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Mitochondrial dysfunction and Parkinson's disease genes: insights from Drosophila

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Parkinson's disease (PD), one of the most common neurodegenerative disorders worldwide, currently lacks a cure. Although most PD cases occur sporadically, studies from rare genetic mutations give significant insights into addressing the pathological mechanism of not only familial PD, but also sporadic PD. Recent PD research focuses on generating genetic mutant animal models that recapitulate the features of human PD patients. Significant advances in PD research have resulted from studying Drosophila mutants of several identified PD-associated genes because they show strikingly visible phenotypes. In particular, previous studies with the Drosophila mutants *parkin* and *PINK1*, which are two common causative genes among PD familial forms, have suggested strongly that mitochondrial dysfunction is the prominent cause for the PD pathogenesis and that these two PD genes are in a common pathway, with Parkin downstream of PINK1. Recent genetic studies have revealed that the PINK1-Parkin pathway is involved in regulating the mitochondrial remodeling process. In addition, PINK1 was recently found to regulate the localization of Parkin through direct phosphorylation. Here, we briefly review these new and exciting findings in Drosophila PD models and discuss how using these models can further advance PD studies.

Parkinson's disease (PD) is characterized by locomotor defects such as rigidity, tremor, bradykinesia of the limbs, and postural instability (reviewed by Lang and Lozano, 1998). In addition, selective degeneration of dopaminergic (DA) neurons in the substantia nigra is the neuropathological hallmark of the disease. PD probably occurs sporadically as the result of many different environmental factors, but it could also occur genetically by mutations in a number of genes such as *α-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 the genes identified in familial cases, *α-synuclein* and *LRRK2* are known as autosomal dominant genes and the rest are known as autosomal recessive genes. The recent identification of these genes has stimulated a search for understanding the mechanisms underlying familial PD pathology, but this in turn would also help us to understand the pathological mechanism of sporadic cases of PD. Before any animal models for these genes were generated, a large number of studies focused on identifying the functions of these gene products through cell culture systems. These studies suggested several possible pathogenic mechanisms, including protein misfolding, abnormal protein accumulation, oxidative stress, mitochondrial dysfunction and caspase activation. In trying to determine the central cause of the disease, more promising clues came from studies of PD animal models, including monkeys, mice, rats, flies and worms. Among these models, recent Drosophila studies have led to major advances in the field by revealing that mitochondrial dysfunction is the prominent cause for the PD pathogenesis.

It is definitely useful to study PD in Drosophila because fly PD models show striking phenotypes that resemble characteristics seen in human patients, such as loss of DA neurons and locomotive defects, whereas other animal models show moderate or no phenotypes. The fly has four representative clusters of DA neurons in the posterior region of the brain (Friggi-Grelin et al., 2003), and these neurons can be detected notably by immunostaining with an anti-tyrosine hydroxylase antibody. In addition, the locomotive phenotype of the flies can be assessed by testing flight ability or by examining climbing ability with negative geotaxis. These locomotive abilities are so robust that it is relatively easy to quantitatively evaluate their behavior. Furthermore, the Drosophila model system has other advantages, such as rapid growth and reproduction; easy genetic manipulation; extensive genetic resources; and over 70% conservation of human disease genes, thus adding to its value as one of the most useful model animals for PD research.

Among the PD genes identified to date, *α-synuclein* is the only gene not conserved in Drosophila. α-synuclein protein is a major component of Lewy bodies, which are the cytoplasmic protein inclusions that are characteristic of PD pathology and that may form as a protective cellular mechanism against oxidative stress (Bence et al., 2001; Kawaguchi et al., 2003; Tanaka et al., 2003; Taylor et al., 2003). Overexpression of human *α-synuclein* in Drosophila induced DA neuronal degeneration and locomotive deficits (Feany and Bender, 2000). Lewy body-like α-synuclein aggregation was also observed in the fly DA neurons. This was the first breakthrough showing that Drosophila could be a robust PD model system.

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One of the most commonly affected PD genes is *parkin*, and its product encodes an E3 ligase that is well conserved in Drosophila and includes functional domains, such as an ubiquitin-like domain and RING finger domains. Several groups have generated *parkin* fly mutants, which showed obvious phenotypes including locomotive defects and DA neurodegeneration (Greene et al., 2003; Pesah et al., 2004; Cha et al., 2005). In addition, these mutants also exhibited defects in wing position and crushed thorax morphology. Further histological examination of the thoracic muscle of the *parkin* mutants showed muscle degeneration, which probably contributes to the locomotive defects with DA degeneration. Interestingly, at higher microscopic levels, mitochondrial swelling was observed in the indirect flight muscles, as well as in the sperm, of the *parkin* mutants, suggesting that mitochondrial dysfunction is an important cause of the disease. This mitochondrial defect is an unexpected finding because several Parkin substrates that were previously identified in vitro were localized mostly in the cytoplasm (reviewed by Moore et al., 2005). Therefore, loss of Parkin was proposed to lead to substrate accumulation and thereby result in ER stress, which in turn may have caused the death of the DA neurons. However, simply characterizing the phenotypes of *parkin* mutants cannot determine whether the mitochondrial swelling is a primary or secondary effect of the loss of the *parkin* gene. Therefore, more studies were required to provide further evidence of the key cause of PD.

DJ-1 is known to function as a cellular protector against oxidative stress, but it has no known functional domain. *DJ-1* fly mutants generated by several groups exhibit locomotive defects in an oxidative stress-sensitive manner (Menzies et al., 2005; Meulener et al., 2005; Park et al., 2005; Yang et al., 2005; Lavara-Culebras and Paricio, 2007), suggesting that DJ-1 is involved in protecting against oxidative stress. However, the mutants did not show a marked overt phenotype, disabling us from studying its molecular functions further.

PINK1 has a mitochondrial targeting motif in the N-terminus, followed by a serine/threonine kinase domain. PINK1 kinase activity is required for its role in PD protection. Interestingly, *PINK1* fly mutants phenocopy almost all of the phenotypes and characteristics of *parkin* mutants, including flight disability, slow climbing ability, indirect flight muscle degeneration and reduced number of DA neurons (Park et al., 2006; Clark et al., 2006; Yang et al., 2006). In addition, mitochondrial swelling occurs in tissues where high energy is required, such as the indirect flight muscle, DA neurons and sperm. This tissue-specific pattern of mitochondrial swelling is very similar to *parkin* mutants. Further genetic analysis with the mitochondrial protein Bcl-2 demonstrates that the mitochondrial defect is the main cause of the defective phenotypes in *PINK1* mutants (Park et al., 2006).

Since the PINK1 and Parkin fly mutants show remarkably similar features, the phenotypic analysis of these PD models led to the prediction that PINK1 and Parkin act in a common pathway. Indeed, subsequent Drosophila genetic studies demonstrated that *parkin* overexpression markedly suppressed the phenotypes of *PINK1* mutants but not vice versa (Park et al., 2006; Clark et al., 2006; Yang et al., 2006), showing the epistatic relationship between the two genes. This finding established that these two different PD-associated proteins, PINK1 and Parkin, are linked in a linear pathway in the protection of mitochondrial integrity and function with Parkin acting downstream of PINK1. Furthermore, this finding suggests strongly that mitochondrial dysfunction is the key cause of PINK1–Parkin-related PD pathogenesis. In parallel with the Drosophila results, Parkin overexpression markedly rescues the mitochondrial dysfunction that is induced by *PINK1* small interfering RNA (siRNA) knockdown in the mammalian system (Exner et al., 2007), demonstrating the conservation of the PINK1-Parkin pathway between flies and mammals.

With the establishment of this pathway, several studies have attempted to investigate the relationship between PINK1 and Parkin. Because *PINK1* mutant phenotypes could be rescued by raising Parkin levels, and because a previous study showed a reduced level of Parkin protein in *PINK1* RNAi flies, one initial hypothesis was that Parkin protein is unstable in the *PINK1* fly mutants (Yang et al., 2006). However, more recent work from our group suggests an alternative mechanism (Kim et al., 2008). Through biochemical analysis in human DA cells and genetic analysis using the Drosophila model system, we found that the primary role of PINK1 is to translocate Parkin to the mitochondria, suggesting that *PINK1* mutants show mitochondrial defects because there is less Parkin localized in the mitochondria. Further experiments demonstrated that the detailed mechanism for this event was direct PINK1 phosphorylation in the RING0 domain (Hristova et al., 2009) of Parkin at threonine (T)175 (T187 in Drosophila). However, in PINK1-null situations, the Parkin protein was not completely absent in the mitochondrial fraction, implicating the possibility that other proteins besides PINK1 regulate Parkin localization to the mitochondria.

As the studies of PINK1 and Parkin fly models showed that the PINK1-Parkin pathway is involved in the protection of mitochondrial integrity and function, the next important step became investigating what particular roles this pathway plays in the mitochondria. Recent studies using fly genetic analyses clearly showed that the PINK1-Parkin pathway regulates the mitochondrial remodeling process, including mitochondrial fusion and fission (Poole et al., 2008; Yang et al., 2008; Deng et al., 2008; Park et al., 2008). Mitochondria are dynamic organelles that constantly fuse and divide, and move to specific subcellular locations where energy demands are high (reviewed by Chan, 2006). Controlling the mitochondria remodeling process is not only important for maintaining mitochondrial morphology but also for mitochondrial function, which in turn may determine various cellular activities and cell survival. There are several genes that have been identified in this process: *MFN1* and *MFN2* in humans (*Marf* and *fuzzy onions* in Drosophila), which are GTPases that are required for mitochondrial outer membrane fusion; *OPA1*/*Opa1*, a GTPase required for inner membrane fusion; and *UTRN* (*Drp1* in Drosophila), a GTPase required for mitochondrial fission. In Drosophila, these genes are also well conserved and their fly mutants show defects that are related to the mitochondrial fusion and fission process (Verstreken et al., 2005; McQuibban et al., 2006; Deng et al., 2008). Surprisingly, increased expression of *Drp1*, or decreased levels of *Opa1* or *Marf*, rescued the PINK1 and Parkin fly mutant phenotypes (Poole et al., 2008; Yang et al., 2008; Deng et al., 2008; Park et al., 2008), suggesting strongly that the PINK1-Parkin pathway plays a role in regulating mito-

chondrial remodeling in the direction of promoting mitochondrial fission. Therefore, loss of PINK1 or Parkin may cause defects in mitochondrial remodeling and, in turn, this mitochondrial dysfunction may induce DA neurodegeneration.

These results contrast with a human cell-based study demonstrating that the PINK1-Parkin pathway promotes mitochondrial aggregation (Kim et al., 2008). Co-expression of PINK1 and Parkin, or sole expression of mitochondria-targeted Parkin, induces mitochondrial aggregation in human DA neuroblastoma cells (Kim et al., 2008). However, mitochondrial fragmentation, or mitochondrial swelling with disorganized cristae, was found in human neuronal cells lacking PINK1 or in the primary cells from human patients with PINK1 mutations (Exner et al., 2007; Wood-Kaczmar et al., 2008). Intriguingly, this disrupted mitochondrial phenotype was observed similarly in the indirect flight muscle and DA neurons of PINK1 and Parkin mutant flies. This is in contrast to the aforementioned fly genetic data with mitochondrial fusion and fission proteins, which suggest strongly that the PINK1-Parkin pathway promotes mitochondrial fission. Currently, without further studies, it is difficult to explain why the PINK1-Parkin pathway promotes mitochondrial fission in flies and fusion in mammals. Thus, the mitochondrial fusion and fission process has emerged recently as a hot issue in PD pathogenesis. One explanation is that this discrepancy might be the result of species-specific differences. Alternatively, the final outcome of the mitochondrial morphology observed in both Drosophila and mammalian cell systems could be the net effect of a disrupted balance between mitochondrial fusion and fission, thereby causing confusion over whether the end results that we observe are attributable to changes in mitochondrial fusion or fission. Genetic analysis using mouse models could be a solution to answer this important question, but the lack of phenotypes in PINK1 or Parkin mouse models limits this study.

A recent fly genetic study revealed additional components in the PINK1-Parkin pathway (Fig. 1). One such component is Rhomboid-7 [known as presenilin-associated, rhomboid-like (PARL) in mammals], a mitochondrial protease known to cleave the precursor form of high temperature requirement A2 (HtrA2, also known as Omi) (Whitworth et al., 2008). Rhomboid-7 may also cleave the mitochondrial targeting motif of PINK1, enabling PINK1 activity not only in the mitochondria, but also in the cytosol. Tain et al. identified Omi/HtrA2 as a possible regulator of the PINK1-Parkin pathway, acting downstream of PINK1 in Drosophila (Tain et al., 2009). However, another study shows that Omi/HtrA2 does not play a role in the pathway (Yun et al., 2008).

Additional fly genetic studies have examined whether other PD gene products converge on the PINK1-Parkin pathway (Fig. 1). Genetic analyses demonstrate that DJ-1, another PD gene product, is not involved in this pathway (Yang et al., 2008). However, cell culture data shows that DJ-1 is somewhat related to mitochondrial function since DJ-1 localizes to the mitochondria under oxidative stress conditions (Canet-Avilés et al., 2004). Further studies must be conducted to determine whether DJ-1 is involved in the PINK1-Parkin pathway in the presence of oxidative stress. Drosophila *LRRK2* mutants have also been generated and exhibit DA neuronal degeneration (Lee et al., 2007; Liu et al., 2008), but it remains unclear whether LRRK2 indeed converges on the PINK1-Parkin pathway. α-synuclein also seems to play a significant role in protecting against mitochondrial dysfunction, as several studies on *α-synuclein* mouse mutants have revealed morphological and functional defects in mitochondria (Song et al., 2004; Martin et al., 2006; Stichel et al., 2007). Moreover, a recent study revealed that α-synuclein has a mitochondrial targeting motif in the N-terminal region and indeed localizes to the mitochondria (Devi et al., 2008; Parihar et al., 2008). Additional studies should be conducted to determine whether α-synuclein acts linearly in the PINK1-Parkin pathway or acts independently on the mitochondria.

The genetic tractability of flies, in combination with the strength of fly PD models in phenocopying human symptoms, has resulted in extensive Drosophila research and, thereby, contributed greatly to understanding the pathological mechanisms of familial PD. These fly models have enabled us to define both the key cause of familial PD, or at least of PINK1–Parkin-associated PD, namely mitochondrial dysfunction, as well as the underlying mechanism, the PINK1-Parkin pathway. In addition, by using mutants that are already available and that cover almost the whole genome, it is possible to conduct a large-scale genomic screen and identify the upstream regulators and downstream targets of the pathway. More importantly,

Advantages of Drosophila as a model for Parkinson's disease

- Flies have rapid growth and reproduction, and genetic tractability
- The fly brain has four distinct and easily identifiable clusters of DA neurons
- The phenotypes of PD fly models resemble characteristics seen in human patients
- Robust locomotive abilities simplify quantitation of behavior and activity

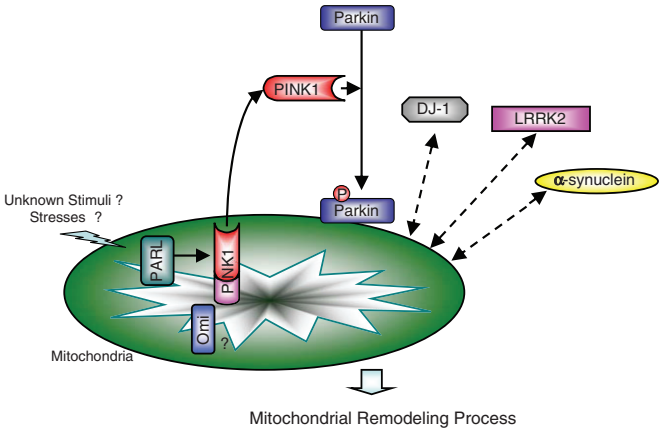

Fig. 1. A schematic model for the PINK1-Parkin pathway on mitochondrial dynamics.

the fly mutants can also be used for primary drug screens to identify promising PD medications. We believe that these powerful genetic models can continue to provide answers to crucial questions about this complex human disease and will be highly useful for dissecting the mechanism of PD.

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COMPETING INTERESTS

The authors declare no competing financial interests.

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