SPOTLIGHT

Who plays the ferryman: ATG2 channels lipids into the forming autophagosome
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Expansion of the autophagosomal membrane requires a mechanism to supply lipids while excluding most membrane proteins. In this issue, Valverde et al. (2019, J. Cell Biol. https://doi.org/10.1083/jcb.201811139) identify ATG2, a member of the autophagy-related protein family, as a lipid transfer protein and provide important novel insights on how autophagosomes grow.

Autophagy, the process of cellular self-eating, is conserved among eukaryotes as a major homeostatic mechanism that provides nutrients during starvation or eliminates damaged organelles and proteins throughout the lifespan of an organism. Deletion of some autophagy genes in whole animals leads to embryonic lethality, but for the majority of autophagy genes their absence renders animals unable to survive past one day after birth. Tissue-specific elimination of autophagy in brain or liver is characterized by accumulation of aggregated proteins and damaged organelles and leads either to neural degeneration (brain) or to hyperplasia (liver; 1).

In analogy to most other cellular trafficking pathways, autophagy is mediated by membranous vesicles termed autophagosomes that sequester cytosolic material and deliver it to lysosomes for degradation. However, unlike conventional transport vesicles originating from a donor membrane and demarcated by a single bilayer, autophagosomes form in the cytosol and contain a characteristic double membrane, i.e., they are demarcated by a double bilayer. The autophagic response displays extremely fast dynamics. Withdrawal of all amino acids from the growth medium leads within a few minutes to formation of tens to hundreds of autophagosomes in each mammalian cell and this can persist for a few hours after reaching a steady-state.

The unusual architecture of autophagosomes together with their rapid induction upon stimulation have engendered a long-lasting debate as to the origin of the autophagosomal membrane and the mechanisms of its formation (2). In some proposed models, autophagosome formation takes place de novo in the cytosol, whereas an alternative view is that preexisting cellular membranes (organelles) give rise to autophagosomes. Recent work, in agreement with earlier studies, has suggested the ER as a significant contributor to autophagosome biogenesis (Fig. 1 A). A likely consensus based on electron microscopy and live imaging studies is that some autophagosomes form within omegasomes, ER-derived compartments enriched in phosphatidylinositol 3-phosphate (PI3P), and in contact with several intracellular organelles such as the ER Golgi intermediate compartiment, mitochondrial, endosomes, and plasma membrane (1, 3).

Irrespective of membrane of origin, it is clear from earlier work that the double bilayer of autophagosomes has unusual characteristics in comparison to other cellular membranes (reviewed and discussed in reference 4). It is virtually devoid of integral membrane proteins and cholesterol, but rich in phospholipids and unsaturated fatty acids. Furthermore, mutations that block late steps in autophagosome biogenesis often produce preautophagosomal structures with multiple bilayers that are devoid of proteins and are stacked together in a multilamellar morphology. All of this has suggested a mechanism of autophagosome growth that depends on lipid supply and excludes for the most part incorporation of membrane proteins. A vesicle-based mechanism would require either protein-poor vesicles or some pathway of protein retrieval operating continuously at the autophagosome formation site. No evidence exists for such a mechanism, and, in fact, lipid transport in cells seldom relies on vesicular carriers. Instead, cellular lipids reach their final destinations via lipid transfer proteins, a large family of transporters that stabilize lipid molecules within their hydrophobic cavities and transport them to various cellular compartments (5). In this issue, Valverde et al. identify such a lipid transfer protein among the ATG protein family and provide important novel insights on how autophagosomes grow (6).

The protein in question is ATG2 (in mammals ATG2A and ATG2B). It had been known since 2011 that the ATG2A/ATG2B proteins, like their yeast counterpart, are required for autophagosome formation at a step downstream of the initiation stage (7). However, the large size of these proteins (∼250 kD) and the fact that they decorate lipid droplets as well as autophagic structures had made further investigation of their function difficult until recently. Valverde et al. noted that the N terminus of ATG2A has structural similarity to the choren_N segment at the N terminus of VPS13 (6), shown previously to function as a lipid transporter at ER sites contacting other organelles (8). Purification of ATG2A in the current work showed that ∼20 glycerolipids were bound to each protein molecule, thus suggesting the possibility that ATG2A can solubilize (and transport lipids). Further in vitro work with purified ATG2A clearly showed that it can transfer...
glycerophospholipids between vesicles, and a low-resolution structure of ATG2A by cryo-electron microscopy revealed an extended conformation with a long cavity voluminous enough to accommodate multiple lipids. The large size of ATG2A precludes its use as a membrane source? This is addressed by localization studies of nonselective autophagy to sequester membrane components to the autophagic vacuole. The family of WIPI proteins (four are known in humans) are involved in the early steps of autophagy and, just like their yeast counterparts ATG8, bind PI3P as well as phosphatidylinositol (3,5)-bisphosphate. Part of the function of the WIPI proteins is to tether the LC3 lipidation machinery to the forming autophagosome in the vicinity of omega-somes. ATG2A associates with the ER and with early autophagic structures positive for WIPI2. In this drawing, ATG2 is tethered to the omegasome via WIPI2, and it transports lipid molecules from the ER to the expanding autophagosome.

As pointed out by Valverde et al. (6), their findings are consistent with recent findings from two other laboratories (9, 10), provide a very satisfying answer to a conundrum in the field: how are lipids supplied to the forming autophagosome? Do these results also help to decide on a membrane source? This is addressed by localization and live imaging studies in the work by Valverde et al. (6). They find that GFP-tagged ATG2A associates with the ER and with early autophagic structures positive for WIPI2. The family of WIPI proteins (four are known in humans) are involved in the early steps of autophagy and, just like their yeast counterparts ATG8, bind PI3P as well as phosphatidylinositol (3,5)-bisphosphate. Part of the function of the WIPI proteins is to tether the LC3 lipidation machinery to the forming autophagosome in the vicinity of omega-somes (2). It is therefore easy to imagine that ATG2 bridges the distance between the ER on one side and the forming autophagosome at the edge of the PI3P domain on the other (Fig. 1B). Remarkably, the live imaging data of Valverde et al. show that ATG2 remains associated with forming autophagosomes and with the ER, even as these serpentine and undulating ER strands move around the cell interior (6).

As pointed out by Valverde et al. (6), their findings are consistent with recent work on the function of the yeast Atg2 protein, both in terms of its ability to transfer lipids as well as its localization between ER and the forming autophagosome (9, 11, 12). It is therefore very likely that despite some notable differences (for example, yeast makes autophagosomes at a time right next to the vacuole whereas mammalian cells make many autophagosomes spread throughout the cell interior), this mechanism of membrane expansion via lipid transport from the ER is conserved across species. Questions left for future studies include the mechanism that ensures unidirectional lipid transfer from the source membrane to the autophagosome, how the process terminates, and the relation of these findings derived from studies of nonselective autophagy to pathways where autophagy sequesters specific cargo in autophagosomes formed specifically for this purpose. Finally, the fact that ATG2 can also regulate lipid droplet formation (7) invites the speculation that some mechanistic connection may be apparent between autophagosome formation and lipid storage.

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