

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

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## Summary

Dysfunction of mitochondria is frequently proposed to be involved in neurodegenerative disease. Deficiencies in energy supply, free radical generation, Ca<sup>2+</sup> 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 neurodegenerative 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  $\alpha$ -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<sup>+</sup>, 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<sup>2+</sup> 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; Kraysberg 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

disease. Below, we briefly summarize the salient features of the proteins encoded by these genes [*SNCA* (encoding  $\alpha$ -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.

### $\alpha$ -Synuclein

$\alpha$ -Synuclein (Fig. 1A) is a natively unfolded protein that upon binding to lipid can form oligomeric or amyloidogenic filaments. Fibrillar forms of  $\alpha$ -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  $\alpha$ -synuclein (A30P, E46K, A53T), and gene duplications and triplications have been identified in familial cases of PD (reviewed in Savitt et al., 2006).  $\alpha$ -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  $\alpha$ -synuclein at synapses has come from studies in mice lacking  $\alpha$ -synuclein or both  $\alpha$ -synuclein and  $\beta$ -synuclein. Although one study demonstrated dramatic loss of synaptic reserve vesicles and an increase in synaptic depression in  $\alpha$ -synuclein-null mice (*Snca<sup>tm1Nbm</sup>*) (Cabin et al., 2002), two other studies only show mild (*Snca<sup>tm1Ros1</sup>*) (Abeliovich et al., 2000) or no effects on these parameters *Snca<sup>tm1Sud</sup>* (Schluter et al., 2003). Also, mice lacking both  $\alpha$ -synuclein and  $\beta$ -synuclein (*Snca<sup>tm1Sud</sup>.Sncb<sup>tm1Sud</sup>*) 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  $\alpha$ -synuclein remains debated, although a role in synaptic integrity seems likely. Indeed, loss of  $\alpha$ -synuclein accelerates degeneration of presynaptic terminals in mice lacking *Dnajc5* (*Dnajc5<sup>tm1Sud</sup>*), encoding the chaperone protein cystein-string protein  $\alpha$  (CSP $\alpha$ ). 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  $\alpha$ -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  $\alpha$ -synuclein in *Drosophila melanogaster* results in adult-onset loss of dopaminergic neurons, the appearance of filamentous intraneuronal inclusions containing  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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).  $\alpha$ -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<sup>tm1Ksh</sup>*) display decreased dopamine release (Ihara et al., 2007). Ihara et al., therefore propose that Sept4 and  $\alpha$ -synuclein are components of presynaptic complexes at the dopaminergic terminals, and that lack of the physiological association with Sept4 facilitates self-aggregation of  $\alpha$ -synuclein.

### Specific sensitivity of dopaminergic neurons to mitochondrial stress

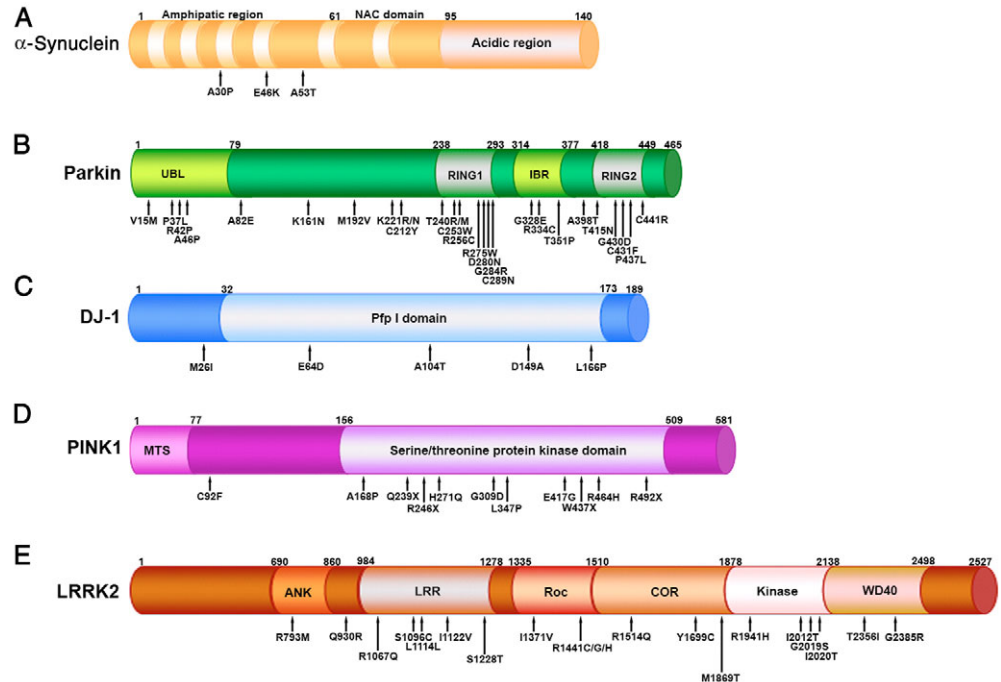
Why are dopaminergic neurons so sensitive to  $\alpha$ -synuclein toxicity? Dopamine is inherently unstable and can generate reactive oxygen species (ROS) and, via monoamine oxidase (MAO), H<sub>2</sub>O<sub>2</sub> (Lotharius et al., 2002). Dopamine is synthesized in the cytosol and rapidly pumped by 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  $\alpha$ -synuclein aggregation (Galvin, 2006) and dopamine- $\alpha$ -synuclein adducts stabilize  $\alpha$ -synuclein protofibrils (Conway et al., 2001).

Although there is no evidence for mitochondrial localization of  $\alpha$ -synuclein, overexpression of mutant  $\alpha$ -synuclein sensitizes neurons to oxidative stress and damage by dopamine metabolites and mitochondrial toxins such as MPP<sup>+</sup> and 6-hydroxydopamine, resulting in increased protein carbonylation and lipid peroxidation in vitro and in vivo (Orth et al., 2003). Interestingly,  $\alpha$ -synuclein-knockout mice (*Snca<sup>tm1Wid</sup>*, *Snca<sup>tm1Sud</sup>*, *Snca<sup>tm1Ros1</sup>*) 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).  $\alpha$ -Synuclein deficiency seems to result in a reduction of oxidative stress and, in addition, it has been proposed that  $\alpha$ -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)  $\alpha$ -Synuclein is a 140 amino acid protein belonging to a family of related synucleins that includes  $\beta$ - and  $\gamma$ -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- $\beta$  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  $\text{Ca}^{2+}$ /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 an 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  $\beta$ -propeller structure that might serve as a rigid scaffold for protein interactions). Approximate positions of missense mutations causing PD are indicated with arrows.

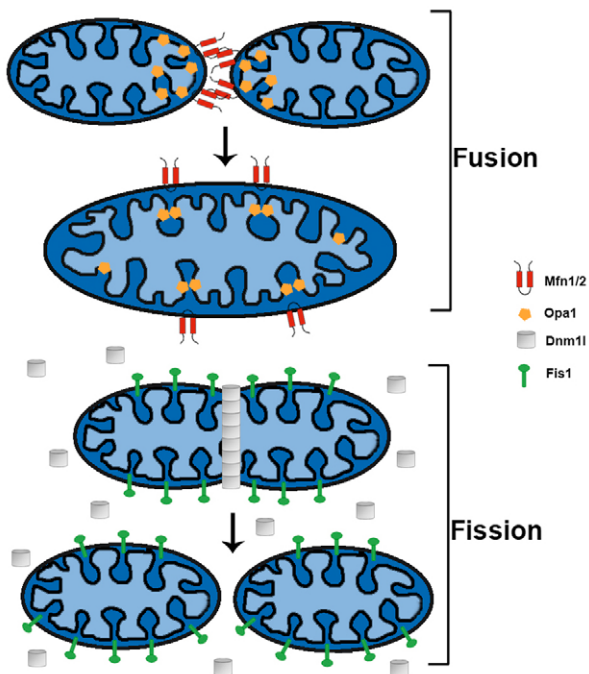


substrates and consequent death of neurons in the SNC (reviewed in Cookson, 2005). In general, no Lewy bodies are observed (Farrer et al., 2001; Mori et al., 1998; Sasaki et al., 2004).

Parkin also impacts on mitochondrial function. The *Drosophila* parkin-null (*park*<sup>25</sup>) 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*<sup>25</sup> flies identified loss-of-function of glutathione S-transferase (*GstS1*) as one of the strongest enhancers of fly death (Greene et al., 2005). *GST1* is an important antioxidant and flies lacking the genes encoding both parkin and *GST1* display progressive degeneration of dopaminergic neurons and increased oxidative damage (Whitworth et al., 2005). Loss of parkin in mice (*Parkin*<sup>tm1sh<sup>m</sup></sup>) 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 *Parkin*<sup>tm1sh<sup>m</sup></sup> 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 (*Parkin*<sup>tm1Tmd</sup>) 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



**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, Dnm1 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<sub>2</sub>H) upon oxidative stress (Canet-Aviles et al., 2004; Mitsumoto and Nakagawa, 2001). DJ-1 can also eliminate H<sub>2</sub>O<sub>2</sub> 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

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 GSK3 $\beta$  (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, *Opal* 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 (Dnm11, 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
<b>A Mutant proteins with primary mitochondrial localization</b>			
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 <i>cristae</i> 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 <sub>1</sub> F <sub>0</sub> 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>B Mutant proteins potentially affecting mitochondrial function</b>			
SOD1	Amyotrophic 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-1 $\alpha$ and p53	Huntington disease	Cytosolic protein whose function remains unclear. However, mutant Huntingtin influences transcriptional activity of p53 and PGC-1 $\alpha$	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 <i>c</i> 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* Dnm11 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. DNMI1 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).

Mitochondrial fission is similarly closely associated with the apoptosis pathway: mitochondrial fragmentation occurs early in apoptosis, and inhibition of DNMI1 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 1 $\alpha$  (PGC-1 $\alpha$ ) (Cui et al., 2006). Repression of this transcription co-activator leads to impaired mitochondrial function and enhances the 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-1 $\alpha$ , 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.

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