Published in final edited form as:
Mitochondrion. 2009 September ; 9(5): 289–298. doi:10.1016/j.mito.2009.06.001.

Mitochondrial Kinases in Parkinson's Disease: Converging Insights from Neurotoxin and Genetic Models

Ruben K. Dagda[1], **Jianhui Zhu**[1], and **Charleen T. Chu**[1,2,†]

[1]Dept. of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA

[2]Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract

Alterations in mitochondrial biology have long been implicated in neurotoxin, and more recently, genetic models of parkinsonian neurodegeneration. In particular, kinase regulation of mitochondrial dynamics and turnover are emerging as central mechanisms at the convergence of neurotoxin, environmental and genetic approaches to studying Parkinson's disease (PD). Kinases that localize to mitochondria during neuronal injury include mitogen activated protein kinases (MAPK) such as extracellular signal regulated protein kinases (ERK) and c-Jun N-terminal kinases (JNK), protein kinase B/Akt, and PTEN-induced kinase 1 (PINK1). Although site(s) of action within mitochondria and specific kinase targets are still unclear, these signaling pathways regulate mitochondrial respiration, transport, fission-fusion, calcium buffering, reactive oxygen species (ROS) production, mitochondrial autophagy and apoptotic cell death. In this review, we summarize accelerating experimental evidence gathered over the last decade that implicate a central role for kinase signaling at the mitochondrion in Parkinson's and related neurodegenerative disorders. Interactions involving α-synuclein, leucine rich repeat kinase 2 (LRRK2), DJ-1 and parkin are discussed. Converging mechanisms from different model systems support the concept of common pathways in parkinsonian neurodegeneration that may be amenable to future therapeutic interventions.

Keywords

autophagy; kinases; mitochondria; neurodegeneration; oxidative stress; Parkinson's disease

1. Introduction

The mitochondrion plays a central role in most eukaryotic metabolic processes. In addition to serving as “powerhouses” to produce the majority of cellular ATP, mitochondria buffer intracellular calcium levels, regulate lipid metabolism, integrate metabolic and apoptotic signaling pathways and represent the major source of intracellular reactive oxygen species (ROS). Mitochondria are dynamic organelles that exhibit bidirectional motility within neurons and plasticity to undergo extensive shape changes mediated by GTPases of the mitochondrial fission/fusion machinery (MFF) (Karbowski & Youle 2003). In neurons, MFF-dependent

†Send proofs and correspondence to: Charleen T. Chu, M.D., Ph.D. Department of Pathology, University of Pittsburgh, 200 Lothrop St., Pittsburgh, PA 15261; email: ctc4@pitt.edu.

transport of mitochondria to dendrites promotes synaptogenesis, while axonal transport of mitochondria to pre-synaptic sites regulates the refilling of neurotransmitter pools (Li *et al.* 2004, Verstreken *et al.* 2005). Given the key role of mitochondria in neuronal function (Mandemakers*et al.* 2007), it is not surprising that disturbances in mitochondrial function, transport, dynamics and turnover have emerged as central mechanisms at the convergence of neurotoxin, environmental and genetic approaches to Parkinson's disease.

2. Mitochondrial dysregulation in Parkinson's and related parkinsonian disorders (PD)

Parkinson's disease is a debilitating, progressive movement disorder that affects ~1 million people in North America. The major motor symptoms can be attributed to degeneration of endogenously pigmented midbrain neurons of the nigrostriatal projection, while involvement of other neuronal populations result in olfactory, autonomic and cognitive dysfunction. While most cases have no known cause, oxidative stress, disordered protein handling/degradation, and mitochondrial dysfunction are mechanistically implicated in sporadic PD, in parkinsonism due to toxin/pesticide exposures, and in several models of familial PD (Giasson *et al.* 2000, Munch *et al.* 2000, Betarbet *et al.* 2002, Dawson & Dawson 2003).

Decreased mitochondrial complex I function has been observed in post-mortem PD midbrain tissues (Schapira *et al.* 1990) and in cybrid cells containing PD patient mitochondria (Swerdlow *et al.* 1996). These data suggest a role for mitochondrial DNA (mtDNA) alterations (Gu *et al.* 2002), although distinguishing potential causative changes remains elusive given the frequency of similar mutations in elderly controls (Simon *et al.* 2004). Cybrid PD lines exhibit rounded, swollen mitochondria with rarefied cristae (Trimmer *et al.* 2000) similar to autophagocytosed mitochondria observed in association with phosphorylated mitogen activated protein kinases (MAPK) in PD/Lewy body disease substantia nigra neurons (Zhu *et al.* 2003). Since substantia nigra DA neurons exhibit decreased basal mitochondrial content compared to other midbrain neurons, it has been proposed that diminished mitochondrial reserves may render them more susceptible to compromise of mitochondrial homeostasis during PD pathogenesis (Liang *et al.* 2007).

2.1 Mitochondria in toxin models of PD

Mitochondria are central to the actions of diverse neurotoxins that preferentially injure dopaminergic neurons. The heroin contaminant 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine (MPTP) causes acute parkinsonian intoxication, and represents one of the earlier models of parkinsonian neuronal injury (Przedborski & Jackson-Lewis 1998). The active metabolite MPP+ is a mitochondrial complex I inhibitor (Sherer *et al.* 2002, Brill & Bennett 2003), as is the pesticide rotenone used to model environmental contributions to PD (Betarbet et al. 2002). 6-OHDA is a redox-active dopamine analog used widely to lesion the DA nigrostriatal system (Zigmond & Keefe 1997). While there are differences in cell death mechanisms elicited by MPP+ and 6-OHDA (Choi *et al.* 1999, Chu *et al.* 2005), mitochondrial oxidative stress (Klivenyi *et al.* 1998, Callio *et al.* 2005), mitogen activated protein kinases (Kulich & Chu 2001, Kuan & Burke 2005, Zhu *et al.* 2007), endoplasmic reticulum stress (Ryu *et al.* 2002), and mitochondrial autophagy (Zhu et al. 2007, Dagda *et al.* 2008) have emerged as common factors.

2.2 Mitochondria in genetic models of PD

The discovery of α-synuclein mutations and later, gene multiplication (Polymeropoulos *et al.* 1997, Singleton *et al.* 2003), as causes of autosomal dominant forms of PD triggered a decade of additional gene discoveries and new efforts to model parkinsonian neurodegeneration. α-Synuclein aggregation and Lewy bodies are observed in both sporadic

and dominant forms of PD. The leucine rich repeat kinase 2 (LRRK2) is the most commonly mutated gene in both familial and sporadic settings (Kachergus *et al.* 2005), accounting for up to a third of cases in some populations. Proteins involved in autosomal recessive parkinsonism include parkin, ATP13A2, DJ-1, and PTEN-induced kinase 1 (PINK1) (Kitada *et al.* 1999, Bonifati *et al.* 2003, Valente *et al.* 2004b, Ramirez *et al.* 2006). PINK1 is the first kinase discovered to be regulated by a canonical N-terminal mitochondrial targeting sequence. The discovery of PINK1 mutations in recessive PD (Valente *et al.* 2004a), combined with observations that DJ-1 localizes to mitochondria during oxidative stress (Dekker *et al.* 2003), presaged the current renaissance of interest in the role of mitochondria in PD. Growing evidence implicates PINK1 and parkin in the regulation of mitochondrial morphology (Exner *et al.* 2007, Yang *et al.* 2008, Dagda *et al.* 2009) and turnover (Narendra *et al.* 2008, Dagda et al. 2009). Both α-synuclein and LRRK2 show at least partial localization to mitochondria (Biskup *et al.* 2006, Devi *et al.* 2008). Thus, as discussed in more detail below, toxin and genetic studies converge on cytoplasmic–mitochondrial signaling and protein trafficking in PD pathogenesis.

3. Overview of mitochondrial transport, dynamics and turnover

Mitochondrial fission is mediated by cytosolic and outer membrane proteins including dynamin-related protein (Drp1) and hFis1, which induce mechanical constriction powered by GTP hydrolysis (Karbowski & Youle 2003). Mitochondrial fission or fragmentation is often associated with cell death, playing an important role in the execution of apoptosis (Youle & Karbowski 2005, Yuan *et al.* 2007), including that elicited by the PD-neurotoxin 6-hydroxydopamine (Gomez-Lazaro *et al.* 2008). Little is known about the posttranslational regulation of mitochondrial dynamics, although sumoylation and phosphorylation of Drp1 have been recently reported (Cribbs & Strack 2007, Wasiak *et al.* 2007). Mitochondrial fusion results in an interconnected network of elongated mitochondria. It is mediated by the inner membrane protein optic atrophy 1 (Opa1) and two outer membrane GTPases termed mitofusion1 and mitofusion2 (Mfn1/2), which facilitate tethering and fusion of outer and inner membranes (Gazaryan & Brown 2007). Enhanced mitochondrial fusion and connectivity is associated with resistance to many forms of cellular injury (Cheung *et al.* 2007, Cribbs & Strack 2007). On the other hand, Drp-1 dependent mitochondrial fission can limit neuronal injury associated with propagating calcium waves (Szabadkai *et al.* 2004) and in models of PINK1 deficiency (Dagda et al. 2009).

Changes in mitochondrial dynamics are integrally linked to trafficking of these organelles to the most distant reaches of neuritic processes where they function to provide critical energy and calcium buffering at synapses (Fig. 1, upper left). In addition to kinesin adapter proteins such as Milton and Miro (Wang & Schwarz 2009), an intact MFF machinery is required for successful trafficking of mitochondria along axons. Disruption of either fission (Li et al. 2004,Verstreken et al. 2005) or fusion (Baloh *et al.* 2007) proteins impair this important process. Alterations in mitochondrial movement are linked to physiologic processes, such as those mediating synaptic plasticity (Li et al. 2004), and to pathologic processes (Chang *et al.* 2006,Orr*et al.* 2008). Interestingly, calcium itself regulates cessation of mitochondrial movement along axons (Wang & Schwarz 2009).

In addition to dynamic changes in mitochondrial fission/fusion and trafficking, autophagic degradation plays a major role in regulating mitochondrial quality and content (Kiselyov *et al.* 2007, Zhang *et al.* 2007). Macroautophagy involves the regulated, membranous engulfment of cytoplasmic cargo destined for lysosomal degradation (Mizushima *et al.* 2002, Cherra & Chu 2008), and represents the only major degradative pathway for organelles and insoluble proteins (Rubinsztein *et al.* 2005). Dysregulation of macroautophagy (Zhu et al. 2003, Alemi *et al.* 2007, Zhu et al. 2007, Dagda et al. 2008) and of chaperone-mediated autophagy (Cuervo

et al. 2004, Martinez-Vicente *et al.* 2008, Yang *et al.* 2009) have been implicated in toxin and genetic models of PD. The autophagy machinery includes conjugating enzymes required for covalent attachment of ubiquitin-fold proteins Atg12 and Atg8/microtubule-associated protein light chain 3 (LC3) to nascent autophagic membranes (Mizushima et al. 2002). RNAi knockdown of Atg conjugation components are effective at inhibiting induction of autophagy and mitophagy (Chu *et al.* 2009). Depolarization, fission and mitochondrial ERK signaling have each been reported to trigger mitochondrial autophagy (Dagda et al. 2008, Gomes & Scorrano 2008, Narendra et al. 2008), with failure of depolarized fragments to re-fuse with the mitochondrial reticulum representing an alternative mechanism governing selective mitophagy (Twig *et al.* 2008). While well-regulated autophagic recycling of damaged mitochondria is beneficial, the outcome most likely also depends upon the degree of damage-induced autophagy and other factors that pre-dispose neurons to autophagic stress (Cherra & Chu 2008) (Fig. 1).

It is important to note that an increase in autophagosomes in degenerating neurons does not necessarily imply increased autophagic activity. The most robust accumulations of autophagosomes are observed experimentally when lysosomal fusion and degradation are inhibited. In human PD brain tissues, increased oxidative damage to mitochondria (Zhang *et al.* 1999) is correlated with a modest increase in autophagosomes containing ERK-labeled mitochondria (Zhu *et al.* 2002, Zhu et al. 2003). Experimental studies demonstrate intact autophagic flux and degradation in several PD models (Zhu et al. 2007, Dagda et al. 2008, Plowey *et al.* 2008, Dagda et al. 2009). In contrast, evidence of mitochondrial autophagy (Moreira *et al.* 2007) is readily identified in AD with robust accumulation of early and intermediate autophagic vacuoles (Nixon *et al.* 2005), potentially attributable to reduced autophagic clearance (Boland *et al.* 2008). Thus, both post-mortem and experimental studies suggest multiple mechanisms by which perturbations in mitochondrial dynamics and turnover could contribute to synaptic dysfunction and neurodegeneration.

4. Kinase signaling to the mitochondrion

Given that only a small fraction of mitochondrial proteins are encoded in the mitochondrial genome and mitochondria rely heavily on synthesis and import of nuclear encoded proteins, mitochondria have undoubtedly evolved complex mechanisms to communicate with the rest of the cell. Despite this central role in cellular metabolism, mitochondria were once though to be unlikely central sites for reversible protein phosphorylation due to compartmentalization from the rest of the cell by multiple membrane layers, and the absence of mitochondrial targeting leader sequences in most signaling proteins (reviewed by (Pagliarini & Dixon 2006)). In yeast, only about seven protein kinases out of 136 (5%) have been identified in mitochondria (Tomaska 2000). However, experimental evidence garnered over the past two decades have demonstrated a clear role for kinases in regulating electron transport chain function, and cytoplasmic kinases can reach not only the outer surface of mitochondria, but also distribute in intermembrane and matrix compartments (Reviewed in (Horbinski & Chu 2005). The discoveries of signaling scaffold proteins that function to target specific kinases to the mitochondrion and of a functional N-terminal mitochondrial targeting sequence in the serine/threonine kinase PINK1 (Feliciello *et al.* 2005) further confirm an important role for kinases in mitochondrial communication with the rest of the cell to include the nucleus (Butow & Avadhani 2004).

As general features of kinase/phosphatase signaling to the mitochondrion have been the subject of several recent reviews (Horbinski & Chu 2005, McBride *et al.* 2006, Pagliarini & Dixon 2006), the subsequent sections will focus upon the role of kinases implicated in PD. In particular, converging roles for specific mitochondrially targeted kinases derived from neurotoxin and genetic models will be emphasized. Regardless of etiology in this multifactorial

disease, these data suggest common pathways of parkinsonian neurodegeneration that are potentially amenable to therapeutic intervention.

5. Extracellular signal regulated protein kinases (ERK1/2)

The extracellular signal-regulated kinases (ERK1/2) are conserved serine/threonine protein kinases that have emerged as important regulators of neuronal responses to both functional and pathologic stimuli (Chu *et al.* 2004). Although ERK1/2 typically translocates between the cytosol and nucleus to mediate well-characterized pro-survival and trophic functions (Yoon & Seger 2006), it is also found in mitochondria of neurons and non-neuronal cells such as in mouse heart (Baines *et al.* 2002); renal epithelial cells (Nowak *et al.* 2006, Zhuang *et al.* 2008); mitochondrial outer membrane and the intermembrane space of rat brain cells (Alonso *et al.* 2004); mouse hippocampus (Rumora *et al.* 2007), B65 cells (Kulich *et al.* 2007), SH-SY5Y cells (Dagda et al. 2008); Leydig cells (Poderoso *et al.* 2008), and human alveolar macrophages (Monick *et al.* 2008). The function of mitochondrial ERK1/2 is still not clear, but it appears to play a central role in regulating mitochondrial function (Nowak et al. 2006, Monick et al. 2008) and survival-death decisions (Kulich et al. 2007, Dagda et al. 2008, Lin *et al.* 2008, Zhuang et al. 2008). In human PD brain and diffuse Lewy body diseases, there are significant increases of phospho-ERK (p-ERK) in the cytoplasm and mitochondria of midbrain dopaminergic neurons (Zhu et al. 2002, Zhu et al. 2003). The punctate mitochondrial distribution of p-ERK in PD is distinct from the diffuse staining pattern observed after cerebral ischemia (Namura *et al.* 2001). Moreover, p-ERK is not elevated in substantia nigra degeneration due to progressive supranuclear palsy (author's unpublished data), indicating that this type of dysregulated ERK1/2 signaling may be relatively specific to PD.

5.1 ERK in parkinsonian neurotoxin models

Alterations in ERK signaling is observed during dopaminergic cell injury elicited by MPTP/MPP, 6-OHDA, rotenone, and toxic doses of dopamine (Kulich & Chu 2001, Gomez-Santos *et al.* 2002, Zhu et al. 2002, Chuenkova & Pereira 2003, Kulich et al. 2007, Zhu et al. 2007, Chen *et al.* 2008, Ren *et al.* 2009). ERK signaling is generally considered a pro-survival pathway (Baines et al. 2002), but increasing evidence suggests that activation of ERK also contributes to cell death (Chu et al. 2004, Zhuang & Schnellmann 2006, Ren et al. 2009). The level of ERK activation or its kinetics may play a role, as inhibiting basal ERK signaling has different effects than inhibiting toxin-induced ERK activation (Gomez-Santos et al. 2002). In the 6-OHDA model, we found that the time course of ERK activation is tightly correlated with mitochondrial ROS production; antioxidants inhibit ERK phosphorylation and rescue from neuronal injury (Kulich et al. 2007). Mitochondrially localized ERK induces autophagy/mitophagy even in the absence of toxin injury, suggesting that mitochondrially localized ERK could act as a sensor downstream of mitochondrial injury induced by toxins.

Factors that could determine the outcome of ERK activation include cell or organ type, nature of the treatments, and the temporal and/or spatial pattern of signaling within the cell (Colucci-D'Amato *et al.* 2003, Chu et al. 2004, Subramaniam & Unsicker 2006, Lin et al. 2008). A rapid and transient activation of ERK in mouse brain and MN9D cells promotes neuronal survival (Weng *et al.* 2007, Lin et al. 2008), while sustained or delayed ERK activation by 6-OHDA, MPP+ or dopamine promote cell death in neuronal cells (Kulich & Chu 2001, Gomez-Santos et al. 2002, Zhu et al. 2007). Detrimental effects of ERK activation are correlated with changes in the nuclear-cytoplasmic ratios of activated signaling phosphoproteins in the ERKRsk-CREB axis (Chen *et al.* 2004, Chalovich *et al.* 2006, Glotin *et al.* 2006, Poderoso et al. 2008). In both the 6-OHDA model and a dopamine toxicity model, only a small amount of p-ERK1/2 activated during injury translocates to the nucleus, with the majority located in the cytoplasm and mitochondria (Zhu et al. 2002, Chen et al. 2004, Dagda et al. 2008). This pattern of p-ERK trafficking causes a decline in neurotrophic transcription accompanied by an increase in

pathologic mitophagy (Fig. 1, right side). While lower levels of mitophagy can confer neuroprotection, prolonged or excessive ERK2-driven mitophagy appears harmful in neuronal cells as MAPK inhibitors or expression of a dominant negative ERK2 reduces cell death (Zhu et al. 2007, Dagda et al. 2008). Thus, addressing the mechanisms underlying altered trafficking of p-ERK may offer insights into potential therapies.

5.2 ERK in parkinsonian genetic models

Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) cause late-onset Parkinson's disease. The mechanisms by which missense alterations in the LRRK2 gene initiate neurodegeneration remain unknown. LRRK2 has putative Ras/GTPase-like, a protein kinase domain, leucine rich domain, and WD40 domains, all suggesting a major role in signaling (Ross & Farrer 2005, Greggio *et al.* 2006). Several mutations in the GTPase and kinase domains have been described. The kinase domain has a catalytic core common to tyrosine and serine/threonine kinases, and is homologous to mitogen activated protein kinase kinase kinases (MAPKKK) or mixed lineage kinases (West *et al.* 2005). The G2019S has been consistently shown to exhibit increased kinase activity. Interestingly, LRRK2 appears to activate ERK1/2 signaling in SH-SY5Y cells (Liou *et al.* 2008). However, in another study, ERK activity was not found to differ significantly in extracts of leukocytes from patients with PD carrying the G2019S mutation, healthy mutation carriers, patients with idiopathic PD, and healthy controls (White *et al.* 2007). We found that G2019S LRRK2, but not wild type LRRK2 or kinase-dead K1906M LRRK2, stimulated neuritic autophagy and neurite retraction by a pathway dependent upon ERK signaling in retinoic acid differentiated SH-SY5Y cells (Plowey et al. 2008). As with the toxin models, it is likely that the potential effects of ERK1/2 signaling will ultimately depend upon timing and compartmentalization of activation, which will determine which downstream pathways predominate in a given pathologic context.

6. c-Jun N-terminal kinases (JNK)

The c-Jun N-terminal kinases (JNK) represent another branch of the MAPK family that is activated by exposure of cells to environmental stress. Phospho-activation of JNK is mediated by MKK4 and MKK7. JNKs phosphorylate a variety of nuclear factors such as c-Jun, ATF2 and Elk1, and also cytoplasmic substrates such as cytoskeletal proteins and mitochondrial proteins including Bcl-2 and Bcl-xl. The spatial-temporal regulation of JNK is differently regulated in multiple intracellular compartments (Bonny *et al.* 2005, Borsello & Forloni 2007). Many studies indicate that JNK could be activated in or translocate to mitochondria, including work in ischemia-injured hippocampus, mouse cardiac mitochondria, H_2O_2-treated rat brain or primary cortical cultures, acetaminophen induced liver injury, HeLa cells treated with paclitaxel, and multiple myeloma cells treated with anti-cancer drugs (Baines et al. 2002, Chauhan *et al.* 2003, Zablocka *et al.* 2003, Brichese *et al.* 2004, Rumora et al. 2007, Hanawa *et al.* 2008, Zhou *et al.* 2008b). While most studies including those discussed below implicate JNK in death signaling, JNK shows neuroprotective effects in hypoxia-reoxygenation studies (Dougherty *et al.* 2004).

6.1 JNK in parkinsonian neurotoxin models

JNK represents one of the major signaling pathways implicated in PD pathogenesis. Increased JNK activity has been reported in MPTP animal models (Saporito *et al.* 1999, Xia *et al.* 2001, Hunot *et al.* 2004, Park *et al.* 2004), MPP+ cell culture model (Xia et al. 2001, Kim *et al.* 2007), rotenone neurotoxicity (Newhouse *et al.* 2004, Klintworth *et al.* 2007), and the 6-OHDA model (Hara *et al.* 2003, Eminel *et al.* 2004, Pan *et al.* 2007). Although the temporal and spatial patterns of JNK activation are different from model to model, activation of JNK almost exclusively leads to cell death. JNK2 and JNK3 mutant mice are more resistant to MPTP as compared with wide type littermates (Hunot et al. 2004). In mice, adenoviral gene transfer

of the JNK binding domain of the scaffold protein JNK-interacting protein-1 inhibited MPTP-induced c-Jun and caspase activation and dopaminergic neuron cell death (Xia et al. 2001). Downstream targets of JNK implicated in MPTP toxicity include cyclooxygenase 2 and the p53 protein (Trimmer *et al.* 1996, Teismann *et al.* 2003, Hunot et al. 2004, Nair 2006). JNK inhibitors and transfection with dominant negative forms of JNK reduce 6-OHDA induced cell death in PC12 cells (Eminel et al. 2004) and rotenone toxicity in SH-SY5Y cells (Newhouse et al. 2004). Similar to pathological ERK activation, antioxidants reduce JNK activation and cell death in 6-OHDA injured neuronal cells (Tian *et al.* 2007). All these experiment suggest JNK plays an important role in mediating parkinsonian cell death.

Exposure of rat primary cortical neurons to H_2O_2 resulted in increased phosphorylated JNK associated with the outer mitochondrial membrane, where causes phosphorylation of pyruvate dehydrogenase (PDH), a key enzyme that links two major metabolic pathways: glycolysis and the tricarboxylic acid cycle. Given that PDH is a matrix-localized proteins, the mechanisms involved are unclear, but phosphorylation of PDH causes a decline in its activity and a shift to anaerobic metabolism and acidosis (Zhou et al. 2008b). Mitochondrial translocation of JNK also causes release of the Second Mitochondria-derived Activator of Caspase and cytochrome c from mitochondria (Chauhan et al. 2003, Eminel et al. 2004), promoting apoptosis and phospho-inactivating Bcl2 and Bcl-x (Kharbanda *et al.* 2000, Brichese et al. 2004). As with pathological ERK activation, the potential mechanisms by which JNK translocate to mitochondria to promote mitochondrial dysfunction and degeneration in PD remains to be fully determined.

6.2 JNK in parkinsonian genetic models

Parkin is an E3 ubiquitin ligase, encoded by *parkin*, the most common gene mutated in autosomal recessive familial parkinsonism. Parkin has been demonstrated to act as a protector of dopaminergic neurons against multiple PD-related toxicities. Overexpression of parkin in SHSY5Y cells significantly attenuated dopamine-induced activation of c-Jun N-terminal kinase (JNK) and caspase-3. It also decreased the level of reactive oxygen species (ROS) and protein carbonyls in the cell (Jiang *et al.* 2004). Conversely, JNK is highly activated in dopaminergic neurons of *parkin* mutants (Cha *et al.* 2005). While it was originally thought that deficits in Parkin biology stimulates aggregation of its substrates to cause cellular stress, Parkin has also been reported to directly inhibit JNK activation via ubiquitination of JNK pathway mediators (Cha et al. 2005) (Fig. 1, top). A recent report suggests that Parkin inactivation of JNK is mediated by multiple mono-ubiquitinations on Hsp70, although the mechanism by which Hsp70 mono-ubiquitination regulates this is not clear (Liu *et al.* 2008). Parkin has three independent microtubule binding domains in addition to its RING domains. Dopaminergic neurons in culture appear are sensitive to rotenone-induced depolymerization of microtubules with subsequent activation of ERK and JNK, and Parkin protects against these effects (Ren et al. 2009). These studies indicate a direct interaction between Parkin and MAPK signaling pathways. JNK has also been implicated in relation to mutations in LRRK2. Protein assays of cell extracts from patients with LRRK2 G2019S-associated PD showed significant reductions in phosphorylation of JNK, Src, and HSP27 compared to healthy controls (White et al. 2007). On the other hand, recent studies indicate that mutant LRRK2 activates pathologic JNK and p38 signaling through phosphorylation of MAPKKs (Gloeckner *et al.* 2009)(Fig. 1, top right). Given dual roles for ERK on survival in different experimental contexts, the role of mutant LRRK2 in modulating JNK signaling deserves further investigation.

7.0 Akt/Protein kinase B

Protein kinase B (PKB) or c-Akt is the downstream kinase that regulates class III phosphoinositide-3-kinase (PI3K) dependent signaling in neurons. Recruitment of cytosolic Akt to the cell membrane via a pleckstrin homology domain (PH) by phosphatidylinositol 1,3,5

triphosphate (PIP3) facilitates its phosphorylation and activation by protein dependent kinase-1 (PDK1), leading to enhanced survival of motor neurons, PC12 cells and in cerebellar granule cells (Namikawa *et al.* 2000, Alvarez-Tejado *et al.* 2001, Bijur & Jope 2003, Leeds *et al.* 2005, Zhong *et al.* 2005, Li *et al.* 2008). Akt also promotes sequestration of Bad and suppresses the pro-apoptotic activity of GSK-3β (Datta *et al.* 1997, del Peso *et al.* 1997). Although mostly cytosolic, a fraction of Akt is recruited to mitochondria upon stimulation of SH-SY5Y cells with growth factors such as insulin (Bijur & Jope 2003). Transient expression of mitochondrially targeted constitutively active Akt protects against staurosporine induced apoptosis (Mookherjee *et al.* 2007). A recent study describes a role for heat shock protein 90 in mediating neuroprotective mitochondrial translocation of Akt (Barksdale & Bijur 2009).

7.1 Akt in parkinsonian toxin models

Several studies demonstrate that upregulation of the Akt pathway is neuroprotective. 6-OHDA treatment of SH-SY5Y cells significantly promotes a decrease in Akt phosphorylation (Li et al. 2008). Likewise, stereotactic injection of adenovirus expressing constitutively active m-Akt into the *substantia nigra* and striatum is strongly neuroprotective against 6-OHDA *in vivo* (Ries *et al.* 2006) (Fig. 1, top). Akt also showed striking trophic effects with increased sprouting of dopaminergic projections and increased substantia nigra neuron sizes. While this study elegantly highlights a potential application of gene therapy for PD, the potential for long-term constitutive Akt activation to promote neoplasia would need to be investigated.

7.2 Akt in parkinsonian genetic models

Stable knockdown of DJ-1 in *Drosophila* is associated with mitochondrial dysfunction and decreased Akt signaling (Yang *et al.* 2005). Furthermore, oxidative stress induces aggregation of α-synuclein, which modulates Akt signaling in neurons (Hashimoto *et al.* 2001, Seo *et al.* 2002). Increased levels of β-synuclein, which seems to antagonize the toxic and aggregating effects of α-synuclein, protected against rotenone toxicity via upregulation of the Akt signaling pathway. (Hashimoto et al. 2001, Uversky *et al.* 2002, Hashimoto *et al.* 2004). Moreover, human genetic studies also support a role for Akt in protecting against PD-type degeneration, as a particular Akt1 haplotype is associated with a decreased risk of developing PD in a Greek cohort of PD cases (Xiromerisiou *et al.* 2008).

8. PTEN-induced kinase 1 (PINK1)

While mitochondrial kinases have been implicated in PD through human tissue studies and parkinsonian toxin models for nearly a decade, the seminal discovery that the PARK6 locus of autosomal recessive, early-onset PD encodes PTEN-induced kinase 1 (PINK1) launched an ongoing period of intensive interest in the regulation of mitochondrial pathobiology by kinases. PINK1 is a serine/threonine kinase with homology to calcium/calmodulin regulated kinases (Valente et al. 2004a). Notably, the primary sequence for PINK1 includes a canonical N-terminal mitochondrial leader sequence (Silvestri *et al.* 2005) and reviewed by (Mills *et al.* 2008), and has been shown to distribute to mitochondria in numerous cell types including human brain (Gandhi *et al.* 2006), where it is predicted to be cleaved by matrix proteases. PINK1 also appears to have cytoplasmic functions, and even cleaved forms can be found in the cytoplasm, suggesting mitochondrial export of the protein for signaling or clearance purposes. There is a putative transmembrane domain thought to arrest import of PINK1 in a manner that allows it to insert in the outer mitochondrial membrane (Zhou *et al.* 2008a). The C-terminal domain of PINK1 regulates its autophosphorylation activity [reviewed in (Mills et al. 2008)].

Multiple point mutations and truncations have been mapped throughout the transmembrane, kinase and C-terminal domains of PINK1. These mutations serve to reduce or impair kinase

activity, promote accelerated degradation, or induce misfolding of PINK1 [reviewed by (Mills et al. 2008)]. The TNF receptor associated protein 1 (TRAP1) was identified as a potential substrate for PINK1, and the serine protease Omi/Htra2 and heat shock proteins, Hsp75 (TRAP1), Hsp90/Cdc37 are potential mitochondrial PINK1 binding partners (Plun-Favreau *et al.* 2007, Pridgeon *et al.* 2007, Moriwaki *et al.* 2008). Given that PINK1 loss of function leads to younger onset ages for parkinsonian neurodegeneration, a better understanding of the normal role(s) of PINK1 may offer important insight applicable to preventing or delaying onset of PD in general.

Although PINK1 is undoubtedly a mitochondrially targeted kinase, the subcellular localization of PINK1 in neurons has been controversial. Some studies suggest a mixed cytosolic/mitochondrial localization or localization in peroxisomes, while others indicate PINK1 is predominantly localized to the mitochondria (Beilina *et al.* 2005, Petit *et al.* 2005, Zhou et al. 2008a). Indeed, only some PINK1 functions appear dependent upon the mitochondrial localization signal, as N-terminal deletions of PINK1 that lead to cytoplasmic localization is sufficient to protect neurons from the classic mitochondrial toxin MPTP (Haque *et al.* 2008). However, a residual pool of mitochondrial leader peptide-truncated PINK1 can associate with mitochondria, possibly through association with the mitochondrial axonal transport proteins Miro and Milton (Weihofen *et al.* 2009). As observed with each of the kinases discussed above, localized activation and/or differential trafficking of different pools of PINK1 likely serve to mediate different physiological roles within neuronal cells.

8.1 PINK1 in parkinsonian toxin models

To date, all studies with PINK1 in toxin models have shown a prominent role for wild type PINK1 in neuroprotection. Transient or stable overexpression of PINK1 protects against a variety of toxic insults including staurosporine, rotenone, proteasome inhibition, MPP+ and 6-OHDA (Dagda & Chu, unpublished data), while RNA interference (RNAi) knockdown of PINK1 has the opposite effect (Deng *et al.* 2005, Petit et al. 2005, Pridgeon et al. 2007, Haque et al. 2008). Proposed prosurvival mechanisms include stabilizing the mitochondrial membrane potential, inhibiting superoxide generation and inhibiting the release of apoptogenic factors such as cytochrome c (Petit et al. 2005, Clark *et al.* 2006, Exner et al. 2007, Wang *et al.* 2007, Wood-Kaczmar *et al.* 2008) (Fig. 1, center).

On the other hand, the regulation of endogenous PINK1 responses by neurotoxic injuries has been less studied. Upon mitochondrial depolarization by oxidative stress, PINK1 in SHSY5Y cells rapidly translocates to mitochondria, is cleaved by matrix proteases and rapidly degraded by the proteasome pathway within minutes of toxin treatment (Lin & Kang 2008). We have observed alterations in PINK1 expression in the MPTP model *in vivo* and in a chronic MPP+ culture model (Zhu, Callio & Chu, unpublished data), which may play into either injury or compensatory mechanisms.

8.2 PINK1 in parkinsonian genetic models

Most PD-associated mutations in PINK1 result in loss of the ability of overexpressed PINK1 to confer neuroprotection against different forms of toxic insults (Wang et al. 2007). While some mutations are directly associated with loss of *in vitro* kinase activity, other mutations promote decreased protein stability or protein misfolding (Beilina et al. 2005). The possibility of an elevated risk for heterozygous PINK1 mutant carriers to develop PD (Valente et al. 2004b, Bonifati *et al.* 2005, Kumazawa *et al.* 2008), show accelerated disease progression (Marongiu *et al.* 2008), or develop neuropsychiatric disorders (Steinlechner *et al.* 2007, Reetz *et al.* 2008), remain controversial. In addition to a growing list of other proteins, PINK1 has been shown to be localized to Lewy bodies in human brain (Gandhi et al. 2006). While it is

attractive to speculate that depletion of functional PINK1 through aggregation may be pathogenic, decreased PINK1 levels in sporadic PD patients has not yet been reported.

Multiple RNAi studies in cultured mammalian cells, Drosophila models, and studies of mutant PINK1 patient primary fibroblasts reveal strikingly aberrant mitochondrial morphology, with loss of membrane potential and increased oxidative stress, implicating PINK1 in the regulation of mitochondrial homeostasis (Clark et al. 2006, Park *et al.* 2006, Wang *et al.* 2006, Yang *et al.* 2006, Exner et al. 2007, Poole *et al.* 2008, Wood-Kaczmar et al. 2008). Interestingly, all these alterations are restored by transient or stable expression of Parkin, leading to the concept of Parkin as a downstream effector of PINK1 neuroprotection.

Recent data also suggest alternative mechanisms for Parkin-mediated complementation of PINK1 deficiency (Narendra et al. 2008, Dagda et al. 2009). We observed that PINK1 loss-of-function resulted in aberrations in mitochondrial morphology, increased mitochondrial ROS, Drp1-dependent mediated mitochondrial fission, and a protective macroautophagy/mitochondrial autophagy responses (Fig. 1, lower left). Interestingly, not only did the MFF machinery mediate mitochondrial fragmentation in stable loss of PINK1 but the autophagic machinery also cooperated in this process. Morever, instead of simply reversing each cellular effect of stable PINK1 knockdown, Parkin overexpression resulted in further amplification of the autophagic/mitophagic response (Dagda et al. 2009), consistent with a recently reported role for Parkin in autophagic clearance of depolarized mitochondria (Narendra et al. 2008).

In contrast to culture and Drosophila studies, the effects of PINK1 deficiency in mouse models has been much more subtle. While there is no frank degeneration in PINK1 shRNA mice or PINK1 knockout mice (Zhou *et al.* 2007, Gautier *et al.* 2008), PINK1 appears to regulate dopamine release, long-term potentiation (Kitada *et al.* 2007) and potentially metabotropic glutamate receptors in medium spiny neurons of the striatum supporting a role of PINK1 at the dendrites and synapse possibly by regulating mitochondrial bioenergetics and dynamics at those compartments (Martella *et al.* 2009). Ultimately, our understanding of mechanisms related to PINK1-associated PD will rely upon a better understanding of the many normal roles of PINK1 in the central nervous system.

9. Conclusions

Data from neurotoxin, environmental and genetic models of parkinsonian neurodegeneration have converged upon a key role for kinases in regulating mitochondrial pathobiology in which disturbances in mitochondrial function, transport, dynamics and turnover are central converging mechanisms (Fig. 1). Altered subcellular localization of signaling proteins and transcription factors is frequently observed in post-mortem tissues and models of several major neurodegenerative diseases [Reviewed in (Chu *et al.* 2007)], with a tendency towards nuclear depletion and cytoplasmic/mitochondrial accumulation. Mitochondrial kinase activity can be mediated by localized ROS-mediated activation at the mitochondria as well as by trafficking and recruitment of signaling proteins activated elsewhere in the cytoplasm. As mitochondria serve as central sensors of metabolic alterations, reverse mitochondrial to nuclear signaling (Dawson & Dawson 2004) may be just as important as traditional pathways mediating communication between extracellular and intracellular environments. With recent impetus and momentum offered by PD-related factors, the basic role of bi-directional kinase signaling involving mitochondrial, as well as nuclear, trafficking represents an important emerging field of study.

Interestingly, neuroprotective autophagy/mitophagy elicited in response to recessive deficiencies (e.g. loss of PINK1, amino acid starvation, insufficient trophic stimulation) are regulated by canonical beclin1-dependent signaling pathway, which serves to prevent

overactivation of autophagy (Pattingre *et al.* 2005). In contrast, autophagy associated with dominant G2019S LRRK2 or MPP+ toxicity occur through beclin1-independent mechanisms (Zhu et al. 2007, Plowey et al. 2008), implying escape from this physiologic regulatory pathway. Hypothetically, either excessive mitochondrial damage or excessive mitophagy induced by neurotoxins, overactive LRRK2, or mitochondrial ERK1/2 (Dagda et al. 2008), could exceed the regenerative capacity of nigral neurons and prove detrimental (Fig. 1).

Although the mechanism(s) by which altered temporal and spatial dynamics of kinases that traffic between cytosolic, nuclear and mitochondrial compartments remain to be elucidated, over-activation and/or mitochondrial translocation of certain serine/threonine kinases (ERK2, JNK1/2, GSK3β, ?LRRK2), and impaired function of others (PINK1, Akt-1), promote PD-related pathogenic mechanisms including aberrations in mitochondrial cytoarchitecture, decreased mitochondrial function, and increased oxidative stress, contributing to protein aggregation. A compensatory mitochondrial autophagic response may represent a double-edged sword depending upon the degree of damage and ability of the neuron to successfully complete autophagic degradation and biogenesis of healthy mitochondria (Cherra & Chu 2008). This exciting new frontier of mitochondrial kinase regulation in PD raises the possibility that administration of known or yet-to-be discovered agents that inhibit kinase activity and/or mitochondrial translocation of ERK2, JNK1/2, LRRK2 or α-synuclein may rescue mitochondrial function, prevent activation of apoptotic or autophagic death pathways and/or prevent neurite degeneration. At the same time, potential therapies that increase kinase activity and/or mitochondrial functions of PINK1, Akt-1 or DJ-1 could aid in stabilizing mitochondrial networks, preventing activation of neurodegenerative and cell death pathways in PD.

ACKNOWLEDGMENTS

The authors'research is supported by the National Institutes of Health (CTC: AG026389, NS053777, DC009120) and the American Parkinson Disease Association (JZ). RKD is supported in part by F32 AG030821

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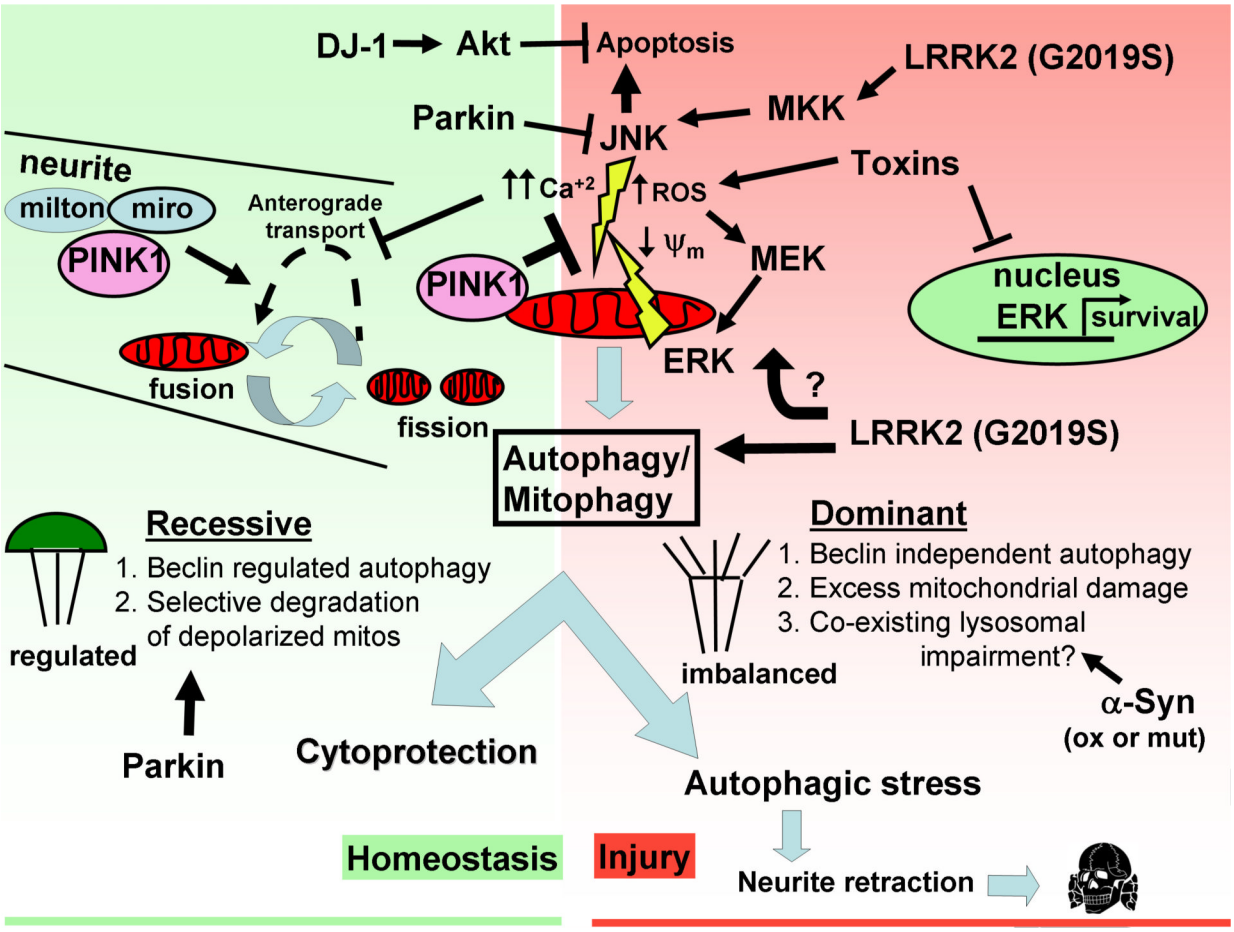

Figure 1. Hypothetical model integrating dominant (genetic or neurotoxin) and recessive (genetic or deficiency) influences on mitochondrial health and the autophagic/mitophagic injury response

Neuronal homeostasis (green background) is mediated by Akt, PINK1 and nuclear ERK/CREB signals that promote transcription of genes that support neuronal survival, differentiation and function (BDNF, Elk1, cJun, Bcl-2). PINK1 promotes maintenance of healthy mitochondrial networks, potentially facilitating mitochondrial trafficking to synapses via its recently reported association with the kinesin adaptor proteins Miro and Milton. Parkin, DJ-1 and Akt oppose the pro-apoptotic effects of JNK, which is activated by neurotoxins and mutant LRRK2 expression. Loss of PINK1 function permits mitochondrial damage involving increased ROS, calcium dysregulation, and decreased respiratory function/membrane potential, which may signal fission and autophagic clearance of damaged mitochondria. Physiologic feedback mechanisms involving beclin 1 interactions and/or parkin-assisted selective clearance of damaged mitochondria serve as safety mechanisms (green parachute) to regulate and prevent overactivation of autophagy.

Dominant neuronal injuries (shaded in red) caused by parkinsonian neurotoxins promote large increases in cytosolic and mitochondrial ROS, activation/translocation of ERK and JNK to mitochondria and induction of beclin 1-independent autophagy, accompanied by decreased nuclear trafficking and neuroprotective transcription. Both JNK and ERK have been shown to contribute directly to mitochondrial dysfunction by suppressing oxidative respiration. An activating mutation in LRRK2 also results in activation of JNK pathways and ERK-dependent neurite retraction mediated by beclin1-independent autophagy. Factors that result in autophagic stress are still incompletely defined, but could hypothetically result from excessive loss of functioning mitochondria or reduced ability to complete lysosomal degradation, as oxidized

or mutant forms of α-synuclein may interfere with certain forms of lysosomal degradation. We propose that excessive autophagy induction relative to the capacity of the neuron to undergo regenerative biosynthesis leads to a harmful state of imbalance that favors neurite retraction, neuronal atrophy and eventually cell death.