RESEARCH HIGHLIGHT

A dual-purpose fusion complex in autophagy

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Degradation of cytoplasm by autophagy requires fusion of autophagosomes with lysosomes, a process driven by SNARE proteins. In a recent paper in *Cell Research*, Jian et al. identify a novel SNAP47-containing SNARE complex which mediates autophagosome–lysosome fusion in both bulk and selective autophagy.

Fusion between cellular membrane entities is mediated by specific sets of SNARE complexes, whose assembly in trans into tetrahelical bundles drives membrane fusion and also contributes to specific membrane recognition.[1] A typical SNARE complex consists of membrane-anchored syntaxin (STX) and VAMP proteins that face each other on the two opposing membranes and a cytosolic SNAP protein that associates with these. A zipper-like complex formation between one SNARE domain each from the STX and VAMP proteins and two from the SNAP protein releases energy that promotes membrane fusion.[1]

The fusion between autophagosomes and lysosomes has been intensively studied, owing to the immense importance of autophagy in physiology and medicine.[2] Autophagosomes are double-membrane vesicles originating from phagophore membranes that enclose portions of cytoplasm. If the phagophore forms around an object such as a damaged organelle, a protein aggregate, or a pathogen, this process is termed selective autophagy. This is in contrast to bulk autophagy, which is typically induced by amino acid starvation and involves sequestration of the phagophore around small volumes of cytosol. Regardless of the autophagic mechanism, when the autophagosome fuses with a lysosome, its sequestered content will be degraded by lysosomal hydrolases.

Previous studies of starvation-induced bulk autophagy have identified two specific SNARE complexes required for autophagosome–lysosome fusion. One complex consists of VAMP7 or VAMP8 on the lysosome membrane and STX17 in association with SNAP29 on the autophagosome membrane,[3] whereas the other contains STX7 on the lysosome membrane and SNAP29 together with the VAMP-like protein YKT6 on the autophagosome membrane.[4] Surprisingly, when Jian et al. studied cells depleted for SNAP29, the common component of these two complexes, degradation of damaged mitochondria, mitophagy, was unaffected.[5] This led the authors to screen for SNAREs that mediate mitophagy. They noticed that, in contrast to SNAP29, SNAP47 is highly enriched on mitophagosome membranes, and depletion of SNAP47 strongly inhibited mitophagy. This suggested that SNAP47 is part of a novel SNARE complex that mediates mitophagosome–lysosome fusion.

Further experiments revealed that SNAP47 appears to be rather universally involved in autophagosome–lysosome fusion, since its depletion not only inhibits mitophagy but also selective autophagy of protein aggregates (aggrephagy) and the endoplasmic reticulum (ER-phagy). Depletion of SNAP47 even inhibited starvation-induced autophagy to some extent, which was further enhanced by SNAP29 depletion. This indicates that SNAP47 is indispensable for selective autophagy and functions in parallel with SNAP29 in bulk autophagy.

To identify the SNARE complex partners of SNAP47, Jian et al. performed co-immunoprecipitation experiments. Unexpectedly, SNAP47 was found to interact with most STXs, but among these, only STX17 was found to be important for mitophagy. Likewise, SNAP47 interacted with several VAMPs, but only depletion of VAMP7 or VAMP8 inhibited mitophagy. Biochemical experiments showed that SNAP47, STX17 and VAMP7/8 can form a ternary complex that has the capability to fuse liposomes in vitro. The authors therefore conclude that this is the SNAP47-containing SNARE complex that mediates autophagosome–lysosome fusion.[5]

SNAP47 is atypical among SNAP family members in that it contains an N-terminal pleckstrin homology (PH) domain. Many PH domains are involved in binding to phosphoinositides, phosphorylated derivatives of phosphatidylinositol.[6] Jian et al. found that the PH domain of SNAP47 binds specifically to phosphatidylinositol 4-5-bisphosphate, $PI(4,5)P_2$. This phosphoinositide is most abundant at the cytosolic face of the plasma membrane,[6] but the authors obtained evidence that it is also formed on autophagic membranes. Importantly, SNAP47 PH domain mutants unable to bind $PI(4,5)P_2$ showed strongly reduced localization to autophagic membranes, indicating that $PI(4,5)P_2$ is involved in SNAP47 recruitment to autophagosomes.

LC3 and other ATG8 family proteins localize to autophagic membranes and play important roles in autophagy.[7] The PH domain of SNAP47 contains two LC3-interacting region (LIR) motifs, and biochemical experiments showed interactions between the SNAP47 PH domain and ATG8 family proteins.[5] Cells lacking all six ATG8 family members are able to form autophagosomes.[8] However, SNAP47 failed to localize to autophagosomes in these cells, and localization could be rescued by transfection with either LC3A, LC3B or GABARAP. This indicates that these ATG8 family proteins participate in recruitment of SNAP47 to phagophores.[5] Since interactions with both $PI(4,5)P_2$ and ATG8 family proteins are required for localization of SNAP47 to autophagic structures, SNAP47 is probably recruited through coincident detection of these molecules.

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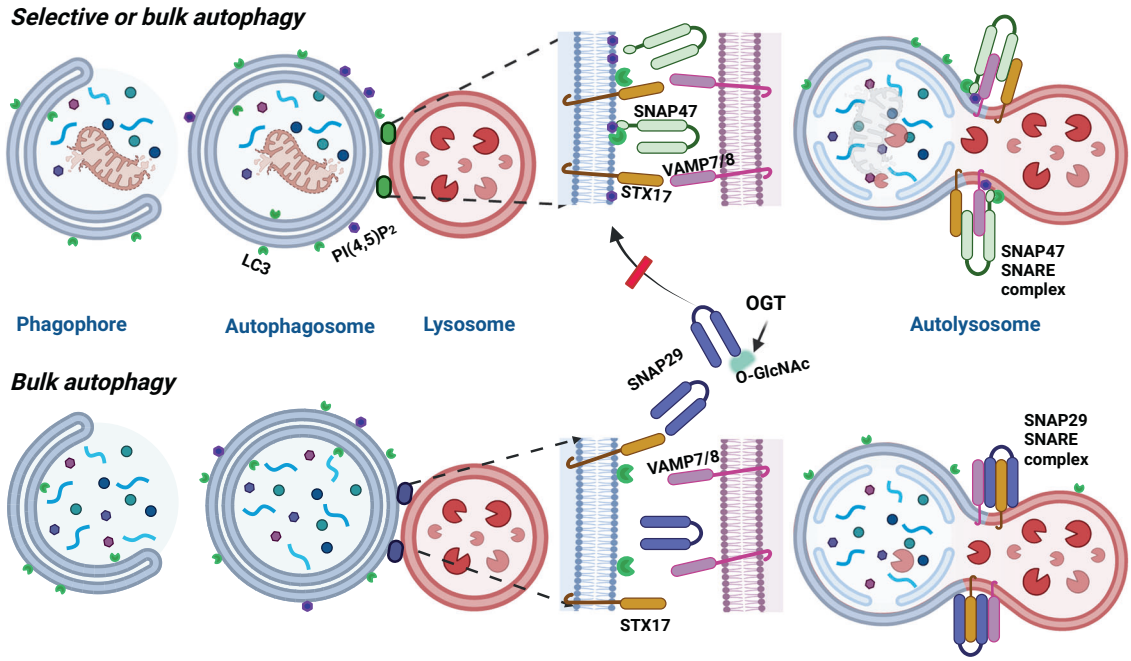

Fig. 1 A novel SNARE complex mediates both bulk and selective autophagy. A SNARE complex formed between STX17, VAMP7/8 and SNAP29 mediates autophagosome–lysosome fusion in bulk autophagy. Another SNAP29-containing complex (not shown) with YKT6 and STX7 has a similar function. The new SNARE complex between STX17, VAMP7/8 and SNAP47 mediates autophagosome–lysosome fusion in both bulk and selective autophagy. SNAP47 is recruited to autophagosomes through coincident detection of LC3 and PI(4,5)P_2, whereas SNAP29 is excluded from participation in selective autophagy by OGT-mediated *O*-GlcNAcylation. Figure generated with BioRender.com.

It is interesting that SNAP47-containing SNARE complexes mediate autophagosome–lysosome fusion in both selective and bulk autophagy, whereas SNAP29-containing complexes only sustain bulk autophagy (Fig. 1). Previous work has shown that SNAP29 is posttranslationally modified with *O*-linked-*N*-acetylglucosaminylation (*O*-GlcNAcylation) at specific threonine and serine residues, mediated by O-GlcNAc transferase (OGT).[9] *O*-GlcNAcylation inhibits the recruitment and function of SNAP29 in autophagosome–lysosome fusion, and a decrease in *O*-GlcNAcylation during starvation thus promotes autophagosome–lysosome fusion. Jian et al. now found that there is no decrease in *O*-GlcNAcylation of SNAP29 during selective autophagy, and *O*-GlcNAcylation of SNAP29 thereby prevents its autophagosomal recruitment during selective autophagy.[5]

Identification of the new SNAP47-containing SNARE complex in autophagosome–lysosome fusion sheds light on previous observations that one of the functions of ATG8 family proteins is to mediate autophagosome–lysosome fusion.[8] Now, this can be explained by their involvement in SNAP47 recruitment. From a broader perspective, identification of the new player in autophagy, SNAP47, provides new possibilities for manipulating autophagy in research and biomedical applications.

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ADDITIONAL INFORMATION

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