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Review article

Autophagy in *C. elegans* development

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ABSTRACT

Autophagy involves the sequestration of cytoplasmic contents in a double-membrane structure referred to as the autophagosome and the degradation of its contents upon delivery to lysosomes. Autophagy activity has a role in multiple biological processes during the development of the nematode *Caenorhabditis elegans*. Basal levels of autophagy are required to remove aggregate prone proteins, paternal mitochondria, and spermatid-specific membranous organelles. During larval development, autophagy is required for the remodeling that occurs during dauer development, and autophagy can selectively degrade components of the miRNA-induced silencing complex, and modulate miRNA-mediated silencing. Basal levels of autophagy are important in synapse formation and in the germ line, to promote the proliferation of proliferating stem cells. Autophagy activity is also required for the efficient removal of apoptotic cell corpses by promoting phagosome maturation. Finally, autophagy is also involved in lipid homeostasis and in the aging process. In this review, we first describe the molecular complexes involved in the process of autophagy, its regulation, and mechanisms for cargo recognition. In the second section, we discuss the developmental contexts where autophagy has been shown to be important. Studies in *C. elegans* provide valuable insights into the physiological relevance of this process during metazoan development.

1. Overview of autophagy

Autophagy, a process of "self-cannibalism", was first described in morphological studies of rat liver cells (DeDuve, 1963, 1964; Deter et al., 1967; Deter and De Duve, 1967; Sachs et al., 1962). It is an evolutionarily conserved stress response pathway that results in the bulk degradation and recycling of cellular components through a lysosomal pathway (Deter et al., 1967; Levine and Klionsky, 2004; Mizushima, 2007). There are three major types of autophagy, which differ with respect to the mode of delivery to the lysosome: chaperone-mediated autophagy (CMA), microautophagy (MI), and macroautophagy (MA). All three forms contribute to the degradation of cellular material by the lysosome.

Chaperone-mediated autophagy (CMA) is a mechanism that allows the direct lysosomal import of proteins, which contain a pentapeptide motif related to KFERQ (Dice et al., 1986; Majeski and Dice, 2004; Massey et al., 2004). Chaperone complexes localized in the cytosol bind to substrates that contain the motif and mediate their transport to the lysosome (Arias and Cuervo, 2011; Dice et al., 1986; Kaushik and Cuervo, 2012). Thereafter, translocation of substrates across the lysosomal membrane occurs via chaperones residing in the lysosomal membrane, such as HSC70, and LAMP2A (Bandyopadhyay et al., 2008; Cuervo and Wong, 2014).

Microautophagy (MI) involves the direct engulfment of target molecules and/or cytoplasm through the invagination of the lysosomal membrane (Ahlberg et al., 1982; Mortimore et al., 1988; Sattler and Mayer, 2000; Uttenweiler et al., 2005). Recent reports in mammals have also described a process of endosomal-microautophagy (e-MI), similar to microautophagy, where cytosolic proteins are internalized into late endosomes, during multivesicular (MVB) formation.

Finally, macroautophagy, referred to as "autophagy", involves the *de-novo* formation of a double-membrane organelle, an autophagosome, which transports cytoplasmic components to the lysosome for their degradation and recycling (Fig. 1) (Levine and Klionsky, 2004; Mizushima, 2007). Cargo known to be degraded by autophagy includes, protein aggregates (aggrephagy), peroxisomes (pexophagy), mitochondria (mitophagy), lipid droplets (lipophagy), ribosomes (ribophagy), bacteria (xenophagy) (Rogov et al., 2014). Defects in autophagy result in the accumulation of polyubiquitinated protein aggregates and damaged organelles, which may contribute to defects in cellular homeostasis (Elmore et al., 2001; Hara et al., 2006; Juhasz et al., 2007a; Khan et al., 2008; Kuma et al., 2004; Lu et al., 2014).

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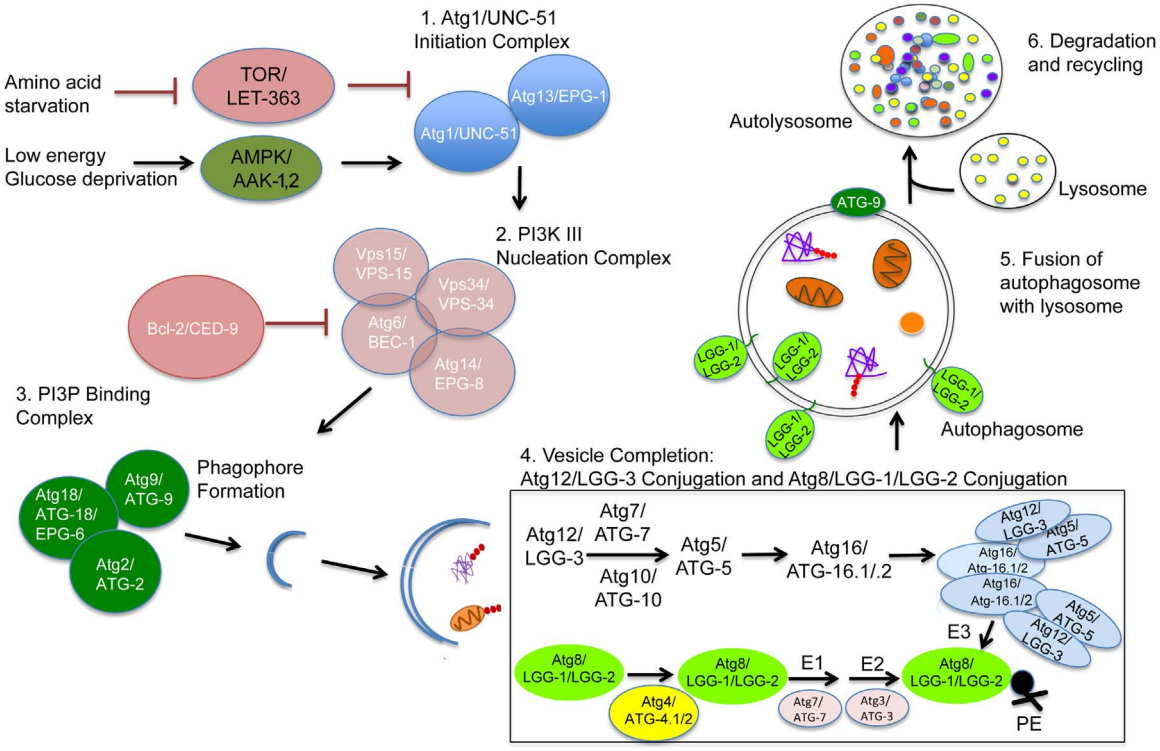

Fig. 1. Autophagy process. Macroautophagy (here referred to as autophagy) involves a series of steps that include autophagy induction, membrane nucleation, phagophore formation, membrane expansion, autophagosome formation, fusion with the lysosome and degradation. These steps are controlled by distinct complexes of proteins: (1) the initiation complex consists of Atg1/UNC-51 and Atg13/EGP-1; (2) the nucleation complex consists of Atg6/BEC-1, VPS-34, and VPS-15; (3) the PI3P binding complex, consists of Atg9/ATG-9, Atg18/ATG-18/EPG-6, and Atg2/ATG-2; (4) vesicle expansion requires the Atg5/ATG-5, Atg12/LGG-3 and Atg16/ATG-16.1/ATG-16.2, part of the conjugation complex and Atg8/LGG-1/LGG-2; (5) fusion with the lysosome containing hydrolases and finally, (6) degradation and recycling. For all proteins, we have indicated the yeast protein name followed by the *C. elegans* ortholog(s). As part of the conjugation systems, Atg8, (in *C. elegans* LGG-1, or LGG-2), is cleaved by Atg4 (in *C. elegans* ATG-4.1 and ATG-4.2), and conjugated with phosphatidylethanolamine (PE) on the autophagosome membrane, which incorporates into pre- and autophagosomal membranes. The autophagosome engulfs mitochondria (in orange), long-lived proteins (in purple), and/or other vesicles and organelles (not shown).

In *Caenorhabditis elegans*, the process of autophagy has been fairly well described. So far there is no genetic data for the existence of microautophagy or CMA in *C. elegans*. In addition, the CMA limiting factor in mammals, the LAMP2A receptor isoform (a lysosomal-associated membrane 2A), appears not to be evolutionarily conserved, since no homolog has been found in yeast, Drosophila or *C. elegans* by sequence similarity. Nevertheless, it is possible that a functional ortholog of LAMP2A exists in these organisms.

Autophagy occurs at low/basal levels during normal growth conditions; however, it is significantly upregulated in response to stress, such as nutrient deprivation, increased temperature, hypoxia, and the accumulation of damaged organelles, DNA damage, reactive oxygen species, intracellular pathogens, and/or protein aggregates (Levine and Klionsky, 2004; Meléndez et al., 2003). These stressors result in the initiation of the autophagy process, which is divided into distinct steps: induction, vesicle nucleation, vesicle elongation, and lysosomal fusion and degradation (Mizushima and Yoshimori, 2007; Nakatogawa et al., 2009; Suzuki et al., 2001; Xie and Klionsky, 2007).

Autophagy is widely known for its pro-survival role during stress conditions, in addition to its role in overall cellular homeostasis. Although autophagy was first described in mammals, most of the core autophagy genes that regulate autophagosome formation were discovered in the yeast model system of *Saccharomyces cerevisiae,* by screening for mutations that decreased survival during starvation, as well as mutations that disrupted the cytoplasm-to-vacuole targeting (*CVT*) process, a selective form of autophagy found in yeast (Harding et al., 1996, 1995; Hutchins and Klionsky, 2001; Klionsky et al., 2003; Thumm et al., 1994; Tsukada and Ohsumi, 1993). Thus, most of our understanding of the process of autophagy comes from studies done in yeast and mammals (Fig. 1 and Table 1). In higher eukaryotes, such as *C. elegans*, the formation of the autophagosome requires conserved Atg proteins (orthologs of yeast Atg proteins), but also several metazoan-specific autophagy genes (genes with no homology in yeast, and conserved in mammals) (Table 1) (Lu et al., 2011; Tian et al., 2010). Although many genes have been identified, how the machinery works to coordinate the formation and maturation of autophagosomes is not well understood.

2. Importance of autophagy

Highlighting the importance of autophagy across species, *S. cerevisiae*, *C. elegans*, and *M. musculus,* display a variety of developmental defects, and in some cases lethality, when containing mutations in autophagy genes and are exposed to stress (Boya et al., 2005; Juhasz et al., 2007b; Komatsu et al., 2005; Kuma et al., 2004; Levine and Kroemer, 2008, 2009; Meléndez et al., 2003; Scott et al., 2004; Tsukada and Ohsumi, 1993; Yue et al., 2003). In humans, as well as in mammals, impaired autophagy is associated with the development of cancer, such as breast, prostate, and lung cancer, as well as neurodegenerative disorders, such as Alzheimer's and Parkinson's disease (Hara et al., 2006; Komatsu et al., 2006; Liu and Debnath, 2016; Marino et al., 2008; Martinez-Vicente et al., 2010; Nixon et al., 2005; Sun, 2016; Yu et al., 2005; Yue et al., 2003). Moreover, in both mammals and yeast, autophagy is required for the maintenance of amino acid pools during starvation, as well as the maintenance of cellular ATP levels, levels of free fatty acids, and glucose levels (Chen et al., 2010; Fader et al., 2012; Karsli-Uzunbas et al., 2014; Kim et al., 2011; Komatsu et al., 2005; Kuma et al., 2004; Mortimore and Schworer, 1977; Onodera and Ohsumi, 2005; Singh et al., 2009b; Suraweera et al., 2012). In summary, autophagy acts as a quality control system to maintain cellular homeostasis and function.

Table 1
Genes involved in the autophagy pathway. Many *C. elegans* genes are conserved in mammals and yeast.

C. elegans gene	Mutant allele	Yeast gene	Mammalian gene	References
atg-2	*bp576*	*ATG2*	*ATG2A, ATG2B*	(Lu et al., 2011)
atg-3 [c]	*bp412*	*ATG3*	*ATG3*	(Zhang et al., 2009b)
atg-4.1	*bp501*	*ATG4*	*ATG4A, ATG4B*	(Wu et al., 2012)
atg-4.2	*tm3948*	*ATG4*	*ATG4C, ATG4D*	(Wu et al., 2012)
atg-5 [c]	*bp484*	*ATG5*	*ATG5*	(Zhang et al., 2013)
bec-1 [ac]	*ok691* *ok700* *bp613* [b]	*ATG6/VPS30*	*BECN1*	(Ames et al., 2017; Ames and Meléndez, 2017; Lapierre et al., 2013b; Meléndez et al., 2003; Ruck et al., 2011; Takacs-Vellai et al., 2005)
atg-7 [c]	*bp422* *bp290*	*ATG7*	*ATG7*	(Ames et al., 2017; Lapierre et al., 2013b; Lu et al., 2011; Meléndez et al., 2003; Zhang et al., 2009b)
lgg-1 [ac]	*bp500* *tm3489*	*ATG8*	*GABARAP*	(Alberti et al., 2010; Manil-Segalen et al., 2014; Meléndez et al., 2003; Tian et al., 2010)
lgg-2 [c]	*tm5755*	*ATG8*	*LC3*	(Manil-Segalen et al., 2014)
atg-9 [c]	*bp564*	*ATG9*	*ATG9A/ATG9B*	(Lu et al., 2011)
atg-10 [c]	*bp421*	*ATG10*	*ATG10*	(Zhang et al., 2009b)
lgg-3 [c]	*tm1462*	*ATG12*	*ATG12*	(Zhang et al., 2009b)
epg-1	*bp417*	*ATG13*	*ATG13*	(Tian et al., 2009)
epg-2 [c]	*bp287* *bp444*	-	-	(Tian et al., 2009)
epg-3 [c]	*bp405*	-	*VMP1*	(Tian et al., 2009)
epg-4 [c]	*bp425*	-	*EI24*	(Tian et al., 2009)
epg-5 [c]	*bp450* *tm3425*	-	*EPG5*	(Tian et al., 2010)
epg-6	*bp242*	*ATG18*	*WIPI3/WIPI4*	(Lu et al., 2011)
epg-7	*tm2508*	-	*FIP200*	(Lin et al., 2013)
epg-8 [a]	*bp251* *ok2561*	*ATG14*	*ATG14L/Barkor*	(Yang and Zhang, 2011)
epg-9	*bp320*	-	*ATG101*	(Liang et al., 2012)
atg-16.1	*gk668615* [b]	*ATG16*	*ATG16L1, ATG16L2*	(Zhang et al., 2013)
atg-16.2	*bp636* *ok3224*	*ATG16*	*ATG16L1, ATG16L2*	(Ames et al., 2017; Zhang et al., 2013)
atg-18 [c]	*gk378* *bp594* [b]	*ATG18*	*WIPI1/WIPI2*	(Ames et al., 2017; Guo et al., 2014b; Minnerly et al., 2017; Tian et al., 2010)
unc-51 [c]	*e369*	*ATG1*	*ULK1/ULK2*	(Meléndez et al., 2003; Ogura et al., 1994; Rowland et al., 2006; Toth et al., 2007)
TOR/let-363 [ac]	*h98* *h111*	*TOR*	*mTOR*	(Hansen et al., 2008; Jia et al., 2004; Toth et al., 2008; Vellai et al., 2003)
vps-34/let-512 [ac]	*h741*	*VPS34*	*PIK3C3*	(Roggo et al., 2002)
vps-15	*or1235* *ok3132*	*VPS15*	*VPS15/PIK3R4*	(Lowry et al., 2015)
rab-7 [c]	*ok511* *tm3300*	*YPT7*	*RAB7*	(Hermann et al., 2005)
rab-10 [c]	*ok1494*	*YPT1*	*RAB10*	(Hansen et al., 2007; Palmisano et al., 2017)
syx-17	-	*SSO1, SSO2*	*SYX17*	(Wilkinson et al., 2015)
snap-29 [c]	*tm2060*	-	*SNAP29*	(Guo et al., 2014b; Sato et al., 2011)
lmp-1 [c]	*nr2045*	-	*LAMP1*	(Kostich et al., 2000; Nilsson et al., 2011)
pgl-3	*bp439*	-	-	(Sato and Sato, 2011)
sepa-1	*bp456*	-	-	(Tian et al., 2009)
sqst-1	*ok2892*	-	SQSTM1/p62	(Lin et al., 2013)
vps-39 [a]	*tm2253* *ok2442*	-	*VPS39*	(Manil-Segalen et al., 2014)
vps-41	*ep402*	-	*VPS4*	(Manil-Segalen et al., 2014)

The following symbols are used: [c] for lethal genes; [b] for known hypomorphic mutations, and [c] for genes with available RNAi clones.

3. Section 1: Processes involved in the autophagy pathway

3.1. Regulation of autophagy

Two main regulators of autophagy are the evolutionarily conserved protein kinase TOR (Target of Rapamycin) and adenosine monophosphate-activated protein kinase (AMPK), which activate anabolic and catabolic pathways, respectively, and interact to control metabolism and maintain energy homeostasis (Fig. 1) (Cafferkey et al., 1993; Heitman et al., 1991; Laplante and Sabatini, 2009; Sabatini et al., 1994). TOR is a serine/threonine protein kinase that forms two signaling complexes, TORC1 and TORC2, distinguished mostly by their association with Raptor or Rictor, respectively. Both TORC1 and TORC2 complexes have been implicated in the regulation of cell growth and autophagy; however, there is a greater understanding for the regulation of autophagy via mTORC1 (Kim et al., 2011; Laplante and Sabatini, 2009). In the presence of amino acids and growth factors, TORC1 becomes activated at the lysosomal membrane. The translocation of TOR, to and from the lysosome, allows the cell to sense and respond to differing nutrient levels, either activating or repressing cell growth (Fingar and Blenis, 2004; Kira et al., 2016; Peli-Gulli et al., 2015; Rohde et al., 2001). TOR localization to lysosomes depends on the evolutionarily conserved Rag GTPases, Gtr1/RAGA/B and Gtr2/RAGC/D (Bar-Peled and Sabatini, 2012; Kim et al., 2014; Sancak et al., 2008). In the presence of amino acids, Rag GTPases recruit TORC1 to the vacuolar/lysosomal membrane, where it becomes activated and inhibits the ULK1/Atg1 induction complex (Peli-Gulli et al., 2015). Upon nutrient deprivation, TORC1 is released from the vacuolar/lysosomal membrane, allowing for the induction of autophagy (Bar-Peled and Sabatini, 2012; Sancak et al., 2008). As part of a positive feedback loop, continued autophagy activity replenishes diminished nutrients, which results in the reactivation of TOR, and inhibition of

autophagy (Yu et al., 2010).

In cooperation with the TOR signaling pathway, the cAMP-dependent protein kinase (PKA) independently targets the Atg1/Atg13 complex to control autophagy (Budovskaya et al., 2005; Stephan et al., 2009; Yorimitsu et al., 2007). Moreover, the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway is another nutrient sensing pathway that can also regulate autophagy activity (Egan et al., 2011). Under nutrient-rich conditions, active mTORC1 phosphorylates Atg1/ULK1, preventing its association and activation by AMPK, keeping Atg1/ULK1 inactive (Egan et al., 2011; Hosokawa et al., 2009a; Jung et al., 2009; Kim et al., 2011; Tian et al., 2015). Forced expression of an activated form of AMPK in *C. elegans* hypodermal cells is sufficient to promote autophagy. Under glucose deprivation, AMPK associates with, and directly phosphorylates Atg1/ULK1 on several sites and this modification is required for Atg1/ULK1 activation (Egan et al., 2011; Hosokawa et al., 2009a; Jung et al., 2009; Kim et al., 2011; Tian et al., 2015). Thus, AMPK, inhibits mTORC1 activity, and positively regulates the Atg1/ULK1 induction complex to induce autophagy (Egan et al., 2011; Hosokawa et al., 2009a; Jung et al., 2009; Kim et al., 2011; Tian et al., 2015). Additionally, mTORC1 inactivation regulates autophagy at the transcriptional level, via the nuclear translocation of the transcription factor, TFEB/HLH-30, a regulator of lysosomal genes and autophagy genes (Martina et al., 2012; Settembre et al., 2013, 2011). TFEB/HLH-30 also regulates genes involved in lipid homeostasis, suggesting that the survival of animals under nutrient deprivation relies on the coordination of pathways involved in lipid metabolism and autophagy.

LET-363 is the *C. elegans* ortholog of mTOR (Long et al., 2002; Robida-Stubbs et al., 2012). Animals deficient in LET-363/TOR activity, or carrying a mutation in *daf-15*/RAPTOR (the regulated associated protein of mTOR), arrest as dauer-like larvae (incomplete dauers) (Jia et al., 2004; Long et al., 2002; Robida-Stubbs et al., 2012; Toth et al., 2008). Animals that have been RNAi depleted against *let-363/TOR* are long-lived and have elevated levels of autophagy (Vellai et al., 2003). LET-363/TOR is part of the insulin/PI3K signaling pathway (Jia et al., 2004; Robida-Stubbs et al., 2012; Vellai et al., 2003). DAF-16 and SKN-1 dependent transcription is essential for the longevity that results from genetic TORC1 inhibition (Robida-Stubbs et al., 2012). Upon reduced insulin levels, DAF-16 and SKN-1 enter the nucleus to control the transcription of stress response genes (Lee et al., 2003; Ogg et al., 1997). One of the genes negatively regulated by DAF-16/FOXO activity is *daf-15/Raptor* (Jia et al., 2004). The inactivation of *daf-15/Raptor* by DAF-16/FOXO results in the induction of autophagy (Guo et al., 2014a; Hansen et al., 2008). Concurrently, SKN-1 in the nucleus regulates the expression of stress response genes, and negatively regulates insulin-like agonists, which may serve as a positive feedback loop to further reduce insulin signaling (Okuyama et al., 2010). SKN-1 is required for increased mitophagy as a result of reduced insulin signaling (Palikaras et al., 2015). Therefore, reduced insulin-signaling results in a cascade of events that inhibit LET-363/TOR activity, and upregulate DAF-16/FOXO and SKN-1, and promotes autophagy induction (Guo et al., 2014a; Hansen et al., 2008). Interestingly, LET-363/CeTORC1 was shown to negatively regulate both DAF-16/FOXO and SKN-1 activity, suggesting that a negative feedback loop exists between LET-363/TOR, DAF-16/FOXO, and SKN-1 (Jia et al., 2004; Robida-Stubbs et al., 2012).

3.2. Autophagy induction

In *S. cerevisiae*, autophagy induction requires the activation of the serine/threonine kinase, Atg1, along with Atg13 (Kamada et al., 2000, 2010; Matsuura et al., 1997). Under nutrient rich conditions, TORC1 hyperphosphorylates Atg13, preventing its association with Atg1, thereby inactivating the autophagy pathway (Kamada et al., 2000, 2010). Under nutrient poor conditions, TORC1 becomes inactivated, leading to the de-phosphorylation and association of Atg13 with Atg1, which results in the activation of Atg1 kinase activity (Cheong et al., 2008; Funakoshi et al., 1997). The Atg1-Atg13 complex then interacts with Atg17, Atg29, and Atg31, autophagy proteins specific for starvation-induced autophagy. Together, these proteins form a ternary complex important for the formation of the pre-autophagosomal structure (PAS) or isolation membrane (IM) (Cheong et al., 2008; Kawamata et al., 2008).

The *C. elegans* ortholog of Atg1 is UNC-51, and its mammalian counterparts are the UNC-51-like kinases 1 and 2 (ULK1 and ULK2) (Kuroyanagi et al., 1998). Irrespective of nutrient conditions, ULK1/2 interacts with ATG13, FIP200 (mammalian ortholog of Atg17), and ATG101, to form the ULK1/2-ATG13-FIP200-ATG101 complex (Hosokawa et al., 2009b; Mercer et al., 2009). In *C. elegans*, only three members of the Ulk1/Atg1 induction complex have been characterized: UNC-51, EPG-1 (ortholog of Atg13/ATG13), and EPG-9 (ortholog of ATG101) (Liang et al., 2012; Ogura et al., 1994; Tian et al., 2009). UNC-51 was discovered as a neuronal specific protein important for axon elongation, and mutations in the *unc-51* gene result in an uncoordinated phenotype (McIntire et al., 1992; Ogura et al., 1994). UNC-51 is essential for autophagy, as *unc-51* mutants have an accumulation of GFP::LGG-1, the marker for autophagosomes, and have several phenotypes associated with defective autophagy (Meléndez et al., 2003; Tian et al., 2009). EPG-1 and EPG-9 were identified in forward genetic screens for genes involved in the degradation of autophagy specific cargo, such as germline P granules, germ cell specific protein aggregates (Schisa et al., 2001; Tian et al., 2009). EPG-9 directly interacts with EPG-1, and defects in either *epg-1,* or *epg-9* function, result in the formation of fluorescently labeled GFP::LGG-1/LC3 foci, or SQST-1::GFP foci, in embryos or larvae, indicating defects in autophagy.

3.3. Vesicle nucleation

Vesicle nucleation requires the class III PI3K (PI3KC3) complex, which consists of Vps34, Atg6/Vps30, and Vps15 (Stack et al., 1993). In yeast, Vps34 is the sole class III PI3-kinase in yeast, and synthesizes phosphatidylinositol (3)-phosphate (PI3P) via phosphorylation of phosphatidylinositol (Schu et al., 1993). Vps15, a serine/threonine kinase, is essential for the phosphorylation and activation of Vps34 (Herman et al., 1991; Stack et al., 1993), and Atg6/Vps30 acts as a scaffold protein that binds to additional proteins and regulates the activity of the core PI3KC3 complex. Atg6/Vps30 can interact with either Atg14 or Vps38, to form distinct subcomplexes (Kametaka et al., 1998; Kihara et al., 2001), where Atg14 confers specificity toward autophagy in complex I, while Vps38 confers specificity toward vacuolar protein sorting (endocytic trafficking to the vacuole) in complex II (Kametaka et al., 1998; Kihara et al., 2001). During autophagy induced conditions, Atg14 is responsible for the localization of complex I to the pre-autophagosomal structures (PAS), resulting in PI3P production, and further localization of autophagy proteins to the developing autophagosome (Kametaka et al., 1998; Obara et al., 2006). One of these proteins includes Atg18, which recycles Atg9, and recruits autophagy proteins needed for autophagosome elongation (Nair et al., 2010).

Vesicle nucleation in mammals is highly regulated. The core PI3KC3 machinery in mammals includes VPS34, p150 (ortholog of Vps15), and BECN1 (ortholog of Atg6/Vps30) (Volinia et al., 1995). BECN1 forms distinct complexes with ATG14L/BARKOR (ortholog of Atg14) and UVRAG (ortholog of Vps38) (Itakura et al., 2008). However, distinct from yeast, the ATG14L/BARKOR-containing and UVRAG-containing PI3K complexes in mammals, regulate both autophagy and endocytosis (Morris et al., 2015). Furthermore, additional regulators of the PI3KC3 complex have been identified in mammals, such as BIF-1 (BAX interacting factor-1), RUBICON (RUN domain and cysteine rich domain containing Beclin 1-interacting protein), and AMBRA1 (Activating molecule in Beclin 1-regulated autophagy 1), all of which directly bind to BECN1 (Fimia et al., 2007; Matsunaga et al.,

2009; Takahashi et al., 2007). BECN1 itself was discovered as a binding partner of the anti-apoptotic protein, B-cell lymphoma 2 (BCL-2), which negatively regulates autophagy (Liang et al., 1999).

In *C. elegans*, components of the core PI3KC3 complex were identified based on sequence similarities to their mammalian and yeast counterparts (Meléndez et al., 2003; Roggo et al., 2002). As in yeast and mammals, BEC-1 (ortholog of BECN1/Atg6/Vps30) physically associates with VPS-34 (ortholog of Vps34/PI3K), and together both are important for PI3P production, normal autophagy function, and phenotypes associated with normal autophagy function, such dauer morphogenesis, lipid homeostasis and longevity (Hansen et al., 2008; Meléndez et al., 2003; Roggo et al., 2002; Ruck et al., 2011; Takacs-Vellai et al., 2005; Zhao et al., 2009). BEC-1 physically interacts with CED-9, the *C. elegans* ortholog of mammalian BCL-2 (Takacs-Vellai et al., 2005). The ortholog of Atg14, EPG-8 is required for the degradation of autophagy substrates, and binds to BEC-1 through its coiled coil domain (Yang and Zhang, 2011). In *C. elegans*, orthologs for AMBRA or RUBICON have not been found on the basis of sequence similarity, however an ortholog for UVRAG exists, but has not been studied. Also, there are two paralogs for Atg18, ATG-18 and EPG-6, both WD40 repeat PtdIns(3)P-binding proteins, which play essential but distinct roles in the formation of autophagosomes (Lu et al., 2011).

3.4. Vesicle elongation

Vesicle elongation and completion of the double-membrane autophagosome requires two ubiquitin-like conjugation systems, Atg8/LC3B/LGG-1 and Atg12/ATG12/LGG-3 (Geng and Klionsky, 2008). Both Atg12/ATG12/LGG-3 and Atg8/LC3B/LGG-1 are classified as ubiquitin-like proteins due to their structural similarities to ubiquitin (Hanada et al., 2007; Kumeta et al., 2010; Matsushita et al., 2007; Suzuki et al., 2005). In yeast, Atg12 is conjugated to Atg5, by an Atg7 E1-like enzyme and Atg10 E2-like conjugating activity (Hanada et al., 2007; Kim et al., 1999; Kuma et al., 2002; Matsushita et al., 2007; Mizushima et al., 1998; Shintani et al., 1999). Interestingly, Atg12-Atg5 conjugation does not require the activity of an E3-like ubiquitin ligase, since Atg10 can directly mediate the conjugation reaction (Yamaguchi et al., 2012). Atg5 interacts with the small alpha helical protein, Atg16, which self-associates to form a coiled-coil dimer (Fujioka et al., 2010; Matsushita et al., 2007). Each monomer of Atg16 interacts with an Atg5-Atg12 conjugate, resulting in formation of an Atg5-Atg12-Atg16 heterohexamer complex (Kuma et al., 2002). Atg16 is required for proper localization of the Atg5-Atg12 conjugate to the pre-autophagosomal structures (PAS) (Hanada et al., 2007; Romanov et al., 2012). Moreover, the Atg5-Atg12-Atg16 complex acts as an E3-like enzyme for the Atg8-Phosphatidylethanolamine (PE) conjugation reaction (Hanada et al., 2007).

The conjugation of Atg8 to PE requires the cleavage of the carboxy terminus of Atg8, by the cysteine protease Atg4, which exposes a glycine residue (Kirisako et al., 2000). This cleavage event results in the formation of a cytosolic form of Atg8, known as Atg8-I (Kabeya et al., 2000; Kirisako et al., 2000). Atg8-I is then processed by Atg7, which in addition to the Atg5-Atg12 conjugation reaction, acts as the E1-like activating enzyme for Atg8 lipidation (Ichimura et al., 2000). However, rather than Atg10 functioning as the E2-like conjugating enzyme, Atg3 is the specific E2-like conjugating enzyme for Atg8 conjugation (Ichimura et al., 2000). Activation of Atg3 conjugation activity is mediated by the Atg5-Atg12-Atg16 complex, which enhances the transfer of the glycine residue of Atg8, from Atg3, to PE (Hanada et al., 2007; Kirisako et al., 1999; Sakoh-Nakatogawa et al., 2013). This results in the lipidated/membrane bound form of Atg8 (Atg8-PE), which associates with the inner and outer membrane of the autophagosome (Kirisako et al., 1999, 2000). On the outer membrane of the autophagosome, Atg8 is deconjugated and recycled by Atg4, a process important for autophagosome biogenesis and maturation (Kirisako et al., 2000; Nair et al., 2012; Nakatogawa et al., 2012; Yu et al., 2012). Atg8 functions in regulating membrane expansion and autophagosome size, inferred from its ability to induce the hemifusion of membranes labeled with PI3P (Nakatogawa et al., 2007; Xie et al., 2008). Additionally, Atg8 also serves as an additional regulator of protein recruitment to the autophagosome, by binding to proteins that contain an Atg8-family interacting motif (AIM), such as Atg1/ULK1, Atg3, or Atg7 (Behrends et al., 2010; Nakatogawa et al., 2012; Noda et al., 2010). AIM motifs contain the consensus sequence, WXXL, where either X is an acidic residue (Johansen and Lamark, 2011; Noda et al., 2010). AIM motifs are also located on cargo adaptor proteins, such as Atg19 in the cytoplasm-to-vacuole targeting (*cvt*) pathway, or Atg32 in mitophagy, which are attached to cargo destined for degradation (Chang and Huang, 2007; Kondo-Okamoto et al., 2012; Noda et al., 2008, 2010; Shintani et al., 2002). Thus, Atg8 links cargo to the autophagosome by binding to the AIM motif on adaptor proteins (Noda et al., 2010).

All members of the two conjugation complexes have been identified in *C. elegans* (Meléndez et al., 2003; Tian et al., 2010; Wu et al., 2012; Zhang et al., 2013). LGG-1 and LGG-2 are both orthologs of yeast Atg8; however, LGG-1 is closely related to mammalian GABARAP, while LGG-2 is closely related to mammalian LC3 (Alberti et al., 2010; Manil-Segalen et al., 2014). Both LGG-1 and LGG-2, which like in yeast and mammals, are found in cytosolic and membrane bound forms, bind to proteins containing a LIR/AIM (LC3-interactive region/ Atg8-interactive motif), and function in distinct steps of the autophagy pathway (Alberti et al., 2010; Manil-Segalen et al., 2014; Wu et al., 2015). LGG-1 functions in the formation of the autophagosome, and both, LGG-1 and LGG-2, function in autophagosome-lysosome fusion (Manil-Segalen et al., 2014; Wu et al., 2015). LGG-1 acts upstream of LGG-2, and is required for the recruitment of LGG-2 to autophagosomes, allowing for autophagosome fusion with the late endosome/lysosome to occur (Manil-Segalen et al., 2014; Wu et al., 2015). In *C. elegans*, there are also two paralogs for the Atg4 cysteine protease, ATG-4.1 and ATG-4.2 (Wu et al., 2012). As in yeast, processing by ATG-4.1 or ATG-4.2 generates LGG-1-I, which is conjugated to phosphatidylethanolamine via the sequential activity of ATG-7 and ATG-3, to generate LGG-1-II. The levels of both forms of LGG-1-I and LGG-1-II can be monitored, based on their differences in molecular weight. LGG-2 has been less characterized. In contrast to LGG-1, LGG-2 is not essential for development and fertility (Alberti et al., 2010; Manil-Segalen et al., 2014; Meléndez et al., 2003). Although, both LGG-1 and LGG-2 can be used to monitor autophagy levels and autophagic flux, this type of analysis has to be done with much caution, as has been previously discussed (Klionsky et al., 2016; Palmisano and Meléndez, 2016; Zhang et al., 2015).

3.5. Cargo recognition

The process of autophagy was once considered to be mainly non-selective, removing non-specific protein aggregates and organelles from the cell. Evidence has accumulated suggesting that autophagy is a very selective process, with specific forms of autophagy present, such as aggrephagy (degradation of protein aggregates), pexophagy (degradation of peroxisomes), mitophagy (degradation of mitochondria), etc (Johansen and Lamark, 2011; Rogov et al., 2014). This selectivity depends on molecular tags that bind to cargo destined for degradation, and also depends on the receptor proteins that bind to both the tag and the autophagosome used to transport the cargo (Kirkin et al., 2009; Rogov et al., 2014).

In yeast, several types of cargo adaptor proteins exist that recognize specific types of cargo. The cytoplasm-to-vacuole targeting (*cvt*) pathway is a selective form of autophagy found in yeast that delivers prApe1 (precursor Aminopeptidase 1) and Ams1 (α-mannosidase 1) to the vacuole (Hutchins and Klionsky, 2001; Klionsky et al., 1992; Klionsky and Ohsumi, 1999; Scott et al., 2001). Atg19 is the cargo adaptor protein for prApe1 and Ams1 (Scott et al., 2001). Yeast mitophagy and pexophagy require the adaptor proteins, Atg32 and Atg36, respectively

(Kanki et al., 2009; Motley et al., 2012). In pexophagy, Atg36 recognizes peroxisomes by binding to the peroxisomal membrane protein, Pex3, and in mitophagy, Atg32 is localized to the mitochondrial outer membrane (Kanki et al., 2009; Motley et al., 2012). In the process of aggrephagy, polyubiquitinated proteins are recognized by the cargo adaptor protein Cue5 (Lu et al., 2014).

Many of the specific forms of autophagy found in yeast exist in mammals, and most require adaptor proteins to sequester specific cargo into the autophagosome. p62/SQSTM1 was identified as a 62-kDa protein that binds to the src homology 2 (SH2) domain of $p56^{lck}$, a tyrosine kinase found in T-cells (Joung et al., 1996; Park et al., 1995). p62/SQSTM1 contains a ubiquitin-binding UBA domain, which binds to K48-linked and K63-linked ubiquitin chains (Long et al., 2008; Vadlamudi et al., 1996; Wooten et al., 2008). Cytoplasmic protein inclusions, such as Lewy bodies, neurofibrillary tangles, Huntington aggregates, etc., are commonly polyubiquitinated and bound to p62/SQSTM1 (Bjorkoy et al., 2005).

3.6. Fusion of autophagosomes with lysosomes

The proper fusion of autophagosomes with lysosomes and the resulting degradation step has to occur for autophagic flux. In general, autophagosomes are delivered to endosomes/lysosomes along microtubules and actin filaments (Kochl et al., 2006; Wu et al., 2015). In addition to its degradative role, the lysosome plays a central role in the release and recycling of the macromolecules produced from the degradation process (Yang et al., 2006). Lysosomes contain various transporters, such as amino acid transporters, on their membrane, which allows for digested molecules to enter into the cytosol (Liu et al., 2012; Rebsamen and Superti-Furga, 2016). Additionally, lysosomes can release digested material by fusing with the plasma membrane, in a process called lysosomal exocytosis (Samie and Xu, 2014). Highlighting the importance of the lysosome, aberrant lysosomal function results in defects in cell homeostasis, which can contribute to lysosomal storage diseases, such as Niemann-Pick disease or Mucolipidosis Type IV (Pryor et al., 2006; Sokol et al., 1988). The mechanism of autophagosome fusion with the vacuole/lysosome is still not fully understood; however, fusion events are thought to occur in four general steps: 1. Priming, 2. Tethering, 3. Docking, and 4. Fusion (Wickner, 2010).

In *C. elegans*, the mechanism of autophagosome-lysosome fusion has been well characterized. Upon completion of autophagosome formation, LGG-1 recruits LGG-2, which in turn binds to VPS-39, a subunit of the HOPS complex (Djeddi et al., 2012; Manil-Segalen et al., 2014). The HOPS complex is also recruited to lysosomes via interaction with ARL-8 (Nakae et al., 2010; Sasaki et al., 2013). VPS-39, found on autophagosomes and endosomes/lysosomes, recruits the RAB-7 protein, which allows EPG-5 to localize to autophagosomes (Wang et al., 2016). EPG-5 promotes the formation of a *trans*-SNARE complex containing STX-17, SNAP-29, VAMP-7/8, which then facilitates autophagosome-lysosome fusion (Djeddi et al., 2012; Manil-Segalen et al., 2014; Wang et al., 2016). A recent report shows that the GTPase RAB-10 is required for autophagic flux. Although the mechanism of action for RAB-10 is not clear, the GTPase cycling activity of RAB-10 was shown to be important (Palmisano et al., 2017).

3.7. Amphisomes in autophagy function

Autophagosomes can fuse with endosomes, forming a hybrid organelle generally known as an amphisome, before fusing with the lysosome (Gordon and Seglen, 1988). The main endocytic compartment that contributes to amphisome formation is the multivesicular body (MVB), a late endocytic vesicle composed of intraluminal vesicles (Gonzalez et al., 2017). The existence of amphisomes has not been documented in yeast, even though they contain MVBs, suggesting that amphisome formation is transient in yeast, or that amphisomes evolved later in higher eukaryotes.

In *C. elegans*, amphisomes can be fluorescently labeled with the GFP::LGG-1 reporter, and reporters for the ESCRT subunits, VPS-27 or VPS-32 (Djeddi et al., 2012; Manil-Segalen et al., 2014). Colocalization between the GFP::LGG-1 and the ESCRT subunit reporter indicates the presence of an amphisome, as there are no specific reporters for amphisomes. Additionally, loss of the autophagy protein, EPG-5, results in the accumulation of amphisomes containing GFP::LGG-1 and mCherry::RAB-7 or RFP::RME-1 (marker for basolateral recycling endosomes) (Wang et al., 2016), suggesting that autophagosomes can fuse with recycling endosomes. In *D. melanogaster*, vesicles in fat body cells are found to contain both GFP-Atg8a and Texas Red-labeled Hrs/Vps27 (Rusten et al., 2007). In addition, amino acid starvation leads to a significant increase in the number vesicles co-labeled with Atg8a and Rab11 in *D. melanogaster* fat body cells (Szatmari et al., 2014).

Highlighting the importance of amphisome formation for autophagy, defects in the endocytic machinery can adversely affect autophagy function. In mammals, loss of various subunits part of the ESCRT complex, or loss of Rab11, hinders autophagosome fusion with multivesicular bodies, resulting in defects in the autophagic degradation of cargo, such as Huntington aggregates (Fader et al., 2008; Filimonenko et al., 2007; Oshima et al., 2016; Szatmari et al., 2014). In *C. elegans*, impairment of the ESCRT machinery increases the number of GFP::LGG-1 and GFP::SQST-1-positive foci (Djeddi et al., 2012). Interestingly, the increase in GFP::LGG-1 and GFP::SQST-1-positive foci was interpreted to be an increase in autophagy activity, since there was no defect in the lysosomal cleavage of the GFP fragments from these reporters (Djeddi et al., 2012). One caveat to this interpretation is that autophagosomes themselves can become acidic, via lysosomal fusion, and can therefore cleave GFP (Stromhaug and Seglen, 1993). In summary, amphisomes are a critical component of the autophagy pathway, and are necessary for the proper degradation of autophagic cargo. Whether amphisome formation is important for autophagy-mediated processes in every cellular context, remains to be investigated. One developmental process that was shown to require amphisome formation is that of allophagy- the autophagic degradation of spermatozoid-inherited organelles.

4. Section 2: Autophagy in *C. elegans* development

C. elegans is a free-living self-fertilizing hermaphrodite nematode species. Males occur spontaneously at a very low frequency and can fertilize hermaphrodites to produce cross progeny. Autophagy functions in several biological processes during *C. elegans* development. The first documented role for autophagy in any metazoan was that of its role in *C. elegans* remodeling during dauer arrest or diapause (Meléndez et al., 2003). Basal levels of autophagy are also required during embryogenesis to remove aggregate-prone proteins (Tian et al., 2010; Zhao et al., 2009), paternal mitochondria and spermatid-specific membranous organelles (MOs) (Al Rawi et al., 2011; Sato and Sato, 2011). During larval development, autophagy modulates miRNA-mediated gene silencing and can regulate cell fate specification (Gibbings et al., 2012; Zhang and Zhang, 2013). Basal levels of autophagy were also recently shown to act cell non-autonomously to promote the proliferation of the germline stem cells (Ames et al., 2017), and cell autonomously in synapse development (Stavoe et al., 2016). Autophagy is required for longevity in several lifespan extension paradigms and is involved in the utilization of lipid droplets during aging (Lapierre et al., 2011), as well as in lipid homeostasis during development (Lapierre et al., 2013b). Studies into the role of autophagy in *C. elegans* development may provide insights as to the physiological roles of autophagy in development of multicellular organisms, including humans.

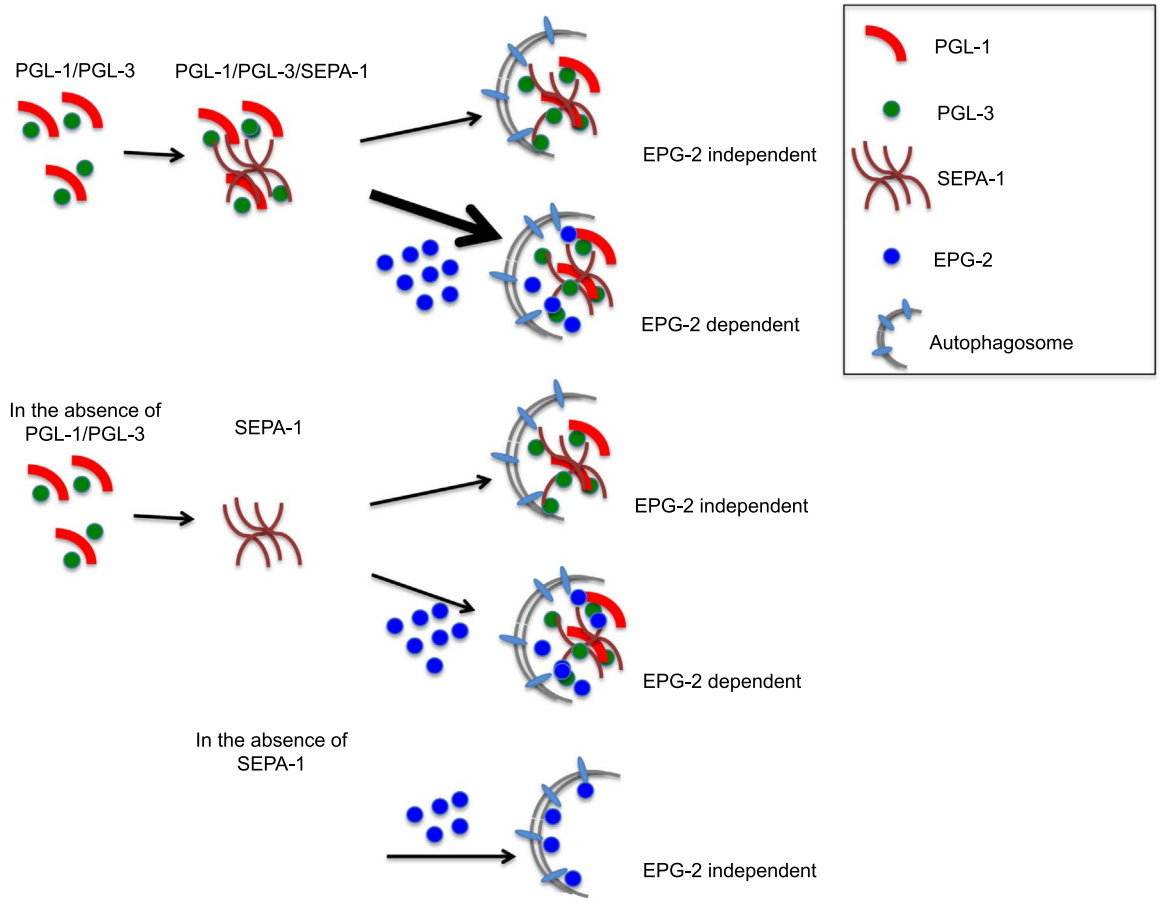

Fig. 2. Aggrephagy in *C. elegans*. A hierarchical recruitment of receptor and scaffold proteins triggers selective degradation of protein aggregates and degradation of PGL granules. The degradation of PGL granules, PGL-1 and PGL-2 are recruited into SEPA-1 aggregates, which associate with multiple ATG proteins. The PGL-1-PGL-2-SEPA-1 complex associates with the scaffold protein, EPG-2. PGL-1, PGL-3, and SEPA-1, are mainly degraded by EPG-2 mediated autophagy. In the absence of PGL-1/PGL-3, SEPA-1 can be degraded by EPG-2, independent of autophagy. Depletion of SEPA-1 activity facilitates the removal of EPG-2.
Figure adapted from figure 4 in Zhang et al. (2017).

4.1. Removal of aggregate-prone proteins in embryonic development

In a newly fertilized embryo, maternally loaded P granules, specialized protein-RNA aggregates, are dispersed throughout the cytoplasm. A series of asymmetric divisions result in the localization of the P granules exclusively in the germline blastomeres P1, P2, P3, and P4, and then finally in two germline precursor cells, Z2 and Z3, which are derived from P4 (Strome, 2005). P granule components, PGL-1 and PGL-3, are partitioned into somatic cells during early cell divisions, and are selectively removed by autophagy, by binding to adaptor proteins, such as SEPA-1 (Zhang et al., 2009b) (Fig. 2). Thus, in autophagy mutants, PGL-1 and PGL-3 co-localize and accumulate in somatic cells, as part of PGL granules. The degradation of P granules highlights the importance of autophagy in the selective removal of protein aggregates.

Genetic screens to identify genes important for the degradation of P granules have identified many conserved autophagy genes, demonstrating the high conservation of the autophagy machinery between yeast and *C. elegans* (Tian et al., 2010; Zhang et al., 2009b). However, these screens have also identified a number of higher eukaryote-specific genes that had not been identified previously in yeast. For example, *epg-2, epg-3, epg-4,* and *epg-5*, for ectopic protein granules, encode autophagy components that are absent in the yeast genome, but exist in higher eukaryotes (Tian et al., 2010; Zhang et al., 2009b).

The degradation of PGL granules by autophagy is mediated by receptor and scaffold proteins, which confer cargo selectivity and degradation efficiency (Fig. 2) (Johansen and Lamark, 2011). These proteins include the receptor proteins, SQST-1 for Sequestome-related protein, and SEPA-1, and the scaffold protein EPG-2 (Tian et al., 2009; Zhang et al., 2009b). SEPA-1 acts as a receptor for the formation of PGL-1 and PGL-3 granules and for their degradation (Zhang et al., 2009b). SEPA-1 directly interacts with PGL-3 and the autophagy protein LGG-1 through its LC3-interactive motif (LIR)(Zhang et al., 2009b). However, SEPA-1 can form aggregates independently of PGL-1 and PGL-3, and is degraded by autophagy also independently of PGL-1 and PGL-3, during embryogenesis (Zhang et al., 2009b). Thus, SEPA-1 acts as a receptor that recruits PGL-1 and PGL-3 into aggregates for degradation by autophagy.

The scaffold protein EPG-2 also binds to embryonic P granules to facilitate their degradation by autophagy (Tian et al., 2009; Zhang et al., 2009b). EPG-2 itself forms aggregates and is degraded by autophagy, however this process is independent of PGL-1, PGL-3 and SEPA-1 (Tian et al., 2010). Thus, the degradation of PGL-1, PGL-3, SEPA-1 and EPG-2 occurs in a linear hierarchical order in wild-type embryos (Tian et al., 2010; Wu et al., 2015; Zhang et al., 2017). A recent report shows that by altering the levels of each component in the PGL-1-PGL-3-SEPA-1-EPG-2 complex, the efficiency of degradation can be significantly altered (Zhang et al., 2017). In *epg-2* mutants, PGL-3 accumulates in large aggregates, however these PGL-3 aggregates are smaller and less frequent in *epg-2;pgl-1* double mutants, suggesting that the loss of PGL-1 renders the degradation of PGL-3 more efficient, and at least partially independent of EPG-2 (Zhang et al., 2017). EPG-2, similarly to SEPA-1, binds to LGG-1 via its LC3-interactive (LIR) motifs (Wu et al., 2015). EPG-2 and SEPA-1 have a direct interaction that is essential for the degradation of PGL granules (Zhang et al., 2017). In *epg-2* mutants, LGG-1 and SEPA-1 aggregates

are separable, however the co-localization of LGG-1 and SEPA-1 increased in *epg-2;pgl-1* double mutants, suggesting that in the absence of PGL-1 or PGL-3, degradation of SEPA-1 is also independent of EPG-2, and may occur via direct interaction with LGG-1 (Zhang et al., 2017). Thus, this report provides evidence that the efficiency of degradation by autophagy is modulated by the composition of the aggregates. A model has been proposed where in wild-type embryos, PGL-1, PGL-3 and SEPA-1 are mainly degraded via an EPG-2 mediated pathway, however an EPG-2 independent pathway is also available. In absence of PGL-1 and PGL-3, SEPA-1 can be degraded independent of EPG-2, and depletion of SEPA-1 facilitates the degradation of EPG-2. In this model, overexpression of SEPA-1 results in the accumulation of PGL-1 and PGL-3 in aggregates and impairs their degradation by the EPG-2 dependent pathway (Zhang et al., 2017). In contrast, the overexpression of EPG-2 facilitates the removal of PGL granules (Zhang et al., 2017).

SQST-1 is the *C. elegans* ortholog of p62/SQSTM1 in mammals (Tian et al., 2010). Human p62/SQSTM1 is a common component of ubiquitinated protein aggregates (Bjorkoy et al., 2006). Although a direct interaction between SQST-1 and ubiquitin has not been investigated in *C. elegans*, there is evidence that SQST-1 is functionally similar to p62/SQSTM1. SQST-1 contains a self-oligomerization domain, co-localizes with LGG-1, contains an LIR motif, and closely associates with accumulated germline P granules (Lin et al., 2013; Lu et al., 2011; Tian et al., 2010). Additionally, SQST-1 itself is degraded by autophagy, and mutations in various autophagy genes, such as *lgg-1* or *atg-9,* result in the accumulation of SQST-1::GFP aggregates in embryos and larvae (Lu et al., 2011; Wu et al., 2015; Zhang et al., 2009b). SQST-1 degradation requires the scaffold protein EPG-7, which interacts with SQST-1 and multiple ATG proteins (Lin et al., 2013). EPG-7 self oligomerizes and can be degraded by autophagy independently of SQST-1 (Lin et al., 2013). EPG-7 directly interacts with SQST-1, as well as with LGG-1, LGG-3/Atg12, ATG-9, and ATG-18 (Lin et al., 2013). Thus, PGL granules and SQST-1 aggregates are of different types, and autophagic degradation mediated by different scaffold proteins is employed to remove different aggregates. The composition and organization of PGL granules appear to be important for clearance, providing another mechanism in the regulation of aggregate degradation (Zhang et al., 2017).

The selective degradation of aggregates by autophagy is now referred to as aggrephagy (Fig. 2) (Johansen and Lamark, 2011). The specificity conferred by scaffold proteins appears to be a general mechanism employed to promote the sequestration of specific cargo/receptor complex into an autophagosome. There are now several examples of scaffold proteins for different cargo degradation: Atg11 in the yeast Cvt pathway interacts with Atg19, a receptor, to transport the prApe1 complex to the PAS (Nair and Klionsky, 2005; Shintani et al., 2002). Also in yeast, Atg11 interacts with a different receptor for mitophagy, the specific degradation of mitochondria. In this case, Atg11 interacts with Atg32, a mitochondrial outer membrane-spanning protein (Okamoto et al., 2009). For pexophagy, Atg11 interacts with PpAtg30, in the removal or peroxisomes (Farre et al., 2008). Another scaffold protein is the phosphatidylinositol 3-phosphate binding protein ALFY (autophagy-linked FYVE protein), which has been implicated in the selective degradation of ubiquitinated protein aggregates by autophagy (Filimonenko et al., 2010). Thus, key factors involved in selective autophagy of protein aggregates are the receptor and have scaffold proteins that connect the cargo- protein aggregates to the autophagy machinery. Autophagy receptors, and scaffold proteins, as well as the substrates, can also be modulated by posttranslational modifications (ubiquitination, phosphorylation, and acetylation), which adds another layer of complexity and regulation (McEwan and Dikic, 2011).

4.2. Removal of paternal mitochondria and sperm MOs

In mammals, inheritance of mitochondria and mtDNA by the offspring is strictly maternal, even if sperm inject much of its mitochondria and mtDNA into the oocyte during fertilization. Paternal mitochondrial degradation is a process that requires the lysosome in *C. elegans*. In this organism, oocytes of the hermaphrodite arrest during meiotic prophase I, and only complete meiosis after fertilization, when the fertilized oocyte moves to the uterus and begins zygotic development (Greenstein, 2005). Mature spermatozoa contain membranous organelles (MOs), mitochondria, and major sperm proteins (MSPs), which concentrate in the pseudopod of the spermatozoa and are thought to be required for sperm motility (L'Hernault, 2006; Ward et al., 1981; Washington and Ward, 2006). Upon fertilization, both MOs and sperm mitochondria enter and remain close to the sperm DNA, at the posterior pole of the embryo. Soon after fertilization, paternal mitochondria, MOs and mtDNA are destroyed by autophagy (Al Rawi et al., 2011; Sato and Sato, 2011). In animals with compromised autophagy, such as *lgg-1* or *lgg-2* RNAi depleted animals, paternal mitochondria and MOs visibly persist until late embryonic stages (Al Rawi et al., 2011; Sato and Sato, 2011). Mutations in *atg-13, bec-1, lgg-1* and *lgg-3,* genes involved in the induction, vesicle nucleation, and vesicle elongation steps of the autophagosome formation, all significantly delayed the removal of paternal mitochondria in fertilized eggs. Depletion of other autophagy proteins, such as ATG-7, the E1-like activating enzyme required for LGG-1 activation, or RAB-7, the small GTPase protein involved in the fusion between autophagosomes and lysosomes, also resulted in the persistence of paternal mitochondria, and MOs, providing evidence that autophagic degradation is required after fertilization (Al Rawi et al., 2011; Sato and Sato, 2011). More evidence was provided, when LGG-1 and LGG-2 positive structures were found to cluster around sperm DNA, 15–30 min post fertilization (Al Rawi et al., 2011). Antibodies against LGG-1 and LGG-2 recognized positive structures clustered around the MOs and paternal mitochondria, and this was corroborated by electron microscopy, indicating that selective uptake of mitochondria by autophagosomes occurs to degrade MOs and paternal mitochondria.

A GFP::ubiquitin reporter and antibodies that recognize ubiquitin were used to determine that MOs are ubiquitinated after sperm enter the oocyte, however sperm mitochondria are not ubiquitinated (Al Rawi et al., 2011; Sato and Sato, 2011). Whether ubiquitination is part of the mechanism for engulfment and degradation in this context remains to be shown. Interestingly, antibodies against two autophagy components showed that autophagy proteins are also recruited to mitochondria in mouse spermatozoa, after fertilization, suggesting that this degradation of paternal mitochondria may be conserved in mammals (Al Rawi et al., 2011).

4.3. Removal of apoptotic cell corpse during development

In certain contexts, autophagy has been shown to be required for engulfment and degradation of apoptotic cell corpses. Clearance of apoptotic cells is essential for metazoan development and tissue homeostasis, as defects in clearance can contribute to inflammation and autoimmunity (Elliott and Ravichandran, 2010; Wickman et al., 2012). Phosphatidylserine (PtdSer) is a lipid that resides exclusively in the inner leaflet of the plasma membrane of healthy cells, but is exposed on the outer leaflet of the apoptotic cell membrane. As part of an “eat-me” signal, PtdSer signals to neighboring cells, or specialized phagocytes, to engulf and degrade the dead cell corpse (Wang et al., 2003). Phagocytes use several receptors that recognize PtdSer or extracellular PtdSer-binding molecules (Hanayama et al., 2004; Park

et al., 2009). Two partially redundant, and evolutionarily conserved, pathways act in the engulfment and degradation of apoptotic cell corpses (Lettre and Hengartner, 2006; Reddien and Horvitz, 2004). One pathway consists of CED-1, CED-6, CED-7 and DYN-1 and is required for the membrane expansion of pseudopods during engulfment and for the degradation inside phagosomes, and the second consists of CED-2, CED-5, and CED-12 proteins, and is required for cytoskeletal reorganization during corpse removal. The nascent phagosome, containing the apoptotic cell corpse, undergoes a stepwise maturation process that involves the dynamic coating of PdtIns(3)P, the sequential recruitment of RAB GTPase proteins, including RAB-5 and RAB-7, and the vacuole protein sorting (HOPS) complex, leading to the formation of phagolysosomes for degradation (Kinchen et al., 2008; Lu and Zhou, 2012; Zhou and Yu, 2008).

In the clearance of apoptotic cell corpses during cavitation in mouse embryoid bodies, genetic deletion of either *Atg5* or *Becn1* was reported to result in the persistence of apoptotic cells and failure to generate the cellular ATP required for PS exposure (Qu et al., 2007). Similarly, during chick retinal development, autophagy proteins contribute to neuronal development by providing energy for cell corpse removal after physiological cell death (Mellen et al., 2008). In both contexts, treatment with methylpyruvate, a cell-permeant substrate for ATP, restored the failed PS exposure. Thus, autophagic machinery was required to provide ATP for PS exposure and the engulfment of apoptotic cell corpses (Mellen et al., 2008; Qu et al., 2007).

In mammals, LC3- associated phagocytosis (LAP) (LC3, microtubule associated protein 1A/1B-light chain 3 is the ortholog of *C. elegans* LGG-1 and yeast Atg8) is a form of non-canonical autophagy where LC3 is conjugated to phagosome membranes using some of the autophagy molecular machinery (Martinez et al., 2011; Sanjuan et al., 2007). LAP was identified as distinct from classical autophagy since LAP lacks the double-membrane vesicle characteristic of classical autophagy (Sanjuan et al., 2007). LAP in microphages, professional phagocytes, is crucial for the clearance of extracellular particles (internalized by the Toll-like receptor 3, TLR3), as well as apoptotic cells or pathogens. Cell corpses generated by different types of cell death, such as apoptosis, necrosis, or even entotic vesicles generated by entosis, can trigger LAP (Florey et al., 2011; Martinez et al., 2011; Sanjuan et al., 2007). LC3 recruitment depends on the PtdIns(3)P-generating nucleation complex, consisting of BECN1 and VPS34, and the two conjugation systems (ATG5 and ATG7), but not the ULK1 complex, which is important for autophagy activation (Martinez et al., 2011; Sanjuan et al., 2007). Similarly, TOR signaling is probably not involved, as rapamycin had no effect (Sanjuan et al., 2007). Binding of a cell corpse via receptors present on the macrophage surface leads to the translocation of autophagy proteins to the phagosome and LC3 conjugation (Martinez et al., 2011). The consequences of LAP deficiency include a reduction in the capacity to clear dying cells, and the potential for autoimmune responses (Heckmann et al., 2017; Savill et al., 2002).

During embryonic development, in *C. elegans*, loss-of-function mutations in autophagy genes acting at distinct steps of the autophagy machinery, including components of the UNC-51/ULK1 complex (UNC-51, EPG-1/ATG13, the nucleation VPS-34 complex (VPS-34, BEC-1, EPG-8), the two conjugation systems (EPG-4, EPG-6, LGG-1, ATG-3, ATG-5, ATG-7, ATG-10, ATG-4.1, ATG-4.2), the retrieval step (ATG-18, ATG-2), and degradation (EPG-5), were all shown to be required for efficient apoptotic cell corpse clearance (Cheng et al., 2013; Huang et al., 2013). The fact that autophagy proteins involved at all steps of the autophagy machinery are required for cell corpse degradation suggests that the canonical autophagy pathway is involved, rather than additional functions of autophagy genes. In contrast, proteins involved in the specific clearance of PGL granules (SEPA-1, EPG-2 or SQST-1), were not involved in cell corpse degradation (Cheng et al., 2013). Phagosomes containing the apoptotic corpse undergo a process of stepwise maturation, where the coating of PtdIns(3)P and recruitment of RAB proteins (including RAB-5, RAB-7, UNC-108/RAB2 and RAB-14) is required before fusion with lysosomes (Guo et al., 2010; Lu et al., 2008). Two lipid kinases, the Class III PtdIns(3)-kinase VPS-34, and the class II PtdIns(3)-kinase PIKI-1, act in parallel to regulate PtdIns3P levels on phagosomes, a step essential for corpse degradation, with VPS-34 having a more important role (Kinchen et al., 2008; Lu et al., 2012). In autophagy mutants, time-course analysis of cell corpses and markers that associate with phagosomes, indicated that engulfment was not affected, nor was PS exposure, however phagosome maturation was impaired (Cheng et al., 2013; Huang et al., 2013). PtdIns(3)P levels were reduced and the association of RAB-5 and RAB-7 with the phagosome was decreased. In summary, genetic and cell biology analyses showed that autophagy proteins act in the same pathway as VPS-34, but in parallel to PIKI-1, to regulate PtdIns3P levels on phagosomes (Cheng et al., 2013). Two models have been proposed, one dictates that the autophagy machinery mediates in part the regulation of PtdIns3P in the phagosomes, and modulates the formation of different VPS-34 complexes, and in a second model, PtdIns3P containing autophagosomes may directly fuse with phagosomes to promote phagosome maturation.

Although the precise mechanism by which autophagy proteins mediate efficient apoptotic cell corpse clearance is not known, other studies during *C. elegans* postembryonic stages also indicate a role in apoptotic corpse degradation, rather than engulfment. In the clearance of the neuroblast Q cell, in the L1 stage larvae, the recruitment of phagosome/lysosome markers RAB5A, RAB7A and CTNS was delayed in *atg-18* or *epg-5* mutants also suggesting a role in phagosome maturation (Li et al., 2012). In the same study, no delay was reported in *unc-51/ULK1* mutants (Li et al., 2012). In addition, Ruck et al. reported that BEC-1, ATG-18, and UNC-51/ULK1 were also required for germ cell corpse clearance in the adult gonad (Ruck et al., 2011). In this study, time-course analysis also indicated a delay in corpse degradation, which would also indicate a role for autophagy proteins in phagosome maturation.

4.4. Dauer development

C. elegans develop through four larval stages, L1-L4, before becoming sexually mature adults, and have a rapid life cycle of 3 days from the egg to adult, at 20 °C (Brenner, 1974). Wild-type animals have a mean life span of ~20 days at 20 °C and have brood sizes of ~300 progeny (Johnson and Wood 1982). In the absence of food, newly hatched larvae (L1 larvae) arrest, and remain viable for about 1–2 weeks (Munoz and Riddle, 2003). Autophagy is required for the survival of L1 animals during starvation, as RNAi of autophagy genes significantly shortens their survival (Kang et al., 2007; Lu et al., 2011; Tian et al., 2009, 2010; Wu et al., 2015; Yang and Zhang, 2011; Zhang and Zhang, 2013).

Under conditions of stress, such as high temperature, high population density, or limited food supply, young larvae (past the L1 stage), arrest at a developmentally specialized L3 larval stage, referred to as the dauer diapause (Albert et al., 1981; Cassada and Russell, 1975; Golden and Riddle, 1984b). Animals can remain as dauers for several months until normal growth conditions resume. Dauer larvae have morphological features that distinguishes them from nondauer animals, which include a thickened cuticle, the constriction and elongation of the body and pharynx, a closed mouth, arrested reproduction, and the presence of a cuticular ridge on the lateral side of the animal called alae (Cassada and Russell, 1975; Golden and Riddle, 1982, 1984a; Vowels and Thomas, 1992). Dauer animals also change their metabolism, they stop feeding, and increase the storage of lipids. The morphological and metabolic changes associated with dauer development promote survival in harsh environmental conditions, such as dehydration and starvation (Cassada and Russell, 1975; Klass and Hirsh, 1976; Vowels and Thomas, 1992). If the environmental conditions improve, dauer larvae exit diapause and resume normal devel-

opment (Cassada and Russell, 1975). *daf-2* in *C. elegans* encodes the insulin IGF-1-like receptor (IIR), and *daf-2(e1370)* temperature sensitive mutants have a dauer constitutive phenotype, when grown at the restrictive temperature (Gems et al., 1998). The dauer constitutive phenotype of *daf-2* mutants requires the activity of DAF-16/FOXO, as the removal of DAF-16/FOXO prevents dauer formation in *daf-2* insulin signaling defective animals, even in harsh environmental conditions (Gottlieb and Ruvkun, 1994; Ogg et al., 1997; Vowels and Thomas, 1992).

In experiments to determine if the cellular process of autophagy was required during dauer development, animals that carry a *daf-2* mutation were RNAi depleted against autophagy genes function (Meléndez et al., 2003). These experiments determined that autophagy was required for the remodeling that occurs during dauer development, and moreover, that dauers have high levels of autophagy, visualized by an increase in GFP::LGG-1 positive structures in hypodermal seam cells, and a higher frequency of autophagosomes detected by transmission electron microscopy (TEM) (Meléndez et al., 2003). The increase in autophagosome frequency during dauer development was interpreted as an increase in activity, since many of the genes in the process are required for the changes associated with dauer development of *daf-2* dauers (Meléndez et al., 2003). Another signaling pathway involved in dauer development is that of DAF-7/TGFβ (Ren et al., 1996; Schackwitz et al., 1996). *daf-7/TGFβ* mutants are also constitutive dauers and require the activity of autophagy genes for dauer development (Meléndez et al., 2003). One major question remaining is which tissues require autophagy to support dauer development. Although cell-autonomous and non cell-autonomous activities for DAF-2/IIR and DAF-16/FOXO have been identified in different contexts, *daf-2* mosaic analysis and tissue specific rescue has shown that neurons are crucial for the decision to enter dauer development (Apfeld and Kenyon, 1998; Wolkow et al., 2000). Similarly, DAF-16/FOXO expression in the nervous system is part of an organism-wide response required for dauer development (Libina et al., 2003). However, other tissues have to also be involved, as the physiological changes associated with the dauer phenotype occur in all tissues. Although the *C. elegans* genome encodes only one insulin/IGF-1 like receptor, there are over 40 Insulin-like ligands (ILPs) (Murphy and Hu, 2013). Thus, it has been proposed that combinations of agonistic and antagonistic ILPs function are integrated in different tissues to prevent or promote dauer formation (Hung et al., 2014).

4.5. Autophagy in synapse development

Autophagy is induced under stress conditions in most cells, however, in neurons, autophagy may function as a constitutive active process (Lee, 2012; Wong and Holzbaur, 2015; Xilouri and Stefanis, 2010). Basal levels of autophagy are required for the survival of neurons, and the specific impairment of autophagic degradation in neurons results in axon degeneration and neuronal cell death (Hara et al., 2006; Komatsu et al., 2007; Yang et al., 2013; Yue et al., 2009). Neurons are highly polarized cells, with long axons, distinct cell soma, dendrites, and presynaptic specializations. In neurons grown in culture, autophagosome biogenesis appears to be spatially compartmentalized and to occur primarily in the distal axons (Shen et al., 2015). In a genetic screen to identify pathways involved in presynaptic assembly, the autophagy protein ATG-9 was found to be required for synapse development in the AIY neurons in *C. elegans* (Stavoe et al., 2016). AIY cells are interneurons that receive and process synaptic input from amphid sensory neurons. AIY modulates behavioral plasticity in response to various sensory modalities, where starvation is paired with thermal or chemical cues (de Bono and Maricq, 2005; Ishihara et al., 2002). ATG-9 is the only integral membrane protein involved in the core machinery of the autophagy pathway (Lang et al., 2000; Noda et al., 2000; Young et al., 2006). Since mutations in several autophagy genes involved at distinct steps in the pathway, such as *epg-8, atg-5, lgg-3/ATG12, lgg-1, lgg-2, epg-3, atg-3, atg-18,* were found to exhibit similar defects to that of *atg-9* mutants, it was concluded that autophagy has a role in synaptic vesicle clustering in presynaptic specializations (Stavoe et al., 2016).

A function for autophagy was not specific for AIY neurons, because disruption of autophagy in a different neuron, the sensory neuron PVD, resulted in a higher rate of axon outgrowth (Stavoe et al., 2016). PVD neurons function in nociception, the feeling of pain, and are highly branched with many sensory dendrites (Albeg et al., 2011; Caterina and Julius, 1999; Halevi et al., 2002; Oren-Suissa et al., 2010; Smith et al., 2010). Autophagy was found to regulate the rate of axon outgrowth in PVD neurons, but not to be required for the morphology or the timing of dendritic branching (Stavoe et al., 2016).

However, autophagy was not required for general neuronal development, since many neurons (HSN, RIA, DA9, and NSM) in autophagy mutants developed normally (Stavoe et al., 2016). Importantly, autophagy gene activity was required cell autonomously to regulate presynaptic assembly and to regulate axon outgrowth. Thus, autophagy serves cell-specific roles in select neurons during development. The study by Stavoe et al. (2016) shows that autophagosome formation is also compartmentalized in neurons of living animals, and that autophagy occurs preferentially in synaptic regions of adult and embryonic animals (Stavoe et al., 2016).

Neurons in *C. elegans* form stereotypic synaptic "*en passant,*" with processes of other neurons as they run along each other (Colon-Ramos et al., 2007; White et al., 1986). The position of the synapses in AIY, including the local organization of the actin cytoskeleton, active zone localization, and synaptic vesicle clustering, is instructed by glia-derived netrins (Colon-Ramos et al., 2007; Stavoe and Colon-Ramos, 2012; Stavoe et al., 2012). Genetically compromising autophagy in AIY resulted in animals with disordered cytoskeletal structures, abnormal active zones, and mislocalized vesicles, phenotypes similar to those displayed by synaptogenic mutants, with defects in actin organization (Stavoe et al., 2016). These findings are consistent with reports in Drosophila, where autophagy is involved in the formation of neuromuscular junctions, and in vertebrates, where changes in axon length requires the degradation of cytoskeletal regulators through autophagy (Ban et al., 2013; Shen et al., 2015). In addition, in actively elongating axons, autophagosomes have been localized at the tip of axons, and found to contain cytoskeletal factors (Hollenbeck and Bray, 1987). Conversely, the induction of autophagy has also been associated with the degradation of cytoskeletal factors, and inhibition of neuron outgrowth (Chen et al., 2013b). In *C. elegans*, the loss of autophagy did not display pleiotropic defects, which would be expected if there were a general loss of cytoskeletal regulation or cellular homeostasis.

In the *C. elegans* model, the local concentration of ATG-9 may act to compartmentalize a section of the neuron where newly formed autophagosomes accumulate (Stavoe et al., 2016). ATG-9 appeared to localize to presynaptic regions, and at the tip of the growing axon. Similarly, in mammalian neurons, Atg9 accumulates in varicosities and colocalizes with synaptic proteins (Tamura et al., 2010). The synaptic vesicle kinesin UNC-104/KIF1A was found to be required for the localization of ATG-9 to the axon, presumably through the transport of vesicles containing ATG-9 (Stavoe et al., 2016). In *unc-104* mutants, impairment of ATG-9 transport resulted in a reduction in the rates of autophagosome biogenesis, and a decrease in the number of animals with autophagosomes in AIY. These findings suggest that transport of the ATG-9 integral membrane protein may directly establish the spatial compartmentalization of autophagosome biogenesis and the localization of autophagosomes to presynaptic compartments and the distal axon of neurons.

The spatial regulation of ATG-9, and autophagosome biogenesis, was proposed to restrain axon outgrowth in PVD and promote presynaptic assembly in AIY (Stavoe and Colon-Ramos, 2012). In *C. elegans*, UNC-104/KIF1A-dependent delivery of ATG-9 to the PVD growth cone is required to remodel the growth cone, presumably by

degradation of growth cone components. This is consistent with reports in cell culture and mammalian neurons, where the disruption of autophagy was shown to result in longer neuronal processes, whereas high levels of autophagy resulted in shorter processes (Ban et al., 2013; Chen et al., 2013b). In both AIY and PVD neurons, spatial regulation of autophagosome biogenesis may act to remodel subcellular structures, such as the cytoskeleton, and facilitate presynaptic formation.

Autophagy has also been implicated in post-developmental events, specifically in synaptic transmission and vesicle recycling (Binotti et al., 2015; Hernandez et al., 2012; Wang et al., 2015). In addition, autophagy may serve in the degradation of postsynaptic receptors such as inhibitory $GABA_A$ receptors, to induce synaptic long-term depression (Rowland et al., 2006). In *C. elegans*, GABA terminals arrange $GABA_A$ receptors into synaptic clusters and, in the absence of presynaptic input, $GABA_A$ receptors get internalized and degraded via autophagy (Rowland et al., 2006). Together, these findings demonstrate a role for autophagy in controlling the balance of neuronal excitation and inhibition, due to selective $GABA_A$ receptor degradation.

A very recent study reported that adult neurons in *C. elegans* can extrude large (approximately 4 μm in diameter) membrane-surrounded vesicles, referred to as exophers, which contain protein aggregates, and organelles (Melentijevic et al., 2017). Inhibition of protein degradation by disruption of autophagy, chaperone protein expression, or proteasome activity, was found to enhance exopher production and compromise mitochondrial quality. Interestingly, neurons that generate exophers appeared to function better than neurons that do not produce exophers, when proteotoxically stressed. Thus, exopher formation may be part of a response to rid cells of neurotoxic components, when proteostasis and mitochondria function are challenged (Melentijevic et al., 2017). In the last several years, there is considerable gained interest in the observation that mammalian neurons can expel protein aggregates associated with Alzheimer's, Parkinson's and prion disease. In mammals, for example, the aggregated poly Q expanded huntingtin can transfer between neurons via tunneling nanotubes, which resemble the connection between exopher and soma (Abounit and Zurzolo, 2012; Costanzo et al., 2013; Gousset et al., 2009; Nath et al., 2012). In Drosophila, poly Q is transferred to glia (Pearce, 2017; Pearce et al., 2015). Similarly in mouse, mitochondria originating in retinal ganglion cells can be shed into neighboring astrocytes for degradation (Davis et al., 2014). This transfer of mitochondria between cells, referred to as transcellular degradation, is likely to occur elsewhere in the CNS, because structurally similar accumulations of degrading mitochondria are also found along neurites in superficial layers of the cerebral cortex (Davis et al., 2014). Thus, this transfer of mitochondria from cells to cell may be a more broadly used mechanism for mitochondrial quality control and neuronal homeostasis.

The *C. elegans* neurons that jettison exophers may provide a model for a type of transcellular degradation. Once outside the neuron, protein aggregates may be taken up by neighboring cells, and this could potentially explain how disease damage can spread in the brain. Thus, exophers in *C. elegans* may provide a novel mechanism by which neurons could (1) transfer cellular material, which is particularly neurotoxic, to other cells, and (2) promote neuronal proteostasis and mitochondrial quality control.

4.6. Autophagy in cell fate specification by miRNA –mediated gene silencing

Micro RNAs (miRNAs) are noncoding RNAs that can repress gene expression posttranscriptionally, by forming either exact or not exact complementary interactions with the 3' untranslated region of of a target mRNA (Ambros, 2004, 2011). miRNA mediated gene silencing is mediated by a miRNA –induced silencing complex (miRISC), which consists of an Argonaute (Ago) protein, the miRNA and a member of the GW182 family of proteins (Ding and Han, 2007). Perfect complementary interactions between the miRNA and the target mRNA result in degradation of the target mRNA, and imperfect complementarity results in the inhibition of translation (Carthew and Sontheimer, 2009). In *C. elegans* development, miRNAs target a variety of mRNA that are involved in developmental decisions, such as miRNAs that regulate heterochronic genes and control the timing of developmental cell lineages (Ambros, 2004). *let-7* encodes an miRNA that targets the *lin-41* mRNA to control the switch from the L4 stage to adulthood (Abbott et al., 2005; Li et al., 2005; Slack et al., 2000). Several miRNAs of the family of *let-7, mir-84, mit-48,* and *mir-241*, inhibit the expression of *hbl-1* and control the development from the L2 to the L3 larval stage (Abbott et al., 2005; Li et al., 2005; Slack et al., 2000). Mutations in the miRNAi pathway, such as mutations in the argonautes *alg-1* or *alg-2,* the Dicer *dcr-1* gene, or *ain-1 and ain-2* (genes that encode the GW182 ortholog), result in a retarded developmental phenotype, referred to as a heterochronic phenotype, where the L2-stage specific division is reiterated at the L3 stage and the terminal differentiation of the late L4 stage is delayed. These animals have an increase in the number of seam cells and have incomplete alae structures, a cuticular ridge that is formed by the seam cells (Ding et al., 2005; Zhang et al., 2007). Although autophagy mutants have no heterochronic phenotypes, autophagy mutations suppress the retarded heterochronic defects associated with *lag-1, dcr-1*, or *ain-1* mutant (Zhang and Zhang, 2013). Reporters of the 3'untranslated region for *hbl-1* and *lin-41* were found to be downregulated in autophagy mutants, and this effect was found to be *let-7* dependent. In contrast, an increase in autophagy, which occurs upon TOR signaling inhibition, was found to increase the expression of the *hbl-1* and *lin-41* 3'UTR reporters. Thus, autophagy modulates miRNA-mediated cell fate specification, as it selectively removes a component of the miRISC complex, the AIN-1 protein (Zhang and Zhang, 2013).

miRNAs have also been shown to have a role in the regulation of other developmental decisions, such as vulval fate specification, or left-right asymmetry. The *let-7* family of miRNAs negatively regulates *let-60/RAS* (Zhang and Zhang, 2013). *let-60/RAS* loss-of-function mutants display a vulvaless (Vul) phenotype, and *let-60/RAS* gain-of-function mutations results in ectopic vulva-like structures, a multivulva (Muv) phenotype (Sternberg, 2005). Loss of autophagy activity similarly suppresses the *let-60/RAS* gain of function multivulva phenotype (Zhang and Zhang, 2013). However, autophagy mutants display normal development of the vulva.

Another miRNA, *lsy-6*, was found to control left-right asymmetry (Johnston and Hobert, 2003). Two ASE taste neurons ASE left and ASE right display left-right asymmetry, since *cog-1* expression is repressed in ASEL (Johnston and Hobert, 2003). *lsy-6* null mutants display a loss of the ASEL fate and the adoption of the ASE right-specific fate (Johnston and Hobert, 2003). Partial loss-of-function mutants in *lsy-6* have a similar phenotype to the null mutants, but with incomplete penetrance. Autophagy gene activity has no effect on ASEL fate specification per se, but the inhibition of autophagy activity suppresses the *lsy-6* partial loss-of-function phenotype (Zhang and Zhang, 2013). In contrast, elevated autophagy activity enhances the ASEL cell fate defect in *lsy-6* partial loss-of-function mutants (Zhang and Zhang, 2013). Thus, selective removal of miRISC components by autophagy has been shown to act in modulating miRNA activity, and miRNA-mediated cell fate specification.

4.7. Autophagy in longevity

Autophagy has been linked to normal physiological aging and with the pathogenesis of many age-related diseases (Levine and Kroemer, 2008, 2009). *C. elegans* is a great model system to study aging, as hermaphrodites will show visible signs of aging, such as slowed movement, decreased reproduction, and develop wrinkled cuticles, before they die as 2–3 week old adults. The rate of aging can be modulated by mutations that affect conserved signaling pathways, suggesting that aging is a regulated process. Mutations in the insulin-

like/IGF-1 receptor (IIR) DAF-2 signaling pathway were the first identified to control aging, as mutations in the *age-1* gene encoding phosphoinositide 3-kinase (PI3K), and *daf-2* gene encoding the insulin/IGF-1 like receptor were found to extend lifespan (Friedman and Johnson, 1988; Kenyon et al., 1993). In response to its ligand, the DAF-2/IIR receptor activates a cascade of kinases that include the class I phosphoinositide 3 kinase AGE-1, PDK, SGK, and AKT, which act to inhibit the nuclear localization of the Forkhead transcription factor DAF-16/FOXO (Lin et al., 1997; Ogg et al., 1997). In *daf-2/IIR* mutants, DAF-16/FOXO is no longer phosphorylated, and translocates to the nucleus, where it regulates the expression of many genes involved in the response to stress, bacterial resistance, and metabolism to prolong lifespan (Murphy et al., 2003).

The first demonstration that autophagy was required to prolong lifespan was reported in *C. elegans* using the *daf-2/IIR* mutants (Meléndez et al., 2003). Autophagy levels, visualized by the GFP::LGG-1 reporter, increased in the *daf-2/IIR* mutants grown at the permissive temperature, and the *bec-1* gene was shown to be required for the long lifespan of *daf-2/IIR* mutants. The autophagy genes *atg-7, atg-12*, and *lgg-1* are also required for the long lifespan of *daf-2/IIR* mutants (Hansen et al., 2008; Hars et al., 2007). Since the first demonstration of the role of autophagy in longevity, other mutations that confer an extended lifespan phenotype have been studied, and in all cases autophagy has been shown to be required (Table 2).

Dietary restriction includes caloric restriction, the reduction in total food intake, limits on specific dietary components, such as protein lipids or carbohydrates, or intermittent fasting. Despite the different protocols utilized to achieve dietary restriction, a common result is the extension of lifespan and healthspan (Greer and Brunet, 2009). One model of dietary restriction is provided by the *eat-2* mutant, which carries a mutation in an acetylcholine receptor normally expressed in pharyngeal muscles, and results in reduced food intake, and extended lifespan (Lakowski and Hekimi, 1998). Direct dilution of the bacteria fed to *C. elegans* also extends lifespan (Greer and Brunet, 2009). Autophagy activity is increased in *eat-2* mutants, and autophagy genes are required for the extension of lifespan of *eat-2* mutants (Jia and Levine, 2007; Hansen et al., 2008; Morck and Pilon, 2006; Toth et al., 2008). RNAi depletion of autophagy genes *unc-51/ULK1*, *bec-1*, *vps-34*, and *atg-7* shortened the lifespan of *eat-2* mutants, but had no significant effect on the lifespan of wild-type animals (Hansen et al., 2008; Hars et al., 2007; Jia et al., 2007; Toth et al., 2008). Dietary restriction does not further extend lifespan in animals with inhibited TOR pathway activity, suggesting that the downregulation of TOR plays an important role in the extension of lifespan by dietary restriction (Hansen et al., 2007, 2008; Vellai, 2003; Kapahi et al., 2004; Kaeberlein et al., 2005; Luong et al., 2006). Autophagy appears to be critical in the intestine to ensure lifespan extension of dietary restricted *eat-2* mutants (Gelino et al., 2016). The protein synthesis machinery, including the ribosomal S6 kinase and translation initiation factors (eIFs), which act downstream of TOR, have also been shown to extend lifespan and improve healthspan (Hansen et al., 2007). Finally, the lifespan extension induced by dietary restriction, or the inhibition of TOR, was also reported to require autophagy (Hansen et al., 2008; Luong et al., 2006; Bergamini et al., 2003). Animals carrying a mutation in the gene that encodes DAF-15/Raptor, a negative regulator of TOR, also have an extended lifespan, and also show an increase in autophagy levels and require autophagy genes for their long-lived phenotype (Hansen et al., 2008).

Signals from the reproductive system have also been shown to influence aging, as the removal of the germline precursor cells by laser ablation, or genetically, results in lifespan extension (Hsin and Kenyon, 1999). However, removal of the entire gonad (somatic and germline derived) does not extend lifespan, suggesting that specific signals from the germline and the somatic gonad affect aging in opposite ways (Hsin and Kenyon, 1999). A model for animals with an ablated germline is

Table 2
Longevity pathways that require autophagy. Conserved longevity pathways include the insulin IGF-1-like signaling, reduced TOR signaling, dietary restriction, germline ablation, and reduced mitochondrial respiration. Interactions between molecules in the pathway are not well elucidated. Several positive regulators of autophagy have been identified including the forkhead FOXO transcription factor DAF-16, the forkhead, the forkhead transcription factor PHA-4, and the Kruppel like family of transcription factors.

Longevity Signaling Pathway	Gene	Mutation	Transcription factors downstream	Tissue where autophagy is required	References
Insulin IGF-like	*daf-2/IIR*	*e1370* *e1368* RNAi	DAF-16/FOXO SKN-1/Nrf KLF-1, KLF-3 HLH-30/TFEB HSF-1	Not required in intestine.	(Chang et al., 2017; Garigan et al., 2002; Hansen et al., 2008; Hansen et al., 2005; Hars et al., 2007; Hsieh et al., 2017; Hsu et al., 2003; Kenyon et al., 1993; Lapierre et al., 2013a; Meléndez et al., 2003; Morley and Morimoto, 2004)
TOR	*let-363/TOR*	*h98* *h502* *ok3018* RNAi	PHA-4/FOXA DAF-16/FOXO SKN-1/Nrf HLH-30/TFEB		(Hansen et al., 2008; Lapierre et al., 2013a; Lapierre et al., 2011; Pan et al., 2007; Toth et al., 2007; Vellai et al., 2003)
	rsks-1/S6K1	*ok1255* *sv31*	PHA-4/FOXA DAF-16/FOXO SKN-1/Nrf HLH-30/TFEB		(Chen et al., 2013a; Hansen et al., 2007; Kapahi et al., 2004; Pan et al., 2007; Robida-Stubbs et al., 2012; Schreiber et al., 2010)
Dietary restriction	*eat-2*	*ad1116*	PHA-4/FOXA DAF-16/FOXO SKN-1/Nrf HLH-30/TFEB KLF-1, KLF-3	Intestine	(Bishop and Guarente, 2007; Carrano et al., 2014; Gelino et al., 2016; Hansen et al., 2007; Hsieh et al., 2017; Lakowski and Hekimi, 1998; Lapierre et al., 2013a)
Germlineless	*glp-1/Notch*	*e2141*	PHA-4/FOXA DAF-16/FOXO HLH-30/TFEB HSF-1	Intestine	(Chang et al., 2017; Hansen et al., 2005; Lapierre et al., 2013a; Lapierre et al., 2011; Wang et al., 2008)
Mitochondrial dysfunction	*clk-1*	*e2519 qm30*	HLH-30/TFEB		(Dillin et al., 2002; Ewbank et al., 1997; Lakowski and Hekimi, 1996; Lapierre et al., 2013a; Toth et al., 2008)
	isp-1	*qm150*			(Dillin et al., 2002; Felkai et al., 1999; Feng et al., 2001; Pujol et al., 2013; Rea et al., 2007; Toth et al., 2008)
	atp-3	*RNAi*			(Dillin et al., 2002; Rea et al., 2007)
	nuo-2	*h82* *tm5258*			(Dillin et al., 2002; Rea et al., 2007; Toth et al., 2008; Tsang et al., 2001)

provided by a loss-of-function mutation in the *glp-1/Notch* receptor gene (Arantes-Oliveira et al., 2002). *glp-1/Notch* mutants lack germline progenitors, resembling germline ablated animals, are sterile, and long-lived. *glp-1* mutants have reduced TOR levels, increased autophagy levels, and require autophagy for their increase in lifespan (Lapierre et al., 2011). Thus, the extension of lifespan in germ line ablated animals may be in part through reduced TOR signaling.

Several *C. elegans* mutants with dysfunctional mitochondrial electron transport chain are long-lived. The *isp-1* gene encodes the Rieske iron sulphur protein, of mitochondrial complex III, and *isp-1* mutants have low oxygen consumption, decreased sensitivity to ROS and increased lifespan (Feng et al., 2001). Another long-lived mutant is the mutant in *nuo-6*, a gene that encodes an NADH ubiquinone oxidoreductase of complex 1 (Pujol et al., 2013). Autophagy levels are increased in animals RNAi depleted against *isp-1* or *nuo-6*. In addition, RNAi depletion of *clk-1*, a coenzyme Q biosynthetic gene, or of *atp-3*, a gene encoding a mitochondrial ATP synthase of complex V, resulted in longer lifespan that was also dependent on the activity of autophagy genes *unc-51/ULK1*, *atg-18* or *bec-1* (Toth et al., 2008). Thus, all long-lived *C. elegans* mutants examined to date, have an increase in autophagy levels, and require autophagy genes for the extension of lifespan observed.

To investigate where autophagy is required for the longevity phenotype, a novel tandem-tagged mCherry/GFP form of LGG-1 in parallel to the GFP::LGG-1 autophagosome marker was used to assess the levels of autophagy in wild-type, as well as in *daf-2/IIR*, and *glp-1/Notch* mutants (Chang et al., 2017). These studies report an age-dependent decline in autophagic activity in the intestine, body-wall muscle, pharynx, and neurons of wild-type animals (Chang et al., 2017). Conversely, *daf-2/IIR* and *glp-1/Notch* loss-of-function mutants were shown to display unique age-dependent and tissue-specific changes in autophagic activity, indicating that the two pathways have distinct effects on autophagy during aging (Table 2) (Chang et al., 2017). Although autophagy appeared active in the intestine of both *daf-2/IIR* and *glp-1/Notch* long-lived mutants, intestinal-specific inhibition of autophagy by *atg-18* RNAi (in intestine-specific RNAi strain), was sufficient to significantly reduce the lifespan extension of *glp-1/Notch* mutants (Chang et al., 2017). In contrast, intestine specific RNAi against *atg-18* or *lgg-1* in *daf-2/IIR* mutants had no effect, whereas whole-body RNAi had an effect, as had been previously reported, suggesting that autophagy activity in the intestine of *daf-2/IIR* mutants is not as important for lifespan extension or that other tissues are more important in this mutant (Table 2).

Another longevity model is represented by the Kruppel-like transcription factors (KLFs), a subfamily of zinc finger transcriptional regulators with roles in cell survival, proliferation, metabolism, and response to stress (McConnell and Yang, 2010). Three KLF proteins exist in *C. elegans*, characterized by the presence of three Krüppel-like zinc fingers at the carboxy (C) terminus, which act to activate or repress transcription. KLFs have been shown to have roles in fat metabolism, muscle attachment, and cell survival (Zhang et al., 2002, 2011, 2009a). Two reports indicate that KLFs are involved in lifespan regulation. In a first report, KLF-1 was found to be the substrate of the HECT ubiquitin E3 ligase WWP-1 (Carrano et al., 2014). KLF-1, and WWP-1, together with the E2 ubiquitin-conjugating enzyme UBC-18, function as positive regulators of the lifespan response to dietary restriction (Carrano et al., 2014, 2009). Overexpression of *klf-1* extended lifespan, when driven by the intestine specific *ges-1* promoter (Carrano et al., 2014). More recently, a second report showed that a double knockdown of *klf-1* and *klf-3* displays a strong reduction in lifespan, and that this KLFs knockdown combination was required for the lifespan extension of several longevity mutants, in addition to dietary restriction (Hsieh et al., 2017). KLF-3 overexpression was found to extend lifespan (Hsieh et al., 2017). This report also connected the KLF-3 overexpression longevity phenotype to autophagy. Loss of function and overexpression experiments suggest that KLFs regulate the transcriptional levels of several autophagy genes. In addition, autophagy genes were shown to be required for the lifespan extension of animals with KLF-3 overexpression (Hsieh et al., 2017). Specifically, RNAi against *bec-1, atg-13, lgg-3,* or *atg-7* were found to either strongly or moderately suppress the lifespan extension of KLF-3 overexpressing animals. In addition, *atg-13* and *bec-1* also suppressed the lifespan extension of KLF-1 overexpressing animals. Consistent with this, promoter analysis of several autophagy genes revealed consensus KLF-binding elements. In summary, KLFs regulate autophagy and the effect of KLFs on lifespan depends on autophagy.

The connection between KLFs and autophagy in the regulation of aging may be conserved in mammals. Overexpression of a mammalian KLF ortholog, KLF4, increased autophagy and improved vessel function in aged mice. Consistent with a role for KLF4 in mammalian vascular aging, Klf4 expression decreases with age in isolated murine cardiac endothelial cells and in human skeletal samples (Hsieh et al., 2017). Interestingly, the decrease in Klf4 expression in the endothelium may underlie several cardiovascular diseases of aging, including hypertension, and atherosclerosis. Experiments with human umbilical vein endothelial cells (HUVECs) found strong induction of KLF4, and increased LC3-1 lipidation, after serum starvation or rapamycin treatment, which increased even further after Bafilomycin treatment, indicating enhanced autophagic flux. These recent findings point to a conserved essential role for KLF4 in the regulation of autophagy in endothelial vascular health and aging. As KLFs have been implicated with lipid transport and metabolism (Zhang et al., 2011, 2009a), and autophagy was previously shown to be involved in lipid homeostasis (see below) (Lapierre et al., 2011, 2013b), it is possible that KLFs mediate lifespan extension and endothelial vascular health through a mechanism that affects lipid metabolism.

4.8. Autophagy in lipid metabolism

Autophagy genes have a complex role in lipid metabolism, as they contribute to lipid storage and breakdown. The autophagy proteins UNC-51, BEC-1, VPS-34, and LGG-1 were found to be required for neutral lipid accumulation during development (Lapierre et al., 2013b). Interestingly, loss-of-function mutants in *daf-2/IIR* and *glp-1/Notch* have a long-lived phenotype, accompanied by an increase in lipid stores, and both phenotypes, in these long-lived mutants, require autophagy (Lapierre et al., 2011, 2013b). LIPL-4 is a triglyceride lipase conserved in humans, which functions as part of an endocrine signaling pathway coordinating reproductive capacity, fat metabolism and longevity (Lapierre et al., 2011; Wang et al., 2008). In response to reduced insulin IGF-1-like signaling, or a lack of germ line in *glp-1/Notch* mutants, *lipl-4* is expressed in the intestine to extend lifespan (Wang et al., 2008). Overexpression of LIPL-4 is sufficient to confer a long lifespan and this longevity requires DAF-16/FOXO activity as well as autophagy gene activity (Lapierre et al., 2011). Thus, the breakdown of lipids via autophagy, a process referred to as lipophagy (Singh et al., 2009a), may be a mechanism by which lifespan can be extended (Lapierre et al., 2011, 2012). The importance of lipophagy, and specifically lysosomal acid lipases, in prolonging lifespan has been highlighted by the fact that several nuclear hormone receptors (NHRs) are involved in lipid metabolism and linked to the extension of lifespan, in *C. elegans* (Wollam and Antebi, 2011). Steroid hormones, such as dafachronic acid, have been implicated in the signaling between the reproductive system and somatic tissues, via the NHR DAF-12 (most homologous to vertebrate farnesoid-X, liver-X and vitamin-D receptors) (Antebi et al., 2000; Gerisch and Antebi, 2004; Motola et al., 2006). In germline-less animals, upregulation of dafachronic acid stimulates DAF-12 dependent activation of miRNAs *mir-84* and *mir-241*, required for longevity (Antebi et al., 2000; Bethke et al., 2009). *mir-84* and *mir-241* were previously implicated in developmental timing circuits, suggesting that components of this circuit used during the L2/L3 temporal transition is co-opted to regulate lifespan (Antebi,

2015; Boehm and Slack, 2005; Gerisch et al., 2001; Shen et al., 2012). NHR-49, related to mammalian peroxisome proliferator- activated receptor (PPAR), is a key regulator of fat metabolism and lifespan, via the activation of beta-oxidation genes upon nutrient deprivation (Van Gilst et al., 2005). In addition, NHR-80 (related to mammalian hepatocyte nuclear factor 4, HNF4) is required for the longevity of germline-less animals, resulting in an increase in the expression of genes involved in lipid desaturation (Goudeau et al., 2011). While overexpression of yolk lipoprotein vitellogenin reduced the lifespan of long-lived animals by impairing autophagy and lysosomal genes, a reduction in vitellogenesis increased lifespan, via induction of autophagy and lysosomal lipid breakdown (Seah et al., 2016). This increase in lifespan due to a decrease in vitellogenesis, or enhanced lipolysis, requires the NHRs, NHR-49 and NHR-80 (Seah et al., 2016).

Omega-6 polyunsaturated fatty acids (PUFAs) promote resistance to starvation, and stimulate autophagy and longevity in conditions of food abundance, highlighting another connection between fatty acid desaturation, autophagy and longevity (O'Rourke et al., 2013). More recently, histone modifiers were shown to influence mono-unsaturated fatty acids (MUFAs) metabolism (Han et al., 2017). A model was proposed where a deficiency in the COMPASS chromatin complex (Greer et al., 2010; Shilatifard, 2012), which trimethylates lysine 4 on histone H3 (H3K4me3), results in the downregulation of specific targets in the germ line (such as the S6 kinase RSKS-1), and triggers a germ line-to-intestine signal, activation of SBP-1 (the *C. elegans* homolog of the mammalian Sterol Regulatory Element Binding Protein (SREBP), expression of delta-9-desaturases, and the accumulation of mono-unsaturated fatty (MUFAs) (Han et al., 2017). Interestingly, in this report, dietary MUFAs, and not their downstream PUFAs, extended lifespan, suggesting that MUFAs could contribute to longevity by minimizing oxidative stress, enhancing energy storage, promoting membrane fluidity, or activating a specific signaling pathway (Han et al., 2017). Noticeably, while evidence for a conserved relationship between lipid metabolism, reproduction and longevity is accumulating (Hansen et al., 2013), the underlying mechanisms for their role in cellular homeostasis, and longevity, remain not fully understood.

4.9. Autophagy in stem cell proliferation

The *C. elegans* reproductive organ consists of two U-shaped arms, where the distal end of each arm has an active stem cell population of germline nuclei that proliferates throughout development and continues to proliferate during adulthood (Kimble and Crittenden, 2005). The distal end contains the mitotic nuclei and from the Distal Tip Cell (DTC), there are approximately 22 cell diameter lengths before germline nuclei transition into meiosis and can differentiate into sperm or oocytes (Fox and Schedl, 2015; Seidel and Kimble, 2015). Although the distal germ line is a syncytium, we refer to the germline nuclei as germ cells, which have incomplete borders and are connected to one another by a central canal called the rachis. The decision of stem cells to proliferate is controlled by several signals that include the nutritional status, and the age of the animal (Hubbard, 2013). The Distal Tip Cell (DTC), a somatic cell that abuts the distal end of the gonad, serves as a niche. The DTC produces a ligand, the Delta-Serrate-LAG-2-like), which activates the GLP-1/Notch receptor on distal progenitor stem cells to promote proliferation, and inhibit differentiation (Austin and Kimble, 1987; Kimble and Crittenden, 2005). Robust expansion of the proliferative stem cell population is regulated by nutrient sensing pathways, such as the insulin IGF-1-like signaling (IIS), TGFβ signaling controlled by the TGFβ ligand DAF-7, and the S6 Kinase (RSKS-1/S6K) (Dalfo et al., 2012; Korta et al., 2012; Michaelson et al., 2010). The activity of autophagy genes was recently shown to be required for the normal accumulation of stem cells (Fig. 3)(Ames et al., 2017). Loss-of-function mutations in *bec-1, atg-16.2*, or *atg-18*, resulted in a significant reduction in the number of germline stem/progenitor cells

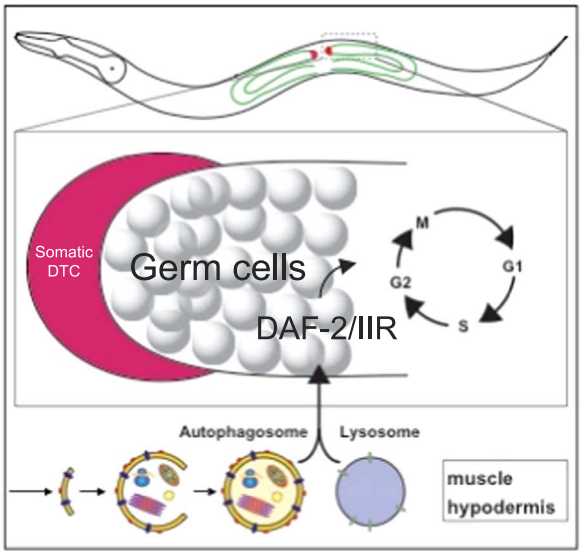

Fig. 3. Autophagy promotes the proliferation of stem cell germline progenitors. Non cell-autonomous BEC-1 mediated-autophagy is required with components of the DAF-2/IIR signaling to directly or indirectly promote cell cycle progression of mitotic cells in the germline.
Figure adapted from figure 4 in Ames et al. (2017).

in the proliferative mitotic zone (Ames et al., 2017). Interestingly, autophagy genes promote cell cycle progression, as *bec-1, atg-18* and *atg-16.2* mutants display a reduction in M phase and S phase index (Ames et al., 2017). More detailed analysis of *bec-1* germline mutants found that the reduction in M- and S-phase indices was accompanied by an extension of the G2 phase (Ames et al., 2017). Surprisingly, BEC-1 activity is required non cell-autonomously to promote germ cell proliferation, as tissue specific BEC-1 expression from a hypodermal, a muscle-specific and, to some extent, from a pan-neuronal promoter, rescued the cell cycle defects of *bec-1* mutants (Ames et al., 2017).

Several signals have been shown to act in promoting the proliferation of germline nuclei in the proliferative mitotic zone. Previous reports have shown that GLP-1/Notch signaling controls the decision to enter the differentiation program, but has no effect on the cell cycle, whereas DAF-2/IIR signaling controls the cell cycle, but has no effect on the differentiation fate, and DAF-7/TGFβ controls the balance between proliferation and differentiation (Michaelson et al., 2010). In contrast, *rsks-1/SK6* affects both the cell cycle and differentiation fate (Hubbard et al., 2013; Korta et al., 2012). In epistasis analysis, autophagy genes exhibited complex interactions with the DAF-2/IIR signaling and the DAF-7/TGFβ signaling pathway to promote cell proliferation (Ames et al., 2017). For example, BEC-1 activity was found to require DAF-18/PTEN and SKN-1/Nrf (nuclear-factor-erythroid-related factor), but independent of the DAF-16/FOXO transcription factor to promote germline proliferation. In contrast, ATG-16.2/ATG16L and ATG-18/WIPI1/2 required the DAF-16/FOXO transcription factor, and the phosphatase and tensin homolog DAF-18/PTEN. Moreover, BEC-1, ATG-16.2 and ATG-18 act independently of the DAF-7/TGFβ pathway, however ATG-7 promotes germline proliferation through DAF-7/TGFβ signaling. These surprising interactions highlight the need for a deeper understanding of the role of autophagy genes in stem cell proliferation, and suggest that autophagy proteins function through autophagy-dependent and independent mechanisms to establish a germline stem cell pool (Ames et al., 2017; Ames and Meléndez, 2017). Clearly, it is important to study mechanisms that promote stem cell proliferation in the context of a whole organism, where cell environment, cellular contacts, and cellular communication may affect the regulation and requirements for autophagy.

5. Conclusion

In conclusion, the study of autophagy in higher eukaryotes such as *C. elegans* has been enabled and empowered by the evolutionary conservation of autophagy genes and their role in the process of biogenesis and maturation of the autophagosome. *C. elegans* are an excellent model system to investigate the role of autophagy under normal physiological conditions as well as under stress. Its powerful genetics continue to reveal molecular and cellular mechanisms underlying developmental processes. Although the conservation of autophagy genes is quite strong, there are differences in the integration of signaling pathways during development. Clearly, investigating how autophagy is regulated during developmental signaling and with the integration of environmental signals, will continue to provide us with insights as to how autophagy dysfunction results in a broad range of human pathologies, such as cancer, neurodegenerative diseases, as well as in metabolic disorders.

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