Molecules and Cells

Minireview

Autophagy and Longevity

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Autophagy is an evolutionally conserved cytoplasmic degradation system in which varieties of materials are sequestered by a double membrane structure, autophagosome, and delivered to the lysosomes for the degradation. Due to the wide varieties of targets, autophagic activity is essential for cellular homeostasis. Recent genetic evidence indicates that autophagy has a crucial role in the regulation of animal lifespan. Basal level of autophagic activity is elevated in many longevity paradigms and the activity is required for lifespan extension. In most cases, genes involved in autophagy and lysosomal function are induced by several transcription factors including HLH-30/TFEB, PHA-4/FOXA and MML-1/Mondo in long-lived animals. Pharmacological treatments have been shown to extend lifespan through activation of autophagy, indicating autophagy could be a potential and promising target to modulate animal lifespan. Here we summarize recent progress regarding the role of autophagy in lifespan regulation.

Keywords: aging, autophagy, *C. elegans*, longevity, transcription factors

INTRODUCTION

Macroautophagy, hereafter referred to as autophagy, is a catabolic process targeting wide varieties of cellular contents. Autophagy occurs at basal level in normal condition, but is accelerated by varieties of stresses such as starvation, accumulation of abnormal proteins, organelle damage and pathogen infection. Autophagy was originally considered to be a bulk and non-selective degradation system. But subsequent studies show autophagy selectively degrades cargos and by doing so contribute to the intracellular homeostasis. During autophagy, a small cisterna, called isolation membrane elongates and surrounds a portion of cytoplasm to form a double-membraned structure, called the autophagosome. Autophagosomes are then transported and fuse with lysosomes to form autolysosomes for the digestion of sequestered contents (Fig. 1). During autophagy, several autophagy-related (ATG) genes are engaged sequentially in a highly regulated manner. Genetic studies in yeast have identified more than 30 ATG genes that are required for autophagy, most of which are conserved from yeast to mammals. Essential ATG genes are organized into at least five functional groups that allow for the initiation, formation, elongation, and fusion of the autophagosome. These functional groups are the Atg1/ULK initiation complex, the class III PI3 Kinase nucleation complex, the phosphatidylinositol 3-phosphate (PI3P) -binding Atg18/Atg2 complex, the Atg5-Atg12 conjugation system, and the Atg8/LC3-PE (Atg8/LC3-phosphatidylethanolamine) conjugation system. First step of autophagy initiates from the activation of Atg1/ULK complex, which lead to the formation of isolation membrane. The next step involves membrane nucleation by the Class III Vps34/PI3-kinase nucleation complex (consisting of Vps34, Atg6/Beclin1, and Vps15/p150) via production of PI3P, to start formation of a double-membrane structure, isolation membrane (or phagophore). In mammals, the isolation membrane originates from the endoplasmic reticulum (ER) - mitochondria contact site and from others including Golgi, endosomes, and plasma membrane (Chan and Tang, 2013;

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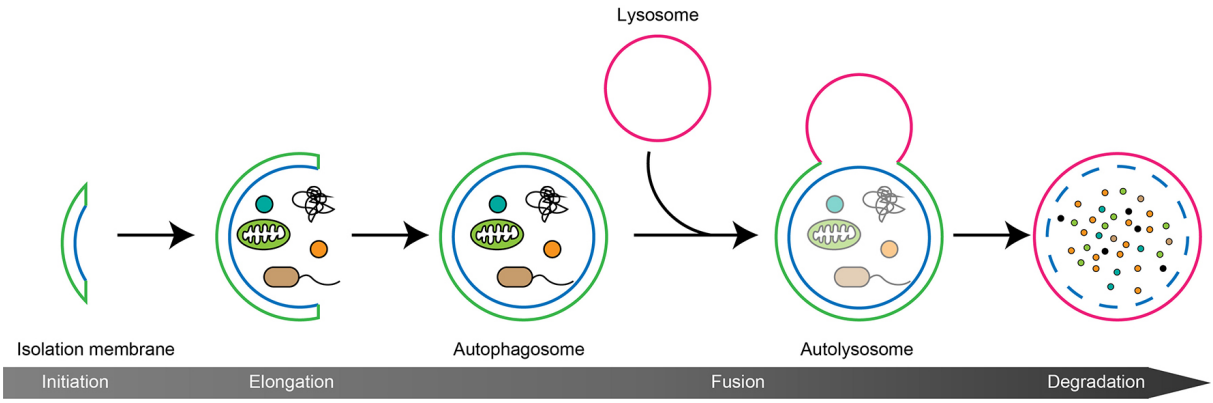

Fig. 1. Overview of macroautophagy. Upon induction of autophagy by stress, cytoplasmic materials are sequestered by a double-membraned structure, called an autophagosome. These autophagosomes fuse with lysosomes to become autolysosomes, in which the sequestered cargos are degraded and recycled for the maintenance of cellular homeostasis.

Hamasaki et al., 2013). To start elongation, the isolation membrane recruits the PI3P-binding complex consisting of Atg18/WIPI and Atg2, which regulates the distribution of Atg9, a transmembrane protein that has been proposed to deliver lipids to the isolation membrane and the growing autophagosome. During the next step, the isolation membrane expands into a double-membrane structure called the autophagosome. Autophagosome elongation is dependent on two ubiquitin-like conjugation systems, the Atg5-Atg12 conjugation system and the Atg8/LC3-PE conjugation system. In Atg5-Atg12 conjugation system, Atg7 and Atg10 (E1- and E2-like enzymes, respectively) conjugate Atg12 to Atg5 and this complex associates with Atg16. Then, the Atg12-Atg5 conjugate promotes the conjugation of phosphatidylethanolamine (PE) to cytosolic Atg8/ LC3, which is formed by cleavage of the ubiquitin-like protein Atg8/LC3 by the protease Atg4. During this process, PE-conjugated LC3 associates with the autophagosomal membrane and therefore LC3 is most commonly used as an experimental marker of autophagosomes (Fujita et al., 2008; Kabeya et al., 2000; Mizushima and Levine, 2010). The autophagosome eventually matures into a closed cargo-containing vesicle, which then fuses with the lysosome to become the autolysosome, and its contents are finally degraded for recycling. Autophagosome fusion step is mediated by HOPS complex, phosphoinositides, Rab proteins and SNEREs. For the detailed molecular mechanism of autophagosome formation and autophagosome-lysosome fusion, please refer to the recent specific review paper (Nakamura and Yoshimori, 2017).

ACTIVITY OF AUTOPHAGY IS ONE OF CONVERGENT MECHANISM OF DIFFERENT LONGEVITY PATHWAYS

Aging represents the functional deterioration of an organism. For long time, aging is not considered as a tightly regulated process. During last 20 decades, the evolutionally conserved molecular mechanisms which delay animal aging and extend lifespan have been identified using several model organisms including yeast, worms, fly and mice. These pathways, for instance, include reduced Insulin/IGF-1 signaling, dietary restriction, reduced TOR signaling, germline removal and reduced mitochondrial respiration. Extensive efforts to identify the downstream mechanism in each longevity pathway reveals that numerous but different sets of factors or biological processes mediate in each longevity pathways, although some factors work in common. Notably, recent studies mainly from *C. elegans* suggest that autophagy is one of convergent downstream mechanisms of all these longevity paradigms. Activity of autophagy is elevated in long-lived animals and is required for their longevity. Below, we summarize the major longevity pathways and their reported relationship with autophagy (Fig. 2 and Table 1).

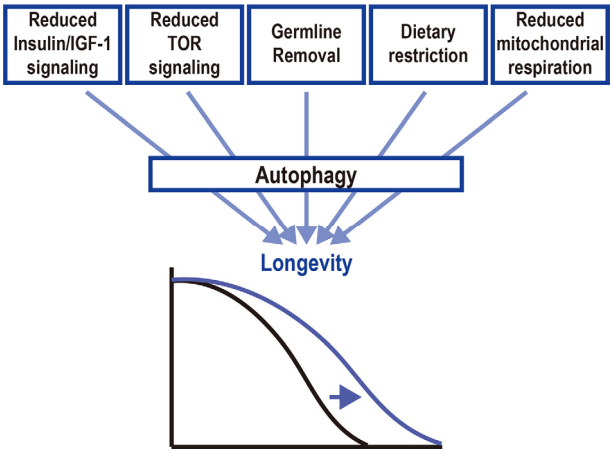

Fig. 2. Autophagy is a convergent mechanism of multiple longevity paradigms. Autophagic activity is commonly elevated in many long-lived animals and is essential for their longevity, suggesting that autophagy is one of convergent mechanisms mediating different longevity paradigms.

Table 1. Longevity through activation of autophagy

Genetic or pharmacological manipulations	Animals	Phenotypes	Epistatic analysis by inhibition of autophagy genes	References
Reduced insulin/IGF-1 signaling	Worm	Lifespan extension, activation of autophagy	Cancelation of longevity	Meléndez et al., 2003
Calorie restriction	Worm	Lifespan extension, activation of autophagy	Cancelation of longevity	Jia et al., 2007
Reduced TOR signaling	Worm	Lifespan extension, activation of autophagy	Cancelation of longevity	Hansen et al., 2008
Reduced mitochondrial respiration	Worm	Lifespan extension, activation of autophagy	Cancelation of longevity	Toth et al., 2008
Germline removal	Worm	Lifespan extension, activation of autophagy	Cancelation of longevity	Lapierre et al.,2011
HLH-30 overexpression	Worm	Lifespan extension, activation of autophagy	Cancelation of longevity	Lapierre et al., 2013
Urolithin A	Worm, mouse	Lifespan extension(worms), improved muscle function(mouse), activation of mitophagy	Cancelation of longevity	Ryu et al., 2016
Resveratrol	Worm	Lifespan extension, activation of autophagy	Cancelation of longevity	Morselli et al., 2010
Spermidine	Worm, *Drosophila*	Lifespan extension, activation of autophagy	Cancelation of longevity	Eisenberg et al., 2009
Rapamycin	*Drosophila*	Lifespan extension, activation of autophagy	Cancelation of longevity	Bjedov et al., 2010
Tomatidine	Worm	Lifespan extension, activation of autophagy	ND	Fang et al., 2017
Brain specific Atg8 overexpression	*Drosophila*	Lifespan extension in female	ND	Simonsen et al., 2008
ATG5 overexpression	Mouse	Lifespan extension, activation of autophagy	ND	Pyo et al., 2013

Mild reduction of Insulin/IGF-1 signaling

Reduced Insulin/IGF-1 signaling has been shown to extend the lifespan in several species (Kenyon, 2010). Initial discoveries were made in *C. elegans*, where mutations in *age-1* and *daf-2* genes were found to extend lifespan. Moreover, first connection between autophagy and longevity has been reported in this Insulin/IGF-1 signaling pathway (Melendez et al., 2003). In *daf-2* mutants, autophagy activity is elevated, as reflected by increased autophagic vesicles by electron microscopy and GFP::LGG-1(a homolog of LC3 in *C. elegans*) puncta, a *C. elegans* autophagosome marker. Importantly, RNAi knockdown of *bec-1/Beclin1* shortens *daf-2* lifespan, indicating that activity of autophagy is essential for *daf-2* longevity. Reduction of Insulin/IGF-1 signaling pathway extends lifespan in *Drosophila* and mice as well. Moreover, human centenarian has mutations in this pathway suggesting that this longevity pathway seems to be conserved up to human. Whether longevity in *Drosophila* and mice depends on autophagy need to be examined in future study. The mechanisms by which *daf-2* mutants regulate autophagy are unclear, but they could include post-translational and transcriptional regulation. The catalytic subunit of the energy regulator AMPK (AAK-2 in *C. elegans*) is essential for lifespan extension in *daf-2*mutants (Apfeld et al., 2004), and it regulates autophagy in both *C. elegans* and mammals (Egan et al., 2011). It is possible that *Ampk/aak-2*- regulated autophagy contributes to lifespan, since AMPK overexpression is sufficient to increase longevity of *Drosophila* in an *Atg1/Ulk1/unc-51*-dependent manner (Ulgherait et al., 2014). *daf-2* mutants also regulate autophagy at the transcriptional level. *daf-2* mutants require a master regulator of autophagy and lysosomal biogenesis, *hlh-30/TFEB* for their long lifespan, display nuclear-localized HLH-30, and have elevated levels of several autophagy-related and lysosomal genes (Lapierre et al., 2013). HLH-30 translocates to the nucleus of intestinal cells following knockdown of mTOR and *daf-2* (Lapierre et al., 2013). Since *mTor* RNAi inhibition in *daf-2* mutants do not extend *C. elegans* lifespan in an additive manner (Vellai et al., 2003), they mediate lifespan extension through at least partially overlapping mechanisms. What is the autophagy cargo relevant for longevity conferred by reduced Insulin/IGF-1 signaling? A recent study suggested that mitophagy is induced in *daf-2* mutants because mitochondria accumulate upon *bec-1* and mitophagy gene inhibition and *daf- 2* mutants require mitophagy genes, including adaptor protein *Bnip3/dct-1*, the E3 ligase *Park/pdr-1* and the kinase *pink-1* for full lifespan extension (Palikaras et al., 2015).

Dietary restriction/ reduced mTOR signaling

Dietary restriction is one of most prominent way to slow aging and extend lifespan in many species. Dietary restriction was first observed to slow down aging in rat about 100 years ago. Since then the beneficial effects to extend lifespan was confirmed in numerous species including yeast, worms, fly, fish, dogs, mice and apes (Mair and Dillin, 2008). Multiple molecular mechanisms have been proposed to mediate the effect of dietary restriction on longevity, including TOR and Insulin/IGF-1 signaling. The lifespan of the budding yeast *S. cerevisiae* can be measured by two methods; replicative lifespan (RLS) and chronological lifespan (CLS). Both RLS and CLS can be modulated in *S. cerevisiae* by reducing nutrients in the growth media (Smith et al., 2007). One method to induce dietary restriction is by amino acid limitation,

which has been shown to extend CLS and also induce autophagy (Alvers et al., 2009a). Similarly, inhibition of the nutrient sensor mTOR by rapamycin (a compound discovered in a soil bacterium on the Easter Island Rapa Nui) increases CLS and autophagy, and autophagy genes are required for rapamycin to extend lifespan (Alvers et al., 2009b). However, the role of autophagy in yeast aging seems complex. Intriguingly deletion of only ATG15, but not other autophagy genes tested, blocks RLS extension induced by glucose limitation (Tang et al., 2008) which is another method of dietary restriction in yeast. Several models of dietary restriction exist in *C. elegans* (Greer and Brunet, 2009), including *eat-2* mutants, which carry an acetylcholine receptor mutation that impairs pharyngeal pumping and reduces food intake. *eat-2* mutants show increased numbers of GFP::LGG-1 in hypodermal seam cells. The longevity of *eat-2* mutants are also abolished when several autophagy genes including *unc-51/ULK1, bec-1/Beclin1, vps-34, atg-18* and *atg-7* are inactivated (Hansen et al., 2008; Jia and Levine, 2007). In *eat-2* animals, some autophagy genes are transcriptionally induced by several transcription factors, including *hlh-30, pha-4* and *nhr-62* (Hansen et al., 2008; Heestand et al., 2013; Lapierre et al., 2013). Recently it has been shown that intestinal autophagy is essential for lifespan extension during dietary restriction (Gelino et al., 2016). How these transcription factors contribute to activation of autophagy and longevity in spatial and temporal manners need to be clarified in future study. Similar to yeast, in *C. elegans,* lifespan extension induced by dietary restriction may be at least partly mediated through TOR, because TOR inhibition in *eat-2* mutants does not further extend lifespan (Hansen et al., 2007). In line with this, similar to dietary-restricted worms, inhibition of TOR extends lifespan in transcription factor, *pha-4* or *hlh-30* dependent manner (Lapierre et al., 2013; Sheaffer et al., 2008). In *Drosophila*, rapamycin treatment results in a modest lifespan extension, and this effect requires the autophagy gene *Atg5* (Bjedov et al., 2010), suggesting that reduction of TOR extends lifespan in *Drosophila* at least partially through autophagy similar to yeast and worms. In 2009, rapamycin treatments have been also shown to extend both median and maximum lifespan of male and female heterogeneous mice (Harrison et al., 2009). After that, other groups also confirmed the positive effect of rapamycin on lifespan in mice using different genetic backgrounds (Lamming et al., 2013). However, the contribution of autophagy to these mice is unclear.

Germline removal

Reproduction is negatively correlated with longevity in many species. Removal of germline stem cells by laser microsurgery or genetic mutation extends lifespan in *C. elegans* and *Drosophila*. In worms, temperature sensitive mutant, *glp-1(e2141)*, which encodes *C. elegans* Notch receptor shows the reduction of germline stem cells and lifespan extension. It has been shown that the numbers of GFP::LGG-1 puncta are in germline deficient *glp-1* animal and autophagy genes are essential for their longevity (Lapierre et al., 2011). In germline deficient animal, several transcription factors including *hlh-30*, *mml-1/mxl-2* and *pha-4* have been shown to induce autophagy genes (Lapierre et al., 2011; 2013; Nakamura et al., 2016). Interestingly, intestine specific knockdown of autophagy genes abolishes *glp-1* longevity, while it is not the case in *daf-2* mutants, indicating critical differences of autophagy regulation in individual tissues between conserved longevity paradigms (Chang et al., 2017). *glp-1* animals have increased lipase activity and *lipl-4* is required for *glp-1* animals to live long (Wang et al., 2008). *Lipl-4* overexpression increases autophagy and lifespan and this animal requires autophagy gene for longevity (Lapierre et al., 2011). These studies indicate lipid turnover by autophagy is essential for longevity.

Reduced mitochondrial respiration

The free radical theory proposes that aging is the cumulative result of oxidative damages to cells and tissues over time. These molecular damages are caused by reactive oxygen species (ROS) which is generated primarily from mitochondrial respiration. Although oxidative damages increase with age, it is still unclear if this is indeed causative effect to organism aging. Importantly, reduced mitochondrial respiration is known to extend lifespan of many organism from yeast to mice (Hur et al., 2010; Kirchman et al., 1999). In worms, the reduction of electron transport chain components extends lifespan, when they are inhibited during larval stages. Several mitochondrial mutants including ubiquinone synthetase mutant *clk-1* and iron-sulfer mutant *isp-1* also show longevity. Larval inhibition of autophagy genes (*vps-34*, *atg-18* and *lgg-1*) specifically shortens the lifespan of *clk-1*and *isp-1* mutants (Lapierre et al., 2013; Toth et al., 2008). Consistent with a role for autophagy, these mutants display increased numbers of GFP::LGG-1 punctae in the hypodermal cells during larval stage L3 (Lapierre et al., 2013). Frataxin is a nuclear-encoded mitochondrial protein involved in the biogenesis of iron-sulphur (Fe-S)-cluster-containing proteins and also involved in the function of the mitochondrial respiratory chain. Partial depletion of *frh-1* has been shown to increase autophagic activity and extends the lifespan of wild-type animals, but not *bec-1*mutants (Schiavi et al., 2013). Moreover, a recent report showed that longevity of *frh-1* mutants requires mitophagy genes for its longevity (Schiavi et al., 2015).

Forced activation of autophagy suffices to extend lifespan?

Loss of autophagic activity has been shown to cause premature aging phenotypes in many species. An unbiased screening for genes involved in chronological lifespan in yeast, identified several short-lived mutants which have mutation in macroautophagy genes (Matecic et al., 2010). Decreased lifespan is also observed in *C. elegans Atg1/unc-51, Atg7, Atg18* and *Beclin1/bec-1* loss of function mutants (Toth et al., 2008). Similar findings are reported in *Drosophila* as well (Simonsen et al., 2008). Although whole body knockout of Atg genes in mice leads to postnatal death, conditional tissue specific knockouts of Atg7 or Atg5 shows several age-associated phenomena including aggregation of inclusion bodies in neurons, accumulation of lysosomes containing lipofuscin pigments, disorganized mitochondria, increased protein oxidation and decreased muscle mass (Rubinsztein

et al., 2011). Moreover, autophagic activity is known to decrease with age in several species (Chang et al., 2017; Del Roso et al., 2003; Donati et al., 2001; Uddin et al., 2012). Based on the correlation between autophagy and aging, it is reasonable to test if the forced activation of autophagy suffices to extend animal lifespan. Overexpression of HLH-30, a master regulator of autophagy and lysosomal biogenesis extends worm lifespan (Lapierre et al., 2013). Consistent with this, the treatment of TFEB agonists have been recently shown to extend lifespan in worms and mitigate metabolic syndromes in mice (Wang et al., 2017). In addition, ATG5 overexpression in mice extend lifespan both in male and female (Pyo et al., 2013). Moreover, neuronal overexpression of Atg8 is sufficient to extend lifespan in *Drosophila* (Simonsen et al., 2008). However why simple overexpression of these autophagy genes lead to the activation of autophagy remains elusive and further studies need to clarify this point.

PHARMACOLOGICAL ACTIVATION OF AUTOPHAGY CONTRIBUTING TO LONGEVITY

In the following section, we summarize several pharmacological treatments which have been shown to extend animal lifespan and healthspan through the activation of autophagy (Table 1).

Spermidine

Administration of a natural polyamine, spermidine provides beneficial for health in a number of species and extends lifespan of yeast, worms, flies and mice (Eisenberg et al., 2009; 2016). Survival of cultured mammalian cells is also promoted by treatment with spermidine, and this is accompanied by epigenetic hypoacetylation of histone H3 via inhibition of histone acetyltransferase activity. This, in turn, correlates with transcriptional upregulation of multiple autophagy-related genes, including *Atg5* and *Lc3/ATG8/lgg-1/2* (Eisenberg et al., 2009). In keeping with this observation, spermidine fails to extend the lifespan of *C. elegans* subjected to *bec-1* RNAi, whereas it increases the expression of DsRed::LGG-1 (Eisenberg et al., 2009) in a *sir-2*-independent fashion (Morselli et al., 2011). In flies, spermidine alters the expression of autophagy markers, protects against age-induced memory loss in an autophagy-dependent manner, and extends the lifespan in an *Atg7*- dependent manner (Gupta et al., 2013). Collectively, these data suggest that the positive effects of spermidine on health and longevity are mediated, at least in part, via autophagy induction.

Resveratrol

Resveratrol is a naturally occurring polyphenolic compound found in grapes and an activator of the NAD-dependent histone deacetylase sirtuin (SIRT1). Administration of resveratrol is known to extend the lifespan of several model organisms (Park et al., 2013). Especially, the lifespan extension in *C. elegans* seems to be dependent on autophagy since resveratrol fails to extend the lifespan of *bec-1* (*RNAi*) treated animals. Additionally, resveratrol increases DsRed::LGG-1 levels in wild-type animals but not in *sir-2.1* loss- of-function mutants (Morselli et al., 2010). These observations are in agreement with findings in mammalian cells, where pharmacological activation of SIRT1 by resveratrol treatment stimulates autophagic flux (Morselli et al., 2010).

Urolithin A

Urolithin A as a first-in-class natural compound that induces mitophagy both *in vitro* and *in vivo* following oral consumption. In *C. elegans*, urolithin A prevents the accumulation of dysfunctional mitochondria with age and extends lifespan (Ryu et al., 2016). Likewise, Urolithin A prolongs normal activity during aging in *C. elegans*, including mobility and pharyngeal pumping, while maintaining mitochondrial respiratory capacity. These effects are observed in rodents, where Urolithin A improves exercise capacity in two different mouse models of age-related decline of muscle function, as well as in young rats.

Tomatidine

Tomatidine, a natural compound abundant in unripe tomatoes, inhibits age-related skeletal muscle atrophy in mice. Recent study shows that tomatidine extends lifespan and healthspan in *C. elegans* (Fang et al., 2017). Tomatidine improves many *C. elegans* behaviors related to healthspan and muscle health, including increased pharyngeal pumping, swimming movement, and reduced percentage of severely damaged muscle cells. Microarray, imaging, and behavioral analyses reveal that tomatidine maintains mitochondrial homeostasis by modulating mitochondrial biogenesis and PINK-1/DCT-1-dependent mitophagy. Detailed analysis shows tomatidine induces mitochondrial hormesis by mildly inducing ROS production, which in turn activates the SKN-1/Nrf2 pathway and possibly other cellular antioxidant response pathways, followed by increased mitophagy. This mechanism occurs in *C. elegans*, primary rat neurons, and human cells.

AUTOPHAGY REGULATORS RELEVANT FOR LONGEVITY

In many cases, autophagic activation at the transcript level seems essential for longevity. Several autophagy and lysosomal genes are regulated by different transcription factors, microRNA and chromatin modifying enzymes, which are described below.

HLH-30/TFEB

TFEB originally identified as a master regulator of lysosomal biogenesis is subsequently is shown to regulate autophagy and fat metabolism (Sardiello et al., 2009; Settembre et al., 2011; 2013a). TFEB is known to be negatively regulated by nutrient sensor TOR. At nutrient rich condition, TFEB is phosphorylated on lysosome. Phosphorylated TFEB is bound to 14-3-3 and is mainly localized on cytosol. Upon starvation, TOR becomes inactivated and TFEB is then dephosphorylated and translocated in the nucleus to initiate the transcription of target genes (Settembre et al., 2013b). *C. elegans* homolog of TFEB, HLH-30 has been shown to regulate genes involved in autophagy and lysosomal function. Essentially, HLH-30 is translocated to the nucleus by inhibition of

Insulin/IGF-1 signaling, mitochondrial respiration, TOR signaling, translation and germline removal, and is required for their longevity. Moreover, overexpression of *hlh-30* is sufficient to extend lifespan of wild type animals. These results indicate that HLH-30/TFEB is a master transcription factor regulating many longevity pathways possibly through transcriptional activation of target genes involved in autophagy and lysosomal function. In future, it is worth examining whether TFEB has a role to regulate aging and lifespan in mammals.

MML-1/Mondo

Other bHLH transcription factor complex, MML-1/MXL-2 has been identified as a novel regulator of longevity (Nakamura et al., 2016). MML-1/MXL-2 belongs to Myc and Mondo family member and their homologs, MondoA/MLX or ChREBP/MXL functions as a glucose sensor. MML-1/MXL-2 is required for the longevity conferred by germline removal, reduced Insulin/IGF-1 signaling, reduced mitochondrial respiration, reduced TOR signaling in *C. elegans*. Interestingly, inhibition of MML-1/MXL-2 impairs HLH-30 nuclear localization and activation of autophagy in germline less long-lived animals, *glp-1*. This is partly through the regulation of *lars-1*, a positive regulator of TOR signaling. Interestingly, in *glp-1*, MML-1/MXL-2 and HLH-30 are mutually regulated each other. Comprehensive transcriptome analysis reveals they have many shared target genes including lysosomal genes, but also have preferential targets. Some autophagy genes including *atg-2/ATG2, atg-9/ATG9* and *epg-9/ATG101* are preferentially regulated by MML-1/MXL-2, while *unc-51/ULK1* and *lgg-1/LC3* are regulated by HLH-30 (Nakamura et al., 2016). Thus, they might distribute the responsibilities to reinforce autophagy and longevity in germline less animals.

Forkhead transcription factors (daf-16/FOXO, pha-4/FOXA)

In *C. elegans*, *Drosophila* and mouse, reduction of Insulin/IGF-1 signaling ultimately activates DAF-16/FOXO function and extends lifespan. In worms, DAF-16 upregulates some of autophagy genes and increases autophagy flux (Jia et al., 2009). Consistent with this, overexpression of DAF-16 increase the number of autophagosomes. However, although *daf-2* and *daf-16* double mutants do not show longevity, these mutants still have increased numbers of autophagosomes. Conceivably, other factors compensate the activity of autophagy or DAF-16 regulates autophagy at other timing. Other forkhead transcription factor, PHA-4/FOXA binds to the promoter region of *unc-51/Ulk1*, *bec-1/Becn1, lgg-1/LC3* which work in early stage of autophagosome formation and upregulates these genes in worms, leading to autophagic activation. *pha-4* is required for the longevity by mTOR inhibition, germline removal and calorie restriction through activation of autophagy.

miR-34

Many microRNA has been shown to regulate animal lifespan. Among them, miR-34 is related to autophagy and aging in some species. In worms, loss of function of miR-34 extends lifespan and this longevity is abolished by RNAi knockdown of several autophagy regulators, *bec-1*, atg-9 and *atg-4.1* (Yang et al., 2013). In long-lived calorie restricted mice, miR-34 expression is reduced. In worms, miR-34 expression increases with age and represses autophagy gene, Atg9a in vitro. In contrary, increased Mir34 levels extend lifespan and reduces the neurodegeneration caused by polyglutamine expansion protein in *Drosophila* (Liu et al., 2012). However, the contribution of autophagy in this context remains elusive.

Sirtuins

NAD-dependent deacetylase SIRT1 (sirtuin 1) is a particularly well-known modulator of aging. Specifically, the life spans of yeast, worms, and flies can be extended by overexpression and/or pharmacological activation of SIRT1 and the lifespan of mice is extended by ubiquitous overexpression of SIRT6, or brain-specific overexpression of SIRT1 (Giblin et al., 2014; Park et al., 2013). In *C. elegans*, the lifespan extension of the SIRT1 activator resveratrol requires the expression of *bec-1* suggesting that autophagy is necessary for this longevity paradigm. SIRT1 regulates autophagy gene expression through histone deacetylation, with lysine 16 on histone H4 (H4K16) (Fullgrabe et al., 2014). SIRT1 is known to co-immunoprecipitate with ATG5, ATG7 and LC3 and deacetylate these in vitro and these interactions could be also essential for autopagy regulation (Lee et al., 2008).

CONCLUSION

As we described above, accumulating evidences show activation of autophagy seems essential for longevity. However, the several fundamental questions remain elusive. How is autophagy regulated during aging? Cells, tissues and timing specific roles of autophagy also need to be considered. Recently and unexpectedly, neuron specific knockdown of autophagy after reproductive period has been shown to extend lifespan in worms (Wilhelm et al., 2017). Thus, it is crucial to understand spatio- and temporal-regulation of autophagy and their physiological relevance to aging. It is essential to determine how autophagy contribute to lifespan extension and which autophagy cargo are relevant for aging and longevity. Clearance of lipids (lipophagy) and mitochondria (mitophagy) are relevance to *C. elegans* ageing (Lapierre et al., 2011; Palikaras et al., 2015; Wang et al., 2008). It remains to be clarified whether such selective autophagy or other autophagy cargos contributes to aging in other species. Additionally, it is necessary to assess which potential autophagy inducers are effective and applicable to humans. One fundamental problem is there is no way to monitor autophagy activity in human. These questions and problems need to be solved in upcoming studies with technical advances.

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