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Parkin-independent mitophagy—FKBP8 takes the stage

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Although the Parkin/PINK1 pathway has received considerable attention in recent years as a key regulator of mitophagy in mammals, it is important to recognize that multiple mitophagy receptors like BNIP3, NIX, and FUNDC1 exist that can promote the selective clearance of mitochondria in the absence of Parkin. In this issue, Bhujabal *et al* expand the repertoire of Parkin-independent mitophagy receptors to include the anti-apoptotic protein, FKBP8. The authors demonstrate that FKBP8 interacts preferentially with LC3A via its LIR motif to destroy damaged mitochondria. During the process, FKBP8 escapes from the destruction presumably to prevent apoptosis during mitophagy [1].

See also **Z Bhujabal *et al*** (June 2017)

Mitophagy refers to the selective degradation of mitochondria by the cell via autophagy. This usually occurs upon mitochondrial damage as a form of intracellular quality control mechanism that helps to maintain the quality of mitochondria as well as to prevent the accumulation of unwanted dysfunctional mitochondria that could otherwise compromise cellular functions. Although mitophagy has in recent years become a "hot" research topic, the degradation of mitochondria by lysosomes, especially upon cellular starvation, has been appreciated for more than half a century. In 1962, Ashford and Porter examined liver cells perfused with glucagon by means of electron microscopy and found that the number of lysosomes was dramatically increased in these cells relative to control preparations and that every lysosome in the glucagon-treated cells almost always contained a mitochondrion (or more) that exhibited a "varying degree of structural decay" [2]. The term "mitophagy" was later coined by Lemasters [3], who alluded to the selective nature of the process. However, until recently, little was known about the proteins regulating the mitophagy process, particularly in mammalian cells. A major breakthrough in our understanding of the molecular mechanisms underlying mitophagy came from the seminal discovery that Parkin, a ubiquitin ligase whose mutations are causative of familial Parkinson's disease (PD), is a key mammalian regulator of the process [4]. Parkin collaborates with another PD-linked gene product known as PINK1, a mitochondrial serine/threonine kinase. Briefly, in the Parkin/PINK1 model [5], a key initial event that occurs upon mitochondrial depolarization is the selective accumulation of PINK1 on the outer membrane of the damaged organelle, which results in the activation and translocation of cytosolic Parkin to the targeted mitochondrion and the recruitment of the autophagy receptors optineurin and NDP52 to trigger mitophagy. Autophagy receptors interact with ATG8 family proteins (LC3A-C, GABARAP, and GABARAPL1-2) via their LC3 interacting region (LIR), which in the case of optineurin and NDP52 facilitates the expansion of LC3-coated phagophore locally on targeted mitochondria to generate the autophagosome necessary for mitophagy. Notwithstanding the critical role that Parkin plays in mediating mitophagy, it is important to note that alternative Parkin-independent pathways exist for the clearance of mitochondria. For example, iron depletion triggers mitophagy in a Parkin-independent manner [6]. Similarly, the mitochondrial-localized BH3-only family member BNIP3L/NIX was found to promote Parkin-independent mitochondrial clearance during reticulocyte maturation [7]. Another outer mitochondrial membrane (OMM) protein, FUNDC1, also functions as a Parkin-independent mitophagy receptor during hypoxia-induced mitophagy [8]. More recently, the Bcl-2-like protein 13 (Bcl2-L-13) was identified to be a mammalian homologue of Atg32 (essential for mitophagy in yeast) that is capable of inducing mitophagy in Parkin-deficient cells [9]. Interestingly, the majority of these mitophagy receptors, including optineurin and NDP52 (but not NIX), mediate their actions through their association with LC3B.

In this issue, Bhujabal *et al* [1] describe a novel interaction between LC3A- and FK506-binding protein 8 (FKBP8, also known as FKBP38), an anti-apoptotic protein that is normally localized to the OMM, and demonstrate that their association is important for FKBP8-mediated mitophagy (Fig 1). Interestingly, the authors have originally isolated FKBP8 as an interacting partner of LC3 via a yeast two-hybrid screen of a human thymus cDNA library using LC3B (i.e., not LC3A) as bait, although their subsequent *in vitro* and cell culture experiments revealed that FKBP8 exhibits significantly stronger interaction with LC3A and related GABARAP members, particularly GABARAPL1. Consistent with the ability of FKBP8 to bind LC3 members, they identify a single LIR motif in the N-terminus of FKBP8 and showed that its mutation reduces the association between FKBP8 and LC3/GABARAP proteins. To determine whether the cellular localization of FKBP8 is important for its interaction with LC3A, the authors created three artificial FKBP8 mutants that restrict their localization to the mitochondria, ER, and cytosol, respectively. All three mutants co-precipitated LC3A in pull-down assays, suggesting that FKBP8 is able to recruit LC3A regardless of its cellular

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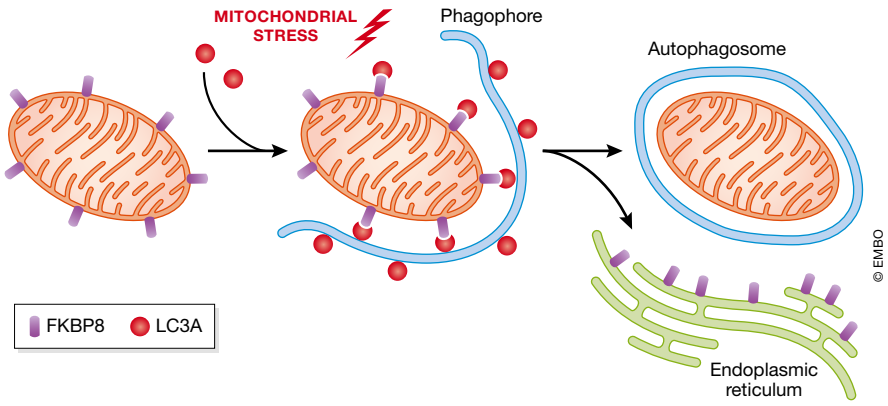

Figure 1. FKBP8 recruits LC3A to mediate mitophagy.
FKBP8 is normally localized to the outer membrane of the mitochondria. In response to mitochondrial depolarization, FKBP8 preferentially recruits the lipidated form of LC3A to initiate mitophagy. During the process, FKBP8 escapes to the endoplasmic reticulum to avoid being degraded, which is apparently important to prevent apoptosis from occurring during mitophagy.

localization, although the mitochondrial-localized mutant co-precipitated the lipidated form of LC3A (i.e., LC3A-II) appreciably more than the other two mutants as well as wild-type FKBP8. Given that FKBP8 is normally localized to the mitochondria, it is intuitive to think that the organelle would represent the preferred site where the phagophore-associated LC3A-II is recruited by the OMM protein. Indeed, ectopic co-expression of FKBP8 and LC3A results in mitophagy in a Parkin-independent manner. When FKBP8 is expressed alone in Parkin-deficient HeLa cells, it promotes mitochondrial fragmentation and peri-nucleus mitochondrial clustering in a manner that is reminiscent of the morphological changes brought about by NIX and BNIP3. FKBP8-induced mitophagy depends on LC3A, which in turn requires FKBP8 to promote mitochondrial acidification, a phenomenon that is dependent on the LIR motif of FKBP8 and is enhanced when FKBP8 expression is restricted to the mitochondria. Notably, despite the apparently widespread degradation of OMM proteins associated with mitophagy, certain OMM proteins can escape the degradation process by translocating from the mitochondria to the ER, which apparently is needed to keep apoptosis suppressed during mitophagy. Interestingly, one of these "escapees" was identified recently by another group to be FKBP8 [10]. Consistent with this, fluorescence imaging studies conducted by Bhujabal *et al* [1] revealed that a significant population (> 95%) of the mitochondrial-localized FKBP8 is redistributed to structures outside but proximal to the mitochondria following 6 hours of CCCP treatment (when targeted mitochondria start to get acidified), again in a Parkin-independent manner. This extra-mitochondrial FKBP8 continues to associate with LC3A in a LIR-dependent manner. In contrast, acidified mitochondrial structures tend to display either no or weak FKBP8 staining. Such a scenario was not observed with the mitochondrial-restricted FKBP8 mutant, which stays localized to acidified mitochondria with no apparent signs of re-distribution to proximal extra-mitochondrial structures. These results support previous findings that FKBP8 escapes from mitophagy [10] and are in stark contrast to the mitophagy process induced by BNIP3 or NIX, where the receptors are degraded along with the mitochondria.

Curiously, the authors found that FKBP8 is not absolutely required for the recruitment of LC3A to the mitochondria as LC3A is present in the mitochondrial fraction even in FKBP8-deficient cells treated with CCCP. However, the presence of FKBP8 augments the recruitment of LC3A to the mitochondria especially in times of mitochondrial stress. Although this is consistent with their finding that mitochondrial-localized FKBP8 is more efficient in binding to LC3A-II, it begs the question on whether additional mitochondrial factors are involved in the recruitment process. Several other pertinent questions also arise from the study: How is FKBP8-LC3A binding normally regulated? Is mitophagy triggered by FKBP8 dependent on PINK1? Is the phenomenon relevant in physiological situations? Notably, the majority of the experiments were performed in cells where the proteins were overexpressed. Notwithstanding these, the discovery by Bhujabal *et al* adds to the growing list of mitophagy receptors that could mediate the selective clearance of mitochondria in a Parkin-independent manner. It is currently unclear why a multitude of mitophagy receptors are required to regulate the same process. One explanation for this is that the diversity allows the cell to respond appropriately to different situations. For example, FUNDC1 is activated to eliminate mitochondria as a response to hypoxia, whereas NIX is recruited to do the job during erythrocyte differentiation. Alternatively, it is possible that multiple mitophagy mechanisms exist to promote functional cooperativity and perhaps functional redundancy as well. Apart from FUNDC, BNIP3 and NIX can also induce mitophagy in response to hypoxic conditions. It is therefore tempting to speculate that the complementary and somewhat redundant roles of the various Parkin-independent mitophagy receptors may in part explain the lack of an overt disease phenotype in Parkin-deficient mice, as well as the substantial number of years needed for patients harboring Parkin mutations to manifest the clinical features of the disease. Clearly, unraveling the interplay between distinct mechanisms of mitophagy and how they potentially cooperate to elicit a coordinated response to various physiological and patho-physiological stimuli would be an exciting area to investigate that holds promise to yield new clues to the mechanisms underlying mitochondrial-related diseases.

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