Drosophila as a Model to Study Mitochondrial Dysfunction in Parkinson's Disease

Ming Guo

Department of Neurology, Department of Molecular and Medical Pharmacology, Brain Research Institute, David Geffen School of Medicine, Los Angeles, California 90095

Correspondence: mingfly@ucla.edu

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Identification of single gene mutations that lead to inherited forms of Parkinson's disease (PD) has provided strong impetus for the use of animal models to study normal functions of these "PD genes" and the cellular defects that occur in the presence of pathogenic PD mutations. *Drosophila* has emerged as an effective model in PD-related gene studies. Important insights into the cellular basis of PD pathogenesis include the demonstration that two PD genes, *PINK1* and *parkin*, function in a common pathway, with *PINK1* positively regulating *parkin*, to control mitochondrial integrity and maintenance. This is accomplished through regulation of mitochondrial fission/fusion dynamics. Subsequent observations in both fly and mammalian systems showed that these proteins are important for sensing mitochondrial damage and recruiting damaged mitochondria to the quality-control machinery for subsequent removal. Here, I begin by reviewing the opportunities and challenges to understanding PD pathogenesis and developing new therapies. I then review the unique tools and technologies available in *Drosophila* for studying PD genes. Subsequently, I review lessons that we have learned from studies in *Drosophila*, emphasizing the *PINK1/parkin* pathway, as well as studies of *DJ-1* and *Omi/HtrA2*, two additional genes associated with PD implicated in regulation of mitochondrial function. I end by discussing how *Drosophila* can be used to further probe the functions of *PINK1* and *parkin*, and the regulation of mitochondrial quality more generally. In addition to PD, defects in mitochondrial function are associated with normal aging and with many diseases of aging. Thus, insights gained from the studies of mitochondrial dynamics and quality control in *Drosophila* are likely to be of general significance.

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with a prevalence second only to that of Alzheimer's disease. There is no cure or treatment that can halt disease progression. A primary pathological hallmark of the disease is degeneration of multiple neuronal types including, most notably, dopaminergic neurons in the substantia nigra

of the midbrain (Dauer and Przedborski 2003; Shulman et al. 2011). However, pathology of many non-dopaminergic neurons including olfactory and brain stem neurons predates that of DA neurons (Braak et al. 2003). Patients with PD present with characteristic "motor symptoms," such as resting tremor, slowness of movement, rigidity, postural instability, and gait difficulty.

Editor: Serge Przedborski

Additional Perspectives on Parkinson's Disease available at www.perspectivesinmedicine.org

Copyright © 2012 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a009944

Cite this article as *Cold Spring Harb Perspect Med* 2012;2:a009944

M. Guo

Although the mainstay of current medical treatment for PD is dopamine replacement, this is not very satisfying. First, the dopamine replacement only alleviates some of the motor symptoms (does not help gait problems), becomes less effective over time, and is often associated with intolerable side effects. Second, PD patients also present with a combination of non-motor symptoms (Simuni and Sethi 2008) including dementia, which occurs in more than one-third of patients; psychiatric symptoms such as depression, anxiety, and obsession; autonomic dysfunction (involving cardiac and digestive systems); sleep disruption; skin lesions; and musculoskeletal abnormalities (Meissner et al. 2011b). Some of these non-motor symptoms may be more debilitating than the motor impairment, but they do not usually respond to dopamine replacement. Third, because of the progressive, debilitating nature of the disease, there is an urgent need to develop disease-modifying therapies that can prevent progression of the illness and perhaps even revert some of the pathology. In summary, PD is a multisystem disease affecting more than DA neurons. Therefore, although therapies targeted to dopaminergic neurons or their targets (such as dopamine replacement, cell transplantation, deep brain stimulation) can provide some therapeutic benefit to patients, particularly with respect to the motor symptoms, we still need to develop therapies that target the underlying cellular defects so as to treat both motor and non-motor symptoms and to prevent disease progression in the first place. A prerequisite for developing these new therapies is that we understand the pathogenesis of PD at the cellular and molecular level in tissues beyond dopaminergic neurons.

IDENTIFICATION OF PD GENES PROVIDES STRONG IMPETUS FOR STUDIES USING ANIMAL MODELS

Vertebrate models have been used in the PD field for many years. Once believed to be solely an environmental disease, much early work focused on toxin models of PD to study disease pathogenesis and develop therapies. Following the landmark discovery that exposure to the mi-

tochondrial toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) leads to loss of dopaminergic neurons and motor-related PD-like symptoms in humans (Langston et al. 1983; Bove et al. 2005), rodent and primate models were developed that used chemical depletion of dopamine using 6-hydroxy-dopamine, or feeding with mitochondrial toxins such as MPTP, rotenone, or paraquat (Bove et al. 2005). The results of these studies led to the important conclusion that dopaminergic neuronal degeneration results from oxidative stress and mitochondrial dysfunction (Abou-Sleiman et al. 2006).

Although the above toxin models show dopaminergic neuronal degeneration, new anti-PD drugs and neuroprotective agents that work well to alleviate symptoms in these animal models have largely failed to yield positive effects in human trials (Linazasoro 2004; Meissner et al. 2011b). These results suggest that the disease mechanisms underlying pharmacological models of PD may be different from those acting in the vast majority of sporadic PD patients.

The identification of genes that mediate familial PD has transformed our understanding of the genetic contribution to PD and is providing insight into the cellular mechanisms underlying PD pathogenesis (Hardy et al. 2009; Martin et al. 2011). Over the past 15 years, mutations in several genes have been definitively shown to mediate familial PD. Mutations in SNCA (encoding α -synuclein to *PARK1* [Polymeropoulos et al. 1997] and *PARK4* [Singleton et al. 2003], *Leucine-rich repeat kinase 2* (*LRRK2*)/*Dardarin* (*PARK8*) [Paisan-Ruiz et al. 2004; Zimprich et al. 2004]) were identified several years ago as being associated with autosomal-dominant forms of the disease. More recently, mutations in *VPS35* (Vilarino-Guell et al. 2011; Zimprich et al. 2011) and *EIF4G1* (Chartier-Harlin et al. 2011) have been reported to cause autosomal-dominant forms of PD. Mutations in *PARKIN* (*PARK2*) (Kitada et al. 1998), *DJ-1* (*PARK7*) (Bonifati et al. 2003), and *PTEN-induced kinase 1* (*PINK1*, *PARK6*) (Valente et al. 2004) are associated with autosomal-recessive forms of PD. Mutations in *ATP13A2* (*PARK9*), which encodes a lysosomal ATPase, have also been found in an atypical, autosomal-recessive parkinsonism

(Ramirez et al. 2006) with distinct clinical manifestations (Schneider et al. 2010). These single-gene, Mendelian forms represent ~10%–15% of all PD cases. Some patients with familial forms of PD show clinical symptoms that are similar to those present in patients with sporadic cases. In addition, genome-wide association studies have provided evidence that polymorphic variants in some of the genes associated with familial PD contribute to sporadic PD (Satake et al. 2009; Simon-Sanchez et al. 2009). Thus, the distinction between sporadic and familial forms of PD has become increasingly blurred, and the knowledge gained from studies of genes associated with inherited forms of PD will likely provide insight into mechanisms underlying sporadic forms of the disease as well.

Although human genetic studies have allowed the identification of PD genes from patient-based studies, ethical and technical considerations constrain the utilization of human subjects as experimental systems. Human cell-based studies are limited by tissue availability and provide only limited information when studying processes at the tissue, organ, or whole-animal levels. Postmortem studies are also limited by tissue availability and seldom allow identification of the initial pathological events during presymptomatic stages. Overall, it is difficult to determine the causality of mechanisms from human studies. Thus, animal models are indispensable tools for the analysis of PD genes.

Why do we need invertebrate models if vertebrate models can be easily generated? Vertebrate models include non-human primates, rodents (mice and rats), zebrafish, frogs, and chickens. Mouse models predominate, because of the large number of genetic technologies available and the long history of cell biology and analysis of behaviors in this organism. It might be assumed that mouse models are superior to invertebrate models, because mice are evolutionarily closer to humans, and we can perhaps more easily relate observations of mouse pathology and behaviors such as locomotion, learning, and memory to their counterparts in humans. However, work from many laboratories has shown that rodent models do not always re-

capitulate human disease pathology (Dawson et al. 2010). *Drosophila* models, however, surprisingly to some, have replicated many pathological processes related to human neurological disorders (Zoghbi and Warren 2010). Next, I outline the advantages that *Drosophila melanogaster* offers as a model organism, and insights that studies in *Drosophila* have provided for PD research.

THE ADVANTAGES OF USING *DROSOPHILA* AS A MODEL SYSTEM TO STUDY PD

The identification of PD genes provides an unprecedented opportunity to understand PD pathogenesis through in vivo studies. The key questions in the field are: What are the normal functions of these PD genes? How do PD pathogenic mutations affect PD gene function? Do these PD genes function in a common pathway, or in parallel pathways?

Among the various model organisms, *Drosophila melanogaster* has emerged as an especially effective tool to study PD genes. *Drosophila* has been used extensively for investigating fundamental biological processes, such as cell death (Hay et al. 2004; Hay and Guo 2006), as well as cell proliferation, growth, and migration (for review, see Bellen et al. 2010). In addition, flies display complex behaviors such as circadian rhythms, learning and memory, sleep, and aggression (Bellen et al. 2010). Some of these were thought to be exclusive to humans, and studies now suggest that the genetic bases of these behaviors are conserved in humans. Accumulated studies from more than a century have left the modern fly field with powerful molecular genetic tools (Adams and Sekelsky 2002; St Johnston 2002; Venken and Bellen 2005). *Drosophila* also has a compact genome size (1/30th of the human genome), limited genetic redundancy, and a short generation time (10 days) and life span (60–80 days). The complete sequence of the *Drosophila* genome has revealed that 77% of human disease genes are conserved in the fly (Rubin 2000; Bier 2005). These features make flies an excellent model system in which to study the function of disease genes including those involved in neurodegenerative diseases (Lessing and Bonini 2009; Lu and Vogel 2009), and in

M. Guo

which to dissect genetic pathways related to these disease genes.

Drosophila has emerged as an effective tool for studying PD pathogenesis (for review, see Dodson and Guo 2007; Lu and Vogel 2009; Guo 2010; Whitworth 2011). The *Drosophila* genome encodes homologs of *DJ-1*, *PINK1*, *PARKIN*, *LRRK2*, and *VPS35*. The adult brain of *Drosophila* contains clusters of dopaminergic neurons (Nassel and Elekes 1992), and these neurons degenerate when flies are fed rotenone (Coulom and Birman 2004), a complex I inhibitor that also triggers dopaminergic neuronal degeneration in mammals (Scherer et al. 2003; Bove et al. 2005). Among the genes that mediate familial PD, only α -synuclein does not have a homolog in *Drosophila*. Nevertheless, expression of human wild-type and PD-causing mutant forms of SNCA in *Drosophila* results in dopaminergic neuronal loss (Feany and Bender 2000; Trinh et al. 2008). In this review, we mainly focus on *Drosophila* homologs of genes associated with recessive forms of PD: *PINK1*, *PARKIN*, and *DJ-1*. We also emphasize the implications that these studies in *Drosophila* have for advancing our understanding of PD in clinical settings. It is important to note that although dopaminergic neurons do show slight degeneration in some *Drosophila* models of PD (Whitworth et al. 2005; Trinh et al. 2008), the significance of *Drosophila* models of PD involves much more than dopaminergic neuron pathology. Flies show defects in multiple systems, reminiscent of the multiple system involvement in PD patients. It is the understanding of the cellular basis of these defects that will provide insight into the pathogenesis of PD.

VARIOUS TOOLS IN *DROSOPHILA*

It would be a chapter in itself to review fully the tools available in *Drosophila*, but here I summarize those that are particularly relevant to building disease models.

Mutagenesis- and Loss-of-Function-Based Studies

Transposons including *P*-element, *piggyBac*, and *Minos* can insert in either coding sequence or

regulatory regions of a gene resulting in loss of function of the gene. The advantage of using *P*-elements, and possibly *Minos*, is that one can generate a null mutation via imprecise excision of *P*-elements located near or in a gene (Adams and Sekelsky 2002). The fly community has generated a large number of transposon insertion lines, and their precise insertion sites in the genome have been determined (Bellen et al. 2004; Thibault et al. 2004). It is estimated that in the next several years, collectively, transposon insertions will be available to disrupt >90% of all *Drosophila* genes. The availability of these transposons has largely replaced the need for chemical mutagenesis, which requires lengthy and tedious mapping strategies. In genomic areas in which transposable elements are lacking, one can perform homologous recombination to generate deletions or disease-related knockin alleles (Rong and Golic 2000; Wesolowska and Rong 2010).

Tissue-Specific Overexpression and Knockdown Can Be Easily Accomplished In Vivo

Temporally controlled, tissue-specific overexpression can be easily accomplished in vivo using the UAS-GAL4 system, a bipartite transcription activation system (Brand and Perrimon 1993). In this system, one transgene consists of a yeast transcription factor (GAL4), driven by a ubiquitous promoter such as tubulin or a tissue-specific promoter such as the dopaminergic neuron-specific TH. The second transgene consists of a gene of interest that is under the control of UAS (upstream activating sequences), which drives transcription in response to GAL4 binding. Therefore, for example, transgenic flies carrying a version of Gal4 driven by the tyrosine hydroxylase promoter (TH-Gal4) and UAS-*PINK1* will express *PINK1* in a dopaminergic neuron-specific pattern. This versatile system allows expression of genes both during development and adulthood, which is very useful for modeling diseases. More recently, several variants on this two-component expression system have been generated. (These are reviewed in detail elsewhere.) Suffice it to say that they allow one to turn gene expression on (or off; see below)

in almost any desired tissue or cell type, in an inducible manner (del Valle Rodriguez et al. 2012). Transposon mutagenesis has also resulted in the creation of a large number of *P*-element-bearing lines that carry a UAS element near one end. These lines, known as EP lines, allow for GAL4-dependent expression of nearby genes, if the UAS element is 5' to the gene, and pointing so as to drive expression of the gene (Rorth 1996).

Tissue-specific and inducible gene silencing can be accomplished through the use of the GAL4 system as well. In brief, flies can be generated that carry UAS-transgenes expressing long double-stranded RNAs (dsRNAs) or microRNAs. When crossed to specific GAL4 drivers or the many variants referenced above, gene silencing is brought about in cells expressing GAL4. Lines of flies carrying UAS-dsRNA or UAS-microRNA transgenes have been, or are being, generated for most *Drosophila* genes (Dietzl et al. 2007; Ni et al. 2011). These lines are publicly available.

Site-Specific Transgenesis Allows Precise Controls over Genetic Background and Expression Levels

Site-specific gene integration can also be brought about using donor plasmids carrying target sites for a site-specific integrase, and fly lines carrying a source of integrase and a “landing pad” target site inserted at specific positions in the genome. Multiple versions of site-specific integration technology and landing pads scattered throughout the genome are available for the fly (Venken and Bellen 2007; del Valle Rodriguez et al. 2012). This technology is important because it allows one to test the activity of a series of mutant proteins, for example, having confidence that all other genomic variables have been kept constant.

Genetics and Compound Screens Provide Unbiased Methods for Identifying Genes and Pathways Important for PD Pathogenesis and Its Suppression

One of the most important tools in the *Drosophila* toolbox lies in the ability to perform large-scale screens for new mutations or conditions of

gene overexpression or silencing that enhance or suppress phenotypes associated with disease models. A number of screen strategies are available (for review, see St Johnston 2002). The goal of unbiased genetic screens is to identify new components that either function in the same genetic pathway of the disease model or in parallel pathways to regulate a common process (the disease state). The power of these screens cannot be understated, because they do not require any existing knowledge related to the function of the disease gene.

In parallel, *Drosophila* has also become a valuable in vivo model in which to perform compound screens for suppressors of disease states (Gladstone and Su 2011; Pandey and Nichols 2011). In vivo systems like the fly are advantageous for several reasons. First, flies are cheap as compared with mammalian disease models such as mice and show disease phenotypes during development or as young adults. Second, many disease states cannot be easily modeled in cell culture. Third, and perhaps most importantly, in vivo screens are unbiased in that they focus on the bottom line—suppression of the disease state. This stands in contrast to many cell-based compound screens, which focus on identifying inhibitors or activators of a particular protein. Although obviously a valuable approach, focus on modifying the activity of a particular protein commits the researcher to that molecule, to the exclusion of all other pathways and molecules, many of which may not be known as regulators of the disease state under study. It may be particularly challenging to implement specific target-based drug screens in cell culture for diseases that result from loss of gene function, as with several of the familial forms of PD, because in these cases one must know the identity of a pharmacologically relevant target in another gene ahead of time.

WHAT LESSONS HAVE WE LEARNED FROM STUDIES IN *DROSOPHILA*?

PINK1 and *Parkin* Function in a Common Pathway to Regulate Mitochondrial Integrity

PARKIN encodes a protein with RING-finger motifs and has E3 ubiquitin ligase activity in

M. Guo

in vitro assays. The initial hypothesis related to *PARKIN* function was that loss of *parkin* resulted in the aberrant accumulation of toxic proteins, perhaps as a result of a failure of the ubiquitin-proteasome system to degrade substrates of Parkin ubiquitination. Consistent with this hypothesis, Parkin catalyzes K48-mediated polyubiquitination, which targets substrates for proteasomal degradation, and multiple proteins have been shown to interact with Parkin and are ubiquitinated in a Parkin-dependent manner in vitro. However, few of these substrates have been shown to accumulate in vivo, in *PARK2* patients, or *parkin* knockout mice, leaving their significance unclear (for review, see West et al. 2007; Dawson and Dawson 2010). Subsequent studies indicated that Parkin also catalyzes other forms of ubiquitination (for review, see West et al. 2007), which can influence cellular processes such as signal transduction, transcriptional regulation, and protein and membrane trafficking, without promoting substrate degradation (Mukhopadhyay and Riezman 2007). However, the exact role of Parkin remained unclear.

The first in vivo indication that *parkin* regulates mitochondrial integrity came from studies of *Drosophila parkin* mutants. Flies lacking *parkin* show dramatic mitochondrial defects in several energy-intensive tissues (Greene et al. 2003; Pesah et al. 2004), and a subset of dopaminergic neurons undergoes degeneration (Whitworth et al. 2005). Subsequent studies in *parkin* knockout mice indicated that although severe defects in mitochondrial morphology are not observed, these animals do display defects in mitochondrial respiration (Palacino et al. 2004).

Key studies that implicate mitochondrial dysfunction as a central mechanism for PD pathogenesis come from studies of *Drosophila PINK1* and its interaction with the fly *parkin*. *PINK1* encodes a protein with a mitochondrial targeting sequence and a serine-threonine kinase domain. We and others reported that a large fraction of *PINK1* is localized to mitochondria (Clark et al. 2006), and *Drosophila* lacking *PINK1* show phenotypes very similar to those of flies lacking *parkin*. *PINK1* and *parkin* mutants are viable but show striking defects in mitochondrial integrity—swollen mitochondria

that have severely broken cristae—in energy-intensive tissues. The males are sterile, adult muscle undergoes degeneration and cell death, and a subset of dopaminergic neurons undergoes degeneration (Clark et al. 2006; Park et al. 2006; Yang et al. 2006). *PINK1* mutants also show reduced ATP levels and mitochondrial DNA (mtDNA) content, and increased stress sensitivity (Clark et al. 2006; Park et al. 2006; Yang et al. 2006). Flies lacking endogenous *PINK1* function but expressing PD-associated mutant forms of *PINK1*, either by overexpression (Yang et al. 2006) or under the control of the endogenous *PINK1* promoter (Yun et al. 2008), show phenotypes similar to those of *PINK1*-null mutants, consistent with *PINK1*-associated disease being the result of loss of function.

What is the relationship between *PINK1* and *parkin*? Stringent genetic studies in *Drosophila* allow one to construct a genetic pathway. *parkin* overexpression in flies suppresses all *PINK1* mutant phenotypes tested (Clark et al. 2006; Park et al. 2006; Yang et al. 2006), whereas *PINK1* overexpression does not compensate for loss of *parkin* function (Clark et al. 2006; Park et al. 2006). Furthermore, double mutants lacking both *PINK1* and *parkin* have phenotypes identical to, rather than stronger than, either single mutant (Clark et al. 2006; Park et al. 2006; Yang et al. 2006). In addition, several groups have reported that Parkin and *PINK1* can physically interact in at least some contexts (Kim et al. 2008; Xiong et al. 2009; Sha et al. 2010). Together, these observations provide compelling in vivo evidence that *PINK1* and *parkin* act in a linear pathway to regulate mitochondrial integrity and maintenance, with *parkin* functioning downstream from *PINK1*.

Several lines of evidence suggest that these observations on *PINK1* and *parkin* function in flies are relevant to humans. First, PD patients who harbor mutations in *PINK1* or *PARKIN* are clinically indistinguishable (Ibanez et al. 2006), and mice lacking both *PINK1* and *parkin* show phenotypes no worse than those of the single mutants (Kitada et al. 2009), consistent with the hypothesis that these genes function in a common genetic pathway. Second, expression of human *PINK1* (Clark et al. 2006; Yang et al. 2006)

or *PARKIN* in *Drosophila* suppresses phenotypes caused by loss of function of *PINK1* or *parkin*, respectively, suggesting that the human and fly proteins are functionally conserved. Third, cells from patients and/or mouse knockout models of *PINK1* or *parkin* also show defects in mitochondrial morphology and/or mitochondrial respiration, particularly in complex I activity in a variety of cell types (Greene et al. 2003; Muftuoglu et al. 2004; Palacino et al. 2004; Exner et al. 2007; Hoepken et al. 2007; Stichel et al. 2007; Gautier et al. 2008; Poole et al. 2008; Wood-Kaczmar et al. 2008; Dagda et al. 2009; Gegg et al. 2009; Morais et al. 2009; Sandebring et al. 2009; Grünwald et al. 2010; Billia et al. 2011; Schmidt et al. 2011; Shim et al. 2011). Fourth, in neurons derived from pluripotent stem cells from PD patients harboring *PINK1* mutations, recruitment of Parkin to mitochondria is impaired (Seibler et al. 2011).

It is important to note that, in contrast to prior toxin models of PD in which dopaminergic neurons are selectively killed, *PINK1* or *parkin* genetic models lead to defects in multiple tissues in addition to those in dopaminergic neurons, which are largely degenerative in nature. Therefore, these genetic models have the potential to uncover new therapies for neuroprotection, as well as targeting the cellular defects that cause cell loss in both dopaminergic neurons and non-dopaminergic tissues.

The *PINK1*/Parkin Pathway Promotes Mitochondrial Fission and/or Inhibits Fusion

How do *PINK1* and *parkin* regulate mitochondrial function? Examination of *PINK1* and *parkin* mutant phenotypes provided an important clue. During *Drosophila* spermatogenesis, mitochondria in spermatids undergo mitochondrial fusion, creating a spherical structure composed of two intertwined mitochondria. Subsequently, the structure unfurls, yielding two mitochondrial derivatives. In both *PINK1* and *parkin* mutants, however, only one mitochondrial derivative is seen, suggesting a defect in mitochondrial fission or an overabundance

of fusion (Riparbelli and Callaini 2006; Deng et al. 2008).

Mitochondria are dynamic and continually undergoing cycles of fission and fusion. Mitochondrial fusion is promoted by *mitofusin* (*mfn*), which is required for outer membrane fusion, and *Optic atrophy 1* (*Opa-1*), which is essential for inner membrane fusion. Mitochondrial fission is promoted by Dynamin-related Protein 1 (Drp1), a predominantly cytoplasmic protein recruited to mitochondria during fission (for review, see Chen and Chan 2009).

Several key findings support the hypothesis that the *PINK1*/*parkin* pathway regulates mitochondrial dynamics. First, mitochondria in *PINK1* or *parkin* mutants are clumped in both dopaminergic neurons and flight muscle. They are also swollen and have disrupted cristae. Second, these cellular defects in mitochondrial morphology, as well as other defects such as the degeneration of flight muscle, cell death, locomotion defects, and a decrease in dopamine levels in fly heads, can be suppressed by increasing the expression of the pro-fission molecules *drp1*, and/or decreasing levels of the pro-fusion molecules *mitofusin* or *opa1* (Deng et al. 2008; Poole et al. 2008; Yang et al. 2008; Park et al. 2009). Third, loss of *PINK1* function leads to defects in assembly of the electron transport chain, which can be rescued by overexpression of *drp1* (Liu et al. 2011). Fourth, heterozygosity for *drp1* is lethal in a *PINK1* mutant background, consistent with the idea that *PINK1* and *drp1* work in the same direction to promote fission (Deng et al. 2008; Poole et al. 2008). That said, it is important to note that the phenotypes associated with loss of *PINK1* or *parkin*, and loss of *drp1*, are distinct (Deng et al. 2008), indicating that *PINK1* and Parkin are not core components of the fission machinery, but instead regulators of the process. Molecular mechanisms of how this is accomplished are suggested in part by studies showing that Parkin can ubiquitinate and degrade Mfn (Poole et al. 2010; Ziviani et al. 2010). Because both cellular defects and organismal defects can be rescued by manipulating mitochondrial dynamics, manipulation of mitochondrial dynamics provides a novel therapeutic strategy.

M. Guo

Observations that point to roles of *PINK1* and *parkin* in regulating mitochondrial morphology have also been obtained in mammalian systems. However, in contrast to the story in *Drosophila*, which is consistent across cell types and laboratories, in mammalian systems various effects have been observed. Some studies suggest that loss of *PINK1* results in fission, with decreased levels of *drp1* resulting in suppression (Exner et al. 2007; Dagda et al. 2009; Lutz et al. 2009; Sandebring et al. 2009). In other studies, however, enlarged mitochondria have been observed in *PINK1* striatal neurons (Gautier et al. 2008), and in COS7 cells mitochondrial phenotypes associated with *PINK1* knockdown are suppressed by *fis1* or *drp1* overexpression, as in *Drosophila* (Yang et al. 2008). Moreover, the *PINK1/parkin* pathway promotes *drp1*-dependent mitochondrial fission and/or inhibits *opa1*-dependent fusion in primary neurons, mammalian hippocampal, and dopaminergic neurons (Yu et al. 2011). In addition, in fibroblasts from patients with *PINK1* or *PARKIN* mutations, Mfn ubiquitination is affected (Rakovic et al. 2011). These studies, in conjunction with those suggesting that Mfn is degraded by Parkin in mammalian cells (Chan et al. 2011; Tanaka et al. 2011), suggest that the *PINK1/parkin* pathway is likely to play a conserved role in mammals. The reasons for the differences in how loss of *PINK1/parkin* results in defects in mitochondrial fusion and fission are not entirely clear. But it is likely that the final mitochondrial morphology phenotype observed in any particular cell type, particularly with respect to the presence or absence of *PINK1/parkin*, will depend on many variables. In any case, what is most important is not the specific morphology observed, but the functional state of the mitochondrial population and the ways in which this is influenced by *PINK1* and *parkin*. Recent observations in mammals and flies detailed below have provided several important insights.

***PINK1* and *Parkin* Promote Mitophagy**

In a series of elegant studies, Youle and colleagues showed that in mammalian cells, Parkin is recruited to mitochondria whose inner mem-

brane has been depolarized (an outcome common to multiple forms of mitochondrial damage), or that have been treated with the herbicide Paraquat, an inducer of complex-1-dependent reactive oxygen species (Narendra et al. 2008). Recruitment of Parkin was followed by removal of these damaged mitochondria through a specialized form of autophagy known as mitophagy, in which mitochondria are specifically degraded following engulfment by autophagosomes (for review, see Goldman et al. 2010). Recent findings suggest that mitophagy is intimately linked with changes in mitochondrial size and shape brought about through fission and fusion (for review, see Hyde et al. 2010). Mitophagy requires both the loss of fusion and the presence of fission. Importantly, decreased mitophagy results in the accumulation of oxidized proteins and decreased cellular respiration, strongly suggesting that the end result of this process is the selective removal of damaged mitochondria (Twig et al. 2008). Mitophagy, but not recruitment of Parkin to depolarized mitochondria, requires Drp1, indicating that Parkin-dependent mitophagy requires fission (Narendra et al. 2008).

Work in flies and mammals further shows that recruitment of Parkin to mitochondria depends on *PINK1* (Narendra et al. 2009; Geisler et al. 2010; Vives-Bauza et al. 2010; Ziviani et al. 2010). Parkin fails to be recruited to depolarized mitochondria in cells lacking *PINK1*. *PINK1*'s ability to recruit Parkin requires *PINK1* kinase activity. In flies, early work suggested a role for *PINK1*-dependent phosphorylation of Parkin as important for recruitment (Kim et al. 2008). Follow-up studies in mammalian cells have yielded conflicting results. In one study, *PINK1* was shown to phosphorylate Parkin, but in a second study, no evidence was found for binding of *PINK1* to Parkin, phosphorylation of Parkin by *PINK1*, or ubiquitination of *PINK1* by Parkin (Vives-Bauza et al. 2010).

How does *PINK1* promote the recruitment of Parkin specifically to damaged mitochondria? *PINK1* protein levels are specifically up-regulated on damaged mitochondria (Narendra et al. 2009; Vives-Bauza et al. 2010; Ziviani et al. 2010). *PINK1* is a membrane protein with its

carboxyl terminus facing the cytoplasm (Zhou et al. 2008). In healthy mitochondria, PINK1 is constitutively cleaved, releasing its carboxy-terminal kinase domain into the cytoplasm, where it is degraded in a proteasome-dependent manner. In damaged mitochondria that have lost their membrane potential, cleavage decreases and full-length PINK1 remains anchored to the membrane (Narendra et al. 2009). Mitochondrial anchorage is all that is required, because tethering of PINK1 to the mitochondrial membrane through other methods is sufficient to recruit Parkin (Narendra et al. 2009). As expected based on these observations, overexpression of *PINK1* in a wild-type background, but not a *parkin* mutant background, is also sufficient to promote Parkin recruitment (Vives-Bauza et al. 2010; Ziviani et al. 2010). In cultured *Drosophila* cells, recruitment of Parkin results in the ubiquitination and removal of Mfn, whereas loss of *PINK1* and *parkin* results in accumulation of Mfn (Poole et al. 2010; Ziviani et al. 2010). Ubiquitination of Mfn may function to prevent outer mitochondrial membrane fusion, thus facilitating the segregation and isolation of damaged mitochondria. Ubiquitinated Mfn may also serve as a signal for mitophagy, because loss of *mitofusin* in *Drosophila* results in decreased recruitment of Parkin to mitochondria (Ziviani et al. 2010), suggesting that these proteins may be involved in recruitment as well.

Once recruited and activated, Parkin has several effects on the mitochondria-associated proteome. In addition to Mfns, many mitochondrial proteins are ubiquitinated and/or degraded in a Parkin-dependent manner. These include Milton and Miro (Chan et al. 2011), Bcl-2 (Chen et al. 2010), components of the TOM mitochondrial protein import complex (Chan et al. 2011; Yoshii et al. 2011), VDAC (Geisler et al. 2010; Narendra et al. 2010a), and Drp-1 (Wang et al. 2011a).

How is PINK1 cleavage regulated? Early work in *Drosophila* showed that PINK1 cleavage required the inner mitochondrial membrane protease rhomboid 7 (Whitworth et al. 2008). This, coupled with an earlier observation that the *rhomboid-7* mutant phenotype includes a loss of mitochondrial fusion (McQuibban et al.

2006), suggested a model in which PINK1's pro-fission activity is negatively regulated through cleavage by rhomboid 7. Early experiments in mammalian systems excluded the rhomboid protease PARL as the protease cleaving PINK1 based on the results of RNAi knockdown (Narendra et al. 2010b). However, more recent observation by the same group (Jin et al. 2010) and others (Deas et al. 2011; Meissner et al. 2011a; Shi et al. 2011) clearly show that PINK1 is cleaved by PARL and that this activity is regulated by mitochondrial membrane potential.

Although these findings are intriguing, it is important to note that the recruitment of Parkin to mitochondria in *Drosophila* and mammalian studies has been performed in cell lines (Ziviani et al. 2010). It remains to be shown that recruitment of Parkin occurs in vivo in tissues that show phenotypes when *PINK1/parkin* are removed, and that this is associated with mitophagy.

The findings that the *PINK1/parkin* pathway regulates mitochondrial dynamics and mitophagy suggest an exciting model in which a failure of mitochondrial quality control lies at the heart of PD pathogenesis. In this model, when mitochondria undergo damage, PINK1 senses the damage, becomes stabilized, and recruits Parkin specifically to the damaged mitochondria. Parkin then promotes degradation of Mitofusin, which prevents these mitochondria from fusing with the network. Parkin-dependent ubiquitination of other substrates then results in mitophagy. In *PINK1/parkin*-mediated PD, damaged mitochondria fail to be cleared, allowing damaged mitochondria, which may carry mutant genomes, to accumulate in cells.

The *PINK1/Parkin* Pathway and Mitochondrial Transport

Observations in *Drosophila* have also led the way in identifying links between *PINK1*, *parkin*, and mitochondrial motility. Mitochondria are not located randomly in cells. They are actively trafficked to regions of the cell where ATP or Ca^{2+} buffering demands are high using a microtubule-based system. Two proteins that mediate microtubule-based transport of mitochondria,

M. Guo

Miro and Milton, were first identified in *Drosophila*, in screens for mutants with defects in synaptic transmission and locomotion (Stowers et al. 2002; Guo et al. 2005). Miro sits in the mitochondrial outer membrane (Fransson et al. 2006), and Milton links Miro-bound mitochondria to Kinesin-1-heavy chain, which promotes mitochondrial movement on microtubules (Glater et al. 2006). Thus, in mutants for these genes, mitochondria accumulate in the soma and fail to be transported to axons and dendrites. Miro also functions as a Ca^{2+} sensor, promoting the release of mitochondria from microtubules in areas of the cell with high levels of intracellular Ca^{2+} (Cai and Sheng 2009).

Recent observations from *Drosophila* and mammalian laboratories show that mitochondrial PINK1 binds Miro (Weihofen et al. 2009; Wang et al. 2011b; Liu et al. 2012). This leads, either through phosphorylation (Wang et al. 2011b) or through a phosphorylation-independent mechanism (Liu et al. 2012), to Parkin-dependent ubiquitination and degradation of Miro, resulting in a loss of mitochondrial movement. Other work has shown that Miro is lost from depolarized mitochondria (Chan et al. 2011), stationary mitochondria are less likely than motile mitochondria to undergo fusion (Twig and Shirihai 2011), and that loss of Miro promotes mitophagy (Liu et al. 2012). Together these observations suggest that PINK1 and Parkin are important regulators of mitochondrial motility, and that one of their functions in mitochondrial quality control is to sequester damaged mitochondria by severing their connections to the microtubule network, preparing them for autophagy. Such an activity is likely to be particularly important in neurons with long processes.

Links between the PINK1/Parkin Pathway and Other PARK Loci

Omi/HtrA2 (PARK13) encodes a serine protease (Vande Walle et al. 2008) that is localized to the mitochondrial matrix. *Omi/HtrA2* has been suggested to function downstream from PINK1 in a common pathway (Plun-Favreau et al. 2007), based on the findings that mammalian

Omi/HtrA2 binds PINK1 and that the phosphorylation of *Omi/HtrA2* is dependent on PINK1 in mammalian cells (Plun-Favreau et al. 2007). One group has also reported the presence of mutations/polymorphisms in *Omi/HtrA2* in sporadic PD patients (Strauss et al. 2005). Loss of *Omi/HtrA2* leads to loss of non-dopaminergic neurons in the striatum (Rathke-Hartlieb et al. 2002; Jones et al. 2003; Martins et al. 2004).

Does *Omi/HtrA2* function as a downstream target of PINK1 in vivo? An overexpression-based genetic interaction is observed between PINK1 and *omi/HtrA2* (Whitworth et al. 2008; Yun et al. 2008). However, in contrast to PINK1 mutants, *omi/HtrA2*-null mutants have normal mitochondrial morphology in both muscle and testes, and a normal number of dopaminergic neurons (Yun et al. 2008). In addition, extensive loss-of-function-based genetic interaction studies fail to provide any in vivo evidence supporting the hypothesis that *Omi/HtrA2* functions in the same pathway, either upstream or downstream of PINK1, to either positively or negatively regulate PINK1. They also do not provide any clear evidence that *Omi/HtrA2* acts in a parallel manner to regulate mitochondrial morphology (Yun et al. 2008). These loss-of-function-based analyses are more relevant to PD than are *Omi/HtrA2* overexpression-based analyses, because reported *Omi/HtrA2* mutations associated with PD are proposed to represent loss-of-function or dominant-negative mutations (Strauss et al. 2005). In addition, mutant forms of the *Drosophila* *Omi/HtrA2* analogous to the reported disease form in sporadic PD patients, and a mutant lacking a serine thought to be a target for PINK1-dependent phosphorylation (Plun-Favreau et al. 2007), retain significant, if not full, *Omi/HtrA2* function in vivo (Yun et al. 2008). Thus, loss-of-function studies strongly suggest that *Omi/HtrA2* does not play an essential role in regulating mitochondrial integrity in the PINK1/parkin pathway in *Drosophila* (Yun et al. 2008). Recent studies in mice show that neurodegeneration in the *Omi/HtrA2* mutant is not rescued by overexpression of *parkin* in mice (Yoshida et al. 2010). The implication of these studies is that *Omi/HtrA2* may not

function in the *PINK1/parkin* pathway. This conclusion is also supported by studies that found no association between mutations in *Omi/HtrA2* and PD, and work showing that the PD-associated mutation in *Omi/HtrA2* occurs with comparable frequency in unaffected populations, suggesting they represent simple polymorphisms (Ross et al. 2008; Simon-Sanchez and Singleton 2008).

Mutations in *DJ-1* (*PARK7*) cause very rare autosomal-recessive forms of PD (Bonifati et al. 2003). Many diverse activities have been proposed for *DJ-1*, including functions as a cysteine protease, a redox-regulated chaperone, a transcription coactivator and RNA-binding protein, and a regulator of survival signaling through interactions with Daxx or the kinase ASK1 (for review, see Wilson 2011). What is clear is that *DJ-1* is cytoprotective and that this activity requires a conserved cysteine. Although *DJ-1* is predominantly cytoplasmic, oxidative stress enhances its association with mitochondria (Canet-Aviles et al. 2004; Blackinton et al. 2005, 2009; Ooe et al. 2005; Lev et al. 2008). In addition, versions of *DJ-1* targeted specifically to mitochondria by fusing a mitochondrial localization sequence to its amino terminus show enhanced cytoprotective functions, suggesting that the mitochondria is one (of perhaps many) important site of *DJ-1* action (Junn et al. 2009). Finally, it is worth noting that triple knockout mice lacking *PINK1*, *parkin*, and *DJ-1* fail to show nigral degeneration, even with aging (Kitada et al. 2009). This somewhat surprising result could reflect action in a common pathway as with *PINK1* and *parkin*. Alternatively, it could reflect developmental compensation, the presence of which is strongly hinted at in experiments using conditional, adult-specific *parkin* knockout mice (Shin et al. 2011; cf. Goldberg et al. 2003; Itier et al. 2003; Von Coelln et al. 2004; Perez and Palmiter 2005).

In *Drosophila*, flies lacking *DJ-1* (deletions) are viable, with their most prominent phenotype being increased sensitivity to oxidative stress as assayed by reduced survival upon Paraquat or rotenone feeding (Menzies et al. 2005; Meulener et al. 2005, 2006). In some studies, overexpression of *DJ-1* fails to rescue *PINK1* mutant muscle phenotypes (Yang et al. 2006)

or male sterility due to lack of *PINK1* or *parkin* (M Guo, unpubl.). In other studies, *Drosophila DJ-1* mutants show mitochondrial defects, but these are in many ways distinct from those associated with loss of *PINK1* or *parkin* (Hao et al. 2010). In addition, although expression of *DJ-1* is capable of rescuing muscle defects due to loss of *PINK1*, it cannot rescue identical-looking muscle defects due to loss of *parkin*. Expression of either *PINK1* or *Parkin* in a *DJ-1* mutant also has the surprising effect of causing organismal lethality. All of these results suggest complex, not linear, interactions between *DJ-1* and the *PINK1/parkin* pathway.

CONTRIBUTIONS OF *DROSOPHILA* AND FUTURE DIRECTIONS

In summary, work in *Drosophila* has provided several key insights in understanding PD. Flies provided the first in vivo evidence that *PINK1* and *parkin* regulate mitochondrial integrity. The stringent epistatic studies provided compelling evidence that *PINK1* and *parkin* function in a common genetic pathway, with *PINK1* positively regulating *parkin*. In contrast, studies have not provided in vivo support for *Omi/HtrA2* as a component of the *PINK1/parkin* pathway. Studies in *Drosophila* also suggest that the *PINK1/parkin* pathway promotes mitochondrial fission and/or inhibits fusion. These studies provide the first demonstration that manipulation of mitochondrial dynamics can suppress *PINK1/parkin* phenotypes both at the cellular level (mitochondrial integrity) and organismal level (muscle degeneration, dopamine levels, and locomotion), thereby providing novel therapeutic targets. More recent work in cultured cells shows that *PINK1* and *parkin* regulate mitophagy in *Drosophila*, paralleling work in mammals and suggesting the exciting possibility that failure of mitophagy, and thus mitochondrial quality control, underlies the pathogenesis of *PINK1/parkin*-mediated PD. The unique attributes of *Drosophila* (robust phenotypes, straightforward genetics providing opportunities for genome-wide genetic screens and drug screens) suggest that *Drosophila* studies will continue to move the field forward and help identify

M. Guo

novel therapies for PD, and potentially other aging-related neurodegenerative disorders. In support of this contention, compounds and a number of genes (either over- or underexpressed, or exogenous) have been identified as suppressors of *PINK1* and/or *parkin* phenotypes in the fly (Greene et al. 2005; Whitworth et al. 2005; Deng et al. 2008; Poole et al. 2008; Yang et al. 2008; Park et al. 2009; Tain et al. 2009; Hao et al. 2010; Imai et al. 2010; Liu and Lu 2010; Fernandes and Rao 2011; Saini et al. 2011; Koh et al. 2012; Vilain et al. 2012). Given that defects in mitochondria accumulate in normal aging as well as in PD, identifying multiple ways of activating mitophagy may be generally useful therapeutically in many diseases of aging. In short, *Drosophila* provides an indispensable and unique opportunity to contribute to the PD field.

ACKNOWLEDGMENTS

I apologize to those whose work has not been cited because of space constraints. I thank the Guo laboratory members and Bruce Hay for discussions. This work is supported by grants and funds from the National Institutes of Health (R01, K02), the Glenn Family Foundation, the Esther A. and Joseph Klingenstein Fellowship (Robert H. Ebert Clinical Scholar), and the McKnight Foundation of Neuroscience.

REFERENCES

- Abou-Sleiman PM, Muqit MM, Wood NW. 2006. Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Nat Rev Neurosci* **7**: 207–219.
- Adams MD, Sekelsky JJ. 2002. From sequence to phenotype: Reverse genetics in *Drosophila melanogaster*. *Nat Rev Genet* **3**: 189–198.
- Bellen HJ, Levis RW, Liao G, He Y, Carlson JW, Tsang G, Evans-Holm M, Hiesinger PR, Schulze KL, Rubin GM, et al. 2004. The BDGP gene disruption project: Single transposon insertions associated with 40% of *Drosophila* genes. *Genetics* **167**: 761–781.
- Bellen HJ, Tong C, Tsuda H. 2010. 100 years of *Drosophila* research and its impact on vertebrate neuroscience: A history lesson for the future. *Nat Rev Neurosci* **11**: 514–522.
- Bier E. 2005. *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat Rev Genet* **6**: 9–23.
- Billia F, Hauck L, Konecny F, Rao V, Shen J, Mak TW. 2011. PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci* **108**: 9572–9577.
- Blackinton J, Ahmad R, Miller DW, van der Brug MP, Canet-Aviles RM, Hague SM, Kaleem M, Cookson MR. 2005. Effects of DJ-1 mutations and polymorphisms on protein stability and subcellular localization. *Brain Res Mol Brain Res* **134**: 76–83.
- Blackinton J, Lakshminarasimhan M, Thomas KJ, Ahmad R, Greggio E, Raza AS, Cookson MR, Wilson MA. 2009. Formation of a stabilized cysteine sulfinic acid is critical for the mitochondrial function of the parkinsonism protein DJ-1. *J Biol Chem* **284**: 6476–6485.
- Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, et al. 2003. Mutations in the *DJ-1* gene associated with autosomal recessive early-onset parkinsonism. *Science* **299**: 256–259.
- Bove J, Prou D, Perier C, Przedborski S. 2005. Toxin-induced models of Parkinson's disease. *NeuroRx* **2**: 484–494.
- Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. 2003. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* **24**: 197–211.
- Brand AH, Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**: 401–415.
- Cai Q, Sheng ZH. 2009. Moving or stopping mitochondria: Miro as a traffic cop by sensing calcium. *Neuron* **61**: 493–496.
- Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D, Petsko GA, Cookson MR. 2004. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc Natl Acad Sci* **101**: 9103–9108.
- Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL, Hess S, Chan DC. 2011. Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Hum Mol Genet* **20**: 1726–1737.
- Chartier-Harlin MC, Dachsel JC, Vilarino-Guell C, Lincoln SJ, LePrete F, Hulihan MM, Kachergus J, Milnerwood AJ, Tapia L, Song MS, et al. 2011. Translation initiator EIF4G1 mutations in familial Parkinson disease. *Am J Hum Genet* **89**: 398–406.
- Chen H, Chan DC. 2009. Mitochondrial dynamics—fusion, fission, movement, and mitophagy—in neurodegenerative diseases. *Hum Mol Genet* **18**: R169–R176.
- Chen D, Gao F, Li B, Wang H, Xu Y, Zhu C, Wang G. 2010. Parkin mono-ubiquitinates Bcl-2 and regulates autophagy. *J Biol Chem* **285**: 38214–38223.
- Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA, Guo M. 2006. *Drosophila pink1* is required for mitochondrial function and interacts genetically with parkin. *Nature* **441**: 1162–1166.
- Coulom H, Birman S. 2004. Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*. *J Neurosci* **24**: 10993–10998.
- Dagda RK, Cherra SJ III, Kulich SM, Tandon A, Park D, Chu CT. 2009. Loss of PINK1 function promotes mitophagy

- through effects on oxidative stress and mitochondrial fission. *J Biol Chem* **284**: 13843–13855.
- Dauer W, Przedborski S. 2003. Parkinson's disease: Mechanisms and models. *Neuron* **39**: 889–909.
- Dawson TM, Dawson VL. 2010. The role of parkin in familial and sporadic Parkinson's disease. *Mov Disord* **25**: S32–S39.
- Dawson TM, Ko HS, Dawson VL. 2010. Genetic animal models of Parkinson's disease. *Neuron* **66**: 646–661.
- Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, Loh SH, Renton AE, Harvey RJ, Whitworth AJ, Martins LM, et al. 2011. PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet* **20**: 867–879.
- del Valle Rodriguez A, Didiano D, Desplan C. 2012. Power tools for gene expression and clonal analysis in *Drosophila*. *Nat Methods* **9**: 47–55.
- Deng H, Dodson MW, Huang H, Guo M. 2008. The Parkinson's disease genes *pink1* and *parkin* promote mitochondrial fission and/or inhibit fusion in *Drosophila*. *Proc Natl Acad Sci* **105**: 14503–14508.
- Dietzl G, Chen D, Schnorrrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblaue S, et al. 2007. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* **448**: 151–156.
- Dodson MW, Guo M. 2007. Pink1, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson's disease. *Curr Opin Neurobiol* **17**: 331–337.
- Exner N, Treske B, Paquet D, Holmstrom K, Schiesling C, Gispert S, Carballo-Carbajal I, Berg D, Hoepken HH, Gasser T, et al. 2007. Loss-of-function of human *PINK1* results in mitochondrial pathology and can be rescued by *parkin*. *J Neurosci* **27**: 12413–12418.
- Feany MB, Bender WW. 2000. A *Drosophila* model of Parkinson's disease. *Nature* **404**: 394–398.
- Fernandes C, Rao Y. 2011. Genome-wide screen for modifiers of Parkinson's disease genes in *Drosophila*. *Mol Brain* **4**: 17.
- Fransson S, Ruusala A, Aspenstrom P. 2006. The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochem Biophys Res Commun* **344**: 500–510.
- Gautier CA, Kitada T, Shen J. 2008. Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. *Proc Natl Acad Sci* **105**: 11364–11369.
- Gegg ME, Cooper JM, Schapira AH, Taanman JW. 2009. Silencing of PINK1 expression affects mitochondrial DNA and oxidative phosphorylation in dopaminergic cells. *PLoS ONE* **4**: e4756.
- Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. 2010. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* **12**: 119–131.
- Gladstone M, Su TT. 2011. Chemical genetics and drug screening in *Drosophila* cancer models. *J Genet Genomics* **38**: 497–504.
- Glater EE, Megeath LJ, Stowers RS, Schwarz TL. 2006. Axonal transport of mitochondria requires Milton to recruit kinesin heavy chain and is light chain independent. *J Cell Biol* **173**: 545–557.
- Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, Meloni EG, Wu N, Ackerson LC, Klapstein GJ, et al. 2003. Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. *J Biol Chem* **278**: 43628–43635.
- Goldman SJ, Taylor R, Zhang Y, Jin S. 2010. Autophagy and the degradation of mitochondria. *Mitochondrion* **10**: 309–315.
- Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. 2003. Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants. *Proc Natl Acad Sci* **100**: 4078–4083.
- Greene JC, Whitworth AJ, Andrews LA, Parker TJ, Pallanck LJ. 2005. Genetic and genomic studies of *Drosophila parkin* mutants implicate oxidative stress and innate immune responses in pathogenesis. *Hum Mol Genet* **14**: 799–811.
- Grünwald A, Voges L, Rakovic A, Kasten M, Vandebona H, Hemmelmann C, Lohmann K, Orolicki S, Ramirez A, Schapira AH, et al. 2010. Mutant Parkin impairs mitochondrial function and morphology in human fibroblasts. *PLoS ONE* **5**: e12962.
- Guo M. 2010. What have we learned from *Drosophila* models of Parkinson's disease? *Prog Brain Res* **184**: 3–16.
- Guo X, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfeld M, Marin L, Charlton MP, Atwood HL, Zinsmaier KE. 2005. The GTPase dMiro is required for axonal transport of mitochondria to *Drosophila* synapses. *Neuron* **47**: 379–393.
- Hao LY, Giasson BI, Bonini NM. 2010. DJ-1 is critical for mitochondrial function and rescues PINK1 loss of function. *Proc Natl Acad Sci* **107**: 9747–9752.
- Hardy J, Lewis P, Revesz T, Lees A, Paisan-Ruiz C. 2009. The genetics of Parkinson's syndromes: A critical review. *Curr Opin Genet Dev* **19**: 254–265.
- Hay BA, Guo M. 2006. Caspase-dependent cell death in *Drosophila*. *Annu Rev Cell Dev Biol* **22**: 623–650.
- Hay BA, Huh JR, Guo M. 2004. The genetics of cell death: Approaches, insights and opportunities in *Drosophila*. *Nat Rev Genet* **5**: 911–922.
- Hoepken HH, Gispert S, Morales B, Wingerter O, Del Turco D, Mulsch A, Nussbaum RL, Muller K, Drose S, Brandt U, et al. 2007. Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. *Neurobiol Dis* **25**: 401–411.
- Hyde BB, Twig G, Shirihai OS. 2010. Organellar vs cellular control of mitochondrial dynamics. *Semin Cell Dev Biol* **21**: 575–581.
- Ibanez P, Lesage S, Lohmann E, Thobois S, De Michele G, Borg M, Agid Y, Durr A, Brice A. 2006. Mutational analysis of the *PINK1* gene in early-onset parkinsonism in Europe and North Africa. *Brain* **129**: 686–694.
- Imai Y, Kanao T, Sawada T, Kobayashi Y, Moriwaki Y, Ishida Y, Takeda K, Ichijo H, Lu B, Takahashi R. 2010. The loss of PGAM5 suppresses the mitochondrial degeneration caused by inactivation of PINK1 in *Drosophila*. *PLoS Genet* **6**: e1001229.
- Itier JM, Ibanez P, Mena MA, Abbas N, Cohen-Salmon C, Bohme GA, Laville M, Pratt J, Corti O, Pradier L, et al. 2003. Parkin gene inactivation alters behaviour and

M. Guo

- dopamine neurotransmission in the mouse. *Hum Mol Genet* **12**: 2277–2291.
- Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. 2010. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *J Cell Biol* **191**: 933–942.
- Jones JM, Datta P, Srinivasula SM, Ji W, Gupta S, Zhang Z, Davies E, Hajnoczky G, Saunders TL, Van Keuren ML, et al. 2003. Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. *Nature* **425**: 721–727.
- Junn E, Jang WH, Zhao X, Jeong BS, Mouradian MM. 2009. Mitochondrial localization of DJ-1 leads to enhanced neuroprotection. *J Neurosci Res* **87**: 123–129.
- Kim Y, Park J, Kim S, Song S, Kwon SK, Lee SH, Kitada T, Kim JM, Chung J. 2008. PINK1 controls mitochondrial localization of Parkin through direct phosphorylation. *Biochem Biophys Res Commun* **377**: 975–980.
- Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. 1998. Mutations in the *parkin* gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**: 605–608.
- Kitada T, Tong Y, Gautier CA, Shen J. 2009. Absence of nigral degeneration in aged parkin/DJ-1/PINK1 triple knock-out mice. *J Neurochem* **111**: 696–702.
- Koh H, Kim H, Kim MJ, Park J, Lee HJ, Chung J. 2012. Silent information regulator 2 (Sir2) and Forkhead box O (FOXO) complement mitochondrial dysfunction and dopaminergic neuron loss in *Drosophila* Pten-induced kinase 1 (PINK1) null mutant. *J Biol Chem* **278**: 12750–12758.
- Langston JW, Ballard P, Tetrud JW, Irwin I. 1983. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**: 979–980.
- Lessing D, Bonini NM. 2009. Maintaining the brain: Insight into human neurodegeneration from *Drosophila melanogaster* mutants. *Nat Rev Genet* **10**: 359–370.
- Lev N, Ickowicz D, Melamed E, Offen D. 2008. Oxidative insults induce DJ-1 upregulation and redistribution: Implications for neuroprotection. *Neurotoxicology* **29**: 397–405.
- Linazasoro G. 2004. Recent failures of new potential symptomatic treatments for Parkinson's disease: Causes and solutions. *Mov Disord* **19**: 743–754.
- Liu S, Lu B. 2010. Reduction of protein translation and activation of autophagy protect against PINK1 pathogenesis in *Drosophila melanogaster*. *PLoS Genet* **6**: e1001237.
- Liu W, Acin-Perez R, Geghman KD, Manfredi G, Lu B, Li C. 2011. Pink1 regulates the oxidative phosphorylation machinery via mitochondrial fission. *Proc Natl Acad Sci* **108**: 12920–12924.
- Liu S, Sawada T, Lee S, Yu W, Silverio G, Alapatt P, Millan I, Shen A, Saxton W, Kanao T, et al. 2012. Parkinson's disease-associated kinase PINK1 regulates Miro protein level and axonal transport of mitochondria. *PLoS Genet* **8**: e1002537.
- Lu B, Vogel H. 2009. *Drosophila* models of neurodegenerative diseases. *Annu Rev Pathol* **4**: 315–342.
- Lutz AK, Exner N, Fett ME, Schlehe JS, Kloos K, Lammermann K, Brunner B, Kurz-Drexler A, Vogel F, Reichert AS, et al. 2009. Loss of *parkin* or *PINK1* function increases Drp1-dependent mitochondrial fragmentation. *J Biol Chem* **284**: 22938–22951.
- Martin I, Dawson VL, Dawson TM. 2011. Recent advances in the genetics of Parkinson's disease. *Annu Rev Genomics Hum Genet* **12**: 301–325.
- Martins LM, Morrison A, Klupsch K, Fedele V, Moiso N, Teismann P, Abuin A, Grau E, Geppert M, Livi GP, et al. 2004. Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol Cell Biol* **24**: 9848–9862.
- McQuibban GA, Lee JR, Zheng L, Juusola M, Freeman M. 2006. Normal mitochondrial dynamics requires rhomboid-7 and affects *Drosophila* lifespan and neuronal function. *Curr Biol* **16**: 982–989.
- Meissner C, Lorenz H, Weihofen A, Selkoe DJ, Lemberg MK. 2011a. The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *J Neurochem* **117**: 856–867.
- Meissner WG, Frasier M, Gasser T, Goetz CG, Lozano A, Piccini P, Obeso JA, Rascol O, Schapira A, Voon V, et al. 2011b. Priorities in Parkinson's disease research. *Nat Rev Drug Discov* **10**: 377–393.
- Menzies FM, Yenisseti SC, Min KT. 2005. Roles of *Drosophila* DJ-1 in survival of dopaminergic neurons and oxidative stress. *Curr Biol* **15**: 1578–1582.
- Meulener M, Whitworth AJ, Armstrong-Gold CE, Rizzu P, Heutink P, Wes PD, Pallanck LJ, Bonini NM. 2005. *Drosophila* DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. *Curr Biol* **15**: 1572–1577.
- Meulener MC, Xu K, Thomson L, Ischiropoulos H, Bonini NM. 2006. Mutational analysis of DJ-1 in *Drosophila* implicates functional inactivation by oxidative damage and aging. *Proc Natl Acad Sci* **103**: 12517–12522.
- Morais VA, Verstreken P, Roethig A, Smet J, Snellinx A, Vanbrabant M, Haddad D, Frezza C, Mandemakers W, Vogt-Weisenhorn D, et al. 2009. Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function. *EMBO Mol Med* **1**: 99–111.
- Muftuoglu M, Elilob B, Dalmizrak O, Ercan A, Kulaksiz G, Ogun H, Dalkara T, Ozer N. 2004. Mitochondrial complex I and IV activities in leukocytes from patients with *parkin* mutations. *Mov Disord* **19**: 544–548.
- Mukhopadhyay D, Riezman H. 2007. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* **315**: 201–205.
- Narendra D, Tanaka A, Suen DF, Youle RJ. 2008. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* **183**: 795–803.
- Narendra D, Tanaka A, Suen DF, Youle RJ. 2009. Parkin-induced mitophagy in the pathogenesis of Parkinson disease. *Autophagy* **5**: 706–708.
- Narendra D, Kane LA, Hauser DN, Fearnley IM, Youle RJ. 2010a. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy* **6**: 1090–1106.
- Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, Cookson MR, Youle RJ. 2010b. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* **8**: e1000298.

- Nassel DR, Elekes K. 1992. Aminergic neurons in the brain of blowflies and *Drosophila*: Dopamine- and tyrosine hydroxylase-immunoreactive neurons and their relationship with putative histaminergic neurons. *Cell Tissue Res* **267**: 147–167.
- Ni JQ, Zhou R, Czech B, Liu LP, Holderbaum L, Yang-Zhou D, Shim HS, Tao R, Handler D, Karpowicz P, et al. 2011. A genome-scale shRNA resource for transgenic RNAi in *Drosophila*. *Nat Methods* **8**: 405–407.
- Ooe H, Taira T, Iguchi-Ariga SM, Ariga H. 2005. Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1. *Toxicol Sci* **88**: 114–126.
- Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, Lopez de Munain A, Aparicio S, Gil AM, Khan N, et al. 2004. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* **44**: 595–600.
- Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, Klose J, Shen J. 2004. Mitochondrial dysfunction and oxidative damage in *parkin*-deficient mice. *J Biol Chem* **279**: 18614–18622.
- Pandey UB, Nichols CD. 2011. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacol Rev* **63**: 411–436.
- Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, et al. 2006. Mitochondrial dysfunction in *Drosophila PINK1* mutants is complemented by *parkin*. *Nature* **441**: 1157–1161.
- Park J, Lee G, Chung J. 2009. The PINK1–Parkin pathway is involved in the regulation of mitochondrial remodeling process. *Biochem Biophys Res Commun* **378**: 518–523.
- Perez FA, Palmiter RD. 2005. Parkin-deficient mice are not a robust model of parkinsonism. *Proc Natl Acad Sci* **102**: 2174–2179.
- Pesah Y, Pham T, Burgess H, Middlebrooks B, Verstreken P, Zhou Y, Harding M, Bellen H, Mardon G. 2004. *Drosophila parkin* mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development* **131**: 2183–2194.
- Plun-Favreau H, Klupsch K, Moiso N, Gandhi S, Kjaer S, Frith D, Harvey K, Deas E, Harvey RJ, McDonald N, et al. 2007. The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. *Nat Cell Biol* **9**: 1243–1252.
- Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, et al. 1997. Mutation in the α -synuclein gene identified in families with Parkinson's disease. *Science* **276**: 2045–2047.
- Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ. 2008. The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci* **105**: 1638–1643.
- Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L. 2010. The mitochondrial fusion-promoting factor Mitofusin is a substrate of the PINK1/Parkin pathway. *PLoS ONE* **5**: e10054.
- Rakovic A, Grunewald A, Kottwitz J, Bruggemann N, Pramstaller PP, Lohmann K, Klein C. 2011. Mutations in PINK1 and Parkin impair ubiquitination of Mitofusins in human fibroblasts. *PLoS ONE* **6**: e16746.
- Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J, et al. 2006. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet* **38**: 1184–1191.
- Rathke-Hartlieb S, Schlomann U, Heimann P, Meisler MH, Jockusch H, Bartsch JW. 2002. Progressive loss of striatal neurons causes motor dysfunction in MND2 mutant mice and is not prevented by Bcl-2. *Exp Neurol* **175**: 87–97.
- Riparbelli MG, Gallaini G. 2006. The *Drosophila parkin* homologue is required for normal mitochondrial dynamics during spermiogenesis. *Dev Biol* **303**: 108–120.
- Rong YS, Golik KG. 2000. Gene targeting by homologous recombination in *Drosophila*. *Science* **288**: 2013–2018.
- Rorth P. 1996. A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc Natl Acad Sci* **124**: 12418–12422.
- Ross OA, Soto AI, Vilarino-Guell C, Heckman MG, Diehl NN, Hulihan MM, Aasly JO, Sando S, Gibson JM, Lynch T, et al. 2008. Genetic variation of Omi/HtrA2 and Parkinson's disease. *Parkinsonism Relat Disord* **14**: 539–543.
- Rubin GM. 2000. Biological annotation of the *Drosophila* genome sequence. *Novartis Found Symp* **229**: 79–82.
- Saini N, Georgiev O, Schaffner W. 2011. The *parkin* mutant phenotype in the fly is largely rescued by metal-responsive transcription factor (MTF-1). *Mol Cell Biol* **31**: 2151–2161.
- Sandebring A, Thomas KJ, Beilina A, van der Brug M, Cleland MM, Ahmad R, Miller DW, Zambrano I, Cowburn RF, Behbahani H, et al. 2009. Mitochondrial alterations in PINK1 deficient cells are influenced by calcineurin-dependent dephosphorylation of dynamin-related protein 1. *PLoS ONE* **4**: e5701.
- Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, et al. 2009. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet* **41**: 1303–1307.
- Schmidt S, Linnartz B, Mendritzki S, Sczepan T, Lubbert M, Stichel CC, Lubbert H. 2011. Genetic mouse models for Parkinson's disease display severe pathology in glial cell mitochondria. *Hum Mol Genet* **20**: 1197–1211.
- Schneider SA, Paisan-Ruiz C, Quinn NP, Lees AJ, Houlden H, Hardy J, Bhatia KP. 2010. ATP13A2 mutations (PARK9) cause neurodegeneration with brain iron accumulation. *Mov Disord* **25**: 979–984.
- Seibler P, Graziotto J, Jeong H, Simunovic F, Klein C, Krainc D. 2011. Mitochondrial Parkin recruitment is impaired in neurons derived from mutant PINK1 induced pluripotent stem cells. *J Neurosci* **31**: 5970–5976.
- Sha D, Chin LS, Li L. 2010. Phosphorylation of Parkin by Parkinson disease-linked kinase PINK1 activates Parkin E3 ligase function and NF- κ B signaling. *Hum Mol Genet* **19**: 352–363.
- Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT. 2003. Mechanism of toxicity in rotenone models of Parkinson's disease. *J Neurosci* **23**: 10756–10764.

M. Guo

- Shi G, Lee JR, Grimes DA, Racacho L, Ye D, Yang H, Ross OA, Farrer M, McQuibban GA, Bulman DE. 2011. Functional alteration of PARL contributes to mitochondrial dysregulation in Parkinson's disease. *Hum Mol Genet* **20**: 1966–1974.
- Shim JH, Yoon SH, Kim KH, Han JY, Ha JY, Hyun DH, Paek SH, Kang UJ, Zhuang X, Son JH. 2011. The antioxidant Trolox helps recovery from the familial Parkinson's disease-specific mitochondrial deficits caused by PINK1- and DJ-1-deficiency in dopaminergic neuronal cells. *Mitochondrion* **11**: 707–715.
- Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, Troconso JC, Dawson VL, Dawson TM. 2011. PARIS (ZNF746) repression of PGC-1 α contributes to neurodegeneration in Parkinson's disease. *Cell* **144**: 689–702.
- Shulman JM, De Jager PL, Feany MB. 2011. Parkinson's disease: Genetics and pathogenesis. *Annu Rev Pathol* **6**: 193–222.
- Simon-Sanchez J, Singleton AB. 2008. Sequencing analysis of OMI/HTRA2 shows previously reported pathogenic mutations in neurologically normal controls. *Hum Mol Genet* **17**: 1988–1993.
- Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, et al. 2009. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet* **41**: 1308–1312.
- Simuni T, Sethi K. 2008. Nonmotor manifestations of Parkinson's disease. *Ann Neurol* **64**: S65–S80.
- Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, et al. 2003. α -Synuclein locus triplication causes Parkinson's disease. *Science* **302**: 841.
- Stichel CC, Zhu XR, Bader V, Linnartz B, Schmidt S, Lubbert H. 2007. Mono- and double-mutant mouse models of Parkinson's disease display severe mitochondrial damage. *Hum Mol Genet* **16**: 2377–2393.
- St Johnston D. 2002. The art and design of genetic screens: *Drosophila melanogaster*. *Nat Rev Genet* **3**: 176–188.
- Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, Schwarz TL. 2002. Axonal transport of mitochondria to synapses depends on Milton, a novel *Drosophila* protein. *Neuron* **36**: 1063–1077.
- Strauss KM, Martins LM, Plun-Favreau H, Marx FP, Kautzmann S, Berg D, Gasser T, Wszolek Z, Muller T, Bornemann A, et al. 2005. Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. *Hum Mol Genet* **14**: 2099–2111.
- Tain LS, Mortiboys H, Tao RN, Ziviani E, Bandmann O, Whitworth AJ. 2009. Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. *Nat Neurosci* **12**: 1129–1135.
- Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M, Youle RJ. 2011. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* **191**: 1367–1380.
- Thibault ST, Singer MA, Miyazaki WY, Milash B, Dompe NA, Singh CM, Buchholz R, Demsky M, Fawcett R, Francis-Lang HL, et al. 2004. A complementary transposon tool kit for *Drosophila melanogaster* using P and piggyBac. *Nat Genet* **36**: 283–287.
- Trinh K, Moore K, Wes PD, Muchowski PJ, Dey J, Andrews L, Pallanck LJ. 2008. Induction of the phase II detoxification pathway suppresses neuron loss in *Drosophila* models of Parkinson's disease. *J Neurosci* **28**: 465–472.
- Twig G, Shirihai OS. 2011. The interplay between mitochondrial dynamics and mitophagy. *Antioxid Redox Signal* **14**: 1939–1951.
- Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, et al. 2008. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* **27**: 433–446.
- Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, et al. 2004. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**: 1158–1160.
- Vande Walle L, Lamkanfi M, Vandenabeele P. 2008. The mitochondrial serine protease HtrA2/Omi: An overview. *Cell Death Differ* **15**: 453–460.
- Venken KJ, Bellen HJ. 2005. Emerging technologies for gene manipulation in *Drosophila melanogaster*. *Nat Rev Genet* **6**: 167–178.
- Venken KJ, Bellen HJ. 2007. Transgenesis upgrades for *Drosophila melanogaster*. *Development* **134**: 3571–3584.
- Vilain S, Esposito G, Haddad D, Schaap O, Dobrev MP, Vos M, Van Meensel S, Morais VA, De Strooper B, Verstreken P. 2012. The yeast complex I equivalent NADH dehydrogenase rescues pink1 mutants. *PLoS Genet* **8**: e1002456.
- Vilarino-Guell C, Wider C, Ross OA, Dachtel JC, Kachergus JM, Lincoln SJ, Soto-Ortolaza AI, Cobb SA, Wilhoite GJ, Bacon JA, et al. 2011. VPS35 mutations in Parkinson disease. *Am J Hum Genet* **89**: 162–167.
- Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, May J, Tocilescu MA, Liu W, Ko HS, et al. 2010. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci* **107**: 378–383.
- Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, Dawson VL, Dawson TM. 2004. Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc Natl Acad Sci* **101**: 10744–10749.
- Wang H, Song P, Du L, Tian W, Yue W, Liu M, Li D, Wang B, Zhu Y, Cao C, et al. 2011a. Parkin ubiquitinates Drp1 for proteasome-dependent degradation: Implication of dysregulated mitochondrial dynamics in Parkinson disease. *J Biol Chem* **286**: 11649–11658.
- Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL. 2011b. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell* **147**: 893–906.
- Weihofen A, Thomas KJ, Ostaszewski BL, Cookson MR, Selkoe DJ. 2009. Pink1 forms a multiprotein complex with Miro and Milton, linking Pink1 function to mitochondrial trafficking. *Biochemistry* **48**: 2045–2052.
- Wesolowska N, Rong YS. 2010. The past, present and future of gene targeting in *Drosophila*. *Fly (Austin)* **4**: 53–59.
- West AB, Dawson VL, Dawson TM. 2007. The role of Parkin in Parkinson's disease. In *Parkinson's disease: Genetics and pathogenesis* (ed. Dawson TM), pp. 199–218. Informa Healthcare, New York.

- Whitworth AJ. 2011. *Drosophila* models of Parkinson's disease. *Adv Genet* **73**: 1–50.
- Whitworth AJ, Theodore DA, Greene JC, Benes H, Wes PD, Pallanck LJ. 2005. Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease. *Proc Natl Acad Sci* **102**: 8024–8029.
- Whitworth AJ, Lee JR, Ho VM, Flick R, Chowdhury R, McQuibban GA. 2008. Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin. *Dis Model Mech* **1**: 168–174.
- Wilson MA. 2011. The role of cysteine oxidation in DJ-1 function and dysfunction. *Antioxid Redox Signal* **15**: 111–122.
- Wood-Kaczmar A, Gandhi S, Yao Z, Abramov AY, Miljan EA, Keen G, Stanyer L, Hargreaves I, Klupsch K, Deas E, et al. 2008. PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PLoS ONE* **3**: e2455.
- Xiong H, Wang D, Chen L, Choo YS, Ma H, Tang C, Xia K, Jiang W, Ronai Z, Zhuang X, et al. 2009. Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. *J Clin Invest* **119**: 650–660.
- Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, Yang L, Beal MF, Vogel H, Lu B. 2006. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. *Proc Natl Acad Sci* **103**: 10793–10798.
- Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel H, Lu B. 2008. Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci* **105**: 7070–7075.
- Yoshida T, Mizuta T, Shimizu S. 2010. Neurodegeneration in *mnd2* mutant mice is not prevented by parkin transgene. *Biochem Biophys Res Commun* **402**: 676–679.
- Yoshii SR, Kishi C, Ishihara N, Mizushima N. 2011. Parkin mediates proteasome-dependent protein degradation and rupture of the outer mitochondrial membrane. *J Biol Chem* **286**: 19630–19640.
- Yu W, Sun Y, Guo S, Lu B. 2011. The PINK1/Parkin pathway regulates mitochondrial dynamics and function in mammalian hippocampal and dopaminergic neurons. *Hum Mol Genet* **20**: 3227–3240.
- Yun J, Cao JH, Dodson MW, Clark IE, Kapahi P, Chowdhury RB, Guo M. 2008. Loss-of-function analysis suggests that Omi/HtrA2 is not an essential component of the PINK1/PARKIN pathway in vivo. *J Neurosci* **28**: 14500–14510.
- Zhou C, Huang Y, Shao Y, May J, Prou D, Perier C, Dauer W, Schon EA, Przedborski S. 2008. The kinase domain of mitochondrial PINK1 faces the cytoplasm. *Proc Natl Acad Sci* **105**: 12022–12027.
- Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, et al. 2004. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* **44**: 601–607.
- Zimprich A, Benet-Pages A, Struhal W, Graf E, Eck SH, Offman MN, Haubenberger D, Spielberger S, Schulte EC, Lichtner P, et al. 2011. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am J Hum Genet* **89**: 168–175.
- Ziviani E, Tao RN, Whitworth AJ. 2010. *Drosophila* parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci* **107**: 5018–5023.
- Zoghbi HY, Warren ST. 2010. Neurogenetics: Advancing the “next-generation” of brain research. *Neuron* **68**: 165–173.

***Drosophila* as a Model to Study Mitochondrial Dysfunction in Parkinson's Disease**

Ming Guo

Cold Spring Harb Perspect Med 2012; doi: 10.1101/cshperspect.a009944 originally published online September 28, 2012

Subject Collection [Parkinson's Disease](#)

Functional Neuroanatomy of the Basal Ganglia

José L. Lanciego, Natasha Luquin and José A. Obeso

Animal Models of Parkinson's Disease: Vertebrate Genetics

Yunjong Lee, Valina L. Dawson and Ted M. Dawson

Innate Inflammation in Parkinson's Disease

V. Hugh Perry

Parkinson's Disease and Parkinsonism: Neuropathology

Dennis W. Dickson

Physiological Phenotype and Vulnerability in Parkinson's Disease

D. James Surmeier, Jaime N. Guzman, Javier Sanchez, et al.

Clinical Approach to Parkinson's Disease: Features, Diagnosis, and Principles of Management

João Massano and Kailash P. Bhatia

The Role of Autophagy in Parkinson's Disease

Melinda A. Lynch-Day, Kai Mao, Ke Wang, et al.

Disruption of Protein Quality Control in Parkinson's Disease

Casey Cook, Caroline Stetler and Leonard Petrucelli

***Drosophila* as a Model to Study Mitochondrial Dysfunction in Parkinson's Disease**

Ming Guo

Parkinsonism Due to Mutations in PINK1, Parkin, and DJ-1 and Oxidative Stress and Mitochondrial Pathways

Mark R. Cookson

Programmed Cell Death in Parkinson's Disease

Katerina Venderova and David S. Park

Genomics and Bioinformatics of Parkinson's Disease

Sonja W. Scholz, Tim Mhyre, Habtom Ressom, et al.

Motor Control Abnormalities in Parkinson's Disease

Pietro Mazzoni, Britne Shabbott and Juan Camilo Cortés

Parkinson's Disease: Gene Therapies

Philippe G. Coune, Bernard L. Schneider and Patrick Aebischer

Functional Neuroimaging in Parkinson's Disease

Martin Niethammer, Andrew Feigin and David Eidelberg

Leucine-Rich Repeat Kinase 2 for Beginners: Six Key Questions

Lauren R. Kett and William T. Dauer

For additional articles in this collection, see <http://perspectivesinmedicine.cshlp.org/cgi/collection/>