MINIREVIEW

Peroxisomes as dynamic organelles: autophagic degradation

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Eukaryotic cells have to regulate peroxisomal metabolism to meet their environmental or developmental requirements. Therefore, the control of peroxisome number, which is mediated not only by proliferation but also by degradation, is an important cellular event. Here we briefly review studies on the autophagic degradation of peroxisomes, a process now termed pexophagy. Recent advances in molecular analyses of both nonselective and selective autophagic pathways have revealed a category of autophagy-related genes (*ATG*), many of which are shared in different pathways. In this review we introduce the functions of the shared *ATG* products, along with their interactions with peroxisomal factors. Physiological functions of this process (especially cellular remodeling) in mammalian cells and in a phytopathogenic fungus are also introduced.

Introduction

The dynamic nature of peroxisomes is supported by various molecular mechanisms of organelle biogenesis (augmentation) and degradation. In this review we focus on the mechanism of autophagic degradation of this organelle. Studies on another aspect of peroxisome dynamics (i.e. peroxisome biogenesis) are elaborately reviewed in the review by Wolf *et al*. in this miniseries [1]. In addition, a comprehensive review on peroxisome dynamics in yeast experimental systems can be found, in this miniseries, in the review by Saraya *et al*. [2].

As with other organelles, such as mitochondria, autophagy is considered to play a major role in the degradation of peroxisomes, and there are reports of lipooxygenase-mediated degradation of peroxisomes [3]. The existence of pexophagy was recognized at a very early stage of cell biology in a milestone report by de Duve and Baudhuin, where 'peroxisomes' were defined [4]. Therein the authors made a statement that 'Like other cytoplasmic components, microbodies (designated as peroxisomes in this report) do occasionally occur within autophagic vacuoles, in which case they are presumably destroyed by the lysosomal enzymes,' which clearly represents the autophagic degradation of peroxisomes.

Biochemical and morphological studies of peroxisome degradation in several methylotrophic yeasts (which are capable of growth on methanol as a sole carbon source) identified the selective mode of autophagic degradation of peroxisomes [5,6], giving birth to the term 'pexophagy'. And in the last decade, more than 30 gene products necessary for pexophagy and/or autophagy have been identified [7]. Among them, we

Abbreviations
ATG, autophagy-related genes; LC3, microtubule-associated protein 1 light chain 3; MIPA, micropexophagic membrane apparatus; PAS, phagophore assembly site; PAS, pre-autophagosomal structure; PE, phosphatidylethanolamine.

herein focus on two groups of such molecular machineries: the core factors for forming pexophagic membrane structures; and interface molecules between peroxisomes and autophagic membrane structures. In the last part of this review, we introduce the physiological roles of pexophagy in mammalian cells and in a phytopathogen that were proposed in recent studies.

Core factors for membrane formation in pexophagy

Ubiquitin-like conjugation system

Autophagy transports various intracellular components from the cytosol to vacuoles – for example peroxisomes (pexophagy), mitochondria (mitophagy), ribosomes (ribophagy) and several vacuolar enzymes including a precursor form of aminopeptidase I (Cvt pathway) – in both a selective and nonselective manner. All of these pathways employ common machinery involving two ubiquitin-like conjugation systems for *de novo* formation of the double-membrane structures to enwrap and transport the targets. These systems produce two covalent conjugates, Atg12–Atg5 and Atg8–phosphatidylethanolamine (PE) [8,9]. The homolog protein of Atg8 in mammalian experimental systems is termed microtubule-associated protein 1 light chain 3 (LC3), and the form conjugated to PE is designated LC3-II. As a portion of the Atg8–PE bond is further cleaved by Atg4, Atg8 can undergo conjugation and deconjugation cycles. During pexophagy, components of the two ubiquitin-like conjugation systems are required to form autophagic membrane structures enwrapping peroxisomes (pexophagosomes; Fig. 1), and Atg8–PE is located on the pexophagosomes [10–13].

These conjugation reactions are also involved in 'micropexophagy', where the vacuolar membrane directly engulfs peroxisomes (Fig. 1). This mode of pexophagy was first discovered in the methylotrophic yeast *Pichia pastoris*, when the organism was cultured in methanol to induce peroxisomes and subsequently transferred to glucose medium where the peroxisomes were no longer needed for methanol metabolism [14]. Under this culture condition, the PE-conjugated Atg8 marked a *de novo*-formed, cup-shaped membrane structure on the peroxisome surface (Fig. 1) [15]. As this peculiar structure was morphologically distinct from pexophagosomes, it was named the micropexophagic membrane apparatus (MIPA). A membrane-fusion event between MIPA and the vacuolar membrane was observed immediately before the completion of peroxisome transport into the vacuole. Therefore, MIPA was assumed to mediate the fusion between the opposing tips of the invaginating vacuole to complete sequestration from the cytosol.

Phagophore assembly site formation

Studies of yeast autophagy show that, for the conjugation processes and subsequent membrane formation to occur, many Atg proteins form a complex at a specific site termed the pre-autophagosomal structure or phagophore assembly site (PAS) [16] [17]. Two Atg proteins, Atg11 and Atg17, function as scaffold proteins for PAS formation [18]. Both Atg11 and Atg17 interact with a pivotal kinase for autophagy, Atg1. The scaffold proteins are required for the recruitment of other Atg proteins to PAS. The recruited proteins include components of the autophagy-specific phosphatidylinositol 3′-kinase complex (Atg14, Vps15, Vps30/Atg6 and Vps34), a putative transmembrane protein (Atg9) and factors involving the ubiquitin-like conjugation system [18].

In *Saccharomyces cerevisiae* and *P. pastoris*, selective transport of the aminopeptidase I precursor into the

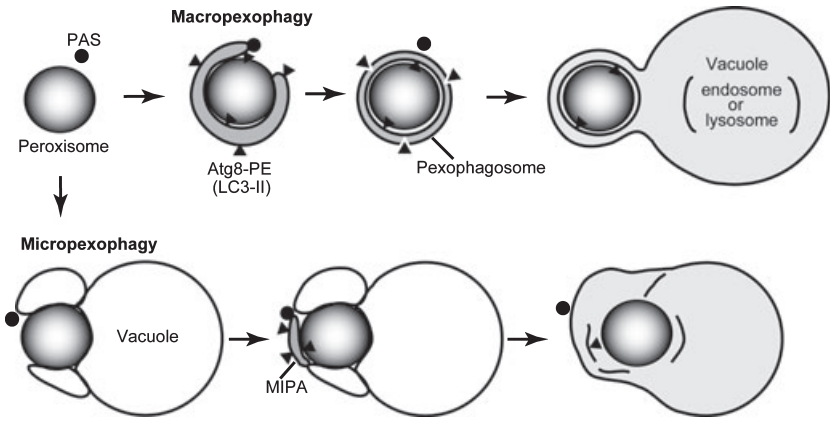

Fig. 1. Two distinct modes of pexophagy: macropexophagy and micropexophagy. In macropexophagy, the peroxisomes are first sequestered from the cytoplasm by an isolation membrane, forming pexophagosomes. In micropexophagy, MIPA, as well as the vacuolar membrane, engulfs the peroxisomes. The counterpart protein and organelles in mammalian systems are indicated in parentheses.

vacuole (Cvt pathway) is dependent on Atg11 [19,20]. Similarly to this Cvt pathway, the pexophagy process in yeast systems requires Atg11 homolog proteins [20,21], suggesting the existence of a common PAS-formation machinery for selective autophagic pathways. Furthermore, a recent study using *P. pastoris* demonstrated that Atg17 was also involved in efficient degradation of peroxisomes, presumably by recruiting the peroxisome–autophagosome interface factor, Atg30 (described below), in combination with Atg11 [22].

In a mammalian system, a putative counterpart of the scaffold protein, FIP200, was found to function in starvation-induced bulk autophagy [23]. Thus, it is questioned whether FIP200 or other yet-unknown counterpart proteins are involved in mammalian pexophagy.

Membrane traffic in *P. pastoris* pexophagy

Recent studies of *P. pastoris* pexophagy suggested the existence of membrane flow from authentic organelles to MIPA or pexophagosomes. One study indicated that a Golgi-resident phosphatidylinositol 4-kinase, Pik1, was necessary for the formation of MIPA, and that MIPA was rich in the product of phosphatidylinositol 4-kinase, (i.e. phosphatidylinositol 4-phosphate) [24]. As Pik1 was not detected in MIPA during micropexophagy, some transport machinery of phosphatidylinositol 4-phosphate was expected, but the molecular details of this transport remain elusive.

Another study showed that a small GTPase, Sar1, previously known to function in vesicle budding from the endoplasmic reticulum, was involved in the pexophagy process of *P. pastoris* [25]. Two mutant derivatives of this protein were utilized in this study: a T34N form that has high affinity for GDP and an H79G form that is impaired in its GTPase activity. While both T34N and H79G mutants inhibited MIPA formation, T34N hampered pexophagosome formation, and H79G expression led to pexophagosomes that were incapable of fusion with the vacuolar membrane. Interestingly, the pexophagosomes formed during H79G expression contained the endoplasmic reticulum marker DsRFP–HDEL, suggesting a traffic link between the endoplasmic reticulum and pexophagosomes.

Interface molecules between peroxisomes and autophagic components

Peroxisomal components for pexophagy

The autophagic selectivity towards peroxisomes is assumed to be defined at the interface of peroxisomal and pexophagosomal membranes. The peroxisomal factor responsible for pexophagy was first identified in the study of the methylotrophic yeast *Hansenula polymorpha* [26]. This study identified Pex14, the gateway membrane protein that imports peroxisomal matrix proteins, as an essential factor for selective organelle degradation, and demonstrated the functional role of its N-terminal region.

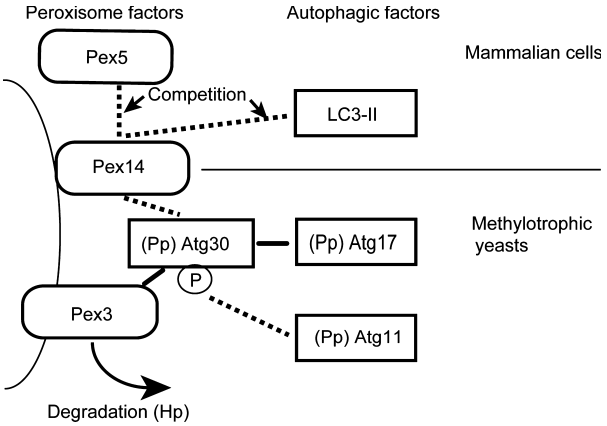

Fig. 2. Interactions between peroxisomal and autophagic factors. The dashed lines indicate the interactions revealed by immunoprecipitation analysis, and the solid lines indicate those confirmed by both immunoprecipitation and yeast two-hybrid analyses. Hp, *Hansenula polymorpha*; P, phosphorylation; Pp, *Pichia pastoris*.

Pex14 was also demonstrated to be involved in mammalian autophagy of peroxisomes following nutrient starvation and resupplementation; in this case, Pex14 formed a complex with LC3–PE (LC3-II), which was dependent on intact microtubules [27]. Interestingly, the Pex14–LC3-II and Pex14–Pex5 interactions seemed to be mutually exclusive, suggesting that the competitive interactions act as a molecular switch that induces peroxisome degradation (Fig. 2).

Pex3 is another peroxisomal membrane factor associated with organelle degradation. In *H. polymorpha*, this protein was rapidly degraded before organelle degradation *per se* [28], and inhibiting Pex3 degradation caused persistence of peroxisomes, even under pexophagic conditions, suggesting that the degradation of Pex3 was necessary for pexophagy in this organism (Fig. 2).

Receptor proteins for pexophagy

In selective autophagy, receptor proteins are often utilized to link the targets and the double-membrane formation machinery. In the *S. cerevisiae* Cvt pathway, the precursor form of aminopeptidase I (the cargo) interacts with a receptor protein, termed Atg19, that

binds to both Atg11 and Atg8 [17]. In the mammalian autophagic system for ubiquitinated protein aggregates, the ubiquitin moiety is captured by p62 (sequestosome 1), which binds to LC3 [17].

The receptor protein for pexophagy was first identified in *P. pastoris* [29]. This protein, termed Atg30, was phosphorylated depending on its interacting partner, Pex3, which enabled it to form complex with Atg11. This protein, which interacts with Atg17 and also Pex14, functions as a pivotal factor between the peroxisomal surface and the autophagic core components (Fig. 2). However, counterpart proteins of Atg30 are not found in the genomes of higher eukaryotes, or even in the genomes of other yeasts, including *S. cerevisiae*; the type of molecule that functions in place of Atg30 in other organisms remains unknown. Recently, artificial tagging of peroxisomal membrane proteins with ubiquitin was found to trigger p62-dependent organelle degradation [30]. Thus, it is plausible to assume that p62 acts as a receptor protein for pexophagy by recognizing endogenously ubiquitinated peroxisomal proteins.

Physiological roles of pexophagy

Pexophagy for quantitative regulation of peroxisomes

In mammalian cells, selective autophagy of peroxisomes was observed after organelle proliferation following stimulation with di-(2-ethylhexyl) phthalate and subsequent chase culture (without the reagent) [10]. Electron microscopy of hepatocytes under this experimental condition revealed selective surrounding of peroxisomes by pexophagosomes and demonstrated a role for pexophagy in control of the organelle quantity in response to the change in cellular metabolism.

Besides such inducible pexophagy responding to metabolic changes, constitutive autophagy of peroxisomes was also observed in cultured mammalian cells [31]. The turnover rate of peroxisomes, which is sensitive to 3-methyladenine, a phosphatidylinositol 3-kinase inhibitor that impairs autophagy, was estimated to be 30% of the total population per day in cells under normal experimental conditions analyzed using the Halo-tag technique, which labels a target protein with a fluorescent label at any given time-point by exogenous addition of a specific ligand. These data are significant in that they show the rate of constitutive turnover of the organelle, yet it has not been fully clarified whether this turnover is the outcome of selective autophagy towards peroxisomes, or of bulk autophagy that eventually transports the organelle into the lysosome.

Pexophagy for cellular remodeling during cell differentiation

A recent study of the plant pathogenic fungus *Colletotrichum orbiculare* demonstrated a novel role of pexophagy in the pathogenicity of this organism [32]. To invade host plants, this fungus forms a specific infection structure termed an appressorium (Fig. 3). While it is known that peroxisome biogenesis is necessary for the formation of a functional appressorium that contains melanin, a pigment essential for phytopathogenicity (pexophagy) in the same structure was found to be required for the subsequent invasion step.

Atg26, a sterol glucosyltransferase, was the key molecule of this pexophagy process. This molecule had been identified as an essential factor for pexophagy in

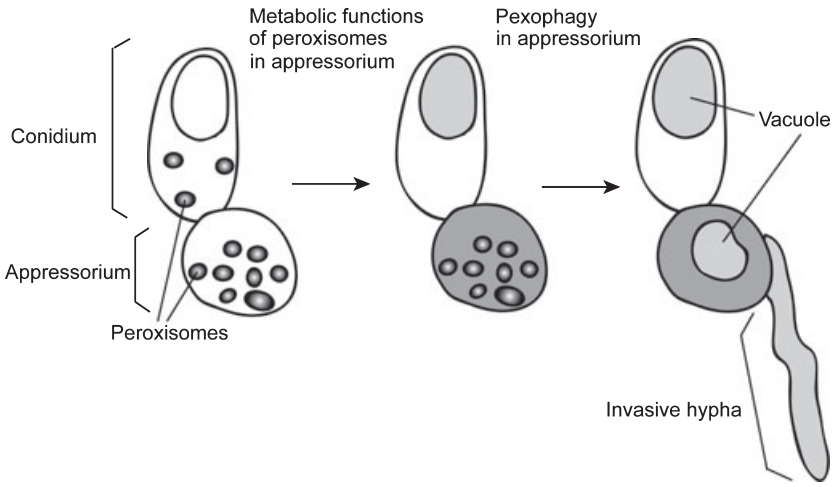

Fig. 3. Cartoons showing cell differentiation of *Colletotrichum orbiculare* for invasion into host plants. First, the conidium (asexual spore) germinates and develops the appressorium whose melanization and functionality depend on peroxisomal metabolic function. In the next step the peroxisomes in the appressorium are degraded by pexophagy, which is necessary for the formation of hypha to invade into the host plant.

methanol-induced *P. pastoris* [22,33]. The GRAM domain (named after glucosyltransferase, Rab-like GTPases, and myotubularins, which harbor this domain) of Atg26 is conserved and involved in pexophagy in both *C. orbiculare* and *P. pastoris*. As this molecule is not required for starvation-induced bulk autophagy [33], the physiological importance of selective autophagy of peroxisomes (pexophagy) is suggested.

The precise molecular mechanism of how pexophagy in the *C. orbiculare* appressorium leads to invasion of the host plant is currently unknown. Notably, the atg26 mutant strain was able to invade heat-shocked plants whose defense responses were impaired and who were more resistant to high concentrations of glycerol, which collapsed the wild-type strain [32]. Taken together, it is suggested that the cell integrity of the appressorium, including the control of turgor pressure, is impaired without pexophagy, which decreases the ability of the fungus to overcome the defense responses of the host plant.

Conclusion and future perspectives

After identification of general *ATG* gene products involved in both nonselective and selective autophagic pathways, pexophagy has been considered to be a good model system for selective autophagy to pursue how the peroxisomes are specifically wrapped by the pexophagic membrane structures. Recent studies revealed the interaction of general *ATG* gene products with some peroxisomal molecules at its membrane. In the upcoming years, progress revealing the detailed mechanism of selectivity acquisition and pexophagy regulation is expected. It will be possible to elucidate the physiological functions of pexophagy at the organism level based on knowledge acquired at the molecular level.

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