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Spatiotemporal dynamics of autophagy receptors in selective mitophagy

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ABSTRACT

Damaged mitochondria are turned over through a process of selective autophagy termed mitophagy. In mitophagy, unhealthy mitochondria are recognized and ubiquitinated by Parkinson disease-linked proteins PINK1 and PARK2. The subsequent recruitment of ubiquitin-binding autophagy receptors leads in turn to the sequestration of the damaged organelles into LC3-positive phagophores, precursors to autophagosomes. The precise identity of these receptors and how they are regulated has been the focus of considerable attention. Our recent work uses live-cell imaging to explore the dynamics and regulation of autophagy receptor recruitment. Utilizing multiple paradigms to induce mitochondrial damage, we identified the rapid, 2-step recruitment of autophagy receptors OPTN, CALCOCO2/NDP52, and TAX1BP1. All 3 receptors are recruited to damaged mitochondria with similar kinetics; however, only OPTN is necessary for efficient formation of a phagophore sequestering damaged mitochondria from the cytosol. OPTN is co-recruited to damaged mitochondria along with its upstream kinase TBK1. Depletion of OPTN or TBK1, or expression of amyotrophic lateral sclerosis (ALS)-linked mutations in either protein, interfere with efficient autophagic engulfment of depolarized mitochondria. These observations suggest that insufficient autophagy of damaged mitochondria may contribute to neurodegenerative disease.

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Mitochondrial homeostasis is maintained through several quality control mechanisms including dynamic fission/fusion, the mitochondrial unfolded protein response, and selective mitochondrial autophagy (mitophagy). In one model for selective mitophagy, depolarized mitochondria are identified and ubiquitinated by a feed-forward cascade triggered by the ubiquitin kinase PINK1 and the E3 ubiquitin ligase PARK2. Ubiquitination functions as a signal to target damaged mitochondria to the autophagosome-lysosome degradation system. Specifically, autophagy receptors are recruited via binding to poly-ubiquitin chains, and in turn mediate autophagic engulfment through binding to lipidated MAP1LC3 family proteins on autophagic phagophore membranes. Currently, at least 5 ubiquitin-LC3 binding autophagy receptors have been implicated in selective mitophagy: OPTN (optineurin), CALCOCO2, TAX1BP1, NBR1, and SQSTM1.

OPTN was the first of these receptors demonstrated to function in selective mitophagy. Since then, knockout studies have investigated the effects of depleting the other receptors, either singly or in combination, and have demonstrated the involvement of CALCOCO2 and TAX1BP1 in mitophagy. In contrast, multiple studies suggest that SQSTM1 is not required.

In our most recent work, we compared the kinetics of OPTN, CALCOCO2, and TAX1BP1 recruitment to mitochondria. Using live-cell imaging in HeLa cells, we tracked the spatiotemporal localization of fluorescently labeled autophagy receptors in response to either global mitochondrial depolarization by the protonophore CCCP or regional reactive oxygen species generation by the matrix-targeted photosensitizer MitoKillerRed. Both paradigms induce the rapid recruitment of OPTN, TAX1BP1, and CALCOCO2 to damaged mitochondria.

Within 15 min of PARK2 recruitment and 30 min of mitochondrial damage, all 3 autophagy receptors weakly associate with the outer membrane of fragmenting mitochondria. This association is dependent on mitochondrial ubiquitination, and blocked by expression of a Parkinson disease-associated inactive PARK2 mutant. At 45 min post-insult, enhanced recruitment and stabilization of autophagy receptors is observed on a subset of mitochondria, simultaneous with the formation of LC3-positive autophagosomes around the damaged organelles. At 90 min post-insult, more than 50% of damaged mitochondrial fragments are encircled by autophagy receptors; nearly half of these end up sequestered within autophagosomes.

The efficiency with which phagophores engulf damaged mitochondria may be controlled by a number of factors, including expression levels of key mitophagy proteins such as PARK2 or OPTN. Indeed, we find that overexpression of exogenous CALCOCO2 more than quadruples the percentage of mitochondria in autophagosomes at 90 min post-CCCP treatment. Despite this, depletion of endogenous CALCOCO2 has no

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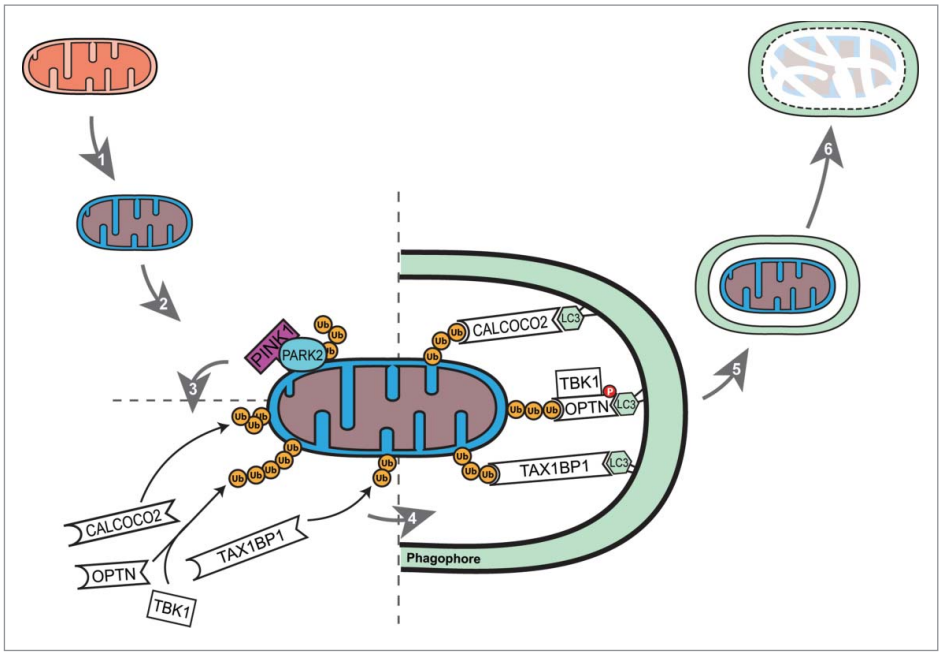

Figure 1. Mitophagy receptors are dynamically recruited to damaged mitochondria in response to PINK1- and PARK2-induced ubiquitination, leading to sequestration of the organelle by an LC3-positive phagophore. (1) An individual mitochondrion is acutely damaged. (2) The damaged mitochondrion is ubiquitinated through the action of Parkinson disease-linked proteins PINK1 and PARK2. (3) Within 15 min of PARK2-dependent ubiquitination, OPTN and its upstream kinase TBK1 are corecruited to the damaged mitochondrion. Simultaneously, autophagy receptors CALCOCO2 and TAX1BP1 translocate to the mitochondrial outer membrane. (4) Upon TBK1 phosphorylation, OPTN, associates with LC3, inducing autophagic engulfment of the damaged organelle. In the absence of TBK1 activity, CALCOCO2 can promote mitophagy. (5) Within 45 min of the initial injury, the damaged mitochondrion is sequestered within an autophagosome and functionally separated from the cytosol. (6) Over several hours, the mitochondrion is degraded within an autolysosome. Whereas Parkinson disease-linked mutations in PARK2 interfere with ubiquitination of damaged mitochondria, ALS-linked mutations in TBK1 and OPTN interfere with the subsequent recruitment of autophagy receptors and LC3-positive membranes.

effect on autophagic engulfment of mitochondria, indicating that CALCOCO2 is not strictly necessary for mitochondrial autophagy. In contrast, depletion of OPTN more than halves the percentage of damaged mitochondria positive for LC3, indicating that OPTN is necessary for initiation of mitophagy under these conditions.

Mutations in both OPTN and its upstream kinase TBK1 have been identified in several cases of familial ALS. Thus, we investigated the interplay between these 2 ALS-linked proteins in mitophagy. We observed robust corecruitment of TBK1 with OPTN to damaged mitochondria. We hypothesize that OPTN is responsible for recruiting TBK1 to the mitochondrial surface, as either depletion of OPTN or expression of an OPTN-binding TBK1 mutant interferes with recruitment of the kinase to depolarized mitochondria.

Once recruited, TBK1 phosphorylates OPTN at position S177, immediately adjacent to the LC3-interacting region motif. S177 phosphorylation activates OPTN, facilitating engulfment by LC3-positive phagophores. Inhibition or knockdown of TBK1 stalls mitophagy. Similarly, cells expressing phosphodeficient S177A OPTN efficiently recruit PARK2 and OPTN, but fail to recruit LC3. This failure can be overcome by overexpressing CALCOCO2, further supporting the hypothesis that expression levels of mitophagy receptors are critical for effective sequestration of damaged organelles, especially in response to cell-wide insults.

To investigate whether specific ALS-associated mutations in OPTN or TBK1 interfere with mitophagy, we depleted endogenous TBK1 and compared the extent of rescue by wild-type TBK1 or the ALS-linked TBK1E696K mutant. Whereas wild-type TBK1 fully restores mitophagy, cells expressing the mutant kinase display a profound mitophagy defect. Similarly, expression of 2 ALS-linked OPTN mutants, OPTNE478G and OPTNQ398X significantly impair mitochondrial autophagy. However, we noted that a third ALS-linked mutant, OPTNR96L, successfully facilitates mitophagy in these assays, indicating more work is required to fully explore the links between defective mitophagy and ALS.

Taken together, this work underscores the importance of OPTN and its upstream kinase TBK1 in the initiation of damage-induced mitophagy. We find that multiple receptors are involved in the recognition of ubiquitinated mitochondria; these receptors are differentially regulated and only partially redundant (Fig. 1). Finally, we observed that multiple neurodegenerative disease-associated mutations interfere with the efficiency of autophagic engulfment of damaged mitochondria, including mutations in PARK2, OPTN, and TBK1. Thus, disordered mitophagy may represent a key pathophysiological mechanism common to both Parkinson disease and ALS.

It is important to note, however, that much of the work investigating the PINK1-PARK2 mitophagy pathway has been carried out in immortalized cell lines. Though immortalized cells represent excellent model systems to investigate the spatiotemporal regulation of molecular pathways, they often carry adaptations that alter their mitochondrial quality control mechanisms. Whether the kinetics and regulation of mitophagy differ substantially between HeLa cells and primary neurons is an exciting future direction for study.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.