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Starved cells use mitochondria for autophagosome biogenesis

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Abbreviations: PE, phosphatidylethanolamine; PS, phosphaditylserine; PSD, phosphaditylserine-decarboxylase; Mfn2, mitofusin 2

Autophagy was first described in the early 1960s as a self-eating pathway, in which double membrane-bounded organelles called autophagosomes sequester cytoplasmic material and deliver it to the lysosome for degradation and recycling.[1] Since then, the origin of the autophagosomal membrane has been the subject of intense debate. In yeast, autophagosome biogenesis occurs predominantly from a discrete element adjacent to the vacuole, called the pre-autophagosomal structure.[2] In mammals, the origin of the autophagosomal membrane is much more ambiguous. Prevailing models implicate potential membrane sources such as the Golgi apparatus, the plasma membrane, the endosomal compartment and, in particular, the endoplasmic reticulum (ER).[3,4] One problem with identifying the membrane source of autophagosomes is that there is no clear signature of membrane origin of these organelles. Instead, nearly all autophagy initiation machinery is comprised of cytosolic proteins, including ATG1, ATG7, ATG5, ATG12 and ATG8/LC3, that are recruited to membranes in response to starvation or other stress conditions. Localization of these proteins to sites of autophagosomal biogenesis depends on the activity of an autophagy-specific phosphatidylinositol 3-kinase (PI3K) complex (i.e., ATG5, 7 and 12) or their lipidation with phosphatidylethanolamine (PE), (i.e., ATG8/LC3).[5]

The ER has been proposed to participate directly in autophagosome biogenesis since a PI(3)P-binding protein normally localized to the ER translocates to a punctate compartment partially co-localizing with autophagy proteins in response to starvation.[6] Strengthening this ER-centric hypothesis, two independent groups observed direct physical linkage of the ER and autophagosomes in electron microscopy tomography studies.[7,8] However, it remains unclear if these events represent the ER being engulfed by autophagosomes, as in ER-phagy, or whether the ER is actually contributing membrane to newly forming autophagosomes.

To clarify where autophagosomal membranes originate in starved cells, we looked by live cell imaging at whether fluorescently-tagged markers of specific organelles, including endoplasmic reticulum (ER), Golgi apparatus, early endosomes, plasma membrane and mitochondria, are transferred into newly forming autophagosomes.[9] Robust transfer was seen for an outer mitochondrial membrane protein, cytochrome b5 (YFP-cb5-Mito), but not for any other organellar marker. Further experiments excluded the possibility that mitophagy (the degradation of mitochondria through autophagy) underlies the co-distribution of YFP-cb5-Mito and autophagosomes: GFP-LC3 labeled autophagosomes co-localized only with the mitochondrial outer membrane marker, YFP-cb5-Mito, not with labeled mitochondrial matrix or intramembrane space proteins. The observed association of YFP-cb5-Mito with starvation-induced autophagosomes suggested that outer mitochondrial membrane is used during autophagosome biogenesis.[9]

Further experiments confirmed this possibility. We found the tail anchor of mitochondrial-targeted cytochrome b5 was sufficient to deliver another outer mitochondria membrane protein, Fis1, to autophagosomes. Repeated photobleaching of mitochondrially-localized YFP-cb5 was sufficient to remove fluorescent signal from newly-forming autophagosomes, suggesting the membranes of these two organelles are transiently shared. Autophagosomes emerging from mitochondrial outer membranes were also seen by transmission electron microscopy. Finally, time-lapse imaging of GFP-tagged ATG5, a protein associated with early autophagosomes, showed localization to autophagosome budding sites on mitochondria.

But why would cells use the outer membrane of mitochondria to form autophagosomes during starvation? One possibility relates to the mitochondria's role in PE production. As mentioned above, PE is needed to conjugate autophagic machinery onto membranes. There are only two intracellular sites for PE production: the ER via the CDP-ethanolamine pathway, and the mitochondria via decarboxylation of phosphatidylserine (PS).[10] During starvation, exogenous ethanolamine and diacylglycerol, needed in the CDP-ethanolamine pathway, might become limiting. PE synthesis in mitochondria may counteract this deficiency by routing mitochondrial-derived PE to lysosomes via autophagy. Once delivered to lysosomes, membrane-trafficking pathways could circulate PE throughout endosomal and secretory membrane systems. In this way, mitochondrial lipid contribution to autophagosomal membranes would contribute both to lipid

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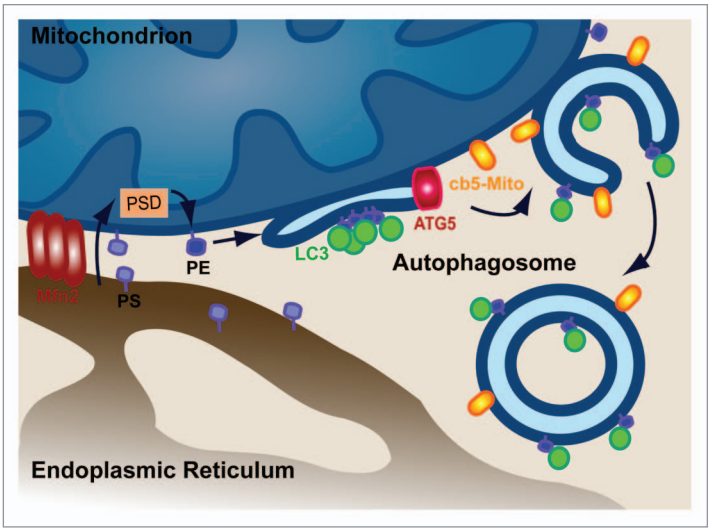

Figure 1. Suggested model for autophagosomal biogenesis from mitochondria: Phosphatidylserine (PS) translocates from ER to mitochondria where it gets converted to phosphatidylethanolamine (PE) through the PS-decarboxylase (PSD). The lipid transfer takes place at close ER-mitochondria association sites, enhanced though Mitofusin 2, a Mito-ER tethering factor. PE on mitochondria then localizes to the growing autophagosome along with the ATG5/12 complex and ATG8/LC3 that gets conjugated to PE. Eventually, the ATG5-12 complex is released from the growing double membrane. Cytochrome b5 (cb5-Mito) relocates from mitochondria to the forming autophagosome while both organellar membranes are in continuity. Finally, the autophagosome closes and gets released from the mitochondrion.

homeostasis and nutrient recycling in starved cells.

For this system to work, mitochondria must continually be supplied with PS, which is converted to PE by the mitochondrially-localized enzyme phosphatidylserine decarboxylase. Since PS is synthesized in the ER, one way this could be achieved is if mitochondria establish close contacts with the ER to facilitate transfer of PS into mitochondria. We tested this possibility by examining autophagosome biogenesis in mouse embryonic fibroblasts that lack Mitofusin 2 (Mfn2), a protein involved in physical tethering of ER and mitochondria.[11] Consistent with this model, an impaired autophagic response to starvation was observed in these cells. Because longer starvation treatments (>6 h) resulted in an autophagic response, additional tethering factors to Mitofusin 2 are likely involved in establishing close contacts between ER and mitochondria for lipid transfer during starvation.

These results raise many important questions for future analysis. One question relates to the regulation of contacts between ER and mitochondria used for lipid transfer during starvation. Are more of these contacts formed in starved cells, or are they pre-existing, involved in calcium transfer from ER to mitochondria and simply more active in response to starvation? Along these lines, phosphorylation of Bcl-2 is directly coupled to calcium retention capacity in the ER,[12] but is also essential to induction of starvation-induced autophagosomes.[13] Another question is whether outer membranes of mitochondria are utilized under other conditions to deliver membrane to organelles, as budding of vesicles from mitochondria that transit to peroxisomes has been seen.[14] The machinery that underlies targeting and fusion of autophagosomes to lysosomes also remains to be clarified. Finally, it is unclear how autophagosomal biogenesis from mitochondria differs from autophagy induced under other conditions, such as DNA damage or ER stress, which do not involve mitochondria outer membranes. Answering these questions regarding the origin, regulation and fate of autophagosomes should keep cell biologists interested in this exciting and important cell homeostatic system occupied for years to come.

References

1. De Duve C. Sci Am 1963; 208:64-72.
2. Reggiori F. Curr Top Dev Biol 2006; 74:1-30.
3. Juhasz G, et al. PLoS Biol 2006; 4:36.
4. Ravikumar B, et al. Nat Cell Biol 2010; 12:747-57.
5. Xie Z, et al. Nat Cell Biol 2007; 9:1102-9.
6. Axe EL, et al. J Cell Biol 2008; 182:685-701.
7. Hayashi-Nishino M, et al. Nat Cell Biol 2009; 11:1433-7.
8. Yla-Anttila P, et al. Autophagy 2009; 5:1180-5.
9. Hailey DW, et al. Cell 2010; 141:656-67.
10. Vance JE. J Lipid Res 2008; 49:1377-87.
11. de Brito OM, et al. Nature 2008; 456:605-10.
12. Bassik MC, et al. EMBO J 2004; 23:1207-16.
13. Wei Y, et al. Mol Cell 2008; 30:678-88.
14. Neuspiel M, et al. Curr Biol 2008; 18:102-8.