ATG2A transfers lipids between membranes in vitro

Takanori Otomo and Shintaro Maeda

Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA

ABSTRACT

ATG2 is a rod-shaped membrane-tethering protein suggested to mediate the endoplasmic reticulum (ER)-phagophore association and the expansion of the phagophore. We recently demonstrated that human ATG2A transfers lipids between membranes in vitro, which led us to propose a model that the phagophore expands upon the transfer of lipids from the ER by ATG2. Here, we summarize our findings and arising issues that need to be addressed to establish the mechanism of phagophore expansion.

The cup-shaped double-membrane phagophore engulfing cytoplasmic materials is a signature of macroautophagy/autophagy. Several models such as those involving organelle maturation and vesicle fusion have been described to explain how the phagophore could form. Although these models are consistent with the fact that the phagophore emerges adjacent to the ER, they lack supporting molecular bases. At the molecular level, recent studies have suggested that the ER and the phagophore edge are tethered by the rod-shaped 200-kDa protein ATG2 (ATG2A/B in mammals and Atg2 in yeast) through membrane interactions at each of its tips. The tip containing a Pfam database-registered ATG2_CAD region (the CAD tip) in the middle of the sequence is directed to the phosphatidylinositol-3-phosphate (PtdIns3P)-enriched phagophore edge through its interaction with the PtdIns3P-binding β-propellers WIPI/Atg18 (WIPI1, WIPI2, WDR45, and WDR45B in mammals and Atg18 in yeast). The other tip (the N tip) composed of the N terminus, which includes the Pfam-registered Chorein_N region, binds to membranes with no apparent lipid specificity. In cells, the N tip binds to the ER, but how it recognizes the ER is unknown. How ATG2-mediated tethering of the phagophore to the ER leads to the expansion of the phagophore has remained unanswered.

Our previous observation that ATG2A tethers 2 membranes like a bridge induced us to ask if lipids could be transferred between tethered membranes through the ATG2A bridge. Meanwhile, VPS13, a homolog of ATG2, was reported to be a lipid transfer protein, suggesting that ATG2 likely catalyzes lipid transfer as well. To characterize the lipid transfer activity of ATG2A, we performed a series of in vitro lipid transfer experiments using the recombinant full-length ATG2A and synthetic liposomes [1]. First, we used liposome flotation assays to demonstrate that ATG2A can extract lipids from membranes, dissociate from the membranes with those lipids, and unload the cargo lipids to other membranes. Second, we performed FRET-based real-time lipid transfer assays and showed that ATG2A transfers lipids between tethered membranes. Third, using the same assay, we showed that the ATG2A-WIPI complexes transfer lipids between PtdIns3P-containing membranes and PtdIns3P-free membranes. Collectively, these results establish that ATG2A can transfer lipids between tethered membranes in vitro and suggest that the ATG2A-WIPI complexes mediate lipid transfer between the ER and the phagophore (Figure 1A).

The lipid transfer activity of ATG2A depends highly on 2 parameters of the substrate membranes: lipid-packing defects and negatively charged lipids, such as phosphatidylinerine (PS) and phosphatidylglycerol (PG). Both factors may be rich at the edge of the phagophore where the membrane is highly curved and contains PtdIns3P. On the ER side, it is likely that at least phosphatidylinositolphospholipids are present as they would be delivered to the phagophore via ATG2A to serve as the precursor of PtdIns3P. The phagophore-associating site of the ER has been reported to be the ER exit site, where the membrane is highly curved, suggesting that the N tip could bind to the ERES by recognizing its lipid-packing defects. The precise values of these parameters in cells will have to be determined for a quantitative understanding of ATG2A-mediated phagophore expansion.

In addition to these parameters, the following three issues must be resolved to establish lipid transfer as a principal mechanism for phagophore expansion. The first is concerned with the rate of lipid transfer, which was estimated to be ~0.017 fluorescent-lipid molecule/protein/s. Because ATG2A most likely transfers non-fluorescent lipids as well, the overall lipid transfer including non-fluorescent lipids is likely faster (up to ~50 fold). However, even with this consideration, the lipid transfer mediated by ATG2A appears to be too slow to account for the overall lipid delivery required for building an autophagosome (tens of millions of lipids in ~5–10 min). The second issue is with the directionality. The ATG2A-mediated lipid transfer demonstrated in vitro is a bidirectional transfer reaction. To expand the phagophore rapidly, the lipid transfer must occur unidirectionally from...
the ER to the phagophore. The third is with the energy source. Unidirectional transport of lipids would require the consumption of energy. The type of energy expended for this process is unknown.

The structural basis of lipid binding by ATG2 and VPS13 has been provided by 2 other groups who also demonstrated lipid transfer by these proteins. Their crystal structures of the yeast Atg2 and Vps13 N-terminal fragments show a unique architecture with a large hydrophobic cavity that can accommodate lipid molecules. As observed in a low-resolution cryo-EM structure, the cavity extends from tip to tip in the full-length ATG2A, suggesting that multiple lipids could occupy the cavity and translocate from one tip to the other. This structural information and the fact that ATG2A can bridge 2 membranes are integrated into a bridge model of lipid transfer, in which the ATG2-WIPI complex stably tethers the PtdIns3P-positive phagophore and the ER and transfers lipids between them (Figure 1B). However, it has also been reported that an ATG2A N-terminal fragment can mediate lipid transfer. As this fragment cannot tether membranes, it must shuttle between membranes to transfer lipids. This notion modifies the bridge model to a ferry model in which the ATG2A-WIPI complex is stably anchored to the phagophore through the WIPI-PtdIns3P interaction, while the N tip interacts with the ER and the phagophore dynamically (Figure 1C). In this model, lipids do not translocate in the cavity. Distinguishing between the two models will require further biophysical studies.

In conclusion, the phagophore expansion model based on ATG2A-mediated lipid transfer explains how lipids could be delivered to the phagophore to serve as building blocks and has uncovered important unknowns that must be clarified to establish the mechanism of phagophore expansion.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by NIH [R01GM092740] to T.O.

**ORCID**

Takanori Otomo [http://orcid.org/0000-0003-3589-238X](http://orcid.org/0000-0003-3589-238X)

**Reference**

[1] Maeda S, Otomo C, Otomo T. The autophagic membrane tether ATG2A transfers lipids between membranes. Elife. 2019;8: Epub 2019/07/05. DOI: 10.7554/eLife.45777. PubMed PMID: 31271352; PMCID: PMC6625793.