Mol. Cells *34*, 7-13, July 31, 2012
DOI/10.1007/s10059-012-0100-8
pISSN: 1016-8478 eISSN: 0219-1032

Molecules
and
Cells
http://molcells.org

Minireview

PINK1 as a Molecular Checkpoint in the Maintenance of Mitochondrial Function and Integrity

Hyongjong Koh[1],*, and Jongkyeong Chung[2],*

Parkinson's disease (PD), the most prevalent neurodegenerative movement disorder, is characterized by an age-dependent selective loss of dopaminergic (DA) neurons. Although most PD cases are sporadic, more than 20 responsible genes in familial cases were identified recently. Genetic studies using *Drosophila* models demonstrate that PINK1, a mitochondrial kinase encoded by a PD-linked gene *PINK1*, is critical for maintaining mitochondrial function and integrity. This suggests that mitochondrial dysfunction is the main cause of PD pathogenesis. Further genetic and cell biological studies revealed that PINK1 recruits Parkin, an E3 ubiquitin ligase encoded by another PD-linked gene *parkin*, to mitochondria and regulates the mitochondrial remodeling process via the Parkin-mediated ubiquitination of various mitochondrial proteins. PINK1 also directly phosphorylates the mitochondrial proteins Miro and TRAP1, subsequently inhibiting mitochondrial transport and mitochondrial oxidative damage, respectively. Moreover, recent *Drosophila* genetic analyses demonstrate that the neuroprotective molecules Sir2 and FOXO specifically complement mitochondrial dysfunction and DA neuron loss in *PINK1* null mutants, suggesting that Sir2 and FOXO protect mitochondria and DA neurons downstream of PINK1. Collectively, these recent results suggest that PINK1 plays multiple roles in mitochondrial quality control by regulating its mitochondrial, cytosolic, and nuclear targets.

INTRODUCTION

Parkinson's disease (PD), the second most common neurodegenerative disease, is characterized by a severe and specific loss of dopaminergic (DA) neurons in the substantia nigra. PD patients typically exhibit movement disorders, including tremor, rigidity, postural instability, and bradykinesia of the limbs (reviewed in Lang and Lozano, 1998). PD mainly occurs sporadically but can also occur genetically. After a mutation in *alpha-synuclein* (PARK1) was identified in a few families with PD (Polymeropoulos et al., 1997), more than 20 PD-associated genes have been discovered. Among them, *parkin* (PARK2) (Kitada et al., 1998), *PTEN-induced kinase 1* [*PINK1* (PARK6)] (Valente et al., 2004), and *DJ-1* (PARK7) (Bonifati et al., 2003) mediate early-onset autosomal recessive parkinsonism (AR-JP). In contrast, *alpha-synuclein* and *leucine-rich repeat kinase 2* [*LRRK2* (PARK8)] (Paisan-Ruiz et al., 2004) mediate autosomal dominant forms. The cloning and characterization of these PD-associated genes initiated our understanding of the molecular mechanisms of the pathology of familial PD, facilitating the study of the underlying pathological mechanisms of sporadic PD.

After Langston et al. (1983) reported that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a selective inhibitor of mitochondrial complex I, causes chronic parkinsonism in humans, numerous studies have strongly implicated mitochondrial dysfunction as an important cause of PD. Other mitochondrial toxins such as rotenone and paraquat could also induce parkinsonism accompanied by a loss of DA neurons in various animal models (Betarbet et al., 2000; Brooks et al., 1999; Coulom and Birman, 2004). Moreover, the activity of mitochondrial complex I is reduced in the brain tissues of patients with sporadic PD (Schapira et al., 1990). These results prompted vigorous investigation of the molecular links between PD pathogenesis and mitochondrial dysfunction, and *Drosophila* genetic studies have resulted in an outstanding breakthrough on PINK1 (Clark et al., 2006; Park et al., 2006; Yang et al., 2006).

MITOCHONDRIAL KINASE PINK1 IS CRITICAL FOR MAINTAINING MITOCHONDRIAL INTEGRITY

PINK1 is a cytosolic serine/threonine kinase mainly localized at mitochondria *via* an N-terminal mitochondrial-targeting sequence (Silvestri et al., 2005; Valente et al., 2004). Cells isolated from patients with a *PINK1* mutation exhibit reduced complex I activity and increased oxidative damage compared with controls (Hoepken et al., 2007). Moreover, the downregulation of PINK1 expression in mammalian neuron cells increases cell death with complex I-inhibiting neurotoxin treatment, which is reversed by PINK1 overexpression. These findings suggest that PINK1 plays a functional role in the protection of mitochon-

---

[1] Department of Pharmacology, Mitochondria Hub Regulation Center (MHRC), Dong-A University College of Medicine, Busan 602-714, Korea, [2] School of Biological Sciences and Institute of Molecular Biology and Genetics, Seoul National University, Seoul 151-742, Korea
*Correspondence: hjkoh@dau.ac.kr (HK); jkc@snu.ac.kr (JC)

Received April 6, 2012; accepted April 24, 2012; published online May 18, 2012

Keywords: FOXO, *Drosophila*, mitochondrial dysfunction, Parkin, Parkinson's disease, PINK1, Sir2

Springer

Table 1. Reported phenotypes of *PINK1* mutant model animals

Model animal	Phenotypes	References
Caenorhabditis elegans	• Reduced mitochondrial cristae length • Increased paraquat sensitivity • Axon pathfinding defects	Sämann et al. (2009)
Drosophila	• Complete male sterility • Downturned wing phenotype with rigidity and crushed thorax • Severe locomotive defects (i.e., slower climbing speed and complete loss of fight ability) • Reduced dopamine levels in brain tissues • Specific loss of DA neurons in 30-day-old flies • Increased sensitivity to paraquat and rotenone • Mitochondrial swelling in sperm, indirect fight muscles, and DA neurons • Decreased mitochondrial content and ATP levels in indirect flight muscles	Clark et al. (2006); Park et al. (2006); Yang et al. (2006)
Mouse	• No change in DA neuron numbers or dopamine levels in the striatum • Decreased dopamine release under electrical stimulation • Impaired mitochondrial respiration in the striatum • Increased sensitivity to H_2O_2 and MPTP • Left ventricular dysfunction • Pathological cardiac hypertrophy	Billia et al. (2011); Gautier et al. (2008); Haque et al. (2012); Kitada et al. (2007)
Zebrafish	• Movement disorders (i.e., reduced swimming activity and impaired response to tactile stimuli) • No significant alteration in the number of DA neurons • Disorganized patterning of DA neurons • Increased susceptibility to MPTP	Sallinen et al. (2010); Xi et al. (2010)

dria and neurons (Deng et al., 2005; Haque et al., 2008; Petit et al., 2005).

On the basis of these cell biological studies, several research groups generated and characterized *PINK1* null animal models (Table 1). Although *PINK1* null mice cannot precisely reproduce human PD symptoms, *Drosophila PINK1* null mutants generate strikingly similar PD-related phenotypes such as the selective loss of DA neurons and locomotive defects, including flight disability and slow climbing speed (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Loss of *PINK1* also induces indirect flight muscle degeneration, which may cause defective wing postures and a crushed thorax. Moreover, mitochondrial swelling accompanied by severe reduction in ATP levels and mitochondrial mass was found in the degenerated indirect flight muscles, and defective mitochondria were also observed in the DA neurons of *PINK1* null fly mutants (Park et al., 2006). These data demonstrate that PINK1 is critical for maintaining mitochondrial integrity and that mitochondrial dysfunction is an important cause of PD. Consistently, the overexpression of Buffy, a *Drosophila* homolog of mitochondrial protein Bcl-2, rescues mitochondrial defects and impaired climbing ability in *PINK1* null mutants, confirming the link between mitochondrial dysfunction and the PD-related phenotypes induced by loss of *PINK1* (Park et al., 2006).

PARKIN PROTECTS MITOCHONDRIA DOWNSTREAM OF PINK1

Parkin is an E3 ubiquitin ligase encoded by *parkin*, the most commonly mutated PD-associated gene (Shimura et al., 2000). Overexpressed Parkin can ubiquitinate and degrade several cytosolic proteins in mammalian cells (reviewed in Dawson, 2006). However, further studies did not find a definite link between these putative Parkin target proteins and PD pathogenesis.

The limitations of cell biological studies were unexpectedly resolved by *Drosophila* genetic studies focusing on *parkin*. The loss of *parkin* in *Drosophila* successfully recapitulated human PD symptoms such as movement disorders and DA neuron degeneration (Cha et al., 2005; Greene et al., 2003; Pesah et al., 2004). Moreover, L-DOPA substantially rescued the reduced climbing ability of *parkin* mutants, confirming that *Drosophila parkin* mutant models accurately simulate human PD patients (Cha et al., 2005). However, the molecular mechanism underlying these phenotypes could not be determined until *Drosophila PINK1* mutants showed remarkably similar PD-associated defects accompanied by mitochondrial dysfunction. In subsequent fly genetic analyses testing whether PINK1 and Parkin are linked in the same pathway, Parkin expression successfully complemented mitochondrial dysfunction and DA neuron loss in *PINK1* mutants. However, PINK1 transgenes could not rescue *parkin* mutant phenotypes (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). In addition, Exner et al. (2007) report that mammalian Parkin can also ameliorate the defective mitochondrial phenotypes induced by *PINK1* deficiency. These data confirm that PINK1 and Parkin act in the same pathway to protect mitochondria and that Parkin functions downstream of PINK1.

The molecular mechanism of the interaction between PINK1 and Parkin was investigated on the basis of their genetic interaction. Using SH-SY5Y neuroblastoma cells and fly mutants, Kim et al. (2008a) demonstrated that PINK1 translocates Parkin to mitochondria in a kinase activity-dependent manner. Additional biochemical analyses show that PINK1 phosphorylates Parkin and promotes its mitochondrial translocalization (Kim et al., 2008a). Further studies using mitochondrial uncoupling agents such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) suggest that PINK1 selectively translocates Parkin to mitochondria with low membrane potential, confirming the exis-

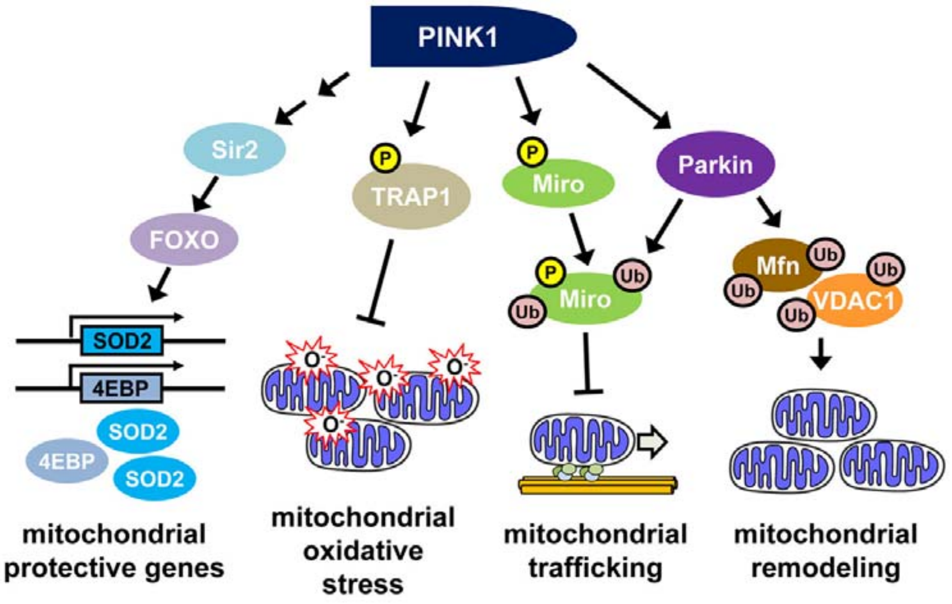

Fig. 1. Mitochondrial protective roles of PINK1. PINK1 regulates the mitochondrial remodeling process *via* the Parkin-mediated ubiquitination of a number of mitochondrial proteins, including Mfn and VDAC1. PINK1 also directly phosphorylates the mitochondrial proteins Miro and TRAP1, subsequently preventing mitochondrial trafficking and mitochondrial oxidative damage, respectively. Moreover, PINK1 can induce the expression of mitochondrial protective genes such as *SOD2* and *4EBP via* the Sir2-FOXO pathway.

tence of PINK1-dependent Parkin translocation (Matsuda et al., 2010; Narendra et al., 2010a; Vives-Bauza et al., 2010). Moreover, CCCP treatment also induces the accumulation of PINK1 protein in depolarized mitochondria (Matsuda et al., 2010; Narendra et al., 2010a; Vives-Bauza et al., 2010). Subsequent studies suggested that mitochondrial depolarization inhibits PINK1 protein degradation induced by mitochondrial protease presenilin-associated rhomboid-like protein (PARL) and promotes the accumulation of full-length PINK1 in the mitochondrial outer membrane to recruit Parkin (Jin et al., 2010). However, other researchers argue that the mitochondrial protease-mediated processing of PINK1 is critical for its mitochondrial protective function (Deas et al., 2011; Shi et al., 2011). Therefore, further investigation is needed to fully understand the molecular activation mechanism of PINK1 in mitochondrial protection as well as the roles of both the full-length and cleaved forms of PINK1 *in vivo*.

PARKIN REMODELS MITOCHONDRIA BY UBQUITINATING ITS MITOCHONDRIAL TARGETS

In addition to locomotive disorder and DA neuron degeneration, *Drosophila PINK1* and *parkin* mutants exhibit complete male sterility (Clark et al., 2006; Greene et al., 2003; Park et al., 2006). Mitochondrial remodeling processes - especially fusion and fission - play critical roles in *Drosophila* spermatogenesis. The mitochondria in each spermatid fuse to form a large onion-like structure called a nebenkern. When the spermatid elongates its tail, the nebenkern also elongates and divides into 2 mitochondrial derivatives that resemble leaf blades. Light microscopic observations of fly spermatids reveal that nebenkerns are swollen and fail to properly divide in *PINK1* and *parkin* mutants. These observations indicate that PINK1 and Parkin control mitochondrial fusion - fission processes (Deng et al., 2008). Moreover, further genetic analyses using mitochondrial fusion - fission regulators demonstrated that *PINK1* and *parkin* mutant phenotypes are successfully rescued by the overexpression of Drp1, a dynamin-related GTPase that promotes mitochondrial fission, or the downeregulation of Opa1 or Marf, which are other dynamin-related GTPases that induce mitochondrial fusion (Deng et al., 2008; Park et al., 2009; Poole et al., 2008; Yang et al., 2008). These findings suggest that PINK1 and Parkin balance mitochondrial dynamics by promoting mitochondrial fission to properly maintain mitochondrial function. However, cell biological studies using human cells give opposite results. The co-expression of PINK1 and Parkin or expression of mitochondria-targeting Parkin causes mitochondria in human DA neuroblastoma cells to aggregate (Kim et al., 2008a). Moreover, *PINK1* deficiency or point mutations induce mitochondrial fragmentation in immortal or primary human neuronal cells, indicating that PINK1 and Parkin inhibit mitochondrial fission in human neuronal cells (Exner et al., 2007; Kim et al., 2008a). Recently, several groups reported that the pro-mitochondrial fusion protein Marf and its human homolog, mitofusin (Mfn), are specifically ubiquitinated and degraded by Parkin in both human and *Drosophila* systems (Gegg et al., 2010; Tanaka et al., 2010; Ziviani et al., 2010). Therefore, the exact molecular mechanisms underlying these different mitochondrial remodeling patterns are expected to be elucidated soon.

Recent cell biological studies using CCCP suggest that PINK1 and Parkin are involved in another mitochondrial remodeling process: mitophagy, which is autophagy selective for mitochondrial degradation. After CCCP treatment, Parkin specifically accumulates on impaired mitochondria and induces their turnover *via* autophagosomes. The deletion of autophagy-related gene 5 (ATG5) or treatment with autophagy inhibitors blocks this mitochondrial degradation, further confirming the Parkin-induced mitophagy (Narendra et al., 2008). Subsequent

studies discovered that PINK1 is selectively translocated to mitochondria damaged by CCCP and activates their degradation *via* Parkin (Matsuda et al., 2010; Narendra et al., 2010a; Vives-Bauza et al., 2010). Moreover, in CCCP-treated cells, Parkin promotes the ubiquitination of impaired mitochondria and the expression of dominant-negative ubiquitin mutants prevents subsequent mitochondrial clearance, demonstrating the functional link between Parkin, ubiquitin, and mitophagy (Geisler et al., 2010; Lee et al., 2010; Matsuda et al., 2010).

It is also reported that Parkin-mediated mitochondrial ubiquitination recruits the ubiquitin-binding autophagic adaptors p62 and HDAC6 to mitochondria and that a deficiency of either inhibits Parkin-dependent mitophagy (Geisler et al., 2010; Lee et al., 2010). In addition, Geisler et al. (2010) identified voltage-dependent anion channel 1 (VDAC1), a channel protein in the mitochondrial outer membrane, as a target for Parkin-mediated ubiquitination and turnover of damaged mitochondria. These data suggest that PINK1 and Parkin induce the ubiquitination of VDAC1 to recruit the autophagic components p62 and HDAC6 for the specific turnover of damaged mitochondria. However, other studies report that p62 and VDAC1 are dispensable for Parkin-induced mitophagy (Narendra et al., 2010b; Okatsu et al., 2010) and that the outer membrane protein degradation caused by the ubiquitin - proteasome system is critical for Parkin-mediated mitophagy (Chan et al., 2011). We expect that more important details of the molecular mechanism of mitophagy mediated by the PINK1 - Parkin pathway will be revealed soon.

In addition to mitochondrial remodeling, PINK1 and Parkin also regulate mitochondrial trafficking. Wang et al. (2011) identified mitochondrial Rho-like GTPase Miro as a PINK1 substrate. Miro links mitochondria to microtubules by anchoring kinesin heavy chain (KHC) to the mitochondrial surface and regulates mitochondrial transport, both *via* its binding partner, Milton (Glater et al., 2006). Interestingly, PINK1 interacts with and phosphorylates Miro upon mitochondrial depolarization. The phosphorylation of Miro induces the Parkin-dependent degradation of Miro and detaches mitochondria from microtubules (Wang et al., 2011). Consistent with these findings, PINK1 and Parkin can inhibit mitochondrial movement in rat hippocampal and *Drosophila* axons (Liu et al., 2012; Wang et al., 2011).

The roles of PINK1 and Parkin in mitochondrial remodeling and trafficking suggest that PINK1 and Parkin repair moderately damaged mitochondria by inducing their fusion to healthy neighbors and remove severely damaged mitochondria by regulating mitochondrial transport and mitophagy.

OTHER MITOCHONDRIAL PARTNERS OF PINK1 IN MITOCHONDRIAL PROTECTION

Several independent proteomics analyses have been performed to elucidate the novel functional partners of PINK1. Pridgeon et al. (2007) report stable complex formation between PINK1 and TNF-receptor-associated protein 1 (TRAP1). TRAP1 is a mitochondrial molecular chaperone also known as heat shock protein 75 (Hsp75) that modulates the permeability transition pore to inhibit mitochondrial-associated cell death (Kang et al., 2007). In PC12 cells, PINK1 binds and co-localizes with TRAP1 in mitochondria and phosphorylates TRAP1. PINK1 depletion reduces TRAP1 phosphorylation and oxidative stress resistance, suggesting that TRAP1 is a key signaling molecule mediating the PINK1-induced protection against oxidative stress. The same research group also reports mitochondrial protease high temperature requirement A2 (HtrA2/Omi) as a direct binding partner and indirect phosphorylation target of PINK1 (Plun-Favreau et al., 2007). However, *Drosophila* genetic studies do not indicate that HtrA2 acts in the same pathway as PINK1 (Yun et al., 2008). Moreover, the molecular link between PINK1 and TRAP1 awaits further genetic confirmation using animal models. Another proteomics analysis found phosphoglycerate mutase 5 (PGAM5) as a PINK1-binding mitochondrial protein (Imai et al., 2010). Although PINK1 cannot phosphorylate PGAM5 and PGAM5 cannot regulate PINK1 kinase activity, the loss of *PGAM5* rescues *PINK1 Drosophila* mutants but not *parkin* mutants. Conversely, PGAM5 overexpression potentiates *PINK1* and *parkin* mutant phenotypes. These data suggest that PGAM5 functions between PINK1 and Parkin or acts downstream of PINK1 parallel to Parkin (Imai et al., 2010). Another *Drosophila* genetic analysis shows that DJ-1 overexpression can ameliorate *PINK1* mutant phenotypes but cannot rescue *parkin* phenotypes (Hao et al., 2010). However, PINK1 or Parkin overexpression rescues the aberrant mitochondrial phenotype in DJ-1–deficient cells, but DJ-1 does not reduce the mitochondrial fragmentation induced by *PINK1* deficiency. These findings suggest that DJ-1 acts independent of PINK1 and Parkin (Exner et al., 2007; Irrcher et al., 2010; Thomas et al., 2011).

SIR2 AND FOXO AS NOVEL PARTNERS OF PINK1

In addition to understanding the physiological functions of PD genes, *Drosophila* genetic models have been very useful for discovering novel components of the related signaling pathways. Venderova et al. (2009) report the genetic interaction between *LRRK2* and *PINK1* in *Drosophila* eyes. Consistently, mutant LRRK2 can activate pathologic JNK signaling via MAPKKs, and Parkin inhibits JNK in an E3 ligase activity-dependent manner; these findings imply crosstalk between LRRK2 and PINK1 (Cha et al., 2005; Gloeckner et al., 2009; Hwang et al., 2010). Another *Drosophila* genetic analysis revealed that *Sir2* knockdown suppresses fly eye phenotypes induced by PINK1 overexpression (Koh et al., 2012). Sir2 is a nicotine amide dinucleotide (NAD)-dependent protein deacetylase that has a neuroprotective function in animal models (Araki et al., 2004; Kim et al., 2007a; Parker et al., 2005). Consistent with these data, Sir2 overexpression ameliorates mitochondrial dysfunction and DA neuron loss in *PINK1* mutants. Further genetic analyses demonstrate that FOXO transcription factor, which is regulated by Sir2-mediated deacetylation (Brunet et al., 2004; Daitoku et al., 2004; van der Horst et al., 2004), mediates the Sir2-induced protection of mitochondria and DA neurons, indicating the mitochondrial protective role of the Sir2–FOXO pathway downstream of PINK1 (Koh et al., 2012). In contrast, Sir2 overexpression does not rescue *parkin* mutant phenotypes, and *Sir2* deletion only inhibits the development of *parkin* mutants but not *PINK1* mutants. Moreover, the mRNA expression of super oxide dismutase 2 (SOD2) and 4E-binding protein (4EBP), the FOXO target genes with mitochondrial protective roles (Kops et al., 2002; Puig et al., 2003; Tain et al., 2009; Zid et al., 2009), are substantially reduced in *PINK1* mutants but are normal in *parkin* mutants (Koh et al., 2012), suggesting that Sir2 and FOXO act in mitochondrial protection in parallel with Parkin. Consistently, *Sir2* and *FOXO* mutants exhibit DA neuron degeneration very similar to that of *PINK1* mutants. Furthermore, *PINK1* deletion induces no further DA neuron loss in *Sir2* or *FOXO* mutants, further supporting the idea that Sir2 and FOXO protect mitochondria and DA neurons downstream of PINK1 and independent of Parkin (Koh et al., 2012).

Although Sir2 is localized in the nucleus and cytosol, it has profound effects on mitochondrial functioning (Finley and Haigis, 2009). Determining the genetic link between Sir2 and PINK1 may provide a key for decoding the signaling mechanism between mitochondria and Sir2. Moreover, PINK1 faces the cytoplasm in the outer membrane (Zhou et al., 2008), and cytosolic PINK1 can protect neurons from MPTP (Haque et al., 2008). Therefore, PINK1 may directly phosphorylate and activate Sir2 in the cytoplasm. Alternatively, PINK1 may also indirectly regulate Sir2 via its upstream regulators such as active regulator of Sirt1 (AROS) (Kim et al., 2007b) or deleted in breast cancer 1 (DBC1) (Kim et al., 2008b; Zhao et al., 2008). This crosstalk between PINK1 and the Sir2-FOXO pathway suggests the novel role of PINK1 in inducing the expression of mitochondrial protective genes by transmitting signals to the cytosol and nucleus.

CONCLUSIONS

Mitochondrial dysfunction has been implicated in PD pathogenesis ever since the first PD studies using MPTP-treated animal models. After decades with little success, pioneering fly genetic studies using *PINK1* mutants finally established the molecular link between mitochondrial damage and PD pathogenesis. Subsequent genetic and cell biological analyses revealed the various molecular roles of PINK1 in maintaining mitochondrial integrity and function, suggesting PINK1 as the mainstay of mitochondrial protection (Fig. 1). Further studies on PINK1 and its partners may provide novel therapeutic targets for more effective treatment of AR-JP and possibly other forms of PD.

ACKNOWLEDGMENTS

This work was supported by the Dong-A University Research Fund (2008-001-18-003) and the National Research Foundation of Korea (NRF) Grant (2009-0093188).

REFERENCES

Araki, T., Sasaki, Y., and Milbrandt, J. (2004). Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. Science *305*, 1010-1013.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., and Greenamyre, J.T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. *3*, 1301-1306.

Billia, F., Hauck, L., Konecny, F., Rao, V., Shen, J., and Mak, T.W. (2011). PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. Proc. Natl. Acad. Sci. USA *108*, 9572-9577.

Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C.J., Squitieri, F., Ibanez, P., Joosse, M., et al. (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science *299*, 256-259.

Brooks, A.I., Chadwick, C.A., Gelbard, H.A., Cory-Slechta, D.A., and Federoff, H.J. (1999). Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res. *823*, 1-10.

Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y.X., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., et al. (2004). Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science *303*, 2011-2015.

Cha, G.H., Kim, S., Park, J., Lee, E., Kim, M., Lee, S.B., Kim, J.M., Chung, J., and Cho, K.S. (2005). Parkin negatively regulates JNK pathway in the dopaminergic neurons of Drosophila. Proc. Natl. Acad. Sci. USA *102*, 10345-10350.

Chan, N.C., Salazar, A.M., Pham, A.H., Sweredoski, M.J., Kolawa, N.J., Graham, R.L.J., Hess, S., and Chan, D.C. (2011). Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. Hum. Mol. Genet. *20*, 1726-1737.

Clark, I.E., Dodson, M.W., Jiang, C.G., Cao, J.H., Huh, J.R., Seol, J.H., Yoo, S.J., Hay, B.A., and Guo, M. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature *441*, 1162-1166.

Coulom, H., and Birman, S. (2004). Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*. J. Neurosci. *24*, 10993-10998.

Daitoku, H., Hatta, M., Matsuzaki, H., Aratani, S., Ohshima, T., Miyagishi, M., Nakajima, T., and Fukamizu, A. (2004). Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. Proc. Natl. Acad. Sci. USA *101*, 10042-10047.

Dawson, T.M. (2006). Parkin and defective ubiquitination in Parkinson's disease. J. Neural Transm. Suppl. *70*, 209-213.

Deas, E., Plun-Favreau, H., Gandhi, S., Desmond, H., Kjaer, S., Loh, S.H.Y., Renton, A.E.M., Harvey, R.J., Whitworth, A.J., Martins, L.M., et al. (2011). PINK1 cleavage at position A103 by the mitochondrial protease PARL. Hum. Mol. Genet. *20*, 867-879.

Deng, H., Jankovic, J., Guo, Y., Xie, W.J., and Le, W.D. (2005). Small interfering RNA targeting the PINK1 induces apoptosis in dopaminergic cells SH-SY5Y. Biochem. Biophys. Res. Commun. *337*, 1133-1138.

Deng, H.S., Dodson, M.W., Huang, H.X., and Guo, M. (2008). The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila. Proc. Natl. Acad. Sci. USA *105*, 14503-14508.

Exner, N., Treske, B., Paquet, D., Holmstrom, K., Schiesling, C., Gispert, S., Carballo-Carbajal, I., Berg, D., Hoepken, H.H., Gasser, T., et al. (2007). Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. J. Neurosci. *27*, 12413-12418.

Finley, L.W.S., and Haigis, M.C. (2009). The coordination of nuclear and mitochondrial communication during aging and calorie restriction. Ageing Res. Rev. *8*, 173-188.

Gautier, C.A., Kitada, T., and Shen, J. (2008). Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. Proc. Natl. Acad. Sci. USA *105*, 11364-11369.

Gegg, M.E., Cooper, J.M., Chau, K.Y., Rojo, M., Schapira, A.H.V., and Taanman, J.W. (2010). Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. Hum. Mol. Genet. *19*, 4861-4870.

Geisler, S., Holmstrom, K.M., Skujat, D., Fiesel, F.C., Rothfuss, O.C., Kahle, P.J., and Springer, W. (2010). PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat. Cell Biol. *12*, 119-131.

Glater, E.E., Megeath, L.J., Stowers, R.S., and Schwarz, T.L. (2006). Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. J. Cell Biol. *173*, 545-557.

Gloeckner, C.J., Schumacher, A., Boldt, K., and Ueffing, M. (2009). The Parkinson disease-associated protein kinase LRRK2 exhibits MAPKKK activity and phosphorylates MKK3/6 and MKK4/7, *in vitro*. J. Neurochem. *109*, 959-968.

Greene, J.C., Whitworth, A.J., Kuo, I., Andrews, L.A., Feany, M.B., and Pallanck, L.J. (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. Proc. Natl. Acad. Sci. USA *100*, 4078-4083.

Hao, L.Y., Giasson, B.I., and Bonini, N.M. (2010). DJ-1 is critical for mitochondrial function and rescues PINK1 loss of function. Proc. Natl. Acad. Sci. USA *107*, 9747-9752.

Haque, M.E., Thomas, K.J., D'Souza, C., Callaghan, S., Kitada, T., Slack, R.S., Fraser, P., Cookson, M.R., Tandon, A., and Park, D.S. (2008). Cytoplasmic Pink1 activity protects neurons from dopaminergic neurotoxin MPTP. Proc. Natl. Acad. Sci. USA *105*, 1716-1721.

Haque, M.E., Mount, M.P., Safarpour, F., Abdel-Messih, E., Callaghan, S., Mazerolle, C., Kitada, T., Slack, R.S., Wallace, V., Shen, J., et al. (2012). Inactivation of Pink1 *in vivo* sensitizes dopamine producing neurons to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and can be rescued by autosomal recessive PD genes, Parkin or DJ-1. J. Biol. Chem. doi: 10.1074/jbc.M112.346437.

Hoepken, H.H., Gispert, S., Morales, B., Wingerter, O., Del Turco, D., Mulsch, A., Nussbaum, R.L., Muller, K., Drose, S., Brandt, U.,

et al. (2007). Mitochondrial dysfunction, peroxidation damage and changes in glutathione metabolism in PARK6. Neurobiol. Dis. *25*, 401-411.

Hwang, S., Kim, D., Choi, G., An, S.W., Hong, Y.K., Suh, Y.S., Lee, M.J., and Cho, K.S. (2010). Parkin suppresses c-Jun N-terminal kinase-induced cell death via transcriptional regulation in Drosophila. Mol. Cells *29*, 575-580.

Imai, Y., Kanao, T., Sawada, T., Kobayashi, Y., Moriwaki, Y., Ishida, Y., Takeda, K., Ichijo, H., Lu, B.W. and Takahashi, R. (2010). The Loss of PGAM5 suppresses the mitochondrial degeneration caused by inactivation of PINK1 in Drosophila. PLoS Genet. *6*, e1001229.

Irrcher, I., Aleyasin, H., Seifert, E.L., Hewitt, S.J., Chhabra, S., Phillips, M., Lutz, A.K., Rousseaux, M.W.C., Bevilacqua, L., Jahani-Asl, A., et al. (2010). Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics. Hum. Mol. Genet. *19*, 3734-3746.

Jin, S.M., Lazarou, M., Wang, C.X., Kane, L.A., Narendra, D.P., and Youle, R.J. (2010a). Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J. Cell Biol. *191*, 933-942.

Kang, B.H., Plescia, J., Dohi, T., Rosa, J., Doxsey, S.J., and Altieri, D.C. (2007). Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. Cell *131*, 257-270.

Kim, D., Nguyen, M.D., Dobbin, M.M., Fischer, A., Sananbenesi, F., Rodgers, J.T., Delalle, I., Baur, J.A., Sui, G.C., Armour, S.M., et al. (2007a). SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. EMBO J. *26*, 3169-3179.

Kim, E.J., Kho, J.H., Kang, M.R., and Um, S.J. (2007b). Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity. Mol. Cell *28*, 277-290.

Kim, Y., Park, J., Kim, S., Song, S., Won, S.K., Lee, S.H., Kitada, T., Kim, J.M., and Chung, J. (2008a). PINK1 controls mitochondrial localization of Parkin through direct phosphorylation. Biochem. Biophys. Res. Commun. *377*, 975-980.

Kim, J.E., Chen, J.J., and Lou, Z.K. (2008b). DBC1 is a negative regulator of SIRT1. Nature *451*, 583-586.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature *392*, 605-608.

Kitada, T., Pisani, A., Porter, D.R., Yamaguchi, H., Tscherter, A., Martella, G., Bonsi, P., Zhang, C., Pothos, E.N., and Shen, J. (2007). Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proc. Natl. Acad. Sci. USA *104*, 11441-11666.

Koh, H., Kim, H., Kim, M.J., Park, J., Lee, H.J., and Chung, J. (2012). Silent information regulator 2 (Sir2) and Forkhead box O (FOXO) complement mitochondrial dysfunction and dopaminergic neuron loss in Drosophila pten-induced kinase 1 (PINK1) null mutant. J. Biol. Chem. *287*, 12750-12758.

Kops, G., Dansen, T.B., Polderman, P.E., Saarloos, I., Wirtz, K. W.A., Coffer, P.J., Huang, T.T., Bos, J.L., Medema, R.H., and Burgering, B.M.T. (2002). Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature *419*, 316-321.

Lang, A.E., and Lozano, A.M. (1998). Parkinson's disease - first of two parts. N. Engl. J. Med. *339*, 1044-1053.

Langston, J.W., Ballard, P., Tetrud, J.W., and Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science *219*, 979-980.

Lee, J.Y., Nagano, Y., Taylor, J.P., Lim, K.L., and Yao, T.P. (2010). Disease-causing mutations in Parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. J. Cell Biol. *189*, 671-679.

Liu, S., Sawada, T., Lee, S., Yu, W., Silverio, G., Alapatt, P., Millan, I., Shen, A., Saxton, W., Kanao, T., et al. (2012). Parkinson's disease-sssociated kinase PINK1 regulates Miro protein level and axonal transport of mitochondria. PLoS Genet. *8*, e1002537.

Matsuda, N., Sato, S., Shiba, K., Okatsu, K., Saisho, K., Gautier, C.A., Sou, Y., Saiki, S., Kawajiri, S., Sato, F., et al. (2010). PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J. Cell Biol. *189*, 211-221.

Narendra, D., Tanaka, A., Suen, D.F., and Youle, R.J. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. *183*, 795-803.

Narendra, D.P., Jin, S.M., Tanaka, A., Suen, D.F., Gautier, C.A., Shen, J., Cookson, M.R., and Youle, R.J. (2010a). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. *8*, e1000298.

Narendra, D.P., Kane, L.A., Hauser, D.N., Fearnley, I.M., and Youle, R.J. (2010b). p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. Autophagy *6*, 1090-1106.

Okatsu, K., Saisho, K., Shimanuki, M., Nakada, K., Shitara, H., Sou, Y., Kimura, M., Sato, S., Hattori, N., Komatsu, M., et al. (2010). p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. Genes Cells *15*, 887-900.

Paisan-Ruiz, C., Jain, S., Evans, E.W., Gilks, W.P., Simon, J., van der Brug, M., de Munain, A.L., Aparicio, S., Gil, A.M., Khan, N., et al. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron *44*, 595-600.

Park, J., Lee, S.B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M.H., Kim, J.M., et al. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature *441*, 1157-1161.

Park, J., Lee, G., and Chung, J. (2009). The PINK1-Parkin pathway is involved in the regulation of mitochondrial remodeling process. Biochem. Biophys. Res. Commun. *378*, 518-523.

Parker, J.A., Arango, M., Abderrahmane, S., Lambert, E., Tourette, C., Catoire, H., and Neri, C. (2005). Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. Nat. Genet. *37*, 349-350.

Pesah. Y., Pham, T., Burgess, H., Middlebrooks, B., Verstreken, P., Zhou, Y., Harding, M., Bellen, H., and Mardon, G. (2004). Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. Development *131*, 2183-2194.

Petit, A., Kawarai, T., Paitel, E., Sanjo, N., Maj, M., Scheid, M., Chen, F.S., Gu, Y.J., Hasegawa, H., Salehi-Rad, S., et al. (2005). Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. J. Biol. Chem. *280*, 34025-34032.

Plun-Favreau, H., Klupsch, K., Moisoi, N., Gandhi, S., Kjaer, S., Frith, D., Harvey, K., Deas, E., Harvey, R.J., McDonald, N., et al. (2007). The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. Nat. Cell Biol. *9*, 1243-1252.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science *276*, 2045-2047.

Poole, A.C., Thomas, R.E., Andrews, L.A., McBride, H.M., Whitworth, A.J., and Pallanck, L.J. (2008) The PINK1/Parkin pathway regulates mitochondrial morphology. Proc. Natl. Acad. Sci. USA *105*, 1638-1643.

Pridgeon, J.W., Olzmann, J.A., Chin, L.S., and Li, L. (2007). PINK1 protects against oxidative stress by phosphorylating mitochondrial chaperone TRAP1. PLoS Biol. *5*, e172.

Puig, O., Marr, M.T., Ruhf, M. L., and Tjian, R. (2003). Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev. *17*, 2006-2020.

Sallinen, V., Kolehmainen, J., Priyadarshini, M., Toleikyte, G., Chen, Y.C., and Panula, P. (2010). Dopaminergic cell damage and vulnerability to MPTP in Pink1 knockdown zebrafish. Neurobiol. Dis. *40*, 93-101.

Sämann, J., Hegermann, J., von Gromoff, E., Eimer, S., Baumeister, R., and Schmidt, E. (2009). Caenorhabditits elegans LRK-1 and PINK-1 act antagonistically in stress response and neurite outgrowth. J. Biol. Chem. *284*, 16482-16491.

Schapira, A.H., Cooper, J.M., Dexter, D., Clark, J. B., Jenner, P., and Marsden, C.D. (1990). Mitochondrial complex I deficiency in Parkinson's disease. J. Neurochem. *54*, 823-827.

Shi, G., Lee, J.R., Grimes, D.A., Racacho, L., Ye, D., Yang, H., Ross, O.A., Farrer, M., McQuibban, G.A., and Bulman, D.E. (2011). Functional alteration of PARL contributes to mitochondrial dysregulation in Parkinson's disease. Hum. Mol. Genet. *20*, 1966-1974.

Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K., et al. (2000). Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat. Genet. *25*, 302-305.

Silvestri, L., Caputo, V., Bellacchio, E., Atorino, L., Dallapiccola, B., Valente, E.M., and Casari, G. (2005). Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. Hum. Mol. Genet. *14*, 3477-3492.

Tain, L.S., Mortiboys, H., Tao, R.N., Ziviani, E., Bandmann, O., and Whitworth, A.J. (2009). Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. Nat. Neurosci. *12*, 1129-1135.

Tanaka, A., Cleland, M.M., Xu, S., Narendra, D.P., Suen, D.F., Karbowski, M., and Youle, R.J. (2010). Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J. Cell Biol. *191*, 1367-1380.

Thomas, K.J., McCoy, M.K., Blackinton, J., Beilina, A., van der Brug, M., Sandebring, A., Miller, D., Maric, D., Cedazo-Minguez, A., and Cookson, M.R. (2011). DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum. Mol. Genet. *20*, 40-50.

Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M.M.K., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A.R., Healy, D.G., et al. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science *304*, 1158-1160.

van der Horst, A., Tertoolen, L.G.J., de Vries-Smits, L.M.M., Frye, R.A., Medema, R.H., and Burgering, B.M.T. (2004). FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). J. Biol. Chem. *279*, 28873-28879.

Venderova, K., Kabbach, G., Abdel-Messih, E., Zhang, Y., Parks, R.J., Imai, Y., Gehrke, S., Ngsee, J., Lavoie, M.J., Slack, R.S., et al. (2009). Leucine-rich repeat kinase 2 interacts with Parkin, DJ-1 and PINK-1 in a Drosophila melanogaster model of Parkinson's disease. Hum. Mol. Genet. *18*, 4390-4404.

Vives-Bauza, C., Zhou, C., Huang, Y., Cui, M., de Vries, R.L.A., Kim, J., May, J., Tocilescu, M.A., Liu, W.C., Ko, H.S., et al. (2010). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc. Natl. Acad. Sci. USA *107*, 378-383.

Wang, X.N., Winter, D., Ashrafi, G., Schlehe, J., Wong, Y.L., Selkoe, D., Rice, S., Steen, J., LaVoie, M.J., and Schwarz, T.L. (2011). PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. Cell *147*, 893-906.

Xi, Y., Ryan, J., Noble, S., Yu, M., Yilbas, A.E., and Ekker, M. (2010). Impaired dopaminergic neuron development and locomotor function in zebrafish with loss of pink1 function. Eur. J. Neurosci. *31*, 623-633.

Yang, Y.F., Gehrke, S., Imai, Y., Huang, Z.N., Ouyang, Y., Wang, J.W., Yang, L.C., Beal, M.F., Vogel, H., and Lu, B.W. (2006). Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused inactivation of Drosophila Pink1 is rescued by Parkin. Proc. Natl. Acad. Sci. USA *103*, 10793-10798.

Yang, Y.F., Ouyang, Y.S., Yang, L.C., Bcal, M.F., McQuibban, A., Vogel, H., and Lu, B.W. (2008). Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. Proc. Natl. Acad. Sci. USA *105*, 17585-17587.

Yun, J., Cao, J.H., Dodson, M.W., Clark, I.E., Kapahi, P., Chowdhury, R.B., and Guo, M. (2008). Loss-of-function analysis suggests that Omi/HtrA2 is not an essential component of the pink1/parkin pathway *in vivo*. J. Neurosci. *28*, 14500-14510.

Zhao, W.H., Kruse, J.P., Tang, Y., Jung, S.Y., Qin, J., and Gu, W. (2008). Negative regulation of the deacetylase SIRT1 by DBC1. Nature *451*, 587-590.

Zhou, C., Huang, Y., Shao, Y.F., May, J., Prou, D., Perier, C., Dauer, W., Schon, E.A., and Przedborski, S. (2008). The kinase domain of mitochondrial PINK1 faces the cytoplasm. Proc. Natl. Acad. Sci. USA *105*, 12022-12027.

Zid, B.M., Rogers, A.N., Katewa, S.D., Vargas, M.A., Kolipinski, M.C., Lu, T.A., Benzer, S., and Kapahi, P. (2009). 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila. Cell *139*, 149-160.

Ziviani, E., Tao, R.N., and Whitworth, A.J. (2010). Drosophila Parkin requires PINK1 for mitochondrial translocation and ubiquitinates Mitofusin. Proc. Natl. Acad. Sci. USA *107*, 5018-5023.