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Review

# Autophagosome formation—The role of ULK1 and Beclin1–PI3KC3 complexes in setting the stage

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ABSTRACT

Autophagy is a conserved and highly regulated degradative membrane trafficking pathway, maintaining energy homeostasis and protein synthesis during nutrient stress. Our understanding of how the autophagy machinery is regulated has expanded greatly over recent years. The ULK and Beclin1–PI3KC3 complexes are key signaling complexes required for autophagosome formation. The nutrient and energy sensors mTORC1 and AMPK signal directly to the ULK complex and affect its activity. Formation and activation of distinct Beclin1–PI3KC3 complexes produces PI3P, a signaling lipid required for the recruitment of autophagy effectors. In this review we discuss how the mammalian ULK1 and Beclin1 complexes are controlled by post-translational modifications and protein–protein interactions and we highlight data linking these complexes together.



## 1. Introduction

Autophagosomes were first identified using electron microscopy in the 1950s and autophagy recognized as a lysosomal degradative pathway by the 1990s (for review of the original publications see [1]). Recently many key findings have revealed the pivotal role played by autophagy in tissue homeostasis and human disease, such as cancer, neurodegeneration, infection, immunity, and aging [2]. Autophagy is also tightly linked to neonatal survival and cellular metabolism [3].

Autophagy is a vesicular process that begins at a structure called the PAS (pre-autophagosomal structure) [4,5] first identified in *Saccharomyces cerevisiae* as the location of autophagy related (Atg) proteins (Fig. 1). The PAS has not been established to exist in mammalian cells and is assumed to be the precursor to the phagophore. The phagophore, or isolation membrane, is often seen in EM as a thin cisterna with a clear lumen, and is the structure that recruits and harbors all the Atg proteins required for the induction of autophagy. The phagophore expands and sequesters cytosolic components, eventually closing to become a double membrane vesicle, the autophagosome. The autophagosome then fuses with endosomes to become an amphisome [6], which then fuses with late endosomes and lysosomes to become an autolysosome. Direct fusion of autophagosomes with late endosomes and lysosomes can also occur. This process results in the degradation of the sequestered material, which can then be re-used by the cell.

The core machinery driving nutrient-regulated autophagy is a group of 36 Atg proteins [7] all so far identified in yeast, but largely conserved in mammals. The starvation-induced autophagic response is downstream of the major homeostatic regulators mammalian Target of Rapamycin complex 1 (mTORC1) and AMPK. In full nutrients mTORC1, the master regulator of cell growth, represses autophagy by the inhibition of ULK1 kinase activity. ULK1 is a serine/threonine kinase found in a complex with Atg13, FIP200, and Atg101 (for review see [8]). The ULK1 complex controls the trafficking of Atg9 [9], the only multi-spanning membrane protein so far identified to be required for autophagy in both yeast and mammalian cells [9,10].

A second key complex is the Beclin1–class III phosphatidylinositol 3-kinase (PI3KC3), which produces an autophagy-specific pool of phosphatidylinositol 3-phosphate (PI3P). Inhibition of the activity or loss of the lipid kinase components inhibits autophagy. The Beclin1–PI3KC3 core complex consists of the mammalian orthologues of Vps34, Vps15, and Atg6/Vps30, which are called Vps34, p150, and Beclin1. Importantly, Beclin1 interacts with a number of proteins, forming multiple functionally distinct complexes, and this allows Beclin1 to respond to different signals regulating autophagy [11,12]. The WIPI proteins, the putative orthologues of Atg18 and Atg21 [13–15], are the major effectors of PI3P produced by Vps34.

Two unique ubiquitin-like (Ub-like) conjugation systems drive downstream events. The first forms the Atg12–5–16 complex. Atg5 is covalently linked to the Ub-like protein Atg12, whereas Atg16L1 associates with the Atg12–5 conjugate to form a larger complex.

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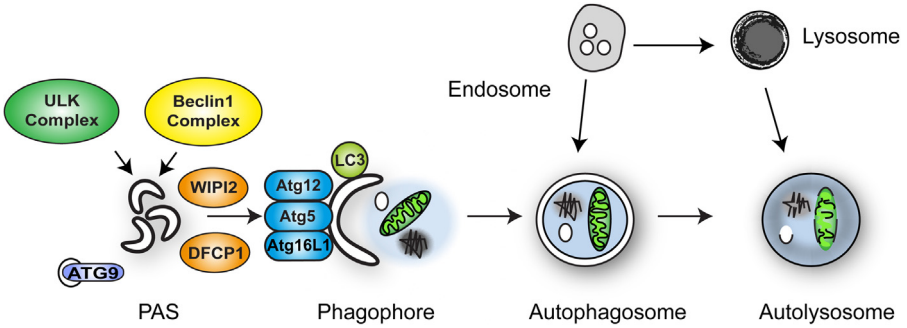


**Fig. 1.** Atg proteins and membranes involved in mammalian autophagy.

The second Ub-like conjugation system involves Atg8, which is covalently modified by the lipid phosphatidylethanolamine (PE). Mammalian cells have several Atg8-like molecules, called LC3, GABARAP, and GATE-16, of which the first two have multiple family members. The best-studied Atg8 protein in mammalian cells is LC3B, which associates with autophagosomal membranes once modified by PE. The function of these two Ub-like complexes are intimately linked: the Atg12–5–16 complex has been shown to recruit the LC3 conjugation machinery [16] and these systems may work together to drive membrane expansion and fusion [17]. In addition Atg8 family members can recruit proteins that contain an LC3-interacting region (LIR) to the autophagosomal membrane [18].

The current data suggest that the ULK1/2 and the Beclin1–PI3KC3 complexes are the first autophagic responders to nutrient depletion. The relocalization of these complexes to the phagophore membrane drives recruitment of Atg proteins and expansion of the phagophore membrane. While substrates of the ULK1/2 complex on the phagophore are not known, at least two PI3P binding proteins, DFCP1 and WIPI2 are recruited to the autophagy-specific pool of PI3P produced in response to amino acid starvation.

The identification of DFCP1 (double FYVE domain containing protein 1) at ER sites of autophagosome formation, called omegasomes, was a significant advance: DFCP1 has been shown to delineate subdomains of the ER which are Atg5 and LC3-positive and form a cradle for the phagophore [19]. Indeed, 3D tomography data support this hypothesis, with putative phagophore membranes found to be sandwiched between two ER cisternae or omegasomes [20,21]. However, while these data are compelling, several recent studies have demonstrated that other organelles (Golgi, plasma membrane and mitochondria) can also contribute to the process [17]. It remains to be understood how these organelles contribute and under what physiological conditions.

## 2. The uncoordinated 51-like kinase 1 (ULK1)

The serine/threonine kinase ULK1 is able to receive upstream signals related to nutrient status and initiate autophagy. The molecular details of this process are being unraveled, but a precise effector mechanism for ULK1 is currently lacking. A role for this kinase, named Apg1p (now Atg1), in autophagy was discovered by loss-of-function studies in yeast [22,23]. In humans there are 5 proteins orthologous to Atg1, namely: ULKs 1–4 and STK36/fused [8]. ULK1 and ULK2 are the orthologues most closely related to the yeast Atg1 protein, and ULK1 was the first to be shown to have a role for autophagy in mammalian cells [8,24]. siRNA knockdown of ULK1 in HEK293A cells and cerebellar granule neurons is sufficient to block autophagy, however in mouse embryonic fibroblasts (MEFs) knockout of both ULK1 and ULK2 was required, suggesting a redundancy between these kinases in particular settings [24–28]. Moreover, overexpression of ULK3 resulted in autophagy induction [29]. The role of ULK4 and STK36 in autophagy, if any, remains to be characterized. Of these 5 orthologues the function of ULK1 in autophagy has been best described and will be the subject of our discussion.

A number of studies revealed that ULK1 is part of a larger complex that remains stable under basal and autophagic conditions [26,30–35]. The ULK1 complex contains ULK1, FIP200, Atg13 and Atg101 and each of these complex members is required for canonical autophagy (Fig. 2). Furthermore, some of the complex members stabilize the others. It is worth noting that non-canonical autophagy that lacks some of the classical autophagy machinery components has also been described [36]. For example, ammonia generated by 24 h glucose starvation seems to induce autophagy independently of ULK1/2 and the upstream regulators mTOR and AMPK [28].

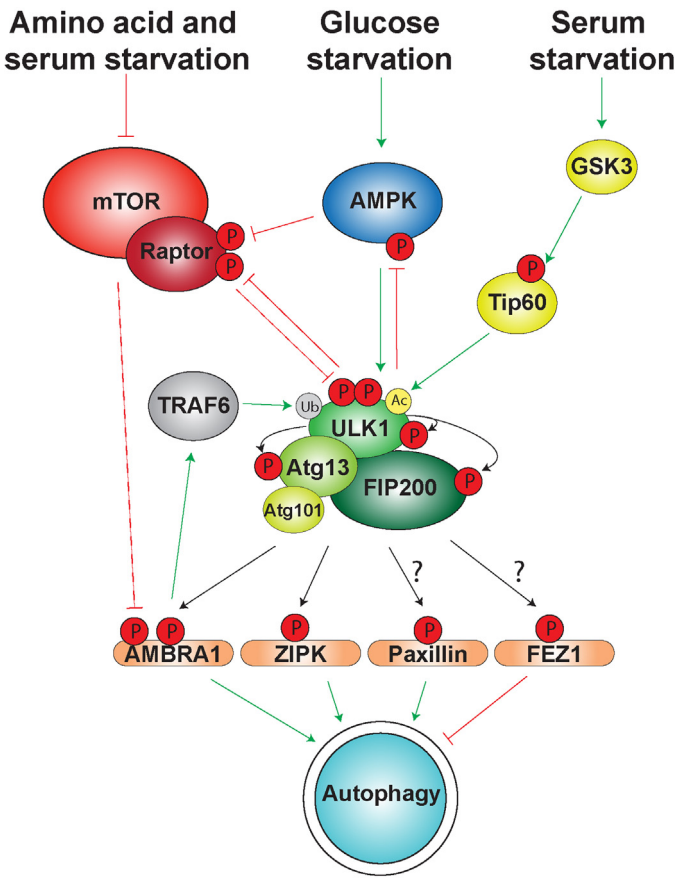


**Fig. 2.** Regulation of and by the ULK1 complex during autophagy activation. Amino acid, glucose and serum starvation activate the ULK1 complex *via* different routes. *Note*: autophosphorylation of ULK1 may maintain it in a conformation that is favorable for autophagy [26].