Cell Insight 3 (2024) 100152

Contents lists available at ScienceDirect

Cell Insight

journal homepage: www.journals.elsevier.com/cell-insight

Review

Recent progresses in the late stages of autophagy

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ARTICLE INFO

Keywords:
Autophagy
Lysosome
Autophagosome-lysosome fusion
Autophagic lysosome reformation
Autophagosomal components recycling

ABSTRACT

Autophagy, a lysosome-dependent degradation process, plays a crucial role in maintaining cell homeostasis. It serves as a vital mechanism for adapting to stress and ensuring intracellular quality control. Autophagy deficiencies or defects are linked to numerous human disorders, especially those associated with neuronal degeneration or metabolic diseases. Yoshinori Ohsumi was honored with the Nobel Prize in Physiology or Medicine in 2016 for his groundbreaking discoveries regarding autophagy mechanisms. Over the past few decades, autophagy research has predominantly concentrated on the early stages of autophagy, with relatively limited attention given to the late stages. Nevertheless, recent studies have witnessed substantial advancements in understanding the molecular intricacies of the late stages, which follows autophagosome formation. This review provides a comprehensive summary of the recent progresses in comprehending the molecular mechanisms of the late stages of autophagy.

Autophagy, a fundamental cellular process conserved from yeast to mammals, plays a pivotal role in maintaining cellular homeostasis by facilitating the degradation and recycling of damaged organelles and proteins (Kraft & Reggiori, 2024; Vargas et al., 2023). Autophagy contributes to crucial cellular functions such as adaptation to nutrient deprivation, organelle turnover, and immunity modulation. Dysregulation of autophagy has been implicated in various diseases, including neurodegenerative disorders, cancer, metabolic syndromes, and infectious diseases (Deretic, 2021; Klionsky et al., 2021; Mizushima & Levine, 2020). Understanding the detailed mechanism of autophagy is thus essential for elucidating its broader implications in health and disease.

This intricate process involves the sequestration of cytoplasmic components within double-membraned autophagosomes, which subsequently fuse with lysosomes for substrate degradation.

Here, we categorize autophagy into two stages: the early stage and the late stage. The early stage of autophagy encompasses autophagosome formation, while the late stage refers to the subsequent stages following autophagosome formation.

1. Autophagosome-lysosome fusion

Once pre-autophagosomes detach from the endoplasmic reticulum (ER) and mature into fully sealed autophagosomes, they begin to acquire tethers and SNARE proteins to initiate their fusion with lysosomes (Yu et al., 2018; Zhao & Zhang, 2019). These tethers and SNARE proteins coordinate in bringing autophagosomes and lysosomes into close contact and the final fusion (Fig. 1 and 2).

1.1. SNAREs

Following the close tethering of autophagosomes to lysosomes, SNARE proteins on opposite membranes come together to form trans-SNARE complexes that facilitate membrane fusion (Jahn & Scheller, 2006). Two distinct SNARE complexes have been identified for autophagosome-lysosome fusion. The first complex consists of STX17, SNAP29, and VAMP7 or VAMP8(Itakura et al., 2012; Takats et al., 2013). The Qa-SNARE STX17 localizes to autophagosomes, while the R-SNARE VAMP7/VAMP8 is on late endosomes/lysosomes. The cytosolic Qbc-SNARE SNAP29 is recruited to autophagosomes (Itakura et al.,

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https://doi.org/10.1016/j.cellin.2024.100152
Received 18 October 2023; Received in revised form 30 January 2024; Accepted 30 January 2024
Available online 8 February 2024

2012). An interesting finding that the incomplete blockage of fusion in STX17 knock-out cells led to the discovery of a second SNARE complex (YKT6-SNAP29-STX7) responsible for autophagosome-lysosome fusion in mammalian cells (Matsui et al., 2018). The R-SNARE YKT6 is recruited to autophagosomes through its N-terminal longin domain, rather than its C-terminal palmitoylation and farnesylation. The Qa-SNARE STX7 is localized to lysosomes. Depletion of any of these SNARE proteins partially inhibits autophagosome-lysosome fusion, while depletion of both STX17 and YKT6 almost completely blocks this fusion (Matsui et al., 2018). (Fig. 1) Although the role of Ykt6 in autophagy is conserved across different species, the mechanism differs in Drosophila and yeast. In Drosophila, Ykt6 localizes to lysosomes and autolysosomes. Both Ykt6 and Vamp7 are essential for autophagosome-lysosome fusion and serve as mutually exclusive subunits within the Syx17-Snap29 complex. It is suggested that Vamp7 is directly involved in membrane fusion, while Ykt6 functions as a non-conventional, regulatory SNARE in this process (Takats et al., 2018). Ykt6 facilitates HOPS recruitment to the autophagosome-lysosome fusion site, which is followed by Ykt6 replacement with Vamp7 (Takats et al., 2018). In budding yeast, the R-SNARE Ykt6 acts on the autophagosome and forms a SNARE bundle with the Q-SNAREs Vam3, Vti1, and Vam7 on the vacuole (Bas et al., 2018; Gao, Reggiori, & Ungermann, 2018). Post-fusion, the trans-SNARE

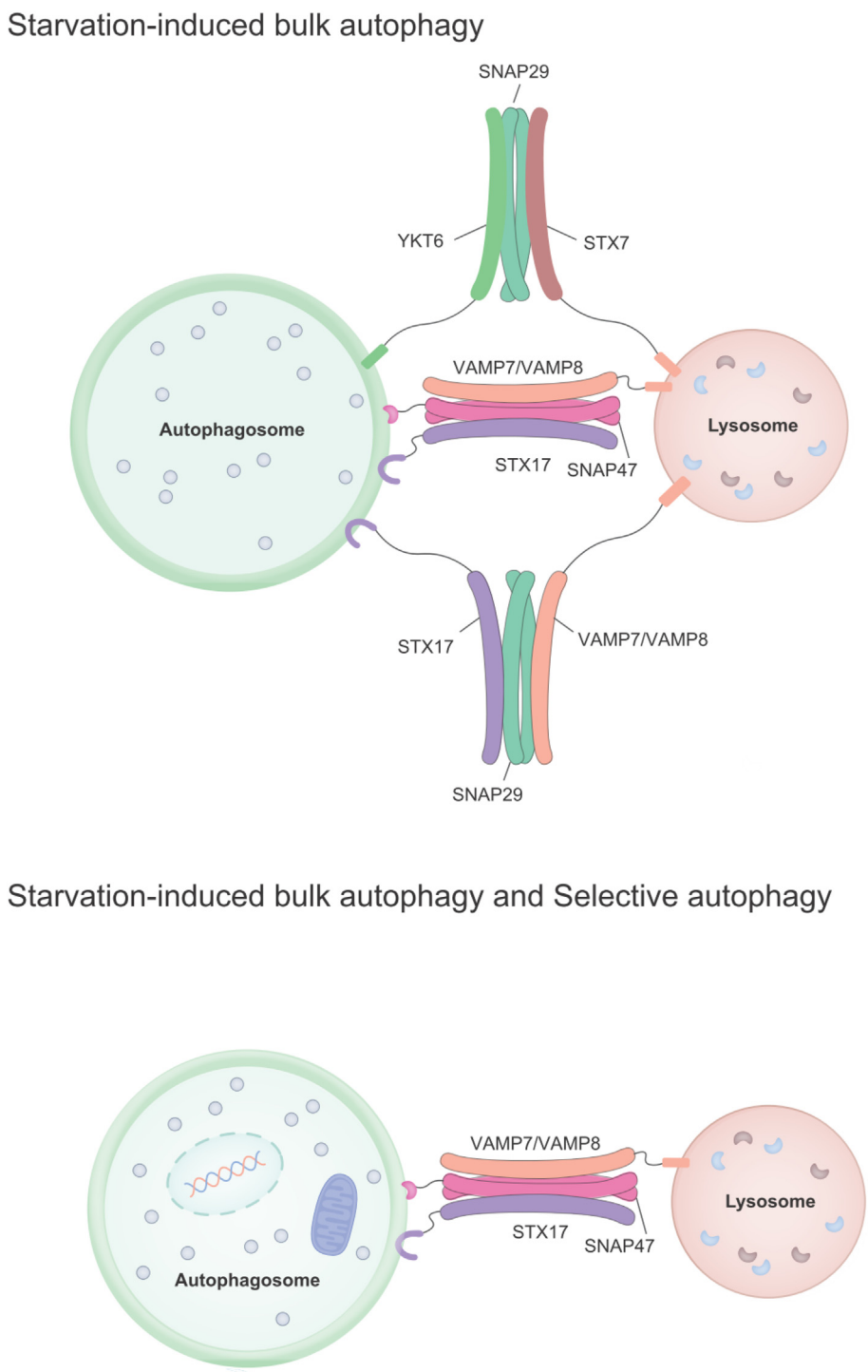

Fig. 1. The SNARE complexes in autophagosome-lysosome fusion.

complex transforms into the cis-SNARE complex and disassembles through the actions of NSF and α-SNAP on autolysosomes (Abada et al., 2017; Ishihara et al., 2001).

Two SNAP29-containing SNARE complexes have been extensively studied in starvation-induced bulk autophagy, while the relevant SNARE complexes in other types of autophagy occurring under non-starvation conditions remains unknown. Recently, we found that autophagosome-lysosome fusion in selective autophagy under non-starvation conditions does not require SNAP29-containing SNARE complexes, but instead requires the STX17-SNAP47-VAMP7/VAMP8 SNARE complex. The STX17-SNAP47-VAMP7/VAMP8 SNARE complex also functions in starvation-induced autophagy. SNAP47 is recruited to autophagosomes following concurrent detection of ATG8s and PI(4,5)P_2 via its Pleckstrin homology domain. By contrast, SNAP29 is excluded from selective autophagy due to inactivation by O-GlcNAcylation under non-starvation conditions. STX17-SNAP47-VAMP7/VAMP8 SNARE complex is a default SNARE complex responsible for autophagosome-lysosome fusion in both selective and bulk autophagy (Jian et al., 2024). (Fig. 1)

1.2. Modifications on SNAREs

O- GlcNAcylation of the SNARE protein SNAP29 plays a role in nutrient-dependent autophagy regulation (Guo et al., 2014). In mammalian cells, either OGT knockdown or mutating SNAP29's O-GlcNAc sites leads to increased SNAP29-containing SNARE complex formation, resulting in enhanced fusion between autophagosomes and endosomes/lysosomes. In *Caenorhabditis elegans*, depletion of *ogt*-1 has a similar influence on autophagy (Guo et al., 2014).

The acetylation of STX17 is under the specific regulation of histone acetyltransferase CREBBP/CBP and deacetylase HDAC2 (Shen et al., 2021). Upon starvation and mTOR inhibition, inactivated CREBBP results in the deacetylation of STX17 within its SNARE domain. This deacetylation enhances the interaction between STX17 and SNAP29 and promotes the assembly of the STX17-SNAP29-VAMP8 SNARE complex via facilitating the interaction between STX17 and the tethering complex HOPS, thereby further enhancing the process of autophagosome-lysosome fusion (Shen et al., 2021).

mTORC1 exerts a negative regulatory influence on the assembly of the SNARE complex (STX17-SNAP29-VAMP8) by phosphorylating VAMP8, thereby impeding autophagosome-lysosome fusion (Huang et al., 2021).

ULK also functions in autophagosome-lysosome fusion stage other than its roles at autophagosome formation stage (Klionsky et al., 2021; Yamamoto et al., 2023). ULK1-dependent phosphorylation of YKT6 within its SNARE domains inhibits its binding to the Qbc-SNARE SNAP29, which hinders the fusion of autophagosomes with lysosomes (Sanchez-Martin et al., 2023). In yeast, Ykt6 is directly phosphorylated by the Atg1 kinase, maintaining this SNARE protein in an inactive state. Ykt6 phosphorylation disrupts its interaction with the vacuolar SNAREs Vam3 and Vti1, preventing premature fusion between autophagosomes and vacuoles (Barz et al., 2020).

The kinase activity of ULK is critically essential for STX17 translocation to autophagosomes (Wang et al., 2023). ULK interacts with STX17 and phosphorylates it at Ser289. This phosphorylated form of STX17 translocates to autophagosomes by interacting with ATG8 family proteins via the actin binding protein FLNA (Wang et al., 2023). Notably, this recruitment process is not dependent on the actin binding activity of FLNA and the role of FLNA is cell type specific. In addition, ULK1 also undergoes phosphorylation at Ser423 by PKCα, leading to reduced binding between ULK1 and STX17. This reduction results in a decrease in fusion events between autophagosomes and lysosomes (Wang et al., 2018). Notably, O-GlcNAcylation of ULK1 at Ser409 and Ser410 counters its phosphorylation at Ser423. The Ser409A and Ser410A double mutants (ULK1-2A) exerts less stable by promoting its interaction with the CMA chaperone HSC70. This ULK1-2A mutant further diminishes the association of ULK1 with STX17, ultimately inhibiting the fusion between autophagosomes and lysosomes (Shi et al., 2022). However, whether the phosphorylation and O-GlcNAcylation of ULK1 also affects the translocation of STX17 to autophagosomes is a question that remains to be investigated.

1.3. HOPS

The HOPS complex, traditionally recognized as a core tether complex involved in vesicle fusion, was originally identified as a tether complex for homotypic fusion of lysosomes/vacuoles (Balderhaar & Ungermann, 2013). It is suggested that the HOPS complex also plays a role in autophagosome-lysosome fusion in mammals and Drosophila (Jiang et al., 2014; Takats et al., 2014). The HOPS complex comprises VPS11, VPS16, VPS18, VPS33A, VPS39, and VPS41. During autophagy, both VPS33A and VPS16 interact effectively with STX17. STX17 participates in their recruitments to autophagosomes, facilitating the tethering of autophagosomes to lysosomes and the subsequent autophagosome-lysosome fusion process (Jiang et al., 2014; Takats et al., 2014).

1.4. ATG8 family proteins (ATG8s)

ATG8s, primarily the GABARAP subfamily, play pivotal roles in the process of autophagosome-lysosome fusion, while the LC3s subfamily has a less prominent role in this particular process (Nguyen et al., 2016). ATG8s bind SNAREs and tether factors on autophagic vacuoles and late endosomes/lysosomes, such as STX17 (Kumar et al., 2018),SNAP47 (Jian et al., 2024), HOPS(Gao, Langemeyer, et al., 2018; Manil-Segalen et al., 2014), Mon1-Ccz1 (Gao, Langemeyer, et al., 2018), EPG5 (Wang et al., 2016), PLEKHM1 (Nguyen et al., 2016), BRUCE(Ebner et al., 2018) and GRASP55 (Zhang et al., 2018, 2019). These interactions serve to tether autophagosomes to lysosomes. GABARAPs also recruit palmitoylated PI4KIIα to autophagosomes. The PI4P generated by PI4KIIα on autophagosomes functions in the subsequent fusion of autophagosomes with lysosomes (Wang et al., 2015).

1.5. EPG5

EPG5, a Vici syndrome protein, is recruited to late endosomes/lysosomes by direct interaction with Rab7 and late endosomal/lysosomal SNARE VAMP7/8. EPG5 also binds LC3/LGG-1 and STX17-SNAP29 binary SNARE complex on autophagosomes, stabilizing and aiding in the assembly of STX17-SNAP29-VAMP7/8 trans-SNARE complexes, thereby promoting STX17-SNAP29-VAMP7/VAMP8 mediated fusion of autophagosome with lysosome (Tian et al., 2010; Wang et al., 2016). WDR45/45B interacts with the tether protein EPG5 and target it to late endosomes/lysosomes to promote autophagosome-lysosome fusion (Ji et al., 2021). TGM2 interacts with EPG5, facilitating EPG5-mediated assembly of QabcR SNARE proteins, thereby controlling the fusion between autophagosomes and lysosomes (Zheng et al., 2023).

1.6. PLEKHM1

PLEKHM1, an effector of Rab7, localizes to late endosomes and lysosomes. PLEKHM1 bridges autophagosomes to lysosomes via binding autophagosomal ATG8 family proteins, particularly GABARAPs. It also recruits the HOPS complex to late endosomes/lysosomes, thus promoting the ultimate fusion of autophagosomes with lysosomes (Ebner et al., 2018; McEwan et al., 2015; Nguyen et al., 2016).

1.7. BRUCE

BRUCE localizes to late endosomes/lysosomes. It bridges autophagosomes with lysosomes by exhibiting a preference for interaction with GABARAP and GABARAPL1 through noncanonical LIR-containing regions, as well as with STX17 (Ebner et al., 2018).

1.8. GRASP55

GRASP55, a Golgi stacking protein, relocates to autophagosomes and late endosomes/lysosomes upon glucose deprivation. In this context, GRASP55 establishes interactions with both LC3-II and LAMP2, serving as a crucial link between LC3-II and LAMP2 to facilitate autophagosome-lysosome fusion. This function is modulated by the negative regulation of GRASP55 through O-GlcNAcylation (Zhang et al., 2018, 2019).

1.9. NRBF2

NRBF2 is a crucial component of the class III phosphatidylinositol 3-kinase complex (PI3KC3), playing a critical role in autophagosome formation. Additionally, NRBF2 contributes to autophagosome-lysosome fusion by enhancing the association of PI3KC3 with the MON1A-CCZ1 complex. This association activates the GEF activity of MON1A-CCZ1 complex and triggers RAB7 activation on the autophagosome (Cai et al., 2021).

1.10. Pacer

Pacer, a recently identified autophagy regulator, localizes to autophagic structures and positively regulates autophagosome maturation. Through anchoring to the autophagosomal SNARE STX17, Pacer recruits the PI3KC3 and HOPS complexes to autophagosomes, facilitating their activation at the specific site. Upon dephosphorylation at serine 157, Pacer undergoes acetylation by TIP60. This dephosphorylation and acetylation event enhances the recruitment of the HOPS complex to autophagosomes and contributes to autophagosome maturation (Cheng et al., 2017, 2019).

1.11. TECPR1 and TECPR2

TECPR1 binds to the Atg12-Atg5 conjugate and phosphatidylinositol 3-phosphate (PI3P) on autophagosomes to facilitate the fusion of autophagosomes with lysosomes (Chen et al., 2012). It has also been shown that TECPR1 binds lysosomal PI4P and LC3C on autophagosomes to promote autophagosome-lysosome fusion (Wetzel et al., 2020). TECPR2 has also been shown to regulate autophagosome-lysosome fusion. It associates with autophagosomes through interactions with ATG8 family proteins using its C-terminal LIR motif, and with lysosomes through interactions with VAMP8, possibly involving the HOPS complex (Fraiberg et al., 2020).

1.12. ATG14

ATG14 serves as an indispensable, autophagy-specific regulator of the PI3KC3 in autophagy initiation (Klionsky et al., 2021; Yamamoto et al., 2023). It also plays a crucial role in autophagosome-lysosome fusion by binding to the SNARE core domain of STX17 through its coiled-coil domain. This interaction stabilizes the STX17-SNAP29 binary t-SNARE complex on autophagosomes, facilitating the fusion of autophagosomes with lysosomes (Diao et al., 2015). In reconstituted proteoliposome assays, ATG14 promotes the tethering of protein-free liposomes to membranes, enhances both hemifusion and full fusion of proteoliposomes reconstituted with STX17, SNAP29 and VAMP8(Diao et al., 2015). RUNDC1, a negative regulator of autophagy, restrains the fusion of autophagosomes with lysosomes by impeding VAMP8 binding. This is achieved by holding the ATG14-STX17-SNAP29 complex and preventing its interaction with VAMP8(Zhang et al., 2023). MARCH7 (membrane-associated ring–CH–type finger 7), an E3 ubiquitin ligase, promotes K6-, K11-, and K63-linked mixed polyubiquitination on ATG14(Shi et al., 2023). This ubiquitination triggers the aggregation of ATG14 and reduces its solubility in cells. Ubiquitinated ATG14 has fewer interactions with STX17, leading to the inhibition of autophagosome-lysosome fusion(Shi et al., 2023). Moreover, ATG14 also interacts with LAMP2B and VAMP8, promoting autophagosome-lysosome fusion independently of STX17 (Chi et al., 2019).

1.13. BORC

The multi-subunit BORC complex, previously associated with the kinesin-dependent transport of lysosomes towards the cell periphery, is essential for the effective fusion of autophagosomes with lysosomes. Deletion of BORC subunits not only reduces encounters between autophagosomes and lysosomes, stemming from the incapacity of lysosomes to migrate to the peripheral cytoplasm where numerous autophagosomes originate, but also impedes the fusion process itself. This hindrance occurs by decreasing the recruitment of the HOPS tethering complex to lysosomes and the assembly of the STX17-VAMP8-SNAP29 trans-SNARE complex, leaving basal mTORC1 activity and autophagy initiation unaffected. In performing these dual functions, BORC integrates the kinesin-dependent movement of lysosomes towards autophagosomes with HOPS-dependent autophagosome-lysosome fusion (Jia et al., 2017).

1.14. STING

STING (Stimulator of Interferon Genes) negatively regulates autophagosome-lysosome fusion in energy stress-induced autophagy. During an energy crisis, the interaction between STING and STX17 is disrupted, allowing STX17 to move towards mature autophagosomes for fusion with lysosomes, thus promoting autophagy flux (Rong et al., 2022). This is a novel function of STING in energy stress-induced autophagy, distinct from its role in non-canonical autophagy induced by DNA or cGAMP. Additionally, STX17 has a function in autophagosome biogenesis 50, TBK1-phosphorylated STX17 at S202 translocates from Golgi to peripheral puncta during induction of autophagy to form mPAS (Kumar et al., 2019). The phosphorylated form of STX17 at Ser202 does not bind to STING and is primarily translocated to phagophores for the biogenesis of autophagosomes, delineating the different roles for each pool.

1.15. SCFD1

SCFD1, a Sec1/Munc18(SM)-like protein, interacts with STX17 and VAMP8, facilitating the assembly of the STX17-SNAP29-VAMP8 SNARE complex (Huang et al., 2021). Acetylation of SCFD1 negatively impacts autophagic flux, primarily by obstructing the formation of the STX17-SNAP29-VAMP8 SNARE complex. During autophagy, SCFD1 acetylation decreases. KAT2B/PCAF is the enzyme responsible for acetylating residues K126 and K515 of SCFD1, and these acetyl groups are removed by SIRT4. Furthermore, AMPK-mediated phosphorylation of SCFD1 significantly disrupts its ability to interact with KAT2B. This phosphorylation ensures that the acetylation level of SCFD1 remains low, promoting autophagosome-lysosome fusion (Huang et al., 2023).

1.16. PACSIN1

PACSIN1 is essential for the fusion of amphisomes with lysosomes during both basal autophagy and specific types of selective autophagy (Oe et al., 2022). It forms interactions with SNAP29 and plays a crucial role in the proper assembly of the STX17 and YKT6 complexes (Oe et al., 2022).

Furthermore, the isoform VAMP7B, which lacks a functional SNARE domain, competes with the functional isoform VAMP7A for binding to STX17, resulting in the inhibition of autophagosome-lysosome fusion (Tian et al., 2020). DIPK2A, an endolysosomal protein, binds to VAMP7B, which disrupts the interaction between VAMP7B and STX17 and enhances the binding of STX17 to VAMP7A. This, in turn, promotes autophagosome-lysosome fusion (Tian et al., 2020). However, it remains unclear whether DIPK2A functions as a kinase to regulate this process. SIGMAR1 and CFTR interact with ATG14, STX17, and VAMP8, or with

STX17 alone, respectively, the precise mechanism underlying the impairment of autophagosome-lysosome fusion due to their depletion remains unclear (Arora et al., 2021; Yang et al., 2019).

1.17. RAB

Rab small GTPases are key regulators of intracellular tethering and vesicle fusion processes (Lurick et al., 2017). Rab proteins can reversibly associate with membranes and bind guanosine-5′-di-(GDP) or -triphosphate (GTP) nucleotides to recruit various effectors (Muller & Goody, 2018). The role of Rab7/Ypt7 in autophagosome-lysosome fusion has been extensively investigated in yeast, flies, and mammals. Rab7 is predominantly located on late endosomes/lysosomes. Deficiency in Rab7 significantly impedes autophagosome-lysosome fusion (Gutierrez et al., 2004). Mechanistically, Rab7 interacts with various tethering proteins, including HOPS(Jiang et al., 2014; Takats et al., 2014), EPG5 (Tian et al., 2010; Wang et al., 2016) and PLEKHM1 (McEwan et al., 2015), to enhance the efficiency and specificity of autophagosome-lysosome fusion. The activity of Rab7 is intricately controlled by its GEF (GTP/GDP exchange factor) and GAP (GTPase-activating protein). A Rab7 GEF complex, Mon1-Ccz1, is directed to autophagosomes through a direct interaction with ATG8, which recruits Rab7 and HOPS to bridge autophagosomes with late endosomes/lysosomes (Gao, Langemeyer, et al., 2018; Hegedus et al., 2016). The Rab7 GAP, Armus, is recruited to autophagosomes by LC3 and aids in autophagosome maturation by regulating Rab7-GTP/Rab7-GDP cycling (Carroll et al., 2013). Rab2 is localized to the autophagosomal side and interacts with Pacer and STX17 to further specify the recruitment of the HOPS complex for autophagosome-lysosome fusion (Ding et al., 2019; Fujita et al., 2017; Lorincz et al., 2017). During starvation, RAB21 is activated by its GEF MTMR13, facilitating the movement of VAMP8 from the plasma membrane to late endosomes/lysosomes. This translocation is crucial for autophagosome-lysosome fusion (Jean et al., 2015).

1.18. Phosphoinositides

Numerous phosphoinositides (PIs) have been identified as participants in the process of autophagosome-lysosome fusion. These include PI3P, PI4P, PI(3,5)P_2, and PI(4,5)P_2. The presence of actin filaments on the lysosomal surface is essential for the fusion between autophagosomes and lysosomes. INPP5E, by decreasing lysosomal PI(3,5)P_2, leads to the stabilization of actin filaments on lysosomes mediated by cortactin, thus facilitating autophagosome-lysosome fusion (Hasegawa et al., 2016). TECPR1 promotes autophagosome-lysosome fusion by binding to PI3P and Atg12-Atg5 conjugate on autophagosomes (Chen et al., 2012). PI4P has also been implicated in the process of autophagosome-lysosome fusion (Chen et al., 2012; Sun et al., 2022). During autophagy, PI4K2A is recruited to autophagosomes by GABARAP, where it generates PI4P, which plays a critical role in autophagosome-lysosome fusion (Chen et al., 2012). TECPR1 selectively binds to PI4P on lysosomes through its PH domain and interacts with LC3C on matured autophagosomes, thereby facilitating the fusion of autophagosomes with lysosomes (Wetzel et al., 2020). PI4K2A and PIP5K1C play a crucial role in regulating the levels of PI(4,5)P_2 at late endosomes/lysosomes, which influences the fusion of autophagosomes with lysosomes. This regulation involves the control of Rab7 and PLEKHM1 cycling on late endosomes/lysosomes (Baba et al., 2019). In response to autophagy induction, the cytosolic 5-phosphatase OCRL relocates to lysosomes, where it facilitates autophagosome-lysosome fusion by decreasing lysosomal PI(4,5)P_2 levels. The elevation in lysosomal PI(4,5)P_2 inhibits MCONL1, and enhancing MCOLN1 activity rescues the autophagosome-lysosome fusion impairment observed in OCRL deficiency (De Leo et al., 2016). A minor fraction of PI(4,5)P_2 in mammalian cells arises from the conversion of PI5P to PI(4,5)P_2. PIP4K2A and PIP4K2B emerge as the most catalytically active PI5P4Ks in this conversion process. The deficiency of PIP4K2A and PIP4K2B, especially in the context of p53 loss, hampers autophagosome-lysosome fusion (Lundquist et al., 2018). In addition, PI(4,5)P_2 produced by PIPKIγi5 not only regulates ATG14 function in autophagy initiation by binding to ATG14, but also plays a role in regulating autophagosome-lysosome fusion (Tan et al., 2016). PI(4,5)P_2, along with ATG8 family proteins, recruits the SNARE protein SNAP47. SNAP47 mediates autophagosome-lysosome fusion by forming a ternary SNARE complex with STX17 and VAMP7/VAMP8(Jian et al., 2024).

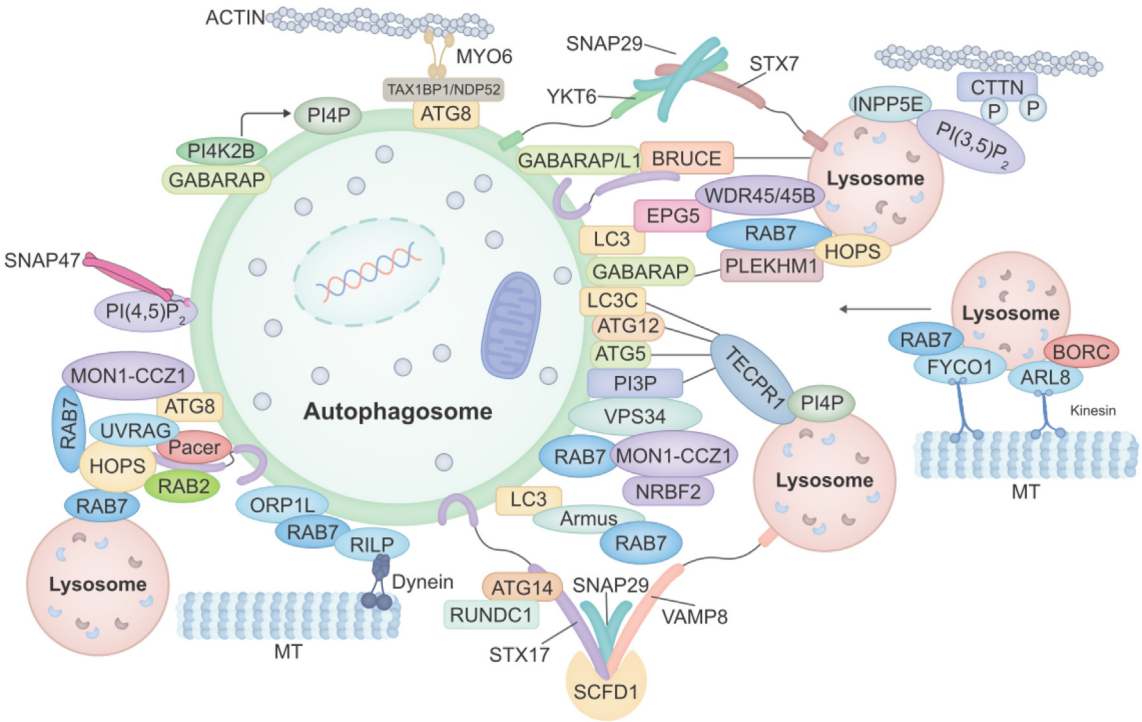

Fig. 2. The molecular mechanism for autophagosome-lysosome fusion.

2. Autolysosomal membrane recycling

Following the fusion of autophagosomes with lysosomes, their internal contents are broken down into small molecules and exported from the autolysosomes for recycling (Klionsky et al., 2021; Yamamoto et al., 2023). However, the cell needs to address the fate of the autolysosome membrane. The components of lysosome membranes are released from autolysosomes through a process involving the tubulation and scission of autolysosomal membranes, known as autophagic lysosome reformation (ALR) (Yu et al., 2010). During ALR, the final scission of tubular autolysosomes results in the formation of proto-lysosomes that lack degradative capabilities (Yu et al., 2010). These proto-lysosomes then mature into functional lysosomes by regaining lysosomal hydrolases and acidity (Yu et al., 2010). (Fig. 3) The components of autophagosomal membranes on autolysosomes are recycled through a process called autophagosomal components recycling (ACR) (Zhou et al., 2022). (Fig. 3) Dysfunction in either ALR or ACR can lead to the inhibition of autophagy (Yu et al., 2010; Zhou et al., 2022). ALR has been implicated in various human diseases, such as lysosome storage disorders, Parkinson's disease, hereditary spastic paraplegia, and spastic ataxia (Nanayakkara et al., 2023). These connections have been summarized in a recent review, we won't delve into those details here (Nanayakkara et al., 2023). Our focus in this review is solely on the molecular mechanism of autolysosomal membrane recycling.

2.1. mTOR signaling to ALR

mTOR, a nutrient-sensing protein, is crucial in autophagy regulation (Liu & Sabatini, 2020). Inhibition of mTOR induces autophagy, whereas the reactivation of mTOR during prolonged starvation triggers the initiation of ALR (Yu et al., 2010). Consistent inhibition of mTOR by rapamycin prevents ALR, resulting in the accumulation of enlarged autolysosomes (Yu et al., 2010). Importantly, mTOR reactivation not only initiates ALR but also concurrently reduces autophagy in a negative feedback manner (Rong et al., 2011; Yu et al., 2010). The degradation of autophagic cargo is necessary for mTOR reactivation during prolonged starvation. The Inhibition of lysosome function prevents ALR, leading to enlarged autolysosomes, as fibroblasts obtained from lysosomal storage disease patients which are characterized by deficiencies in specific lysosomal enzymes exhibit impaired mTOR reactivation and deficient ALR, supporting the connection between lysosome degradation and mTOR's reactivation (Yu et al., 2010).

Spinster, a lysosomal transporter, plays a crucial role in regulating ALR by influencing the reactivation of mTOR[67]. Spinster function as a hypothetical H^+/carbohydrate uniporter or lysolipid transporter within lysosomes (Dermaut et al., 2005; He et al., 2022). Deficiency in Spinster leads to profound disruption in lysosomal function, characterized by the abnormal accumulation of sugars and lysolipids, as well as excessive acidification within lysosomes (Dermaut et al., 2005; He et al., 2022; Rong et al., 2011). The block of ALR resulting from Spinster deficiency can be attributed to the defective production of amino acids, which is critical for mTOR reactivation during prolonged periods of nutrient deprivation[67]. In addition, BLOC1S1, a shared subunit of BROC and BLOC-1 complexes, also contributes to ALR regulation, partially through its modulation of mTOR activity (Wu et al., 2021).

To date, only a limited number of downstream substrates of mTOR associated with autophagic lysosome reformation have been identified. Notably, a study has reported that mTOR's phosphorylation of UVRAG, resulting in a reduction of UVRAG-VPS34 activity, which promotes ALR (Munson et al., 2015). This finding suggests the existence of a feedback loop in the late stages of autophagy, serving to initiate ALR while simultaneously terminating autophagy by employing UVRAG-VPS34 as a substrate of mTOR. Additionally, mTOR has been observed to phosphorylate the lysosomal Ca^{2+} efflux channel TRPML1, leading to the inhibition of TRPML1 activity in vivo (Onyenwoke et al., 2015), while TRPML1 activity is involved in ALR (Li et al., 2016). This suggests the

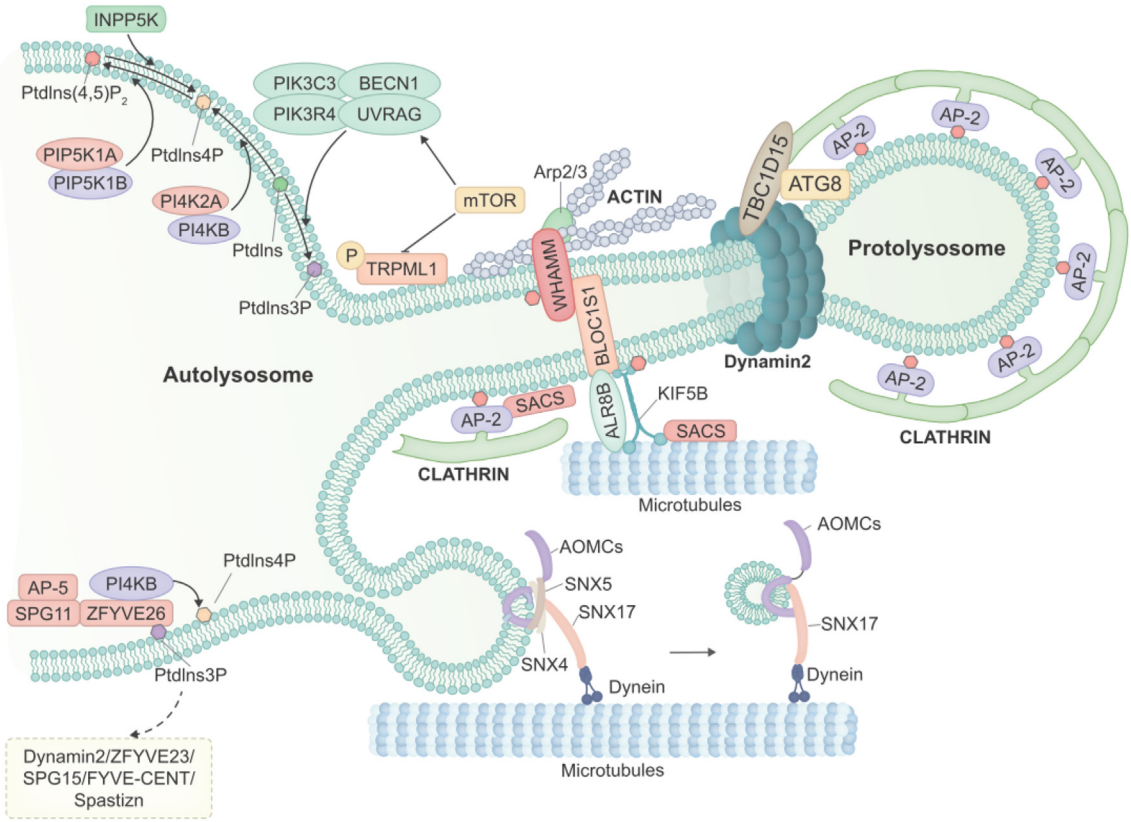

Fig. 3. The molecular mechanism for autolysosomal components recycling.

existence of a potential signaling pathway involving mTOR-TRPML1-ALR. However, it is crucial to note that the role of dephosphorylation mutants of TRPML1 in ALR remains unexplored. Intriguingly, when assessing whole-lysosome TRPML1 currents in an endolysosomal electrophysiology assay, it was observed that mTOR inhibition did not activate these currents (Li et al., 2016). It's possible that other intracellular components, which may be absent in this semi-in vitro assay, cooperate in the regulation of TRPML1 activity. Therefore, further comprehensive investigations are required to explore this complex interplay in greater detail.

2.2. *The important but complicated roles of phosphoinositides on autophagic lysosome reformation*

ALR is finely regulated by multiple phosphoinositides through transient and reversible interconversions in precise spatiotemporal patterns. During autophagy, there is a significant increase in the level of PI4P on lysosomes, which is observed on both spherical and tubular autolysosomes (Rong et al., 2012). PI4P is converted into PI(4,5)P_2 by a phosphatidylinositol-4-phosphate 5-kinase, PIP5K1B(Rong et al., 2012). PI(4,5)P_2 plays a crucial role in recruiting AP2 and clathrin to the autolysosomal membrane, generating membrane curvature that facilitates autolysosome tubulation (Rong et al., 2012). WHAMM, a nucleation-promoting factor (NPF) protein, induces actin assembly by activating Arp2/3 (Campellone & Welch, 2010). It is recruited to autolysosomes through autolysosomal PI(4,5)P_2, where it promotes actin assembly (Dai et al., 2019). The branched actin network and WHAMM are localized on the main body of autolysosomes and at the base of reformation tubules (Campellone & Welch, 2010), suggesting a likely role for WHAMM in initiating autolysosome tubulation by exerting pushing forces through the branched actin network. The plus-end-directed motor protein KIF5B is also essential for ALR and is recruited to the autolysosomal membrane by PI(4,5)P_2 (Du et al., 2016). Clathrin functions in generating PI(4,5)P_2-enriched microdomains on autolysosomes, leading to the clustering of KIF5B. These KIF5B clusters may serve as sites to initiate tubulation (Du et al., 2016). It's worth noting that other kinesin family proteins may also participate in autolysosome tubulation. BLOC1S1, another player in autolysosomal tubulation, engages ALR8B, which recruits KIF5B to autolysosomes. Additionally, BLOC1S1 interacts with KIF5B and WHAMM, both of which are PI(4,5)P_2 binding proteins, suggesting the involvement of BLOC1S1 in initiating and regulating autolysosomal tubules (Wu et al., 2021). However, it has not been determined whether BLOC1S1 regulates ALR through the BLOC complex, the BROC complex, or both, as it is a shared subunit of both complexes (Di Pietro et al., 2006; Pu et al., 2015; Setty et al., 2007). In nanoparticle-treated hepatocytes, cells are unable to convert PI4P into PI(4,5)P_2. The exogenous supplementation of PI(4,5)P_2 suppresses the enlargement of autolysosomes (Zhang et al., 2017). Dynamin2 is recruited to autolysosomes and generates proto-lysosomes by scissoring autolysosome tubules (Schulze et al., 2013). Another phosphatidylinositol-4-phosphate 5-kinase, PIP5K1A, also play a role in this scission process, as indicated by the presence of hyperextended tubules in PIP5K1A knockdown cells (McGrath et al., 2021; Rong et al., 2012). However, further research is needed to determine which specific PIs are involved in the recruitment of dynamin2 to autolysosomes, given its ability to interact with PI3P, PI4P, and PI(4,5)P_2, all of which are present on autolysosomes.

PI4P is synthesized on autolysosomes by PI4K2A and PI4K3B (Khundadze et al., 2021; Sridhar et al., 2013). Depletion of either PI4K2A and PI4K3B results in the prolongation of autolysosome tubules and increased clathrin and dynamin2 presence on autolysosomes (Khundadze et al., 2021; Sridhar et al., 2013). Overexpression of PI4K2A suppresses autolysosome tubulation, albeit accompanied by an increased localization of clathrin and dynamin2 on autolysosomes, suggesting the involvement of PI4P in autolysosome tubulation (Khundadze et al., 2021; Sridhar et al., 2013).

In PIP5K1B knockdown cells with inhibited autolysosome tubulation, this inhibition can be rescued by depleting PI4K3B[82]. This rescue was attributed to the suppressive role of PI4P in autolysosome tubulation. Nevertheless, the possibility of an independent PI(4,5)P_2-driven tubulation mechanism cannot be ruled out. Additionally, it's conceivable that the scission process, which may require bidirectional interconversion between PI4P and PI(4,5)P_2, is compromised. Supporting this hypothesis, the deficiency of the inositol polyphosphate-5-phosphatase, INPP5K, leading to increased intracellular PI(4,5)P_2, inhibits ALR and extends autolysosomal tubules (McGrath et al., 2021). In addition, both SPG11 and SPG15 interact with PI4K3B, and either SPG11 or SPG15 depletion suppresses the tubulation induced by PI4K3B depletion, suggesting a role of SPG11 in the initiation of ALR (Chang et al., 2014). However, the coordination between PI4K3B and SPG11 remains unclear.

PI3P has also been implicated in ALR (Munson et al., 2015). Mild inhibition of VPS34 through a PI3KC3 inhibitor or a loss-of-function mutant of UVRAG leads to significant lysosomal tubulation (Munson et al., 2015), indicating a role of PI3P in the scission process of ALR (Munson et al., 2015). Notably, this tubulation is blocked by mTOR inhibition. However, the specific effector of PI3P in ALR and the mechanism by which PI3P regulates this scission process remain unresolved. Candidates for the effector of PI3P in ALR may include Dynamin2 (Munson et al., 2015) and ZFYVE26/SPG15/FYVE-CENT/spastizin (Chang et al., 2014; Hirst et al., 2013, 2021; Sagona et al., 2010), given their demonstrated binding activity to PI3P. Furthermore, severe inhibition of VPS34 activity results in enlarged lysosomes rather than lysosomal tubulation (Munson et al., 2015). All these findings underscore the critical need for tight regulation of PI3P, PI4P, and PI(4,5)P_2 levels to ensure proper ALR functionality. Although it is evident that distinct phospholipids play different roles in various stages of ALR, it remains to be determined whether these phospholipids act coordinately to regulate ALR or if each one exerts its unique regulatory influence on ALR.

2.3. *Other genes involved in ALR*

Deficiencies in spastic paraplegia proteins SPG15/spastizin and SPG11/spatacsin lead to the accumulation of enlarged autolysosomes. However, it's important to note that these deficiencies are expected to operate downstream of mTOR, as mTOR inactivation and reactivation appears to proceed normally in cells lacking SPG11 and SPG15 (Chang et al., 2014). In a human neuronal model with a deficiency in the LYST protein, a conspicuous lysosome depletion occurs alongside the emergence of hyperelongated tubules extending from enlarged autolysosomes. This observation indicates a critical role for LYST in the scission of autolysosomal tubules (Serra-Vinardell et al., 2023). SACS, a causative gene for recessive ataxia, is also required for ALR, possibly through its functions in microtubule dynamics and/or binding to the AP2-clathrin complex and KIF5B(Francis et al., 2022). The protein RME-8/DNAJC13 has now been recognized for its conserved but previously unidentified role in autolysosome reformation (Swords et al., 2023). This role likely involves influencing the initiation and/or severing of ALR tubules in both *C. elegans* mechanosensory neurons and primary mouse cortical neurons. Depletion of RME-8/DNAJC13 in these systems results in the accumulation of excessively elongated autolysosomal tubules (Swords et al., 2023). Dysfunction in the retromer complex has a profound impact on endo-lysosomal health and homeostasis within human neuroglioma cells. This dysfunction leads to widespread changes in the lysosomal proteome, including inefficiencies in autophagic lysosome reformation (Daly et al., 2023).

Furthermore, a recent study has revealed that autophagic lysosome reformation also participate in the repair of damaged lysosomes (Bhattacharya et al., 2023). After lysosomal damage occurs, TBC1D15 interacts with ATG8 proteins on the damaged lysosomes, serving as a scaffold for assembling and stabilizing the autophagic lysosomal reformation machinery, such as clathrin, dynamin2, and KIF5B(Bhattacharya et al., 2023). This process enhances the formation of lysosomal tubules

and subsequent dynamin-2-dependent scission (Bhattacharya et al., 2023).

2.4. Autophagosomal components recycling

A recently identified recycling process involving autolysosomes is known as autophagosomal components recycling (ACR) (Wang et al., 2022; Wu et al., 2023; Zhou et al., 2022). Autophagosomes, acquiring membranes from diverse origins, fuse with lysosomes to form autolysosomes. Within autolysosomes, the inner components of autophagosomes are degraded, while the outer membrane components of autophagosomes, such as STX17 or ATG9A, are recycled through ACR. ACR occurs before ALR and is mediated by a protein complex known as the recycler, composed of SNX4, SNX5, and SNX17 (Zhou et al., 2022) (Fig. 3).

Both autophagic lysosome reformation and autophagosomal components recycling represent critical late-stage processes within the autophagic pathway. Since their discovery, significant progress has been made in unraveling the cellular and molecular mechanisms that underlie these processes. Nevertheless, our understanding of ALR and ACR remains limited, and numerous questions remain unanswered. These unresolved questions include:

1) How do newly formed proto-lysosomes acquire the necessary acidity and lysosomal hydrolases to mature into functional lysosomes?
2) The specific effectors responsible for PI4P and PI3P in ALR have been rarely identified.
3) Given the heterogeneity of lysosomes and the tissue-specific context, is it possible that multiple mechanisms coexist for autolysosome initiation, tubulation, and scission?
4) ALR disruption is observed in various diseases, while its direct involvement in the pathogenesis has yet to be rigorously tested. If it does play a role, could the targeted rescue of ALR provide therapeutic benefits?
5) What is the ultimate destination and fate of the vesicles generated during ACR?
6) The underlying mechanism of ACR largely remains unknown.
7) What are the physiological and pathological functions of ACR, and is it linked to any human diseases?

Collectively, there has been a rapid expansion of research into the molecular mechanisms governing autolysosomal recycling. Several disorders, including severe neurodegenerative conditions and muscular dystrophy, have been associated with dysfunction in ALR. Autolysosomal recycling holds potential as a target for intervention in disease treatments and drug development.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work was supported by grants from NSFC 92254302, 91854116, 32170685 and 31771529 (to Y.R.). The work was partially supported by the Fundamental Research Funds for the Central Universities 5003510089 and 2023BR028 (to Y.R.).

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