For Parkin, it's not all or nothing

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Mitochondria accumulate damage over time, which can lead to impairment of cell function by excessive production of reactive oxygen species. The PINK1/Parkin pathway therefore monitors the quality of the mitochondrial population and stimulates the elimination of depolarized organelles by mitophagy. In this issue of *The EMBO Journal*, McLelland *et al* (2014) show that this pathway also responds to mild oxidative damage, and instead of inducing mitophagy, allows oxidized proteins to be sorted into mitochondria-derived vesicles (MDVs) that are transported to lysosomes.

See also: G-L McLelland *et al* (February 2014)

If a pear in your refrigerator is going bad, will you just cut out the moldy part or dump the whole thing down the garbage disposal? That might depend on just how bad it is. Cells, it seems, face a similar decision when dealing with an impaired mitochondrion: trash the whole organelle by mitophagy or selectively remove damaged proteins. Mitochondria are particularly prone to damage because electron transport in the inner mitochondrial membrane (IMM) can generate reactive oxygen species (ROS) from the incomplete reduction of molecular oxygen. When produced in excess, these ROS will not only damage the mitochondrion but its cellular milieu as well. This phenomenon may be particularly problematic in long-lived cells with high energy demands such as neurons where mitochondrial dysfunction has been increasingly implicated in neurodegeneration (Schon & Przedborski, 2011). Cells, therefore, recognize and eliminate damaged mitochondria. The process is orchestrated by the PINK1/Parkin pathway, which detects mitochondria that have a compromised

membrane potential and triggers their engulfment by autophagosomes. The importance of mitochondrial quality control is clear: mutations in PINK1 and Parkin cause Parkinson's Disease. In this issue of *The EMBO Journal*, McLelland *et al* (2014) show that PINK1 and Parkin can act in a subtler way to protect mitochondrial quality, by the selective vesicular transport of oxidized cargoes to lysosomes.

PINK1 is a Ser/Thr kinase and Parkin is an E3 ubiquitin ligase. Loss of mitochondrial membrane potential, which can be induced with depolarizing drugs such as carbonyl cyanide m-chlorophenyl hydrazone (CCCP), stabilizes PINK1 on the outer mitochondrial membrane (OMM) and thereby recruits and activates Parkin. Parkin then ubiquitinates a plethora of OMM proteins (Sarraf *et al*, 2013) some of which, such as Mitofusin and Miro1/RhoT1, are then degraded by the proteasome (Tanaka *et al*, 2010; Wang *et al*, 2011). In a manner not yet understood, massive ubiquitination of the mitochondrion recruits the autophagy machinery. Subsequently autophagosomes containing mitochondria fuse with lysosomes to complete mitophagy (Fig 1) (Narendra *et al*, 2012 and references therein).

A less drastic way to preserve mitochondrial quality has recently been uncovered that avoids degrading the whole organelle. Mitochondrial oxidative stress generates mitochondria-derived vesicles (MDV) that are 70–100 nm in diameter and preferentially contain oxidized cargoes that are transported to lysosomes (Soubannier *et al*, 2012a,b). MDVs can contain both matrix and IMM proteins. MDVs are not a form of mitophagy; they are produced and degraded independently of core autophagy genes such as ATG5, beclin-1 and Rab9. Now, McLelland *et al* (2014) show that PINK1 and

Parkin mediate MDV generation (Fig 1). Upon non-depolarizing treatment with antimycin A, a blocker of the electron transport chain and a potent inducer of mitochondrial ROS, a population of MDVs became co-localized with Parkin. Parkin was preferentially in vesicles that were close to mitochondria, suggesting an early role in the budding or sorting of cargoes. Catalytically inactive Parkin^{C431F}, a mutant allele found in PD patients, did not support the formation of vesicles, indicating that ubiquitination is necessary for MDV formation. Knock-down of PINK1 also suppressed the production of MDVs. When does the PINK1/Parkin pathway induce complete mitophagy versus selective removal of damage via MDVs? The answer apparently lies in the extent of the damage. When impairment was severe, such as upon CCCP treatment, massive depolarization caused mitophagy. Mild damage, as with antimycin A treatment, may produce many ROS but is only modestly depolarizing and produced MDVs instead. When both loss of membrane potential and ROS production occurred, both mitophagy and MDV production were observed. The authors conclude that levels of ROS insufficient to decrease membrane potential will nonetheless activate PINK1, which will stimulate, via Parkin, MDV formation and traffic to the lysosome. The MDV pathway may clear damaged mitochondrial components on an on-going basis while mitophagy is reserved for extreme situations.

The paper raises many questions. How can ROS activate PINK1 in the absence of mitochondrial depolarization? In the established model, PINK1 is constitutively imported to mitochondria, cleaved, and then retrotranslocated into the cytosol for proteasomal degradation (Narendra *et al*, 2012) and is only stabilized when the mitochon-

Fig 1. Depending on the nature of mitochondrial damage, PINK1 and Parkin will induce mitophagy or the formation of mitochondria-derived vesicles (MDVs).

If the damage is severe and depolarizing, mitophagy will be induced. If the damage produces ROS but does not depolarize the mitochondrion, the MDV pathway will be activated instead. PINK1 acts upstream of Parkin in both cases, but with different consequences: either the recruitment of the phagophore to the mitochondrion or the formation MDVs that carry oxidized cargoes to lysosomes. Thus depolarizing insults cause degradation of the entire organelle but ROS triggers degradation of only the selected portions sequestered to the MDVs.

drion is depolarized. One possibility is that, if the matrix is not isopotential, ROS can diminish membrane potential locally to stabilize PINK1. Alternatively, ROS may modify PINK1 directly to activate it and prevent its degradation. What is the mechanism

of cargo selection and vesicle budding? The MDVs contained primarily matrix components and lacked outer membrane proteins such as Tom20. Is Parkin necessary for the sorting of cargoes, the budding of the vesicles, for their traffic, or all of the above?

Moreover, since both pathways entail PINK1 and Parkin activation, how do they achieve such distinct results for the organelle? Finally, how important are ROS-triggered MDVs compared to bulk mitophagy? A recent study in *Drosophila* compared the rate of *in vivo* protein turnover in flies mutant for PINK1, Parkin and ATG7, where the latter should abrogate only the mitophagy pathway (Vincow *et al*, 2013). Consistent with a role for the MDVs in constitutive mitochondrial protein turnover, the authors found both autophagy-dependent and autophagy-independent proteins whose turnover was influenced by Parkin and PINK1. Did the autophagy-independent pathway, which was implicated particularly for respiratory chain components, correspond to the MDV pathway? Detailed proteomic analysis of MDVs may clarify this issue.

The MDV pathway may be particularly beneficial in neurons where, compared to other cell types, it may be more costly and slow to destroy and replace mitochondria in distal axons and dendrites. Far from the site of most protein and lipid synthesis, the “economics” may strongly favor mitochondrial repair over replacement. Thus it is uncertain at present whether the neurodegenerative consequences of PINK1 and Parkin mutations are more due to failure of the MDV or mitophagy pathways. Moreover, while the PINK1/Parkin pathway helps to keep a healthy mitochondrial population, other pathways surely figure in mitochondrial turnover as well. Parkin- and PINK1-null mutant mice show very mild phenotypes, and human cases of Parkin and PINK1 mutations cause slow and selective neurodegeneration, sparing many cell types. Other mechanisms must exist that control mitochondrial number and health. The linkage of defective mitochondrial clearance to neurodegenerative diseases, however, strongly suggests that understanding mitochondrial quality control is an essential goal.

Conflict of interest

The authors declare that they have no conflict of interest.

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