

# The many faces of mitophagy

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**Failure to maintain mitochondrial integrity is linked to age-related conditions, such as neurodegeneration. Two genes linked to Parkinson's disease, *PINK1* and *Parkin*, play a key role in targeting the degradation of dysfunctional mitochondria (mitophagy). However, the mechanisms regulating the *PINK1*/*Parkin* pathway and other processes that impinge on mitochondrial turnover are poorly understood. Two articles in *EMBO reports*, by the Przedborski and Ganley groups [1, 2], shed light on a new role for processed, cytoplasmic *PINK1*, and show that depletion of cellular iron levels stimulates *PINK1*/*Parkin*-independent mitophagy.**

Mitochondrial homeostasis is a tightly controlled process that involves mitochondrial dynamics, trafficking and degradation. The discovery of a link between autosomal-recessive forms of Parkinson's disease (PD) and mitochondrial quality control has sparked intense interest in understanding these pathways. The serine-threonine kinase *PINK1* and the E3-ubiquitin ligase *Parkin* act in a common pathway to promote the degradation of failing mitochondria through selective autophagy—a process known as mitophagy. The prevailing model posits that under basal conditions, 'healthy' mitochondria import *PINK1*, which undergoes rapid proteolysis, export and degradation. This process constitutively represses a key degradation signal. Upon mitochondrial damage—modeled by the dissipation of mitochondrial membrane potential ( $\Delta\Psi_m$ ) with CCCP or valinomycin—*PINK1* import is blocked, precluding its proteolytic processing and resulting in the stabilization of full-length *PINK1* on the outer mitochondrial membrane (OMM). This stimulates the recruitment of cytosolic *Parkin* to the mitochondrial surface, where it ubiquitinates multiple OMM targets. The mechanism by which this occurs is currently unclear, but ubiquitinated mitochondria are

segregated from the network and targeted for safe removal by mitophagy.

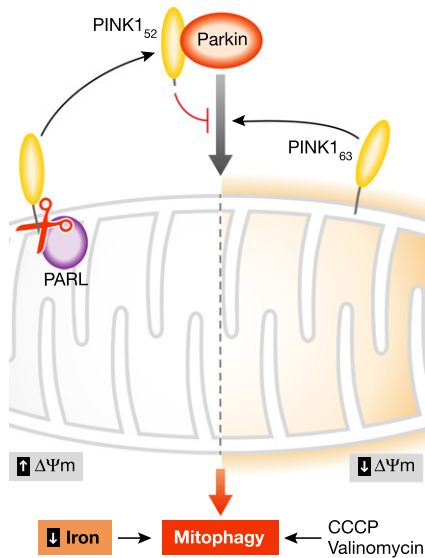
“... iron depletion specifically triggers mitophagy in a *PINK1*/*Parkin*-independent manner”

The details of *Parkin* recruitment remain to be elucidated, but one particular aspect of the pathway has been the subject of much debate; the localization and functional relevance of *PINK1* isoforms. Full-length *PINK1* is approximately 63 kDa (*PINK1*<sub>63</sub>); its import into mitochondria leads to processing by several proteases, including the inner mitochondrial membrane (IMM) protease *PARL* [3], which generates a short *PINK1* isoform of approximately 52 kDa (*PINK1*<sub>52</sub>). *PINK1*<sub>63</sub> is localized to mitochondria, consistent with the clear mitochondrial targeting sequence, however, *PINK1*<sub>52</sub> localization is more dynamic. *PARL*-mediated cleavage severs *PINK1*'s transmembrane domain anchor, enabling its re-distribution to other cellular compartments and the possibility for extra-mitochondrial functions.

Early studies considered *PINK1*<sub>52</sub> as 'mature' *PINK1*, thought to be the major mediator of *PINK1* functionality. Supporting this view was evidence that cytosolic *PINK1*<sub>52</sub> was protective against mitochondrial stressors [4], and may perform a distinct role from mitochondrial *PINK1* [5]. However, subsequent data argued that OMM stabilization of *PINK1*<sub>63</sub>, and not *PINK1*<sub>52</sub>, is required for *Parkin* recruitment, E3-ligase activation and mitophagy [6, 7]. In addition, *PINK1*<sub>52</sub> is very short-lived in mammalian cells, being rapidly degraded by the proteasome. In fact, cleavage by *PARL* exposes an N-terminal phenylalanine residue, promoting N-end rule proteasomal degradation [8]. Thus, *PINK1*<sub>52</sub> has recently been considered a non-functional intermediate.

Challenging this view, a study in this issue of *EMBO reports* by Przedborski and colleagues suggests that *PINK1*<sub>52</sub> has a direct role in regulating *Parkin* activity [1]. The authors re-assess the subcellular distribution of *PINK1* isoforms using multiple approaches. Under basal conditions, both *PINK1* isoforms seem to reside on the OMM, with *PINK1*<sub>52</sub> more loosely associated than *PINK1*<sub>63</sub>. However, *PINK1*<sub>52</sub> spontaneously exits mitochondria and an N-truncated form (*PINK1* <sup>$\Delta$ 1–103</sup>, representing cytoplasmic *PINK1*<sub>52</sub>) was found to physically interact with the *Parkin* RING1 domain. Cytosolic *PINK1*<sub>52</sub> was also shown to inhibit *Parkin* translocation. Specifically, the authors show that promoting cytosolic *PINK1*<sub>52</sub> accumulation through proteasome inhibition prior to valinomycin treatment significantly decreases *Parkin* translocation and mitophagy (Fig 1). A similar effect is observed upon *PINK1* <sup>$\Delta$ 1–103</sup> overexpression, suggesting that *PINK1*<sub>52</sub> confers a dominant-negative effect (Fig 1).

Hence, Przedborski and colleagues propose a novel function for cytosolic *PINK1*<sub>52</sub> in negatively regulating the *PINK1*/*Parkin*-mitophagy pathway. Although the main claim certainly warrants independent verification, the approach used does not perfectly recapitulate the physiological situation. For instance, *PINK1* <sup>$\Delta$ 1–103</sup> would not be subject to N-end rule degradation, increasing its stability, and potentially overestimating the influence of *PINK1*<sub>52</sub> under physiological conditions. Similarly, stabilizing endogenous *PINK1*<sub>52</sub> with proteasome inhibitors will of course have non-specific effects, so such experiments should be interpreted with caution. Curiously, the authors find that under basal conditions *PINK1* <sup>$\Delta$ 1–103</sup> actually promotes *Parkin* translocation and mitophagy, which is in conflict with the proposed negative regulation of *Parkin* by *PINK1*<sub>52</sub>, so further work is required to resolve these details. Nevertheless, the current data suggest that *PINK1*<sub>52</sub> may have a distinct cellular function to *PINK1*<sub>63</sub> in the cytoplasm.



**Fig 1. Effects of PINK1 cleavage and low iron on mitophagy.**

In 'healthy' mitochondria with high membrane potential ( $\Delta\Psi_m$ ), PINK1 is imported cleaved by PARL and other proteases, and processed PINK1<sub>52</sub> released into the cytosol. Upon mitochondrial damage and loss of  $\Delta\Psi_m$ , induced *in vitro* by CCCP or valinomycin, full-length PINK1<sub>63</sub> is stabilized on the outer surface and stimulates the recruitment of Parkin, leading to mitophagy. Przedborski and colleagues present evidence that cytoplasmic PINK1<sub>52</sub> can inhibit Parkin recruitment. A chemical screen by Ganley and colleagues found that depletion of cellular iron can trigger mitophagy in a new mechanism that does not require PINK1 or Parkin, or the loss of  $\Delta\Psi_m$ .

Not only are additional mechanisms that regulate PINK1/Parkin-mediated mitophagy being identified, but also alternative pathways that influence mitophagy that seem not to rely on these molecules. In the December issue of *EMBO reports*, Ganley and colleagues reported that iron depletion specifically triggers mitophagy in a PINK1/Parkin independent manner [2; Fig. 1]. Not only does this study indicate that mitochondrial turnover responds to intracellular iron levels, it presents a novel mechanism through which mitophagy can be initiated. If defective mitophagy is the key underlying defect in PINK1/Parkin-related PD, stimulating

mitophagy via iron depletion may offer a therapeutic approach.

Allen *et al* [2] performed a screen for chemical inducers of mitophagy using an elegant mitophagy sensor system consisting of an OMM-bound, tandem GFP-mCherry. Upon lysosomal localization of mitochondria, GFP is quenched whilst the mCherry signal persists, providing a simple but effective read-out of mitophagy. In this screen, in addition to known inducers of mitophagy—such as CCCP and valinomycin—the iron chelator Deferiprone (DFP) robustly induced mitophagy. An increase in transferrin receptor levels and a rescue by the addition of exogenous iron support the notion that iron depletion stimulates mitophagy.

One intriguing feature of DFP-induced mitophagy is that affected organelles maintain their  $\Delta\Psi_m$ , in contrast to the PINK1/Parkin-mediated mitophagy discussed above, which requires the dissipation of  $\Delta\Psi_m$ . Membrane potential is generally regarded as a read-out of mitochondrial health, therefore, DFP-treated cells would appear to have a healthy mitochondrial network and, indeed, ATP-levels remain stable. However, the cells had switched from oxidative phosphorylation to glycolysis, previously suggested to be a permissive event in the induction of mitophagy [9].

The maintenance of  $\Delta\Psi_m$  in DFP-induced mitophagy is immediately at odds with the model for the PINK1/Parkin pathway, suggesting this is triggered by a separate mechanism. Ganley and colleagues show that DFP-induced mitophagy does not lead to PINK1<sub>63</sub> stabilization on the OMM, and occurs in cells lacking PINK1 or Parkin. These results firmly uncouple this mechanism from the PINK1/Parkin pathway. However, notably DFP still potently induces mitophagy in these cells, supporting the idea that this may provide a therapeutic angle.

These findings have broader implications in the context of PD, as dysregulated iron metabolism is associated with PD and other neurodegenerative diseases [10], through its involvement in generating oxidative radicals and in the biosynthesis of iron-sulfur clusters

and heme in mitochondria. Here Ganley and colleagues suggest another way in which iron dysregulation may impact on neuroprotective mechanisms.

Taken together, these two studies provide new insights in our understanding of mitophagy and its induction. PINK1/Parkin-mediated mitophagy is clearly a complex pathway and much still needs to be resolved, particularly in physiological settings. It is encouraging that mitophagy can be pharmacologically stimulated, although the physiological impact of this will require careful consideration.

### Conflict of interest

The authors declare that they have no conflict of interest.

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