# 1

# Autophagosome and Phagosome

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## Summary

Autophagy and phagocytosis are evolutionarily ancient processes functioning in capture and digestion of material found in the cellular interior and exterior, respectively. In their most primordial form, both processes are involved in cellular metabolism and feeding, supplying cells with externally obtained particulate nutrients or using portions of cell's own cytoplasm to generate essential nutrients and energy at times of starvation. Although autophagy and phagocytosis are commonly treated as completely separate biological phenomena, they are topologically similar and can be, at least morphologically, viewed as different manifestations of a spectrum of related processes. Autophagy is the process of sequestering portions of cellular interior (cytosol and intracellular organelles) into a membranous organelle (autophagosome), whereas phagocystosis is its topological equivalent engaged in sequestering cellular exterior. Both autophagosomes and phagosomes mature into acidified, degradative organelles, termed autolysosomes and phagolysosomes, respectively. The basic role of autophagy as a nutritional process, and that of phagocytosis where applicable, has survived in present-day organisms ranging from yeast to man. It has in addition evolved into a variety of specialized processes in metazoans, with a major role in cellular/cytoplasmic homeostasis. In humans, autophagy has been implicated in many health and disease states, including cancer, neurodegeneration, aging and immunity, while phagocytosis plays a role in immunity and tissue homeostasis. Autophagy and phagocytosis cooperate in the latter two processes. In this chapter, we briefly review the regulatory and execution stages of both autophagy and phagocytosis.

**Key Words:** Autophagy; Phagocytosis; Atg; Vps34; Tor.

## 1. Autophagosome

Autophagy is an evolutionarily highly conserved cellular homeostatic process whereby cells control their cytoplasmic biomass, organellar abundance, and distribution, remove potentially harmful protein aggregates, eliminate intracellular pathogens such as bacteria, protozoans, and viruses, and process self or

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foreign proteins for antigen presentation ***(1–10)***. Autophagy comes in several forms: macroautophagy (bulk degradation of cytoplasmic components including proteins and whole organelles), microautophagy (a morphologically distinct form of autophagy often seen in yeast), and chaperone-mediated autophagy (a more subtle form of protein degradation whereby individual proteins are imported directly into lysosomes for degradation). There is also a growing number of function- or target-based classifications of autophagy with terms such as mitophagy (autophagy of mitochondria), pexophagy (autophagy of peroxisomes), ER-phagy (autophagy of the endoplasmic reticulum), xenophagy (autophagy of invading microorganisms), immunophagy (autophagy in innate and adaptive immunity), and virophagy (autophagy of cytoplasmic viruses). Unless otherwise specified, we refer in this volume to macroautophagy simply as autophagy.

All cells in our bodies are capable of undergoing autophagy. Its induction is controlled by a specialized regulatory cascade, with the Ser/Thr kinase Tor at its center. When Tor is active, cells increase their biomass and proliferate, while when Tor is inhibited, cells reduce their biomass by autophagy. A short list of the inputs funneled through Tor into the regulation of autophagy is shown in **Fig. 1**. Once autophagy is induced, morphologically detectable execution processes **(Fig. 2)** begin to unfold with the following visually discernible stages: (1) initiation, whereby membranous structures (isolation membrane or phagophore) form in the cytoplasm, giving appearance of crescents; (2) elongation, during which membranes increase in size, wrap themselves around sections of the cytosol or a targeted organelle, culminating in phagophore closure and formation of a typical double membrane autophagosome characterized by two lipid bilayers ***(11)***; and (3) maturation, whereby a newly formed autophagosome fuses with endosomal organelles, forming a hybrid organelle referred to as amphisomes ***(11)***, and lysosomes, forming a terminal, fully acidified, and degradative organelle termed the autolysosome. There are also classification systems in which the autophagic pathway has been separated in additional substages, with nucleation being considered as a well-defined step in yeast. In this organism, a specialized organelle, termed the preautophagosomal structure (PAS), is identifiable as a distinct sorting station supplying components to newly initiated phagophores. A PAS equivalent has been elusive in metazoan cells, although membrane trafficking steps, theoretically leading to and from a putative PAS equivalent, can be recapitulated in mammalian cells ***(12)***.

The autophagically captured material is eventually degraded in autolysosomes. Depending on the targeted cellular component, this results in (1) removal of damaged organelles (e.g., spuriously compromised mitochondria lest cells undergo an unscheduled apoptosis); (2) trimming of an organelle (e.g., ER) to its appropriate size; (3) generation of free amino acids and energy sources to maintain essential cellular anabolic needs at times of nutrient or energy

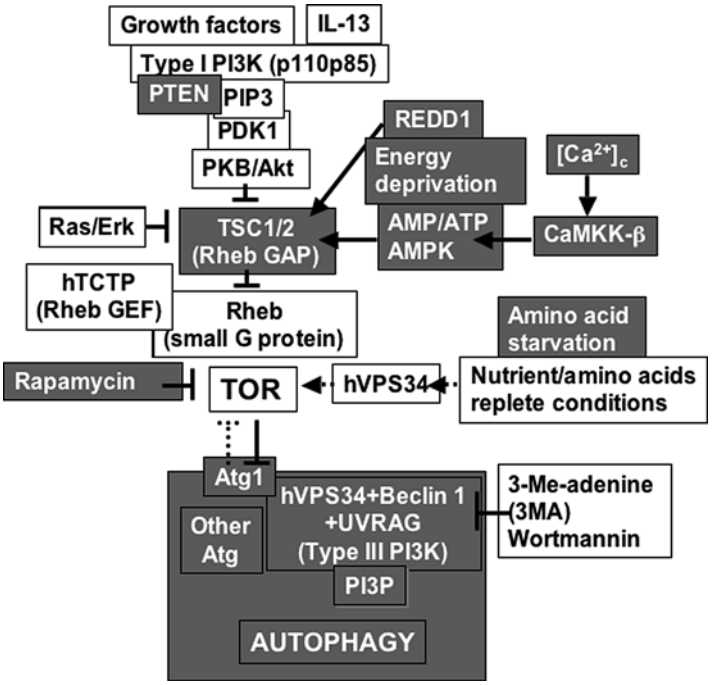


Fig. 1. Core signaling pathways controlling autophagy. Activators of Tor (black letters, white boxes) suppress autophagy, while inhibitors of Tor promote autophagy (white letters, gray boxes). In yeast, Tor negatively regulates Atg1, a factor that sets off the autophagy execution cascade. Hence, when Tor is inhibited by rapamycin, autophagy is induced. Tor is normally regulated by a small GTPase, Rheb, which binds to the N-terminal portion of the Tor kinase domain. As with other small signaling GTPases, which act as molecular switches, the GDP-bound form of Rheb is in the OFF position, while the GTP bound form is in the ON position. Rheb activity is regulated by the GTPase activating protein (GAP) TSC1/2, which receives and integrates various upstream inputs ***(28)*** (**Fig. 3**) that come from (1) growth factor receptor signaling via the Akt/PKB pathway, inhibiting autophagy; (2) energy status via AMPK, a kinase that responds to the AMP/ATP ratio, with active AMPK-stimulating autophagy ***(29)***, and the recently recognized contributor REDD1, which acts independently of AMPK ***(30)***; and (3) $Ca^{2+}$ effects on CaMKK-β and phosphorylation and activation of AMPK and thus induction of autophagy ***(31)***. The best understood pathway controlling TSC1/2 is the one stimulated by growth factors. Binding of growth factors to receptors activates type I PI3K p110/p85, which generates phosphatidylinositol-(3,5)-P3 (PIP3). PIP3 recruits PDK1 and Akt/PKB to the plasma membrane, where PDK1 phosphorylates and activates Akt/PKB. Active PKB phosphorylates and inactivates TSC1/2. By inactivating the TSC1/2 GAP, this cascade enhances Rheb-GTP–dependent activation of Tor and phosphorylation of Tor targets. This in turn inhibits autophagy. If growth factors or amino acids are withheld,

deprivation; (4) removal of intracellular pathogens including bacteria, protozoans, and viruses; (5) capture and processing of self antigens for endogenous antigen presentation in an MHC II–restricted fashion; etc. Many aspects of the molecular machinery controlling autophagy have been delineated, and specific Atg factors have been characterized. In yeast, they number in excess of 30 genes, but in mammalian cells their orthologs, identifiable through bioinformatics, are limited to just over a dozen (not counting multiple isoforms), with many more clearly remaining to be identified as the studies progress. Some of these factors are shown in **Fig. 2**.

## 2. Phagosome

In contrast to autophagy (a process of phagocytosis of objects/targets already in the cellular interior), conventional phagocytosis ***(13)*** represents uptake and internalization of objects initially located outside of the cell **(Fig. 3)**. The two processes (autophagy and phagocytosis) are initiated and driven by different mechanisms. Nevertheless, there is an overlap during the terminal stages in both pathways (**Figs. 2** and **3**), when autophagosomes become autolysosomes or when conventional phagosomes mature into phagolysosomes. While all cells in our body are capable of undergoing autophagy, most cells, apart from the specialized phagocytic cells, are normally not particularly active in phagocytosis. Even so, they can be "coerced" into doing so in some cases: (1) often, in tissues during organ development, neighboring cells may phagocytose bystander dying cells in the process of organ cavity formation ***(14)***; (2) in vitro manipulations can make a nonphagocytic cell actively phagocytic, often requiring expression of a receptor or additional molecules ***(15)***; (3) bacteria and other pathogens can stimulate processes in host cells to make them engulf and take up microbes ***(16)***. Nevertheless, a strong, prominent phagocytic function is reserved for the cells of the reticuloendothelial system, and macrophages are usually considered to be the prototypic phagocytic cells.

Fig. 1. *(Continued)* autophagy is augmented. Recent work ***(32)*** has identified a long missing nucleotide exchange factor for Rheb, which loads Rheb with GTP, thus providing another arm of Rheb regulation, and hence Tor activation (which should result in autophagy inhibition), which yet remains to be explored. Recognized only recently, but probably representing an ancient signaling mechanism, hVPS34 appears to transduce the amino acid–replete conditions to Tor and in this capacity plays a negative role in signaling upstream of autophagy initiation ***(33,34)***. However, once autophagy is initiated, hVPS34 complexed with Beclin 1 and PI3P play an essential role in the execution of autophagy. Autophagy can be induced by rapamycin or amino acid starvation and inhibited by 3-methyl adenine, an inhibitor of PI3K.

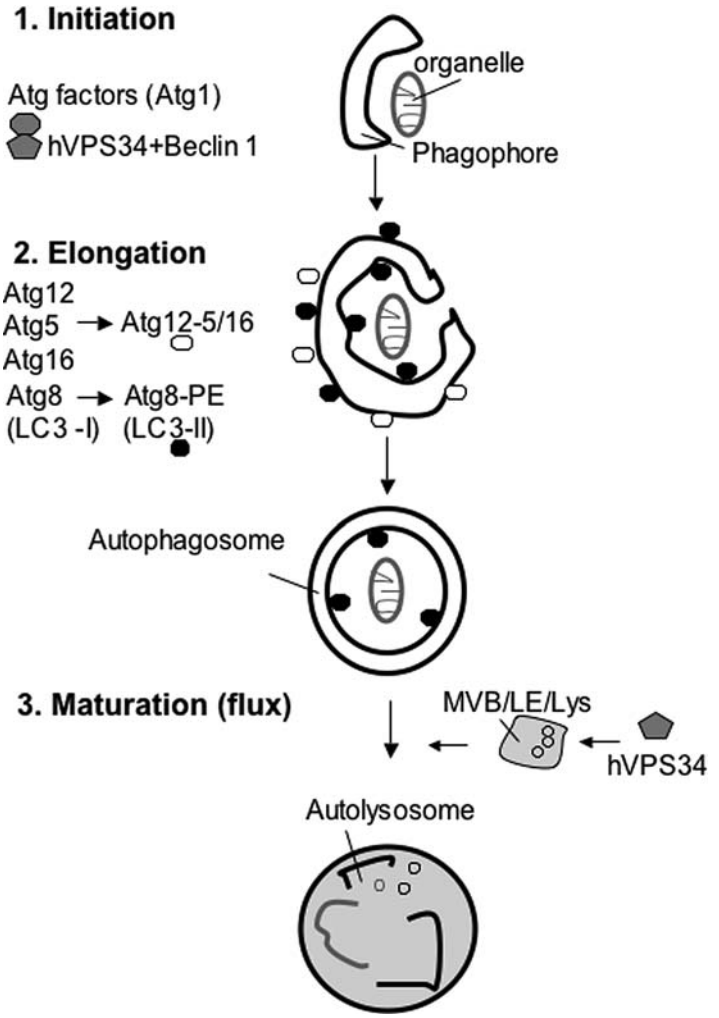


Fig. 2. Execution stages of autophagy. Initiation: A nascent autophagosomal organelle, termed a phagophore (isolation membrane), corrals an organelle or a section of the cytoplasm. Elongation: The phagophore elongates and bends, wrapping around the cytoplasmic target to be sequestered. At this stage, Atg factors form two distinct protein–protein or protein–lipid conjugates: (1) Atg5 is covalently linked to Atg12, and the resulting Atg5-Atg12 conjugate associates with Atg16; (2) Atg8 (LC3) is converted from its cytosolic LC3-I form into a C-terminally phosphatidylethanolamine (PE) conjugated form, LC3-II. This stage culminates with phagophore closure and formation of double-membrane (two lipid bilayers) autophagosome. Maturation (flux): During this stage, the autophagosomal pathway merges with the endosomal/lysosomal pathway. Autophagosomes fuse with endosomes (generating amphisomes) and lysosomes, finally being converted into fully lytic, acidified organelles termed autolysosomes. The inner

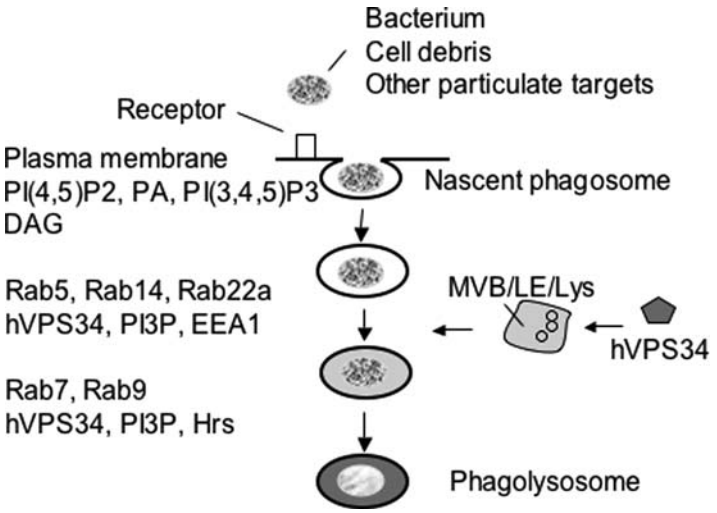


Fig. 3. Conventional phagosomes. Depicted are stages in the formation and maturation of a conventional phagosome in a macrophage ***(25)***. Several small GTPases from the family of Rab proteins have been implicated at different stages of phagolysosome biogenesis. The phagolysosome represents the default terminal stage of phagosomal maturation pathway, unless it is blocked by factors produced by intracellular pathogens. Phosphoinositides implicated at different stages are shown. PA, phosphatidic acid; DAG, diacylglycerol. EEA1 and Hrs are PI3P-binding proteins acting at different stages of phagosomal maturation.

Phagocytosis studies can be subdivided into investigations of (1) particle recognition by receptors and particle uptake and (2) phagolysosome biogenesis, which involves maturation of the initially formed, nondegradative phagosome into a degradative organelle. It is, however, hard not to draw a parallel with autophagy, if one simply substitutes external space with particles (the principal target of phagocytosis) for an internal space with an organelle or cytosol (the conventional targets of autophagy). There are also physiological overlaps, because phagocytosis and autophagy have common functions when it comes to eliminating intracellular pathogens ***(17–19)*** and antigen

Fig. 2. *(Continued)* membrane has been disrupted, and the autolysosome has only the outer, delimiting membrane. The density of the material in autophagosomes resembles that in the surrounding cytosol, but autolysosomal lumen appears denser. Often, internal membranes can be seen, representing remnants of organelles captured by autophagy. MVB, multivesicular bodies; LE, late endosome; Lys, lysosome.

presentation ***(20,21)***. The two pathways start, based on our current understanding, using different machinery, but end in a similar way with a degradative organelle and engagement of similar regulators such as the phosphatidylinositol-3-kinase hVPS34. It is of note that phosphoinositides ***(22)***, and in particular phosphatidylinositol-3-phosphate (PI3P), play an essential role in phagolysosome biogenesis ***(23–25)***. Furthermore, PI3P is a key element in both initiation and maturation of autophagosomes.

The lipid kinase hVPS34 has specific interacting partners (e.g., Beclin1 ***(26)***) that modulate its function: in yeast there are two hVPS34 complexes, one involved in autophagy, and the other controlling conventional endosomal pathway. The hVPS34 complexes in mammalian cells may be similarly specialized or perhaps exist in more than two forms, regulating various processes including autophagy, the endosomal pathway, and maturation of conventional phagosomes. The mammalian factors participating in hVPS34 complexes are beginning to be identified, and presently include UVRAG ***(27)*** and at least three additional elements with or without obvious equivalents in the yeast.

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