Expanding insights of mitochondrial dysfunction in Parkinson's disease

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Abstract | The quest to disentangle the aetiopathogenesis of Parkinson's disease has been heavily influenced by the genes associated with the disease. The α-synuclein-centric theory of protein aggregation with the adjunct of parkin-driven proteasome deregulation has, in recent years, been complemented by the discovery and increasing knowledge of the functions of *DJ1*, *PINK1* and *OMI/HTRA2*, which are all associated with the mitochondria and have been implicated in cellular protection against oxidative damage. We critically review how these genes fit into and enhance our understanding of the role of mitochondrial dysfunction in Parkinson's disease, and consider how oxidative stress might be a potential unifying factor in the aetiopathogenesis of the disease.

Complex I
Reduced NADH–ubiquinone reductase is an enzyme complex consisting of more than 40 polypeptides that spans the inner mitochondrial membrane. It oxidizes NADH, resulting in the transfer of electrons from NADH to ubiquinone.

Mitochondrial electron transport chain
A collective term describing the mitochondrial enzymes (also known as complexes I–IV) that are needed to generate the electron and proton 'gradient' that is used by complex V to generate ATP.

Dopamine transporter
(DAT). A monoamine transporter, the function of which is the clearance of the neurotransmitter dopamine out of a synapse into a presynaptic neuron or a glial cell.

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder, affecting ~1% of the population above the age of 60. The classical form of the disease is characterized clinically by rigidity, resting tremor, bradykinesia and postural instability. Its pathological hallmarks are the preferential loss of dopaminergic neurons of the substantia nigra pars compacta and formation of Lewy bodies — intracytoplasmic inclusion bodies that are mainly composed of fibrillar α-synuclein[1]. The clinical symptoms of PD arise by a threshold effect, whereby denervation of the corpus striatum by dopaminergic neuronal loss reduces dopamine levels to below 70% of wild type[2].

Mitochondrial dysfunction has long been implicated in the pathogenesis of PD. Evidence first emerged following the accidental exposure of drug abusers to 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) — an environmental toxin that results in an acute and irreversible parkinsonian syndrome[3]. The active metabolite of MPTP, the 1-methyl-4-phenylpyridinium ion (MPP^+) is an inhibitor of complex I of the mitochondrial electron transport chain and a substrate for the dopamine transporter (DAT). It therefore accumulates in dopaminergic neurons, where it confers toxicity and neuronal death through complex I inhibition[4]. This has many deleterious consequences, including increased free radical production and oxidative stress; and decreased ATP production, which causes increased intracellular calcium concentration, excitotoxicity and nitric oxide related cellular damage. MPP^+ also causes enhanced dopamine release, which leads to further oxidative damage[5]. Crucially, a biochemical link between MPTP toxicity and idiopathic PD was established when several groups worldwide reported that complex I was decreased in the substantia nigra, skeletal muscle and platelets of patients with PD[6–9]. However, it remained to be shown whether this systemic complex I deficiency, as observed in patients with PD, was causally related to dopaminergic cell loss in PD. Greenamyre's laboratory showed that rats administered the highly selective complex I inhibitor rotenone developed a PD-like syndrome characterized by neuronal degeneration and the formation of α-synuclein-rich inclusion bodies, however, the degree of complex I inhibition was partial and not sufficient to impair brain mitochondrial function; the effects were therefore more likely to be conferred by increased production of free radicals and oxidative stress[10].

Mitochondrial DNA (mtDNA) encodes 13 proteins that are all components of the electron transport chain, and there have been several reports of mtDNA mutations in rare maternally-inherited pedigrees of parkinsonism, including the *12SrRNA* gene in one family with parkinsonism, deafness and neuropathy[11]. More recently, mutations in the nuclear encoded mitochondrial gene, DNA polymerase γ (*POLG*), were reported in families with parkinsonism associated with progressive external ophthalmoplegia and multiple mitochondrial deletions in affected members[12]. However, the clinicopathological phenotype of such pedigrees is distinct from that of idiopathic PD, and there is little convincing evidence to support a causal role of mtDNA variants in the pathogenesis of idiopathic PD.

Despite the evidence of complex I inhibition and concomitant free radical production resulting in increased oxidative stress in cellular, animal and human studies,

Table 1 | **Parkinson's disease-associated genes**

Locus	Gene	Inheritance	Function	Phenotype
*PARK1/4	α-Synuclein	Autosomal dominant	Involved in synaptic vesicle formation	Age of onset: 30–60 years Lewy bodies: ++
PARK2	Parkin	Autosomal recessive	An E3 ligase	Age of onset: ~30 years ‡Lewy bodies: –
PARK6	Phosphatase and tensin homologue (PTEN)-induced kinase 1 (*PINK1*)	Autosomal recessive	A mitochondrial kinase	Age of onset: 30–50 years Lewy bodies: ?
PARK7	Parkinson's disease (autosomal recessive, early onset) 7 (*DJ1*)	Autosomal recessive	Involved in oxidative stress response	Age of onset: 20–40 years Lewy bodies: ?
PARK8	Leucine-rich repeat kinase 2 (*LRRK2*)	Autosomal dominant	A protein kinase	Age of onset: 40–60 years Lewy bodies: + variable pathology
Unmapped	HtrA serine peptidase 2 (*HTRA2*, also known as *OMI*)	Autosomal dominant? Predisposition	A serine protease and/or involved in stress response	Age of onset: 44–70 years Lewy bodies: ?

*PARK1 and 4 share an entry because they have been shown to be caused by the same gene. ‡There has been one reported case of a parkin-positive patient with Lewy bodies. ++ Fulminant Lewy body pathology. + Lewy bodies present.

it remained unclear whether this observed dysfunction was a primary process in the pathogenesis of the disease or a secondary process.

A major leap in our understanding of the aetiopathogenesis of the disease came when mutations were identified in α-synuclein in 1997, followed by mutations in parkin a year after that[13,14]. The demonstration that α-synuclein is the main constituent of Lewy bodies in the same year suggested a primary role for α-synuclein aggregation, however, later studies revealed close interplay between α-synuclein aggregation and oxidative stress in the pathogenesis of PD[1,15]. The identification of mutations in *DJ1* (Parkinson's disease (autosomal recessive, early onset) 7, a possible redox sensor) in 2003 and phosphatase and tensin homologue (PTEN)-induced kinase 1 (*PINK1*, a mitochondrial kinase) in 2004 provided strong evidence that mitochondrial dysfunction and oxidative stress might have a primary role in the pathogenesis of PD, although how mutations in these genes cause neuronal degeneration is still unclear[16,17]. So, although classically regarded as an archetypical non-genetic disease due to the high proportion of sporadic cases, hugely significant advances in our understanding of PD have stemmed directly from the study of these genes associated with a small proportion of familial cases.

So far, five PD-associated genes have been conclusively identified (TABLE 1). A sixth gene, ubiquitin carboxyl-terminal esterase L1 (*UCHL1*), has been associated with the disease, but the genetic evidence for its pathogenicity is weak as only a single mutation has been identified in one family[18]. Recently, variation and mutation of the HtrA serine peptidase 2 (*HTRA2*, also known as *OMI*) gene has also been tentatively associated with PD[19].

In this review, we present an overview of the published functional data on α-synuclein, parkin, *DJ1*, *PINK1*, *OMI/HTRA2* and leucine-rich repeat kinase 2 (*LRRK2*) in order to place them in the context of the current theories on the aetiopathogenesis of the disease. However, rather than attempting to review the entire known biochemistry of PD, we focus on potential areas of overlap between the different pathways. As a consequence, we highlight the involvement of primarily oxidative stress and, by association, the mitochondria in all of the parkinsonian pathways described so far. We therefore conclude that oxidative stress is a good candidate as the unifying factor for several diverse but overlapping pathways to PD, and suggest that an improved understanding of the mechanisms involved in the production of reactive oxygen species (ROS) and the protection against them, which is primarily mitochondrial, might result in improved treatment of the disease.

α-Synuclein, protein aggregation and PD

During the early 1990s, the relevance of rare Mendelian forms of PD to idiopathic PD remained under-appreciated and the prevailing view was that environmental factors were the most important influence in disease aetiology. In 1997, the discovery of an alanine-to-threonine missense mutation (A53T) in α-synuclein prompted a shift in this belief[13]. This shift gained significant momentum by the discovery that α-synuclein was a main component of Lewy bodies in both familial and sporadic disease[1], which suggests that abnormalities of α-synuclein might be crucial for the pathogenesis of both rare and common forms of PD.

Although the function of α-synuclein is still unclear, exciting recent work has revealed that α-synuclein can rescue mice from neurodegeneration caused by deletion of the synaptic co-chaperone, cysteine-string protein-α (CSPα), and that it might act with CSPα to serve a protective role against injury at nerve terminals[20]. Given that α-synuclein assumes a fibrillar β-pleated sheet structure in Lewy bodies in PD and related α-synucleinopathies[21], the leading hypothesis for its pathogenicity is the formation of toxic aggregates. Under certain *in vitro* conditions, wild-type or mutant α-synuclein can form protofibrillar

Complex I deficiency
A reduction in the enzymatic activity of complex I compared with the remaining respiratory chain complexes, as determined by *in vitro* biochemical assays.

(oligomeric) or fibrillar conformations. However, controversy remains about which species is the toxic culprit[22,23]. The missense mutants A53T and A30P (alanine to phenylalanine) in α-synuclein both promote protofibril formation, but only the A53T mutation promotes fibril formation, and, in fact, A30P inhibits conversion to fibrils[23]. How might protofibrils be toxic? α-Synuclein has been shown to bind synaptic vesicles, and protofibrils can form pores that could lead to permeabilization of the vesicle membranes, thereby releasing excess dopamine into the cytosol[24]. Formation of protofibrils is enhanced and stabilized by dopamine quinones derived from the oxidation of dopamine, and this could account for the selective toxicity of α-synuclein in the substantia nigra[15]. Protofibril formation is also enhanced by missense and triplication mutants of α-synuclein. Protofibrils were recently reported *in vivo,* in brains from patients with PD who had the α-synuclein triplications[25].

Toxicity associated with increased α-synuclein expression has important ramifications for the genetic predisposition to idiopathic PD, as α-synuclein promoter polymorphisms have been associated with the disease[26]. There is also accumulating evidence that phosphorylation of α-synuclein promotes the formation of α-synuclein protofibrils and filaments *in vitro*, and α-synuclein is extensively and selectively phosphorylated at serine residue 129 *in vivo* in the brains of patients with PD and related synucleinopathies[27]. Recently, Chen and Feany found that phosphorylation of α-synuclein at Ser129 significantly enhanced α-synuclein toxicity *in vivo* in a *Drosophila* model of PD, and, interestingly, showed that blockade of Ser129 phosphorylation was associated with reduced toxicity and inclusion formation[28]. In *Drosophila*, the G-protein-coupled receptor kinase 2 (GPRK2) phosphorylated α-synuclein at Ser129 (REF. 28), however, the identity of the kinase responsible for α-synuclein phosphorylation in the human brain remains unknown.

The *Drosophila* model of α-synuclein also lends weight to the fibril hypothesis, as expression of either wild-type or mutant A30P or A53T α-synuclein resulted in flies with progressive dopaminergic cell loss, motor deficits, premature death and fibrillar α-synuclein-positive inclusions similar to Lewy bodies[29]. Almost all of the transgenic α-synuclein mouse models have failed to recapitulate all the features of PD, most notably the dopaminergic cell loss. Nevertheless, expression of wild-type or A53T α-synuclein in mice generally results in the neurological dysfunction and early death associated with the formation of widespread α-synuclein-positive inclusions that can be fibrillar[30,31]. However, transgenic expression of the protofibrillogenic A30P α-synuclein resulted in no phenotype in mice, in contrast to parallel A53T mouse lines that were associated with widespread α-synuclein inclusions and death[32]. The reason for discordance in phenotypes between the A30P mice and *Drosophila* remains unknown.

It is still not clear whether the aggregates in PD are toxic or protective. In α-synuclein-mutant flies, the chaperone heat-shock protein 70 (HSP70) suppressed the PD phenotype and reduced dopaminergic neuronal degeneration. However, there was no difference in the frequency or distribution of α-synuclein inclusions in surviving flies[33]. Moreover, the work of Chen and Feany also suggests that, at least in flies, the formation of α-synuclein inclusions might be a protective response to α-synuclein toxicity *in vivo*[28]. The ubiquitin ligase parkin has also been shown to reduce α-synuclein toxicity *in vitro* and *in vivo*, but this is associated with an enhancement of α-synuclein aggregation, including an increase in phosphorylated inclusions in rats transduced with lentiviruses expressing the A30P α-synuclein mutation[34,35]. Although these latter studies do not rule out the possibility that protofibrils are toxic in these systems and that the protective response is mediated by their conversion to non-toxic fibrillar forms, they do at least raise the possibility that additional cellular defects might occur secondary to altered α-synuclein expression.

α-Synuclein is localized predominantly in synaptic terminals and in the cytosol of the cell body[36]. Although there is no evidence for mitochondrial localization, dysfunction of α-synuclein has been shown to indirectly but significantly impact on neuronal mitochondrial function. There is accumulating evidence for a close relationship between α-synuclein and oxidative damage; overexpression of mutant α-synuclein sensitizes neurons to oxidative stress and damage by dopamine and mitochondrial toxins such as MPP^+ and 6-hydroxydopamine, resulting in increased protein carbonylation and lipid peroxidation *in vitro* and *in vivo*[37,38]. Interestingly, α-synuclein-knockout mice were initially found to have marked resistance to MPTP, and a recent study also showed resistance to other mitochondrial toxins, including malonate and 3-nitropropionic acid[39,40]. The mechanism of this resistance seems to be due to α-synuclein deficiency resulting in a reduction of oxidative stress — α-synuclein has previously been shown to modulate the release of dopamine from synaptic vesicles into the cytosol, where it auto-oxidizes, leading to increased production of free radicals and oxidative stress[41]. Moreover, the generation of ROS and oxidative stress is likely to exacerbate the toxic effect of the α-synuclein mutations, including aggregation in an amplification loop (see below). Therefore, it can be envisaged that cellular pathways in dopamine neurons can conspire to cause catastrophic neuronal demise in the presence of defects in either the α-synuclein or oxidative stress pathways.

Parkin

Homozygous mutations in the parkin gene were discovered in families with autosomal recessive PD (ARPD)[14]. In contrast to α-synuclein, parkin mutations are common, and account for almost half of all cases of ARPD, especially those with onset before 21 years of age[42,43]. The 465 amino acid protein contains two RING (really interesting new gene) fingers separated by an in-between RING (IBR) domain at the carboxyl (C) terminus, which, like other RING finger proteins, functions as an E3 ubiquitin ligase[44]. The amino (N) terminus bears a ubiquitin-like domain that binds to the RPN10 subunit of the 26S proteasome[45]. In the cell, E3 ubiquitin ligases are one component of the ubiquitin–proteasome system

Autosomal recessive PD
(ARPD). A familial form of PD with an autosomal recessive mode of inheritance.

RING finger proteins
Specialized zinc finger proteins that bind two atoms of zinc. Proteins containing RING fingers are involved in mediating protein–protein interactions.

26S proteasome
Macromolecules composed of many subunits that are involved in the degradation of proteins.

(UPS), a main cellular pathway that promotes removal of damaged or misfolded proteins[46]. E3 ligases catalyse the addition of ubiquitin molecules to lysine residues of damaged target proteins, and the presence of a polyubiquitin chain provides a signal for its removal and degradation by the proteolytic complex, the 26S proteasome[46].

A striking variety of homozygous and compound heterozygous mutations have been reported, including gene rearrangements and missense mutations[42,43]. Despite the fact that there have been few postmortem studies in parkin-related ARPD, it is becoming clearer that the nature of the parkin mutation is crucial for interpreting the pathology, and that mutations that abolish parkin activity seem to be associated with a lack of Lewy bodies. However, in mutations that reduce but do not abolish parkin activity, Lewy bodies can occur[47].

The lack of Lewy bodies in ARPD patients led some to postulate that parkin-related ARPD was a distinct clinical syndrome from sporadic PD. However, recent insights in parkin suggest that the two types of PD might have shared aetiological pathways. Complex I is selectively reduced in peripheral leukocytes of patients with parkin-related ARPD[48], and parkin is generally found in Lewy bodies of patients with sporadic and familial PD[49,50]. A *Drosophila* model has revealed a role for parkin in maintaining mitochondrial function and preventing oxidative stress — pathways heavily implicated in sporadic PD. Parkin null mutants had severe mitochondrial pathology associated with reduced lifespan, apoptosis, flight muscle degeneration and male sterility[51]. Microarray analysis in these flies revealed upregulation of genes involved in oxidative stress and electron transport, including a homologue of the mammalian peripheral benzodiazepine receptor. A genomic screen for modifiers of lifespan in the parkin null flies found the strongest modifier to be loss-of-function mutations of glutathione S-transferase (GSTS1)[52]. Reanalysis of the same flies revealed progressive degeneration of a select cluster of dopaminergic neurons and evidence of increased oxidative damage with increased protein carbonyls compared with controls[53]. Furthermore, neurodegeneration was enhanced in GSTS1 null mutants, whereas GSTS1 overexpression significantly rescued the parkin phenotype[53].

Mammalian models also support a role for parkin in maintaining mitochondrial function. Deletion of exon 3 of parkin in mice results in nigrostriatal dysfunction and reduced expression of several proteins involved in mitochondrial function and oxidative stress, including subunits of complexes I and IV. The mice also had decreased mitochondrial respiratory capacity and showed evidence of increased oxidative damage[54]. Intriguingly, parkin deficiency in these mice did not cause dopaminergic degeneration, which is also observed in exon 2 and other exon 3 deletion models[55–57]. It will therefore be interesting to determine whether neurodegeneration in these models requires an environmental insult such as an oxidative stressor. A similar mechanism might operate in parkin heterozygotes, as they seem to be at increased risk of PD and have nigrostriatal dysfunction, as visualized by positron emission tomography (PET)[58].

The mechanism by which parkin might regulate mitochondrial function is unclear. Parkin might be directly involved in maintaining mitochondrial integrity: it has been localized to the outer mitochondrial membrane (OMM), where it has a crucial role in preventing mitochondrial swelling and rupture secondary to ceramide toxicity[59]. Furthermore, the *Drosophila* homologue of the peripheral benzodiazepine receptor (PBR) was upregulated in parkin mutant flies, and PBR is a component of the mitochondrial permeability transition pore (mPTP), where it is important in mPTP opening, thereby regulating oxidative damage secondary to mitochondrial dysfunction and OMM rupture[60]. It is not known whether PBR is a substrate for parkin. Ubiquitylation also mediates the insertion of mitochondrial proteins into the OMM and parkin might also have a role in this[61]. However, further studies are required to confirm mitochondrial localization of parkin. So far, most studies have localized parkin to other subcellular organelles, most notably the endoplasmic reticulum, where it has been shown to have a neuroprotective role against endoplasmic reticulum stress[62].

It is equally likely that parkin could indirectly maintain mitochondrial function. As free radical-induced oxidative damage is a normal consequence of the electron transport chain, parkin might have a role in removing oxidatively damaged proteins and mutant parkin might lead to the accumulation of such proteins, which might lead to further oxidative stress and apoptosis. This mechanism would be most compatible with the known function of E3 ligases in the UPS. Moreover, the related E3 ubiquitin ligase, HOIL1, has been shown to degrade its oxidized substrate, iron responsive element-binding protein 2 (IRP2)[63]. Parkin expression is upregulated after exposure to the complex I inhibitor MPP^+ in neuronal cells[64]. However, parkin function itself can be modified by oxidative stress: exposure to nitric oxide generates free radicals that modify cysteine residues in RING1, which results in significant alteration of parkin's E3 ligase activity, including inactivation[65,66]. Furthermore, nitrosylated forms of parkin are detectable in both the brains of patients with PD and the brains from MPTP and rotenone treated animals, which suggests that inactivation of parkin might be a crucial step in the pathogenesis of sporadic PD[65,66]. Moreover, dopamine has recently been shown to covalently modify and functionally inactivate parkin E3 ligase activity by increasing parkin insolubility *in vitro* and in the brains of patients with PD[67].

Another recent study also found that a wide array of oxidative stressors, including rotenone, MPP^+, 6-hydroxydopamine, paraquat, nitric oxide and iron, as well as dopamine, all altered parkin solubility and caused parkin aggregation, thereby suggesting a mechanism for parkin dysfunction in the pathogenesis of idiopathic PD[68]. Interestingly, modulation of parkin's E3 ligase activity has also been shown for the interactor, BCL2-associated athanogene 5 (BAG5). BAG5 is a member of the BAG family of proteins and has been shown to interact with parkin, as well as the chaperone HSP70. Moreover, BAG5 seems to promote neurodegeneration by inhibiting parkin's E3 ligase activity and promoting sequestration of parkin into aggregates[69].

Ever since parkin was found to be an E3 ligase, it has been proposed that mutations of parkin or parkin dysfunction might lead to the toxic accumulation of its substrate. E3 ligases confer specificity in the UPS, usually by targeting one protein[70]. Surprisingly, many disparate substrates for parkin have been discovered, with strong replicated evidence for the septin CDCREL1 and PAELR[71]. Moreover, overexpression of CDCREL1 and PAELR *in vivo* mediates dopaminergic neurodegeneration[72,73], and both also accumulate in the brains of patients with parkin-related ARPD[74,75]. However, neither of these substrates accumulates in *Drosophila* or mammalian parkin-knockout models, and it is not known whether any of these substrates are oxidatively modified following oxidative stress or can be appropriately degraded by parkin. By contrast, the parkin substrate, aminoacyl-tRNA synthetase cofactor p38, is upregulated in the midbrain of parkin null mice as well as in the brains of patients with ARPD and idiopathic PD[76]. Moreover, adenovirus-mediated overexpression of p38 in the substantia nigra of mice induced loss of dopaminergic neurons[76].

α-Synuclein might unify the oxidative stress and substrate accumulation hypotheses for the mediation of parkin dysfunction. Parkin can protect against α-synuclein-induced neurotoxicity *in vitro* and *in vivo*[73,77]. However, there is no replicated data that parkin interacts directly with α-synuclein. Parkin has been shown to interact with a glycosylated form of α-synuclein (known as sp22)[78], however, it is not clear whether α-synuclein toxicity is mediated by sp22. The interaction of parkin and α-synuclein might be direct if parkin can degrade oxidatively modified forms of α-synuclein. Alternatively, the interaction might be indirect and α-synuclein has been shown to inhibit the proteasome (although it is not clear how), and it has been observed that parkin can rescue neurons from α-synuclein induced proteasomal dysfunction[77]. What is becoming clear from numerous studies is that loss of parkin function might impact on many cellular pathways, rendering dopaminergic neurons sensitive to neurotoxicity and death. Although UPS dysfunction and mitochondrial dysfunction might be the main pathogenetic pathways, the discovery of a wide range of substrates implicates additional pathways. Future studies should illuminate these pathways, and it will be of significant interest to see whether and how these additional pathways impact and/or converge on the two currently accepted pathways of UPS and oxidative stress.

DJ1

Mutations of *DJ1* were identified in two consanguineous European families with early-onset ARPD[16], and several pathogenic mutations have since been identified, including homozygous and heterozygous missense mutations and exonic deletions. In terms of the overall mutation burden in PD, *DJ1* mutations seem to be quite rare, accounting for only an estimated 1–2% of early-onset cases[79].

The precise cellular distribution and subcellular localization of the protein has been hotly contested since it was first associated with PD. It is certain that *DJ1* is widely expressed in both the brain and peripheral tissues. Beyond that, reports are conflicting[80–83] — the only study carried out on the endogenous protein so far has detected *DJ1* expression in both neurons and glia, although expression in the latter was weak. Subcellular distribution of the endogenous protein is primarily cytoplasmic with a smaller pool of mitochondrial-associated protein[83].

Structurally, DJ1 is a member of the ThiJ/PfpI/DJ1 superfamily and is highly conserved across species. It has limited homology to several prokaryotic proteins, including heat shock protein chaperones and ThiJ/PfpI proteases[84]. DJ1 has been ascribed various functions[85,86], but perhaps the most relevant in terms of the pathogenesis of PD is its potential role in oxidative stress response, either as a redox sensor or antioxidant protein[87,88]. At present, there are two lines of evidence to support this theory, the first of which is that in mammalian cells exposed to an oxidative stressor, such as paraquat or H_2O_2, DJ1 undergoes an acidic shift in pI-value by modifying its cysteine residues, which quench ROS and protect cells against stress-induced death. Its *Drosophila* homologues DJ1α and DJ1β are also oxidized at their cysteine residues after exposure to the same stresses both *in vivo* and in culture[89,90]. Further evidence has been obtained from various model systems. Mammalian cell cultures, and mouse and *Drosophila* knockouts all indicate that the ablation of functional DJ1 either by small interfering RNA (siRNA) or gene deletion sensitizes cells to oxidative stress. These cells can be rescued by overexpression of wild-type but not mutant (L166P) DJ1 (REF. 91). Therefore, loss of DJ1 increases levels of intracellular ROS and increases susceptibility to dopaminergic neuron degeneration *in vivo* following exposure to exogenous sources of oxidative stress[92]. However, it is unlikely that DJ1 exerts its protective function through simple antioxidation. Its ability to quench ROS is modest[82,89], and several lines of evidence indicate that it is more likely to be implicated in the regulation of apoptosis. It is plausible that, through modification of its cysteine residues on exposure to H_2O_2, DJ1 is acting as a sensor of cellular ROS levels. DJ1 functions in the phosphatidylinositol 3-kinase (PI3K) survival pathway as a negative regulator of PTEN. The PTEN tumour suppressor regulates the PI3K pathway by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate, which is required for the activation of a survival kinase, protein kinase B (PKB, also known as Akt)[93]. In cultured cells, tissue from patients with cancer and *Drosophila* DJ1α RNAi knockdowns, DJ1 expression correlates with the phosphorylation of PKB. In keeping with a possible modulatory effect, it has been shown that increased PI3K/Akt signalling capacity in *Drosophila* DJ1α RNAi knockdowns reduced cell death, whereas reduced PI3K/Akt expression enhanced the phenotype[89]. How DJ1 interacts with the PI3K–PTEN–Akt pathway remains to be determined. One possibility, given that PTEN function can be modulated by exposure to H_2O_2, is that DJ1 exerts a redox effect on PTEN through either its protease activity or redox-sensitive chaperone activity[93].

ThiJ/PfpI/DJ1 superfamily
Proteins that share sequence homology to the bacterial ThiJ domain. Functions include protein chaperones, catalases, proteases and ThiJ kinases.

pI
(Isoelectric point). The pH of a solution at which a dissolved charged molecule has no electric charge and will therefore not move in an electric field.

DJ1 is further implicated in both apoptosis and cellular response to oxidative stress through its binding partners in the brain, death-associated protein 6 (DAXX)[82], 54 kDa nuclear RNA-binding protein (p54NRB), pyrimidine tract-binding protein-associated splicing factor (PSF)[94] and topoisomerase I binding protein (TOPORS, also known as p53BP3)[95]. DJ1 acts as a potent inhibitor of the DAXX–ASK1 (apoptosis signal-regulating kinase 1) pathway by sequestering DAXX to the nucleus, away from its cytoplasmic effector, and also inhibits PSF-induced apoptosis by cooperating with p54NRB to activate PSF-silenced transcription[82].

DJ1 mutations disrupt protein activity by either destabilizing the protein or affecting its subcellular localization. Wild-type DJ1 exists as a homodimer *in vitro*[96–99]. However, the L166P mutation impairs the ability of DJ1 to self-interact, which results in a highly unstable protein, which is degraded by the 20S/26S proteasome[99–101]. The reduced protein levels result in loss of function, as demonstrated by the impaired regulation of the DAXX–ASK1 pathway seen in L116P mutants[82]. Incorrect subcellular localization might also result in loss of DJ1 function — the L166P, M26I and D149A mutations all show reduced nuclear localization in favour of mitochondrial localization[16,94]. The reduced access to nuclear proteins, such as p54NRB and PSF might increase PSF-induced apoptosis as a result of the increased mitochondrial localization of the mutants[82]. As the mitochondrial function of DJ1 remains to be determined, it is not clear whether the mutagenecity associated with increased mitochondrial localization is due to a mitochondrial gain of function or to a loss of access to binding partners in different cellular compartments. In addition to cellular response to oxidative damage and apoptosis, DJ1 also functions as a redox sensitive molecular chaperone that is capable of preventing the aggregation of α-synuclein and the neurofilament subunit NFL[102]. However, as the majority of these studies have been carried out on cell lines overexpressing DJ1, which are prone to artefact, these putative roles remain to be confirmed *in vivo*.

PINK1 and *OMI/HTRA2*

Mutations in *PINK1* were initially identified in three large consanguineous families with ARPD — one Spanish and two Italian[17]. The gene had previously been cloned by two groups of researchers who independently analysed differential expression profiles of cancer cell lines[103,104]. *PINK1* was shown to be transcriptionally activated by PTEN, but its expression was not sufficient to suppress the growth of cancer cells[103]. It is noteworthy that DJ1 has recently been identified as a suppressor of PTEN function[93].

PINK1 encodes a ubiquitously expressed 581 amino acid protein, which consists of an N-terminal mitochondrial targeting motif, a highly conserved serine/threonine kinase domain and a C-terminal autoregulatory domain[17,105].

Mutation reports from around the world indicate that the frequency of *PINK1* mutations lies somewhere between those of *DJ1* and parkin, with the notable exception of the L347P *PINK1* mutation, for which a carrier frequency of 8% has been reported in the Philippino population[106–109]. The reported mutations do not show any obvious clustering within the gene, and most are distributed throughout the kinase domain with a subset located in the N-terminal region between the mitochondrial targeting motif and the kinase domain (approximately amino acid residues 30–150). At present, it is not clear how the mutations located outside the kinase domain affect enzyme function. One possibility is that they could disrupt mitochondrial localization or processing. Most nuclear encoded mitochondrial proteins contain a cleavable N-terminal 'pre-sequence' of between 20 and 60 amino acid residues that directs the protein towards its target after translation on cytoplasmic ribosomes (for a recent review on mitochondrial import, see REF. 110). The mechanism of PINK1 mitochondrial import remains unknown, and the peptidase cleavage sites unmapped. These mutations might either disrupt interaction with the translocase complex of the inner membrane (TIM complex) or be cleaved by the mitochondrial processing peptidase (MPP). Western blot analysis of cells overexpressing PINK1 suggests that the processed protein is ~10 kDa smaller than the pre-protein, and the 10 kDa fragment translates to a cleavage product of ~100 amino acids[108], potentially placing mutations such as C92F at or around the cleavage site.

As the kinase domain is the only functional domain in PINK1, and the site of most of the mutations, disruption of the kinase activity is the most probable disease mechanism. Kinases are defined by 12 highly conserved subdomains that fold to form a common catalytic core structure that is required for phosphorylation[111]. Alignments of the PINK1 protein against other known kinases indicate that it has all the necessary subdomains to form an active serine/threonine kinase. At present, confirmation of the bioinformatic prediction is limited to studies that use *in vitro* autophosphorylation assays; these studies demonstrate that PINK1 is active[104,105,108]. However, these assays are too crude to distinguish any significant functional effects of the mutations on kinase activity. Some functional data has been obtained from indirect assays, which show that loss of PINK1 function adversely affects mitochondrial function and cell viability under stress. Mitochondrial membrane potential ($\Delta\psi m$) (FIG. 1) and levels of cell death were measured in a neuroblastoma cell line overexpressing G309D PINK1 after exposure to an exogenous source of cellular stress, MG-132, a proteasome inhibitor. Cells overexpressing G309D PINK1 had significantly reduced $\Delta\psi m$ compared with the wild type and increased levels of cell death following exposure to stress, but not under basal conditions. Moreover, cells overexpressing wild-type PINK1 had higher $\Delta\psi m$ and lower levels of cell death than cells transfected with vector alone[17]. Consistent with these results, overexpression of wild-type PINK1 was subsequently shown to reduce the release of cytochrome *c* from mitochondria under basal conditions and staurosporine-induced stress. The reduced cytochrome *c* levels also result in a decrease in the production of the pro-apoptotically cleaved caspase 3, caspase 7, caspase 9

Mitochondrial membrane potential
($\Delta\Psi m$). A chemiosmotic gradient of protons across the inner mitochondrial membrane. The energy this creates is used for ATP synthesis by the electron transport chain.

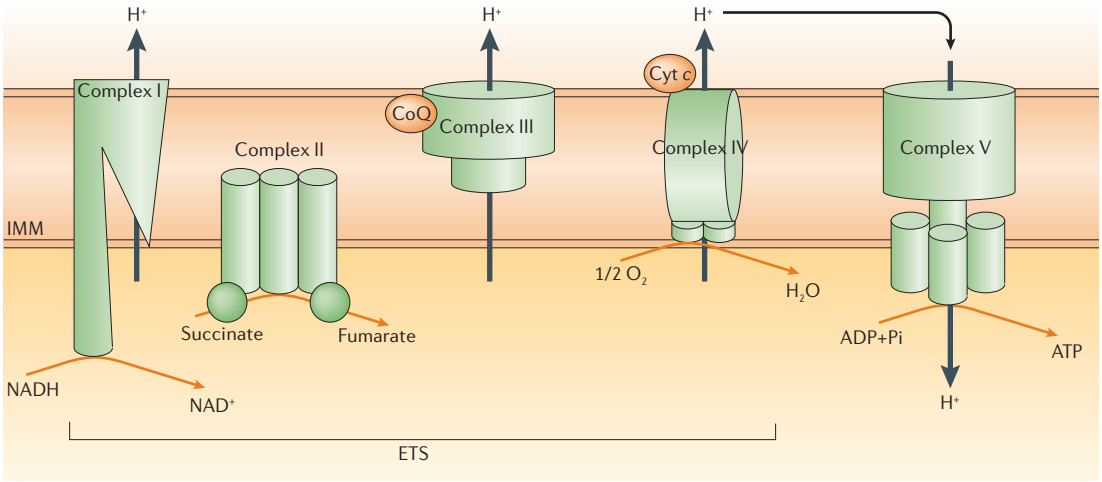

Figure 1 | **Schematic representation of the mitochondrial electron transport system.** Mitochondria are the main providers of cellular energy, which is generated through the flow of electrons down the electron transport system (ETS). The ETS is located on the inner mitochondrial membrane (IMM) and consists of 4 membrane spanning enzyme complexes. These comprise complex I (NADH–ubiquinone reductase), which oxidizes NADH, complex II (succinate–ubiquinone oxidoreductase), which oxidizes $FADH_2$, complex III (ubiquinol cytochrome *c* oxidoreductase) and complex IV (cytochrome *c* oxidase). The ETS also contains two hydrophobic electron carriers, coenzyme Q10 (CoQ) and cytochrome *c* (Cyt *c*), both of which are encoded by nuclear DNA. The ETS transfers electrons through a series of oxidation–reduction reactions, culminating in the reduction of oxygen to produce water. The oxidation–reduction reactions are coupled to the transfer of protons (H^+) across the IMM, and this proton efflux creates a proton electrochemical gradient known as the protomotive force[140]. The protomotive force consists mainly of an electrical component called the mitochondrial membrane potential ($\Delta\Psi m$) and a transmembrane pH gradient. The $\Delta\Psi m$, which is maintained at about –150 to –180 mV negative to the cytosol, is central to mitochondrial function and provides the force that drives the influx of protons and Ca^{2+} into the mitochondria as well as determining the generation of O_2^-. Protons traverse the mitochondrial membrane through a proton channel of the F_1F_0-ATP synthase (also known as complex V). The re-entry of protons depolarizes the $\Delta\Psi m$ and induces the phosphorylation of matrix ADP to generate ATP. Therefore, the transfer of electrons, generation of the $\Delta\Psi m$ and ATP synthesis are all closely coupled. Pi, phosphate group.

and poly (ADP-ribose) polymerase (PARP). Significantly, two PINK1 mutations, E240K and L489P, were shown to abrogate the protective effect[112]. Finally, the L347P mutation decreases the half-life of PINK1 from ~2 h to ~0.25 h, which supports the idea that loss of PINK1 function might contribute to PD[108].

Studies on PINK1 mutations strongly support a direct involvement of mitochondria in the pathogenesis of idiopathic PD. The relationship between PINK1 and the $\Delta\psi m$ remains to be determined. It might centre around the electron transport chain or, more likely, the mitochondrial pro-apoptotic pathways. Regulation of the electron transport chain might involve phosphorylation of at least two of its subunits, complexes I and V (REFS 113,114), in order to maintain the electrochemical gradient that generates the $\Delta\psi m$. Depolarization of the $\Delta\psi m$ is associated with opening of the mitochondrial permeability transition pore (mPTP), which occurs in necrotic and apoptotic cell death (see hypothetical model in FIG. 2). Both the voltage-dependent anion channel (VDAC) and the adenine nucleotide translocator (ANT) undergo phosphorylation in response to stress and might represent potential areas for PINK1 involvement. Recent studies of isolated mitochondria from selective ANT-knockout mice revealed preservation of mPTP activation and cytochrome *c* release, suggesting that ANT is a non-essential structural component of the mPTP. However, ANT might still have a role in the regulation of mPTP opening, and any post-translational modifications of ANT, such as phosphorylation, might be important for this regulation[115].

One example of a procaspase that is released from the intermembrane space by the opening of the mPTP is the OMI/HTRA2 protein. It has previously been implicated in neurodegeneration, and recently associated with predisposition to PD[19]. OMI/HTRA2, a PDZ domain-containing serine protease, also contains an N-terminal mitochondrial-targeting motif and a reaper-like motif. OMI/HTRA2 is thought to localize to the mitochondrial intermembrane space, where it is released into the cytosol during apoptosis to relieve the inhibition of caspases by binding to inhibitor of apoptosis proteins (IAPs). OMI/HTRA2 is also able to induce cell death through its proteolytic activity.

OMI/HTRA2 was initially shown to interact with proteins associated with Alzheimer's disease, such as presenillin 1 and amyloid-β[116,117]. Recently, OMI/HTRA2-knockout mice have been shown to display parkinsonian phenotypes, including rigidity and tremor[118], which, together with its localization in the PARK3 (Parkinson's disease 3) locus made it a candidate for mutation screening in patients with PD. Mutations in OMI/HTRA2 were not found in the PARK3 families. However, a mutation at Gly399 was found in four

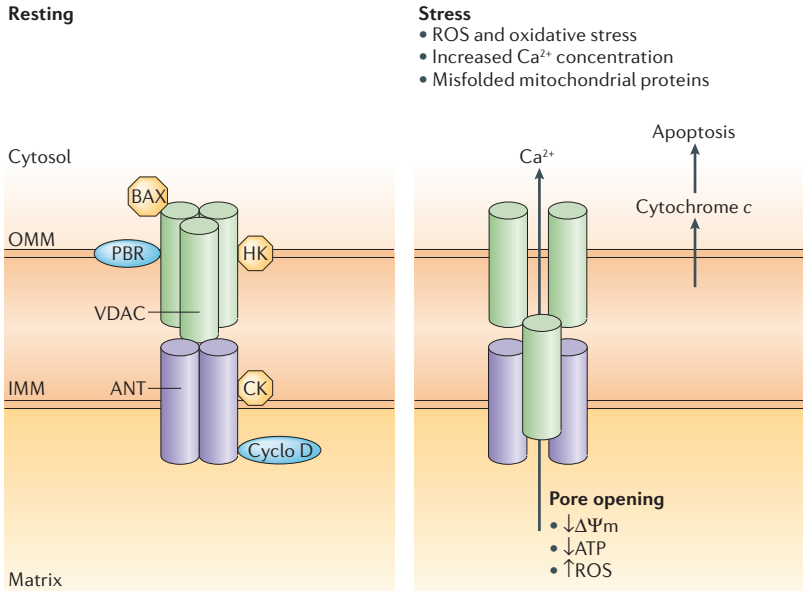

Figure 2 | **Hypothetical schematic of the mitochondrial permeability transition pore.** The mitochondrial permeability transition pore (mPTP) is a conductance pore that spans the inner (IMM) and outer (OMM) mitochondrial membranes. It consists of membranous elements such as the voltage-dependent anion channel (VDAC) on the OMM, the adenine nucleotide translocator (ANT) on the IMM, and cyclophilin D (Cyclo D) in the matrix. Other proteins, such as the peripheral benzodiazepine receptor (PBR), hexokinase (HK) and creatine kinase (CK), might also be associated with the mPTP. It is not clear whether the mPTP has a role in normal mitochondrial physiology. However, under a combination of pathophysiological conditions, such as high Ca^{2+} concentration, increased oxidative stress, low ATP, and mitochondrial depolarization, the complex forms an open pore between the inner and outer membranes, allowing free diffusion of solutes across the membranes. The opening of the mPTP ultimately results in mitochondrial swelling, mitochondrial Ca^{2+} efflux and the release of apoptogenic proteins, such as cytochrome *c* and procaspases, from the intermembrane space[141]. ANT-knockout studies suggest that it might not be an essential structural component but might have a role in the regulation of mPTP opening[115]. ΔΨm, mitochondrial membrane potential; BAX, BCL2-associated X protein; ROS, reactive oxygen species.

patients with sporadic PD, and a polymorphism at Ala141 was found at higher frequencies in patients with PD[19]. Both mutations are predicted to affect regulation of the proteolytic activity of OMI/HTRA2, thereby modulating cell death. At the cellular level, the mutations have been shown to increase susceptibility to stress, as shown by decreased Δψm after exposure to staurosporine. It is tempting to speculate that PINK1 and OMI/HTRA2 share a common pathway in the mitochondrial response to cellular stress and modulation of apoptosis, perhaps with OMI/HTRA2 as a downstream target for PINK1, which initiates apoptosis when levels of oxidative stress become too high. However, whether such interaction exists in PD remains to be determined.

Leucine-rich repeat kinase 2

Mutations of *LRRK2* have recently been shown to cause autosomal dominant PD previously linked to the PARK8 locus[119,120]. *LRRK2* mutations are estimated to account for 5–6% of cases with a positive family history, and a significant minority of apparently sporadic cases (up to 1.6%)[121]. *LRRK2* encodes a complex multi-domain protein that consists of N-terminal leucine-rich repeats, a GTPase ROC/COR domain, a mitogen-activated protein kinase kinase kinase (MAPKKK) and C-terminal WD40 repeats[120]. At present, little is known about LRRK2 function, however, some interesting preliminary data has begun to emerge from *in vitro* overexpression systems. LRRK2 encodes a kinase and is capable of autophosphorylation. It might be associated with the outer mitochondrial membrane (OMM)[122,123] and can bind parkin[124]. Significantly, three PD-associated mutations, two in the kinase domain (G2019S and I2020T) and one in the ROC/COR GTPase domain (R1441C) increase LRRK2 autophosphorylation, hinting at a dominant gain-of-function mechanism[122,123]. Indeed, overexpression of R1441C, Y1699C or G2019S LRRK2 was sufficient to induce neuronal degeneration in mouse primary cortical neurons[124]. Although these data still require validation *in vivo*, the delineation of the LRRK2 signalling pathway holds great promise for furthering our understanding of the aetiology of the disease.

Pathways of cell death in PD

The discovery of PD genes has raised important questions, including what the principal pathways are and how the genes fit into them. Important clinicopathological and epidemiological observations had already marked out the main pathways before the recent advances in genetics. Pathological studies show that the loss of dopaminergic neurons is ubiquitous in all forms of human parkinsonism. Considerable evidence shows that the mitochondria-dependent apoptotic pathway is predominantly activated[125], although this has been controversial, as there have been many studies that have also failed to confirm the presence of apoptosis in the substantia nigra of patients with PD (for a review, see REF. 126). The identification of Lewy bodies in the brains of people with PD suggested that aggregation was also important in the pathogenesis of dopaminergic cell death. Interestingly, recent pathological insights from families with LRRK2 mutations have revealed distinct pathology with neurofibrillary tangles, which suggests that mutant LRRK2 might have a role in tau phosphorylation and processing[120].

As the apoptotic pathway is substantially conserved in all cells, primary dysfunction in this cascade alone was not thought likely to account for the selective cell loss in patients with PD. Moreover, despite many studies, it was still unclear whether Lewy bodies were toxic or protective for dopamine neurons. Therefore, additional pathways, perhaps upstream of these two pathological endpoints, were thought more important. Epidemiological studies unambiguously demonstrated that ageing is the greatest risk factor in the development of sporadic PD[127]. The molecular mechanisms underlying ageing are complex, but elegant studies in *Drosophila* and *Caenorhabditis elegans*, in which the expression of superoxide dismutase (SOD) was manipulated, as well as the positive effects of caloric restriction in mammals, suggest that oxidative stress is a main cellular pathway in the ageing process[128,129]. Postmortem studies of the brains of patients with sporadic PD reveal increased

WD40 repeats
Short (~40) amino acid motifs that form beta-propeller structures, which are thought to serve as rigid scaffolds for protein interactions. WD40 repeat-containing proteins can therefore coordinate the assembly of multi-protein complexes.

oxidative damage of lipids (peroxidation) and proteins (carbonylation)[127], and the finding of decreased complex I activity in the brains of people with PD suggested that mitochondrial dysfunction might exacerbate the oxidative stress in PD. The remarkably exclusive degeneration of dopaminergic neurons following exposure to mitochondrial neurotoxins (such as MPTP and rotenone) suggests that dysfunction of the mitochondrial pathway could confer the selective vulnerability of dopaminergic neurons in PD, although the mechanism remains obscure[3,10]. One possible mechanism is the pro-oxidant environment in dopaminergic neurons due to the presence of the oxidant neurotransmitter dopamine that is normally packaged in vesicles. Exposure of neurons to increased cytosolic dopamine has many deleterious effects, including increases in oxidative stress as well as promotion and stabilization of potentially toxic α-synuclein protofibrils[15].

Ubiquitin–proteasome system and mitochondria. Although α-synuclein and parkin mutations confirm that protein misfolding and UPS dysfunction are part of a significant upstream pathway *en route* to dopaminergic degeneration, the discovery of *PINK1*, *DJ1* and *OMI/HTRA2* mutations confirmed that mitochondrial dysfunction is another main upstream pathway to parkinsonism. An intriguing question is whether all of the known genes converge to form a common pathogenetic pathway. In view of their differential subcellular localizations, direct interactions seem unlikely. However, they might interact via overlapping pathways, and there is significant evidence for a close relationship between the UPS and mitochondrial function. For example, proteasomal stress can result in increased sensitivity of neurons to MPTP and, conversely, complex I defects can result in decreased proteasome activity[130,131].

The mechanism through which mitochondrial and proteasomal impairment lead to dopamine cell loss is becoming clearer, and the generation of oxidative stress might be common to both and might be allied to apoptotic cell death in PD (FIG. 3). Evidence of increased oxidative damage has been demonstrated following mitochondrial or proteasomal impairment *in vivo*[132,133].

Furthermore, the pathways might be interdependent, which would result in either feedback or feedforward loop mechanisms between the UPS and mitochondria such that dysfunction of one pathway would have inevitable deleterious consequences for the other. As the UPS requires ATP, mitochondrial dysfunction and ATP depletion are likely to lead to UPS dysfunction. Treatment of human neuroblastoma cell lines with the complex I inhibitor rotenone was associated with ~20% ATP depletion, an increase in ROS and oxidized proteins, and a marked reduction in proteasome activity[134]. Inactivation of the proteasome is therefore likely to create a feedforward amplification loop, with further damage from failure to clear the excess oxidized protein species leading to further ROS generation. Detergent-insoluble aggregates of α-synuclein accumulate in cells following mitochondrial inhibition with rotenone or oligomycin, and disappear after subsequent washout of inhibitors paralleling recovery of mitochondrial metabolism[32]. Perhaps the most compelling evidence for this interplay occurring in PD is the demonstration that chronic rotenone exposure in rats results in the formation of Lewy body-like aggregates in addition to parkinsonism[10].

Evidence is also accumulating for a converse mechanism, whereby UPS dysfunction can result in secondary mitochondrial dysfunction and damage. Treatment of cultured primary rat cortical neurons with proteasome inhibitors was sufficient to induce the redistribution of cytochrome *c* into the cytosol, and this was associated with depolarization of the Δψm, which led to activation of caspase 3 and resulted in apoptotic cell death[135]. Of particular relevance to PD, when the expression of A30P mutant α-synuclein was induced in PC12 cells treated with a proteasome inhibitor, cells underwent mitochondrial apoptosis and Δψm depolarization. Both Δψm depolarization and apoptosis were blocked with cyclosporin A, which suggests that proteasome inhibition resulted in Δψm depolarization associated with opening of the mPTP[136] (FIG. 2).

The mechanism by which proteasome inhibition leads to mitochondrial injury and apoptosis remains unclear. Several pro-apoptopic proteins, such as p53 and BCL2 (B-cell leukaemia/lymohoma 2) family members, including BAX, BID and SMAC, are normally degraded by the UPS[137]. Therefore, the UPS has a crucial role in homeostasis by preventing the accumulation of these potentially toxic molecules. Proteasome inhibition leads to the accumulation of these proteins, which result in mPTP opening, Δψm depolarization and apoptosis[137]. In addition, p53 has been shown to transcriptionally activate the expression of several target genes, including *BAX*, *NOXA* (adult T cell leukaemia-derived PMA-responsive) and *PUMA* (BCL2 binding component 3), which can, in turn, cause mitochondrial dysfunction[138]. Further studies are required to determine the significance of p53 alterations in proteasome inhibition, particularly in PD and *in vivo* models of the disease.

The interplay between the UPS and mitochondria might also be mediated in part by the known PD genes. Parkin seems to be crucial for maintenance of mitochondrial function, as parkin-knockout mice develop mitochondrial deficits and oxidative damage, which does not lead to neurodegeneration. Although mitochondrial proteins are not targeted by the UPS, parkin might regulate proteins on the OMM that are accessible to the UPS. Parkin in neuronal PC12 cell lines localized to the OMM, where it protected cells from the damaging effects of ceramides by delaying mitochondrial swelling and cytochrome *c* release[59]. We have also shown in neuroblastoma cells that proteasome inhibition induces Δψm depolarization and apoptosis, and that wild-type PINK1 protein, but not the missense mutant G309D, protected cells from this stress[17]. Similarly, RNAi knockdown of DJ1 also sensitized neurons to proteasomal inhibiton[91].

DJ1 has recently been identified as a regulator of p53 transcriptional activity by binding TOPORS/p53BP3, and might, therefore, represent a molecular bridge between the UPS and the mitochondrial stress

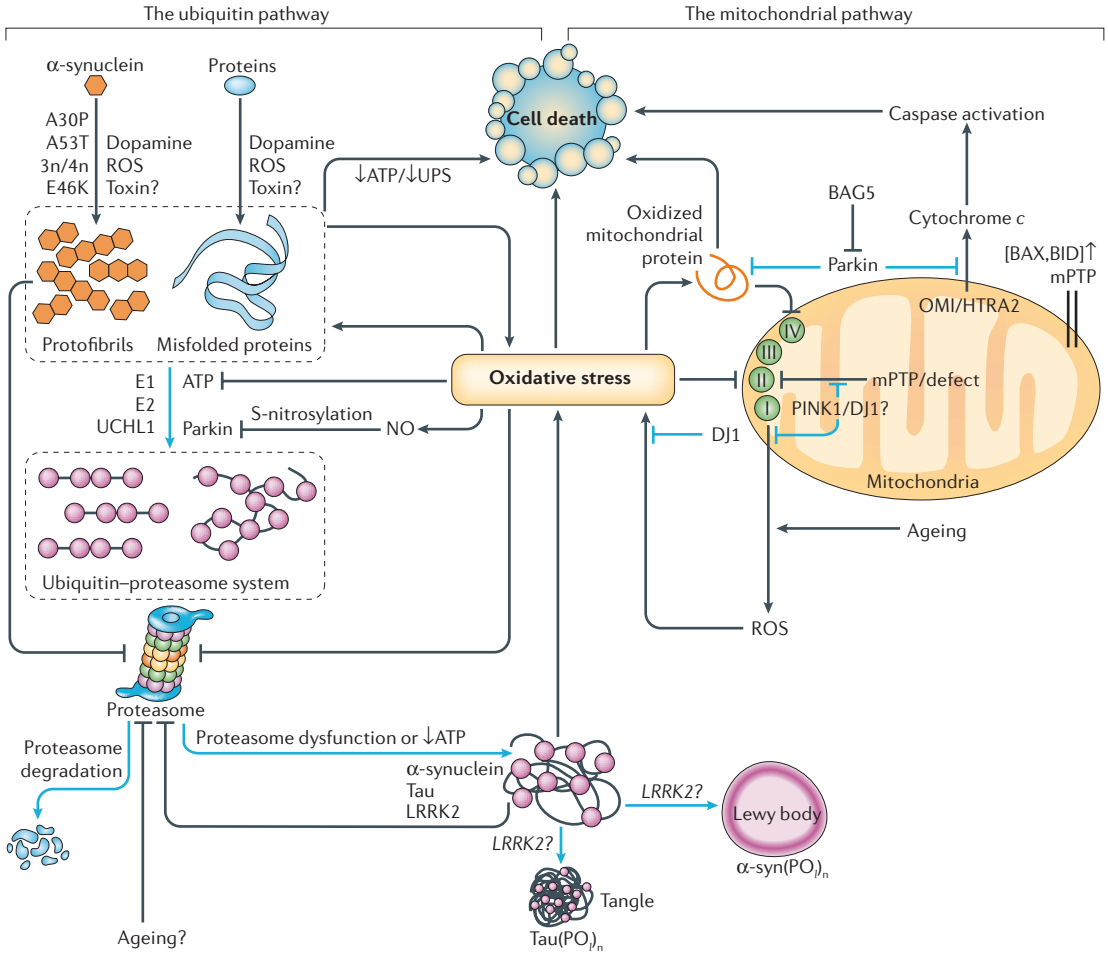

Figure 3 | **Pathways to parkinsonism.** The discovery of Mendelian inherited genes has enhanced our understanding of the pathways that mediate neurodegeneration in Parkinson's disease. One main pathway of cell toxicity arises through α-synuclein, protein misfolding and aggregation. These proteins are ubiquitinated and initially degraded by the ubiquitin–proteasome system (UPS), in which parkin has a crucial role. However, there is accumulation and failure of clearance by the UPS over time, which leads to the formation of fibrillar aggregates and Lewy bodies. α-Synuclein protofibrils can also be directly toxic, leading to the formation of oxidative stress that can further impair the UPS by reducing ATP levels, inhibiting the proteasome, and by oxidatively modifying parkin. This leads to accelerated accumulation of aggregates. Phosphorylation of α-synuclein-containing or tau-containing aggregates might have a role in their pathogenicity and formation, but it is not known whether leucine-rich repeat kinase 2 (LRRK2) mediates this. Another main pathway is the mitochondrial pathway. There is accumulating evidence for impaired oxidative phosphorylation and decreased complex I activity in Parkinson's disease, which leads to reactive oxygen species (ROS) formation and oxidative stress. In parallel, there is loss of the mitochondrial membrane potential. This leads to opening of the mitochondrial permeability transition pore (mPTP), release of cytochrome *c* from the intermembrane space to the cytosol, and activation of mitochondrial-dependent apoptosis resulting in caspase activation and cell death. There is evidence that recessive-inherited genes, such as phosphatase and tensin homologue (PTEN)-induced kinase 1 (PINK1), Parkinson's disease (autosomal recessive, early onset) 7 (DJ1) and HtrA serine peptidase 2 (HTRA2, also known as OMI), might all have neuroprotective effects against the development of mitochondrial dysfunction, although the exact site of their action remains unknown. Parkin has also been shown to inhibit the release of cytochrome *c* following ceramide-induced stress, and is itself modified by the interacting protein BCL2-associated athanogene 5 (BAG5). Dysfunction of both pathways leads to oxidative stress, which causes further dysfunction of these pathways by feedback and feedforward mechanisms, ultimately leading to irreversible cellular damage and death. I–IV, mitochondial electron transport chain complexes I–IV; α-syn$(PO_4)_n$, phospho-α-synuclein; A30P, alanine to proline substitution at α-synuclein amino acid residue 30; A53T, alanine to threonine subsitution at α-synuclein residue 53; E_1, ubiquitin activating enzyme; E_2, ubiquitin conjugating enzyme; E46K, glutamic acid to lysine substitution at α-synuclein residue 46; NO, nitric oxide; 3n/4n, 3 or 4 copies of α-synuclein; Tau$(PO_i)_n$,Tau $(PO_i)_n$, phospho-Tau; UCHL1, ubiquitin carboxyl-terminal esterase L1.

response[95]. Parkin might also be an important bridging protein between these two systems, which are important for ubiquitinating and degrading oxidized mitochondrial proteins, thereby ameliorating oxidative stress. Parkin is itself sensitive to oxidative stress, and is inactivated by nitric oxide-mediated nitrosylation, which could lead to a simultaneous increase in UPS and mitochondrial dysfunction[65,66].

Despite this, parkin's exact role in mitochondria is unknown and, in particular, no mitochondrial substrates have been identified. Interestingly, LRRK2 has been shown to associate with the OMM, and the same research group have also reported that LRRK2 interacts with parkin, although this appears to occur in the cytoplasm[122,124]. Moreover, parkin appeared to increase the amount of ubquitinated LRRK2-containing aggregates in cells[124]. It is still not clear whether parkin might interact with the recently discovered PINK1, DJ1 or OMI/HTRA2 (REFS 16,17,19). Parkin has been localized to the OMM, and overexpressed DJ1 is translocated from the cytosol to the OMM after oxidative stress, which might mediate DJ1's neuroprotective function[88]. Furthermore, parkin has been found to associate with mutant DJ1, which might enhance its stability *in vitro*. The detergent-insoluble levels of DJ1 seem to be differentially regulated by parkin *in vivo*, although parkin does not seem to ubiquitinate DJ1 directly[139]. Recently, endogenous DJ1 was detected in the matrix and intermembranous space of mitochondria, where it is thought it might have a role in the maintenance of mitochondrial function[83]. Deleting DJ1 expression in mammalian systems *in vivo* does not lead to dopaminergic degeneration, which suggests that DJ1 is not crucial for mitochondrial integrity. However, it might protect neurons from exposure to oxidative stress, as DJ1-knockout mice were more sensitive to MPTP neurotoxicity[93]. This suggests that DJ1 acts as a free radical scavenger, which prevents the accumulation of free radicals derived from the mitochondrial electron transport system. This would be consistent with its localization in the intermembranous space[93]. It will be interesting to investigate the effect of ageing on DJ1-knockout mice.

Conclusions

Delineating the mitochondrial pathways involved in PD will require a lot of further work, and identifying the substrates of the known genes will provide an ideal starting point. Although mitochondria might be key to neuronal integrity and survival, defects in disparate areas of mitochondrial signalling are likely to have deleterious effects that converge due to feedback and feedforward mechanisms. It is likely that the main dysfunctional pathway will centre around the response to oxidative stress and its numerous associated facets. These could include the deregulation of the electron transport chain, which can cause a dual effect of reduced ATP production and increased oxidative stress. The reduction of ATP might lead to UPS dysfunction, which, in combination with oxidative stress, is likely to lead to further mitochondrial dysfunction, leading to pathological activation of pathways involved in aggregation and apoptosis (FIG. 3). The initial defects might be mild, but could increase through the actions of feedback mechanisms and the effects of ageing. Furthermore, oxidative stress could affect mitochondrial integrity, which is maintained by the mitochondrial membrane potential and the mPTP. This might be the second main site of regulation after the electron transport chain, and an important molecular link between mitochondria and the UPS. The death of dopaminergic neurons, which results in PD, is almost certainly due to a combination of exogenous stressors (which probably include dopamine itself) and a genetic predisposition (which renders the cells less capable of dealing with the stress). The common endpoint to these cellular insults might converge on the initiation of the mitochondrial apoptotic pathways and release of pro-apoptotic proteins from the intermembranous space, including cytochrome *c* and SMAC, that induce caspase-dependent cell death; and apoptosis inducing factor (AIF) and endonuclease G that translocate to the nucleus and induce caspase-independent nuclear DNA fragmentation[126]. We now have an understanding of the basic molecular framework to the aetiopathogenesis of the disease, further research into the mitochondrial pathways should lead to more clarity for designing effective drugs to treat PD.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

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