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Three Distinct Types of Microautophagy Based on Membrane Dynamics and Molecular Machineries

*Masahide Oku and Yasuyoshi Sakai**

Microautophagy is originally defined as lysosomal (vacuolar) membrane dynamics to directly enwrap and transport cytosolic components into the lumen of the lytic organelle. Molecular details of microautophagy had remained unknown until genetic studies in yeast identified a set of proteins required for the process. Subsequent studies with other experimental model organisms resulted in a series of discoveries that accompanied an expansion of the definition of microautophagy to also encompass endosomal membrane dynamics. These findings, however, still impose puzzling, non-integrated images as to the molecular mechanism of microautophagy. By reviewing recent studies on microautophagy in various experimental systems, we propose the classification of microautophagy into three types, as the basis for developing a comprehensive view of the process.

1. Introduction

Autophagy, defined as a collection of transport systems for movement of cytoplasmic components into the lysosome (vacuoles), plays versatile physiological, and pathological roles.[1] Based on morphological and mechanistic features, the autophagic systems (pathways) found are categorized into three types: macroautophagy, microautophagy, and chaperone-mediated autophagy.[1] Among them, the molecular mechanism of macroautophagy has been elucidated to the greatest extent. This type generates a double-membrane structure, termed the autophagosome, which enwraps the target cytosolic components and whose outer membrane fuses with the endosomal or lysosomal membrane, eventually releasing the inner membrane along with the targets into the lumen of the fused organelles. Although macroautophagic pathways hold variations in terms of target specificities or induction conditions, they all utilize common molecular machinery exerted by "core" Autophagy-related (*ATG*) gene products to generate the autophagosome,[2] which facilitates our comprehensive understanding of the molecular machinery. The chaperone-mediated autophagy transports cytosolic proteins into the lysosome though the target recognition by a chaperone protein Hsc70, followed by transfer of the targets to the lysosomal LAMP-2A protein.[3]

In contrast, microautophagy has remarkably more diverse morphologies and molecular machineries.[4] Historically, it was noticed in a pioneering EM observation as an extension and engulfment of part of the lysosomal membrane to enwrap a "micro" potion of the cytoplasm.[5] A similar vacuolar membrane morphology was observed in a yeast *Pichia pastoris* to capture peroxisomes for degradation.[6] Subsequent studies with several yeasts identified vacuolar membrane invagination to incorporate multiple organelles. In mouse embryonic cells, invagination of a lysosome-derived organelle (apical vacuole) toward the endosomes was reported.[7] Furthermore, recent studies demonstrated that invagination of the endosomal membrane known as formation of intraluminal vesicles by the endosomal sorting complexes required for transport (ESCRT) machinery also contributes to incorporation of cytoplasmic proteins into the lysosome, and thus is regarded as an alternative pathway of microautophagy.[8] Considering these findings, we now need to reconsider the concept of microautophagy as a unified form of membrane dynamics. We herein propose to categorize microautophagy into three types: Type 1, microautophagy with lysosomal protrusion; Type 2, with lysosomal invagination; and Type 3, with endosomal invagination. Below we describe the morphological and molecular basis revealed to date for each type of microautophagy (**Table 1**).

2. Type 1, Microautophagy with Lysosomal Protrusion

2.1. In Mammalian Cells

In pioneering EM analyses, lysosomes were observed to change their shapes in mouse macrophages[9] and rat liver cells:[10] the extension and flattening of the lysosomes wrapped around a portion of the

Dr. M. Oku, Prof. Y. Sakai
Division of Applied Life Sciences
Graduate School of Agriculture
Kyoto University
Kyoto 606-8502, Japan
E-mail: ysakai@kais.kyoto-u.ac.jp
Prof. Y. Sakai
Research Unit for Physiological Chemistry
The Center for the Promotion of Interdisciplinary Education and Research
Kyoto University
Kyoto 606-8502, Japan

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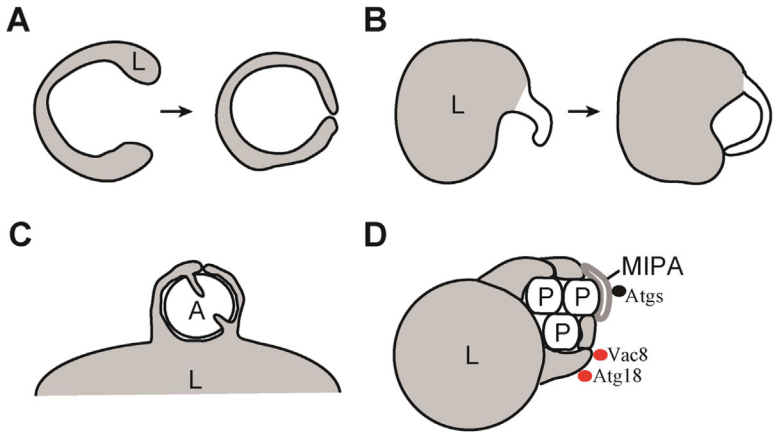

Figure 1. Type 1 microautophagy with lysosomal protrusion. A) A schematic model of lysosomal wrapping mechanism in mammalian cells. L: Lysosome. B) Microautophagy through an arm-like protrusion of the lysosome in mammalian cells. The luminal portion of the arm structure is often stained less dense in EM analyses, compared with the rest of the lysosomal lumen. C) Extension of the lysosomal/vacuolar (L) membrane engulfs anthocyanin aggregate in *Arabidopsis thaliana*. Some of the vacuolar-membrane extensions reach the inner part of the aggregate. A: Anthocyanin aggregate. D) Membrane structures observed in microautophagic degradation of peroxisomes in the methylotrophic yeast *Pichia pastoris*. P: Peroxisome. MIPA, MIcroPexophagy-specific membrane Apparatus. Protrusion of the lysosomal/vacuolar (L) membrane requires Vac8 and Atg18 proteins, both of which are localized on the vacuolar membrane (shown as red dots). MIPA is formed by actions of Atg proteins, and harbors several Atg proteins on it.

cytoplasm along with other organelles, which strongly suggested turnover of the wrapped components inside the lysosomes (Figure 1A). This process, termed the Lysosomal Wrapping Mechanism (LWM), is thought to constitute an autophagic pathway, but the molecular mechanism of this process remains unknown.

Other EM studies demonstrated that arm- or flap-like protrusions of partial lysosomal membranes surrounded a portion of the cytoplasm in mouse and rat liver cells[11,12] (Figure 1B). A similar morphology had been reported for isolated rat liver lysosomes incubated with Percoll particles.[13] The observation frequencies of secondary lysosomes whose protrusions sequestered cytoplasmic portions were correlated with basal protein-turnover rates when liver cells were subjected to perfusion experiments in which they were supplied with a sufficient amount of amino acids, suggesting that this microautophagic pathway contributes predominantly to the basal protein turnover under these conditions.[14]

It is of note that the luminal portion of the lysosomal protrusions often exhibited less electron density, compared with the rest of the lysosomal lumen.[11,12] A recent study identified a similar structure during the lysosome-reformation process, which was induced after fusion of the lysosome with autophagosomes.[15] The tubular extension of the lysosome, eventually released from the remainder of the organelle during the lysosome reformation, contained fewer luminal hydrolases, suggesting segregated properties from the main portion of the lysosome. The reformation process was driven by reactivation of mTOR activity, and curiously, similar re-activation of yeast Tor kinase activity was found to induce microautophagy,[16] although it was morphologically classified as Type 2 (with vacuolar invagination, see below) (Table 1).

2.2. In Plant Cells

Anthocyanin vacuolar inclusions are an aggregated form of the flavonoid compound found in the vacuoles of plant cells. Since the biosynthesis of anthocyanin occurs in the cytoplasm, several systems for transporting the aggregates into the vacuole have been proposed. A recent study of *Arabidopsis thaliana* and *Eustoma grandiorum* demonstrated that the aggregate was directly engulfed by extensions of the vacuolar membrane before being transported into the vacuolar lumen (Figure 1C), and consistent with this observation, the anthocyanin vacuolar inclusion was surrounded by a single membrane most likely derived from the vacuolar membrane transported along with the aggregate during microautophagy.[17] This process was independent of Atg5, a pivotal factor for macroautophagy,[18] showing that the machinery for this type of microautophagy is distinct from that of the macroautophagy system.

2.3. In Yeast Cells (*Pichia pastoris*)

The methylotrophic yeast *Pichia pastoris* (*Komagataella phaffii*) has been used as a model organism to extensively study peroxisome dynamics, owing to the drastic augmentation or degradation of the organelle in response to changes in carbon source.[19] EM of yeast cells that were shifted from methanol to glucose medium enabled the discovery of protrusions of the partial vacuolar membrane to sequester peroxisomes, which appeared morphologically similar to the membrane dynamics seen in the above-mentioned higher eukaryotes[6] (Figure 1D). Random-mutagenesis approaches identified several genes required for vacuolar-membrane dynamics.

One of the identified genes encoded Vac8, a protein anchored to the vacuole through palmitoylation and mirystoylation at its N-terminal region.[20] Expression of a Vac8 mutant devoid of its characteristic armadillo domain and C-terminal region failed to form the vacuolar membrane protrusion, and instead formed round vacuoles under microautophagy-inducing condition.[21] This protein had been originally identified to be functional in the transfer of the vacuole portion from a mother cell to the daughter cell during yeast cell division (vacuole inheritance),[20] which may reflect similar vacuole dynamics between microautophagy and vacuole inheritance.

Atg18 is another protein required for the protrusion of the vacuolar membrane portion during microautophagy.[22] This protein was found associated both to the vacuolar membrane and to the pre-autophagosomal structure/phagophore assembly site (PAS), which is pivotal for the biogenesis of the autophagosome during macroautophagy. It bound to a phosphoinositide form, phosphatidylinositol 3′, 5′-bisphosphate (PI3,5P2) for its localization on the vacuolar membrane and its function in the vacuolar-membrane protrusion. Notably, phosphorylation of Atg18 suppressed its binding to the phosphoinositide.[23] These observations suggest that phosphoinositides (PI3,5P2 and its

Table 1. Summary of three types of microautophagy

Type	Membrane deformation site	Morphology of membrane deformation	Molecular machineries
Type 1	Lysosome (Vacuole)	Protrusion	1. Unknown for mammalian process (Figures 1A,B) 2. Atg5 dispensable for the transport of anthocyanin aggregate in plants (**Figure 1**C) 3. Direct function of Vac8 and Atg18 in the protrusion step for microautophagy of peroxisomes in the yeast *Pichia pastoris* (Figure 1D)
Type 2	Lysosome (Vacuole)	Invagination	1. Rab7 required for degradation of endosomes by apical vacuole in mouse VE cells 2. Indirect action of multiple Atg proteins (for autophagosome formation) in uptakes of the cytoplasm, portion of the nucleus, and lipid droplets in the yeast *Saccharomyces cerevisiae* undergoing formations of vacuolar microdomains (Figure 2A-C) 3. Indirect action of ESCRT protein (Vps4) in the uptake of lipid droplets induced during nutrient starvation in *S. cerevisiae* (**Figure 2**C) 4. Direct action of ESCRT proteins for degradation of the vacuolar membrane proteins in *S. cerevisiae* (Figure 2D)
Type 3	Endosome	Invagination	1. Independent of Atg proteins (Atg7 in a murine dendritic cell line; Atg 5, 7, and 12 in the fly Drosophila melanogaster) 2. Direct action of ESCRT proteins (**Figure 3**) 3. Direct action of Hsc70 in the target capture and/or the membrane deformation process (Figure 3)

precursor form phosphatidylinositol 3′-phosphate, PI3P) are important for the protrusion process of the vacuolar membrane.

Completion of the peroxisome incorporation into the vacuole after vacuolar membrane protrusion requires a sealing membrane structure termed MIPA (micropexophagy-specific membrane apparatus)[24] (Figure 1D). This structure is formed by the action of core *ATG* gene products in a similar way to the autophagosome formation, and thus the whole process of this type of microautophagy requires many *ATG* genes that function in macroautophagy.[25] Independent of this MIPA formation, several *ATG* gene products, Atg4, Atg8, and Atg24, were involved in regulation of vacuolar dynamics for microautophagy through their direct actions on the vacuolar membrane.[26,27]

3. Type 2, Microautophagy with Lysosomal Invagination

3.1. In Mammalian Cells

Mouse embryogenesis requires polarized cells surrounding the epiblast and extraembryonic ectoderm, termed the visceral endoderm (VE), for transmitting proper signals and normal patterning for cell differentiation.[7] The VE possesses a very large specialized organelle termed the apical vacuole that contains lysosomal enzymes and lysosomal membrane proteins. This organelle was found to incorporate endosomes through a microautophagic invagination process, which is dependent on the small GTPase Rab7.[7] Since genetic loss of Rab7 in the VE caused embryonic lethality, the physiological importance of microautophagy in embryogenesis is strongly suggested.

3.2. In Yeast Cells (*Saccharomyces cerevisiae*)

In extensive studies with the yeast *Saccharomyces cerevisiae*, various organelle-specific microautophagic pathways have been reported to accompany the vacuole invagination process, and the molecular machineries underlying the membrane dynamics have been revealed to the greatest extent beyond other experimental systems. The target organelles of these microautophagic pathways include the cytoplasm,[28] ER,[29] portions of the nucleus (piecemeal microautophagy of the nucleus, or PMN),[30] mitochondria,[31] and lipid droplets.[32,33] In addition, recent studies demonstrated that vacuolar membrane proteins are degraded through this type of microautophagy.[34,35] Below we summarize three aspects of the molecular machineries discovered in these yeast studies.

3.2.1. Dependency on Atg Gene Products

Several of the identified microautophagic pathways require *ATG* genes for the efficient process of target degradation. One pathway, characterized by tubular invagination of the vacuolar membrane under nitrogen-starvation conditions, was partially dependent on *ATG* genes (*ATG1, 3,4,5,8,* and *13*) as demonstrated by an in vitro analysis.[36] In this study the authors argued that *ATG* genes were indirectly involved in the microautophagic membrane dynamics, most likely through membrane fusion of the autophagosomes (formed by the *ATG* gene products) with the vacuolar membrane, which supplies a sufficient source of membrane required for the formation of the invagination. While PMN requires all the core *ATG* genes (*ATG1* through *18*)[37] and microautophagy of lipid droplets (microlipophagy) requires the core factors except Atg11,[33,38] none of these studies demonstrated the de novo synthesis of membrane structures targeting the nucleus or lipid droplets. Notably, a recent study on microlipophagy showed that the localization of Niemann-Pick Type C proteins (Ncr1 and Npc2), functional in sterol transport within the vacuole and necessary for the microlipophagy, were perturbed by loss of either of *ATG 1,2,3,5,7,8,* or *18*.[39] This finding also implies an indirect involvement of *ATG* genes, or macroautophagy, in the process of microautophagic membrane dynamics.

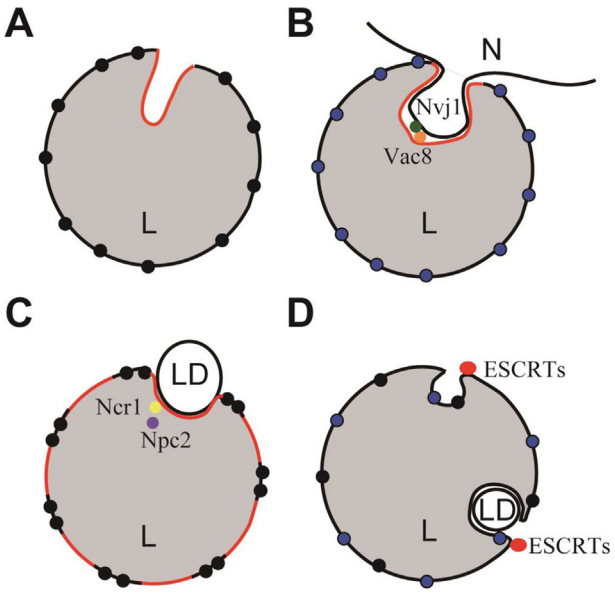

Figure 2. Type 2 microautophagy with lysosomal invagination. A) Tube-like invagination (in red) of the yeast vacuole formed under nitrogen-starved conditions contains few transmembrane proteins (illustrated as the black dots). L, Lysosome/vacuole. B) The nucleus-vacuole junction (shown in red), where nuclear Nvj1(in green) and vacuolar Vac8 (in orange) proteins tether the opposing organelle membranes, lacks a vacuolar transmembrane protein, Vph1 (illustrated as the blue dots). N, Nucleus. C) The vacuoles in the yeast cells under nutrient-starved conditions possess sterol-rich lipid microdomains (in red) that exclude most of the vacuolar transmembrane proteins (illustrated as the black dots). LD, Lipid Droplet. Ncr1 (shown in yellow, localized on the inner surface of the vacuole) and Npc2 (in purple, localized in the vacuolar lumen) support the development of the microdomains. D) ESCRT-driven microautophagy of LD and the vacuolar-membrane proteins (Vph1shown as the blue dots and others as the black dots). ESCRT proteins, illustrated as the red dots, are localized onto the vacuolar surface to exert the invagination.

3.2.2. Lipid Microdomain Formation

In any of the microautophagic pathways undergoing tubular invagination, piecemeal microautophagy of the nucleus, or microlipophagy under starvation conditions, the invaginated vacuolar-membrane part was found to form microdomain architecture (Figure 2). The microautophagic tubes, whose diameters range from 200 to 300 nm, were segregated from the rest of the vacuolar membrane by their neck (ring) parts, and contained very small amounts of transmembrane proteins[28] (Figure 2A). The vacuolar invagination during PMN occurs at the nucleus-vacuole junction formed by an interaction between nuclear Nvj1 and vacuolar Vac8 proteins.[40] This junction site was found to exclude the transmembrane subunit of the V-ATPase, Vph1[41] (Figure 2B). Furthermore, recent studies have shown that the microdomain of ordered-phase lipids (also called 'membrane rafts'), which is rich in ergosterol, occupied the vacuolar-membrane interface with lipid droplets during microlipophagy under nutrient-starvation conditions,[38,42] and the development of the microdomain depended on the sterol transporting Niemann-Pick Type C proteins[39] (Figure 2C). It is notable that in all of these pathways membranes undergo invaginations at the micrometer scale, and thus we can speculate that such huge invaginations must require lipid microdomain formation as a motive force to bend the membrane.

3.2.3. Dependency on the ESCRT Machinery

Microlipophagy requires ESCRT components, but not the core Atg proteins for the autophagosome formation, when it is induced either by inhibition of phosphatidylcholine biosynthesis[43] or by diauxic shift of the culture[34] (Figure 2D). Furthermore, microautophagy for the degradation of vacuolar membrane proteins utilizes the ESCRT machinery under different culture conditions.[34,35] These findings seem reasonable in light of the direct activity of the ESCRT machinery on membrane curvature and formation of luminal vesicles in endosomes, membrane dynamics that are similar to the invagination process of microautophagy. Yet we have to be aware of another possibility: that the blockade of the ESCRT machinery suppresses endocytic membrane transport to the vacuole, which alters the vacuolar compositions and inhibits microautophagy. Such an indirect involvement of the ESCRT machinery was proposed in studies of microautophagy of the ER[29] and microlipophagy induced under nitrogen-starvation conditions.[39] In the case of microautophagy for degradation of vacuolar membrane proteins, the components of the ESCRT machinery were localized on the vacuolar membrane at the time of microautophagy induction, strongly suggesting the direct

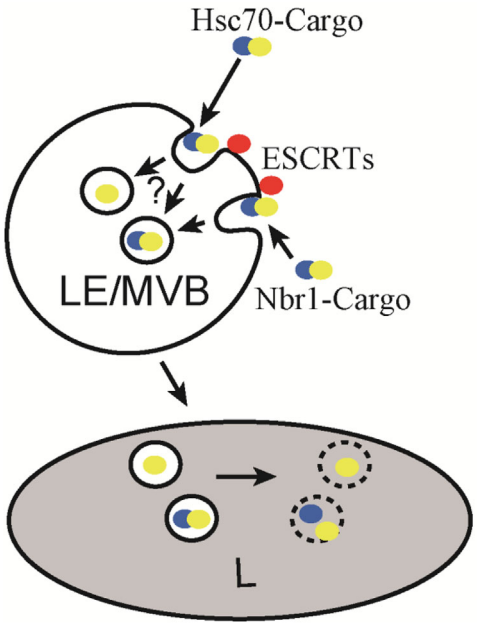

Figure 3. Type 3 microautophagy with endosomal invagination. ESCRT proteins (shown as the red dots) are responsible for the luminal vesicle formation. Adaptor proteins (Hsc70 and Nbr1) are shown in blue, and their cargos are illustrated with the yellow dots. It is unclear whether Hsc70 is incorporated into the luminal vesicles, while Nbr1 enters the vesicles and reaches the lysosome/vacuole (L) along with its cargos. LE/MVB, Late Endosome/MultiVesicular Body.

action of the ESCRT machinery in inducing membrane curvature and luminal vesicle formation[34,35] (Figure 2D). The endosomal luminal vesicles formed by the ESCRTs are less than 100 nm in diameter, and vesicles of similar size were detected in vacuoles undergoing microautophagy for degradation of vacuolar membrane proteins.[35] Notably, an in vitro study with the ESCRT components demonstrated the formation of vesicles with diameters of micrometer scale,[44] and hence it will be important to examine in future studies whether the ESCRTs directly generate vesicles of greater size from the vacuolar membrane in microlipophagy or other organelle-incorporating microautophagy. At this stage, it is still unclear whether the ESCRT-driven microautophagic processes accompany microdomain formation, yet the microautophagic degradation of vacuolar membrane proteins induced by diauxic shift seems to precede the microdomain formation and to be independent of sterol biosynthesis (Oku et al., unpublished data).

4. Type 3, Microautophagy with Endosomal Invagination

This type of microautophagy, termed endosomal microautophagy, was recently identified and studied with a murine dendritic cell line[8] and fruit fly (*Drosophila melanogaster*),[45,46] and a mechanically similar pathway functional in the transport of several vacuolar hydrolases was reported in the fission yeast *Schizosaccharomyces pombe*.[47] The membrane dynamics (invagination) rely on the endosomal membrane dynamics by the ESCRT machinery to generate intraluminal vesicles, called the multivesicular body (MVB) pathway. In addition, this type of microautophagy possesses several adaptor proteins for selective recruitment of cytosolic proteins onto the endosomal surface. Below we summarize the molecular functions of the two receptors, Nbr1 and Hsc70 (Figure 3).

4.1. Nbr1

In *S. pombe*, this protein was shown to recruit two vacuolar hydrolases (Ape2 and Lap2) onto the endosomal membrane, where these proteins were captured inside the luminal vesicles formed by the ESCRT machinery, leading to the biosynthetic transport of the hydrolases into the vacuole.[47] The mammalian counterpart of this protein has N-terminal PB1, C-terminal UBA domains, and LC3-Interracting regions, in addition to the zinc finger and FW (four W [tryptophan]) domains that are characteristic of Nbr1.[48] Extensive studies have demonstrated that mammalian Nbr1 is one of the main adaptor proteins operating selective macroautophagic pathways, owing to its affinity to LC3 (autophagosome-associating protein) and ubiquitin chain.[48] In contrast, *S. pombe* Nbr1 was co-localized with the ESCRT-0 complex components for efficient ubiquitination of the cargo protein on the endosomal surface, and indeed was ubiquitinated for transport into the vacuole.[47] While the targets of ESCRT-0-driven ubiquitination in the canonical MVB pathway are endocytosed membrane proteins, the ubiquitination of Nbr1 during microautophagy is unique in that the targets are cytoplasmic proteins.

4.2. Hsc70

This protein had originally been identified as a key factor in the chaperone-mediated autophagy, through interaction with the target proteins and with the lysosomal membrane protein LAMP-2A.[3] In the case of microautophagy, Hsc70 was shown to be recruited to late endosomes via electrostatic interaction between this protein and a phospholipid phosphatidylserine.[8] This molecular scheme of microautophagy is well conserved in *D. melanogaster*, although the organism seemed to lack chaperone-mediated autophagy.[46] Interestingly, the *D. melanogaster* Hsc70 responsible for the endosomal microautophagy, Hsc70-4, exhibited a membrane-deforming activity, independent from its chaperone activity.[45] This membrane-deforming activity is needed for microautophagy, synaptic protein turnover, and efficient neurotransmitter release activity, implying the physiological importance of microautophagy.[45]

5. Conclusions and Future Perspectives

Categorization of microautophagic pathways by the morphology (protrusion or invagination) and location (the lysosome/vacuole or the endosome) of membrane dynamics facilitates our understanding of the mechanistic scheme of microautophagy, and provides a better view of the dynamics than considering only the target proteins/organelles or selectivity of the microautophagic pathways. Notably, several molecular machineries span different types of microautophagy. Formation of membrane microdomains is a characteristic of both mammalian Type 1 microautophagy and yeast Type 2 microautophagy, and clearly, the ESCRT machinery is key to a subset of yeast Type 2 microautophagy as well as to Type 3. These points may hold primary importance for comprehensive elucidation of the microautophagy mechanism, and should also be considered in studying other types of microautophagy. In addition, we should pay careful attention to linkage between microautophagy and other membrane dynamics, especially the other types of autophagy pathways, since multiple factors such as Atg proteins and Hsc70 are shared in the processes.

Abbreviations

EM, electron microscopy; ER, endoplasmic reticulum; ESCRT, endosomal sorting complexes required for transport; MVB, multivesicular body; PMN, piecemeal microautophagy of the nucleus.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

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[1] N. Mizushima, M. Komatsu, *Cell* **2011**, *147*, 728.
[2] Z. Xie, D. J. Klionsky, *Nat. Cell Biol*. **2007**, *9*, 1102.
[3] S. Kaushik, A. M. Cuervo, *Trends Cell Biol*. **2012**, *22*, 407.
[4] D. Mijaljica, M. Prescott, R. J. Devenish, *Autophagy* **2011**, *7*, 673.
[5] C. De Duve, R. Wattiaux, *Annu. Rev. Physiol*. **1966**, *28*, 435.
[6] D. L. Tuttle, W. A. Dunn Jr, *J. Cell Sci* **1995**, *108*, 25.
[7] N. Kawamura, G. H. Sun-Wada, M. Aoyama, A. Harada, S. Takasuga, T. Sasaki, Y. Wada, *Nat. Comm*. **2012**, *3*, 1071.
[8] R. Sahu, S. Kaushik, C. C. Clement, E. S. Cannizzo, B. Scharf, A. Follenzi, I. Potolicchio, E. Nieves, A. M. Cuervo, L. Santambrogio, *Dev. Cell* **2011**, *20*, 131.
[9] M. Sakai, K. Ogawa, *Histochem*. **1982**, *76*, 479.
[10] T. Saito, K. Ogawa, *Acta Histochem. Cytochem*. **1974**, *7*, 1.
[11] G. E. Mortimore, N. J. Hutson, C. A. Surmacz, *Proc. Natl. Acad. Sci. USA* **1983**, *80*.
[12] E. J. de Waal, H. Vreeling-Sindelarova, J. P. Schellens, J. M. Houtkooper, J. James, *Cell Tissue Res*. **1986**, *243*, 641.
[13] L. Marzella, J. Ahlberg, H. Glaumann, *Exp. Cell Res*. **1980**, *129*, 460.
[14] G. E. Mortimore, B. R. Lardeux, C. E. Adams, *J. Biol. Chem*. **1988**, *263*, 2506.
[15] L. Yu, C. K. McPhee, L. Zheng, G. A. Mardones, Y. Rong, J. Peng, N. Mi, Y. Zhao, Z. Liu, F. Wan, D. W. Hailey, V. Oorschot, J. Klumperman, E. H. Baehrecke, M. J. Lenardo, *Nature* **2010**, *465*, 942.
[16] F. Dubouloz, O. Deloche, V. Wanke, E. Cameroni, C. De Virgilio, *Mol. Cell* **2005**, *19*, 15.
[17] A. Chanoca, N. Kovinich, B. Burkel, S. Stecha, A. Bohorquez-Restrepo, T. Ueda, K. W. Eliceiri, E. Grotewold, M. S. Otegui, *Plant Cell* **2015**, *27*, 2545.
[18] N. Mizushima, T. Noda, T. Yoshimori, Y. Tanaka, T. Ishii, M. D. George, D. J. Klionsky, M. Ohsumi, Y. Ohsumi, *Nature* **1998**, *395*, 395.
[19] M. Oku, Y. Sakai, *Biochim. Biophys. Acta* **2016**, *1863*, 992.
[20] Y. X. Wang, N. L. Catlett, L. S. Weisman, *J. Cell Biol*. **1998**, *140*, 1063.
[21] M. Oku, T. Nishimura, T. Hattori, Y. Ano, S. Yamashita, Y. Sakai, *Autophagy* **2006**, *2*, 272.
[22] J. Guan, P. E. Stromhaug, M. D. George, P. Habibzadegah-Tari, A. Bevan, W. A. Dunn, Jr., D. J. Klionsky, *Mol. Biol. Cell* **2001**, *12*, 3821.
[23] N. Tamura, M. Oku, M. Ito, N. N. Noda, F. Inagaki, Y. Sakai, *J. Cell Biol*. **2013**, *202*, 85.
[24] H. Mukaiyama, M. Baba, M. Osumi, S. Aoyagi, N. Kato, Y. Ohsumi, Y. Sakai, *Mol. Biol. Cell* **2004**, *15*, 58.
[25] H. Mukaiyama, M. Oku, M. Baba, T. Samizo, A. T. Hammond, B. S. Glick, N. Kato, Y. Sakai, *Genes Cells* **2002**, *7*, 75.
[26] N. Tamura, M. Oku, Y. Sakai, *J. Cell Sci*. **2010**, *123*, 4107.
[27] Y. Ano, T. Hattori, M. Oku, H. Mukaiyama, M. Baba, Y. Ohsumi, N. Kato, Y. Sakai, *Mol. Biol. Cell* **2005**, *16*, 446.
[28] O. Muller, T. Sattler, M. Flotenmeyer, H. Schwarz, H. Plattner, A. Mayer, *J. Cell Biol*. **2000**, *151*, 519.
[29] S. Schuck, C. M. Gallagher, P. Walter, *J. Cell Sci*. **2014**, *127*, 4078.
[30] P. Roberts, S. Moshitch-Moshkovitz, E. Kvam, E. O'Toole, M. Winey, D. S. Goldfarb, *Mol. Biol. Cell* **2003**, *14*, 129.
[31] I. Kissova, B. Salin, J. Schaeffer, S. Bhatia, S. Manon, N. Camougrand, *Autophagy* **2007**, *3*, 329.
[32] C. H. Moeller, W. W. Thomson, *J. Ultrastruct. Res*. **1979**, *68*, 38.
[33] T. van Zutphen, V. Todde, R. de Boer, M. Kreim, H. F. Hofbauer, H. Wolinski, M. Veenhuis, I. J. van der Klei, S. D. Kohlwein, *Mol. Biol. Cell* **2014**, *25*, 290.
[34] M. Oku, Y. Maeda, Y. Kagohashi, T. Kondo, M. Yamada, T. Fujimoto, Y. Sakai, *J. Cell Biol*. **2017**, *216*, 3263.
[35] L. Zhu, J. R. Jorgensen, M. Li, Y. S. Chuang, S. D. Emr, *eLife* **2017**, *6*, e26403.
[36] T. Sattler, A. Mayer, *J. Cell Biol*. **2000**, *151*, 529.
[37] R. Krick, Y. Muehe, T. Prick, S. Bremer, P. Schlotterhose, E. L. Eskelinen, J. Millen, D. S. Goldfarb, M. Thumm, *Mol. Biol. Cell* **2008**, *19*, 4492.
[38] C. W. Wang, Y. H. Miao, Y. S. Chang, *J. Cell Biol*. **2014**, *206*, 357.
[39] T. Tsuji, M. Fujimoto, T. Tatematsu, J. Cheng, M. Orii, S. Takatori, T. Fujimoto, *eLife* **2017**, *6*, e25960.
[40] E. Kvam, D. S. Goldfarb, *J. Cell Sci*. **2004**, *117*, 4959.
[41] R. Dawaliby, A. Mayer, *Mol. Biol. Cell* **2010**, *21*, 4173.
[42] A. Y. Seo, P. W. Lau, D. Feliciano, P. Sengupta, M. A. L. Gros, B. Cinquin, C. A. Larabell, J. Lippincott-Schwartz, *eLife* **2017**, *6*, e21690.
[43] J. D. Vevea, E. J. Garcia, R. B. Chan, B. Zhou, M. Schultz, G. Di Paolo, J. M. McCaffery, L. A. Pon, *Dev. Cell* **2015**, *35*, 584.
[44] T. Wollert, J. H. Hurley, *Nature* **2010**, *464*, 864.
[45] V. Uytterhoeven, E. Lauwers, I. Maes, K. Miskiewicz, M. N. Melo, J. Swerts, S. Kuenen, R. Wittocx, N. Corthout, S. J. Marrink, S. Munck, P. Verstreken, *Neuron* **2015**, *88*, 735.
[46] A. Mukherjee, B. Patel, H. Koga, A. M. Cuervo, A. Jenny, *Autophagy* **2016**, *12*.
[47] X. M. Liu, L. L. Sun, W. Hu, Y. H. Ding, M. Q. Dong, L. L. Du, *Mol. Cell* **2015**, *59*, 1035.
[48] T. Johansen, T. Lamark, *Autophagy* **2011**, *7*, 279.