FORUM REVIEW ARTICLE

Beyond Mitophagy: Cytosolic PINK1 as a Messenger of Mitochondrial Health

Erin K. Steer,¹ Michelle K. Dail,^{1,2} and Charleen T. Chu^{1,2}

Abstract

Significance: Disruptions in mitochondrial homeostasis are implicated in human diseases across the lifespan. Recessive mutations in *PINK1*, which encodes the mitochondrially targeted PTEN-induced putative kinase 1 (PINK1), cause an autosomal recessive form of Parkinson's disease. As with all kinases, PINK1 participates in multiple functional pathways, and its dysregulation has been implicated in a growing number of diseases. **Recent Advances:** In addition to its heavily studied role in mitophagy, PINK1 regulates mitochondrial respiratory function, reactive oxygen species generation, and mitochondrial transport. Moreover, recent studies implicate processed PINK1 in cytosolic signaling cascades that promote cell survival and neuron differentiation. Cytosolic PINK1 is also capable of suppressing autophagy and mitophagy. We propose a working hypothesis that PINK1 is released by functional mitochondria as a signal to coordinate cell growth and differentiation in response to mitochondrial status. **Critical Issues:** PINK1 biology needs to be better understood in primary neurons, as the stability and subcellular localization of PINK1 is differentially regulated in different cell types. Delineating factors that regulate its mitochondrial import/export, processing by different peptidases, kinase activity, subcellular localization, and degradation will be important for defining relevant downstream kinase targets. **Future Directions:** It is becoming clear that different subcellular pools of PINK1 mediate distinct functions. Future studies will undoubtedly expand on the spectrum of cellular functions regulated by PINK1. Continued study of cytosolic PINK1 may offer novel insights into how functional mitochondria communicate their status with the rest of the cell. *Antioxid. Redox Signal.* 22, 1047–1059.

PARKINSON'S DISEASE (PD) IS A debilitating age-related neurodegenerative disorder that affects 4–5 million people in the world's most populous nations (28). PD pathology is characterized by: (i) Lewy body inclusions, which contain alpha-synuclein and ubiquitin, in autonomic, brainstem and cortical regions (10), and (ii) the loss of pigmented brain stem neurons from the substantia nigra and locus ceruleus. Degeneration of dopaminergic nigral neurons account for the classic motor signs, although other neuron groups are also affected. While the majority of PD cases are idiopathic, studies of gene products implicated in familial PD implicate common pathways that are likely to be important for disease progression (101).

Mutations in *PINK1*, which encodes PTEN-induced putative kinase 1 (PINK1), represent the second most common

cause of autosomal recessive parkinsonism (48, 103). *PINK1* families exhibit an earlier onset of disease (range of 9–52 years), accompanied by sustained responses to levodopa treatment (9, 57, 64, 103). Autopsy findings have been reported from one family, showing classic Lewy body pathology (92). Pathways regulated by PINK1 appear to interact with other PD-linked genes (11, 20, 91), and PINK1 enhances the resistance of neurons to toxic/environmental models of PD (43, 99). Alterations in PINK1 have also been shown to affect the severity of cardiovascular and pulmonary diseases (8, 14, 121).

As noted in *Drosophila* (20, 86), *PINK1* genetically interacts with *PARK2*, which is also mutated in familial recessive parkinsonism. *PARK2* encodes the ubiquitin ligase Parkin, whose overexpression restores many of the

¹Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

²Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

pathological effects of PINK1 loss of function (24, 29). In the past 5 years, the PINK1-Parkin pathway has been heavily studied with respect to depolarization-induced mitochondrial degradation, which occurs *via* mitophagic, proteasomal, and vesicular pathways (51, 76, 119). However, a number of recent studies also implicate PINK1 in the regulation of mitochondrial function, particularly in relation to complex I or IV activity (37, 71, 80). Moreover, cytosolic pools of PINK1 trigger pro-survival and trophic signaling pathways (26, 83), suppressing autophagy and mitophagy (24, 30) to promote dendritic extension (26). This review focuses on these emerging functions of PINK1. We propose that, rather than functioning solely as a sensor for damaged mitochondria, PINK1 is processed and released from healthy mitochondria as an intrinsic signal to promote neuronal differentiation, function, and survival.

Structure and Processing of PINK1

PINK1 is a 581-amino-acid serine/threonine kinase (Fig. 1). Human PINK1 has relatively weak *in vitro* kinase activity, which may reflect the need for PINK1 phosphorylation (58) or other activation mechanisms. In addition to a conserved kinase domain, PINK1 exhibits an N-terminal mitochondrial targeting sequence (MTS), a putative transmembrane anchor, and a C-terminal regulatory tail [reviewed in Sim *et al.* (97)]. In healthy mitochondria, PINK1 is imported and processed through interactions with the core TOM and TIM machinery (7, 66, 77, 98, 112) (Fig. 2). An alternate pathway of PINK1 import that does not require TOM40 has also been described in a cell-free import system (55).

The submitochondrial localization of PINK1 and fate of endogenously processed PINK1 remains more controversial, due in part to difficulties studying untagged PINK1. Several studies suggest localization to the inner membrane or intermembrane space (IMS) based on protease protection assays or location of putative kinase targets (32, 74, 77, 82, 87, 96) (Fig. 2a). Indeed, PINK1 has a hydrophobic sequence following the MTS that could function as a stop transfer signal (Fig. 1), as observed in proteins known to insert in the inner mitochon-

drial membrane (IMM). However, other studies have found that this hydrophobic sequence functions to tether PINK1 to the outer mitochondrial membrane (OMM) with the kinase domain accessible to the cytosol (122) (Fig. 3). A significant fraction of processed PINK1 is also found in the cytoplasm (7, 24, 26, 30, 43, 66, 77, 98, 112). A recent study using a cell-free import system may help unify these observations (6). This study showed that PINK1 residues 1–115 are sufficient to mediate complete translocation of a smaller marker protein, or of C-terminally truncated PINK1, through the OMM into the IMS. Given its length, wild-type PINK1 may require additional chaperone systems to mediate its complete import; saturation of such a system, or impaired membrane potential as observed in the FCCP/CCCP model (75, 84), would favor its OMM retention. Alternatively, we speculate that incomplete import of PINK1 could serve to facilitate its retro-translocation back into the cytosol for signaling purposes (Fig. 2b), as discussed below.

Once imported, the N-terminal region of PINK1 may be processed by several mitochondrial peptidases. One of the first to be implicated is the presenilin-associated rhomboid-like protein (PARL) (27, 39, 50, 77, 114). This integral IMM protease cleaves overexpressed PINK1 between alanine-103 and phenylalanine-104 in the putative transmembrane domain (27). The resultant product may be targeted for proteasomal degradation by the N-end rule (118), although many such proteins are only conditionally destabilized due to incorporation into functional protein complexes or interaction with chaperones (107). Steady-state levels of PINK1 are regulated by chaperones (81, 112) and vary considerably by cell type (authors' unpublished data). In an RNAi study of endogenous PINK1 processing, the mitochondrial processing peptidase (MPP) and two ATP-dependent proteases, matrix-AAA (m-AAA) and caseinolytic mitochondrial matrix peptidase (ClpX) were also identified as potential PINK1 processing enzymes (39). While it is possible that some of these act indirectly to regulate PINK1 cleavage, the cleavage of PINK1 by m-AAA produces a fragment of approximately the same size as the PARL truncated form (~55 kDa). The cleavage patterns

FIG. 1. Domain structure of PTEN-induced putative kinase 1 (PINK1) and homology of autophosphorylation sites. PINK1 is composed of a canonical N-terminal mitochondrial targeting sequence, putative transmembrane domain, serine/threonine kinase domain, and a C-terminal regulatory tail. Eleven conserved subdomains have been described within the kinase domain (96). Subdomains I–IV coordinate the orientation and docking of ATP, whereas the subdomains VI–XI are responsible for detection of target peptide and phosphotransfer. Two evolutionarily conserved autophosphorylation sites within the kinase domain have been shown to promote parkin recruitment to depolarized mitochondria.

also support the prediction that conserved regions of PINK1 at residues 8–18 and 67–77 contain potential MPP cleavage motifs (97), although the involvement of MPP has been controversial (6, 55). AFG3L2 (m-AAA catalytic subunit) has been suggested as the protease producing the ΔN_2 fragment (~45 kDa band) (39, 100). Although the precise cleavage sites

FIG. 3. Functions attributed to unprocessed PINK1 at the outer mitochondrial membrane (OMM). Depolarized mitochondria are unable to import mitochondrial proteins, resulting in the accumulation of unprocessed PINK1 on the mitochondrial surface (a). This form of PINK1 triggers several important cellular responses to mitochondrial damage. OMM-tethered PINK1 activates mitochondrial ubiquitylation through phosphorylation of ubiquitin and the recruitment and phosphorylation of Parkin. Phosphorylation of the mitochondrial fusion protein Mitofusin 2 (a) or the mitochondrial transport adaptor Miro (b) promotes ubiquitination by Parkin. Degradation of these proteins results in mitochondrial fission and cessation of the outer transport, preparing the damaged mitochondrion for recognition *via* ubiquitin-binding cargo adaptors for mitophagy. Mitochondrial depolarization also triggers cytoprotective responses *via* PINK1-dependent phosphorylation of the anti-apoptotic protein Bcl-XL, which prevents its inactivation by cleavage (c).

of these other proteases remain unknown, endogenous PINK1 may consist of a mixture of differentially processed species.

PINK1 forms protein complexes with many other mitochondrial and cytosolic proteins (6, 61, 66, 113, 117). In particular, interactions with heat shock protein chaperones (66, 81, 112), or the bcl2-associated athanogene 5 (BAG5),

FIG. 2. Schematic illustrating the import and processing of PINK1 by functional mitochondria. A canonical N-terminal mitochondrial targeting sequence of PINK1 is recognized by the translocases of the outer mitochondrial membrane (TOM) and the inner mitochondrial membrane (TIM). Depending on the experimental system, the PINK1 kinase domain may be protected from externally applied proteases (a), or remain partially exposed outside the mitochondrion (b). Mitochondrial processing peptidase (MPP), presenilin-associated rhomboid-like protein (PARL), and other mitochondrial peptidases appear to sequentially cleave the N-terminal domain, resulting in release of mature, processed PINK1 to the intermembrane space (a) or its retrotranslocation to the cytosol (b).

play important roles in regulating its stability (110). As discussed next, determining the fate of PINK1 in terms of processing, submitochondrial localization, and release to the cytosol is critically important for understanding its signaling role within the cell.

PINK1 in the Regulation of Mitochondrial Function and ROS

Decreased mitochondrial membrane potential ($\Delta\Psi_m$) is frequently reported in PINK1 loss-of-function studies (Fig. 4), with variable effects on respiratory complex activity and ATP levels depending on the cell system studied [reviewed in Chu (18)]. Mitochondria isolated from PINK1 knockout mouse embryonic fibroblasts (MEFs) exhibit reduction in substrate oxidation, rather than proton leak, as the mechanism for reduced membrane potential (2). Reductions in the activities of mitochondrial complex I (1, 2, 34, 35, 79), II (2, 34, 35), III (1), and IV (37, 71) have been reported in PINK1-deficient neurons and MEFs. Increased opening of the mitochondrial permeability transition pore (34) and deficient activity of the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCLX) (33) have also been suggested to contribute to reduced membrane potential in PINK1 knockout cells.

While some of the effects of PINK1 deficiency on respiration may be due to impaired PINK1-Parkin mediated turnover of respiratory chain proteins (108), PINK1 siRNA causes a more rapid and severe deficit in mitochondrial function than observed with autophagic or lysosomal im-

pairment. Recent data suggest a more proximal mechanism by which PINK1 supports electron transport chain activity. PINK1^{-/-} mice exhibit a decrease in phosphorylation of the complex I subunit NdufA10, which is located on the IMS side of complex I and is required for ubiquinone reduction (80). Although it is unclear whether or not PINK1 itself is the direct kinase for NdufA10, post-translational regulation of respiratory function would represent an efficient mechanism to respond to rapidly changing metabolic demand in neurons. PINK1 deficiency also affects substrate availability through regulation of the plasma membrane glucose transporter (33), components of the citric acid cycle (35), or the ability of mitochondrial calcium to stimulate ATP synthesis (45). Interestingly, Vitamin K₂ is able to reverse ATP deficiencies in PINK1 null flies by acting as an electron carrier downstream of complex I (109). PINK1 may also regulate mitochondrial biology through activation of protein kinase A (PKA) (26). PKA regulates the biosynthesis of mtDNA encoded proteins, and enhances complex I activity through direct phosphorylation of the NDUF54 subunit (85). Notably, AKAP1-mediated targeting of endogenous PKA to mitochondria reverses the respiratory and ATP deficits in PINK1-deficient mammalian cells (25).

Given that PINK1 deficiency results in dysfunctional respiration and disruption in calcium homeostasis, it is not surprising that increased markers of oxidative stress are observed in patient-derived fibroblasts from patients with disease-causative mutations (47, 73). In mammalian cells, PINK1 RNAi elicits an increase in mitochondrial superoxide,

FIG. 4. Mitochondrial changes observed in PINK1 loss-of-function models. PINK1-deficient cells typically show decreases in mitochondrial membrane potential (Ψ_m), decreased oxygen consumption rates, and increased mitochondrial ROS production. Respiratory chain defects contribute to reduced levels of cytosolic ATP observed in some PINK1-deficient systems, and may result in part from decreased phosphorylation of the complex I subunit NdufA10. Loss of PINK1 also impairs NCLX-mediated calcium efflux, resulting in the prolonged elevation of mitochondrial calcium concentrations. Reduced Ψ_m and elevated levels of mitochondrial ROS and calcium promote susceptibility to permeability transition pore (PTP) opening in PINK1 loss-of-function models. Enlarged, rounded mitochondria that are isolated from the mitochondrial network are observed in PINK1-deficient cells. Phosphorylation of the fission protein Drp1 by protein kinase A (PKA) inhibits its mitochondrial translocation. In PINK1-deficient cells, increased activation of the phosphatase calcineurin releases this negative regulation of Drp1, leading to fragmentation of the mitochondrial network. Altered degradation of mitofusins and mitochondrial swelling may also contribute to observed alterations in mitochondrial morphology.

which is essential for triggering compensatory mitophagy and mitochondrial fission (24). In mice, loss of PINK1 results in enhanced sensitivity to oxidative stress (35). These loss-of-function studies suggest that PINK1 may act to reduce production of ROS, perhaps through better maintenance of electron transfer chain function, and/or serve to promote detoxifying pathways. Indeed, PINK1 is necessary for the induction of hypoxia-response pathways in cortical neuron cultures (67). Antioxidant effects of PINK1 are also observed in other organ systems, as endothelial PINK1 is necessary for protection against hyperoxic lung injuries (121).

Dual Roles of PINK1 in Mitochondrial Turnover

Probably, the most studied aspect of PINK1 function lies in the regulation of mitochondrial degradation by mitophagy. Much of this data has been collected in the context of mitochondria that have been rendered nonfunctional through treatment with uncoupling drugs. While this has been useful for detailed delineation of a pathway for mitophagy, the more nuanced interplay of potentially conflicting pathways activated in the context of alternative or less severe injuries are beginning to emerge. It is important to keep in mind that PINK1 exhibits a dual role in regulating mitochondrial content and mitophagy. The less studied capacities of PINK1 to support electron transfer chain function, reduce ROS production, and suppress autophagy enable it to adjust the anabolic-catabolic balance in response to the bioenergetic status of the neuron.

In the most intensely studied pathway, complete loss of membrane potential due to treatment with FCCP, CCCP, or antimycin results in the inability of mitochondria to import and process PINK1 (66). This pathway has been the subject of many excellent reviews (41, 44, 105), and can be triggered by photoirradiation of mitochondria (4), expression of proteins that result in mitochondrial depolarization (40), or overexpression of mutant matrix proteins to induce the mitochondrial unfolded protein response (52).

In brief, full-length PINK1 builds up on the surface of mitochondria to recruit Parkin (75, 84) (Fig. 3). The recruitment of Parkin, an E3 ubiquitin ligase, results in the ubiquitylation of numerous mitochondrial proteins (13, 93). Among these, the degradation of the mitochondrial transport protein Miro (70, 111) and of mitofusin (36, 125) is believed to facilitate cessation of transport and the onset of mitophagy (102). Indeed, Drp1-dependent fission is necessary for mitophagy (24). Full-length PINK1 and Parkin also interact with the autophagy regulator Beclin 1 (16, 78). Recently, it was shown that PINK1 phosphorylates ubiquitin to activate Parkin activity (53, 56, 59). Adapter proteins such as p62 or optineurin are recruited to ubiquitinated mitochondria (38, 115). These proteins interact with the autophagy protein microtubule associated protein 1 light chain 3 (LC3) to promote autophagic sequestration. Depolarization also triggers exposure of the phospholipid cardiolipin to the mitochondrial surface, where it also interacts with LC3 to mediate entry of damaged mitochondria into autophagosomes (19).

Although it had been assumed that autophagy occurs mostly within the neuronal soma, autophagosomes are continually formed at the distal tip of axons (72), sufficient to support mitophagy of photo-irradiated mitochondria (4). In this study, 10% of photo-irradiated mitochondria showed

parkin translocation. Antimycin-stimulated axonal mitophagy was not observed in *Park2*^{-/-} axons. In *Pink1*^{-/-} axons, however, mitophagy induced by antimycin was reduced by only ~50%. This suggests that PINK1 augments, but is not necessary for mitophagy, consistent with observations that mitophagy is induced in PINK1-deficient cells (24). Strikingly, another ubiquitin ligase implicated in PD has recently been linked to mitophagy (11). The F-box protein Fbxo7 undergoes a similar PINK1-dependent mitochondrial translocation as Parkin, and can rescue Parkin deficiency in *Drosophila*. PINK1 may also recruit Fbxo15 to downregulate mitochondrial cardiolipin in pulmonary epithelial cells (14).

It has become clear that there are multiple pathways regulating classical mitophagy (5) (Fig. 5), defined as lysosomal degradation of mitochondrial components that is dependent on the macroautophagic Atg8-lipidation machinery. These include direct engagement of the autophagy machinery protein LC3 by mitochondrial transmembrane receptors (68) or by cardiolipin translocated from the IMM to the OMM in neurons treated with parkinsonian toxins (19). The relationship between these pathways and the Parkin or Fbxo7 pathways remains unclear, as inhibiting cardiolipin redistribution suppressed about 50% of depolarization-induced mitophagy. In contrast, neurotoxin-triggered mitophagy does not require accumulation of PINK1 and Parkin on the mitochondria, although a possible role for ubiquitin ligases was not excluded (19). Steroid metabolism and ceramides may also play a role in PINK1-Parkin dependent and -independent mitophagy pathways (49, 94).

In addition, there are several alternative pathways to mitochondrial protein degradation. PINK1-Parkin not only activates mitophagy but also triggers cell death (120), proteasomal degradation of outer membrane proteins (13, 119), and delivery of mitochondria-derived vesicles to lysosomes (76). The latter pathway may prove particularly important in neurons, as neurons and other cells that are metabolically dependent on mitochondria do not engage the Parkin mitophagy pathway to the same extent as tumor cells (12, 104). The engagement of one or more of these pathways is likely to be regulated in a cell-type and context-dependent manner; it remains to be discovered how the different outcomes of Parkin activation are determined. Ultimately, neuronal function or dysfunction will likely be determined by the sum total of degradative *versus* biosynthetic forces (124) and the appropriate transport of mitochondria to pre- and post-synaptic sites.

A *Drosophila* study indicates that PINK1 and Parkin are involved in turnover of mitochondrial proteins *in vivo* (108). Interestingly, while Parkin-regulated proteins correlated well with Atg7-regulated proteins, PINK1-regulated proteins did not follow this pattern. This finding could support an important role for Atg7-independent "micromitophagy" (63, 76). Alternatively, the effects of PINK1 could be masked due to a dual role in promoting and suppressing mitochondrial degradation, as discussed later. It is important to note that the proteomic method used did not directly measure degradation, but inferred turnover rates from relative abundance of heavy and light peptides. Increased protein synthesis could also result in decreased ratios due to dilutional effects. Interestingly, data suggest that parkin and PINK1 may regulate mitochondrial biogenesis (37, 60, 95) through effects on nuclear transcription. PINK1 also promotes the activation of

FIG. 5. Molecular mechanisms underlying cargo recognition for selective mitophagy. In yeast, the transmembrane autophagy-related protein 32 (Atg32) acts as a mitophagy receptor through an association with Atg8 (**a**). This association may occur directly through its WXXL-like LC3-interacting region (LIR) motif or *via* the adaptor protein Atg11. Although there are no structural homologs of these proteins in humans, analogous transmembrane LIR motif proteins serve similar purposes. During erythrocyte differentiation, hypoxia, or metabolic stress, NIP3 like protein X (Nix), Bcl-2/adenovirus E1B 19kD-interacting protein 3 (Bnip3), and FUN14 domain containing protein 1 (FUNDC1) act as mitophagy receptors (**b**) [reviewed in Feng *et al.* (31)]. Dissipation of the mitochondrial membrane potential, or other insults that interfere with mitochondrial protein import, can trigger the accumulation of PINK1 on the OMM, resulting in recruitment and activation of parkin (**c**). Parkin mediates the poly-ubiquitination of mitochondrial proteins, including the voltage-dependent anion channel (VDAC). Sequestosome-1-like LIR motif adaptor proteins such as p62 and optineurin (OPTN) serve potentially redundant roles to bind these poly-ubiquitin chains, promoting the sequestration of damaged mitochondria into autophagosomes. An enzyme-dependent translocation of cardiolipin from the inner leaflet of the inner mitochondrial membrane (IMM) to the outer leaflet of the OMM mediates selective mitophagy in response to staurosporine and the parkinsonian toxins rotenone and 6-hydroxydopamine (**d**). Although drawn as separate pathways, selective mitophagy in mammals mostly likely involves redundant and/or additive effects of more than one pathway (**b-d**) activated by a given stimulus or injury.

CREB (26), a positive regulator of both nuclear and mtDNA transcription (62).

In contrast to its clearance role for nonfunctional mitochondria, there is also a body of evidence demonstrating that PINK1 plays an important role in suppressing the turnover of functioning mitochondria (Fig. 6). PINK1 deficiency often causes a loss of mitochondrial mass and/or increased evidence of mitophagy (21, 23, 24, 69, 88), which can be reversed by stimulating mitochondrial biogenesis (21). The damaged mitochondria in PINK1-deficient cells often appear swollen with loss of cristae ultrastructurally (24). Thus, a decrease in functional mass may be accompanied by preservation or even an increase in the volume occupied by mitochondria. For this reason, measures of mitochondrial content should be carefully defined in comparing different studies. In mammalian cells, loss of endogenous PINK1 triggers mitochondrial degradation through Atg7-dependent delivery to autophagosomes and autolysosomes (24). Pulsing PINK1-deficient cells with bafilomycin to trap newly formed autophagosomes reveals that autophagosomes in PINK1-deficient cells are formed at a significantly higher rate than in control cells, resulting in selective sequestration of mitochondria (17). Restoration of PINK1 expression suppresses mitophagy, and overexpression of PINK1 inhibits toxin-induced mitophagy (24).

There are several mechanisms by which PINK1 may suppress mitophagy. PINK1 has been shown to drive the degradation of endogenous, but not overexpressed, Parkin

(90). PINK1 can also bind Parkin in the cytosol to prevent its translocation to damaged mitochondria (30). PINK1 may act indirectly to reduce the triggers for mitophagy by preserving mitochondrial membrane potential, reducing ROS, and promoting calcium homeostasis. PINK1 activates Akt (83), which typically inhibits autophagy. PINK1 may also suppress autophagy through PKA (26), which, in turn, phosphorylates and inactivates the autophagy protein LC3 (15) and the fission protein Drp1 (22), both of which are necessary for most forms of mitophagy.

We have proposed that the key to regulating this functional switch of PINK1 with respect to autophagy/mitophagy lies in the processing or subcellular targeting of PINK1 (42, 123, 124). While OMM-tethered full-length PINK1 is required for Parkin recruitment, N-terminally truncated, cytosolic PINK1, which is only generated in cells with functioning mitochondria, is sufficient to suppress mitophagy triggered by PINK1-deficiency, 6-hydroxydopamine, or CCCP (24, 26, 30). Moreover, as discussed next, mitochondrial and cytosolic pools of PINK1 appear to have distinct effects (26, 30).

Cytosolic PINK1 as an Intrinsic Neurotrophic Signal?

Although postmitotic neurons do not proliferate in response to growth factor signals, neurotrophic factors play a key role in stimulating intracellular signaling cascades that promote survival, differentiation, and function of neurons. Likewise, PINK1 is capable of promoting neuronal survival

FIG. 6. Proposed dual role of PINK1 as both a pro-growth messenger of mitochondrial health and a sensor for mitochondrial damage. We propose a working hypothesis that the processing of PINK1 by mitochondria functions as a switch (*inset a*) to determine neurotrophic (*left*) versus degradative (*right*) effects of PINK1. Neurons with high levels of functioning mitochondria produce sufficient mature, processed PINK1 to support mitochondrial complex I activity (*inset b*), reducing mitochondrial ROS formation and regulating mitochondrial dynamics (fission/fusion, transport) to ensure proper mitochondrial homeostasis and distribution in neurons. Cytosolic PINK1, most likely stabilized from degradation by chaperones, acts to enhance neuronal differentiation through multiple mechanisms. These include engagement of Akt and PKA signaling pathways, as well as unidentified pathways that regulate nuclear transcription. In addition to these growth-promoting effects, cytosolic PINK1 acts to suppress autophagic degradation through indirect effects on LC3 and Drp1 phosphorylation, the activation of Akt and/or cytosolic sequestration of Parkin. In contrast, individual damaged mitochondria that are unable to import, process, and release PINK1 would trigger the PINK1-Parkin pathways of mitochondrial degradation by accumulating OMM-tethered PINK1 (*right*). Recruitment and activation of Parkin and ubiquitin to the mitochondrial surface results in degradation of regulatory proteins to result in arrested anterograde axonal transport, mitochondrial fission, and mitochondrial degradation through mitophagy, the proteasome, or mitochondria-derived vesicles. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

(3), differentiation (26), and synaptic function (79). In this sense, PINK1 has neurotrophic effects. While other neurotrophic signaling pathways originate outside of the neuron, we propose that cytosolic PINK1 functions as an intracellular trophic signal that is initiated by well-functioning mitochondria.

While many studies have demonstrated that PINK1 is present in both mitochondrial and cytosolic compartments, Haque *et al.* were the first to suggest that cytoplasmic activities of PINK1 may be important for its neuroprotective function (43). A PINK1 construct lacking the first 111 amino acids (Δ N-PINK1) protected against MPP⁺ toxicity *in vitro* and against MPTP toxicity *in vivo*. Although there is a minor component of Δ N-PINK1 that can co-purify with mitochondria (6, 113), the predominant location of this form of PINK1 is cytosolic. PINK1 also activates the cytosolic mammalian target of rapamycin complex 2 (mTORC2)/Akt pathway (83), as well as enhancing mitochondrial, cytosolic, and nuclear signaling downstream of PKA (26). Interestingly, a phosphoproteomic study of PINK1-deficient cells

reveals that PINK1 regulates the phosphorylation state of nuclear proteins (89). Of course, the phosphorylation of many of these proteins may be mediated by other kinases. The use of insect homologs of PINK1 and artificial activating substrates (46, 116) may help accelerate discovery of direct PINK1 targets.

Among the binding partners for PINK1 are Miro and Milton, adapter proteins for the transport of mitochondria along microtubules (113). Activation of the PINK1-Parkin pathway serves to downregulate Miro, leading to a decrease in mitochondrial transport in axons (70, 111). Alternatively, processed cytoplasmic PINK1 increases the density of mitochondria in dendrites (26). These differences may relate to fundamental differences in the regulation of microtubule-dependent transport between axons and dendrites (54, 106). Notably, the interaction of PINK1 with Miro does not require the N-terminal MTS (113). Moreover, the ability to undergo Drp1-dependent fission is essential for transport of mitochondria into dendrites (65). This indicates that the ability of PINK1 and Parkin to modulate mitochondrial fission/fusion

may be important not only for preparing damaged mitochondria for mitophagy but also for regulating mitochondrial transport under more physiological conditions.

The recent discovery that cytosolic PINK1 promotes neuron differentiation provides a particularly compelling argument for conceptualizing PINK1 not only as a mitochondrial kinase but also as a trophic signal released by mitochondria (26) (Fig. 6). PINK1-deficient primary cortical and midbrain neurons exhibit much reduced dendrite lengths and complexity. This can be rescued by reintroduction of either full length or Δ N-PINK1. Moreover, expression of either full length or Δ N-PINK1 in undifferentiated SH-SY5Y cells induces neurite extension and expression of differentiation markers by Western blot. In contrast, a PINK1 construct targeted to the OMM had no effects on neurite length, despite having access to cytosolic targets. This indicates that a residual association of Δ N-PINK1 with mitochondria is unlikely to explain the potency of Δ N-PINK1 in stimulating neurite outgrowth. Moreover, it lends further support to the concept that distinct pools of PINK1 exist that mediate functions beyond the mitochondrion. Although the mechanism has not been completely elucidated, it appears that the PINK1 and PKA signaling pathways cooperate to promote neuron differentiation (26). In this sense, PINK1 may be considered a cellular signal, activated and released by healthy mitochondria to trigger other intracellular signaling pathways.

Dual Roles for PINK1 as a Messenger of Mitochondrial Function and Dysfunction

In summary, advances in understanding PINK1 biology have been occurring at an explosive rate. Much has been learned about the role of PINK1 in sensing mitochondrial dysfunction to trigger quality control clearance by mitophagy and other degradative mechanisms. A new emerging body of data indicates that PINK1 is a multifunctional protein participating not only in the regulation of mitochondrial function and quality control but also in coordinating broader cellular functions of survival, differentiation, intracellular transport, and nuclear signaling. It is likely that the differentially located subcellular pools of PINK1—whether of the IMS, OMM, or cytosol—have different substrates and functions in regulating mitochondrial respiration, ROS generation, mitochondrial dynamics, mitochondrial quality control, and mitochondrial retrograde signaling.

We propose a working hypothesis conceptualizing PINK1 in dual roles to sense both mitochondrial function and dysfunction. This concept may help encourage alternative lines of investigation regarding the role of PINK1 in neurons, which we consider crucial for the field to advance. Healthy mitochondria import and process PINK1, resulting in stabilization of cristae structure, complex I function, and preservation of functional mitochondrial networks (Fig. 6, left). Processed PINK1 may also be released to orchestrate more distant effects necessary for neuron differentiation and function. When these signals cannot be effectively generated, due to the inability to import and process PINK1, or presumably to genetic mutations underlying PD, than the balance switches to favor catabolism and neurodegeneration (Fig. 6, right). Given that PINK1 is unique among the large multidomain protein kinases in possessing a canonical MTS,

it is ideally situated to act as an intrinsic signal responsive to mitochondrial status. Much additional study is needed to experimentally test and refine this concept.

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Address correspondence to:
Dr. Charleen T. Chu
Division of Neuropathology
Department of Pathology
University of Pittsburgh School of Medicine
S701 Scaife Hall
200 Lothrop St.
Pittsburgh, PA 15213
E-mail: ctc4@pitt.edu

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Abbreviations Used

AKAP1 = A kinase (PRKA) anchor protein 1
 BAG5 = bcl2-associated athanogene 5
 CCCP = carbonyl cyanide m-chlorophenyl hydrazine
 ClpX = caseinolytic mitochondrial matrix peptidase
 FCCP = carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone
 IMM = inner mitochondrial membrane
 IMS = intermembrane space
 m-AAA = matrix AAA protease
 MEF = mouse embryonic fibroblasts
 MPP = mitochondrial processing peptidase
 MTS = mitochondrial targeting sequence
 NCLX = mitochondrial Na⁺/Ca²⁺ exchanger
 OMM = outer mitochondrial membrane
 PARL = presenilin-associated rhomboid-like protein
 PD = Parkinson's disease
 PINK1 = PTEN-induced putative kinase 1
 PKA = protein kinase A