DUBs counteract parkin for efficient mitophagy

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The Parkinson's disease-associated ubiquitin E3 ligase parkin impacts various cellular processes including the autophagic clearance of defective mitochondria. In this issue of *The EMBO Journal*, Durcan *et al* (2014) reveal a novel control mechanism of parkin-mediated mitophagy via the selective removal of atypical K6-linked ubiquitin chains from parkin by the deubiquitinase USP8. Together with recent studies on USP15 and USP30, this establishes a functional role for deubiquitination in mitophagy regulation.

See also: **TM Durcan *et al*** (November 2014)

Parkin's disease (PD) is a common neurodegenerative disorder characterized by the selective loss of dopamine-producing neurons from the substantia nigra, and the presence of aggregated inclusion bodies called Lewy bodies that are rich in ubiquitin and α -synuclein (Goedert *et al*, 2012). Accumulating evidence suggest that defective mitochondria contribute to PD pathology. Both parkin and the Ser/Thr kinase PINK1 are key regulators of mitochondrial quality control, and mutations in *PARK2* and *PINK1* are the most common causes of autosomal recessive PD (Scarffe *et al*, 2014).

Parkin is a RING-HECT hybrid E3 ubiquitin ligase that modifies mitochondrial outer membrane (MOM) proteins and promotes removal of dysfunctional mitochondria by selective autophagy (mitophagy) (Stolz *et al*, 2014). Parkin E3 ligase activity is tightly controlled by associated proteins, post-translational modifications and self-regulation through intramolecular interactions (Walden & Martinez-Torres, 2012). In response to mitochondrial depolarization, PINK1 phosphorylates Ser 65 in the N-terminal

ubiquitin-like (Ubl) domain of parkin (Kondapalli *et al*, 2012), thereby releasing the inhibitory interaction between the Ubl domain and the C-terminal RING-between-RING (IBR)-RING (RBR) domain. Recent structural analyses of parkin in an autoinhibited state have provided further insights into how parkin is activated (Trempe *et al*, 2013; Wauer & Komander, 2013). In addition, several groups have shown that PINK1 also modifies ubiquitin at Ser65, which is homologous to the site phosphorylated in parkin's Ubl domain (Kane *et al*, 2014; Kazlauskaitė *et al*, 2014; Koyano *et al*, 2014). Both phosphorylation events are necessary to unlock autoinhibition of parkin, allowing parkin autoubiquitination and its efficient recruitment to damaged mitochondria.

In this issue of *The EMBO Journal*, Fon and colleagues present a novel regulator of parkin, namely the ubiquitin-specific protease (USP) 8. USP8 has profound effects on endosomal morphology and organization (Clague *et al*, 2013) but has never been linked to mitochondrial quality control before. The authors demonstrate in their current study that USP8 directly deubiquitinates parkin and opposes its autoubiquitination. RNAi-mediated knockdown of USP8 in various cell lines resulted in delayed parkin recruitment to depolarized mitochondria and in accumulation of ubiquitinated parkin, which persisted longer at damaged mitochondria and caused a delay in their clearance by mitophagy. In contrast, loss of USP8 had no impact on, for example, mitochondrial dynamics, depolarization or PINK1 accumulation, further supporting that USP8 controls parkin activity via deubiquitination of the E3 ligase.

A key finding of the paper is that USP8 selectively removed K6-linked ubiquitin

chains from parkin, although it shows no preference for this linkage type when processing unattached ubiquitin dimers *in vitro* (Faesen *et al*, 2011). Expression of an ubiquitin mutant that cannot assemble K6-linked ubiquitin conjugates (Ub^{K6R}) in USP8-depleted cells was able to rescue delayed parkin recruitment to damaged mitochondria and impaired mitophagy as compared to wild-type ubiquitin. Importantly, expression of Ub^{K6-only}, a mutant only producing K6-linked ubiquitin polymers, mimicked the effect of USP8 knockdown and resulted in delayed parkin recruitment although USP8 was present. Thus, the authors concluded that removal of K6 linkages from parkin is critical for mitophagy to proceed efficiently. However, more detailed investigations are necessary, as it is not understood how K6-linked ubiquitin chains exert their negative effect.

This phenomenon underlines the importance of deubiquitinases (DUBs) and the precise regulation of ubiquitin signals for efficient mitophagy. Interestingly, two other DUBs were recently linked to mitophagy as well. USP15 and the mitochondrial DUB USP30 were shown to oppose parkin-mediated ubiquitination of MOM proteins and to slow down subsequent clearance of depolarized mitochondria (Bingol *et al*, 2014; Cornelissen *et al*, 2014). In contrast to USP8, USP15, and USP30, neither affect the ubiquitination status of parkin nor its recruitment to damaged mitochondria.

The central observation made by Cornelissen *et al* (2014) is that loss of USP15 enhanced parkin-mediated ubiquitination of depolarized mitochondria and accelerated mitophagy in human dopaminergic neuronal SH-SY5Y cells and primary fibroblasts. By using linkage-specific anti-ubiquitin antibodies, they detected an increase in K48- and

Figure 1. The parkin-mediated clearance of depolarized and fragmented mitochondria by autophagy is regulated by the deubiquitinases USP8, USP15 and USP30.

K63-linked ubiquitin conjugates at mitochondria. In the complete absence of parkin, USP15 had no effect on mitophagy. The authors further demonstrated the antagonistic functions of parkin and USP15 *in vivo*. Knockdown of *CG8334*, the closest homologue of USP15 in *Drosophila*, largely rescued the mitochondrial defects of *parkin* RNAi flies.

Bingol *et al* (2014) used slightly different approaches to analyze the effect of USP30 in mitophagy. Essentially, USP30 also deubiquitinated MOM proteins, for example, MIRO1 and TOM20, and thereby counteracted removal of dysfunctional mitochondria. The authors showed that ubiquitination of TOM20 promoted mitochondrial clearance and that deubiquitination of TOM20 by USP30 explains at least in part how USP30 inhibited mitophagy. In addition, knockdown of USP30 in *Drosophila* and subsequent crossing with *parkin* or *pink* mutant flies revealed that loss of USP30 restored mitochondrial morphology defects. Finally, knockdown of USP30 in dopaminergic neurons protected flies against the effects of the mitochondrial toxin paraquat.

So far it remains unclear how the action of USP8, USP15, and USP30 is orchestrated during mitophagy, and what are the consequences of deregulated DUB function for PD pathology. It is not known whether these DUBs impact clearance of damaged mitochondria independently or synergistically, and whether the same MOM proteins are deubiquitinated by USP15 and USP30. Potentially, these DUBs can be targeted in PD patients with defective parkin to promote mitochondrial clearance and quality control. The use of USP DUB inhibitors in anticancer treatment strategies is currently investigated (Pal *et al*, 2014), and development of more selective small-molecule DUB inhibitors is in progress.

Future studies on parkin activation and ubiquitin-mediated regulation of mitophagy will shed light not only on a better understanding of the pathogenesis of hereditary forms of PD, but will also provide a rational for design of novel therapies against PD.

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