Topic Introduction

Detection of Autophagy in *Caenorhabditis elegans*

Nicholas J. Palmisano^{1,2} and Alicia Meléndez^{1,2,3}

¹Department of Biology, Queens College-CUNY, Flushing, New York 11367; ²The Graduate Center, The City University of New York, New York 10016

Autophagy is a dynamic and catabolic process that results in the breakdown and recycling of cellular components through the autophagosomal-lysosomal pathway. Many autophagy genes identified in yeasts and mammals have orthologs in the nematode *Caenorhabditis elegans*. In recent years, gene inactivation by RNA interference (RNAi) and chromosomal mutations has been useful to probe the functions of autophagy in *C. elegans*, and a role for autophagy has been shown to contribute to multiple processes, such as the adaptation to stress, longevity, cell death, cell growth control, clearance of aggregation-prone proteins, degradation of P granules during embryogenesis, and apoptotic cell clearance. Here, we discuss some of these roles and describe methods that can be used to study autophagy in *C. elegans*. Specifically, we summarize how to visualize autophagy in embryos, larva, or adults, how to detect the lipidation of the ubiquitin-like modifier LGG-1 by western blot, and how to inactivate autophagy genes by RNAi.

AUTOPHAGY IN *C. ELEGANS*

Autophagy is a lysosome-mediated pathway resulting in the degradation and recycling of long-lived proteins, protein aggregates, as well as damaged and old organelles (Levine and Klionsky 2004). It is highly conserved and has been shown to be a fundamental catabolic process in eukaryotes that is required for key developmental and pathological events. Autophagy was first described in mammals, through morphological studies of rat liver cells (Deter et al. 1967). However, it was in the budding yeast *Saccharomyces cerevisiae* where many autophagy genes (*ATG* genes) were discovered by screening for mutations that decreased the survival of yeast cells under starvation, as well as mutations that disrupted the cytoplasm-to-vacuole targeting (Cvt) process (Tsukada and Ohsumi 1993; Thumm et al. 1994; Harding et al. 1995, 1996; Hutchins and Klionsky 2001; Klionsky et al. 2003).

The process of autophagy comprises several distinct steps: formation of a phagophore (also referred to as an isolation membrane or preautophagosomal structure); elongation and closure of the phagophore to form the double-membrane autophagosome; transport and fusion of the autophagosome with a lysosome; and finally degradation of the autophagosomal contents and recycling of degraded material (Fig. 1) (Mizushima 2007; Xie and Klionsky 2007; Nakatogawa et al. 2009). In addition to fusing with a lysosome, an autophagosome can also fuse with an endosome to form a hybrid organelle called the amphisome (Liou et al. 1997; Jing and Tang 1999). When an amphisome or autophagosome fuses with a lysosome, it is referred to as an autophagolysosome (or an autolysosome).

The evolutionary conservation of autophagy genes between budding yeast and *Caenorhabditis elegans* allowed for the identification of genes that encode core components of the autophagic ma-

³Correspondence: alicia.melendez@qc.cuny.edu

FIGURE 1. Autophagy in the nematode *Caenorhabditis elegans*. (A) The process of autophagy has been delineated by studies in yeast and mammalian cells. It is presumed that induction of autophagy begins with the activation of UNC-51, via loss of signaling from the *C. elegans* ortholog of the target of rapamycin (TOR), LET-363. (B) Autophagosome formation requires the integral protein ATG-9, thought to contribute membrane to the developing autophagosome. (C) Nucleation requires the class III phosphoinositide 3-kinase (PI3K) complex (BEC-1–VPS-34–ATG-14), which recruits downstream autophagy proteins to the isolation membranes (IMs) in mammals or preautophagosomal structure (PAS) in yeast, through the production of phosphatidylinositol 3-phosphate (PI3P; pale purple). (D) Two conjugation complexes (LGG-1 and ATG-12) are required for elongation of the IMs and completion of the developing autophagosome. LGG-1 conjugated to phosphatidylethanolamine (PE, red) binds to both the inner and outer membranes of the autophagosome. LGG-1 also has the ability to bind to autophagic adaptor proteins, such as SQST-1 (encoded by *T12G3.1*), which bind to polyubiquitylated aggregates. (E) The complete autophagosome eventually fuses with the lysosome (depicted in a red circle), leading to the degradation of cargo within the autophagosome.

chinery in *C. elegans* on the basis of genomic sequence homology (Table 1) (Meléndez et al. 2003; Meléndez and Levine 2009). Genetic screens for mutations that disrupt the degradation of P granules led to the discovery of autophagy genes not previously identified in *C. elegans* on the basis of sequence homology, including: *epg-1*, the ortholog of yeast *ATG13*, and *epg-8*, the ortholog of yeast *ATG14* (Table 1) (Tian et al. 2009; Yang and Zhang 2011). Although, the similarities between *S. cerevisiae*, mammals and *C. elegans* autophagy proteins suggest that the molecular mechanisms of autophagosome formation might be conserved (Fig. 1; Table 1) (Meléndez and Levine 2009), the presence of genes recently identified in *C. elegans* that do not have a yeast ortholog could indicate that autophagy involves more-complex membrane dynamics in higher eukaryotes. Hence, it is important to uncover further details about the roles of autophagy genes in autophagosome formation and maturation in *C. elegans*, and uncover further details about the function of these genes in the different settings where autophagy is required.

The role of autophagy genes in the development of *C. elegans* has emerged from studies using chromosomal mutations or RNA interference (RNAi) against autophagy genes. Chromosomal mutations exist for many of the autophagy genes found in *C. elegans*, and many RNAi clones are available (Table 1).

TABLE 1. Autophagy genes in the nematode *Caenorhabditis elegans*

<i>C. elegans</i> Atg gene	Allele	Yeast/mammalian ortholog	Phenotype in <i>C. elegans</i>	References
<i>let-363^a</i>	<i>h98</i>	<i>TOR1.2/mTOR</i>	Let, LL	Brown et al. (1994); Noda and Ohsumi (1998); Vellai et al. (2003); Jia et al. (2004); Hansen et al. (2007, 2008)
<i>unc-51^a</i>	<i>e369</i>	<i>ATG1/Ulk1/2</i>	Unc, AbD, Pg, Egl	Hedgecock et al. (1985); Ogura et al. (1994); Matsuura et al. (1997); Kuroyanagi et al. (1998); Meléndez et al. (2003); Zhang et al. (2009)
<i>epg-1^a</i>	<i>bp414</i>	<i>ATG13/Atg13</i>	Dv, Pg	Funakoshi et al. (1997); Chan et al. (2009); Tian et al. (2009)
<i>bec-1^a</i>	<i>ok691</i>	<i>ATG6/VPS30/bec1lin 1</i>	Let, AbD, St, SL, Pg pQ	Seaman et al. (1997); Kametaka et al. (1998); Kihara et al. (2001); Meléndez et al. (2003); Takacs-Vellai et al. (2005); Jia et al. (2007); Hansen et al. (2008); Zhao et al. (2009); Ruck et al. (2011)
<i>let-512/ vps-34^a</i>	<i>h797</i>	<i>VPS34/Vps34</i>	Let, SL, Pg	Seglen and Gordon (1982); Volinia et al. (1995); Roggo et al. (2002); Zhao et al. (2009); Ruck et al. (2011)
<i>ZK930.1^a</i>	<i>ok3132</i>	<i>VPS15/p150</i>	ND	Panaretou et al. (1997); Kihara et al. (2001); Kovács et al. (2003)
<i>epg-8^a</i>	<i>bp251</i>	<i>ATG14/Atg14L, Barkor</i>	Dv, Pg	Kihara et al. (2001); Obara et al. (2006); Sun et al. (2008); Fan et al. (2011); Yang and Zhang (2011)
<i>epg-6</i>	<i>bp242</i>	<i>-NIP14</i>	Dv, Pg	Lu et al. (2011)
<i>epg-3^a</i>	<i>bp405</i>	<i>-NMP1</i>	Dv, Pg	Tian et al. (2010)
<i>epg-4^a</i>	<i>bp425</i>	<i>-E124,PIG8</i>	Dv, Pg	Tian et al. (2010)
<i>atg-3</i>	<i>bp412</i>	<i>ATG3/Atg3</i>	Pg	Tanida et al. (2002); Zhang et al. (2009)
<i>atg-4.1^{a,b}</i>	<i>tm3949</i>	<i>ATG4/Atg4</i>	Pg	Kirisako et al. (2000); Tanida et al. (2004); Zhang et al. (2009)
<i>atg-4.2^{a,b}</i>	<i>tm3948</i>	<i>ATG4/Atg4</i>	ND	Kirisako et al. (2000); Tanida et al. (2004); Zhang et al. (2009)
<i>atg-5</i>	<i>bp545</i>	<i>ATG5/Atg5</i>	ND	Mizushima et al. (1998, 2001); Tian et al. (2010)
<i>atg7/M7.5^a</i>	<i>tm831</i>	<i>ATG7/Atg7</i>	AbD, SL, Pg, pQ	Kim et al. (1999); Tanida et al. (2001); Meléndez et al. (2003)
<i>lgg-1^a</i>	<i>bp500, tm3489</i>	<i>ATG8/LC3</i>	Let, Dv, AbD, SL, Pg	Kirisako et al. (2000); He et al. (2003); Meléndez et al. (2003); Zhang et al. (2009); Alberti et al. (2010)
<i>lgg-2^a</i>	–	<i>ATG8/LC3</i>	Let, Dv, AbD, SL, Pg	Kirisako et al. (2000); He et al. (2003); Meléndez et al. (2003); Zhang et al. (2009); Alberti et al. (2010)
<i>atg-10^a</i>	<i>bp588</i>	<i>ATG10/Atg10</i>	ND	Shintani et al. (1999); Mizushima et al. (2002); Meléndez et al. (2003); Hars et al. (2007)
<i>lgg-3R^a</i>	<i>gk1857</i>	<i>ATG12/Atg12</i>	SL, Pg	Mizushima et al. (1998); Meléndez et al. (2003); Hars et al. (2007)
<i>atg-16.1^{a,b}</i>	–	<i>ATG16/Atg16L1</i>	ND	Kuma et al. (2002); Mizushima et al. (2003); Tian et al. (2010)
<i>atg-16.2^{a,b}</i>	<i>ok3224</i>	<i>ATG16/Atg16L1</i>	ND	Kuma et al. (2002); Mizushima et al. (2003); Tian et al. (2010)
<i>atg-2</i>	<i>bp576</i>	<i>ATG2/Atg2</i>	ND	Shintani et al. (2001); Wang et al. (2001); Lu et al. (2011)
<i>atg-9^b</i>	<i>bp564</i>	<i>ATG9/Atg9</i>	Let, AbD, Pg, pQ	Noda et al. (2000); Yamada et al. (2005); Reggiori and Klionoky 2006)
<i>atg-18^b</i>	<i>gk378</i>	<i>ATG18/WIP1/2</i>	Pg	Barth et al. (2001); Meléndez et al. (2003); Jia et al. (2007); Polson et al. (2010); Tian et al. (2010)
<i>epg-2</i>	<i>bp287</i>	–	Dv, Pg	Tian et al. (2010)
<i>epg-5^b</i>	<i>bp450</i>	–/ KIAA1632	Pg	Zhang et al. (2009)
<i>sepa-1</i>	<i>bp402</i>	–	Pg	Tian et al. (2010); Lu et al. (2011)
<i>sqst-1</i>	<i>ok2892</i>	–/p62(SQSTM1)		

Let, lethal; Unc, uncoordinated; Dv, decreased viability of L1s during starvation; AbD, abnormal dauer; St, sterile; LL, long lifespan; SL, short lifespan; Pg, P granule accumulation; Egl, egg laying defective; pQ, polyQ expansion susceptibility; ND, not determined;

^aRNAi clone available; ^bParalogs in *C. elegans*.

AUTOPHAGY IN *C. ELEGANS* DEVELOPMENT AND AGING

L1 Arrest after Starvation

Autophagy plays a role mediating the developmental changes associated with survival during extracellular and/or intracellular stress, such as starvation (Levine and Klionsky 2004). In the absence of food, L1 larvae undergo a reversible developmental arrest and can survive for 1–2 wk (Johnson et al. 1984). The insulin-like/IGF-1 signaling (IIS) pathway, which comprises the insulin-like/IGF-1 receptor *daf-2* and the Forkhead box protein O transcription factor *daf-16*, is involved in regulating L1 arrest triggered by starvation (Gems et al. 1998; Baugh and Sternberg 2006; Fukuyama et al. 2006). Interestingly, reduced levels of autophagy have been shown to greatly decrease the survival of starved L1 larvae, emphasizing the importance of autophagy during early stages of development (Kang et al. 2007; Tian et al. 2009, 2010; Alberti et al. 2010; Lu et al. 2011; Yang and Zhang 2011).

Dauer Development

During the first larval stages, animals that are exposed to a limited food supply develop into an alternative L3 larval stage, termed dauer (Albert et al. 1981). Dauer development is associated with morphological and behavioral changes that allow for survival under harsh conditions and stress (Cassada and Russell 1975; Golden and Riddle 1984). The regulation of dauer development has been well characterized and requires the insulin-like/IGF-1, guanylyl cyclase, and transforming growth factor- β signaling pathways, as mutations in any of these pathways can result in a dauer-constitutive phenotype (Daf-c) or a dauer-defective phenotype (Daf-d) (Estevez et al. 1993; Thomas et al. 1993; Gottlieb and Ruvkun 1994; Ren et al. 1996; Schackwitz et al. 1996; Patterson et al. 1997; Birnby et al. 2000; Inoue and Thomas 2000; da Graca et al. 2004). Dauer development is associated with an increase in autophagy, which appears to be required for the cell remodeling associated with proper dauer formation (Meléndez et al. 2003).

Longevity Pathways

In *C. elegans*, aging is controlled by multiple longevity pathways, such as insulin-like growth factor signaling, target of rapamycin (TOR) signaling, dietary restriction, mitochondrial activity, and germline signaling (Antebi 2007). Recent genetic studies suggest that autophagy interacts with many of these longevity signals to regulate *C. elegans* aging (Meléndez et al. 2003; Hansen et al. 2008; Toth et al. 2008; Lapierre et al. 2011). Mutants in the insulin-like/IGF-1 receptor (IIR) gene, *daf-2*, display an increase in autophagy, as detected by an increase in the number of punctate structures labeled with the autophagy marker, GFP::LGG-1, in hypodermal seam cells, a cell type commonly used to visualize autophagy in *C. elegans* (Meléndez et al. 2003; Hansen et al. 2008). A reduction in autophagy during development, or only during adulthood, shortens the long life span of *daf-2* mutants (Meléndez et al. 2003; Hars et al. 2007; Hansen et al. 2008). Reduced food intake without malnutrition, otherwise referred to as dietary restriction, occurs in *eat-2* mutants (Avery 1993). These animals lack a nicotinic acetylcholine receptor specific to the pharynx, thereby showing reduced pharyngeal pumping, and have a phenotype of extended life span (Raizen et al. 1995; Lakowski and Hekimi 1998). Consistent with a role for TOR in dietary restriction, *eat-2* mutants have reduced TOR signaling, display an increase in autophagy, and require autophagy for their phenotype of long life span (Jia and Levine 2007; Hansen et al. 2008; Toth et al. 2008). The reduction in mitochondrial respiration in *isp-1* mutants extends life span (Dillin et al. 2002; Lee et al. 2003), and this phenotype is also dependent on autophagy (Toth et al. 2008). Finally, *glp-1/Notch* germline-less mutants induce autophagy, and require autophagy for life span extension (Lapierre et al. 2011). Interestingly, protein HLH-30, the ortholog of the mammalian TFEB transcription factor, is required for the life span extension associated with the longevity pathways described above and also regulates autophagy (Lapierre et al. 2013). In conclusion, autophagy is required as part of most longevity pathways in *C. elegans*, the only

exception thus far being the longevity associated with a reduction in protein translation (Pan et al. 2007; Hansen et al. 2008).

Degradation of Paternal Mitochondria

Directly after fertilization, autophagy is induced, resulting in the elimination of spermatozoon-specific organelles, including paternal mitochondria (Al Rawi et al. 2011; Sato and Sato 2011). Whether autophagy also acts in higher eukaryotes to degrade paternal mitochondria is not known; however, an increase in ubiquitylation and the localization of the Atg8-family protein LC3 (*Map1lc3a*) near the sperm mid-piece at the point of entry are suggestive that this is the case in fertilized mouse zygotes (Al Rawi et al. 2011).

Autophagy in Apoptosis, Necrosis, and Cell Clearance

Although autophagy has a role in homeostasis as an important prosurvival mechanism in response to stress, an excess in autophagy can result in cell death (Kang et al. 2007). Autophagy is also required for necrotic cell death, a type of cell death characterized by the loss of integrity of the cell plasma membrane (Toth et al. 2007; Samara et al. 2008). Additionally, similar to the situation in mammalian cells, BEC-1, a component of the class III phosphoinositide 3-kinase complex (Fig. 1), interacts with an antiapoptotic ortholog of Bcl-2, CED-9, suggesting the existence of cross talk between autophagy and apoptosis (Takacs-Vellai et al. 2005; Erdelyi et al. 2011). Autophagy proteins have been shown to play a role in the proper degradation of apoptotic cell corpses in *C. elegans* as, in autophagy-deficient animals, apoptotic cells are internalized but not properly degraded (Ruck et al. 2011; Li et al. 2012). Interestingly, rescue experiments indicate that autophagy genes are required within the engulfing cell to promote apoptotic cell degradation (Li et al. 2012).

DETECTING AUTOPHAGY IN *C. ELEGANS*

Autophagy can be monitored by transmission electron microscopy, fluorescent image analysis of the GFP::LGG-1 reporter or other autophagy reporters (Table 2), and by western blotting. The latter technique takes advantage of changes in the level of lipidation of LGG-1 that occur during autophagy and result in the protein running faster on a gel in its lipidated form. It should be noted that an increase in the number of autophagosomes does not necessarily reflect an induction of autophagy (Klionsky 2012), and it is therefore important to distinguish between induction of autophagy, an increase in autophagic flux, and the accumulation of autophagosomes due to inefficient or blocked autophagy (Klionsky 2012). Usually, it is useful to infer the turnover of autophagosomes in the presence and absence of lysosomal degradation. In *C. elegans*, this can be achieved by RNAi knock-down of genes with lysosomal function, such as *cup-5* (Kostich et al. 2000; Fares and Greenwald 2001; Sun et al. 2011), or the addition of inhibitors such as bafilomycin A1, or chloroquine, routinely used in mammalian cells to inhibit fusion between autophagosomes and lysosomes, which have also been successful in *C. elegans* (Oka and Futai 2000; Ji et al. 2006; Pivtoraiko et al. 2010). Clearly, the use of multiple assays to verify an increase in functional autophagy is recommended. A comprehensive list of guidelines was recently reported (Klionsky 2012). Here, we describe four protocols for the basic study of autophagy in *C. elegans*: first, we present a method for the detection of autophagy by using LGG-1 tagged with the fluorescent marker GFP (see Protocol: **Detection of Autophagy in *Caenorhabditis elegans* Using GFP::LGG-1 as an Autophagy Marker** [Palmisano and Meléndez 2015a]); second, we give a methodology for monitoring the degradation of P granules, which is dependent on autophagy in embryos (see Protocol: **Detection of Autophagy in *Caenorhabditis elegans* Embryos Using Markers of P Granule Degradation** [Palmisano and Meléndez 2015b]); third, we show how western blotting can be used to evaluate the lipidation of LGG-1 and discuss how caution has to be applied to the interpretation of the results of such an analysis (see Protocol: **Detection of Autophagy in *Caenorhabditis elegans* by Western Blotting Analysis of LGG-1** [Palmisano and Meléndez 2015c]); and

TABLE 2. Fluorescent reporters for monitoring autophagy in the nematode *Caenorhabditis elegans*

Protein	Function	Tissue expression ^a	Transgenes ^b	References
LGG-1	Microtubule-associated protein-1/ubiquitin-like protein	Intestine, hypodermis, muscle, pharynx, neurons, vulva, somatic gonad, germline	<i>adls1222[P_{lgg-1}::GFP::LGG-1; rol-6(su1006)]jizEx1[P_{lgg-1}::GFP::LGG-1; rol-6(su1006)]jizEx5[P_{lgg-1}::GFP::LGG-1; Podr-1::RFP]</i> <i>vkEx1093[P_{nhx-2}::mCherry::LGG-1]dks399[P_{pie-1}::GFP::lgg-1, unc-119 (+)]ls(P_{pie-1}::GFP::mCherry::LGG-1; unc-119(+))Ex[P_{lgg-1}::DsRED::LGG-1; Pmyo-2::GFP]</i>	Meléndez et al. (2003); Kang et al. (2007); Samara et al. (2008); Gosai et al. (2010); Manil-Segalen et al. (2014)
LGG-2	Ubiquitin-like protein	Hypodermis, intestine, vulva, pharynx, neurons, muscle	<i>RD108 Ex[P_{lgg-2}::GFP::LGG-2; rol-6(su1006)]RD217 unc119(ed3)III; Ex[unc-119(+); P_{pie-1}::gfp::mCherry::lgg-1]VIg9 unc119(ed3)III; ls[unc-119(+); P_{lgg-2}::gfp::lgg-2]</i> <i>bpls168[P_{nfya-1}::DFCP1::GFP; unc-76(+)]</i>	Alberti et al. (2010); Manil-Segalen et al. (2014)
DFCP1	Double FYVE-containing protein	Head, tail, vulva, neurons		Derubeis et al. (2000); Cheung et al. (2001); Axe et al. (2008); Tian et al. (2010)
ATG-16.1	WD repeat-containing protein	Intestine, head, pharynx, muscle, neurons	<i>[Patg-16.1::ATG-16.1::GFP; rol-6(su1006)]</i>	Zhang et al. (2013)
ATG-16.2	WD repeat-containing protein	Intestine, head, pharynx, muscle, neurons	<i>[Patg-16.2::ATG-16.2::GFP; rol-6(su1006)]</i>	Zhang et al. (2013)
ATG-9	Integral membrane protein	Head, tail, vulva, neurons	<i>bpls211[P_{nfya-1}::ATG-9::GFP; unc-76(+)]</i>	Noda et al. (2000); Lu et al. (2011); Liang et al. (2012); Lin et al. (2013)
EPG-1	Atg13 homolog	Neurons, pharynx, muscle	<i>bpls175[P_{pegg-1}::EPG-1::GFP; rol-6(su1006)]</i>	Tian et al. (2009)
EPG-9	Atg101 homolog	Intestine, pharynx, neurons	<i>bpls214[P_{pegg-9}::EPG-9::GFP; unc-76(+)]</i>	Liang et al. (2012)
BEC-1	Coiled-coil protein	Intestine, hypodermis, vulva, neurons, somatic gonad	<i>swEx520 [P_{bec-1}::BEC-1::GFP; rol-6(su1006)]grEx129 [P_{bec-1}::BEC-1::mRFP; lin-15(+)]Ex[P_{ced-1}::mCherry::BEC-1; rol-6(su1006)]Ex[P_{egl-1}::mCherry::BEC-1; rol-6(su1006)]</i>	Takacs-Vellai et al. (2005); Rowland et al. (2006); Ruck et al. (2011); Huang et al. (2012)
SQST-1	p62/autophagy adaptor protein	Hypodermis, neurons, intestine, vulva, muscle	<i>bpls151[P_{sqsst-1}::SQST-1::GFP; unc-76(+)]</i>	Hunt-Newbury et al. (2007); Pankiv et al. (2007); Tian et al. (2010)
SEPA-1	Autophagy adaptor protein	Intestine, head, tail	<i>bpls131[P_{sepa-1}::SEPA-1::GFP; unc-76(+)]</i>	Zhang et al. (2009); Tian et al. (2010)
PGL-1	RNA-binding protein/P-granule component	P-granules, intestine	<i>bpls1[P_{pie-1}::GFP::PGL-1; unc-119(+)]bpls26 [P_{elt-2}::PGL-1::GFP; Pmyo-2::mCherry]</i>	Cheeks et al. (2004); Zhang et al. (2009); Updike et al. (2011)

^aTissue expression can vary depending on the specific promoter used.^bThe transgenes shown are those found in autophagy studies or those that might be beneficial in autophagy studies; additional transgenes might be available for each gene.

finally, we present a protocol for investigating the function of autophagy genes by RNAi, a technique that has the advantage of circumventing some of the drawbacks associated with strong loss-of-function mutations (see Protocol: RNAi-Mediated Inactivation of Autophagy Genes in *Caenorhabditis elegans* [Palmisano and Meléndez 2015d]).

ACKNOWLEDGMENTS

We thank all members of the Meléndez laboratory for helpful discussions and comments on the manuscript. We extend special thanks to Melissa Silvestrini and Kristina Ames for comments on an earlier draft. The work in the Meléndez laboratory is supported by a National Science Foundation Research Initiation Award (0818802) and a National Institutes of Health 1 R15 GM102846-01 award; A.M. is an Ellison Medical Foundation New Scholar in Aging and N.J.P. is supported by a CUNY Graduate Center Dissertation award.

REFERENCES

- Al Rawi S, Louvet-Vallee S, Djeddi A, Sachse M, Culetto E, Hajjar C, Boyd L, Legouis R, Galy V. 2011. Postfertilization autophagy of sperm organelles prevents paternal mitochondrial DNA transmission. *Science* 334: 1144–1147.
- Albert PS, Brown SJ, Riddle DL. 1981. Sensory control of dauer larva formation in *Caenorhabditis elegans*. *J Comp Neurol* 198: 435–451.
- Alberti A, Michelet X, Djeddi A, Legouis R. 2010. The autophagosomal protein LGG-2 acts synergistically with LGG-1 in dauer formation and longevity in *C. elegans*. *Autophagy* 6: 622–633.
- Antebi A. 2007. Genetics of aging in *Caenorhabditis elegans*. *PLoS Genet* 3: 1565–1571.
- Avery L. 1993. The genetics of feeding in *Caenorhabditis elegans*. *Genetics* 133: 897–917.
- Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Kistakis NT. 2008. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol* 182: 685–701.
- Barth H, Meiling-Wesse K, Eppe UD, Thumm M. 2001. Autophagy and the cytoplasm to vacuole targeting pathway both require Aut10p. *FEBS Lett* 508: 23–28.
- Baugh LR, Sternberg PW. 2006. DAF-16/FOXO regulates transcription of cki-1/Cip/Kip and repression of lin-4 during *C. elegans* L1 arrest. *Curr Biol* 16: 780–785.
- Birnby DA, Link EM, Vowels JJ, Tian H, Colacurcio PL, Thomas JH. 2000. A Transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. *Genetics* 155: 85–104.
- Brown EJ, Albers MW, Bum Shin T, Ichikawa K, Keith CT, Lane WS, Schreiber SL. 1994. A mammalian protein targeted by G1-arresting rapamycin–receptor complex. *Nature* 369: 756–758.
- Cassada RC, Russell RL. 1975. The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. *Dev Biol* 46: 326–342.
- Chan EY, Longatti A, McKnight NC, Tooz SA. 2009. Kinase-inactivated ULK proteins inhibit autophagy via their conserved C-terminal domains using an Atg13-independent mechanism. *Mol Cell Biol* 29: 157–171.
- Cheeks RJ, Canman JC, Gabriel WN, Meyer N, Strome S, Goldstein B. 2004. *C. elegans* PAR proteins function by mobilizing and stabilizing asymmetrically localized protein complexes. *Curr Biol* 14: 851–862.
- Cheung PC, Trinkle-Mulcahy L, Cohen P, Lucocq JM. 2001. Characterization of a novel phosphatidylinositol 3-phosphate-binding protein containing two FYVE fingers in tandem that is targeted to the Golgi. *Biochem J* 355: 113–121.
- da Graca LS, Zimmermann KK, Mitchell MC, Kozhan-Gorodetska M, Sekiewicz K, Morales Y, Patterson GI. 2004. DAF-5 is a Ski oncoprotein homolog that functions in a neuronal TGF β pathway to regulate *C. elegans* dauer development. *Development* 131: 435–446.
- Derubeis AR, Young MF, Jia L, Robey PG, Fisher LW. 2000. Double FYVE-containing protein 1 (DFCP1): Isolation, cloning and characterization of a novel FYVE finger protein from a human bone marrow cDNA library. *Gene* 255: 195–203.
- Deter RL, Baudhuin P, De Duve C. 1967. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *J Cell Biol* 35: C11–C16.
- Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C. 2002. Rates of behavior and aging specified by mitochondrial function during development. *Science* 298: 2398–2401.
- Erdelyi P, Borsos E, Takacs-Vellai K, Kovacs T, Kovacs AL, Sigmond T, Hargitai B, Pasztor L, Sengupta T, Dengg M, et al. 2011. Shared developmental roles and transcriptional control of autophagy and apoptosis in *Caenorhabditis elegans*. *J Cell Sci* 124: 1510–1518.
- Estevez M, Attisano L, Wrana JL, Albert PS, Massague J, Riddle DL. 1993. The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. *Nature* 365: 644–649.
- Fan W, Nassiri A, Zhong Q. 2011. Autophagosome targeting and membrane curvature sensing by Barkor/Atg14(L). *Proc Natl Acad Sci* 108: 7769–7774.
- Fares H, Greenwald I. 2001. Regulation of endocytosis by CUP-5, the *Caenorhabditis elegans* mucolipin-1 homolog. *Nat Genet* 28: 64–68.
- Fukuyama M, Rougvie AE, Rothman JH. 2006. *C. elegans* DAF-18/PTEN mediates nutrient-dependent arrest of cell cycle and growth in the germline. *Curr Biol* 16: 773–779.
- Funakoshi T, Matsuura A, Noda T, Ohsumi Y. 1997. Analyses of APG13 gene involved in autophagy in yeast, *Saccharomyces cerevisiae*. *Gene* 192: 207–213.
- Gems D, Sutton AJ, Sundermeyer ML, Albert PS, King KV, Edgley ML, Larsen PL, Riddle DL. 1998. Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* 150: 129–155.
- Golden JW, Riddle DL. 1984. The *Caenorhabditis elegans* dauer larva: Developmental effects of pheromone, food, and temperature. *Dev Biol* 102: 368–378.
- Gosai SJ, Kwak JH, Luke CJ, Long OS, King DE, Kovatch KJ, Johnston PA, Shun TY, Lazo JS, Perlmutter DH, et al. 2010. Automated high-content live animal drug screening using *C. elegans* expressing the aggregation prone serpin alpha1-antitrypsin Z. *PLoS ONE* 5: e15460.
- Gottlieb S, Ruvkun G. 1994. *daf-2*, *daf-16* and *daf-23*: Genetically interacting genes controlling Dauer formation in *Caenorhabditis elegans*. *Genetics* 137: 107–120.
- Hansen M, Taubert S, Crawford D, Libina N, Lee SJ, Kenyon C. 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell* 6: 95–110.
- Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. 2008. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet* 4: e24.

- Harding TM, Morano KA, Scott SV, Klionsky DJ. 1995. Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. *J Cell Biol* 131: 591–602.
- Harding TM, Hefner-Gravink A, Thumm M, Klionsky DJ. 1996. Genetic and phenotypic overlap between autophagy and the cytoplasm to vacuole protein targeting pathway. *J Biol Chem* 271: 17621–17624.
- Hars ES, Qi H, Ryazanov AG, Jin S, Cai L, Hu C, Liu LF. 2007. Autophagy regulates ageing in *C. elegans*. *Autophagy* 3: 93–95.
- He H, Dang Y, Dai F, Guo Z, Wu J, She X, Pei Y, Chen Y, Ling W, Wu C, et al. 2003. Post-translational modifications of three members of the human MAP1LC3 family and detection of a novel type of modification for MAP1LC3B. *J Biol Chem* 278: 29278–29287.
- Hedgecock EM, Culotti JG, Thomson JN, Perkins LA. 1985. Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. *Developmental Biol* 111: 158–170.
- Huang S, Jia K, Wang Y, Zhou Z, Levine B. 2012. Autophagy genes function in apoptotic cell corpse clearance during *C. elegans* embryonic development. *Autophagy* 9: 138–149.
- Hunt-Newbury R, Viveiros R, Johnsen R, Mah A, Anastas D, Fang L, Halfnight E, Lee D, Lin J, Lorch A, et al. 2007. High-throughput in vivo analysis of gene expression in *Caenorhabditis elegans*. *PLoS Biol* 5: e237.
- Hutchins MU, Klionsky DJ. 2001. Vacuolar localization of oligomeric α -mannosidase requires the cytoplasm to vacuole targeting and autophagy pathway components in *Saccharomyces cerevisiae*. *J Biol Chem* 276: 20491–20498.
- Inoue T, Thomas JH. 2000. Suppressors of transforming growth factor- β pathway mutants in the *Caenorhabditis elegans* dauer formation pathway. *Genetics* 156: 1035–1046.
- Ji YJ, Choi KY, Song HO, Park BJ, Yu JR, Kagawa H, Song WK, Ahn J. 2006. VHA-8, the E subunit of V-ATPase, is essential for pH homeostasis and larval development in *C. elegans*. *FEBS Lett* 580: 3161–3166.
- Jia K, Levine B. 2007. Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. *Autophagy* 3: 597–599.
- Jia K, Chen D, Riddle DL. 2004. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. *Development* 131: 3897–3906.
- Jia K, Hart AC, Levine B. 2007. Autophagy genes protect against disease caused by polyglutamine expansion proteins in *Caenorhabditis elegans*. *Autophagy* 3: 21–25.
- Jing Y, Tang XM. 1999. The convergent point of the endocytic and autophagic pathways in leydig cells. *Cell Res* 9: 243–253.
- Johnson TE, Mitchell DH, Kline S, Kemal R, Foy J. 1984. Arresting development arrests aging in the nematode *Caenorhabditis elegans*. *Mech Ageing Dev* 28: 23–40.
- Kametaka S, Okano T, Ohsumi M, Ohsumi Y. 1998. Apg14p and Apg6/Vps30p form a protein complex essential for autophagy in the yeast, *Saccharomyces cerevisiae*. *J Biol Chem* 273: 22284–22291.
- Kang C, You YJ, Avery L. 2007. Dual roles of autophagy in the survival of *Caenorhabditis elegans* during starvation. *Genes Dev* 21: 2161–2171.
- Kihara A, Noda T, Ishihara N, Ohsumi Y. 2001. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae*. *J Cell Biol* 152: 519–530.
- Kim J, Dalton VM, Eggerton KP, Scott SV, Klionsky DJ. 1999. Apg7p/Cvt2p is required for the cytoplasm-to-vacuole targeting, macroautophagy, and peroxisome degradation pathways. *Mol Biol Cell* 10: 1337–1351.
- Kirisako T, Ichimura Y, Okada H, Kabeya Y, Mizushima N, Yoshimori T, Ohsumi M, Takao T, Noda T, Ohsumi Y. 2000. The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *J Cell Biol* 151: 263–276.
- Klionsky DJ. 2012. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* 8: 1–100.
- Klionsky DJ, Cregg JM, Dunn WA Jr, Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, et al. 2003. A unified nomenclature for yeast autophagy-related genes. *Dev Cell* 5: 539–545.
- Kostich M, Fire A, Fambrough DM. 2000. Identification and molecular-genetic characterization of a LAMP/CD68-like protein from *Caenorhabditis elegans*. *J Cell Sci* 113: 2595–2606.
- Kovács AL, Vellai T, Müller F. 2003. Autophagy in *Caenorhabditis elegans*. In *Autophagy* (Klionsky D), pp. 219–225.
- Kuma A, Mizushima N, Ishihara N, Ohsumi Y. 2002. Formation of the approximately 350-kDa Apg12-Apg5-Apg16 multimeric complex, mediated by Apg16 oligomerization, is essential for autophagy in yeast. *J Biol Chem* 277: 18619–18625.
- Kuroyanagi H, Yan J, Seki N, Yamanouchi Y, Suzuki Y, Takano T, Muramatsu M, Shirasawa T. 1998. Human ULK1, a novel serine/threonine kinase related to UNC-51 kinase of *Caenorhabditis elegans*: cDNA cloning, expression, and chromosomal assignment. *Genomics* 51: 76–85.
- Lakowski B, Hekimi S. 1998. The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc Natl Acad Sci* 95: 13091–13096.
- Lapierre LR, Gelino S, Melendez A, Hansen M. 2011. Autophagy and lipid metabolism coordinately modulate life span in germline-less *C. elegans*. *Curr Biol* 21: 1507–1514.
- Lapierre LR, De Magalhães Filho CD, McQuary PR, Chu CC, Visvikis O, Chang JT, Gelino S, Ong B, Davis AE, Irazoqui JE, et al. 2013. The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in *Caenorhabditis elegans*. *Nat Commun* 4: 2267.
- Lee CY, Clough EA, Yellon P, Teslovich TM, Stephan DA, Baehrecke EH. 2003. Genome-wide analyses of steroid- and radiation-triggered programmed cell death in *Drosophila*. *Curr Biol* 13: 350–357.
- Levine B, Klionsky DJ. 2004. Development by self-digestion: Molecular mechanisms and biological functions of autophagy. *Dev Cell* 6: 463–477.
- Li W, Zou W, Yang Y, Chai Y, Chen B, Cheng S, Tian D, Wang X, Vale RD, Ou G. 2012. Autophagy genes function sequentially to promote apoptotic cell corpse degradation in the engulfing cell. *J Cell Biol* 197: 27–35.
- Liang Q, Yang P, Tian E, Han J, Zhang H. 2012. The *C. elegans* ATG101 homolog EPG-9 directly interacts with EPG-1/Atg13 and is essential for autophagy. *Autophagy* 8: 1426–1433.
- Lin L, Yang P, Huang X, Zhang H, Lu Q, Zhang H. 2013. The scaffold protein EPG-7 links cargo-receptor complexes with the autophagic assembly machinery. *J Cell Biol* 201: 113–129.
- Liou W, Geuze HJ, Geelen MJ, Slot JW. 1997. The autophagic and endocytic pathways converge at the nascent autophagic vacuoles. *J Cell Biol* 136: 61–70.
- Lu Q, Yang P, Huang X, Hu W, Guo B, Wu F, Lin L, Kovacs AL, Yu L, Zhang H. 2011. The WD40 repeat PtdIns(3)P-binding protein EPG-6 regulates progression of omegasomes to autophagosomes. *Dev Cell* 21: 343–357.
- Manil-Segalen M, Lefebvre C, Jenzer C, Trichet M, Boulogne C, Satiat-Jeunemaitre B, Legouis R. 2014. The *C. elegans* LC3 acts downstream of GABARAP to degrade autophagosomes by interacting with the HOPS subunit VPS39. *Dev Cell* 28: 43–55.
- Matsuura A, Miki T, Wada Y, Ohsumi Y. 1997. Apg1p, a novel protein kinase required for the autophagic process in *Saccharomyces cerevisiae*. *Gene* 192: 245–250.
- Meléndez A, Levine B. 2009. Autophagy in *C. elegans*. *WormBook* doi: 10.1895/wormbook.1.147.1.
- Meléndez A, Tallozy Z, Seaman M, Eskelinen EL, Hall DH, Levine B. 2003. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* 301: 1387–1391.
- Mizushima N. 2007. Autophagy: Process and function. *Genes Dev* 21: 2861–2873.
- Mizushima N, Sugita H, Yoshimori T, Ohsumi Y. 1998. A new protein conjugation system in human the counterpart of the yeast Apg12p conjugation system essential for autophagy. *J Biol Chem* 273: 33889–33892.
- Mizushima N, Yamamoto A, Hatano M, Kobayashi Y, Kabeya Y, Suzuki K, Tokuhisa T, Ohsumi Y, Yoshimori T. 2001. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J Cell Biol* 152: 657–667.
- Mizushima N, Yoshimori T, Ohsumi Y. 2002. Mouse Apg10 as an Apg12-conjugating enzyme: Analysis by the conjugation-mediated yeast two-hybrid method. *FEBS Lett* 532: 450–454.
- Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, Yoshimori T. 2003. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J Cell Sci* 116: 1679–1688.
- Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y. 2009. Dynamics and diversity in autophagy mechanisms: Lessons from yeast. *Nat Rev Mol Cell Biol* 10: 458–467.

- Noda T, Ohsumi Y. 1998. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J Biol Chem* 273: 3963–3966.
- Noda T, Kim J, Huang WP, Baba M, Tokunaga C, Ohsumi Y, Klionsky DJ. 2000. Apg9p/Cvt7p is an integral membrane protein required for transport vesicle formation in the Cvt and autophagy pathways. *J Cell Biol* 148: 465–480.
- Obara K, Sekito T, Ohsumi Y. 2006. Assortment of phosphatidylinositol 3-kinase complexes—Atg14p directs association of complex I to the pre-autophagosomal structure in *Saccharomyces cerevisiae*. *Mol Biol Cell* 17: 1527–1539.
- Ogura K, Wicky C, Magnenat L, Tobler H, Mori I, Muller F, Ohshima Y. 1994. *Caenorhabditis elegans unc-51* gene required for axonal elongation encodes a novel serine/threonine kinase. *Genes Dev* 8: 2389–2400.
- Oka T, Futai M. 2000. Requirement of V-ATPase for ovulation and embryogenesis in *Caenorhabditis elegans*. *J Biol Chem* 275: 29556–29561.
- Palmisano NJ, Meléndez A. 2015a. Detection of autophagy in *Caenorhabditis elegans* using GFP::LGG-1 as an autophagy marker. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot086496.
- Palmisano NJ, Meléndez A. 2015b. Visualization of autophagy in *Caenorhabditis elegans* embryos using markers of P granule degradation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot086504.
- Palmisano NJ, Meléndez A. 2015c. Detection of autophagy in *Caenorhabditis elegans* by western blotting analysis of LGG-1. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot086512.
- Palmisano NJ, Meléndez A. 2015d. RNAi-mediated inactivation of autophagy genes in *Caenorhabditis elegans*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot086520.
- Pan KZ, Palter JE, Rogers AN, Olsen A, Chen D, Lithgow GJ, Kapahi P. 2007. Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* 6: 111–119.
- Panaretou C, Domin J, Cockcroft S, Waterfield MD. 1997. Characterization of p150, an adaptor protein for the human phosphatidylinositol (PtdIns) 3-kinase. *J Biol Chem* 272: 2477–2485.
- Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkoy G, Johansen T. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem* 282: 24131–24145.
- Patterson GI, Kowek A, Wong A, Liu Y, Ruvkun G. 1997. The DAF-3 Smad protein antagonizes TGF- β -related receptor signaling in the *Caenorhabditis elegans* dauer pathway. *Genes Dev* 11: 2679–2690.
- Pivtoraiko VN, Harrington AJ, Mader BJ, Luker AM, Caldwell GA, Caldwell KA, Roth KA, Shacka JJ. 2010. Low-dose bafilomycin attenuates neuronal cell death associated with autophagy-lysosome pathway dysfunction. *J Neurochem* 114: 1193–1204.
- Polson HE, de Lartigue J, Rigden D, Reedijk M, Urbé S, Clague M, Tooze S. 2010. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy* 6: 506–522.
- Raizen DM, Lee RY, Avery L. 1995. Interacting genes required for pharyngeal excitation by motor neuron MC in *Caenorhabditis elegans*. *Genetics* 141: 1365–1382.
- Reggiori F, Klionsky DJ. 2006. Atg9 sorting from mitochondria is impaired in early secretion and VFT-complex mutants in *Saccharomyces cerevisiae*. *J Cell Sci* 119: 2903–2911.
- Ren P, Lim CS, Johnsen R, Albert PS, Pilgrim D, Riddle DL. 1996. Control of *C. elegans* larval development by neuronal expression of a TGF- β homolog. *Science* 274: 1389–1391.
- Roggo L, Bernard V, Kovacs AL, Rose AM, Savoy F, Zetka M, Wymann MP, Muller F. 2002. Membrane transport in *Caenorhabditis elegans*: An essential role for VPS34 at the nuclear membrane. *EMBO J* 21: 1673–1683.
- Rowland AM, Richmond JE, Olsen JG, Hall DH, Bamber BA. 2006. Presynaptic terminals independently regulate synaptic clustering and autophagy of GABAA receptors in *Caenorhabditis elegans*. *J Neurosci* 26: 1711–1720.
- Ruck A, Attonito J, Garces KT, Nunez L, Palmisano NJ, Rubel Z, Bai Z, Nguyen KC, Sun L, Grant BD, et al. 2011. The Atg6/Vps30/Beclin1 ortholog BEC-1 mediates endocytic retrograde transport in addition to autophagy in *C. elegans*. *Autophagy* 7: 386–400.
- Samara C, Syntichaki P, Tavernarakis N. 2008. Autophagy is required for necrotic cell death in *Caenorhabditis elegans*. *Cell Death Differ* 15: 105–112.
- Sato M, Sato K. 2011. Degradation of paternal mitochondria by fertilization-triggered autophagy in *C. elegans* embryos. *Science* 334: 1141–1144.
- Schackwitz WS, Inoue T, Thomas JH. 1996. Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. *Neuron* 17: 719–728.
- Seaman MNJ, Marcussen EG, Cereghino JL, Emr SD. 1997. Endosome to Golgi retrieval of the vacuolar protein sorting receptor, Vps10p, requires the function of the VPS29, VPS30, and VPS35 gene products. *J Cell Biol* 137: 79–92.
- Seglen PO, Gordon PB. 1982. 3-Methyladenine: Specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. *Proc Natl Acad Sci* 79: 1889–1892.
- Shintani T, Mizushima N, Ogawa Y, Matsuura A, Noda T, Ohsumi Y. 1999. Apg10p, a novel protein-conjugating enzyme essential for autophagy in yeast. *EMBO J* 18: 5234–5241.
- Shintani T, Suzuki K, Kamada Y, Noda T, Ohsumi Y. 2001. Apg2p functions in autophagosome formation on the perivacuolar structure. *J Biol Chem* 276: 30452–30460.
- Sun Q, Fan W, Chen K, Ding X, Chen S, Zhong Q. 2008. Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proc Natl Acad Sci* 105: 19211–19216.
- Sun T, Wang X, Lu Q, Ren H, Zhang H. 2011. CUP-5, the *C. elegans* ortholog of the mammalian lysosomal channel protein MLN1/TRPML1, is required for proteolytic degradation in autolysosomes. *Autophagy* 7: 1308–1315.
- Takacs-Vellai K, Vellai T, Puoti A, Passannante M, Wicky C, Streit A, Kovacs AL, Muller F. 2005. Inactivation of the autophagy gene *bec-1* triggers apoptotic cell death in *C. elegans*. *Curr Biol* 15: 1513–1517.
- Tanida I, Tanida-Miyake E, Ueno T, Kominami E. 2001. The human homolog of *Saccharomyces cerevisiae* Apg7p is a protein-activating enzyme for multiple substrates including human Apg12p, GATE-16, GABARAP, and MAP-LC3. *J Biol Chem* 276: 1701–1706.
- Tanida I, Tanida-Miyake E, Komatsu M, Ueno T, Kominami E. 2002. Human Apg3p/Aut1p homologue is an authentic E2 enzyme for multiple substrates, GATE-16, GABARAP, and MAP-LC3, and facilitates the conjugation of hApg12p to hApg5p. *J Biol Chem* 277: 13739–13744.
- Tanida I, Sou YS, Ezaki J, Minematsu-Ikeguchi N, Ueno T, Kominami E. 2004. HsAtg4B/HsApg4B/autophagin-1 cleaves the carboxyl termini of three human Atg8 homologues and delipidates microtubule-associated protein light chain 3- and GABAA receptor-associated protein-phospholipid conjugates. *J Biol Chem* 279: 36268–36276.
- Thomas JH, Birnby DA, Vowels JJ. 1993. Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. *Genetics* 134: 1105–1117.
- Thumm M, Egner R, Koch B, Schlumpberger M, Straub M, Veenhuis M, Wolf DH. 1994. Isolation of autophagocytosis mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 349: 275–280.
- Tian E, Wang F, Han J, Zhang H. 2009. *epg-1* functions in autophagy-regulated processes and may encode a highly divergent Atg13 homolog in *C. elegans*. *Autophagy* 5: 608–615.
- Tian Y, Li Z, Hu W, Ren H, Tian E, Zhao Y, Lu Q, Huang X, Yang P, Li X, et al. 2010. *C. elegans* screen identifies autophagy genes specific to multicellular organisms. *Cell* 141: 1042–1055.
- Toth ML, Simon P, Kovacs AL, Vellai T. 2007. Influence of autophagy genes on ion-channel-dependent neuronal degeneration in *Caenorhabditis elegans*. *J Cell Sci* 120: 1134–1141.
- Toth ML, Sigmond T, Borsos E, Barna J, Erdelyi P, Takacs-Vellai K, Orosz L, Kovacs AL, Csikos G, Sass M, et al. 2008. Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. *Autophagy* 4: 330–338.
- Tsukada M, Ohsumi Y. 1993. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 333: 169–174.
- Udipke DL, Hachey SJ, Kreher J, Strome S. 2011. P granules extend the nuclear pore complex environment in the *C. elegans* germ line. *J Cell Biol* 192: 939–948.
- Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F. 2003. Genetics: Influence of TOR kinase on lifespan in *C. elegans*. *Nature* 426: 620.
- Volinia S, Dhand R, Vanhaesebroeck B, MacDougall LK, Stein R, Zvelebil MJ, Domin J, Panaretou C, Waterfield MD. 1995. A human

N.J. Palmisano and A. Meléndez

- phosphatidylinositol 3-kinase complex related to the yeast Vps34p-Vps15p protein sorting system. *EMBO J* 14: 3339–3348.
- Wang CW, Kim J, Huang WP, Abeliovich H, Stromhaug PE, Dunn WA Jr, Klionsky DJ. 2001. Apg2 is a novel protein required for the cytoplasm to vacuole targeting, autophagy, and pexophagy pathways. *J Biol Chem* 276: 30442–30451.
- Xie Z, Klionsky DJ. 2007. Autophagosome formation: Core machinery and adaptations. *Nat Cell Biol* 9: 1102–1109.
- Yamada T, Carson AR, Caniggia I, Umebayashi K, Yoshimori T, Nakabayashi K, Scherer SW. 2005. Endothelial nitric-oxide synthase antisense (NOS3AS) gene encodes an autophagy-related protein (APG9-like2) highly expressed in trophoblast. *J Biol Chem* 280: 18283–18290.
- Yang P, Zhang H. 2011. The coiled-coil domain protein EPG-8 plays an essential role in the autophagy pathway in *C. elegans*. *Autophagy* 7: 159–165.
- Zhang Y, Yan L, Zhou Z, Yang P, Tian E, Zhang K, Zhao Y, Li Z, Song B, Han J, et al. 2009. SEPA-1 mediates the specific recognition and degradation of P granule components by autophagy in *C. elegans*. *Cell* 136: 308–321.
- Zhang H, Wu F, Wang X, Du H, Wang X, Zhang H. 2013. The two *C. elegans* ATG-16 homologs have partially redundant functions in the basal autophagy pathway. *Autophagy* 9: 1965–1974.
- Zhao Y, Tian E, Zhang H. 2009. Selective autophagic degradation of maternally-loaded germline P granule components in somatic cells during *C. elegans* embryogenesis. *Autophagy* 5: 717–719.

Cold Spring Harbor Protocols

Detection of Autophagy in *Caenorhabditis elegans*

Nicholas J. Palmisano and Alicia Meléndez

Cold Spring Harb Protoc; doi: 10.1101/pdb.top070466

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

[C. elegans](#) (45 articles)

[Cell Biology, general](#) (1382 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>