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The PINK1/Parkin pathway: a mitochondrial quality control system?

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Abstract Significant insight into the mechanisms that contribute to dopaminergic neurodegeneration in Parkinson disease has been gained from the analysis of genes linked to rare heritable forms of parkinsonism such as *PINK1* and *parkin*, loss-of-function mutations of which cause autosomal recessive parkinsonism. *PINK1* encodes a mitochondrially targeted Ser/Thr kinase and *parkin* encodes a ubiquitin-protein ligase. Functional studies of PINK1 and Parkin in animal and cellular model systems have shown that both proteins play important roles in maintaining mitochondrial integrity. Genetic studies of PINK1 and Parkin orthologs in flies have shown that PINK1 acts upstream from Parkin in a common pathway that appears to regulate mitochondrial morphology. Mitochondrial morphology is regulated by mitochondrial fission and fusion-promoting proteins, and is important in a variety of contexts, including mitochondrial trafficking and mitochondrial quality control. In particular, mitochondrial fission appears to promote the segregation of terminally dysfunctional mitochondria for degradation in the lysosome through a process termed mitophagy. Recent work has shown that Parkin promotes the degradation of dysfunctional mitochondria in vertebrate cell culture. Here we postulate a model whereby the PINK1/Parkin pathway regulates mitochondrial dynamics in an effort to promote the turnover of damaged mitochondria.

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The mechanisms underlying dopamine neuron degeneration in Parkinson's disease (PD) are incompletely understood, although increasing evidence suggests that mitochondrial dysfunction is a major contributor: several different mitochondrial complex I inhibitors elicit parkinsonian-like syndromes in humans and animal models (Betarbet et al. 2002; Corti et al. 2005); a high proportion of sporadic PD patients exhibit systemic mitochondrial defects (Schapira 2007); mitochondrial DNA deletions are present at high abundance in dopamine neurons from aged individuals, and are even more prevalent in PD individuals (Bender et al. 2006; Kraytsberg et al. 2006); and functional studies of the genes causative for simple Mendelian forms of PD suggest that many have mitochondrial roles (Abou-Sleiman et al. 2006). Although, there are reports in the literature that potentially implicate all of the PD-related genes in mitochondrial dysfunction, the evidence is perhaps strongest for the *PINK1* and *Parkin*, genes, which are the focus of this review.

Loss-of-function mutations in *PINK1* and *parkin* are the primary cause of early-onset autosomal recessive forms of PD (Gasser 2009). The *PINK1* gene encodes a mitochondrially targeted Ser/Thr kinase, whereas *parkin* encodes an E3 ubiquitin-protein ligase. The subcellular distribution of PINK1 and Parkin is a matter of some dispute, although there is broad agreement that at least some fraction of PINK1 protein localizes to mitochondria with its kinase domain facing the inter-membrane space or the cytoplasm (Gandhi et al. 2006; Zhou et al. 2008), and that Parkin is

predominantly a cytosolic protein. To understand the mechanism by which PINK1 and Parkin influence dopamine neuron integrity, much of the early work on these factors involved a search for their substrates. To identify these substrates investigators sought proteins that bind to PINK1 and Parkin, and also tested candidate proteins implicated in PD pathogenesis. While these approaches have led to the identification of a number of putative PINK1 and Parkin substrates, *in vivo* evidence supporting a pathogenic role for many of these substrates is lacking, and only a subset of these putative substrates have been independently validated in multiple model systems.

Using a complementary approach to understand the biological roles of PINK1 and Parkin, animal models of PD were created by inactivating the *PINK1* and *parkin* genes in worms, flies, fish and mice and studying the consequences of these knockouts. In this review we focus on the work performed in mice and flies, which have had the biggest impacts on our current thinking about the biological roles of PINK1 and Parkin. Although mice bearing knockouts of the *PINK1* and *parkin* genes have mild phenotypes and do not recapitulate the dopamine neuron loss observed in humans, these mice do have nigrostriatal physiological defects suggesting a role for PINK1 and Parkin in dopamine neurotransmission (Goldberg et al. 2003; Kitada et al. 2007, 2009a, b). The *PINK1* and *parkin* knockout mice have also proven useful in experiments to test the validity of putative substrates of PINK1 and Parkin, and to explore models of pathogenesis (e.g., see (Goldberg et al. 2003) and (von Coelln et al. 2006)). By contrast, flies bearing knockouts of the *PINK1* and *parkin* genes have dramatic phenotypes, including progressive degeneration of a subset of dopamine neurons in the brain, degeneration of flight muscle tissue, and defective sperm cell development (Greene et al. 2003; Clark et al. 2006; Park et al. 2006). Interestingly, enlarged and swollen mitochondria are a prominent and early feature of all of the tissues affected by loss of PINK1 and Parkin, suggesting that these factors act to influence mitochondrial integrity. Although this finding was not particularly surprising for PINK1 given that it bears a targeting sequence directing it to mitochondria, these studies provided the first evidence that Parkin promotes mitochondrial integrity (Greene et al. 2003).

Owing to the striking similarity of the Drosophila *PINK1* and *parkin* mutant phenotypes studies were performed to test whether PINK1 and Parkin act in a common pathway. These studies yielded three lines of evidence in support of this conclusion: First, *PINK1*:*parkin* double mutants have similar phenotypes as the respective single mutants (Clark et al. 2006; Park et al. 2006). Second, overexpression of *parkin* was able to compensate for loss of *PINK1* but not vice versa, suggesting that Parkin acts downstream from PINK1 (Clark et al. 2006; Park et al. 2006; Yang et al. 2006).; Third, null mutations of *parkin* partially suppress a *PINK1* overexpression phenotype (Poole et al. 2008; Whitworth et al. 2008), providing additional support for the model that PINK1 acts upstream from Parkin in a common pathway that regulates mitochondrial integrity. These important findings have since been validated in mammalian cellular models (Exner et al. 2007; Dagda et al. 2009). A major question remaining from these studies was precisely how PINK1 and Parkin influence mitochondrial integrity.

An important clue to the possible mechanism by which PINK1 and Parkin influence mitochondrial integrity was provided by the finding that mutations in Drosophila *PINK1* and *parkin* influence the morphology of mitochondria. Although mitochondria are often depicted as static kidney bean shaped organelles, mitochondria are highly motile and fuse with one another to form interconnected tubular networks. These dynamic networks undergo continual cycles of fission and fusion controlled by evolutionarily conserved fission- and fusion-promoting factors (Detmer and Chan 2007). Among the known mitochondrial fission and fusion promoting factors are the large dynamin-related GTPases Dynamin-related protein 1 (Drp1), Optic Atrophy 1 (Opa1) and Mitofusin (Mfn) (Detmer and Chan 2007). Drp1 is a cytosolic factor that assembles with mitochondria to promote mitochondrial fission, whereas Opa1 and Mfn reside in the inner and outer mitochondrial membranes, respectively, where they act to promote mitochondrial fusion (Detmer and Chan 2007). Given that mutations in *PINK1* and *parkin* influence mitochondrial morphology, we and several other laboratories tested the hypothesis that PINK1 and Parkin regulate mitochondrial morphology. These studies revealed that removing a single copy of the fission-promoting factor *Drp1* in *PINK1* or *parkin* mutants dramatically reduces their viability. In contrast, *PINK1* and *parkin* mutant phenotypes are suppressed by overexpressing *Drp1* to enhance mitochondrial fission, or by introducing loss-of-function mutations in genes encoding the fusion-promoting factors *Opa1* and *Mfn* (Deng et al. 2008; Poole et al. 2008; Yang et al. 2008; Park et al. 2009). Together, these findings suggest that the PINK1/Parkin pathway promotes mitochondrial fission and/or inhibits mitochondrial fusion. However, two observations indicate that PINK1 and Parkin are not obligatory components of the mitochondrial morphogenesis machinery: first, Opa1, Drp1 and Mfn are conserved from yeast to mammals, but PINK1 and Parkin are only found in metazoans; second, null mutations in *PINK1* and *parkin* result in less severe phenotypes than null mutations in *Opa1*, *Drp1* and *Mfn*, Thus, PINK1 and Parkin may regulate the mitochondrial morphogenesis machinery in biological contexts that are particularly relevant to metazoans.

While the biological roles of mitochondrial fission have not yet been extensively studied in metazoans, previous work suggests several possible mechanisms by which derangements in mitochondrial fission could impact tissue viability. One possible mechanism derives from genetic studies of the fission-promoting factor *Drp1* in Drosophila. Loss-of-function mutations in Drosophila *Drp1* result in a failure to efficiently traffic mitochondria to presynaptic terminals in neurons, which in turn impairs calcium buffering and synaptic transmission (Verstreken et al. 2005). While these findings are consistent with some of the phenotypes documented in PINK1 and Parkin-deficient flies and mice, the distribution of mitochondria in motor neurons appears to be unaffected in PINK1-deficient flies (Morais et al. 2009), and a mitochondrial trafficking defect does not readily account for the flight muscle and male germline defects of PINK1 and Parkin deficient flies, and the selective vulnerability of dopaminergic neurons to loss of PINK1 and Parkin activity.

Another possible model by which defective mitochondrial fission could impact tissue viability derives from a recent study involving live-cell imaging of mitochondrial dynamics in cultured vertebrate cells (Twig et al. 2008). This study showed that while many products of mitochondrial fission rapidly fuse again with the mitochondrial network, a proportion of the fission products exhibit a decreased membrane potential and a decreased probability of fusion. These defective fission products are frequently targeted to the lysosome for degradation through a process termed mitophagy (Twig et al. 2008). Thus, these findings raise the possibility that derangements in PINK1 and Parkin could impair the selective turnover of damaged and dysfunctional mitochondria. Recent work provides support for this hypothesis by showing that Parkin is selectively recruited from the cytoplasm to damaged mitochondria upon treatment of cultured cells with mitochondrial damaging agents, and that Parkin is required for the turnover of these damaged mitochondria (Narendra et al. 2008). While these findings require independent replication, and further work will be required to test whether PINK1 is required for the mitophagy-promoting activity of Parkin, the findings of Narendra et al. are attractive for several reasons: first, they offer an explanation for the variety of mitochondrial defects that have been documented in PINK1 and Parkin-deficient cell lines, including decreased membrane potential, deficits in the electron transport chain complexes, reduced ATP synthesis, decreased mitochondrial DNA synthesis and aberrant mitochondrial calcium efflux (Gandhi et al. 2009; Gegg et al. 2009; Morais et al. 2009) by suggesting that these pleiotropic phenotypes derive from

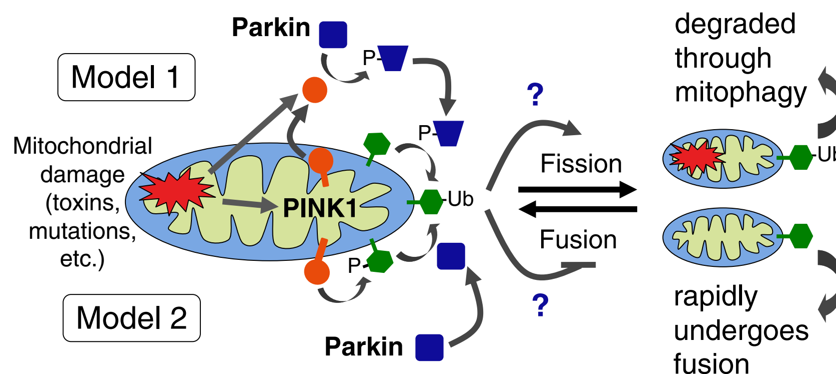

Fig. 1 Proposed models by which the PINK1/Parkin pathway influences mitochondrial integrity. Both models postulate that the PINK1 kinase directly or indirectly senses mitochondrial damage resulting from mitochondrial DNA mutations, toxins, oxidative stress, or other sources and communicates this information to its substrates. Models 1 and 2 differ from one another in terms of the subcellular distribution of PINK1, which has been reported to localize to the intermembrane space (Pridgeon et al. 2007), to the cytoplasm as a soluble protein (Beilina et al. 2005; Lin and Kang 2008; Weihofen et al. 2008), and anchored to mitochondria with its kinase domain facing the cytoplasm (Zhou et al. 2008), and in terms of the possible substrates of PINK1. In model 1, a proteolytically processed, soluble form of PINK1 is exported from the inter membrane space of damaged mitochondria to phosphorylate (P) its possible substrates in the cytoplasm. In model 2, PINK1 anchored to mitochondria with its kinase domain facing the cytoplasm phosphorylates its cytoplasmic substrates upon mitochondrial damage. In Model 1, PINK1 phosphorylates cytoplasmically localized Parkin, thereby inducing Parkin to translocate to mitochondria and ubiquitinate (Ub) its mitochondrial target proteins. In Model 2, PINK1 phosphorylates proteins residing on the outer mitochondrial membrane, which in turn elicits the recruitment of Parkin to ubiquitinate these phosphorylated mitochondrial proteins. It should be emphasized that the PINK1 substrates in the two models are potentially interchangeable (i.e., soluble PINK1 in Model 1 could proceed to phosphorylate mitochondrial proteins, and membrane localized PINK1 in model 2 could phosphorylate Parkin). Excellent candidate targets of PINK1/Parkin include the fission-promoting factor Fis1 and the fusion-promoting factor Mfn, both of which reside on the outer mitochondrial membrane. Ubiquitination of Fis1 could serve to activate its fission-promoting function, which could involve the recruitment of Drp1 from the cytoplasm to initiate the fission event. Alternatively, ubiquitination of mitofusin could lead to its inactivation to promote the segregation of terminally damaged mitochondria that are unable to re-fuse with the mitochondrial network, while simultaneously labeling them for degradation by mitophagy

the accumulation of damaged mitochondria in the absence of a functional mitochondrial quality control system; second, these findings would explain the protective effects of PINK1 and Parkin overexpression from exposure to mitochondrial toxins (Rosen et al. 2006; Paterna et al. 2007; Haque et al. 2008); and third, the abundant mitochondrial DNA mutational load of dopaminergic neurons (Bender et al. 2006; Kraytsberg et al. 2006) would neatly account for the selective vulnerability of this population of cells to the loss of a mitochondrial quality control system.

A model that combines work from flies showing that PINK1 acts upstream of Parkin to promote mitochondrial fission and the work of Narendra et al. demonstrating a role for Parkin in mitophagy is shown in Fig. 1. While this model is consistent with much of the literature on PINK1 and Parkin, it is important to point out that the molecular mechanisms by which PINK1 and Parkin regulate mitochondrial fission and mitophagy, and indeed the very role of PINK1 in mitophagy are currently unknown, so this model should be considered tentative. Moreover, several recent findings have challenged features of this model. In particular, some of the studies of PINK1 in vertebrate systems have found that reduced PINK1 activity results in mitochondrial fragmentation (Exner et al. 2007; Dagda et al. 2009; Lutz et al. 2009; Sandebring et al. 2009), suggesting that PINK1 may promote mitochondrial fusion—exactly the opposite of the conclusion drawn from studies in flies. Another recent study has found that RNAi-mediated knockdown of PINK1 results in increased mitochondrial autophagy (Dagda et al. 2009), a finding that conflicts with a prediction of our model in Fig. 1. Indeed, even the work of Narendra et al. upon which our model is heavily based, suggests that Parkin is required for mitophagy at a step downstream of mitochondrial fission, thus raising the possibility that the beneficial effects of promoting increased fragmentation in Drosophila *PINK1* and *parkin* mutants stems from the mitophagy promoting effects of mitochondrial fission.

While additional work will likely invoke revisions to the model presented in Fig. 1, it is important to point out that this model is primarily intended as a guide to future investigation. However, it is also important to add that some of the apparently discordant findings have multiple interpretations, not all of which conflict with our proposed model. For example, the finding of Narendra et al. suggesting that Parkin plays a post-fission role in mitochondrial turnover could be explained by a molecular event that marks the mitochondria for destruction while simultaneously inhibiting mitochondrial fusion. For example, Parkin-mediated ubiquitination of Mfn could inactivate the fusion-promoting function of Mfn, thus triggering mitochondrial fragmentation, and the ubiquitinated Mfn could simultaneously serve as a tag marking the mitochondria for destruction by mitophagy (Fig. 1). In potential support of this model, recent work has shown that ubiquitin can serve as a tag for the destruction of peroxisomes (Kim et al. 2008). Additionally, the finding that mitochondrial fragmentation and enhanced mitophagy result from reduced PINK1 activity in vertebrate systems may simply reflect a compensatory global induction of these processes in PINK1-deficient vertebrate cells. In potential support of this model is the finding that while the mitochondrial fragmentation seen in PINK1-deficient vertebrate cells can be suppressed by inactivating Drp1, this manipulation enhances the cell death associated with PINK1-deficiency (Dagda et al. 2009), a finding that is entirely consistent with work in flies. Future work should resolve these conflicts and clarify the mechanism by which PINK1 and Parkin influence mitochondrial integrity.

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