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SPOTLIGHT

Mitophagy is induced by short ubiquitin chains on mitochondria

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Mitophagy has a critical role in maintaining cellular homeostasis by removing damaged mitochondria. In this issue, Yamano et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201912144) uncover that a novel complex of the autophagy adaptor optineurin and the membrane protein ATG9A specifically regulate ubiquitin-induced mitophagy.

Mitophagy is a process of removing damaged mitochondria by lysosomes via selective autophagy (1). Damaged organelles need to be rapidly removed to maintain cellular homeostasis; thus, such a selective autophagic process is crucial. To date, we understand that ubiquitination of the outer mitochondrial membrane (OMM) proteins of damaged mitochondria by the E3 ligase Parkin with a concerted action of the mitochondrial kinase, PTEN-induced kinase 1 (PINK1), plays a critical role (2; Fig. 1, left). Open questions were whether ubiquitination is sufficient for the process and how the process is regulated. In this issue, Yamano et al. address these questions and come to interesting observations (3). They demonstrated that if OMM proteins are polyubiquitinated (by a nonbranched chain and preferentially a dimer), mitophagy is initiated independently of PARKIN or PINK1 in mammalian cells (Fig. 1, right). These chains are recognized by an autophagy adaptor, Optineurin (OPTN), originally named for "optic neuropathy inducing" protein (4), which makes a novel complex with ATG9A in mammalian cells (3).

Technically, it is not very simple to specifically target a substrate for ubiquitination in cells. The authors approached this point in two elegant ways. First, they used a tandem repeat of ubiquitin moieties tagged with a mitochondrial targeting sequence. The trick was to use ubiquitin mutants in different lengths in which all branching sites are mutated, so that the endogenous ubiquitin machinery cannot elongate or branch the chain. In this way, they can control the length of ubiquitin chains. This enabled them to clarify that only short and nonbranched ubiquitin chains initiate mitophagy and they are sufficient for the event. The second approach was to use a synthetic hybrid molecule called "specific and nongenetic inhibitor of apoptosis (IAP)–dependent protein erasers" (SNIPERs; 5). They used a version of SNIPER, which binds to the ubiquitin E3 ligase (IAP) and a substrate CRABP-II. By using SNIPER in combination with a CRABP-II fusion protein with an OMM protein Hexo kinase 1 or TOMM20, ubiquitination of CRABP-II by endogenous IAP occurs at OMM. Again, mitophagy was induced in these cells even when PINK1 was knocked down, concluding that ubiquitination of OMM proteins is sufficient to initiate mitophagy.

Once the cargo (e.g., damaged mitochondria) is ubiquitinated, autophagy adaptors such as OPTN, NDP52, p62, NBR1, and TAX1BP1 bridge the cargo via ubiquitin recognition and autophagosomes via binding to LC3. Autophagy adaptors link the cargo to autophagosomes; however, understanding of the precise functional differences of these adaptors in different types of selective autophagy is still developing. Here, the authors showed that among those autophagy adaptors, OPTN was the only adaptor that was able to rescue ubiquitin-initiated mitophagy in autophagy adaptor–deficient cells (Penta knockout HeLa; 6). This indicates that OPTN is the critical adaptor for initiating ubiquitin-induced mitophagy, although OPTN and NDP52 were both shown to be more important in the process than the others.

So, what is so special about OPTN? Is the affinity to LC3s higher than other adaptors? It was not the case that OPTN binds more to LC3 proteins in cells in comparison to p62 and NBR1, which was determined by the Fluoppi protein–protein interaction technique. Importantly, this OPTN–ATG9A colocalization is independent from ATG5, an essential autophagy regulator, or FIP200, a known interacting protein of NDP52 (7, 8). At the molecular level, they found that OPTN has a leucine zipper where ATG9A binds, and this binding is crucial to induce mitophagy. The region is independent from interactions with TBK1, an important partner kinase of OPTN, indicating that the OPTN–ATG9A interaction is important. Recruitment of ATG9A was not observed in NDP52-reconstituted Penta knockout cells, further confirming the specific function of OPTN.

In conclusion, Yamano et al. clarified a long-standing question whether ubiquitination is sufficient for mitophagy initiation and demonstrated that this is independent

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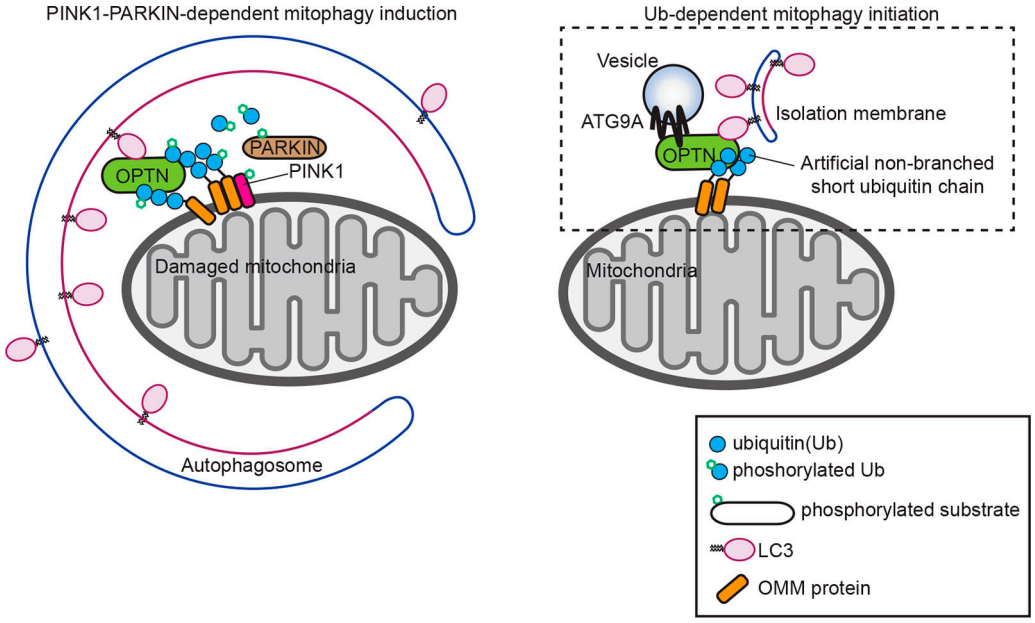

Figure 1. **Mitophagy is regulated by various players.** Left: A model of PINK1-PARKIN–dependent mitophagy is shown. The kinase PINK1 accumulates on the outer mitochondrial membrane (OMM) of damaged mitochondria and phosphorylates itself, ubiquitin, and the PARKIN ubiquitin E3 ligase. An autophagy adaptor, such as OPTN, bridges ubiquitin-coated damaged mitochondria and an LC3-integrated autophagosome, leading to engulfment of mitochondria. Right: Players for ubiquitin-induced mitophagy initiation found in this study are shown. Short non-branched ubiquitin chains on OMM proteins bind OPTN, which links to an ATG9A-positive vesicle, independently from PINK1 or PARKIN. OPTN-LC3 interaction bridges them to an isolation membrane, a source of autophagosomes.

from PINK1 and PARKIN. Importantly, they identified that the OPTN–ATG9A complex formation is a critical mechanism to initiate mitophagy, which has a larger effect than OPTN–LC3 binding based on the loss of function mutant experiments.

As is typical for excellent studies, this brings up a lot of questions for us to solve. For example, the authors showed that "short" ubiquitin chains on the OMM proteins are preferred, and branched ubiquitin chains seem to prevent mitophagy initiation. It was previously shown that ubiquitin chain length is rather short on damaged mitochondria in cells (9), which is in line with the working model based on the current study (3). However, do these observations suggest that PARKIN or any other E3 ligase manages to control the ubiquitin chain length or branching for efficient mitophagy induction? Is this due to PINK1-dependent phosphorylation of ubiquitin at the damaged mitochondria? Or do deubiquitinases for editing ubiquitin chain length and branching contribute to the event? Why are branched chains not good at initiating mitophagy although branches with K63-linked/linear ubiquitin chains should be able to recruit OPTN? Another aspect pertains to the OPTN–ATG9A complex. Does "OPTN–ATG9A complex-dependent selective autophagy" happen also for other ubiquitinated cargos? Interestingly, a recent study showed that the OPTN–ATG9A complex formation in mammalian cells is involved in the immune signaling cascade (10), suggesting it also has a role in other types of biology. This study by Yamano et al. has opened a door for exciting research to discover more about both mitophagy and ubiquitin.

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