delettre, C., Landes, T., Arnaune-Pelloquin, L., Emorine, L. J., Mils, V., Daloyau, M., Hamel, C., Amati-Bonneau, P. et al. (2006). Mitochondrial dynamics and disease, OPA1. *Biochim. Biophys. Acta* **1763**, 500-509.

Olzmann, J. A., Brown, K., Wilkinson, K. D., Rees, H. D., Huai, Q., Ke, H., Levey, A. I., Li, L. and Chin, L. S. (2004). Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function. *J. Biol. Chem.* **279**, 8506-8515.

Orth, M., Tabrizi, S. J., Schapira, A. H. and Cooper, J. M. (2003). Alpha-synuclein expression in HEK293 cells enhances the mitochondrial sensitivity to rotenone. *Neurosci. Lett.* **351**, 29-32.

Palacino, J. J., Sagi, D., Goldberg, M. S., Krauss, S., Motz, C., Wacker, M., Klose, J. and Shen, J. (2004). Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J. Biol. Chem.* **279**, 18614-18622.

Park, J., Kim, S. Y., Cha, G. H., Lee, S. B., Kim, S. and Chung, J. (2005). Drosophila DJ-1 mutants show oxidative stress-sensitive locomotive dysfunction. *Gene* **361**, 133-139.

Park, J., Lee, S. B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M., Kim, J. M. et al. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature* **441**, 1157-1161.

Perez, F. A. and Palmiter, R. D. (2005). Parkin-deficient mice are not a robust model of parkinsonism. *Proc. Natl. Acad. Sci. USA* **102**, 2174-2179.

Petit, A., Kawarai, T., Paitel, E., Sanjo, N., Maj, M., Scheid, M., Chen, F., Gu, Y., Hasegawa, H., Salehi-Rad, S. 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.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R. et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045-2047.

Pyle, A., Foltynie, T., Tiangyou, W., Lambert, C., Keers, S. M., Allcock, L. M.,

Davison, J., Lewis, S. J., Perry, R. H., Barker, R. et al. (2005). Mitochondrial DNA haplogroup cluster UKJT reduces the risk of PD. *Ann. Neurol.* **57**, 564-567.

Ramsey, C. P., Glass, C. A., Montgomery, M. B., Lindl, K. A., Ritson, G. P., Chia, L. A., Hamilton, R. L., Chu, C. T. and Jordan-Sciutto, K. L. (2007). Expression of Nrf2 in neurodegenerative diseases. *J. Neuropathol. Exp. Neurol.* **66**, 75-85.

Rossignol, R., Faustin, B., Rocher, C., Malgat, M., Mazat, J. P. and Letellier, T. (2003). Mitochondrial threshold effects. *Biochem. J.* **370**, 751-762.

Rotig, A., de Lonlay, P., Chretien, D., Foury, F., Koenig, M., Sidi, D., Munnich, A. and Rustin, P. (1997). Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat. Genet.* **17**, 215-217.

Rugarli, E. I. and Langer, T. (2006). Translating m-AAA protease function in mitochondria to hereditary spastic paraplegia. *Trends Mol. Med.* **12**, 262-269.

Sasaki, S., Shirata, A., Yamane, K. and Iwata, M. (2004). Parkin-positive autosomal recessive juvenile Parkinsonism with alpha-synuclein-positive inclusions. *Neurology* **63**, 678-682.

Savitt, J. M., Dawson, V. L. and Dawson, T. M. (2006). Diagnosis and treatment of Parkinson disease: molecules to medicine. *J. Clin. Invest.* **116**, 1744-1754.

Schluter, O. M., Fornai, F., Alessandri, M. G., Takamori, S., Geppert, M., Jahn, R. and Sudhof, T. C. (2003). Role of alpha-synuclein in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in mice. *Neuroscience* **118**, 985-1002.

Sherer, T. B., Kim, J. H., Betarbet, R. and Greenamyre, J. T. (2003). Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. *Exp. Neurol.* **179**, 9-16.

Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K. et al. (2000). Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat. Genet.* **25**, 302-305.

Silvestri, L., Caputo, V., Bellacchio, E., Atorino, L., Dallapiccola, B., Valente, E. M. and Casari, G. (2005). Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum. Mol. Genet.* **14**, 3477-3492.

Smith, W. W., Pei, Z., Jiang, H., Moore, D. J., Liang, Y., West, A. B., Dawson, V. L., Dawson, T. M. and Ross, C. A. (2005). Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration. *Proc. Natl. Acad. Sci. USA* **102**, 18676-18681.

Smith, W. W., Pei, Z., Jiang, H., Dawson, V. L., Dawson, T. M. and Ross, C. A. (2006). Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat. Neurosci.* **9**, 1231-1233.

Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M. and Goedert, M. (1998). alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc. Natl. Acad. Sci. USA* **95**, 6469-6473.

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jager, S., Handschin, C., Zheng, K., Lin, J., Yang, W. et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* **127**, 397-408.

Stowers, R. S., Megeath, L. J., Gorska-Andrzejak, J., Meinertzhagen, I. A. and Schwarz, T. L. (2002). Axonal transport of mitochondria to synapses depends on milton, a novel Drosophila protein. *Neuron* **36**, 1063-1077.

Strauss, K. M., Martins, L. M., Plun-Favreau, H., Marx, F. P., Kautzmann, S., Berg, D., Gasser, T., Wszolek, Z., Muller, T., Bornemann, A. et al. (2005). Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum. Mol. Genet.* **14**, 2099-2111.

Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S. M., Takahashi, K. and Ariga, H. (2004). DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Rep.* **5**, 213-218.

Takahashi, K., Taira, T., Niki, T., Seino, C., Iguchi-Ariga, S. M. and Ariga, H. (2001). DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor. *J. Biol. Chem.* **276**, 37556-37563.

Truant, R., Atwal, R. and Burtnik, A. (2006). Hypothesis: huntingtin may function in membrane association and vesicular trafficking. *Biochem. Cell Biol.* **84**, 912-917.

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A. R., Healy, D. G. et al. (2004a). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**, 1158-1160.

Valente, E. M., Salvi, S., Ialongo, T., Marongiu, R., Elia, A. E., Caputo, V., Romito, L., Albanese, A., Dallapiccola, B. and Bentivoglio, A. R. (2004b). PINK1 mutations are associated with sporadic early-onset parkinsonism. *Ann. Neurol.* **56**, 336-341.

van der Walt, J. M., Nicodemus, K. K., Martin, E. R., Scott, W. K., Nance, M. A., Watts, R. L., Hubble, J. P., Haines, J. L., Koller, W. C., Lyons, K. et al. (2003). Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *Am. J. Hum. Genet.* **72**, 804-811.

Verstreken, P., Ly, C. V., Venken, K. J., Koh, T. W., Zhou, Y. and Bellen, H. J. (2005). Synaptic mitochondria are critical for mobilization of reserve pool vesicles at Drosophila neuromuscular junctions. *Neuron* **47**, 365-378.

Vila, M. and Przedborski, S. (2003). Targeting programmed cell death in neurodegenerative diseases. *Nat. Rev. Neurosci.* **4**, 365-375.

Votruba, M. (2004). Molecular genetic basis of primary inherited optic neuropathies. *Eye* **18**, 1126-1132.

West, A. B. and Maidment, N. T. (2004). Genetics of parkin-linked disease. *Hum. Genet.* **114**, 327-336.

West, A. B., Moore, D. J., Biskup, S., Bugayenko, A., Smith, W. W., Ross, C. A., Dawson, V. L. and Dawson, T. M. (2005). Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc. Natl. Acad. Sci. USA* **102**, 16842-16847.

West, A. B., Moore, D. J., Choi, C., Andrabi, S. A., Li, X., Dikeman, D., Biskup, S., Zhang, Z., Lim, K. L., Dawson, V. L. et al. (2007). Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum. Mol. Genet.* **16**, 223-232.

Whitworth, A. J., Theodore, D. A., Greene, J. C., Benes, H., Wes, P. D. and Pallanck, L. J. (2005). Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a Drosophila model of Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **102**, 8024-8029.

Wilson, R. B. (2006). Iron dysregulation in Friedreich ataxia. *Semin. Pediatr. Neurol.* **13**, 166-175.

Wilson, R. B. and Roof, D. M. (1997). Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. *Nat. Genet.* **16**, 352-357.

Xu, J., Zhong, N., Wang, H., Elias, J. E., Kim, C. Y., Woldman, I., Pifl, C., Gygi, S. P., Geula, C. and Yankner, B. A. (2005). The Parkinson's disease-associated DJ-1 protein is a transcriptional co-activator that protects against neuronal apoptosis. *Hum. Mol. Genet.* **14**, 1231-1241.

Yang, Y., Gehrke, S., Haque, M. E., Imai, Y., Kosek, J., Yang, L., Beal, M. F., Nishimura, I., Wakamatsu, K., Ito, S. 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.

Yang, Y., Gehrke, S., Imai, Y., Huang, Z., Ouyang, Y., Wang, J. W., Yang, L., Beal, M. F., Vogel, H. and Lu, B. (2006). Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. *Proc. Natl. Acad. Sci. USA* **103**, 10793-10798.

Yokota, T., Sugawara, K., Ito, K., Takahashi, R., Ariga, H. and Mizusawa, H. (2003). Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. *Biochem. Biophys. Res. Commun.* **312**, 1342-1348.

Yoon, Y., Krueger, E. W., Oswald, B. J. and McNiven, M. A. (2003). The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Mol. Cell. Biol.* **23**, 5409-5420.

Yu, T., Fox, R. J., Burwell, L. S. and Yoon, Y. (2005). Regulation of mitochondrial fission and apoptosis by the mitochondrial outer membrane protein hFis1. *J. Cell Sci.* **118**, 4141-4151.

Zuchner, S., Mersiyanova, I. V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E. L., Zappia, M., Nelis, E., Patitucci, A., Senderek, J. et al. (2004). Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat. Genet.* **36**, 449-451.

Journal of Cell Science