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## Parkin and Parkinson's Disease

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### 1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder affecting millions of predominantly elderly individuals worldwide. Originally described as "The Shaking Palsy" in 1817 by the British physician, James Parkinson, the disease is attended by a constellation of motoric deficits including bradykinesia (slowness in movements), postural instability, rigidity and tremor that ultimately result in near total immobility. Although pathological changes are distributed in the PD brain, the principal neuropathology that underlies the characteristic motor phenotype of PD patients is unequivocally the loss of midbrain dopaminergic (DA) neurons in the *substantia nigra pars compacta* (SNpc), which results in a severe depletion of striatal dopamine and thereby an impaired nigrostriatal system that otherwise allows an individual to execute proper, coordinated movements. This specific pattern of neurodegeneration in PD is often accompanied by the presence of eosinophilic intracytoplasmic inclusions known as Lewy bodies (LBs) in surviving neurons in the SN as well as in other affected brain regions such as the dorsal motor nucleus of the vagus, locus ceruleus (LC) and olfactory nuclei (Braak et al., 2003). In advanced stages of the disease, LB pathology can also be found in the limbic structures and neocortex (Braak et al., 2003). Non-motor features arising from these extra-nigral neuronal lesions, including autonomic, sensory and cognitive dysfunctions, present additional sources of considerable consternation and disability for affected individuals (Olanow et al., 2008). Thus, although SN pathology is often regarded as the most important hallmark of PD, the disease is increasingly being recognized as a multi-system disorder affecting not only dopaminergic, but also noradrenergic, cholinergic and serotonergic systems (Alexander, 2004).

Despite intensive research, the etiology of PD remains poorly understood and no current treatments can unequivocally slow or stop the degenerative process. Current therapies for PD are palliative at best and the mainstay is pharmacologic intervention via DA replacement (e.g. through L-DOPA administration). Major drawbacks with current therapies include the inevitable loss of effectiveness and increasing drug-induced side effects as the disease progresses. Invariably, the debilitating nature and morbidity of the disease present significant healthcare, socio-economic and emotional problems. As the world population rapidly ages, these problems undoubtedly would also increase. Notably, a recent study by Dorsey et al projected that the number of PD cases in Europe's five most

and the world's ten most populous countries would increase to 9 million in year 2030 (Dorsey et al., 2007). This is definitely a worrying trend, and one that aptly emphasizes the urgency to develop more effective treatment modalities for the PD patient.

In large part, the difficulty in identifying disease-modifying drugs for PD is due to: i) our current limited understanding of the molecular mechanism(s) that underlies the pathogenesis of PD (the illumination of which would allow the identification and therapeutic exploitation of key molecules involved in the pathogenic process), and ii) the lack of a truly representative mammalian model of PD that recapitulates accurately the progressive process as well as the nature and distribution of the human pathology. However, during the last decade or so, the identification and functional characterization of several genes whose mutations are causative of rare familial forms of PD have provided tremendous insights into the molecular events underlying neurodegeneration in PD. Alongside, several genetic models of PD have also been generated that replicate the disease phenotype to varying degrees of success. Collectively, these studies implicate aberrant mitochondrial and protein homeostasis as key contributors to the development of PD, with oxidative stress likely acting an important nexus between the two pathogenic events.

## 2. Parkin and Parkinson's disease

Mutations in *α-synuclein*, *parkin*, *DJ-1*, *PINK1*, and *LRRK2* are currently recognized to be unequivocally linked to familial parkinsonism (Table 1). Whereas *α-synuclein* and *LRRK2* mutations are causative of dominant parkinsonism, mutations in *parkin*, *DJ-1*, *PINK1* are associated with recessive parkinsonism (Thomas and Beal, 2007).

Locus	Chromosome	Gene	Inheritance	Function
PARK1 & 4	4q21-23	<i>α-synuclein</i> ( <i>SNCA</i> )	Dominant	Unclear (presynaptic protein)
PARK2	6q25.2-27	<i>Parkin</i> ( <i>PRKN</i> )	Recessive	Ubiquitin ligase
PARK3	2p13	<i>Unknown</i>	Dominant	-
PARK5	4p14	<i>UCHL1</i>	Dominant	Ubiquitin hydrolase
PARK6	1p35-36	<i>PINK1</i>	Recessive	Putative serine/threonine kinase
PARK7	1p36	<i>DJ-1</i>	Recessive	Redox sensor
PARK8	12p11.2-q13.1	<i>LRRK2/dardarin</i>	Dominant	Putative serine/threonine kinase
PARK9	1p36	<i>ATP13A2</i>	Recessive	Lysosomal P-type ATPase
PARK10	1p32	<i>Unknown</i>	Unknown	-
PARK11	2q37.1	<i>Unknown</i>	Dominant	-
PARK12	Xq21-q25	<i>Unknown</i>	Unknown	-
PARK13	2p12	<i>Omi/HtrA2</i>	Dominant	Mitochondrial serine protease
PARK14	22q13.1	<i>PLA2G6</i>	Recessive	Phospholipase
PARK15	22q12-q13	<i>FBXO7</i>	Recessive	Component of SCF E3 complex
PARK16	1q32	<i>Unknown</i>	Unknown	-

Table 1. PD-linked genes

Apart from these, mutations in *UCHL1*, *Omi/HtrA2* and *ATP13A2* have also been suggested to cause parkinsonism, but their relevance to PD is currently unclear because the purported disease-causing mutation is either extremely rare in occurrence (e.g. *UCHL1* I93M) (Healy et al., 2004) or present in control population at similar frequencies (e.g. *Omi/HtrA2* G399S) (Simon-Sanchez and Singleton, 2008) or otherwise results in a clinical phenotype that is rather divergent from classical PD (e.g. *ATP13A2*-linked parkinsonism that is characterized by mild parkinsonism and prominent cognitive defects) (Ramirez et al., 2006). More recently, two other genes, *PLA2G6* encoding a phospholipase and *FBXO7* encoding a component of the Skp-Cullin-Fbox (SCF) ubiquitin ligase complex, have also been identified to be associated with recessive parkinsonism (Di Fonzo et al., 2009; Paisan-Ruiz et al., 2009). The function of these various disease-associated genes, especially the unequivocal ones, has been extensively characterized. Interestingly, it turned out that these seemingly functionally disparate PD-linked proteins that have no clear relationship with each other at first glance may all influence either protein or mitochondrial homeostasis, with some, like parkin, being directly involved in both. Accordingly, in the absence of functional parkin, aberrations in both protein and mitochondrial quality control (QC) would ensue that may eventually trigger the demise of susceptible neurons. Indeed, the pivotal role parkin plays in maintaining DA neuronal survival is underscored by our current recognition that parkin dysfunction represents not only a predominant cause of familial parkinsonism but also a formal risk factor for the more common, sporadic form of PD. Here, we shall discuss the role of parkin in PD and why a better understanding of parkin function can help elucidate potential therapeutic strategies for the disease.

## 2.1 Parkin mutations and parkinsonism

Mutations in parkin were originally identified in Japan more than a decade ago to be causative of autosomal recessive juvenile parkinsonism (ARJP) (Kitada et al., 1998). Following this discovery, several ethnically diverse individuals with early-onset PD (age < 45 years) in other parts of the world were also found to carry parkin mutations, which occur at a frequency of about 10-20% and 50% in sporadic and familial early-onset cases respectively (Lucking et al., 2000; Mata et al., 2004; Periquet et al., 2003). Clinically, parkin-associated parkinsonism is often quite indistinguishable from idiopathic PD, with bradykinesia, rigidity and rest tremor being variably combined in both disorders. However, parkin-related patients tend to exhibit slower disease progression and better response to L-DOPA (and also a higher tendency to develop L-DOPA-induced dyskinesia) than those with parkinsonism of different etiologies. Additionally, other less typical features common to parkin cases include prominent dystonia, presence of hyperreflexia and more symmetrical presentation of symptoms, although none of these features are sufficiently specific to be diagnostic of parkin mutations. Limited post-mortem studies of known parkin cases conducted to date demonstrated neuronal loss predominantly in the SN and LC regions of the brain that is accompanied by gliosis. Importantly, the majority of parkin-related cases are devoid of classic LBs (Hayashi et al., 2000; Mori et al., 1998; Pramstaller et al., 2005), suggesting that parkin-associated parkinsonism is pathologically distinct from idiopathic PD, and as such may represent a phenocopy of the latter. However, this is debatable as LBs are found in at least two cases of parkin-related PD (Farrer et al., 2001; Pramstaller et al., 2005). An attractive corollary to this is that functional parkin may facilitate LB formation, which we favor as a working hypothesis (see section 2.2).

Structurally, the 465 amino acid-containing human parkin protein is comprised of a ubiquitin-like (UBL) domain at its N-terminus, a RING1-IBR-RING2 domain at its C-terminus and a unique middle segment that links the two domains (Fig. 1). Similar to other RING finger-containing proteins, parkin functions as an E3 ubiquitin ligase associated with the ubiquitin-proteasome system (UPS). To date, more than 100 different parkin mutations have been described with over 50 of these being missense/nonsense substitutions (Fig. 1). Although most of the missense mutations occur within the RING1-IBR-RING2 catalytic moiety, many of them are also found along the length of the protein outside this region. Further, exonic mutations are also frequently observed with deletion of exon 3 and/or 4 being the most common (Hedrich et al., 2004).

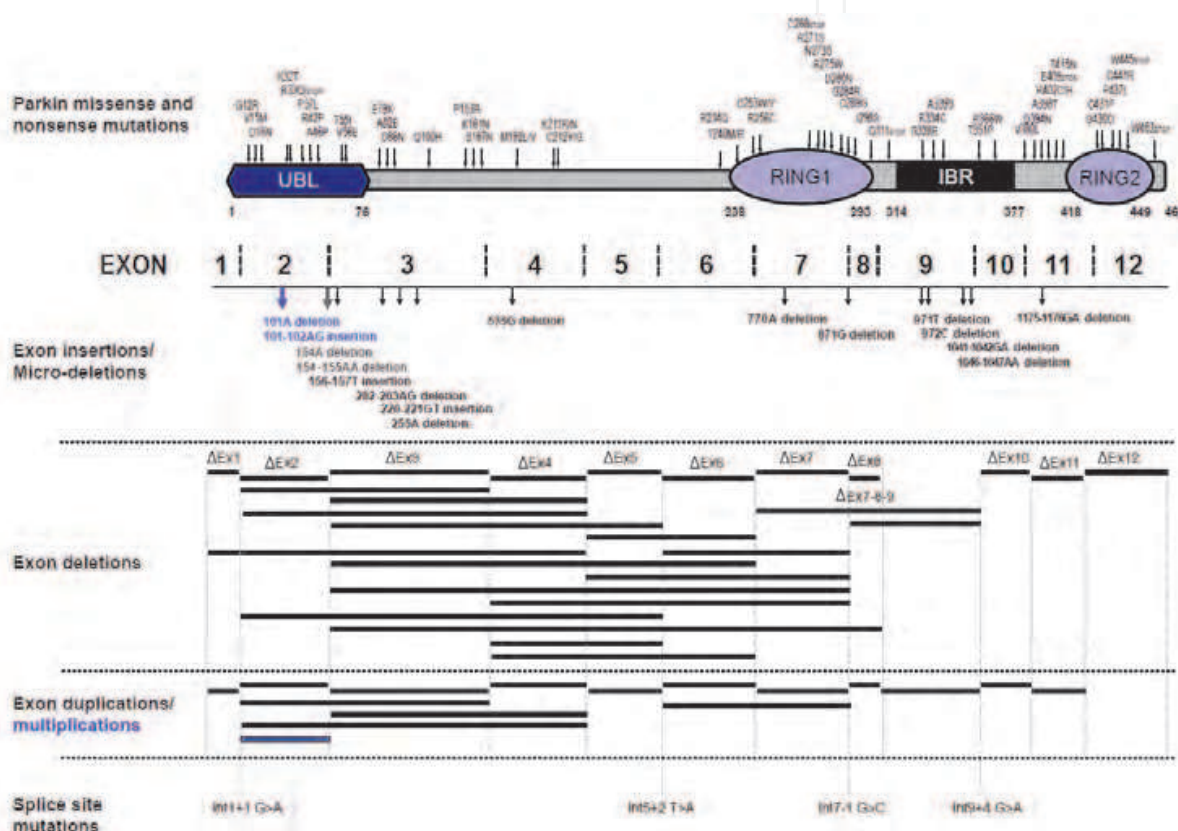


Fig. 1. Schematic depiction of parkin structure and associated disease-linked mutations including missense/nonsense substitutions, insertions, microdeletion as well as exonic multiplications and deletions.

Curiously, despite the heterogeneity, there is no discernable difference in the clinical manifestations among PD patients carrying different parkin mutations. This suggests that substitutions of amino acids resulting from missense mutations are as detrimental to parkin function as are truncation and deletion mutations. Another curious, albeit controversial feature of parkin-related cases is that a single allelic hit might be sufficient to cause disease, which contradicts the widely accepted notion that parkin mutations transmit in a classical autosomal recessive manner (Klein et al., 2007). Supporting this, Hilker and colleagues demonstrated via positron emission tomography that asymptomatic carriers of a single parkin mutation exhibit significantly reduced fluorodopa uptake in the striatal regions compared to control subjects, suggesting preclinical dopaminergic dysfunction (Hilker et al.,

2001). In concordance with this, a subsequent transcranial sonography-based study revealed SN hyperechogenicity in 5 out of 7 asymptomatic carriers of parkin mutations (Walter et al., 2004). Moreover, fMRI analysis of heterozygous parkin carriers demonstrated reorganization of striatocortical motor loops that possibly reflect a compensatory effort to overcome latent nigrostriatal dysfunction (Buhmann et al., 2005). Notwithstanding these evidences, the pathogenicity of single heterozygous parkin mutation is a subject of ongoing debate. An outstanding issue is whether heterozygous parkin mutations may have arisen incidentally. Several studies have sought to address this, but the findings have thus far been inconsistent, i.e. heterozygosity for parkin mutations was reported to be similar between patients and controls in some studies (Kay et al., 2007; Lincoln et al., 2003) but also found to be unique to PD cases in others (Clark et al., 2006b). Another issue is how mutations occurring on a recessive gene can contribute to disease pathogenesis. Given the neuroprotective role of parkin function (discussed in later section), one could envisage that parkin haploinsufficiency could increase the risk of heterozygous parkin mutation carriers for PD. Supporting this, a promoter variant of parkin associated with a lower expression of parkin was found to occur more commonly in PD cases than in controls (West et al., 2002). Alternatively, emerging evidence suggest that certain parkin mutations may also contribute to neurotoxicity directly. The role of single heterozygous parkin mutations thus remains unresolved.

Notwithstanding the controversy surrounding the role of heterozygous parkin mutations, how parkin mutations disrupt the function of the protein was a topic of intense research. Intuitively, one would posit that disease-associated mutations of parkin result in the loss of its enzymatic activity. Although this is true for mutations that occur on parkin's RING2 domain, several groups including ours have demonstrated that the majority of parkin missense mutants outside its RING2 domain retain their catalytic competency (Chung et al., 2001; Hampe et al., 2006; Matsuda et al., 2006; Sriram et al., 2005; Wang et al., 2005). Instead, misfolding of parkin triggered by these mutations appears to be the major mechanism underlying parkin inactivation. Notably, missense parkin mutations frequently alter the protein solubility and concomitantly promote its aggregation into inclusion bodies (Ardley et al., 2003; Cookson et al., 2003; Gu et al., 2003; Hampe et al., 2006; Muqit et al., 2004; Sriram et al., 2005; Wang et al., 2005b). Amongst the aggregation-producing parkin mutations is the R275W substitution, which frequently occurs in the heterozygous state. Alternatively, some mutations of parkin compromise its function by destabilizing the protein and accelerating its degradation via the proteasome (Schlehe et al., 2008).

## **2.2 Parkin-mediated ubiquitination and protein homeostasis**

As mentioned earlier, parkin functions as an E3 ligase associated with the UPS, a major proteolytic machinery that normally identifies and degrades unwanted intracellular proteins. In this system, proteins that are destined for proteasome-mediated degradation are added a chain of ubiquitin via a reaction cascade that involves the ubiquitin-activating (E1), -conjugating (E2) and -ligating (E3) enzymes. Through the sequential and repetitive actions of these enzymes, successive isopeptide linkages are formed between the C-terminal glycine carboxyl group (G76) of the ubiquitin moiety being added and the  $\epsilon$ -amino group of a free lysine (most commonly K48) on the ubiquitin that is attached to the protein. The (G76-K48) polyubiquitinated substrate is then recognized by the 26S proteasome as a target for degradation. It is important to highlight that the ubiquitin sequence contains seven lysine residues (at positions 6, 11, 27, 29, 33, 48 and 63) and that polyubiquitin chain assembly can

occur at any of these lysine residues (Peng et al., 2003). In addition, proteins can also be monoubiquitinated. Notably, both K63-linked polyubiquitination and monoubiquitination of proteins are not typically associated with proteasome-mediated ubiquitination.

Shortly after the discovery of parkin as a PD-linked gene, three independent groups demonstrated that parkin-mediated ubiquitination is linked to protein degradation and that disease-associated parkin mutations compromise its role as an E3 enzyme (Imai et al., 2000; Shimura et al., 2000; Zhang et al., 2000). A logical and popular hypothesis that ensued is that loss of parkin function could lead to a toxic accumulation of one or several of its substrates, thereby leading to neurodegeneration. This had fuelled intense effort by many laboratories around the world to identify the culprit substrate(s) involved. To date, no less than 25 substrates (or putative substrates) of parkin has been reported since the identification of CDCrel1 as the first putative substrate for the ubiquitin ligase (Zhang et al., 2000) (Table 2). However, none of the parkin substrates identified thus far is exclusively expressed in DA neurons, which raises the question on why DA neurons in familial parkinsonism cases linked to parkin mutations are selectively vulnerable to deficient parkin function. Further, few from the laundry list fulfil an important criterion expected of an "authentic" parkin substrate: accumulation in the brains of ARJP patients and parkin-deficient models (Table 2). Although the pace of substrate identification has slowed down considerably in recent years, new parkin substrates continue to emerge periodically. Amongst the most recently isolated parkin substrates is the zinc finger-containing protein called PARIS (ZNF746) (Shin et al., 2011), a major transcriptional repressor of PGC-1 $\alpha$  expression, which in turn regulates the transcription of many genes involved in cellular metabolism. Importantly, unlike most previously identified parkin substrates, PARIS accumulates in post-mortem brain tissues derived from ARJP and sporadic PD patients, as well as in the ventral midbrain region of mice that is conditionally ablated of parkin expression (Shin et al., 2011). Moreover, stereotactic injection of viral vector encoding PARIS into the SN of mice results in a selective loss of TH-positive DA neurons that can be rescued by either parkin or PGC-1 $\alpha$  co-expression (Shin et al., 2011). Together, these results suggest that PARIS is an "authentic" parkin substrate and that its upregulation may underlie neurodegeneration due to parkin inactivation. Notwithstanding this and the fact that a few other substrates have similarly been reported to fulfil the criterion of a parkin substrate (Table 2), what constitutes an "authentic" substrate for parkin is really debatable, as discussed below.

Although protein ubiquitination is classically associated with proteasome-mediated degradation, the existence of non-classic ubiquitin modifications such as K63-linked polyubiquitination would caution against the fixation on the traditional view that substrates of a ubiquitin ligase must exhibit an accelerated, proteasome-dependent turnover in the presence of the enzyme. This is particularly relevant to parkin, which we and others have demonstrated to be a multifunctional enzyme capable of mediating alternative ubiquitin topologies such as monoubiquitination and K63-linked polyubiquitination - modifications that are typically uncoupled from the proteasome and often considered as "non-proteolytic" (Doss-Pepe et al., 2005; Hampe et al., 2006; Lim et al., 2005; Matsuda et al., 2006) (Fig. 2). Our results would argue that the catalytic function of parkin is not limited to targeting substrate for degradation by the proteasome. Thus, the lack of accumulation of an identified parkin substrate in the brains of ARJP patients and parkin-deficient models does not necessarily mean that it is less than an "authentic" substrate. For example, we have previously reported that parkin-mediated polyubiquitination of synphilin-1 (an interactor of  $\alpha$ -synuclein) normally occurs via K63-linked chains, which does not appear to affect its steady-state turnover (Lim et al., 2005). Not surprisingly, the level of synphilin-1 is neither appreciably altered in ARJP brains nor in brain

tissues derived from parkin null mice (Ko et al., 2005). A corollary to this is that proteasome-independent pathways may also be relevant to parkin-related neurodegeneration.

Substrates	Ub Type	Elevated in			Reference
		KO mice	ARJP Brain	PD brain	
CDCrel-1	-	Yes/No	Yes/No	-	(Ko et al., 2005)
CDCrel-2a	-	-	Yes	-	(Choi et al., 2003)
Synaptotagmin XI	-	Yes/No	-	-	(Periquet et al., 2005)
Synphilin-1	K63	No	No	-	(Chung et al., 2001; Ko et al., 2005; Lim et al., 2005)
P38/AIMP2	Multiple Mono	Yes	Yes	Yes	(Corti et al., 2003; Hampe et al., 2006; Ko et al., 2005; Periquet et al., 2005)
FBP1	-	Yes	Yes	Yes	(Ko et al., 2006)
PARIS (ZNF746)	K48	Yes	Yes	Yes	(Shin et al., 2011)
Cyclin E	-	No	Yes/No	Yes	(Ko et al., 2005; Staropoli et al., 2003)
PDCP2-1	-	-	Yes	Yes	(Fukae et al., 2009)
Pael-R	-	No	Yes/No	-	(Ko et al., 2005)
$\alpha/\beta$ tubulin	-	Yes/No	No	-	(Ko et al., 2005; Ren et al., 2003)
Hsp70	Multiple Mono	No	No	Yes	(Moore et al., 2008)
PICK1	Mono	No	-	-	(Joch et al., 2007)
VDAC1	K27, Mono	Yes	-	-	(Geisler et al., 2010; Narendra et al.; Periquet et al., 2005)
Ataxin-2	-	Yes	-	-	(Huynh et al., 2007)
O-glycosylated $\alpha$ -synuclein	-	-	Yes	-	(Shimura et al., 2001)
RanBP2	-	-	-	-	(Um et al., 2006)
Lim Kinase 1	-	-	-	-	(Lim et al., 2007)
Eps15	Mono	-	-	-	(Fallon et al., 2006)
DJ-1 L166P	K63	-	-	-	(Olzmann et al., 2007)
Bcl-2	Mono	-	-	-	(Chen et al., 2010)
Drp1	K48	-	-	-	(Wang et al., 2011)
Mitofusin	-	-	-	-	(Poole et al., 2010; Ziviani et al., 2010)
Dopamine Transporter	-	-	-	-	(Jiang et al., 2004)
Phospholipase C $\gamma$ 1	-	Yes	-	-	(Dehvari et al., 2009)
Ataxin3 polyQ79	-	-	-	-	(Tsai et al., 2003)

Table 2. List of parkin substrates/putative substrates



By virtue of its apparent dissociation from the proteasome, we have originally proposed that parkin-mediated K63-linked ubiquitination may be involved in cargo diversion during proteasomal stress and accordingly, in the biogenesis of inclusion bodies associated with neurodegenerative diseases (Lim et al., 2006). Our proposal is consistent with the concept of aggresomes, which are juxtannuclear inclusion bodies formed in the presence of proteasomal stress and that have been suggested to act as staging grounds for the disposal of protein aggregates via the autophagic route (Kopito, 2000). Supporting our hypothesis, we found that parkin-mediated K63 polyubiquitination of synphilin-1 promotes its aggregation into aggresome-like inclusion bodies (Lim et al., 2005). Corroborating our findings, Olzmann et al demonstrated that parkin-mediated K63 polyubiquitination of misfolded DJ-1 couples the protein to the dynein motor complex via the histone deacetylase 6 (HDAC6) adaptor, thereby promoting its sequestration into aggresomes (Olzmann et al., 2007). Importantly, our recent work identified K63-linked polyubiquitin as a novel cargo selection signal for macroautophagy-mediated clearance of aggresomes (Tan et al., 2008a; Tan et al., 2008b). By being capable of mediating both proteasome-associated K48-polyubiquitination and macroautophagy-associated K63-linked polyubiquitination, parkin may potentially act as an important triage between the two major cellular degradation systems. This multifunctionality of parkin may in part help explain its apparent broad neuroprotective properties, as the flexibility of ubiquitin linkage usage presumably would allow the enzyme to adapt rapidly to changes in cellular environment.

### 2.3 Parkin-mediated ubiquitination and mitochondrial homeostasis

One of the first hints that parkin may play a role in mitochondrial homeostasis aside from its role as a regulator of protein turnover came from a study in fruit flies. Greene and colleagues analyzed adult *Drosophila parkin* null mutant and observed that the most prominent pathology is not in the brain but in the flight musculature of these mutant flies, which is plagued by muscle degeneration and pronounced mitochondrial lesions (Greene et al., 2003). Interestingly, *PINK1* null flies were subsequently found to phenocopy their *parkin*-deficient counterparts and importantly, *parkin* over expression in *PINK1*<sup>-/-</sup> flies is able to rescue all the mutant phenotypes tested, although the reverse, does not happen (Clark et al., 2006a; Park et al., 2006), suggesting that parkin acts in the same pathway but downstream of PINK1. We now know from several follow-up studies in flies and other model systems that the parkin/PINK1 pathway is a key regulator of mitochondrial dynamics, although it is currently controversial whether the pathway promotes mitochondrial fission or fusion [For a recent review, see (Burbulla et al., 2010)].

In an exciting development, Narendra and colleagues from Youle laboratory have recently demonstrated that parkin plays an essential role in removing damaged mitochondria from the cell via a specialized form of autophagy known as mitophagy (Narendra et al., 2008). This has fuelled widespread interest amongst many researchers directed at elucidating the mechanism underlying parkin-mediated mitophagy, which is reminiscent of the initial excitement shared by many to uncover novel parkin substrates. A model (Fig. 2) that has emerged from a flurry of follow-up studies proposed that a key initial event that occurs upon mitochondrial depolarization is the selective accumulation of PINK1 in the outer membrane of the damaged organelle (presumably after its segregation by fission). Notably, PINK1 accumulation in healthy mitochondria is prevented by a proteolytic event that rapidly cleaves the protein (Narendra et al., 2010; Vives-Bauza et al., 2010). Upon recruitment to depolarized mitochondria by PINK1, parkin then becomes activated

(Matsuda et al., 2010) and promotes the ubiquitination and subsequent p97 AAA-ATPase-regulated degradation of mitofusins (Poole et al., 2010; Ziviani et al., 2010), the elimination of which will prevent unintended fusion events involving damaged mitochondria and thereby their re-entry into undamaged mitochondrial network from occurring (Tanaka et al., 2010). The event is then followed by the induction of mitophagy, which is also parkin-dependent. Parkin-mediated K63 ubiquitination is apparently important here, as mitochondrial substrates that are modified by K63 polyubiquitin can presumably help in the recruitment of autophagy receptors such as HDAC6 and p62 to the damaged organelle. Notably, both HDAC6 and p62 are ubiquitin-binding proteins that preferentially recognize K63 ubiquitin chains (Lim and Lim, 2010; Olzmann et al., 2007; Tan et al., 2008a). The end result is the formation of “mito-aggresomes” and their subsequent clearance by lysosomes. The whole process thus resembles the formation and autophagic clearance of aggresomes.

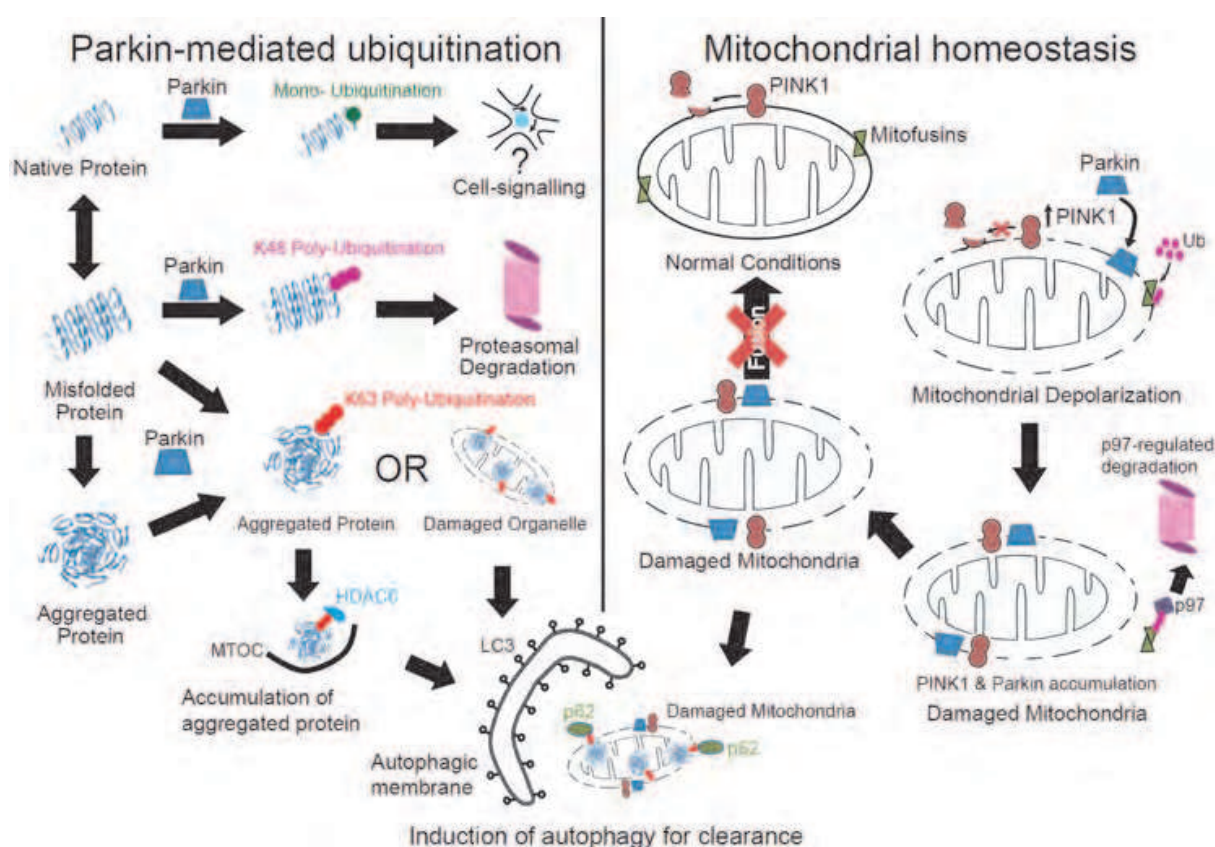


Fig. 2. Role of parkin in protein and mitochondrial QC. (Left) Parkin is a unique E3 enzyme capable of mediating various types of ubiquitin modification on its substrates that would result in different outcomes. (Right) A model of parkin/PINK1-mediated mitophagy.

Despite the logic and experimental support for the events surrounding parkin-mediated mitophagy as described above, the model is not without controversy. Because the majority of studies related to parkin-mediated mitophagy were carried out in cells grown in glucose-containing medium, a potential confounding factor is that these cells generate most of their ATP via glycolysis from glucose present in the medium, and not via oxidative phosphorylation. Notably, mitophagy is blocked in yeast that are made obligatorily dependent on mitochondrial metabolism, even under severe starvation conditions (Kanki and Klionsky, 2008). According to a recent study by Van Laar and colleagues, parkin-

mediated mitophagy does not seem to take place appreciably in primary neurons treated with mitochondrial uncouplers such as CCCP (Van Laar et al., 2011). Unlike other cell types, neurons are strictly dependent on mitochondrial respiration. In HeLa cells (grown in glucose medium) where parkin-mediated mitophagy was originally observed, the phenomenon is similarly attenuated when these cells were forced into dependence on mitochondrial respiration (i.e. cultured in glucose-free medium) (Van Laar et al., 2011). Thus, parkin-mediated mitophagy appears to be dependent on the bioenergetic status of the cell. Presumably, additional mechanisms are involved in the regulation of mitophagy in neurons and other cell types that derive their energy from oxidative phosphorylation. Notwithstanding this, it is important to highlight that at least two groups have found that parkin accumulation on mitochondria does occur in primary neurons treated with CCCP (Narendra et al., 2008; Vives-Bauza et al., 2010). The reason for this discrepancy is presently unclear, but the conundrum obviously needs to be resolved as whether parkin-mediated mitophagy takes place appreciably in neurons has significant implications for its role in PD pathogenesis.

Interestingly, parkin is also involved in mitochondrial biogenesis. Kuroda and colleagues previously demonstrated that parkin over-expression enhances the transcription and replication of mitochondrial DNA by associating with mitochondrial transcription factor A (TFAM) and promoting TFAM-mediated transcription (Kuroda et al., 2006). Accordingly, the event is attenuated by parkin expression silencing with siRNA. However, an important caveat is that parkin-induced mitochondrial biogenesis only occurs in proliferating but not differentiated cells (Kuroda et al., 2006). Hence, its relevance to post-mitotic cells like neurons is unclear. From the recent work by Shin and colleagues, we now know that parkin can also potentially regulate mitochondrial biogenesis by regulating PGC-1 $\alpha$  expression indirectly through its ability to down-regulate PARIS (which represses PGC-1 $\alpha$  expression) (Shin et al., 2011). PGC-1 $\alpha$  regulates a variety of genes that play important roles in mitochondrial biogenesis. Amongst these PGC-1 $\alpha$ -regulated genes is nuclear respiratory factor-1 (NRF-1), whose activity contributes to the expression of respiratory subunits and mitochondrial transcription factors (Scarpulla, 2008). Notably, both PGC-1 $\alpha$  and NRF-1 expression are reduced in the SN of PD brains (Shin et al., 2011), suggesting that impaired renewal of neuronal mitochondria may contribute to DA neurodegeneration in parkin-related cases. Based on these results, it is also logical to speculate that parkin might assist in the replenishment of exited mitochondria following extensive mitophagy. Thus, parkin appears to be involved in the entire spectrum of mitochondrial dynamics, i.e. from biogenesis to clearance.

#### **2.4 Parkin is a potent neuroprotectant**

Given the critical role of parkin in regulating protein and mitochondrial homeostasis, it is perhaps unsurprising to note that parkin is capable of protecting neurons against a wide variety of insults, including those mediated by  $\alpha$ -synuclein or mutant LRRK2, as well as those generated by dopamine quinones, neurotoxins or metallic ions (Feany and Pallanck, 2003; Winklhofer, 2007). Yet, it remains rather amazing at the same time to note that a single ubiquitin ligase could do all that. Moreover, several studies have also demonstrated that parkin can afford protection against neurotoxicity elicited by agents that are not directly related to PD. For example, parkin is apparently capable of mitigating cytotoxicity induced by  $\beta$ -amyloid or by expanded polyglutamine-containing proteins through facilitating their

elimination from the cell (Rosen et al., 2010; Tsai et al., 2003). While the removal of aberrant proteins or damaged mitochondria from cells undergoing various forms of stress may be common denominators underlying the broad neuroprotective capacity of parkin, recent studies also implicated a more direct role for parkin in neuroprotective signalling. Indeed, parkin-mediated ubiquitination is important for the activation of major cellular pro-survival pathways such as the NF- $\kappa$ B pathway (Henn et al., 2007). Henn and colleagues demonstrated in cells treated with kainate (an excitotoxin) or rotenone (a mitochondrial complex I inhibitor) that parkin-mediated protection against cell death is dependent on NF- $\kappa$ B activity (Henn et al., 2007). Interestingly, the authors further showed that the activation of NF- $\kappa$ B pathway by parkin is associated with its K63 polyubiquitination activity (Henn et al., 2007). Notably, several studies have also reported the ability of parkin to suppress stress-related mitogen-activated protein kinase (MAPK) signalling (e.g. JNK and p38) (Cha et al., 2005; Ren et al., 2009), although the effect may be an indirect consequence of reduced oxidative stress level in the presence of parkin.

Besides its catalytic role, parkin can also promote neuroprotection in an ubiquitination-independent manner. For example, Ren and colleagues have demonstrated that parkin does not require its ligase activity to stabilize microtubules and that this activity-independent function of parkin is important in the protection of DA neurons against microtubule-depolymerizing toxins (Ren et al., 2009). In a separate development, a provocative report suggested that parkin possesses ubiquitination-independent transcriptional activity, which in this case acts as a repressor of the tumor suppressor p53 expression. The repression of p53 expression by parkin ultimately leads to decreased Bax transcription, caspase 3 activity and apoptosis (da Costa et al., 2009). Although the subject of parkin's nuclear residency is currently still controversial, it is noteworthy that parkin localization to the nucleus has been independently observed by at least one other group (Kao, 2009a). Kao showed that parkin translocates to the nucleus following DNA damage (Kao, 2009a), apparently to assist in DNA repair (Kao, 2009b). Since parkin lacks a defined nucleus localization signal, precisely how the translocation of parkin occurs under different conditions remains to be elucidated. Notwithstanding the gaps in our current knowledge regarding the mechanisms underlying parkin-mediated protection, it is clear that parkin functions as a broad-spectrum neuroprotectant. Conceivably, parkin gene delivery could offer a novel avenue of PD therapy. Indeed, virus-mediated delivery of parkin has been shown to prevent DA neurodegeneration in rats either overexpressing  $\alpha$ -synuclein (Lo Bianco et al., 2004; Yamada et al., 2005) or treated with parkinsonian neurotoxins (Paterna et al., 2007), or in a non-human primate model of  $\alpha$ -synuclein overexpression (Yasuda et al., 2007). Another way to harness parkin's protective function is to look for molecules that can functionally compensate for loss of parkin function. Notably, Tain and colleagues have recently demonstrated that pharmacological activation of *4E-BP* (*Thor*) expression in parkin null flies by rapamycin can suppress all their pathologies (Tain et al., 2009), suggesting that rapamycin can somehow mimic parkin neuroprotection in the absence of the ubiquitin ligase.

## 2.5 Mammalian models of parkin-related parkinsonism

Given that parkin-related parkinsonism transmits in a largely recessive fashion and that parkin function is clearly important for neuronal homeostasis, one would intuitively be optimistic about generating a representative model of parkin-related parkinsonism through

the ablation of endogenous parkin in animals. Although *Drosophila parkin* null mutants do exhibit prominent parkinsonian features (including DA neuronal loss and locomotive defects) that progress with age, germline-derived *parkin* knockout mice generally fail to exhibit robust signs of parkinsonism (Dawson et al., 2010; Perez and Palmiter, 2005). On the other hand, biochemical and proteomic analyses of brain samples prepared from these mice revealed deficits in dopamine handling, reduced mitochondrial respiration and antioxidant capacity, as well as increased ROS-mediated damage, all of which are consistent with the implicated cellular roles of parkin (Goldberg et al., 2003; Itier et al., 2003; Palacino et al., 2004). Conceivably, other proteins like PINK1 and DJ-1 that have overlapping functions with parkin may help to mitigate the overt manifestation of phenotypes in *parkin* null mice. However, it is noteworthy that even *parkin/PINK1/DJ-1* triple knockout mice do not exhibit any evidence of nigral degeneration or dramatic pathology, at as late as 24 months of age (Kitada et al., 2009).

Why conventional *parkin* knockout mice (and for that matter the majority of genetic mouse models of PD generated via traditional means) consistently failed to produce significant nigral cell loss and associated phenotypes remains a puzzle, although the controlled environment that these animals frequently lived in may deny them the entry of unknown disease-promoting factor(s) that might be present in the environment at large. Notably, even individuals with overt parkin mutations typically require a few decades for the disease to surface, suggesting pathogenic interaction between genetic mutations and age-associated or other factors. Alternatively, compensatory mechanisms that kicked in during development may influence the onset of disease symptoms in humans and mouse alike. Supporting this, virus-mediated delivery of PD-linked genes into the SN of adult rodents via stereotactic injections, which effectively bypass developmental compensation, usually results in marked DA neurodegeneration (Ulusoy et al., 2008). As mentioned earlier, such an approach was taken by Shin and colleagues, who generated a conditional *parkin* knockout mice by which exon 7 of the *parkin* gene flanked by loxP sites was removed from the SN of adult mice following stereotactic injection of a Cre recombinase-containing lentiviral vector (Shin et al., 2011). This elegant approach leads to a dramatic loss of parkin expression from the ventral midbrain of injected mice that is accompanied by an age-dependent loss of DA neurons, although the motor parameters of these mice were not reported. Thus, loss of parkin function in adult mice does promote DA neuronal loss, albeit in a conditional-dependent manner. At the same time, the study also provided indirect evidence supporting that the pathogenicity arising from the loss of parkin function can be developmentally compensated (at least in mice). Such compensatory mechanisms may conceivably operate in humans and may account for the age-dependency and the heterogeneity of age of onset of PD. At the least, we now have a model of parkin-linked recessive parkinsonism that exhibits substantial and progressive nigral cell loss.

A different approach was taken by Lu and colleagues in modelling parkin dysfunction in mice. In view of the possibility that single parkin mutations may also contribute to the disease, the group developed a BAC (bacterial artificial chromosome) transgenic mouse model expressing a C-terminal truncated human mutant parkin (Parkin-Q311X) in DA neurons under the direction of a dopamine transporter promoter (Lu et al., 2009). Expression of Parkin-Q311X, an aggregation-prone mutant, in mice produces age-dependent loss of SN DA neurons that is accompanied by progressive hypokinetic motor deficits. These results suggest that certain parkin mutants may exert direct neurotoxicity and support the

role of heterozygous parkin mutations in disease pathogenesis. Notably, previous studies conducted in the *Drosophila* by the same group as well as by our group also arrived at the same suggestion (Sang et al., 2007; Wang et al., 2007). Indeed, transgenic flies expressing human parkin mutants such as R275W and Q311X display age-dependent, selective loss of DA neurons that is accompanied by progressive locomotion deficits (Sang et al., 2007; Wang et al., 2007).

## 2.6 Relevance of parkin to sporadic PD

That parkin mutations transmit in a recessive manner would suggest that the loss of parkin function predisposes DA neurons to degeneration. An important corollary to this is that any post-translational event that promotes the loss of parkin function could potentially mimic the effects brought about by overt mutations and be just as detrimental. Further, in view of the suggested contribution of parkin haploinsufficiency to disease risk, it is reasonable to assume that the down-regulation of normal parkin function mediated by such events need not result in the total abolition of its enzymatic activity to elicit a pathogenic effect.

We and others have found that a wide variety of PD-linked stressors, including those that produce oxidative and nitrosative stress, induce parkin solubility alterations and thereby its aggregation in a manner analogous to that brought about by several of its missense mutations (LaVoie et al., 2005; Wang et al., 2005; Winklhofer et al., 2003). Remarkably, dopamine also modifies parkin in a similar fashion (LaVoie et al., 2005; Wang et al., 2005). Furthermore, parkin appears to be uniquely susceptible to dopamine-induced modifications compared to several other related E3 members such as HHARI, Cbl and CHIP (LaVoie et al., 2007; LaVoie et al., 2005; Wong et al., 2007). Accordingly, detergent-insoluble parkin, but not HHARI, Cbl and CHIP, accumulates in the PD brain (LaVoie et al., 2005; Wang et al., 2005; Wong et al., 2007). As optimal level of soluble parkin is important for neuronal survival, the immobilization of the parkin within aggregates would not only deprive the protein of its function but also effectively reduce the pool of functional parkin available to the cell. Like haploinsufficiency, the biochemical depletion of soluble parkin levels is expected to increase the vulnerability of susceptible neurons to degeneration. Interestingly, normal parkin in the brain also becomes progressively more detergent-insoluble with aging (Pawlyk et al., 2003), which may provide an explanation to why age represents a risk factor for PD.

Besides stress-induced modifications, parkin phosphorylation is another post-translational modification that is linked to its inactivation. Notably, serine phosphorylation of parkin by casein kinase 1 (CK1) or cyclin-dependent kinase 5 (Cdk5) reportedly down-regulates its activity (Avraham et al., 2007; Yamamoto et al., 2005), and compound phosphorylation of parkin by both kinases further leads to its aggregation (Rubio de la Torre et al., 2009). While the physiological role of parkin phosphorylation remains unclear, it is conceivable that aberrant CK1 or Cdk5 activity can promote loss of parkin function and play a role in sporadic PD pathogenesis. Supporting this, parkin phosphorylation is elevated in distinct regions of sporadic PD brains and correlates with increased levels of p25, the activator of CDK5 (Rubio de la Torre et al., 2009). More recently, Ko and colleagues have demonstrated that tyrosine phosphorylation of parkin at residue 143 (Y143) by the src family kinase member, c-Abl, similarly inactivates its enzyme activity and compromises its protective function both *in vitro* and *in vivo* (Ko et al., 2010). They further showed that parkin is tyrosine-phosphorylated at Y143 in human post-mortem PD brains and that this modification of parkin is accompanied by the accumulation of parkin substrates such as

AIMP2. Together, the results by Ko and colleagues suggest a pathophysiologic regulation of parkin by c-Abl in sporadic PD (Ko et al., 2010).

Other than the above-mentioned post-translational modifications, a number of parkin interactors have also been demonstrated to be capable of inactivating its E3 ligase activity. For example, the bcl-2-associated athanogene 5 (BAG5) associates with and inhibits parkin activity (Kalia et al., 2004). Further, BAG5 promotes the sequestration of parkin within protein aggregates and compromises its neuroprotective function. Accordingly, targeted expression of BAG5 in the SNpc of mice enhances DA neurodegeneration following axotomy-induced DA neuronal injury or MPTP administration (Kalia et al., 2004). Although the authors suggested that BAG5 may serve as a useful therapeutic target for PD, the mechanism by which BAG5 inhibits parkin E3 activity remains unclear to this date. It is also curious why DA neuronal injury would induce BAG5 expression, an event that inhibits rather than stimulates the neuroprotective activity of parkin, which invariably will worsen the condition. Notably, besides BAG5, several other parkin interactors have also been identified as modulators of its catalytic activity. These include 14-3-3 $\eta$ , a chaperone-like protein, and Nrdp1, a RING-finger containing ubiquitin ligase (Sato et al., 2006; Zhong et al., 2005). Whereas 14-3-3 $\eta$  interacts with and reduces parkin activity directly (Sato et al., 2006), Nrdp1 promotes parkin degradation and depletes the availability of parkin to the cell (Zhong et al., 2005). Both events if unregulated would obviously be detrimental to parkin's protective function.

Taken together, it is apparent that loss of parkin function is not limited to those induced by disease-causing mutations, but also includes several biochemical and protein-protein modifications that can either alter the catalytic function of the E3 ligase directly, or indirectly through promoting its aggregation or degradation. Collectively, these mutation-independent modifications that inactivate parkin activity provide a mechanism for parkin dysfunction that is relevant to the pathogenesis of sporadic PD.

### 3. Conclusion

It is evident from the above discussion that parkin plays a key role in maintaining DA neuronal homeostasis and that its dysfunction is relevant to not just familial parkinsonism but also to sporadic PD. Parkin thus represents a neuroprotective PD-linked gene whose function can be and arguably should be exploited. Accordingly, a better understanding of parkin-mediated neuroprotective pathways would offer opportunities to uncover molecular targets that may be of therapeutic value to both familial and sporadic PD. The *Drosophila* model, with its powerful genetics as well as its amenability to drug screening, will be particularly useful here. Alternatively, given that the loss of parkin function can apparently be developmentally compensated (at least in mice), the identification and validation of components of the compensatory network will represent another approach to elucidate rational therapeutic strategies. Indeed, from a therapeutics point of view, it may be equally (if not more) beneficial to understand how parkin-related cases as well as PD in general are kept in check for decades before disease onset than to map the precise pathogenic events that occur after disease onset.

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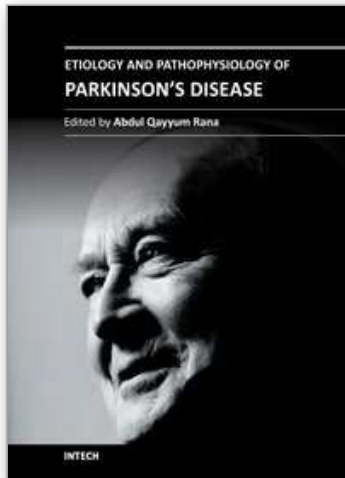
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## **Etiology and Pathophysiology of Parkinson's Disease**

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This book about Parkinson's disease provides a detailed account of etiology and pathophysiology of Parkinson's disease, a complicated neurological condition. Environmental and genetic factors involved in the causation of Parkinson's disease have been discussed in detail. This book can be used by basic scientists as well as researchers. Neuroscience fellows and life science readers can also obtain sufficient information. Beside genetic factors, other pathophysiological aspects of Parkinson's disease have been discussed in detail. Up to date information about the changes in various neurotransmitters, inflammatory responses, oxidative pathways and biomarkers has been described at length. Each section has been written by one or more faculty members of well known academic institutions. Thus, this book brings forth both clinical and basic science aspects of Parkinson's disease.

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