# Drosophila as a model system to study autophagy

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# *Drosophila* as a model system to study autophagy

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## Abstract

Originally identified as a response to starvation in yeast, autophagy is now understood to fulfill a variety of roles in higher eukaryotes, from the maintenance of cellular homeostasis to the cellular response to stress, starvation, and infection. Although genetics and biochemical studies in yeast have identified many components involved in autophagy, the findings that some of the essential components of the yeast pathway are missing in higher organisms underscore the need to study autophagy in more complex systems. This review focuses on the use of the fruitfly, *Drosophila melanogaster* as a model system for analysis of autophagy. *Drosophila* is an organism well-suited for genetic analysis and represents an intermediate between yeast and mammals with respect to conservation of the autophagy machinery. Furthermore, the complex biology and physiology of *Drosophila* presents an opportunity to model human diseases in a tissue specific and analogous context.

### Keywords

*Drosophila*; Autophagy; Atg; Model system

## The process of autophagy

There are three morphologically distinct forms of autophagy in mammalian cells: macroautophagy, microautophagy, and chaperone-mediated autophagy [13]. This review focuses on macroautophagy, an evolutionarily conserved mechanism for bulk degradation of organelles and long-lived proteins [61, 76]. Upon autophagy induction, a flat membrane cistern, called the isolation membrane or phagophore, envelops a portion of cytoplasm, eventually forming a closed double-membrane bound vesicle containing cytoplasm and organ-elles. The completed vesicle, called the autophagosome, fuses with the lysosome, where its inner membrane and contents are degraded by hydrolases. The resulting degradation products are transported back to cytoplasm where they can be reused for protein synthesis and adenosine triphosphate (ATP) production.



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Studies in yeast have identified 33 *ATG* (autophagy-related) genes, involved in autophagy, many of which are conserved in higher organisms [55, 115]. However, several large gaps in our understanding of the process remain. In particular, it is not clear how the upstream signaling events known to trigger autophagy connect to the molecular machinery of autophagosome formation. Furthermore, although the core autophagy machinery appears to be conserved [110], there are significant differences between the genetics of autophagy in yeast and higher organisms. For example, the Atg1 kinase complex regulates autophagy in yeast, but several components of the complex are not conserved outside of fungi [79]. This suggests that there are a set of genes that have not yet been identified that are critical to the regulation of autophagy in mammals and other higher eukaryotes. These observations underscore the need to study autophagy in more complex genetically tractable systems.

## Conservation of the core autophagy machinery in *Drosophila*

The molecular mechanism of autophagy can be divided into three major steps: (1) induction; (2) autophagosome nucleation; and (3) membrane expansion and completion (reviewed in Xie and Klionsky [110]). Figure 1 outlines the *Drosophila* proteins involved in these processes and the link between the Insulin/Tor pathway and the autophagy machinery. In all eukaryotes, autophagy is induced via the autophagy-related gene 1 (Atg1) complex. Autophagosomal membrane nucleation involves a complex containing Vps34 (the class III PI3K). Expansion of the autophagosome membrane requires two distinct sets of ubiquitin-like protein conjugation systems, Atg8 and Atg5-Atg12. Following autophagosome completion, the vesicles fuse with lysosomes, forming autolysosomes. Table 1 lists the core autophagy genes from Saccharomyces cerevisiae, humans, and their *Drosophila* orthologs.

Atg1, the only serine/threonine kinase among the identified Atg proteins, is conserved in higher organisms and required for autophagy in *Drosophila* [70, 94] and mammals [69, 93, 94, 111, 112]. However, the Atg1 complex, comprised of several other Atg proteins [45], differs significantly both in its composition, and in its function, between yeast, fruitflies, and humans. In yeast, TOR regulates formation of an Atg1-Atg13-Atg17 complex [45] via phosphorylation of Atg13. Reduced TOR activity during starvation conditions causes Atg13 dephosphorylation, which increases its affinity for Atg1-Atg17, leading to autophagy induction [45]. Neither *Drosophila* nor humans possess clear orthologs for Atg17 or the Atg17-interacting proteins Atg29 and Atg31 [49, 50]. Rather, in *Drosophila*, Atg1 and Atg13 form a stable complex regardless of Tor activity. Likewise, the mammalian Atg1 ortholog, Unc-51-like kinase (Ulk1) interacts with Atg13 independent of nutrient conditions [30, 71]. In addition to Atg13, the mammalian Ulk1 complex also contains Atg101 and FIP200 [27, 31, 71], both of which are required for autophagosome formation. *Drosophila* orthologs of both Atg101 and FIP200 have been identified, but not yet tested. Importantly, although they contain many of the same core components, the *Drosophila* Atg1 and mammalian Ulk1 complexes do not function identically. For example, over-expression of *Drosophila* Atg1 induces autophagy, while overexpression of mammalian Ulk1 inhibits autophagy [6, 93]. It is not clear how this difference arises; speculation has focused on the influence of additional regulatory proteins, Atg13-Atg1 stoichiometry, and feedback from Atg1 to Tor. See Mizushima [8] and Chang and Neufeld [73] for more in-depth examinations of Atg1 regulation.

In yeast, *Drosophila*, and mammalian cells, following autophagy induction by the Atg1 complex, a phosphatidylinositol-3-phosphate (PI3P)-enriched structure appears at the site of autophagosome formation. PI3P is produced by phosphatidylinositol 3-kinase (PI3K), but its function at the nascent autophagosome is not yet clear. Possibly, PIP3 recruits additional factors, consistent with the observation that several yeast Atg proteins bind PIP3 and localize to the autophagosome in a PI3K-dependent manner [100]. However, of these

proteins, only Atg18 has a *Drosophila* and mammalian ortholog, with only the former having been confirmed to be required for autophagy [3, 94].

The single yeast PI3K, Vps34, is required for a variety of membrane trafficking events, including autophagy [28]. Vps34, and the other components of the yeast PI3K complex, Vps15, Atg6, and Atg14, are conserved in both *Drosophila* and mammals, where they have an essential role in autophagosome formation [99]. It is important to note that unlike yeast, *Drosophila* and mammals possess three types of PI3K. The more familiar class I PI3K functions downstream of insulin signaling, and inhibits autophagy through activation of Tor [35]. As noted above, Vps34, the class III PI3K, activates autophagy through production of PIP3. Mammals have a further layer of complexity in that Vps34-Vps15-Atg6 is found associated with Atg14, Ambra1, UVRAG, or Rubicon, depending on the context [99]. *Drosophila* has orthologs of UVRAG, Rubicon and Atg14, however it is not yet clear how they function in the Vps34 complex.

Vesicle expansion is mediated by two ubiquitin-like groups, Atg5–Atg12–Atg16, and Atg8, both of which are highly conserved from yeast to mammals [84]. The Atg16 complex localizes to the autophagosome, and is required for membrane biogenesis [21, 59]. Atg12 is covalently attached to Atg5 in a ubiquitin conjugation-like process, involving Atg10 and Atg7, the latter homologous to the E1 ubiquitin-activating enzyme [75]. Atg10 functions like an E2 ubiquitin conjugating enzyme, although it is not homologous to those in the ubiquitin system [96]. Atg5-Atg12 is then noncovalently linked to Atg16, forming the completed complex [59]. *Drosophila* orthologs exist for each of these proteins, although neither Atg10 (CG12821) nor Atg16 (CG31033) have been shown to function in the autophagy pathway.

The second conjugation system involves the ubiquitin-like protein, Atg8, which is linked via an amide bond to the lipid phosphatidylethanolamine (PE) [33]. The conjugation process begins when the cysteine protease Atg4 cleaves Atg8, which is then bound by Atg7 [54]. Atg8 is then transferred to Atg3, an E2-like enzyme, which catalyzes the conjugation to PE. Humans possess four orthologs of yeast *ATG8*, *MAP1LC3*, *GATE16*, *GABARAP*, and *ATG8L*, each of which is conjugated to PE as occurs in yeast [29, 44, 105, 107]. *Drosophila* possesses two *Atg8* genes (*Atg8a* and *Atg8b*), both of which localize to autophagosomes [39, 91, 94]. These proteins likely have some redundancy, as *Atg8a* loss of function alleles give a milder phenotype than would be expected for such an essential component [3, 93]. Orthologs of *Atg3*, *Atg4*, and *Atg7* also exist in *Drosophila*, and have been shown to function in the autophagy pathway [38, 93, 94, 108].

## Autophagy as an adaptive response to nutrient deprivation and cell stress

Like the ubiquitin-proteosomal degradation of short-lived proteins, autophagy is deployed at basal levels to recycle large protein complexes and damaged organelles. This nutrient recycling function is particularly important during periods of starvation. In nutrient rich conditions, yeast autophagy is almost undetectable. Within 30 min of nitrogen starvation, however, autophagosome formation is dramatically up-regulated [103]. Similarly, within one hour of starvation, autophagosomes appear in the *Drosophila* fat body, a nutrient storage organ analogous to the liver [94]. Likewise, in mice, autophagy increases in most organs under starvation conditions, with muscles showing a particularly clear response [78]. Interestingly, following birth, autophagy is up-regulated in various tissues in mouse neonates, apparently as an adaptive response to the severe starvation resulting from abrupt separation from the placenta [57]. Thus, the protective response to nutrient deprivation is a fundamental and well-conserved function of autophagy in eukaryotes.

In response to starvation, yeast cells regulate autophagy through the nutrient-sensing Tor pathway [35]. Higher eukaryotes also regulate autophagy through the insulin/class I phosphoinositide 3-kinase (PI3K) pathway upstream of Tor [12]. Recent studies have confirmed that both the Tor and Insulin pathways control autophagy in the *Drosophila* larval fat body (Fig. 1). Starvation, rapamycin treatment, or genetic inactivation of the Tor pathway induces a rapid autophagic response in fat body cells. [91, 93, 94]. Similarly, loss of PI3K or Insulin receptor function strongly induces autophagy in this tissue [91, 93, 94]. Thus, the signaling pathways controlling the autophagic response to starvation are conserved in *Drosophila*. Importantly, orthologs of several yeast ATG genes (*Atg1, Atg5, Atg7, and Atg12*) are required for the formation of autophagosomes in the fat body model, demonstrating that *Drosophila* autophagy utilizes conserved components of the ATG machinery.

Two recent studies demonstrate the conservation of Tor mediated autophagy regulation in *Drosophila* and mammalian cells, as well as the value of a complementary approach using both organisms. Kim et al. identified Rag GTPases as Tor activators in response to amino acid signals [52]. Using both *Drosophila* and mammalian cell culture they observed that reduced *Rag* gene expression suppressed the stimulatory effect of amino acids on Tor. In vivo analysis in the *Drosophila* fat body was then used to demonstrate a functional role for Rag in TOR-mediated regulation of autophagy and cell size regulation. The same group followed up these results with a similar analysis of Rab and Arf family GTPases, demonstrating that these also regulate Tor activity and autophagy, although unlike Rag this does not involve direct interation with Tor [62].

## Modeling autophagy and disease in drosophila

In addition to autophagic recycling of essential nutrients and energy during periods of starvation, autophagy also protects the cell from injuries caused by oxidative stress, pathogenic infection, misfolded proteins, growth factor deprivation, and hypoxia. For example, mammalian cardiac muscles subjected to oxygen deprivation accumulate large numbers of autophagosomes, which appear to facilitate their survival [113]. This type of protective role has significant consequences for tumor cells, where autophagy may both promote survival [15] and inhibit growth [68]. Neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and Huntington's disease are accompanied by the accumulation of large mutant protein aggregates. Of all of the autophagy associated diseases in humans, those dealing with abnormal protein accumulations have the most highly developed model systems in *Drosophila*. The increased autophagosome formation observed in these diseases may play a protective role by degrading misfolded proteins [117]. Consistent with this notion, decreased neurodegeneration and huntingtin aggregate accumulation were reported in *Drosophila* following treatment with a rapamycin analog that induces autophagy [90]. Several recent reports have used *Drosophila* neurodegenerative disease models to explore the role of autophagy in Amyloid beta 42, polyQ repeat, and TDP-43 toxicity [26, 66, 88, 89]. Genetic analysis of the *Drosophila* ortholog of Alfy (autophagy-linked FYVE protein) using an eye-degeneration model of Huntington's disease has been essential in confirming the in vivo function of Alfy in clearance of ubiquitin-positive inclusions and suppression of neuronal degeneration [19, 97, 98].

### Innate immunity and autophagy

The usefulness of *Drosophila* as a model system for immune system diseases is limited by the fact that insects lack the adaptive immune response typical of vertebrates. Thus, *Drosophila* studies cannot provide direct insight into the role of autophagy in T cell survival or the delivery of antigens to MHC class II compartments. However, in an evolutionarily conserved response to infection, autophagosomes can also directly envelop bacterial and

viral pathogens. Several microbes, including group A streptococci and *Mycobacterium tuberculosis* are destroyed via the autophagy-lysosome system [25, 80]. Induction of autophagy has been shown to protect against sindbis virus in a mouse model of encephalitis [64]. Some pathogens, such as poliovirus and rhinoviruses, are able to avoid autophagic degradation, instead using the autophagic machinery to replicate [36].

The case of *Listeria* infection illuminates both the advantages and disadvantages of modeling bacterial autophagy in *Drosophila*. *Listeria monocytogenes* induces anti-bacterial autophagy in *Drosophila* cells in a manner dependant upon the peptidoglycan recognition protein LE [114]. In contrast, *Listeria* appears able to evade autophagy in mammalian macrophages, both in the cytoplasm via the actin-based motility protein ActA, and by Listeriolysin O, a pore-forming toxin that promotes the formation of non-degradative spacious Listeria-containing phagosomes [4, 72, 116]. These *Listeria*-containing phagosomes restrict bacterial replication and appear to form from abortive attempts at autophagosome/lysosome fusion. Genetic analysis of *Listeria* infection in *Drosophila* is therefore limited by the different response of autophagy machinery to the pathogen. Nonetheless, studying *Listeria* and autophagy in *Drosophila* cells is likely to reveal significant information related to the ability of *Listeria* to evade the full effects of autophagy.

Recent work by Cherry and colleagues [9, 95] has demonstrated the usefulness of *Drosophila* as a model for studying viral autophagy. This group showed that vesicular stomatitis virus (VSV) infects *Drosophila* cells both in culture, and in vivo, causing autophagy induction. Furthermore, RNAi-mediated knockdown of core autophagy genes greatly increased the virulence of VSV infection. Experimental manipulation of the insulin signaling pathway also impacted viral replication, consistent with the known influence of the PI3K/Tor pathway on autophagy. To date, this is the only work yet published that directly addresses viral autophagy in *Drosophila*. This model should be an attractive one for further research given the rapidity of *Drosophila* genetics and the fact that the evolutionary distance of host and virus allows for relatively straightforward identification of pathogen-autophagy interactions [16].

## Tools and techniques

### Autophagy detection

As is the case in human studies, autophagy in *Drosophila* is most commonly visualized by detection of the conserved lipid-conjugated ubiquitin-like protein Atg8, which localizes to autophagosomes in yeast, flies and mammals [75]. However, *Drosophila* Atg8 antibodies that are effective in immunocytochemistry are not readily available. Thus, several groups have constructed GFP-tagged versions of Atg8 that can be expressed using the tissue specific Gal4/UAS system, or under control of a heat shock promoter [39, 91, 94]. These transgenes have proved reliable indicators of autophagosome formation in a number of studies, but it is important to note that concerns have been raised about this reagent [58]. In brief, Kuma et al. found that in mammalian studies Atg8 tends to be incorporated into intracellular protein aggregates, independent of autophagy. Importantly, this association with aggregates includes endogenous Atg8 as well as ectopically expressed Atg8-GFP fusion protein. Thus, an Atg8 or Atg8-GFP-positive punctate dot can represent either an aggregate or a bone fide autophagosome. Given this issue, many *Drosophila* researchers supplement Atg8-based autophagosome detection with assays based on lysosomal markers Lyso Tracker Red and LAMP1 (lysosome associated membrane protein 1). Similarly, transcriptional upregulation of Atg genes has been used as a correlative measurement of autophagy activity [24, 40, 60]. Although it is non-quantitative, electron microscopy is the

most conclusive demonstration of the presence of autophagosomes, and has been used in autophagy studies on both larval and adult *Drosophila* tissues.

Autophagosome detection approaches provide a snapshot of the autophagy activity of a cell but can sometimes be difficult to interpret. For example, an increase in autophagosome number could indicate either an increase in autophagy, or a functional decrease in autophagy due to failure of autophagosome-lysosme fusion. This becomes particularly important when determining the contribution of autophagy to disease pathogenesis. Thus, efforts are currently being made to measure autophagic flux, that is, the passage of organelles, cytoplasm, and other cargo through the autophagy-lysosmal degradation system. For example, a recent study used Western blots to examine the temporal profile of insoluble ubiquitinated aggregates isolated from *Drosophila* brain samples [14]. The researchers showed that as autophagic activity decreases in aging brains, ubiquitinated protein levels increase. Mutations in *Atg8* exacerbated the build-up, while ectopic Atg8 expression prevented aggregate accumulation, demonstrating that the level of ubiquitinated proteins indicates changes in the rate of autophagic flux.

## Autophagy mutants and RNAi

One great advantage of *Drosophila* as a model system is the extensive set of genetic tools readily available to the researcher. Loss-of-function alleles (point mutations, deletions, and transposon insertions) exist for nearly all of the core autophagy genes, and most of these have been tested for their function in the autophagy pathway in a variety of contexts. Two key exceptions are the *Drosophila* orthologs of Atg101 and FIP200, both newly identified components of the Atg1-Atg13 complex, for which there are no mutant alleles. Detailed information about all extant autophagy alleles and transgenic constructs can be found at Flybase, a comprehensive *Drosophila* genetics database.

Many of the tissues most affected by autophagy in *Drosophila*, such as fat, muscle, and neurons, are postmitotic, making clonal analysis difficult. Given the pleitropic function of autophagy genes, it is therefore not possible to clearly assay their tissue specific functions using classical alleles. Here, RNAi technology has been essential, as it allows for the spatial and temporal control of gene knockdown. In *Caenorhabditis elegans*, in vivo genome-wide RNAi screens of gene function are common practice based on feeding methods [46]. In *Drosophila*, as RNAi feeding is not possible, numerous efforts have been made to generate transgenes that express snapback/hairpin constructs. The most commonly used method relies on the Gal4/UAS system [5, 17, 82] to induce the specific expression of a doublestranded RNAi hairpin that triggers the posttranscriptional silencing of target genes. Based on the success of transgenic RNAi, large-scale efforts to generate genome-scale set of hairpin lines have been initiated [17]. In our lab we recently developed a system based on targeted integration of the hairpin constructs [81, 82]. Using this method, in collaboration with the *Drosophila* RNAi Screening Center, we are currently establishing a “second generation library”. Both groups have generated transgenic RNAi lines targeting most of the known core autophagy genes, which are available to the community through Flybase.

## Genetic screens

The full potential of *Drosophila* genetics has not yet been realized in the field of autophagy. As noted above, the *Drosophila* tissues most often used as models for autophagy are polyploid and postmitotic, making the standard mosaic analyses much less efficient. Furthermore, it is a challenge to devise a screen for a cellular process like autophagy that has no easily scored morphology. RNAi-based analysis of autophagy in established *Drosophila* cell lines has shown promise, but the biological relevance of studies in such cell lines is

questionable. Below, we discuss some of the approaches that have been applied to autophagy screening in *Drosophila*.

Simonsen et al. [98] performed a classic genetic modifier screen, taking advantage of a dominant eye phenotype associated with ectopic expression of the gene *blue cheese* (*bchs*). Bchs and its mammalian ortholog Alfy (autophagy-linked FYVE protein) are essential for the autophagic clearance of protein aggregates through an unknown mechanism [19]. Using deficiency stocks and select mutant alleles, the researchers found that mutations in lysosmal trafficking genes, as well as mutations in the SUMO and ubiquitin signaling pathways modified the dominant Bchs eye phenotype. Importantly, *Atg1, Atg6*, and *Atg18* were identified as enhancers of the phenotype, suggesting that this type of approach could successfully identify genes involved in the autophagy pathway. In an attempt to directly screen autophagy regulators, Arsham and Neufeld [1] combined standard mosaic analysis with live-cell imaging of LysoTracker Red and fixed-cell imaging of autophagy-specific fluorescent protein markers. The FLPFRT recombination system was used to generate homozygous mutant clones for each of 383 lethal transposon insertions on chromosome 2 L. Mosaic fat bodies from the larvae were then dissected, and immediately stained with LysoTracker Red to compare the number of acidic lysosomes in the mutant cells versus surrounding wild-type tissue. Using this approach the reearchers identified 79 transposon insertions that caused an increase in lysosmal activity. The insertions were enriched in genes involved in protein synthesis, folding, transport, and degradation, and mitochondrial function and morphology.

Given the difficulty of performing large-scale mutagenesis screens for autophagy in *Drosophila*, several labs have turned to transcriptional and proteomic analysis to identify new factors involved in the pathway. Two studies published at the same time [24, 60] involved genome-wide analyses of transcripts from salivary glands undergoing autophagic cell death. Using microarrays, Lee et al. found several fly ATG genes (*Atg2, Atg4, Atg5*, and *Atg7*) that were transcriptionally up-regulated. A later report from the same lab demonstrated that dynein *light chain 1*, a gene that was previously detected in their microarray study, is required for autophagy induction during during salivary gland cell death [2]. Gorski et al. performed serial analysis of gene expression (SAGE) on salivary glands undergoing autophagic cell death. In addition to previously known autophagy and cell death genes, this group identified more than 732 differentially expressed genes with unknown functions. Another more recent study used microarrays to analyze autophagy induction in the larval fat body [40]. This group found that *FK506-binding protein of 39 kDa* (*FKBP39*) was down-regulated during fat body autophagy, and showed that it functions in vivo as an inhibitor of autophagy, likely through modulation of the transcription factor Foxo.

High-throughput proteomic techniques have also been used to identify proteins expressed during salivary gland and fat body autophagy in *Drosophila* [56, 67]. Martin et al. used a shotgun proteomics approach to identify proteins that are expressed during autophagic programmed cell death of the larval salivary glands. There was significant overlap between the resulting protein set and the previous microarray and SAGE studies. However, they also identified proteins not previously known to be expressed in dying salivary glands, including *warts, a* serine/threonine kinase in the Hippo signaling pathway. Subsequent work has shown that Warts is required for salivary gland programmed cell death via regulation of autophagy [18]. Kohler et al. used Isotope coded affinity tag and mass spectrometry to use identify components of starvation-induced autophagic responses in the *Drosophila* fat body. By comparing the proteins from starved versus wild-type fat body, they found 110 proteins that were differentially regulated. Among these, the lipid desaturase Desat1 was up-regulated in the starved sample, and was found to be required for starvation-induced autophagy and to localize to Atg5-and Atg8-positive structures.

*Drosophila* has proven an excellent system for systematic genome-wide cell-based RNAi high-throughput screens (RNAi HTS). Existing *Drosophila* cell culture lines (S2, Kc, Cl8, and BG3) rapidly take up long dsRNAs added to the medium, causing efficient target knockdown [11]. Furthermore, comprehensive dsRNA libraries allow for large-scale screens to systematically interrogate the function of all genes predicted from genomic sequencing. Thus, it is somewhat surprising that this system has not yielded a published report of a genome-wide autophagy screen. Nonetheless it is clear that *Drosophila* cell culture can be used to model the role of autophagy in immunity, cell death and starvation [32, 53, 95, 114]. In one moderately scaled screen, Chittaranjan et al. assayed 460 genes previously identified by expression studies of autophagic cell death in the salivary gland [10]. The authors induced autophagic cell death in a tumorous *Drosophila* hemocyte cell line by application of the hormone, ecdysone, which promotes metamorphosis and autophagic cell death in vivo. Screening for dsRNAs that significantly increased or decreased cell survival, they identified 25 genes for further analysis. Knockdown of *Atg2, Atg3, Atg5, Atg6, Atg7, Atg8a, Atg8b* caused decreased viability irrespective of ecdysone application.

## Conclusions

There is an impressive repertoire of tools available to *Drosophila* researchers for studying autophagy. We have discussed how classical genetic approaches, RNAi both in vivo and in culture, transcription analyses, and a growing number of proteomic techniques have been applied to autophagy in a range of contexts. It is remarkable how conserved the autophagy machinery is between fruitfly and human, and cross-species studies have proven the value of the *Drosophila* model system in uncovering new autophagy pathway components and interactions. Given the establishment of cultured cell models of bacterial autophagy, viral autophagy and autophagic cell death, the next few years are likely to see a greater number RNAi screens focused on these phenomena. If previous experience is a guide, such research will provide insights into the molecular mechanisms underlying autophagy in both *Drosophila* biology and human disease.

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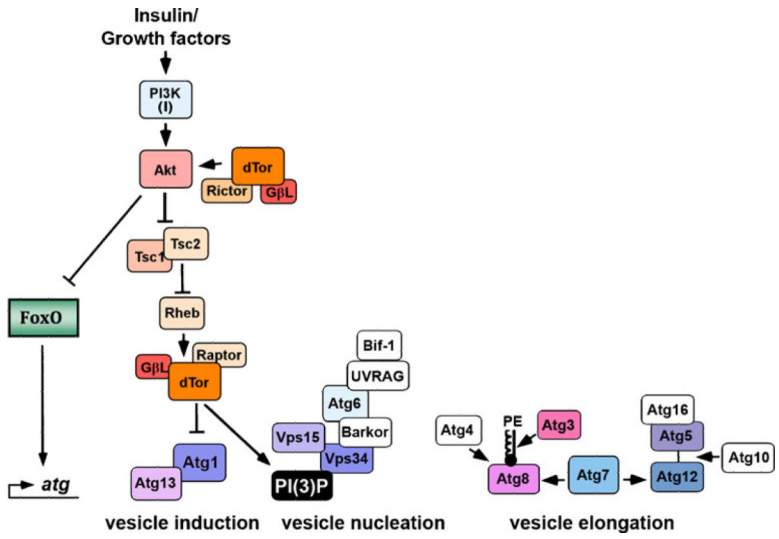


**Fig. 1.**

Connecting the Insulin pathway to the *Drosophila* autophagy machinery. Both branches of the insulin pathway downstream of Akt regulate autophagy in *Drosophila*. Insulin signaling inhibits Tor, allowing for autophagic vesicle induction by a complex containing Atg1 and Atg13. The mechanism of autophagy induction by FOXO remains unknown, although it likely involves transcriptional regulation of autophagy-related (*Atg*) genes. Vesicle nucleation by the Class III PI3K/Vps34 complex is regulated, in part, by Tor/Atg1 activity. Elongation and completion of the autophagosome require the Atg8-PE and Atg12 ubiquitin-like conjugation systems. Many of the components of these complexes have been demonstrated to be active in *Drosophila* autophagy (colored boxes). However, the function of several conserved autophagy proteins (white boxes) in *Drosophila* remains undetermined

**Table 1**

Conservation of genes involved in autophagosome induction, nucleation, and expansion

| | *S. cerevisiae gene* | *D. melanogaster gene* | *Homo sapiens gene* | References |
|---|---|---|---|---|
| Induction | *TOR* | *dTOR* | *mTOR* | [23, 30, 41, 83, 94] |
| | *ATG1* | *Atg1* | *ULK1, ULK2* | [69, 93, 94, 111, 112] |
| | *ATG13* | *Atg13* | *HARBI1* | [6, 7, 22] |
| | *ATG17* | – | – | [42, 45] |
| | *ATG29* | – | – | [50] |
| | *ATG31* | – | – | [43] |
| | – | *CG1347* | *FIP200, RB1CC1* | [27] |
| | – | *CG7053* | *ATG101* | [31, 71] |
| Nucleation | *ATG6* | *Atg6* | *BECN1* | [39, 48, 51, 87, 94, 118] |
| | *VPS34* | *Pi3K59F* | *PIK3C3* | [39, 51, 86] |
| | *VPS15* | *ird1* | *PIK3R4* | [51, 65, 109] |
| | *ATG14* | *CG11877* | *ATG14 (barkor)* | [34, 48, 51, 101] |
| | – | *CG6116* | *UVRAG* | [34, 63] |
| | – | endoB | *SH3GLB1* | [102] |
| | – | *buffy* | *BCL2* | [32, 85, 92] |
| | – | – | *AMBRA1* | [20] |
| Expansion | *ATG3* | *Atg3* | *ATG3* | [33, 37, 106] |
| | *ATG4* | *Atg4* | *ATG4A,B,C,D* | [29, 54, 95] |
| | *ATG5* | *Atg5* | *ATG5* | [47, 75, 77, 91, 94] |
| | *ATG7* | *Atg7* | *ATG7* | [38, 54, 75, 77, 104] |
| | *ATG8* | *Atg8a, Atg8b* | *LC3, GABARAP, GABARAPL2* | [3, 29, 39, 44, 91, 94, 105, 106] |
| | *ATG10* | *CG12821* | *ATG10* | [75, 77] |
| | *ATG12* | *Atg12* | *ATG12* | [75, 77, 94] |
| | *ATG16* | *CG31033* | *ATG16L1, ATG16L2* | [59, 74] |