

## Understanding the Role of Parkin in Parkinson's Disease

Lim Kah Leong *BSc (Hons), PhD*,<sup>1,2</sup> Jeanne MM Tan *BSc (Hons), PhD*<sup>1</sup>

<sup>1</sup> Neurodegeneration Research Laboratory, National Neuroscience Institute

<sup>2</sup> Department of Biological Sciences, National University of Singapore

### ABSTRACT

The recent linkage of genetic mutations in the parkin gene to familial Parkinson's disease (PD) and the seminal discovery that parkin functions as an ubiquitin ligase associated with the ubiquitin-proteasome system have been instrumental in shaping our understanding of PD pathogenesis. Currently, mutations in parkin are recognised as the most common cause of familial PD. Emerging evidence also suggests a role for parkin in dopaminergic neuronal dysfunction as well as Lewy body biogenesis in the more common sporadic PD. The relevance of parkin in PD is thus compelling. In this review, we highlight several significant discoveries made recently regarding parkin function that support its role in both familial and idiopathic PD. We will also discuss the phenotypes of a number of mouse models of parkin function that have been generated based on the targeted ablation of the parkin gene.

*Keywords:* Lewy body, PARK 2, proteasome, ubiquitin ligase

### INTRODUCTION

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. Its occurrence crosses geographic, racial and social boundaries, affecting mainly the elderly population above the age of 65.<sup>1</sup> Currently, the prevalence of PD is highest among Caucasians in North America and Europe (0.98 to 0.194%) and lowest among blacks in Africa (0.01%).<sup>2</sup> In Singapore, the prevalence of PD has been found to be comparable to that of Western countries.<sup>3</sup>

Clinically, most PD patients present with a motoric disorder and typically exhibit one or more cardinal symptoms of PD that include resting tremor, rigidity, bradykinesia and postural instability. The underlying neuropathology giving rise to this constellation of

motor deficits is the relatively selective loss of midbrain dopaminergic neurons in the substantia nigra pars compacta (SNpc). This specific pattern of neuronal degeneration is often accompanied by eosinophilic intracytoplasmic inclusions known as Lewy bodies (LBs) that are present in surviving neurons in the substantia nigra as well as the locus coeruleus and other brain areas.<sup>4</sup> LBs are thought to be a pathognomonic feature of PD. Hitherto, there are no proven neuroprotective or neurorestorative therapies for the PD patient.<sup>5</sup> Current treatment options for PD patients focus mainly on providing symptomatic relief and as such, do not halt disease progression.

Although the pathogenesis of PD has been the subject of intensive research, the aetiology of the nigral neuronal loss in PD remains poorly understood. Favoured hypotheses for sporadic PD include combinations of genetic propensity and environmental exposures leading to intracellular oxidative stress. Supporting this hypothesis are findings from human postmortem tissue and animal models indicating the participation of reactive oxygen species and mitochondrion complex 1 dysfunction in the pathogenesis of sporadic PD.<sup>6,7</sup>

### Abbreviations

PD, Parkinson's disease; SNpc, substantia nigra pars compacta; LC, locus coeruleus; NE, norepinephrine; ARJP, autosomal recessive, juvenile onset PD; LB, Lewy body; UCHL1, ubiquitin C-terminal hydrolase; PINK1, PTEN-induced kinase; *PACRG*, parkin co-regulated gene; *huParkin*, human parkin gene; *fuparkin*, Fugu parkin gene; UPS, ubiquitin-proteasome system; UBC, ubiquitin conjugating enzyme; RING, Really interesting new gene.

Table 1. Loci and genes linked to familial Parkinson's disease.

Locus	Gene	Chromosome	Mode of Inheritance
PARK1	<i>α-synuclein</i>	4q21-q23	Autosomal Dominant
PARK2	<i>Parkin</i>	6q25.2-q27	Autosomal Recessive
PARK3	Unknown	2p13	Autosomal Dominant
PARK4	<i>α-synuclein</i>	4q	Autosomal Dominant
PARK5	<i>UCHL1</i>	4p14	Autosomal Dominant
PARK6	<i>PINK1</i>	1p35-p36	Autosomal Recessive
PARK7	<i>DJ-1</i>	1p36	Autosomal Recessive
PARK8	Unknown	12p11q13.1	Autosomal Dominant
PARK9	Unknown	1p36	Autosomal Recessive
PARK10	Unknown	1p32	Late-onset Susceptibility Gene

Table 2. Clinical features of ARJP\*.

Age of Onset	Typically Before 40, Ranging from 7-58
Common Signs and Symptoms	Rigidity Tremor Bradykinesia Postural Instability Good Response to L-DOPA L-DOPA Induced Dyskinesia
Other Signs and Symptoms	Hyperflexia Sleep Benefit Impassive Facial Expression Painful Dystonia
CSF Biochemistry	Frontal Atrophy
PET	Decreased [ <sup>18</sup> F]-DOPA Uptake in Putamen and Caudate Decreased Striatal [ <sup>11</sup> C]-raclopride Dopamine D2 Receptor Binding

\*Adapted from Zhang *et al*, 2001<sup>48</sup>

A major advance in the understanding of PD comes from recent studies of rare monogenically inherited forms of the disease. To date, at least 10 distinct gene loci (PARK 1-PARK 10) have been linked to familial forms of PD (Table 1). Of these, 5 candidate genes have been mapped. These familial PD-linked genes include *α-synuclein*, *parkin*, *ubiquitin C-terminal hydrolase L1* (*UCHL1*), *DJ-1* and a newly identified gene known as *PINK1*.<sup>8,9,10</sup> Mutations in *α-synuclein* and *UCHL1* are linked to autosomal dominant familial PD while mutations in *parkin*, *DJ-1* and *PINK1* cause autosomal recessive PD. The functional characterisation of these genes has provided tremendous insights into the molecular mechanisms of neurodegeneration in PD. In particular, the seminal discovery of *parkin* function as a ubiquitin ligase associated with intracellular protein homeostasis has been instrumental in shaping our understanding of PD pathogenesis.<sup>11-13</sup>

Currently, mutations in *parkin* are recognised as the most common cause of familial PD.<sup>14,15</sup> The recent association of *parkin* haploinsufficiency with idiopathic PD further implicates a role for *parkin* in the more common form of PD. This observation aligns with increasing evidence suggesting that the expression level of *parkin* is crucial for neuronal functioning.<sup>16</sup> Indeed, *parkin* appears to serve as a multipurpose neuroprotective agent, a role that has obvious implication in the development of PD therapeutics.<sup>17</sup> The relevance of *parkin* in PD research is thus compelling.

In this review, we highlight recent significant discoveries made regarding *parkin* function and dysfunction that have led to advances in our understanding of the role of *parkin* in PD. We will also discuss briefly the various challenges confronting researchers and clinicians in the PD field and speculate

on some possible solutions to circumvent these problems.

### MUTATIONS IN PARKIN CAUSE AUTOSOMAL RECESSIVE PD

Mutations in the parkin gene are causal of autosomal recessive, juvenile onset PD (ARJP).<sup>18</sup> This form of inherited parkinsonism was originally described in Japan and is characterised pathologically by a severe loss of nigral dopaminergic neurons and the absence of classic LBs. Clinically, ARJP occurs at an early age (typically before 40 years of age) and is usually associated with dystonia and diurnal fluctuations as well as early and severe L-DOPA-induced dyskinesia and sleep benefit (Table 2).

Following the discovery of parkin mutations in Japanese ARJP patients, several families with recessively inherited PD throughout the world were also found to carry parkin mutations.<sup>14,15</sup> This is in stark contrast to the restricted occurrence of  $\alpha$ -synuclein, UCHL1 and DJ-1 mutations.<sup>9</sup> Indeed, mutations in parkin are currently considered to be the main contributor to familial PD. Familial PD-linked mutations in parkin are heterogeneous, comprising various exonic deletion, duplication and triplication as well as several missense/nonsense mutations (Fig. 1). This heterogeneity in parkin mutations may be, in part, responsible for the wide age of disease onset in ARJP, which ranges from 7 to 58 years.

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 the truncation and deletion mutations. Although PD due to parkin mutations is classically transmitted in an autosomal recessive inheritance, the existence of patients with single mutations raises the question of a possible pseudo-dominant effect of the mutations, or alternatively, an expanded risk associated with *parkin* haploinsufficiency.<sup>16,19</sup>

Heterozygous loss of parkin function has been demonstrated to influence the rate of dopaminergic cell death in patients. In asymptomatic carriers of a single parkin mutation with a normal allele, significantly reduced fluorodopa uptake in the striatal regions of these carriers compared to control subjects were observed, suggesting preclinical dopaminergic dysfunction.<sup>20</sup> Parkin expression variability could thus be a risk factor in the development of PD, and its relationship to idiopathic PD is further supported by

the recent association of parkin gene promoter with late-onset PD.<sup>21</sup>

### PARKIN GENE ORGANISATION AND REGULATION

The human parkin gene (*huParkin*) locus, spanning about 1.4 Mb, is one of the largest in the human genome.<sup>22,23</sup> It contains 12 exons and codes for a 4.5 kb transcript. Despite its huge size, the coding sequences of *huParkin* occupy only 0.1% of the gene length. Thus, *huParkin* appears to have one of the highest ratio of non-coding to coding DNA lengths in the human genome. The role of the large introns which account for about 99.9% of *huParkin* is not known.

Interestingly, the promoter region of *huParkin* is very short. Its 5' flanking gene, *PACRG*, resides just 204 bp upstream on the opposite strand.<sup>24</sup> Given the close head-to-head linkages of the 2 genes, it is not surprising that their promoters overlap. It is possible that the two genes share some regulatory elements. Despite its key role in familial PD, very little is known about the transcriptional regulation of *huParkin*. However, it is apparent that many *cis*-acting elements controlling the parkin gene expression exist.

Besides expressing in varying amounts in different tissues, parkin transcriptional level could also be modulated by several conditions and agents. For example, parkin mRNA and protein expression levels in cell culture models increase several-folds during cellular unfolded protein stress.<sup>12</sup> In rat striatum, the mRNA level of parkin also increases following acute and chronic administration of haloperidol, a dopamine-D2 receptor antagonist, but decreases following the administration of a neurotoxic dose of methamphetamine.<sup>25,26</sup> Given the apparent association of parkin expression variability and PD, it would be important to elucidate the regulatory elements on *huParkin* that govern its expression.

Although the core promoter region of *huParkin* has recently been identified, its structure did not reveal significant insights into the complexity of parkin transcriptional regulation.<sup>22,23</sup> Further, since *PACRG* resides just 204bp upstream from the start codon of *huParkin*, the resulting short intergenic interval between the two genes is likely to pose some structural constraints. In contrast, the situation is quite different with the very large size of the introns in *huParkin*, which averages 100kb each. As it is not uncommon for regulatory elements to be located in the introns, it is conceivable that additional *huParkin* regulatory regions may potentially be located within its introns.

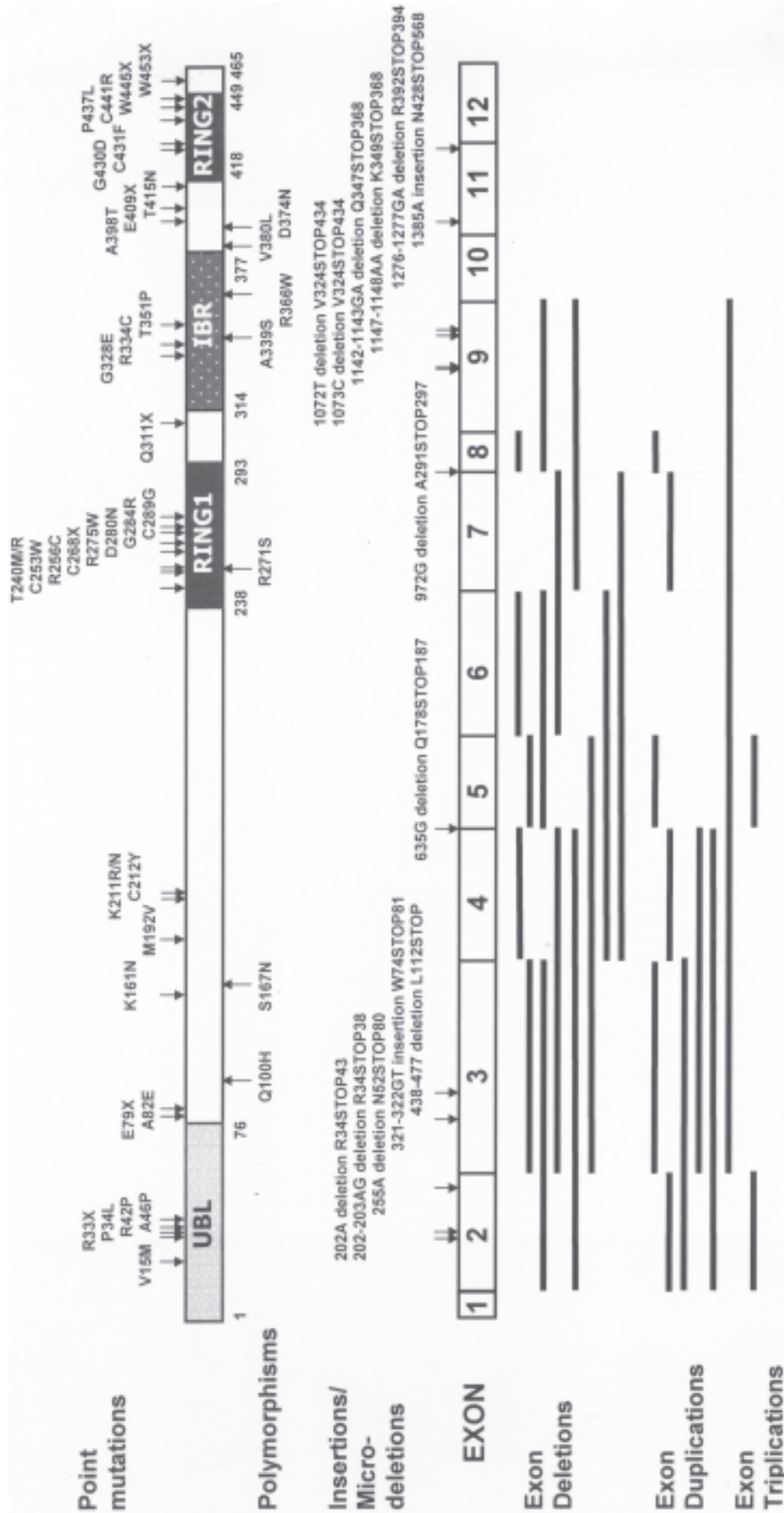


Fig. 1. Parkin structure and mutations. Schematic depiction of the modular architecture of the parkin protein (*top panel*) and its corresponding exon structure (*bottom panel*). Various heterogeneous parkin mutations published to date are indicated.

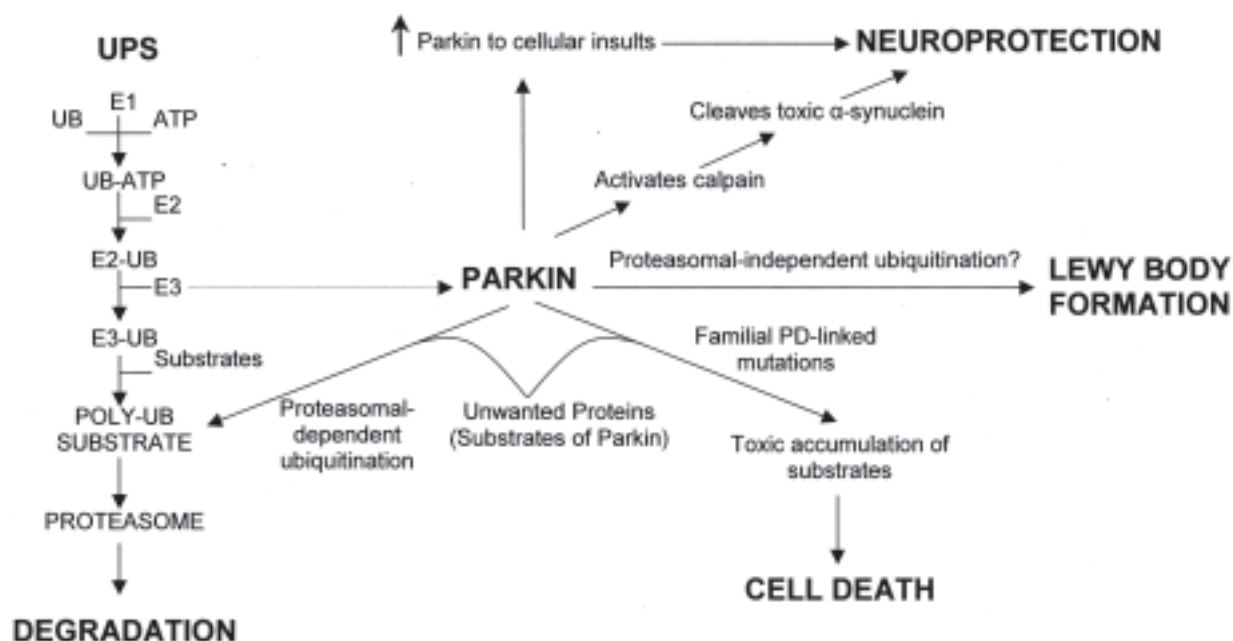


Fig. 2. Model of the UPS and parkin function. Free ubiquitin (UB) is activated in an ATP-dependent manner by the activity of a ubiquitin-activating enzyme (E1) before being transferred to a targeted protein substrate via the sequential actions of distinct ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3). The process is repeated many times to allow the formation of a polyubiquitin chain (POLY-UB) on the substrate, which acts as a targeting signal for proteasomal degradation. Parkin functions as an E3 ligase. Proteasomal-dependent ubiquitination mediated by parkin helps remove unwanted proteins, whose toxic accumulation as a result of parkin mutations would lead to cell death. Parkin also subserves other cellular functions like neuroprotection and Lewy body biogenesis, which may take place via non-classical routes for an E3 ligase.

However, to experimentally verify the existence of such elements in any of *huParkin*'s gigantic introns would be a laborious and daunting task. One solution to circumvent this problem is to perform comparative functional genomics of parkin in a vertebrate whose genome is more compact and amenable to such studies. For this purpose, we have isolated and characterised the pufferfish (*Fugu*) ortholog of *huParkin*. Interestingly, we found that the size of the *Fugu* parkin gene (*fuparkin*) is dramatically compressed by about 400-fold compared to its human ortholog, which is beyond the 8- to 10-fold compression we initially expected.<sup>27</sup> We envisaged that the dramatically compact *fuparkin*, which is amenable to *in vitro* manipulation, would offer an attractive alternative to uncover functionally relevant regulatory elements.

### PARKIN AND THE UBIQUITIN-PROTEASOME SYSTEM

Structurally, the parkin protein has a modular organisation consisting of an ubiquitin-like (UBL) domain at its N-terminus, a RING box domain at its C-terminus and a unique middle segment that links the two domains (Fig. 1). The RING box, containing two RING finger motifs (termed RING 1 and RING

2) flanking a Cys-rich in-between RING (IBR), is important for the enzymatic activity of parkin.<sup>11-13</sup> Notably, many clinically relevant point mutations on parkin occur on the RING box region (Fig. 1).

Parkin functions as a ubiquitin ligase associated with the ubiquitin-proteasome system (UPS), a cellular machinery that plays an important role in maintaining intracellular homeostasis through the clearance of unwanted proteins (Fig. 2). In this system, proteins destined for degradation are covalently tagged with ubiquitin, a 76 amino acid residue protein, by the sequential actions of ubiquitin-activating (E1), -conjugating (E2) and ligating (E3) enzymes.<sup>28</sup> The ligation process is usually repeated many times to form a polyubiquitin chain in which the C-terminus glycine residue of each ubiquitin unit is linked to a specific lysine (K) residue (most commonly K-48) of the previous ubiquitin (Fig. 2). Polyubiquitin chain formation is important in identifying the target molecules for degradation by the proteasome. Substrates ubiquitinated by parkin would hence be removed via this system. The function of parkin as an E3 ligase thus indicates that defects in ubiquitination is a crucial mechanism behind parkin mutations leading to PD.

Table 3. Putative Parkin substrates and their functions.

Substrate	Function
CDCrel-1 ( <u>C</u> ell <u>D</u> ivision <u>C</u> ontrol <u>R</u> elated protein 1)	A septin-GTPase required for the completion of cytokinesis. Predominantly expressed in the nervous system and is associated with synaptic vesicles.
CDCrel-2A	CDCrel-1 homolog
Pael-R ( <u>P</u> arkin-associated <u>e</u> ndothelin-receptor-like <u>R</u> eceptor)	Novel G-protein coupled receptor that tends to be misfolded when overexpressed, resulting in the unfolded protein stress.
Glycosylated $\alpha$ -synuclein	Unknown
Synphilin-1	An $\alpha$ -synuclein interacting protein that associates with synaptic vesicles.
Cyclin E	Cell cycle-related protein; accumulation results in neuronal apoptosis.
$\alpha/\beta$ Tubulin	Main constituent of microtubules which cooperates with other cytoskeletal proteins to maintain cell architecture.
p38 aminoacyl-tRNA	Essential for the structure of aminoacyl-tRNA synthetase which catalyse the ligation of cognate amino acids to tRNAs for protein biosynthesis.
Synaptotagmin XI	An integral synaptic vesicle membrane protein that is highly expressed in the brain; speculated to be involved in vesicular trafficking and exocytosis.

Consistent with this suggestion, several reports have shown that pathological mutations of parkin disrupt parkin binding either to its cognate E2s (UBCH7 and UBCH8) or to its substrates, resulting in a failure of parkin to ubiquitinate its target substrates.<sup>11-13,29</sup> It is therefore conceivable that a loss of parkin function would result in a toxic accumulation of its substrates, eventually killing the cell (Fig. 2). Supporting this speculation are the observations that numerous reported substrates of parkin accumulate in brains of PD patients carrying parkin mutations.<sup>30-32</sup> To date, at least 9 putative parkin substrates have been described (Table 3). However, since none of the parkin substrates are found exclusively in the substantia nigra, it remains unclear why the nigral dopaminergic neurons are preferentially susceptible to degeneration when parkin is mutated. Nonetheless, the direct involvement of parkin in the ubiquitination of proteins supports the current popular hypothesis that the UPS plays a key role in the pathogenesis of PD.

### **PARKIN AS A MULTIPURPOSE NEUROPROTECTIVE AGENT**

Several recent reports have implicated neuroprotective roles of parkin in response to diverse cellular insults including  $\alpha$ -synuclein toxicity, proteasomal dysfunction, unfolded protein stress, kainate-induced excitotoxicity and ceramide-induced mitochondrion apoptosis.<sup>17,33</sup> Additionally, parkin may also promote the clearance of expanded polyglutamine proteins that are characteristic of the triplet repeat disorders.<sup>34</sup> Taking all these findings together, it thus seems reasonable to assume that the catalytic activity of parkin is required for the proper maintenance of

dopaminergic neuronal function. It remains a challenge, however, to acknowledge that defects in a single ubiquitin ligase could result in a specific and devastating pathological outcome.

Given the large number of ubiquitin ligases within the cell that perform similar roles, one would tend to think that the ubiquitination machinery has some degree of redundancy. Indeed, for synphilin-1 alone (a parkin substrate), there are at least two other E3 ligases besides parkin that are known to promote its degradation through ubiquitination.<sup>29,35,36</sup> The mechanism by which parkin protects against toxicity mediated by mutant forms of  $\alpha$ -synuclein is also not clear, since  $\alpha$ -synuclein is not a target of parkin-mediated ubiquitination except when it becomes modified in rare circumstances.<sup>29,30,37</sup> Further, the neuroprotective effect of parkin in this case does not involve a decrease in  $\alpha$ -synuclein levels, but nonetheless requires its functional activity.<sup>37</sup> Thus, parkin appears to be capable of protecting cells against a protein it does not degrade.

This is seemingly paradoxical, and begs the speculation that parkin may subserve other cellular functions that are proteasomal-independent. Aligning with this speculation is a recent report indicating that the overexpression of parkin in embryonic hippocampal cells induces the activation of calpain, an intracellular cysteine protease that cleaves  $\alpha$ -synuclein.<sup>38</sup> The cytoprotective effect of parkin on  $\alpha$ -synuclein-mediated toxicity in this case is significantly inhibited by calpain-specific inhibitors but not by proteasomal inhibitors.<sup>38</sup> Although the exact mechanism by which parkin overexpression leads to the activation of calpain remains to be established, the findings opened the

possibility that parkin may have functions that are non-classical for a ubiquitin ligase.

### PARKIN AND LEWY BODY FORMATION

A very intriguing feature of parkin-proven PD cases is the apparent lack of LBs reported in a number of, albeit limited, detailed pathological examinations.<sup>15</sup> As LBs are thought to represent sites within which toxic or damaged proteins are sequestered by the cell in an inactive form, the lack of LBs may promote neurotoxicity and account for the earlier age of onset in PD patients carrying parkin mutations.

A popular speculation that ensues is that functional parkin is required for the formation of LBs. The identification of synphilin-1, a LB component, as a parkin substrate, together with the demonstration that parkin mediates ubiquitination of proteins within LB-like inclusions formed by the ectopic expression of  $\alpha$ -synuclein and synphilin-1, align with this speculation.<sup>29</sup> However, although the results implicating the involvement of parkin-mediated ubiquitination in LB formation parallel the consistent findings of ubiquitinated inclusions in neurodegenerative lesions, how proteins within inclusion bodies escaped proteasomal degradation despite being heavily ubiquitinated remains a conundrum.

A logical explanation is that parkin-mediated LB formation takes place in the presence of an impaired proteasome. Supporting this, several reports have demonstrated the accumulation of various proteins, including parkin, in aggresomes in response to proteasome inhibition.<sup>39,40</sup> Further, structural and functional defects of the proteasome with accumulation and aggregation of potentially cytotoxic abnormal proteins have also been identified in the SNpc of patients with sporadic PD.<sup>41</sup>

Alternatively, we wondered if the formation of protein inclusions could be a result of proteasomal-independent ubiquitination. Mechanistically, this idea is attractive to us as it would also explain how protein aggregates are seemingly stabilised within such inclusions. In the case of parkin-mediated LB formation, this would imply that parkin must be able to catalyse this unique form of ubiquitination, in addition to its established proteasomal-dependent ubiquitination activity. Indeed, our preliminary results suggest that parkin function may be associated with

proteasomal-independent ubiquitination and this unique form of post-translational modification could enhance the formation of protein inclusions mediated by the co-expression of synphilin-1 and  $\alpha$ -synuclein (Lim KL and Dawson TM, unpublished observations\*). Potentially, proteasomal-independent ubiquitination could contribute to LB biogenesis.

### PARKIN AND AGEING

It is widely accepted that age is the unequivocal risk factor in the development of neurological disorders, including PD. For a multipurpose neuroprotectant like parkin, it is conceivable that its activity could be modified in an age-dependent manner, thereby promoting the chance of neuronal cell death in response to external insults.

Using sequential extraction of varying strength, our collaborators have shown that the extractibility of parkin in the frontal cortex of 3 young human cases differed from parkin in the frontal cortex of the elderly human cases in that significant amounts of parkin were recovered from the high salt fraction of young cases but not in old cases.<sup>42</sup> In aged human brains, parkin is predominantly found in the SDS-extractable fraction, suggesting an altered solubility.<sup>42</sup> This shift in extractibility in parkin with ageing indicates an age-dependent modification of parkin or other molecules that interact with parkin. Presumably, the alteration in the biochemical property of parkin by age would deplete the pool of functional parkin needed by the cells to counteract against external insults and concomitantly increases its susceptibility to apoptosis.

In a manner highly reminiscent of the age-dependent modification of parkin, we recently found that several familial-PD linked point mutations on parkin produce the same alteration in the protein extractibility (Wang C *et al*, manuscript in preparation\*). Taken together, our results suggest that the alteration in the solubility of parkin may underlie the molecular basis of the loss of parkin function caused by mutations or by age.

### ANIMAL MODELS OF PARKIN FUNCTION

Most disease-causing mutations of parkin are thought to be loss of function-mutations, leading to the failure of parkin substrates to be ubiquitinated and degraded by the proteasome, and ultimately in dopaminergic neuronal degeneration. To test this hypothesis, a number of parkin null mice have been generated via the targeted ablation of selected parkin exons. Two groups described parkin exon 3-deleted mice, which have modest deficits in dopaminergic and glutamatergic

\* The text-stated unpublished observations as well as the manuscript in preparation by Wang C *et al* are now respectively published in the *Journal of Neuroscience* (Feb 23, 2005) and the *Journal of Neurochemistry* (in press).

neurotransmission, as well as subtle behavioural abnormalities.<sup>43,44</sup> However, no significant neuronal loss or pathology was observed with the exon 3-deleted mice.<sup>43,44</sup>

In contrast, a recent report by the Dawson's lab demonstrated that the targeted deletion of parkin exon 7 leads to a significant loss of catecholaminergic neurons in the locus coeruleus (LC) and an accompanying loss of norepinephrine (NE) in discrete brain regions.<sup>45</sup> Moreover, the exon 7-deleted parkin null mice show a marked impairment of the NE-dependent startle response.<sup>45</sup> The reason for the surprising discrepancy between the phenotype of the exon 3-deleted parkin mice and the exon 7-deleted mice is unclear.

A likely explanation is the presence of enzymatically active splice variants of parkin in exon 3-deleted, but not exon 7-deleted, mice. Indeed, exon 3-5 of the parkin gene are normally spliced out in human peripheral leukocyte.<sup>46</sup> Further, alternative splicing events are well documented to be the cause of hypomorphs in animals whose genes were thought to be completely knocked out.<sup>47</sup> Nonetheless, it is interesting that the primary phenotype of the exon 7-deleted parkin null mice is the derangement of the central noradrenergic system, and not the dopaminergic system in the SNpc. However, patients with parkin mutations also have loss of LC neurons, and neuronal loss in the LC is often more pronounced in patients with sporadic PD. Hence, it is conceivable that the degeneration of LC neurons occurs earlier than SNpc neurons, a phenomenon that is recapitulated by the exon 7-deleted parkin null mice. Interestingly, in all the three parkin null models described, none of the reported parkin substrates examined show an increase in their endogenous levels.

## CONCLUSION

Although the elucidation of parkin function has provided tremendous insights into the molecular mechanisms underlying dopaminergic cell death and LB formation in PD, it is important to recognise that neurodegeneration in idiopathic PD is probably a result of a cascade of events rather than a single pathogenic event. Nevertheless, the association of parkin mutations with PD positions the UPS prominently in the limelight as a key felon in PD pathogenesis. Currently, UPS disruption, together with oxidative stress, are thought to represent major focal points in the circle of events leading to nigral cell death.

Since a tapestry of events appear to be responsible in PD pathogenesis, therapies aimed at curing this debilitating disease may require a cocktail concoction targeted at major problem centres in the pathogenic cascade. Although stem cell-based neurorestorative strategies provide attractive therapeutic options for the degenerating brain, there is a pertinent need to know more about the characteristics of stem cells and also the safety of using these pluripotent cells as molecular medicine before we could see their widespread applications. Given the multi-neuroprotective roles of functional parkin, it may be worthwhile to explore its utility in providing some intervention against the progression of the disease in PD patients.

## ACKNOWLEDGEMENTS

This work was supported by grants from the National Medical Research Council (NMRC 0776/2003) and the National Healthcare/SingHealth Group (NHG RPR/03019).

## REFERENCES

1. Siderowf A, Stern M. Update on Parkinson disease. *Ann Intern Med* 2003; 138:651-8.
2. Zhang ZX, Roman GC. Worldwide occurrence of Parkinson's disease: an updated review. *Neuroepidemiology* 1993; 12:195-208.
3. Tan LC, Venketasubramanian N, Hong CY, Sahadevan S, Chin JJ, Krishnamoorthy ES, et al. Prevalence of Parkinson disease in Singapore: Chinese vs Malays vs Indians. *Neurology* 2004; 62:1999-2004.
4. Forno LS. Neuropathology of Parkinson's disease. *J Neuropathol Exp Neurol* 1996; 55:259-72.
5. Dawson TM, Dawson VL. Neuroprotective and neurorestorative strategies for Parkinson's disease. *Nat Neurosci* 2002; 5 Suppl:1058-61.
6. Zhang Y, Dawson VL, Dawson TM. Oxidative stress and genetics in the pathogenesis of Parkinson's disease. *Neurobiol Dis* 2000; 7:240-50.
7. Jenner P. Oxidative stress in Parkinson's disease. *Ann Neurol* 2003; 53:S26-38.
8. Lim KL, Lim TM. Molecular mechanisms of neurodegeneration in Parkinson's disease: clues from Mendelian syndromes. *IUBMB Life* 2003; 55:315-22.
9. Lim KL, Dawson VL, Dawson TM. The genetics of Parkinson's disease. *Curr Neurol Neurosci Rep* 2002; 2:439-46.
10. Valente EM, Sleiman PM, Caputo V, Muqit MMK, Harvey K, Gispert S. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 2004; 304:1158-60.
11. Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc Natl Acad Sci USA* 2000; 97:13354-9.
12. Imai Y, Soda M, Takahashi R. Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. *J Biol Chem* 2000; 275:35661-4.

13. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet* 2000; 25:302-5.
14. Lucking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetics Study Group. *N Engl J Med* 2000; 342:1560-7.
15. Mata IF, Lockhart PJ, Farrer MJ. Parkin genetics: one model for Parkinson's disease. *Hum Mol Genet* 2004; 13 Spec No 1:R127-33.
16. West A, Periquet M, Lincoln S, Lucking CB, Nicholl D, Bonifati V, et al. Complex relationship between Parkin mutations and Parkinson disease. *Am J Med Genet* 2002; 114:584-91.
17. Feany MB, Pallanck LJ. Parkin. A multipurpose neuroprotective agent? *Neuron* 2003; 38:13-6.
18. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998; 392:605-8.
19. Foroud T, Uniacke SK, Liu L, Pankratz N, Rudolph A, Halter C, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. *Neurology* 2003; 60:796-801.
20. Hilker R, Klein C, Ghaemi M, Kis B, Strotmann T, Ozelius LJ, et al. Positron emission tomographic analysis of the nigrostriatal dopaminergic system in familial parkinsonism associated with mutations in the parkin gene. *Ann Neurol* 2001; 49:367-76.
21. West AB, Maraganore D, Crook J, Lesnick T, Lockhart PJ, Wilkes KM, et al. Functional association of the parkin gene promoter with idiopathic Parkinson's disease. *Hum Mol Genet* 2002; 11:2787-92.
22. West A, Farrer M, Petrucelli L, Cookson M, Lockhart P, Hardy J. Identification and characterization of the human parkin gene promoter. *J Neurochem* 2001; 78:1146-52.
23. Asakawa S, Tsunematsu K, Takayanagi A, Sasaki T, Shimizu A, Shintani A, et al. The genomic structure and promoter region of the human parkin gene. *Biochem Biophys Res Commun* 2001; 286:863-8.
24. West AB, Lockhart PJ, O'Farrell C, Farrer MJ. Identification of a novel gene linked to parkin via a bi-directional promoter. *J Mol Biol* 2003; 326:11-9.
25. Nakahara T, Gotoh L, Motomura K, Kawanami N, Ohta E, Hirano M, et al. Acute and chronic haloperidol treatments increase parkin mRNA levels in the rat brain. *Neurosci Lett* 2001; 306:93-6.
26. Nakahara T, Kuroki T, Ohta E, Kajihata T, Yamada H, Yamanaka M, et al. Effect of the neurotoxic dose of methamphetamine on gene expression of parkin and Pael-receptors in rat striatum. *Parkinsonism Relat Disord* 2003; 9:213-9.
27. Yu WP, Tan MM, Chew CMK, Oh T, Kolatkar P, Venkatesh B, et al. The 350-fold compacted Fugu parkin gene is structurally and functionally similar to human Parkin. *Gene* in press.
28. Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001; 70:503-33.
29. Chung KK, Zhang Y, Lim KL, Tanaka Y, Huang H, Gao J, et al. Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease. *Nat Med* 2001; 7:1144-50.
30. Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, et al. Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* 2001; 293:263-9.
31. Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, Takahashi R. An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. *Cell* 2001; 105:891-902.
32. Choi P, Snyder H, Petrucelli L, Theisler C, Chong M, Zhang Y, et al. SEPT5\_v2 is a parkin-binding protein. *Brain Res Mol Brain Res* 2003; 117:179-89.
33. Cookson MR. Neurodegeneration: how does parkin prevent Parkinson's disease? *Curr Biol* 2003; 13:R522-4.
34. Tsai YC, Fishman PS, Thakor NV, Oyler GA. Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. *J Biol Chem* 2003; 278:22044-55.
35. Liani E, Eyal A, Avraham E, Shemer R, Szargel R, Berg D, et al. Ubiquitylation of synphilin-1 and alpha-synuclein by SIAH and its presence in cellular inclusions and Lewy bodies imply a role in Parkinson's disease. *Proc Natl Acad Sci USA* 2004; 101:5500-5.
36. Ito T, Niwa J, Hishikawa N, Ishigaki S, Doyu M, Sobue G. Dofrin localizes to Lewy bodies and ubiquitylates synphilin-1. *J Biol Chem* 2003; 278:29106-14.
37. Petrucelli L, O'Farrell C, Lockhart PJ, Baptista M, Kehoe K, Vink L, et al. Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. *Neuron* 2002; 36:1007-19.
38. Kim SJ, Sung JY, Um JW, Hattori N, Mizuno Y, Tanaka K, et al. Parkin cleaves intracellular alpha-synuclein inclusions via the activation of calpain. *J Biol Chem* 2003; 278:41890-9.
39. Ardley HC, Scott GB, Rose SA, Tan NG, Markham AF, Robinson PA. Inhibition of proteasomal activity causes inclusion formation in neuronal and non-neuronal cells overexpressing Parkin. *Mol Biol Cell* 2003; 14:4541-56.
40. McNaught KS, Bjorklund LM, Belizaire R, Isacson O, Jenner P, Olanow CW. Proteasome inhibition causes nigral degeneration with inclusion bodies in rats. *Neuroreport* 2002; 13:1437-41.
41. McNaught KS, Belizaire R, Isacson O, Jenner P, Olanow CW. Altered proteasomal function in sporadic Parkinson's disease. *Exp Neurol* 2003; 179:38-46.
42. Pawlyk AC, Giasson BI, Sampathu DM, Perez FA, Lim KL, Dawson VL, et al. Novel monoclonal antibodies demonstrate biochemical variation of brain parkin with age. *J Biol Chem* 2003; 278:48120-8.
43. Itrier JM, Ibanez P, Mena MA, Abbas N, Cohen-Salmon C, Bohme GA, et al. Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. *Hum Mol Genet* 2003; 12:2277-91.
44. Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, et al. Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. *J Biol Chem* 2003; 278:43628-35.
45. Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, et al. Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc Natl Acad Sci USA* 2004; 101:10744-9.
46. Sunada Y, Saito F, Matsumura K, Shimizu T. Differential expression of the parkin gene in the human brain and peripheral leukocytes. *Neurosci Lett* 1998; 254:180-2.
47. Huang PL, Dawson TM, Brecht DS, Snyder SH, Fishman MC. Targeted disruption of the neuronal nitric oxide synthase gene. *Cell* 1993; 75:1273-86.
48. Zhang Y, Dawson VL, Dawson TM. Parkin: Clinical aspects and neurobiology. *Clinical Neuroscience Research* 2001; 1:467-82.