Cell PRESS

Connections between SNAREs and autophagy

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Autophagy involves the sequestration of portions of cytoplasm by double-membraned autophagosomes, which are then trafficked to lysosomes. After autophagosome–lysosome fusion, the contents of the autophagosomes are degraded by lysosomal hydrolases. SNAREs [soluble N-ethylmaleimide-sensitive fusion (NSF) attachment protein receptors] are molecules that mediate vesicular fusion events. Here, we review recent data implicating SNAREs as having key roles both in the genesis of autophagosomes, as well as in autophagosome–lysosome fusion, and we discuss the implications of these findings in the context of a long-standing mystery: the origin of autophagosomes.

Autophagy machinery

Macroautophagy (here referred to as autophagy) is a catabolic process that promotes cellular homeostasis through degradation of intracytoplasmic proteins and organelles. Dysregulation of autophagy has been associated with diverse pathologies such as cancer, inflammation, and neurodegeneration [1–3]. Autophagy starts with the formation of small double-membraned, cup-shaped structures called phagophores. The edges of phagophores elongate, engulfing proteins and/or organelles, and then fuse to form completed autophagosomes. Autophagosomes are then trafficked and fused to lysosomes, allowing degradation of the autophagic cargoes.

The core autophagy machinery comprises a set of 35 autophagy-related genes (*ATG*; see Glossary) that were originally identified in yeast genetic screens. Most of these genes have mammalian orthologues [3–5]. Phagophore formation requires the activity of the class III phosphoinositide 3-kinase (PI3K) VPS34 in order to generate phosphatidylinositol-3-phosphate (PI3P). VPS34 acts in a large complex along with ATG6/BECLIN-1, ATG14/BARKOR, and VPS15/p150. The function of PI3P in autophagy is still unclear, but it appears to aid the recruitment of WIPI-1 (the mammalian orthologue of ATG18) to the autophagosomal membrane. The activity of VPS34 is enhanced by its interaction with BECLIN-1, which is further regulated by other binding partners, such as AMBRA-1, UV radiation resistance-associated gene (UVRAG), BIF-1, inositol 1,4,5-triphosphate receptor (IP3R), and the antiapoptotic proteins BCL-2 and BCL-X_L [3–5].

Phagophore maturation and subsequent autophagosome formation requires membrane expansion and fusion, which are regulated by two ubiquitylation-like reactions. In the first reaction, the ubiquitin-like molecule ATG12 is conjugated to ATG5 by a reaction that involves ATG7, similar to an E1 ubiquitin-activating enzyme (E1-like), and ATG10, which acts like an E2 ubiquitin-conjugating enzyme (E2-like). Subsequently, the ATG12–ATG5 complex interacts noncovalently with ATG16L1, and the ATG12–ATG15–ATG16L1 complex associates with the nascent phagophore. This complex is essential for the elongation of the preautophagosomal membrane, but it dissociates from fully formed autophagosomes. In the second ubiquitylation-like reaction, ubiquitin-like molecules of the ATG8 family (microtubule-associated protein 1 light chain 3 (MAP-LC3 or LC3), GABARAP or GATE-16) are conjugated to the lipid phosphatidylethanolamine (PE). LC3, the best characterised member of the ATG8 family, is synthesised as a precursor form and is cleaved at its C terminus by ATG4B, resulting in the cytosolic form LC3-I.

Glossary

Acb: Acyl-CoA binding protein.
AMBRA: activating molecule in Beclin-1 regulated autophagy.
ATG: autophagy-related (*Atg*) gene.
Autophagosome: double-membrane vesicle formed by the elongation of phagophore and fusion of phagophore edges.
BARKOR: Beclin-1 associated autophagy-related key regulator.
Bcl: B-cell lymphoma.
BIF: Bax-interacting factor.
GABARAP: GABA-A receptor-associated protein.
GATE16: Golgi-associated ATPase enhancer of 16 kDa.
GRASP: General receptor for phosphoinositide 1-associated scaffold protein.
Hrb: HIV Rev-binding protein.
MCOLN: mucolipin.
Phagophore: isolation membrane formed by the homotypic fusion of autophagic precursors. Elongation of the phagophore leads to the formation of autophagosome.
Phagophore precursors: single-membrane vesicles whose homotypic fusion leads to the formation of phagophore.
SNARE: acronym derived from SNAP (soluble NSF attachment protein) receptor that relates to members of a large protein superfamily with more than 60 members (found in yeast and mammalian cells). The primary role of SNARE proteins is to mediate vesicle fusion.
SNAP: synaptosomal associated protein.
Sso: Suppressor of Sec1.
TFEB: transcription factor EB.
Tlg: t-SNARE affecting a late Golgi compartment.
TSG: tumor suppressor gene.
UVRAG: UV radiation resistance-associated gene protein.
Vam: vacuolar morphology.
VPS: vacuolar protein sorting.
VTI: vesicles transport through interaction with t-SNARE homolog.
WIPI: WD-repeat protein interacting with phosphoinositides.
Ypt1: yeast protein transport 1.

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Keywords: autophagy; SNARE; lysosome.

LC3-I is then conjugated to PE by ATG7 (E1-like) and ATG3 (E2-like) to form LC3-II, the autophagosome-associated LC3 form. The ATG12–ATG5–ATG16L1 complex may bring LC3 to the site of lipidation and enhance LC3–PE conjugation, acting like an E3-ubiquitin ligase. In contrast to the ATG12–ATG15–ATG16L1 complex, LC3-II remains associated with the completed autophagosome. After autophagosomal fusion with the lysosome, the LC3-II inside the autolysosome is degraded, whereas the LC3-II on the cytoplasmic face can be recycled by an ATG4-dependent delipidation process.

The most obvious vesicle fusion events in autophagy are those between autophagosome and lysosome. Vesicle fusions are often mediated by SNAREs, which include members of a large protein superfamily with more than 60 members (found in yeast and mammalian cells). SNAREs are transmembrane proteins that can assemble in high-affinity *trans* complexes between two opposing membranes to drive the fusion process [6]. SNARE function requires an ordered dynamic interaction between different SNAREs with consecutive rounds of assembly, membrane fusion, and disassembly of post-fusion SNARE *cis* complexes [7]. SNARE proteins are classified as Q-SNAREs [in which glutamine (Q) is the central amino acid of the SNARE motif] or R-SNAREs [in which the central amino acid is arginine (R)], and fusion-competent SNARE complexes generally consist of four-helix bundles composed of three Q-SNAREs and one R-SNARE. For a long time, it was assumed that SNAREs did not play a role in autophagosome formation and that phagophore elongation was dependent on *de novo* lipid addition. However, recent studies have suggested that SNAREs have additional roles in autophagy other than simply mediating autophagosome–lysosome fusion. Here, we discuss recent work that reveals how SNAREs influence autophagosome biogenesis, and autophagosome secretion, in addition to autophagosome–lysosome fusion.

Role of SNAREs in autophagosome formation

The membrane sources for the formation of autophagosomes has been the subject of intense research over the past 30 years. Recent work has suggested that autophagosomes may derive membranes from multiple nonmutually exclusive sources, including the endoplasmic reticulum (ER), Golgi, plasma membrane, and mitochondria [8–13]. Clathrin-mediated endocytosis, as well as a form of clathrin-independent endocytosis, contributes membrane to phagophore precursors, which mature to form phagophores and subsequently autophagosomes, because plasma-membrane-binding probes associate with autophagosome precursor structures as well as with completed autophagosomes [10,14].

SNAREs have recently been shown to regulate autophagosome formation in both mammalian cells and in yeast [15,16]. In mammalian cells, the SNAREs vesicle-associated membrane protein (VAMP)7, syntaxin-7, syntaxin-8, and VTI1B regulate the homotypic fusion of phagophore precursors [15,17]. These fusion events enable the growth of these structures into a tubular network leading to the formation of phagophores and autophagosomes [15]. Indeed, the fusion of small, apparently single-membrane phagophore precursor vesicles to form tubulovesicular structures may be a mechanism contributing to the genesis of double-membraned phagophores or autophagosomes (Figure 1, Table 1). Supporting this idea, inhibition of SNARE-dependent fusion decreases the size of autophagic precursors and their subsequent maturation into autophagosomes.

Some SNAREs are present in diverse cellular locations and work in collaboration via the formation of SNARE complexes. One of the pools of SNAREs required for autophagosome formation may originate at the plasma membrane, since blocking VAMP7 endocytosis by knocking down Hrb, its adaptor at the plasma membrane, leads to the same phenotype as VAMP7 knockdown [15]. Interestingly, VAMP7 and its partners have also been implicated in the subsequent step of autophagosome–lysosome fusion [18,19], which will be described later, suggesting that these SNAREs may be present from the beginning to the end of the process of autophagy.

In yeast, SNAREs that are required for exocytosis (Sec9p and Sso2p) were shown to be important for autophagosome biogenesis, because they regulate the formation of tubulovesicular structures that are positive for Atg9, a transmembrane protein that is required for autophagy but whose functions are still largely elusive (Figure 2, Table 2) [16]. The absence of these SNAREs abolishes the formation of this tubular network and leads to the formation of small ATG9 vesicles, which appear not to undergo homotypic fusions, suggesting that these SNAREs are involved in mediating homotypic fusion of Atg9 vesicles [16]. Other SNAREs that are not involved in exocytosis (Tlg2, Sec22p, and Ykt6p) have also been implicated in this process [16]. Interestingly, the Sso2p (Qa), Tlg2 (Qa), Sec9p (Qb and c), Sec22p (R), and Ykt6p (R) complex would be orthologous to mammalian syntaxin-1 (Qa), syntaxin-16 (Qa), SNAP23 (Qb and c), SEC22B (R), YKT6 (R). However, it is likely that the yeast SNAREs regulate a different process required for autophagosome formation compared to VAMP7, syntaxin-7, syntaxin-8, and VTI1B discussed above, and that the formation of ATG9-positive vesicles is distinct from the homotypic fusion of ATG5–12/ATG16L1 vesicles. It is also possible that additional SNAREs regulating autophagosome formation have yet to be discovered in both yeast and mammalian systems, such as the SNAREs regulating the incorporation of Atg9 vesicles into autophagosome precursors. Some of these may have eluded discovery using genetic screens due to the redundancy that can exist in SNARE complexes [20]. We also cannot exclude the possibility that these differences may reflect differences between yeast and mammalian autophagy. Nevertheless, these data in yeast and mammalian cells are compatible with the concept that many organelles, such as the plasma membrane, Golgi, ER, and mitochondria, may contribute to the biogenesis of autophagosomes, and that SNAREs are key players that may help to integrate that process (Figures 1 and 2). Although these data suggest that a key role for SNAREs is in autophagosome formation, important SNARE-mediated events are also critical for subsequent steps in autophagy that enable delivery of autophagosomes to lysosomes.

Role of SNAREs in autophagosome maturation

The progress of autophagy subsequent to autophagosome formation involves many different membrane fusion steps,