SYT6 interacts with components of the plant autophagic machinery

Cardeñas Echevarría, Carlos[1], Moya-Barrientos, Miriam, Sánchez-Vera, Victoria y Ruiz-López, Noemí

[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC) 29010, Departamento de Biología Molecular y Bioquímica, Málaga, Spain.

ABSTRACT: Membrane contact sites (MCSs) are fundamental hubs of inter-organelle communication, mediating the non-vesicular transfer of lipids, ions, and metabolites to maintain cellular homeostasis. Synaptotagmins (SYTs) are membrane-bridging proteins that facilitate lipid exchange at these sites. Particularly, SYT6, an endoplasmic reticulum (ER)-anchored protein, has been proposed to localize at ER-trans-Golgi network (TGN) contact sites, although its role is not yet known.

Our preliminary experiments indicate that SYT6 putatively associates with the autophagy protein ATG18a, suggesting a potential link to this recycling pathway. To investigate this, we transiently co-expressed SYT6 with either ATG8a or ATG18a (two key autophagy proteins) in *Nicotiana benthamiana* leaves and analyzed interactions using Bimolecular Fluorescence Complementation (BiFC) and co-immunoprecipitation (Co-IP). BIFC images were quantified using an AI-based segmentation pipeline (Ilastik) and Fiji.

distinct patterns: SYT6–ATG8a vesicles were significantly smaller (0–10 μm^2) and more numerous, whereas SYT6–ATG18a complexes formed larger structures (often >50 μm^2). Co-IP further validated the specific physical association of SYT6 with both autophagy proteins.

These findings support that SYT6 physically associates with key components of the autophagy machinery and suggest it may play distinct roles at different stages of autophagosome formation, potentially linking ER membranes to autophagic vesicles.

Bibliography: Huercano, C., Moya-Barrientos, M., Cuevas, O., Cardenas, C., Salas, J. J., Sanchez-Vera, V., & Ruiz-Lopez, N. (2025). The plant lipid contactome: emerging roles of inter-organelle contact sites in lipid metabolism. *Progress in lipid research*, *101*, 101372. Advance online publication. https://doi.org/10.1016/j.plipres.2025.101372

1. Molecular components: From SYT6 to ATG machinery

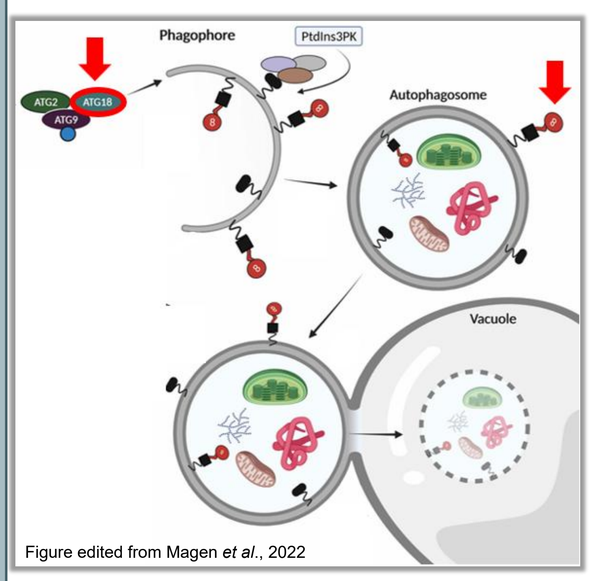


Figure edited from Magen *et al*., 2022

Autophagy is a conserved eukaryotic pathway essential for cellular homeostasis, stress adaptation, and organelle quality control. A central yet unresolved question in the field concerns how lipids are supplied to sustain the rapid membrane expansion required for autophagosome biogenesis.

Autophagosome formation follows a coordinated sequence:

1.PI3P production at the nascent phagophore
2.ATG18 recruitment to PI3P-enriched membranes
3.ATG8 lipidation, driving membrane expansion and curvature
4.Phagophore closure and autophagosome maturation

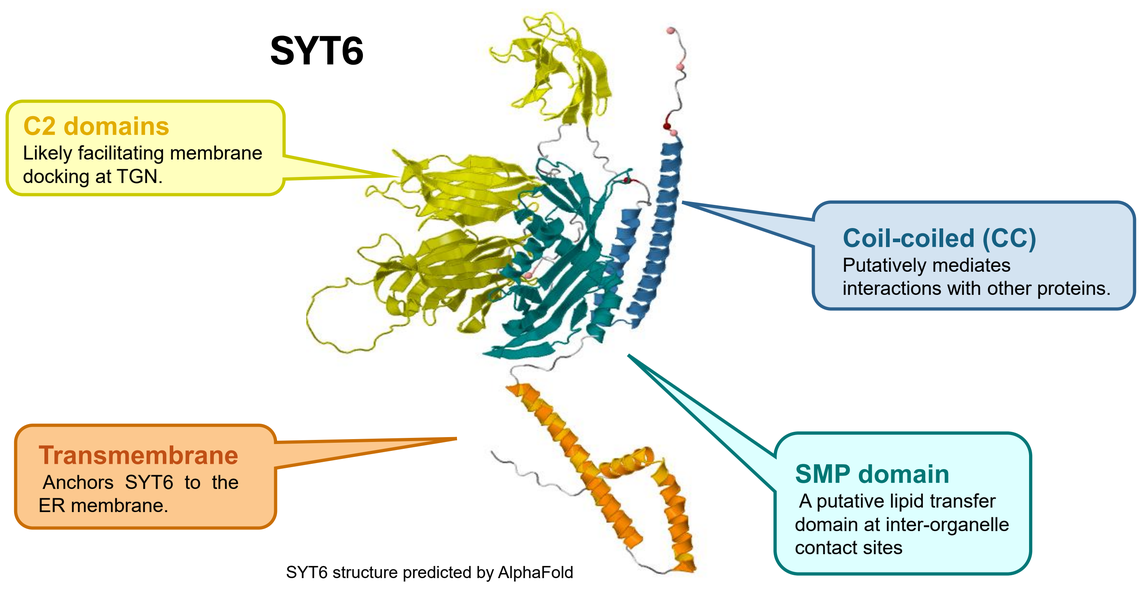
While the core ATG machinery has been extensively characterized, how lipid transfer is spatially coupled to this process remains unclear.

Emerging evidence suggests that ER membrane contact sites may act as platforms for lipid delivery during this process. Proteins harboring SMP lipid transfer domains are strong candidates to mediate this coupling.

ATG8a: A ubiquitin-like protein essential for the biogenesis of the autophagosomal membrane and the recruitment of the core autophagic machinery.

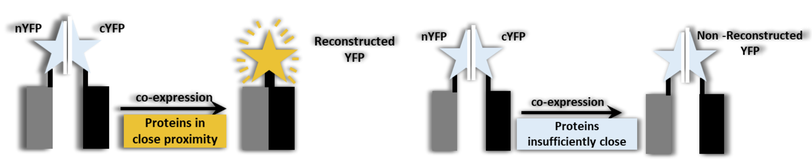
ATG18a: Phosphoinositide-binding protein that interacts with ATG2 to facilitate membrane expansion during autophagosome formation.

Working hypothesis
We propose that the ER-anchored protein SYT6 functions at membrane contact sites to facilitate lipid transfer, thereby promoting ATG recruitment and phagophore expansion.

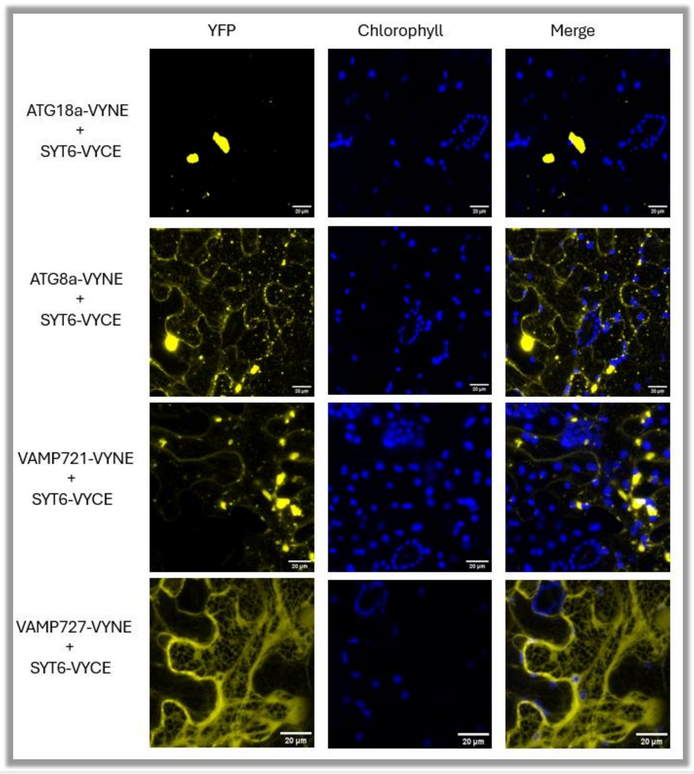


SYT6 structure predicted by AlphaFold

2. SYT6 and ATG proteins converge at vesicular compartments

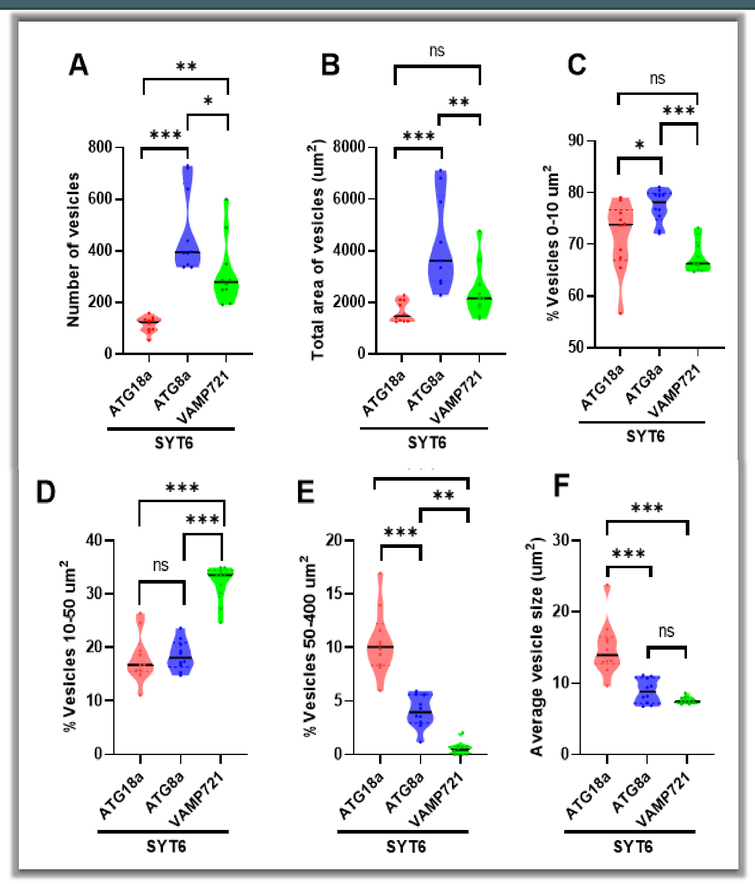


BiFC assays are based on the reconstitution of a functional YFP fluorophore from two non-fluorescent fragments (VYNE and VYCE) to detect *in vivo* protein–protein proximity. Importantly, reconstitution of YFP fluorescence occurs only when the two proteins are in very close proximity, reflecting a direct or near-direct interaction.



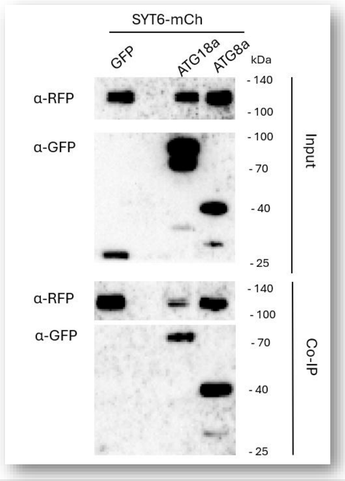
***In vivo* BiFC analysis of SYT6 interactions**. Confocal micrographs suggest the spatial proximity of SYT6 with ATG18a, ATG8a, and VAMP721 (positive control) at vesicular compartments, whereas no association is observed with VAMP727 (negative control). SYT6–ATG8a complexes also localize at the plasma membrane.

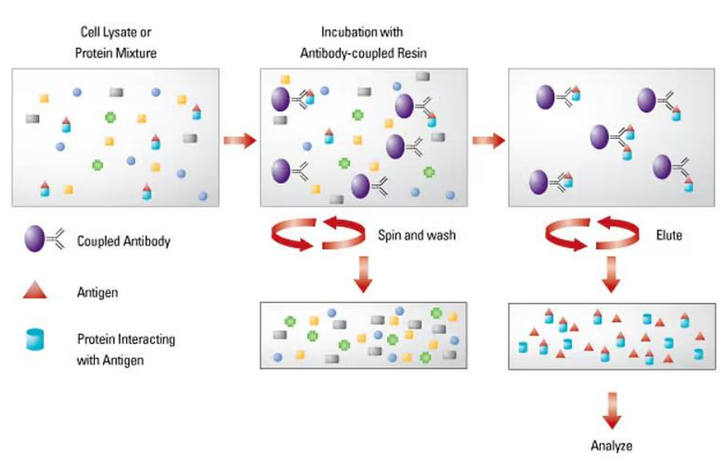
3. Vesicle population dynamics: ATG8a vs ATG18a



Morphometric quantification of SYT6-associated vesicular structures. Violin plots display the size distribution and density of complexes formed with ATG8a and ATG18a. SYT6-ATG8a interactions yield a significantly higher number of small vesicles (0-10 μm^2), whereas SYT6-ATG18a complexes predominantly form larger vesicular populations (50-400 μm^2). The differential size distribution observed suggested that the SYT6-ATG8a interaction might correlate with mature, functional autophagosomes (~1.8 μm^2), whereas the larger SYT6-ATG18a complexes potentially represent early phagophore nucleation platforms or late-stage vacuolar fusion events.

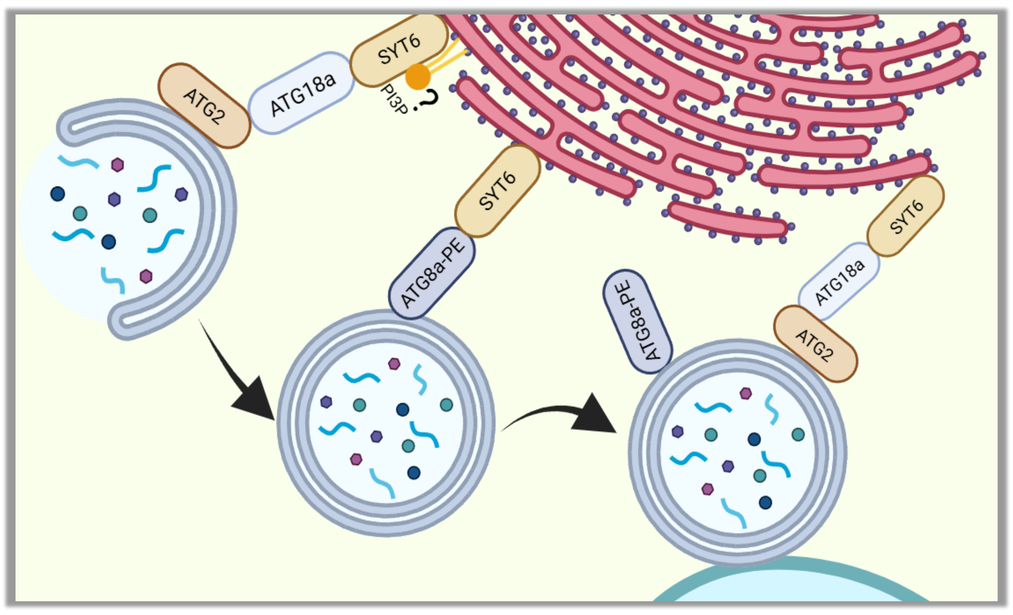
4. SYT6 forms stable complexes with ATG18a and ATG8a





Co-immunoprecipitation (Co-IP) was performed to validate protein–protein interactions *in vivo*. SYT6–mCherry was immunoprecipitated using anti-RFP beads, and co-precipitation of ATG8a-GFP and ATG18a-GFP was assessed to determine their association within the same protein complex. Western blot analysis of the IP fraction shows that SYT6 specifically associates with ATG8a and ATG18a, supporting an *in vivo* interaction between the synaptotagmin and components of the autophagic machinery. Schematic adapted from Thermo Fisher Scientific.

5. SYT6 as a putative lipid transfer protein at ER-autophagosome contact sites



Proposed model for SYT6-mediated lipid transfer. Our findings support a model in which SYT6 functions as an ER–phagophore tether during autophagosome biogenesis. We propose that SYT6 senses ER-localized PI3P and facilitates glycerolipid transfer through its interaction with ATG18a. In parallel, its association with ATG8a may contribute to phagophore expansion and maturation, thereby promoting efficient autophagosome formation.

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