Autophagosome maturation and lysosomal fusion

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Abstract
Compartmentalization is essential in the eukaryotic cell and this is most often achieved by sequestering specific components that perform a related function in a membrane-bound organelle. To function normally these organelles must transiently fuse with other compartments in order to transfer protein and lipid that is needed for them to function. These events must be highly coordinated otherwise non-specific fusion could occur leading to loss of compartment identity and function. The autophagosome is a specialized membrane compartment that delivers cytosolic components to the lysosome for degradation. Likewise, this delivery is coordinated so that only when the autophagosome is fully formed is it imparted with the information to allow it to specifically fuse with the endocytic system and deliver its contents to the lysosome. In the present chapter, I discuss our current understanding of how this happens.

Keywords:
actin, autophagosome, endosome, fusion, lysosome, maturation, microtubule, Rab, SNARE, tether.

Introduction
Lysosomes are the degradative organelles of the cell and contain an acidic lumen to allow specialized hydrolases to break down protein, lipid, carbohydrate and nucleic acids [1,2]. Material to be degraded can come from the outside, cell surface or inside of the cell and is delivered to

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the lysosome in a series of inter-related transport pathways (Figure 1). As lysosomes are the final destination, inhibition of lysosomal function will disrupt all of these pathways. Of these, the endocytic pathway is the most important as it is central to lysosomal biogenesis and function; the pathway supplies not only the hydrolases, but also proteins such as the vacuolar ATPases and glycosylated LAMPs that acidify and allow the organelle to cope with its digestive lumenal environment respectively. Given this, the endocytic pathway is also central to autophagy. The endocytic pathway itself starts at the cell surface, where external solutes and plasma membrane components are internalized and delivered to a series of sorting compartments where the majority of protein and membrane is recycled. Components such as hydrolases and those destined for destruction are further sorted to the lysosome (for a review see [3]). Disruption of this pathway at multiple points leads to impaired lysosomal function and hence autophagosome turnover. Surprisingly this is not only due to the accumulation of undegraded autophagic components in the lysosome, but also because the autophagosomes themselves fail to fuse with the lysosome. For example, disruption of early endosomal function by depletion of COPI (coatamer protein I) (see Table 1 for a brief description of proteins discussed below) leads to the accumulation of autophagic structures that fail to reach the lysosome [4]. Likewise, loss of function of the ESCRT (endosomal sorting complex required for transport) that is required for

**Figure 1. Summary of lysosomal degradation pathways**

Lysosomes are the digestive organelles of the cell and the source of the material to be degraded determines which pathway is taken. Intracellular material is taken up by the autophagic pathway (shown at the top), whereas small extracellular molecules and cell-surface receptors are delivered to the lysosome by the endocytic pathway (shown in the centre). In a similar fashion, bulk uptake of extracellular material (pinocytosis) or pathogens proceeds via the phagocytic pathway (shown at the bottom). Once material has been sequestered, delivery to the lysosome proceeds by a similar mechanism for all the pathways, with autophagosomes and phagosomes essentially merging with endosomes en route to lysosomes. Hence the endocytic pathway is central to all the lysosomal degradation pathways.
The generation of intraluminal vesicles in later endosomal structures not only blocks endocytic degradation, but also leads to the accumulation of autophagosomes [5]. Taken together, this highlights the importance of endocytosis in autophagy. These data also raise another important question: where exactly does the autophagosome fuse with the endocytic system? It seems likely that autophagosomes can fuse with the endocytic system at multiple points. By fusing with both early and late endosomes they form a hybrid organelle termed an amphisome, which then goes on to fuse with lysosomes to form autolysosomes. It is, however, possible that autolysosomes are formed directly by autophagosome–lysosome fusion. It should also be noted that the endocytic pathway is not just important for autophagosomal maturation and turnover, but as discussed in Chapter 3, is also important for autophagosome biogenesis.

Not only is the autophagosomal fusion step essential for autophagy, it also must be taken into account when measuring the process experimentally; an increase in autophagosomes does

Table 1. Glossary of proteins implicated in autophagosome–lysosome fusion

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function in endocytosis and autophagy</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPI</td>
<td>Vesicle coat protein complex involved in endosomal and Golgi sorting and early endosomal fusion with autophagosomes</td>
</tr>
<tr>
<td>ESCRT</td>
<td>Series of protein complexes involved in sorting cargo into multivesicular bodies and required for autophagosome fusion</td>
</tr>
<tr>
<td>ATG8</td>
<td>Family of ubiquitin-like modifiers, including LC3, that are essential for autophagosome maturation</td>
</tr>
<tr>
<td>Rab5</td>
<td>Small GTPase involved in early endosomal function and autophagosome formation</td>
</tr>
<tr>
<td>Rab7</td>
<td>Small GTPase involved in endosomal/autophagosome maturation</td>
</tr>
<tr>
<td>Rab33b</td>
<td>Small GTPase involved in autophagosome formation</td>
</tr>
<tr>
<td>OATL1/TBC1D25</td>
<td>Rab33b GAP</td>
</tr>
<tr>
<td>RILP</td>
<td>Rab7 effector protein that binds to dynein motors</td>
</tr>
<tr>
<td>FYCO1</td>
<td>Rab7 effector that binds to kinesins</td>
</tr>
<tr>
<td>HDAC6</td>
<td>Recruits actin to aid in specific autophagosome fusion</td>
</tr>
<tr>
<td>HOPS</td>
<td>Endosome–autophagosome tethering complex [six subunits: VPS11, VPS16 (interacts with UVRAG), VPS18, VPS33, VPS39 (Rab7 GEF) and VPS41]</td>
</tr>
<tr>
<td>VPS34</td>
<td>Class III PI3P required for autophagy and endocytosis</td>
</tr>
<tr>
<td>UVRAG</td>
<td>Activator of VPS34 and VPS16 interactor</td>
</tr>
<tr>
<td>Rubicon</td>
<td>Inhibitor of UVRAG and VPS34</td>
</tr>
<tr>
<td>TECPR1</td>
<td>Autophagosomal-lysosomal tether</td>
</tr>
<tr>
<td>Syntaxin 7, syntaxin 8, Vti1b</td>
<td>Q-SNAREs implicated in endosome–lysosome fusion</td>
</tr>
<tr>
<td>VAMP7, VAMP8</td>
<td>R-SNAREs implicated in endosome–lysosome fusion</td>
</tr>
<tr>
<td>Syntaxin 17, SNAP-29</td>
<td>Q-SNAREs implicated in autophagosome–lysosome fusion</td>
</tr>
<tr>
<td>VAMP3, VAMP8</td>
<td>R-SNAREs implicated in autophagosome–lysosome fusion</td>
</tr>
</tbody>
</table>
not necessarily mean increased autophagy as it might equally result from a block in autophagosome fusion. This means that as well as autophagosome formation, lysosomal fusion and turnover must also be measured: the so-called autophagic flux. A very elegant method to visualize flux in cells was developed by Yoshimori and co-workers [6] in an assay that relies on a tandem-tagged RFP–GFP–LC3 (light-chain 3) (Figure 2). The chemical properties of these two fluorophores mean that in the cytosol and on autophagosomes both will fluoresce; however, on autophagosome fusion with lysosomes the acidic environment quenches the GFP signal, but not the RFP signal. Therefore immature autophagosomes will fluoresce both red and green, whereas autolysosomes will only fluoresce red.

![Figure 2. Visualizing autophagosomes fusing with lysosomes](image)

Fluorescence micrograph showing immortalized human breast epithelial cells expressing a tandem mRFP (red) and GFP (green)-tagged version of the autophagosomal marker LC3. Cells have been deprived of amino acids for 2 h to trigger autophagy, as visualized by LC3 puncta. Early autophagosomes and phagophores are positive for both green and red fluorescence (examples are indicated by arrowheads in the magnified right-hand panels). However, once autophagosomes have fused with lysosomes to form autolysosomes, the GFP signal is quenched and the LC3 fluoresces red only (examples are indicated by arrows in the right-hand panels). The nuclei of the cells are stained blue using DAPI and the scale bar is 10 μm.
Maturation of the autophagosome

Once the autophagosome has formed it must travel to the endocytic system to fuse and deliver its contents for degradation. The timing of this is very important and must only happen once the phagophore has sealed – if the fusion machinery is recruited and activated before this, then the cytosolic cargo will be released or left attached to the cytosolic side of the lysosomal membrane, unable to be degraded. Therefore these fusion factors must only recognize the mature autophagosome and how this is achieved is currently unknown. One potential signal may come from the ATG8 family of proteins. As discussed in Chapter 5, this family of ubiquitin-like proteins is thought to be involved in sealing the phagophore to form the autophagosome. Work from yeast has suggested that loss of Atg8 from the outer autophagosomal membrane is a signal for fusion competency by allowing removal of the autophagy initiating machinery [7,8]. Indeed, immunoelectron microscopy suggests that the majority of Atg8 is present on the inner membrane of mature autophagosomes [9]. It is not clear whether a similar requirement of ATG8 removal exists in mammalian cells, especially as some forms, such as LC3, may be required for movement of autophagosomes towards lysosomes (see below). However, proteins associated with the phagophore, such as ATG16 and the ULK1 (uncoordinated-51-like kinase 1) complex, are absent from the mature autophagosome, implying that they are removed before fusion with the lysosome. This mechanism and whether it is a prerequisite for fusion is currently unknown.

Travelling to endosomes and lysosomes: role of the cytoskeleton

The cytoskeleton has many cellular functions that range from structural maintenance of cell shape to cell division and movement. Of relevance, microtubules and actin filaments affect membrane transport in the following manner: these structures form an interconnected network that act as highways on which vesicles and organelles, with the aid of motor proteins, move along to reach their destinations. It is therefore no surprise that microtubules and actin filaments have been implicated in the movement of autophagosomes and fusion with the endocytic system (Figure 3) [10].

Microtubules

Microtubules are a dynamic polymer consisting of α- and β-tubulin dimers that form a hollow cylindrical structure. Microtubules are polar with two distinct ends: a minus end that is often attached to the centrosome, and a plus end that stretches towards the periphery of the cell. The plus end is the more dynamic of the two and can undergo rapid cycles of polymerization/depolymerization. Movement of protein or organelle cargo along the surface of microtubules is driven by the kinesin and dynein motor protein families, which are powered by ATP hydrolysis, permitting them to move in a stepwise fashion [11]. Kinesins are plus-end-directed motors; they transport their cargoes towards the plus end of microtubules, whereas dyneins are minus-end-directed motors. In this way, movement can be directed either towards the periphery or centre of the cell.

Surprisingly, microtubules appear to be dispensable for autophagy in yeast. Forced microtubule depolymerization using the chemical nocodazole or deletion of the tub2 gene (β-tubulin)
does not affect bulk autophagy and fusion with the vacuole [9]. However, the situation appears slightly different in mammalian cells and although microtubules may not be absolutely essential, they likely facilitate this fusion step [10]. Autophagosomes are thought to form randomly throughout the cytoplasm, yet late endosomes and lysosomes are predominantly perinuclear. Therefore movement of autophagosomes to the proximity of these organelles will increase fusion efficiency and, indeed, live-cell microscopy has shown that mature autophagosomes move along microtubule tracks [12–15]. Perhaps the most striking evidence for this has come from studies in primary neurons that shows autophagosomes form distally, but migrate inwards along the axon in a microtubule- and dynein-dependent manner until fusion with endosomes and lysosomes [16]. The positive role of dynein has also been linked to autophagosomal fusion in studies showing that its loss leads to accumulation of LC3-II and reduction in the autophagic clearance of aggregate-prone proteins [17].

Given the more perinuclear distribution of late endosomes and lysosomes, kinesins that move in the opposite direction may counteract the actions of dynein. However, it is likely that both operate in combination to optimize movement of autophagosomes, given that depletion of the kinesin KIF5B blocked autophagy and resulted in tight perinuclear clustering of autophagosomes [18]. It is interesting to note that localization of lysosomes themselves can determine the rate of autophagosomal fusion. Increasing the perinuclear localization of lysosomes by depletion of the kinesins KIF1B-β and KIF2A led to increased autophagosomal fusion, whereas dispersion of lysosomes to the periphery by overexpression of the motors reduced autophagosomal fusion [19].
How then are autophagosomes connected to microtubule proteins? An important player is the small GTPase Rab7. Rabs act as molecular switches and are master coordinators of multiple stages of membrane trafficking (see below and [20]). Rab7 localizes to late endosomes and lysosomes and is involved in their motility and fusion. For example, Rab7 recruits dynein motors to endosomes through the interaction of its effector protein RILP (Rab-interacting lysosomal protein) [21]. Given that Rab7 appears to be essential for autophagosomal fusion with lysosomes [22], it is possible, although evidence is currently lacking, that a similar mechanism with respect to RILP recruitment occurs with autophagosomes. Rab7 has also been linked to recruitment of kinesin motors to autophagosomes from a study on the protein FYCO1 (FYVE and coiled-coil domain containing 1) [23]. FYCO1 is recruited to membranes by direct interaction with GTP-bound Rab7 as well as interacting with the lipid PI3P (phosphatidylinositol 3-phosphate). FYCO1 also contains an LC3-interacting motif that, in combination with Rab7 and PI3P binding, specifically recruits it to autophagosomes. The study also showed that over-expression or depletion of FYCO1 redistributed autophagosomes to the periphery or cell centre respectively, consistent with a role in kinesin recruitment (Figure 3).

**Actin filaments**

Actin filaments comprise polymerized actin monomers and, similar to microtubules, this polymerization is dynamic. However, actin filaments are shorter and appear to have a more random orientation, although they do have polarity with filament ends termed either barbed or pointed. As with microtubules, actin filaments can act as tracks to move various intracellular cargoes via the myosin family of motor proteins [24].

Evidence suggests that actin is involved in selective autophagy induction in yeast, but there are few data to suggest involvement in fusion of autophagosomes with the vacuole [10]. In contrast, actin may be involved in mammalian autophagosomal fusion. Recent work has demonstrated that HDAC6 (histone deacetylase 6) helps to recruit cortactin, a protein that can activate actin polymerization, to autophagosomes involved in clearing protein aggregates [25]. The authors found that loss of HDAC6, cortactin or actin polymerization led to a block in the fusion of autophagosomes with lysosomes. Surprisingly, this block was specific to autophagosomes targeting protein aggregates as general starvation-induced autophagy was unaffected. This is an important observation as it suggests that not all autophagosomes are made equal; the itinerary of the autophagosome could be dependent on the cargo it contains. Actin motors have also been implicated as loss of myosin VI leads to the accumulation of autophagosomes [26]. The authors also showed that myosin VI interacts with Tom1, a component of the ESCRT machinery, suggesting that the actin motor is important for fusion with ESCRT-containing endosomes (Figure 3).

**Fusion of the autophagosome**

Once the autophagosome has arrived at its destination then it must fuse with the endocytic system. Our knowledge of the machinery involved in this process stems from our understanding of general intracellular membrane trafficking events, which involve the coordination of three sets of protein families: Rab GTPases, membrane-tethering complexes and SNAREs (soluble N-ethylmaleimide-sensitive factor-attachment protein receptor). In this way, specific fusion events can be coordinated as Rab proteins localize to specific membranes and recruit
tethering molecules that act as bridges to bring the compartments intended for fusion into close proximity. The tethers in turn prime SNARE proteins that physically drive the fusion of opposing lipid bilayers. This is discussed in more detail below and summarized in Figure 4.

**Rabs: master coordinators of membrane trafficking**

Rabs are the largest family of small GTPases with over 60 members in humans and regulate multiple stages of membrane trafficking. They recruit specific effector proteins such as cargo adaptors into forming transport vesicles, motor proteins to allow movement of vesicles to their target membrane and tethers to aid the fusion machinery in delivery of vesicular cargo when it reaches its destination [20]. Rabs localize to distinct membrane compartments and in this way they are thought to provide specificity in membrane trafficking. Membrane-associated Rabs are activated by specific GEFs (guanine nucleotide-exchange factors) that drive GTP binding. On binding GTP Rabs undergo a conformational change that allows interaction with its
effector proteins. Rabs are in turn inactivated by a specific GAP (GTPase-activating protein) that stimulates hydrolysis of the bound GTP to GDP causing loss of effector binding and extraction from membranes.

As well as being involved in autophagosome movement, Rab7 also has a more direct role in the fusion event. Rab7 is essential for late endosomal/lysosomal fusion in conjunction with the multi-subunit HOPS (homotypic fusion and vacuolar protein sorting) tethering complex (see below and [3]). It must be remembered, however, that blockage of endocytosis will also indirectly block autophagosomal fusion. Therefore a factor may not be involved in autophagy, yet still cause a block. Given Rab7’s involvement in endosome maturation and fusion, it could be one such factor. However, evidence suggests a more direct role for Rab7 in autophagy. A recent study showed that the compound thapsigargin (an inhibitor of the SERCA calcium pump) blocked recruitment of Rab7 to mature autophagosomes and inhibited their fusion with the endocytic system, yet did not block recruitment to endosomes, or block endosomal fusion [27]. This implies a specific role for Rab7 in autophagy. A critical factor therefore is likely to be recruitment of the Rab protein to the mature autophagosome. How this happens with respect to autophagosomes is currently unknown, but is likely to involve the coordination of multiple Rab effector proteins and exchange factors [20]. An intriguing possibility is through a process called Rab conversion, where an upstream Rab recruits a GEF that enhances association and activation of a downstream Rab. In this way, maturation of a membrane compartment can be coordinated and has been elegantly shown in the switch of early endosomes to late endosomes [28,29]. Here, early endosomal Rab5 recruits the HOPS complex, the VPS39 subunit of which acts as a GEF to activate Rab7. In turn, negative Rab5 feedback occurs and the endosome matures to a Rab7-positive Rab5-negative structure that can then go on to fuse with the lysosome. As Rab7 has been linked to autophagosomal fusion, a similar mechanism could operate here, and in support of this, Rab5 has been linked to autophagy induction [30,31]. However, there could be an alternate upstream Rab. Rab33b is a Golgi-localized Rab that plays a role in autophagosome formation through interaction with its autophagy effector protein ATG16 [32]. Interestingly, OATL1/TBC1D25 has been identified as a GAP for Rab33b and its overexpression inhibits autophagosomal–lysosomal fusion [33]. It is possible that OATL1 acts in the negative feedback during Rab conversion to allow the autophagosome to mature to a Rab33b-free form. Whether Rab33b interacts with the HOPS complex/Rab7, or an as yet unidentified Rab/tether combination, is currently unknown. Regardless, Rab7 is an important factor in both endosomal and autophagic maturation.

Lipid signalling also plays an important role in Rab activation and membrane fusion. The PI3K (phosphoinositide 3-kinase) VPS34 catalyses the formation of PI3P and is important for endosomal Rab5–Rab7 conversion mentioned previously [29]. VPS34 exists in multiple complexes within the cell, but one complex containing the protein UVRAG (UV radiation resistance-associated gene) is directly linked to endosomal and autophagic maturation. UVRAG stimulates VPS34 and also binds to VPS16, a subunit of the HOPS complex. The UVRAG interaction with VPS16 is thought to aid in membrane recruitment of the HOPS complex which further activates Rab7 through its VPS39 GEF subunit [34]. In support of this, the protein Rubicon, which binds to UVRAG and negatively regulates VPS34 activity, inhibits endosomal and autophagosomal fusion with lysosomes. Interestingly, active Rab7 displaces the UVRAG–Rubicon interaction, freeing up UVRAG to bind to HOPS complex that, in turn, further activates Rab7 [35,36]. This provides a strong feed-forward signal to drive the fusion machinery.
Membrane tethers: bridges to fusion

Membrane tethers are thought to provide another level of specificity and facilitate the docking and fusion process by bridging the opposing membranes and/or stimulating SNARE complex formation. As mentioned, Rab proteins function to recruit and activate tethering factors, of which there are two broad types: long coiled-coil domain-containing proteins, or multi-subunit tethering complexes [37]. As discussed previously, the HOPS complex is thought to act as a tether between late endosomes and lysosomes. Although direct evidence is lacking for a HOPS role in autophagosomal fusion, it is an attractive candidate given its interaction with Rab7. The HOPS complex falls into the multi-subunit family of tethers and contains six subunits linking Rab7 activation through its VPS39 subunit, as well as interaction with UVRAG through its VPS16 subunit [37]. In yeast, the HOPS complex has also been shown to bind to and 'proofread' the vacuolar SNAREs Vam3p, Vam7p and Vti1p to enhance efficiency of fusion [38]. Whether this is the case with the mammalian complex is not clear, neither is it clear if this only happens in endosomal–lysosomal fusion or can also be applicable to autophagosomal–lysosomal fusion. Regardless, by interacting with Rab7 on the incoming autophagosome/endosome and the SNARE protein on the target endosome/lysosome, the HOPS complex bridges the two compartments to facilitate their fusion.

In addition to the HOPS complex, another molecule has been highlighted recently that could perform a tethering function in autophagosome fusion with the endocytic system. TECPR1 (tectonin β-propeller repeat-containing protein 1) was identified as a protein that interacts with the autophagy-initiating protein ATG5 during bacterial infection, implying a role in specific autophagosome formation [39]. However, recent studies have shown that TECPR1 localizes to mature autophagosomes and lysosomes and its loss leads to autophagosome accumulation. Interestingly TECPR1 binds to PI3P, the product of the VPS34 reaction, in an ATG5–ATG12-dependent manner. This PI3P binding appears essential for TECPR1 function [40]. Whether TECPR1 interacts with any SNARE or Rab proteins remains to be seen.

SNAREs: the driving force of membrane fusion

SNAREs are membrane-anchored proteins localized on opposing membrane compartments that can interact with each other to form a highly energetically favourable complex. It is the energy released on forming this complex that is thought to provide the driving force behind membrane fusion. SNAREs are essential for vesicular transport and cell function, this is highlighted by the fact that the most toxic protein known to man, botulinum toxin, is an inhibitor of the neuronal SNARE SNAP-25 (25 kDa synaptosome-associated protein). For SNAREs to drive membrane fusion they must form a trans-SNARE complex consisting of one R-SNARE [with a key arginine (R) residue in the SNARE motif] on the donor membrane and three Q-SNAREs [with a key glutamine (Q) residue in the SNARE motif] on the acceptor membrane [41]. It is the 'zipping-up' into a four-helical bundle of multiple SNARE complexes that brings the opposing lipid bilayers together to allow fusion to occur. The Q-SNAREs syntaxin 7, syntaxin 8 and Vti1b, along with the R-SNAREs VAMP7 and VAMP8, have been linked to the fusion of late endosomes and lysosomes and thus play a role in autophagosome maturation indirectly [42]. It is possible that these SNAREs also function in autophagosomal fusion,
indeed VAMP8 and Vti1b, but not VAMP7, syntaxin 7 or syntaxin 8, were shown to be involved in autophagosomal fusion during clearance of intracellular bacteria [43]. In contrast, another study suggested that VAMP3 and VAMP7 are required sequentially to allow autophagosomes to fuse with endosomes and then lysosomes [44]. As these SNAREs are common to the endocytic pathway, interpretation can be difficult due to the essential role of endocytosis in autophagy. However, a recent study has identified a Q-SNARE that is specific for autophagy. Syntaxin 17 is recruited to autophagosomes and its loss disrupts autophagosome fusion with lysosomes, but not endosome fusion [45]. This work also demonstrated that syntaxin 17 interacted with another Q-SNARE, SNAP-29, as well as the R-SNARE VAMP8. In support of these data, work in Drosophila melanogaster has also shown that syntaxin 17 is required for autophagosomal fusion with lysosomes [46]. Given that the block in fusion is specific for autophagy, this likely represents a \textit{bona fide} autophagosomal SNARE complex. However, more work is needed to clarify this in light of a recent study suggesting that syntaxin 17 can operate at an earlier step in autophagosome biogenesis [47].

\section*{Conclusion}

Lysosomal fusion is not the final stage of autophagy as the autophagosomal contents must then be degraded and the resultant metabolites transported into the cytosol. However, lysosomes are the terminal point and therefore delivery and fusion of autophagosomes to these organelles is critical. As discussed, much of the machinery required for endosome maturation and fusion is shared with the autophagic system and essentially the mature autophagosome can be considered as a specialized endosome with respect to lysosomal fusion. Even so, there is still a lot of ambiguity with regards to autophagosome maturation. Is it a 'one-size-fits-all' scenario for autophagosomes in terms of fusion with the endocytic pathway? Does the type of autophagy (specific or non-specific), or for example the source of the phagophore membrane, determine at which point in the endocytic system the autophagosome fuses and which Rabs/tethers/SNAREs are utilized? Additionally, autophagosome formation is tightly controlled, especially under stresses such as nutrient starvation; however, it remains to be seen whether the fusion step is also regulated, or is it that once autophagosomes have formed they are essentially on a conveyor belt to the lysosome. A deeper understanding of these events will hopefully allow specific manipulation of the autophagic pathway to treat certain medical conditions, something which is becoming increasingly attractive given the links between autophagy and disease (see later chapters).

\section*{Summary}

\begin{itemize}
  \item Lysosomes and hence endocytosis are essential for autophagy.
  \item Disruption of endocytosis will block autophagy.
  \item Autophagosomes can fuse with the endocytic system at early and late points.
  \item Microtubules and actin filaments enhance fusion by bringing autophagosomes, endosomes and lysosomes into close proximity.
  \item The general rules that govern endocytic fusion likely apply to autophagic fusion: Rabs recruit tethers recruit SNAREs.
\end{itemize}
I am thankful to the Ganley laboratory for critically reading the chapter and fruitful discussions.

References

• Many components of the endocytic and autophagic fusion machinery are shared such as Rab7 and the HOPS complex, yet certain SNAREs may be unique to one process.
• Specific compared with non-specific autophagosomes may have different fusion itineraries.

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