

PINK1 and Parkin to control mitochondria remodeling

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Abstract: Parkinson's disease (PD), one of the most common neurodegenerative diseases, is characterized by movement disorders and a loss of dopaminergic (DA) neurons. PD mainly occurs sporadically, but may also result from genetic mutations in several PD-linked genes. Recently, genetic studies with *Drosophila* mutants, *parkin* and *PINK1*, two common PD-associated genes, demonstrated that Parkin acts downstream of PINK1 in maintaining mitochondrial function and integrity. Further studies revealed that PINK1 translocates Parkin to mitochondria and regulates critical mitochondrial remodeling processes. These findings, which suggest that mitochondrial dysfunction is a prominent cause of PD pathogenesis, provide valuable insights which may aid in the development of effective treatments for PD.

Key words: PINK1, Parkin, Mitochondria, Parkinson's disease

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Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease in the world, is characterized by locomotor disorders including rigidity, tremor, bradykinesia, and postural instability (Lang & Lozano, 1998). In addition, massive and selective degeneration of dopaminergic (DA) neurons in the substantia nigra is the neuropathological hallmark of the disease. The majority of PD cases are sporadic; however, familial forms have also been reported. Over the past decade, mutations linked to familial forms of PD have been identified in a number of genes such as *alpha-synuclein* (also known as *SNCA*) (Polymeropoulos *et al.*, 1997), leucine-rich repeat kinase 2 (*LRRK2*) (Paisán-Ruíz *et al.*, 2004), *parkin* (also known as *PARK2*) (Kitada *et al.*, 1998), PTEN-induced putative kinase 1 (*PINK1*) (Valente *et al.*, 2004), *DJ-1*

(also known as *PARK7*) (Bonifati *et al.*, 2003), and *ATP13A2* (Ramirez *et al.*, 2006). Among these, *alpha-synuclein* and *LRRK2* mediate autosomal dominant forms of PD, and the others mediate autosomal recessive forms. Discovery of these PD-linked genes has enabled an understanding of the molecular mechanisms underlying familial PD pathology, providing valuable insight into the pathological mechanisms involved in sporadic cases.

Mitochondrial dysfunction has been heavily implicated in PD pathogenesis (Henchcliffe & Beal, 2008). The activity of complex I, a major component of the mitochondrial respiratory chain, is decreased in substantia nigra and other tissues in PD patients (Keeny *et al.*, 2006; Parker *et al.*, 2008). Moreover, several complex I inhibitors successfully reproduce key features of PD such as loss of DA neurons and motor deficits. Exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes parkinsonism in humans (Langston *et al.*, 1983). Administration of rotenone or paraquat also induces selective loss of DA neurons and produces locomotor defects in various animal models (Betarbet *et al.*, 2000; Coulom & Birman, 2004; Cicchetti *et al.*, 2005). Recent findings that *parkin* and *PINK1* have

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critical roles in maintaining mitochondrial function and integrity have suggested that mitochondrial dysfunction is the prominent cause of PD pathogenesis, enabling investigation of the pathological mechanisms of PD at the molecular level (Clark *et al.*, 2006; Park *et al.*, 2006; Yang *et al.*, 2006). The following is a brief review of the recent findings related to the roles of *parkin* and *PINK1* in mitochondria.

Parkin is critical in maintaining mitochondrial integrity

Parkin is an E3 ubiquitin ligase encoded by *parkin*, the most commonly affected PD gene conserved in various organisms from *Drosophila* to humans (Shimura *et al.*, 2000). Parkin is composed of an ubiquitin-like domain in its N-terminus and two RING-finger domains in its C-terminus. In mammalian cell-based studies, Parkin can ubiquitinate and degrade several proteins including CDCrel-1 (Zhang *et al.*, 2000), parkin-associated endothelin receptor-like receptor (Pael-R) (Imai *et al.*, 2001), and cyclin E (Staropoli *et al.*, 2003). From these results, endoplasmic reticulum (ER) stress resulting from accumulated Parkin substrates was proposed as the cause of DA neuronal death by loss of *parkin*. However, further studies failed to establish a meaningful relationship between these putative Parkin substrates and PD pathogenesis. To overcome the limitations of the cell-based study, several groups generated and characterized *parkin* null animal models. Although *parkin* null mice could not reproduce human PD symptoms, *Drosophila parkin* mutants demonstrated obvious phenotypes including locomotive defects and DA neuron degeneration (Greene *et al.*, 2003; Pesah *et al.*, 2004; Cha *et al.*, 2005). In addition, administration of L-DOPA substantially rectified the behavioral defects of the *parkin* mutants, further confirming that *parkin* mutant fly models successfully parallel human PD patients (Cha *et al.*, 2005). These mutants also showed defective wing posture and a crushed thorax. Histological examination of the *parkin* mutants demonstrated indirect flight muscle degeneration, which probably contributed to the locomotive defects along with DA neuron degeneration. Furthermore, mitochondrial swelling was found in the indirect flight muscles of the *parkin* mutants, suggesting that mitochondrial dysfunction may be an important cause of PD. However, these data cannot confirm whether mitochondrial swelling is a primary or secondary effect of *parkin* mutation.

Further evidence is needed to confirm the importance of mitochondrial dysfunction in PD pathogenesis.

PINK1 and Parkin act in a common pathway in mitochondrial protection

PINK1 is a serine/threonine kinase localized to the mitochondrial membrane via a mitochondrial targeting motif in its N-terminus (Valente *et al.*, 2004). Most of the currently reported mutations are located in its kinase domain, indicating that PINK1 kinase activity is required for its role in PD protection (Klein & Lohmann-Hedrich, 2007). Interestingly, *PINK1* fly mutants demonstrated phenotypes remarkably similar to *parkin* mutants, including flight disability, slow climbing speed, indirect flight muscle degeneration and a reduced number of DA neurons (Clark *et al.*, 2006; Park *et al.*, 2006; Yang *et al.*, 2006). Moreover, mitochondrial swelling was also observed in the indirect flight muscles and DA neurons (Fig. 1). Upon further genetic analysis, over-expression of mitochondrial protein Bcl-2 was found to rescue mitochondrial dysfunction and defective phenotypes in *PINK1* mutants, indicating that mitochondrial defects are the main cause of PD-related phenotypes in *PINK1* mutants (Park *et al.*, 2006).

Because of the marked phenotypic similarities between *parkin* and *PINK1* mutants, subsequent *Drosophila* genetic analysis were performed to test whether PINK1 and Parkin act in a common pathway. Transgenic expression of *parkin* markedly ameliorated the phenotypes of *PINK1* mutants; however, *parkin* mutant phenotypes could not be recovered by over-expression of PINK1 (Clark *et al.*, 2006; Park *et al.*, 2006; Yang *et al.*, 2006; Fig. 2). These data established that PINK1 and Parkin are linked in the same pathway to protect mitochondrial integrity and function with Parkin acting downstream of PINK1. In addition to the *Drosophila* results, over-expression of *parkin* successfully rescued the mitochondrial dysfunction induced by *PINK1* knockdown in the mammalian system, demonstrating that the PINK1-Parkin pathway is conserved in flies and mammals (Exner *et al.*, 2007).

Following the establishment of the PINK1-Parkin pathway, researchers have been trying to investigate the relationship between these two proteins. Using human DA cells and *Drosophila* models, Kim *et al.* demonstrated that PINK1 translocates Parkin to mitochondria in its kinase

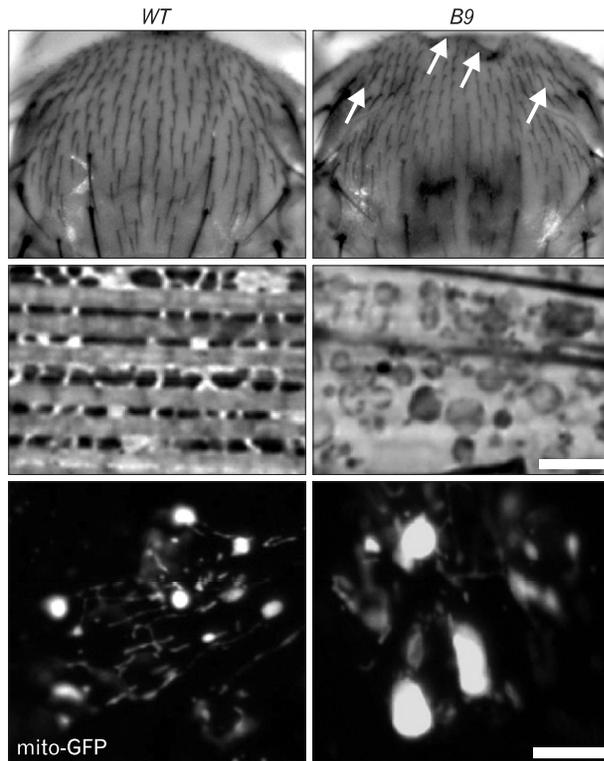


Fig. 1. *PINK1* mutation induces mitochondrial defects. *Drosophila PINK1* mutants (*B9*) demonstrated the crushed-thorax phenotype (top panel, white arrows), an indicator of flight muscle degeneration, and mitochondrial swelling in indirect flight muscles (middle panel) and DA neurons (bottom panel). Toluidine blue staining of longitudinal thorax sections revealed mitochondrial morphology of indirect flight muscles (middle panel). Expression of mitochondria-targeted green fluorescent protein (mito-GFP, green) showed mitochondrial shape and size in the DA neurons of adult fly brains (bottom panel). Wild type controls (*WT*) showed an intact thorax structure and mitochondrial morphology. Scale bar=yellow, 5 μ m.

activity-dependent manner (Kim *et al.*, 2008). Further analysis suggested that PINK1 phosphorylates Parkin on its linker region and promotes its mitochondrial translocation. Additional studies showed that PINK1 selectively translocates Parkin to impaired mitochondria, confirming the PINK1-dependent Parkin translocation that Kim *et al.* reported (Matsuda *et al.*, 2010; Narendra *et al.*, 2010).

PINK1 and Parkin remodel mitochondria

Since fly genetic analysis clearly demonstrated that the PINK1-Parkin pathway is involved in the protection of mitochondrial integrity and function, various efforts have concentrated on investigating the particular role of this

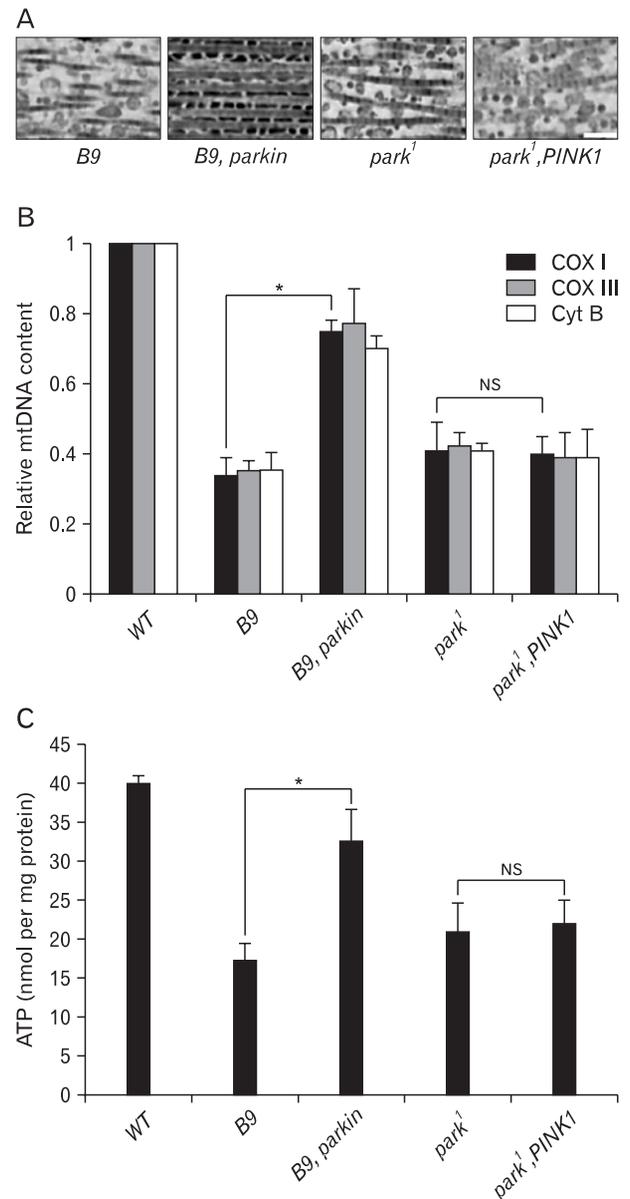


Fig. 2. Transgenic expression of *parkin* ameliorates *PINK1* mutant phenotypes, but not vice versa. Over-expression of *parkin* successfully rescued mitochondrial swelling, mtDNA content, and ATP levels in *PINK1* mutants. However, the *PINK1* transgene failed to restore these defects in *parkin* mutants. (A) Mitochondria in indirect flight muscles from *PINK1* mutants (*B9*), Parkin-expressing *PINK1* mutants (*B9, parkin*), *parkin* mutants (*park¹*), and *PINK1*-expressing *parkin* mutants (*park¹, PINK1*). (B) Quantification of mtDNA in fly thoraces. Quantitative real-time PCR was performed using primers from the mtDNA sequences of the following mitochondrial genes: Cox I, cytochrome *c* oxidase subunit I; Cox III, cytochrome *c* oxidase subunit III; Cyt B, cytochrome *b*. Results are expressed as fold change compared relative to *WT* controls. (C) Comparison of ATP content in fly thoraces. The relative ATP level was calculated by dividing the measured ATP concentration by the total protein concentration. In mtDNA and ATP assays, averages \pm SDs are from three experiments. Significance was determined by one-way ANOVA (* P <0.01; NS, not significant). Error bars indicate mean \pm SD. Scale bar=yellow, 5 μ m.

pathway in mitochondria. Recent fly genetic studies showed that the PINK1-Parkin pathway regulates the mitochondrial remodeling process including mitochondrial fusion and fission. Mitochondria are dynamic organelles that constantly fuse and divide. Uncontrolled fusion-fission processes lead to severe damage of mitochondria morphology and function, causing the impairment of various cellular process and even cell death (Chan 2006). Surprisingly, in *Drosophila*, over-expression of Drp1, a guanosine triphosphatase (GTPase) for mitochondrial fission, or down-regulation of Opa1 or Marf expression, GTPases for mitochondrial fusion, rescued *PINK1* and *parkin* mutant phenotypes, suggesting that the PINK1-Parkin pathway regulates mitochondria remodeling by promoting mitochondrial fission (Deng *et al.*, 2008; Poole *et al.*, 2008; Yang *et al.*, 2008; Park *et al.*, 2009). Therefore, loss of *PINK1* or *parkin* may induce uncontrolled mitochondrial remodeling leading to severe mitochondrial dysfunction and subsequent DA neuronal degeneration. Results from other human cell-based studies, however, are not in agreement. Co-expression of PINK1 and Parkin, or expression of mitochondria-targeted Parkin induces mitochondrial aggregation in human DA neuroblastoma cells (Kim *et al.*, 2008). Moreover, mitochondrial fragmentation was found in human neuronal cells lacking *PINK1* or in the primary cells from human patients with *PINK1* mutations (Exner *et al.*, 2007; Kim *et al.*, 2008; Wood-Kaczmar *et al.*, 2008). To account for these different patterns in mitochondrial remodeling, researchers are attempting to dissect the exact mechanism involved in PINK1 and Parkin-mediated mitochondrial remodeling.

In addition to balancing between mitochondrial fusion and fission processes, the PINK1-Parkin pathway may also be involved in another mitochondrial remodeling process: mitophagy, the specific autophagic turnover of mitochondria. Narendra *et al.* reported that Parkin is selectively translocated to damaged mitochondria upon treatment of mitochondria damaging agents, and induces the turnover of damaged mitochondria through an autophagy-related gene 5 (ATG5)-dependent mechanism (Narendra *et al.*, 2008). This finding elicited further studies to test whether PINK1 is required for the mitophagy-promoting activity of Parkin. In human DA neuroblastoma cells, co-expression of PINK1 and Parkin induces the formation of perinuclear mitochondrial clusters surrounded by autophagic vacuoles (Vives-Bauza *et al.*, 2010). Moreover, knock-down or knock-out of *PINK1* successfully inhibits mitochondrial damage-induced mitophagy

(Matsuda *et al.*, 2010; Narendra *et al.*, 2010). Further analysis determined that PINK1 is selectively stabilized on impaired mitochondria, and activates the autophagic degradation of these mitochondria by recruiting Parkin (Matsuda *et al.*, 2010; Narendra *et al.*, 2010). These data provide the functional link between PINK1, Parkin, and the selective autophagy of mitochondria, suggesting that PINK1 and Parkin maintain mitochondrial integrity and function by selectively removing impaired mitochondria.

Mitochondrial targets of the PINK1-Parkin pathway in mitochondrial remodeling

In biochemical studies using Parkin proteins containing disease causing mutations, the ubiquitin ligase activity of Parkin is critical for normal physiological activities. Moreover, Parkin-mediated mitochondrial ubiquitination was observed in mitochondrial damaging agent-treated cells. Over-expression of dominant negative ubiquitin mutants prevented Parkin-induced mitophagy, demonstrating that ubiquitination links between Parkin and mitophagy (Geisler *et al.*, 2010; Lee *et al.*, 2010; Matsuda *et al.*, 2010). To identify a putative Parkin target on mitochondria, molecular weight changes of various mitochondrial proteins upon mitochondrial damaging-agent treatment were evaluated. Surprisingly, a molecular weight shift of voltage-dependent anion channel 1 (VDAC1) was observed. Further biochemical analysis identified VDAC1 as a target for Parkin-mediated mitochondrial ubiquitination and subsequent mitophagy (Geisler *et al.*, 2010). The ubiquitin-binding autophagic component p62 and HDAC6 were also essential for Parkin-dependent mitophagy (Geisler *et al.*, 2010; Lee *et al.*, 2010), suggesting that ubiquitinated VDAC1 may be an important adapter molecule for p62 and HDAC6. In addition, Ziviani *et al.* reported that the pro-mitochondrial fusion protein Marf is ubiquitinated by Parkin, inferring that Parkin prevents refusion of damaged mitochondria to support proper mitophagic degradation (Ziviani *et al.*, 2010). These mitochondrial Parkin targets further confirmed the role of the PINK1-Parkin pathway in mitochondrial remodeling, suggesting a mitochondrial quality control system driven by PINK1 and Parkin (Whitworth & Pallanck, 2009). Under mitochondrial damaging stress, PINK1 selectively translocates Parkin to impaired mitochondria. In mitochondria, Parkin ubiquitinates its targets, prevents refusion of damaged mitochondria, and finally removes damaged mitochondria

to protect mitochondrial function and integrity. These novel Parkin targets have only been studied in cell-based PD models and are yet to be confirmed in animal PD models. Therefore, further studies are required to confirm and refine the molecular mechanisms of PINK1 and Parkin with regards to regulating the mitochondrial remodeling process.

Concluding remarks

After the cloning of PD-associated genes, their roles in protection against PD were investigated in cell-based PD models. However, pioneering work using a genetic fly model revealed that the most affected PD gene, *parkin*, acts downstream of another PD gene, *PINK1*, to protect mitochondria, confirming the link between mitochondrial dysfunction and PD pathogenesis. Additional studies based on this finding revealed the exact role of the PINK1-Parkin pathway in mitochondria: balancing between mitochondria fusion and fission, and selective mitophagy of damaged mitochondria. Further studies investigating the molecular mechanisms of *PINK1* and *parkin* in regulating mitochondrial remodeling will provide an enhanced understanding of PD pathogenesis and the development of an effective treatment strategy for PD.

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