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Pexophagy and Its Novel Membrane Dynamics in the Methylotrophic Yeast *Pichia pastoris*

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Autophagy is a cellular process in which proteins and organelles are delivered to and degraded in vacuoles (or lysosomes). Two modes of membrane dynamics exist for autophagy: microautophagy and macroautophagy[1]. Microautophagy is the sequestration of targeted cytosolic components (including organelles) by enclosure in vacuolar membranes; during this process, vacuoles form invaginations or protrusions by generating new compartments. Macroautophagy refers to the sequestration of cytosol by membranes to generate autophagosomes that then fuse with vacuoles, releasing autophagic bodies that are degraded within.

The number of organelles (e.g., peroxisomes) per cell varies as cells adapt to surrouding stimuli. This is controlled by a combination of organellar proliferation and degradation. The selective degradation of peroxisomes, called "pexophagy", is a type of autophagy. As with general autophagy, there are two types of pexophagy: macropexophagy and micropexophagy.

Micropexophagy is a dynamic event in the methylotrophic yeast *Pichia pastoris*, in which vacuolar membranes engulf and sequester a cluster of giant peroxisomes directly and formation of the isolation membrane has been believed not to occur[2]. Nevertheless, multiple genes necessary for micropexophagy were shown to overlap with *ATG* genes required for macroautophagy[3]. We found that a novel cup-shaped membrane structure was formed after the onset of micropexophagy to which a ubiquitin-like protein Atg8 (GFP-Atg8) is localized[4]. Just before contact is made by opposing vacuolar membranes, GFP-Atg8 was localized exclusively between them. We suggest that the cup-shaped membrane structure is formed as a "micropexophagy-specific membrane apparatus (MIPA)" and that it mediates the fusion between opposing vacuolar membranes to sequester peroxisomes from the cytosol.

Atg26 (Paz4/Ugt51) was essential for pexophagy, but not for macroautophagy[5]. Atg26, which synthesizes sterol glucoside, contained a GRAM domain as well as a PH and a catalytic domain. The GRAM domain was suggested to play a role in membrane traffic and pathogenesis, but its significance in any biological processes was not known. During micropexophagy, Atg26 was associated with the MIPA, and this localization depended on the GRAM domain and was required for pexophagy. Recently, we found that the PpAtg26 GRAM domain is a novel binding motif to phosphatidylinositol, which is a key signaling molecule in diverse cellular processes. In contrast, deletion of the catalytic domain did not impair protein localization, but abolished pexophagy. These results indicated that not only sterol glucoside synthesis but also phosphatidylinositol signaling are

required for pexophagy.

References

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