Front. Biol. 2014, 9(1): 18–34
DOI 10.1007/s11515-014-1293-3

REVIEW

Structural biology of the macroautophagy machinery

Leon H. CHEW, Calvin K. YIP (✉)

Department of Biochemistry and Molecular Biology, The University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada

Abstract **Macroautophagy is a conserved degradative process mediated through formation of a unique double-membrane structure, the autophagosome. The discovery of autophagy-related (Atg) genes required for autophagosome formation has led to the characterization of approximately 20 genes mediating this process. Recent structural studies of the Atg proteins have provided the molecular basis for their function. Here we summarize the recent progress in elucidating the structural basis for autophagosome formation.**

Keywords macroautophagy, autophagy, Atg proteins, structural biology, X-ray crystallography, single-particle electron microscopy

Introduction

Degradation of intracellular components through delivery to the lysosome/vacuole by autophagy constitutes one of the primary mechanisms regulating cellular homeostasis (Suzuki and Ohsumi, 2007; Nakatogawa et al., 2009; Reggiori and Klionsky, 2013). In addition to delineating the roles of autophagy in homeostatic functions, the study of autophagy is of particular importance due to its involvement in diverse physiological processes such as growth, development, differentiation, apoptosis, and its association with pathological conditions including neurodegeneration, myopathies, cellular immunity, aging, and cancer (Choi et al., 2013).

Macroautophagy (hereafter autophagy) is the most extensively studied form of autophagy, and is thought to be the primary form in cells (Suzuki and Ohsumi, 2007; Nakatogawa et al., 2009; Mizushima et al., 2011; Reggiori and Klionsky, 2013). Autophagy can be both nonselective and selective for specific cargo. Selectivity is achieved by adaptor proteins that function to sequester specific components such as protein aggregates or whole organelles (Weidberg et al., 2011b; Suzuki, 2013). Various forms of selective autophagy have been named based on the recruited cargoes, with examples that include but not limited to, mitophagy (degradation of mitochondria, Kondo-Okamoto et al., 2012; Ashrafi and Schwarz, 2013), lipophagy, (degradation of lipids, Liu and Czaja, 2013) and ribophagy (degradation of ribosomes, Kraft et al., 2008). In comparison, nonselective autophagy has been characterized as a bulk degradation process, which functions independent of specialized cargo-receptor proteins.

Central to both nonselective and selective autophagy is the *de novo* synthesis of double-membrane vesicles known as autophagosomes (Fig. 1). Autophagosome formation is thought to be initiated at a single nucleation region in yeast cells: the preautophagosomal structure or phagophore assembly site (PAS) in yeast cells, but an analogous structure has not been identified in mammalian cells. Autophagy proceeds through expansion of an initial precursor structure into a cup-shaped membrane sac, termed an isolation membrane or phagophore. Cytoplasmic content becomes sequestered into this growing membrane structure until the vesicle is sealed, forming the double-membrane autophagosome. Mature autophagosomes are subsequently delivered to the lysosomal compartment (or the vacuolar compartment in yeast cells), where the outer membrane of the autophagosome fuses with the lysosomal membrane, leading to the translocation of the inner vesicle encapsulating the cargo into the lumen of the lysosome. The inner membrane vesicle, termed an autophagic body, is subsequently degraded by hydrolytic enzymes in the lysosome, and the resulting metabolites are recycled for anabolic pathways.

Received October 1, 2013; accepted December 22, 2013
Correspondence: Calvin K. YIP
E-mail: calvin.yip@ubc.ca

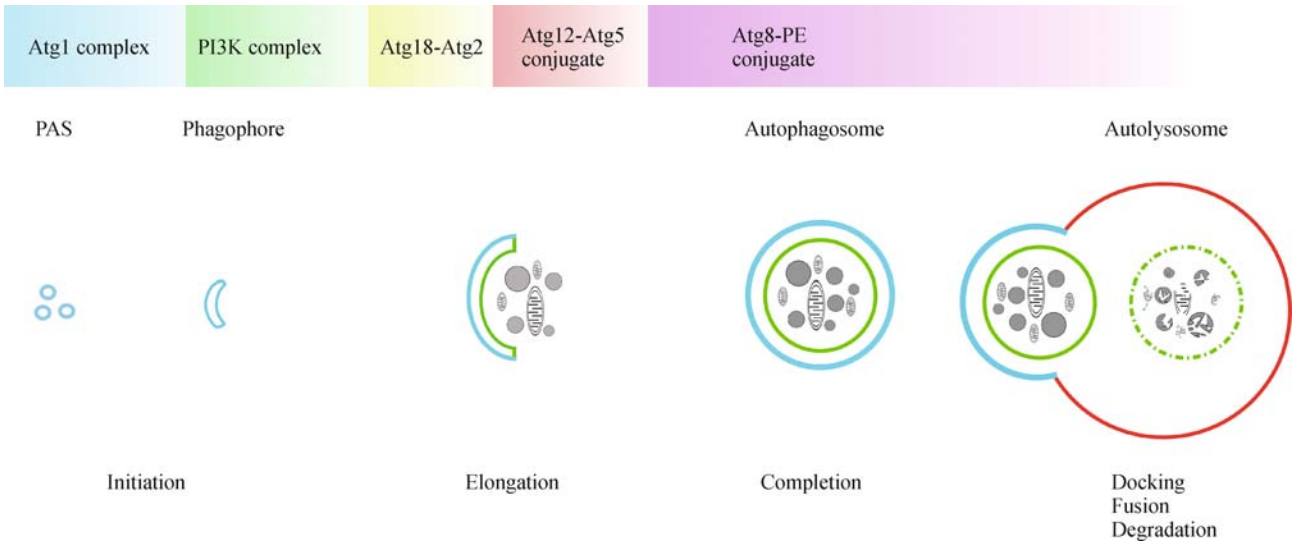

Figure 1 Overview of macroautophagy in *Saccharomyces cerevisiae*. Autophagosome formation begins at the preautophagosomal structure (PAS). Expansion of the PAS leads to formation of a cup-shaped membrane known as a phagophore. Elongation of the phagophore sequesters cytoplasmic content until the vesicle is sealed off, forming a complete mature autophagosome. The autophagosome is subsequently targeted to the lysosome where the inner membrane and its content would be degraded by hydrolytic enzymes. The overall contributions of each Atg functional group to the different steps of autophagosome biogenesis are depicted.

Research on the autophagy pathway was restricted to morphological studies until the early 1990s when Ohsumi and colleagues discovered that the baker's yeast *Saccharomyces cerevisiae* mounts an autophagic response upon starvation (Takeshige et al., 1992; Tsukada and Ohsumi, 1993). Subsequent genetic screens carried out in *S. cerevisiae* and other fungal species led to the identification of 38 Atg (autophagy-related) genes (Mizushima et al., 2011). Approximately 20 Atg proteins are essential for autophagosome formation in yeast and can be classified into five functional groups based primarily on identified protein–protein interactions (Table 1): (i) the Atg1 kinase complex, (ii) the autophagy-specific class III phosphatidylinositol 3-kinase (PI(3)K) complex, (iii) Atg9 and the Atg2-Atg18 complex, (iv) the Atg12 conjugation system, and (v) the Atg8 conjugation system. Importantly, the autophagy pathway and core machineries were found to be evolutionarily conserved, with more than half of the Atg proteins in yeast having orthologs in higher eukaryotes (Mizushima et al., 2011, Reggiori and Klionsky, 2013) (Table 1).

Over the past 15 years, extensive research has gone into delineating the biological functions of the core Atg proteins and determining how these proteins cooperate with one another to mediate and regulate autophagosome biogenesis, the central step of the autophagy pathway. Recent X-ray crystallographic, nuclear magnetic resonance (NMR), and single-particle electron microscopy (EM) studies have shed novel insights in the functions of various Atg proteins and protein complexes. In this review, we highlight key findings from these studies and discuss their implications in advancing our understanding of the structural basis of autophagosome biogenesis.

The Atg1 kinase complex

The yeast Atg1 kinase complex consists of the serine/threonine kinase Atg1, two proteins that regulate Atg1's kinase activity, Atg13 and Atg17, and two non-conserved regulatory subunits, Atg31 and Atg29 (Matsuura et al., 1997; Kamada et al., 2000; Cheong et al., 2005; Kabeya et al., 2005; Kabeya et al., 2009). The yeast Atg1 kinase complex is functionally analogous to the mammalian ULK1 complex, which is composed of ULK1, mATG13, FIP200, orthologs of Atg1, Atg13 and Atg17, respectively, and a unique component ATG101 (Hosokawa et al., 2009; Ganley et al., 2009; Mercer et al., 2009). Hierarchical genetic analysis in yeast has shown that the core components of the Atg1 kinase complex acts upstream of other Atg proteins and plays a central role in initiating autophagosome formation (Suzuki et al., 2007). Notably, Atg17, in complex with Atg31 and Atg29, is constitutively localized to the PAS and serves as a platform for assembly of the full Atg1 complex (Cheong et al., 2008; Kabeya et al., 2009). The kinase activity of Atg1, though not essential for the early stages of autophagosome formation, is critical in regulating the localization of downstream Atg proteins and further disassociating these proteins from the PAS (Cheong et al., 2008).

The nutrient status of the cell regulates autophagosome formation through different kinases. Under nutrient rich conditions, the nutrient-sensing target of rapamycin (TOR) kinase and protein kinase A (PKA) inhibit Atg1 complex activation through phosphorylation of Atg1 and Atg13 (Kamada et al., 2000; Kijanska et al., 2010; Kim et al., 2011, Yeh et al., 2011). Starvation induces partial dephosphorylation of Atg13, and this event activates the Atg1

Table 1 Functional groups for autophagosome formation

Functional group	Role in autophagosome formation	Yeast	Human	Structural biology reference	Technique	Species studied
Atg1 complex	Atg1 is a serine/threonine kinase with a role in tethering membranes. Atg17-Atg31-Atg29 acts a scaffold for assembling the Atg1 complex	Atg1	ULK1			
		Atg13	ATG13	Jao et al., 2013	X-ray crystallography	*Lachancea thermotolerans*
		Atg17	FIP200	Ragusa et al., 2012	X-ray crystallography	*Lachancea thermotolerans*
				Chew et al., 2013	EM	*Saccharomyces cerevisiae*
		Atg29	N/A	Ragusa et al., 2012	X-ray crystallography	*Lachancea thermotolerans*
				Chew et al., 2013	EM	*Saccharomyces cerevisiae*
		Atg31	N/A	Ragusa et al., 2012	X-ray crystallography	*Lachancea thermotolerans*
				Chew et al., 2013	EM	*Saccharomyces cerevisiae*
			ATG101			
PI(3)K complex	Produces Ptdins(3)P on autophagic membranes. Atg14 and Atg6 have shown membrane binding capabilities.	Vps34	VPS34	Miller et al., 2010	X-ray crystallography	*Drosophila melanogaster*
		Vps15	VPS15	Heenan et al., 2009	X-ray crystallography	*Saccharomyces cerevisiae*
		Atg14	ATG14L/BARKOR			
		Vps30/Atg6	Beclin1	Oberstein et al., 2007	X-ray crystallography	*Homo sapiens*
				Feng et al., 2007	X-ray crystallography	*Homo sapiens*
				Huang et al., 2012	X-ray crystallography	*Homo sapiens*
				Li et al., 2012	X-ray crystallography	*Rattus norvegicus*
				Noda et al., 2012	X-ray crystallography	*Saccharomyces cerevisiae*
		Atg38				
Atg9	ntegral membrane protein. Provides early membrane source for PAS formation.	Atg9	Atg9			
Atg18–Atg2 complex	Atg18 specifically binds PI(3)P . Recruits ubiquitin-like conjugation system.	Atg18	WIPI1-4	Krick et al., 2012	X-ray crystallography	*Kluyveromyces lactis (Hsv2)*
				Watanabe et al., 2012	X-ray crystallography	*Kluyveromyces marxianus (Hsv2)*
				Baskaran et al., 2012	X-ray crystallography	*Kluyveromyces lactis (Hsv2)*
		Atg2	Atg2			

(*Continued*)

Functional group	Role in autophagosome formation	Yeast	Human	Structural biology reference	Technique	Species studied
Atg12 conjugation	Atg12 is conjugated torAtg5 through a ubiquitin-like cascade. Atg12-Atg5 conjugates form a complex with Atg16. Atg5 can bind membranes and may tether membranes through dimerization of Atg16.	Atg12	ATG12	Suzuki et al., 2005	X-ray crystallography	*Arabidopsis thaliana*
		Atg7	ATG7	Taherbhoy et al., 2011	X-ray crystallography	*Saccharomyces cerevisiae*
				Noda et al., 2011	X-ray crystallography	*Saccharomyces cerevisiae*
					NMR	
				Hong et al., 2011	X-ray crystallography	*Saccharomyces cerevisiae*
				Kaiser et al., 2012	X-ray crystallography	*Saccharomyces cerevisiae*
				Yamaguchi et al., 2012a	X-ray crystallography	*Kluyveromyces marxianus*
						Arabidopsis thaliana
		Atg10	ATG10	Yamaguchi et al., 2012a	X-ray crystallography	*Kluyveromyces marxianus*
				Kaiser et al., 2012	X-ray crystallography	*Saccharomyces cerevisiae*
				Hong et al., 2012	X-ray crystallography	*Saccharomyces cerevisiae*
				Yamaguchi et al., 2012b	X-ray crystallography	*Kluyveromyces marxianus*
		Atg5	ATG5	Matsushita et al., 2007	X-ray crystallography	*Saccharomyces cerevisiae*
				Otomo et al., 2013	X-ray crystallography	*Homo sapiens*
				Yamaguchi et al., 2012b	X-ray crystallography	*Kluyveromyces marxianus*
					NMR	
				Noda et al., 2013	X-ray crystallography	*Saccharomyces cerevisiae*
				Metlagel et al., 2013	X-ray crystallography	*Homo sapiens*
		Atg16	ATG16L1/2	Fujioka et al., 2010	X-ray crystallography	*Saccharomyces cerevisiae*
Atg8 conjugation	Atg8 is lipidated to PE through an ubiquitin-like cascade. Atg4 proteolytically activates Atg8 followed by conjugation to the enzymes Atg7 (E1), Atg3 (E2).	Atg8	LC3A/B/C GABARAP GATE-16	Paz et al., 2000	X-ray crystallography	*Bos taurus (GATE-16)*
				Coyle et al., 2002	X-ray crystallography	*Homo sapiens (GABARAP)*
				Knight et al., 2002	X-ray crystallography	*Homo sapiens (GABARAP)*
				Sugawara et al., 2004	X-ray crystallography	*Rattus norvegicus (LC3)*
				Schwarten et al., 2010	NMR	*Saccharomyces cerevisiae*
				Kumeta et al., 2010	NMR	*Saccharomyces cerevisiae*
				Noda et al., 2011	X-ray crystallography	*Saccharomyces cerevisiae*
				Hong et al., 2011	X-ray crystallography, NMR	*Saccharomyces cerevisiae*
		Atg4	ATG4A-D	Sugawara et al., 2005	X-ray crystallography	*Homo sapiens*
				Kumanomidou et al., 2006	X-ray crystallography	*Homo sapiens*
				Satoo et al., 2009	X-ray Crystallography	*Homo Sapiens*
		Atg7	ATG7	see above		
		Atg3	ATG3	Yamada et al., 2007	X-ray crystallography	*Saccharomyces cerevisiae*

complex through promoting Atg1 complex formation and Atg1 kinase activity. Initial studies in yeast suggested that Atg1 is dynamically recruited to dephosphorylated Atg13 upon starvation (Kamada et al., 2000). However, recent evidence showed that Atg1 and Atg13 are constitutively associated, a phenomenon similar to what has been observed in higher eukaryotes (Kraft et al., 2012). Although the order of assembly of the Atg1 complex remains poorly defined, recent structural studies have revealed novel functions for several components of the Atg1 complex.

The yeast Atg17-Atg31-Atg29 subcomplex (hereafter Atg17-Atg31-Atg29) is an important organizer of the Atg1 complex at the PAS during autophagy. Biochemical studies have shown that this sub-complex exists as a constitutive dimer independent of the nutrient status (Kabeya et al., 2009). Recent X-ray crystallographic and single-particle EM analyses of *Lachancea thermotolerans* and *S. cerevisiae* Atg17-Atg31-Atg29 confirmed the inherent dimeric state of this complex (Ragusa et al., 2012; Chew et al., 2013). From the crystal structure, a single Atg17 protomer was found to consist of four α-helices forming a three-helix bundle. Atg31, on the other hand, adopts a β-sandwich motif with a single carboxyl-terminus α-helix mediating interaction with Atg17 to form a four-helix bundle. In contrast, Atg29 is predominantly unstructured and only a single N-terminal β-strand that is engaged in an interaction interface with the β-sandwich of Atg31 could be clearly defined from the electron density map. Although Atg31and Atg29 form stable heterodimers, the interaction of Atg29 with Atg17 is mediated through Atg31.

The overall architecture of Atg17-Atg31-Atg29 is that of an S-shaped dimer mediated through interaction of the C terminus of Atg17. The dimerization of Atg17 is functionally important for autophagy induction (Ragusa et al., 2012). The N-terminal region of Atg17 generates the characteristic curvature with Atg31-Atg29 binding to the concave side of the arc. Single-particle EM analysis of Atg17 alone suggested that Atg31-Atg29 association may function to establish and maintain the characteristic curvature of Atg17 (Chew et al., 2013). Furthermore, dissociation of Atg31-Atg29 from Atg17, through truncation of Atg31's C terminus α-helix, resulted in loss of autophagy function *in vivo*, indicating an important role for Atg31-Atg29. Indeed, it was recently shown that the C terminus of Atg29 constituted a regulatory domain that interacts with the scaffold protein Atg11 in a phosphorylation-dependent manner (Mao et al., 2013). Comparison of the available EM and crystal structure models of Atg17-Atg31-Atg29 suggests that the C-terminal region of Atg29 projects away from the central scaffold, making it potentially accessible to protein kinases and Atg11.

Its unique arc dimension and strikingly structural resemblance to BAR domain-containing proteins, led Hurley and colleagues to hypothesize that Atg17-Atg31-Atg29 could be involved in tethering the Atg9 vesicles, which contain the transmembrane protein Atg9 and are highly curved because their diameters range from 30 to 60 nm. Atg9 vesicles are thought to be a major source of membrane during the early stage of autophagosome formation in yeast (Yamamoto et al., 2012). However, Atg17-Atg31-Atg29 could not bind liposomes of any size *in vitro*. Instead, these investigators found that a C-terminal region of Atg1 (residues 562–831) termed the EAT (early autophagy targeting/tethering) domain is capable of binding and tethering small vesicles (diameter $<$ 50 nm) possessing highly curved membranes. Curiously, in the context of a pentameric assembly that consists of Atg17-Atg31-Atg29 and a C-terminal fragment of Atg13 (residues 350–550), the Atg1 EAT domain can no longer bind lipid vesicles and appears to be locked into an inhibited state. These observations led to a working model of how the Atg1 kinase complex mediates tethering of Atg9 vesicles (Fig. 2B). It is important to note that the membrane binding experiments were conducted with fragments of Atg1 and Atg13 (Ragusa et al., 2012) and may not fully reflect the inherent property of the full length Atg1 kinase complex. Furthermore, the proposed model remains to be validated *in vivo,* and future studies should focus on examining the capacity of the fully assembled Atg1 kinase complex in binding and tethering purified or reconstituted Atg9 vesicles. The subsequent fusion of Atg9 vesicles into larger vesicles may require other factors from conventional vesicular trafficking pathway, such as the TRAPPIII tethering complex and the SNARE proteins (Nair et al., 2011; Kakuta et al., 2012; Moreau et al., 2013). Future study should determine whether or not the Atg1 complex also participates in the vesicle fusion process.

The fully assembled Atg1 complex also recruits additional Atg proteins to the PAS to mediate vesicle nucleation, expansion, and closure. One of the first complexes targeted by the Atg1 complex is the autophagy-specific class III PI(3)K complex. Hierarchical analysis showed that Atg17 and Atg13 are the specific Atg1 complex subunits required for the recruitment of the Atg14 component of the class III PI(3)K complex to the PAS (Suzuki et al., 2007). Recent crystallographic analysis of the N-terminal domain of Atg13 (residues 1-260) from *L. thermotolerans* provided insights into how Atg13 might mediate this targeting (Jao et al., 2013). This previously unidentified Atg13 domain shows unexpected structural homology to the HORMA (Hop1p, Rev1p, and Mad2) domain family of proteins that are involved in recognizing DNA damage. The Atg13 HORMA domain specifically consists of three α-helices and two anti-parallel β-sheets arranged in an α/β motif. The α-helices are packed along one side of the β-sheet and are held together primarily through hydrophobic interactions while a large loop extends out from the opposite side of the same β-sheet. A non-conserved anti-parallel β-sheet lies above the α/β sandwich. Importantly, deletion of Atg13 HORMA domain results in the loss of Atg14 localization to the PAS and a defect in autophagy. The presence of a basic binding pocket on the side of the β-sheet opposite to the α-helices led to the hypothesis that Atg13 HORMA domain may function as a phosphate

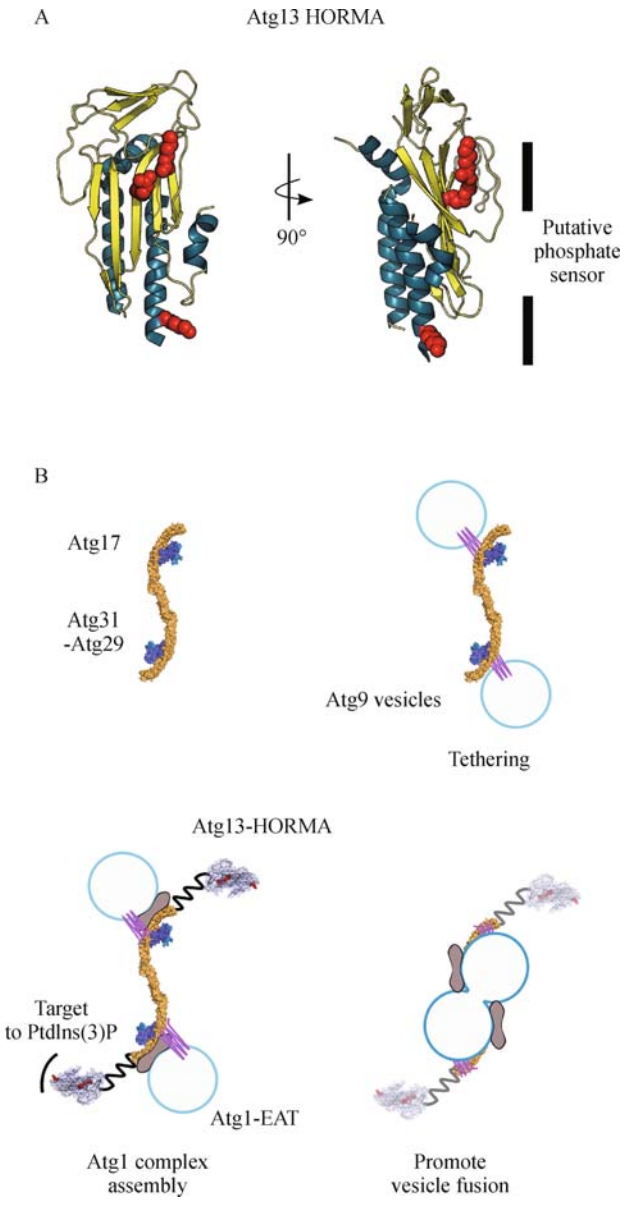

Figure 2 Model of Atg1 kinase complex function. (A) Crystal structure of the Atg13-HORMA domain (PDB: 4J2G). Residues in red highlight the putative phosphate-sensing motif. (B) Model of vesicle tethering mediated by the Atg1 kinase complex. Atg17 (orange) and Atg31-Atg29 (blue) are constitutively formed during nutrient-rich conditions and are localized to the PAS. Upon autophagy induction, Atg17 recruits Atg9 vesicles through direct interaction with Atg9 and may function as a tether for these vesicles. Atg13 and Atg1 are recruited to the PAS where the C-terminal domain of Atg1 (Atg1-CTD) can function to bind the high-curved Atg9 vesicles. The dimerization of Atg1-CTD may promote fusion of Atg9 vesicles. PDB codes for structures depicted: 4HPQ (Atg17-Atg31-Atg29), 4J2G (Atg13 HORMA domain).

sensor. This is supported by observations that two arginine residues (*L. thermotolerans*: R118 and R205) coordinate a sulfate ion in the crystal structure of the HORMA domain. These two residues are conserved in *S. cerevisiae* Atg13 (R120 and R213) and are essential to Atg13's molecular function and induction of autophagy *in vivo* (Fig. 2A). Building on the structural work on Atg13 HORMA domain, future studies should focus on determining whether Atg13 binds directly to Atg14 and/or other components of the autophagy-specific PI 3-kinase complex in promoting the recruitment of this complex to the PAS.

The structures of other HORMA domain containing proteins provide further mechanistic insights into the function of Atg13. Notably, the HORMA domain containing Mad2 protein has the ability to transition between an open state and a closed state, with the closed form being induced upon binding its physiological ligand Mad1 (Sironi et al., 2002). The crystallized form of Atg13 HORMA resembles the closed conformation of Mad2, and the putative phosphate sensor of Atg13 HORMA would only form in this state. An important objective of future study would be to determine whether Atg13 HORMA adopts the open conformation.

The fact that Atg13 could potentially contain a phosphate-sensing switch raised the question as to what this sensor actually targets. A recent study on human mATG13 protein investigated Atg13's capacity to associate with acidic lipids (Karanasios et al., 2013). Using whole cell lysate and recombinantly expressed human mATG13, these authors showed that a highly basic region at the extreme N terminus of ATG13 could associate with phosphatidic acid and mono-phosphorylated phosphatidylinositol (PtdIns3P and PtdIns4P). However, this basic region is not conserved in the primary sequence of yeast Atg13, and is mapped to the first α-helix, which is distal to the putative phosphate sensor of yeast Atg13 (Fig. 2A). Functionally, mutations to this region only affected mATG13 association with puncta and its ability to association with ULK1 complex components. It is currently not known whether yeast Atg13 can associate with phosphatidylinositides or whether the mechanism observed is conserved in lower eukaryotes of which a structure of the human mATG13 HORMA domain would provide great insight.

Despite recent advances in structural understanding of the yeast Atg1 kinase complex, there is a general lack of structural information on the orthologous mammalian ULK1 complex. Furthermore, mechanistic differences exist between the mammalian and fungal autophagy pathways, and the mammalian ULK1 complex likely functions in different contexts compared to the yeast Atg1 complex. Notably, yeast autophagosome formation occurs at a single nucleation point, the PAS, while a well-defined PAS has not yet been identified in mammalian cells. Instead multiple autophagosomes can form at different sites throughout the cell (Mizushima et al., 2011; Lamb et al., 2013). Compared to yeast, the initial source of membrane for early autophagosome formation has not been clearly defined in mammalian cells, with several sources, endoplasmic reticulum (ER), mitochondria, mitochondria-ER contact sites, and plasma membrane being highly touted candidates (Axe et al., 2008; Hayashi-Nishino

et al., 2009; Ylä-Anttila et al., 2009; Hailey et al., 2010; Ravikumar et al., 2010; Moreau et al., 2011; Hamasaki et al., 2013; Puri et al., 2013). Furthermore, the role of human mATG9 vesicles in PAS formation is less well understood with additional membrane sources, such as ATG16L vesicles, having a role in forming and elongating the phagophore (Moreau et al., 2011; Puri et al., 2013). The ULK1 complex may serve as a tethering factor for ATG16L vesicles as ULK1 has also been shown to associate with membranes and FIP200, the human ortholog of Atg17 has recently been shown to associate with ATG16L (Chan et al., 2009; Gammoh et al., 2013; Nishimura et al., 2013). Development of methods to isolate the relatively large-sized individual components of the ULK1 complex and to reconstitute the full complex would be critical to future biochemical and structural analyses of this important autophagy regulator.

The phosphatidylinositide-3-kinase complex

Generation of phosphatidylinositol 3-phosphate (PtdIns(3)P) on autophagic membranes is essential for the elongation and completion of autophagosomes (Kihara et al., 2001; Obara et al., 2008a). Several core Atg proteins are capable of binding PtdIns(3)P, suggesting that this lipid has a role in the recruitment of autophagy-specific proteins to the growing autophagosome membrane (Noda et al., 2010). Interestingly, both electron and fluorescent microscopy based studies in yeast cells have shown PtdIns(3)P is enriched on the inner surface of the autophagosome membrane (Obara et al., 2008a), and inhibiting the production of PtdIns(3)P completely abolishes the autophagic response during starvation conditions (Obara et al., 2008a) .

In yeast, PtdIns(3)P is produced by the sole class III phosphatidylinositide-3-kinase (PI(3)K), Vps34. Two distinct class III PI(3)K complex exist: complex I and complex II. These two complexes share three core subunits: Vps34, the catalytic PI(3) kinase, Vps15, a regulatory serine/threonine kinase, and Atg6(Vps30) (Kihara et al., 2001; Obara et al., 2006) (Fig. 3B). Each complex contains a unique component that determines its localization in the cell. The autophagy-related complex I contains Atg14 and associates with the PAS (Kihara et al., 2001; Obara et al., 2006) while the Vps (Vacuole protein sorting)-related complex (Complex II) contains Vps38 and localizes to vacuolar and endosomal membranes (Kihara et al., 2001; Burda et al., 2002). In yeast, Vps15 is a myristoylated protein that contains both a kinase and a WD repeat domain required for recruitment and phosphorylation-dependent activation of Vps34 kinase activity (Panaretou et al., 1997; Heenan et al., 2009). Atg6 mediates interaction with Atg14 (Noda et al., 2012; Fogel et al., 2013). Recently, Atg38 was shown to mediate complex integrity by bridging the interaction of Vps15-Vps34 with Atg14-Atg6 through a predicted C-terminal coiled-coil domain (Araki et al., 2013). Atg38 does not interact with Vps38 indicating that it has a distinct role in autophagy.

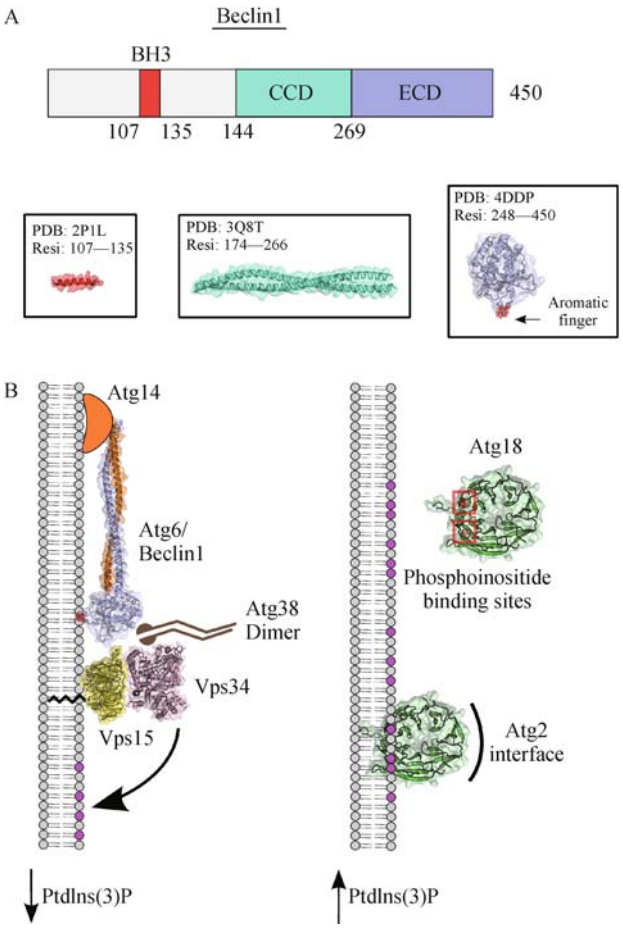

Figure 3 Components of the autophagy specific class III PI(3)K complex. (A) Structural organization of Beclin1. Beclin1 is a central hub for regulation of class III PI(3)K complex. Beclin1 can be subdivided into three functional domains; the N-terminal Bcl-2 homology (BH3) domain (PDB: 2P1L, residues 107-135), the central coiled-coil domain (CCD; PDB: 3Q8T, residues 144-269), and the evolutionarily conserved domain (ECD; PDB: 4DDP, residues 248-450). (B) Model of class III PI(3)K formation. This complex is composed of Vps34 (red), Vps15 (yellow), Atg6/Beclin1 (blue), Atg14 (orange), and Atg38 (brown). Complex formation occurs through interaction of Atg14 with Atg6/Beclin1 and Vps34 with Vps15, which is further stabilized by association with Atg38. The class III PI(3)K complex produces PtdIns(3)P at the autophagosome membrane through the catalytic activity of the PI(3) kinase Vps34. This complex is targeted and anchored to membranes by myristoylated Vps15 and the intrinsic membrane binding ability of Atg14 and Atg6/Beclin1. Following PtdIns(3)P production, Atg18 is recruited through two phosphoinositide binding sites. A small hydrophobic loop may facilitate its insertion into the membrane. Atg2 can be recruited in an Atg18 dependent or independent mechanism to interact with effectors. PDB codes for structures depicted: 3GRE (Vps15), 4DDP (Beclin1 ECD), 3Q8T (Beclin1 CCD), 2X6H (Vps34), 4EXV (Hsv2/Atg18).

Regulation of PtdIns(3)P is more complex in mammalian cells due to the presence of multiple PI(3)K complexes.

Although both class II and class III PI(3)K complexes have capacity to synthesize PtdIns(3)P, only class III PI(3)K complexes are autophagy specific and are thought to be the major generator for this lipid modification. The mammalian autophagy specific class III PI(3)K complexes consist the core subunits: hVPS34 kinase, hVPS15 kinase, and Beclin1 (an Atg6 ortholog). Similar to the yeast complex, the mammalian class III PI(3)K complexes are localized to different compartments due to association with the human Atg14 ortholog, ATG14L/Barkor that localizes the class III PI(3)K complex to the ER while the Vps38 ortholog UVRAG localizes the complex to late endosomal membranes (Itakura et al., 2008; Matsunaga et al., 2009; Zhong et al., 2009; Fan et al., 2011). A third class III PI(3)K complex contains rubicon, which associates with UVRAG containing complexes (Matsunaga et al., 2009; Zhong et al., 2009). These additional complexes are physiologically important for autophagosome formation with ATG14L/Barkor and UVRAG-containing complexes promoting autophagy and Rubicon-containing complexes inhibiting autophagy.

In mammalian cells, the class III PI(3)K complex serves as an essential hub for autophagy induction. Central to regulation is Beclin1, which interacts with a multitude of proteins including but not limited to ATG14L, UVRAG, Rubicon, and Bcl-2 (Aita et al., 1999; Liang et al., 1999; Furuya et al., 2005; Itakura et al., 2008). These critical interactions are mediated through distinct domains of Beclin1: the N-terminal Bcl-2 homology 3 (BH3) domain (residues 107–135), a central coiled-coil domain (CCD residues 144–269), and an evolutionarily conserved domain (ECD residues 248–450) at its C terminus (Fig. 3A).

The non-conserved BH3 domain of Beclin1 interacts with the Bcl-2 family protein Bcl-XL (Liang et al., 1998). This interaction inhibits autophagy presumably through sequestering Beclin1 from the class III PI(3)K complex. NMR and crystallographic analyses have shown that the α-helical Beclin1 BH3 peptide forms a tight interaction with an N-terminal region of Bcl-XL (Feng et al., 2007; Oberstein et al., 2007). Notably, the binding pocket in Bcl-XL is a hydrophobic groove formed by four α helices, and both polar and hydrophobic interactions mediate the interaction between Beclin1 BH3 and Bcl-XL. Available crystal structures of Bcl-XL in complex with BH3 domain from other non-autophagy related proteins showed that the Bcl-XL binding pocket is flexible with a single α-helix (α4) capable of adopting different conformation to accommodate different substrates (Sattler et al., 1997; Petros et al., 2000; Liu et al., 2003). Interestingly, biochemical studies showed that the Beclin BH3 domain exhibited the weakest interaction among interacting partners of Bcl-XL. The importance of the interaction between Beclin1 BH3 domain and Bcl-2 proteins in autophagy is further highlighted by its exploitation by the viral Bcl-2 protein M11 (Ku et al., 2008). The crystal structure of murine gamma Herpes virus 68 M11 protein in complex with Beclin1 BH3 domain revealed strong interactions analogous to those observed in other Bcl-2–BH3 complexes. However, isothermal titration calorimetry (ITC) studies showed that Beclin1 BH3 domain binds M11 with an affinity 20 orders of magnitude higher than with endogenous Bcl-2. The binding of M11 to Beclin1 leads to autophagy inhibition but exactly how this interaction affects the outcome of viral infection is not known.

The CCD of Beclin1, on the other hand, mediates interaction with ATG14L and UVRAG, and serves as a critical platform for complex assembly. This domain also mediates Beclin1 homo-dimerization. A recent crystallographic analysis revealed that the Beclin1 CCD (residues 174–266) forms a canonical anti-parallel coiled coil of approximately 129 Å in length and this coiled coil is held together by13 heptad repeats (Li et al., 2012). This crystal structure further uncovered the presence of polar residues at the center of the dimer interface. These polar residues destabilize the coiled coil structure, resulting in Beclin dimers that readily disassociate at physiologic temperature. It has therefore been suggested that the metastable coiled coil structure is an inherent property that allows Beclin1 to dynamically regulate autophagy induction through the heterodimerization with ATG14L and UVRAG and the formation of their respective regulatory complexes (Fig. 3B left panel). Interestingly, mutagenesis together with ITC and co-immunoprecipitation (co-IP) studies showed that the Beclin1 CCD binds more strongly to UVRAG compared to ATG14L (Li et al., 2012).

The third functional domain of Beclin1 is the C-terminal ECD, whose definition is quite broad in the autophagy field and can be confusing. Some studies refer the ECD as the entire C-terminal region after CCD while others excluded the extreme C-terminal region. For clarity, the domain boundaries for each of the ECD would be listed. It has been initially demonstrated that ECD (244–337) mediates Beclin1's interaction with Vps34 and activation of Vps34 kinase activity (Furuya et al., 2005). This domain is essential to both autophagy and Beclin1's tumor suppressive function (Liang et al., 1999). The recent crystal structures of human Beclin1 ECD (residues 248–450) and yeast Atg6 ECD (319–540) have revealed additional roles for this domain (Huang et al., 2012; Noda et al., 2012). Human Beclin1 ECD consists of four α-helices and three anti-parallel β-sheets, arranged into three α/β repeats showing three fold symmetry. The α-helices form an internal three-helix bundle surrounded by the three β-sheets, with additional loops and the last α-helix are found around the perimeter of the α/β repeats. A prominent loop, connecting β-sheet 2 to α-helix 3, contained a triad of hydrophobic aromatic residues (Phe359, Phe360, and Trp361) that are directly adjacent to a hydrophobic cleft. This "aromatic finger" motif was shown to be a membrane binding domain associated with cardiolipin-enriched liposomes. Membrane association of Beclin1 ECD may be strictly driven by direct interaction with membranes exhibiting high curvature, such as cardiolipin-containing mem-

branes, which induce negative curvature in membranes (Renner and Weibel, 2011). Although the aromatic finger is absent in the yeast Beclin1 ortholog Atg6, a recent study found that the C-terminal domain of yeast Atg6 (Atg6-CTD) is required for its localization to the PAS (Fogel et al., 2013). Furthermore, domain deletion studies showed that the ECD of yeast Atg6 may have an additional role in regulating the size and number of autophagosomes (Fogel et al., 2013).

Recently, the autophagy-specific protein Atg14L/Barkor has also been found to associate with highly curved membranes (< 100 nm) enriched in PtdIns(3)P through a predicted C-terminal amphipathic α-helix domain (Fan et al., 2011). Although only the N-terminal region of Atg14L is essential to autophagy, the C-terminal domain is required to support a normal level of autophagy through regulating autophagosome size and number (Obara et al., 2006). Thus, several components of the class III PI(3)K complex may function in binding and tethering membranes to further promote expansion and elongation of the growing autophagosome.

Modulation of autophagy with small-molecule inhibitors is important both for studying the molecular mechanisms of autophagy and for developing therapeutics for autophagy-related diseases. 3-methyladenine (3-MA) is the first autophagy inhibitor identified and is still widely used in the autophagy field (Seglen and Gordon, 1982). 3-MA targets both the class I and III PI(3) kinases (Wu et al., 2010). The crystal structure of *Drosophila melanogaster* Vps34 catalytic domain has provided structural insights into 3-MA inhibition of Vps34 and has generated a framework for the development of new generation of autophagy-specific class III PI(3)K inhibitors (Miller et al., 2010). Notably, this crystal structure features an N-lobe catalytic domain surrounded by a helical solenoid domain and a regulatory C-terminal domain. Several features required for catalysis are clearly observed from the crystal structure: the P-loop for binding ATP, the activation loop for recognition of PtdIns, and the catalytic loop. Interestingly, the crystal structure showed that an amphipathic C-terminal helix, previously identified to be essential for Vps34 kinase activity *in vivo*, is obstructing the catalytic loop and substrate binding loop. It is thought that when Vps34 is localized to membranes, this C-terminal helix can be displaced to associate with the membrane region and promote Vps34 activity.

Structures of the Vps34 catalytic domain in complex with several PI3K inhibitors enabled researchers to examine the P-loop binding pocket more closely. Notably, the interaction of 3-MA in the binding pocket is mediated by a hydrophobic ring unique to the P-loop of Vps34. Also, compared to Class I PI(3)Ks, the ATP binding pocket of Vps34 exhibits a smaller overall volume and higher rigidity, an observation that suggests higher selectivity for potential inhibitors. These structures led to identification of improved compounds such as PT210, which had increased specificity to Vps34 compared to other PI(3)K, and provide a framework for rational design of inhibitors that exploit the unique features of the Vps34 ATP binding pocket (Miller et al., 2010).

Atg9

Atg9 is the only transmembrane protein required for autophagy (Noda et al., 2000). Atg9 has six predicted transmembrane segments with both termini projecting to and localized in the cytoplasm. Atg9 is present in high curvature 30–60 nm lipid vesicles known as Atg9 vesicles, which are thought to be derived from Golgi-endosome sources (Yamamoto et al., 2012). In yeast, biogenesis of Atg9 vesicles is dependent on Atg23 and Atg27. During nutrient rich conditions, Atg9 vesicles exist as a pre-PAS. Upon induction of autophagy, several Atg9 vesicles are trafficked to the vacuole to form the initial isolation membrane and provide the first membrane source for the autophagosome (Yamamoto et al., 2012). It is thought that as little as three Atg9 vesicles participate in this early autophagosome biogenesis event (Yamamoto et al., 2012). As mentioned previously, the Atg1 complex may have a role in mediating Atg9 vesicle tethering and fusion (Reggiori et al., 2004; Sekito et al., 2009). Because Atg9 vesicles likely contribute only a small amount of membranes to autophagosomes, these specialized lipid vesicles may possess alternative functions. Indeed, recent studies revealed that Atg9 recruits the TRAPPIII vesicle tethering complex and Ypt1 Rab GTPase to the PAS (Kakuta et al., 2012; Lipatova et al., 2012).

Atg18-Atg2

In yeast, Atg18 and Atg2 are peripheral membrane proteins that interact with Atg9. A downstream effector of class III PI(3)K, Atg18 localizes to the PAS through binding to PtdIns(3)P (Obara et al., 2008b). Atg18 forms a complex with Atg2 that together localize to the autophagosome membrane (Obara et al., 2008b). Previous work has shown that Atg2 localization to the phagophore requires Atg18, however, a recent study showed that Atg2 could localize to the PAS independent of Atg18 (Obara et al., 2008b; Rieter et al., 2013). The exact mechanism through which Atg2 localizes to the PAS is still not clearly defined, but the importance of Atg2 localization is highlighted by the observation that artificially inducing PI(3)P targeting of Atg2 can rescue the autophagy defect resulting from the absence of Atg18 (Kobayashi et al., 2012). The exact biological functions of Atg18 and Atg2 are currently not known but the Atg2-Atg18 complex may facilitate the recruitment of Atg proteins in the ubiquitin-like conjugation system to the PAS and may further regulate cycling of Atg9 (Reggiori et al., 2004; Sun et al., 2013).

Atg18 is a member of the PROPPIN (β-propeller that bind phosphoinositide species) family. PROPPIN proteins are predicted to adopt a seven-bladed β propeller structure and bind PtdIns(3)P and PtdIns(3,5)P through a conserved FRRG

motif (Dove et al., 2004). In yeast, Atg21 and Hsv2 a conserved paralogues of Atg18. Atg21 is not essential to autophagy but is required for the Cvt (cytoplasm-to-vacuole targeting) pathway (Meiling-Wesse et al., 2004; Strømhaug et al., 2004) while Hsv2 may have a role in specific autophagy of the nucleus (Krick et al., 2008). Three recent crystal structures of the Hsv2 paralog from *Kluyveromyces lactis* and *Kluyveromyces marxianus* have provided structural insights into PtdIns(3)P recognition by the PROPPIN proteins (Baskaran et al., 2012; Krick et al., 2012; Watanabe et al., 2012). These structures showed that Hsv2 forms a seven-bladed β-propeller with each blade consisting of 4 antiparallel β-strands. The two arginine residues of the FRRG motif were found to participate in a different binding groove. Hsv2 contains two phosphoinositide binding sites: site 1 in blade 5 and site 2 in blade 6. Another interesting feature of Hsv2 is a large loop consisting of aromatic residues extending from β-strands 3 and 4 of blade 6. This loop was shown to be necessary for binding liposomes. When these motifs were studied in the context of Atg18, it was shown that phosphoinositide binding sites and the aromatic loop are required for localization of Atg18 to the PAS and proper autophagy function. The location of the phosphoinositide binding sites and the aromatic loop suggest that Atg18 is likely oriented with its edge against the membrane. This orientation positions blade2, which interacts specifically with Atg2, to the most distal end of the β-propeller, and allows the resulting Atg18-Atg2 complex to be in an optimal position for interacting with downstream effectors (Fig. 3B).

In mammals, the WIPI (WD-repeat protein interacting with phosphoinositides) family of proteins serve a similar function to Atg18. Four isoforms, WIPI1, WIPI2, WIPI3, WIPI4, have been identified (Proikas-Cezanne et al., 2004; Polson et al., 2010; Mauthe et al., 2011). Phylogenetic analysis revealed that WIPI1 and WIPI2 are orthologous to Atg18 while WIPI3 and WIPI4 are orthologous to Hsv2. Functionally, WIPI1 and WIPI2 have a role in autophagy in mediating localization of mATG2 and promoting formation of LC3-positive autophagosomes (Polson et al., 2010). All WIPI family proteins are predicted to adopt a seven-bladed β propeller structure. Additionally, the mechanisms in which WIPIs associate with PtdIns(3)P appears to be conserved from the yeast system. A recent study of WIPI1identified that indeed residues which are predicted to form blade 5 and blade 6 were important in associating with autophagic membranes through binding PtdIns(3)P (Gaugel et al., 2012).

The ubiquitin-like conjugation systems

The two ubiquitin-like conjugation systems in the autophagy pathway are highly conserved and are thought to mediate expansion and completion of the autophagosome (Mizushima et al., 1998; Ichimura et al., 2000). Each system has a unique ubiquitin-like protein, Atg8 or Atg12, that are conjugated to phosphatidylethanolamine (PE) or Atg5, respectively. The conjugation event occurs through an ubiquitin like-cascade that involves a common E1-like enzyme, Atg7, and two unique E2-like enzymes: Atg10 for the Atg12 conjugation system and Atg3 for the Atg8 conjugation system. The Atg8-PE and Atg12-Atg5 conjugates localize to the PAS and are required for autophagy. The structural aspects of the Atg8 and Atg12 ubiquitin-like cascade have been discussed extensively in a recent review (Noda et al., 2009) and will not be covered here. Instead, we will focus on describing the potential roles of the Atg8-PE and Atg12-Atg5 conjugates in autophagosome biogenesis.

Atg8-PE conjugate

Central to the completion of autophagosome formation is the lipidation of Atg8 with PE. This conjugation event occurs through an ubiquitin-like cascade. First, nascent Atg8 is proteolytically processed at a C-terminal arginine by the Atg4 cysteine protease to expose a glycine residue. Next, this glycine residue is activated by adenylation to form a thioester bond with Cys507 of the E1-like enzyme Atg7. Atg8 is then transferred to a cysteine residue of the E2-like enzyme Atg3. In the final step, Atg8 is conjugated to the amino group of PE, which is mediated by the E3-like activity of the Atg12-Atg5-Atg16 complex (see below). Recent studies have uncovered possible roles of Atg8-PE in autophagosome formation.

In yeast, Atg8 expression and lipidation are increased during starvation-induced autophagy. A result of Atg8 lipidation is localization of this protein to the expanding PAS where Atg8-PE has a role in mediating expansion and size of the autophagosome (Xie et al., 2008). During autophagosome formation, Atg8-PE is distributed symmetrically to both the inner and outer membranes and is the only Atg component associated with mature autophagosomes (Kabeya et al., 2000). Furthermore, Atg8 functions to recruit specific cargo and core autophagy machinery through interaction with a conserved motif, the LC3-interacting region (LIR) or the Atg8-family interacting motif (AIM), which associates with Atg8 in a specific hydrophobic pocket (Noda et al., 2008; Yamaguchi et al., 2010; Birgisdottir et al., 2013) (Fig. 4A). During the later stages of autophagosome formation, Atg8 is deconjugated from PE by Atg4. This event is important for recycling other core Atg components and for completing the autophagosome biogenesis process (Nair et al., 2012; Nakatogawa et al., 2012; Yu et al., 2012). Interestingly, multiple Atg8 orthologs exist in mammalian cells, and these include LC3, GABARAP, and GATE-16. All of these proteins associate with autophagosome and they appear to participate in different steps of autophagosome formation (Kabeya et al., 2004; Weidberg et al., 2010).

Yeast Atg8-PE regulates autophagosome size through its activity as both a membrane tether and a fusogen, as demonstrated by *in vitro* tethering and liposome fusion assays (Nakatogawa et al., 2007). Although mutagenesis

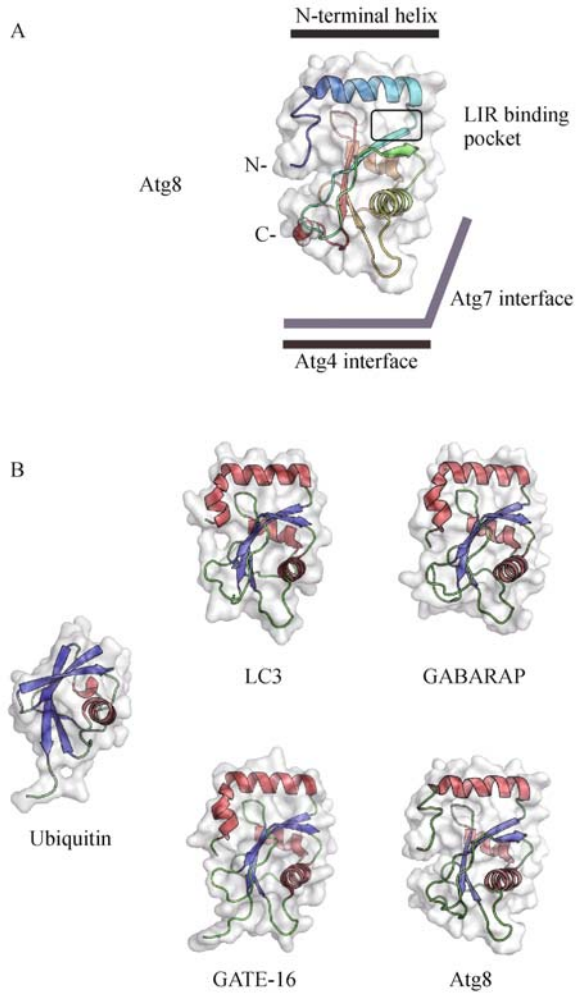

Figure 4 Atg8-family of ubiquitin-like proteins. (A) NMR structure of yeast Atg8. Several interaction interfaces are labeled. (B**)** Comparison of Atg8-family protein structures exhibiting the ubiquitin-like fold. PDB Code for structure depicted: 2KQ7 (Atg8), 1UGM (LC3), 1GNU (GABARAP), 1EO6 (GATE-16), 1UBI (ubiquitin).

studies further localized the tethering function of Atg8-PE to the N-terminal region of Atg8 (Nakatogawa et al., 2007; Weidberg et al., 2011a), the membrane fusion function of Atg8-PE remains a controversial topic in the field. Notably, a recent study argued that non-physiologic amounts of PE used in the *in vitro* experiments could artificially promote Atg8-PE's ability to fuse unstable vesicles (Nair et al., 2011). Thus, Atg8-PE's capacity to tether and hemifuse autophagosomal membranes requires further experimental validation.

NMR and crystallographic analyses have shown that despite low sequence similarity, Atg8 homologs from mammalian, protozoan, and fungal cells all adopt an ubiquitin-like fold made of a four-stranded β-sheet and two α-helices (Paz et al., 2000; Coyle et al., 2002; Knight et al., 2002; Sugawara et al., 2004; Schwarten et al., 2010; Kumeta et al., 2010) (Fig. 4A, 4B). Compared to canonical ubiquitin, the highly conserved N-terminal helical domain, exhibits different physiochemical properties among different Atg8 paralogs (Fig. 4A). For example, it is relatively basic in LC3 but acidic in GABARAP/GATE-16. The N-terminal domain of Atg8 is implicated in Atg8-PE's ability to tether membranes (Weidberg et al., 2011a). The crystal structure of GABARAP showed that Atg8-family proteins may have the ability to adopt an open and closed conformation with the N-terminal helix mediating an intermolecular interaction with another Atg8 molecule (Coyle et al., 2002). In support of this hypothesis, NMR has demonstrated the structural flexibility of the N-terminal region of yeast Atg8 (Schwarten et al., 2010; Kumeta et al., 2010). The potential of Atg8 to form higher order homo-oligomers via the dynamic N-terminal tail may be the mechanism that underlies the putative membrane tethering function of membrane-anchored Atg8-PE and testing this tantalizing hypothesis will be an area for future investigation.

The Atg12-Atg5-Atg6 complex

The process of Atg12 conjugation to Atg5 can be separated into several distinct events. First, the C-terminal Gly186 residue of Atg12 forms a thioester linkage with Cys507 of the E1-like enzyme Atg7. Atg12 is then transferred to the E2-like enzyme Atg10 before finally conjugated to Lys149 of Atg5. The Atg12-Atg5 conjugate, through Atg5, can further form a non-covalent complex with Atg16. The Atg12-Atg5-Atg16 complex, which is capable of forming higher-order oligomers, localizes to the concave side of the expanding phagophore and dissociates prior to autophagosome completion (Kuma et al., 2002).

The crystal structure of Atg12 from *Arabidopsis thaliana* (AtAtg12) revealed that AtAtg12 adopts a canonical ubiquitin-like fold (Suzuki et al., 2005). AtAtg12 is structurally similar to Atg8 and these two proteins both possess a similar hydrophobic patch, which may mediate association with the shared E1-like Atg7 enzyme. AtAtg12 contains a second region, which has been shown to interact with Atg3. In yeast, formation of the full Atg12-Atg5-Atg16 complex has been shown to further promote Atg12's ability to bind Atg3 (Romanov et al., 2012).

Recently, it was discovered that yeast Atg5 could bind membranes, with this ability being enhanced when in complex with Atg12 and Atg16 (Romanov et al., 2012). *In vitro* experiments showed that conjugation of Atg5 to Atg12 inhibits its membrane binding ability while complex formation with Atg16 promotes membrane binding. Importantly, a charge dependence for Atg5 membrane association was identified, and key residues (K160, R171) mediating this interaction were shown to be essential for autophagy and the related Cvt (cytoplasm to vacuole targeting) pathway.

The crystal structures of the yeast Atg5-Atg16 complex (Matsushita et al., 2007) and of human and yeast ATG12-ATG5 in complex with a short N-terminal peptide from ATG16 (Otomo et al., 2013; Noda et al., 2013) have revealed

the mechanism of complex formation and how this may promote membrane tethering. In particular, Atg5 was found to consist of two ubiquitin-like domains joined by a helix-rich domain while a short N-terminal fragment of Atg16 forms an α-helix. Atg12 associates with the Atg5-Atg16 complex through Atg5 and does not make direct contacts with Atg16, and together these proteins assemble into a relatively compact structure. These structures also revealed that Atg12 does not change conformation upon binding Atg5, and that these two proteins interact through extensive hydrophobic/hydrophilic interactions in addition to their covalent linkage. Conjugation appears to further stabilize Atg12 and promote interaction with Atg5.

As mentioned, Atg12 contains a region required for association with Atg3. In the human crystal structure, this was proposed to be mediated by a basic region in Atg12 (K54, K72, W73) opposite to the interaction interface of Atg5 (Otomo et al., 2013). However, analysis of *S. cerevisiae* and *A. thaliana* Atg12 has attributed the Atg3 interacting region to lying within the interface between Atg12 and Atg5 (Noda et al., 2013). Thus a large conformational change in Atg12 would be required to promote interaction with Atg3. These models likely differ due to inherent differences between mammalian and fungal/plant species. More recently, a study of human ATG12-ATG5-ATG16 using NMR and X-ray crystallography isolated an ATG3 peptide that co-crystallized with ATG12-ATG5-ATG16 (Metlagel et al., 2013). These results provide support that the acidic Atg3 peptide (residues 153-157) does in fact associate with the basic patch on Atg12, which further supported Otomo et al.'s model (Fig. 5C). Atg12 adopts a ubiquitin-like fold, interestingly, Atg12's interaction with Atg3 was remarkably similar to the interaction observed with Atg8-family proteins and LIR (LC3-interacting region) peptides (Fig. 5B).

The Atg5 residues for membrane binding localize to an interface between Atg12 and Atg16, which would position both proteins parallel to the membrane (Romanov et al., 2012). A recent crystal structure showed that the C-terminal domain of yeast Atg16 adopts a parallel coiled coil structure and mediates dimerization of the Atg12-Atg5-Atg16 complex (Fujioka et al., 2010). This structural feature of Atg16 may confer the ability of the Atg12-Atg5-Atg16 complex to tether membranes through Atg5 (Fig. 5C), and future experiments should focus on testing this exciting hypothesis.

As opposed to the yeast autophagy pathway, ATG16L containing vesicles in mammalian cells appear to be a key source of membranes for growing autophagosomes, which are derived from plasma membranes and endosomal compartment (Moreau et al., 2011; Puri et al., 2013). While it remains to be experimentally validated, the dimerization ability of ATG16L may serve to tether and promote homotypic fusion of ATG16L containing vesicles. The observation that ATG16 associates with FIP200, a component of the ULK1 complex, suggests that the ULK1 complex could be potentially involved in mediating tethering and/or fusion of the ATG16L vesicles (Gammoh et al., 2013; Nishimura et al., 2013).

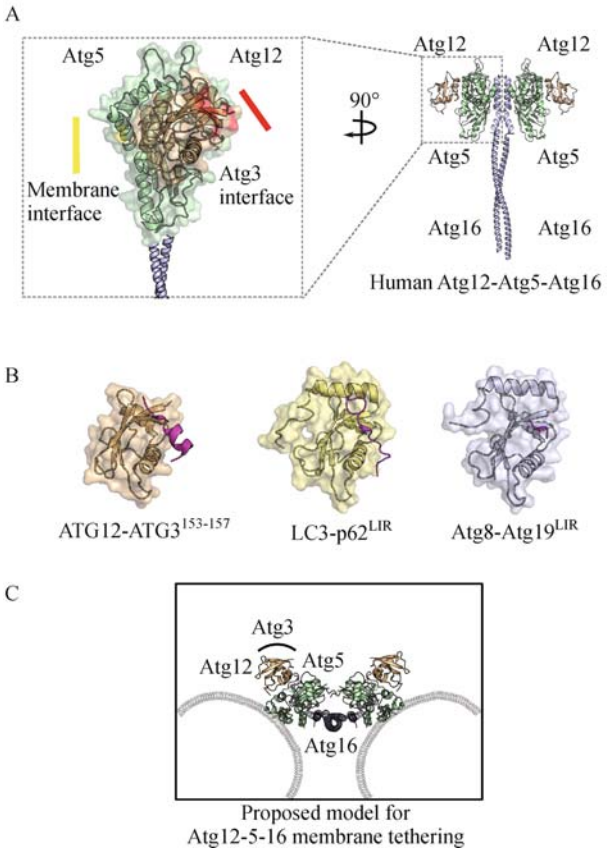

Figure 5 Model of Atg12-Atg5-Atg16 complex. (A) Dimer model of the Atg12-Atg5-Atg16 complex. Atg12 is covalently linked to Atg5. Atg16 is bridged through Atg5 and has a C terminus coiled-coil dimerization domain that mediates dimerization of this complex. Atg12 contains an Atg3 binding region highlighted in red. Atg5 has a membrane binding region highlighted in yellow. (B) Comparison of the Atg3 binding pocket of Atg12 to LC3-interacting region (LIR) peptides in complex with Atg8-family members. The peptide is depicted in magenta. Atg12 (4NAW), LC3 (2K6Q), Atg8 (2ZPN) (Noda et al., 2008). (C) A model for Atg12-Atg5-Atg16 membrane tethering. Membrane binding occurs through Atg5. This positioning of the complex allows for the freely exposed Atg3 binding region on Atg12. Structures were made from PDB files: 4GDK (Atg12-Atg5-Atg16) and 3A7O (Atg16 dimer).

Conclusions

Autophagosome formation, the central step of the autophagy pathway, is a dynamic and complex process. Over the past decade, the field has made significant strides in understanding this pathway at the molecular level. A common theme established is that several core Atg proteins have inherent capabilities to interact with and remodel membranes. However, the molecular mechanisms through which these Atg proteins regulate membrane dynamics and contribute to autophagosome biogenesis are still largely unknown, which is

due in part to the dynamic nature of this process. Future experiments should focus on establishing *in vitro* systems to examine the intermediate steps of the autophagosome formation process and determining how the core Atg proteins coordinate with other non-Atg proteins, such as SNARE proteins, TRAPPIII tethering complex, and the regulatory Rab GTPases, to carry out the different membrane shaping and remodelling steps.

The availability of structural information for several core Atg proteins from yeast to humans has advanced our current understanding of the function of these proteins and their putative yet conserved roles in autophagosome formation. However, we have only scratched the surface, and the majority of published structures provide only a static picture of these proteins in action. Capturing conformational dynamics of Atg proteins and determining how they interact with one another in the context of assemblies using hybrid structural approaches that combine X-ray crystallography/NMR with single-particle EM and single-molecular approaches will therefore be critical goals in future autophagy research. In the long run, the accumulation of high-resolution structural data on the various Atg proteins from yeast to humans will contribute to the identification of novel autophagy-modulating compounds that can be potentially developed as therapeutics for the treatment of a broad range of human diseases, and help resolve many mysteries of the autophagy pathway.

Compliance with ethics guidelines

Leon H. Chew and Calvin K. Yip declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Aita V M, Liang X H, Murty V V, Pincus D L, Yu W, Cayanis E, Kalachikov S, Gilliam T C, Levine B (1999). Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. Genomics, 59(1): 59–65

Araki Y, Ku W C, Akioka M, May A I, Hayashi Y, Arisaka F, Ishihama Y, Ohsumi Y (2013). Atg38 is required for autophagy-specific phosphatidylinositol 3-kinase complex integrity. J Cell Biol, 203(2): 299–313

Ashrafi G, Schwarz T L (2013). The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ, 20(1): 31–42

Axe E L, Walker S A, Manifava M, Chandra P, Roderick H L, Habermann A, Griffiths G, Ktistakis N T (2008). Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol, 182(4): 685–701

Baskaran S, Ragusa M J, Boura E, Hurley J H (2012). Two-site recognition of phosphatidylinositol 3-phosphate by PROPPINs in autophagy. Mol Cell, 47(3): 339–348

Birgisdottir A B, Lamark T, Johansen T (2013). The LIR motif - crucial for selective autophagy. J Cell Sci, 126(Pt 15): 3237–3247

Burda P, Padilla S M, Sarkar S, Emr S D (2002). Retromer function in endosome-to-Golgi retrograde transport is regulated by the yeast Vps34 PtdIns 3-kinase. J Cell Sci, 115(Pt 20): 3889–3900

Chan E Y W, Longatti A, McKnight N C, Tooze S A (2009). Kinase-inactivated ULK proteins inhibit autophagy via their conserved C-terminal domains using an Atg13-independent mechanism. Mol Cell Biol, 29(1): 157–171

Cheong H, Nair U, Geng J, Klionsky D J (2008). The Atg1 kinase complex is involved in the regulation of protein recruitment to initiate sequestering vesicle formation for nonspecific autophagy in *Saccharomyces cerevisiae*. Mol Biol Cell, 19(2): 668–681

Cheong H, Yorimitsu T, Reggiori F, Legakis J E, Wang C W, Klionsky D J (2005). Atg17 regulates the magnitude of the autophagic response. Mol Biol Cell, 16(7): 3438–3453

Chew L H, Setiaputra D, Klionsky D J, Yip C K (2013). Structural characterization of the Saccharomyces cerevisiae autophagy regulatory complex Atg17-Atg31-Atg29. Autophagy, 9: 1467–1474

Choi A M K, Ryter S W, Levine B (2013). Autophagy in human health and disease. N Engl J Med, 368(7): 651–662

Coyle J E, Qamar S, Rajashankar K R, Nikolov D B (2002). Structure of GABARAP in two conformations: implications for GABA(A) receptor localization and tubulin binding. Neuron, 33(1): 63–74

Dove S K, Piper R C, McEwen R K, Yu J W, King M C, Hughes D C, Thuring J, Holmes A B, Cooke F T, Michell R H, Parker P J, Lemmon M A (2004). Svp1p defines a family of phosphatidylinositol 3,5-bisphosphate effectors. EMBO J, 23(9): 1922–1933

Fan W, Nassiri A, Zhong Q (2011). Autophagosome targeting and membrane curvature sensing by Barkor/Atg14(L). Proc Natl Acad Sci USA, 108(19): 7769–7774

Feng W, Huang S, Wu H, Zhang M (2007). Molecular basis of Bcl-xL's target recognition versatility revealed by the structure of Bcl-xL in complex with the BH3 domain of Beclin-1. J Mol Biol, 372(1): 223–235

Fogel A I, Dlouhy B J, Wang C, Ryu S W, Neutzner A, Hasson S A, Sideris D P, Abeliovich H, Youle R J (2013). Role of membrane association and Atg14-dependent phosphorylation in beclin-1-mediated autophagy. Mol Cell Biol, 33(18): 3675–3688

Fujioka Y, Noda N N, Nakatogawa H, Ohsumi Y, Inagaki F (2010). Dimeric coiled-coil structure of Saccharomyces cerevisiae Atg16 and its functional significance in autophagy. J Biol Chem, 285(2): 1508–1515

Furuya N, Yu J, Byfield M, Pattingre S, Levine B (2005). The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. Autophagy, 1(1): 46–52

Gammoh N, Florey O, Overholtzer M, Jiang X (2013). Interaction between FIP200 and ATG16L1 distinguishes ULK1 complex-dependent and -independent autophagy. Nat Struct Mol Biol, 20(2): 144–149

Ganley I G, Lam H, Wang J, Ding X, Chen S, Jiang X (2009). ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem, 284(18): 12297–12305

Gaugel A, Bakula D, Hoffmann A, Proikas-Cezanne T (2012). Defining

regulatory and phosphoinositide-binding sites in the human WIPI-1 β-propeller responsible for autophagosomal membrane localization downstream of mTORC1 inhibition. J Mol Signal, 7(1): 16

Hailey D W, Rambold A S, Satpute-Krishnan P, Mitra K, Sougrat R, Kim P K, Lippincott-Schwartz J (2010). Mitochondria supply membranes for autophagosome biogenesis during starvation. Cell, 141(4): 656–667

Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, Amano A, Yoshimori T (2013). Autophagosomes form at ER-mitochondria contact sites. Nature, 495(7441): 389–393

Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A (2009). A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat Cell Biol, 11(12): 1433–1437

Heenan E J, Vanhooke J L, Temple B R, Betts L, Sondek J E, Dohlman H G (2009). Structure and function of Vps15 in the endosomal G protein signaling pathway. Biochemistry, 48(27): 6390–6401

Hong S B, Kim B W, Kim J H, Song H K (2012). Structure of the autophagic E2 enzyme Atg10. Acta Crystallogr D Biol Crystallogr, 68(Pt 10): 1409–1417

Hong S B, Kim B W, Lee K E, Kim S W, Jeon H, Kim J, Song H K (2011). Insights into noncanonical E1 enzyme activation from the structure of autophagic E1 Atg7 with Atg8. Nat Struct Mol Biol, 18 (12): 1323–1330

Hosokawa N, Sasaki T, Iemura S, Natsume T, Hara T, Mizushima N (2009). Atg101, a novel mammalian autophagy protein interacting with Atg13. Autophagy, 5(7): 973–979

Huang W, Choi W, Hu W, Mi N, Guo Q, Ma M, Liu M, Tian Y, Lu P, Wang F L, Deng H, Liu L, Gao N, Yu L, Shi Y (2012). Crystal structure and biochemical analyses reveal Beclin 1 as a novel membrane binding protein. Cell Res, 22(3): 473–489

Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M, Noda T, Ohsumi Y (2000). A ubiquitin-like system mediates protein lipidation. Nature, 408(6811): 488–492

Itakura E, Kishi C, Inoue K, Mizushima N (2008). Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. Mol Biol Cell, 19(12): 5360–5372

Jao C C, Ragusa M J, Stanley R E, Hurley J H (2013). A HORMA domain in Atg13 mediates PI 3-kinase recruitment in autophagy. Proc Natl Acad Sci USA, 110(14): 5486–5491

Kabeya Y, Kamada Y, Baba M, Takikawa H, Sasaki M, Ohsumi Y (2005). Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy. Mol Biol Cell, 16(5): 2544–2553

Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y, Yoshimori T (2004). LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. J Cell Sci, 117(Pt 13): 2805–2812

Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, Yoshimori T (2000). LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J, 19: 5720–5728

Kabeya Y, Noda N N, Fujioka Y, Suzuki K, Inagaki F, Ohsumi Y (2009). Characterization of the Atg17-Atg29-Atg31 complex specifically required for starvation-induced autophagy in *Saccharomyces cerevisiae*. Biochem Biophys Res Commun, 389(4): 612–615

Kaiser S E, Mao K, Taherbhoy A M, Yu S, Olszewski J L, Duda D M, Kurinov I, Deng A, Fenn T D, Klionsky D J, Schulman B A (2012). Noncanonical E2 recruitment by the autophagy E1 revealed by Atg7-Atg3 and Atg7-Atg10 structures. Nat Struct Mol Biol, 19(12): 1242–1249

Kakuta S, Yamamoto H, Negishi L, Kondo-Kakuta C, Hayashi N, Ohsumi Y (2012). Atg9 vesicles recruit vesicle-tethering proteins Trs85 and Ypt1 to the autophagosome formation site. J Biol Chem, 287(53): 44261–44269

Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, Ohsumi Y (2000). Tor-mediated induction of autophagy via an Apg1 protein kinase complex. J Cell Biol, 150(6): 1507–1513

Karanasios E, Stapleton E, Manifava M, Kaizuka T, Mizushima N, Walker S A, Ktistakis N T (2013). Dynamic association of the ULK1 complex with omegasomes during autophagy induction. J Cell Sci, 126(Pt 22): 5224–5238

Kihara A, Noda T, Ishihara N, Ohsumi Y (2001). Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae*. J Cell Biol, 152, 519–30

Kijanska M, Dohnal I, Reiter W, Kaspar S, Stoffel I, Ammerer G, Kraft C, Peter M (2010). Activation of Atg1 kinase in autophagy by regulated phosphorylation. Autophagy, 6(8): 1168–1178

Kim J, Kundu M, Viollet B, Guan K L (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol, 13(2): 132–141

Knight D, Harris R, McAlister M S B, Phelan J P, Geddes S, Moss S J, Driscoll P C, Keep N H (2002). The X-ray crystal structure and putative ligand-derived peptide binding properties of gamma-aminobutyric acid receptor type A receptor-associated protein. J Biol Chem, 277(7): 5556–5561

Kobayashi T, Suzuki K, Ohsumi Y (2012). Autophagosome formation can be achieved in the absence of Atg18 by expressing engineered PAS-targeted Atg2. FEBS Lett, 586(16): 2473–2478

Kondo-Okamoto N, Noda N N, Suzuki S W, Nakatogawa H, Takahashi I, Matsunami M, Hashimoto A, Inagaki F, Ohsumi Y, Okamoto K (2012). Autophagy-related protein 32 acts as autophagic degron and directly initiates mitophagy. J Biol Chem, 287(13): 10631–10638

Kraft C, Deplazes A, Sohrmann M, Peter M (2008). Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nat Cell Biol, 10(5): 602–610

Kraft C, Kijanska M, Kalie E, Siergiejuk E, Lee S S, Semplicio G, Stoffel I, Brezovich A, Verma M, Hansmann I, Ammerer G, Hofmann K, Tooze S, Peter M (2012). Binding of the Atg1/ULK1 kinase to the ubiquitin-like protein Atg8 regulates autophagy. EMBO J, 31(18): 3691–3703

Krick R, Busse R A, Scacioc A, Stephan M, Janshoff A, Thumm M, Kühnel K (2012). Structural and functional characterization of the two phosphoinositide binding sites of PROPPINs, a β-propeller protein family. Proc Natl Acad Sci USA, 109(30): E2042–E2049

Krick R, Muehe Y, Prick T, Bremer S, Schlotterhose P, Eskelinen E L, Millen J, Goldfarb D S, Thumm M (2008). Piecemeal microautophagy of the nucleus requires the core macroautophagy genes. Mol Biol Cell, 19(10): 4492–4505

Ku B, Woo J S, Liang C, Lee K H, Hong H S, e X, Kim K S, Jung J U, Oh B H (2008). Structural and biochemical bases for the inhibition of

autophagy and apoptosis by viral BCL-2 of murine gammaherpesvirus 68. PLoS Pathog, 4(2): e25

Kuma A, Mizushima N, Ishihara N, Ohsumi Y (2002). Formation of the approximately 350-kDa Apg12-Apg5.Apg16 multimeric complex, mediated by Apg16 oligomerization, is essential for autophagy in yeast. J Biol Chem, 277(21): 18619–18625

Kumanomidou T, Mizushima T, Komatsu M, Suzuki A, Tanida I, Sou Y S, Ueno T, Kominami E, Tanaka K, Yamane T (2006). The crystal structure of human Atg4b, a processing and de-conjugating enzyme for autophagosome-forming modifiers. J Mol Biol, 355(4): 612–618

Kumeta H, Watanabe M, Nakatogawa H, Yamaguchi M, Ogura K, Adachi W, Fujioka Y, Noda N N, Ohsumi Y, Inagaki F (2010). The NMR structure of the autophagy-related protein Atg8. J Biomol NMR, 47(3): 237–241

Lamb C A, Yoshimori T, Tooze S A (2013). The autophagosome: origins unknown, biogenesis complex. Nat Rev Mol Cell Biol, 14(12): 759–774

Li X, He L, Che K H, Funderburk S F, Pan L, Pan N, Zhang M, Yue Z, Zhao Y (2012). Imperfect interface of Beclin1 coiled-coil domain regulates homodimer and heterodimer formation with Atg14L and UVRAG. Nat Commun, 3: 662

Liang X H, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature, 402(6762): 672–676

Liang X H, Kleeman L K, Jiang H H, Gordon G, Goldman J E, Berry G, Herman B, Levine B (1998). Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. J Virol, 72 (11): 8586–8596

Lipatova Z, Belogortseva N, ZhangX Q, Kim J, Taussig D, Segev N (2012). Regulation of selective autophagy onset by a Ypt/Rab GTPase module. Proc Natl Acad Sci U S A, 109: 6981–6986

Liu K, Czaja M J (2013). Regulation of lipid stores and metabolism by lipophagy. Cell Death Differ, 20(1): 3–11

Liu X, Dai S, Zhu Y, Marrack P, Kappler J W (2003). The structure of a Bcl-xL/Bim fragment complex: implications for Bim function. Immunity, 19(3): 341–352

Mao K, Chew L H, Inoue-Aono Y, Cheong H, Nair U, Popelka H, Yip C K, Klionsky D J (2013). Atg29 phosphorylation regulates coordination of the Atg17-Atg31-Atg29 complex with the Atg11 scaffold during autophagy initiation. Proc Natl Acad Sci USA, 110(31): E2875–E2884

Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, Maejima I, Shirahama-Noda K, Ichimura T, Isobe T, Akira S, Noda T, Yoshimori T (2009). Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat Cell Biol, 11(4): 385–396

Matsushita M, Suzuki N N, Obara K, Fujioka Y, Ohsumi Y, Inagaki F (2007). Structure of Atg5.Atg16, a complex essential for autophagy. J Biol Chem, 282(9): 6763–6772

Matsuura A, Tsukada M, Wada Y, Ohsumi Y (1997). Apg1p, a novel protein kinase required for the autophagic process in *Saccharomyces cerevisiae*. Gene, 192, 245–250

Mauthe M, Jacob A, Freiberger S, Hentschel K, Stierhof Y D, Codogno P, Proikas-Cezanne T (2011). Resveratrol-mediated autophagy requires WIPI-1-regulated LC3 lipidation in the absence of induced phagophore formation. Autophagy, 7(12): 1448–1461

Meiling-Wesse K, Barth H, Voss C, Eskelinen E L, Epple U D, Thumm M (2004). Atg21 is required for effective recruitment of Atg8 to the preautophagosomal structure during the Cvt pathway. J Biol Chem, 279(36): 37741–37750

Mercer C A, Kaliappan A, Dennis P B (2009). A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. Autophagy, 5(5): 649–662

Metlagel Z, Otomo C, Takaesu G, Otomo T (2013). Structural basis of ATG3 recognition by the autophagic ubiquitin-like protein ATG12. Proc Natl Acad Sci USA, 110(47): 18844–18849

Miller S, Tavshanjian B, Oleksy A, Perisic O, Houseman B T, Shokat K M, Williams R L (2010). Shaping development of autophagy inhibitors with the structure of the lipid kinase Vps34. Science, 327 (5973): 1638–1642

Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George M D, Klionsky D J, Ohsumi M, Ohsumi Y (1998). A protein conjugation system essential for autophagy. Nature, 395(6700): 395–398

Mizushima N, Yoshimori T, Ohsumi Y (2011). The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol, 27(1): 107–132

Moreau K, Ravikumar B, Renna M, Puri C, Rubinsztein D C (2011). Autophagosome precursor maturation requires homotypic fusion. Cell, 146(2): 303–317

Moreau K, Renna M, Rubinsztein D C (2013). Connections between SNAREs and autophagy. Trends Biochem Sci, 38(2): 57–63

Nair U, Jotwani A, Geng J, Gammoh N, Richerson D, Yen W L, Griffith J, Nag S, Wang K, Moss T, Baba M, McNew J A, Jiang X, Reggiori F, Melia T J, Klionsky D J (2011). SNARE proteins are required for macroautophagy. Cell, 146(2): 290–302

Nair U, Yen W L, Mari M, Cao Y, Xie Z, Baba M, Reggiori F, Klionsky D J (2012). A role for Atg8-PE deconjugation in autophagosome biogenesis. Autophagy, 8(5): 780–793

Nakatogawa H, Ichimura Y, Ohsumi Y (2007). Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. Cell, 130(1): 165–178

Nakatogawa H, Ishii J, Asai E, Ohsumi Y (2012). Atg4 recycles inappropriately lipidated Atg8 to promote autophagosome biogenesis. Autophagy, 8(2): 1–10

Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009). Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol, 10(7): 458–467

Nishimura T, Kaizuka T, Cadwell K, Sahani M H, Saitoh T, Akira S, Virgin H W, Mizushima N (2013). FIP200 regulates targeting of Atg16L1 to the isolation membrane. EMBO Rep, 14(3): 284–291

Noda N N, Fujioka Y, Hanada T, Ohsumi Y, Inagaki F (2013). Structure of the Atg12-Atg5 conjugate reveals a platform for stimulating Atg8-PE conjugation. EMBO Rep, 14(2): 206–211

Noda N N, Kobayashi T, Adachi W, Fujioka Y, Ohsumi Y, Inagaki F (2012). Structure of the novel C-terminal domain of vacuolar protein sorting 30/autophagy-related protein 6 and its specific role in autophagy. J Biol Chem, 287(20): 16256–16266

Noda N N, Kumeta H, Nakatogawa H, Satoo K, Adachi W, Ishii J, Fujioka Y, Ohsumi Y, Inagaki F (2008). Structural basis of target recognition by Atg8/LC3 during selective autophagy. Genes Cells, 13(12): 1211–1218

Noda N N, Ohsumi Y, Inagaki F (2009). ATG systems from the protein structural point of view. Chem Rev, 109(4): 1587–1598

Noda N N, Satoo K, Fujioka Y, Kumeta H, Ogura K, Nakatogawa H, Ohsumi Y, Inagaki F (2011). Structural basis of Atg8 activation by a homodimeric E1, Atg7. Mol Cell, 44(3): 462–475

Noda T, Kim J, Huang W P, Baba M, Tokunaga C, Ohsumi Y, Klionsky D J (2000). Apg9p/Cvt7p is an integral membrane protein required for transport vesicle formation in the Cvt and autophagy pathways. J Cell Biol, 148(3): 465–480

Noda T, Matsunaga K, Taguchi-Atarashi N, Yoshimori T (2010). Regulation of membrane biogenesis in autophagy via PI3P dynamics. Semin Cell Dev Biol, 21(7): 671–676

Obara K, Noda T, Niimi K, Ohsumi Y (2008a). Transport of phosphatidylinositol 3-phosphate into the vacuole via autophagic membranes in *Saccharomyces cerevisiae*. Genes Cells, 13(6): 537–547

Obara K, Sekito T, Niimi K, Ohsumi Y (2008b). The Atg18-Atg2 complex is recruited to autophagic membranes via phosphatidylinositol 3-phosphate and exerts an essential function. J Biol Chem, 283 (35): 23972–23980

Obara K, Sekito T, Ohsumi Y (2006). Assortment of phosphatidylinositol 3-kinase complexes—Atg14p directs association of complex I to the pre-autophagosomal structure in *Saccharomyces cerevisiae*. Mol Biol Cell, 17(4): 1527–1539

Oberstein A, Jeffrey P D, Shi Y (2007). Crystal structure of the Bcl-XL-Beclin 1 peptide complex: Beclin 1 is a novel BH3-only protein. J Biol Chem, 282(17): 13123–13132

Otomo C, Metlagel Z, Takaesu G, Otomo T (2013). Structure of the human ATG12~ATG5 conjugate required for LC3 lipidation in autophagy. Nat Struct Mol Biol, 20(1): 59–66

Panaretou C, Domin J, Cockcroft S, Waterfield M D (1997). Characterization of p150, an adaptor protein for the human phosphatidylinositol (PtdIns) 3-kinase. Substrate presentation by phosphatidylinositol transfer protein to the p150.Ptdins 3-kinase complex. J Biol Chem, 272(4): 2477–2485

Paz Y, Elazar Z, Fass D (2000). Structure of GATE-16, membrane transport modulator and mammalian ortholog of autophagocytosis factor Aut7p. J Biol Chem, 275(33): 25445–25450

Petros A M, Nettesheim D G, Wang Y, Olejniczak E T, Meadows R P, Mack J, Swift K, Matayoshi E D, Zhang H, Thompson C B, Fesik S W (2010). Rationale for Bcl-xL/Bad peptide complex formation from structure, mutagenesis, and biophysical studies. Protein Sci, 9: 2528–2534

Polson H E J, de Lartigue J, Rigden D J, Reedijk M, Urbé S, Clague M J, Tooze S A (2010). Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy, 6(4): 506–522

Proikas-Cezanne T, Waddell S, Gaugel A, Frickey T, Lupas A, Nordheim A (2004). WIPI-1alpha (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy. Oncogene, 23 (58): 9314–9325

Puri C, Renna M, Bento C F, Moreau K, Rubinsztein D C (2013). Diverse autophagosome membrane sources coalesce in recycling endosomes. Cell, 154(6): 1285–1299

Ragusa M J, Stanley R E, Hurley J H (2012). Architecture of the Atg17 complex as a scaffold for autophagosome biogenesis. Cell, 151(7): 1501–1512

Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein D C (2010). Plasma membrane contributes to the formation of pre-autophagosomal structures. Nat Cell Biol, 12(8): 747–757

Reggiori F, Klionsky D J (2013). Autophagic processes in yeast: mechanism, machinery and regulation. Genetics, 194(2): 341–361

Reggiori F, Tucker K A, Stromhaug P E, Klionsky D J (2004). The Atg1-Atg13 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure. Dev Cell, 6(1): 79–90

Renner L D, Weibel D B (2011). Cardiolipin microdomains localize to negatively curved regions of Escherichia coli membranes. Proc Natl Acad Sci USA, 108(15): 6264–6269

Rieter E, Vinke F, Bakula D, Cebollero E, Ungermann C, Proikas-Cezanne T, Reggiori F (2013). Atg18 function in autophagy is regulated by specific sites within its β-propeller. J Cell Sci, 126(Pt 2): 593–604

Romanov J, Walczak M, Ibiricu I, Schüchner S, Ogris E, Kraft C, Martens S (2012). Mechanism and functions of membrane binding by the Atg5-Atg12/Atg16 complex during autophagosome formation. EMBO J, 31: 4304–4317

Sattler M, Liang H, Nettesheim D, Meadows R P, Harlan J E, Eberstadt M, Yoon H S, Shuker S B, Chang B S, Minn A J, Thompson C B, Fesik S W (1997). Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. Science, 275: 983–986

Satoo K, Noda N N, Kumeta H, Fujioka Y, Mizushima N, Ohsumi Y, Inagaki F (2009). The structure of Atg4B-LC3 complex reveals the mechanism of LC3 processing and delipidation during autophagy. EMBO J, 28(9): 1341–1350

Schwarten M, Stoldt M, Mohrlüder J, Willbold D (2010). Solution structure of Atg8 reveals conformational polymorphism of the N-terminal domain. Biochem Biophys Res Commun, 395(3): 426–431

Seglen P O, Gordon P B (1982). 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. Proc Natl Acad Sci USA, 79(6): 1889–1892

Sekito T, Kawamata T, Ichikawa R, Suzuki K, Ohsumi Y (2009). Atg17 recruits Atg9 to organize the pre-autophagosomal structure. Genes Cells, 14(5): 525–538

Sironi L, Mapelli M, Knapp S, De Antoni A, Jeang K T, Musacchio A (2002). Crystal structure of the tetrameric Mad1-Mad2 core complex: implications of a 'safety belt' binding mechanism for the spindle checkpoint. EMBO J, 21(10): 2496–2506

Strϕmhaug P E, Reggiori F, Guan J, Wang C W, Klionsky D J (2004). Atg21 is a phosphoinositide binding protein required for efficient lipidation and localization of Atg8 during uptake of aminopeptidase I by selective autophagy. Mol Biol Cell, 15(8): 3553–3566

Sugawara K, Suzuki N N, Fujioka Y, Mizushima N, Ohsumi Y, Inagaki F (2004). The crystal structure of microtubule-associated protein light chain 3, a mammalian homologue of *Saccharomyces cerevisiae* Atg8. Genes Cells, 9(7): 611–618

Sugawara K, Suzuki N N, Fujioka Y, Mizushima N, Ohsumi Y, Inagaki F (2005). Structural basis for the specificity and catalysis of human Atg4B responsible for mammalian autophagy. J Biol Chem, 280(48): 40058–40065

Sun L L, Li M, Suo F, Liu X M, Shen E Z, Yang B, Dong M Q, He W Z, Du L L (2013). Global analysis of fission yeast mating genes reveals new autophagy factors. PLoS Genet, 9(8): e1003715

Suzuki K (2013). Selective autophagy in budding yeast. Cell Death Differ, 20(1): 43–48

Suzuki K, Kubota Y, Sekito T, Ohsumi Y (2007). Hierarchy of Atg

proteins in pre-autophagosomal structure organization. Genes Cells, 12(2): 209–218

Suzuki K, Ohsumi Y (2007). Molecular machinery of autophagosome formation in yeast, *Saccharomyces cerevisiae*. FEBS Lett, 581(11): 2156–2161

Suzuki N N, Yoshimoto K, Fujioka Y, Ohsumi Y, Inagaki F (2005). The crystal structure of plant ATG12 and its biological implication in autophagy. Autophagy, 1(2): 119–126

Taherbhoy A M, Tait S W, Kaiser S E, Williams A H, Deng A, Nourse A, Hammel M, Kurinov I, Rock C O, Green D R, Schulman B A (2011). Atg8 transfer from Atg7 to Atg3: a distinctive E1-E2 architecture and mechanism in the autophagy pathway. Mol Cell, 44(3): 451–461

Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y (1992). Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. J Cell Biol, 119(2): 301–311

Tsukada M, Ohsumi Y (1993). Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. FEBS Lett, 333(1-2): 169–174

Watanabe Y, Kobayashi T, Yamamoto H, Hoshida H, Akada R, Inagaki F, Ohsumi Y, Noda N N (2012). Structure-based analyses reveal distinct binding sites for Atg2 and phosphoinositides in Atg18. J Biol Chem, 287(38): 31681–31690

Weidberg H, Shpilka T, Shvets E, Abada A, Shimron F, Elazar Z (2011a). LC3 and GATE-16 N termini mediate membrane fusion processes required for autophagosome biogenesis. Dev Cell, 20(4): 444–454

Weidberg H, Shvets E, Elazar Z (2011b). Biogenesis and cargo selectivity of autophagosomes. Annu Rev Biochem, 80(1): 125–156

Weidberg H, Shvets E, Shpilka T, Shimron F, Shinder V, Elazar Z (2010). LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. EMBO J, 29(11): 1792–1802

Wu Y T, Tan H L, Shui G, Bauvy C, Huang Q, Wenk M R, Ong C N, Codogno P, Shen H M (2010). Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. J Biol Chem, 285(14): 10850–10861

Xie Z, Nair U, Klionsky D J (2008). Atg8 controls phagophore expansion during autophagosome formation. Mol Biol Cell, 19(8): 3290–3298

Yamada Y, Suzuki N N, Hanada T, Ichimura Y, Kumeta H, Fujioka Y, Ohsumi Y, Inagaki F (2007). The crystal structure of Atg3, an autophagy-related ubiquitin carrier protein (E2) enzyme that mediates Atg8 lipidation. J Biol Chem, 282(11): 8036–8043

Yamaguchi M, Matoba K, Sawada R, Fujioka Y, Nakatogawa H, Yamamoto H, Kobashigawa Y, Hoshida H, Akada R, Ohsumi Y, Noda N N, Inagaki F (2012a). Noncanonical recognition and UBL loading of distinct E2s by autophagy-essential Atg7. Nat Struct Mol Biol, 19(12): 1250–1256

Yamaguchi M, Noda N N, Nakatogawa H, Kumeta H, Ohsumi Y, Inagaki F (2010). Autophagy-related protein 8 (Atg8) family interacting motif in Atg3 mediates the Atg3-Atg8 interaction and is crucial for the cytoplasm-to-vacuole targeting pathway. J Biol Chem, 285(38): 29599–29607

Yamaguchi M, Noda N N, Yamamoto H, Shima T, Kumeta H, Kobashigawa Y, Akada R, Ohsumi Y, Inagaki F (2012b). Structural insights into Atg10-mediated formation of the autophagy-essential Atg12-Atg5 conjugate. Structure, 20(7): 1244–1254

Yamamoto H, Kakuta S, Watanabe T M, Kitamura A, Sekito T, Kondo-Kakuta C, Ichikawa R, Kinjo M, Ohsumi Y (2012). Atg9 vesicles are an important membrane source during early steps of autophagosome formation. J Cell Biol, 198(2): 219–233

Yeh Y Y, Shah K H, Chou C C, Hsiao H H, Wrasman K M, Stephan J S, Stamatakos D, Khoo K H, Herman P K (2011). The identification and analysis of phosphorylation sites on the Atg1 protein kinase. Autophagy, 7: 716–726

Ylä-Anttila P, Vihinen H, Jokitalo E, Eskelinen E L (2009). 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy, 5(8): 1180–1185

Yu Z Q, Ni T, Hong B, Wang H Y, Jiang F J, Zou S, Chen Y, Zheng X L, Klionsky D J, Liang Y, Xie Z (2012). Dual roles of Atg8-PE deconjugation by Atg4 in autophagy. Autophagy, 8(6): 1–10

Zhong Y, Wang Q J, Li X, Yan Y, Backer J M, Chait B T, Heintz N, Yue Z (2009). Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. Nat Cell Biol, 11(4): 468–476