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Autophagy as a promoter of longevity – insights from model organisms

Malene Hansen[1], David C. Rubinsztein[2,3], and David W. Walker[4,5]

[1]Sanford Burnham Prebys Medical Discovery Institute, Program of Development, Aging and Regeneration, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA.

[2]Cambridge Institute for Medical Research, Department of Medical Genetics;

[3]UK Dementia Research Institute, University of Cambridge, Hills Road, Cambridge CB2 0XY, UK.

[4] Department of Integrative Biology and Physiology, University of California, Los Angeles, CA 90095, USA;

[5]Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA.

Correspondence:

MH (mhansen@sbpdiscovery.org), DWW (davidwalker@ucla.edu), DCR (dcr1000@cam.ac.uk)

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Glossary

Aggrephagy: The selective removal of cytosolic aggregates by autophagy.

Autophagosome: A cytosolic double membrane-bound vesicle, capable of sequestering cytoplasmic inclusions and organelles destined for degradation in the autolysosome.

Autolysosome: A cytosolic vesicle resulting from fusion between an autophagosome and acidic lysosomes in which degradation of the inner membrane and sequestered material in the autophagosome takes place.

Glomerulus: A key structure of a nephron, the functional unit of the kidney.

Hormesis/Hormetic heat shock: Beneficial effects of a treatment that at a higher intensity is harmful. In one form of hormesis, non-lethal exposure to elevated temperature induces a response that results in increased stress resistance and longevity.

Lipophagy: Selective degradation of lipid droplets by lysosomes contributing to lipolysis (breakdown of triglycerides into free fatty acids).

Lysophagy: Selective degradation of lysosomes by autophagy.

Lysosome: A degradative organelle in higher eukaryotes that compartmentalizes a range of hydrolytic enzymes and maintains a highly acidic pH.

Mitophagy: Selective degradation of mitochondria by autophagy.

mTOR: Mechanistic Target of Rapamycin (mTOR) is an evolutionarily conserved protein kinase that negatively regulates autophagy.

Nucleophagy: Selective removal of nuclear material from a cell by autophagy.

Podocytes: Highly specialized cells of the kidney glomerulus that wrap around capillaries.

Proximal tubule: The most populous cell type in the kidney that accounts for resorption of nearly two-thirds of all filtered water, sodium, and chloride.

Ribophagy: Selective degradation of ribosomes by autophagy.

Sarcopenia: Degenerative loss of muscle mass, quality, and strength associated with ageing.

Septate junction: An intercellular occluding junction found in invertebrate epithelia.

S6K: Ribosomal protein S6 kinase (S6K) is a downstream effector of the mTOR pathway. mTOR/*S6K* signaling modulates protein synthesis, autophagy, and ageing.

Urolithin A: A metabolite produced by gut microbes from ellagic acid. Urolithin A induces mitophagy.

Xenophagy: The selective degradation of intracellular pathogens by autophagy; is part of the cell-autonomous innate immunity defense.

Abstract

Autophagy is a conserved process that catabolizes intracellular components to maintain energy homeostasis and protect the cell against stressful conditions. Accordingly, it has been shown to play critical roles not only during development and disease, but accumulating evidence over the past decade also supports a direct role for autophagy in the ageing process. In particular, elegant studies using yeast, worms, flies, and mice have demonstrated a broad requirement for autophagy-related genes in the long lifespan observed in a number of conserved longevity paradigms. Moreover, several new and interesting concepts relevant to autophagy and its role in modulating longevity have been highlighted: (i) tissue-specific overexpression of single autophagy genes is sufficient to extend lifespan, (ii) selective types of autophagy may be critical for longevity, and (iii) autophagy can act in cell non-autonomous ways to influence organismal health and ageing. Understanding these mechanisms will be critical for modulating autophagy in approaches aimed at improving human healthspan.

Introduction

Autophagy is an evolutionarily conserved catabolic process that plays an essential role in cellular homeostasis by facilitating lysosomal degradation and recycling of intracellular macromolecules and organelles, also referred to as cargo. Autophagy was first discovered as a survival mechanism in yeast subjected to nutrient deprivation, a condition that potently induces the process over basal levels. Since then, studies in several different organisms have established critical roles for autophagy in a variety of biological processes ranging from development to ageing[1]. In turn, autophagy is often found perturbed in disorders such as cancer, diabetes, and neurodegenerative diseases, which all display age-linked onsets[2]. Three types of autophagy have been distinguished based on the mechanism of cargo sequestration: microautophagy (sequestration of cytoplasmic components directly into the lysosome, where

acidic hydrolases mediate degradation), chaperone-mediated autophagy (selective degradation of unique, motif-containing cargo proteins recognized and delivered to the lysosome by a chaperone complex), and macroautophagy (degradation of cytosolic material via sequestration into double-membrane vesicles called autophagosomes that subsequently fuse with lysosomes). This review will focus on macroautophagy (hereafter termed autophagy), which has been extensively studied in the context of ageing in invertebrate models.

A number of autophagy-related (Atg) proteins function in the autophagy process, which can be divided into at least five sequential steps: (1) initiation, (2) double-membrane nucleation and formation of a pre-autophagosome or phagophore, (3) phagophore elongation and sequestration of cytoplasmic cargo, (4) fusion of the autophagosome (the fully enclosed phagophore) to a lysosome, and (5) degradation of sequestered cargo in the autolysosome (**Figure 1**)[3]. Key upstream regulators of this multi-step process include the highly conserved nutrient sensors mTOR (mechanistic Target of Rapamycin) and AMP-activated kinase (AMPK) (which are also critical longevity determinants, see **Box 1**), which directly phosphorylate ULK1 (Atg1 in yeast), a key upstream-acting kinase[4]. Another set of key autophagy proteins to highlight are the LC3/GABARAP family in mammals (Atg8 in yeast). Fluorescently-tagged or endogenous LC3/GABARAP/Atg8 proteins are commonly used as steady-state autophagy markers in many species to facilitate microscopic visualization of phagophores and autophagosomes in the cell[5]. LC3/GABARAP/Atg8 are proteolytically processed and attached to autophagosomal membranes, where they participate in cargo recognition and recruitment to the phagophore by interacting with various cargo receptors bound to proteins or organelles. Prominent examples of cargo receptors are SQSTM1/p62, which recognizes ubiquitinated proteins or organelles targeted for degradation[6], and BNIP3, a receptor for mitochondria destined for degradation by mitophagy[7]. Clearance of such specific types of cargo, including additional macromolecules like lipids and organelles such as ribosomes, is collectively referred to as selective autophagy. Notably, damaged macromolecules and organelles are known to

accumulate over time, likely contributing to the functional decline experienced during ageing. Below, we discuss the current literature linking autophagy, including selective types of autophagy, to organismal, tissue and cellular ageing in model organisms.

Autophagy in organismal ageing

Different lines of evidence indicate that ageing modulates the autophagy process. Autophagy reporter analyses and gene expression studies in different species indicate a decline in autophagy over time, whereas genetic experiments carried out in multiple short-lived model organisms to modulate autophagy gene activity indicate that autophagy induction plays an important role in ensuring lifespan extension, as summarized below.

Observations in ageing animal models

Many organisms show signs of a decrease in autophagic capacity with age. For example, levels of lysosomal protease activity decline with age in the nematode *C. elegans*[8], autophagy gene transcripts decrease with age in tissues of the fruit fly *Drosophila*, including the brain (*Atg2, Lc3/Atg8a, Wipi/Atg18, Alfy/bchs*)[9] and muscle (*Ulk1/Atg1, Atg5, Becn1/Atg6, Atg7,* and *Lc3/Atg8a*)[10,11], LC3/Atg8 and ATG7 protein levels decline with age in mouse hypothalamus[12], and in mouse and human muscle[13], and lysosomal-associated membrane protein type 2a (LAMP2a) as well as chaperone-mediated lysosomal activity decline in rat liver[14]. Consistent with such changes in the levels of key autophagy components, assays monitoring the autophagy process indicate a decline in autophagic capacity over time in several species. For example, a recent spatiotemporal analysis of autophagy in *C. elegans* using fluorescently-tagged LC3/Atg8/LGG-1 proteins as markers of autophagosomes and autolysosomes in combination with autophagy inhibitors (i.e., so-called 'flux assays') shows an age-dependent increase in decline in autophagic vacuoles in four major tissues (intestine, body-wall muscle, pharyngeal muscle, and neurons), with possible tissue-specific kinetic differences still to be

153 determined; this accumulation of autophagic structures likely reflects impaired autophagic
154 activity [15]. Another recent study similarly reported a reduction in autophagic activity in whole-
155 body extracts of aged *C. elegans*[16]. Moreover, electron-microscopy analysis of rat livers shows
156 an increase in autophagic vacuoles with age, and flux assays used to estimate autophagic
157 activity also here indicate that aged animals have a decreased ability to turn over autophagic
158 vesicles[17]. Consistently, quantification of proteolysis of long-lived proteins in the livers of rats
159 indicates an age-dependent decline in autophagic function and lysosomal degradation[17,18],
160 whereas the lifespan-extending intervention of dietary restriction, i.e., reduction in food intake
161 without malnutrition (see **Box 1**) prevents this decline[19,20]. Thus, evidence from multiple model
162 organisms shows that autophagy gene expression and protein levels decrease with age, at least
163 in some contexts causing an accumulation of autophagic structures, and possibly limiting
164 autophagic capacity to maintain cellular homeostasis. Further studies of tissue- and cell type-
165 specific differences will be required to better understand the exact contribution of each tissue to
166 systemic ageing.
167
168 Genetic links

169 Autophagy has also been directly linked to ageing via genetic experiments in multiple model
170 organisms (see **Table 1**), showing a broad and critical role for autophagy genes in several
171 conserved longevity paradigms (**Box 1**). Specifically, multiple autophagy-related genes are
172 required for the long lifespan observed in longevity models, including inhibition of mTOR, an
173 inhibitor of autophagy in eukaryotes. Indeed, various autophagy genes in yeast (*Ulk1/Atg1*,
174 *Atg7*, *Atg11*)[21], worms (*Ulk1*/*Atg1/unc-51, Becn1/Atg6/bec-1, Wipi/Atg18)*[22,23], and flies (*Atg5*)[24]
175 are required for mTOR-mediated longevity (**Table 1**). Similarly, lifespan extension by dietary
176 restriction is abrogated in yeast (*Atg15,* and fusion-related v-SNARE genes *Vam3, Vam7*)[25] and
177 in worms (*Ulk1/Atg1/unc-51, Becn1/Atg6/bec-1*, *Vps34, Atg7*)[22,23,26] with compromised
178 autophagy. Moreover, the long lifespan of animals overexpressing AMPK, an activator of

autophagy, is reduced in autophagy-deficient backgrounds in worms (*Wipi/atg-18*, Hansen lab, unpublished results) and flies (*Atg1*)[27]. Finally, lifespan extension obtained by media supplementation with the polyamine spermidine is blocked in yeast (*Atg7*), worm (*Becn1*/*Atg6/bec-1*), and fly (*Atg7*)[28] autophagy mutants. Similarly, autophagy genes are required for numerous conserved longevity paradigms in *C. elegans*, including reduced insulin/IGF-1 signaling (IIS)[22,23,29,30], reduced S6K signaling[31], reduced mitochondrial respiration[22,32,33], germline ablation[34], hormetic heatshock[35], the plant phenol resveratrol[36] and the human microbiome metabolite urolithin A[37]; likewise, the autophagy gene *Lc3/Atg8* is required for the long lifespan of flies with reduced TGF-beta signaling[11] (**Table 1**; see also [38] for additional genetic links between autophagy and specific long-lived *C. elegans* mutants). Indeed, impairment of autophagy genes in young adult animals abrogates lifespan extension in all long-lived mutants of any species tested so far (see Discussion for later-in-life impairments), but generally has small or no effects on the lifespan of normal animals[27,38,39]. The latter observations likely reflect that residual autophagy gene expression is sufficient to support basal autophagy in RNAi-compromised wild-type animals. This is in contrast to autophagy impairments carried out during development, which generally causes sickly and short-lived animals irrespective of their genetic background, reflecting important developmental roles for autophagy[1,40]. Notably, where analyzed, long-lived mutants also display increased steady-state markers of autophagy, consistent with these animals possessing increased autophagic activity. This has been directly assessed by flux assays in long-lived *C. elegans* with reduced insulin/IGF-1 signaling and in mutants lacking a germline; these mutants generally show increased autophagic capacity compared to wild-type *C. elegans*, yet with notable tissue-specific differences[15] (see also discussion below). Moreover, several long-lived worms and flies display increased expression of multiple autophagy-related and lysosomal genes (reviewed in [41]). Collectively, these observations suggest a model in which increased autophagic activity plays a causal role in promoting lifespan extension in long-lived animals. It should be noted, however, that for a

proportion of the studies, especially the work in *Drosophila*, the conclusions are based on knockdown of single autophagy genes.

In further support of a direct role for autophagy genes in lifespan determination, overexpression of specific autophagy genes can extend lifespan in several species (**Table 1**). For example, overexpression of fly *Lc3/Atg8a* in the nervous system[9], or in the muscle[11] is sufficient to extend fly lifespan. Similarly, neuron-specific overexpression of *Ulk1/Atg1* in flies[27], and ubiquitous overexpression of *Atg5* in mice is sufficient to stimulate autophagy, improve markers of health, and extend lifespan[42]. While all of these lifespan extensions are accompanied by increases in autophagy markers and improved healthspan parameters (see section on tissue-specific roles for autophagy below), it remains to be formally tested if the observed longevity requires the autophagy process. In this regard, it is noteworthy that overexpression of the helix-loop-helix transcription factor TFEB/HLH-30, a conserved regulator of many autophagy-related and lysosomal genes[41,43], extends lifespan in *C. elegans* in an autophagy-dependent fashion[32]. Collectively, these observations indicate, but do not prove, that up-regulation of autophagy may be an effective approach to delay ageing and promote healthspan, in diverse species including mammals.

Importantly, genetic and age-related loss of autophagic and lysosomal function has also been linked to the development of several age-related diseases, including neurodegenerative diseases and cancer (**Table 1**). For example, loss-of-function mutations in several genes with autophagy-related functions (e.g., *Becn1/Atg6, Atg5,* and *Atg7*) result in decreased autophagy along with accumulation of dysfunctional organelles and disordered and aggregated proteins in mammalian models of neurodegenerative disorders such as Huntington's disease (Huntingtin, HTT), Alzheimer's disease (Aβ and Tau), and Parkinson's disease (α-synuclein) (reviewed in [44]). Importantly, Mendelian mutations in autophagy regulators can cause neurodegenerative diseases, including spastic paraplegia[45,46], and ataxia[47], and loss-of-activity of autophagy receptors/selective autophagy can cause Parkinson's disease[48,49] or forms of motor-neuron

disease[50-52]. Overall, accumulating evidence supports a beneficial role for autophagy in ageing and age-related diseases, although the underlying mechanisms of autophagy regulation are not fully understood.

Selective types of autophagy in ageing

While the above genetic links indicate involvement of the general autophagy process, different variations of autophagy may play critical roles in ageing. The turnover of such specific cargoes via specific autophagy receptors is referred to as selective autophagy. Below, we discuss studies suggesting that selective forms of autophagy play important roles in ageing and lifespan determination (**Figure 2**).

Mitophagy

The accumulation of dysfunctional mitochondria is a shared hallmark of ageing and numerous diseases of old age[53-56]. As organisms age, mitochondrial function decreases causing an increase in electron leakage and generation of reactive oxygen species. Aged mitochondria may also have an increased susceptibility to apoptotic signaling. Although the underlying mechanisms that lead to an age-related loss of mitochondrial function remain incompletely understood and may involve numerous processes, it has been suggested that a decline in mitophagy may contribute[55-57]. In mammals, the degradation of damaged mitochondria is mediated by a pathway comprised of PTEN-induced putative protein kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin. In recent years, the molecular mechanisms of mitophagy have been elucidated in some detail from studies in mammalian cell culture and genetic studies in model organisms[58-60]. Disruptions in mitophagy have been implicated in the pathophysiology of age-related diseases such as cardiac senescence[61], retinopathy[62], fatty liver disease[63], pulmonary hypertension[64], kidney disease[65] and neurodegenerative disorders, including Parkinson's disease, motor neuron disease/amyotrophic lateral sclerosis (ALS)[66] and Alzheimer's

disease[58,67] (**Figure 2**). However, as with all forms of selective autophagy described below, it is very challenging to demonstrate causality for the selective autophagy in human disease in a direct sense, as opposed to links or associations.

Studies in worms[57], flies[39], mice and humans[68,69] have reported a decline in mitophagy markers in aged animals. This may be relevant to age-related pathologies, as loss of *pink1* or *parkin* leads to early-onset behavioral decline and shortened lifespan in flies[70,71]. Two recent studies, in *C. elegans*, have investigated the importance of mitophagy in longevity assurance[33,57]. *dct-1* (DAF-16/FOXO Controlled) is a putative orthologue to the mammalian NIX/BNIP3L and BNIP3 (Nip3-like protein X/Bcl-2 and adenovirus E1B interacting protein 3, respectively), which act as mitophagy receptors in mammals[59]. Inhibition of *dct-1* leads to an increase in mitochondrial content, indicating that DCT-1 is the nematode orthologue of NIX/BNIP3L and functions as a key regulator of mitophagy[57]. Moreover, inhibition of *Nix/Bnip3L/dct-1* or *pink-1* shortens the lifespan of long-lived *InR/daf-2* mutants and dietary-restricted *eat-2* mutants[57], whereas inhibition of *Nix/Bnip3L/dct-1* or *pink-1* impairs lifespan extension in several long-lived *C. elegans* models of moderate mitochondrial dysfunction[33,57] (**Table 1**). Collectively, these studies indicate that mitophagy plays a causal role in these modes of lifespan extension.

A number of studies have examined the impact of enhancing mitophagy on ageing and lifespan. Critically, ubiquitous or neuron-specific, adult-onset upregulation of Parkin extends *Drosophila* lifespan[72] (**Table 1**). Moreover, it was recently reported that a midlife shift towards a more elongated mitochondrial morphology is linked to impaired mitophagy and the accumulation of dysfunctional mitochondria in aged *Drosophila* flight muscle[39]. Promoting Dynamin-related protein 1 (Drp1)-mediated mitochondrial fission in midlife restores mitochondrial morphology to a youthful state, facilitates mitophagy and improves mitochondrial-respiratory function. Importantly, transient, midlife induction of Drp1 improves markers of organismal health, delays age-onset pathology and prolongs fly lifespan in an *Ulk1/Atg1*-dependent fashion (**Table 1**).

283 Furthermore, upregulating Drp1 specifically in neurons or the intestine, from midlife onwards,
284 is sufficient to prolong fly lifespan[39]. These findings indicate that a midlife decline in
285 mitophagy, due to a shift in mitochondrial dynamics, contributes to age-onset mitochondrial
286 dysfunction and is limiting for lifespan.

287 Given the findings above, it has been proposed that pharmacological interventions that
288 stimulate mitophagy may prove effective in delaying age-onset health decline[73]. Consistent with
289 this model, dietary treatment of *C. elegans* with the human microflora-metabolite urolithin A
290 induces mitophagy and prolongs worm lifespan[37]. More specifically, short-term urolithin A
291 treatment in worms induces mitochondrial fragmentation and reduces mitochondrial content in
292 an autophagy-dependent fashion. Urolithin A treatment improves a number of markers of *C.*
293 *elegans* healthspan and maintains mitochondrial-respiratory capacity during ageing, and the
294 lifespan-extending effects of urolithin A treatment require the mitophagy genes *pink-1* and
295 *Nix/Bnip3L/dct-1* (**Table 1**). Importantly, urolithin A treatment is also beneficial in rodents, where
296 it improves exercise capacity in two different mouse models of age-related decline of muscle
297 function, as well as in young rats[37], overall suggesting conserved beneficial effects of inducing
298 mitophagy.

299
300 Lipophagy

301 Studies in diverse organisms have suggested that specific alterations in lipid metabolism are
302 associated with different prolongevity interventions[74]. In recent years, the contribution of
303 autophagy to intracellular lipid droplet degradation has been identified[75]. The first clear
304 demonstration that lipid droplets could be turned over via autophagy came from studies in
305 cultured hepatocytes with reduced Atg5 levels[76]. The fact that autophagy can regulate lipid
306 metabolism expands the physiological relevance of autophagy to modulate the cellular energetic
307 balance directly. Furthermore, alterations in lipophagy could impact cell physiology, indirectly,
308 via alterations in the regulatory activities that lipids exert inside cells. As a result, it has been

proposed that alterations in lipophagy may underlie the metabolic syndrome of ageing[77] (**Figure 2**), a constellation of features including obesity, dysregulated lipoprotein metabolism, abnormal glucose handling and high blood pressure. Lipophagy has also been linked to cancer[78] and atherosclerosis[79].

Recent studies in *C. elegans* have linked lipophagy to longevity. Specifically, germline-less *glp-1* mutants require both a lysosomal lipase LIPL-4[80] and autophagy genes[34] for their lifespan extension, and increased autophagy and LIPL-4-dependent lipolysis work interdependently to promote longevity[34] (**Table 1**). Furthermore, increased lysosomal lipolysis has been directly linked to lifespan extension in worms[80-82]. Although the molecular mechanisms involved are not fully understood, overexpression of LIPL-4 has been shown to induce nuclear translocalization of a lysosomal lipid chaperone LBP-8, consequently promoting longevity by activating the nuclear hormone receptors NHR-49 and NHR-80[83]. Of further note, aged worms display a deposition of lipids in non-adipose tissues, including the nervous system[84]. Interestingly, interventions that promote longevity, such as dietary restriction, reduce this ectopic fat accumulation, whereas inhibition of autophagy-related genes, including *hlh-30*/TFEB, increase fat accumulation[84], overall indicating a role for lipophagy in ectopic fat deposition in *C. elegans*.

Aggrephagy

Aggrephagy describes the selective recruitment of protein aggregates, or possibly oligomeric forms of proteins that are destined to form aggregates. These include proteins like tau, alpha-synuclein and mutant huntingtin, which accumulate and cause toxicity in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease (**Figure 3**). These proteins are autophagy substrates[85-87] and autophagy upregulation by chemical[88], genetic[89], or environmental means, i.e., by a small beneficial heatshock (referred to as hormesis)[35] can ameliorate signs in a wide range of animal models of these diseases including, *C. elegans*, *Drosophila*, zebrafish and mice (reviewed in [90]). Furthermore, alpha-synuclein[91] and

many mutant polyglutamine-expanded proteins, like mutant huntingtin[92] can inhibit autophagy. This could potentially introduce a feed-forward loop into disease pathogenesis as inhibition of autophagy accelerates disease by accumulating the disease-causing aggregate prone proteins[115]. Thus, any age-dependent decrease in autophagy in the brain will have a major impact in these conditions. Indeed, age is a major risk factor in most neurodegenerative diseases.

Lysophagy

The selective degradation of damaged lysosomes appears to be an important mechanism, which would shield the cytoplasmic contents from leakage of lysosomal hydrolases[93]. This mechanism protects against acute kidney injury in mice[93] (**Figure 2**). It is interesting to speculate that any age-dependent loss of autophagy may reduce this mechanism of cellular protection against lysosomal enzyme leakage into the cytoplasm and would thus predispose to kidney damage and chronic renal failure, two age-related conditions. While compromised autophagy enhances kidney damage with age in mice[94], it is interesting that basal autophagy is increased in kidney proximal tubules in older versus younger mice[94]. However, starvation-induced autophagy in kidney proximal tubules is blunted in aged mice[94]. Interestingly, autophagy appears to be less active in podocytes (the most vulnerable cells in the glomerulus)[95] compared to the proximal tubule, but no age-dependent change in podocye autophagy was observed[94]. Thus, in this case, autophagic capacity may correlate inversely with cell-type vulnerability to damage in the kidneys. While this may be mediated in part by lysophagy, it is likely that altered clearance of other autophagy substrates may contribute, including mitochondria. The links of lysophagy to physiology and disease are still largely limited to studies in the kidney likely due to its relatively recent characterization although recent studies have also proposed links with muscle disease and neurodegeneration[96]. However, it is possible that this mechanism may have much broader importance and thus impact many other organ systems.

Autophagy in tissue-specific ageing

While ageing is linked to a decline in physiological functions at both the tissue and organismal level, it remains unclear how ageing of individual tissues may limit the lifespan of the organism. Thus, it is of interest to understand tissue-specific roles for autophagy in ageing, including selective types of autophagy in individual tissues and cell types of model organisms (**Table 2**), as reviewed below.

Intestine

Intestinal barrier dysfunction is a common feature of ageing organisms and has been linked to a number of human diseases[97]. In *Drosophila*, age-onset intestinal barrier dysfunction is linked to microbial dysbiosis, increased immune gene expression, loss of motor activity, systemic metabolic defects and is a harbinger of mortality [98,99]. Together with data showing that the intestine represents a critical target organ for genetic interventions that prolong lifespan[100], these findings support the idea that maintaining intestinal integrity during ageing is critical for organismal health and viability. Dietary restriction delays the onset of intestinal barrier dysfunction in both *C. elegans*[101] and *Drosophila*[99,102]; likewise, short-term protein restriction has recently been linked to improved markers of intestinal barrier function in adult pigs[103]. While direct tests are needed, improved intestinal barrier function may be a phenotype shared by multiple conserved longevity paradigms, as *C. elegans* mutants with reduced insulin/IGF-1 signaling also have improved intestinal barrier function[101].

Intestinal expression of autophagy genes has been shown to be critical for the lifespan extension observed in several longevity paradigms (**Tables 1, 2**), including dietary restriction in *C. elegans*[101]. Indeed, various reporters and flux analyses indicate that autophagy is induced in the intestine of long-lived *eat-2* mutants, a genetic model of dietary restriction, and intestine-specific RNA interference (RNAi) of two *Atg8* homologs, *lgg-1* and *lgg-2*, or of Wipi homolog *atg-18* significantly decreases the long lifespan of such mutants. Consistent with a role for

autophagy in dietary restriction, *Wipi/atg-18* mutants do not display lifespan extension upon bacterial dilution (an independent dietary restriction protocol), and intestine-specific expression of *Wipi/atg-18* in these mutants restores dietary restriction-mediated lifespan extension[104]. Moreover, either whole body or intestine-specific RNAi of autophagy genes impairs the improvements in age-related intestinal barrier function in dietary-restricted *eat-2* mutants[101]. Collectively, these findings suggest that autophagy induction in the intestine of dietary-restricted animals can act to maintain intestinal barrier function during ageing, and that this may be important for lifespan extension. While dietary restriction has been reported to increase autophagy markers in multiple tissues and organs of mice[105-107], it remains unknown whether modulation of autophagy, systemically or in specific organ systems, plays a causal role in dietary restriction-mediated lifespan extension in mammals.

How may intestinal autophagy, induced by for example dietary restriction, improve intestinal barrier function? In *Drosophila*, there is an altered localization and expression of septate-junction proteins in the aged intestine, which may contribute to age-onset barrier dysfunction[108]. Although a direct role for autophagy dysfunction has not been shown, defects in autophagy-related proteins have been linked to the pathogenesis of Crohn's disease, which is characterized by intestinal barrier dysfunction[109]. Moreover, it has been shown that autophagy selectively reduces epithelial tight-junction permeability by lysosomal degradation of the tight-junction protein claudin-2[110]. Therefore, it is possible that investigating the interplay between autophagy, junction-protein localization, and ageing may provide novel therapeutic approaches to maintain intestinal health during ageing.

Additional links exist between the intestine, autophagy and longevity. For example, flux analysis in germline-less *C. elegans* mutants (i.e., that carry a mutation in the GLP-1/Notch receptor) indicate induced autophagy in the intestine, and knockdown of *Wipi/atg-18* in the intestine of adult animals abrogates the lifespan extension observed in *glp-1* mutants[15] (**Table 1**). In contrast, the same adult intestinal RNAi treatment did not significantly shorten the lifespan

413 of *C. elegans daf-2* insulin/IGF-1 receptor (InR) mutants, indicating that intestinal autophagy
414 may not play a key role in lifespan extension in this longevity paradigm[15]. In contrast, another
415 recent *C. elegans* study expressed *Wipi/atg-18* tissue-specifically in short-lived,
416 developmentally-impaired *Wipi/atg-18* loss-of-function mutants carrying the same *InR/daf-2*
417 mutation and found that such intestinal reintroduction of WIPI/ATG-18 rescued the short lifespan
418 and fully extended lifespan of these animals[104]. The latter experiment does not discriminate
419 between the role of WIPI/ATG-18 during development versus ageing, and more experiments are
420 needed to fully address the role of intestinal autophagy in *InR/daf-2* mutants. Consistent with
421 these observations linking intestinal autophagy to longevity in *C. elegans*, intestinal
422 overexpression of AMPK in *Drosophila* induces markers of autophagy and autophagy gene
423 expression in the intestine, and extends fly lifespan[27], collectively indicating an important role for
424 intestinal autophagy in lifespan determination.

425 This *Drosophila* study also highlighted, as noted above, cell non-autonomous effects of
426 tissue-specific autophagy induction. Specifically, neuronal overexpression of *Ulk1/Atg1*, or of
427 AMPK, causes increases in autophagy markers and autophagy gene expression in the intestine,
428 whereas intestinal overexpression of AMPK causes alterations in autophagy in the brain[27].
429 Importantly, neuronal *Ulk1/Atg1* overexpression also improved intestinal barrier function,
430 indicating a beneficial role for such autophagy induced in a non-cell autonomous manner.
431 Although these inter-tissue effects are associated with reduced insulin-like peptide levels in the
432 brain, the causal mechanisms involved remain to be elucidated. It is also worth considering that
433 ablation of the insulin-like peptide-producing median neurosecretory cells in the brain can
434 prolong fly lifespan[111]. Therefore, it is possible that the prolongevity effects of neuronal
435 *Ulk1/Atg1* overexpression may involve altered insulin-like peptide signaling.

436 Indeed, it is interesting to speculate as to whether induction of intestinal or neuronal
437 autophagy can impact systemic autophagy levels to prolong lifespan, e.g., upon dietary
438 restriction. In support of this model, inhibition of autophagy genes in the intestine significantly

impairs motility, presumed to be a marker of neuromuscular function, in long-lived, dietary-restricted *C. elegans eat-2* mutants[101]. Collectively, these studies indicate an important role for autophagy in the intestine of multiple organisms; it will therefore be interesting to investigate the requirement for autophagy in the intestine of ageing mammals, including the role of autophagy in intestinal integrity.

Nervous system

Several studies have shown that the nervous system plays an important role in modulating lifespan, yet the cellular mechanisms involved are not well understood[112,113]. There are a number of suggestions that autophagic activity may be compromised with age in the brains of different species. As noted above, autophagy is decreased in mouse hypothalamic neurons[12,114], and the mRNA expression of a number of autophagy genes is decreased in aged human brains[115]. However, further work is required to test the hypothesis that autophagic activity may decline in an age-dependent fashion more rigorously with alternative approaches.

Neuronal autophagy has been linked to organismal ageing in several *Drosophila* studies in which overexpression of single autophagy genes has been shown to increase longevity (**Tables 1, 2**). Specifically, pan-neuronal overexpression of *Lc3/Atg8* throughout life extends *Drosophila* lifespan and improves neuronal proteostasis and organismal oxidative stress response[9]. Likewise, adult-onset, pan-neuronal overexpression of *Ulk1/Atg1* extends fly lifespan[27]. As noted above, such adult-onset induction of ULK1/Atg1, or AMPK, in the fly nervous system is also linked to a delay in intestinal barrier dysfunction during ageing, and both the cell autonomous and non-cell-autonomous effects of ULK1/Atg1 and AMPK on intestinal integrity during ageing are linked to an increase in autophagy markers and autophagy gene expression in the intestinal epithelium[27], indicating cell autonomous and non-autonomous effects of neuronal autophagy.

In *C. elegans*, restoring expression of *Wipi/atg-18* in neurons of *InR/daf-2; Wipi/atg-18* double mutants fully rescues the short lifespan of these animals[104]. Moreover, expression of *Wipi/atg-18* exclusively in chemosensory neurons is sufficient to mediate InR/DAF-2-mediated longevity signals. In the same study, it was reported that *Wipi/atg-18* expression in chemosensory neurons does not rescue the lifespan of *Wipi/atg-18* mutants[104]. Therefore, it would appear that *Wipi/atg-18* expression in chemosensory neurons plays a more significant role in mediating *InR/daf-2* longevity signals than maintaining normal lifespan. Although it was shown that the ability of *Wipi/atg-18* expression in chemosensory neurons to mediate *InR/daf-2* longevity depends genetically on the release of neurotransmitters, the underlying physiological mechanisms are not known.

How may autophagy contribute to brain function during ageing? An age-related decline in memory formation has been reported in both model organisms and humans[116], yet the underlying mechanisms are not well understood. Interestingly, recent studies have linked autophagy to cognitive functions in *Drosophila* treated with polyamines such as spermidine and putrescine. These compounds promote lifespan in diverse species by augmenting autophagy[28] (**Table 1**). Notably, dietary spermidine suppresses age-induced memory impairment in an autophagy-dependent manner in *Drosophila*[117]. Most recently, it has been reported that spermidine counteracts age-related changes affecting the size and function of a specific synaptic compartment, the presynaptic active zone, to maintain memory in aged flies[118] (**Table 2**). Together, these findings support a model in which an age-dependent decline in autophagy contributes to cognitive ageing. Autophagy may impact many processes in the central-nervous system and other tissues that contribute to ageing including degradation of aggregate-prone intracytoplasmic proteins (aggrephagy) and dysfunctional mitochondria (mitophagy), as discussed in detail above.

Muscle

Recent work in mammals and *Drosophila* indicates that maintaining muscle integrity and function is critical for systemic aging and lifespan determination[119]. Although the mechanisms involved are not fully understood, emerging evidence suggests that muscle-derived growth factors and cytokines, known as myokines, can modulate systemic physiology[119]. Interestingly, of the tissues examined, the greatest change in autophagy markers occurs in the body-wall muscle of *C. elegans*[15], potentially reflecting muscle as a tissue with especially active autophagy.

Muscle-specific autophagy has been linked to longevity in studies in *C. elegans* and in *Drosophila*. In worms, inhibition of autophagy genes *Lc3/Atg8/lgg-1* and *Wipi/atg-18* in the body-wall muscle of adult animals is sufficient to shorten the lifespan of both dietary-restricted *eat-2* mutants[101], as well as *InR/daf-2* mutants[15]. In turn, overexpression of *Lc3/Atg8* in the body-wall muscle of flies increases lifespan[11] (**Table 1**). In mice, muscle-specific *Atg7* deficiency causes impaired muscle function and reduced lifespan[13]. While interpreting lifespan-shortening interventions can prove challenging[120], this study suggests that loss of muscle function during ageing, due to impaired autophagy, may limit lifespan in mice.

How could autophagy be important for muscle function? The muscle of animals is critical for mobility, which declines with age due to sarcopenia, or age-related muscle loss[121]. A recent follow-up study in animals lacking *Atg7* in the muscle showed that autophagy plays a critical role in maintaining the neuromuscular junction and muscle strength, at least in part by improving mitochondria number and function[13] (**Table 2**). This is consistent with work in mice, in which overexpression of ATG7 in muscle prevents age-associated myofiber degeneration and mitochondrial dysfunction[13].

Autophagy may also play important roles in specialized cells of the muscle. Mammalian muscle contains muscle stem cells, also referred to as satellite cells. Satellite cells are usually present in a quiescent state, but require autophagy to become activated, i.e., to proliferate and differentiate into muscle fibers, likely in order to provide nutrients for this metabolically

demanding event[122,123]. Of note, the ability to activate satellite cells declines with ageing, and impaired autophagy was recently shown to play a causal role in this phenotype. Specifically, autophagy is used to maintain stemness of satellite cells by preventing cellular senescence, likely via mechanisms that at least in part relate to mitochondrial maintenance[124] (**Table 2**). Notably, induction of autophagy by the mTOR inhibitor rapamycin can reverse senescence and restore regenerative functions of both aged murine and human satellite cells[124]. In conclusion, autophagy plays important protective roles in the muscle, and it will be interesting to investigate whether boosting autophagy can alleviate sarcopenia and improve mobility in aged animals.

Other tissues

Additional tissue types show autophagy chances with age, for example in the immune system. Immune senescence is a risk factor for numerous age-onset diseases, including cancer. Recent studies have revealed that autophagy-deficient immune cells show numerous aging phenotypes, and that autophagy-inducing agents can improve the immune responses in the elderly[125]. Hence, autophagy has emerged as a novel target to treat age-onset diseases associated with immune senescence. To this point, it is interesting to note that autophagy appears to be better maintained in immune cells of exceptionally long-lived humans[126]. At present, however, it is not known whether it is possible to induce autophagy specifically in immune cells and improve immune surveillance.

Another tissue that displays changes in autophagy over time is hematopoietic stem cells (HSCs). These stem cells differentiate into multiple types of blood cells in vertebrates, but HSCs lose their ability to regenerate the blood system over time. Recent studies have implicated autophagy as a key pathway in homeostasis of the blood system[127]. Indeed, it has been reported that autophagy is essential for maintaining the replicative quiescence of HSCs throughout life by limiting the number of active mitochondria[128]. It remains to be tested if the autophagy process in the immune system and HSCs are directly linked to organismal lifespan.

Conclusions and perspectives

Evidence has been mounting over the last decade that autophagy and ageing are closely linked. In particular, work in model organisms from yeast to mice has shown that multiple autophagy-related genes are required for the long lifespan of conserved longevity paradigms. Combined with gene expression data and autophagy-marker analyses generally indicating that such animals also have increased levels of autophagy, these observations indicate that long-lived animals may boost autophagy and this contributes to their extended lifespan. In turn, autophagy appears to become limiting with normal ageing, possibly in a tissue-specific fashion and involving selective types of autophagy.

How could autophagy be declining in most tissues with age? One general idea would involve an alteration in the activity of key regulators of autophagy, such as the nutrient sensor mTOR. The activity of mTOR, which negatively regulates autophagy, has been reported to increase over time in at least some tissues of mice, with some notable exceptions[129]. Likewise, while autophagic activity appears to decline in most settings, increases in autophagy have been observed in an ageing subset of hemaepoetic stem cells[128], as mentioned above. In tissues where mTOR activity may be increased with age, it remains to be investigated which mTOR-controlled steps of autophagy are changed with ageing, i.e., phosphorylation of Atg1/ULK1 as well as regulation of the transcription factor TFEB. Lastly, what are the consequences of these changes, and what exact step of autophagy may ultimately become limiting for lifespan? One possibility could be impairment of lysosomal acidification, as observed in yeast[130]. Consistent with this idea, the activity of several lysosomal proteases decrease with age in *C. elegans*[8]. Another possibility might involve an age-dependent impairment of autophagic vesicle transport, as observed in neurons[131]. Regardless of the mechanism, stalled autophagy could become detrimental to the cells as certain types of cellular components might accumulate to potentially toxic levels and possibly in a tissue-specific fashion. As an example, accumulation of

autophagosomes or the autophagic machinery have been observed in *C. elegans*[15,16] and in mouse livers[17]**.** It is an important objective for future research to understand the regulation of age-associated changes in autophagic activity. Since autophagy is tightly linked to another major proteostatic process, the ubiquitin-proteasomal system, it will likely also be important to understand how the coordination of such systems changes over time.

As noted above, mounting evidence suggests that different longevity paradigms require functional autophagy. Since at least two of such conserved paradigms, namely reduced insulin/IGF-1 signaling and germline removal, affect autophagy regulation differently at the tissue-specific level in *C. elegans*[15], there may be multiple ways to increase lifespan by autophagy modulation. Since several of the longevity paradigms are additive for lifespan extension[132], it will be interesting to similarly investigate if combining the paradigms can produce synergistic results in regards to induction of the autophagy process. Moreover, it will be valuable to further examine the cell-autonomous and cell-non-autonomous roles of autophagy in long-lived animals. To this end, it will be important to address tissue requirements for autophagy in longevity models in mammalian systems, e.g., in response to dietary restriction.

Both the ageing and the autophagy research fields have been driven forward tremendously by the use of genetically-tractable model organisms, with many new concepts emerging from research in these systems. While a lot of research is still needed to consolidate such new and exciting findings in additional species, it is interesting to ponder what new unexpected findings that have yet to come? Surely, it is possible that more types of specific cargo in addition to the ones discussed here (i.e., lipophagy, mitophagy, aggrephagy, and lysophagy) will prove to be relevant to ageing, since other types of selective autophagy have been reported (e.g., ERphagy, ribophagy, xenophagy, and nucleophagy). In addition, it will be important to fully address the functional role of autophagy not only in different tissues but also over time. To this end, a very recent study, surprisingly, reported that late-life inhibition of autophagy genes with functions in the phagophore nucleation complex causes a potent lifespan extension in wild-type *C.*

595 *elegans*[16]. This is in stark contrast to the effects of inhibiting the same genes early in adult life,
596 when autophagy gene RNAi generally has no or a small lifespan-shortening effect in wild-type
597 *C. elegans*[38]. Thus, the multi-step autophagy process may affect ageing in a much more
598 complex manner than previously anticipated, and future experiments are needed to address this
599 important point. Another interesting avenue to explore further may be the interplay between
600 autophagy, commensal homeostasis, and organismal health and ageing, given recent attention
601 on links between microbiota dynamics and host ageing[133,134]. Likewise, as non-conventional
602 roles for autophagy-related genes for example in secretion are becoming increasingly
603 recognized[135], it also remains to be addressed if any of such non-conventional functions of
604 autophagy genes may be linked to ageing. Finally, it will be very attractive to explore the
605 growing number of pharmacological interventions that can induce autophagy for possible effects
606 on longevity (**Box 2**).
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BOX 1. Conserved longevity paradigms linked to autophagy.

Ageing is a complex physiological process characterized by the progressive failure of tissue- and cellular functions, ultimately leading to death of the organism. Interestingly, extensive research efforts using model organisms from yeast to mice have identified a number of genetic pathways and environmental interventions that can delay ageing and thus extend organismal lifespan in a conserved fashion (see Table Box). These interventions are also referred to as longevity paradigms. The first example of such a longevity paradigm was described in the 1930's, when reduced food intake without malnutrition (called dietary restriction) was shown to extends the lifespan of rats, a treatment shown later to have beneficial effects in several other organisms[136]. Similarly, reducing the activity levels of two major nutrient-sensing pathways, the mTOR[137], and insulin/IGF-1[138] signaling pathways extend lifespan in a number of species, whereas overexpression of the nutrient-sensor AMPK extends lifespan in worms and flies[139]. Other interventions also extend lifespan in at least yeast, worms and flies, including reduced levels of mitochondrial respiration[140], and hormetic heat shock[141]. Lastly, a number of pharmacological interventions extends lifespan in a common fashion, for example the polyamine spermidine[142], and the plant phenol resveratrol[143]. The study of these longevity paradigms have long focused on identifying the underlying molecular culprits, including roles for different transcription factors[132]. In turn, a common theme is that all of the above mentioned conserved longevity paradigms require autophagy-related and lysosomal genes for their lifespan extension in one or more organisms (indicated with * in Table Box; these links, and reports of lifespan extension by overexpression of autophagy genes can be found in **Table 1**).

Genetic Longevity paradigms		
	Organism	References
Dietary restriction	Yeast* Worms* Flies Mice	136
mTOR inhibition (e.g., rapamycin)	Yeast* Worms* Flies* Mice	137
Reduced insulin/IGF-1 signaling	Yeast Worms* Flies Mice	138
Increased AMPK activity	Yeast Worms* Flies* Mice	139
Reduced mitochondrial respiration	Yeast Worms* Flies	140
Hormetic heat shock	Yeast Worms* Flies	141
Germline removal	Worms* Flies	74
Reduced TGF-beta signaling	Worms Flies*	11,144
Pharmacological Longevity paradigms		
	Organism	Reference
Spermidine	Yeast* Worms* Flies* Mice	142
Resveratrol	Yeast Worms* Flies Mice	143
Urolithin A	Worms* (Mice)	37

*, Organisms in which genetic links between autophagy and aging have been observed (see Table 1)

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BOX 2. Pharmacological interventions that upregulate autophagy, and which may be relevant to longevity.

Agents that enhance autophagy by inducing autophagosome biogenesis can be considered in small-molecule and non-small-molecule categories. In the former, one can divide such agents into those acting via inhibition of the nutrient sensor and major autophagy regulator mTOR, and those acting via mTOR-independent pathways. Rapamycins, which target mTOR, have shown lifespan benefits in model organisms ranging from yeast to mice[145], and it is possible that some of these are via effects on autophagy. While there are side-effects caused by rapamycins, like immunosuppression and poor glucose tolerance, and these are large molecules that do not penetrate the blood-brain barrier well, intermittent mTOR inhibition with agents that may be able to selectively target mTOR and get into the brain may be a tractable objective. Numerous mTOR-independent molecules with autophagy-inducing effects have been described elsewhere and include metformin and trehalose[90]. While the general impact of such drugs and their target pathways have not been widely studied in model organisms and in relation to conserved longevity paradigms, such experiments may be useful and informative, particularly since autophagy induction with Atg5 overexpression lengthens lifespan in mice[42].

Autophagy can also be induced with non-small molecule approaches, including an autophagy-inducing peptide based on Beclin 1[146]; it will be interesting to test if this peptide modulates organismal lifespan.

In addition to targeting pathways impacting autophagosome biogenesis, it may be beneficial to identify drugs that act at the level of the lysosome. For example, the transcription factor TFEB, which is a master regulator of lysosomal function, also regulates autophagy[43]. The *C. elegans* orthologue of TFEB positively regulates lifespan, at least in part via autophagy[32]. Thus, it will be interesting to identify drugs targeting this transcription factor, and investigate effects on autophagy and longevity.

FIGURE LEGENDS

Figure 1. The macroautophagy process.

Schematic depicting the regulatory machinery of macroautophagy (referred to as autophagy). The conserved metabolic sensors and longevity determinants mTOR (mechanistic Target of Rapamycin) and AMP-activated kinase (AMPK) regulate autophagy. When autophagy is induced, cytoplasmic material (i.e., cargo) is sequestered in double-membrane vesicles, or autophagosomes, which subsequently fuse with acidic lysosomes in which the cargo is degraded. Autophagy is a multi-step process that includes (1) initiation, (2) membrane nucleation and phagophore formation, (3) phagophore elongation, (4) lysosome fusion, and (5) degradation, which correspondingly are regulated by multiple protein complexes: the ULK/Atg1 initiation complex; the PI3-kinase nucleation complex; the PI3P-binding complex, which directs the distribution of the machinery that enables autophagosome formation, and includes the Atg12- and the LC3/Atg8-conjugation systems. In the latter system, LC3/Atg8 is cleaved by the protease Atg4 to form LC3-I/Atg8-I, which is then conjugated with phosphatidylethanolamine to form LC3-I/Atg8-II. This conjugate is incorporated into pre-autophagosomal and autophagosomal membranes. For simplicity, only the names of yeast gene products are depicted in the figure. Symbols depicted inside phagophore: Green diamond, LC3/Atg8; orange oval, autophagy receptor; blue circle, cargo.

Figure 2. Selective types of autophagy linked to organismal ageing.

Schematic summarizing selective types of autophagy linked to organismal ageing in model organisms. In these selective types of autophagy, autophagosomes recruit mitochondria (mitophagy), lipid droplets (lipophagy), aggregate-prone proteins (aggrephagy), and lysosomes (lysophagy). This is generally mediated by so-called autophagy receptors that bridge the cargo (i.e., substrates/organelles) and the autophagy machinery. Possible consequences of deficiencies in these types of selective autophagy for age-related diseases are listed. Note that while this figure illustrates possible links between forms of selective autophagy and diseases, it is very challenging to demonstrate causality for the selective autophagy in disease in a direct sense, as opposed to links or associations. For example, PINK1, which is mutated in a rare form of recessive Parkinsonism, has been shown to be involved in models of mitophagy in tissue culture models, leading to the assumption that loss of PINK1 causes disease via defect in mitophagy. However, recent work suggests that loss of PINK1 in mice does not affect mitophagy, thus challenging the model[147].

Table 1. Summary of autophagy genes linked to organismal ageing in model organisms and to age-related disorders in humans.

Gene	Function in autophagy	Reported function in lifespan determination	References
S. cerevisiae			
ATG1/Ulk1	Autophagy initiation	Gene required for longevity induced by rapamycin	[21]
ATG11	Autophagosome-vacuole fusion; selective autophagy	Gene required for longevity induced by rapamycin	[21]
ATG7	E1-like enzyme for ATG5-12 and ATG8 conjugation systems	Gene required for longevity induced by dietary restriction, rapamycin, and spermidine	[21,28,148]
ATG5	Conjugated by ATG12	Gene required for longevity induced by dietary restriction (Met)	[148]
ATG8	Phagophore elongation, cargo recruitment	Gene required for longevity induced by dietary restriction (Met)*	[148]
VAM3	SNARE protein, fusion	Gene required for longevity induced by dietary restriction	[25]
VAM7	SNARE protein, fusion	Gene required for longevity induced by dietary restriction	[25]
ATG15	Putative lipase required for intravacuolar disintegration of autophagic bodies	Gene required for longevity induced by dietary restriction	[25]
C. elegans			
unc-51/ Atg1/Ulk1	Autophagy initiation	Gene required for longevity induced by mTOR inhibition, dietary restriction, germline ablation, and reduced mitochondrial respiration	[22,34]
bec-1/ Atg6/Pi3c3	Allosteric regulator of VPS34	Gene required for longevity induced by mTOR inhibition, dietary restriction, germline ablation, reduced mitochondrial respiration, spermidine, resveratrol, and urolitin A**	[23,28,30,32,34,36,37]
vps-34/ Vps34	Kinase that produces PI(3)P to enable recruitment of machinery that forms autophagosomes	Gene required for longevity induced by mTOR inhibition, dietary restriction, germline ablation, reduced mitochondrial respiration, and urolitin A	[23,26,34,37]
atg-9	Phagophore formation	**	[149]
atg-18/ Wipi	Phagophore formation	Gene required for longevity induced by inhibition of IIS (m), dietary restriction (I, m, n), germline ablation (i), reduced mitochondrial respiration, AMPK overexpression***, and inhibition of S6K (Overexpression of this gene by the endogenous promoter does not extend lifespan)	[15,31,34,101,104]
atg-12	Ubiquitin-like modifier of ATG5	Gene required for longevity induced by inhibition of IIS, and dietary restriction**	[30]
atg-7	E1-like enzyme for ATG5-12 and ATG8 conjugation systems	Gene required for longevity induced by inhibition of IIS	[26,30]
lgg-1/Atg8/	Phagophore	Gene required for longevity induced by inhibition	[23,34]

Lc3/ Gabarap	elongation, cargo recruitment	of IIS (l, m), germline ablation, mitochondrial respiration, and AMPK overexpression**, *** (Overexpression of this gene from the endogenous promoter does not extend lifespan)	
atg-4.1	ATG8 processing to make it conjugation-competent, and ATG8 delipidation	**	[149]
vha-16	Subunit of vacuolar proton-translocating ATPase	Gene required for longevity by germline ablation	[32]
C08H9.1	Lysosomal degradation	Gene required for longevity induced by inhibition of IIS	[150]
lipl-1	Lysosomal lipolysis	Overexpression of this gene from the endogenous promoter extends lifespan	[82]
lipl-3	Lysosomal lipolysis	Overexpression of this gene from the endogenous promoter extends lifespan	[82]
lipl-4/HLAL	Lysosomal lipolysis	Gene required for longevity induced by inhibition of IIS and germline ablation Overexpression of this gene from endogenous and intestinal-specific promoters extends lifespan, lifespan extension by *lipl-4* promoter overexpression is autophagy dependent	[80]
dct-1/ Nix/Bnip3L	Mitochondrial receptor protein	Gene required for longevity induced by inhibition of IIS, dietary restriction, mitochondrial dysfunction, and urolitin A**	[33,37,57]
pink-1	Kinase that enables mitophagy	Gene required for longevity induced by IIS, dietary restriction, mitochondrial dysfunction, and urolitin A**	[33,37,57]
sqst-1/ Sqstm1	Receptor protein	Gene required for longevity induced by mitochondrial dysfunction and urolitin A**	[33,37]
hlh-30/Tfeb	Transcription factor regulating lysosomal biogenesis and autophagy	Gene required for longevity induced by mTOR inhibition, inhibition of IIS, dietary restriction, germline ablation, reduced mitochondrial respiration, and inhibition of S6K Overexpression of this gene from the endogenous promoter extends lifespan in autophagy-dependent fashion	[31,32]
Drosophila			
Atg1/Ulk1	Autophagy initiation	Gene required for longevity induced by AMPK overexpression (n) **** Overexpression of this gene from neuronal-specific promoter during adulthood extends lifespan	[27]
Atg7	E1-like enzyme for ATG5-12 and ATG8 conjugation systems	Gene required for longevity induced by spermidine	[28]
Atg5	Conjugated by ATG12	Gene required for longevity induced by rapamycin	[24]
Atg8/Lc3/ Gabarap	Phagophore elongation,	** Overexpression of this gene from a neuronal- and	[9,11]

	cargo recruitment	a muscle-specific promoter extends lifespan	
parkin	E3 ubiquitin ligase that facilitates mitophagy	Overexpression of this gene from ubiquitous and neuronal-specific promoters during adulthood extends lifespan	[72]
Drp1	Dynamin-related protein that promotes mitochondrial fission, facilitates mitophagy	Overexpression of this gene from ubiquitous, intestine-specific and neuronal-specific promoters in midlife extends lifespan in an autophagy-dependent fashion	[39]
M. musculus			
Atg7	E1-like enzyme for ATG5-12 and ATG8 conjugation systems	Depletion of this gene in the muscle impairs muscle function and shortens lifespan	[13]
Atg5	Conjugated by Atg12	Overexpression of this gene from a ubiquitous promoter extends lifespan	[42]
Human			
BECN1	Allosteric regulator of VPS34	Variants in this gene causes have been associated with breast cancer prognosis	[151]
WDR45	Phagophore formation	Mutations in this gene causes neurodegeneration with brain iron accumulation	[152,153]
ATG7	E1-like enzyme for ATG5-12 and ATG8 conjugation systems	Variants in this this gene have been proposed to impact age at onset of Huntington disease	[154]
ATG5	Conjugated by ATG12	Mutations in this gene causes ataxia and developmental delay	[47]
ATG16L1	LC3/ATG8 lipidation	Mutation T300A in this gene increases risk for Crohn's disease	[155,156]
TECPR2	Interacts with LC3/ATG8	Mutations in this gene causes spastic paraparesis	[45]
SPG15	Autophagosome maturation	Mutations in this gene causes spastic paraplegia	[46]
EPG5	Autophagosome-lysosome fusion	Mutations in this gene causes Vici syndrome	[157]
Parkin	E3 ubiquitin ligase that facilitates mitophagy	Mutations in this gene causes autosomal recessive Parkinson's disease	[48]
PINK1	Kinase that facilitates mitophagy	Mutations in this gene causes autosomal recessive Parkinson's disease	[49]
SQSTM1	Receptor protein	Mutations in this gene causes Paget disease of bone and motor-neuron disease	[50,51]
TBK1	Kinase that phosphorylates autophagy receptors	Mutations in this gene causes motor-neuron disease	[52]

Table summarizes autophagy-related and lysosomal genes and their role in conserved longevity paradigms (yeast, worms, flies and mice), or in age-related diseases (humans).

#, requirement assessed by gene deletion or RNAi treatment in combination with longevity paradigm.

*, dietary restriction by methionine (Met) restriction.

**, additional longevity paradigms, e.g., calcineurin, frataxin, and *miR-34* depletion require this autophagy gene in *C. elegans* (see [38] for additional links). Moreover, reduced Activin signaling require *Atg8a* in *Drosophila*[11].

***, unpublished data from Hansen lab.

****, longevity induced by overexpression of the mitochondrial protein Drp1 require this autophagy gene in *Drosophila*[39].

Abbreviations, IIS, insulin/IGF-1 signaling.

Table 2. Tissue-specific functions of autophagy in ageing.

	Intestine	Muscle	Nervous system
Autophagy genes linked to aging	Yes (worms, flies)	Yes (worms, flies, mice)	Yes (worms, flies)
Functions of autophagy in tissue	Intestinal barrier function (worms, flies)	Motility (worms) Mitochondrial homeostasis (flies, mice) Muscle contractility maintenance of neuro-muscular junction (mice)	Learning/memory (flies) Proteostasis (worms, flies, mice)
Longevity interventions improves tissue function in autophagy-dependent fashion	Dietary restriction (worms, flies, mice) Midlife Drp1 overexpression (flies)	Dietary restriction (worms) Midlife Drp1 overexpression (flies)	Hormetic heart shock (worms) Spermidine (flies) Neuronal Atg8 overexpression (flies)
Cell non-autonomous effects and possible signals	Intestine-> muscle/neuro-muscular junctions (worms) Intestine-> neurons (flies)		Neurons -> intestine (worms, flies) Neurotransmitters, peptide release Insulin-like peptides? Other neuropeptides?

Table summarizes links between ageing and autophagy in specific tissues and cell types of model organisms. Genetic links have been established in the intestine, neurons, muscle and blood, and studies have indicated a functional role for autophagy in these tissues that may be relevant to ageing. Longevity interventions that can improve tissue-specific functions in an autophagy-dependent manner are listed, along with possible cell non-autonomous mechanisms. See text for details.

FIG. 1

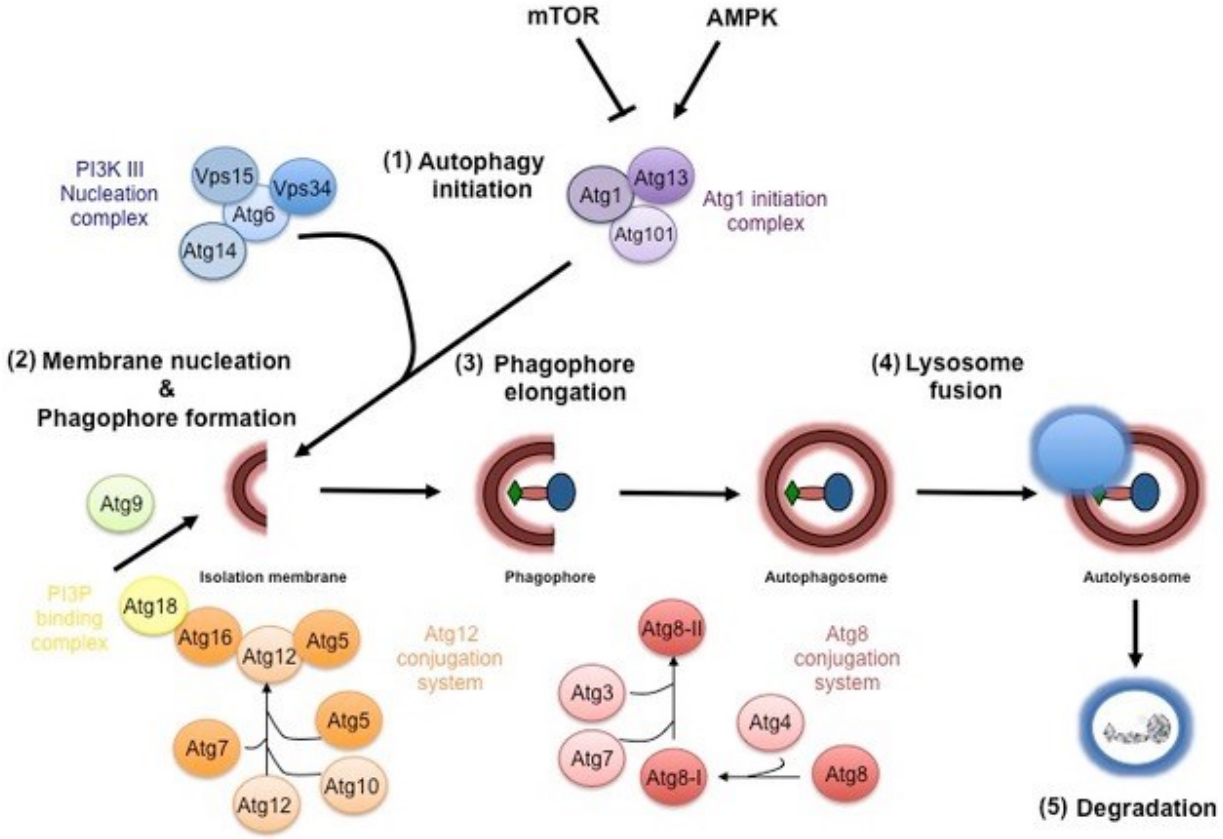
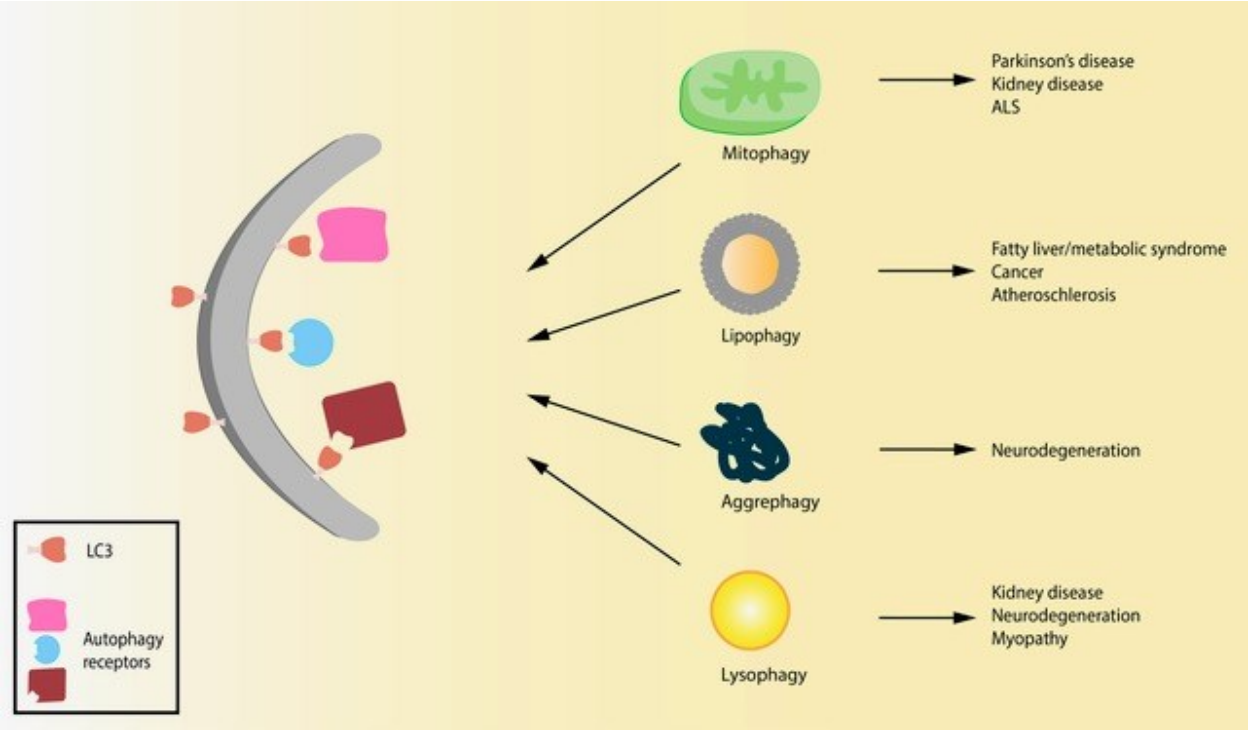

mTOR
AMPK
PI3K III
Nucleation
complex
Vps15
Vps34
Atg6
Atg14
(1) Autophagy
initiation
Atg1
Atg13
Atg101
Atg1 initiation
complex
(2) Membrane nucleation
&
Phagophore formation
(3) Phagophore
elongation
(4) Lysosome
fusion
Atg9
PI3P
binding
complex
Atg18
Isolation membrane
Phagophore
Autophagosome
Autolysosome
Atg16
Atg12
Atg5
Atg12
conjugation
system
Atg7
Atg5
Atg10
Atg12
Atg8-II
Atg3
Atg7
Atg8-I
Atg4
Atg8
Atg8
conjugation
system
(5) Degradation

FIG 2.


Parkinson's disease
Kidney disease
ALS
Mitophagy
Fatty liver/metabolic syndrome
Cancer
Atheroschlerosis
Lipophagy
Neurodegeneration
Aggrephagy
Kidney disease
Neurodegeneration
Myopathy
Lysophagy
LC3
Autophagy receptors