Published in final edited form as:

Curr Opin Cell Biol. 2016 April ; 39: 61–68. doi:10.1016/j.ceb.2016.02.010.

Structure and Function of the ULK1 Complex in Autophagy

Mary G. Lin¹ and James H. Hurley^{1,2}

¹Department of Molecular and Cell Biology, University of California, Berkeley, California 94720 and California Institute for Quantitative Biosciences, University of California, Berkeley, California 94720

²Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Abstract

The ULK1 complex initiates autophagosome formation, linking cellular nutrient status to downstream events in autophagy. Recent work suggests that the ULK1 complex might also be activated in selective autophagy independent of nutrient or energy status. In this review we will discuss our current understanding of how the ULK1 complex is regulated by different signals, as well as how this complex then regulates other components of the autophagy machinery. Recently obtained structural data both on ULK1 and the orthologous yeast Atg1 complex are beginning to shed light on the higher-order organization of ULK1 complex. Ultimately, these insights might make it possible to understand how cargo organization and structure recruits and regulates ULK1 in selective autophagy initiation.

Introduction

Autophagy is an essential cellular process in all eukaryotes. A wide range of substrates are degraded by autophagy, from protein aggregates to organelles to bacteria [1]. Autophagosomes form *de novo* at sites proximal to the endoplasmic reticulum, and expand via vesicular traffic from organelles including the Golgi, mitochondria, and recycling endosomes [2,3]. The growing double-membraned phagophore engulfs cytoplasmic contents and the completed autophagosome fuses with the lysosome, resulting in degradation of cargo and release of free amino acids and other byproducts. At a basal level, autophagy serves a recycling role, maintaining cellular homeostasis. It can also be induced in response to nutrient deprivation, bacterial invasion or other stresses, thus serving a cytoprotective role. Defects in autophagy are linked to disease phenotypes including cancer, neurodegenerative disease, and infectious disease [4].

The core machinery of autophagy consists of ~40 proteins in yeast [5], and more in human cells [6]. Autophagy is initiated through the ULK1 (unc-51 like autophagy activating kinase 1) complex (Atg1 complex in yeast) [7]. The human ULK1 complex consists of the ULK1

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

protein kinase itself, the FIP200 (FAK family kinase interacting protein of 200 kDa; also known as RB1CC1) scaffold, and the HORMA (Hop/Rev7/Mad2) domain-containing proteins ATG13 and ATG101 [8–15]. Mammals have four ULK1 homologs, with ULK2 showing the most extensive conservation in the kinase and C terminal domains [16]. ULK1 and ULK2 show high functional redundancy; both must be knocked out in mouse embryonic fibroblasts to robustly inhibit nutrient-dependent autophagy [17]. ULK1 and ATG13 have orthologs in the yeast Atg1 complex, and FIP200 is the functional counterpart of the two yeast scaffold proteins Atg17 and Atg11. ATG101 is absent from budding yeast, while the budding yeast Atg1 complex subunits Atg29 and Atg31 have no orthologs in the mammalian complex. Downstream of the ULK1/Atg1 complex, the autophagy-specific class III phosphatidylinositol 3-kinase (PI3KC3) complex, the ATG9 transmembrane protein, the WIPI (WD repeat domain phosphoinositide-interacting) proteins (Atg18, Atg21, and Hsv2 in yeast) and ATG2 function in vesicle nucleation. The ubiquitin-like LC3/Atg8-family proteins and ATG12 and their conjugation systems contribute to the expansion of the autophagosome membrane and recruitment of cargo [18].

Autophagy can degrade substrates both in bulk and selectively. In yeast, the cytoplasm-to-vacuole (Cvt) transport pathway uses autophagy machinery to selectively transport hydrolases to the vacuole to be cleaved and activated [19]. Cargos are recognized and linked to the autophagosome membrane protein LC3/Atg8 by adaptor proteins, including Atg19 in yeast, and p62, NBR2, and optineurin in mammals [20]. The best-known mammalian autophagy adaptors bind to ubiquitin modifications on cargo, but cargo can also be recognized directly or via other modifications [20]. Taking together data from yeast and mammalian systems, we will frame the current picture for how ULK1 integrates signals that promote both bulk and selective autophagy.

Upstream regulation of the ULK1 complex

ULK1 is regulated by amino acid and energy status via the mTORC1 (mechanistic target of rapamycin-1) and AMPK (AMP-activated protein kinase) kinases. mTORC1 integrates input from growth factors, oxygen levels, amino acid and energy status and promotes protein synthesis and other anabolic processes involved in cell growth and metabolism [21]. When mTORC1 is active, it inhibits autophagy by phosphorylating both ULK1 and ATG13 (Table 1), reducing ULK1 kinase activity [11–13,22,23]. Chemical inhibition of ULK1 prevents rapamycin from triggering autophagy, demonstrating that regulation of ULK1 is a key step in autophagy induction downstream of mTORC1 inhibition [24]. When mTORC1 is inhibited under starvation conditions, it dissociates from ULK1 [12]. ULK1 is also regulated by AMPK, which senses cellular energy status and is activated when intracellular AMP increases, reflecting decreased availability of ATP. By inactivating mTORC1, AMPK indirectly activates the ULK1 complex. AMPK also directly phosphorylates ULK1 at multiple sites in the linker region between the kinase and C-terminal domains, and this has been shown to stimulate autophagy in most cases, although one study has shown an inhibitory effect [22,25–27]. AMPK also phosphorylates ATG13 [23].

In yeast, TORC1 regulation of autophagy initiation is thought to occur on the level of Atg1 complex assembly. Atg13 is an TORC1 substrate [28], and its dephosphorylation upon

TORC1 inhibition promotes the assembly of the Atg1 complex and subsequent progression of autophagy. Alanine substitution of TORC1 phosphorylation sites on Atg13 is sufficient to trigger Atg1 complex assembly in nutrient-rich conditions [28].

Selective autophagy can be induced even in non-starved conditions during which mTORC1 may be active. How is the ULK1 complex activated in these cases? Rui *et al.* identified the protein Huntingtin as a scaffold that activates selective autophagy in fly and mammalian cells [29]. Huntingtin is encoded by the gene mutated in Huntington's disease, and itself is a substrate of autophagy [30]. Huntingtin specifically functions in selective autophagy by interacting with cargo adaptor protein p62 [31,32] and enhancing its recognition of ubiquitin K63-marked cargo. Huntingtin was found to promote three different selective autophagy pathways (mitophagy, aggrephagy, and lipophagy) by directly binding ULK1 and competing it away from mTORC1 [29].

In an example of a scaffold promoting Atg1 activation in yeast, Kamber *et al.* found that the Atg1 kinase is activated via Atg11 in selective autophagy [33]. They deduced that Atg1 exists in a complex with Ape1, a selective autophagy substrate, and its receptor, Atg19, with Atg11 bridging the interaction between Atg1-Atg13 and Atg19-Ape1. Cells lacking Ape1, Atg19, or Atg11 showed reduced Atg1 kinase activation under nourished conditions, as measured by autophosphorylation. They reconstituted activation of the Atg1 kinase through Atg19-Atg11 interactions and showed that damaged peroxisomes and peroxisome-receptor Atg36 operate analogously, again using Atg11 to activate the Atg1 kinase.

In mitophagy, ULK1 is recruited to mitochondria downstream of autophagy adaptors. The ubiquitin kinase PINK1 is involved in marking mitochondria for uptake and destruction by mitophagy. PINK1 triggers the recruitment of the adaptors optineurin and NDP52 to mitochondria; this in turn triggers the recruitment of core autophagy machinery, including ULK1 [34]. The mechanism whereby optineurin and NDP52 communicate with ULK1 and other core autophagy components is an important question. The mitochondrial outer-membrane protein FUNDC1 also recruits ULK1 to damaged mitochondria, where ULK1 then phosphorylates FUNDC1 [35]. These studies begin to offer mechanistic insight into how ULK1/Atg1 can be activated in response to a range of signals – from nutrient or energy stress to the presence of specific substrates (Figure 1).

Substrates and inhibitors of the ULK1 complex

How does ULK1 then transduce cellular signals to downstream events in autophagy? Russell *et al.* found that ULK1 phosphorylates Ser14 in BECN1, activating the PI3KC3 complex and promoting autophagy [36]. Egan *et al.* determined the ULK1 consensus phosphorylation motif and found that ULK1 has a strong preference for serine as the phosphoacceptor and for hydrophobic residues surrounding the phosphorylation site. This group identified ULK1 substrates including components of the ULK1 complex, with two phosphorylation sites in ATG101 and multiple sites in FIP200 and ATG13 [37]. The ATG101 phosphorylation sites occur at the extreme N- and C-termini of the protein, at Ser11 and Ser203. Ser11 is at the start of the ATG101 HORMA domain, and Ser203 in a flexible region just past the end of the HORMA domain. The functional consequences of these phosphorylations are unknown.

In the downstream VPS34 complex, two additional ULK1 phosphorylation sites were identified in BECN1, and the previously identified Ser14 site was confirmed. VPS34 was also found to contain a single robust ULK1 phosphorylation site at Ser249. Ser249 is in a loop connecting the catalytic domain of VPS34 to a motif called the CHIL domain, which seems to anchor the catalytic domain to the rest of the complex [38]. The location of this site suggests it could affect how the catalytic domain binds to the rest of the PI3KC3 complex, and thence regulate its activity. However, no functional role for this site has been demonstrated to date. In addition to phosphorylating proteins involved in autophagosome formation, ULK1 was recently found to phosphorylate adaptor protein p62 upon proteotoxic stress, increasing the binding affinity of p62 for ubiquitin [39].

The past year saw several reports of ULK1 inhibitors [24,37,40,41]. Petherick *et al.* describe the compounds MRT67307 and MRT68921, and used them to conclude that ULK1 kinase activity is important in autophagosome maturation as well as initiation. Egan *et al.* identified a small molecule inhibitor of ULK1, SBI-0206965, which inhibited phosphorylation of previously identified ULK1 sites in VPS34 and BECN1, and selectively inhibited endogenous ULK1 kinase activity in vivo. MRT67307 and several inhibitors developed by Lazarus and Shokat inhibit both ULK1 and ULK2, whereas SBI-0206965 selectively inhibits ULK1. Because ULK1 and ULK2 show functional redundancy, molecules that target both may be more potent inhibitors. Molecules that are selective may be used to decipher differences in ULK1 and ULK2 function and regulation. Small-molecule inhibitors of ULK1/2 have potential use in cancer treatment, as some tumors become “addicted” to autophagy [42]. There has been less discussion of the possible use of ULK1/2 activators, but in principle these might be useful in neurodegeneration and infection, where autophagic function is compromised by disease [43,44].

In yeast, Atg1 regulates the localization of Atg9 to the phagophore assembly site [45]. Atg9 decorates vesicles that are thought to contribute to nucleation of the autophagosome [46,47]. Papinski *et al.* used peptide-array based consensus screening to determine the Atg1 consensus site, which, similar to the ULK1 site, is characterized by preference for hydrophobic residues surrounding a serine phosphoacceptor, and identified Atg9 as a direct substrate of Atg1 [48]. The phosphorylation of Atg9 is essential for its recruitment of Atg18 and Atg8 to the PAS and the subsequent expansion of the isolation membrane. It is unclear whether human Atg9 is also an ULK1 substrate, particularly given the lack of amino acid conservation between the cytosolic domains of yeast versus mammalian Atg9.

Structural aspects of ULK1 complex components

How is the ULK1 complex turned on in response to starvation or selective autophagy signals? How does the ULK1 complex carry out its non-catalytic functions in organizing phagophore biogenesis? How does the complex interact with other regulatory partners, from mTORC1 upstream, to its many substrates downstream, to its partners in the core autophagy machinery? How do all of these events lead to the higher-order remodeling of membranes to form the phagophore? These are inherently structural questions, and their answers ultimately need structural biology approaches.

ULK1 has a serine-threonine kinase domain at its N terminus and two microtubule-interacting and transport (MIT) domains at its C-terminus. Both the kinase and MIT domains are conserved among yeast and mammals [49]. The first crystal structure of ULK1 bound to inhibitors was recently reported [40]. As with all eukaryotic kinases, it shows a kinase fold similar to that of protein kinase A, with conserved catalytic and regulatory residues. It also contains a large, positively charged activation loop between N and C terminal lobes that may function in substrate recognition and regulation of kinase activity. In the crystalized on-state, from protein expressed in bacteria, phosphorylation of Thr180 in the activation loop is observed. Substitution of this residue with alanine blocks kinase activity, showing that autophosphorylation is important in regulation of ULK1 kinase activity. As kinases show conformational plasticity [50], the key question to be elucidated is how interactions with other components of the complex, such as FIP200 or ATG13, activate kinase activity, possibly by induced conformational changes. However, it should be noted that the kinase domain itself represents only 10% of the mass of the complex, and the ULK1 complex may have a non-catalytic role independent of the kinase domain, as in the yeast Atg1 complex. The C-terminal early autophagy targeting/tethering (EAT) domain consists of two three-helix bundles arranged as microtubule-interacting and transport (MIT) domains [51]. These MIT domains bind to ATG13 and may also mediate interactions with membranes [8,10,52].

Both ULK1 and Atg1 interact with Atg8/LC3 through a conserved motif (LIR, LC3-interacting region in humans; AIM, Atg8-interacting motif in yeast) in their intrinsically disordered regions [53–55]. Human ATG13 also contains a LIR motif [55]. The crystal structures of the ATG13 LIR bound to LC3A, B, and C have recently been determined [56]. The structure showed that the FVMI peptide sequence of ATG13 binds in an essentially identical manner to the corresponding WTHL peptide of p62. Thus LC3 family proteins use very similar interactions to bind both cargo adaptors and core autophagy initiation components.

Recently, crystal structures were determined for the ATG13-ATG101 heterodimer [57,58]. ATG13 has a HORMA domain at its N terminus [59]. The HORMA domain mediates protein-protein interactions and has been well characterized in mitotic spindle checkpoint protein Mad2, where it can fold into two structures, acting as a conformational switch [60,61]. ATG101 is a HORMA domain in its entirety, and also has a protruding loop of conserved phenylalanine and tryptophan residues termed the WF finger [57]. The WF finger was crystalized in both a protruding and a sequestered conformation, suggesting that it may have a regulatory function in binding interaction partners. It will be important to identify the interaction partners of the WF finger and ATG13 HORMA domain.

It is currently thought that assembly of the human ULK1 complex is constitutive; however, the possibility of a regulatory mechanism like that in yeast bears further study. In yeast, Atg17-Atg31-Atg29 form a 2:2:2 subcomplex that is constitutive regardless of nutrient status and that scaffolds the PAS assembly [62,63]. Atg17 dimerizes via its C-terminus, forming a S shape with a radius of curvature of around 10 nanometers [52,64]. The Atg1-Atg13 subcomplex then binds Atg17-Atg31-Atg29, via Atg13, with affinity two orders of magnitude lower than Atg1 binding to Atg13 [65]. This suggests that in yeast, regulation

may occur at the level of Atg1-Atg13 binding Atg17-Atg31-Atg29. Interestingly, in mammalian cells, it has recently been reported that autophagy occurs even when the ULK1–ATG13 interaction is disrupted [66], suggesting that ATG13 may function independently of ULK1 and challenging the view of the ULK1 complex as necessarily constitutive.

Using small-angle x-ray scattering, Kofinger *et al.* modelled the Atg1 complex to exist as a tetramer [67]. Unpartnered Atg17 and Atg13 binding sites at the ends of each tetramer suggest that they may function in branched interactions, potentially tethering Atg9-positive vesicles at the PAS. This is supported by recent findings that the yeast Atg13 N terminal HORMA domain mediates Atg9 recruitment and that the interaction between Atg13 HORMA and Atg9 is enhanced upon autophagy induction [68]. The implication is that human ULK1 complex organization may be similar, with FIP200 also as an S-shaped scaffold, but more structural information will be needed (Figure 2). At least one autophagy adaptor, p62, has been shown to form a higher-order assembly [69]. It would not be completely far-fetched if assembly of higher-order assemblies of adaptors were to promote oligomerization and activation of ULK1.

Conclusions and perspective

Recent studies have shown that ULK1 can be activated and/or recruited by inputs from both nutrient and energy status as well as selective autophagy substrates. It then phosphorylates a growing list of substrates. The functional significance of ULK1 phosphorylations is fairly clear for BECN1 and FUNDC1, and for Atg9 in yeast, but has yet to be elucidated in many other cases. A low-resolution solution model for the Atg1 complex assembly has been obtained in yeast. The pieces are beginning to come together to build up an analogous understanding of the structural organization of the human ULK1 complex. FIP200 would appear to be an ideal candidate for transmitting cargo signals to the ULK1 kinase. For this reason, a better structural understanding of FIP200 is particularly pressing.

The analogies to the role of Atg17 in scaffolding the yeast Atg1 complex and the newfound role of Atg11 in activating Atg1 in selective autophagy are exciting but essentially untested. More specifically, it will be important to resolve how intermolecular interactions within the ULK1 complex affect the conformation of the ULK1 kinase domain and thereby control its activity. Additionally, our expanded understanding of ULK1 regulation beyond mTORC1 and AMPK raises the question of how the kinase is differentially activated by nutrients or selective cargoes.

Acknowledgments

We thank members of the Hurley lab for helpful discussions and comments on the manuscript. This work was supported by the National Institutes of Health grant GM111730 (J. H. H).

References

1. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell*. 2011; 147:728–741. [PubMed: 22078875]
2. Lamb CA, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol*. 2013; 14:759–74. [PubMed: 24201109]

3. Graef M, Friedman JR, Graham C, Babu M, Nunnari J. ER exit sites are physical and functional core autophagosome biogenesis components. *Mol Biol Cell*. 2013; 24:2918–31. [PubMed: 23904270]
4. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*. 2008; 451:1069–75. [PubMed: 18305538]
5. Reggiori F, Klionsky DJ. Autophagic processes in yeast: mechanism, machinery and regulation. *Genetics*. 2013; 194:341–61. [PubMed: 23733851]
6. Behrends C, Sowa ME, Gygi SP, Harper JW. Network organization of the human autophagy system. *Nature*. 2010; 466:68–76. [PubMed: 20562859]
7. Mizushima N. The role of the Atg1/ULK1 complex in autophagy regulation. *Curr Opin Cell Biol*. 2010; 22:132–9. [PubMed: 20056399]
8. Chan EYW, Longatti A, McKnight NC, Tooze SA. Kinase-inactivated ULK proteins inhibit autophagy via their conserved C-terminal domains using an Atg13-independent mechanism. *Mol Cell Biol*. 2009; 29:157–71. [PubMed: 18936157]
9. Chan EYW, Kir S, Tooze SA. siRNA screening of the kinome identifies ULK1 as a multidomain modulator of autophagy. *J Biol Chem*. 2007; 282:25464–74. [PubMed: 17595159]
10. Hara T, Takamura A, Kishi C. FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. *J Cell Biol*. 2008; 181:497–510. [PubMed: 18443221]
11. Jung CH, Jun CB, Ro S-H, Kim Y-M, Otto NM, Cao J, Kundu M, Kim D-H. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell*. 2009; 20:1992–2003. [PubMed: 19225151]
12. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y. Nutrient-dependent mTORC1 Association with the ULK1-Atg13-FIP200 Complex Required for Autophagy. *Mol Biol Cell*. 2009; 20:1981–1991. [PubMed: 19211835]
13. Ganley IG, Lam DH, Wang J, Ding X, Chen S, Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem*. 2009; 284:12297–305. [PubMed: 19258318]
14. Mercer CA, Kaliappan A, Dennis PB. A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. *Autophagy*. 2009; 5:649–62. [PubMed: 19287211]
15. Hosokawa N, Sasaki T, Iemura S, Natsume T, Hara T, Mizushima N. Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy*. 2009; 5:973–9. [PubMed: 19597335]
16. Chan EY, Tooze SA. Evolution of Atg1 function and regulation. *Autophagy*. 2009; 5:758–65. [PubMed: 19411825]
17. McAlpine F, Williamson LE, Tooze SA, Chan EYW. Regulation of nutrient-sensitive autophagy by uncoordinated 51-like kinases 1 and 2. *Autophagy*. 2013; 9:361–73. [PubMed: 23291478]
18. Mizushima N, Yoshimori T, Ohsumi Y. The role of atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol*. 2011; 27:107–132. [PubMed: 21801009]
19. Lynch-Day MA, Klionsky DJ. The Cvt pathway as a model for selective autophagy. *FEBS Lett*. 2010; 584:1359–66. [PubMed: 20146925]
20. Stolz A, Ernst A, Dikic I. Cargo recognition and trafficking in selective autophagy. *Nat Cell Biol*. 2014; 16:495–501. [PubMed: 24875736]
21. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012; 149:274–93. [PubMed: 22500797]
22. Kim J, Kundu M, Viollet B, Guan K-L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*. 2011; 13:132–41. [PubMed: 21258367]
23. Puente C, Hendrickson RC, Jiang X. Nutrient-Regulated Phosphorylation of ATG13 Inhibits Starvation-Induced Autophagy. *J Biol Chem*. 2016; 291:1074–1084. [PubMed: 2689646]
- 24*. Petherick KJ, Conway OJL, Mpamhanga C, Osborne SA, Kamal A, Saxty B, Ganley IG. Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. *J Biol Chem*. 2015; 290:11376–83. Identifies specific ULK1 inhibitors and shows that ULK1 activity is involved in autophagosome initiation and maturation. [PubMed: 25833948]

25. Egan DF, Shackelford DB, Mihaylova MM, Gelino SR, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. [date unknown]. 10.1126/science.1196371
26. Shang L, Chen S, Du F, Li S, Zhao L, Wang X. Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. *Proc Natl Acad Sci U S A*. 2011; 108:4788–93. [PubMed: 21383122]
27. Mack HID, Zheng B, Asara JM, Thomas SM. AMPK-dependent phosphorylation of ULK1 regulates ATG9 localization. *Autophagy*. 2012; 8:1197–214. [PubMed: 22932492]
28. Kamada Y, Yoshino K, Kondo C, Kawamata T, Oshiro N, Yonezawa K, Ohsumi Y. Tor directly controls the Atg1 kinase complex to regulate autophagy. *Mol Cell Biol*. 2010; 30:1049–1058. [PubMed: 19995911]
- 29**. Rui Y-N, Xu Z, Patel B, Chen Z, Chen D, Tito A, David G, Sun Y, Stimming EF, Bellen HJ, et al. Huntingtin functions as a scaffold for selective macroautophagy. *Nat Cell Biol*. 2015; 17:262–275. Shows that Huntingtin interacts with ULK1 and p62 in mammalian cells, promoting selective autophagy of K63-ubiquitylated substrates. [PubMed: 25686248]
30. Jeong H, Then F, Melia TJ, Mazzulli JR, Cui L, Savas JN, Voisine C, Paganetti P, Tanese N, Hart AC, et al. Acetylation targets mutant huntingtin to autophagosomes for degradation. *Cell*. 2009; 137:60–72. [PubMed: 19345187]
31. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun J-A, Outzen H, Øvervatn A, Bjørkøy G, Johansen T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem*. 2007; 282:24131–45. [PubMed: 17580304]
32. Bjørkøy G, Lamark T, Johansen T. p62/SQSTM1: a missing link between protein aggregates and the autophagy machinery. *Autophagy*. 2:138–9. [PubMed: 16874037]
- 33**. Kamber RA, Shoemaker CJ, Denic V. Receptor-Bound Targets of Selective Autophagy Use a Scaffold Protein to Activate the Atg1 Kinase. *Mol Cell*. 2015 Shows how receptor-bound selective substrates can potentially bypass TORC1 inhibition of autophagy to drive selective autophagy in fed cells and activate Atg1 directly using Atg11 as a scaffold. 10.1016/j.molcel.2015.06.009
- 34**. Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, Sideris DP, Fogel AI, Youle RJ. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature*. 2015; 524:309–14. Shows ULK1 is recruited to sites of mitophagy downstream of the adaptors NDP52 and optineurin. [PubMed: 26266977]
35. Wu W, Tian W, Hu Z, Chen G, Huang L, Li W, Zhang X, Xue P, Zhou C, Liu L, et al. ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy. *EMBO Rep*. 2014; 15:566–575. [PubMed: 24671035]
36. Russell RC, Tian Y, Yuan H, Park HW, Chang Y-Y, Kim J, Kim H, Neufeld TP, Dillin A, Guan K-L. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol*. 2013; 15
- 37**. Egan DF, Chun MGH, Vamos M, Zou H, Rong J, Miller CJ, Lou HJ, Raveendra-Panickar D, Yang C-C, Sheffler DJ, et al. Small Molecule Inhibition of the Autophagy Kinase ULK1 and Identification of ULK1 Substrates. *Mol Cell*. 2015; 59:285–297. Identifies several autophagy proteins as ULK1 substrates and a small-molecule inhibitor of ULK1, SBI-0206965. Defines the ULK1 substrate consensus sequence. [PubMed: 26118643]
38. Baskaran S, Carlson L-A, Stjepanovic G, Young LN, Kim DJ, Grob P, Stanley RE, Nogales E, Hurley JH. Architecture and dynamics of the autophagic phosphatidylinositol 3-kinase complex. *Elife*. 2014; 3
39. Lim J, Lachenmayer ML, Wu S, Liu W, Kundu M, Wang R, Komatsu M, Oh YJ, Zhao Y, Yue Z. Proteotoxic stress induces phosphorylation of p62/SQSTM1 by ULK1 to regulate selective autophagic clearance of protein aggregates. *PLoS Genet*. 2015; 11:e1004987. [PubMed: 25723488]
- 40*. Lazarus MB, Novotny CJ, Shokat KM. Structure of the Human Autophagy Initiating Kinase ULK1 in Complex with Potent Inhibitors. [date unknown]. Identifies ULK1 inhibitors and reports the first structure of the ULK1 catalytic domain. 10.1021/cb500835z

41. Lazarus MB, Shokat KM. Discovery and structure of a new inhibitor scaffold of the autophagy initiating kinase ULK1. *Bioorg Med Chem*. 2015; 23:5483–8. [PubMed: 26275681]
42. Galluzzi L, Pietrocola F, Bravo-San Pedro JM, Amaravadi RK, Baehrecke EH, Cecconi F, Codogno P, Debnath J, Gewirtz DA, Karantza V, et al. Autophagy in malignant transformation and cancer progression. *EMBO J*. 2015; 34:856–80. [PubMed: 25712477]
43. Nixon RA. The role of autophagy in neurodegenerative disease. *Nat Med*. 2013; 19:983–97. [PubMed: 23921753]
44. Rubinsztein DC, Codogno P, Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov*. 2012; 11:709–30. [PubMed: 22935804]
45. Reggiori F, Tucker KA, Stromhaug PE, Klionsky DJ. The Atg1-Atg13 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure. *Dev Cell*. 2004; 6:79–90. [PubMed: 14723849]
46. Yamamoto H, Kakuta S, Watanabe TM, Kitamura A, Sekito T, Kondo-Kakuta C, Ichikawa R, Kinjo M, Ohsumi Y. Atg9 vesicles are an important membrane source during early steps of autophagosome formation. *J Cell Biol*. 2012; 198:219–33. [PubMed: 22826123]
47. Mari M, Griffith J, Rieter E, Krishnappa L, Klionsky D, Reggiori F. An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis. *J Cell Sci*. 2010; 190:1005–22.
- 48*. Papinski D, Schuschnig M, Reiter W, Wilhelm L, Barnes CA, Maiolica A, Hansmann I, Pfaffenwimmer T, Kijanska M, Stoffel I, et al. Early Steps in Autophagy Depend on Direct Phosphorylation of Atg9 by the Atg1 Kinase. *Mol Cell*. 2014 Identifies the Atg1 kinase substrate sequence and shows that Atg1 phosphorylation of Atg9 functions in the expansion of the phagophore membrane. 10.1016/j.molcel.2013.12.011
49. Yan J, Kuroyanagi H, Kuroiwa A, Matsuda Y, Tokumitsu H, Tomoda T, Shirasawa T, Muramatsu M. Identification of mouse ULK1, a novel protein kinase structurally related to *C. elegans* UNC-51. *Biochem Biophys Res Commun*. 1998; 246:222–7. [PubMed: 9600096]
50. Huse M, Kuriyan J. The Conformational Plasticity of Protein Kinases. *Cell*. 2002; 109:275–282. [PubMed: 12015977]
- 51*. Fujioka Y, Suzuki SW, Yamamoto H, Kondo-Kakuta C, Kimura Y, Hirano H, Akada R, Inagaki F. Structural basis of starvation-induced assembly of the autophagy initiation complex. *Nat Struct Mol Biol*. 2014; 21:513–521. Crystal structure of yeast Atg1 EAT domain bound to Atg13 MIMs is probably a good model for the ULK1-Atg13 complex. [PubMed: 24793651]
52. Ragusa MJ, Stanley RE, Hurley J. Architecture of the Atg17 complex as a scaffold for autophagosome biogenesis. *Cell*. 2012; 151:1501–1512. [PubMed: 23219485]
53. Kraft C, Kijanska M, Kalie E, Siergiejuk E, Lee SS, Semplicio G, Stoffel I, Brezovich A, Verma M, Hansmann I, Ammerer G, Hofmann K. Binding of the Atg1/ULK1 kinase to the ubiquitin-like protein Atg8 regulates autophagy. *EMBO J*. 2012; 31:3691–3703. [PubMed: 22885598]
54. Okazaki N, Yan J, Yuasa S, Ueno T, Kominami E, Masuho Y, Koga H, Muramatsu M. Interaction of the Unc-51-like kinase and microtubule-associated protein light chain 3 related proteins in the brain: possible role of vesicular transport in axonal elongation. *Brain Res Mol Brain Res*. 2000; 85:1–12. [PubMed: 11146101]
55. Alemu EA, Lamark T, Torgersen KM, Birgisdottir AB, Larsen KB, Jain A, Olsvik H, Øvervatn A, Kirkin V, Johansen T. ATG8 family proteins act as scaffolds for assembly of the ULK complex: sequence requirements for LC3-interacting region (LIR) motifs. *J Biol Chem*. 2012; 287:39275–90. [PubMed: 23043107]
56. Suzuki H, Tabata K, Morita E, Kawasaki M, Kato R, Dobson RCJ, Yoshimori T, Wakatsuki S. Structural basis of the autophagy-related LC3/Atg13 LIR complex: recognition and interaction mechanism. *Structure*. 2014; 22:47–58. [PubMed: 24290141]
- 57*. Suzuki H, Kaizuka T, Mizushima N, Noda N. Structure of the Atg101-Atg13 complex reveals essential roles of Atg101 in autophagy initiation. *Nat Struct Mol Biol*. 2015; 22:572–580. Reports crystal structure of fission yeast Atg13-Atg101 complex and identifies Atg101 WF finger as important for autophagy function. [PubMed: 26030876]
- 58*. Qi S, Kim J, Stjepanovic G, Correspondence JHH. Structure of the Human Atg13-Atg101 HORMA Heterodimer: an Interaction Hub within the ULK1 Complex. *Struct Des*. 2015;

23:1848–1857. Reports high-resolution crystal structure of human Atg13-Atg101 heterodimer and suggests that the ATG101 WF finger may have a regulatory role in binding interaction partners.

59. Jao CC, Ragusa MJ, Stanley RE, Hurley JH. A HORMA domain in Atg13 mediates PI 3-kinase recruitment in autophagy. *Proc Natl Acad Sci U S A*. 2013; 110:5486–91. [PubMed: 23509291]
60. Mapelli M, Musacchio A. MAD contortions: conformational dimerization boosts spindle checkpoint signaling. *Curr Opin Struct Biol*. 2007; 17:716–25. [PubMed: 17920260]
61. Rosenberg SC, Corbett KD. The multifaceted roles of the HORMA domain in cellular signaling. *J Cell Biol*. 2015; 211:745–55. [PubMed: 26598612]
62. Suzuki K, Kubota Y. Hierarchy of Atg proteins in pre-autophagosomal structure organization. *Genes to Cells*. 2007; 12:209–218. [PubMed: 17295840]
63. Kabeya Y, Noda NN, Fujioka Y, Suzuki K, Inagaki F, Ohsumi Y. Characterization of the Atg17-Atg29-Atg31 complex specifically required for starvation-induced autophagy in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun*. 2009; 389:612–5. [PubMed: 19755117]
64. Chew L, Setiaputra D, Klionsky D, Yip C. Structural characterization of the *Saccharomyces cerevisiae* autophagy regulatory complex Atg17-Atg31-Atg29. *Autophagy*. 2013; 9:1467–1474. [PubMed: 23939028]
- 65*. Stjepanovic G, Davies CW, Stanley RE, Ragusa MJ, Kim DJ, Hurley JH. Assembly and dynamics of the autophagy-initiating Atg1 complex. [date unknown]. Solution structure and dynamic study of Atg1 EAT domain highlights its high affinity for Atg13 and its ability to dimerize, suggesting that the ULK1 EAT may have similar properties. 10.1073/pnas.1407214111
66. Hieke N, Löffler AS, Kaizuka T, Berleth N, Böhler P, Drießen S, Stuhldreier F, Friesen O, Assani K, Schmitz K, et al. Expression of a ULK1/2 binding-deficient ATG13 variant can partially restore autophagic activity in ATG13-deficient cells. *Autophagy*. 2015; 11:1471–83. [PubMed: 26213203]
- 67*. Köfinger J, Ragusa MJ, Lee I-H, Hummer G, Hurley JH. Solution structure of the Atg1 complex: implications for the architecture of the phagophore assembly site. *Structure*. 2015; 23:809–18. Small-angle x-ray scattering structure of Atg1 complex suggests that tetramers of Atg1 pentamers scaffold the phagophore assembly site. Implies a model for higher order oligomerization of ULK1. [PubMed: 25817386]
68. Suzuki S, Yamamoto H, Oikawa Y, Kondo-Kakuta C, Kimura Y, Hirano H, Ohsumi Y. Atg13 HORMA domain recruits Atg9 vesicles during autophagosome formation. *PNAS*. 2015; 112:3350–3355. [PubMed: 25737544]
69. Ciuffa R, Lamark T, Tarafder AK, Guesdon A, Rybina S, Hagen WJH, Johansen T, Sachse C. The selective autophagy receptor p62 forms a flexible filamentous helical scaffold. *Cell Rep*. 2015; 11:748–58. [PubMed: 25921531]

Figure 1. ULK1 in bulk and selective autophagy initiation

ULK1 is integral in initiating autophagy in response to various signals. The initiation of bulk autophagy is inhibited when ULK1 and ATG13 are phosphorylated by mTORC1. ULK1 phosphorylates the PI3KC3 complex subunit BECN1 and activates PI3KC3. AMPK stimulates autophagy through inactivation of mTORC1 and direct phosphorylation of ULK1. In some cases of selective autophagy, ULK1 has been shown to be activated or recruited to the vesicle-nucleation site through scaffolds including Huntingtin and Atg11, and adaptors such as optineurin, NDP52, and p62, which bind ubiquitinated substrates and LC3/Atg8 on

the autophagosome membrane. It is likely that a great many other ULK1 substrates also have important roles in autophagy initiation and/or autophagosome maturation. By analogy to yeast, ULK1 is also likely to have essential non-catalytic roles in scaffolding autophagosome formation.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Structure of the ULK1 complex

Model of the ULK1 complex. The ULK1 (orange) kinase and EAT domains are conserved in mammals and yeast. ATG13 (green) and ATG101 (cyan) heterodimerize through their HORMA domains, and a newly discovered WF finger in ATG101 (cyan) may regulate interactions with partner proteins. On the basis of the yeast Atg17 structure, FIP200 (yellow) has been modeled as an elongated S-shaped scaffold. Crystal structures are shown for the human ATG13-ATG101 HORMA dimer (pdb: 5C50), with the WF finger residues shown as sticks; the human ATG13 LIR bound to LC3B (pdb: 3WAO), with the LIR shown in a stick model; the human ULK1 catalytic domain (pdb: 4WNP), bound to N~2~-(1H-benzimidazol-6-yl)-N~4~-(5-cyclobutyl-1H-pyrazol-3-yl)quinazoline-2,4-diamine ("compound 6"), with the compound shown in a space-filling sphere model in the active site; yeast Atg17 (pdb: 4HPQ), with the Atg29 and Atg31 subunit omitted, shown as a stand-in for FIP200, whose structure is unknown; and the yeast Atg1 EAT domain-Atg13 MIM complex (pdb: 4P1N), as a model for the likely structure of the ULK1 EAT domain. ULK1 is likely a dimer in this complex; however, only the EAT domain of the second ULK1 molecule is shown for clarity. By analogy to the yeast Atg1 complex, a second ULK1 dimer (not shown) might associate with the opposite tip of FIP200, leading to a molecular chain. There are many phosphorylation sites in the complex, as listed in Table 1.

Table 1

Protein	Residue	Kinase	Location in structure	Functional effect	Reference
ULK1	T180	ULK1 autophosphorylation	Activation loop in catalytic domain	Stimulates ULK1 kinase activity	40
	S317	AMPK	IDR	Stimulates ULK1 kinase activity	22
	S467	AMPK	IDR	Required for mitochondrial homeostasis during starvation	25
	S555	AMPK	IDR	Required for mitochondrial homeostasis during starvation, regulates Atg9 localization	25, 26, 27
	T574	AMPK	IDR	Required for mitochondrial homeostasis during starvation	25
	S637	mTORC1	IDR	Promotes ULK1-AMPK interaction	26
		AMPK		Required for mitochondrial homeostasis during starvation, regulates Atg9 localization	25,27
	S659	AMPK	IDR	Regulates Atg9 localization	27
	S757	mTORC1	IDR	Inhibits ULK1 kinase activity	22, 25, 26
	S777	AMPK	IDR near N-terminus of EAT domain	Stimulates ULK1 kinase activity	22, 25
ATG13	S224	AMPK	IDR	Inhibits ULK1 kinase activity	23
	S258	mTORC1	IDR	Inhibits ULK1 kinase activity	23
	S389	ULK1	IDR	Unknown	37
ATG101	S11	ULK1	HORMA	Unknown	37
	S203	ULK1	Flexible region just past C-terminus of HORMA	Unknown	37
FIP200	S943	ULK1	Coiled coil	Unknown	37
	S989	ULK1	Coiled coil	Unknown	37
	S1323	ULK1	Coiled coil	Unknown	37