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## Mitochondrial dynamics in Parkinson's disease

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### Abstract

The unique energy demands of neurons require well-orchestrated distribution and maintenance of mitochondria. Thus, dynamic properties of mitochondria, including fission, fusion, trafficking, biogenesis, and degradation, are critical to all cells, but may be particularly important in neurons. Dysfunction in mitochondrial dynamics has been linked to neuropathies and is increasingly being linked to several neurodegenerative diseases, but the evidence is particularly strong, and continuously accumulating, in Parkinson's disease (PD). The unique characteristics of neurons that degenerate in PD may predispose those neuronal populations to susceptibility to alterations in mitochondrial dynamics. In addition, evidence from PD-related toxins supports that mitochondrial fission, fusion, and transport may be involved in pathogenesis. Furthermore, rapidly increasing evidence suggests that two proteins linked to familial forms of the disease, parkin and PINK1, interact in a common pathway to regulate mitochondrial fission/fusion. Parkin may also play a role in maintaining mitochondrial homeostasis through targeting damaged mitochondria for mitophagy. Taken together, the current data suggests that mitochondrial dynamics may play a role in PD pathogenesis, and a better understanding of mitochondrial dynamics within the neuron may lead to future therapeutic treatments for PD, potentially aimed at some of the earliest pathogenic events.

### Keywords

Parkinson disease; Parkinson's disease; mitochondria; mitochondrial dynamics; mitochondrial fission; mitochondrial fusion; parkin; PINK1; mitophagy

### Parkinson's disease and mitochondrial involvement

Parkinson disease (PD) is the second most common neurodegenerative disorder, and the most common neurodegenerative movement disorder. Clinically, it consists of progressive rigidity, bradykinesia, tremor, and gait abnormalities, as well as symptoms involving non-motor brain functions, including cognitive and autonomic functions (Barbas, 2006; Pallone, 2007; Weintraub et al., 2008; Weintraub and Stern, 2005). Pathologically, it is classically characterized by the loss of pigmented dopaminergic neurons in the substantia nigra in the midbrain and the presence of proteinaceous cytoplasmic inclusions called Lewy bodies (see Samii et al., 2004). While loss of the nigral dopaminergic neurons and terminals are responsible

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for the movement disorders associated with PD, it has become clear that additional neuronal populations throughout the brain are also affected in the disease (Braak et al., 2004).

Over the last several decades, evidence has accumulated that mitochondrial dysfunction is associated with PD. This evidence has been detailed in numerous comprehensive reviews (e.g. see Banerjee et al., 2008; Beal, 2003; Beal, 2007; Orth and Schapira, 2002; Schapira, 2008), and will only be briefly summarized here. A mild deficiency in mitochondrial respiratory electron transport chain NADH dehydrogenase (Complex I) activity was first found in the substantia nigra of patients with PD (Mann et al., 1994; Schapira et al., 1989), followed by studies identifying a similar Complex I deficit in platelets (Blandini et al., 1998; Haas et al., 1995; Krige et al., 1992; Parker et al., 1989), lymphocytes (Barroso et al., 1993; Yoshino et al., 1992), and, less consistently, in muscle tissue (Penn et al., 1995; Taylor et al., 1994) from PD patients. These results suggest there is a systemic, low-grade inhibition of Complex I activity associated with PD. Consistent with this, chronic systemic administration of rotenone, a specific Complex I inhibitor and pesticide, results in neuropathologic and behavioral changes in rats that is similar to human PD (Alam and Schmidt, 2002; Betarbet et al., 2000; Fleming et al., 2004; Sherer et al., 2003). MPTP, a meperidine analog found to cause parkinsonism in humans, has been found to exert its toxic effects through metabolism to 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), another Complex I inhibitor (Langston et al., 1999; Dauer and Przedborski, 2003). These compounds have long been used as animal models of PD.

In addition, oxidative stress has been linked to the neurodegeneration of PD, and alterations in levels of antioxidants and oxidized targets have been reported in PD (see Jenner, 2003). As the mitochondrial electron transport chain is a known source of reactive oxygen species generation, and inhibition of mitochondrial complexes can increase production of free radicals (Fiskum et al., 2003; Orth and Schapira, 2002; Turens, 2003), this has lent further evidence to a role for mitochondrial dysfunction in PD. Mitochondrial protein expression and/or abundance have also been found to be altered in PD. A recent proteomic analysis of mitochondria-enriched fractions from post mortem PD substantia nigra revealed differential expression of multiple mitochondrial proteins in PD brain as compared to control, including subunits of Complex I, mitochondrial creatine kinase, and the chaperone mortalin/GRP75/mtHSP70 (Jin et al., 2006). Also, decreased immunostaining for mitochondrial alpha-ketoglutarate, a protein of the tricarboxylic acid cycle, was noted in post mortem PD brain (Mizuno et al., 1994). While cause and effect is difficult to determine in post mortem studies, these findings are suggestive that mitochondrial function may be altered in PD.

More recently, the identification of familial forms of PD caused by genetic mutations has further supported a critical role for mitochondrial function. PD-linked mutations have been identified in genes encoding both mitochondrially targeted proteins and proteins involved in mitochondrial function and/or oxidative stress responses, including mutations in PINK-1, DJ-1, parkin, and possibly Omi/HtrA2 (see Thomas and Beal, 2007, and discussed in more detail below). Together, the body of evidence suggests an important role for mitochondria in PD pathogenesis, yet detailed exploration of the mechanisms involved is still ongoing.

## Mitochondrial dynamics and neurodegeneration

### Mitochondrial dynamics

Mitochondria are dynamic organelles, and these dynamic functions are emerging as critical to neuronal function and neurodegeneration. Mitochondria actively divide (fission), fuse with one another (fusion) and, in neurons, are actively transported throughout axons and dendrites (Hollenbeck and Saxton, 2005; Okamoto and Shaw, 2005). Mitochondrial fission and fusion are important in maintaining integrity of mitochondria, electrical and biochemical connectivity, in turnover of mitochondria, and in segregation, stabilization, and protection of mitochondrial

DNA (mtDNA) (Westermann, 2002). In addition to normal mitochondrial maintenance functions, mitochondrial fission, as well as mitochondrial fusion factors, have been found to play a critical role in apoptotic mechanisms as well (reviewed in (Suen et al., 2008).

Mediators of mitochondrial fission and fusion are best described in yeast, but many yeast mediators have mammalian homologues (Okamoto and Shaw, 2005). In mammalian cells, the GTPase Dynamin-related protein 1 (Drp1; yeast Dnm1) translocates to the outer mitochondrial membrane, and in conjunction with outer membrane proteins such as hFis1 as well as others, mediates mitochondrial fission (Hoppins et al., 2007). Mitochondrial fusion is mediated in mammalian cells in part by the outer membrane dynamin-like GTPases mitofusin-1 and -2 (Mfn1 and Mfn2) and the inner membrane optic atrophy protein (Opa1) (Cervený et al., 2007; Detmer and Chan, 2007).

### Importance of mitochondrial dynamics in neurons

Whereas mitochondrial fission and fusion have critical functions in all cells, these dynamic processes may be of particular importance in neurons, perhaps due to unique features such as their post-mitotic state and their long processes with higher energy requirements. Along these lines, specific mutations in fusion genes result in human neuropathies (Alexander et al., 2000; Delettre et al., 2000; Zuchner et al., 2004), while mutations in both fission and fusion genes have been associated with central nervous system disease (Brockmann et al., 2008; Waterham et al., 2007). In neurons, the mitochondrial fission/fusion machinery is intimately and critically involved in formation of synapses and dendritic spines. Preventing mitochondrial fission leads to a loss of mitochondria from dendritic spines and a reduction of synapse formation, whereas increasing fission increases synapse formation (Li et al., 2004). In addition, the absence of the mitochondrial fission protein Drp1 has been shown to prevent mitochondria from distributing to synapses and to lead to synaptic dysfunction (Verstreken et al., 2005).

In addition, maintenance of mtDNA cannot take place without mitochondrial fusion (Rapaport et al., 1998), and fusion of mitochondria has been directly implicated in preventing the accumulation of damaged mtDNA (Nakada et al., 2001; Ono et al., 2001). More recently, mitochondrial fission has also been shown to be critical to mtDNA maintenance (Parone et al., 2008), suggesting that fission and fusion may function in a coordinated manner to maintain and protect mtDNA. Since mtDNA mutations accumulate in the brain with age (Corral-Debrinski et al., 1992), these functions may have particular importance in neurons and neurodegenerative disease.

Also important in proper mitochondrial localization, maintenance, and function, and specific to neurons, is the active transport of mitochondria through axons and dendrites to sites of increased energy requirement. Mitochondria are transported in both anterograde and retrograde directions, by a process regulated in part by cytoskeletal and mitochondrial proteins and by extrinsic signaling mechanisms, including growth factor signaling (Hollenbeck and Saxton, 2005). In addition, mitochondrial movement is affected by several intrinsic components of mitochondrial bioenergetics, including ATP synthesis and changes in mitochondrial membrane potential (Rintoul et al., 2003; Miller and Sheetz, 2004), suggesting links between mitochondrial bioenergetic functions and mitochondrial transport and distribution. In *Drosophila*, specific genetic disruption of mitochondrial transport has been shown not only to lead to the absence of mitochondria at nerve terminals but also to result in synaptic dysfunction (Stowers et al., 2002). Disruption of mitochondrial movement has been noted in excitotoxicity and oxidative stress (Rintoul et al., 2003), two factors implicated in cellular stress and neuronal death.

## Mitochondrial dynamics in neurodegenerative diseases other than PD

As briefly mentioned above, mutations in the GTPase proteins comprising the mitochondrial fusion machinery are directly linked to neuropathy and neurodegeneration. Mutations in OPA1 result in autosomal dominant optic atrophy, or ADOA, a progressive degeneration of the optic nerve and retinal ganglion (Alexander et al., 2000; Delettre et al., 2000), whereas *mfn-2* mutations are linked to the peripheral neuropathy Charcot-Marie-Tooth subtype 2A, which involves axonal degeneration of motor and sensory neurons (Zuchner et al., 2004). In addition, *mfn-2* mutations may cause central nervous system white matter disease (Brockmann et al., 2008). Recently, a mutation in the fission gene *drp1* was found in an infant with lethal abnormal brain development, emphasizing the importance of the fission machinery in neuronal maintenance (Waterham et al., 2007).

In addition to the associations of genetic diseases of the fission/fusion machinery with neurodegeneration, evidence is suggesting possible involvement of mitochondrial dynamics in the pathogenesis of several chronic neurodegenerative diseases of aging. While this review focuses on PD, mitochondrial dysfunction has also been associated with Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) (see Beal, 2007; Kwong et al., 2006), and there is evidence that the dynamic functions of mitochondria may be involved.

Much of the evidence in AD, HD, and ALS relies on observations of morphologic changes in mitochondria. In an animal model of ALS, for example, expression of the mutant (G93A) human SOD1 in NSC-34 motoneuronal-like cells resulted in a fragmented mitochondrial network and mitochondrial swelling, though the mechanism is unknown (Raimondi et al., 2006).

In AD models, the amyloid beta peptide (A $\beta$ ), a component of AD-related neurodegenerative plaques, has been shown to induce increased mitochondrial fragmentation in rat cortical neurons (Barsoum et al., 2006). Also, overexpression of the amyloid precursor protein (APP) and the familial AD-causing APPsw mutation can lead to increased fragmentation and perinuclear aggregation of mitochondria in M17 cells (Wang et al., 2008b). APP and an A $\beta$ -derived diffusible ligand had similar effects on the mitochondria of primary hippocampal neurons (Wang et al., 2008b).

With regard to HD, a neurodegenerative disorder of striatal neurons caused by a triplication repeat mutation in the huntington gene (*Htt*), aggregates of mutant *Htt* protein were found to impair trafficking and mobility of mitochondria in rat primary cortical neurons (Chang et al., 2006), possibly by blocking trafficking pathways in the neuron. Recent findings also suggest that mutant *Htt* can impact mitochondrial fission and fusion. Wang et al. (2008b) demonstrated that HeLa cells expressing mutant *Htt* exhibited mitochondria with reduced movement and increased Drp1-dependent fragmentation. Using a transgenic *C. elegans* model of HD, the authors found that RNAi-mediated knock-down of Drp1 rescued the mutant *Htt*-induced motility defect in the worms, suggesting *Htt*-mediated dysfunction can be rescued by preventing fragmentation of mitochondria (Wang et al., 2008a).

## Mitochondrial dynamics and PD: Why might mitochondrial dynamics be particularly important in PD?

Although there is increasing evidence linking mitochondrial dynamics to many neurodegenerative diseases, we will detail below that the evidence is particularly strong, and rapidly accumulating, in PD. There are several features of the pathogenesis of PD that may help to explain this.

## Selective vulnerability in PD

One key question is why only specific sets of neurons die in PD. Interestingly, several features of PD neurodegeneration suggest that mitochondrial dynamics could be important. It has become clear that the degeneration of PD affects not only dopaminergic neurons, but many other neurons in the CNS, including populations in the brainstem as well as in subcortical and cortical regions (Braak et al., 2003). What makes these particular sets of disparate neurons selectively vulnerable in PD is not known, but may be key to understanding the underlying mechanisms of neurodegeneration in PD. Strikingly, they have in common two features: they have long and thin axons, and the axons have little or no myelination (Braak et al., 2004). The high energy requirement of these selectively vulnerable neurons, and the long distance between axon terminals and cell bodies, is likely to provide clues to the underlying mechanisms involved. Neurons with these features are likely to be particularly dependent on proper mitochondrial dynamics. In addition, Liang et al. (2007) recently found that the cytoplasmic area occupied by mitochondria in the dopaminergic neurons in the substantia nigra (susceptible to PD degeneration) is lower than in neighboring non-dopaminergic neurons or in dopaminergic neurons of the ventral tegmental area (resistant in PD), suggesting the possibility that the vulnerable neurons may be more susceptible to subtle changes in mitochondrial maintenance.

It is also generally believed that the degenerative process in PD begins at the terminals rather than the cell body (e.g., see Braak et al., 2004). Whether 'classic' apoptotic programmed cell death mechanisms occur in PD is still controversial, and there is evidence that axonal/dendritic degenerative mechanisms may be different than the classic mechanisms of programmed cell death (Finn et al., 2000; Raff et al., 2002; Whitmore et al., 2003). Given the importance of mitochondrial dynamics for mitochondrial distribution to synapses, synaptic function, and overall mitochondrial function, dysfunction in these dynamic processes could be an early step in PD neurodegeneration.

Additionally, as detailed above, mitochondrial fusion and fission maintains mtDNA stability and perhaps helps to reduce the burden of damaged mtDNA. PD is a disease of aging brain, and mtDNA damage has been found to increase with age (Corral-Debrinski et al., 1992). Evidence regarding mtDNA damage in the etiology of PD is still conflicting, but several studies have suggested an association with mtDNA mutations and PD (e.g. Parker and Parks, 2005; Winkler-Stuck et al., 2005). In addition, cybrid cell lines with normal nuclear genomes but mtDNA from PD patients have been reported to exhibit a Complex I deficit, a higher sensitivity to MPP<sup>+</sup> (the toxic metabolite of MPTP), and generation of Lewy body-like inclusions, suggesting that possible genetic defects in mtDNA genes encoding Complex I subunits are associated with PD pathogenesis (Sheehan et al., 1997; Swerdlow et al., 1996; Trimmer et al., 2004). Thus, it is possible that mitochondrial fusion-related stabilization of mtDNA integrity is an important modulator in pathogenesis of PD.

## Toxin models of PD and mitochondrial dynamics

Evidence from toxin-induced PD models supports a role for mitochondrial fission/fusion and transport. Exposure to sublethal concentrations of the PD-related toxin rotenone, the mitochondrial electron transport chain Complex I inhibitor, resulted in profound mitochondrial morphologic changes in HeLa cells, causing mitochondria to become more 'donut'-shaped (Benard et al., 2007). In human fibroblasts, acute exposure to high-dose rotenone resulted in decreased mitochondrial membrane potential and caused fragmented morphology (Mortiboys et al., 2008). In cultured rat cortical neurons, acute rotenone exposure was found to cause rapid mitochondrial fragmentation and cell death in a manner dependent on the fission protein Drp1 (Barsoum et al., 2006). Similar results were obtained from rat-derived dopaminergic N27 cells exposed to rotenone or MPP<sup>+</sup> (Barsoum et al., 2006). These studies suggest that rotenone



influences maintenance of mitochondrial morphology, and that, at least for acute toxicity, this may involve the mitochondrial fission machinery.

Using real-time microscopy, rotenone-induced swelling and decreased motility of mitochondria in fibroblasts was also observed (Pham et al., 2004). In addition, chronic exposure to rotenone was reported to reduce mitochondrial movement in differentiated dopaminergic SH-SY5Y cells (Borland et al., 2008). Together, these results suggest that PD-related toxins exert effects on fission/fusion and transport.

## Genetics evidence of mitochondrial involvement in PD: PINK1 and parkin

The strongest evidence of a role for mitochondrial dynamics in PD, however, is rapidly emerging from studies of several genes whose mutated forms are associated with familial PD.

### Parkin and mitochondrial function and morphology

Mutations in the *parkin* gene (*PARK2*) are associated with autosomal recessive inheritance of juvenile-onset PD (Kitada et al., 1998). The gene codes for parkin, a cytosolic ubiquitin E3 ligase protein that plays a role in the ubiquitin-dependent proteasome pathway (Shimura et al., 2000). Parkin loss-of-function mutants in *Drosophila* exhibited increased sensitivity to oxygen radical stress, dopaminergic cell loss, and severe mitochondrial damage in muscle and germline tissues that included swollen mitochondria and fragmented cristae (Greene et al., 2003; Pesah et al., 2004). It was later found that functional parkin was necessary for proper mitochondrial organization and morphology throughout spermatid development in *Drosophila* (Riparbelli and Callaini, 2007). Further, *Drosophila* overexpressing one pathogenic parkin mutation, R275W, were found to exhibit dopaminergic degeneration and mitochondrial abnormalities similar to parkin knockout flies, including mitochondria with large vacuoles, concentric membranous structures, disorganized cristae, or degenerated membranes (Wang et al., 2007). These studies suggest parkin plays a key role in maintaining mitochondrial stability in certain cell types.

Additionally, mitochondrial respiratory defects and morphological abnormalities have been noted in brains of *parkin*-knockout and *parkin*-mutant transgenic mice (Palacino et al., 2004; Stichel et al., 2007) and in leukocytes from PD patients with parkin mutations (Mortiboys et al., 2008; Muftuoglu et al., 2004). Primary fibroblasts from patients carrying mutations in parkin, or control fibroblasts treated with siRNA against parkin, exhibited lower mitochondrial membrane potential, Complex I activity, lower ATP levels, decreased complex I-dependent ATP production, and increased susceptibility to rotenone toxicity (Mortiboys et al., 2008). The fibroblasts also exhibited mitochondrial morphological abnormalities, revealing mitochondria that were longer and more highly branched. Interestingly, there was a correlation between decreased Complex I-dependent ATP production and increased length and branching, as well as between Complex I activity and branching. Further, parkin-mutant cells were more susceptible to rotenone toxicity.

As parkin is not specifically a mitochondrial protein and must be translocated to the organelle, its effects on mitochondrial morphology are likely tied into a larger pathway mediating mitochondrial maintenance. Recent studies have not only begun to elucidate such a pathway, but have collectively pointed to mitochondrial dynamics as a critical target, as will be detailed below.

### PINK1 and mitochondrial function and morphology

The PTEN-induced putative kinase 1 protein, PINK1, is a nuclear-expressed mitochondrially-targeted kinase. Mutations in the *PINK1* (*PARK6*) gene are associated with autosomal recessive inheritance of PD (Valente et al., 2004), directly linking a mitochondrial protein with

PD pathogenesis. In *PINK1* knock-out mice, loss of PINK1 significantly impairs mitochondrial respiration selectively in the striatum (Gautier et al., 2008). While no gross changes in the ultrastructure or the total number of mitochondria were observed in the striatum, the number of larger mitochondria was found to be selectively increased.

Several independent research groups developed transgenic *Drosophila* fruit fly lines that either expressed loss-of-function PINK1 mutants or were PINK1-deficient. In all cases, the flies exhibited increased susceptibility to stress, decreased cellular ATP levels, reduced mtDNA content, and mitochondrial morphological defects (Clark et al., 2006; Park et al., 2006; Yang et al., 2006; Yang et al., 2008). Interestingly, the mitochondrial morphologies and tissues in which they were exhibited were noted to be remarkably similar to those of parkin mutant *Drosophila*. Specifically, PINK1 mutant flies, like parkin mutant flies, exhibited disorganized indirect flight muscle fibers, swollen mitochondria with disorganized cristae in muscle tissue and dopaminergic neurons, and dopaminergic neuron degeneration (Clark et al., 2006; Park et al., 2006; Yang et al., 2006).

### Linking PINK1 and parkin together and to mitochondrial dynamics

Noting the mitochondrial morphologic similarities, the studies mentioned above tested whether ectopic expression of parkin would influence the PINK1 mutant phenotype. In all cases, parkin overexpression completely rescued the effect of the loss of PINK1 activity (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Interestingly, PINK1 overexpression did not rescue the phenotype of *parkin* knockout flies, while double *PINK1/parkin* knockouts exhibited the same level of deficits as either model alone. These studies suggest that the PD-related proteins parkin and PINK1 participate in a related pathway to affect mitochondrial function and stability, in which parkin is downstream of PINK1.

Mitochondrial effects were also observed in HeLa cells in which PINK1 expression was knocked down via siRNA (Exner et al., 2007). In these cells, PINK1 deficiency resulted in mitochondrial fragmentation, disorganized cristae, and functional deficiencies. As in the transgenic *Drosophila* models, wildtype parkin overexpression, but not PD-related *parkin* mutants, rescued the phenotype. The added observation of mitochondrial fragmentation suggests that mitochondrial fission/fusion machinery may be involved in the effects of the PINK1/parkin pathway on mitochondria. These results suggest that mutations in parkin may be adversely influencing mitochondrial stability, downstream of PINK1, and may be a pathway by which these proteins contribute to PD pathogenesis.

Reasoning that the mitochondrial morphological deficits may be the result of dysfunctional fission and/or fusion, several research teams investigated the relationship of these two proteins further in the *Drosophila* transgenic lines. By modulating expression of fission and fusion machinery in the fly lines, it was found that the PINK1/parkin pathway promotes fission and/or inhibits fusion of mitochondria (Deng et al., 2008; Park et al., 2009; Poole et al., 2008; Yang et al., 2008). Specifically, overexpression of Drp1, the mitochondrial fission protein, was protective against the PINK1 or parkin mutant phenotypes, but not overexpression of the inactive form of Drp1. The independent groups also found that loss of fusion machinery, Opa1 or Mfn, (knockdown or mutation) also partially rescued either parkin or PINK1 mutant phenotypes. Park et al. (2009) also showed that overexpression of Opa1 or Mfn alone, presumably upregulating mitochondrial fusion, resulted in mitochondrial swelling, and did not rescue the PINK1 mutant phenotype. However, parkin overexpression did rescue the Opa1 overexpression phenotype. Interestingly, Yang et al (2008) showed that the PINK1/parkin interaction with mitochondrial fusion and fission occurs not only non-neuronal tissues but in dopaminergic neurons as well. Together, these findings illustrate a role for the PINK1/parkin pathway promoting fission and/or inhibiting fusion in *Drosophila* muscle and neuronal mitochondria.

However, the above findings also create a notable discrepancy between findings in flies and some studies in mammalian cells. As mentioned above, siRNA-knockdown of PINK1 in HeLa cells was observed to increase mitochondrial fragmentation, or presumably promote fission (Exner et al., 2007). Similar results were observed in neuroblastoma cells with siRNA knockdown of PINK1 and parkin (Park et al., 2009) and primary cells cultured from patients carrying PINK1 mutations (Exner et al., 2007; Wood-Kaczmar et al., 2008). Similarly, preliminary work from (Dagda et al., 2008) found that stable PINK1 expression in SH-SY5Y cells increased interconnectivity of mitochondria, while PINK1 RNAi knockdown or expression of kinase-inactive PINK1 increased mitochondrial fragmentation. Although mitochondrial morphology alone does not necessarily predict the expected effects on fission and fusion (Berman et al., 2009), these results would suggest that the PINK1/parkin pathway could also *promote* fusion and/or *inhibit* fission in mammalian cells, the exact opposite of the findings from *Drosophila* models. Conversely, Mortiboys et al. (2008) recently demonstrated that cultured fibroblasts from patients carrying parkin mutations exhibited mitochondrial morphological abnormalities, being longer and more branched than controls. In addition, overexpression of PINK1 in COS-7 cells resulted in punctuate mitochondria, whereas suppression of PINK1 with shRNA resulted in long, tubular mitochondria, a phenotype inhibited by the overexpression of the fission proteins hFis1 or Drp1 (Yang et al., 2008). Thus, these observations would support the findings from the *Drosophila* models. While clearly, further studies are necessary to understand these conflicts, it is entirely possible that given the dynamic and interrelated regulation between fission and fusion, PINK1/parkin effects may differ depending on the cell type and/or cellular conditions.

## What potential mechanisms could explain the effects of PINK1 and parkin on mitochondrial dynamics?

What has been consistently found is that parkin acts downstream of PINK1 in effects on mitochondrial morphology and function. Although the mechanisms of the interaction between PINK1 and parkin and the actions of parkin on mitochondrial dynamics are far from clear, some lines of evidence suggest intriguing possibilities (Summarized in Figure 1).

### PINK1 influencing parkin localization?

Because in most cases, parkin appears to be largely cytosolic under basal conditions (Darios et al., 2003; Shimura et al., 1999), it would seem that parkin effects on mitochondrial fission and fusion might occur by 1) parkin translocating to mitochondria and exerting its actions there, or 2) acting in the cytosol on other proteins that then translocate to mitochondria (Figure 1B). There is some evidence that PINK1 might influence parkin localization. In neuroblastoma cells, it was found that overexpression of parkin alone resulted in cytosolic localization, but that co-expression with PINK1 resulted in parkin translocation to mitochondria (Kim et al., 2008). This was dependent on PINK1's kinase activity, since a kinase-dead mutant did not cause parkin translocation. Parkin localization to the mitochondria appeared to be mediated by phosphorylation at a linker-region threonine, apparently directly phosphorylated by PINK1 (Kim et al., 2008). Whether this occurs under physiologic conditions is as yet unknown.

For the suggested direct interaction to occur, however, the two proteins must be in proximity to one another. The subcellular localization of PINK1, as well as parkin, is a subject of controversy. There is some evidence that parkin interacts with the outer mitochondrial membrane (Darios et al., 2003), and in some cases is found inside the mitochondria (Kuroda et al., 2006b). PINK1 is a mitochondrially targeted protein possessing a transmembrane domain, and it has been found localized to the inner mitochondrial membrane (Gandhi et al., 2006; Muqit et al., 2006; Pridgeon et al., 2007; Silvestri et al., 2005), the intermembrane space (Plun-Favreau et al., 2007; Pridgeon et al., 2007; Silvestri et al., 2005), and spanning the outer



membrane (Gandhi et al., 2006; Zhou et al., 2008), with its kinase domain facing the cytosol (Zhou et al., 2008). PINK1 has also been described in the cytosol (Beilina et al., 2005; Haque et al., 2008; Takatori et al., 2008; Weihofen et al., 2008). There is also the possibility that the two interact via intermediary protein targets. Thus, there are multiple ways parkin and PINK1 may potentially interact, both at the mitochondria and in the cytosol (Figure 1B).

### Role of ubiquitin ligase activity of parkin?

Parkin, as an E3 ubiquitin ligase (Shimura et al., 2000), could affect mitochondrial dynamics either by ubiquitination-induced proteasomal degradation or by ubiquitination-mediated cell signaling. Although many potential protein targets of parkin's ubiquitin ligase activity have been identified (Lee and Liu, 2008), no obvious direct connection with factors influencing mitochondrial fission and fusion has been identified. However, other ubiquitin ligases play a critical role in regulating mitochondrial fission, fusion and trafficking (Neutzner et al., 2008). In yeast, an inactive mutant of a homologue of the NEDD4/NEDD-like ubiquitin ligase family induced abnormal mitochondrial morphology, distribution, and inheritance, which was rescued by overexpression of ubiquitin (Fisk and Yaffe, 1999). In addition, outer mitochondrial membrane-located E3 ubiquitin ligases, MARCH-V/MITOL, MULAN, and MRF1, have been recently shown to influence mitochondrial membrane dynamics in mammalian cells (Karbowski et al., 2007; Li et al., 2008; Nakamura et al., 2006; Neutzner et al., 2008; Yonashiro et al., 2006). Mitochondrial fission and fusion factors, such as the fission factors Drp1 and hFis1, have been shown to be directly ubiquitinated by some of these E3 ligases (Nakamura et al., 2006; Yonashiro et al., 2006), and there is evidence that mitochondrial fission/fusion proteins might be degraded by the ubiquitin proteasomal pathway (Neutzner et al., 2008). However, E3 ligase-related activity does not always result in changes in levels of fission/fusion factors, suggesting that ubiquitination as a cell-signaling, rather than degradation, mechanism could be occurring (Karbowski et al., 2007). This suggests the possibility that parkin could induce similar regulation of mitochondrial fission and fusion factors after translocation to mitochondria, or potentially, even in the cytosol.

### Parkin, mitochondrial dynamics, and mitophagy?

The role of parkin in mitochondrial dynamics may go beyond regulating fission and fusion. A recent elegant study suggests an entirely new role for parkin, targeting dysfunctional mitochondria for autophagic degradation (Narendra et al., 2008). The authors showed that mitochondrial depolarization with the protonophore CCCP resulted in recruitment of parkin to mitochondria in several mammalian cell types, and that subsequently, these mitochondria undergo fission and mitophagy. This is particularly interesting in light of the evidence suggesting that regulated fusion/fission can target mitochondria for degradation (Twig et al., 2008). Although one previous study showed parkin being *released* nonspecifically from mitochondria after exposure to CCCP in proliferating dopaminergic SH-SY5Y cells (Kuroda et al., 2006a), these new findings suggest the possibility that one of the functions of parkin in mitochondrial dynamics could be to function as a regulator of mitochondrial maintenance and homeostasis, surveying the cell and responding to specific mitochondrial stressors by targeting damaged mitochondria for degradation (Figure 1C). Considering the proposed role for a PINK1/parkin pathway in regulation fission and fusion, it is tantalizing to speculate that the two pathways are linked, and that PINK1 and parkin work in tandem to initiate fission of depolarized mitochondria, and subsequently target any resulting dysfunctional mitochondria for mitophagy. This relationship, however, remains to be explored.

### Parkin and PINK1 affecting mitochondrial transport?

Parkin may even influence mitochondrial transport and distribution within the neuron. Parkin was shown to bind to and stabilize microtubules (Yang et al., 2005). Since mitochondrial

transport is dependent on the microtubule system in neuronal processes (Hollenbeck and Saxton, 2005), one could hypothesize that parkin, or mutations in parkin, could affect mitochondrial transport throughout the neuron.

New evidence suggests that PINK1 may also have a role in mitochondrial transport. Weihofen et al. (2009) recently reported that PINK1 forms a complex with mitochondrial proteins Miro and Milton, both key in mitochondrial transport. Interestingly, abnormal mitochondrial morphology associated with a loss of functional PINK1 was ameliorated by Milton and Miro overexpression. Thus, dysregulation of proper mitochondrial transport may play a role in the effects of PINK1 mutations on altered mitochondrial morphology and PD-associated pathogenesis.

### Other potential protein interactions?

Conflicting evidence examines whether PINK1 participates in a pathway with another putative PD-related protein Omi/HtrA2 (Plun-Favreau et al., 2008). Omi/HtrA2 is a mitochondrial serine protease, and mutations affecting protease activity have been linked to increased risk of PD (PARK13) (Bogaerts et al., 2008; Strauss et al., 2005), though recent evidence has questioned this finding (Ross et al., 2008; Simon-Sanchez and Singleton, 2008). Omi/HtrA2 knockout mice and mice expressing a protease-inactive mutant form Omi/HtrA2, found that both demonstrated premature death associated with parkinsonian-like neurodegeneration (Jones et al., 2003; Martins et al., 2004). In one study, PINK1 was identified as a binding partner for Omi/HtrA2, and it was found that Omi/HtrA2 phosphorylation was decreased in brain tissue from PD patients who had PINK1 mutations (Plun-Favreau et al., 2007). Phosphorylation appeared to increase serine protease activity of Omi/HtrA2 and was necessary for Omi/HtrA2-mediated protection of mitochondria when cells were exposed to various toxins (Plun-Favreau et al., 2007). One study in *Drosophila* has reported that Omi/HtrA2 genetically interacts with, and downstream of, PINK1 in a pathway independent from parkin (Whitworth et al., 2008). In that study, overexpression of PINK1 alone or PINK1 and Omi/HtrA2 together in the fly eye resulted in a disorganized eye phenotype, and the downregulation of Omi/HtrA2 protected against PINK1 overexpression. However, some of the results of that study have been directly contradicted by a genetic loss-of-function study recently reported, which found no strong evidence for *in vivo* interaction between Omi/HtrA2 and PINK1 (Yun et al., 2008). Given this conflicting evidence, it is unclear whether this mitochondrial protease will prove to be involved in the PINK1 pathway of mitochondrial dynamics.

Another studied mitochondrial target is the intermembrane protease Rhomboid-7, which has not been previously linked to PD pathogenesis. Rhomboid-7 was demonstrated to be important for normal mitochondrial fusion in *Drosophila* (McQuibban et al., 2006). Overexpression of Rhomboid-7 has also been shown to enhance the PINK1 overexpression phenotypes in *Drosophila*, and has been suggested to function upstream of PINK1 and parkin (Whitworth et al., 2008). The relationship of these findings to PD pathogenesis, if any, remain to be studied, but this work does provide a step toward further defining the pathway by which PINK1 and parkin affect mitochondrial dynamics.

### Conclusions

Accumulating evidence supports the possibility that changes in mitochondrial dynamics could critically influence PD-related cellular degeneration. One could hypothesize that these are the types of changes that could cause damage first in distal portions of neurons, where mitochondrial distribution, fusion, and fission play an important role and where early pathogenesis is thought to occur (Figure 2).

When considering the role of mitochondrial dynamics in neurodegeneration, it is important to take into account that mitochondrial fission and fusion are not isolated events in the cell, but surely are interrelated with other mitochondrial maintenance functions such as biogenesis and degradation (e.g. Berman et al., 2009). Thus, influencing one feature of this system will likely influence the entire balance of dynamic mitochondrial maintenance. It is also important to consider that the specific pathways by which PINK1 and parkin are mediating mitochondrial dynamics are not fully characterized. Both proteins, particularly parkin, have identified functions beyond mitochondrial dynamics, and thus may further affect mitochondrial function via other pathways. Additionally, the specific regulatory roles these proteins play in fission and fusion may differ between differing cell types or cell-specific situations. This may explain the apparent contradictions between studies as to the effect of PINK1 and parkin on mitochondrial morphology.

In familial forms of PD in which mutations in PINK1 or parkin occur, the evidence thus far suggests that mutations could lead to problems in the interaction of these proteins, and potentially the recruitment of parkin to the mitochondria. This could lead to dysregulation of normal mitochondrial dynamics, disrupting pathways necessary for the mitochondria to maintain health. In addition, dysfunctional parkin, or the inability to recruit parkin, could also prevent proper targeting of irreparably-damaged mitochondria for mitophagy, leading to a buildup of toxic, dysfunctional mitochondria, ultimately leading to death in more susceptible neurons.

But what about sporadic PD? The above scenario is harder to fit to an otherwise normally-functioning system. It is possible that other factors, such as toxic exposure, increased oxidative stress, or contributing genetic factors, could limit the availability of parkin and other proteins for proper mitochondrial maintenance. It has been shown that parkin is a target for covalent modification and inactivation by dopamine quinone, which may contribute to the increased susceptibility of the dopaminergic neurons (LaVoie et al., 2005). Alternatively, these toxic, metabolic, or genetic factors may directly or indirectly influence mitochondrial dynamics themselves, leading to degeneration through similar mechanisms. As a result, cells such as the dopaminergic neurons of the substantia nigra, which have a lower mitochondrial mass and increased stress in general, may not be able to maintain a distribution of healthy mitochondrial and eventually succumb to degeneration, contributing to PD pathogenesis.

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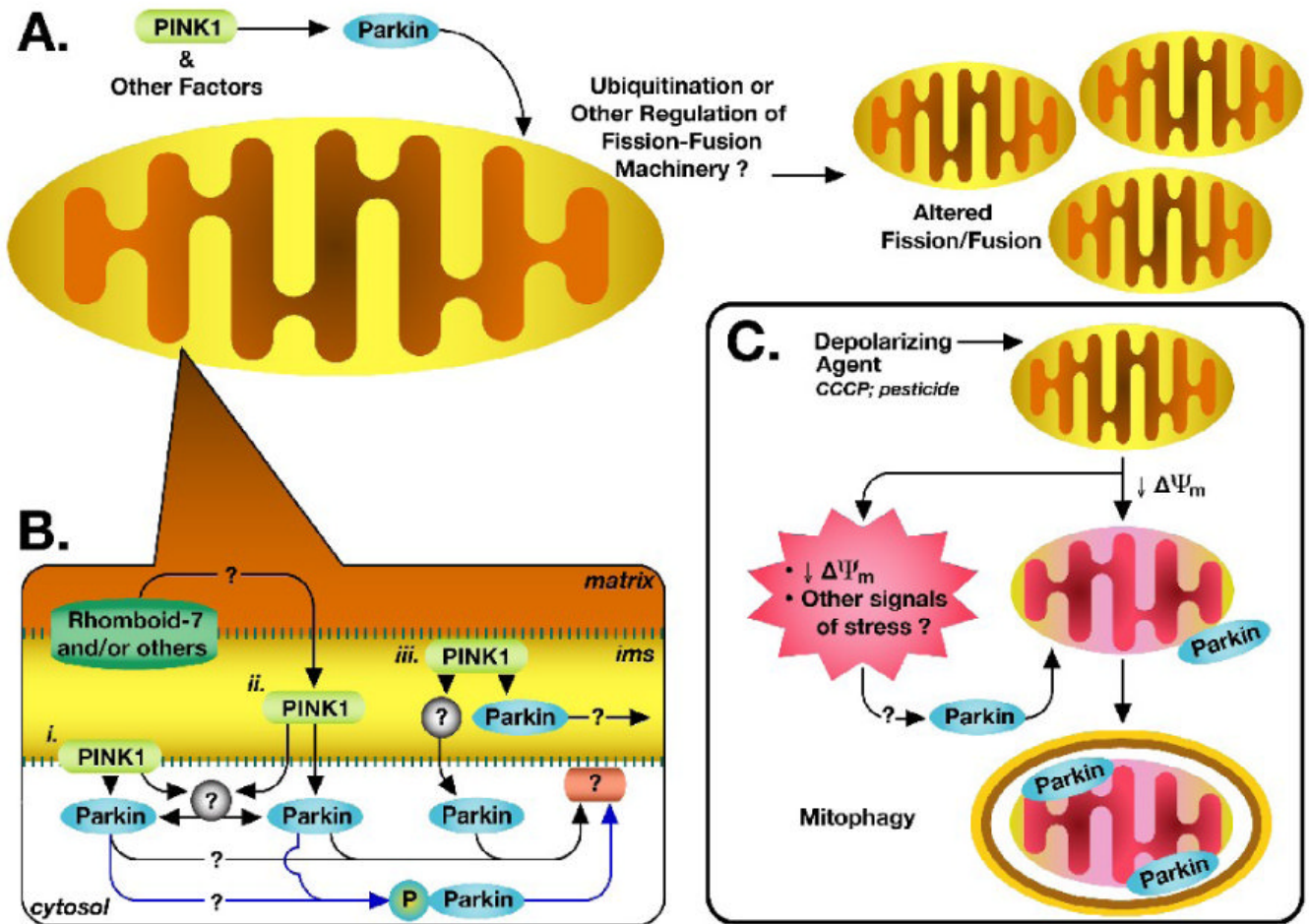
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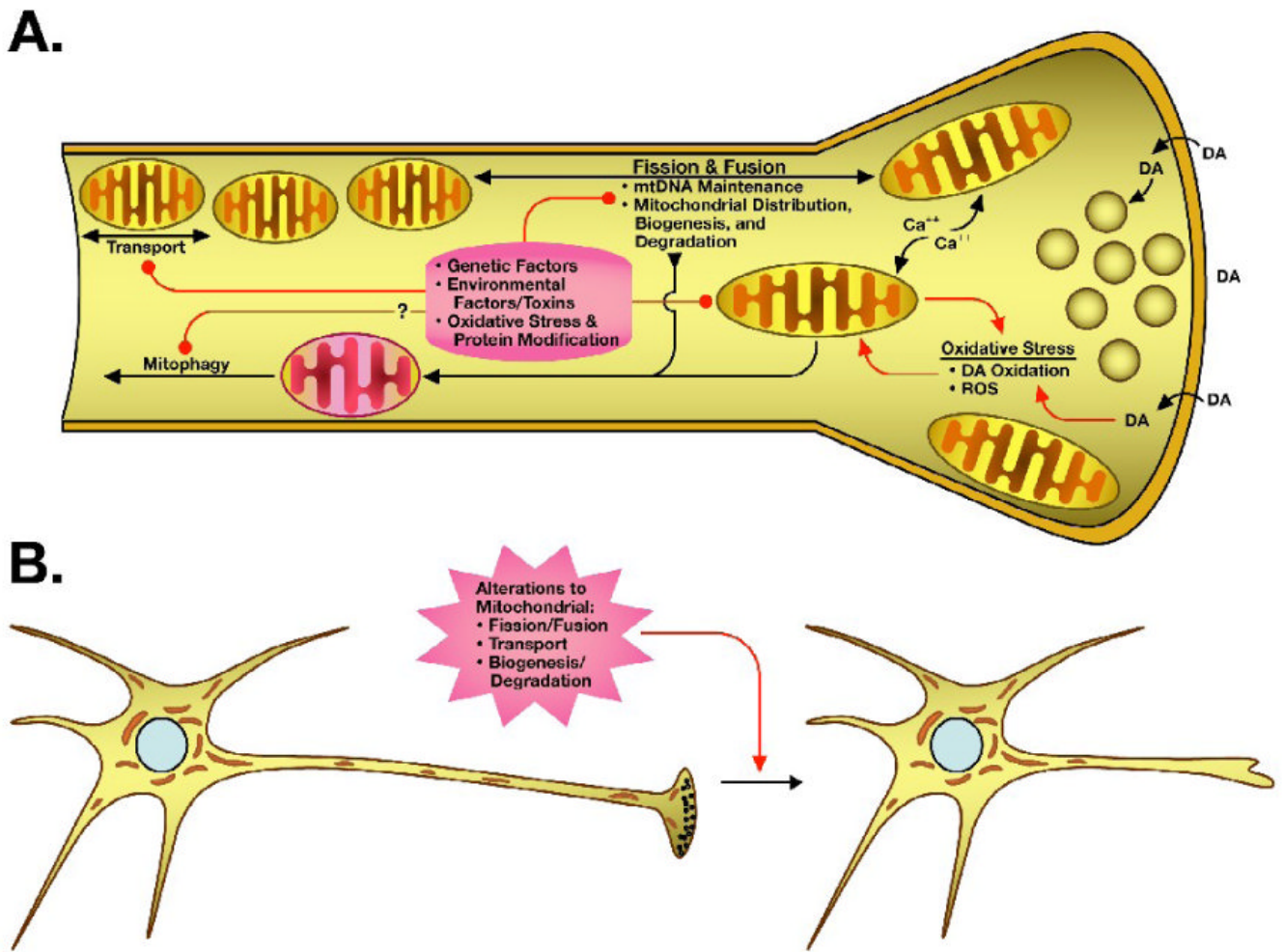
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**Figure 1. The emerging role for parkin and PINK1 in mitochondrial dynamics and homeostasis**  
**A.** Mounting evidence suggests that parkin operates downstream of PINK1 in a common pathway to regulate mitochondrial dynamics. In many cells, it appears to promote fission and/or inhibit fusion, though fission/fusion regulatory effects may differ by cell type or cellular environment. Such regulation may involve parkin-mediated regulation of mitochondrial fission/fusion machinery, either directly or indirectly, as through protein ubiquitination. Regulation may also involve some as yet unidentified parkin-mediated regulatory or protein-interaction pathway. **B.** How and through what pathway PINK1 and parkin interact is not known. The specific localization of both proteins is subject to controversy. Given current evidence, PINK1 may directly interact with and recruit parkin either, *i.* at the mitochondrial outer membrane, *ii.* in the cytosol, following cleavage and/or translocation of PINK1 out of the mitochondria, or *iii.* within the mitochondria. Alternatively, PINK1 may act through an intermediary (grey circles) to modify and/or recruit parkin to the mitochondria. PINK1 was also shown to directly phosphorylate cytosolic parkin, recruiting it to the mitochondria (blue pathways). However, where and how parkin exerts its effects on the mitochondria, whether inside the mitochondria, at the surface, or by interacting with surface proteins (pink ovoid), is unknown. **C.** Parkin was recently shown to localize to depolarized mitochondria, targeting them for mitophagy. The signals that target parkin to the damaged mitochondria, however, are not known. Whether this pathway is related to the fission/fusion regulatory functions of the PINK1/parkin pathway is also not known, though mitochondrial fission was previously shown

to be an apparent prerequisite for mitophagy. See text for related references. ims = intermembrane space



**Figure 2. Potential susceptibility of neurons to impaired mitochondrial dynamics**

The unique energy demands of axons and nerve terminals, particularly those of the dopaminergic nigrostriatal pathway, are dependent on proper mitochondrial distribution and function. At the terminus, mitochondria are critical for calcium buffering and energy for maintaining the vesicular pool. Environmental and genetic factors may contribute to increased mitochondrial and oxidative stress, impacting mitochondrial function, fission and fusion, transport, and perhaps even mitochondrial maintenance systems (red connectors). The dopaminergic neurons of the substantia nigra are known to have increased oxidative stress, due in part to the oxidative nature of the neurotransmitter dopamine (DA). Mitochondrial dysfunction also has the potential to set up a vicious cycle of increased oxidative stress, through production of reactive oxygen species (ROS) and decreased energy availability (red arrows). B. Altered or disrupted mitochondrial dynamics, which may result in an accumulation of damaged mitochondria or decreased distribution of healthy mitochondria at the nerve terminal and within the axon, may ultimately lead to stress and terminal loss, propagating back to the cell body.