

Microautophagy – distinct molecular mechanisms handle cargoes of many sizes

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ABSTRACT

Autophagy is fundamental for cell and organismal health. Two types of autophagy are conserved in eukaryotes: macroautophagy and microautophagy. During macroautophagy, autophagosomes deliver cytoplasmic constituents to endosomes or lysosomes, whereas during microautophagy lytic organelles take up cytoplasm directly. While macroautophagy has been investigated extensively, microautophagy has received much less attention. Nonetheless, it has become clear that microautophagy has a broad range of functions in biosynthetic transport, metabolic adaptation, organelle remodeling and quality control. This Review discusses the selective and non-selective microautophagic processes known in yeast, plants and animals. Based on the molecular mechanisms for the uptake of microautophagic cargo into lytic organelles, I propose to distinguish between fission-type microautophagy, which depends on ESCRT proteins, and fusion-type microautophagy, which requires the core autophagy machinery and SNARE proteins. Many questions remain to be explored, but the functional versatility and mechanistic diversity of microautophagy are beginning to emerge.

KEY WORDS: Microautophagy, Membrane fission, Membrane fusion, ESCRT machinery, SNAREs, Core autophagy machinery

Introduction

“What’s in a name? That which we call a rose by any other name would smell as sweet!” cries Shakespeare’s Juliet as she implores Romeo to renounce his name so that the two star-crossed lovers can escape the violent rivalry of their families. Nowadays, this quote is used to convey that the nature of a thing is unaffected by the name we attach to it. This Review highlights that microautophagy, despite an ill-fitting name, is a fascinating collection of mechanistically distinct cellular processes.

Autophagy is the transport of cytoplasmic constituents into lysosomes. Autophagic cargoes include cytosolic proteins, membrane-enclosed organelles, parts of the nucleus and even lysosomes themselves. In contrast, extracellular material and plasma membrane components are delivered into lysosomes by endocytosis. Owing to its broad scope, autophagy has many functions, for example in metabolic adaptation, cell homeostasis, differentiation and immunity, and is relevant for numerous human diseases (Levine and Kroemer, 2019; Morishita and Mizushima, 2019).

Two types of autophagy exist in nearly all eukaryotes: macroautophagy and microautophagy (Fig. 1). Both processes

separate autophagic cargo from the remaining cytoplasm by membranes. To understand the membrane rearrangements involved, it is helpful to differentiate between morphological and topological transformations. Morphological transformations are changes in membrane shape through bending, whereas topological transformations are changes in membrane connectivity through fission and fusion (Knorr et al., 2017). During macroautophagy, a phagophore is generated, which is a vesicle consisting of one continuous membrane. Phagophore biogenesis requires a set of proteins called the core autophagy machinery (Mizushima et al., 2011; Wen and Klionsky, 2016). The phagophore then undergoes a morphological transformation into a cup-shaped structure that engulfs a portion of the cytoplasm. Next, the phagophore closes to become an autophagosome, which consists of two separate inner and outer membranes. Conversion of a one-membrane phagophore into a two-membrane autophagosome requires the topological transformation of membrane fission (Knorr et al., 2015). Phagophore maturation involves so-called endosomal sorting complex required for transport (ESCRT) proteins (Spitzer et al., 2015; Takahashi et al., 2018), which mediate membrane budding and scission in many cellular processes. The next topological transformation is fusion of the outer autophagosomal membrane with an endosome or lysosome to generate a hybrid lytic organelle. This step depends on SNARE proteins, the main mediators of membrane fusion (Yim and Mizushima, 2020). As a result, a one-membrane vesicle called a macroautophagic body is released into the lytic organelle and degraded. In contrast, microautophagy does not involve autophagosomes as transport intermediates. Instead, an endosome or lysosome directly engulfs and takes up autophagic cargo. In a morphological transformation, its membrane first invaginates and forms a bud that sequesters cytoplasmic material. In a subsequent topological transformation, the invaginated membrane pinches off into the organelle lumen as a microautophagic body. Detachment of the bud from the limiting organelle membrane is topologically equivalent to phagophore closure and requires membrane fission.

The following discussion is divided into four parts. The first summarizes the history of research on microautophagy, the second provides an inventory of microautophagic processes, the third introduces a new classification of microautophagic mechanisms, and the fourth outlines the physiological roles of microautophagy.

The history of microautophagy research

The term microautophagy was invented by Christian de Duve, the discoverer of the lysosome. When discussing how multivesicular lysosomes might form, he suggested “internalization by ‘microautophagy’ of small cytoplasmic buds in shrinking lysosomes” as one hypothetical possibility (de Duve and Wattiaux, 1966). Ultrastructural observations in mammals and yeast in the 1970s indeed revealed import of cytoplasm into lysosomes by inward budding of the lysosomal membrane (Saito and Ogawa,

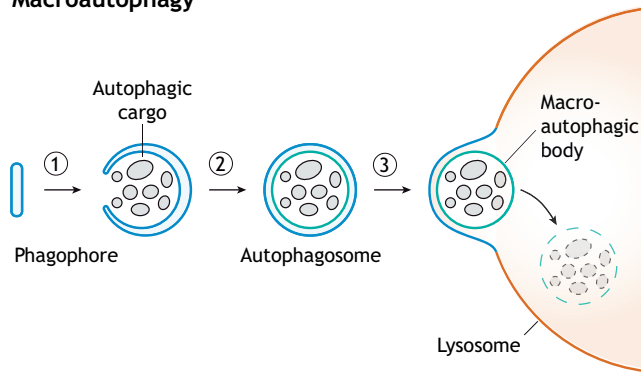
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A Macroautophagy



B Microautophagy

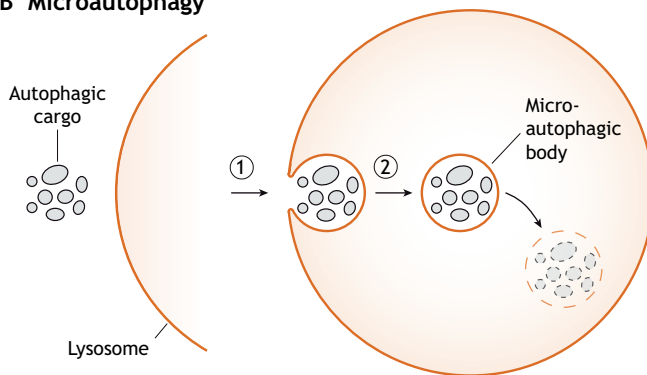


Fig. 1. Membrane transformations during macroautophagy and microautophagy. The lysosome is depicted here as being much larger than an autophagosome, as is the case in yeasts and plants, but not mammals. (A) During macroautophagy, a phagophore forms, expands and bends into a cup-shaped structure that engulfs autophagic cargo (1). The one-membrane phagophore closes through membrane fission to become a two-membrane autophagosome (2). The outer autophagosomal membrane then fuses with the lysosome (3) to release a macroautophagic body. (B) During microautophagy, the lysosomal membrane invaginates to sequester autophagic cargo (1). Membrane fission releases a microautophagic body (2).

1974; Moeller and Thomson, 1979b). Compelling evidence for microautophagy was then provided from studies of isolated rat liver lysosomes, which took up and degraded soluble proteins by invagination, followed by the generation and breakdown of intralysosomal vesicles (Marzella et al., 1980; Ahlberg and Glaumann, 1985).

After the initial characterization of microautophagy in mammals in the 1980s, much of the progress in the next two decades came from research in yeast. These investigations uncovered microautophagy of peroxisomes (Tuttle et al., 1993; Sakai et al., 1998), cytosol (Chiang et al., 1996; Müller et al., 2000), mitochondria (Campbell and Thorsness, 1998), parts of the nucleus (Roberts et al., 2003), lipid droplets (van Zutphen et al., 2014), endoplasmic reticulum (ER) (Schuck et al., 2014), certain cytosolic enzymes (Liu et al., 2015) and vacuole membrane proteins (Yang et al., 2020). Many of these cargoes are large structures. Therefore, the term ‘micro’ autophagy is a misnomer, unless it is taken to mean that the process can handle micrometer-sized cargo. Moreover, the studies in yeast showed that microautophagy can target virtually any cellular structure, implying that it has a variety of functions.

In the 2010s, further microautophagic processes were discovered, including microautophagy of certain cytosolic proteins and ER in animals (Sahu et al., 2011; Omari et al., 2018; Loi et al., 2019), and of pigment aggregates and chloroplasts in plants (Chanoca et al., 2015;

Nakamura et al., 2018). This development resembles the history of research on macroautophagy. Macroautophagy was also first found in mammals, but progress was slow until breakthroughs in yeast sparked a rapidly growing interest (Ohsumi, 2014). Many microautophagic processes have now been described. Their mechanisms differ profoundly, suggesting that the term microautophagy, as currently used, conflates distinct types of autophagy.

An inventory of microautophagic processes

This section covers those microautophagic processes for which at least some molecular machinery has been identified (Table 1 and Fig. 2). The discussion focusses on the core autophagy machinery, ESCRTs and SNAREs, which are all conserved in eukaryotes. Information on additional factors is provided in Table S1. Both macroautophagy and microautophagy can selectively degrade certain organelles. A straightforward nomenclature for these autophagic processes is macro/micro-X-phagy, with X indicating the target organelle. Microautophagic processes that do not target particular organelles are collected under the umbrella term ‘general microautophagy’. Nevertheless, these processes can also be selective.

Yeast

General microautophagy

The yeast equivalent of lysosomes is the vacuole. Its diameter in proliferating *Saccharomyces cerevisiae* is $\sim 2 \mu\text{m}$, or four times larger than the diameter of a typical lysosome in mammals. Hence, the volumes of yeast vacuoles and mammalian lysosomes differ by nearly two orders of magnitude (Klionsky and Eskelinen, 2014). During starvation, the vacuole invaginates and produces microautophagic bodies up to 500 nm in diameter (Oku et al., 2017). Microautophagic bodies frequently contain lipid droplets (LDs), which may result from selective microautophagy of LDs (see below) or reflect a spatial preference due to the proximity of LDs and the vacuole (van Zutphen et al., 2014). General microautophagy does not need the core autophagy machinery but depends on ESCRTs (Oku et al., 2017; Iwama and Ohsumi, 2019). The ESCRT machinery is best understood in the context of multivesicular body (MVB) formation in the endocytic pathway. The ESCRT-0 complex recognizes ubiquitylated cargo on endosomes, such as endocytosed plasma membrane proteins, and recruits ESCRT-I, ESCRT-II and ESCRT-III complexes. Multimeric ESCRT-III assemblies, together with the AAA-ATPase Vps4, remodel endosomes to generate intraluminal vesicles (Adell et al., 2017; McCullough et al., 2018; Vietri et al., 2020). MVB formation and microautophagy share many similarities and are topologically equivalent. Intraluminal vesicles in yeast MVBs are $\sim 25 \text{ nm}$ in diameter, but ESCRTs can also constrict much wider membrane necks, for example during mammalian cytokinesis (Vietri et al., 2020), and can therefore produce large microautophagic bodies. The first insight into how ESCRTs are differentially deployed for MVB formation and microautophagy has recently been obtained. Starvation inhibits TOR kinase, leading to dephosphorylation of the ESCRT-0 component Vps27, recruitment of the ESCRT-0 complex to the vacuole and initiation of microautophagy (Hatakeyama et al., 2019; Morshed et al., 2020). This change in Vps27 phosphorylation does not affect ESCRT activity at MVBs and specifically turns on microautophagy (Hatakeyama and De Virgilio, 2019).

Starvation also induces microautophagic bodies whose generation requires similar machinery but whose size is closer to that of intraluminal vesicles in MVBs. The autophagic cargo in this case consists of vacuole membrane proteins that are tagged with ubiquitin for recognition by ESCRTs and are degraded to remodel

Table 1. Overview of microautophagic processes

Organism	Process	Machinery required			Classification
		ATG	ESCRT	SNARE	
Yeast	General microautophagy	No	Yes	No	Fission type
	Micro-ER-phagy	No	Yes	n.t.	Fission type
	Micropexophagy	Yes	n.t.	n.t.	Fusion type
	Micronucleophagy	Yes	n.t.	Yes ¹	Fusion type
	Micromitophagy	Yes	n.t.	Yes ¹	Fusion type?
	Microlipophagy	Yes ¹	Yes ¹	n.t.	?
Plants	General microautophagy	No	n.t.	No	?
	Microchlorophagy	Yes	n.t.	n.t.	Fusion type?
Animals	General microautophagy	No ²	Yes	n.t.	Fission type
	Micro-ER-phagy	No ²	Yes	No	Fission type

¹indirect role possible; ²some ATG components needed for cargo selection. ATG, core autophagy machinery; n.t., not tested.

the vacuole (Zhu et al., 2017; Yang et al., 2020). Thus, these small microautophagic bodies carry out selective degradation of membrane proteins, and their high surface-to-volume ratio may help to minimize the off-target destruction of soluble proteins. Intriguingly, small microautophagic bodies sometimes appear to be connected (Li et al., 2019). Concatenated intraluminal vesicles are normally rare in yeast but are abundant in plant endosomes (Buono et al., 2017). It is possible that their formation is induced under certain conditions.

In addition to the vacuole invaginations described so far, peculiar structures called autophagic tubes can form in starving *S. cerevisiae* (Müller et al., 2000). These autophagic tubes extend deep into the lumen of the vacuole; they are often branched, contain few membrane proteins in their distal parts and release microautophagic bodies from their tips. Whether autophagic tube formation involves ESCRTs has not been tested. It is reduced, however, upon disruption of the core autophagy machinery (Müller et al., 2000; Sattler and Mayer, 2000). This finding suggests an indirect role of the core autophagy machinery, possibly resulting from an interplay between macroautophagy and microautophagy in maintaining vacuole membrane homeostasis (Müller et al., 2000). Finally, constitutive ESCRT-dependent microautophagy delivers newly synthesized hydrolases from the cytosol into endosomes in *Schizosaccharomyces pombe* (Liu et al., 2015). The autophagy receptor Nbr1, which has homologs in *S. cerevisiae* and mammals, ensures selectivity by linking cargo to the ESCRT machinery. This pathway is also independent of the core autophagy machinery.

In summary, general microautophagy can non-selectively or selectively degrade cytoplasm and vacuole membrane proteins. The only topological transformation required for general microautophagy is membrane fission, which is mediated by ESCRTs. The core autophagy machinery is not needed, and SNAREs were found not to have a direct role whenever the requirement for them was tested rigorously (Sattler and Mayer, 2000; Zhu et al., 2017). The diverse morphologies of vacuole membrane invaginations and microautophagic bodies likely influence the efficiency, capacity and selectivity of microautophagy. Understanding how these morphologies arise is a key challenge.

Micro-ER-phagy

Parts of the ER are degraded by selective micro-ER-phagy during ER protein folding stress. Micro-ER-phagy involves the conversion of stacked ER sheets into spherical whorls with diameters of ~500 nm (Schuck et al., 2014). Microautophagy of these whorls does not require the core autophagy machinery but depends on ESCRTs for vacuole membrane scission (Schäfer et al., 2020).

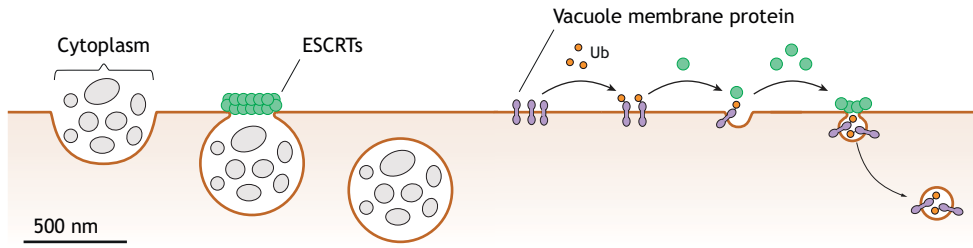
Thus, the mechanisms of micro-ER-phagy and general microautophagy appear to be similar. ER whorls are depleted of many transmembrane proteins, so that micro-ER-phagy entails the selective degradation of special ER subdomains (Schäfer et al., 2020). How cells recognize whorls for degradation is unknown.

Micropexophagy

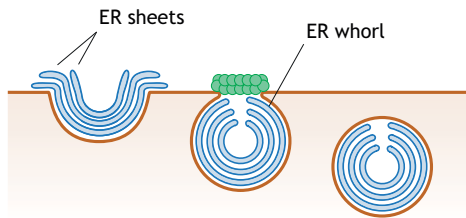
The yeast *Pichia pastoris* develops large peroxisomes during growth on methanol. When glucose becomes available, cells initiate selective microautophagy of peroxisome clusters, whose diameters can exceed 1 µm (Tuttle et al., 1993). Micropexophagy differs fundamentally from general microautophagy, possibly to accommodate an unusually large cargo. First, peroxisomes are not taken up into simple vacuole invaginations, but vacuole extensions form around peroxisome clusters (Tuttle et al., 1993). Second, these extensions are sealed by a peroxisome-associated, phagophore-like structure called the micropexophagy-specific membrane apparatus (MIPA) (Mukaiyama et al., 2004). Micropexophagy requires the core autophagy machinery (Strømhaug et al., 2001; Mukaiyama et al., 2002). This machinery is modular; the Atg1 kinase complex and the Atg14-containing phosphatidylinositol 3-kinase (PI3K) complex initiate phagophore assembly, Atg9-containing vesicles and the Atg2–Atg18 complex supply lipids to phagophores, and two conjugation systems link Atg8 to phosphatidylethanolamine (PE) in the phagophore membrane. Lipidated Atg8 promotes phagophore expansion and binds to autophagy receptors to include selected cargoes into autophagosomes (Xie et al., 2008; Farré and Subramani, 2016). During micropexophagy, core autophagy proteins help to produce vacuole extensions (Guan et al., 2001; Chang et al., 2005). The building blocks for these extensions are vacuole fragments that are generated by Atg18-mediated membrane scission (Mukaiyama et al., 2002; Tamura et al., 2013). Subsequent fusion of the fragments requires the vacuole membrane protein Vac8 (Fry et al., 2006; Oku et al., 2006), which also has a general role in SNARE-mediated homotypic vacuole fusion (Wang et al., 2001). The MIPA forms at the side of peroxisome clusters facing away from the vacuole and then appears to fuse with vacuole extensions to complete the enclosure of peroxisome clusters (Mukaiyama et al., 2004). Cargo selectivity is ensured by the autophagy receptor Atg30, which binds to peroxisomes, the scaffold protein Atg11 on vacuole extensions, and Atg8 on the MIPA (Farré et al., 2008, 2013). Thus, micropexophagy achieves selectivity in a manner similar to various macroautophagic processes (Farré and Subramani, 2016).

Overall, micropexophagy in *P. pastoris* requires the core autophagy machinery for vacuole membrane remodeling and MIPA formation. The process involves homotypic fusion to create

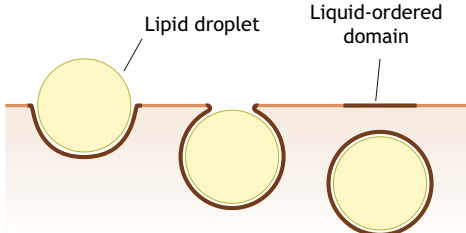
General microautophagy



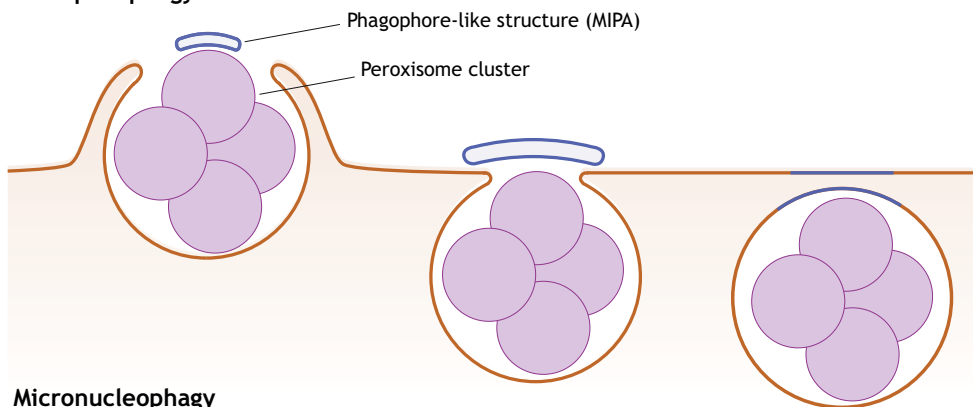
Micro-ER-phagy



Microlipophagy



Micropexophagy



Micronucleophagy

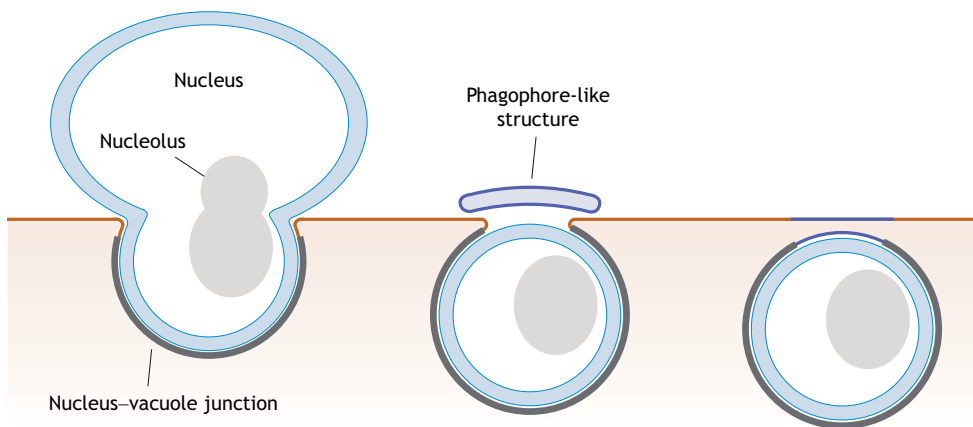


Fig. 2. Microautophagic processes in yeast. The vacuole membrane is shown as a flat surface. Other organelles are drawn approximately to scale to illustrate the relative sizes of different cargoes. General microautophagy involves ESCRT-dependent generation of large microautophagic bodies containing cytoplasm or small microautophagic bodies containing vacuole membrane proteins tagged with ubiquitin (Ub). Micro-ER-phagy entails ESCRT-dependent vacuolar uptake of ER whorls that arise from stacked ER sheets. Microlipophagy takes place at liquid-ordered membrane domains, but the mechanism for vacuolar uptake of lipid droplets is unclear. Micropexophagy of peroxisome clusters occurs by means of a phagophore-like structure called the micropexophagy-specific membrane apparatus (MIPA). Micronucleophagy of portions of the nucleus is mediated by the nucleus–vacuole junction, preferentially targets nucleolar components and, as is the case for micropexophagy, involves a phagophore-like structure. Micronucleophagic bodies consist of three membranes, which are derived from the inner nuclear membrane, the outer nuclear membrane and the vacuole membrane. The mechanism of micromitophagy is uncertain, and this process is therefore not depicted.

vacuole extensions and heterotypic fusion of the MIPA with the vacuole. A requirement for SNAREs in either fusion event has yet to be shown but is implied by the function of Vac8 in the formation of vacuole extensions. A role for ESCRTs in micropexophagy remains to be tested directly but appears unlikely because genes encoding ESCRTs are non-essential and should have surfaced during the extensive screens for micropexophagy genes (Strømhaug et al., 2001; Mukaiyama et al., 2002).

Micronucleophagy

Piecemeal microautophagy of the nucleus requires the nucleus–vacuole junction (NVJ), an organelle contact site formed by an interaction between Vac8 and the nuclear envelope protein Nvj1 (Pan et al., 2000). Upon starvation, the NVJ expands, buds towards the vacuole lumen and gives rise to a complex microautophagic body that is ~1 μm in diameter and consists of three membranes derived from the inner nuclear membrane, the outer nuclear

membrane and the vacuole membrane (Roberts et al., 2003). Micronucleophagy requires the core autophagy machinery and involves an intermediate stage at which detached nuclear fragments are contained within open vacuole invaginations (Krick et al., 2008). Atg8 accumulates at the necks of these invaginations, indicating the presence of a phagophore-related structure similar to the MIPA. In addition, micronucleophagy requires Atg11 and the autophagy receptor Atg39 (Otto and Thumm, 2020). Thus, micronucleophagy is mechanistically related to micropexophagy. SNAREs may mediate fusion of the phagophore-like structure with the vacuole, and micronucleophagy indeed requires vacuolar SNAREs (Krick et al., 2008). However, it has not been ruled out that this is an indirect consequence of vacuole fragmentation in SNARE mutants. Micronucleophagy selectively occurs at the NVJ. In addition, it preferentially targets the nucleolus, but spares nucleolus-embedded rDNA, and thus has sub-organelle specificity (Mostofa et al., 2019).

Micromitophagy

When *S. cerevisiae* is grown under respiratory conditions to induce mitochondrial biogenesis and is then starved of nitrogen, mitochondria are degraded predominantly by micromitophagy, rather than macromitophagy. This microautophagic process depends on the core autophagy machinery (Kiššová et al., 2007). Atg8 forms clusters on or near mitochondria under these conditions, and it has been proposed that Atg8 is anchored in the mitochondrial outer membrane (Vigié et al., 2019). A speculative alternative idea would be that these Atg8 clusters reflect the formation of a mitochondria-associated membrane structure analogous to the MIPA. The selectivity of micromitophagy appears to be provided by Atg32, which, under different conditions, serves as a receptor for macromitophagy (Fukuda and Kanki, 2018; Vigié et al., 2019).

Microlipophagy

Yeast LDs are ~500 nm in diameter and are degraded by microlipophagy during prolonged starvation. Remarkable early studies showed large-scale lipid domain segregation in the vacuole membrane of quiescent *S. cerevisiae* (Moeller and Thomson, 1979a; Moeller et al., 1981). This segregation yields coexisting liquid-ordered and liquid-disordered domains (Toulmay and Prinz, 2013). Liquid-ordered domains are rich in sterols and are the sites of microlipophagy (Moeller and Thomson, 1979b; Wang et al., 2014). Line tension at the interface of two immiscible lipid domains can drive so-called domain-induced budding. In addition, adhesion of particles to membranes can change their spontaneous curvature and promote budding (Lipowsky, 2014). Association of LDs with vacuolar liquid-ordered domains could thus facilitate budding, although presumably not fission. Microlipophagy requires the core autophagy machinery, ESCRTs, sterol transporters and vacuolar proteases. All of these factors contribute to proper lipid domain organization and could affect microlipophagy indirectly (van Zutphen et al., 2014; Toulmay and Prinz, 2013; Wang et al., 2014; Tsuji et al., 2017). For example, the autophagy receptor Atg32 is needed for microlipophagy (Wang et al., 2014), but how this integral mitochondrial membrane protein could directly participate in LD uptake into the vacuole is difficult to imagine. In contrast, the Atg6 and Atg14 subunits of the autophagy-specific PI3K complex associate with vacuolar liquid-ordered domains and may have direct roles (Wang et al., 2014; Seo et al., 2017). In other studies, microlipophagy was found not to require the core autophagy machinery; it may therefore be needed only under certain conditions (Oku et al., 2017; Iwama and Ohsumi, 2019).

Alternatively, vacuolar uptake of LDs may have resulted from non-selective general microautophagy (see above). Finally, how the selectivity of microlipophagy is achieved is unknown.

Plants

The diameter of plant vacuoles can exceed 10 μm . Microautophagy in plants has been reviewed recently (Siefko et al., 2020), and only the two best-characterized examples are presented here. First, pigment aggregates are transported from the cytosol into the vacuole by microautophagy in *Arabidopsis thaliana* seedlings. This process involves engulfment of pigment aggregates by elaborate vacuole extensions. Furthermore, it does not require the core autophagy machinery or vacuolar SNAREs (Chanoca et al., 2015). These requirements are congruent with those of general microautophagy in yeast, but the possible roles of ESCRTs have not been tested. Second, intense light induces microautophagy of photodamaged chloroplasts in *A. thaliana* leaves. This process relies on the core autophagy machinery and may involve a phagophore-like structure analogous to the MIPA (Nakamura et al., 2018; Nakamura and Izumi, 2019). Thus, microchlorophagy may resemble micropexophagy in yeast.

Animals

The complexity of the endocytic system in mammals often blurs the distinction between late endosomes and lysosomes. In addition, typical late endosomes and lysosomes in mammals both have diameters of around 500 nm and are considerably smaller than autophagosomes (Klionsky and Eskelinen, 2014). This makes microautophagy more difficult to detect than macroautophagy and also places constraints on the size of microautophagic cargo.

General microautophagy

Amino acid deprivation in human cells rapidly triggers the degradation of certain cytosolic proteins via microautophagy at endosomes (Mejlvang et al., 2018). Their uptake requires ESCRT-III and VPS4, but not ESCRT-0, ESCRT-I and ESCRT-II. Hence, the mechanism of ESCRT-III recruitment in this case is distinct from that during regular MVB biogenesis. Interestingly, the most efficiently degraded proteins include receptors for selective macroautophagy, suggesting that this fast microautophagic response shapes the subsequent macroautophagic response. Degradation is unaffected by inhibition of ULK1 (Atg1 in yeast) or PI3K. Moreover, degradation of some target proteins is insensitive to the removal of ATG5 or ATG7, showing that general microautophagy is, in principle, independent of the core autophagy machinery. Nevertheless, a subset of target proteins requires lipidated LC3 family proteins (hereafter denoted LC3; Atg8 in yeast) for their recruitment to endosomes. Hence, LC3 here does not act in phagophore biogenesis but retains a role in selective cargo capture. Finally, syntaxin-17, a SNARE protein important for autophagosome fusion with lysosomes, is not needed, nor is the Hsc70 chaperone that is discussed below (Mejlvang et al., 2018).

A related variant of general microautophagy, termed endosomal microautophagy, constitutively takes up cytosolic proteins into late endosomes in murine dendritic cells (Sahu et al., 2011). This process requires ESCRTs but not the core autophagy machinery. Here, selectivity is provided by Hsc70 (also known as HSPA8), which recognizes proteins with KFERQ motifs and binds to endosomes via phosphatidylserine (Sahu et al., 2011). Of note, the recognition of KFERQ motifs by Hsc70 also is a key step in chaperone-mediated autophagy, a distinct type of autophagy in animals that involves the lysosomal import of unfolded proteins by a

membrane-embedded translocation complex (Tekirdag and Cuervo, 2017). However, Hsc70 is more than a cargo receptor because it can bend membranes, and this capacity is critical for ESCRT-mediated microautophagy in fly synapses (Uytterhoeven et al., 2015). Hsc70-dependent microautophagy occurs constitutively but is induced, at least in the fly fat body, by long-term starvation, oxidative and genotoxic stress. It therefore likely has multiple roles in cellular damage control (Mukherjee et al., 2016; Mesquita et al., 2020).

Micro-ER-phagy

ER exit sites containing misfolded procollagen and core autophagy proteins can be directly taken up by lysosomes in mouse osteoblasts (Omari et al., 2018). Furthermore, mouse fibroblasts recovering from ER stress send ER-derived vesicles to late endosomes or lysosomes for microautophagic degradation (Loi et al., 2019). This cargo delivery requires ESCRT-III, VPS4 and the LC3/Atg8 lipidation system but not other components of the core autophagy machinery, including ULK/Atg1 and PI3K. The SNAREs syntaxin-17 and VAMP8 are also dispensable (Loi et al., 2019). In a theme familiar from starvation-induced general microautophagy (Mejlvang et al., 2018), LC3 is needed for cargo capture by binding to the ER protein SEC62 (Fumagalli et al., 2016). Intriguingly, LC3 is found on the ER-derived vesicles (Loi et al., 2019). Perhaps LC3 becomes lipidated and anchored in the membrane of the lytic organelle just before microautophagic cargo uptake occurs.

Others

Cells of the visceral endoderm in mouse embryos contain unusually large lysosomes that internalize early endosomes through selective microautophagy (Kawamura et al., 2012). Furthermore, there are indications of piecemeal micromitophagy (Yogalingam et al., 2013). Beyond these processes, alternative ways for microautophagy of large cargoes in animals can be envisaged. Lysosomes fuse with endosomes as well as each other (Ward et al., 1997; Luzio et al.,

2014), and this may generate lytic organelles large enough to accommodate unwieldy cargoes. Autophagosome fusion with lysosomes creates autolysosomes, which would also have the requisite size. Structures analogous to the MIPA in yeast have not been observed in animals so far.

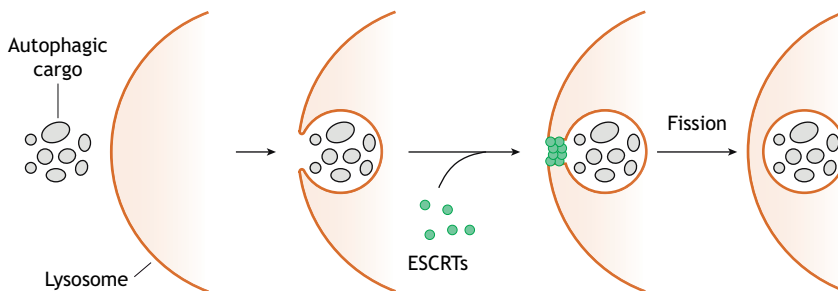
A classification of microautophagic processes

The diversity of microautophagic processes raises the question of how many truly distinct types of microautophagy there are. Classifications can be based on the site of microautophagy (endosomes versus lysosomes) or the morphology of uptake intermediates (membrane invaginations versus extensions) (Oku and Sakai, 2018). Considering the new information gathered in the past few years, I propose a different classification based on the molecular mechanism of cargo uptake. The locations and morphologies of cellular events are ultimately determined by the underlying molecular mechanisms, and these mechanisms should, whenever possible, form the basis for defining biological processes. This classification distinguishes two principal types of microautophagy: fission-type and fusion-type microautophagy (Table 1 and Fig. 3).

Fission-type microautophagy occurs by invagination and fission of endosomal or lysosomal membranes (Fig. 3A). It requires ESCRTs, likely for membrane scission, but possibly also for membrane budding (Schäfer et al., 2020). The generation of new membrane structures by the core autophagy machinery or membrane fusion by SNAREs are not needed. Processes in this class are general microautophagy and micro-ER-phagy in yeast and animals. Selectivity is achieved by direct binding of ubiquitylated cargo to ESCRTs, or by factors such as Nbr1, LC3 and Hsc70, which recognize cargo and recruit it to uptake sites by binding to ESCRTs or membranes.

Fusion-type microautophagy produces microautophagic bodies by a different mechanism – membrane invaginations or extensions are generated and then sealed by fusion with phagophore-like

A Fission-type microautophagy



B Fusion-type microautophagy

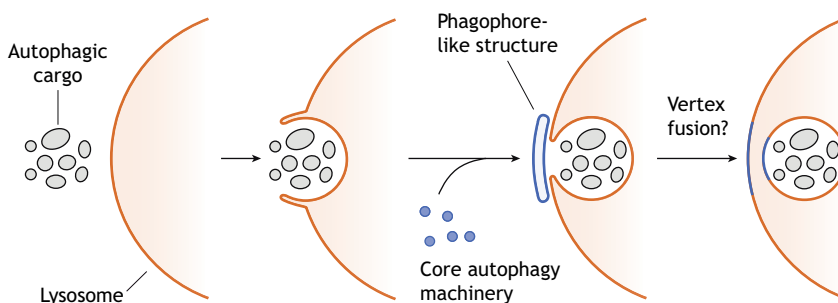


Fig. 3. Principal types of microautophagy. (A) Fission-type microautophagy requires ESCRT proteins for fission of the lysosomal membrane. (B) Fusion-type microautophagy requires the core autophagy machinery for the biogenesis of a phagophore-like structure. This structure seals lysosomal membrane invaginations, possibly by SNARE-mediated vertex fusion.

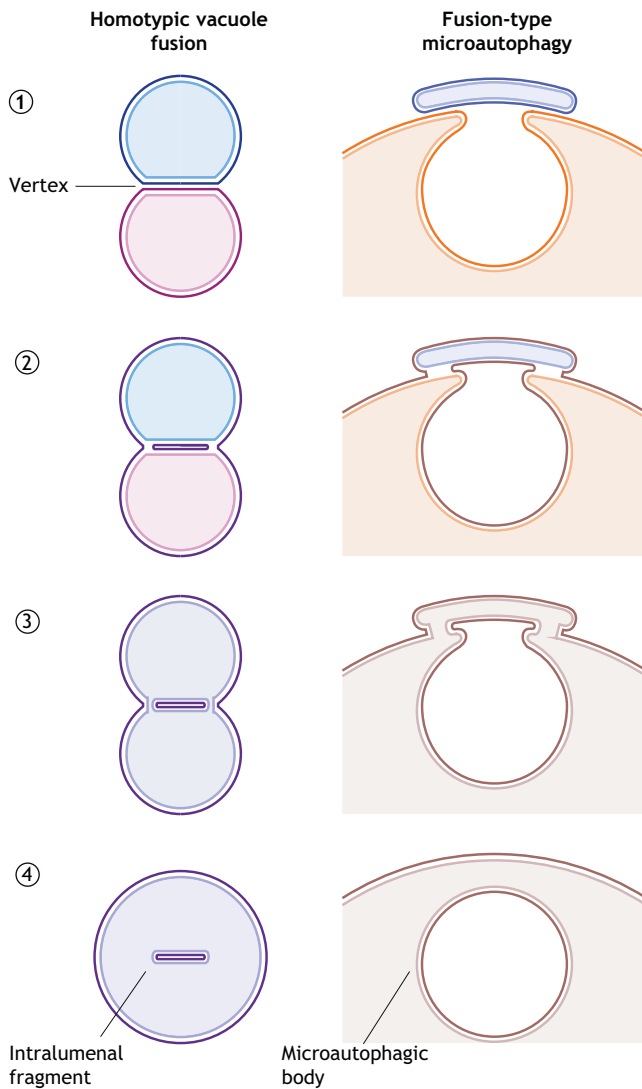


Fig. 4. Vertex fusion during vacuole fusion and microautophagy. The schematic illustrates mechanistic similarities between homotypic vacuole fusion in yeast and the hypothetical fusion of a phagophore-like membrane structure with the lysosomal membrane during fusion-type microautophagy. Each line represents a lipid monolayer. Steps include (1) docking so that the apposed membranes form a vertex ring, (2) fusion of the outer membrane leaflets, resulting in lipid mixing and a hemifusion intermediate, (3) fusion of the inner membrane leaflets, resulting in content mixing, and (4) release of an intraluminal fragment and a microautophagic body, respectively.

structures such as the MIPA (Fig. 3B). Sealing could occur by vertex fusion, which is characteristic of homotypic vacuole fusion (Wang et al., 2002). During vertex fusion, two apposed vesicles do not fuse at a single point but along their vertex ring (Fig. 4). Importantly, vertex fusion does not change the number of membranes and is a coupled fusion–fission reaction that produces one membrane nested inside another (Wickner, 2010). The notion of microautophagy by vertex fusion is untested; however, fusion at a single point, as occurs in most membrane fusion events, would not seal invaginations but simply enlarge the invaginated organelle. Fusion-type microautophagy depends on the core autophagy machinery to generate sealing membrane structures and may require SNAREs. Processes in this class are micropexophagy and micronucleophagy in yeast and, possibly, micromitophagy in yeast and microchlorophagy in plants. Selectivity is ensured by receptors,

such as Atg30 and Atg39, which link cargo to the core autophagy machinery.

Whether a microautophagic process entails invagination of the sequestering organelle or membrane extensions may depend on cargo size. Microautophagy of large peroxisome clusters in *P. pastoris* involves vacuole extensions, but small peroxisome clusters can also be internalized through simple vacuole invagination (Mukaiyama et al., 2002). Lipid domains could be important for any type of microautophagy. According to the concept of domain-induced budding, lateral segregation of lipids can, at a certain critical length of the domain boundary, drive budding (Lipowsky, 2014). There is plenty of evidence that the sites of microautophagy are compositionally distinct membrane subdomains. For instance, autophagic tubes are depleted of membrane proteins, and their formation is inhibited by the sterol-sequestering drug nystatin (Müller et al., 2000; Kunz et al., 2004). Microlipophagy occurs at sterol-rich liquid-ordered domains (Tsuji et al., 2017), and micronucleophagy depends on the NVJ, which is also a raft-like lipid domain (Dawaliby and Mayer, 2010). General microautophagy in mammals is sensitive to cholesterol depletion (Sahu et al., 2011; Mejlvang et al., 2018). Clearly, the notion of lipid domains promoting microautophagy merits further investigation.

Physiological roles of microautophagy

The known functions of microautophagy relate to biosynthetic transport, metabolic adaptation, organelle remodeling and quality control. Microautophagy carries out biosynthetic delivery of certain newly synthesized hydrolases in yeast and pigments in plants (Liu et al., 2015; Chanoca et al., 2015). Starving yeast cells activate multiple microautophagic processes to acquire nutrients and remove redundant vacuolar transporters (Oku et al., 2017; Iwama and Ohsumi, 2019; Yang et al., 2020). In humans, fast starvation-induced microautophagy targets receptors for selective macroautophagy; this may render the following macroautophagic response non-selective and ensure that nutrients are derived from all cytoplasmic constituents (Mejlvang et al., 2018). Examples of microautophagic organelle remodeling include the removal of superfluous peroxisomes in yeast (Tuttle et al., 1993), downsizing of the yeast vacuole upon exit from quiescence (Dubouloz et al., 2005) and degradation of excess ER in mice after episodes of ER stress (Loi et al., 2019). Finally, microautophagy likely mediates stress-induced quality control during micromitophagy and micro-ER-phagy in yeast (Nowikovsky et al., 2007; Schuck et al., 2014), microchlorophagy in plants (Nakamura et al., 2018) and general microautophagy of damaged cytosolic proteins in flies, worms and mammals (Bohnert and Kenyon, 2017; Bae et al., 2019; Mesquita et al., 2020). Constitutive general microautophagy in fly synapses may serve as a pre-emptive quality control mechanism by rejuvenating the proteome (Uytterhoeven et al., 2015).

Macroautophagy and microautophagy of the same organelle are often activated simultaneously, as seen for pexophagy, ER-phagy and nucleophagy in yeast (Monastryska et al., 2004; Schäfer et al., 2019; Otto and Thumm, 2020). The benefit of such parallel targeting is not obvious, especially as even the cargo receptors are sometimes the same (Farré et al., 2008; Otto and Thumm, 2020). An interesting idea is that macroautophagy and microautophagy are coupled to ensure homeostasis of the vacuole membrane, which would expand in case of unopposed macroautophagy and shrink in case of excessive microautophagy (Müller et al., 2000). However, the yeast vacuole does not shrink in macroautophagy mutants upon activation of microautophagy by TOR inhibition (Chan and Marshall, 2014), which is at odds with the coupling idea.

Outlook

Our understanding of microautophagy has progressed substantially over the past few years, but many open issues remain. Perhaps the most urgent challenge is to identify the molecules and mechanisms that are unique to each microautophagic process. This would allow to specifically disrupt different microautophagic processes and assess their physiological functions. At present, there are not even any unambiguous ways to block the two principal types of microautophagy. For example, simple ablation of ESCRTs abolishes fission-type microautophagy but also impairs lysosome biogenesis and phagophore maturation (Vietri et al., 2020). Furthermore, considering the large size of many microautophagic membrane invaginations, it is obvious to ask which forces drive their formation. The lysosomal membrane is protected from digestion, presumably by glycoproteins at the luminal side (Neiss, 1984). This protection must be disabled during the formation of microautophagic bodies to allow their degradation, yet how this is achieved is unclear. As microautophagy entails partial self-digestion of a lytic organelle, it is a dangerous process that needs to be carefully controlled. However, little is known about the regulation of microautophagy, except that it sometimes, but not always, involves TOR signaling (Dubouloz et al., 2005; Mejlvang et al., 2018; Yang et al., 2020). Finally, it will be important to understand how macroautophagy and microautophagy cooperate and why these two fundamentally different types of autophagy have co-evolved. Given these intriguing questions and the emerging conceptual framework for distinct microautophagic mechanisms, research on microautophagy is set to blossom.

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Supplementary information

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