Chapter 11
Autophagy in Aging and Longevity

Shuhei Nakamura, Tatsuya Shioda, and Tamotsu Yoshimori

Abstract Macroautophagy (autophagy) is an evolutionally conserved cytoplasmic degradation system in which varieties of materials are sequestered by a double membrane structure, called autophagosome, and delivered to the lysosomes for the degradation. Due to the wide varieties of targets, autophagic activity is essential for cellular homeostasis and survival. Accumulating evidences suggest that the activity of autophagy decreases with age, whereas several interventions which induce activation of autophagy promote longevity and prevents age-related diseases. Here we summarize recent progress regarding the role of autophagy in animal aging and life span regulation.

Keywords Autophagy · ATGs · Longevity · Rubicon · MML-1/Mondo · HLH-30/TFEB · Autophagosome · Lysosome

11.1 Overview of Autophagy

Autophagy is a conserved lysosomal degradation essential for cellular homeostasis and stress resistance. Autophagy can be classified into three distinct types depending how cytoplasmic materials are delivered to lysosomes: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Among these, this review particularly focuses on macroautophagy, since its roles and regulation in aging and age-related diseases are well documented. 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 several stresses such as starvation, accumulation of abnormal proteins, organelle damage, and pathogen infection. During autophagy, a small cisterna, called isolation membrane (also called isolation membrane or phagophore), elongates and surrounds a portion of cytoplasm to form a double-membraned structure, called the

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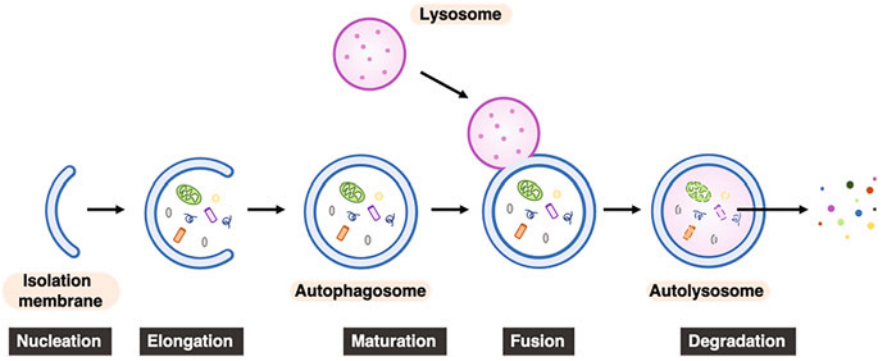

Fig. 11.1 Overview of autophagy. Upon induction of autophagy by stress, cytoplasmic materials are sequestered by a double-membraned structure, called an autophagosome. These autophagosomes fuse with lysosomes to become autolysosomes, in which the sequestered cargos are degraded and recycled for the maintenance of cellular homeostasis. Autophagy can be divided into several steps: formation of the isolation membrane (nucleation), elongation of the isolation membrane (elongation), completion and transport of the autophagosome (maturation), docking and fusion between autophagosome and lysosome (fusion), and degradation of the cargos inside the autolysosome (degradation)

autophagosome. Autophagosomes are then transported and fuse with lysosomes to form autolysosomes for the digestion of sequestered contents (Fig. 11.1). Autophagy is originally considered to be a bulk and nonselective degradation system. But subsequent studies show autophagy selectively degrades cargos and by doing so contribute to the intracellular homeostasis. Many cargos such as damaged mitochondria, damaged lysosomes, invading bacteria, lipid droplet, and aggregated proteins are selectively sequestered and degraded by specific selective autophagy, called mitophagy, lysophagy, xenophagy, lipophagy, and aggrephagy, respectively. 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 six functional groups that allow for the nucleation, elongation, maturation, and fusion of the autophagosome. These functional groups are the Atg1/ULK initiation complex, the class III PI3 kinase nucleation complex, Atg9 vesicles, the phosphatidylinositol 3-phosphate (PI3P)-binding Atg18/Atg2 complex, the Atg5-Atg12 conjugation system, and the Atg8/LC3-PE (Atg8/LC3-phosphatidylethanolamine) conjugation system. The first step of autophagy initiates from the activation of Atg1/ULK complex, which leads 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, Atg14L, and Vps15/p150) via the production of PI3P, to start the formation of a double-membrane structure, isolation membrane (or phagophore). In mammals, the isolation membrane originates from the endoplasmic reticulum (ER), the mitochondrial contact site, and from others including the Golgi, endosomes, and plasma membrane (Chan and Tang 2013; 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. 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 the 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 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-lysosome fusion step is mediated by HOPS complex, phosphoinositides, Rab proteins, and SNEREs. In addition, autophagosome lysosome fusion step is negatively regulated by Rubicon which comprises different class III PI3K complex including Beclin1, UVRAG, Vps34, and Vps15 (Matsunaga et al. 2009). The detailed molecular mechanism of autophagosome formation and autophagosome-lysosome fusion is summarized in recent specific review paper (Nakamura and Yoshimori 2017; Nakatogawa 2020). As described in the following section, recent genetic evidence indicates that autophagy has a crucial role in the regulation of animal life span. The basal level of autophagic activity is elevated in many longevity paradigms, and importantly its activity is required for life span extension. On the other hand, the activity of autophagy decreases with age in many organisms. Pharmacological treatments have been shown to extend life span through the activation of autophagy, indicating autophagy could be a potential and promising target to modulate animal life span.

11.2 Activation of Autophagy Is One of the Convergent Mechanisms of Animal Longevity

Aging represents the functional deterioration of an organism. For a long time, aging is not considered as a tightly regulated process. During last twenty decades, the evolutionally conserved molecular mechanisms which delay animal aging and extend life span 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 mTOR signaling, germline removal, and reduced mitochondrial respiration. Extensive efforts to identify the downstream mechanism in each longevity pathway reveals that numerous but

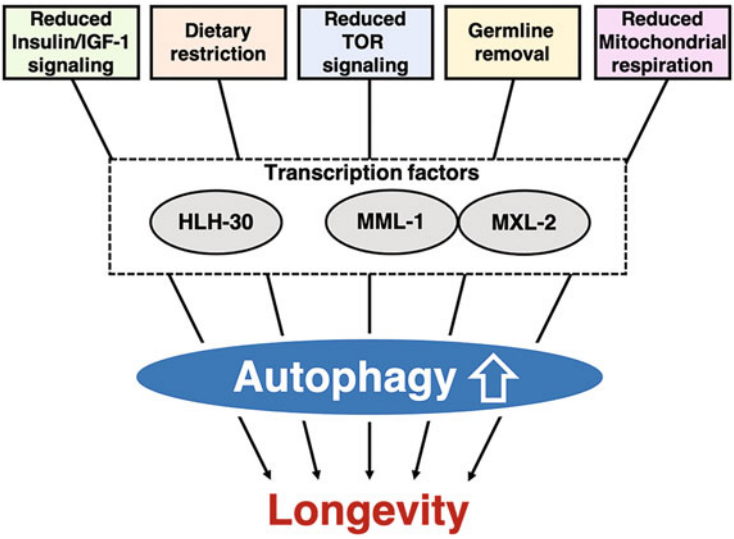

Fig. 11.2 Activation of autophagy is one of the convergent mechanisms of several longevity pathways. Several transcription factors such as HLH-30/TFEB and MML-1/Mondo are commonly activated in multiple longevity pathways. These regulate autophagic activity and extend its life span

different sets of factors or biological processes mediate in each longevity pathways, although some factors work in common. Notably, recent studies suggest that autophagy is one of the convergent downstream mechanisms of all these longevity paradigms. The activity of autophagy is elevated by several transcription factors in long-lived animals and is required for their longevity (Fig. 11.2, Table 11.1).

Reduced insulin/IGF-1 signaling has been shown to extend the life span in several species (Kenyon 2010). The first connection between autophagy and longevity has been reported in this insulin/IGF-1 signaling pathway in *C. elegans* (Meléndez et al. 2003). In long-lived *daf-2* (encoding *C. elegans* insulin/IGF-1 receptor) 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* life span, indicating that the activity of autophagy is essential for *daf-2* longevity. Reduction of insulin/IGF-1 signaling pathway extends the life span in *Drosophila* and mice as well. In *Drosophila*, life span extension with deletion of the insulin receptor substrate chico was completely abrogated by the knockdown of Atg5 (Bjedov et al. 2020). Moreover, human centenarian has mutations in this pathway, suggesting that this longevity pathway seems to be conserved up to human. The exact mechanisms of autophagic activation in *daf-2* mutants are unclear, but they could include posttranslational and transcriptional regulation. For instance, the catalytic subunit of the energy regulator AMPK (AAK-2 in *C. elegans*) is essential for life span 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

Table 11.1 Genetic modulation of autophagic activity which leads to longevity

Procedure for lifespan extension	Model organisms	Phenotypes	Correlation between autophagy and longevity	References
Reduced insulin/IGF-1 signaling pathway (*daf-2* KO)	*C. elegans*	Lifespan extension, enhanced autophagy	*bec-1* KD abolished DAF-2-mediated lifespan extension	Meléndez et al. (2003)
Dietary restriction (DR)	*C. elegans*	Lifespan extension, enhanced autophagy	*bec-1*, *atg-7*, *unc-51*, or *vps-34* KD abolished DR longevity Intestine-specific *atg-18* or *lgg-1/lgg-2* KD abolished DR longevity	Jia et al. (2007), Hansen et al. (2008), Tóth et al. (2008), Gelino et al. (2016)
Reduced TOR signaling	*C. elegans*	Lifespan extension, enhanced autophagy	*bec-1 or hlh-30* KD abolished the longevity by reduced TOR signaling	Hansen et al. (2008), Lapierre et al. (2013)
Reduced mitochondrial respiration (*atp-3* KD)	*C. elegans*	Lifespan extension, enhanced autophagy	*bec-1, unc-51*, or *atg-18* KD abolished ATP-3-mediated longevity	Tóth et al. (2008)
Germline removal	*C. elegans*	Lifespan extension, enhanced autophagy	*bec-1*, *vps-34*, *lgg-1*, *unc-51, atg-18, hlh-30, or mml-1* KD abolished germline-mediated longevity Intestine-specific *atg-18* KD abolished germline-mediated longevity	Lapierre et al. (2011), Chang et al. (2017), Lapierre et al. (2013), Nakamura et al. (2016)
Overexpression of HLH-30/TFEB	*C. elegans*	Lifespan extension, enhanced autophagy	No data	Lapierre et al. (2013)
Hexosamine pathway activation (*gfat-1* KO)	*C. elegans*	Lifespan extension, enhanced autophagy	*atg-18* KD abolished GFAT-1-mediated longevity	Denzel et al. (2014)
Frataxin silencing (*frh-1* KO)	*C. elegans*	Lifespan extension, enhanced mitophagy	*sqst-1*/p62 KD abolished FRH-1-mediated longevity	Schiavi et al. (2015)
Overexpression of KLF-3	*C. elegans*	Lifespan extension, enhanced autophagy	*bec-1* KD abolished *klf-3*-mediated longevity	Hsieh et al. (2017)
Overexpression of HSF-1	*C. elegans*	Lifespan extension, enhanced autophagy	*unc-51, bec-1*, or *lgg-1* KD abolished HSF-1-mediated longevity	Kumsta et al. (2017)
Knockdown of *xpo-1*	*C. elegans*	Lifespan extension, enhanced autophagy	*atg-7* or *atg-18* KD abolished XPO-1 mediated longevity	Silvestrini et al. (2018)

(continued)

Table 11.1 (continued)

Procedure for lifespan extension	Model organisms	Phenotypes	Correlation between autophagy and longevity	References
Mutation of mir-83	*C. elegans*	Lifespan extension, enhanced autophagy	No data	Zhou et al. (2019)
Low temperature	*C. elegans*	Lifespan extension, enhanced autophagy	*bec-1* KD abolished the lifespan extension at low temperature	Chen et al. (2019)
Muscle-specific overexpression of dFOXO	*D. melanogaster*	Lifespan extension, enhanced proteostasis at least in part via autophagy	No data	Demontis et al. (2010)
Reduced muscle activin signaling	*D. melanogaster*	Lifespan extension, enhanced autophagy	Atg8a KD abolished the longevity by reduced muscle activin signaling	Bai et al. (2013)
Knockdown of AcCoAS	*D. melanogaster*	Lifespan extension, enhanced autophagy	No data	Eisenberg et al. (2014)
Midlife Drp1 induction	*D. melanogaster*	Extension of healthy lifespan, enhanced mitophagy	Atg1 KD abolished Drp1-mediated longevity	Rana et al. (2017)
Overexpression of SQST-1/p62	*C. elegans* *D. melanogaster*	Lifespan extension, enhanced autophagy/mitophagy	*lgg-1* KD abolished *sqst-1*-mediated longevity (*C. elegans*) Atg1 KD abolished p62-mediated longevity (*D. melanogaster*)	Kumsta et al. (2019), Aparicio et al. (2019)
Knockdown of *rub-1*/Rubicon	*C. elegans* *D. melanogaster*	Lifespan extension, enhanced autophagy	Neuron-specific *atg-18* KD abolished RUB-1-mediated longevity	Nakamura et al. (2019)
Overexpression of Sestrin in intestinal stem cells	*D. melanogaster*	Lifespan extension, enhanced autophagy	No data	Lu et al. (2020)
Overexpression of Atg5	Mice	Lifespan extension, enhanced autophagy	No data	Pyo et al. (2013)
Beclin1(F121A)knock-in mutation	Mice	Lifespan extension, enhanced autophagy	No data	Fernández et al. (2018)

Ampk/aak-2-regulated autophagy contributes to life span, since AMPK overexpression is sufficient to increase the longevity of *Drosophila* in an *Atg1/Ulk1/unc-51*-dependent manner (Ulgherait et al. 2014). *daf-2* mutants also displays lower expression of key autophagy-related genes. They require a master regulator of autophagy and lysosomal biogenesis, *hlh-30/TFEB*, for their long life span, to 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* life span in an additive manner (Vellai et al. 2003), they mediate life span 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 life span extension (Palikaras et al. 2015).

Dietary restriction is one of the most prominent ways to slow aging and extend the life span 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 life span 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 life span of the budding yeast *S. cerevisiae* can be measured by two methods: replicative life span (RLS) and chronological life span (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, the 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 life span (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 life span 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*, life span extension induced by dietary restriction may be at least partly mediated through TOR, because TOR inhibition in *eat-2* mutants does not further extend life span (Hansen et al. 2007). In line with this, similar to dietary-restricted worms, the inhibition of TOR extends life span in a 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 life span extension, and this effect requires the autophagy gene *Atg5* (Bjedov et al. 2010), suggesting that the reduction of TOR extends the life span in *Drosophila* at least partially through autophagy similar to yeast and worms. Rapamycin extends mammalian life span and ameliorates neurodegeneration and osteoarthritis in mice (Harrison et al. 2009; Li et al. 2014). Other groups also confirmed the positive effect of rapamycin on the life span in mice using different genetic backgrounds (Lamming et al. 2013). However, the contribution of autophagy to these mice is unclear.

Reproduction is negatively correlated with longevity in many species. Removal of germline stem cells by laser microsurgery or genetic mutation extends life span 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 life span extension. It has been shown that the numbers of GFP::LGG-1 puncta are increased 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 life span, and this animal requires autophagy gene for longevity (Lapierre et al. 2011). These studies indicate lipid turnover by autophagy is essential for longevity.

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 a causative effect to organism aging. Importantly, reduced mitochondrial respiration is known to extend the life span 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 life span, when they are inhibited during larval stages. Several mitochondrial mutants including ubiquinone synthetase mutant *clk-1* and iron-sulfur mutant *isp-1* also show longevity. Larval inhibition of autophagy genes (*vps-34*, *atg-18*, and *lgg-1*) specifically shortens the life span of *clk-1* and *isp-1* mutants (Lapierre et al. 2013; Tóth 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-sulfur (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 life span of wild-type animals, but not *bec-1* mutants (Schiavi et al. 2013). Moreover, a recent report showed that the longevity of *frh-1* mutants requires mitophagy genes for its longevity (Schiavi et al. 2015).

In addition to the role of autophagy in longevity, the loss of autophagic activity has been shown to cause premature aging phenotypes in many species. An unbiased screening for genes involved in chronological life span in yeast identified several short-lived mutants which have mutation in macroautophagy genes (Matecic et al. 2010). Decreased life span is also observed in *C. elegans Atg1/unc-51*, *Atg7*, *Atg18*, and *Beclin1/bec-1* loss of function mutants (Tóth 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 show 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).

11.3 Autophagic Activity Declines with Age

Autophagic activity is known to decrease with age in several species (Chang et al. 2017; Chapin et al. 2015; Del Roso et al. 2003; Donati et al. 2001; Uddin et al. 2012). Interestingly, the study using centenarians shows the general increase of autophagy genes (Xiao et al. 2018) and also increased circulating Beclin1 (Emanuele et al. 2014). Based on these correlations between autophagy and aging, it is reasonable to test if the forced activation of autophagy suffices to extend animal life span (Table 11.1). Indeed, the overexpression of HLH-30/TFEB, a master regulator of autophagy and lysosomal biogenesis, extends worm life span (Lapierre et al. 2013). Consistent with this, the inhibition of HLH-30/TFEB nuclear export or the treatment of HLH-30/TFEB agonists have been recently shown to extend the life span in worms and mitigate metabolic syndromes in mice (Silvestrini et al. 2018; Wang et al. 2017). In addition, ATG5 overexpression or Beclin1 gain of function in mice extends life span (Fernández et al. 2018; Pyo et al. 2013). Moreover, the neuronal overexpression of Atg8 or mild upregulation of Atg1 is sufficient to extend life span in *Drosophila* (Bjedov et al. 2020; Simonsen et al. 2008). Although the molecular mechanism underlying age-dependent autophagic decline has remained elusive, the recent study suggests that age-dependent accumulation of autophagy negative regulators; Rubicon is one of such mechanisms (Matsunaga et al. 2009; Nakamura et al. 2019). Rubicon is increased in *C. elegans*, *Drosophila*, and mouse tissues such as the liver and kidney, and importantly knockdown of Rubicon increases life span in an autophagy-dependent manner and/or ameliorate several age-associated phenotypes,

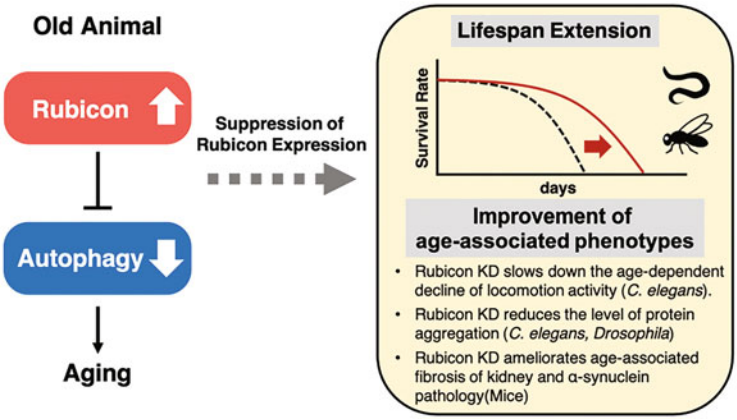

Fig. 11.3 The increase of Rubicon is a signature of aging and autophagic decline. Rubicon expression increases with age. This causes a decrease in autophagic activity, which curtails life span and leads to aging. Knockdown of Rubicon increases life span and improves age-associated phenotypes in many species

such as kidney fibrosis and α-synuclein pathology in these animals (Fig. 11.3). Specific microRNA is also involved in the autophagic decline across tissues during aging in *C. elegans* (Zhou et al. 2019b). *mir-83* is upregulated by the transcription factor hsf-1/HSF1 in the intestine during aging and transported across tissues. *mir-83* disrupts autophagy in intestines and muscles by downregulating lysosomal calcium channel cup-5/TRPML1 essential for the induction of autophagy. Lysosomal morphology and activity decrease age in *C. elegans* and are regulated in many longevity pathways (Sun et al. 2020), which partly explain autophagic decline with age. In addition, a key autophagy-negative regulator, the mTOR activity, has been shown to increase over time in some mouse tissues, which might also affect age-dependent autophagic impairment (Baar et al. 2016). On the other hand, another upstream regulator of autophagy, the AMPK activity, is constant during aging, but AMPK activation by an activator, such as AICAR or exercise, is blunted by aging (Reznick et al. 2007).

11.4 Autophagy and Age-Related Neurodegenerative Diseases

Autophagy is essential to prevent many age-associated diseases. Among them autophagy activity in neuronal cells is particularly essential, since neuronal cells are post-mitotic and they cannot segregate and dilute the proteotoxic damage. Therefore, proteostasis function by autophagic activity is essential to prevent the accumulation of several aggregation-prone proteins which lead to neurodegeneration. Indeed, the impairment of the autophagy pathway is involved

in many age-associated neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson disease (PD) (Hara et al. 2006; Komatsu et al. 2006; Mizushima et al. 2008).

AD involves the deposition of extracellular β-amyloid (Aβ) plaque and intracellular neurofibrillary tangles containing hyperphosphorylated tau. Both Aβ and Tau are known to be substrates of autophagy. The loss of autophagy by conditional knockout of ATG7 in mouse brain leads to phosphor-tau accumulation (Inoue et al. 2012). On the other hands, autophagic activation decreases tau levels (Krüger et al. 2012). The heterozygous deletion of Beclin1 increases both intracellular and extracellular Aβ (Pickford et al. 2008). The accumulation of autophagic vacuoles is observed in AD neurons (Nixon et al. 2005), suggesting that autophagy is impaired in this pathological condition.

PD is characterized by the accumulation of α-synuclein. α-synuclein overexpression impairs autophagy due to mislocalization of ATG9 (Winslow et al. 2010). Accumulation and propagation of α-synuclein aggregation in the brain can be suppressed by autophagy activation by an autophagy-negative regulator, Rubicon (Nakamura et al. 2019). The loss of function of Parkin, an E3 ubiquitin ligase, and PINK1, a mitochondrial protein kinase, causes autosomal-recessive and sporadic juvenile onset PD (Kitada et al. 1998; Valente et al. 2001, 2004). Parkin and PINK1 are known to regulate mitophagy to clear the damaged mitochondria in the cell culture. PINK1 recruits Parkin on damaged mitochondria to induce mitophagy. However, PINK1 and Parkin knockout mice fail to develop PD-associated phenotypes (Gautier et al. 2008; Palacino et al. 2004), suggesting that PD could be caused by other mechanism.

11.5 Molecular Mechanism Regulating Autophagy and 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. The bHLH transcription factor, TFEB originally identified as a master regulator of lysosomal biogenesis, is subsequently shown to regulate autophagy and fat metabolism (Sardiello et al. 2009; Settembre et al. 2011, 2013). TFEB is known to be negatively regulated by nutrient sensor mTOR. 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 (Martina et al. 2012; Settembre et al. 2012). The dephosphorylation of TFEB is mediated by calcineurin activated upon calcium efflux through TRPML1 (Medina et al. 2015). The *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 (Lapierre et al. 2013). Moreover, the overexpression and activation of *hlh-30* is sufficient to extend the life span of wild-type animals (Lapierre et al. 2013). These results indicate that HLH-30/TFEB is a master transcription factor downstream of many longevity pathways possibly through the transcriptional activation of target genes involved in autophagy and lysosomal function.

Other bHLH transcription factor complex, MML-1/MXL-2, has been identified as a novel regulator of longevity (Johnson et al. 2014; 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, and reduced TOR signaling in *C. elegans*. Interestingly, the 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 mutually regulated each other. Comprehensive transcriptome analysis reveals that 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. Thus, they might distribute the responsibilities to reinforce autophagy and longevity in germline less animals.

In *C. elegans*, *Drosophila*, and mouse, the reduction of insulin/IGF-1 signaling ultimately activates DAF-16/FOXO function and extends life span. DAF-16 could regulate autophagy partly through regulating autophagy and lysosomal genes (Li and Zhang 2016). Consistent with this, the overexpression of DAF-16 increases the number of autophagosomes during bacterial infection (Jia et al. 2009). However, although *daf-2* and *daf-16* double mutants do not show longevity, these mutants still have increased numbers of autophagosomes (Hansen et al. 2008). Possibly other factors compensate the activity of autophagy, or DAF-16 regulates autophagy at other timing. Interestingly, DAF-16 interacts with HLH-30 and cooperates target gene expression, promoting stress resistance and longevity (Lin et al. 2018). Other forkhead transcription factor, PHA-4/FOXA, binds to the promoter region of *unc-51/Ulk1*, *bec-1/Becn1*, and *lgg-1/LC3* which work in the 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 the activation of autophagy (Hansen et al. 2008; Lapierre et al. 2011).

11.6 Intervention of Aging via Modulating Autophagy

In addition to the abovementioned rapamycin, several pharmacological treatments have been shown to extend animal life span and health span through the activation of autophagy (Table 11.2). Administration of a natural polyamine, spermidine, is

Table 11.2 Pharmacological modulation of autophagic activity which leads to longevity

Chemical	Model organisms	The effect of autophagy	The lifespan extension was abolished by	References
Resveratrol	*C. elegans*	Enhanced autophagy	Knockdown of *bec-1*	Morselli et al. (2010)
GlcNAc	*C. elegans*	Lifespan extension, enhanced autophagy	No data	Denzel et al. (2014)
α-ketoglutarate	*C. elegans*	Increased LGG-1/LC-3 puncta	No data	Chin et al. (2014)
Metformin	*C. elegans*	Lifespan extension, increased mRNA levels of ATGs	No data	Chen et al. (2017)
Tomatidine	*C. elegans*	Median lifespan extension, enhanced mitophagy	No data	Fang et al. (2017)
Ikarugamycin	*C. elegans*	Lifespan extension, enhanced autophagy	No data	Wang et al. (2017)
Salicylic acid	*C. elegans*	Lifespan extension, enhanced autophagy	Knockdown of *bec-1* or *hlh-30*	Shamalnasab et al. (2018)
Nordihydroguaiaretic acid	*C. elegans*	Median lifespan extension, enhanced autophagy	No data	Tezil et al. (2019)
Myo-inositol	*C. elegans*	Lifespan extension, enhanced mitophagy	Knockdown of *pink-1*	Shi et al. (2020)
4,4′-dimethoxychalcone	*C. elegans* *D. melanogaster*	Lifespan extension, enhanced autophagy	Knockdown of *atg-5* (*C. elegans*) Knockdown of Atg7 (*D. melanogaster*)	Gutierrez et al. (2019)
Spermidine	*C. elegans* *D. melanogaster* Mice	Lifespan extension, enhanced autophagy	Knockdown of *bec-1* (*C. elegans*) Knockdown of Atg7 (*D. melanogaster*)	Eisenberg et al. (2009, 2016)
Rapamycin	*C. elegans* *D. melanogaster* Mice	Lifespan extension, enhanced autophagy	Knockdown of Atg5 or Atg1 (*D. melanogaster*)	Bjedov et al. (2010), Schinaman et al. (2019)
Urolithin A	*C. elegans* Mice	Lifespan extension, enhanced mitophagy	Knockdown of *bec-1, vps-34*, or *pink-1*	Ryu et al. (2016)

beneficial for the health in a number of species and extends the life span of yeast, worms, flies, and mice (Eisenberg et al. 2009, 2016). The survival of cultured mammalian cells is also promoted by treatment with spermidine, and this is accompanied by epigenetic hypoacetylation of histone H3 via the inhibition of histone acetyltransferase activity. This, in turn, correlates with the 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 life span 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 life span 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 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 life span of several model organisms (Park et al. 2013). The life spans of yeast, worms, and flies can be extended by the overexpression and/or pharmacological activation of SIRT1, and the life span of mice is extended by brain-specific overexpression of SIRT1 (Giblin et al. 2014). Especially, the life span extension in *C. elegans* seems to be dependent on autophagy since resveratrol fails to extend the life span 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 the pharmacological activation of SIRT1 by resveratrol treatment stimulates autophagic flux. In contrast, SIRT1 −/− fibroblasts show suppressed autophagy during starvation and show elevated acetylation of key autophagy protein (Lee et al. 2008). Thus, SIRT1 promotes autophagy via the deacetylation of proteins involved in the autophagy pathways. Recent evidence suggests that Beclin1 is acetylated by p300 and deacetylated by SIRT1 (Sun et al. 2015). Acetylated Beclin1 recruits Rubicon, leading to the inhibition of autophagosome maturation, and SIRT1 might promote autophagy through deacetylation of Beclin1. SIRT1 is also known to co-immunoprecipitate with ATG5, ATG7, and LC3 and deacetylate these in vitro, and these interactions could be also essential for autophagy regulation (Lee et al. 2008).

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 life span (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, a natural compound abundant in unripe tomatoes, inhibits age-related skeletal muscle atrophy in mice. Recent study shows that tomatidine

extends life span 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. A 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.

The biguanide metformin extends healthspan and life span in several models including *C. elegans* and mice and the risk of dementia in humans (Barzilai et al. 2016). Metformin has pleiotropic roles and inhibits mitochondrial respiration, mTOR, and activates AMPK, leading to the activation of autophagy (Song et al. 2015; Xie et al. 2011). Whether metformin-mediated life span extension requires autophagy activity needs to be clarified.

11.7 Conclusion

Accumulating evidence suggest that the activation of autophagy is one of the common mechanisms of many longevity paradigms. Moreover, the forced activation of autophagy suffices to extend life span and ameliorates age-associated phenotype in model organism, implying autophagy activation is one of the promising methods to delay human aging. However, actual roles of autophagy contributing to longevity need to be clarified further. It is also critical to establish the method to measure autophagic activity in human. In addition, recent evidence also suggests that autophagy activation in some context becomes detrimental rather than beneficial. For instance, too much upregulation of autophagy genes becomes rather detrimental (Bjedov et al. 2020) and elevated autophagy shortens life span when the mitochondria permeability increases (Zhou et al. 2019a). The neuronal inhibition of some autophagy genes during post reproductive period extends life span (Wilhelm et al. 2017). In addition, autophagy inhibition is necessary in adipocyte for proper function (Yamamuro et al. 2020). Further studies to reveal tissue and timing-specific roles of autophagy are required to make autophagy modulation a promising antiaging therapy in the next decades.

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