Parkin promotes cell survival via linear ubiquitination

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Mitochondrial dysfunction has been linked to the pathogenesis of Parkinson's disease (PD). PD-associated mutants of Parkin show defects in mitophagy, suggesting that Parkin exerts its neuroprotective role by removal of damaged mitochondria. Müller-Rischart *et al* (2013) now uncover an autophagy-independent pathway involving Parkin-mediated activation of the LUBAC ubiquitin ligase to prevent mitochondrial impairment and thereby to promote survival.

PD represents the most common movement disorder and is caused by dopaminergic neurodegeneration. As the majority of cases arise sporadically, the identification of monogenetic variants marked the beginning of a new era in PD research.

Loss-of-function mutations mapped to the Parkin ubiquitin ligase and PINK1 kinase loci were shown to cause autosomal recessive juvenile parkinsonism (Kitada *et al*, 1998).

The generation of PINK1 and Parkin deletion mutants in *Drosophila* illustrated their requirement for the maintenance of mitochondrial integrity (Clark *et al*, 2006) and supported the notion that mitochondrial dysfunction is a major etiological factor in PD. These reports also demonstrated the genetic epistasis of PINK1 and Parkin, placing them in a common or converging pathway. Further studies revealed their involvement in the autophagic removal of damaged mitochondria (Narendra *et al*, 2008). PINK1 is selectively retained and stabilized at the

Figure 1 Two alternative Parkin-mediated pathways to promote mitochondrial integrity are shown. (A) Parkin-mediated mitophagy: upon stress induction mitochondria are fragmented and PINK1 gets stabilized at the outer membrane. PINK1 retention recruits Parkin and induces its E3 ligase activity towards mitochondrial substrates. The ubiquitin signal then ultimately is decoded by the autophagy machinery and results in autophagic degradation. (B) The novel described pathway by Müller-Rischart *et al* (2013) demonstrates that fragmented and impaired mitochondria are rescued by Parkin-induced fusion. By driving LUBAC-mediated linear ubiquitination of NEMO, Parkin increases OPA1 transcription and protein levels. OPA1 allows mitochondrial inner-membrane fusion following Mitofusin-dependent outer-membrane fusion.

outer membrane of depolarized mitochondria and serves as a signal for Parkin recruitment and activation. Subsequent ubiquitination of mitochondrial proteins by Parkin drives affected mitochondria into autophagosomes (Geisler *et al*, 2010). In addition, mounting evidence also suggests that Parkin confers anti-apoptotic protection under different stress conditions (Dawson and Dawson, 2010). As Parkin-induced sequestration of damaged mitochondria could potentially account for these observations, Müller-Rischart *et al* (2013) analysed if Parkin's pro-survival function indeed can be attributed to its well-known role in mitophagy.

Surprisingly, the authors found that the stress-protective effects of Parkin did not depend on mitophagy. In support of an alternative pathway being at play, they could show that protection by Parkin required functional NF κ B signalling. Moreover, OPA1, which is essential for mitochondrial fusion and for inhibition of cytochrome c release (Frezza *et al*, 2006), is an NF κ B target gene and a critical downstream mediator of Parkin's anti-apoptotic potential.

In pursuit of the mechanistic links between Parkin and NF κ B, Winklhofer and her team discovered a connection between Parkin and the linear ubiquitin chain assembly complex (LUBAC). So far, LUBAC is the only known E3 ligase able to generate linear ubiquitin chains, which are conjugates of ubiquitin linked by their N- and C-termini (Walczak *et al*, 2012). LUBAC is composed of the catalytic core subunit HOIP and its activators HOIL-1 and Sharpin (Gerlach *et al*, 2011; Ikeda *et al*, 2011; Tokunaga *et al*, 2011). Importantly, previous studies revealed a fundamental role of LUBAC in the NF κ B-signalling pathway by linear ubiquitinating NEMO (Gerlach *et al*, 2011; Ikeda *et al*, 2011; Tokunaga *et al*, 2011).

By using quantitative proteomics Müller-Rischart *et al* show that depletion of Parkin leads to a substantial decrease in linear ubiquitin chains. Immunoprecipitation experiments further demonstrated that HOIP and Parkin interact and that this interaction is stimulated under cellular stress. It was recently shown that HOIP resides in an autoinhibited state, which can be relieved by HOIL-1L binding (Stieglitz *et al*, 2012). As Parkin and HOIL-1 show similar domain architectures, it suggests that Parkin might activate HOIP in an analogous way. However, in contrast to HOIL-1L, which binds HOIP via its ubiquitin-like domain (UBL), a UBL deletion mutant of Parkin retains HOIP binding, but is unable to stimulate linear ubiquitin formation. Unlike the case for HOIL-1L, a catalytic-dead mutant of Parkin does not harbour anti-apoptotic potential implicating Parkin's ligase activity in the stimulation of LUBAC. The observed differences between HOIL-1 and Parkin-driven activation of HOIP raise the question of how LUBAC function is influenced by Parkin on a molecular level. The exact interaction mode of HOIP and Parkin remains to be characterized along with the question whether both proteins interact directly. Based on these unexplored issues, it thus cannot be ruled out that

Parkin increases linear ubiquitination by LUBAC indirectly involving other effectors.

Being a central player in survival signalling, it is plausible that LUBAC has different composition and activators depending on the cell type. Sharpin-deficient mice show increased cell death in keratinocytes and develop chronic proliferative dermatitis (Ikeda *et al*, 2011). Parkin on the other hand might be a prominent mediator of LUBAC in the nervous system.

Based on the finding that Parkin-deficient cells are defective in TNF α induced NF κ B signalling, Winklhofer and colleagues propose a role for Parkin in cytokine mediated NF κ B activation. Although Parkin knockout mice do not show any obvious phenotype and thus no PD pathology, it is noteworthy that a previous study observed lipopolysaccharide-mediated degeneration of dopaminergic neurons in these animals (Frank-Cannon *et al*, 2008). As LUBAC also acts downstream of toll-like receptors (Ikeda *et al*, 2011) this raises the issue whether the above-mentioned phenotype relates to the findings provided by Müller-Rischart *et al* (2013).

It has previously been shown that Parkin promotes mitochondrial fission during mitophagy (Ziviani *et al*, 2010). In this study, the Winklhofer group describes Parkin as a stress-responsive inducer of mitochondrial fusion. Seemingly contradictory, these findings nicely correspond to the expectations for a guardian of mitochondrial integrity. When impaired mitochondria are beyond repair, organelle fission facilitates autophagic sequestration and removal. In cases where a point of no return has not been reached yet, it is advantageous to stimulate complementation by fusion of defective with undamaged mitochondria (Figure 1B).

Similarly to Parkin, PINK1 is cytoprotective. Given the genetic epistasis of both genes, future work needs to address whether PINK1 similarly functions independently of mitophagy and cross-talks with the NF κ B signalling cascade. Genetic, biochemical, and cell biological evidence link PINK1 and Parkin to mitophagy and suggest a defect in this pathway to be a possible cause for the development of PD. Still, mitophagy has yet to be demonstrated at endogenous levels of PINK1 and Parkin *in vivo*. The study by Winklhofer and colleagues thus puts forward an exciting additional explanation for the observed neuroprotective function of these PD-associated genes. Future work addressing the *in vivo* relevance of Parkin-dependent signalling events will undoubtedly help to advance our knowledge in the pathogenesis of PD.

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Conflict of interest

The authors declare that they have no conflict of interest.

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