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Damaged mitochondria get a Parkin ticket

In 2008, Narendra et al. revealed that a protein associated with Parkinson's disease promotes the turnover of dysfunctional mitochondria.

Loss-of-function mutations in *PARK2*, the gene encoding the ubiquitin ligase Parkin, are the most common cause of autosomal recessive Parkinson's disease. But just seven short years ago, the cellular function of Parkin was completely unknown; even the enzyme's subcellular localization was uncertain. That all changed, however, when Richard Youle and colleagues demonstrated that Parkin is specifically recruited to damaged mitochondria, promoting their engulfment and removal by autophagosomes (1). The discovery led to an explosion of interest in how defects in mitochondrial quality control lead to neurodegeneration.

As an E3 ubiquitin ligase, Parkin was first proposed to target misfolded proteins to the proteasome for degradation, an idea that fit nicely with the role played by protein aggregation in neurodegenerative diseases. But some evidence suggested that Parkin might have a mitochondrial function. Studies in *Drosophila* revealed that the ligase interacts genetically with PINK1, a mitochondrial kinase that is also linked to early-onset Parkinson's disease. Flies lacking either protein developed swollen mitochondria and gradually lost their indirect flight muscles (2, 3).

"I was working on the role of mitochondria in apoptosis," explains Youle, from the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland. "I had a medical student, Derek Narendra, join the lab for one year. He was more interested in clinically related things, so I had him look at Parkin. I suggested he fuse it to GFP and do some cell biology."

GFP-tagged Parkin was predominantly cytosolic, even in cells undergoing apoptosis. However, Narendra then treated cells with the mitochondrial toxin paraquat, an herbicide linked to the development of Parkinson's-like symptoms in farm workers. "In certain cases, Parkin translocated from the cytosol to the mitochondria," Youle recalls.

The uncoupling agent carbonyl cyanidem-chlorophenylhydrazone (CCCP), which depolarizes the mitochondrial inner membrane, had an even stronger effect, prompting Parkin's complete relocalization to mitochondria.

By using a cell line containing a mixed population of healthy and dysfunctional mitochondria, Narendra et al. found that Parkin was selectively recruited to the mitochondria with lower membrane potentials.

"Then Derek made a really amazing discovery," Youle recalls. "You only have to add the uncoupler for 30 minutes to see Parkin translocate to mitochondria. However, he also left the uncoupler on the cells for 48 hours. When he came back, the mitochondria were gone. I'd never seen anything like it in my life!" Narendra and colleagues confirmed that, after 48 hours in the presence of CCCP, mitochondria were completely eliminated from cells expressing Parkin. But the organelles persisted in cells lacking the ubiquitin ligase, indicating that Parkin is required for the removal of damaged mitochondria.

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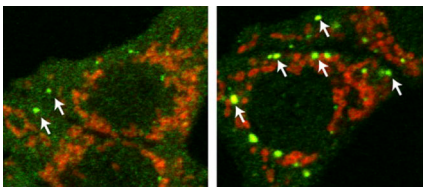
Previous studies had shown that dysfunctional mitochondria are eliminated by autophagy, in which double-membraned autophagosomes engulf fragments of the organelle and deliver them to lysosomes for degradation (4). Narendra et al. found that Parkin promotes the recruitment of autophagosomes to depolarized mitochondria and their subsequent removal via the autophagy pathway.

"This was a big discovery and it was a competitive field, so we wanted to get it out quickly," Youle says. In a follow-up paper, the researchers found that the PINK1 kinase, which is normally imported into healthy mitochondria and rapidly degraded, is stabilized on the surface of depolarized mitochondria, where it promotes the recruitment of Parkin (5). More recently, Youle and colleagues have demonstrated that PINK1 switches on Parkin's ubiquitin ligase activity by phosphorylating ubiquitin itself (6).



Parkin (green) is mostly cytosolic in control cells (left) but is recruited to mitochondria (red) in the presence of the uncoupling agent CCCP (right).

"The story has moved quickly since 2008," says Youle. "It's been exciting."

Once activated, Parkin ubiquitinates numerous mitochondrial outer membrane proteins (7). "For me, a lot of the outstanding questions now are downstream," Youle says. "How do the ubiquitin chains generated by Parkin recruit the autophagy machinery?"

Disease-associated mutations in *PINK1* and *Parkin* block this pathway and cause defective mitochondria to accumulate inside cells (5), a process that could be especially deleterious for long-lived cells such as neurons. "We and a number of other groups are interested in drugging this pathway," Youle explains. "If you could activate it, you might improve mitochondrial quality control, particularly in patients with mitochondrial diseases or in aging and Parkinson's disease."

"This landmark publication by Richard Youle's group illustrates the tremendous power of cell biology to uncover both basic and disease-linked mechanisms," says Jodi Nunnari, an academic editor at *JCB* and expert in mitochondrial biology. "In this particular case, the experiments opened up an entirely new way of thinking about Parkinson's and provided new tools that have now unraveled how defective mitochondria are eliminated from the cell by mitophagy."

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