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Autophagic degradation of peroxisomes in mammals

Zientara-Rytter Katarzyna and **Subramani Suresh**[1]

Section of Molecular Biology, Division of Biological Sciences, University California, San Diego, CA 92093-0322, USA

Abstract

Peroxisomes are essential organelles required for proper cell function in all eukaryotic organisms. They participate in a wide range of cellular processes including the metabolism of lipids and generation, as well as detoxification, of hydrogen peroxide. Therefore, peroxisome homeostasis, manifested by the precise and efficient control of peroxisome number and functionality, must be tightly regulated in response to environmental changes. Due to the existence of many physiological disorders and diseases associated with peroxisome homeostasis imbalance, the dynamics of peroxisomes have been widely examined. The increasing volume of reports demonstrating significant involvement of the autophagy machinery in peroxisome removal leads us to summarize current knowledge of peroxisome degradation in mammalian cells. In this review we present current models of peroxisome degradation. We particularly focus on pexophagy - the selective clearance of peroxisomes through autophagy. We also critically discuss concepts of peroxisome recognition for pexophagy, including signaling and selectivity factors. Finally, we present examples of the pathological effects of pexophagy dysfunction and suggest promising future directions.

Keywords

peroxisome; pexophagy; autophagy; pexophagy receptor/adaptorAMPK

Peroxisomes and their life-cycle

Peroxisomes are organelles involved in hydrogen peroxide (H_2O_2) metabolism [1]. These dynamic organelles contain various enzymes such as: (1) acyl-CoA oxidase, alcohol oxidase, glycolate oxidase, urate oxidase, which are involved in reducing oxygen to H_2O_2 as a consequence of substrate oxidation; (2) catalase, which converts H_2O_2 to water and oxygen, as well as (3) enzymes involved in fatty acid β-oxidation [1, 2]. Heterogeneity of enzymes in peroxisomes links them to multiple metabolic pathways, including the breakdown of various carboxylates, synthesis of bile acids, docosahexaenoic acid (DHA) or ether-phospholipids [3] and underlies the necessity of peroxisomes for normal cell development and physiology. Peroxisomes are the main organelles responsible for cellular metabolism of H_2O_2, which is a type of Reactive Oxygen Species (ROS). It is an oxidant that is toxic for cells [4, 5] but also

[1]Corresponding author: Phone 858-534-3132, FAX 858-534-0053.

a key molecule involved in redox signaling [6, 7]. Because of dual role of H_2O_2 [8–10], peroxisome homeostasis is critical for cell function.

The physiological importance of peroxisome homeostasis is demonstrated by the existence of many physiological disorders associated with peroxisomal deficiencies or dysfunctions. Because many of them are related to peroxisome biogenesis disorders (PBDs) [11] the mechanism of peroxisome biogenesis and proliferation has been examined extensively. The focus on peroxisome homeostasis is more recent, with an emphasis on peroxisome degradation mechanisms, as a healthy peroxisome population requires not only their effective formation but also efficient removal of obsolete and dysfunctional peroxisomes.

Peroxisome degradation mechanisms

The estimated half-life time of mammalian peroxisomes is between 1.3–2.2 days [12–15]. This requires a balance between two processes: (1) new peroxisomes need to be formed and (2) obsolete, abnormal or dysfunctional peroxisomes must be removed. While some peroxisomes are degraded non-selectively, selectivity insures that only defective/redundant peroxisomes are destroyed. Three mechanisms are involved in peroxisome degradation: (1) matrix proteins degradation by the Lon protease; (2) 15-lipoxygenese (15-LOX)-mediated autolysis, and (3) degradation by the autophagy-lysosome pathway (pexophagy) (Fig. 1).

Lon proteases, first characterized in bacteria (reviewed in [16]), are homo-oligomeric ATP-dependent proteases with chaperone-like activity. They are present in eukaryotic mitochondria, chloroplasts and peroxisomes [17–19]. Two isoforms exist in mammals - one each in mitochondria and peroxisomes.

The peroxisomal Lon protease is involved in processing and activating a specific regulatory protein belonging to peroxisome targeting signal (PTS) 1-containing proteins [20]. The peroxisomal Lon may also be involved in the degradation of induced peroxisomal enzymes such as acyl-CoA oxidase, bifunctional protein and thiolase (involved in fatty acid β-oxidation) [21]. Both roles are evolutionarily conserved and are preserved in a plant peroxisomal Lon protease. This protease from *Arabidopsis*, AtLON2, is necessary for sustained import of matrix proteins into peroxisomes and assists in the degradation of dysfunctional peroxisomal matrix proteins [17, 22]. Interestingly, a knock-out of *AtLON2* that prevents the degradation of specific matrix proteins enhances another pathway of peroxisome destruction – pexophagy [22, 23].

In LOX15-dependent peroxisome autolysis, whole peroxisomes, and not individual proteins, are eliminated. LOX15 is a member of the lipoxygenase enzyme family, which integrates into the membranes of organelles to convert poly-unsaturated fatty acids into conjugated hydroperoxides [24]. As a result of membrane lipid peroxidation, lumenal and integral membrane proteins are released for digestion by cytosolic proteases [25]. 15-LOX was postulated to be important for programmed organelle degradation in reticulocytes, hepatocytes, keratinocytes and lens cells [25–27]. In these cells, expression of 15-LOX was observed prior to organelle degradation [25] and 15-LOX colocalized with several, but not all, peroxisomes [26]. Interestingly, LOX-derived oxidized phospholipids are effective

substrates for lipidation of Atg8-like family proteins crucial for autophagy, such as the mammalian LC3 and yeast Atg8 [28]. 15-LOX is only expressed in selected cells and mice deficient in 12/15-LOX (homolog of 15-LOX) are generally healthy [29, 30]. Additionally, since both Lon-dependent and 15-LOX-dependent degradation mechanisms still exhibit pexophagy, it is more likely that pexophagy is the principal mode of peroxisome turnover in mammals.

Pexophagy

Autophagy is one of the main degradation pathways in cells. Contrary to the ubiquitin-proteasome system (UPS), which digests predominantly short-lived, soluble, poly-ubiquitylated proteins, autophagy is involved in the removal of many cellular component including protein aggregates, invading pathogens or even organelles. These cargoes are sequestered and delivered to lysosomes for destruction and recycling of macromolecular constituents. Three major types of autophagy are present in mammals (1) chaperone-mediated autophagy (CMA), (2) microautophagy and (3) macroautophagy.

CMA does not require vesicle formation or major changes in the lysosomal membrane as do the other two types of autophagy, nor does it require the core autophagy machinery. Instead, soluble cytosolic intracellular proteins containing a KFERQ-like motif are targeted by a cytosolic chaperone complex, consisting of the heat shock cognate protein of 73 kDa (cyt-hsc70) and its cochaperones, to the lysosomal membrane. Then proteins are unfolded and translocated into the lysosomal lumen via interaction with the lysosome-associated membrane protein type 2A (LAMP-2A) [31], and they are rapidly degraded (for details see [32]).

In micro- and macroautophagy, substrates for lysosomal degradation are enclosed in vesicles. The main difference between them is morphology, as well as the origin of the membrane used to enclose cargo. During macroautophagy, a double-membrane vesicle, called the autophagosome, is formed from lipids derived from multiple sources [33]. Each mature autophagosome is subsequently transported using microtubules and finally fused with endosomes/lysosomes (for more information about the autophagy machinery see reviews: [34–36]). In contrast, during microautophagy, engulfment of cargo (e.g. a portion of the cytoplasm) occurs by invagination, protrusion and/or septation of the lysosomal membrane [37]. Macroautophagy, or rather pexophagy, a type of selective macroautophagy where a peroxisome is selectively sequestered into a specialized autophagosome, is the prevalent mode of peroxisome destruction in mammals.

Multiple lines of evidence exist for mammalian pexophagy. First, autophagic degradation of peroxisomes is induced after treatment of cultured cells or animals with hypolipidemic drugs that cause hepatomegaly and proliferation of peroxisomes accompanied by remarkable elevation of catalase activity [38–41], but all effects are reversible after discontinuation of drug treatment [42]. The peroxisome number rapidly returns to basal levels in an autophagy- and lysosome-dependent manner and is inhibited by leupeptin [43], an inhibitor of lysosomal cysteine proteases [44]. The stages of autophagosome formation around peroxisomes have been documented [45]. Peroxisome degradation via the autophagy

machinery was also confirmed by using other autophagy inhibitors such as 3-methyladenine (3-MA) [13, 46, 47]. Finally, the involvement of the autophagy machinery in peroxisome degradation was proven in mice whose livers were deficient in Atg7, an E1-like enzyme involved in phagophore (pre-autophagosomal membrane) maturation. In autophagy-deficient ($Atg7^{-/-}$) mouse hepatocytes, pexophagy was impaired upon removal of the peroxisome proliferation stimulus [48].

Pexophagy receptors

All selective autophagy pathways require specific cargo receptor(s) and/or adaptors. These proteins simultaneously recognize specific cargo destined for destruction by autophagy and bind autophagy effectors belonging to the LC3 or GABARAP subfamilies of the Atg8-like protein family. Their physiological role is to bridge selected substrates to the autophagy machinery. Selective autophagy cargo receptors/adaptors have modular domains responsible for interactions with other proteins [49]. Many such receptors/adaptors exist in mammals [50]. Based on their predisposition to ubiquitin (Ub) binding, autophagy cargo receptors can be divided into two groups: those that do or do not bind ubiquitylated cargo through defined ubiquitin-binding domains (UBDs). Members of the latter group usually contain a transmembrane (TM) or other domain/motif that allows their interaction with substrate.

As of now, four receptors/adaptors for pexophagy have been proposed among eukaryotes: yeast Atg30 (from *P. pastoris*) [51] and Atg36 (from *S. cerevisiae*) [52, 53], which cannot bind ubiquitin but possess other motifs for substrate recognition, as well as mammalian NBR1 [54] and p62 [55], which recognize ubiquitin (Fig. 2).

Despite low sequence similarity between PpAtg30 and ScAtg36 proteins, both are required for pexophagic receptor protein complex (RPC) formation and are pexophagy-specific. Both proteins are attached to peroxisomes via interaction with at least one peroxin and recruit the autophagy machinery via interaction with Autophagy Related (Atg) proteins. ScAtg36 is recruited to peroxisomes in a Pex3–dependent manner [53] and interacts with ScAtg11, an adaptor protein that interacts with the core autophagy machinery. ScAtg36 also interacts with ScAtg8, an ubiquitin-like protein crucial for autophagosome maturation by expansion of the phagophore membrane. PpAtg30 interacts with PpPex3 and PpPex14 [56] on the peroxisome membrane, as well as with PpAtg11, PpAtg8 [51] and another component of the autophagy machinery, PpAtg17, responsible for pre-autophagsomal structure (PAS) organization [51, 57]. Recently, a new protein in *P. pastoris*, Atg37, which is evolutionarily conserved in higher eukaryotes and regulates the assembly of the pexophagic RPC was described [57]. PpAtg37 is an integral peroxisomal membrane protein binding PpPex3 as well as PpAtg30 and enables recruitment of PpAtg11 to the RPC by positive regulation of PpAtg30 phosphorylation status [57, 58]. It is unknown which kinase is involved in PpAtg30 phosphorylation crucial for pexophagy. However, in *S. cerevisiae,* Hrr25 kinase, a member of casein kinase 1δ family, triggers pexophagy by phosphorylating ScAtg36 at the Atg11–binding site [59–61]. It is worth mentioning that the phosphoregulation of PpAtg30 and ScAtg36 [62] is a general mechanism for activation of cargo-selective autophagy and is not restricted to yeast, but is also true in mammals [63, 64].

Unfortunately PpAtg30 or ScAtg36 homologs are not found outside of fungi [51, 53]. Until now, only NBR1 and p62 a proven to act as adaptors for pexophagy in mammalian cells. However, contrary to PpAtg30 and ScAtg36, mammalian NBR1 and p62 are multifunctional proteins involved in various processes [65]. NBR1 is required for degradation of protein aggregates, midbody rings and in endocytic trafficking [66–69]. The p62 protein is involved in the degradation of soluble proteins and aggregates, mitochondria, midbody rings and even bacteria [65, 66, 70–76].

Both NBR1 and p62 share similar domain architecture (Fig. 2) but the absence of the JUBA domain in p62 protein might explain why NBR1, and not p62, acts as an endogenous pexophagy adaptor. Instead, p62 supports and co-operates with NBR1 [54] or is colocalized only with ubiquitylated peroxisomes [55, 77] (Fig. 3). The NBR1 JUBA domain recognizes phosphatidylinositol-phosphates (PIPs) and phosphatidic acid (PA) lipids, which is consistent with the fact that amphipathic α-helices can insert into membranes. The JUBA domain is essential for the localization of NBR1 to late endocytic vesicles [68], as well as peroxisomes [54]. Interestingly, because these PIPs are components of diverse membranes, the JUBA structure alone cannot discriminate among different compartments but, in co-operation with the UBA domain, it can distinguish late endocytic vesicles and Ub-tagged peroxisomes [54].

Peroxisome recognition for pexophagy

Little is known regarding how pexophagy is triggered or how dysfunctional peroxisomes are recognized and selected. Recent data indicate that ubiquitylation of peroxisomal membrane-associated proteins might trigger pexophagy. First, evidence of such a mechanism was the demonstration that pexophagy in mammalian cells is enhanced by the fusion of ubiquitin to the peroxisomal membrane proteins, PMP34 and PEX3 [78] (Fig. 4A). In these proteins, ubiquitin facing the cytosol was recognized by ubiquitin-binding autophagy adaptors. However, it was unclear if such ubiquitylation occurs in physiological conditions. Even the recent data reporting that overexpression of PEX3 causes its ubiquitylation and pexophagy [55] does not address the physiological relevance, especially because pexophagy was still observed when ubiquitylation of PEX3 was abrogated [55]. However, new data focusing on PEX5 ubiquitylation has shed light on peroxisome recognition by the autophagy machinery, albeit under certain specific conditions.

Several yeast peroxins (Pex4, Pex5, Pex7 and Pex20) are ubiquitylated [79] during the peroxisomal protein import cycle but in mammals only PEX5 was proven [80]. During this import cycle, *S. cerevisiae* Pex5 is ubiquitylated in two different ways. Under normal physiological conditions, mono-ubiquitylation of Pex5 on C6 by the E2 enzyme, Pex4, and the E3 ligases, Pex12 and Pex10, is required for receptor recycling to the cytosol. This process requires the AAA-ATPases, Pex1 and Pex6, to export Pex5 in an ATP-dependent fashion, from peroxisomes to the cytosol, where Pex5 is deubiquitylated (by Ubp15) to enable another round of import. However, malfunction of the Pex5 recycling machinery induces the RADAR (Receptor Accumulation and Degradation in the Absence of Recycling) mechanism, wherein Pex5 becomes poly-ubiquitylated on lysines (K18 and K24 in *S. cerevisiae* Pex5) by the E2 enzymes, Ubc4 or Ubc5, and the E3 ligase, Pex2. Poly-

ubiquitylated Pex5 is then extracted from the peroxisomal membrane and degraded via the UPS [81].

Two independent reports indicate that ubiquitylation of mammalian PEX5 also triggers pexophagy. The first report showed that export-incompetent PEX5 (PEX5-EGFP), generated by PEX5 fusion to a bulky C-terminal tag, activates pexophagy in SV40 large T antigen-transformed mouse embryotic fibroblasts [82]. This PEX5 variant was properly mono-ubiquitylated at C11 (equivalent to *S. cerevisiae* C6) but its recycling was impaired (Fig. 4B). Interestingly, expression of mutants $PEX5^{C11K}$-EGFP and $PEX5^{C11A}$-EGFP stimulated or inhibited pexophagy, respectively, indicating that mono-ubiquitylation of PEX5-EGFP at C11 is crucial for triggering ubiquitin-dependent pexophagy [82].

The second report demonstrated that upon activation by ROS, Ataxia-telangiectasia mutated (ATM) kinase, in response to ROS, phosphorylates human PEX5 at S141, thereby stimulating its mono-ubiquitylation at K209 [77]. This, in turn, activates pexophagy via p62 binding to mono-ubiquitylated PEX5. It is unknown if K209 ubiquitylation is only characteristic for ROS-induced pexophagy or is a general pexophagy mechanism in mammals. Nevertheless, the mechanism of pexophagy activation by ROS signaling sheds new light on peroxisome recognition during pexophagy.

ATM is a kinase that is directly activated by ROS. The oxidized form of this kinase forms an active disulphide-crosslinked dimer [83, 84]. ATM is present in the nucleus, where it works as a DNA damage response sensor [85–87], but it is also localized to peroxisomes upon ROS activation [88, 89]. ATM localization to peroxisomes is mediated by PEX5 binding to an internal PTS1-like sequence in ATM and its transport is accelerated in response to H_2O_2. Interaction between PEX5 and active ATM causes PEX5 phosphorylation at S141 and subsequent PEX5 ubiquitylation at K209. However, it is unknown when PEX5 is phosphorylated by ATM and which E2 is responsible for K209 ubiquitylation. It is proposed that peroxisomal RING proteins (PEX2, PEX10, PEX12) might be the E3 ligases. Once ATM reaches peroxisomes, it induces autophagy through two pathways: mono-ubiquitylated PEX5 (at K209) binds p62 to target peroxisomes for pexophagy and ATM represses mammalian target of rapamycin complex 1 (mTORC1) - a negative autophagy regulator [77]. ATM signaling at peroxisomes activates AMP activated protein kinase (AMPK), which subsequently stimulates unc51-like protein kinase 1 (ULK1) [90, 91] essential for autophagy in mammalian cells [92]. Active ATM also activates the tuberous sclerosis complex (TSC) by phosphorylation of TSC2, which represses mTORC1 activity [93] (Fig. 4C). It should be emphasized here that, like ATM, TSC signaling nodes, TSC1 and TSC2, interact with PEX19 and PEX5 and therefore, in response to ROS, are localized to peroxisome membranes [94].

Physiological role of pexophagy

Because peroxisomes are involved in multiple metabolic pathways, they influence many biological processes [95, 96]. Peroxisomes have been linked to apoptosis [7, 97, 98], cellular aging [99, 100], cancer development [42, 101], diabetes [102, 103], inflammation [104] and innate immunity [105, 106]. Peroxisome homeostasis disruption may be the basis of several

human diseases and could be related to organism physiology. This link to diseases is also true for pexophagy. For example, an endotoxin-induced stress study revealed that pexophagy prevents the accumulation of functionally compromised peroxisomes, altered redox balance and renal damage in human and mice vascular endothelial cells exposed to lipopolysaccharides [107, 108]. Additionally, a mutated form of the pexophagy-specific protein, ACBD5 is linked to cone cell retinal dystrophy [109].

The cellular aging process nicely illustrates one physiological role of pexophagy. It is widely known that genetic inhibition of autophagy induces degenerative changes in mammalian tissues that resemble those associated with aging. Pexophagy is linked to this process. During cell senescence, progressive disruption of H_2O_2 metabolism affects peroxisomes. During aging, peroxisomes lose their ability to import matrix proteins, like catalase, and their number increases [110].

Concluding remarks and future directions

Pexophagy is the main degradation mechanism maintaining peroxisome homeostasis in mammalian cells. Unfortunately, despite its important role in cell physiology, pexophagy is just starting to be explored in mammals. While yeast cells are excellent model systems to decipher the mechanisms and molecular players underlying pexophagy, the molecular basis for regulation and specificity of these processes, and even the players, are poorly understood in mammalian cells. As of now, most of the gathered data refers to ubiquitin-dependent pexophagy and point to ubiquitin as a factor in triggering pexophagy. Although work from two groups has identified PEX5 as a putative physiological target for ROS-induced pexophagy, it is unclear how exactly this works because other sites for ubiquitylation on PEX5 have been described, each leading to different physiological fates [111]. These details remain to be sorted out.

Another important unanswered question is whether NBR1 and p62 are the only adaptors for pexophagy in mammals. These ubiquitin-binding proteins may not be a sufficient mechanism for all physiological forms of pexophagy [111]. It was recently shown that down-regulation of PEX14 partly protects peroxisomes from NBR1-induced pexophagy [54]. Although the authors postulated impairment of PEX5 recruitment to the peroxisomes via PEX14 as a cause, their results do not unequivocally prove PEX5 recognition by NBR1, since PEX14 directly interacts with LC3-II under nutrient starvation conditions [112, 113] (Fig. 5A).

Furthermore, the evolutionary conservation of PpAtg37 in mammals also suggests other mechanisms for pexophagy induction. This protein is crucial in *P. pastoris* for proper phosphoregulation and therefore activation of the pexophagy receptor, PpAtg30 [57]. The mammalian orthologue of PpAtg37, ACBD5, is located on peroxisomes and is involved in pexophagy [57] (Fig. 5B). Unfortunately, ACBD5 has not been characterized well yet and its protein partners are unknown.

New pexophagy machinery components, even including pexophagy regulators, may be established by studying genes, expression of which is regulated by Hypoxia-inducible factor

2α (HIF-2α) that promotes pexophagy in mice [114]. HIFs are heterodimeric transcription factors composed of O_2-regulated α subunits (HIF1A/HIF-1α or Endothelial PAS domain-containing protein 1 (EPAS1)/HIF-2α) [115]. It was already proved that during hypoxia, HIF-1α triggers autophagic clearance of mitochondria by activating selective autophagy receptors, NIX and BNIP3. Also, as described earlier, ATM activates TSC2 activity, which leads to repression of mTORC1 in response to hypoxia, by directly phosphorylating HIF-1α on Ser696 [116]. It remains to be seen whether a similar mechanism operates in hypoxia-induced pexophagy regulated by HIF-2α.

Finally, the reports of organelle degradation in a macroautophagy-independent manner [117] suggest the involvement of other cell- and/or condition-specific mechanisms in peroxisome degradation. For example, peroxisome degradation in mammals may occur in certain cell types or conditions through other mechanisms such as micropexophagy, or by one of alternative autophagy pathways. Moreover, currently described autophagy pathways that are independent of key players of the autophagy machinery might also be alternatives for mammalian pexophagy. Two such alternative macroautophagic pathways exist in mammals. The first is an Atg5/Atg7-independent pathway and the second is the non-canonical autophagy pathway, which is independent of Beclin 1 [117].

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Abbreviations

AMP	activated protein kinase
ATM	Ataxia-telangiectasia mutated
CMA	chaperone-mediated autophagy
EGFP	enhanced green fluorescent protein
HIF-2α	hypoxia-inducible factor 2α
15-LOX	15-lipoxygenase
mTORC1	mammalian target of rapamycin complex 1
PAS	preautophagosomal structure
PIP	phosphatidylinositol-phosphate
PTS	peroxisome targeting signal
ROS	reactive oxygen species
RPC	receptor protein complex
TSC	tuberous sclerosis complex

UBA	ubiquitin-associated
ULK1	unc51-like protein kinase 1

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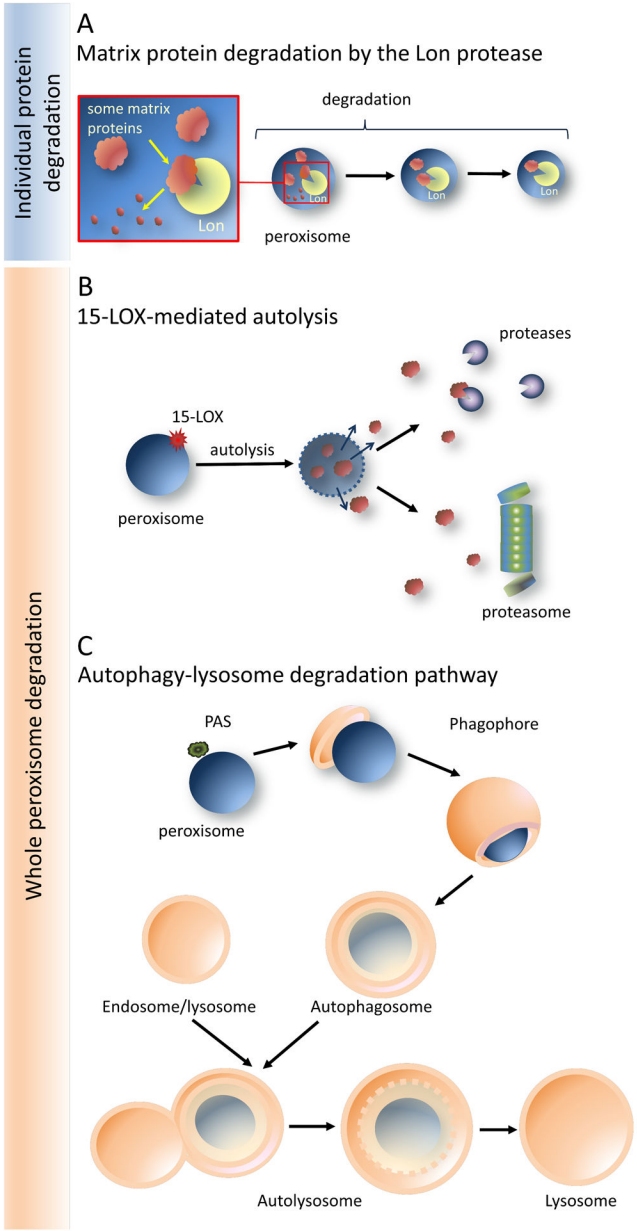

Fig. 1. Peroxisome degradation pathways in mammalian cells

The degradation of peroxisomal proteins and whole peroxisomes is accomplished by at least three distinct mechanisms. (A) Lon protease-dependent proteolysis degrades individual peroxisomal matrix proteins and might cause a reduction in peroxisome mass, depicted by a smaller peroxisome. (B) 15-lipoxygenase (15-LOX)-mediated autolysis and (C) autophagy-mediated lysosomal degradation (pexophagy) pathways remove whole peroxisomes by protein destruction via the UPS and cytosolic proteases or lysosomal proteases, respectively.

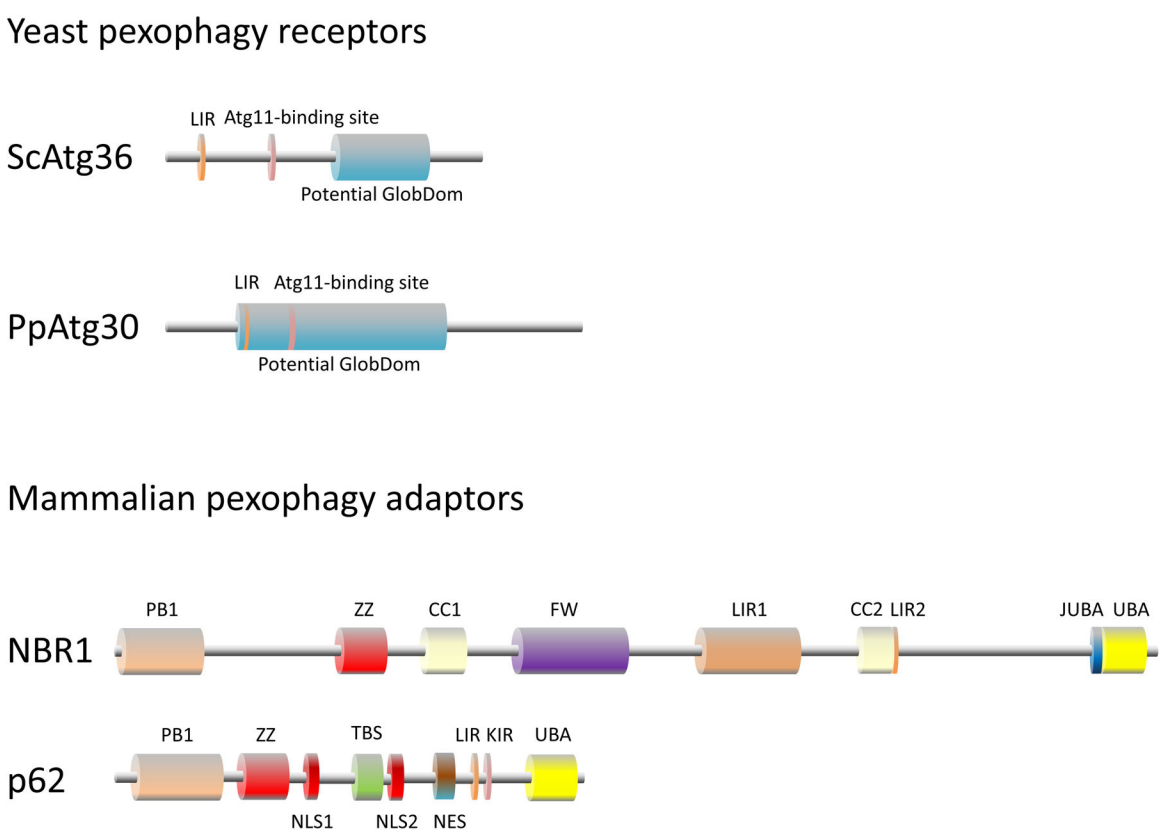

Fig. 2. Domain and motif organization of yeast and mammalian pexophagy receptors/adaptors

Yeast ScAtg36 and PpAtg30 are pexophagy receptors that directly recognize proteins on the peroxisome surface and interact with the autophagy machinery via their LIR motifs and Atg11-binding sites. Regions responsible for peroxisome recognition (e.g. Pex3 binding) are still undefined in these proteins. Potential globular domains (GlobDom) by the Russell/Linding definition were predicted by us using GlobPlot 2.3. Mammalian NBR1 and p62 are adaptors that bind ubiquitylated cargo through defined ubiquitin-binding domains. Both proteins contain several conserved domains which include: Phox/Bem1p domain (PB1) at the N-terminus, which allows for interaction with another p62 molecule, ZZ-type zinc finger domain in the middle and ubiquitin-associated domain (UBA) at the C-terminus to bind ubiquitylated cargo. Additionally, NBR1 contains two coiled-coil regions (CC1 and CC2) for homodimerization, a weakly characterized FW domain also called the NBR1 box, which is conserved in all NBR1-like proteins following the ZZ domain and an amphipathic α-helical structure (JUBA) involved in lipid binding and located near the C-terminus, upstream of the UBA domain. Additionally, p62 has a TRAF6 binding (TBS) domain, a KIR motif (Keap 1-interacting) and two basic monopartite nuclear localization signals (NLS1 and NLS2) and one nuclear export signal (NES). All proteins are drawn to scale.

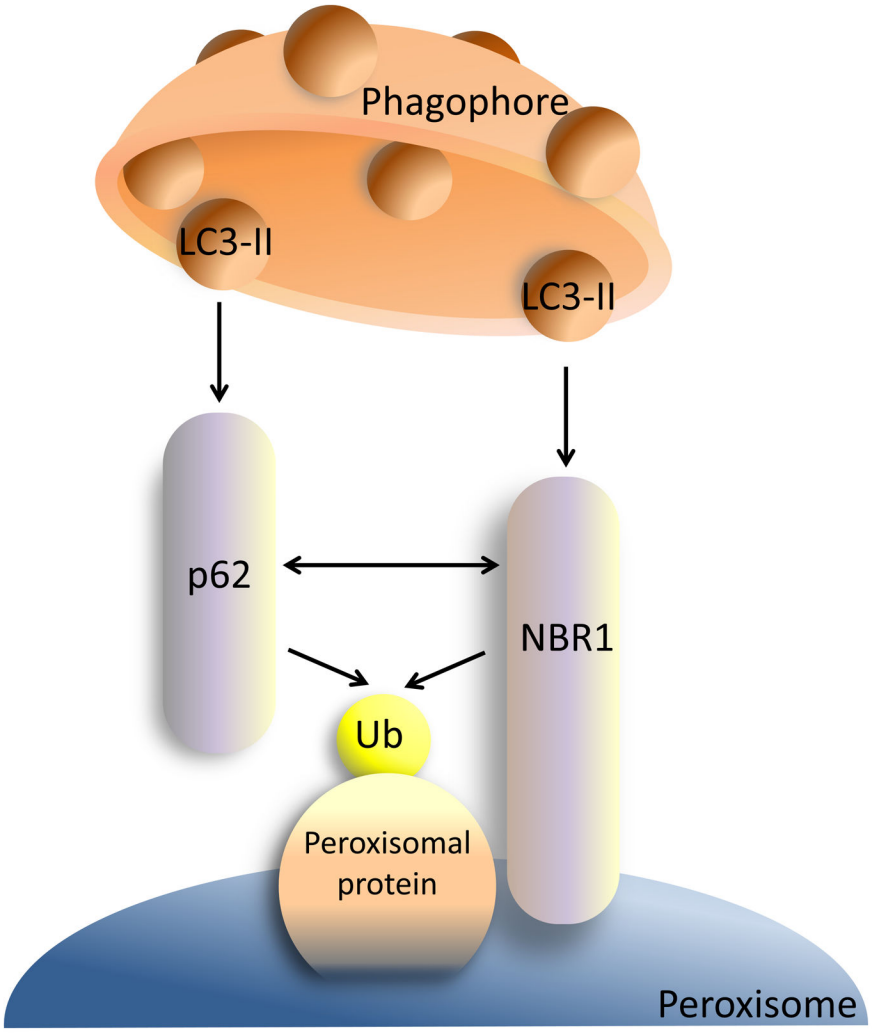

Fig. 3. Interplay between NBR1 and p62 in peroxisome recognition

NBR1 localizes on peroxisome where it binds lipids and ubiquitylated proteins via the JUBA and the UBA domains, respectively. The absence of JUBA may account for its lack of binding to peroxisome membranes, but the UBA domain recognizes ubiquitylated peroxisomal proteins. Both NBR1 and p62 support each other and cooperate. Forms of pexophagy have been described in which NBR1 or p62 act alone, or in concert, to bind an ubiquitylated peroxisomal target, and in some cases neither adaptor appears to be involved [111].

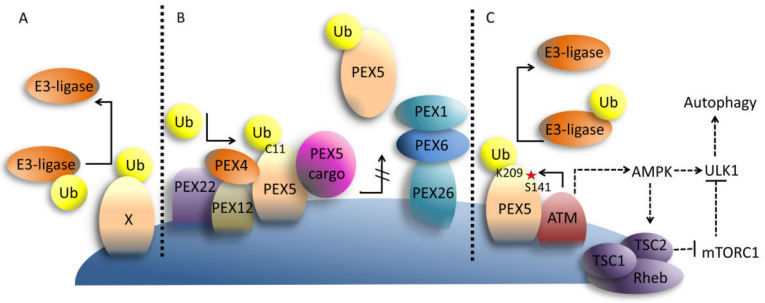

Fig. 4. Ubiquitin-dependent peroxisome recognition for pexophagy in mammalian cells

At least three different mechanisms have been proposed to trigger ubiquitin-dependent pexophagy. A) Ubiquitylation of overexpressed peroxisomal membrane-associated proteins (X), such as PEX3 and PMP34, has been shown to induce pexophagy. B) The pexophagy target is PEX5, which is mono-ubiquitylated on C11 in SV40 T-antigen transformed cells [82]. This serves as a pexophagy signal when PEX5 recycling from peroxisomes to the cytosol by the receptor recycling complex (AAA-ATPases, PEX1 and PEX6, anchored at peroxisomes via PEX26) is blocked. C) In ROS-mediated pexophagy, PEX5 is the pexophagy target, but mono-ubiquitylation at K209 is necessary for pexophagy [77]. As descried in the text, ATM phosphorylates PEX5 at S141 (red star), which allows ubiquitylation at K209 by RING-domain E3 ligases associated with peroxisomes. ATM simultaneously inhibits mTORC1 and activates ULK1, via AMPK, to stimulate autophagy (dotted arrows). For more information, see section “Peroxisome recognition for pexophagy”.

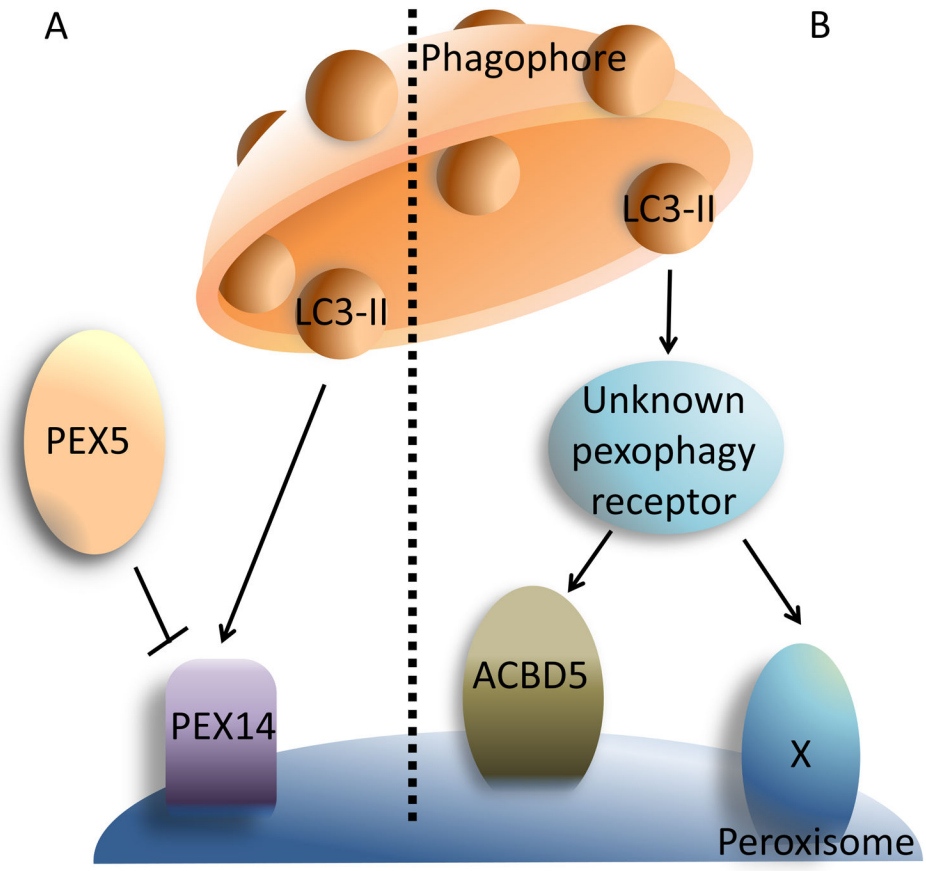

Fig. 5. Models of ubiquitin-independent pexophagy in mammalian cells

Two proposed mechanisms for pexophagy activation without peroxisome ubiquitylation. A) PEX14 directly binds LC3-II and engages the autophagy machinery. The binding of PEX5 and LC3-II to PEX14 is competitive. In nutrient-rich conditions, peroxisome biogenesis would be needed and PEX5 would bind to PEX14, thereby limiting pexophagy. In contrast, under starvation conditions, peroxisome biogenesis would be inhibited and LC3-II would bind PEX14, allowing pexophagy to proceed [112, 113]. B) ACBD5 is the only pexophagy-specific protein known to date [57, 58]. Since it does not interact directly with LC3-II, it could be involved in recruitment of the pexophagy-specific receptor/adaptor. Alternatively, another peroxisome-localized protein (X) might function as a pexophagy-specific adaptor.