

## **Autophagy and endocytosis: interconnections and interdependencies**

Ása B. Birgisdóttir<sup>1,2\*</sup> and Terje Johansen<sup>3\*</sup>

<sup>1</sup>The Heart and Lung Clinic, University Hospital of North Norway, 9037 Tromsø, Norway

<sup>2</sup>Clinical Cardiovascular Research Group, Department of Clinical Medicine, University of Tromsø – The Arctic University of Norway, 9037 Tromsø, Norway.

<sup>3</sup>Molecular Cancer Research Group, Department of Medical Biology, University of Tromsø – The Arctic University of Norway, 9037 Tromsø, Norway

\*Authors for correspondence ([aasa.birna.birgisdottir@uit.no](mailto:aasa.birna.birgisdottir@uit.no) or [terje.johansen@uit.no](mailto:terje.johansen@uit.no))

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## **ABSTRACT**

Autophagy and endocytosis are membrane-vesicle-based cellular pathways for degradation and recycling of intracellular and extracellular components, respectively. These pathways have a common endpoint at the lysosome where their cargo is degraded. In addition, the two pathways intersect at different stages during vesicle formation, fusion and trafficking, and share parts of the molecular machinery. Accumulating evidence shows that autophagy is dependent upon endocytosis and *vice versa*. The emerging joint network of autophagy and endocytosis is of vital importance for cellular metabolism and signaling, and thus also highly relevant in disease settings. In this Review, we will discuss examples of how the autophagy machinery impacts on endocytosis and cell signaling and highlight how endocytosis regulates the different steps in autophagy in mammalian cells.. Finally, we will focus on the interplay of these pathways in the quality control of their common end point, the lysosome.

## **Introduction**

Autophagy is an evolutionary conserved intracellular renovation process where cells degrade and recycle their own cytoplasmic materials (Mizushima and Komatsu, 2011). Autophagy is responsible for degradation and recycling of dysfunctional or misfolded proteins, as well as aged and/or damaged organelles. Autophagy plays a central role in stress management, development, infection, inflammation, immunity, aging and in major diseases including cancer and neurodegeneration (Deretic et al., 2013; Dikic and Elazar, 2018; Lahiri et al., 2019; Levine and Kroemer, 2019). The endpoint of autophagy is at lysosomes, acidic, hydrolytic organelles, where degradation of the cytoplasmic material occurs. Autophagy processes are distinguished based on the entry mode of the cargo destined for degradation. Macroautophagy (hereafter autophagy) involves engulfment of cytoplasmic contents (cargo) into a double membrane vesicle termed the autophagosome. The autophagosome fuses with vesicles from the endolysosomal compartment, before becoming an autolysosome, in which its cargo is degraded (Yu et al., 2018). Other autophagy processes that do not involve formation of double membrane autophagosomes, are microautophagy, endosomal microautophagy, and chaperone-mediated autophagy (CMA). These processes proceed with a direct engulfment of cargo into the endolysosomal compartment, either through production of intraluminal vesicles (Marzella et al., 1981; Sahu et al., 2011) or direct import through the lysosomal membrane aided by the cytoplasmic chaperone HSC70 (heat shock-cognate protein of 70 KDa) and the lysosomal membrane protein LAMP2A (CMA) (Tekirdag and Cuervo, 2018) (Fig. 1).

Endocytosis is a vital cellular process that also can terminate with lysosomal degradation, but here extracellular cargo is internalized by the plasma membrane (PM) (Fig. 1). PM lipids, integral membrane proteins and extracellular fluid become internalized through invagination or extrusion of the PM. After internalization, the cargo traffics through and is sorted by a highly dynamic series of tubulovesicular compartments, collectively called endosomes (Scott et al., 2014). Internalized cargo is either recycled back to the PM, sent to the trans-Golgi network (TGN) via retrograde trafficking, or delivered to lysosomes for degradation. Cells communicate with and adapt to their environment through their PM. Thus, endocytosis plays key roles in a variety of processes, including nutrient uptake, cell signaling, cell adhesion and migration, pathogen entry, receptor downregulation, antigen presentation, cell polarity, and cell division, as well as growth and differentiation. Cytosolic proteins and multi-subunit complexes orchestrate the formation and maturation of endocytic compartments

(Doherty and McMahon, 2009).

Autophagy and endocytosis pathways intersect at several stages throughout vesicle formation, transport and fusion and share a number of the components of the molecular machineries involved (Table 1). A particular form of phagocytosis (type of endocytosis) provides an excellent example of an intersection between the two pathways (see **Box 1**). Indeed, as discussed here, the endocytic compartment and essential components thereof regulate different steps of the autophagy pathway (Tooze et al., 2014). The interplay between autophagy and endocytosis ensures the biogenesis and quality control of lysosomes, which are a prerequisite for functionality of both pathways. There is also a substantial crosstalk between exosome biogenesis, autophagy and the endolysosomal system (Hessvik and Llorente, 2018; Xu et al., 2018). Due to space limitations, we will not address this important aspect here. The aim of this review is to highlight the crosstalk and interdependence between autophagy and endocytosis in mammalian cells that ensures the efficient function of these pathways.

## Overview of the autophagy pathway

The autophagy process **consists of several steps involving** evolutionary conserved autophagy-related (ATG) proteins (the core autophagic machinery, identified by pioneering studies in yeast), orchestrating multiple membrane remodeling processes (Ohsumi, 2014) (Fig. 1). Autophagy is initiated with the nucleation of a crescent-shaped double membrane termed the phagopore. In yeast, autophagosomes are generated at a single site called **the phagophore** assembly site (PAS) (Suzuki et al., 2007). In multicellular organisms, autophagosomes are formed simultaneously at different locations. In response to autophagy initiation by amino acid starvation or mammalian target of rapamycin kinase complex 1 (mTORC1) inhibition (see **Box 2**), nucleation is triggered by active unc-51-like kinase (ULK) complex (ULK1 and ULK2, ATG13, ATG101 and the FAK family–interacting protein of 200 kD (FIP200)) (Zachari and Ganley, 2017), a serine-threonine kinase complex that phosphorylates several components of the class III phosphatidylinositol 3-kinase complex 1 (PI3KC3-C1). This specific complex consists of PIK3C3 (VPS34), PIK3R4/p150 (VPS15), BECN1/Beclin 1 and ATG14. Another variant of this complex, PI3KC3-C2, contains UV radiation resistance associated (UVRAG) instead of ATG14. After ULK-mediated activation, PI3KC3-C1 produces local pools of phosphatidylinositol 3-phosphate (PI3P); this defines the future phagophore **assembly** site and recruits PI3P-binding proteins (Mizushima et al., 2011). ER subdomains enriched in PI3P, termed omegasomes owing to their omega-like shape (Axe

et al., 2008), act as phagophore **assembly** sites. The expansion of the phagophore is a more elusive process that involves diverse membrane sources, with ER, mitochondria, Golgi, PM, recycling endosomes, as well as ATG9 vesicles, all being implicated (Lamb et al., 2013). A supply of lipids is crucial for phagophore expansion. Recently, ATG2 was identified as a lipid-transfer protein mediating transport of lipid molecules from ER to the expanding phagophore (Maeda et al., 2019; Valverde et al., 2019). Expansion of the phagophore depends on two ubiquitin-like (Ubl) conjugation systems, the ATG5–ATG12 conjugation system and ATG8 conjugation to phosphatidylethanolamine (PE). In mammalian cells there are two ATG8 subfamilies, the microtubule-associated protein 1 light chain 3 (LC3) -A, -B, -B2 and -C and GABA type A receptor associated protein (GABARAP) and GABARAP-like; GABARAPL1 and GABARAPL2 (Shpilka et al., 2011). After processing by the cysteine protease ATG4, ATG8s are conjugated to PE for membrane insertion. The PI3P effector WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) recruits ATG16L1 to the phagophore to specify sites of ATG8-conjugation (Dooley et al., 2014). The PE-conjugated forms of ATG8s are present on both the inner and outer membrane of the growing phagophore and recruit proteins with an LC3-interacting region (LIR) motif (Birgisdottir et al., 2013; Johansen and Lamark, 2019). ATG8s promote phagophore expansion and closure of the autophagosome, and are crucial for its fusion with the lysosome (Weidberg et al., 2010; Mizushima et al., 2011; Shpilka et al., 2011; Nguyen et al., 2016; Tsuboyama et al., 2016). Maturation of autophagosomes to degradative autolysosomes occurs through fusion with endolysosomal compartments (Zhao and Zhang, 2019). Prior to fusion, the autophagy machinery dissociates from the outer autophagosomal membrane. Delipidation of ATG8s on the outer membrane is mediated by ATG4 isoforms (Kauffman et al., 2018). In contrast to yeast, ATG4-mediated delipidation in human cells is not essential for autophagosome formation and fusion with lysosomes (Agrotis et al., 2019). Originally, autophagy was described as a non-selective bulk degradation pathway during nutrient deprivation (De Duve and Wattiaux, 1966). However, more recent findings regarding the selective elimination of aberrant protein aggregates, RNA bodies, lipid droplets, mitochondria, dysfunctional organelles, as well as invading pathogens, has revealed the existence of selective autophagy pathways (Johansen and Lamark, 2019). Instrumental in these processes are selective autophagy receptors (SARs), which can be soluble or membrane-anchored, that direct the degradation of specific cellular components; they bind cargo, attract core autophagy components and interact with one or more of the ATG8 homologs at the inner membrane of the phagophore via their LIR motifs (Rogov et al., 2014; Johansen and Lamark, 2019). The

first identified mammalian SAR was p62 (also known as sequestosome-1 (SQSTM1)), a soluble autophagy receptor that binds to ubiquitinated cargo (Bjørkøy et al., 2005; Pankiv et al., 2007).

## **Overview of endocytosis**

There are numerous co-existing endocytic pathways, broadly categorized as clathrin-dependent or clathrin-independent; this refers to the clathrin coat protein, a key component of the endocytic machinery. Clathrin-mediated endocytosis (CME) is the best characterized endocytic mechanism (Doherty and McMahon, 2009) (Fig. 1). Clathrin is recruited to sites of endocytosis by adaptor protein (AP) complexes. Transmembrane proteins (cargo) contain sorting motifs within their cytoplasmic tails that interact with AP complexes for inclusion into vesicles (Park and Guo, 2014). Clathrin polymerizes into the trimeric clathrin “triskelion”, which forms polygonal lattices, known as the clathrin coat, that surrounds the nascent vesicle formed by PM invagination into a deep invaginated pit. The GTPase dynamin is responsible for scission of the coated vesicle from the PM (Ferguson and De Camilli, 2012). The resulting vesicle is uncoated and participates in intracellular membrane trafficking events. Uncoating also releases proteins of the endocytic machinery back to the cytosolic pool (Sousa and Lafer, 2015). The initial endocytic vesicles undergo homotypic fusion (i.e. membranes of the same type merge) and are rapidly directed to early endosomes (EEs). Sorting events initiated at EEs determine the fate of internalized cargo molecules, such as recycling to PM, degradation in lysosomes or delivery to the TGN (Naslavsky and Caplan, 2018). Cargo destined for fast recycling to the PM is transported from EE-originating tubular-vesicular structures directly back to the PM; this is coordinated by Rab4 (van der Sluijs et al., 1992; Mohrmann et al., 2002). A slower recycling circuit involves a route through Rab11-positive recycling endosomes (REs) before returning to the PM (Sonnichsen et al., 2000) (Fig.1). EEs have a complex and dynamic structure with a central cavity with tubular membrane extensions; intraluminal vesicles (ILVs) can also form by inward budding of the endosomal membrane mediated by the endosomal sorting complex required for transport (ESCRT) machinery (Wenzel et al., 2018). **The ESCRT subcomplexes** specifically recognize ubiquitinated cargo and facilitate loading of cargo destined for degradation into ILVs. The ESCRT machinery comprises four multimeric protein core complexes; ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, plus the VPS4 ATPase complex, as well as additional accessory proteins. ESCRTs are usually localized in the cytoplasm and are recruited to different membranes by various adaptors. The ESCRT machinery creates an inverse membrane deformation away

from the cell cytoplasm, without providing a coat around the nascent membrane bud (Vietri et al., 2019). The subcomplex that is most directly involved in membrane reshaping is ESCRT-III (Christ et al., 2017). EEs mature into late endosomes (LE) (Stoorvogel et al., 1991) through multiple rounds of cargo sorting and ILV formations (Wenzel et al., 2018). More specifically, vesicles termed multivesicular bodies (MVBs) that contain multiple ILVs, presumably detach from EE and gradually mature into LE (Gruenberg, 2020). **MVBs can also fuse with the PM and release their ILVs as exosomes (Hessvik and Llorente, 2018).** Rab GTPases (a subfamily of the Ras superfamily of small GTPases) are small evolutionary conserved GTPases that act as molecular switches regulating vesicular membrane trafficking (Zhen and Stenmark, 2015). EEs are characterized by the presence of Rab5, which is essential for EE biogenesis and endosomal fusion (Zeigerer et al., 2012). During the endosomal maturation process, Rab5 is replaced with Rab7 on LEs and lysosomes (Rink et al., 2005). Both Rab5 and Rab7 activate the UVRAG-containing PI3KC3-C2 complex to produce PI3P on endosomes, resulting in the recruitment of PI3P effectors that coordinate endosome maturation and trafficking (Langemeyer et al., 2018). LEs have numerous ILVs and either mature into lysosomes themselves by acquiring hydrolytic competence, or fuse with lysosomes (Pillay et al., 2002); this results in the degradation of cargo-loaded ILVs by lysosomal acidic hydrolases (Bright et al., 2016). Lysosomes are acidic organelles with a luminal pH of 4.5 to 5. The lysosomal membrane contains a proton-importing V-type ATPase that maintains the acidic pH, which corresponds to the optimum pH of most of the over 60 luminal hydrolases. The lysosome membrane also contains ion transporters and channels, as well as multiple other transporters for export of degradation products and several highly-glycosylated lysosome-associated membrane proteins (LAMPs). In addition, tethering factors and soluble N-ethylmaleimide-sensitive-factor attachment receptors (SNAREs) are present for interactions and fusion with other organelles (Pu et al., 2016).

### **Impact of autophagy on endocytosis and signaling**

**Components of the autophagy machinery may have direct effects on endocytosis as clearly illustrated by LC3-associated phagocytosis (LAP) (see Box 1).** A crucial role of endocytosis is to sort, recycle and degrade signaling receptors and their ligands. Cells need to regulate trafficking of activated receptors to ensure adequate signaling propagation and duration. There is increasing evidence that autophagy is involved **in receptor recycling and signaling.**

### **Involvement of ATG8s in clathrin-mediated endocytosis**

Factors that are essential for endocytosis interact with the ATG8s. The AP2 complex has a LIR motif and its interaction with LC3 is important for clathrin-mediated phagocytosis of Alzheimer precursor protein (APP) (Tian et al., 2013). In addition, clathrin heavy chain contains a LIR motif with unknown functional role (Mohrluder et al., 2007). Furthermore, adaptor-associated kinase 1 (AAK1), which is involved in the phosphorylation of AP2 for more efficient clathrin-dependent endocytosis (Conner and Schmid, 2002; Henderson and Conner, 2007), interacts with ATG8s and contains putative LIR motifs (Loi et al., 2016). Hence, clathrin-mediated endocytosis might depend on ATG8s.

### **Involvement of the autophagy machinery in receptor recycling and signaling**

Recycling of the glucose transporter 1 (GLUT1) involves the autophagy machinery (Roy et al., 2017). The retromer and retriever (both highly conserved multi-protein complexes) and their associated protein complexes facilitate the recycling of transmembrane proteins (Cullen and Steinberg, 2018). TBC1D5 (the GAP for Rab7A, see below) inhibits the retromer complex. However, during metabolic stress, TBC1D5 binds to LC3 on autophagosomes; this relieves its inhibition of the retromer, which then associates with endosomal membranes, resulting in the recycling of endocytosed GLUT1 to the PM to facilitate glucose uptake (Roy et al., 2017). Furthermore, autophagy maintains endosomal homeostasis through recognition and targeting of damaged EEs. Hence, loss of autophagy disrupts recycling of epidermal growth factor receptor (EGFR) to the PM and perturbs EGF-mediated signaling (Fraser et al., 2019).

The Notch signaling pathway is a key regulator of stem cells and crucial for development of most tissues. The Notch receptor is cleaved after ligand binding; this releases the Notch intracellular domain (NICD), which translocates to the nucleus to activate target genes (Bray and Bernard, 2010). The canonical degradation of the Notch1 receptor is through endocytosis (Bray, 2006). However, autophagy impacts on Notch signaling through uptake of Notch1 receptor into ATG16L-containing vesicles, resulting in autophagy-mediated degradation of Notch1 (Wu et al., 2016). Here, autophagy impairment in Atg16L1 hypomorph mice results in retarded Notch 1 dependent stem cell differentiation. In addition, p62 can bind to Notch1 intracellular domain (NICD1) and promote its autophagic degradation. Thus, autophagy can also affect the transcriptional activity necessary for Notch1 signaling (Zhang, T. et al., 2018).



## **Autophagosome formation and its intersection with the endosomal system**

Numerous membrane sources are implicated in formation of autophagosomes (Kawabata and Yoshimori, 2016). In particular, ATG9- and ATG16-containing membrane vesicles, which are formed by endocytosis, traffic through the endocytic compartment and carry autophagy mediators to sites of autophagosome formation (Fig. 2A).

### **ATG9A vesicles**

Of the two mammalian ATG9 homologs, ATG9A and ATG9B, ATG9A exhibits the most ubiquitous expression (Yamada et al., 2005). ATG9A is usually localized in Golgi membranes and endosomes, and undertakes complex trafficking routes that involve both endocytic and secretory pathways (Young et al., 2006). ATG9A is routed to the PM and returns through clathrin-dependent endocytosis, **traveling** through the early endocytic compartment and recycling endosomes. ATG9 interacts with AP2, which together with the Rab GAP TBC1D5 (see below), are required for proper sorting of ATG9 towards sites of autophagosome formation (Popovic and Dikic, 2014). ATG9-containing vesicles are mobilized from Rab11-positive recycling endosomes (Takahashi et al., 2016), and from the Golgi (Marcassa et al., 2017). During amino acid starvation, ATG9A is found on vesicles and on tubular vesicular structures, termed the ATG9 compartment (Itakura et al., 2012b; Orsi et al., 2012) that engages in transient interactions at phagophore **assembly** sites. ATG9A is involved in supplying lipids and proteins to the phagophore (Noda, 2017) (Fig. 2A). Rab1b also localizes to ATG9A-containing vesicles and participates in formation of autophagosomes (Kakuta et al., 2017). ATG9A vesicles that originate from the Golgi deliver the Golgi-resident PI4-kinase PI4KIII $\beta$  to the site of autophagosome initiation. Here, PI4KIII $\beta$  and the resulting PI4P **may** recruit the ULK1 complex through ATG13 (Judith et al., 2019).

### **ATG16L1 vesicles**

ATG16L1 is essential for correct localization of the ATG12-5 conjugate to the phagophore (Fujita et al., 2008). ATG16L1 is a peripheral membrane protein present on vesicles formed by clathrin-dependent endocytosis. Clathrin heavy chain interacts with the N-terminal region of ATG16L1 (Ravikumar et al., 2010). ATG16L-containing vesicles are distinct from those that have ATG9A and they bypass EEs (Fig. 2A). The membrane-curvature-inducing protein Annexin A2 enhances formation and homotypic fusion of ATG16L1-positive vesicles

preceding their integration into the expanding phagophore (Morozova et al., 2015). The homotypic fusion is dependent on the SNARE VAMP7 (Moreau et al., 2011). ATG16L1-containing vesicles coalesce with those that have ATG9A into Rab11-positive recycling endosomes. Here, VAMP3 mediates the fusion event (Puri et al., 2013). The sorting nexin SNX18 and Rab11 are important for recruitment of ATG16L1 to recycling endosomes (Knaevelsrud et al., 2013). Furthermore, SNX18 mediates the transport of ATG9 and ATG16L1 from recycling endosomes to the phagophore through interaction with the GTPase Dynamin-2 (Soreng et al., 2018). In addition, the PI3P-binding protein WIPI2b recruits ATG16L1 to the omegasome (Dooley et al., 2014). Here, the E3-like ATG12-ATG5:ATG16L complex conjugates ATG8 proteins to PE on the growing phagophore.

### **Endocytic components involved in phagophore formation and closure**

During amino acid starvation, membranes at or close to rough ER and/or ER-mitochondria contact sites (MAMs) mediate phagophore formation at PI3P-positive domains (Ktistakis, 2019). However, PI3P is found on both early and late endosomes (Gillooly et al., 2000) and Rab5 is involved in phagophore formation by activating PIK3C3 (VPS34) and promoting ATG5-ATG12 conjugation (Ravikumar et al., 2008). Rab5-positive early endosomes have been reported to sequester depolarized mitochondria into single-membrane structures (Hammerling et al., 2017). Furthermore, Rab11A-enriched recycling endosomes can act as primary platforms for phagophore formation. Here, co-incidence detection of PI3P and Rab11A by WIPI2 is suggested to mediate autophagosome formation (Puri et al., 2018). Rab11A-containing recycling endosomes have also been suggested to be sites of autophagosome formation during viral infection (Kuroki et al., 2018). This highlights the possible role of the endosomal system in phagophore formation.

ESCRT-mediated ILV formation during endosome maturation resembles the topological membrane transformation involved in phagophore closure (Knorr et al., 2015). Indeed, the ESCRT-III component CHMP2A was identified to regulate phagophore closure (Takahashi et al., 2018; Zhen et al., 2019), demonstrating an involvement of the endocytic machinery. Interestingly, components of ESCRT-I, -II and -III, including VPS4 and the accessory protein ALIX, are involved in the engulfment of cargo in endosomal microautophagy (Tekirdag and Cuervo, 2018). **Moreover**, upon acute amino acid starvation, endosomal microautophagy leads to a selective and rapid degradation of certain autophagy receptors and ATG8 proteins. The mechanism requires the activities of the ESCRT-III component CHMP4B and VPS4 in late endosomes (Mejlvang et al., 2018). These examples

reveal that complex interconnections and interdependence exist between the endocytic compartment and different forms of autophagy for cargo engulfment and degradation.

Taken together, autophagosome formation appears tightly coupled to components of the endosomal system, not only with regard to the delivery of components and membranes through ATG9A- and ATG16L-containing vesicles, but also via a role of ESCRT-III in phagophore closure.

### **Autophagosome maturation is dependent on fusion with the endolysosomal system**

Autophagosome maturation involves fusions with different parts of the endolysosomal compartment. Autophagosomes can fuse with early or late endosomes, forming amphisomes (Gordon and Seglen 1988), before fusing with lysosomes. Autophagosomes also directly fuse with lysosomes (Mizushima et al., 2011). Phosphatidylinositol-3-phosphate (PI3P) is a key player in membrane dynamics and participates in nearly all aspects of endosomal function, as well as in autophagy, including fusion with lysosomes (Nascimbeni et al., 2017). PI4P generation on autophagosomes is also critically important for their fusion with lysosomes. GABARAPs bind to and recruit phosphatidylinositol 4-kinase II $\alpha$  (PI4KII $\alpha$ ), a lipid kinase that generates PI4P, to autophagosomes (Wang et al., 2015). The importance of the endocytic compartment for autophagosome maturation is evident from experiments showing that inhibition of early endosome function (Razi et al., 2009), or ESCRT disruption on late endosomes (Lefebvre et al., 2018), results in accumulation of autophagosomes and inhibition of autophagic degradation. The main fusion machinery consists of Rab GTPases, tethering factors, SNAREs and other auxiliary proteins that govern fusion of the outer autophagosomal membrane with membranes of the endolysosomal system (Fig. 2B). The main components of the fusion machinery are briefly described below.

#### **Rab GTPases**

Rab GTPases exist in an inactive cytosolic GDP-bound form and a membrane-anchored, active GTP-bound form that subsequently interacts with effector proteins. Guanine nucleotide exchange factors (GEFs) catalyze GDP dissociation to allow replacement with GTP, while GTPase-activating proteins (GAPs) mediate hydrolysis of GTP to GDP (Zhen and Stenmark, 2015; Pfeffer, 2017). Approximately 60 different Rab isoforms are present in mammals (Pfeffer et al., 2017). Rab GAPs contain the highly conserved Tre2/Bub2/Cdc16 (TBC)

domain that inactivates Rabs by stimulating hydrolysis of GTP to GDP (Frasa et al., 2012). ATG8s interact with a number of TBC1-domain-containing Rab GAPs involved in endocytosis (Popovic et al., 2012). Rab7 is essential for late endosome trafficking and lysosome biogenesis (Bucci et al., 2000). Rab7 also has a key role in the maturation of autophagosomes to autolysosomes mediated by fusion events with lysosomes (Gutierrez et al., 2004; Jager et al., 2004). The Mon1-Ccz1 complex acts as a GEF for Rab7 (Kinchen and Ravichandran, 2010; Gerondopoulos et al., 2012) and activates Rab7 only on late endosomes in mammalian cells (Yasuda et al., 2016). The GAPs regulating Rab7 are TBC1D2A (Armus), TBC1D2B, TBC1D5 and TBC1D15, and they interact with LC3 on autophagosomes (Stroupe, 2018). A recent analysis of mammalian Rab7 knockout (KO) cells suggests that Rab7 is involved in autolysosome maturation rather than the fusion step itself (Kuchitsu et al., 2018). Furthermore, Golgi-resident Rab2 is involved in autophagosome maturation by recruiting the homotypic fusion and protein sorting (HOPS) complex (see below) (Ding et al., 2019) (Fig. 2B).

### **Tethering factors**

Tethering factors enhance the specificity and efficiency of membrane fusion and are recruited to specific membranes through coordinated binding to Rab proteins, phospholipids, ATG8s and SNAREs (Kriegenburg et al., 2018) (Fig. 2B). The HOPS complex (VPS33A, VPS16, VPS11, VPS18, VPS39 and VPS41) is the core-tethering factor mediating autophagosome-lysosome fusion. HOPS is recruited to endolysosomal membranes by binding to the Rab7 effectors pleckstrin homology domain containing protein family member 1 (PLEKHM1) and Rab7a-interacting lysosomal protein (RILP) (Wijdeven et al., 2016). PLEKHM1 binds to ATG8s, preferentially GABARAPs, on autophagosomes and promotes SNARE-complex assembly (see below) (McEwan et al., 2015). HOPS can be recruited to autophagosomes by interacting with the SNARE syntaxin 17 (STX17) (Jiang et al., 2014). The tethering factor EPG5 is localized to the endolysosomal compartment through Rab7 and interacts with autophagosomes via ATG8s and the SNAREs STX17 and synaptosome associated protein 29 (SNAP29) to facilitate fusion (Wang et al., 2016). ATG14, a component of PI3KC3-C1, can also function as a tethering factor on the nascent autophagosome by interacting with STX17 to stabilize the STX17-SNAP29 complex and promote membrane fusion (Diao et al., 2015). Furthermore, ATG14 can interact with GABARAPs through a C-terminal LIR motif (Birgisdottir et al., 2019), and this interaction might also play a role in the fusion step. Similar to EPG5, other tethering factors interact with ATG8s for capturing autophagosomes.

Baculovirus IAP repeat-containing ubiquitin-conjugating enzyme (BRUCE), which is present on the endolysosomal compartment, promotes autolysosome formation by interacting with ATG8s as well as STX17-SNAP29 (Ebner et al., 2018). Golgi Reassembly and Stacking Protein 55 (GRASP55) contains a LIR motif and simultaneously binds to ATG8s on the autophagosome and LAMP2 on late endosomes /lysosomes to facilitate fusion (Zhang, X. et al., 2018). Finally, tectonin  $\beta$ -propeller repeat containing protein 1 (TECPR1) is localized on mature autophagosomes and lysosomes and has been implicated in tethering by mediating autophagosome fusion with the endocytic system, as depletion of TECPR1 results in autophagosome accumulation (Chen et al., 2012).

### SNAREs

SNAREs are membrane-anchored proteins that mediate membrane fusion by forming a trans-SNARE complex comprised of a parallel four-helix bundle that contains one R-SNARE helix and three Q-SNARE helices (named after conserved arginine (R) and glutamine (Q) residues, respectively) to bridge the opposing membranes (Jahn and Scheller, 2006). Two cognate SNARE complexes function in parallel to drive fusion of autophagosomes with late endosomes/lysosomes. One is composed of the Q-SNARE STX17 in the autophagosomal membrane bound to the cytosolic Q-SNARE SNAP29 (contains two helices), which interacts with either the R-SNARE vesicle associated membrane protein 8 (VAMP8) or VAMP7 in the endolysosomal membrane (Itakura et al., 2012a). The other complex consists of the R-SNARE YKT6 in the autophagosomal membrane, which interacts with SNAP29 bound to the Q-SNARE STX7 in the endolysosomal membrane (Matsui et al., 2018) (Fig.2B). Notably, YKT6 can also form a complex with STX17 and SNAP29 that could be involved in the fusion of autophagosomes at different maturation stages. STX17 and YKT6 are not found on the phagophore and are recruited independently to the autophagosome (Matsui et al., 2018). STX17 recruitment is accompanied by closure of the nascent autophagosome and allows coordination of closure and fusion. Immunity-related GTPase M (IRGM) in human cells facilitates efficient targeting of STX17 to autophagosomes through a direct interaction with STX17 and ATG8s via a non-canonical LIR motif. Interestingly, STX17 also interacts with ATG8s through a LIR motif within its SNARE domain (Kumar et al., 2018). **However, the recruitment of STX17 is not affected by depletion of all the ATG8s (Nguyen et al., 2016) and absence of the ATG8 conjugation system still mediated STX17 recruitment, although at a reduced rate (Tsuboyama et al., 2016). An unanticipated early role for STX17 in autophagy is that phosphorylation of serine 202 by TBK1 controls formation of the mammalian pre-**

autophagosomal structure (mPAS) in response to induction of autophagy (Kumar et al., 2019).

The motifs responsible for the recruitment of SNAREs to membranes must be exposed to allow the assembly of trans-SNARE complexes. SNAP29 engages in SNARE complex formation through interaction with membrane bound STX17 or STX7 (Hohenstein and Roche, 2001; Diao et al., 2015). Endocytic internalization and endolysosomal trafficking regulated by Rab21 mediates the targeting of the R-SNARE VAMP8 to the late endosome/lysosome membrane (Jean et al., 2015). Similarly, phosphatidylinositol binding clathrin assembly protein (PICALM) regulates the endocytosis of SNAREs, such as VAMP2, VAMP3 and VAMP8, thereby affecting autophagy at different stages (Moreau et al., 2014).

After fusion is completed, the individual SNARE molecules are released from their complex by the enzyme *N*-ethylmaleimide sensitive factor (NSF) and its adaptor soluble NSF-attachment protein alpha ( $\alpha$ SNAP) (Baker and Hughson, 2016). Interestingly, GABARAP interacts with NSF (Kittler et al., 2001) and thus could be involved in facilitating the detachment. In addition to STX17 (Kumar et al., 2018), several other SNAREs bind to ATG8s via LIR motifs, with STX16 and STX17 acting synergistically in several types of selective autophagy (Gu et al., 2019). STX16 is also important for lysosomal biogenesis, and ATG8s regulate its localization to endosomal and lysosomal compartments (Gu et al., 2019). Thus, it is becoming increasingly clear that ATG8s serve important roles in autophagy and beyond as membrane scaffolds (Johansen and Lamark, 2019).

## **Vesicle transport governs fusion between autophagosomes and the endolysosomal system**

Autophagosomes are typically formed throughout the cytoplasm (Jahreiss et al., 2008), but nutrient starvation leads to a perinuclear clustering of lysosomes, which has been assumed to favor autophagy initiation and autophagosome-lysosome fusion (Kimura et al., 2008; Korolchuk et al., 2011). Motor proteins mediate bidirectional transport of autophagosomes and lysosomes between center and periphery of the cell along microtubules (Fig. 3A). The minus-end-directed dynein-dynactin motor complex transports its cargos to the perinuclear region, whereas plus-end-directed kinesin motor proteins mediate transport towards the cell periphery (Gennerich and Vale, 2009). Rab GTPases and their effectors govern interactions with motor proteins. Each Rab regulates a specific step of vesicular trafficking. Rab7 controls transport of autophagosomes, late endosomes and lysosomes (Guerra and Bucci, 2016). Their minus-end-directed transport towards the cell center is regulated by a complex of Rab7, RILP,

the cholesterol sensor oxysterol-binding protein-related protein 1; (ORP1L, also known as OSBPL1A) and dynein-dynactin (Rocha et al., 2009; Wijdeven et al., 2016). ORP1L is located on late endosomes, lysosomes, amphisomes and autolysosomes where cholesterol levels dictate its ability to engage in complex formation. At low cholesterol levels, ORP1L interacts with an ER-membrane protein; this suppresses the interaction of the motor with the Rab7-RILP complex, resulting in vesicle retention in the cell periphery (Rocha et al., 2009). Rab7 can also facilitate plus-end-directed transport of autophagosomes and late endosomes through its effector FYVE and coiled-coil (CC) domain-containing protein (FYCO1) that binds to LC3 and PI3P on autophagosomes (Pankiv et al., 2010). **In the presence of amino acids FYCO1-mediated transport of lysosomes towards the cell periphery promotes mTORC1 signaling and suppresses autophagy (Hong et al., 2017).** The JNK-interacting, motor scaffolding protein JIP1 directs both plus-end and minus-end transport of autophagosomes in neurons. JIP1 binds to LC3 via a LIR motif to promote a switch to JIP1-mediated transport of autophagosomes to the perinuclear region for their efficient fusion with lysosomes (Fu et al., 2014). The multi-subunit protein complex BLOC-1-related complex (BORC) interacts with the small GTPase ADP-ribosylation factor-like protein 8 (ARL8) on lysosomes. This promotes ARL8-dependent association with kinesin-5 and lysosome movement towards the cell periphery (Pu et al., 2015). The BORC-ARL8 interaction is inhibited by low levels of amino acids, resulting in the perinuclear accumulation of lysosomes (Filipek et al., 2017; Pu et al., 2017). Knockout of BORC subunits causes accumulation of lysosomes in the perinuclear area without affecting basal mTORC1 activity (see **Box 2**); this decreases encounters between peripheral autophagosomes and lysosomes resulting in inhibition of autophagic flux. Furthermore, depletion of BORC impairs fusion of autophagosomes with lysosomes, likely due to a lack of BORC-mediated, ARL8-dependent recruitment of the HOPS complex to lysosomes (Jia et al., 2017). This way, the movement of both autophagosomes and lysosomes affects their fusion rate and the recruitment of tethering factors. Thus, motor protein-mediated trafficking comprises a crucial part of the encounter and fusion between autophagosomes and lysosomes.

## **Lysosome quality control**

In addition to its major role in cellular catabolism, the lysosome has a central role as a nutrient- sensing and metabolic signal-transduction platform (**Box 2**). Quality control of the lysosome is crucial for propagation of autophagy and endocytosis, and as discussed below, the

machinery governing both pathways is involved in lysosome homeostasis.

### **Reformation of lysosomes**

Reformation of lysosomes from endolysosomes and autolysosomes maintains lysosome homeostasis by restoring the level of free lysosomes. However, the mechanistic details are not well known. In autophagic lysosome reformation (ALR), which is crucial for maintaining autophagic flux, functional lysosomes are regenerated from autolysosomes. During ALR, long tubular structures extend from the autolysosome and small proto-lysosomes bud from the tips of the tubules. Proto-lysosomes are initially pH-neutral and mature into functional lysosomes (Fig. 3B). Essential factors of ALR are clathrin, PI(4,5)P<sub>2</sub>-related kinase PIP5K1B and the kinesin motor KIF5B (Chen and Yu, 2018). PIP5K1B converts PI4P to PI(4,5)P<sub>2</sub> and AP2 recruits clathrin to PI(4,5)P<sub>2</sub> on the autolysosomal membrane resulting in clathrin-coated buds on the surface (Rong et al., 2012). These buds serve as sites of tubule generation with KIF5B providing a pulling force along the microtubules (Du et al., 2016). Tubule generation is regulated by mTORC1 in an unknown manner (Yu et al., 2010; Rong et al., 2011). The large GTPase Dynamin 2 is responsible for scission at the tip of the tubules, which mediates proto-lysosome formation (Fig. 3B) (Schulze et al., 2013). The phospholipids PI4P, PI(4,5)P<sub>2</sub> and PI3P play important roles as signals and adaptors during this process. PI3P, which is generated by the active PI3KC3-C2 complex, is important for the scission step (Chen and Yu, 2015; Munson et al., 2015). Many unanswered questions remain regarding ALR, **but the mechanisms involved rely on many molecular components important for both endocytosis and autophagy.**

### **Damage repair and removal of lysosomes: ESCRTs, galectins and autophagy**

Damage or rupture of lysosomes occur incidentally, caused by their transported or accumulated cargo, or intentionally through incoming pathogens. The release of protons, reactive oxygen species (ROS), calcium and cathepsins from lysosomes interferes with cellular processes and damages intracellular structures (Papadopoulos and Meyer, 2017). Furthermore, lysosomal membrane permeabilization can elicit cell death pathways (Wang et al., 2018). Lysosomal damage thus has detrimental consequences for the cell and protective mechanisms are activated to maintain and restore lysosomal membrane integrity. Recent studies implicate the ESCRT machinery in membrane repair during limited damage of the lysosomal membrane (Fig. 3C). Here, small perforations trigger Ca<sup>2+</sup>-dependent recruitment of the ESCRT machinery for membrane resealing (Radulovic et al., 2018; Skowyra et al.,



2018). These findings demonstrate a dual role of the ESCRT machinery in the endolysosomal compartment, as it is important for sorting of cargo into intraluminal vesicles targeted for degradation and ensuring the integrity of lysosomes in order to maintain biochemical activity. Upon lysosomal membrane damage, luminal glycosylated proteins are exposed as a mark of injured organelles (Aits et al., 2015). Severely damaged lysosomes are subsequently removed and recycled by selective autophagy termed lysophagy (Hung et al., 2013; Maejima et al., 2013) (Fig. 3C). A group of cytosolic galectins (galectins-1, -3, -8 and -9) acts as specific sensors of lysosomal damage by binding to the exposed glycosylated proteins (Thurston et al., 2012; Johannes et al., 2018). Galectins-3 and -8 mediate lysophagy by binding to tripartite motif (TRIM) 16 and the autophagy receptor nuclear dot protein 52 kDa (NDP52), respectively (Thurston et al., 2012; Chauhan et al., 2016). In addition, galectins-8 and -9 can promote autophagy by inhibition of mTORC1 and activation of AMP-activated protein kinase (AMPK), respectively (Jia et al., 2018). In lysophagy, damaged lysosomes, as well as membrane remnants originating from complete lysosome rupture are engulfed by autophagosomes (Papadopoulos and Meyer, 2017). Very recently, it has been reported that galectins-3, -8 and -9 coordinate a repair, removal, and replacement program for damaged lysosomes. Here, galectin-3 recruits ESCRT components for repair of lysosomal membranes. When ESCRT-mediated lysosome repair fails, galectin-3 recruits TRIM16 for removal of unsalvageable lysosomes by autophagy, aided by the autophagy inducing effects of galectins-8 and -9 (Jia et al., 2020). In this way, components of the endosomal system and the autophagy machinery are instrumental for the homeostasis of lysosomes.

## Conclusions and perspectives

Autophagy and endocytosis mediate the degradation and recycling of intracellular and extracellular components, respectively. Both pathways involve the gradual maturation and fusions of vesicles, which traffic to their final destination, the lysosome. The emerging extensive crosstalk between these two pathways is therefore not surprising. Many questions regarding the identity and composition of various intracellular membrane compartments, as well as differential control of their intersections and the protein complexes involved still remain unanswered. Novel high-resolution live-cell imaging approaches combined with proteomics and CRISPR-based screens will undoubtedly provide further insight. Elucidation of the dynamic interplay between autophagy and endocytosis in the regulation of cell signaling is likely to provide exciting avenues for development of new therapeutic

approaches.

### **Competing interests**

The authors declare no competing interests.

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## Boxes

### Box 1: LC3-associated phagocytosis (LAP) and LAP-like processes

Phagocytosis is the endocytosis of large-molecular-weight antigens or intact microbes. PM protrusions surround and internalize the extracellular particles into single-membrane, confined internalized structures called phagosomes, which are then trafficked to the lysosome for content degradation (Flannagan et al., 2012). LC3-associated phagocytosis (LAP) involves the conjugation of LC3 to the single-membrane phagosome (LAPosome) through the engagement of components of the autophagy machinery (Fig. 2A). These phagosomes can carry bacterial and fungal pathogens, or apoptotic and necrotic cells. LAP-like LC3 lipidation can also occur during macropinocytosis and entosis (live-cell engulfment) (Florey et al., 2015a; Cadwell and Debnath, 2018; Heckmann and Green, 2019). **The binding of a variety of ligands, including pathogen moieties, dying cells and immune complexes, to surface receptors triggers LC3-recruitment to phagosomes. LAP thus serves as a host-defense mechanism against several pathogens and also has key roles in inflammation and immunity (Heckmann et al., 2017; Herb et al., 2019).** Unlike canonical autophagy, LAP does not require the ULK complex or ATG9. LC3-decorated phagosomes display enhanced lysosomal fusion, resulting in a more effective degradation of their contents **compared to LC3-lacking phagosomes.** LAP depends upon NADPH-oxidase (NOX2) to generate reactive oxygen species (ROS), PI3KC3-C2 for PI3P generation, as well as the components for LC3 conjugation (Sanjuan et al., 2007; Martinez et al., 2011; Martinez et al., 2015). **The signaling events that link NOX2 activity and ROS production to LC3 lipidation remain elusive. However, ROS inside the LAPosome lumen promote oxidative damage and thus can be of high importance in destruction of certain microbes (Heckmann et al., 2018).** Importantly, the WD repeat-containing C-terminal domain of ATG16L1 is crucial for LC3 recruitment to endolysosomal membranes, but dispensable for canonical autophagy (Fletcher et al., 2018). Interestingly, LAP-like processes that result in lipidation of LC3 at single-membrane compartments of the endolysosomal system also occur upon treatment with **known modulators of canonical autophagy**; drugs with lysosomotropic (**accumulate in acidic compartments**) or ionophore (**drive exchange of ions across membranes**) properties, including monensin, CCCP (carbonyl cyanide m-chlorophenyl hydrazine) and chloroquine, **induce an osmotically driven swelling of single-membrane endolysosomal compartments and activate noncanonical LC3 lipidation** (Florey et al., 2015b; Jacquin et al., 2017). **This highlights that LC3 is not a specific autophagosome marker.** In this context, more recently, LC3-associated endocytosis (LANDO) has been described, in which  $\beta$ -amyloids, whose deposition is causative of Alzheimer's Disease (**AD**), are cleared in

microglia and A $\beta$  receptors are returned back to the PM (Heckmann et al., 2019). Here, LANDO is dependent upon specific components of the autophagy machinery and acts as an important regulator of immune-mediated aggregate removal and microglial activation. The overall effect is protection against neuronal loss and memory impairment in a murine AD model.

### **Box 2: Nutrient sensing and mTORC1 regulation to maintain lysosome homeostasis and autophagic flux**

When nutrients are available, the main growth regulator mammalian target of rapamycin kinase complex 1 (mTORC1) protein kinase is recruited and activated at the lysosome, resulting in autophagy inhibition. The nutrient-sensing mTORC1 complex detects both cytosolic and intra-lysosomal amino acids through distinct mechanisms to inhibit autophagy (Condon and Sabatini, 2019). Subsequently, downstream anabolic signaling pathways are initiated to promote cell growth and survival (Saxton and Sabatini, 2017). A group of small lysosome-localized and amino acid-regulated GTPases, the Ras-related GTP-binding (Rag) GTPases recruit mTORC1. Rheb, a small GTP-binding protein regulated by growth factors, activates mTORC1, which in turn phosphorylates and inhibits ULK1 and transcription factor EB (TFEB), a master regulator of both lysosomal biogenesis and autophagy (Hosokawa et al., 2009; Settembre et al., 2011). During nutrient deprivation, the tuberous sclerosis complex (TSC) is recruited to lysosomes, resulting in Rheb inactivation and thus mTORC1 inhibition and release (Inoki et al., 2005). Calcineurin, a phosphatase activated by starvation-induced lysosomal calcium release, dephosphorylates TFEB (Medina et al., 2015). Dephosphorylated TFEB translocates from the cytosol to the nucleus and induces transcription of the coordinated lysosomal expression and regulation (CLEAR) gene network, including several key autophagy and lysosomal genes (Puertollano et al., 2018). This promotes the formation of autophagosomes and their fusion with lysosomes. Interestingly, TFEB is a target of chaperone-mediated autophagy (CMA) (Schneider et al., 2015), pointing to an interconnection between different autophagy pathways. During prolonged starvation, mTORC1 is reactivated and autophagy attenuated while lysosome reformation is induced (Yu et al., 2010). In addition, TFEB promotes the expression of endocytic genes and induces trafficking of mTORC1 activators to the lysosome in an autophagy-independent manner. TFEB-triggered endocytosis is thus required for re-activation of mTORC1 and for maintaining autophagic flux (Nnah et al., 2019).





**Table 1.** Overview of known common effectors of mammalian autophagy and endocytosis and their main impact on autophagosomes and endosomes/lysosomes

<b>Protein</b>	<b>Effect on autophagosomes</b>	<b>Effect on endosomes/lysosomes</b>
ATG3	Involved in phagophore elongation.  (Mizushima et al., 2011)	Required for LAP.  (Sanjuan et al., 2007; Martinez et al., 2015, 2016)
ATG4B	Involved in phagophore elongation.  (Mizushima et al., 2011)	Obligatory for LAP and LANDO.  (Sanjuan et al., 2007; Martinez et al., 2015, 2016; Heckmann et al., 2019)
ATG5	Required for phagophore elongation,  (Mizushima et al., 2011)	Involved in LAP and LANDO.  (Sanjuan et al., 2007; Martinez et al., 2015, 2016; Heckmann et al., 2019)
ATG7	Required for phagophore elongation.  (Mizushima et al., 2011)	Crucial for targeting of damaged early endosomes. Required for LAP and LANDO.  (Fraser et al., 2019; Sanjuan et al., 2007; Martinez et al., 2015, 2016; Heckmann et al., 2019)
ATG8s	Promote elongation of the phagophore membrane as well as its closure. Crucial for fusion with the lysosome.  (Weidberg et al., 2010; Mizushima et al., 2011; Shpilka et al., 2011; Nguyen et al., 2016; Tsuboyama et al., 2016)	Involved in LAP and LAP-like processes such as LANDO. Interacts with several central players in clathrin-mediated endocytosis, such as AP2.  (Tian et al., 2013; Florey et al., 2015a; Cadwell and Debnath, 2018; Heckmann and Green, 2019; Heckmann et al., 2019)
ATG9A	Localized on endocytic and Golgi derived vesicles that deliver lipids and proteins to the forming phagophore.  (Noda, 2017; Judith et al., 2019)	Localized on endocytic vesicles that fuse and contribute to the EE and RE.  (Noda, 2017)
ATG12	Mediates phagophore elongation.  (Mizushima et al., 2011)	Required for LAP.  (Sanjuan et al., 2007; Martinez et al., 2015, 2016)

ATG14L	<p>Component of the PI3KC3-C1 complex and crucial for phagophore nucleation. Interacts with STX17 and promotes fusion of autophagosomes with LE/lysosomes.</p> <p>(Mizushima et al., 2011; Baskaran et al., 2014; Diao et al., 2015)</p>	<p>Binds to the SNARE effector protein Snapin to facilitate endosome maturation.</p> <p>(Kim et al., 2012)</p>
ATG16L1	<p>Localized on endocytic vesicles and is delivered to the forming phagophore. Mediates phagophore elongation.</p> <p>(Fujita et al., 2008; Ravikumar et al., 2010; Moreau et al., 2011; Soreng et al., 2018)</p>	<p>Localized on endocytic vesicles that fuse and contribute to the RE. Required for LAP.</p> <p>(Sanjuan et al., 2007; Martinez et al., 2015, 2016; Fletcher et al., 2018)</p>
Beclin 1	<p>Core component of PI3KC3-C1 complex and crucial for phagophore nucleation</p> <p>(Mizushima et al., 2011; Baskaran et al., 2014)</p>	<p>Core component of PI3KC3-C2 complex and promotes PI3P production on endosomal membranes. Involved in EE localization, maturation and LE formation. Required for LAP and LANDO.</p> <p>(Rohatgi et al., 2015; McKnight et al., 2014; Sanjuan et al., 2007; Martinez et al., 2015, 2016; Heckmann et al., 2019)</p>
BIF-1	<p>Regulates ATG9 vesicle formation from Golgi and Rab11 positive endosomal membranes. Involved in phagophore nucleation.</p> <p>(Takahashi et al., 2011; 2016)</p>	<p>Involved in degradative endocytic traffic.</p> <p>(Thoresen et al., 2010)</p>
Rubicon	<p>Inhibits autophagosome-lysosome fusion.</p> <p>(Matsunaga et al., 2009; Zhong et al., 2009)</p>	<p>Controls EE to LE maturation. Involved in LAP and LANDO.</p> <p>(Sun et al., 2010; Sanjuan et al., 2007; Martinez et al., 2015, 2016; Heckmann et al., 2019)</p>
UVRAG	<p>Component of the PI3KC3-C2 complex and involved in autophagosome maturation</p>	<p>Component of the PI3KC3-C2 complex and involved in PI3P production on endosomal</p>

	and fusion with LE/lysosomes.  (Itakura et al., 2008; Matsunaga et al., 2009; Cheng et al., 2017)	membranes. Involved in degradative endocytic traffic. Involved in lysosome recycling.  (Liang et al., 2008; Thoresen et al., 2010)
VPS34	Core component of PI3KC3-C1 complex and crucial for phagophore nucleation.  (Itakura et al., 2008; Matsunaga et al., 2009; Baskaran et al., 2014)	Core component of PI3KC3-C2 complex and mediates PI3P production on endosomal membranes. Involved in degradative endocytic traffic. Important in LAP and LANDO.  (Sanjuan et al., 2007; Liang et al., 2008; Thoresen et al., 2010; Martinez et al., 2015, 2016; Heckmann et al., 2019)
VPS15	Core component of PI3KC3-C1 and -C2 complexes. Crucial for phagophore nucleation.  (Itakura et al., 2008; Matsunaga et al., 2009; Baskaran et al., 2014)	Core component of PI3KC3-C2 complex and mediates PI3P production on endosomal membranes. Involved in degradative endocytic traffic. Important in LAP.  (Sanjuan et al., 2007; Liang et al., 2008; Thoresen et al., 2010; Martinez et al., 2015, 2016; Heckmann et al., 2019)
ESCRT complexes	Involved in phagophore closure.  (Takahashi et al., 2018; Zhen et al., 2019)	Important for sorting of cargo into intraluminal vesicles targeted for degradation. Involved in cargo engulfment in endosomal microautophagy. Involved in lysosome damage repair.  (Christ et al., 2017; Tekirdag and Cuervo, 2018); Mejlvang et al., 2018; Skowyra et al., 2018)
Rab5	Involved in phagophore nucleation.  (Ravikumar et al., 2008)	Involved in EE formation and the transition of EE to LE.  (Zeigerer et al., 2012)
Rab7	Important role in autophagosome trafficking and autolysosome maturation.  (Guerra and Bucci, 2016; Kuchitsu et al., 2018)	Key mediator of endosome maturation and LE/lysosome trafficking.  (Bucci et al., 2000)
Rab11A	Enriched on RE membranes	Involved in RE-to-plasma

	<p>where it can promote phagophore nucleation. Important for recruitment of ATG16L1 to RE.</p> <p>(Puri et al., 2018; Knaevelsrud et al., 2013)</p>	<p>membrane transport.</p> <p>(Ullrich et al., 1996)</p>
HOPS complex	<p>A core tethering effector that is recruited to autophagosomes to mediate fusion with lysosomes.</p> <p>(Jiang et al., 2014; Gao et al., 2018)</p>	<p>Rab7 effectors recruit HOPS to endolysosomal membranes to mediate fusion events.</p> <p>(Wijdeven et al., 2016)</p>
VAMP3	<p>R-SNARE that regulates heterotypic fusion of ATG9A and ATG16L1-containing vesicles. Impacts on phagophore nucleation.</p> <p>(Puri et al., 2013)</p>	<p>Mediates transport from endosomes to TGN,</p> <p>(Mallard et al., 2002; Ganley et al, 2008)</p>
VAMP7	<p>R-SNARE that regulates homotypic fusion of ATG16L1-positive vesicles. Mediates fusion between autophagosomes and lysosomes.</p> <p>(Moreau et al., 2011; Saleeb et al., 2019)</p>	<p>Mediates fusion of endosomes with lysosomes.</p> <p>(Pryor et al., 2004)</p>
STX16	<p>Important for fusion of autophagosomes with lysosomes.</p> <p>(Gu et al., 2019)</p>	<p>Involved in transport from endosomes to TGN. Involved in lysosome biogenesis.</p> <p>(Mallard et al., 2002; Ganley et al, 2008)</p>
SNX18	<p>Mediates transport of ATG9 and ATG16L1 from RE to the phagophore. Impacts on phagophore nucleation.</p> <p>(Knaevelsrud et al., 2013; Soreng et al., 2018)</p>	<p>Important for recruitment of ATG16L1 to RE.</p> <p>(Knaevelsrud et al., 2013; Soreng et al., 2018)</p>

## Figure legends

### **Fig 1. Overview of autophagy and endocytosis.**

**Upper:** Key steps in autophagy (selective autophagy is depicted) are initiation and nucleation, followed by expansion, cargo recruitment and closure culminating in maturation and fusion. Initiation is mediated by an active ULK complex that subsequently activates the **PI3KC3-C1 complex**, resulting in the formation of a crescent-shaped double membrane structure called the phagophore (nucleation). The formation and expansion of the phagophore involves lipids from diverse membrane sources, including the endoplasmic reticulum (ER), mitochondria (M), trans-Golgi network (TGN), plasma membrane (PM) and recycling endosomes (RE) (dotted arrows). Phagophore expansion depends upon lipidation and insertion of ATG8-family proteins (ATG8 conjugation) on both the inner and outer membrane of the growing phagophore. The conjugated ATG8s recruit proteins, including selective autophagy receptors (SARs) that bring cargo destined for degradation to the phagophore. The phagophore finally encloses its intracellular cargo, resulting in the double-membrane autophagosome. The autophagosome then matures through fusions with different endolysosomal compartments, culminating in the formation of the autolysosome where the cargo is degraded and recycled to the cytosol. N, nucleus. Blue dotted arrows indicate effects of the endosomal compartment on autophagy.

**Lower:** Endocytosis involves inward invagination of the plasma membrane, giving rise to small intracellular vesicles that contain plasma-membrane constituents and extracellular components (clathrin-mediated endocytosis is depicted). These small vesicles fuse and form the compartment termed the early endosome (EE). Here, sorting events direct the fate of the internalized cargo for either return to the plasma membrane or degradation. The cargo can be directly and rapidly returned to the plasma membrane from early endosomes via Rab4-mediated transport. Slower recycling to the plasma membrane involves vesicle transport from EE to the plasma membrane through the Rab11-containing recycling endosome (RE). The early endosome, characterized by the presence of Rab5, gradually matures into a late endosome (LE) **mainly associated with Rab7, through a structure termed multivesicular body (MVB)**. Here, multiple intraluminal vesicles (ILVs) are formed by inward budding of the endosomal membrane. The ILVs contain the cargo destined for degradation. The LE finally matures into a lysosome or fuses with an existing lysosome, resulting in an endolysosome, in which the cargo-loaded ILVs are degraded. MVBs can also fuse with the plasma membrane in a process termed exocytosis, releasing ILVs as exosomes to the extracellular environment.

**Insets:** Other forms of autophagy termed microautophagy and chaperone mediated autophagy (CMA) involve the direct engulfment of cargo into lysosomes through either inward budding

of the lysosomal membrane or HCS70 (heat shock-cognate protein of 70 KDa)-mediated membrane translocation through LAMP2A, respectively. Direct cargo engulfment by late endosomes can also occur and is termed endosomal microautophagy.

**Fig. 2. Intersections between endocytosis and autophagy. (A) Vesicle formation and components of the autophagy machinery.** ATG9- and ATG16L-containing vesicles are formed through **clathrin-dependent endocytosis**. ATG9A vesicles travel through the early endosome (EE) to the recycling endosome (RE) where they converge with ATG16L vesicles that bypass the EE. ATG9A and ATG16L vesicles are mobilized from the RE and supply lipids and proteins to the forming phagophore. ATG9A is also mobilized from the TGN and is involved in transient membrane interactions at the phagophore. ATG16L is recruited to the phagophore and engages in the ATG8 conjugation complex to mediate ATG8 membrane insertion. ESCRT-III located at endolysosomal membranes is transiently recruited and involved in mediating phagophore closure, giving rise to the autophagosome. Blue arrows indicate contribution from endocytosis to early steps of autophagosome formation. **LC3-associated phagocytosis (LAP)** involves engulfment of large extracellular particles; this results in a single-membrane containing structure, termed a LAPosome (**See Box 1**). Here, lipidated LC3 is inserted in a mechanism that is dependent on ATG16L. Notably, the recruitment of ATG16L to the LAPosome is distinct from recruitment to the phagophore **where the WD repeat-containing C-terminal domain is crucial for LAP but dispensable for canonical autophagy.** **(B) The endocytic compartment and effects on late steps of autophagy.** The autophagosome can either fuse directly with the lysosome to form the autolysosome, or first merge with early or late endosomes. This results in the single-membrane structure termed the amphisome that subsequently fuses with the lysosome. The fusion between the autophagosome/amphisome and the endosome/lysosome is mediated by Rab GTPases, tethering factors and SNARE complexes (depicted in the insets). **In the zoomed in capture of the two membranes fusing, ATG8 is illustrated bound to the tether. It should be noted that the HOPS complex does not interact with ATG8 in mammalian cells.**

**Fig. 3. Vesicle trafficking, lysosome reformation and quality control**

**A) Transport of autophagosomes and endosomes/lysosomes.** The motor proteins kinesin and dynein/dynactin are responsible for vesicle transport on microtubules towards the cell periphery (plus-end transport) and the perinuclear region (minus-end transport), respectively. Rab7 interacts with its effectors FYCO1 and RILP to promote motor-mediated vesicle

movements in opposite directions. FYCO1 can also bind to LC3 on the autophagosome membrane. ORP1 facilitates the interaction between the motor and the Rab7-RILP complex and mediates perinuclear directed movement when endosome/autophagosome membrane cholesterol levels are high. The motor scaffolding protein JIP1 interacts with LC3 on autophagosomes and promotes their perinuclear transport via dynein-dynactin in neurons. The protein complex BORC-ARL8 facilitates kinesin-mediated endosome/lysosome movement towards the cell periphery. **(B) Autophagic lysosome reformation (ALR).** A clathrin-coated bud on the surface of an autolysosome acts as the site of tubule generation for lysosome reformation. The tubule is extended through kinesin activity, which provides a pulling force along the microtubule. Dynamin 2 GTPase is responsible for vesicle scission at the tip of the tubule, giving rise to a protolysosome that subsequently matures into a functional lysosome. **(C) Lysosome damage, repair and removal.** Damage of lysosomes in the form of small perforations is recognized by cytosolic galectins that act as damage sensors; such a limited damage triggers the recruitment of the ESCRT machinery for membrane resealing and repair. Upon more extensive lysosomal membrane damage, the autophagy machinery is recruited by galectins; this removes damaged lysosomes in a process termed lysophagy.



Figure 1

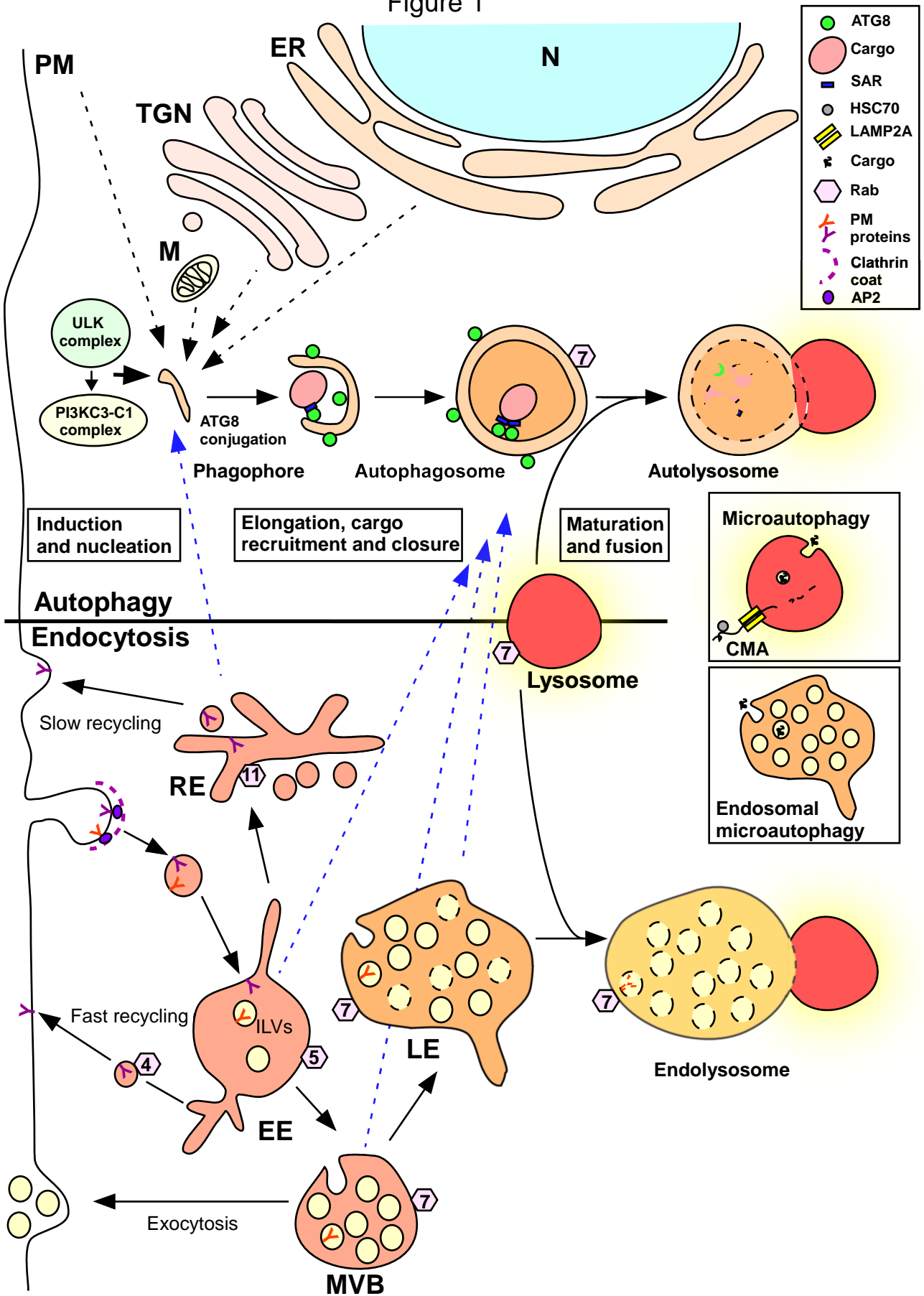


Figure 2

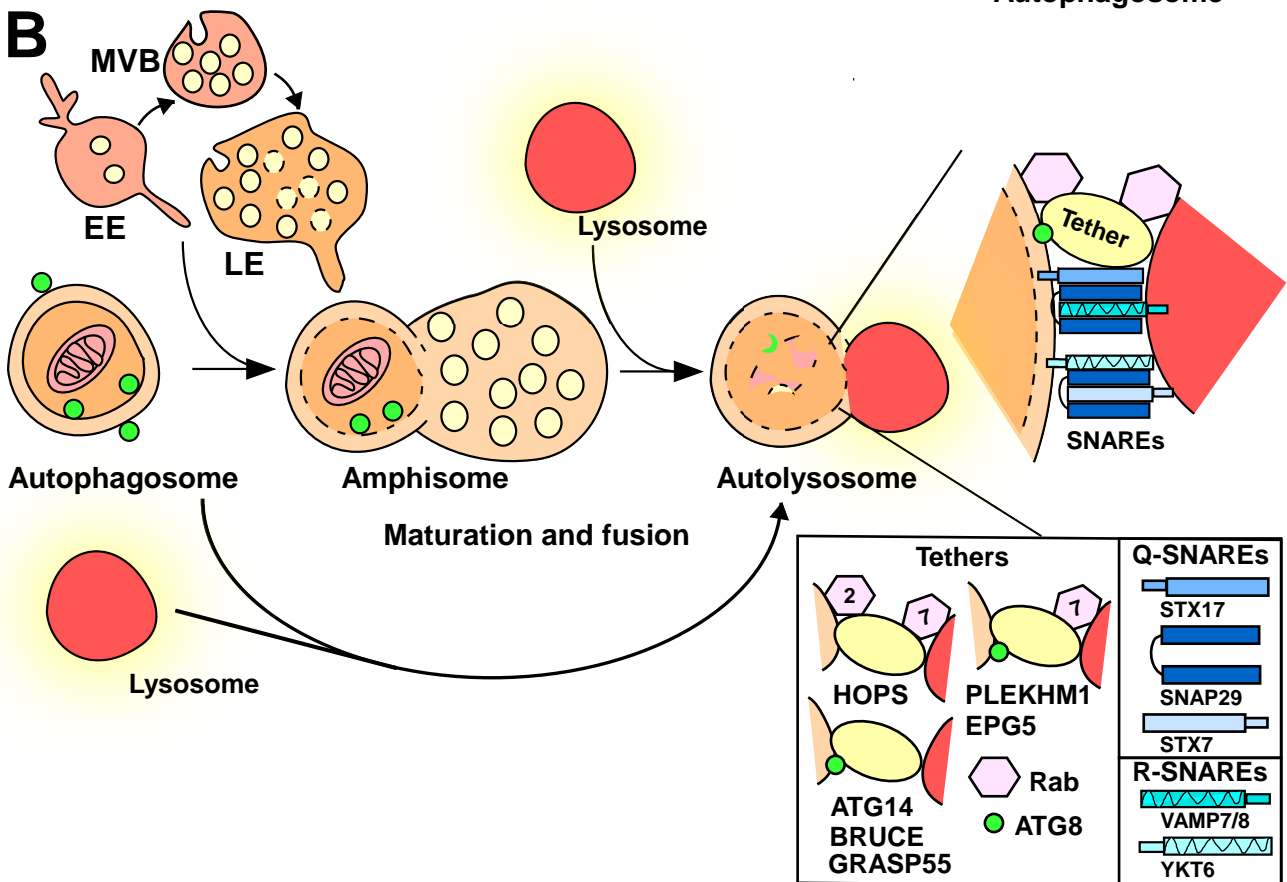
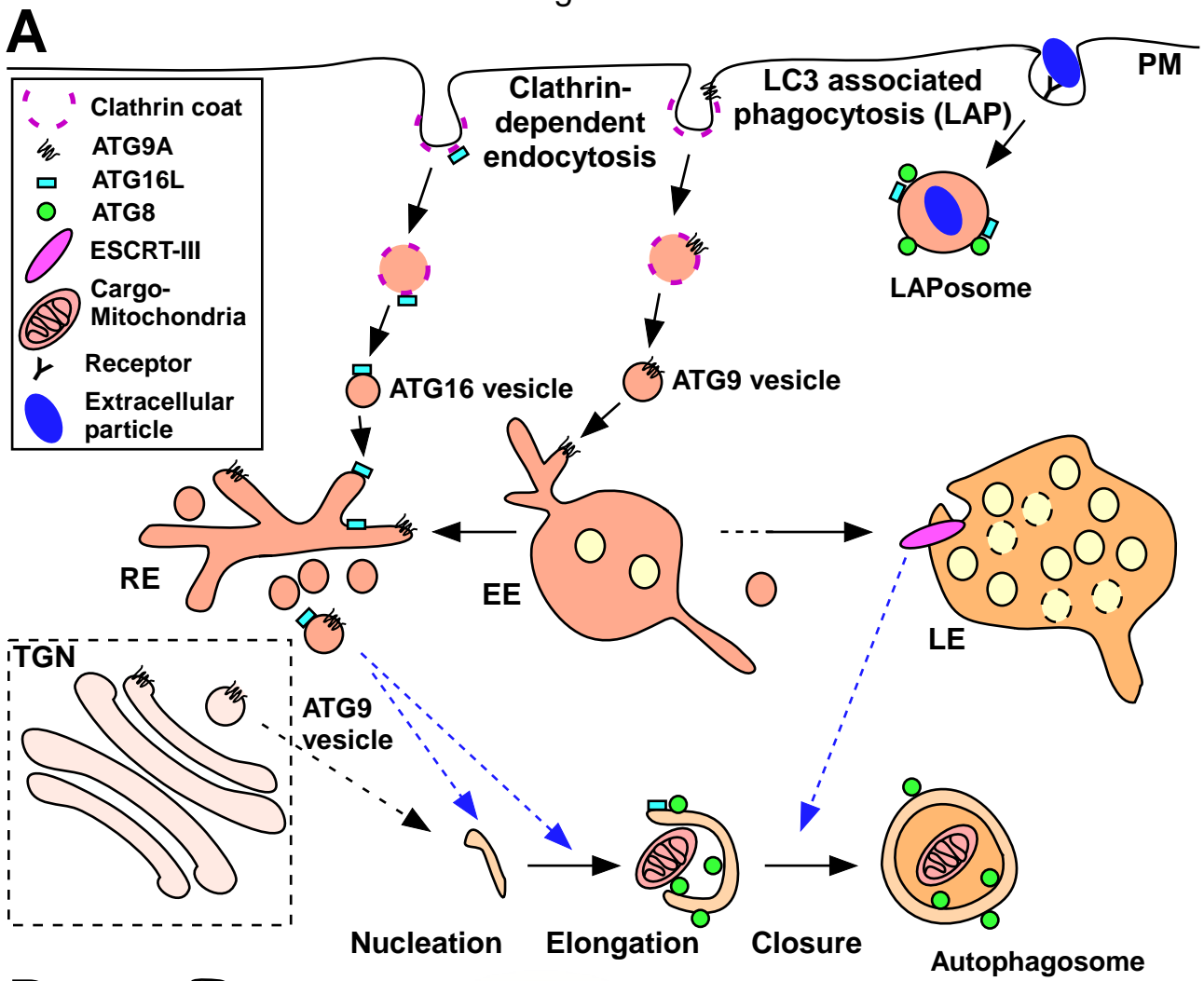


Figure 3

