Chapter

Intracellular Lipid Homeostasis and Trafficking in Autophagy

Shreya Goyal, Meaghan R. Robinson, Verónica A. Segarra and Richard J. Chi

Abstract

In eukaryotes, lipids are not only an important constituent of the plasma membrane but also used to generate specialized membrane-bound organelles, including temporary compartments with critical functions. As such, lipids play a key role in intracellular homeostasis—the ability of a cell to maintain stable internal conditions upon changes in its extracellular environment. Autophagy, one of the cellular processes through which eukaryotic cells strive for survival under stress, is heavily dependent on lipid and membrane trafficking through the *de novo* formation of autophagosomes—temporary, large, and double-bilayered organelles in which materials are encapsulated for recycling. This chapter discusses what we know about lipid homeostasis and trafficking during autophagy and autophagosome formation and comments on future directions of the field.

Keywords: lipids, lipid homeostasis, lipid trafficking, autophagy, autophagosomes

1. Introduction

Careful control of cellular lipid pathways plays an important role in a cell's ability to maintain stable internal conditions in the face of an ever-changing extracellular environment. This is particularly true as it relates to cellular self-eating or autophagy, a process brought about by proteins collectively known as the Atg (in yeast) or ATG (in mammals, **A**u**T**opha**G**y) proteins [1]. Macroautophagy (herein referred to as autophagy) is the catabolic process by which unneeded or damaged cellular components are sequestered as cargo into unique double-membrane vesicles called autophagosomes [1]. Once made, autophagosomes deliver their contents for breakdown by docking and fusing with the cell's degradative organelle, the lysosome in animal cells or the vacuole in plant and yeast cells. Following the breakdown of these materials, the components can undergo efflux to enable recycling and reuse by cells. Autophagy can be activated in many ways, including by starvation and cellular damage. When induced by starvation, autophagy allows for the recycling of nutrients to sustain metabolism in the absence of extracellular nutrients. Under other conditions, such as when specific cellular components are damaged, autophagy can take more specific forms, targeting the damaged components for sequestration into autophagosomes and delivery to lysosomes/vacuoles for destruction and recycling of the generated materials. The damaged components sequestered by more targeted forms of autophagy include mitochondria (mitophagy), ribosomes (ribophagy), and peroxisomes (pexophagy) [2–4].

Dysregulation of autophagy has been linked to a variety of human disease states, including cancer, neurodegenerative disease, and heart disease [5]. Mutations in ATG genes can result in autosomal recessive human genetic conditions including diseases like Niemann-Pick Type C1, a progressive lipid storage disorder associated with impaired autophagosome maturation and characterized by neurodegeneration [6], Gaucher disease, a disorder related to the inability to breakdown specific cargo once autophagosomes fuse with the lysosome and characterized by hematologic symptoms [7], and Pompe disease, commonly coupled with glycogen accumulation in autophagic compartments and lysosomes, often resulting in myopathies [8, 9]. These relationships between autophagy and human disease have generated much interest in attaining a better understanding of autophagic processes, especially its hallmark—autophagosome formation [1, 5].

Autophagosomes differ from smaller, more traditional types of transport vesicles in that they form *de novo* (anew) rather than budding off of a pre-existing donor organelle, range from 600 to 900 nm rather than 60–100 nm in diameter, and are delimited by two bilayer membranes rather than by a single bilayer [1]. Autophagosome formation takes place at distinct cellular locations called pre-autophagosomal structures (PAS). Anew, membrane materials from different intracellular lipid sources are brought to the PAS to ultimately nucleate, form, and complete autophagosome biogenesis—highlighting the important role of lipid homeostasis in autophagy. Much effort has been dedicated to understanding the lipid trafficking events leading to autophagosome formation, the unique hallmark of autophagy. In this review, we discuss the progress that has been made in understanding this relationship between autophagosome formation and lipid homeostasis.

We begin this discussion by surveying the diversity of lipids present inside cells, including evidence on the role of each lipid type in autophagy. We discuss how membrane composition helps establish organelle identity, in order to place autophagosome formation within a larger context of lipid homeostasis. After considering how these lipids relate to autophagy, we discuss the membrane trafficking events leading to autophagosome formation. We conclude by commenting on the questions that remain unanswered at the intersection of autophagy and lipid trafficking.

2. Structure/function diversity of lipids and membranes in eukaryotes

Lipids are the only biologically relevant macromolecules that cannot be categorized as a polymer—their diversity is not dependent on monomer sequence like that of complex carbohydrates, proteins, or nucleic acids. The diversity of lipids is instead dependent on their chemical structure and the unique properties that are attained by combining different lipid molecules in the context of biological membrane bilayers [10–12]. The structural diversity of lipids in biological membranes can be divided into three different groups: glycerophospholipids (also often referred to as phospholipids), sphingolipids, and sterols (**Figure 1**). These three types of amphipathic lipids, when combined in different ratios and leaflet asymmetries, give rise to a variety of biological membrane bilayer properties such as fluidity and curvature. Lipids in biological membranes can be covalently modified through the attachment of carbohydrate or phosphate moieties through glycosylation or phosphorylation, respectively. These modified lipids also contribute to the functional diversity of biological membranes. The properties of a membrane bilayer are also influenced by the proteins embedded within it. Some transmembrane proteins can be post-translationally modified to contain chemical groups like carbohydrates

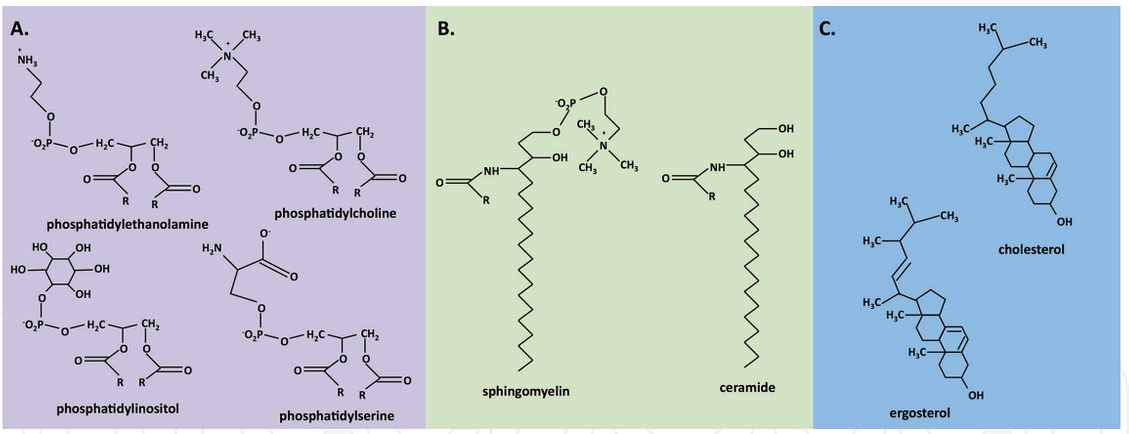

Figure 1.
Chemical structures of selected cell membrane lipids. Examples of some of the different types of lipids that can be found in cell membranes, namely (A) phospholipids, (B) sphingolipids, and (C) sterols.

(glycoproteins) and phosphate groups (phosphoproteins) that also inform membrane function. Some membrane and peripheral membrane proteins can be modified by covalent attachment to lipid groups as well.

2.1 Glycerophospholipids

Glycerophospholipids or phospholipids are the major component of biological membranes and their name evokes the building blocks used for their synthesis, including a three-carbon glycerol backbone (**Figure 1A**). This glycerol molecule is modified so that two of its hydroxyl groups (the ones attached to carbons 1 and 2) are esterified to covalently attach long-chain fatty acids to its backbone [10–12]. The fatty acid esterified to carbon 1 is often saturated (no carbon to carbon double-bonds) and the fatty acid esterified to carbon 2 is often unsaturated (with carbon to carbon double bonds present) [10–12]. Carbon to carbon double bonds in fatty acids have bends associated with them, which modify interactions with adjacent molecules and contribute to the fluidity of the bilayer [10–12]. The hydroxyl group on the third carbon of the glycerol backbone is covalently attached or esterified to phosphoric acid, adding a highly polar head group to the phospholipid and contributing to its amphipathic nature [10–12].

Glycerophospholipid diversity is determined by the specific fatty acids and potentially alcohol-modified phosphoric acid groups attached to the glycerol backbone. A cell can generate a large variety of more than 100 glycerophospholipids, each with a different combination of fatty acids on carbons 1 and 2 and a head group on carbon 3 [10–12]. Glycerophospholipids are named depending on the head group attached to carbon 3. Common glycerophospholipids in cells include: phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI) [10–12]. PE is the simplest phospholipid, with just a phosphoric acid moiety as a head group, while the other common types have alcohol-modified phosphoric acid head groups [10–12]. PI in particular is the kind of lipid that plays a major role in determination of organelle identity, as described later in this review [10–12].

Certain phospholipids and lipid-modifying enzymes are key for autophagy. For example, the PI3Kinase complex (Class III and potentially Class II) and its substrate PI are essential for autophagosome formation [13, 14]. Moreover, soluble autophagy-related proteins known to be required for the induction of autophagosome formation like Atg1 are known to be recruited to membrane enriched with PI and PI3P [13, 14].

Another lipid component essential for autophagosome formation is PE [13, 14]. PE lipids present in the autophagosome are important for the ability of this structure to associate with Atg8, another essential autophagy protein. This is because successful recruitment of Atg8 to autophagosomes involves Atg8 lipidation to the head group of PE, ultimately yielding a mature autophagosome that is decorated by lipidated Atg8 [13, 14]. In fact, PE is the lipid that contributes the most to autophagosome expansion [13, 14].

2.2 Sphingolipids

The backbone of sphingolipids is not glycerol but sphingosine, an amino alcohol synthesized from palmitoyl-CoA and serine [10–12]. The covalent attachment of the sphingosine backbone to a fatty acid through an amide linkage yields an amphipathic sphingolipid (**Figure 1B**) [10–12]. Common examples of this type of lipid in cells include ceramides (the simplest form of sphingolipid), phosphosphingolipids (also known as sphingomyelins, the only phospholipids without a glycerol backbone), and glycosphingolipids [10–12]. The differences and diversity between these sphingolipids is dependent on the type of fatty acid and additional head groups attached to the sphingosine backbone. Sphingolipids were discovered and can be predominantly found in nervous tissue. This type of lipid can also be found in lower eukaryotes such as budding yeast [10–12].

Sphingolipids such as sphingosine-1-phosphate (S1P) and ceramides have been found to have an effect on autophagy [13, 14]. S1P, as part of its well-known role in fostering proliferation and cell survival, can induce autophagy in a way that engages elements usually associated with apoptosis [13, 14]. Ceramides, in their well-known role in promoting cell cycle arrest, promote cell death while engaging elements associated with autophagy [13, 14].

2.3 Sterols

Sterols are four-ringed steroid molecules that contain a hydroxyl functional group at position 3 and as well as a variety of potential side chains (**Figure 1C**) [10–12]. These lipids can be present in plant, animal, and microbial cells such as budding yeast. The predominant form of sterol lipid in animal cells is cholesterol. In other organisms, the lipid forms most similar to cholesterol in function are called by different names (**Figure 1C**). For example, the functional yeast equivalent of cholesterol is referred to as ergosterol (**Figure 1C**).

Sterols in eukaryotes have been shown to influence specific forms of autophagy including chaperone-mediated autophagy (CMA), pexophagy (autophagy of peroxisomes) and lipophagy (autophagy of lipid droplets) [13, 14]. These types of specific autophagy are beyond the scope of this review and are therefore not discussed.

2.4 Lipid contributions to membrane structure/function

2.4.1 Membrane curvature

The concept of membrane curvature refers to the lipid composition asymmetry between the two leaflets of a membrane bilayer [11, 15, 16]. The membrane curvature of an area can be changed, not only by altering its lipid composition, but also through the function of specialized proteins that can bind and remodel membranes. Early autophagosomes, often referred to as phagophores, have a high degree of curvature and are enriched for PI and PI3P [13, 14]. This combination of curvature

and lipid composition is thought to help recruit a collective of autophagy-related proteins such as Atg1 and Atg3 to the site of autophagosome formation [13, 14].

2.4.2 Membrane fluidity

Membrane fluidity refers to the ability of a membrane to sustain diffusion-driven movement of molecules within it [11, 17]. In membranes, diffusion takes place laterally [11, 15, 17]. The presence of unsaturated and sterol lipid structures enhances membrane fluidity, ultimately allowing for more lateral diffusion of molecules and rendering it easier to bend and deform [11, 17].

This relationship between membrane fluidity and bending is highly relevant to autophagosome membranes [13, 14]. Early autophagosomal structures are characterized by high levels of curvature, suggesting that fluidity is key for autophagosome formation [13, 14]. In fact, autophagosomes are enriched for unsaturated lipids, and abrogation of enzyme functions that mediate desaturation can have inhibitory effects on autophagy [13, 14].

2.4.3 Lipid microdomains

Membranes are not even in composition throughout. Instead, they can have areas of differential lipid composition such as lipid rafts. Lipid rafts are generally more tightly packed (less fluid) than neighboring membrane material and their edges are usually rich in sphingolipids and cholesterol [11, 15, 17, 18]. Lipid rafts, because of their differential lipid composition, can act as scaffolds for non-clathrin-mediated internalization dynamics [11, 15, 17, 18].

While no specific lipid microdomains have been identified as characteristic of autophagosome membranes, it is known that lipid rafts associated with the endoplasmic reticulum and mitochondria can contribute to autophagosome formation from these lipid sources [13, 14].

3. Organelle lipid identity

Each membrane-bound organelle in the cell is characterized by a membrane bilayer with a particular lipid composition, leading to unique physical properties and the ability to recruit a specific set of interacting partners (**Table 1**). For this reason, organelle identity is not only defined by the collection of lipids displayed by the membrane of a particular organelle, but also by the set of proteins and molecules that are able to associate and interact with it. It is also important to recognize that these membranes can be remodeled and change their properties over time as cells strive to respond to stimuli.

Organelle Membrane	Lipid Content
Plasma membrane	ceramide, sphingosine, S_1P, diglyceride (also known as diacylglycerol), PI_4P, $PI_{(4,5)}P_2$, $PI_{(3,4)}P_2$,$PI_{(3,4,5)}P_3$
Endoplasmic Reticulum	PC, PE, PI, PS, PA, ceramide, galactosylceramide, cholesterol and ergosterol.
Mitochondria	PE, PG, cardiolipin, PA
Golgi	PE, PS, PC, PI_4P, sphingolipid
Late endosome	$PI_{(3,5)}P_2$, PI_3P, PS
Lipid Droplets	triacylglycerol esters and steryl esters, PE

Table 1.
Lipid content of different organelle membranes.

The two main molecular determinants of organelle membrane identity in cells are lipid content, specifically PIs, and association with activated small GTPases (such as the Rab family of proteins) [19, 20]. These membrane elements can easily be remodeled and changed, in contrast to transmembrane proteins and other integral membrane elements, allowing for the dynamic regulation and sculpting of membranes. Small GTPases exhibit diversity that correlates with specific subcellular locations, such that Rab 1 (ER and Golgi), Rab 2 (cis Golgi), Rab 4/11 (Recycling endosomes), and Rab 5 (Early endosomes, plasma membrane, clathrin coated vesicles) each localize to the membranes of different organelles [19, 20].

Small GTPases like Rabs exist in two forms: a GTP-bound active and membrane associated form, and a GDP-bound inactive cytosolic form. While Rabs can be lipidated (prenylated) at their C-terminus, facilitating their anchoring to the membrane, what is thought to determine Rab-membrane interaction specificity are the proteins that facilitate their GDP/GTP exchange [19, 20].

Organelle identity is also determined by specific forms of PI, with phosphate groups covalently linked to positions 3, 4, or 5 of their inositol ring [11, 12, 15, 19–22]. The plasma membrane contains predominantly PI4P and PI(4,5)P2, with the latter synthesized from the former [19–22]. These two can be phosphorylated by PI3-kinases (PI3K) to generate signaling lipids [19–22]. PI(4,5)P2 can also be cleaved to generate diacylglycerol (DAG; which can activate autophagic response) and I(1,4,5)P3 second messengers [19–22]. PI4P can also be found in the Golgi [19, 20]. Early endosomes are characterized by the presence of PI(3)P through the function of PI3-OH kinases like Vps34, which require small GTPases like Rab5 to function at the proper location [13, 14, 19–22]. This requirement for a Rab GTPase to produce the corresponding PI species characteristic of the compartment highlights the close functional relationship between the two key determinants of organelle identity [19–22]. The presence of PI(3,5)P (synthesized from PI3P) is characteristic of late endosomes [19–22].

The lipid composition of organelles is influenced by exchange of materials between them. There are different ways in which such exchanges can take place, including vesicle-mediated membrane trafficking between organelles, lipid droplet function, and direct exchange of lipid species via membrane contact sites between organelles such as the ER and Golgi. While the ER is the central site for intracellular lipid synthesis, lipid trafficking is essential for lipids to move from the ER to different organelles in order to maintain homeostatic membrane composition required for morphology, signaling and cellular processes. Moreover, it is common for organelles like the Golgi and endosome to change their lipid and membrane compositions as they mature.

Much of what we know about the lipid content of intracellular organelles or compartments is due to lipidomics studies that have served as a tool to identify lipid species in these membranes [23–27]. Biochemical fractionation and/or extraction techniques are used to isolate organellar membranes of interest and subject them to mass spectrometry for lipid identification and lipidome determination, similar to proteomics studies that have been used to catalog the protein content of many of these organelles [27]. Different efficiencies and membrane targets can be obtained depending on how the samples are prepared and treated during extraction leading up to ionization and mass spectrometry analysis [27]. Different types of data processing allow for a range of lipidomics applications, from species identification and quantification to pathway and network analysis [27]. These techniques have been thoroughly reviewed elsewhere and outside of the scope of this review article [27].

While proteomic studies have shed light on the proteins that reside in autophagosomes [28–31], an autophagosome lipidome has yet to be experimentally determined. Through microscopy and biochemical methods, we know that the lipid identity of autophagosomes is defined largely by the presence of PE, PI and PI3P.

In the following section of this review, we discuss in detail the molecular processes that bring about lipid homeostasis and trafficking during autophagy, particularly in autophagosome formation.

4. Understanding autophagosomes: a non-traditional membrane vesicle

Current efforts to better understand autophagy are focused on the molecular details leading to the formation of autophagosomes, a double-membraned vesicle unlike any other in size and structure, that serves as the hallmark of autophagy. These approaches have mainly focused on understanding the intracellular trafficking of autophagy-related transmembrane proteins that are thought to help deliver lipids to the growing autophagosome via vesicle transport. Insights have also been obtained from assessing the influence of lipid-dependent enzymes as well as lipid-synthesis and transport pathways on autophagy. For example, mutation of PI3K enzymes severely impairs autophagy [32, 33], pointing at the importance PI and some of its phosphorylated forms in autophagic processes. In these ways, we have identified that PE and PI lipids play an important role in autophagy (**Table 2**) [14, 34–41]. We discuss these findings below.

4.1 Membrane dynamics during autophagy

The process of autophagy is often thought about to happen in different stages—initiation or nucleation of phagophores (cup-shaped, double-membraned autophagosome precursors), expansion of phagophores, completion or closure of autophagosomes, fusion of autophagosomes with the lysosome/vacuole, and the efflux of materials from the lysosome/vacuole for reuse (**Figure 2**). Because our review aims to focus on the lipid homeostasis and trafficking during autophagy, we focus our discussion below on the first four stages of autophagy (initiation through fusion).

4.1.1 Initiation

During the initial stages of autophagy, essential Atg proteins are recruited to PAS structures to nucleate the gathering of membrane *de novo* and to generate

Donor Membrane	Lipid Contributions	Autophagic Process/Step Benefited	References
Endoplasmic Reticulum	PI_3P	Autophagosome formation	34
Mitochondria	PE	Autophagosome formation	35
Plasma Membrane	PI_3P	Autophagosome and ATG16 vesicle formation	36
Golgi	PI_4P, PE, PS	ATG9 vesicles formation, autophagosome fusion with vacuole/lysosome	37-39
Late Endosome	PI_3P, $PI_{(3,5)}P_2$, PS	Autophagosome formation, autophagosome fusion with vacuole/lysosome	39, 40
ER-Golgi Intermediate compartments	PE	LC3/Atg8 lipidation	41
ER-Mitochondria contact sites	PE	Autophagosome formation	42
ER-Plasma membrane contact site	PI_3P	Autophagosome formation	43

Table 2.
Organellar lipid contributions to autophagic processes and autophagosome formation.

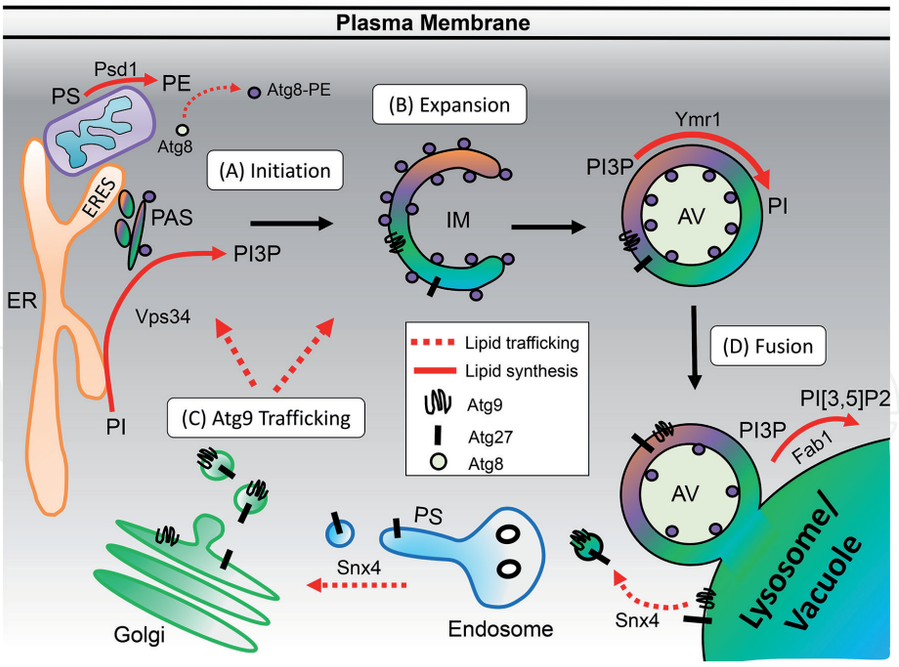

Figure 2.
Lipid biosynthesis and trafficking during autophagosome biogenesis and fusion. The relative lipid contributions via lipid trafficking or lipid biosynthesis from each organelle are shown. (A) upon autophagy induction, membrane is sequestered from pools of lipid donors such as the ER, mitochondria, Golgi, endosome and vacuole to form the pre-autophagosomal structure (PAS) or isolation membrane (IM). (B) PI in the PAS is converted to PI3P via Vps34 and Atg8-PE is enriched, allowing for IM expansion and closure. (C) Additional lipid membrane from Atg9-Atg27 vesicle trafficking allows for further expansion of the IM. (D) Autophagosome (AV) maturation is completed once PI3P is converted into PI by myotubularin phosphatases such as Ymr1. The mature AV can fuse to the vacuole which has converted PI3P into PI(3,5)P2 via Fab1 kinase. PI(3,5)P2 permits fusion of AV and endosomes. Vacuole lipid homeostasis is further aided by Snx4 trafficking of PS and Atg27 from the endosome and vacuole.

autophagosome precursors called phagophores. In mammalian cells, phagophores can be formed at PAS sites proximal to ER, mitochondria, or plasma membrane [13, 14]. In yeast however, PAS structures are perivacuolar in nature, leading phagophores to originate at locations proximal to the vacuole with membrane contributions with other cellular locations like discussed below. While mammalian cells can display many PAS structures at steady state, yeast often display one of these structures at any given time.

Initially, activated or triggered by upstream inhibition of mTOR, core autophagy proteins collect at the PAS structure. These core factors include the ULK1 complex, ATG13, FIP200, ATG101, (yeast Atg1, Atg13, Atg17, Atg29 and Atg31) [13, 14]. The mobilization of these factors ultimately leads to the PAS recruitment and activation of the PI3K/Vps34 kinase complex which allows for the generation of PI3P at this site (from PI) (**Figure 2A**) [13, 14, 30, 31]. This enzyme activity drives forward phagophore initiation/nucleation, allowing additional recruitment of other proteins like WIPI proteins (Atg18 in yeast). WIPI proteins are key PI3P effectors. PI3K regulator proteins like ATG14 (Atg14 in yeast) are also recruited to the PAS, allowing for the modulation of initiation/nucleation of phagophore formation [13, 14]. ATG14 is also able to mediate homotypic fusion of single-membrane vesicles at the PAS, allowing more traditional membrane vesicles delivered to the PAS to fuse and contribute to the nucleation and growth of the phagophore [13, 14]. In this way, the phagophore has been found to accept lipid inputs from the ER, ER exit sites (ERES), the Golgi, the plasma membrane and recycling endosomes for its growth and expansion (**Figure 2A**) [13, 14].

Generation of PI3P at PAS sites functions as a scaffold for ATG/Atg proteins to associate with the PAS, helping in the expansion, elongation and curvature generation needed for autophagosome formation to proceed successfully [13, 14].

4.1.2 Expansion

The lipidation of Atg8 (LC3) onto PE lipid molecules in the growing phagophore membrane is key for autophagosome formation. Other Atg proteins like ATG16L1 (yeast Atg16) as well as ATG12-ATG5 are required for Atg8 lipidation with PE. Interestingly, some of these required Atg factors like ATG16L1 partly reside in the recycling endosome and have been found to localize to the PAS in a PX-BAR/SNX protein-dependent manner (for example, SNX18) [13, 14]. While not considered core Atg proteins, PX-BAR/SNX proteins are membrane remodeling proteins that have also been found to be required for autophagosome formation.

While the exact lipid composition of the phagophore/autophagosome is still under debate, because of the findings described above, PI3P is thought to be one of the most abundant lipid species present in these structures. In fact, recent findings identify the ATG protein ATG2A as a lipid shuttle factor that facilitates PI3P-dependent autophagosome growth [42–44]. Other phosphoinositides such as PI4P, PI(4,5)P2 and PI(3,5)P2 have also been suggested to play a role in the expansion of the phagophore [13, 14]. Apart from these, as mentioned above, sphingosine-1-phosphate (S1P) and ceramide, also contribute to autophagy. And while, Atg8/LC3 lipidation resulting in LC3-PE is important for the expansion and closure of the autophagosome, the intracellular membranes that are the source of this PE still remain to be identified. PE is produced from phosphatidylserine (PS) in mitochondria, which might be one of the sources of PE (**Figure 2A**). PE might also be shuttled from the ER, plasma membrane and recycling endosomes. It has been recently shown by Ma et al. [39], that PS trafficking by sorting nexin Snx4 in yeast is required for maintaining the correct lipid composition of the vacuole for autophagosome fusion in yeast (**Figure 2C**). Thus, maintenance of organelle lipid identity through proper trafficking and lipid homeostasis are crucial for autophagy as well.

4.1.3 ATG9 trafficking

ATG9 (Atg9 in yeast) is a six-transmembrane protein that is required for autophagosome formation [45–52]. ATG9 trafficking is one of the most studied topics in the autophagy field, highlighting the important role it plays in autophagy—the autophagic phenotypes of null mutants are very penetrant. While this is the case, we still lack detailed information about the function of this protein during autophagy. Yeast studies have shown that Atg9 is localized to PAS as well as cytoplasmic vesicles of 30–60 nm diameter that bud-off of the late Golgi. It is believed that Atg9 shuttles between the PAS and its cytoplasmic vesicle pool upon autophagy induction (**Figure 2C**). At the PAS, Atg9 associates with Atg1, Atg2 as well as Atg18. Since Atg9 is a transmembrane protein, its trafficking directly affects the funneling or channeling of membrane to the PAS and the growing autophagosome. The Atg9 vesicles are thought to originate at the Golgi and contain fusion factors such as subunits of the TRAPIII (Trs85) complex that are responsible for fusion with the growing autophagosome [45, 46, 52]. Once Atg9 reaches the lysosome/vacuole as autophagosomes fuse, some studies suggest that the protein can be recycled out of the vacuolar membrane for reuse in new rounds of autophagosome formation. These studies are synergistic with findings that Atg9 traffics through endosomal compartments, as this might be an intermediary step important for recycling.

This recycling model is supported by the observation that mutations in the retromer complex, when combined with mutations in tethering factor Trs85, can abrogate trafficking of Atg9 to the PAS. Similarly the combination mutations with GARP subunits that are responsible for tethering vesicles to the Golgi from the endosome, with Trs85 result in defective autophagy. Atg9 trafficking is influenced by other autophagy proteins such as Atg27 and Atg23 [53–56]. Atg23 is a peripheral membrane protein that facilitates the anterograde trafficking of Atg9 from the Golgi to the PAS. Atg27, another transmembrane protein that facilitates formation of Atg9 vesicles at the late Golgi, is itself observed to be present in early/late endosomes, PAS, Golgi and vacuolar membrane. Atg27 can be retrieved from the vacuole in a process that is Snx4-dependent. Furthermore, earlier studies identified a C terminal tyrosine (YSAV) motif in Atg27 that is important for the proper delivery of Atg27 to the vacuole as well as to maintain Atg9 pools at the endosome that can be mobilized to different compartments during autophagy (**Figure 2C**). Taken together, the complexity of Atg9 trafficking synergizes with the hypothesis that a collection of different membranes such as endosome and Golgi all contribute to the lipid identity of the autophagosome.

4.1.4 Autophagosomal fusion with the lysosome/vacuole

After the autophagosome is completed, the double membrane vesicle is ready for fusion with the lysosome/vacuole. The fusion of the autophagosome not only requires components of the fusion machinery as well as PI3P turnover, but it also requires the disassembly or retrieval of several ATG proteins from the autophagosome. In yeast, phosphoinositide phosphatases, including those from the myotubularin protein family, like Ymr1, along with Sjl2 and Sjl3 are important for removal of PI3P from completed autophagosomes, making autophagosomes fusion-competent (**Figure 2D**). In mammalian cells, PI3P phosphatase MYM-3 acts similarly to Ymr1 in promoting autophagosome maturation and fusion. Fusion of autophagosomes with the lysosome is mediated by RAB7-like protein Ypt7 along with the HOPS (Homotypic fusion and vacuole protein sorting) tethering complex and SNARE (Soluble Ethylmaleimide-Sensitive Factor Attachment Protein Receptor) proteins. Three Q–SNAREs: Vam3, Vti1 and Vam7 have been identified in yeast along with R-SNARE Ykt6 as key for this fusion step. Vamp7 is a sorting nexin family protein containing a PX domain that interacts with PI3P. Studies suggest that Vam7 interacts with Atg17-Atg29-Atg31 trimer complex via Atg17 interaction. In mammalian cells, SNAREs such as SYN12, SNAP29 and VAMP7/8 are responsible for autophagosome fusion with the lysosome. Interestingly, the PI3K VPS34 has also been linked with later stages of autophagy, including lysosomes returning to normal or regenerating once fusion with autophagosomes has taken place. Other phosphorylated lipid species like PI4P and PI(4,5)P2 can also facilitate lysosomal regeneration, allowing for new rounds of autophagy to occur. This might also trigger the formation of recycling vesicles packaging ATG protein cargo for recycling off the lysosomal membrane.

Another aspect that is key for late autophagy stages to proceed is intact lysosomal/vacuolar lipid homeostasis and lipid identity. For example, yeast vacuoles have unique lipid composition that is different from other membrane organelles within the cell. Vacuoles are enriched with lipids such as, myo-inositols, PI3P, and typically PI3,5P2, among other lipids such as ergosterol, diacylglycerol and some sphingolipids (**Figure 2D**). The specific lipid identity of vacuoles is important for the recruitment of fusion factors such as SNARES Ypt7 and HOPS that allow fusion of autophagosomes. Thus, the specific lipid identity of vacuole is important for its physiological function and its fusion with the late endosome and autophagosome.

5. Conclusion

While progress has been made in understanding the molecular underpinnings of autophagosome formation, our understanding has been primarily advanced by understanding the functions of proteins that lead and are required for this autophagic vesicle to form [28–31]. Because the extensive level of membrane remodeling that takes place during autophagy formation, much will be gained by investigating the process using methods that focus on the membrane and lipid biology of the process [23–27]. This new perspective has the potential of changing the way we have conventionally understood the remodeling of membranes for vesicle formation.

Table 2 describes the lipid contributions of different intracellular organelles or donor membranes to the progression of autophagy and/or autophagosome formation. The autophagy processes or steps benefiting from these contributions are also indicated.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

Atg/ATG proteins	autophagy-related proteins
PAS	pre-autophagosomal structure
PA	phosphatidic acid
PG	phosphatidylglycerol
PE	phosphatidylethanolamine
PC	phosphatidylcholine
PS	phosphatidylserine
PI	phosphatidylinositol
PI3P	phosphatidylinositol-3-phosphate
PI (3,5) P2	phosphatidylinositol-3, 5-biphosphate
PI4P	phosphatidylinositol-3-phosphate
PI(4,5)P2	phosphatidylinositol-4, 5-biphosphate
PI3K	phosphatidylinositol-3-kinase
S1P	sphingosine-1-phosphate

Author details

Shreya Goyal[1], Meaghan R. Robinson[2], Verónica A. Segarra[2*] and Richard J. Chi[1*]

1 Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC, USA

2 Department of Biology, High Point University, High Point, NC, USA

*Address all correspondence to: vsegarra@highpoint.edu and richard.chi@uncc.edu

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