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Seeking an *In Vivo* Neuronal Context for the PINK1/Parkin Pathway

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Review of Sung et al.

Despite the passage of nearly two centuries since James Parkinson penned his seminal work, “An Essay on the Shaking Palsy,” the neurobiology of the disease that now shares his name remains unclear (Parkinson, 2002). Although the majority of Parkinson's disease (PD) cases are idiopathic, advances in sequencing technology at the turn of the century allowed for the identification of five genes mutated in familial PD. These include *PARK2*, which encodes the E3 ubiquitin ligase Parkin, and *PARK6*, which encodes phosphatase and tensin homolog-induced kinase 1 (PINK1) (Kitada et al., 1998; Valente et al., 2004). In the last two decades, researchers have sought to understand the role of these proteins in normal neuronal systems and PD pathogenesis.

Investigations into the function of PINK1 and Parkin have revealed a complex signaling pathway for targeted removal of damaged mitochondria through autophagy machinery, or mitophagy (Nguyen et al., 2016). However, much of the investi-

gation into PINK1/Parkin activity has been performed in non-neuronal cells. Because PD is fundamentally a disease affecting neurons in the substantia nigra pars compacta, understanding PINK1/Parkin activity in neurons is a current challenge in the field.

A large body of *in vitro* data suggests that PINK1 and Parkin mutations heavily disrupt mitochondrial dynamics. Mitochondria form a complex and dynamic network throughout the cell. This network is carefully maintained through a balance of fission and fusion events (van der Bliek et al., 2013). The mitochondrial network is able to shift the balance between fragmented (more fission, less fusion) and filamentous (more fusion, less fission) in response to the needs of the cell (Youle and van der Bliek, 2012). To respond to stress conditions, the E3 ubiquitin ligase activity of Parkin is activated by PINK1 and targets a plethora of mitochondria-associated proteins (Pickrell and Youle, 2015). Activation of the PINK1/Parkin pathway leads to an increase in mitochondrial fragmentation, thought to be caused by the targeted degradation of key mitochondrial fusion proteins (mitofusins), the large GTPases Mfn1 and Mfn2 (Tanaka et al., 2010).

Previous work in cultured neurons suggested that PINK1 and Parkin primarily regulate the last stage of mitochondrial lifespan, namely, mitophagy. Although

PINK1/Parkin-mediated mitophagy was shown to occur in axons of cultured neurons (Ashrafi et al., 2014; Bingol et al., 2014), there is still debate about whether these mechanisms take place in neurons *in vivo*. In *Drosophila*, Devireddy et al. (2015) presented evidence to argue that, unlike in *in vitro* systems, mitophagy does not occur in the axons *in vivo*: loss of PINK1 did not lead to axonal accumulation of mitochondria or drastic changes in mitochondrial morphology. To extend this work, Sung et al. (2016) characterized motor neurons lacking Parkin using GFP targeted to the mitochondria (mito-GFP) in the intact nervous system of dissected *Drosophila* larvae, a preparation accepted to replicate *in vivo* conditions. They report three major findings: (1) loss of Parkin resulted in a lower mitochondrial density in motor neuron axons and neuromuscular junctions; (2) changes in mitochondrial morphology resulting from Parkin loss were limited to the neuronal cell body; and (3) Parkin-dependent mitophagy was absent in axons.

Because previous literature suggested that Parkin is responsible for both mitochondrial quality control and trafficking of axonal mitochondria (Wang et al., 2011; Liu et al., 2012b; Saxton and Hollenbeck, 2012), the authors speculated that, if these two functions are related, loss of Parkin could lead to a buildup of defunct mitochondria in the axon, unable to be

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transported back to the cell body. Instead, they found that mitochondrial trafficking was reduced in both anterograde and retrograde directions, but mitochondrial motility characteristics were unchanged in Parkin-null neurons (Sung et al., 2016, their Fig. 1; their Table 2). After analyzing mitochondria density, the authors deduced that the reduction of mitochondrial transport in motor neuron axons was due to an overall decrease in mitochondrial density in both larval motor neuron axons and sensory axons of adult flies (Sung et al., 2016, their Fig. 2).

The authors characterized the Parkin-null phenotype further by examining the smaller mitochondria population present in the axon in greater depth. They assessed mitochondria health by measuring mitochondrial membrane depolarization and mitochondria length, which they hypothesized would increase due to Parkin-mediated degradation of mitofusins (Deng et al., 2008; Poole et al., 2010; Ziviani et al., 2010). The authors were surprised to find that these markers of mitochondria health did not indicate the presence of defunct mitochondria in their system (Sung et al., 2016, their Fig. 4). In contrast, Sung et al. (2016) found that neurons cultured *in vitro* did display both of these markers for dysfunctional mitochondria (their Fig. 9), which they argue provided evidence for an alternate role for Parkin in neurons *in vivo*.

As most mitochondrial biogenesis is thought to occur in the cell body, with mitochondria then transported distally (Davis and Clayton, 1996), the authors speculated that lower levels of axonal mitochondria in Parkin-null *Drosophila* could result from mitochondrial dysfunction in the soma. Sung et al. (2016) found that the cell bodies of neurons lacking Parkin contained mitochondrial networks with higher degrees of connectivity, presumably as a result of some combination of fission inhibition and fusion promotion (their Fig. 5). Without fission, no new mitochondria can be produced. Thus, the authors speculated that either a lack of mitochondria biogenesis or an inability to transport interconnected mitochondria explained the low levels of axonal mitochondria.

Sung et al. (2016) next examined Parkin-dependent mitophagy. In addition to mito-GFP, the authors tagged autophagy-related protein 8 (Atg8), a marker for autophagy-associated membranes, with red fluorescent protein and looked for areas of colocalization in both the cell bodies and axons of *Drosophila*

neurons. They compared wild-type and Parkin-null neurons, as well as neurons in larvae grown in basal and starvation conditions (Sung et al., 2016, their Fig. 6). Surprisingly, the authors did not detect the expected colocalization events representative of mitophagy in either wild-type or Parkin-null flies under any conditions. Importantly, while starvation induces a form of nonspecific autophagy called macroautophagy (Gomes et al., 2011; Rambold et al., 2011; Ghosh et al., 2012), Parkin-dependent mitophagy is thought to not be induced under starvation conditions (Chen et al., 2010; Wang and Klionsky, 2011). To selectively increase Parkin-mediated mitophagy *in vivo*, the authors could attempt to mutate the mitochondrial DNA polymerase γ (Trifunovic et al., 2004; Kujoth et al., 2005; Vermulst et al., 2008) or incubate in an oxygen-deficient environment (Liu et al., 2012a).

Although our knowledge of PINK1/Parkin function in non-neuronal cells has been greatly expanded in the last two decades, the role of these proteins in neurons *in vivo* is still poorly understood. Expanding on neuronal work *in vitro*, Sung et al. (2016) provide more insight into the effects of Parkin loss in larval *Drosophila* neurons by demonstrating that, rather than accumulate dysfunctional mitochondria, axons have less mitochondria overall. Although Parkin was thought to maintain the mitochondrial population through axonal quality control, their results questioned this role for Parkin *in vivo* and shifted the authors' focus to the soma. There, the authors observed that Parkin-null flies appear to have an overly connected mitochondrial network, suggesting that Parkin may serve to facilitate mitochondrial biogenesis in the soma and/or transport to the axon.

As an E3 ubiquitin ligase, Parkin is thought to promote fission *in vitro* through ubiquitin-mediated degradation of mitofusins (Tanaka et al., 2010). Further, Parkin ubiquitin ligase activity has been shown to halt mitochondria motility by facilitating the degradation of Miro, a key adapter protein required to link mitochondria to kinesin motors (Wang et al., 2011). The observed increase in connectivity of cell body mitochondria and the lower levels of axonal mitochondria could be explained by a lack of Parkin-mediated degradation of mitofusins and Miro in the cell body, respectively.

Increased refinement of PD model systems beyond *in vitro* cultures was also recently achieved by Pickrell et al. (2015) in

mice. In this study, researchers used a previously established model for accelerated aging in mice, a homozygous deficiency in the proofreading ability of the mitochondrial DNA polymerase γ , to examine how the largest risk factor for PD, aging, is exacerbated by Parkin knock-out (KO). These prematurely aging mice, called Mutator mice, were crossed with Parkin KO mice; their offspring (Mutator Parkin KO mice) displayed many of the characteristic PD pathologies, such as loss of dopaminergic neurons, lower striatal dopamine content, and impaired motor function (Pickrell et al., 2015, their Fig. 1). Although the mouse model used in this study may be limited in its malleability and long growth times in a way *Drosophila* is not, it does provide a powerful mammalian context for neuronal systems *in vivo*. While mitochondrial dynamics have not been examined in these mice, future studies to assess mitophagy and mitochondrial dynamics and expand on the *in vivo* neuronal work by Sung et al. (2016) could be done by crossing Mutator Parkin KO mice with mice expressing genetic markers of mitophagy, such as mito-QC (McWilliams et al., 2016) and mt-Keima (Sun et al., 2015) mice. Because PD pathology is selective to substantia nigra pars compacta neurons, levels of mitophagy between these neurons and other neurons should be compared.

PD is a complex disease, with the PINK1/Parkin pathway a prominent factor in disease pathology. Although *in vitro* studies of this pathway have led to important insights into protein function and activation, more studies benefiting from the varied advantages of different organismal neuronal models *in vivo* are necessary to understand the role of this pathway in PD pathogenesis.

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