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Molecular mechanism and physiological role of pexophagy

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Abstract

Pexophagy is a selective autophagy process wherein damaged and/or superfluous peroxisomes undergo vacuolar degradation. In methylotropic yeasts, where pexophagy has been studied most extensively, this process occurs by either micro- or macropexophagy: processes analogous to micro- and macroautophagy. Recent studies have identified specific factors and illustrated mechanisms involved in pexophagy. Although mechanistically pexophagy relies heavily on the core autophagic machinery, the latest findings about the role of auxiliary pexophagy factors have highlighted specialized membrane structures required for micropexophagy, and shown how cargo selectivity is achieved and how cargo size dictates the requirement for these factors during pexophagy. These insights and additional observations in the literature provide a framework for an understanding of the physiological role(s) of pexophagy.

Introduction

Pexophagy is a turnover pathway in which peroxisomes are selectively degraded by the autophagy machinery in response to specific environmental cues [1-5]. Other selective autophagy pathways include mitophagy, ribophagy, ER-phagy, micronucleophagy and the Cvt pathway in yeasts [3]. One of the earliest descriptions of mitophagy and pexophagy comes from a comparison of the diurnal rhythms of lysosomal degradation of subcellular organelles in rat liver, kidney and pancreas [6].

Model systems used to examine pexophagy

Many model systems, encompassing yeast, fungal, plant and mammalian cells, have been exploited for the study of the mechanisms of pexophagy [3,7-10]. However, methylotrophic yeasts have provided most of the important details regarding the selectivity of pexophagy, which is the primary focus of this mini-review. Specific genetic screens using UV, chemical or insertional mutagenesis strategies in yeasts have elucidated genes required for pexophagy [11-17]. Because selective autophagy pathways rely on components of the core autophagic machinery, these screens have revealed components of the core autophagy machinery as well as unique selectivity factors that adapt the core autophagy machinery for pexophagy [14,16].

The work in methylotrophic yeasts, especially *Pichia pastoris* and *Hansenula polymorpha*, has characterized two modes of pexophagy, which are related morphologically to macroautophagy

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and microautophagy [18-20] (Fig. 1A). In macropexophagy, individual peroxisomes are engulfed by double-membrane structures known as pexophagosomes, which fuse with lysosomes (or the vacuole in yeast) to deliver pexophagic bodies into the vacuole lumen for degradation and recycling (Fig. 1B). In micropexophagy, peroxisome clusters are engulfed by vacuolar sequestering membranes (VSMs) and by the micropexophagy specific apparatus (MIPA) [21], which forms a lid over the cup-shaped VSMs cradling the peroxisomes [3] (Fig. 1C). Heterotypic fusion between the VSMs and the MIPA delivers the pexophagic body into the vacuole lumen for turnover and recycling of building blocks. Both the MIPA and the pexophagosome are assembled at the phagophore assembly site (PAS) from which the isolation membrane expands. Thus the PAS and its organization are central to the early steps of pexophagy.

Nutrient conditions that trigger pexophagy

Pexophagy can be triggered experimentally by switching yeast or mammalian cells from media causing peroxisome proliferation to an environment where peroxisome biogenesis is repressed. In *Saccharomyces cerevisiae*, pexophagy is activated by moving cells from oleate, which induces peroxisomes, to glucose lacking nitrogen [22]. In *P. pastoris*, peroxisomes are induced on methanol, oleate or amines. The transfer of cells from methanol to ethanol, or from either oleate or methylamine to glucose, triggers macropexophagy [23,24]. In contrast, the transfer of cells from methanol to glucose induces micropexophagy [20]. Interestingly, in *H. polymorpha* similar nutrient adaptation (a shift to glucose as carbon source) triggers macropexophagy [25].

Steps and components of the core autophagy machinery in yeast

The discovery of many of the autophagy genes (termed *ATGs*) and early mechanistic advances regarding autophagy and autophagy-related pathways were made in *S. cerevisiae*. The core autophagy machinery (reviewed elsewhere [26], including articles in this issue) is comprised of the yeast proteins required for following steps –

1. Signaling proteins required for the induction of autophagy (e.g. Tor1 kinase, PKA, Sch9, Tap42 and PP2A)
2. Cargo packaging (e.g. Atg19, Atg11 and Atg8).
3. Organization of the PAS (e.g. Atg1, Atg11, Atg13, Atg17, Atg29 and Atg31).
4. Vesicle nucleation (e.g. Atg6, Atg9 and PtdIns 3 kinase subunits).
5. Vesicle expansion and completion (e.g. Atg3-5, Atg6, Atg7, Atg8, Atg10, Atg12, Atg14, Atg16).
6. Protein retrieval (e.g. Atg1, Atg2, Atg18, Atg23, Atg27).
7. Homotypic fusion of the isolation membrane (e.g. Tlg2).
8. Docking and heterotypic fusion between the autophagosome and the vacuole (e.g. v- and t-SNAREs, Ccz1, Mon1, HOPS complex).
9. Intra-vacuolar vesicle breakdown (e.g. Atg15, proteinase A, proteinase B)

Pexophagy also requires most of these components of the core autophagic machinery, but in addition requires over half a dozen selectivity factors that adapt the autophagy machinery for pexophagy [3].

Selectivity factors required for pexophagy

Some of the principles regarding selective autophagy pathways come from studies of the cytosolto-vacuole transport (Cvt) pathway, which delivers the precursor of aminopeptidase (prApe1) to the vacuole to generate mature Ape1 (mApe1) in yeasts (Fig. 2A) [15,27]. The Cvt pathway targets selective cargoes such as prApe1, α-mannosidase1 (Ams1) and leucine aminopeptidase III (Lap3) [28] to the vacuole, whereas general autophagy is non-selective. Unique components uncovered in studies on selective autophagy include a cargo receptor, which is Atg19 for prApe1 and Ams1, and a second protein Atg11, which is a selectivity factor that bridges the cargo receptor and the core autophagy machinery [29]. The cargo receptor has a dual function – to bind cargo and to interact with one or more key components of the autophagy machinery at the PAS [29,30]. In *S. cerevisiae*, the cargo receptor, Atg19, interacts with Atg11, which is a component that organizes the PAS [30]. Additionally, Atg19 also interacts with Atg8, a key protein required for nucleation of isolation membranes at the PAS [29]. The region of Atg8 that interacts with Atg19 has been defined via crystallography, and is comprised of a WxxL motif on Atg19 [31]. Interestingly, in mammalian systems, proteins that interact with the yeast Atg8 homolog, LC3 (or related family members), have an LC3-interacting region (LIR), which is also comprised of a WxxL (or a related) motif, often preceded by some acidic residues and/or phosphorylation sites [31-33]. Recently, several additional LIR-containing proteins involved in selective autophagy have been discovered, including the mitophagy receptors, Atg32 in yeast [34,35] and NIX in mammals [36], as well as the autophagy proteins, p62/SQSTM1 and NBR1 in mammals [32,33]. This theme of cargo receptors necessary to bridge the cargo and components that organize and nucleate membrane expansion at the PAS is also true of pexophagy.

The *P. pastoris* peroxisome receptor, Atg30, interacts with peroxisomes (the selective cargo) via two peroxisomal membrane proteins, Pex3 and Pex14, and with autophagy machinery via Atg11 and Atg17, which organize the PAS [37] (Fig. 2B). By analogy, the newly-discovered *S, cerevisiae* mitophagy receptor, Atg32, interacts with both Atg8 and Atg11 [34,35] (Fig. 2C). In contrast to Atg32, Atg30 lacks a LIR and does not seem to interact with Atg8. It remains to be seen if some other protein containing a LIR bridges peroxisomes to Atg8.

The three selectivity factors (Atg19, Atg30 and Atg32) described up to date interact directly with the adaptor protein Atg11, identifying Atg11 as a common component of all known selective autophagy pathways. During pexophagy, phosphorylation of Atg30 is a prerequisite for its association with Atg11 and this interaction probably occurs at the PAS, their only common localization [37]. The phosphorylation of another autophagy receptor, Atg19, has been described, but its physiological role has not been studied [38]. Additionally, Atg19 also gets ubiquitinated which is important for efficient ApeI processing [39]. Thus, the emerging theme encompasses the following steps: (a) a signal for selective autophagy (if required), (b) modification of the receptor, (c) interaction of the receptor with the adaptor Atg11, (d) Atg11-dependent organization of the PAS where the interaction occurs, and (e) interaction with Atg8 to “guide” the elongation of the phagophore membrane around the cargo. Surprisingly, however, the three receptors described above for the Cvt, pexophagy and mitophagy pathways appear to be species specific.

A second theme common to inducible, selective forms of autophagy relates to signaling systems in the cell that activate these processes. In yeasts, the Cvt pathway is constitutively active, and may not require such selectivity factors. However, since most forms of organelle turnover are triggered only under special conditions, wherein superfluous or damaged organelles need to be removed, it seems likely that such inducible forms of selective autophagy will require signaling proteins. An example of such a protein for micropexophagy in *P. pastoris* is the alpha subunit of the enzyme phosphofructokinase, Pfk1, whose catalytic activity is not necessary for

glucose-induced micropexophagy, but whose presence is essential [40]. There are also reports that cell surface glucose sensors, Gpr1 (a G-protein-coupled receptor) and the G-protein, Gpa2, involved in the cAMP-dependent activation of PKA, are involved in glucose sensing during pexophagy in *S. cerevisiae* [41].

A third set of proteins necessary for pexophagy is related to cargo size [23]. Autophagy-related pathways engulf a variety of cargoes ranging in size from protein complexes (e.g. the Cvt-complex) to organelles or even bacteria and viruses. During this process, the isolation membrane has to expand to engulf these cargoes. In the case of macropexophagy, Atg11 and Atg26 (a sterol glucosyltransferase) are necessary for the turnover of large and medium/large peroxisomes, respectively, but not for pexophagy of small peroxisomes [23]. In *S. cerevisiae*, it has been shown that the level of Atg8 at the PAS determines the size of the autophagosomes [42]. During pexophagy, the level of Atg8 itself does not change dramatically, but the amount of Atg8 conjugated to PE does increase 2–fold (our unpublished observations). If Atg11 and Atg26 regulate, directly or indirectly, the efficiency of Atg8-PE formation, they might contribute partly to the formation of pexophagosomes of larger size. Additionally, the interactions between Atg30 on the peroxisome surface and Atg11 may also provide a template for the expansion of the isolation membrane.

Additional proteins required for pexophagy include PtdIns-3P-binding proteins, such as Atg24, which binds PtdIns-3P via a PX domain [43]. In fact, several pairs of such PX-domain proteins play roles, most likely at the final fusion steps between autophagosome/pexophagsomes/Cvt vesicles and the vacuole. For example, Atg20 and Atg24 are required for the Cvt pathway [44]. There is also evidence that other proteins may be necessary to recruit these selectivity factors to specific subcellular locations during pexophagy. This is exemplified by the PtdIns 4 kinase, Pik1, which is proposed to generate PtdIns-4P at the PAS during pexophagy [45, 46]. This lipid recruits Atg26, which has a GRAM domain that binds PtdIns-4P, to the PAS where it plays a role in MIPA and pexophagosome formation. Another example of a selectivity factor is the coiled-coil protein, Atg25, required for macropexophagy in *H. polymorpha* [47]. Atg25 is present on pexophagosomes and it is involved in the completion of sequestration of peroxisomes or in the fusion of pexophagosomes with the vacuolar membrane [5].

Finally, because micropexophagy requires novel membrane structures, such as the MIPA, that have not been described for macroautophagy or the Cvt pathway, it would not be surprising if certain proteins were uniquely required for MIPA formation.

The pexophagy-specific PAS

The origin and formation of the isolation membrane for all autophagy-related pathways is unusual in that only a single yeast membrane protein, Atg9, has been identified at the PAS, but by the time the autophagosome is formed, this protein is depleted from autophagosomes by a retrieval machinery [48-50]. All the other components of the core autophagy machinery are recruited in a temporally-orchestrated, hierarchical manner to the PAS [51,52]. This hierarchy of protein assembly at the PAS has been studied in both yeasts and mammals, and has led to the concept of a Cvt- and autophagy-specific PAS that dictates whether selective or non-selective cargoes will be subjected to encapsulation and subsequent vacuolar delivery. Thus, the autophagy-specific PAS requires the function of Atg17, Atg29 and Atg31 and the Cvt-specific PAS requires the function of Atg11 and Atg19 [51,53,54]. Interestingly, the pexophagy-specific PAS requires the function of both Atg11 and Atg17, as well as Atg30 [23]. In *S. cerevisiae*, the single mutants, *atg29* and *atg31* are reported to be required for pexophagy but are proficient in the Cvt pathway [55-57], but we predict that the double mutant of *atg29 atg31* will be affected in the Cvt pathway, based on the finding that in *P. pastoris*, Atg28, the homolog of *S. cerevisiae* Atg29 and Atg31, is needed for all autophagy-related

pathways [23]. It has been found that Atg28 has homologies to both Atg29 (at its N-terminus) and Atg31 (at its C-terminus) and may therefore fulfill the functions of these *S. cerevisiae* proteins. Consistent with this idea, many yeasts that have Atg29 and Atg31 have no Atg28 and vice versa [23].

Role of phosphoinositides in pexophagy

It is well known now that PtdIns 3 kinase is critical for all autophagy-related pathways in yeast and mammals [26]. Yeasts have a unique catalytic subunit, Vps34, endowed with this activity, whereas in mammals it is the Class III PtdIns 3 kinase that is required for autophagy. Studies in both yeast and mammals have revealed at least two complexes containing Vps34 [58,59]. Complex I, involved in autophagy, is comprised of *S. cerevisiae* Vps15, Vps34, Vps30/Atg6 and Atg14. Complex II, involved in the vacuolar protein sorting (VPS) pathway, shares the first three subunits with Complex I, but has Vps38 (instead of Atg14) as the unique subunit [58]. Similar complexes have been reported in mammals where Atg14 and a Vps38-like protein, UVRAG, are unique to Complexes I and II, respectively [59]; however, several other proteins are also associated with these complexes, most likely involved in the regulation of their activities [60,61]. In *P. pastoris*, an UVRAG homolog has been described, and it plays no role in autophagy-related pathways, but is involved instead in the VPS pathway [62]. No Atg14 homolog has been described to date in *P. pastoris*.

As part of the core autophagy machinery, the PtdIns 3 kinase Complex I plays a role in the vesicle nucleation step at the PAS [3,26]. Our analysis of this using time-lapse microscopy has shown that pexophagosome formation during macropexophagy in *P. pastoris* proceeds via the following steps, all dependent on PtdIns-3P generation at the PAS [62]: (1) Atg6 localizes to several peri-vacuolar dots; (2) Atg8 starts to localize in one of these structures, defining this structure as the PAS; (3) the isolation membrane begins to elongate from the PAS; (4) the isolation membrane engulfs one peroxisome, creating a pexophagosome. Atg6 stays at the PAS during the entire process.

Using a fusion of the FYVE domain, which binds PtdIns-3P, to GFP, the Ohsumi lab localized PtdIns-3P to only the concave surface of the isolation membrane during autophagy [63]. This is also where the other PtdIns-3P-binding proteins, such as Atg18, localize [64]. How the PtdIns-3P is restricted to only the concave surface of the double-membrane structure is unknown. Additionally, it is unclear at present whether the actual expansion of the isolation membrane is restricted to fusion events at the PAS, or whether incoming vesicles can fuse anywhere along the isolation membrane to cause its expansion. Because additional components are required for the efficient formation of the pexophagosome, it is interesting to hypothesize that these PtdIns-3P binding proteins provide an additional/extended scaffold to engulf large cargoes [23]. It can be envisaged that by interacting with the cargo (peroxisomes), this framework might stabilize the completion of pexophagosome around larger peroxisomes. Thus extension of the isolation membrane during the elongation step might proceed in a zipper-like fashion and pexophagosome formation completion might occur by homotypic fusion.

Peroxisomal components necessary for pexophagy

In *P. pastoris,* Atg30 interacts with two proteins, Pex3 and Pex14, residing in the peroxisomal membrane [37]. We have always believed that for efficient peroxisome homeostasis (and also applicable to other organelles) the biogenesis and turnover of the organelle must be coordinated. Most intriguingly, the interaction partners of Atg30 comprise the heart of the peroxisome biogenesis machinery. Pex3 is essential for the biogenesis of all peroxisomal membrane proteins and Pex14 is the central protein necessary for all peroxisomal matrix protein import [65]. It will be interesting to determine how this communication between the peroxisome biogenesis and turnover is achieved mechanistically.

In *H. polymorpha*, Pex14, and specifically its N-terminal 64 amino acids, is required for pexophagy [66,67]. Additionally, it has been reported that during macropexophagy in *H. poymorpha* Pex3 is removed from peroxisomes and not subjected to degradation by autophagy [68]. How Pex3 is removed from the peroxisome membrane is not understood. However, previous studies have shown that Pex3 is essential for the stability of the RING subcomplex of the peroxisomal importomer [69], so this step may destabilize some of the preformed subcomplexes in the peroxisome membrane, in addition to inhibiting peroxisome biogenesis.

In mammals, it is known that insoluble ubiquitinated protein aggregates that are recalcitrant to degradation via proteasomes can be degraded by autophagy. A protein, p62/SQSTM1, required for autophagy, binds ubiquitinated proteins via an UBA domain, and p62/SQSTM1 also has LIRs allowing interaction with LC3, a component of the autophagy machinery [33]. Monoubiquitination of both cytosolic and peroxisomal proteins in mammalian cells is sufficient to cause their turnover by targeting to autophagosomes and lysosomes [70]. This process is sensitive to inhibitors of autophagy (3-methyladaenine) and requires *bona fide* autophagy proteins, such as Atg12 and p62/SQSTM1. However, because pexophagy in this study was triggered by artificially incorporating an ubiquitinated protein into the peroxisome membrane, it remains to be seen if this mechanism is used during physiological pexophagy in mammals.

Signaling events and involvement of the cytoskeleton in pexophagy

Although one could have imagined that the pexophagy receptor might be synthesized only under pexophagy conditions, in *P. pastoris*, Atg30 is actually induced along with peroxisomes, but is activated for pexophagy by phosphorylation [37]. This is indeed a more versatile solution because of our finding that even under peroxisome-induction conditions, there is a basal level of Atg30 phosphorylation that we have hypothesized to target excess and/or dysfunctional peroxisomes to the vacuole for turnover. Consistent with this hypothesis, the overexpression of Atg30 is sufficient to create a peroxisome biogenesis defect by causing newly-synthesized peroxisomes to be targeted for degradation prematurely [37]. At present, the kinase that phosphorylates Atg30 is unknown, but our view is that it must be tied in to the cellular signaling system that senses pexophagy conditions. Such a signaling system would need to be activated via cell-surface receptors, such as Gpr1 described earlier [41], which sense the extracellular environment and translate that to a readout that determines whether or not peroxisomes are necessary under those conditions. Alternatively, unknown intracellular signaling events that sense damaged or dysfunctional peroxisomes might activate pexophagy.

During pexophagy in yeast, Atg9 moves from a peripheral compartment (PC) (adjacent to, but not coincident with, mitochondria) to the PAS transiently, and subsequently returns to the PC via retrieval mechanisms [49,50,71]. This movement requires the actin cytoskeleton (inhibited by Latrunculin A) and proteins of the Arp2/3 complex [72]. Additionally, peroxisomes may also need to be transported to the PAS and pexophagosomes may have to be delivered to the vacuole prior to fusion event. Details of how these events are orchestrated and coordinated are unknown at present.

Physiological role of pexophagy

Pexophagy has emerged recently as an important process for the vitality of several fungi under different growth conditions. For example, *H. polymorpha* peroxisomes are constitutively degraded by autophagy during the growth of cells in methanol medium despite the continuous induction of peroxisome proliferation. The lack of Atg1 leads to accumulation of peroxisomes and decreased viability of *H. polymorpha* cells in methanol medium. Some of these peroxisomes have decreased activity of peroxisomal catalase that is associated with increased cellular levels of reactive oxygen species (ROS) [73,74]. A similar phenotype is also observed

in a strain lacking the peroxisomal Lon protease, Pln, required for the degradation of unfolded and non-assembled peroxisomal matrix proteins. Since the Δ*pln* Δ*atg1* double mutant accumulated these defects in a cumulative manner [73], peroxisomal proteolysis and constitutive autophagy of peroxisomes might represent two independent mechanisms protecting cells from accumulation of peroxisomal protein aggregates and ROS under peroxisome proliferation conditions.

Pexophagy is responsible for the degradation of excess peroxisomes induced by phthalate esters after discontinuation of the drug administration in mice [4]. Interestingly, the accumulation of peroxisomes was also reported in aging human cells [75]. Their peroxisomes have a reduced capacity to import PTS1-containing enzymes, especially catalase. As a consequence, the older cells produce increased amounts of hydrogen peroxide. The increased load of ROS further reduces peroxisomal matrix protein import and worsens the effects of aging [75]. The phenotype of aging human cells resembles the phenotype of the *H. polymorpha* Δ*atg1* mutant in methanol medium [73] and suggests that an age-related decline in pexophagy might be responsible for changes seen in peroxisome population of human cells. Therefore, pexophagy might play an important role in preserving the functional integrity of the peroxisome population and maintaining cell viability in both lower and higher eukaryotes.

Direct studies on physiological role of pexophagy became possible recently after the discovery of the selectivity factors essential for pexophagy. These are the peroxisome receptor-adaptors complex, Atg30-Atg11-Atg17, and the sterol glucosyltransferase, Atg26 [23,37,46]. The first important insight into the physiological role of pexophagy was made with the *atg26* mutant of the cucumber anthracnose fungus *Colletotrichum orbiculare* [76]. Asexual spores (conidia) of *C. orbiculare* germinate and develop specific infection structures (appressoria) that are able to invade the cucumber leaves. The abundant peroxisomes present in conidia before germination are selectively degraded in the vacuoles during this infection-related morphogenesis. The *C. orbiculare atg26* mutant, specifically affected in pexophagy, develops appresoria with increased number of peroxisomes, and these appressoria fail to penetrate the host plant [76]. Therefore, pexophagy is physiologically important for phytopathogenicity of this infectious fungus.

The role of pexophagy was also directly addressed with the *atg11* mutant of the opportunistic human fungal pathogen *Candida glabrata* [7]. To disseminate and establish the infection, *C. glabrata* cells must survive their internalization by macrophages. Phagocytosed *C. glabrata* cells suffer from mild oxidative stress, sustained carbon starvation and transiently induce peroxisomes. However, at later stages of engulfment, peroxisomes are degraded by pexophagy, which plays an important role in prolonging the survival of engulfed cells, since the *C. glabrata atg11* mutant specifically blocked in pexophagy has significantly reduced survival rates in mouse macrophages [7]. These results indicate that the mobilization of peroxisomal resources via pexophagy contributes to virulence of important plant and human pathogens.

Future prospects

Most or all selective autophagy pathways share the same core machinery, superimposed on which are selectivity factors. The unresolved questions in pexophagy are the functions performed by the core machinery and the selectivity factors. Moreover, pexophagy is a tightly regulated event that can be activated by the transfer of carbon sources, but the signals leading to this activation remain unknown. Besides the degradation of superfluous organelles that are no longer needed, damaged peroxisomes could also be eliminated by pexophagy, but what constitutes damaged peroxisomes and the mechanisms for detecting and eliminating these organelles have not been described. The peroxisomes are abundant and distributed in the cytosol, but the mechanisms that deliver these organelles from the cytosol to the PAS (or the

PAS to the peroxisomes) and the pexophagosome to the vacuole are completely unknown. Another important question is how the isolation membrane expands around the peroxisome surface while excluding cytosolic contents. In this context, we know that the peroxisomal protein, Atg30 interacts with Atg11 and Atg17, but none of these proteins are present on the isolation membrane. We can speculate that Atg30 interacts with an unknown protein in the phagophore membrane or that an unknown protein on the peroxisome surface interacts with Atg8 present on the isolation membrane. Additionally, the identification of pexophagy-specific Atg proteins in multicellular organisms would improve our understanding of the physiological role of pexophagy in mammals.

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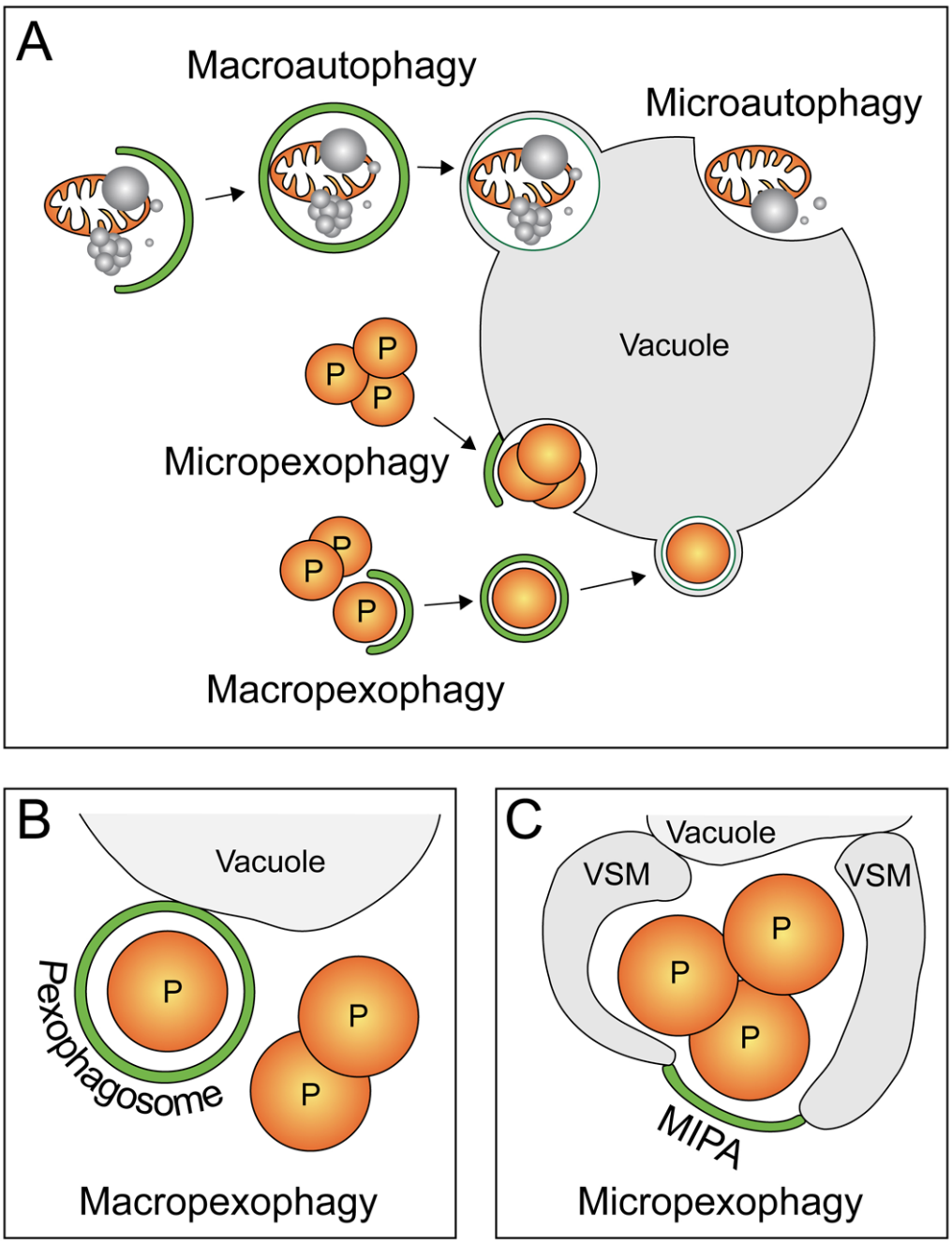

Fig. 1. Modes of general autophagy and pexophagy

As described in the text, general autophagy involves sequestration and degradation of nonspecific cargo (cytosolic proteins and organelles) either by engulfment into autophagosomes (macroautophagy) or by the invagination of the vacuolar membrane around these cargoes (microautophagy) (Fig. 1A). In an analogous fashion, pexophagy involves the engulfment of specific cargo, the peroxisomes (P) by either macro- or micropexophagy. While the formation of a pexophagosome around a peroxisome is the hallmark of macropexophagy (Fig. 1B), micropexophagy is morphologically characterized by vacuolar extensions (vacuolar sequestration membranes, VSM) and the cup-shaped micropexophagy apparatus (MIPA) that capture the peroxisome cluster (Fig. 1C).

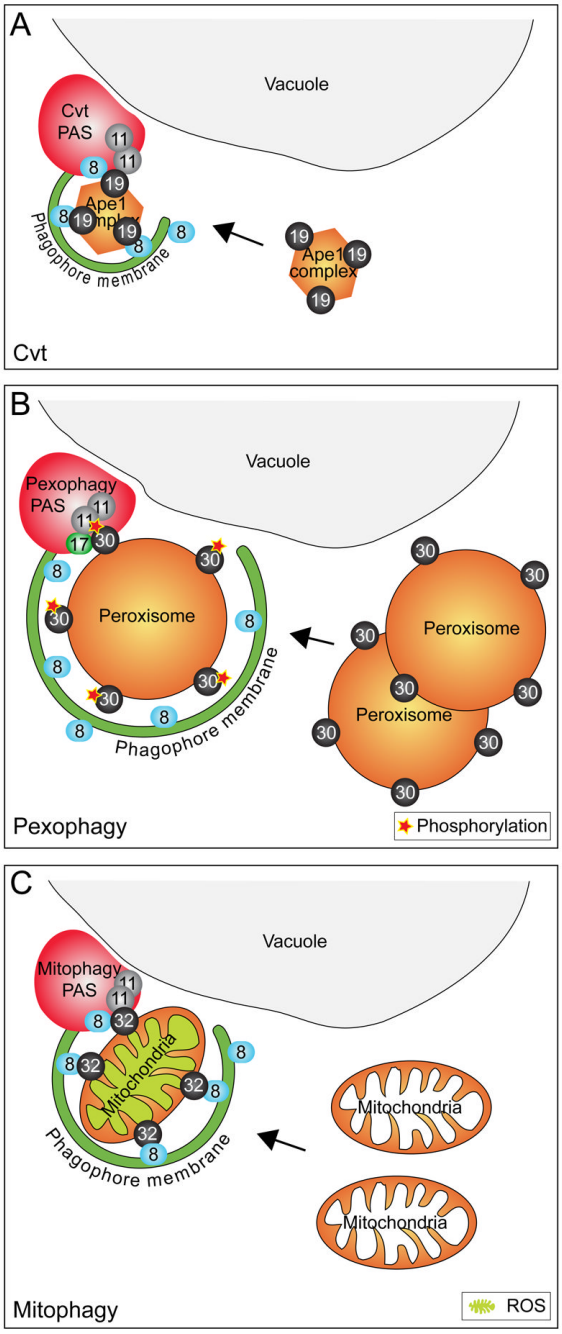

Fig. 2. Mechanistic views of selective autophagy pathways

Atg19 is the receptor for the Cvt pathway that sequesters the aminopeptidase I complex (Ape1 complex). At the Cvt-specific PAS, it interacts with Atg11, while its interaction with Atg8 is responsible for phagophore expansion (Fig. 2A). The phosphoprotein Atg30 is the peroxisome receptor for pexophagy. This protein is present on the peroxisomal membrane and phosphorylated during pexophagy. It then interacts with the autophagy adaptor proteins, Atg11 and Atg17 at the pexophagy-specific PAS. Atg30 is required for the formation of the phagophore membrane that sequesters peroxisomes during pexophagy (Fig. 2B). During mitophagy, ROS-damaged mitochondria are selectively captured for autophagic degradation

using the mitophagy receptor, Atg32. Atg32 interacts with Atg11 at the mitophagy-specific PAS and also plays a role in phagophore expansion by engaging Atg8 (Fig. 2C).