

Membrane curvature response in autophagy

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Membrane trafficking is key for signal transduction, cargo transportation, and in the case of autophagy, delivering cytoplasmic substrates to the lysosome for degradation. Autophagy requires the formation of a unique double membrane vesicle, the autophagosome. However, the mechanism by which the autophagosome forms is unknown. Our recent study focused on the role of Barkor/Atg14(L) in targeting the autophagy-specific class III phosphatidylinositol-3-kinase (PtdIns3KC3) complex to the early autophagosome has implicated this complex in autophagosome formation. This study found that the BATS domain of Barkor targets the PtdIns3KC3 complex to early autophagic structures and senses highly curved membranes enriched in phosphatidylinositol-3-phosphate (PtdIns(3)P). Consequently, this study uncovered an exciting new role for the PtdIns3KC3 complex as a potential inducer of autophagosome formation.

Why is Membrane Curvature Important?

Vesicle formation from a flat membrane into a highly curved structure involves drastic morphological changes. The creation of specific membrane microenvironments that subsequently recruit proteins that deform the membrane and form the stabilizing coat regulates this process. In some cases, one protein can perform both of these steps. For instance, N-BAR domain-containing proteins can induce membrane curvature by inserting N-terminal amphipathic alpha helices into the lipid bilayer and stabilizing this curvature using C-terminal BAR domain dimers. In

contrast, other processes require separate proteins to perform both curvature induction and stabilization. For example, in the endocytosis pathway, clathrin self-oligomerizes and forms a coat that stabilizes curvature but lacks membrane-binding and deformation activity; it depends on its accessory proteins, such as Epsin, Ap2 and Ap180 to deform the membrane.

Amphipathic alpha helices are often implicated in inducing or sensing membrane curvature. These helices are defined by the localization of hydrophobic and hydrophilic amino acids on opposite sides of the helix. This characteristic allows for insertion of the hydrophobic half into the lipid bilayer; it is thought that this direct penetration generates membrane curvature by increasing the surface area of only one bilayer. In helices that induce curvature, electrostatic interactions between the polar half of the helix with lipid headgroups are usually required. However, in some cases, the polar side of the helix is not highly charged, such as with the ArfGAP1 lipid packing sensor (ALPS). In these motifs, the weakly charged polar face of the amphipathic alpha helix is enriched in serines and threonines and associates with membranes of high curvature, serving as curvature sensors, not curvature inducers.

Characterization of the PtdIns3KC3 complex component Barkor/Atg14(L) by our group identified the Barkor/Atg14(L) autophagosome targeting sequence (BATS) domain. This domain is required for Barkor/Atg14(L) and PtdIns3KC3 complex recruitment to LC3 puncta upon autophagy induction. This domain also colocalizes with early autophagic markers, again implicating it in autophagosome formation. Upon further analysis, we found that the highly conserved BATS domain

Key words: autophagy, PtdIns3KC3, Barkor, Atg14(L), BATS, membrane curvature, ALPS, amphipathic alpha helix, autophagosome, PtdIns(3)P

Submitted: 05/27/11

Revised: 06/01/11

Accepted: 06/08/11

DOI:

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Punctum to: Fan W, Nassiri A, Zhong Q. Autophagosome targeting and membrane curvature sensing by Barkor/Atg14(L). *Proc Natl Acad Sci USA* 2011; 108:7769-74; PMID: 21518905; DOI: 10.1073/pnas.1016472108.

contains a predicted amphipathic α helix. Interestingly, this helix fits the model for ALPS motifs with its weakly charged polar face. Additionally, we found that BATS domain preferentially binds highly curved liposomes enriched in PtdIns(3)P through the ALPS motif, and therefore serves as a membrane curvature sensor. This discovery has consequently suggested a previously unknown role of Barkor/Atg14(L) and the PtdIns3KC3 complex in autophagosome formation.

How does the Autophagosome Form?

Among all of the autophagy machinery that has been characterized, few of these proteins exhibit any known membrane curvature-sensing or -inducing motifs. This has consequently left a huge gap in our understanding of autophagy initiation. A recent discovery of Bif-1 as a protein that associates with the PtdIns3KC3 complex through UVRAG shed some light on the topic. Bif-1 is a N-BAR domain-containing endophilin family protein. Upon interaction with the PtdIns3KC3 complex, Bif-1 regulates PtdIns3KC3 kinase activity; elimination of Bif-1 expression compromises autophagosome formation. These results imply that Bif-1 could function like other N-BAR domain proteins and induce and stabilize autophagosome membrane curvature. However, since Bif-1 associates with the PtdIns3KC3 complex through UVRAG, which is implicated in later autophagic steps such as autophagosome maturation, it is an unlikely major component of autophagosome formation.

Consequently, the discovery of the amphipathic alpha helix in the BATS

domain establishes another candidate for autophagosome formation. Although the membrane origin of autophagosomes is still highly debated, increasing evidence has pointed to ER-associated membrane structures, such as the omegasome, as at least one of the membrane sources for autophagosomes. In our model, Barkor/Atg14(L) associates with ER membrane in unstressed conditions. Autophagic stimuli then promote the formation of the omegasome, an ER-associated autophagic membrane structure. Due to its high binding affinity to highly curved membrane, the ALPS motif of BATS localizes and clusters at membrane regions of high curvature, such as at the tip of the omegasome. This Barkor activation then recruits the rest of the PtdIns3KC3 complex to the curved membrane, activating PtdIns3 kinase activity, creating more PtdIns(3)P. This PtdIns(3)P-enriched membrane microenvironment recruits more PtdIns3KC3 complexes that associate with the highly curved membrane through BATS/PtdIns(3)P interactions. This results in a feed-forward loop of PtdIns(3)P production and membrane curvature that could eventually be stabilized by the additional recruitment of PtdIns(3)P binding proteins (DFCP1 and WIPI1/2) or coat-forming proteins (Atg12-Atg5-Atg16), allowing the omegasome to develop into a mature autophagosome.

This study also proposes a critical role of PtdIns(3)P in membrane curvature sensing and stabilization during autophagosome formation, which is supported by several lines of evidence. PtdIns(3)P production is essential for the biogenesis of autophagosomes. Depletion of PtdIns(3)P decreases membrane

curvature and impedes membrane expansion. PtdIns(3)P generation and subsequent removal from the autophagosome membrane could constitute a pivotal regulatory factor for the membrane curvature response, which could subsequently control autophagosome biogenesis and fusion processes.

Perspectives and Future Directions

Although the BATS domain is capable of sensing membrane curvature, it is not clear whether it can also induce membrane curvature. It is likely that other factors in the PtdIns3KC3 complex, especially at high local concentrations, may stabilize and direct membrane deformation that was initiated by ALPS motif insertion. Finding and characterizing the cofactors that are required for curvature induction will be critical for deciphering the mechanism of autophagosome formation.

Overall, this intriguing new role of the PtdIns3KC3 complex has uncovered a target for further understanding of the biochemical mechanism of autophagosome formation. To accomplish this, we must better understand the interplay between the PtdIns3KC3 complex and the early autophagosome membrane and also decipher how other early autophagic machinery are recruited to the membrane and interact with each other to induce autophagosome formation.

Acknowledgements

Q.Z. is supported by a New Investigator Award for Aging from the Ellison Medical Foundation, Hellman Family Fund and NIH RO1(CA133228).