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Autophagosomes

Cytosolic double-membrane-bound vesicles 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 autophagosomal material takes place.

mTOR

An evolutionarily conserved protein kinase that negatively regulates autophagy.

Autophagy receptors

Proteins that facilitate recruitment of cargo to the phagophore for subsequent lysosomal degradation.

Hypothalamus

A region of forebrain that coordinates the autonomic nervous system and the activity of the pituitary, which controls various homeostatic systems, including body temperature.

Lysosomal-associated membrane glycoprotein 2a

(LAMP2A). A lysosomal protein with a key role in chaperone-mediated autophagy.

Insulin/IGF1 signalling

A multi-component signalling pathway that regulates metabolism and longevity in a conserved fashion.

Spastic paraplegia

An inherited disorder characterized by spasticity of the legs.

Ataxia

Loss of control over bodily movements.

Mitochondrial fission

The separation of a single mitochondrion into two or more daughter organelles.

Urolithin A

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

Lipid droplet

A cellular organelle that regulates the storage and hydrolysis of neutral lipids.

Lipid chaperone

A fatty acid-binding protein important in the transport of lipids inside and between cells.

Hormetic heat shock

An aspect of hormesis, meaning beneficial effects of a treatment for which higher intensity is harmful. During hormetic heat shock, non-lethal exposure to elevated temperature induces a response that results in increased stress resistance and longevity.

Proximal tubules

The segment of the kidney that is responsible for reabsorption of nearly two-thirds of all filtered water, sodium and chloride.

Podocytes

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

Glomerulus

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

Microbial dysbiosis

The condition of having imbalances in the microbial communities either in or on the body.

Septate junction

An intercellular occluding junction found in invertebrate epithelia.

Crohn's disease

A chronic inflammatory bowel disease.

Insulin-like peptide

A type of peptide with homology to insulin, a hormone produced in the pancreas that regulates glucose levels in the blood.

Autophagy as a promoter of longevity: insights from model organisms

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Abstract

Autophagy is a conserved process that catabolizes intracellular components to maintain energy homeostasis and to protect cells against stress. Autophagy has crucial roles during development and disease, and evidence accumulated over the past decade indicates that autophagy also has a direct role in modulating ageing. In particular, elegant studies using yeasts, worms, flies and mice have demonstrated a broad requirement for autophagy-related genes in the lifespan extension observed in a number of conserved longevity paradigms. Moreover, several new and interesting concepts relevant to autophagy and its role in modulating longevity have emerged. First, select tissues may require or benefit from autophagy activation in longevity paradigms, as tissue-specific overexpression of single autophagy genes is sufficient to extend lifespan. Second, selective types of autophagy may be crucial for longevity by specifically targeting dysfunctional cellular components and preventing their accumulation. And third, autophagy can influence organismal

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Chemosensory neurons
Sensory neurons responsive to chemical stimuli.

Presynaptic active zone
The part of the nerve terminal from which neurotransmitters are released by synaptic vesicle exocytosis.

Sarcopenia
The degenerative loss of muscle mass, quality and strength associated with ageing.

Senescence
Loss of the ability of a cell to divide, differentiate and grow.

Beclin 1
Mammalian orthologue of yeast autophagy-related 6 (Atg6), which forms part of the class iii phosphatidylinositol 3-kinase complex involved in activating autophagy.

ERphagy
The selective degradation of the endoplasmic reticulum by autophagy.

Ribophagy
The selective degradation of ribosomes by autophagy.

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

Nucleophagy
The selective removal of nuclear material from a cell by autophagy.

health and ageing even non-cell autonomously, and thus, autophagy stimulation in select tissues can have beneficial, systemic effects on lifespan. Understanding these mechanisms will be important for the development of approaches to improve human healthspan that are based on the modulation of autophagy.

Autophagy is an evolutionarily conserved catabolic process that has an essential role in cellular homeostasis by facilitating lysosomal degradation and recycling of intracellular macromolecules and organelles (collectively referred to as autophagic cargo). Autophagy was first discovered as a survival mechanism in yeasts subjected to nutrient deprivation, a condition that potently stimulates 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]. Interestingly, autophagy is often found perturbed in age-related disorders such as cancer, diabetes and neurodegenerative diseases[2,3]. Accordingly, autophagy is important for the maintenance of organismal health, which prominently declines with ageing.

Three types of autophagy have been distinguished on the basis of 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 lyso-some 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 focuses on macroautophagy (hereafter termed autophagy), which has been extensively studied in the context of ageing, particularly in invertebrate models.

The autophagy process is mediated by a number of autophagy-related (ATG) proteins and can be divided into at least five sequential steps: first, initiation; second, double-membrane nucleation and formation of a preautophagosome or phagophore; third, phagophore elongation and sequestration of cytoplasmic cargo; fourth, fusion of the autophagosome (the fully enclosed phagophore) to a lysosome; and fifth, degradation of sequestered cargo in the autolysosome. Different ATG molecules are implicated in these steps[4] (Fig. 1). Key upstream regulators of this multistep process include the highly conserved nutrient sensors mTOR and AMP-activated kinase (AMPK) — which notably are also critical longevity determinants (see BOX 1) — which directly phosphorylate Unc-51-like kinase 1 (ULK1; Atg1 in yeasts), a conserved kinase that serves as the key upstream initiator of autophagy[5]. Another set of key autophagy proteins is the family comprising microtubule-associated protein light chain 3 (LC3) proteins and γ-aminobutyric acid receptor-associated proteins (GABARAPs) in mammals (Atg8 in yeasts). Fluorescently tagged or endogenous LC3/GABARAP family proteins are commonly used as steady-state auto-phagy markers in many species to facilitate microscopic visualization of phagophores and autophagosomes in the cell[6]. LC3/GABARAP family proteins are proteolytically processed and attached to autophagosomal membranes, where they participate in cargo recognition and recruitment to the phagophore by interacting with various autophagy receptors or cargo receptors bound to proteins or organelles. Prominent examples of autophagy receptors are p62 (also known as

SQSTM1), which recognizes ubiquitylated proteins or organelles targeted for degradation[7], and BCL-2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), a receptor for mitochondria destined for degradation by mitophagy[8]. Such specific clearance of cargo, including organelles and macromolecules, 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. Here, we discuss the current literature linking autophagy, including selective types of autophagy, to organismal, tissue and cellular ageing. These data have been accumulated from studies of model organisms, including yeasts, worms, flies and mice, showing conservation of autophagy as a molecular mechanism important for longevity, with potential implications for healthspan in humans.

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 activation can be used as a strategy to promote longevity, as summarized below.

Autophagy decline in ageing animal models.

Many organisms show signs of decreased autophagic capacity with age. For example, levels of lysosomal protease activity decline with age in the nematode *Caenorhabditis elegans*[9]; autophagy gene transcripts decrease with age in tissues of the fruitfly *Drosophila melanogaster*, including in the brain (*Atg2*, *Atg8a* (*LC3/GABARAP* in mammals), *Atg18* (*WIPI1* and *WIPI2* in mammals) and *bchs* (*ALFY* in mammals))[10] and in (*Atg1* (*ULK1* in mammals), *Atg5, Atg6* (*BECN1* in mammals), *Atg7* and *Atg8a*[11–12]; protein levels of LC3 and ATG7 decline with age in mouse hypothalamus[13] and in mouse and human muscle[14]; and lysosomal-associated membrane protein type 2a (LAMP2A) as well as chaperone-mediated autophagy decline in rat liver[15]. 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 protein LGG-1 (LC3/GABARAP in mammals and Atg8 in yeasts) as a marker of autophagosomes and autolysosomes in combination with autophagy inhibitors (an approach known as a flux assay) shows an age-dependent increase in the number of autophagosomes and autolysosomes in four major tissues (intestine, body-wall muscle, pharyngeal muscle and neurons), with possible tissue-specific kinetic differences still to be determined. This accumulation of autophagic structures likely reflects impaired autophagic activity[16]. Another recent study similarly reported a reduction in autophagic activity in whole-body extracts of aged *C. elegans*[17]. Moreover, electron microscopy analysis of mouse and rat livers shows an accumulation of autophagic vacuoles with age, and chemical alteration of the process indicates that aged animals have a decreased ability to turn over autophagic vesicles[18,19]. Consistently, proteolysis of long-lived proteins is impaired in the livers of old rats[18,20], whereas the lifespan-extending intervention of dietary restriction, that is, reduction

in food intake without malnutrition (see BOX 1) prevents this decline [21,22], suggesting an age-dependent decline in autophagic function and lysosomal degradation. Thus, evidence from multiple model organisms shows that autophagy gene expression and protein levels decrease with age, at least in some contexts, causing an accumulation of autophagic structures and possibly limiting autophagic capacity to maintain cellular homeostasis. Further studies of tissue-specific and cell type-specific differences will be required to better understand the exact contribution of autophagy defects in each tissue to systemic ageing.

Genetic links of autophagy to ageing.

Autophagy, and more specifically, requirement of different *ATG* genes, has been directly linked to ageing via genetic experiments in multiple model organisms (TABLE 1; see also REF[23] for additional genetic links between autophagy and specific long-lived *C. elegans* mutants), showing a broad and critical role for autophagy genes in several conserved longevity paradigms (BOX 1). Indeed, impairment of autophagy genes by RNAi in young adult animals abrogates lifespan extension in all long-lived mutants of any species tested so far (see BOX 1 for longevity paradigms and discussion below for the effects oflater-in-life impairments of autophagy on lifespan) but generally has small or no effects on the lifespan of normal invertebrate animals[23–25]. The latter observations likely reflect the fact that residual expression of autophagy genes in RNAi approaches is sufficient to support the basal auto-phagy required for fitness of wild-type animals. This is in contrast to the rapid lethality of mice following auto-phagy gene knockout in adulthood[26] or the sickly and short-lived invertebrate animals resulting from auto-phagy impairments during development, irrespective of their genetic background. This reflects critical roles for autophagy in adult mammals, particularly as a buffer against neurodegeneration and various infections[26], and important developmental roles for autophagy in invertebrates[1,27]. Notably, where analysed, long-lived invertebrate mutants also display increased steady-state markers of autophagy, indicating increased autophagic activity in these mutants and providing evidence that autophagy activation is generally associated with lifespan extension in various genetic backgrounds. This has been directly assessed by flux assays in long-lived *C. elegans* with reduced insulin/iGF1 signalling (*daf-2* mutants, which carry mutations in the insulin/IGF1-like receptor) and in mutants lacking a germline (*glp-1* mutants, which carry mutations in the Notch receptor); these mutants generally show increased autophagic capacity compared with wild-type *C. elegans*, yet with notable tissue-specific differences[16] (see also below). Moreover, several long-lived worms and flies display increased expression of multiple ATG and lysosomal genes (reviewed in REF[28]). Collectively, these observations suggest a model in which increased autophagic activity has 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 *D. melanogaster*, 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 *Atg8a* in the nervous system[10] or in the muscle[12] is sufficient to extend fly lifespan. Similarly, neuron-specific overexpression of *Atg1* in flies[24], and ubiquitous overexpression of *Atg5* in mice, is sufficient to stimulate autophagy, improve

motor function and extend lifespan[29]. While all these lifespan extending manipulations are accompanied by increases in autophagy markers and improved healthspan parameters (see section on tissue-specific roles for auto-phagy below), it remains to be formally tested whether the observed longevity requires the autophagy process in all cases. In this regard, it is noteworthy that overexpression of the helix-loop-helix transcription factor *hlh-30* (*TFEB* in mammals), a conserved regulator of many ATG and lysosomal genes[28,30], extends lifespan in *C. elegans* in an autophagy-dependent fashion[31]. Collectively, these observations indicate, but do not prove, that upregulation 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 auto-phagic 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 of several *ATG* genes (for example, *BECN1* (*ATG6* in yeasts), *ATG5* and *ATG7*) result in decreased autophagy along with accumulation of dysfunctional organelles and disordered and aggregated proteins in mammalian models of neurodegenerative disorders, including Huntington disease (Huntingtin (HTT) accumulation), Alzheimer disease (amyloid-β (Aβ) and Tau accumulation) and Parkinson disease (α-synuclein accumulation) (reviewed in REF [32]). Importantly, Mendelian mutations in auto-phagy regulators can cause neurodegenerative diseases, including spastic paraplegia[33,34] and ataxia[35], and loss of activity of autophagy receptors as well as other regulators of selective autophagy (see also next section) can cause Parkinson disease[36,37] or other forms of motor neuron disease[38–40]. Overall, accumulating evidence supports a beneficial role for autophagy in counteracting ageing and age-related diseases, although the underlying mechanisms are not fully understood.

Selective autophagy in ageing

While the above genetic links indicate involvement of the bulk autophagy in ageing processes, there is now evidence that the impairment of the turnover of specific cargoes via selective autophagy might have important roles in age-related pathologies and in ageing. Below, we discuss studies implicating the different types of selective autophagy in ageing and lifespan determination (FIG. 2).

Mitophagy.

The accumulation of dysfunctional mitochondria is a shared hallmark of ageing and numerous diseases of old age[41–44]. Although the underlying mechanisms that lead to age-related loss of mitochondrial function remain incompletely understood and may involve numerous processes, it has been suggested that a decline in mitophagy has a key role[43–45]. In mammals, the degradation of damaged mitochondria is mediated by a pathway comprising PTEN-induced putative protein kinase 1 (PINK1) and the E3 ubiquitin-protein 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[46–48]. Disruptions in mitophagy have been implicated in the pathophysiology of age-related diseases such as heart disease[49], retinopathy[50], fatty liver disease[51], pulmonary hypertension[52], kidney disease[53] and neurodegenerative disorders, including Parkinson

disease, amyotrophic lateral sclerosis[54] and Alzheimer disease[46,55] (FIG. 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[45], flies[25], mice and humans[56,57] have reported a decline in mitophagy markers in aged animals. This decline may be relevant to age-related pathologies, as loss of *Pink1* or *Parkin* leads to early-onset behavioural decline and shortened lifespan in flies[58,59]. Two recent studies in *C. elegans* have investigated the importance of mitophagy in longevity assurance[45,60]. The gene *dct-1* is a putative orthologue to the mammalian proteins BNIP3 and BNIP3-like (BNIP3L), which act as mitophagy receptors in mammals[47]. Inhibition of *dct-1* leads to an increase in mitochondrial content, indicating that DAF-16/FOXO controlled germline tumour affecting-1 (DCT-1) is the nematode orthologue of BNIP3L and functions as a key regulator of mitophagy[45]. Moreover, inhibition of *dct-1* or *pink-1* shortens the lifespan of long-lived *daf-2* mutants and *eat-2* mutants (which recapitulate experimental dietary restriction paradigms[45]) and in several long-lived *C. elegans* models of moderate mitochondrial dysfunction (where mild mitochondrial stress signalling evokes beneficial effects on longevity)[45,60] (TABLE 1). Collectively, these studies indicate that mitophagy indeed has a causal role in lifespan extension, at least in long-lived *C. elegans* models.

A number of studies have examined the impact of increased mitophagy on ageing and lifespan. Critically, ubiquitous or neuron-specific, adult-onset upregulation of Parkin extends the lifespan of *D. melanogaster*[61] (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 the flight muscle of aged *D. melanogaster*[25]. 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 gut pathology and prolongs fly lifespan in an *Atg1*-dependent fashion (TABLE 1). Furthermore, upregulating Drp1 specifically in neurons or the intestine (see also section below on tissue-specific effects of autophagy induction), from midlife onwards, is sufficient to prolong fly lifespan[25]. These findings indicate that a midlife decline in mitophagy, resulting at least in part from a shift in mitochondrial dynamics, contributes to ageing-related onset of mitochondrial dysfunction and limits lifespan, at least in *D. melanogaster*.

Given the findings above, it has been proposed that pharmacological interventions that stimulate mitophagy may prove effective in delaying the health decline associated with ageing[62]. Consistent with this model, dietary treatment of *C. elegans* with the human microflora-metabolite urolithin A induces mitophagy and prolongs worm lifespan[63]. More specifically, short-term urolithin A treatment in worms induces mitochondrial fragmentation and reduces mitochondrial content in an autophagy-dependent fashion. Urolithin A treatment improves a number of markers of *C. elegans* healthspan and maintains respiratory capacity of mitochondria during ageing. These lifespan-extending effects of urolithin A treatment require the mitophagy genes *pink-1* and *dct-1* (TABLE 1). Importantly, urolithin A treatment is also beneficial in rodents, where it was shown to improve exercise capacity in

two different mouse models of age-related decline of muscle function, as well as in young rats[63], overall suggesting conserved beneficial effects of inducing mitophagy for organismal fitness.

Lipophagy.

Studies in diverse organisms have suggested that specific alterations in lipid metabolism are associated with different pro-longevity interventions[64]. In recent years, the contribution of autophagy to intracellular lipid droplet degradation, referred to as lipophagy, has been identified[65]. The first clear demonstration that lipid droplets could be turned over via autophagy came from studies in cultured hepatocytes with reduced ATG5 levels[66] (we note that chaperone-mediated autophagy has also been linked to lipid metabolism; reviewed in REF[67]). The fact that autophagy can regulate lipid metabolism expands the physiological relevance of autophagy to modulate the cellular energetic balance directly. Furthermore, alterations in lipophagy could impact cell physiology indirectly 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[68] (FiG. 2), which comprises a constellation of features including obesity, dysregulated lipoprotein metabolism, abnormal glucose handling and high blood pressure. Lipophagy has also been linked to cancer[69] and atherosclerosis[70].

Recent studies in *C. elegans* have provided further evidence for the role of lipophagy in longevity. Specifically, lifespan extension in germline-less *glp-1* mutants requires both the lysosomal lipase LIPase-like 4 (LIPL-4) (REF [71]) and autophagy genes[72], whereby autophagy-dependent lypolysis and LIPL-4-dependent lipolysis promote longevity independently[72] (TABLE 1). Furthermore, increased lysosomal lipolysis has been directly linked to lifespan extension in worms[71,73,74]. Although the molecular mechanisms involved are not fully understood, overexpression of *lipl-4* has been shown to induce nuclear translocation of a lysosomal lipid chaperone lipid-binding protein 8 (LBP-8), consequently promoting longevity by activating nuclear hormone receptor 49 (NHR-49) and NHR-80 (REF.[75]). Of further note, aged worms display a deposition of lipids in non-adipose tissues, including the nervous system[76]. Interestingly, dietary restriction, an intervention that promotes longevity, reduces this ectopic fat accumulation, whereas inhibition of *ATG* genes, namely, *lgg-1* (*LC3/GABARAP* family in mammals, *ATG8* in yeasts) and *hlh-30* (homologue of mammalian *TFEB*), increases fat accumulation[76], which is consistent with a role for aberrant 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, to autolysosomes. Aggregation-prone proteins, including tau, α-synuclein and mutant HTT, accumulate as aggregates in ageing neurons, which is thought to contribute to toxicity in neurodegenerative diseases such as Alzheimer disease, Parkinson disease and Huntington disease (FiG. 2). These proteins are autophagy substrates[77–79], and autophagy upregulation by chemical[80], genetic[81] or environmental means (by hormetic heat shock[82]) can ameliorate signs of these diseases in a wide range of animal models including *C. elegans*, *D. melanogaster*, zebrafish and mice

(reviewed in REF.[83]). Furthermore, α-synuclein[84] and many mutant polyglutamine-expanded proteins, like mutant HTT[85], can inhibit autophagy. This could potentially introduce a feedforward loop supporting disease pathogenesis. Accordingly, inhibition of auto-phagy accelerates neurodegenerative disease by accumulating disease-causing aggregate-prone proteins, like mutant HTT and α-synuclein[115]. Thus, any age-dependent decrease in autophagy in the brain will have a major impact on protein aggregation in these mutant backgrounds. Indeed, age is a major risk factor in most neurodegenerative diseases.

Lysophagy.

The selective degradation of damaged lysosomes (lysophagy) appears to be an important mechanism, which has been suggested to shield the cytoplasmic contents from leakage of lysosomal hydrolases[86], and lysophagy has been shown to be protective against acute kidney injury in mice[86] (FiG. 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 increases kidney damage with age in mice[87], it is interesting that basal auto-phagy is increased in kidney proximal tubules in older versus younger mice[87]. However, starvation-induced autophagy in kidney proximal tubules is blunted in aged mice[87]. Interestingly, autophagy appears to be less active in podocytes (the most damage-prone cells in the glomerulus)[88] than in cells in the proximal tubule, but no age-dependent change in podocyte autophagy was observed[87]. Thus, in this case, autophagic capacity may correlate inversely with cell type-specific vulnerability to damage in the kidneys and may suggest that autophagy is a protective process in this organ. While these differences in susceptibility to damage may be mediated in part by differential capacity for lysophagy, it is likely that altered clearance of other autophagy substrates may contribute, including removal of damaged mitochondria. The links of lysophagy to physiology and disease are still largely limited to studies in the kidney, likely owing to recent characterization of lysophagy, although the most recent studies have also proposed links between decreased lysophagy and muscle disease as well as neurodegeneration[89]. However, it is possible that efficient clearance of dysfunctional lysosomes may have much broader importance for determining cellular fitness that is progressively compromised upon accumulation of defects in autophagic clearance during ageing and that aberrations in lysophagy may be relevant to many other organ systems.

Tissue-specific autophagy and ageing

While ageing is linked to a decline in physiological functions at both the tissue and the 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, whereby the ability to prevent the passage of harmful intraluminal entities is compromised, is a common feature of ageing organisms and has been

linked to a number of human diseases[90]. In *D. melanogaster*, ageing-related intestinal barrier dysfunction is linked to microbial dysbiosis, increased immune gene expression, loss of motor activity and systemic metabolic defects and is a harbinger of mortality[91,92]. Together with data showing that the intestine represents a critical target organ for genetic interventions that prolong lifespan[93], 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*[94] and *D. melanogaster*[92,95]; likewise, short-term protein restriction has recently been linked to improved intestinal barrier function in adult pigs[96]. Although direct tests are needed, improved intestinal barrier function may be a phenotype shared by multiple conserved longevity paradigms. In support of this, long-lived *C. elegans daf-2* mutants also have improved intestinal barrier function[94].

Intestine-specific expression of autophagy genes has been shown to be critical for lifespan extension observed in several longevity paradigms (TABLES 1,2), including dietary restriction in *C. elegans*[94]. Indeed, various reporters of autophagic activity and flux analyses indicate that autophagy is induced in the intestine of long-lived *eat-2* mutants. Furthermore, intestine-specific RNAi of two LC3/GABARAP family homologues, *lgg-1* and *lgg-2* or of the *WIPI* homologue *atg-18* significantly decreases the extended lifespan of these mutant worms. Consistent with a role for intestinal autophagy in longevity mediated by dietary restriction, *atg-18* mutants do not display lifespan extension upon food dilution (a direct dietary restriction protocol), and intestine-specific expression of *atg-18* in these mutants restores dietary restriction-mediated lifespan extension[97]. Moreover, either whole-body or intestine-specific RNAi of autophagy genes abrogates the improvements in age-related intestinal barrier function in *eat-2* mutants[94]. Collectively, these findings suggest that autophagy induction in the intestine of animals subjected to dietary restriction can act to maintain intestinal barrier function during ageing and that this is important for lifespan extension. Even though dietary restriction has been reported to increase autophagy markers in multiple tissues and organs of mice[98–100], it remains unknown whether modulation of autophagy, systemically or in specific organ systems, such as the intestine, has 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 *D. melanogaster*, intestines of aged animals are characterized by altered expression and localization of septate junction proteins, which may contribute to the impairment of epithelial integrity and increased tissue permeability and consequently may be linked to ageing-related intestinal barrier dysfunction[101]. Although a direct role for autophagy impairment has not yet been shown to promote intestinal barrier dysfunction in organisms other than *C. elegans*, defects in ATG proteins have been linked to the pathogenesis of Crohn's disease, which is characterized by intestinal barrier dysfunction in humans[102]. Moreover, it has been shown that autophagy selectively reduces epithelial tight junction permeability by lysosomal degradation of the tight junction protein claudin 2 (REF.[103]). Therefore, it is possible that investigating the interplay between autophagy, epithelial junction proteins and ageing may provide novel therapeutic approaches to maintain intestinal health during ageing.

Additional links exist between the intestine, auto-phagy and longevity. For example, flux analysis in germline-lessglp-1 *C. elegans* mutants indicates induced autophagy in the intestine of these animals, and RNAi-mediated knockdown of *atg-18* in the intestine of adult animals abrogates the lifespan extension observed in *glp-1* mutants[16] (TABLE 1). Curiously, the same RNAi treatment did not significantly shorten the lifespan of *C. elegans daf-2* mutants, indicating that intestinal autophagy may not be contributing to the lifespan extension resulting from reduced insulin/IGF1 signalling[16]. However, in another recent study it was found that intestinal reintroduction of ATG-18 into *daf-2* and *atg-18* double mutants extended lifespan of these animals to match that of *daf-2* mutants[97]. Notably, this latter study does not discriminate between the role of AT G-18 during development versus during ageing, and more experiments are needed to fully address the role of intestinal autophagy in *daf-2* mutants. Consistent with these observations linking intestinal autophagy to longevity in *C. elegans*, intestinal overexpression of *AMPKalpha* (AMPK catalytic subunit, hereafter referred to as *AMPK*) in *D. melanogaster* induces markers of autophagy and autophagy gene expression in the intestine and extends fly lifespan[24], collectively indicating an important role for intestinal autophagy in lifespan determination of flies.

Interestingly, intestinal overexpression of *AMPK* promotes autophagy not only in the intestine itself but also in the fly brain[24]. Similarly, neuronal overexpression of *Atg1*, or of *AMPK* (see also next subsection), is associated with increased autophagy markers and autophagy gene expression in the fly intestine[24]. Importantly, neuronal *Atg1* overexpression also improves intestinal barrier function, indicating that autophagy can act non-cell autonomously and even across tissues to promote longevity. However, mechanisms of inter-tissue pro-longevity effects remain to be elucidated. One observation is that neuronal *Atg1* overexpression is associated with reduced insulin-like peptide levels in the brain[24]. It is also worth considering that ablation of the insulin-like peptide-producing median neurosecretory cells in the brain lowers systemic insulin signalling and can prolong fly lifespan[104]. Therefore, it is possible that the pro-longevity effects of autophagy stimulation in neurons may involve decreased insulin-like peptide signalling.

It is interesting to speculate as to whether induction of intestinal or neuronal autophagy can impact systemic autophagy levels to prolong lifespan, for example, upon dietary restriction. Inhibition of autophagy genes in the intestine of worms considerably impairs organismal motility, which is presumed to be a marker of neuro-muscular function, in *C. elegans eat-2* mutants[94]. However, the question of whether intestine-specific inhibition of autophagy genes, under dietary restriction, impacts autophagy in neurons or muscle remains unanswered. Collectively, these studies indicate an important role for autophagy in the intestines of multiple organisms; it will therefore be interesting to investigate the requirement for autophagy in the intestines of ageing mammals, including the role of autophagy in intestinal integrity.

Nervous system.

Several studies have implicated the nervous system in modulating lifespan, yet the cellular mechanisms involved are not well understood[105,106.] There are a number of suggestions that autophagic activity is compromised with age in the brains of different species. As noted

above, autophagy is decreased in mouse hypothalamic neurons[13,107], and the mRNA levels of a number of *ATG* genes are decreased in aged human brains[108]. However, further work is required to more rigorously test the hypothesis that autophagic activity may decline in an age-dependent fashion in the nervous system.

Neuronal autophagy has been linked to lifespan in several studies in flies, in which overexpression of single autophagy genes has been shown to promote longevity (TABLES 1,2). Specifically, pan-neuronal over-expression of *Atg8a* throughout life extends the lifespan of *D. melanogaster* and improves neuronal proteostasis and organismal response to oxidative stress[10]. Likewise, adult-onset, pan-neuronal overexpression of *Atg1* extends fly lifespan[24]. As noted above, such adult-onset induction of Atg1 or AMPK in the fly nervous system prevents intestinal barrier dysfunction during ageing, and both the cell-autonomous and the non-cell autonomous effects of Atg1 and AMPK overexpression on the intestine are linked to an increase in autophagy markers and *ATG* gene expression in the intestinal epithelium[24].

In *C. elegans*, restoring expression of *atg-18* in neurons of *atg-18;daf-2* double mutants fully rescues the short lifespan of these animals[97]. Moreover, expression of *atg-18* exclusively in chemosensory neurons is sufficient to unleash the beneficial effects of reduced insulin/IGF1 signalling in *daf-2* mutants. Interestingly, in the same study, it was reported that *atg-18* expression in chemosensory neurons alone does not rescue the shortened lifespan of *atg-18* only mutants[97]. This suggests that *atg-18* expression in chemosensory neurons is important for lifespan extension in response to metabolic alterations but is not sufficient to support normal animal lifespan. Although it was shown that extended lifespan resulting from the rescue of *atg-18* expression in chemosensory neurons in *atg-18*;*daf-2* mutants depends genetically on the release of neurotransmitters from these neurons, the underlying physiological mechanisms for how rescuing autophagy in only a subpopulation of neurons can impact the lifespan of the entire organism remain to be investigated.

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[109], yet the underlying mechanisms are not well understood. Interestingly, recent studies have linked autophagy to cognitive functions in *D. melanogaster* treated with polyamines such as spermidine and putrescine. These compounds promote lifespan in diverse species by augmenting autophagy[110] (TABLE 1). Notably, dietary spermidine suppresses age-induced memory impairment in an autophagy-dependent manner in flies[111]. Most recently, it has been reported that spermidine counteracts age-related alterations in the size and function of a specific synaptic compartment, the presynaptic active zone, to maintain memory in aged flies[112] (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 cytoplasmic proteins (aggrephagy) and dysfunctional mitochondria (mitophagy), as discussed in detail above.

Muscle.

Recent work in mammals and flies indicates that maintaining muscle integrity and function is critical for counteracting systemic ageing and for lifespan determination[113]. Although the mechanisms involved in muscle-mediated modulation of systemic ageing are not fully understood, emerging evidence suggests that muscle-derived growth factors and cytokines, known as myokines, can modulate systemic physiology[113]. Interestingly, of the four major tissues examined in *C. elegans*, the greatest change in autophagy markers occurs in the body-wall muscle[16], potentially reflecting muscle as a tissue with especially high autophagic activity.

Muscle-specific autophagy has been linked to longevity in studies in *C. elegans* and in *D. melanogaster*. In worms, inhibition of *lgg-1* and *atg-18* in the body-wall muscle of adult animals is sufficient to shorten the lifespan of both *eat-2* mutants[94] and *daf-2* mutants[16]. In turn, overexpression of *Atg8a* in fly muscle increases lifespan[12] (TABLE 1). Furthermore, in *D. melanogaster*, overexpression of the transcription factor forkhead box, subgroup O (FOXO) maintains proteostasis during muscle ageing, at least in part by promoting autophagy, which is linked to extended lifespan[11,12].

In mice, muscle-specific ATG7 deficiency causes impaired muscle function and reduced lifespan[14]. While interpreting lifespan-shortening interventions can prove challenging[114], the latter study suggests that loss of muscle function during ageing, owing to impaired autophagy, limits lifespan in mice.

How could autophagy be important for muscle function? The muscle of animals is critical for mobility, which declines with age owing to sarcopenia, or age-related muscle loss[115]. A recent study in mice lacking *Atg7* in the muscle showed that autophagy has a critical role in maintaining the neuromuscular junction and muscle strength, at least in part by increasing the number and improving the function of mitochondria, which are highly abundant at neuromuscular junctions[14] (TABLE 2). Indeed, overexpression of *Atg7* in muscle prevents age-associated myofibre degeneration[14].

Autophagy may also have 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, resulting in their proliferation and differentiation into muscle fibres. Autophagy likely provides nutrients for this metabolically demanding event[116,117]. Of note, the ability to activate satellite cells declines with ageing, and impaired autophagy was recently shown to have 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[118] (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[118]. In conclusion, autophagy has important protective roles in the ageing muscle, and it will be interesting to investigate whether boosting autophagy can alleviate sarcopenia and improve mobility in aged animals.

The blood and the immune system.

One aspect of blood ageing is related to the decline in immunity. Immune cell senescence that progressively occurs during ageing is associated with decreased immune surveillance and is a risk factor for numerous ageing-related diseases, including cancer. Recent studies have revealed that autophagy-deficient immune cells show numerous ageing phenotypes and that autophagy-inducing agents can improve the immune responses in elderly people[119]. Hence, autophagy has emerged as a novel target to treat late-onset diseases associated with immune cell senescence. To this point, it is interesting to note that autophagic capabilities appear to be better maintained in immune cells of exceptionally long-lived humans than in the cells of those with an average lifespan[120]. At present, however, it is not known whether it is possible to induce autophagy specifically in immune cells and improve immune function.

Another blood compartment that displays changes in autophagy over time is that of the haematopoietic stem cells (HSCs). These stem cells differentiate into multiple types of blood cells in vertebrates throughout their lives but progressively lose this regenerative capacity over time. Recent studies have implicated autophagy as a key pathway in homeostasis of the blood system[121]. Further, it has been reported that autophagy is essential for maintaining the quiescence of HSCs throughout life, as it restricts their proliferation and prevents premature exhaustion by limiting the number of active mitochondria[122]. It remains to be tested whether the autophagy processes in the immune system and HSCs are directly linked to organismal lifespan.

Conclusions and perspectives

Evidence has been mounting over the past decade that autophagy and ageing are closely linked. In particular, work in model organisms ranging from yeasts to mice has shown that multiple *ATG* genes are required for the long lifespan induced by conserved longevity paradigms. Combined with gene expression data and autophagy-marker analyses generally indicating that long-lived animals also have increased levels of autophagy, these observations indicate that increased autophagy contributes to extended lifespan. In turn, autophagy appears to become limited with normal ageing, possibly occurring in a tissue-specific fashion and involving selective types of autophagy.

Why would autophagy decline with age in most tissues, and what could be the mechanism for this decline? One general mechanism could involve alterations 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, but with some notable exceptions[122,123]. In tissues where mTOR activity may be increased with age, it remains to be investigated which mTOR-controlled steps of autophagy, prominently including phosphorylation of ULK1 and regulation of TFEB, are altered with ageing and whether these alterations are directly linked to mTOR upregulation. Lastly, it will be important to address what the consequences of these changes in autophagy are and what exact step(s) of the autophagy process may ultimately become limiting for lifespan. One possibility could be impairment of lysosomal acidification, as observed in yeasts[124]. Consistent with this idea, the activity of several lysosomal proteases decrease with age in *C. elegans*[9]. Another possibility might involve age-dependent impairment of

autophagic vesicle transport, as observed in neurons[125]. Regardless of the mechanism, stalled autophagy could become detrimental to 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 has been observed in aged *C. elegans*[16,17] and in livers from aged mice[18]. It is an important objective for future research to understand the regulation of age-associated changes in autophagic activity. Because 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 these two systems changes over time.

As noted above, mounting evidence suggests that different longevity paradigms require functional autophagy. Because at least two such conserved paradigms, namely, reduced insulin/IGF1 signalling and germline removal, affect autophagy regulation differently at the tissue-specific level in *C. elegans*[16], there may be multiple ways to increase lifespan by autophagy modulation. Given that several of the longevity paradigms are additive for lifespan extension[126], it will be interesting to investigate whether combining the paradigms can produce synergistic results with respect to induction of the autophagy process. Moreover, it will be valuable to further examine the cell-autonomous and non-cell 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.

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. Although a lot of research is still needed to consolidate these new and exciting findings and to investigate their conservation across species, it is interesting to consider future prospects. Surely, it is possible that more types of specific autophagic cargo in addition to those discussed here (mitochondria, protein aggregates, lipid droplets and lysosomes) will prove to be relevant to ageing, particularly because selective autophagy has been shown to be a widespread process that and also includes eRphagy, ribophagy, xenophagy and nucleophagy. In addition, it will be important to fully address the functional role of auto-phagy not only in different tissues but also over time. To this end, a very recent study surprisingly reported that late-life inhibition of *ATG* genes with functions in the phagophore nucleation complex causes a potent lifespan extension in wild-type *C. elegans*[17]. This is in stark contrast to the effects of inhibiting the same genes early in adult life, which has no or small lifespan-shortening effects in wild-type *C. elegans*[23]. Thus, the multistep autophagy process may affect ageing in a much more complex manner than previously anticipated, and future experiments are needed to address this important point. Another interesting avenue to explore further may be the interplay between autophagy, commensal bacteria and their homeostasis and organismal health and ageing, given recent attention regarding links between microbiota dynamics and host ageing[127,128]. Likewise, as non-conventional roles for *ATG* genes, for example, in protein secretion, are becoming increasingly recognized[129], it also remains to be addressed whether any of these non-conventional functions may be linked to ageing. Finally, it will be very attractive to explore the growing number of pharmacological interventions that can induce autophagy with possible effects on longevity (BOX 2), opening up the possibilities for lifespan extension in humans.

Note added in proof: A recent study showed that decreased interaction between Beclin1 and its negative regulator Bcl-1increases autophagic flux and extends health- and lifespan in mice[162].

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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 ranging from yeasts 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 the table). These interventions are also referred to as longevity paradigms. the first example of such a longevity paradigm was described in the 1930s, when reduced food intake without malnutrition (called dietary restriction) was shown to extend the lifespan of rats, a treatment shown later to have beneficial effects in several other organisms[130]. similarly, reducing the activity levels of two major nutrient-sensing pathways, the mTOR[131] and insulin/iGF1 (REF.[132]) signalling pathways, extends lifespan in a number of species, and overexpression of the nutrient sensor AMP-activated protein kinase (AMPK) extends lifespan in worms and flies[133]. Other interventions also extend lifespan in at least yeasts, worms and flies, including reduced levels of mitochondrial respiration[134] and hormetic heat shock[135]. Lastly, a number of pharmacological interventions extend lifespan in a number of species, for example, the polyamine spermidine[136] and the plant phenol resveratrol[137]. the study of these longevity paradigms has long focused on identifying the underlying molecular mechanisms mediating lifespan extension, including roles for different transcription factors[126]. 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 by * in the table; these links, and reports of lifespan extension by overexpression of autophagy genes, can be found in TABLE 1).

Longevity paradigm	Organism	Refs
Genetic longevity paradigms		
Dietary restriction	• Yeasts* • Worms (*eat-2* mutants)* • Flies • Mice	130
mTOR inhibition (for example, rapamycin)	• Yeasts* • Worms (*daf-15* heterozygotes)* • Flies* • Mice	131
Reduced insulin/IGF1 signalling	• Yeasts • Worms (*daf-2* mutants)* • Flies • Mice	132

Longevity paradigm	Organism		Refs
Increased AMPK activity	•	Yeasts	133
	•	Worms*	
	•	Flies*	
	•	Mice	
Reduced mitochondrial respiration	•	Yeasts	134
	•	Worms*	
	•	Flies	
Hormetic heat shock	•	Yeasts	135
	•	Worms*	
	•	Flies	
Germ-line removal	•	Worms (*glp-1* mutants)*	64
	•	Flies	
Reduced TGFβ/Activin signalling	•	Worms	12,138
	•	Flies*	
Pharmacological longevity paradigms			
Spermidine	•	Yeasts*	136
	•	Worms*	
	•	Flies*	
	•	Mice	
Resveratrol	•	Yeasts	137
	•	Worms*	
	•	Flies	
	•	Mice	
Urolithin A	•	Worms*	63
	•	Mice (of note, in this case, animal healthspan and not longevity was evaluated)	

TGFβ, transforming growth factor-β.

Box 2 |

Autophagy inducers relevant to lifespan extension

Pharmacological agents that increase 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 yeasts to mice[139], and it is possible that some of these are via effects on autophagy. while there are side effects caused by rapamycins, such as immunosuppression and poor glucose tolerance, and they 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 feasible approach for lifespan extension. Numerous mTOR-independent molecules with autophagy-inducing effects have been described elsewhere and include metformin and trehalose[83]. 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 because autophagy induction with *ATG5* overexpression, which is a more specific genetic approach, lengthens lifespan in mice[29].

Autophagy can also be induced with non-small-molecule approaches, including an autophagy-inducing peptide based on beclin 1 (REF[140]); it will be interesting to test whether 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, transcription factor EB (TFEB), which is a master regulator of lysosomal function, also regulates autophagy[30]. The *Caenorhabditis elegans* orthologue of TFEB positively regulates lifespan, at least in part via autophagy[31]. Thus, it will be interesting to identify drugs targeting this transcription factor and to investigate their effects on autophagy and longevity across species.

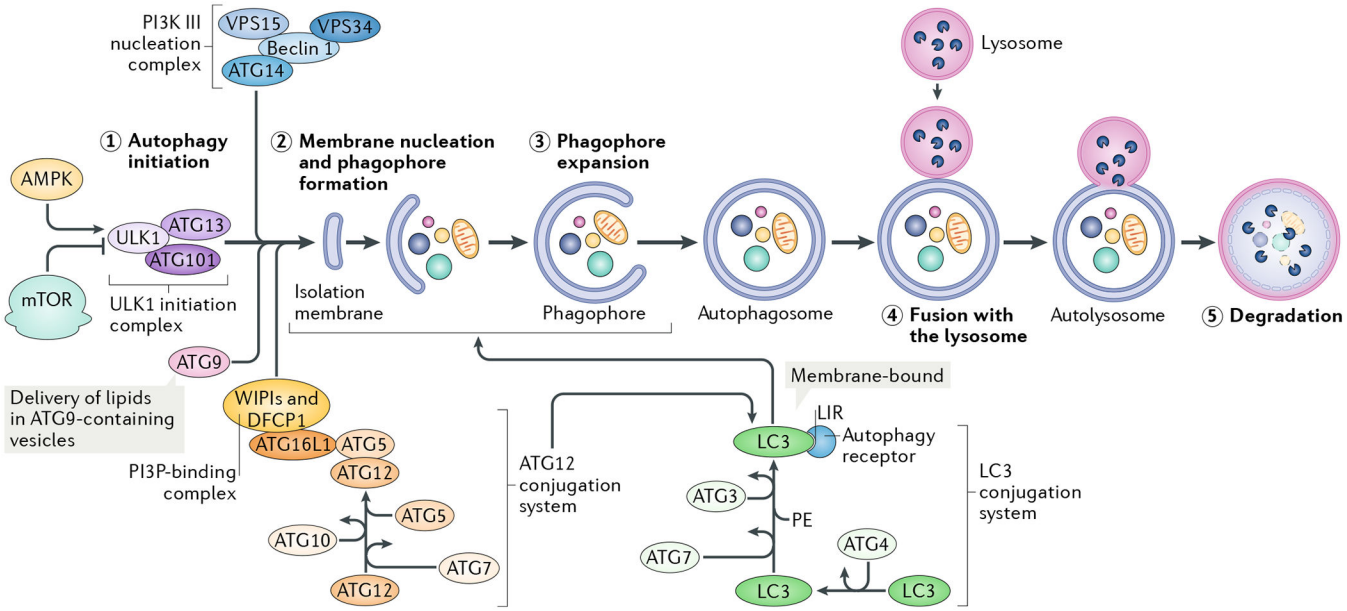

Fig. 1 |. The macroautophagy process.

A schematic depicting the process and main regulatory machinery of macroautophagy (referred to as autophagy) is shown. The conserved metabolic sensors and longevity determinants mTOR and AMP-activated kinase (AMPK) are the main regulators of autophagy, with mTOR acting as an inhibitor and AMPK as an activator. When autophagy is induced, cytoplasmic material (the autophagic cargo) is engulfed by double membranes, starting from the formation of a cup-shaped structure called the phagophore to the sequestration into double-membrane vesicles, called autophagosomes, which subsequently fuse with acidic lysosomes and form autolysosomes, where cargo is degraded. Autophagy is a multistep process that includes (1) initiation, (2) membrane nucleation and phagophore formation, (3) phagophore expansion, (4) fusion with the lysosome, and (5) degradation, which correspondingly are regulated by multiple proteins, referred to as autophagy-related proteins (ATGs). ATGs assemble into several complexes: the Unc-51-like kinase 1 (ULK1; Atg1 in yeasts) initiation complex, the class III PI3K nucleation complex and the phosphatidylinositol 3-phosphate (PI3P)-binding complex, which directs the distribution of the machinery that enables autophagosome formation, and includes the ATG12 and the microtubule-associated protein light chain 3/γ-aminobutyric acid receptor-associated proteins (LC3/GABARAPs; Atg8 in yeasts) conjugation systems (for simplicity, only LC3 is noted in the figure). In the ATG12 conjugation system, ATG12 is attached to ATG5, which is then attached to ATG16L1 (Atg16 in yeasts), followed by dimerization (not shown) and interaction with the PI3P-binding complex (formed by WD repeat domain phosphoinositide-interacting proteins (WIPIs; Atg18 in yeasts) and zinc-finger FYVE domain-containing protein 1 (DFCP1). The ATG12-ATG5-ATG16L1 complex then promotes conjugation of LC3 (or GABARAP), whereby LC3 is cleaved by the protease ATG4 to form LC3-I, which is then conjugated with phosphatidylethanolamine (PE) to form LC3-II. This conjugate is incorporated into pre-autophagosomal and autophagosomal membranes, where LC3 can interact with cargo receptors, which harbour LC3-interacting motifs (LIRs). Membranes for phagophore expansion are delivered, at least in part, by ATG9-containing vesicles. For

simplicity, only the names of vertebrate ATGs are shown. VPS15, PI3K regulatory subunit 4 (also known as PIK3R4 in humans); VPS34, phosphatidylinositol 3-kinase catalytic subunit type 3 (also known as PIK3C3 in humans).

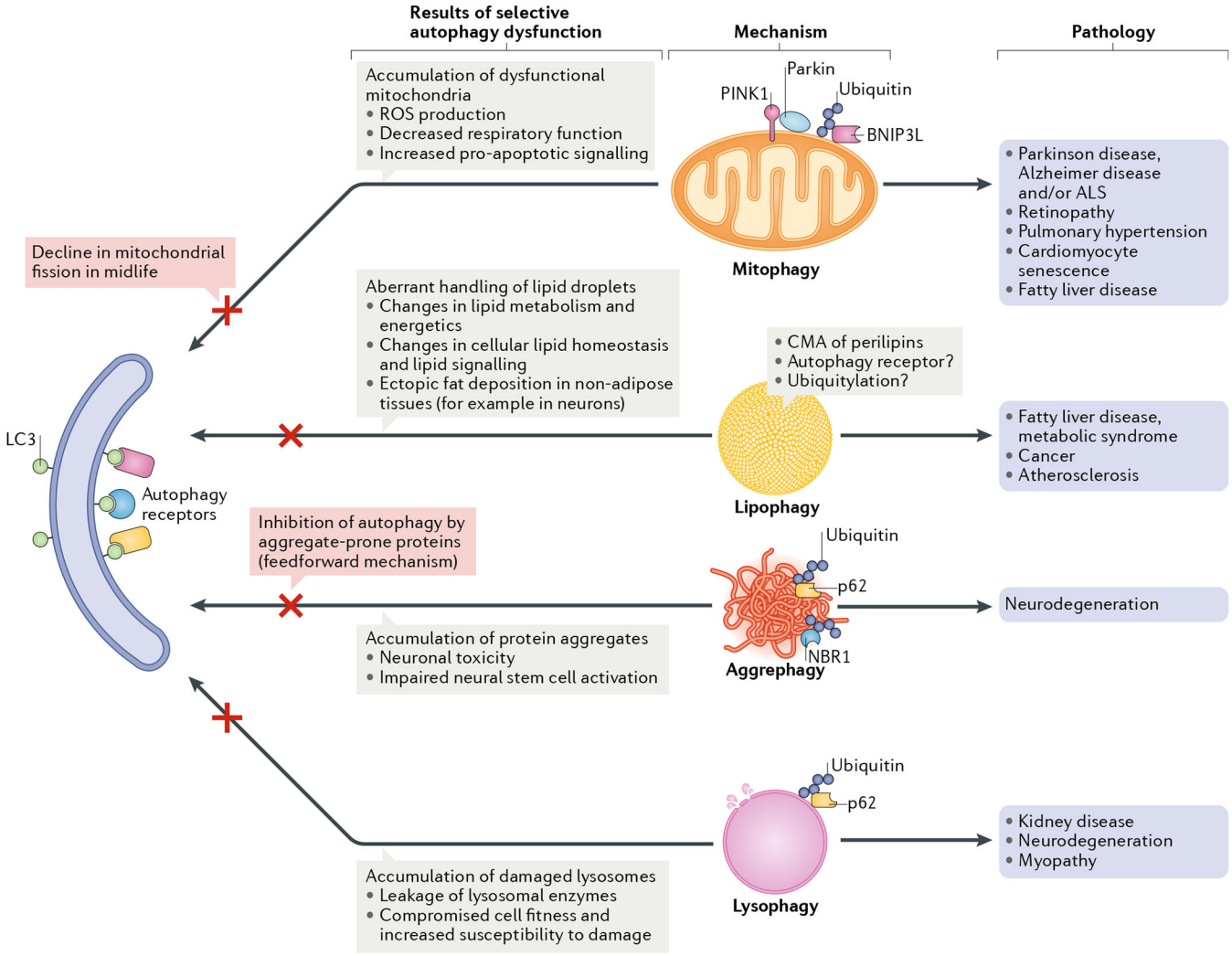

Fig. 2 |. Selective types of autophagy linked to organismal ageing.

A schematic summarizing selective types of autophagy linked to pathologies of 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 and the autophagy machinery (some examples of autophagy receptors that have been indicated to function in the context of ageing are depicted, but most likely other receptors are involved). Consequences of deficiencies in these types of selective autophagy and their links to 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, PTEN-induced putative protein kinase 1 (PINK1), which is mutated in a rare form of recessive Parkinsonism, has been implicated in stress-induced mitophagy in tissue-culture models, leading to the assumption that loss of PINK1 causes disease via defects in mitophagy. However, recent work suggests that loss of PINK1 in mice does not affect mitophagy, thus challenging the model[141]. ALS, amyotrophic

lateral sclerosis; BNIP3L, BCL-2/adenovirus E1B 19 kDa protein-interacting protein 3-Like; CMA, chaperone-mediated autophagy; LC3, microtubule-associated protein light chain 3; NBR1, next to BRCA1 gene 1 protein; ROS, reactive oxygen species.

Table 1 |

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

Gene (mouse orthologue)	Function in autophagy	Association with lifespan determination or age-related diseases in humans	Refs
Saccharomyces cerevisiae			
ATG1(*Ulk1*)	Autophagy initiation	Required for longevity induced by rapamycin	142
ATG11	Autophagosome-vacuole fusion; selective autophagy	Required for longevity induced by rapamycin	142
ATG7	El-like enzyme for the Atg5-Atg12 complex and the Atg8 conjugation systems	Required for longevity induced by dietary restriction, rapamycin and spermidine	110,142,143
ATG5	Conjugated by Atg12	Required for longevity induced by dietary restriction by methionine restriction	143
ATG8	Phagophore elongation and cargo recruitment	Required for longevity induced by dietary restriction by methionine restriction	143
VAM3	SNARE protein involved in vacuolar fusion	Required for longevity induced by dietary restriction	144
VAM7	SNARE protein involved in vacuolar fusion	Required for longevity induced by dietary restriction	144
VMA2	Vacuolar ATPase subunit	Required for longevity induced by dietary restriction	123
ATG15	Putative lipase required for intravacuolar disintegration of autophagic bodies	Required for longevity induced by dietary restriction	144
Caenorhabditis elegans			
unc-51 (*Ulk1*)	Autophagy initiation	Required for longevity induced by mTOR inhibition, dietary restriction, germline ablation and reduced mitochondrial respiration	72,145
bec-1(*Becn1*)	Allosteric regulator of VPS-34	Required for longevity induced by mTOR inhibition, dietary restriction, germline ablation, reduced mitochondrial respiration, spermidine, resveratrol, urolithin A and other paradigms[a]	31,63,72,110,146–149
vps-34	Kinase that produces PI3P to enable recruitment of machinery that forms autophagosomes	Required for longevity induced by mTOR inhibition, dietary restriction, germline ablation, reduced mitochondrial respiration and urolithin A	63,72,148,150
atg-9	Phagophore formation	Other paradigms[a]	151
atg-18 (*Wipl*)	Phagophore formation	Required for longevity induced by inhibition of insulin/IGF1 signalling (M), dietary restriction (I, M and possibly N), germline ablation (I), reduced mitochondrial respiration, AMPK overexpression[c], inhibition of a downstream effector of the mTOR pathway, S6K and other paradigms[a] (but overexpression from the endogenous promoter does not extend lifespan)	16,72,94,97,152
lgg-3 (*Atg12*)	Ubiquitin-like modifier of ATG-5	Required for longevity induced by inhibition of insu1in/IGF1 signalling, dietary restriction and other paradigms[a]	147
atg-7	El-like enzyme for the ATG-5-ATG-12 complex and the ATG-8 conjugation systems	Required for longevity induced by inhibition of insu1in/IGF1 signalling	147,150
lgg-1 (*Lc3/Gabarap* family genes)	Phagophore elongation and cargo recruitment	Required for longevity induced by inhibition of insu1in/IGF1 signalling (M), germline ablation, mitochondrial respiration, AMPK overexpression[c] and other	72,148

Gene (mouse orthologue)	Function in autophagy	Association with lifespan determination or age-related diseases in humans	Refs
		paradigms[a] (but overexpression from the endogenous promoter does not extend lifespan)	
atg-4.1	ATG-8 processing to make it conjugation-competent, and ATG-8 delipidation	Other paradigms[a]	151
vha-16	Subunit of vacuolar proton-translocating ATPase	Required for longevity by germline ablation	31
C08H9.1	Lysosomal degradation	Required for longevity induced by inhibition of insu1in/IGF1 signalling	153
lipl-1	Lysosomal lipolysis	Overexpression from the endogenous promoter extends lifespan	74
lipl-3	Lysosomal lipolysis	Overexpression from the endogenous promoter extends lifespan	74
lipl-4 (*Hla-l*)	Lysosomal lipolysis	• Required for longevity induced by inhibition of insu1in/IGF1 signalling and germline ablation • Overexpression from endogenous and intestinal-specific promoters extends lifespan and lifespan extension by *lipl-4* promoter overexpression is autophagy-dependent	71
dct-1 (*Bnip3L*)	Mitochondrial receptor protein	Required for longevity induced by inhibition of insu1in/IGF1 signalling, dietary restriction, mitochondrial dysfunction, urolithin A and other paradigms[a]	45,60,63
pink-1	Kinase that enables mitophagy	Required for longevity induced by inhibition of insulin/IGF1 signalling, dietary restriction, mitochondrial dysfunction, urolithin A and other paradigms[a]	45,60,63
sqst-1 (*Sqstml*)	Receptor protein	Required for longevity induced by mitochondrial dysfunction, urolithin A and other paradigms[a]	60,63
hlh-30 (*Tfeb*)	Transcription factor regulating lysosomal biogenesis and autophagy	• Required for longevity induced by mTOR inhibition, inhibition of insulin/IGF1 signalling, dietary restriction, germline ablation, reduced mitochondrial respiration and inhibition of S6K • Overexpression from the endogenous promoter extends lifespan in an autophagy-dependent fashion	31,152
Drosophila melanogaster			
Atg1 (*Ulk1*)	Autophagy initiation	Required for longevity induced by AMPK overexpression (N) (overexpression from a neuronal-specific promoter during adulthood extends lifespan); also required for longevity induced by overexpression of the mitochondrial protein dynamin-related protein 1 (Drp1)	24,25
Atg7	E1-like enzyme for the Atg5-Atg12 complex and the Atg8 conjugation systems	Required for longevity induced by spermidine	110
Atg5	Conjugated by Atg12	Required for longevity induced by rapamycin	154
Atg8a (*Lc3/Gabarap* family genes)	Phagophore elongation and cargo recruitment	Overexpression from a neuronal-specific and a muscle-specific promoter extends lifespan[b]	10,12
Parkin	E3 ubiquitin ligase that facilitates mitophagy	Overexpression from ubiquitous and neuronal-specific promoters during adulthood extends lifespan	61

Gene (mouse orthologue)	Function in autophagy	Association with lifespan determination or age-related diseases in humans	Refs
Drp1	Dynamin-related protein that promotes mitochondrial fission and facilitates mitophagy	Overexpression from ubiquitous, intestine-specific and neuronal-specific promoters in midlife extends lifespan in an autophagy-dependent fashion	25
Mus musculus[d]			
Atg7	E1-like enzyme for the ATG5-ATG12 complex and the ATG8 conjugation systems	Depletion in the muscle impairs muscle function and shortens lifespan	14
Atg5	Conjugated by ATG12	Overexpression from a ubiquitous promoter extends lifespan	29
Human			
BECN1	Allosteric regulator of VPS34	Variants have been associated with breast cancer prognosis	155
WDR45	Phagophore formation	Mutations cause neurodegeneration with brain iron accumulation	156,157
ATG7	E1-like enzyme for the ATG5-ATG12 complex and the ATG8 conjugation systems	Variants have been proposed to impact age at onset of Huntington disease	158
ATG5	Conjugated by ATG12	Mutations cause ataxia and developmental delay	35
ATG16L1	LC3 lipidation	Mutation T300A increases risk of Crohn's disease	159,160
TECPR2	Interacts with LC3	Mutations cause spastic paraparesis	33
ZFYVE26	Autophagosome maturation	Mutations cause spastic paraplegia	34
EPG5	Autophagosome-lysosome fusion	Mutations cause Vici syndrome	161
PRKN	E3 ubiquitin ligase that facilitates mitophagy	Mutations cause autosomal recessive Parkinson disease	36
PINK1	Kinase that facilitates mitophagy	Mutations cause autosomal recessive Parkinson disease	37
SQSTM1	Receptor protein	Mutations cause Paget disease of bone and motor neuron disease	38,39
TBK1	Kinase that phosphorylates autophagy receptors	Mutations cause motor neuron disease	40

The table summarizes autophagy-related (ATG) and lysosomal genes and their role in conserved longevity paradigms (in yeasts, worms, flies and mice) or in age-related diseases (in humans). AMPK, AMP-activated kinase; GABARAP, γ-aminobutyric acid receptor-associated protein; E1-like, ubiquitin-activating enzyme-like; I, intestinal-specific RNAi in adult animals; LC3, microtubule-associated protein light chain 3; M, muscle-specific RNAi in adult animals; N, neuronal-specific RNAi in adult animals; PI3P, phosphatidylinositol 3-phosphate; PRKN, Parkin; RSK, ribosomal protein S6 kinase; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; Sqstm1, sequestosome-1; VPS34, phosphatidylinositol 3-kinase catalytic subunit type 3.

[a] Additional longevity paradigms, for example, calcineurin, frataxin and miR-34 depletion, require this autophagy gene in *C. elegans* (see REF.[23] for additional links).

[b] Moreover, reduced Activin signalling requires *Atg8a* is required for longevity associated with the loss of Activin signalling in muscle[12].

[c] Unpublished observations from M.H.'s laboratory.

[d] See note added in proof and REF.[162].

Table 2 |

Tissue-specific functions of autophagy in ageing

Intestine	Muscle	Nervous system
Links between autophagy genes and ageing have been demonstrated[a]		
Yes (worms and flies)	Yes (worms, flies and mice)	Yes (worms and flies)
Functions of autophagy in the tissue related to ageing		
Maintenance of intestinal barrier function (worms and flies)[24,94]	• Maintenance of muscle function (worms, flies and mice; in mice, related to the maintenance of neuromuscular junctions)11,12,14,24,60,93 • Preventing senescence and promoting activation of satellite cells (mice)[116–118]	Supporting learning and memory processes (flies)[111,112]
Longevity paradigms in which tissue-specific autophagy has a ***role***		
• Dietary restriction (worms)[94] • Midlife Drp1 overexpression (flies)[25] • Intestinal AMPK overexpression (flies)[24]	• Dietary restriction (worms)[94] • Muscle-specific FOXO overexpression (flies)[11] • Reduced Activin signalling (flies)[12]	• Hormetic heat shock (worms)[82] • Dietary spermidine (flies)[110] • Neuronal Atg8a overexpression (flies)[10] • Neuronal AMPK overexpression (flies)[24] • Neuronal Atg1 overexpression (flies)[24] • Midlife Drp1 overexpression (flies)[25] • Neuronal Parkin overexpression (flies)[61]
Tissue-specific mechanisms of autophagy linked to ageing		
Maintenance of tight junctions and regulation of tight junction permeability (human intestinal cell line)[103]	• Improved mitochondrial homeostasis (flies and mice)[14,25,61] • Improved proteostasis and clearance of protein aggregates (flies)[10–12,24,25,61] • Supply of nutrients for stem cell activation (mouse and human muscle stem cells)[116,117]	• Improved proteostasis and clearance of protein aggregates (flies)[10–12,25,61] • Mitochondrial homeostasis (flies)[25,61]
Inter-tissue communication and systemic effects of autophagy on ageing		
• Communication to muscle and/or neuromuscular junctions: maintenance of muscle function and motility (worms)[94] • Communication to neurons: promoting autophagy in the brain (flies)[24]	• Improved mobility (worms)[94] • Improved systemic proteostasis (flies)[11]	Communication to the intestine: improved intestinal barrier function (worms and potentially also flies)[24,94]
Potential mechanisms supporting non-tissue autonomous effects of autophagy on ageing		

Intestine	Muscle	Nervous system
Not known	Decrease in insulin-like peptide secretion and reduced systemic insulin-like peptide signalling (flies)[11,12]	Sustained neurotransmitter release from chemosensory neurons (worms)[97] and reduced levels of neuropeptides and/or insulin-like peptides (worms[97] and flies[24])

Atg1, autophagy-related gene 1 (*ULK1* in mammals); Atg8a, autophagy-related 8a; Drp1, dynamin-related protein 1; FOXO, forkhead box protein.

[a] See TABLE 1.

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