Review

Mitophagy: the latest problem for Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative disorder of unknown cause. Some familial forms of PD are provoked by mutations in the genes encoding for the PTEN (phosphatase and tensin homolog)-induced putative kinase-1 (PINK1) and Parkin. Mounting evidence indicates that PINK1 and Parkin might function in concert to modulate mitochondrial degradation, termed mitophagy. However, the molecular mechanisms by which PINK1/Parkin affect mitophagy are just beginning to be elucidated. Herein, we review the main advances in our understanding of the PINK1/Parkin pathway. Because of the phenotypic similarities among the different forms of PD, a better understanding of PINK1/Parkin biology might have far-reaching pathogenic and therapeutic implications for both the inherited and the sporadic forms of PD.

Parkinson's disease

Parkinson's disease (PD) is one of the most common neurodegenerative disorders affecting 1% of the population over the age of 65 [1]. Clinically, PD is characterized mainly by motor manifestations such as bradykinesia, resting tremor, rigidity and postural instability [1]. One of the most salient neuropathological features of PD is the loss of the dopamine-containing neurons in the substantia nigra pars compacta (SNpc), which is responsible for the profound reduction of dopamine in the striatum [2]. The presence of cytoplasmic proteinaceous inclusions called Lewy bodies, in the few surviving dopaminergic neurons, is another striking pathological feature of PD [3]. Although the cause of PD remains unknown, genetic predisposition factors together with environmental agents are thought to trigger a cascade of deleterious events that are believed to involve mitochondrial dysfunction, oxidative stress and failure of the protein degradation machinery, which, ultimately, lead to the demise of the SNpc dopaminergic neurons [2]. In addition to these proposed scenarios, a defect in autophagy, especially of mitochondria (mitophagy), has emerged as the newest pathogenic hypothesis in PD [4–7]. However, this novel and exciting twist in our quest to unravel the mechanisms responsible for neurodegeneration in PD is still in its nascent stage, and major gaps remain in our understanding of this molecular pathway and how defects in mitophagy might cause PD. Herein, we review the available literature on PD pathogenesis and alterations in mitochondrial biology as it relates to defects in mitochondrial dynamics and autophagy. We will discuss these important topics in light of recent advances made by the discoveries of gene mutations causing familial forms of PD, and which seem to be linked to alterations in mitochondrial dynamics and/or autophagy. Through a survey of the literature, we will stress what new information has been acquired about how damaged mitochondria are cleared from the cells and how defects in such a quality control mechanism caused by PD-linked proteins might ultimately lead to neurodegeneration. We will also discuss some of the most salient outstanding issues surrounding the question of how damaged mitochondria are selectively targeted for degradation in the context of PD. Thus far, our incomplete understanding of the mechanisms of neurodegeneration in PD has hindered the development of effective treatments for this debilitating disease. We believe that clarifying the role of altered mitochondrial dynamics and mitophagy in the demise of dopaminergic neurons might have far-reaching implications, not only for familial PD, but also for all forms of this neurodegenerative condition.

Mitophagy

As reminded by DiMauro and Schon [8], the story of mitochondria in eukaryotic cells is fascinating. Indeed, a billion years ago, aerobic bacteria colonized anaerobic eukaryotic cells. Through this symbiotic relationship, bacteria evolved into mitochondria and the host cells acquired the ability to metabolically use oxygen. By all accounts, it seems that eukaryotic cells have lived 'happily ever after' with mitochondria. For instance, each eukaryotic cell from multicellular organisms is inhabited, not by just a few, but by a couple of thousand mitochondria. It is now also well established that mammalian cells rely on mitochondria for a host of crucial functions such as pyruvate oxidation, the Krebs cycle, calcium homeostasis, the metabolism of amino acids, fatty acids, steroids and the generation of energy as ATP [8]. Mitochondria also play a important role in several mechanisms of cell death because they are the repositories of pro-apoptotic molecules such as cytochrome *c* and are decorated with proteins of the Bcl-2 family such as Bax [9].

Furthermore, not only are the quality and the number of mitochondria critical to the well-being of eukaryote cells, but the recruitment of these functionally versatile organelles to the most active subcellular regions is also important. As reviewed by Detmer and Chan [10], this singular intracellular distribution is particularly well documented in

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neurons, in which imaging studies indicate that mitochondria are recruited to subcellular regions with high energy demands, such as active growth cones, presynaptic sites and postsynaptic sites. The accumulation of mitochondria in specific subcellular regions is molecularly controlled and depends on mitochondrial trafficking along cytoskeletal tracks [10]. For instance, in eukaryotic cells mitochondria are transported along microtubules, which involves kinesin family members and cytoplasmic dynein for their anterograde and retrograde movements, respectively [10]. It has also been demonstrated that two additional proteins, Milton and Miro, are required for anterograde mitochondrial transport in neurons: Milton interacts directly with kinesin and Miro is a mitochondrial outer membrane protein that has GTPase and Ca^{2+}-binding EF-hand domains [10]. How cells modulate mitochondrial trafficking remains ill-defined; however, it is worth noting that mitochondria with high membrane potential (used as a proxy for mitochondrial health) seem to preferentially migrate in the anterograde direction, whereas mitochondria with low membrane potential (used as a proxy for mitochondrial damage) seem to move in the retrograde direction [11]. Should these observations be correct, it might be envisioned that neurons possess the machinery needed to modulate the mitochondrial kinesin/Milton/Miro-dependent motor and/or the dynein-dependent retrograde motor so that active mitochondria would be specifically transported to distal regions with high-energy requirements, and impaired mitochondria would be specifically returned to the cell soma for repair or destruction.

It is also important to remember that cells can destroy damaged mitochondria, but they cannot produce new mitochondria *per se*. Instead, following the loss of mitochondria, a cell maintains the number of mitochondria – as well as their lengths and shapes – by altering the balance between fission and fusion [10]. In contrast to yeast, the core machineries controlling mitochondrial fission and fusion are only partially understood [12]. In mammals, mitochondrial fission seems to be controlled primarily by the cytosolic protein DRP1 (dynamin-related protein 1) and by the integral outer mitochondrial membrane protein FIS (mitochondrial fission protein). Whether there is inner membrane fission machinery in mammals remains to be established. Also to be elucidated is how the different fission proteins operate in mammalian cells to fragment mitochondria. Thus far, we mostly know that DRP1 translocates to mitochondria in response to cellular and mitochondrial cues, and then several DRP1 molecules form oligomeric rings that constrict mother mitochondrial tubes to give rise to two or more daughter mitochondria [12]. As for mitochondrial fusion in mammals, this is controlled primarily by the GTPase proteins mitofusin- (MFN-) 1 and 2, which are anchored to the outer mitochondrial membrane, and the dynamin-like GTPase protein optic atrophy 1 (OPA1), which is located in the mitochondrial intermembrane space in a soluble form or tightly attached to the inner mitochondrial membrane. Here, in contrast to fission, there is a distinct submitochondrial localization of key fusion proteins consistent with the idea that the combination of two mitochondria into a single organelle occurs in a two-step process, where the outer and inner mitochondrial membranes fuse by separate events [12]. In addition to maintaining the size of the mitochondrial population, fusion/fission is believed necessary to refurbish 'old mitochondria' with fresh functional units and to preserve the correct balance among mitochondrial sizes, metabolic requirements and motility. For instance, the genetics of Charcot-Marie-Tooth Neuropathy type 2A and of Dominant Optic Atrophy show that mutations in MFN-2 and OPA1 cause neurodegeneration [13,14]. Thus, it is probable that the equilibrium between mitochondrial fusion and fission is crucial for the well-being of postmitotic cells such as neurons, maybe because of their high-energy demands and large cytoplasmic masses. Dopaminergic neurons have a propensity to accumulate high levels of mtDNA deletions [15] perhaps because of their higher calcium conductance [16]. It can thereby be speculated that the number of mtDNA molecules carrying deletions per mitochondrion is kept in check thanks, at least in part, to the fusion/fission machinery. Indeed, this process, by constantly mixing mitochondria and their contents, might ensure that there are always sufficient numbers of wild type mtDNA molecules to compensate for the loss of function associated with mutated mtDNA.

The loss of mitochondrial membrane potential ($\Delta\Psi m$) is often seen as a functional correlate to mitochondrial damage. In mammalian cells, the dissipation of $\Delta\Psi m$ seems to be a common feature associated with mitophagy, suggesting that damaged mitochondria are specific targets for removal by macroautophagy [17–19]. From this view, the cell would selectively induce mitophagy to eliminate malfunctioning mitochondria, thereby ridding the cell of troublesome sources of reactive oxygen species, apoptosis-inducing factors or unnecessary metabolic burdens. Like other forms of autophagy, mitophagy utilizes the core autophagic machinery (reviewed in [20]). Although autophagy was initially demonstrated in mammals, it was genetic screens in yeast that led to the discovery of the autophagy-related (Atg) genes, and it is by reversed genetics, again in yeast, that the roles of many of the different Atg proteins were initially identified [21]. From these seminal investigations, it emerged that the core autophagy machinery is comprised of the: Atg1–Atg13–Atg17 kinase complex; class III phosphatidylinositol 3-kinase complex I (which includes Atg6 and Atg14); ubiquitin-like protein conjugating systems Atg12 and Atg8; and Atg9 and its cycling system [21]. By now, it has been shown that this core machinery is conserved among eukaryotes and that the mammalian homologs of many of the core Atg proteins do exist. For example, the serine/threonine-protein kinases ULK1 and ULK2 are mammalian homologs of Atg1, whereas Beclin 1 is the homolog of Atg6, and LC3 (microtubule-associated protein 1 light chain 3) and GABARAP (gamma-aminobutyric acid receptor-associated protein) are the homologs of Atg8 [21]. In addition to the core autophagy machinery, mitophagy relies on other Atg proteins to acquire its mitochondrial specificity. Although much remains to be elucidated about the molecular mechanisms of mitophagy, recent genetic screens performed in yeast have led to the discovery of an outer mitochondrial membrane protein that is instrumental in mitochondrial disposal and has been named Atg32 [22,23]. Its silencing abrogates mitochondrial degradation without affecting the clearance of long-lived proteins or

other organelles such as peroxisomes [22,23]. Atg32 links mitochondria to the autophagy machinery via interaction with the core protein Atg8 and with Atg11, a yeast adaptor protein for selective autophagy [24]. Thus far, no mammalian Atg32 homolog has been discovered, but several of the Atg32 structural characteristics have pointed to the mammalian mitophagy factor Nix/BNIP3L [25] as a potential candidate. Indeed, Nix, which is a noncanonical BH3 member of the Bcl-2 family of proteins, like Atg32, is an outer mitochondrial membrane protein that binds Atg8 and is essential for mitochondrial destruction [26–29]. However, despite the fact that Nix seems to be ubiquitously expressed in mammals, up to now its role in mitophagy has only been documented in the hematopoietic system during the maturation of reticulocytes in erythrocytes [26–29].

Familial forms of PD point to a relation between PD and mitophagy

PD commonly arises sporadically but, in some cases, the disease is inherited. PARK6/PINK1 is one of the gene products associated with familial PD [30,31]. This 581-amino acid polypeptide is a serine/threonine kinase with a high degree of homology to the Ca^{2+}/calmodulin kinase family that localizes to mitochondria. Loss-of-function mutations in the gene encoding PARK2/Parkin (an E3 ubiquitin ligase) can cause an autosomal recessive form of familial PD [31,32]. *Drosophila* carrying *pink1* mutations exhibit an abnormal mitochondrial morphology [33,34]. In *Drosophila*, Parkin is thought to operate within the same molecular pathway as PINK1 [33–35]. Although the initial interpretation of these observations in *Drosophila* was that the PINK1/Parkin pathway modulates mitochondrial dynamics, mounting evidence, from primarily mammalian cells, indicates that this pathway might in fact modulate mitophagy [4–6,36,37]. These hypotheses, however, are not mutually exclusive because fusion/fission affects mitochondrial turnover by autophagy.

To our knowledge, the first experimental evidence supporting a role for the Parkin in mitophagy originated from the work of Narendra and collaborators [36]. These investigators found that Parkin, in both HEK293 and HeLa cells, is selectively recruited through an unknown mechanism to depolarized mitochondria, and is followed by a profound mitochondrial disappearance in cultured cell lines [36]. Within 48 h of exposure to the protonophore *m*-chlorophenylhydrazone (CCCP), which collapses the $\Delta\Psi m$, mitochondrial markers such as Tom20, cytochrome *c* and TRAP1 (tumor necrosis factor type 1 receptor-associated protein-1) were all lost, suggesting that mitochondria were eliminated (Figure 1). The specificity of mitochondrial elimination was remarkable because peroxisomes remained intact. Narendra and colleagues [36] also provided data supporting the idea that depolarized mitochondria were eliminated by autophagy. Indeed, mitochondria were seen within intracellular LC3-positive vesicular structures. In addition, they showed that CCCP-triggered mitochondrial disappearance was abrogated in HeLa cells treated with the lysosomal inhibitor bafilomycin A1 and in mutant mouse embryonic fibroblasts (MEFs) deficient in the autophagic factor Atg5.

Do PINK1 and Parkin modify each other?

Parkin translocation to mitochondria relies on PINK1 expression (Figure 1), and if wild type but not functionally deficient mutated PINK1 is overexpressed, Parkin can be recruited to mitochondria with normal $\Delta\Psi_m$ [4–6,37]. The latter observation suggests that PINK1, probably through its kinase activity, is a key signaling molecule in mitophagy

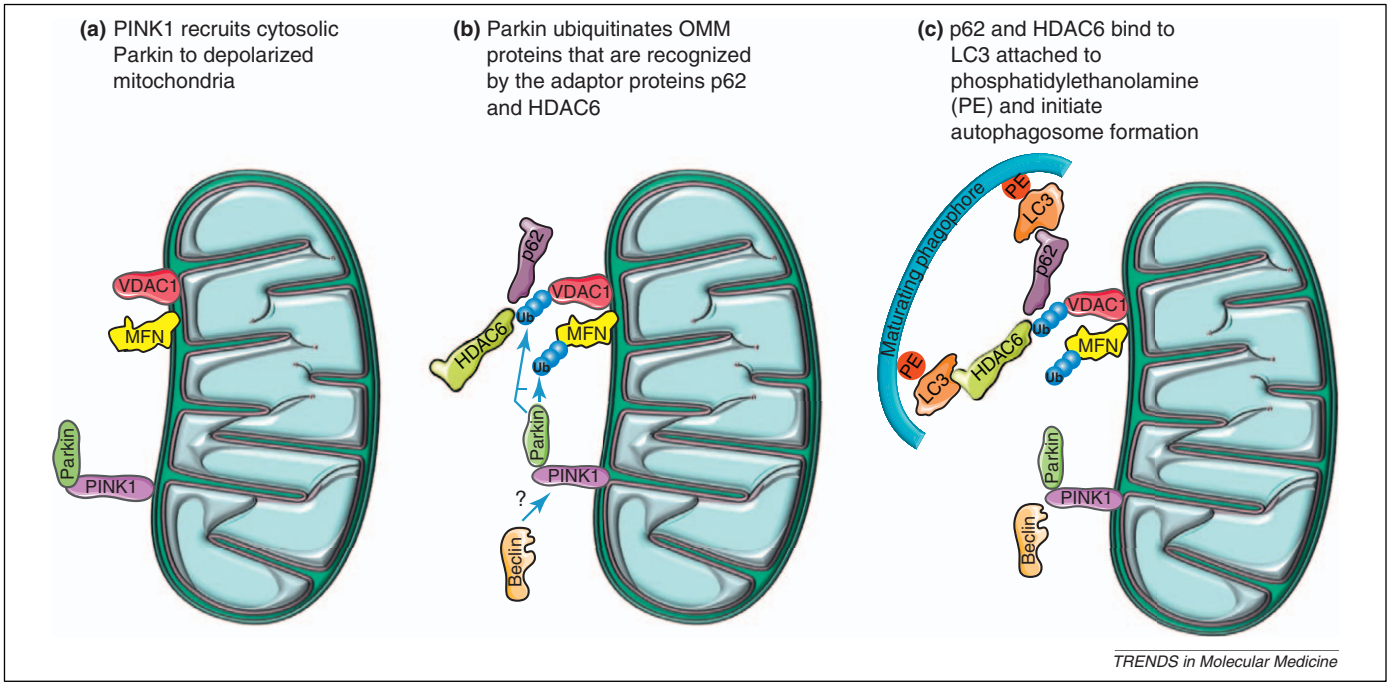

Figure 1. The PINK1/Parkin signaling pathway. Damaged mitochondria lose their membrane potential, which inhibits PINK1 cleavage. The mitochondrial content of full-length PINK1 thereby increases, which enhances PINK1 signaling. By an unknown mechanism, this triggers either directly or indirectly **(a)** Parkin recruitment to mitochondria. **(b)** VDAC1 and/or MFN are ubiquitinated in a Parkin-dependent manner. Then, mitochondrial aggregation occurs because of the Parkin-mediated ubiquitination of mitochondrial outer membrane proteins. **(c)** Both p62 and HDAC6 link polyubiquitinated mitochondria with LC3, initiating mitophagy.

and that it operates downstream to the intramitochondrial molecular alterations provoked by the loss of $\Delta\Psi_m$. PD-linked loss-of-function mutations in either protein abolishes Parkin recruitment to mitochondria and the ensuing association of mitochondria with autophagic and lysosomal markers [4–6,37]. Because PINK1 spans the outer mitochondrial membrane with its kinase domain facing the cytosol [38], and because PINK1 and Parkin can physically interact [4,39], it is reasonable to speculate that PINK1 phosphorylates Parkin. Consistent with this view, Sha and collaborators [40] reported that wild type but not mutant PINK1 phosphorylated wild type Parkin in an *in vitro* kinase assay. They also showed that, in SH-SY5Y cells, the phosphorylation of overexpressed Parkin was increased by wild type PINK1 overexpression and decreased by *PINK1* silencing [40]. Notably, these authors claim that Parkin was modified by PINK1 only in the presence of calcium [40], whereas previously, in the case of the phosphorylation of TRAP1 by PINK1, they did not mention any calcium requirement [41]. Could it be that different substrates can be modified by PINK1 via distinct kinase activities? Nonetheless, under kinase assay conditions similar to those used successfully by others to compare wild type and mutant PINK1 kinase activity [42,43], we have not been able to confirm the phosphorylation of Parkin by PINK1 [4]. Because neither Sha and collaborators nor our group have studied endogenous proteins, it is possible that differences in the levels of overexpression of Parkin and PINK1 after transfection account for the discrepancy, especially if the phosphorylation signal is not robust. Perhaps relevant to this issue are the results of Kim and collaborators [44] showing that the highly conserved threonine residue of Parkin T175, but not residue T217, is specifically phosphorylated by PINK1 in BE(2)C human neuroblastoma cells. These authors also showed that Parkin T175 phosphorylation is pivotal for Parkin translocation to mitochondria in *Drosophila* [44]. Subsequently, another group substituted those conserved threonine residues of Parkin with either alanine (a nonphosphorylatable residue) or glutamic acid (to mimic a permanently phosphorylated residue) and found, upon transfecting these mutants in HeLa cells, that T175 and T217 seem necessary, but not sufficient, for Parkin translocation to mitochondria [5]. Given the above data, it is fair to conclude that even if it can be agreed upon that Parkin is phosphorylated during PINK1/Parkin-dependent mitophagy, whether Parkin is a direct substrate of PINK1 and how, once phosphorylated, it is targeted specifically to damaged mitochondria remains to be established. Furthermore, it is important to mention that a small number of proteins other than Parkin have also been reported to be phosphorylated by PINK1 or in a PINK1-dependent manner, including TRAP1 and the serine protease HtrA2/Omi [41,45]. At this point, whether TRAP1 is a *bona fide* substrate of PINK1 and plays a functional role in PINK1/Parkin signaling is still unknown. As for HtrA2/Omi, a series of *in vivo* genetic interaction studies in *Drosophila* [46–48] suggest that HtrA2/Omi and Parkin might act downstream of PINK1 within distinct parallel pathways; this potentially important notion awaits confirmation in living mammalian organisms.

Although one report failed to find any evidence of Parkin-catalyzed PINK1 ubiquitination [4], two other studies [6,49] provided data supporting a model in which Parkin promotes the polyubiquitination of specific mitochondrial proteins. Consistent with our findings [4], these two reports confirm that, following Parkin translocation to mitochondria, Parkin-decorated mitochondria assemble as large clusters primarily in the lysosome-rich perinuclear area [6,49]. These authors also show that these mitochondrial clusters are ubiquitin-positive and are ubiquitinated in a Parkin-dependent manner [6,49]. For Geisler and collaborators, the specific mitochondrial protein modified by Parkin is the voltage-dependent anion channel 1 (VDAC1) [6]. These authors, however, do not prove that VDAC1 ubiquitination has any functional significance in the PINK1/Parkin mitochondrial pathway. In fact, although Narendra and colleagues confirmed that VDAC1 (and not VDAC2) is ubiquitinated in HeLa cells in the presence of Parkin following mitochondrial depolarization, they demonstrated that the polyubiquitination of VDAC1 is dispensable for PINK1/Parkin-dependent mitochondrial clustering and mitophagy [49]. If VDAC1 were not the pivotal mitochondrial substrate of Parkin, do we know of any other potential candidate? Relevant to this important issue is the demonstration, in two independent studies, that the PINK1/Parkin pathway mediates the ubiquitination of the outer membrane mitochondrial fusion protein Mfn [50–52]. Because mitochondrial fission might be necessary to trigger mitophagy [19], the modification of Mfn by Parkin might be crucial to the disposal of damaged mitochondria. Indeed, it is possible that rather than being a part of the mitophagy machinery *per se*, Parkin merely facilitates mitophagy by depleting the pool of functional Mfn via proteasomal degradation and/or inactivation of this key fusion factor, thereby enhancing fission and, in turn, engulfing mitochondria in autophagosomes.

Polyubiquitin chains articulated around different lysine (Lys) residues of ubiquitin, such as Lys 27 or Lys 63, have specific consequences for tagged substrates and are linked to diverse cellular functions [53]. The proteins that recognize and convert these signals into functional responses are responsible for this diversity [54]. Through its ubiquitin-associated domain, p62/SQSTM1 (p62 hereafter) [55] is thought to link ubiquitinated substrates to LC3 to facilitate the autophagic degradation of ubiquitinated proteins [56]. Several studies have reported that Parkin promotes the recruitment of p62 to clustered mitochondria and that p62 knockdown does not affect Parkin translocation to depolarized mitochondria [6,49,57,58]. Yet, although Geisler and collaborators found that p62 is necessary for Parkin-dependent mitophagy provoked by $\Delta\Psi$m dissipation in HeLa cells [6], others found, in both HeLa cells in which *p62* was silenced by siRNA and in mutant MEF $p62^{-/-}$ cells, that p62 has no effect on Parkin-dependent mitochondrial degradation [49,57,58]. Thus, further studies are needed to clarify the role of p62 in Parkin-mediated mitochondrial quality control. Aside from the fact that Parkin recruits p62, Lee and colleagues showed that Parkin also recruits the ubiquitin-binding protein histone deacetylase 6 (HDAC6) to mitochondria [59]. HDAC6 is implicated in aggresome formation, which is a concentration of toxic protein aggregates

that are subsequently cleared by autophagy [59–62]. Of further support to the idea that perinuclear mitochondrial clusters are reminiscent of aggresomes is that, like these, mitochondrial clusters are driven to the perinuclear area by the microtubule dynein motor. Indeed, treatment with the microtubule-destabilizing reagent nocodazole or overexpression of dynamitin, which inhibits dynein motor activity, significantly reduced the formation of perinuclear mitochondrial aggregates but did not affect Parkin translocation to mitochondria [4,59]. Of possible relevance to this biased retrograde movement of Parkin-decorated mitochondria is the reported interaction between PINK1 and Miro/Milton [63], suggesting that by acting on these motor adaptor molecules, PINK1 could, in response to cellular and/or mitochondrial cues, favor the trafficking of damaged mitochondria toward the lysosome-rich perinuclear area. Importantly, nocodazole treatment significantly inhibited the clearance of depolarized Parkin-positive mitochondria [59], indicating that dynein motor-dependent aggregate formation is required for the efficient elimination of impaired mitochondria (Figure 1).

Thus, we believe that the available data do not definitively conclude that PINK1 and Parkin modify each other, but instead indicate that the catalytic activities of both proteins are necessary for the function of the PINK1/Parkin signaling pathway. Even the data discussed above require further investigations, especially because, in living mammalian organisms, they strongly suggest that PINK1 and Parkin each have several mitochondrial substrates and that the regulation of mitophagy by the PINK1/Parkin pathway is multifaceted.

Signaling and regulation of PINK1 and Parkin

Although we are gaining insights into the PINK1/Parkin molecular pathway, several important outstanding questions remain (Figure 1). For instance, regarding PINK1 biology, we still do not know how the loss of $\Delta\Psi m$ engages PINK1 signaling or how PINK1 triggers Parkin recruitment. Some recent observations might help clarify these intriguing questions. In mitochondria with normal $\Delta\Psi m$, PINK1, which has a short half-life, is present in low amounts [5,37]. However, a loss in $\Delta\Psi m$ is associated with an increase in PINK1 levels in mitochondria [4,5,37]. Because this effect on PINK1 is observed within an hour of the dissipation of $\Delta\Psi m$ and occurs even in the presence of an inhibitor of protein synthesis [5], we predict that this corresponds to a post-translational response.

PINK1 is cleaved, and its cleavage seems to play a role in its protective function against various stressors [64,65]. Moreover, full-length PINK1, but not its cleaved form, interacts with the pro-autophagic protein Beclin 1 to enhance autophagy [66]. Furthermore, it seems that the cleaved fragment of PINK1 exhibits a shorter half-life than full-length PINK1 does [65] and that the cleaved fragment of PINK1 is, of the two main forms of mitochondrial PINK1, the one that is preferentially degraded by a proteasomal-like activity [38,65]. The intramembrane protease rhomboid-7, known as presenilin-associated rhomboid-like (PARL) protease in mammals, is important for normal mitochondrial fusion in *Drosophila* [67] and is, thus far, the only proposed candidate protease for the cleavage of PINK1, at least in *Drosophila* [46]. Intriguingly, rhomboid-7/PARL is thought to reside inside the mitochondrial inner membrane [68], and yet, the only predicted transmembrane domain around the cleavage site of PINK1 is thought to be embedded in the mitochondrial outer membrane [38]. Perhaps the work of Jim and collaborators provides the answer to this apparent topological dilemma [69].

Although there are several reports on the putative extramitochondrial biology of Parkin, nothing is known yet about the mechanism underlying Parkin translocation from the cytosol to mitochondria. Whether Parkin translocates alone or in the company of other cytosolic proteins, such as DRP1 or Bax [70,71], which can also relocalize to mitochondria upon $\Delta\Psi m$ dissipation, is unknown. It also remains unclear whether the post-translational modification of Parkin is required for redistribution to mitochondria, such as in the case of DRP1 [71]. Related to this latter point is the observation made by Matsuda and colleagues that the Parkin ubiquitin ligase activity is repressed by an unidentified mechanism, whereas the protein is cytosolic, but upon mitochondrial translocation, the enzymatic activity of Parkin is recovered [37].

It seems that the mitochondrial content of PINK1 is pivotal in allowing Parkin to be recruited to mitochondria and for the ensuing mitophagy. In addition, it seems that the mitochondrial content of PINK1 is primarily regulated by a voltage-dependent cleavage. However, how this proteolytic activity is regulated by $\Delta\Psi m$, how PINK1 triggers Parkin translocation and whether PINK1, Parkin or both operate within the mitophagy machinery *per se* or rather within other molecular pathways (e.g. mitochondrial dynamics) that are necessary for the successful clearance of damaged mitochondria represent some of the burning outstanding questions surrounding the PINK1/Parkin biology.

Mitophagy and fusion/fission

In connection to one of the above issues, one might wonder whether PINK1/Parkin modulates mitophagy through the fusion/fission machinery. Indeed, it is reasonable to believe that the smaller the mitochondrion, the better it can be handled by the autophagy machinery. Consistent with this speculation are reports that knocking down the fission proteins DRP1 and FIS1 or overexpressing the fusion protein OPA1 reduces mitophagy [19,36]. By contrast, an excess of fission, driven by the overexpression of DRP1 or FIS1, leads to mitochondrial disappearance [72,73]. At first glance, these data suggest that mitochondrial fission is required for mitophagy. However, Twig and coworkers examined the role of fission and fusion in mitophagy by following several individual mitochondrion through rounds of fission and fusion [19]. Depolarized mitochondria could not re-fuse and were preferentially segregated in LC3-positive structures [19,74]. Mitochondrial depolarization and the ensuing fragmentation occurred well before autophagy. Similarly, Gomes and Scorrano found that the overexpression of a mutant form of FIS1, which does not cause fission but can provoke mitochondrial dysfunction, was as efficacious as wild type FIS1 at promoting mitophagy [73]. Furthermore, in neurons induced to undergo apoptosis but prevented

from dying using caspase inhibitors, mitochondria are fragmented and lose their $\Delta\Psi_m$ prior to disappearing [75,76]. Thus, the above data raise the question: is fission sufficient or even necessary to trigger mitophagy?

Emerging united pathogenic theme

The multiplicity of genetic defects giving rise to similar PD phenotypes has prompted researchers to consider the possibility that a common pathogenic cascade could underlie neurodegeneration in many if not all forms of familial PD. To date, however, there is no clear and compelling pathway unifying these different PD-linked mutant proteins. Only PINK1 and Parkin seem to converge functionally. In familial forms of PD, owing to mutations in either of these genes, impaired Parkin recruitment to mitochondria could lead to the accumulation of damaged mitochondria, with a consequent increase in oxidative stress and toxic burden that could lead to a specific kind of cell death of the most susceptible neurons.

Other PD-related proteins play a role in other types of autophagy. For example, Leucine-Rich Repeat Kinase 2 (LRRK2), a multidomain protein containing a kinase and a GTPase domain, is located in membrane microdomains, in multivesicular bodies (MVBs) and in autophagic vesicles (AVs) [77]. Mutations in LRRK2 induce autophagic stress characterized by the accumulation of abnormal MVBs and enlarged AVs with high levels of p62. LRRK2 depletion impairs the autophagy/lysosomal pathway as evidenced by the accumulation of lipofuscin granules and altered levels of LC3 and p62 [78]. α-Synuclein, which upon mutation and overexpression leads to an autosomal dominant form of PD, is degraded, at least in part, by chaperone-mediated autophagy [79]. However, neither mutated α-synuclein nor dopamine-modified α-synuclein can be properly degraded by this process [79,80]. Alternatively, one recent report [81] suggests that α-synuclein, perhaps because of its unique capacity to interact with membranes, inhibits mitochondrial fusion in SH-SY5Y human neuroblastoma cells and that this effect is alleviated by the overexpression of PINK1, Parkin and even DJ1, whose gene mutations have also been linked to a familial form of PD [82]. Likewise, DJ1 deletion was also associated with mitochondrial fragmentation and depolarization in both cultured human neuroblastoma cells [83] and *Drosophila* [84]. If confirmed, these findings will suggest that, perhaps through different mechanisms, all of the different PD-related proteins might contribute to the pathogenesis of this common neurodegenerative disorder by a combination of effects on mitochondrial dynamics and autophagy.

Concluding remarks

The identification and understanding of PD-related proteins are valuable for providing insight into the pathogenic mechanisms of this neurodegenerative disorder. Impairment in quality control autophagy (i.e. autophagy triggered to enforce intracellular quality control by eliminating toxic protein aggregates or damaged organelles) has emerged as a prominent new pathogenic mechanism. Whether the scenario proposed for PINK1/Parkin familial PD (Figure 1) can be extrapolated to sporadic PD remains to be determined. It is possible that other factors such as toxin exposure, increased oxidative stress or genetic factors could limit the availability of Parkin and other proteins for proper mitochondrial maintenance. In fact, Parkin can be covalently modified and inactivated by dopamine quinone, which might contribute to the increased susceptibility of the dopaminergic neurons [85]. Another possibility is that environmental factors such as rotenone or even paraquat might directly or indirectly influence mitochondrial dynamics and/or mitophagy, thereby recapitulating the key pathogenic hypotheses discussed above in the context of familial PD.

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