1 Solicited opinion/review to Nature Ageing
2 **Autophagy in healthy ageing and disease**
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Abstract

Autophagy is a fundamental cellular process that eliminates subcellular components via lysosome mediated degradation to promote homeostasis, differentiation, development, and survival. While autophagy is intimately linked to health, the intricate relationship between autophagy, ageing and disease, remains unclear. Furthermore, the broad spectrum of substrates associated with autophagy (nucleic acids, proteins, lipids, organelles, and pathogens) raises a key question: how do distinct autophagic mechanisms influence tissue and organismal homeostasis in the long-term? This review examines several emerging features of autophagy and postulates how they may be linked to ageing as well as to the development and progression of disease. In addition, we discuss the current pre-clinical evidence arguing for the use of autophagy modulators as suppressors of age-related pathologies, such as neurodegenerative diseases. Finally, we highlight key questions and propose novel research avenues that will likely reveal new links between autophagy and the hallmarks of ageing. Understanding the precise interplay between functionally active autophagy and the risk of age-related pathologies will elucidate the ageing landscape across organisms and eventually facilitate the development of clinical applications that promote long-term health.

1. Ageing and autophagy

Ageing is a biological process that is characterised by a time-dependent cellular and functional decline, resulting in reduced quality of life for the organism[1]. Consistent with this, ageing is the primary risk factor for the development of many disorders, including cardiovascular disease (e.g., stroke), cancer and neurodegenerative disease (e.g., Alzheimer's disease (AD)). Collectively, age-related ailments result in a formidable global socio-economic burden and a significant healthcare challenge[2,3]. Therefore, identifying therapeutic interventions that promote "healthy ageing" (i.e., the maintenance of functional ability in old age, enabling the elderly to independently carry out daily tasks) and simultaneously halt the progression of multiple age-related pathological conditions, is of paramount importance[2].

Amongst many molecular changes associated with old age, altered autophagy has emerged as a feature of ageing across diverse species. However, recent advances in understanding the numerous substrates of autophagy, and the temporal and spatial effects of impaired autophagy regulation on tissue homeostasis, have revealed a complex and multi-factorial relationship between autophagy and ageing. Here we examine the relationship between autophagy, ageing, and disease; and propose novel links between specific autophagic processes and long-term tissue health, as well as possible implications for anti-ageing therapeutic interventions.

2. Compromised autophagy is a hallmark of ageing

Research over the last decade has revealed that the process of autophagy can take many different forms. Autophagy (from the Greek words "*auto*", meaning "*self*," and "*phagein*", meaning "*to eat*") is a highly conserved pathway that degrades cellular components, such as defective organelles and aggregates of misfolded proteins[4], through the lysosomes. The process of autophagy was first described in the 1960s, but it was the identification of autophagy-related genes (*ATG*) in the 1990s that propelled the major breakthroughs in unravelling the mechanistic complexities of autophagy[5-12]. There are three major types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (**Fig. 1a-c**), all of which involve delivery of substrates to the lysosome for degradation (detailed reviews[13,14]). Macroautophagy (hereafter referred to as autophagy) was originally thought of as a non-selective, bulk degradation process (**Fig. 1a-I**). However, the discovery of selective autophagy receptors, with p62/SQSTM1 being the first, changed this notion[15,16]. Today, autophagy is recognised as a highly selective cellular clearance pathway that is associated with the maintenance of cellular and tissue homeostasis[17,18]. Selective autophagy can be further classified into many sub-types based on the specific cargos involved. These include various macromolecules (glycophagy, lipophagy) (**Fig. 1a-II to Fig. 1a-V))**, mitochondria (mitophagy) (**Fig. 1a-VI**), endoplasmic reticulum (ER) (ER-phagy) (**Fig. 1a-VII**), parts of the nucleus (nucleophagy) (**Fig. 1a-VIII**), pathogens (xenophagy) (**Fig. 1a-IX**), and lysosomes themselves (lysophagy) (**Fig. 1a-X**). Below, we will discuss the links between these selective autophagy pathways, ageing and disease. The core process of macroautophagy has been described in detail elsewhere[14,19]. However, in brief, amongst the core autophagy process is initiated following inhibition of the mechanistic target of rapamycin (mTOR) or activation of 5' AMP-activated protein kinase (AMPK), both of which are canonical inducers of autophagy in response to stress (e.g., starvation, elevated temperatures) and physical exercise. In addition, Transcription Factor EB (TFEB) is an important positive regulator of autophagy and lysosomal biogenesis, whose nuclear translocation is coupled to the activity of both mTOR (via phosphorylation) and AMPK (via Folliculin (FLCN))[20-23]. Upon activation of autophagy, the process is initiated by membrane nucleation and phagophore formation, and elongation and maturation, are initiated, before the autophagosome fuses with the lysosome for cargo degradation and recycling. The key proteins involved in each step are presented in **Fig. 2**.

A growing body of evidence suggests that autophagic activity declines with age in diverse organisms[1]. Studies in *Caenorhabditis elegans* (*C. elegans*), rodents, and human cells have demonstrated an age-dependent reduction in lysosomal proteolytic function, thereby impairing

128 autophagic flux[24-27], exacerbating cellular impairment and contributing to the development of
129 age-related diseases[1,28,29]. Further evidence stemming from *Drosophila* has revealed that
130 ageing is associated with the reduced expression of several *Atg* genes (*Atg2*, *Atg8a*, blue
131 cheese gene / *Bchs*), which are pivotal for both autophagy initiation and activity[30]. In aged wild-
132 type (WT) mice, autophagy is diminished in neuronal cells as evidenced by decreased rates
133 of autophagolysosomal fusion and impaired delivery of autophagy substrates to lysosomes in
134 the hypothalamus[31]. Moreover, a decrease in autophagic processes was observed in 18–25-
135 month-old murine brain tissue, as demonstrated by a reduction in *Atg5-Atg12* and *Becn1*
136 levels, elevated mTOR activity, and increased ferritin H levels (Ferritin H is mainly removed
137 from cells by the autophagy-lysosome pathway)[32]. In addition, emerging findings in aged-rats
138 have also highlighted an age-associated decline in the expression of the autophagy related
139 protein Becn1 in whole brain tissue, as well as in the hippocampus of naked mole-rats and
140 Wistar rats, respectively[33,34]. Consistent with observations in rodent models, findings in
141 humans have suggested that the expression of autophagy-related genes, such as *ATG5*,
142 *ATG7* and *BECN1*, declines with age[35]. Moreover, the development and progression of several
143 human pathologies is highly associated with age-dependent autophagy deficits[19,36,37].
144 Collectively these studies demonstrate that a gradual decline in autophagy-related proteins,
145 and reduced delivery of cargo to lysosomes, occurs with age, implicating compromised
146 autophagy as a cardinal feature of organismal ageing.

148 Consistent with a causal role for autophagy in the ageing process[14], genetically impairing non-
149 selective or selective autophagy results in accelerated tissue functional decline and disease in
150 a range of experimental models. Transcriptomic profiling in the Brewer's yeast *Saccharomyces*
151 *cerevisiae,* has provided evidence for defective autophagy amongst short-lived, compared to
152 long-lived, mutants[38]. In addition, selective mutation(s) and/or knockdown of genes encoding
153 proteins of the autophagic machinery in *C. elegans* (*ATG8/ lgg-1*, *ATG1*/*unc-51*, *bec-1*, *atg-7*,
154 *atg-12* and *atg-18*), *Drosophila* (*Atg3* and *Atg8a*) and mice (*Atg5*, *Atg7*, and *Becn1*) shorten
155 lifespan and healthspan[1,14,30,39]. Consistent with these observations, the systemic genetic
156 knockout of the autophagy components (*Becn1*, *Atg5*, *Atg9,* and *Atg13*) are lethal in mice,
157 thereby highlighting the importance of autophagy in development[40]. Furthermore, knockdown
158 of genes encoding transcription factors that regulate autophagy, such as *TFEB* (orthologue in
159 *C. elegans hlh-30*) and Forkhead Box O (*FOXO*; orthologue in *C. elegans daf-16*) shortened
160 lifespan in both wild-type (WT) and long-lived *daf-2* (insulin/IGF-1 receptor) mutants[41].

162 In contrast, studies in long-lived mutant animals have shown that increased autophagy is
163 associated with delayed ageing. In particular, the extended lifespan of *C. elegans daf-2* loss-
164 of-function mutants is dependent on autophagic genes, such as *bec-1*, *lgg-1*, *atg-7* and *atg-*
165 *12*[1,14,42]. Furthermore, hlh-30/TFEB is required for the long lifespan of multiple longevity
166 mutants, including not only *daf-2*/InR reduced insulin/insulin-like signalling mutants, but also
167 germline-less *glp-1(e2141)* mutants, dietary-restricted *eat-2(ad1116)* mutants, mitochondrial
168 respiration defective *clk-1(e2519)* mutants, and mRNA translation impaired *rsks-1(sv31)*
169 mutants[43]. These findings coincide with impaired induction of autophagosome formation and
170 lysosomal degradation upon loss of *hlh-30/TFEB*, suggesting that HLH-30/TFEB promotes
171 longevity by regulating the autophagy process downstream of multiple lifespan extension
172 paradigms[43]. In addition, the formation of long-lived dauer worms, a larval hibernation stage,
173 is also associated with increased autophagy, and is dependent on the autophagy genes *atg-*
174 *1*, *atg-7*, *lgg-1* and *atg-18,* underlining the essential role of autophagy in organismal adaptation
175 during challenging conditions[42].

177 Consistent with observations from long-lived mutants, genetic or pharmacological upregulation
178 of autophagy promotes longevity in animals. Autophagy induction by overexpression of *Atgs*
179 in *Drosophila* (*Atg1* and *Atg8a*) and mice (*Atg5*) extended lifespan[30,44,45]. Similarly, *BCL2*
180 mutations that disrupt the BECN1-BCL2 complex, increase basal autophagic flux, which
181 results in long-lived male and female mice with improved healthspans[46]. Additionally,
182 overexpression of autophagic regulators in *C. elegans* and *Drosophila*, such as AMPK, further

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183 facilitates autophagy in diverse tissues, and in turn extends longevity[14,45]. Furthermore, *hlh-*
184 *30/TFEB* overexpression enhances autophagy and promotes lifespan extension in *C.*
185 *elegans*[43], and silencing of the nuclear export protein, Exportin-1 (XPO1/XPO-1), enhances
186 autophagy by enrichment of HLH-30/TFEB in the nucleus, which is accompanied by
187 proteostatic benefits and improved longevity[47]. Moreover, rapamycin, an inhibitor of the mTOR
188 pathway, fed late in life, has been shown to extend the median and maximal lifespan of both
189 female and male mice[48].
190
191 Accumulating evidence in aged mice, as well as rodent models recapitulating characteristic
192 features of human diseases, shows that compromised autophagy is amongst the most
193 common factors contributing to the collapse of tissue homeostasis. In particular, age-
194 associated dysregulation of autophagy (demonstrated by the accumulation of
195 autophagosomes), possibly due to impaired lysosomal fusion and/or degradation, is
196 associated with cellular dysfunction and/or death, which contributes to neurodegeneration, as
197 well as cardiac and skeletal muscle ageing[49-53]. In hematopoietic stem cells (HSC), autophagy
198 has been shown to delay ageing via activation of downstream sirtuin-3 (SIRT3), a key
199 mitochondrial protein capable of rejuvenating blood and protecting against oxidative stress in
200 mice and human HSC-enriched cells[54].
201
202 Moreover, autophagy appears to be a critical mechanism to maintain immune memory in mice,
203 and levels of the endogenous autophagy-inducing metabolite spermidine fall in human T cells
204 with age. In fact, spermidine supplementation to T cells from old donors restores autophagy
205 levels to that observed in young donors via the translation factor eIF5A and transcription factor
206 TFEB[55]. Furthermore, spermidine administration in a mouse model of mild cognitive
207 impairment (MCI), a transitional phase between AD and healthy ageing, causes an
208 improvement in degradation of misfolded proteins, and an accompanying delay in age-related
209 memory deficits, thereby implicating autophagy as a pathophysiological mechanism of action
210 [56].
211
212 Whilst the dysregulation of autophagy underlies ageing and disease phenotypes, excessive
213 autophagy may also contribute to the deterioration of cellular function in some contexts. Recent
214 evidence demonstrates that an age-dependent decline in Rubicon, a negative regulator of
215 autophagy, exacerbates metabolic disorders in adipocytes[57]. In addition to the possibility that
216 hyper-upregulated autophagy may exacerbate metabolic disorders, this finding may also be
217 attributed to autophagy-independent changes in metabolism. Furthermore, elevated
218 autophagy has been found to shorten lifespan in *C. elegans* mutants lacking
219 serum/glucocorticoid regulated kinase-1 (*sgk-1*). The loss of *sgk-1* results in increased
220 mitochondrial permeability, leading to excessive autophagy and reduced organismal fitness in
221 worms and mice[58]. Conversely, reducing the levels of autophagy in *sgk-1* mutants, or
222 suppressing the opening of the mitochondrial permeability transition pore, restores normal
223 lifespan[58]. Similarly, suppressing autophagy exclusively in the intestine of post-reproductive
224 adults at higher temperatures has been proposed to prevent the emergence of age-related
225 pathologies in *C. elegans*[59]. However, it should be noted that this is in direct contrast to findings
226 in long-lived mutants, where intestinal autophagy is enhanced[60,61]. Another study in *C. elegans*
227 showed that siRNA-based reduction of VPS-34/BEC-1/EPG-8 autophagic nucleation complex
228 in aged post-reproductive worms, extended lifespan, and improved neuronal integrity[29].
229 However, detailed data on knockdown efficiency in aged worms, as well as an understanding
230 of the remaining levels of neuronal autophagy are necessary to ensure accurate in-depth data
231 interpretation. Collectively, these observations suggest that the maintenance of functional
232 autophagy is essential for healthy cellular and organismal ageing, and that dysregulation of
233 autophagy in either direction, whether insufficient or excessive, contributes to cellular deficits
234 and functional organismal decline.
235
236 A summary of autophagy-related genes linked to longevity and disease is provided in **Table 1**
237 and **Supplementary Table 1**. Furthermore, several interventions known to promote lifespan,

238 including dietary restriction and treatment with pharmacological agents, such as rapamycin,
239 spermidine, and NAD$^+$ precursors, require an intact autophagic machinery. In totality, these
240 findings reinforce the notion that autophagy stimulation is necessary and sufficient to sustain
241 organismal homeostasis and extend longevity in multiple model organisms (discussed in detail
242 below)[1]. An overview of autophagy inducers linked to enhanced longevity and improved health
243 is presented in **Table 2**.

245 Together, numerous studies provide evidence that: (i) autophagy is compromised during the
246 process of ageing; (ii) dysfunction of autophagy shortens lifespan in various experimental
247 animal models; and (iii) promotion/restoration of autophagy contributes to lifespan and
248 healthspan extension of diverse organisms. This suggests that autophagy is a central regulator
249 of ageing. However, an important and fundamental question remains unanswered: How does
250 autophagy facilitate long-term cell and tissue health?

252 **3. The multi-faceted role of autophagy in health and ageing**
253 **3.1. Autophagy and protein homeostasis**
254 Protein homeostasis (proteostasis) collapse is a central hallmark of ageing and disease that is
255 characterized by the appearance of misfolded, mislocalised and aggregated proteins. While
256 the age-related loss of proteostasis is documented in numerous tissues, age-dependent
257 protein aggregation is strongly linked to neurodegenerative pathologies, such as AD,
258 Parkinson's disease, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS)[62,63].

260 Along with molecular chaperones and the ubiquitin proteasome system (UPS), autophagy is a
261 central regulator of cellular proteostasis that operates to: (1) degrade soluble misfolded or
262 oligomeric proteins via CMA (the selective degradation of ubiquitin-tagged protein aggregates
263 by chaperone-assisted selective autophagy) and (2) remove bulk protein aggregates by
264 macroautophagy[13,19,64]. Consistently, genetic perturbation of core components or regulators of
265 the autophagy machinery accelerates age-related protein aggregation, shortens lifespan, and
266 exacerbates pathological features in worm, fly and mouse models of disease. Conversely,
267 increasing autophagy, genetically or pharmacologically, suppresses protein aggregation and
268 promotes health and longevity[48,65,66] (and reviewed by [67-69]).

270 In *C. elegans,* loss of function mutations in *Beclin/bec-1* or *WIPI2/atg-18,* or RNAi against *bec-*
271 *1, atg-9* or *ATG8/lgg-1,* increases susceptibility to protein aggregation, accelerates the onset
272 of age-related paralysis and shortens lifespan[39,70]. Similarly, mutations in the core autophagy
273 components *atg8* or *atg7* in *Drosophila,* increase the levels of insoluble protein aggregates and
274 reduce longevity[30,71]. Finally, knockout of *ATG5* or *ATG7* in mouse neurons leads to the
275 appearance of cytoplasmic inclusion bodies in the brain and early onset neurodegeneration[72-
276 74], while knockout of LAMP2A (the primary receptor for CMA) in the liver, results in altered
277 proteostasis and hepatic dysfunction with age[75].

279 Conversely, enhanced proteostasis and extended lifespan in *C. elegans* occurs when the
280 lysosome-autophagy transcription factor TFEB/HLH-30*,* or the selective autophagy receptor,
281 p62/SQSTM-1*,* are upregulated. Likewise, in *Drosophila,* overexpression of Ref(2)P (p62
282 orthologue) or the autophagy activator, FOXO, reduces protein aggregation in various tissues
283 and extends lifespan*[43,76-79]*. Pharmacological (e.g., clonidine, rilmenidine and rapamycin) and
284 genetic (e.g., *atg5*) upregulation of autophagy in zebrafish, harbouring the rare tau variant
285 p.A152T, ameliorates tau pathology[80]. Increased autophagy is also associated with enhanced
286 clearance of protein aggregates in mammals, as systemic overexpression of *Atg5,* or *Becn1*
287 mutations that disrupt BECN1-BCL2 binding, improves proteostasis and promotes longevity in
288 mice [44,81], while overexpression of the selective autophagy mediator, BAG3, suppresses tau
289 accumulation in neurons[82].

291 As a complement to the genetic modulation of autophagy, treatment of *Drosophila* with the
292 mTOR inhibitor rapamycin, suppresses age-related protein aggregation and extends lifespan

in an autophagy-dependent manner[83]. Furthermore, in cell culture and fly models, rapamycin suppresses toxicity associated with neurodegenerative disease-associated proteins, including mutant huntingtin, polyalanine expansion proteins and tau[84]. Several other pharmacological autophagy-inducers, such as spermidine and nicotinamide, have also been reported to protect against proteostasis collapse and proteotoxicity in various models of HD, AD, PD and ALS[67].

Autophagy has also been linked to stem cell function, with the autophagy-mediated clearance of protein aggregates central to the activation of quiescent neuronal stem cells. Activating autophagy by overexpression of TFEB, or rapamycin supplementation, inhibits age-related protein aggregation and enhances neuronal and muscle stem cell function in aged mice [85-87]. Given the fact that stem cell exhaustion is intimately linked to age-related tissue dysfunction, these findings suggest that enhancing proteostasis specifically in stem cells may preserve many aspects of healthy tissue function during ageing. Collectively, these observations strongly support the notion that autophagy promotes healthy ageing by protecting cells against toxic misfolded and aggregated proteins.

3.2. Autophagy regulation of macromolecule availability
Another important role for autophagy in cellular homeostasis and organismal ageing is to ensure the availability of metabolites, including amino acids, lipids, carbohydrates and nucleic acids, especially during states of stress, such as nutrient starvation **(Fig. 1a)**. Under challenging conditions, autophagy promotes cellular metabolism and survival by recycling amino acids, which are generated from the degradation of cytosolic substrates, to replenish nutrients, produce energy and promote protein synthesis. The inability to properly recycle amino acids through autophagy is linked to growth and developmental defects in *Atg5* deficient mice, and impaired growth during nitrogen starvation in *atg*-deficient yeast[88-90]. Autophagy can also be tailored to mediate the availability of carbohydrates, lipids and nucleic acids through three main cellular processes: glycophagy, lipophagy and RNA/DNA-phagy, respectively.

3.2.1. Glycophagy
Glucose is the primary energy source for cellular metabolism. It is stored as glycogen, and metabolism of glucose is tightly regulated in a tissue-dependent manner (i.e., liver: maintain blood glucose level; muscle: source of cellular energy). However, various conditions resulting in metabolic stress, such as starvation, stimulate glycogen breakdown in order to augment cellular glucose levels and promote metabolic activity[91]. Glycogen can be degraded in the cytosol through the activity of glycogen phosphorylase and glycogen debranching enzymes (detailed in[92]), or in the lysosome via autophagy. The selective clearance of glycogen via autophagy, referred to as glycophagy, plays a crucial role in glucose homeostasis. In response to nutrient-deficiency, the energy sensor AMPK is activated, which in turn inhibits the mTOR complex 1 (mTORC1), leading to activation of the ULK1 kinase, which is important for induction of autophagy (AMPK-mTORC1-ULK1 triad)[91]. Recent findings in yeast demonstrated that Atg11 is necessary to facilitate the interaction between AMPK homolog Snf1 with ULK1 homolog Atg1 upon glucose starvation to promote autophagy[93]. The LC3-interacting region (LIR) motif, also known in yeast as Atg8 family interacting motif (AIM), in starch-binding domain-containing protein 1 (STBD1) may allow cells to physically link glycogen to GABARAPL1, facilitating the transport of glycogen to lysosomes for degradation **(Fig. 1a-III)**[94]. In parallel to glycophagy, other pathways, such as β-oxidation, may maintain cellular bioenergetics to compensate for glucose deprivation[95]. Autophagy also plays a pivotal role in maintaining cell function, not only in glucose starvation, but also in conditions of excess glucose. High glucose levels were associated with mitochondrial dysfunction, generation of reactive oxygen species (ROS), and induction of autophagy in endothelial progenitor cells (EPCs)[96].

Under conditions of impaired autophagy, the accumulation of glycogen contributes to the pathogenesis of age-related diseases. Pompe disease, a lysosomal storage disorder has an impaired ability of the lysosome to degrade glycogen, due to deficiency in the lysosomal

348 hydrolytic enzyme acid α-glucosidase (GAA). This results in accumulation of lysosomal
349 glycogen in many tissues, predominantly in skeletal and cardiac tissues, leading to progressive
350 lethal skeletal myopathy, respiratory and cardiac defects[97]. Impaired tissue function results
351 from an inability of the lysosome to degrade glycogen leading to energy deficiency in skeletal
352 muscle. For infantile-onset Pompe disease[98], a promising therapeutic intervention is
353 administration of recombinant human GAA. Furthermore, dysfunctional autophagy-mediated
354 accumulation of glycogen has been demonstrated to be the cause of neurodegeneration in a
355 mouse model of Lafora disease, which is suppressed when glycogen synthase is deleted[99].
356 These findings indicate that glycogen accumulation might be a cause, rather than a
357 consequence, of impaired autophagy, resulting in impaired cellular function and disease.
358 Glycophagy, therefore, is essential for cellular function and survival, suggesting that levels of
359 glycophagy could determine organismal health, and possibly longevity.
360
361 *3.2.2. Lipophagy*
362 The intracellular storage and utilization of lipids are critical to maintain cellular energy
363 homeostasis. In response to starvation, triglycerides stored in lipid droplets are hydrolysed by
364 specific lipases into free fatty acids for energy metabolism. Lipid droplets can also undergo
365 selective degradation by autophagy, termed lipophagy, as an alternative mechanism for
366 regulating lipid homeostasis **(Fig. 1a-IV)**[100]. To date, a specific receptor coupling lipid droplets
367 to autophagosomes, and trafficking to lysosomes, has not yet been identified, although LC3-
368 mediated engulfment of lipid droplets has been observed[101]. Moreover, CMA has been
369 implicated in degradation of the lipid droplet-associated proteins perilipin 2 (PLIN2) and
370 perilipin 3 (PLIN3)[102]. The lysosomal acid lipases are involved in the degradation of lipid
371 droplets; in particular, lipolysis is conducted primarily by adipose triglyceride lipase (ATGL) and
372 hormone-sensitive lipase (HSL), and selective knockdown of ATGL and HSL in mice results in
373 selective inhibition of lipid droplet degradation, whilst other autophagy processes (i.e.,
374 degradation of proteins and organelles) serve as a compensatory mechanism to replenish the
375 reduced availability of energy substrates [103]. An age-dependent decline in basal autophagy in
376 the liver may underlie the accumulation of hepatic lipids, which in turn has been proposed to
377 contribute to metabolic conditions as well as impairing autophagy, a vicious cycle promoting
378 ageing[100]. For example, the age-dependent reduction of CMA is likely due to alterations in the
379 lipid composition of discrete microdomains at the lysosomal membrane, including altered
380 dynamics and stability of the CMA receptor, LAMP-2A, in the lysosome[104]. Additional
381 mechanisms by which age-related alterations in lipid composition and/or levels may impair
382 autophagy, remain unknown. Further, age-dependent accrual of lipid droplets and ectopic fat
383 deposition are highly interconnected with the age-dependent decline of autophagy and/or
384 autophagic defects[105,106]. Autophagy and LIPL-4/hLAL-dependent lipolysis are both
385 upregulated in germline-less *C. elegans,* and work interdependently to prolong lifespan[107]. The
386 mammalian homologue of the worm LIPL-4 is lysosomal acid lipase (LIPA), a key enzyme
387 involved in the hydrolysis of cholesterol via autophagy[108,109]. Cellular supplementation with
388 NAD+, which stimulates autophagy and subtypes of autophagy, including mitophagy, and
389 stimulates the activity of the NAD+-dependent SIRT1 and SIRT3 pathways, reduced fat
390 accumulation and increased lifespan in high-fat fed and progeroid animals[110-112], highlighting
391 the importance of autophagic degradation of lipids in healthspan and lifespan.
392
393 In pathological conditions, such as alcoholic fatty liver disease (AFLD), impaired lipophagy has
394 been shown to be the basis of lipid peroxidation and cellular damage. AFLD results from
395 excessive consumption of alcohol, leading to damage caused to the liver in the form of
396 oxidative stress, excessive lipid droplet accumulation in the cytoplasm of hepatocytes
397 (steatosis), mitochondrial damage and cell death. Acute exposure to ethanol triggers
398 lipophagy, which acts as a defence mechanism against lipid peroxidation, thereby protecting
399 hepatocytes. However, chronic exposure to ethanol leads to mTOR-mediated inhibition of
400 lipophagy, which in turn, contributes to lipid peroxidation and cell death[22,113,114]. In fact,
401 inhibition of mTOR-mediated suppression of TFEB, using torin-1, resulted in enrichment of
402 TFEB levels in the liver and protection against steatosis and ethanol-induced liver injury[115].

403 Genetic overexpression of TFEB in the liver was shown to increase lysosomal biogenesis and
404 mitochondrial bioenergetics, which served as a protective mechanism against ethanol-induced
405 liver injury in mice. In line with these findings, knockdown of TFEB in the liver of mice resulted
406 in more severe liver injury in response to increased ethanol consumption[115]. In addition,
407 lipophagy is key for the differentiation of several cell types, including hepatocytes[116] and
408 neutrophils[117]. Knocking out Atg7 in hematopoietic stem cells leads to an accumulation of
409 immature neutrophils resembling the myeloid bias of an aging hematopoietic system. Their
410 differentiation can be rescued by exogenous free fatty acids used for β-oxidation, further
411 demonstrating that lipophagy usually provides these during the energy intensive process of
412 differentiation. Further studies on the molecular mechanisms of lipophagy, including
413 identification of lipid-specific autophagy receptors, and their impact on cellular homeostasis,
414 will shed light on the relationship between autophagy, metabolism, and ageing.
415
416 *3.2.3. Autophagic degradation of nucleic acids: RNAs (RNautophagy / RNAphagy) and DNAs*
417 *(DNautophagy / DNAphagy)*
418 Nucleic acids are degraded via multiple mechanisms (the complete description of which is
419 beyond the scope of this review (see details in[118,119])), including autophagy. RNA/DNA are
420 targeted for lysosomal degradation via several pathways, including Atg8/LC3-dependent
421 autophagic degradation of stress granules (condensates of proteins and RNAs)[120], p62 and
422 NDP52-dependent autophagic degradation of retrotransposon RNA[121], lysosomal membrane
423 protein LAMP2C-dependent direct binding to RNA (also for DNA[122]) for lysosomal
424 degradation[123], and a lysosomal putative RNA/DNA transporter, SIDT2 (SID1 transmembrane
425 family, member 2)-that mediates direct uptake of RNA (and DNA[124]) for lysosomal
426 degradation[125]. At present, little is known about whether, and how, RNAphagy and DNAphagy
427 affect health and ageing. However, it is reasonable to suggest that nucleic acid turnover is
428 essential for health, as accumulation of damaged or unnecessary DNA and RNA in the cytosol
429 promotes inflammation, cancer and even accelerated ageing[68,126,127]. DNA damage triggers
430 autophagy and subtypes of autophagy that are considered as cell survival responses[128];
431 however, genetic or age-dependent impairment of DNA repair leads to genomic instability,
432 cellular dysfunction, cell death and accelerated ageing[68]. Exogenous DNA or RNA (e.g.
433 microbial) or endogenous nuclear or mitochondrial DNA in the cytoplasm may trigger
434 autophagy. Nuclear DNA (including extranuclear chromatin) could be aberrantly released into
435 the cytoplasm due to impaired nuclear envelope integrity, nuclear envelope blebbing, or
436 nuclear export processes[129]; mitochondrial DNA could leak into the cytoplasm due to
437 mitochondrial damage and inefficient elimination of damaged mitochondria via
438 mitophagy[126,127]. The cyclic GMP-AMP (cGAS)-stimulator of interferon genes (STING) or RIG-
439 I/ MAVS signalling axis detects these nucleic acid fragments to initiate an innate immune
440 reaction, linking it to autoimmunity, inflammation, senescence, and autophagy[129]. Collectively,
441 genomic instability, accumulation of mitochondrial DNA leakage into the cytoplasm, and
442 increased levels of cellular stress granules, are linked to inflammation, accelerated ageing and
443 a broad range of neurodegenerative diseases[120,121,126]. Although maintenance of DNA/RNA
444 homeostasis is critical for healthy ageing, the contribution of RNAphagy and DNAphagy to
445 long-term tissue health and pathology requires further exploration.
446
447 **3.3. Autophagy of sub-cellular organelles: mitophagy, ER-phagy, nucleophagy,**
448 **lysophagy**
449 Ageing is associated with an accumulation of damage to subcellular organelles. The timely
450 and efficient disposal and recycling of dysfunctional organelles is necessary to maintain
451 cellular function and viability. Selective autophagy is the common mechanism underlying the
452 clearance of sub-cellular damaged and/or superfluous organelles, such as, mitochondria
453 (mitophagy), endoplasmic reticulum (reticulophagy or ER-phagy), nucleus (nucleophagy) and
454 lysosomes (lysophagy)[17]. Both membrane-bound and soluble selective autophagy receptors
455 are involved in the selective organelles degradation[18,130].
456

457 Among the different types of autophagy targeting sub-cellular organelles, the most investigated
458 is mitophagy. Mitophagy is the selective autophagic elimination of defective or surplus
459 mitochondria. The PINK1-PARKIN mediated pathway for degradation of heavily depolarised
460 mitochondria is best understood and involves Ser-65 phosphorylated ubiquitin that attracts
461 soluble selective autophagy receptors NDP52, optineurin and p62, which then recruit the core
462 autophagy machinery for autophagosome formation on the damaged mitochondria[131]. In
463 addition, other basal-, developmental or stress-induced mitophagy pathways involve binding
464 of LC3 to a series of LIR-containing mitochondrial outer membrane proteins, such as NIX
465 (BNIP3L), BNIP3, FKBP8, FUNDC1, BCL2L13, PHB2, AMBRA1 and also Atg8/LC3-binding
466 mitochondrial lipids like cardiolipin[37] (**Fig. 1a-VI**, left). While whole mitochondria can be
467 degraded via mitophagy, it appears that organelles with minor damage can be 'repaired' by
468 other quality control mechanisms such as the piecemeal mitophagy pathway, which is a basal
469 housekeeping mitophagy pathway that involves degradation of mitochondrial proteins in an
470 LC3C- and p62-dependent manner[132] (**Fig. 1a-VI**, right). In addition, other mitochondrial
471 degradation pathways include the mitochondria-derived vesicle (MDV) pathway, where
472 damaged cargo (e.g., impaired mitochondrial proteins) are delivered to the lysosome for
473 degradation in a process dependent on Syntaxin-17, PINK1 and Parkin[133]. A recent study in
474 *C. elegans* shows that damaged subcellular components, including mitochondria among
475 others, can be budded off from certain neurons via membrane-bound vesicles (termed
476 'exophers')[134]. Once in the extracellular space, these damaged organelles can be engulfed and
477 digested by surrounding cells[134]. This cellular release of 'exophers' is also conserved in
478 mammals, as cardiomyocytes release exophers (containing mitochondria) to be received and
479 eliminated by adjacent macrophages[135].
480
481 Accumulating evidence highlights that mitophagy is a critical contributor to cellular physiology
482 and organ homeostasis. First, there is an increase of mitophagy from juvenile to adulthood,
483 followed by a dramatic reduction in old animals. For example, there is an increase of basal
484 mitophagy in fly flight muscle from the ages of 1 week to 4 weeks[136]; in mice, mitophagy in the
485 dentate gyrus (DG), a region that is essential for memory, was reduced by approximately 70%
486 between 3 and 21-months of age[137]. Mitophagy is also impaired in high-fat feeding
487 conditions[137] and in neurodegenerative diseases (reviewed in[37]). Indeed, mitophagy is reduced
488 in mice with AD (by approximately 50% in the hippocampus vs. healthy controls)[138], PD
489 (reviewed in[139]) and HD (by over 70% in the DG region of HTT expressing mice versus WT
490 controls)[137]. Second, intact mitophagic machinery is required for longevity. Since there are
491 several redundant mitophagy pathways, dysfunction of isolated individual mitophagy pathways
492 may not affect lifespan [140,141]. However, mitophagy is essential for longevity under conditions
493 of low insulin/IGF-1 signalling (*C. elegans daf-2* mutants) and dietary restriction (*C. elegans*
494 *eat-2* mutants)[140,142], as well as for the maintenance of neuronal functions in response to
495 stressful conditions[126]. Third, mitophagy induction is sufficient to improve healthspan and
496 extends lifespan in several model organisms, rescues age-associated neurodegenerative
497 phenotypes in AD[138,143] and prolongs lifespan in nematode and fly models of accelerated
498 ageing[66,111,144]. Moreover, functional mitophagy is essential for restraining innate immunity, as
499 mitochondrial stress can lead to the release of damage-associated molecular patterns
500 (DAMPs) that activate innate immunity. Inflammation resulting from excessive exercise in
501 *Pink1* and *Parkin* knockout mice has been shown to be suppressed by loss of STING, a central
502 regulator of the type I Interferon response to cytosolic DNA[126].
503
504 Other autophagic pathways that target subcellular organelles include ER-phagy, nucleophagy,
505 and lysophagy. In yeast, Atg39 regulates perinuclear ER-phagy and nucleophagy, while Atg40
506 is necessary for cortical/cytoplasmic ER-phagy[145] (**Fig. 1a-VII**). ERphagy is conserved in
507 mammalian cells through specific ERphagy receptors, such as FAM134B, SEC62, RTN3L,
508 CCPG1, ATL3 and TEX264 (reviewed in[146]). Nucleophagy is conserved in mammalian cells[147]
509 with a nuclear LC3B-lamin B1 interaction-based nuclear-to-cytoplasmic degradation, which
510 may be a guarding mechanism protecting cells from tumorigenesis[148] (**Fig. 1a-VIII**). Lysophagy
511 is regulated by both ubiquitin-dependent (Galectin-3-TRIM16-ULK1/ATG16L-autophagy

receptor-LC3, the F-box protein FBXO27 and UBE2QL1) as well as -independent (Galectin-8-autophagy receptor-Atg8/LC3) pathways (reviewed in[149]) (**Fig. 1a-X**). Maintenance of functional and effective lysosomes, via timely and efficient lysophagy, is essential for cell survival. In particular, dysfunction in lysosomal membrane proteins such as SCAV-3, the *C. elegans* homolog of human LIMP-2, has been linked to reduced lifespan, implicating lysosome integrity as a defining factor of longevity[150,151 25]. Moreover, dysfunctional lysosomal membrane proteins coupled to leakage of proteolytic enzymes (i.e., cathepsin D) into the cytosol has been associated with ageing, and pathological ageing, in a broad range of neurodegenerative diseases[152]. Thus, maintaining physiological lysophagy is critical for many cellular processes and is presumably important for health and longevity, as lysosomal rupture triggers endo-lysosomal damage responses, and even lysosomal cell death, which links to ageing and diseases[152,153].

Collectively, imbalanced quality surveillance system of sub-cellular organelles, such as mitochondria, ER, small nuclear fractions, and lysosomes, might be a causative factor for age-related pathologies as well as premature ageing. Further studies on mitophagy, ER-phagy, nucleophagy, and lysophagy to decipher their multi-layer regulatory network, and their association with ageing and health, are necessary. In particular, studies to address how these processes change with age, and how they influence age-related tissue function, will lead to critical insights with broad relevance to human health and quality of life.

3.4 Xenophagy
Xenophagy (from the Greek meaning "to eat foreign matter") is the process by which autophagy targets pathogens[154]. Many pathogens are known to be degraded by autophagy, while others take over core autophagy components for their own benefit[155] (**Fig. 1a-IX**). Indeed, several studies have revealed that autophagy can target bacteria like *Rickettsia conorii[156]*, *Listeria monocytogenes[157]* *Streptococcus pyogenes*[158], and *Mycobacterium tuberculosis[159,160]*. Xenophagy may also protect the body against invasion by viruses and parasites.

Upon intake by inhalation, alveolar macrophages capture *Mycobacterium tuberculosis*. However, this bacterium has evolved to be able to impair phagosome maturation (which in normal conditions would lead to phagocytosis), and ends up hijacking the macrophage[161]. Later on, using ESX-1 (6 kDa early secretory antigenic target/ESAT-6 secretion system 1) secretions, the bacterium is able to break free from the phagosome and enters the cytosol. Here, xenophagy comes into action. cGAS detects the bacterial DNA[162], which results in ubiquitination of the invading bacteria by Smurf1 (or Parkin)[163]. NBR1 (or p62) attaches to these ubiquitin chains, resulting in the recruitment of ATG8/LC3B, and finally autophagic degradation occurs[164]. Indeed, the absence of autophagic machinery components namely, ULK1[165], BECN1/Beclin-1[166], p62[167], ATG7[168] and TBK1[167] may promote the proliferation of the bacterium. The mechanism is similar for specific viruses. BECN1/Beclin-1, p62, and selective autophagy of viral capsids can be protective against Sindbis virus[169,170]. However, other viruses, such as herpes simplex virus type 1, have evolved to inhibit autophagy by targeting BECN1[171]. In addition, several studies have highlighted the importance, and possible therapeutic relevance, of autophagy for controlling SARS-CoV-2, the coronavirus that causes COVID-19 [172-174]. Regarding parasites, autophagy can control *Toxoplasma gondii*. Knockout of ATG5, ATG7 or ATG16L1 renders mice more likely to succumb to parasites[175]. A detailed review between parasites and autophagy is available[176].

Although there is not much data available on a direct link between xenophagy and ageing or lifespan, it is conceivable that blocking the infection of exogenous intruders is required for maintenance of a healthy state and reduced inflammation[151,177]. Further work to investigate the molecular mechanisms of xenophagy and their association with ageing and longevity is required.

3.5. Tissue-specific autophagy in ageing

As ageing is associated with a functional decline at both the tissue and organismal level, it is important to understand how ageing within individual tissues affects, and is affected by, ageing across the entire organism. Evidence from nematodes, flies, and mice has revealed that autophagy may have tissue-specific roles in regulating ageing[14]. Inhibition of *lgg-1/Atg8* and *atg-18* specifically in the body-wall muscle of adult worms, is sufficient to shorten the lifespan of both dietary-restricted *eat-2* and insuln/IGF-1 receptor *daf-2* long-lived mutants[28,178]. In addition, the shortened lifespan of *atg-18* mutants (ATG-18 is a member of the WIPI protein family, homologous to mammalian WIPI1 and WIPI2) can be suppressed by tissue-specific restoration of ATG-18 function: pan-neuronal- or intestine-specific expression of *atg-18*, fully restored the lifespan of *atg-18* mutants to that of wild-type worms, while muscle- or hypodermis-specific rescue of ATG-18 had little to no ability to restore lifespan to wild-type levels[179]. In flies, the promotion of autophagy in muscle tissue via overexpression of Atg8a or the transcription factor, FOXO, was sufficient to extend lifespan[78,180], while in mice, inhibition of autophagy through muscle-specific ATG7 deficiency, resulted in impaired muscle function (possibly via mitochondrial dysfunction) and lifespan decrease[181]. Furthermore, enhancing autophagy specifically in the intestine, maintains intestinal barrier function and promotes longevity and healthspan in worms and flies[45,178]. Given that our tissues age unevenly, with some tissues presenting faster degeneration than others[182], it will be interesting to determine how closely rates of ageing and autophagy are correlated in different tissues throughout life.

4. Defective autophagy in diseases associated with accelerated ageing, neurodegenerative diseases and inflammaging

Accumulating evidence from studies using laboratory animals and human samples supports an essential role for autophagy in embryonic development, tissue health and lifespan through the suppression of age-associated inflammation (inflammaging), maintenance of genomic integrity, preservation of cellular/tissue homeostasis and rejuvenation of stem cells (**Fig. 3a**, references[13,14,68,69]). While autophagy is tightly regulated by multiple molecular pathways involving central modulators of energy metabolism, such as AMPK, mTORC1, sirtuins, and calcineurin (**Fig. 3b**), several interventions such as dietary restriction, exercise, and supplementation of small chemical compounds (detailed below) stimulate autophagy[69]. Recent preclinical studies link impaired general autophagy or subtypes of autophagy (in some cases, while a sub-type of selective autophagy may be impaired, there is no change, or even an increase, in general autophagy in some diseases) to pathological states, such as progeria and a series of accelerated ageing diseases[68] (**Fig. 3c**), neurodegenerative diseases[19,37] (**Fig. 3d**), and other disorders[13,14,68,69]. For example, the maintenance of CMA in aged cells sustains haematopoietic stem-cell function[183] and prevents collapse of the neuronal metastable proteome[184]. Similarly, mitophagy, which is reduced in both normal ageing and in AD, extends healthspan[140] and suppresses Aβ- and pTau-induced memory loss when stimulated in aged tissues[138].

Understanding the relationship between compromised autophagy and other hallmarks of ageing will provide a better understanding of the molecular events that promote ageing and disease[14,68,69]. Among the many age-related changes previously described, inflammation is linked to autophagy, as impaired autophagy drives inflammation, and has emerged as a major driver of age-related tissue damage.[63,69,185,186]. Inflammation is an evolutionary conserved protective mechanism designed to maintain organismal homeostasis against acute and local perturbations, and serves as an adaptive response to infection or injury[187]. Chronic, systemic inflammation develops progressively with age and contributes to organismal deterioration through a process termed "inflammaging"[186].

Autophagy has been identified to be amongst the pivotal pillars that orchestrates the differentiation and metabolic state of innate immune cells. In particular, the balance between mTOR and AMPK activation plays a central role in immune cell maintenance and function. Upon mTOR activation, autophagic flux is reduced, accompanied by increased cellular glycolytic activity, giving rise to proliferative and pro-inflammatory phenotype of macrophages.

In contrast, AMPK activation drives autophagy and promotes the OXPHOS-dependent function of non- or anti-inflammatory macrophages[188].

Autophagy also regulates the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, which is an intracellular protein complex that activates caspase-1, which in turn catalyses the cleavage, activation, and subsequent release of pro-inflammatory cytokines (i.e., IL-1β) that can induce neurodegeneration[186,189]. The NLRP3 inflammasome has been identified as a critical component of the innate immune response (i.e., microbial motifs, endogenous danger signals and environmental irritants) and orchestrates host immune homeostasis[189]. Defective autophagy, for example in models of selective knockout or knockdown of genes/proteins involved in the autophagic core machinery (i.e., ATG5, ATG7, BECN1 and MAP1LC3B), results in unrestricted inflammasome activation and consequent inflammation. Likewise, promotion of autophagy through starvation or pharmaceuticals (i.e., rapamycin) inhibits the inflammasome[190]. In addition, evidence stemming from an APP/PS1 mouse model of AD demonstrated mitophagy-induced inhibition of the NLRP3 inflammasome, thus reducing neuroinflammation[138]. These findings imply an important role for autophagy in the regulation of inflammation, and in turn, ageing and neurodegenerative diseases.

5. Anti-ageing effects of autophagy modulators
The mounting evidence that an imbalance of autophagy is an important age-associated characteristic has driven extensive research into the development of compounds that can promote autophagy[1]. Pharmacological agents promoting autophagy can be classified based on their effect on the mTOR pathway[191]. mTOR inhibition by rapamycin has been shown to reduce protein synthesis and promote autophagy, both of which contribute to extended lifespan in yeast, nematodes, flies, and mice (**Table 2**). In addition, rapamycin has been demonstrated to protect against neurodegenerative diseases, including AD, via promotion of autophagy; however, rapamycin treatment was observed to be detrimental in the case of models of amyotrophic lateral sclerosis (ALS), possibly due to non-autophagy-related side effects[191]. Other pharmacological agents reported to promote autophagy via direct interaction with mTOR include Torin-1 and PP242[192]. The mTOR-independent promoters of autophagy mainly act via the AMPK pathway. Examples include metformin and trehalose, which have been demonstrated to be effective in enhancing autophagy, extending lifespan, and protecting against neurodegeneration in experimental models[191].

Compounds such as resveratrol and spermidine modulate the acetylation state of proteins to regulate autophagy and promote longevity. Resveratrol is a natural polyphenol that reportedly promotes lifespan in *C. elegans* and healthspan in mice via activation of NAD⁺-dependent deacetylase, sirtuin-1 (SIRT1)[112,193,194]. Spermidine is a polyamine that extends the lifespan of yeast, worms, flies and mice via enhancing autophagy through inhibition of EP300 (E1A-binding protein p300) acetyltransferase[195], among other mechanisms[55,196-198]. The longevity-extending effects of spermidine are abolished upon depletion or deletion of essential autophagy genes such as *BECN1/ bec-1* in *C. elegans* and *Atg7* in yeast and flies[197,199]. Furthermore, pharmacological inhibition of XPO-1 results in enhanced autophagy (evidenced by an increase in the frequency of autophagosomes and autolysosomes) and increased lifespan in *C. elegans*. These effects were mediated by nuclear enrichment of HLH-30/TFEB, which occurred in an mTOR-independent manner[47]. Additional modulators of TFEB that regulate autophagy, and which have also been demonstrated to protect against pathophysiological ageing, include ouabain and fisetin. Ouabain is a cardiac glycoside that enhances activation of TFEB through inhibition of the mTOR pathway and induces downstream autophagy-lysosomal gene expression and cellular restorative properties[200]. Ouabain has been shown to reduce the accumulation of abnormal toxic tau both *in vitro* and *in vivo*[200]. Fisetin is a flavonol, which was shown to facilitate the clearance of endogenous tau via TFEB (through inhibition of mTOR kinases) and Nrf2 activation[20].

677 Other small molecules that induce subtypes of autophagy, especially mitophagy, also enhance
678 longevity and suppress age-associated diseases. These include NAD$^+$, a fundamental
679 metabolite in energy metabolism, redox homeostasis, mitochondrial function, or the arbitration
680 of cell survival and death[185]. NAD$^+$-activated SIRTs stimulate autophagy via mTOR inhibition
681 and deacetylation of several key autophagy proteins (ATG5, ATG7 and ATG8/LC3B) [201,202]. In
682 addition, the NAD$^+$-SIRT axis activates mitophagy by increasing the activity of a series of
683 mitophagy-related proteins, such as PINK1, Parkin, NIX (in *C. elegans* DCT-1) and BNIP3[66,203].
684 Supplementation with NAD$^+$ precursors, such as nicotinamide (NAM), nicotinamide riboside
685 (NR), or nicotinamide mononucleotide (NMN), can increase lifespan and/or improve
686 healthspan in worms, flies, and mice[111,204-206]. NAD$^+$ augmentation also prevents memory loss
687 in both Aβ and Tau *C. elegans* and mouse models of AD, in a mitophagy-dependent manner
688 (requiring *pink-1*, *pdr-1*, or *dct-1*)[138]. Over seven human clinical trials have shown the safety
689 and bioavailability of NR (1 -2 g/day for up to 3 months); there have been more than 30 on-
690 going clinical trials on the use of NR to treat premature ageing and other age-related diseases
691 (see review in[185]). Another clinically promising mitophagy inducer is Urolithin A (UA), a
692 metabolite of ellagitannins from the gut microflora. UA extends healthspan and lifespan in *C.*
693 *elegans*, with lifespan extension depending on genes involved in autophagy (i.e., *bec-1*, *sqst-*
694 *1*, *vps-34*) and mitophagy (*pink-1*, *dct-1*, and the non-specific Nrf2/*skn-1*)[207]. Intriguingly, UA
695 inhibits memory loss in both Aβ and Tau *C. elegans* and mouse models of AD in a mitophagy-
696 dependent manner (*pink-1*, *pdr-1*, or *dct-1*)[138]. UA (500 mg and 1,000 mg /day for 4 weeks)
697 was also shown to be safe in a stage I clinical trial[208]. A summary of different lifespan/health-
698 benefit mitophagy inducers can be found in **Table 2**. Encouraged by the clinical safety of NR
699 and UA, their effects on healthspan and lifespan in the elderly deserve further investigation.
700 Despite recent progress in the identification of novel as well as well-known autophagy-inducing
701 compounds, it is also of great importance to highlight the pleiotropic effects of these
702 pharmacological interventions and to completely understand the full complement of targets
703 that they interact with in order to use them safely for therapeutic intervention.
704
705 While experimental/empirical evidence indicates that autophagy is defective in the elderly, it is
706 conceivable that exposing individuals to autophagy inducers, dietary restriction, and exercise
707 late in life could boost autophagy and result in benefits to tissue function [209,210] (**Fig. 4a**). Based
708 on preclinical data, it is presumed that autophagy stimulation (ideally to increase autophagy to
709 the levels observed early in adulthood) may be sufficient to provide benefits (**Fig. 4b**).
710
711
712 **6. Conclusions and future perspectives**
713 Mounting evidence from studies using laboratory animals, human tissues, and related clinical
714 trials support that: a) there is an age-dependent decline of autophagy, b) autophagy is a crucial
715 determinant of cellular health and organismal longevity and c) impairment/imbalance in
716 autophagy promotes pathological ageing and disease. Given the broad spectrum of unique
717 properties associated with autophagy, we propose 'compromised autophagy' is a central
718 feature of normal ageing. Although the relationship between autophagy and ageing is often
719 described as "decreased autophagy is detrimental" and "increased autophagy is beneficial",
720 this may be too simplistic a picture. Instead, long-term health benefits will likely arise from
721 achieving the right balance of autophagy, which itself will depend upon the tissue and
722 organismal age. For example, in *C. elegans*, impairing autophagy early in life has a negative
723 effect on longevity, whereas knock down of specific subset of autophagy genes in adulthood
724 may have beneficial effects on lifespan[59]. Similarly, increased autophagy through a
725 hypermorphic allele of *atg-5,* has differential effects on polyglutamine aggregation in muscles
726 and neurons of *C. elegans[211]*. In flies, a mild increase in autophagy extends lifespan, while
727 strongly increasing autophagy shortens lifespan[212]. It should also be noted that autophagy
728 induction may also result in unwanted effects, such as multiple senescent pathologies[59] and
729 resistance to cancer therapy (reviewed in [213]). Collectively, these observations suggest that
730 the level and balance among the different forms of autophagy in each tissue is highly specified
731 for each stage of life and an understanding of this will be crucial for healthy ageing. Thus,

732 while different types of autophagy may influence ageing to different extents, a central goal for
733 promoting health will be to find approaches that can fine-tune autophagy, to the right levels, at
734 the right time, in the right tissues, to enhance health (**Fig. 4**). In order to achieve this, it will be
735 critical to develop novel interventions that allow for the controlled delivery of autophagy
736 modulators into specific tissues or cell types at precise stages of life. Such therapeutic
737 strategies could then be administered chronically, acutely, or in a pulsed fashion as and when
738 required. Additionally, it may be necessary to specifically induce either general or selective
739 autophagy, in order to provide overall long-term health benefits[66]. For example, the premature
740 ageing diseases, such as A-T, XPA, and CS, exhibit increased general autophagy but impaired
741 mitophagy; therefore, specifically stimulating mitophagy, rather than general autophagy, would
742 be the most efficient way to counteract disease pathological features, while avoiding
743 detrimental side-effects[66].
744
745 To this end, there remain many outstanding questions related to autophagy in ageing that need
746 to be addressed. What are the intricate mechanisms that orchestrate distinct autophagic
747 pathways? How is autophagy spatially- and temporally-regulated and how does the disruption
748 of this regulation suppress or promote disease? Are some aspects of autophagy more
749 important in an age- and/or tissue-dependent manner? What are the determining factors that
750 dictate the route of degradation via the UPS or autophagy? How are clearance mechanisms
751 balanced with synthesis and folding through the proteostasis network? What are the thresholds
752 of life-benefit and life-detrimental autophagy? In line with the traditional Chinese 'Yin-Yang'
753 philosophy, autophagy must be balanced, as diminished autophagy results in the accumulation
754 of toxic subcellular components while excessive autophagy can lead to organ atrophy and
755 other side effects[14,37,59,69,212]. Furthermore, compensatory responses between proteolytic
756 systems (e.g., between autophagy and the UPS[214]) play a critical role in determining the onset
757 and rate of age-related tissue deterioration, and should be considered in future experimental
758 design and data interpretation. Finally, are there any conditions or diseases where we should
759 be cautious about inducing autophagy in that protection against one form of pathology
760 increases the risk for another? E.g., pancreatic cancer cells may hijack autophagy processes
761 to obtain nutrients for growth; hence, in this condition autophagy inhibition in combination with
762 cancer chemotherapies may inhibit pancreatic cancer growth[215,216]. Addressing these
763 questions will facilitate our understanding of the ageing process and, more importantly, enable
764 us to identify novel targets that may be manipulated for therapeutic intervention in age-
765 associated diseases.
766
767

Declaration of Interests
E.F.F. has CRADA arrangement with ChromaDex, and is consultant to Aladdin Healthcare Technologies, Vancouver Dementia Prevention Centre, and Intellectual Labs. AKS is a consultant to Oxford Healthspan. D.C.R. is a consultant for Aladdin Healthcare Technologies Ltd, Drishti Discoveries and Nido Biosciences. All other authors declare no competing interests.

Figure Legends

Figure 1. Different mechanisms of autophagy. (a) Macroautophagy: The non-selective process of macroautophagy will target macromolecules or sub-cellular organelles in bulk. Cytoplasmic material is sequestered into an autophagosome and delivered to the lysosome (or endo-lysosome) for degradation **(I)**. Selective macroautophagy involves recognition of specific cytoplasmic cargo via autophagy receptors that also interact with LC3 in the autophagic membrane, leading to cargo sequestration into autophagosomes that are delivered to a lysosome (or endo-lysosome) for degradation. This includes **II) Aggrephagy**: aggregated proteins are ubiquitinated and targeted by ubiquitin-binding autophagy receptors such as p62 (or NBR1). **III) Glycophagy**: STBD1 (Genethonin-1) binds to glycogen and GABARAP, facilitating lysosomal glycogen break down into non-phosphorylated glucose by enzymes like glycogen-hydrolyzing acid α-glucosidase (GAA). **IV) Lipophagy**: the unknown receptor/s (yellow) involved in sequestration of lipid droplets is yet to be determined. Lysosomal lipids are degraded into free fatty acids, which are then converted into ATP. **V) Granulophagy**: sequestration of stress granules (RNA + proteins) is mediated by Cdc48/VCP, allowing the stress granule to be delivered to the lysosome for degradation. **VI) Mitophagy**: Damaged mitochondria can be fully engulfed by mitophagy (left). Soluble or membrane-bound mitophagy receptors can bind to the mitochondrion as well as to LC3, leading to engulfment of the mitochondrion into an autophagosome and subsequent delivery to a lysosome for degradation. Piecemeal mitophagy (right) allows degradation of parts of the mitochondria via binding of MTX1 to LC3C, resulting in the recruitment of p62 and autophagosome formation. **VII) ER-phagy:** In mammals it uses the specific receptors FAM134B, RTN3L, ATL3, SEC62, CCPG1 & TEX264, which are located in different parts of the ER. These receptors bind to LC3, leading to sequestration of ER into an autophagosome and lysosomal degradation of the ER. **VIII) Nucleophagy**: In mammals, when nucleophagy is triggered, nuclear LC3 binds to lamin B1. These form a bulge which is pinched off to the cytoplasm where degradation by autophagy will occur. **IX) Xenophagy A**: A bacterium's DNA is detected by c-GAS, sensors which trigger a process of ubiquitination via Smurf1. This is followed by the attachment of NBR1 to the ubiquitin chains. The receptor NBR1 binds to LC3 in order to continue with the autophagy process for the degradation of the bacterium. **X) Xenophagy B:** A bacterium damages the membrane of the phagosome, exposing interior glycans. These glycans recruit Galectin-8 (Gal-8). NDP52 recognizes Gal-8 and then recruits TBK1, LC3C, Nap and Sintbad. Receptors optineurin, p62 and NDP52 interact with the ubiquitin on the pathogen and recruit the autophagic engulfment system, the engulfed pathogen is then brought for degradation. **XI) Lysophagy**: occurs upon lysosomal membrane permeabilization and can be achieved with or without ubiquitination. Recruitment of Galectin-3 (Gal-3) to damaged lysosomes further recruits TRIM16 and autophagic proteins like ULK1 and ATG16L1. Furthermore, ubiquitination on the lysosome results in the recruitment of p62 that binds to LC3 to facilitate the autophagic process. In a parallel ubiquitin-independent process, Gal-8 is recruited to damaged lysosomes, which is capable of directly binding to the receptor NDP52 that interacts with LC3 to continue the autophagic process. **b) Microautophagy:** involves capture of cytoplasmic components through a direct invagination of endo-lysosome membranes and can be **XII) non-specific (bulk)** or highly specific. Examples of selective microautophagy in mammalian cells include **XIII) Micro-ER-phagy**: which uses the receptor SEC62 and involves ER capture and degradation by invagination of the lysosome/endolysosome and **XIV) endosomal microautophagy of proteins** having the pentapeptide motif KFERQ in a process requiring the chaperone HSC70. **(c) Chaperone-mediated autophagy (CMA)**: also involves **XV) targeting of proteins** containing a KFERQ pentapeptide related motif by HSC70 and other cochaperones such as HSP40. Then, the substrate is imported into lysosomes through the receptor LAMP2A, for further degradation. The LAMP2A receptor is modulated by the glial fibrillary acidic protein (GFAP). Finally, in a CMA-like manner, **XVI) DN/RNAutophagy** can occur: nucleic acids (DNA/RNA) bind to the receptor LAMP2C (orange) which also binds to lysosomes. This process allows nucleic acids to be taken up by the lysosomes. It has been

858 proposed that a transporter called SIDT2 (green) might play a role in direct uptake of nucleic
859 acids by the lysosomes as well.
860
861
862 **Figure 2. Core machinery of Autophagy**. The initiation of autophagy requires the ULK1
863 kinase complex, which is tightly regulated by AMPK and mTOR, acting as an activator and
864 inhibitor, respectively. AMPK activates ULK1 through phosphorylation. The ULK1 complex,
865 composed of FIP200, ATG13 and ATG101, stimulates the class III phosphatidylinositol 3-
866 kinase complex (PIK3C3 complex), which includes BECN1 (which can be inhibited by BCL2),
867 AMBRA1, ATG14L, VPS15 and VPS34. This complex then produces a pool of PtdIns3P, which
868 leads to the recruitment of WIPI proteins, which will recover ATG-9 vesicles from previous
869 membranes, as well as recruiting ATG5-ATG12-ATG16L1 (E3). LC3 is first cleaved by an
870 ATG4 protease to form cytosolic LC3-I, which is further recognized by E1 (ATG7), E2 (ATG3)
871 and E3 leading to its conjugation to phosphatidylethanolamine (PE). After this process, LC3-I
872 is referred as LC3-II. LC3-II binds to LIR-containing autophagy receptors (AR, such as p62)
873 bound to cargos targeted for degradation. Fusion of autophagosomes with lysosomes is mainly
874 mediated by the assistance of Rab proteins, SNARE proteins and a HOPS complex. After
875 fusion, cargo is degraded by lysosomal hydrolases and the degraded products can be reused
876 by the cell. LC3-II bound to the outer membrane is cleaved by ATG4 to be reused for a new
877 round of lipidation.
878
879
880 **Figure 3. Autophagy in health and disease. (a)** Autophagy participates in multiple
881 processes that are essential for longevity. **(b)** A brief summary of some of the major known
882 mechanisms that regulate autophagy in multiple organisms, and their influences in the
883 process. **(c)** A list summarizing premature ageing diseases with impaired mitophagy as a
884 cause of mitochondrial dysfunction, which contributes to short lifespan (LS) and healthspan
885 (HS). These premature ageing diseases are Ataxia Telangiectasia (AT), Cockayne
886 Syndrome (CS), Fanconi Anemia (FA), Hutchinson-Gilford Syndrome (HG), Werner
887 Syndrome (WS), Xeroderma Pigmentosum (XP, especially group A). Changes of autophagy
888 and mitophagy in HG are elusive. **(d)** Autophagy (including sub-types of selective autophagy,
889 like mitophagy) is impaired in broad neurodegenerative diseases, where it may drive or
890 exacerbate disease progression. Alzheimer's Disease (AD), Parkinson's Disease (PD),
891 Huntington's Disease (HD), Amyotrophic Lateral Sclerosis (ALS), and Frontotemporal
892 Dementia (FTD). We emphasize that these are not the only drivers of the disease and other
893 processes may play roles leading the pathology and symptomatology. '?', information
894 unknown.
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897 **Figure 4. Maintaining autophagy through lifestyle and medical interventions pro-**
898 **longevity. (a)** Potential interventions to stimulate autophagy: autophagy inducers, dietary
899 restriction, exercise, and genetic approaches. **(b)** Autophagy induction could positively
900 impact human health.

901

902 **Table 1. A summary of autophagy genes/proteins that can be pro-longevity.**

Protein (names in different species)	Functions	Modification effect in longevity
Y: ATG1 W: UNC-51 F: Atg1 M: ULK1 H: ULK1	Kinase required for the formation of the autophagosome[217].	(W) mutations in the gene (whole life) cause the organism to age faster[39]. (F, Y, W) Essential for longevity when using approaches such as mTOR suppression, ↑AMPK, dietary restriction, rapamycin and others[39,45,218,219].
Y: ATG2 W: ATG-2 F: Atg2 M: ATG2A and ATG2B H: ATG2A and ATG2B	Lipid transport protein crucial for the formation of the autophagosome[220].	(F) Knockdown reduces lifespan[221]. The levels significantly decrease with age[30].
Y: ATG4 W: ATG-4.1 and ATG-4.2 F: Atg4b M: ATG4A to ATG4D H: ATG4A to ATG4D	Protease required for conjugation/deconjugation of Atg8 proteins to phosphatidylethanolamine[222].	(W) essential for longevity when using approaches such as mir-34 loss-of-function[223].
Y: ATG5 W: ATG-5 F: Atg5 M: ATG5 H: ATG5	Part of the E3 complex required for Atg8 lipidation[224].	(M) Ubiquitous ↑ in transgenic mice increase lifespan[44]. (F, Y) gene is essential for longevity induced by methionine restriction and rapamycin[225,226].
Y: Vps30/Atg6 W: BEC-1 F: Atg6 M: BECN1(Beclin-1) H: BECN1 (Beclin-1)	Subunit of the class III PI3K complex required for autophagosome formation[224].	(F, W, Y) mutations in the gene (whole life) cause the organism to age faster. Essential for longevity when using approaches such as mTOR suppression, *mir-34* loss-of-function, treatment by spermidine and urolithin A, and dietary restriction[39,61,199,207,223,227].
Y: ATG7 W: ATG-7 F: Atg7 M: Atg7 H: ATG7	E1 enzyme required for Atg8 lipidation[224].	(M, W) absence of the gene decreases lifespan, increases atrophy and inflammation[181,228]. (F, W, Y) important for longevity when using approaches, such as spermidine, dietary restriction and methionine restriction[199,225,227]. (H) significantly reduced in the muscle of sarcopenic adults[181]. (M) significantly reduced in the muscles of older mice[181].
Y: ATG8 W: LGG-1 and LGG-2 F: Atg8a M: LC3/GABARAP H: LC3/GABARAP	Small ubiquitin protein conjugated to PE in autophagic membranes. Interacts with protein containing AIM/LIR motifs.	(F) ↑ in neurons increases lifespan[30]. (F) ↑ in muscles increases lifespan[180]. (F) mutations in the gene produce neurodegeneration and reduces lifespan[30]. (Y) essential for longevity when using approaches such as methionine restriction**[225]**. (F) Crucial for the formation of the autophagosome. At week 4, it is downregulated up to 60%[30]
Y: ATG9 W: ATG-9 F: Atg9 M: ATG9A H: ATG9A	Transmembrane protein required for autophagosome formation[229].	(W) essential for longevity when using approaches such as mir-34 loss-of-function[223].
Y: ATG12 W: LGG-3 F: Atg12 M: ATG12 H: ATG12	Forms a complex with ATG5 and ATG16L1 (W: ATG-16.1 & H/M: ATG16L1)[230].	(W) ↓ from egg lay (RNAi) reduces lifespan[228].
Y: ATG15 W: ? F: ? M: ? H: ?	Required for the lysis of subvacuolar vesicles[231].	(Y) essential for longevity when using approaches such as dietary restriction[232].
Y: ATG18 W: ATG-18 F: Atg18 M: WIPI-1/2 H: WIPI-1/2	PtdIns3P binding proteins essential for autophagy[233].	(W) mutations in the gene (whole life) cause the organism to age faster and loss of function reduces lifespan[28,39]. Its expression in the neurons and intestine is essential for maintaining wild type lifespan[179]. ↓ S6K increases its levels[234]. Essential for longevity when using approaches such as mTOR suppression and

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		dietary restriction[39,179]. (F) Significantly decreases with age[30].
Y: ? W: PDR-1 F: Parkin M: Parkin H: PRKN	Ubiquitin E3 ligase that ubiquitinates outer mitochondrial membrane (OMM) proteins, promoting mitophagy[235].	(F) ↑ ubiquitously or in neurons (during ageing) increases lifespan[236].
Y: ? W: PINK-1 F: Pink1 M: PINK1 H: PINK1	Mitochondrial kinase that phosphorylates ubiquitin and recruits Parkin upon mitochondria depolarization[237].	(W) essential for longevity when using multiple approaches, such as nicotinamide riboside and urolithin A treatment among others[140,207,238].
Y: ? W: SQST-1 F: ref(2)P M: p62 and NBR1 H: P62/SQSTM1 and NBR1 (many other receptors exist)	Ubiquitin-binding autophagy receptor involved in selective autophagy[239].	(W) Strains that ↑ the gene have increased lifespan[76,77]. Essential for longevity and for mitophagy inducers (e.g., urolithin A)-induced lifespan/healthspan improvement[76,207,238].
Y: ? W: HLH-30 F: ? M: TFEB H: TFEB HLH-30 (W), MITF (F), TFEB (M & H)	Transcription factor for genes involved in autophagy, lysosome, and phagocytosis[43].	(W) Strains that have ↑ have increased lifespan[43].
Y: VPS34 W: VPS-34 F: Pi3K59F M: VPS34 H: PIK3C3	PI3K required for autophagosome formation[240].	(W) essential for longevity when using multiple approaches, such as urolithin A treatment and dietary restrictions[61,207].

Abbreviations: Yeast (Y), Worms (W), Flies (F), Mice (M), Humans (H), ↑ (Overexpression), ↓ (Reduced expression)

Table 2. Summary of autophagy inducers which extend healthspan and increase lifespan in laboratory animals

Pharmacological Agents	Health benefits	Modes of action
Metformin	(W, M), ↑ lifespan, healthspan	activates AMPK and other mechanisms[241] (also reviewed in [242])
Rapamycin	(W, F, M), ↑ lifespan, different healthspan parameters	Direct autophagy induction via mTOR inhibition (reviewed in [242])
Resveratrol	(Y, W, F, M[1]) ↑ lifespan, different healthspan parameters	SIRT1-dependent induction of autophagy, and non-autophagy pathways[112] (reviewed in[68])
Spermidine	(W, F, M, R), ↑ median lifespan, different healthspan parameters	Autophagy, anti-inflammation, arginine and NO metabolism[196,199]
NR/NMN	(W, F, M) ↑ lifespan, (W, F, M) healthspan, (M) memory	Autophagy/mitophagy-dependent and also-independent pathways (reviewed in[185,243])
Urolithin A	(W) ↑ lifespan, healthspan, (W, M) memory	Autophagy/mitophagy induction[138,207,208]
Actinonin	(W, M) ↑ memory	Autophagy/mitophagy-dependent pathway[138]

Tomatidine	(W) ↑ lifespan, healthspan	Mitophagy induction via the SKN-1/Nrf2 pathway[142]
Trehalose	(W) ↑ lifespan, healthspan[244]	?
myo-inositol (MI)	(W) ↑ lifespan, healthspan	PINK1-dependent mitophagy induction[245]
XPO1 inhibitors	(W, F) ↑ lifespan, improved conditions in neurodegenerative models	Induction of nuclear localization of HLH-30/TFEB[47]

909

910 Abbreviations: Yeast (Y), Worms (W), Flies (F), Mice (M), Humans (H), Rat (R), ↑
911 (Overexpression), ↓ (Reduced expression), NR (nicotinamide riboside), NMN
912 (nicotinamide mononucleotide),
913 [1]No extension in WT mice with normal diet, but extended lifespan in the high-fat diet
914 fed mice[112].

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Supplementary Table 1. Progeria syndromes and neurodegenerative diseases show altered autophagy
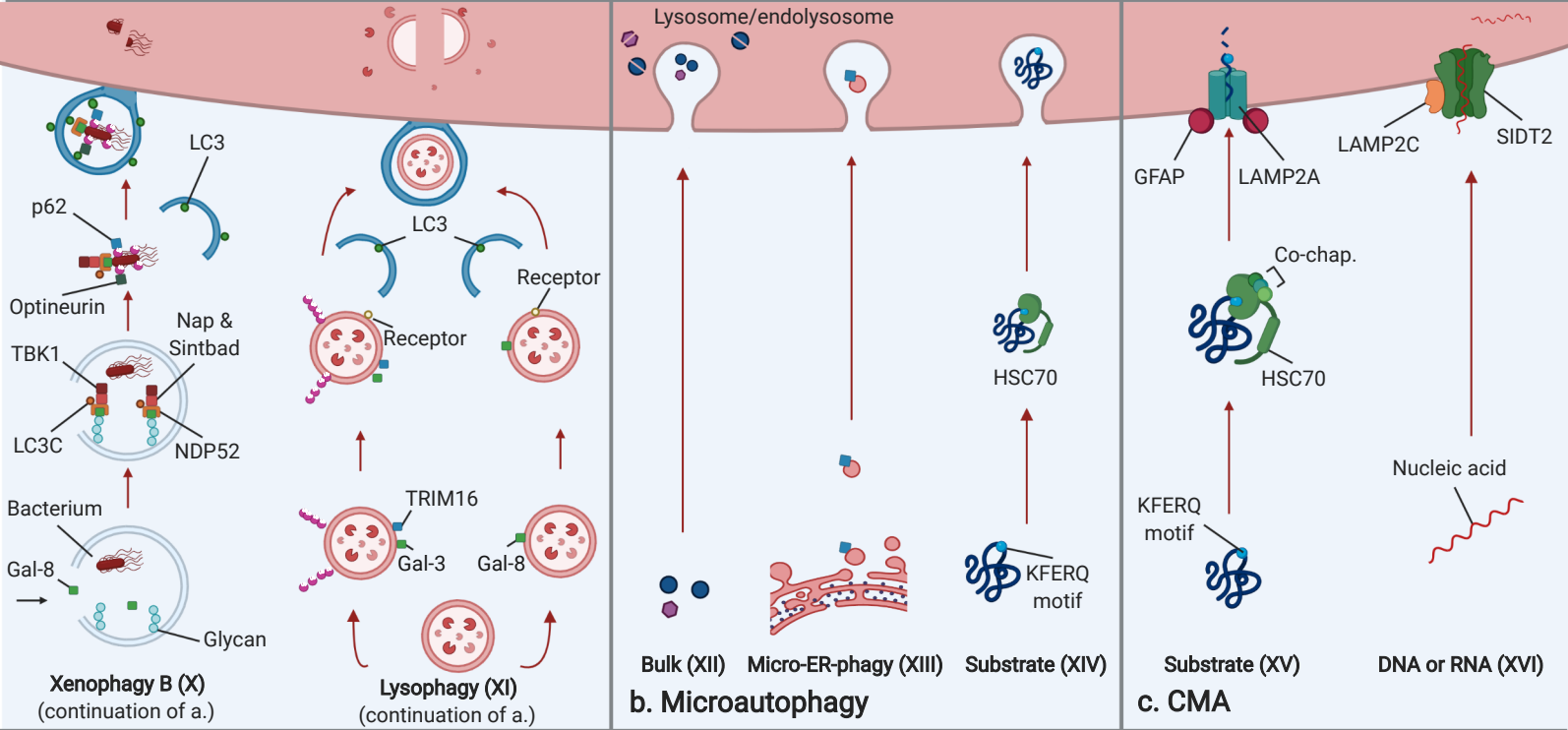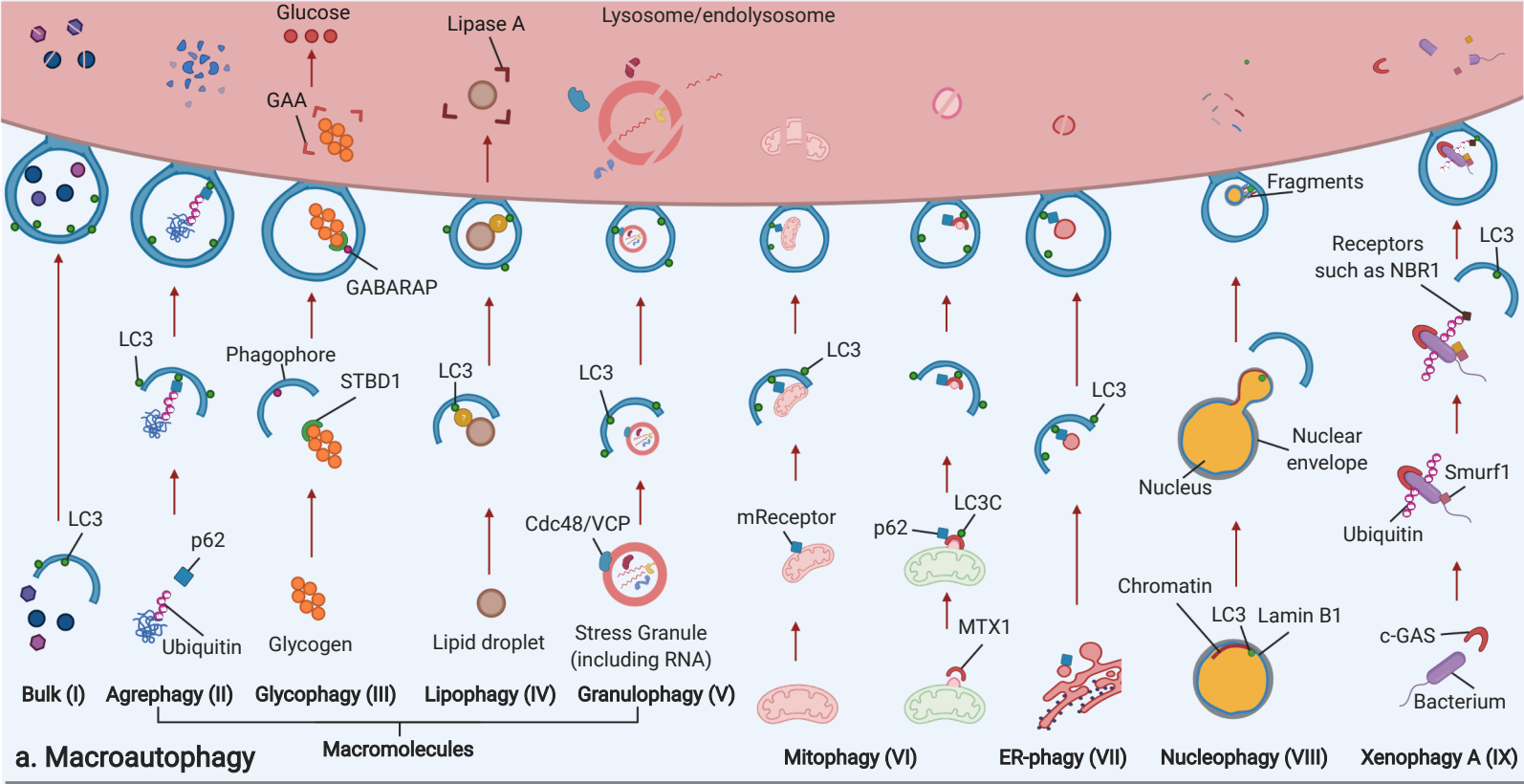
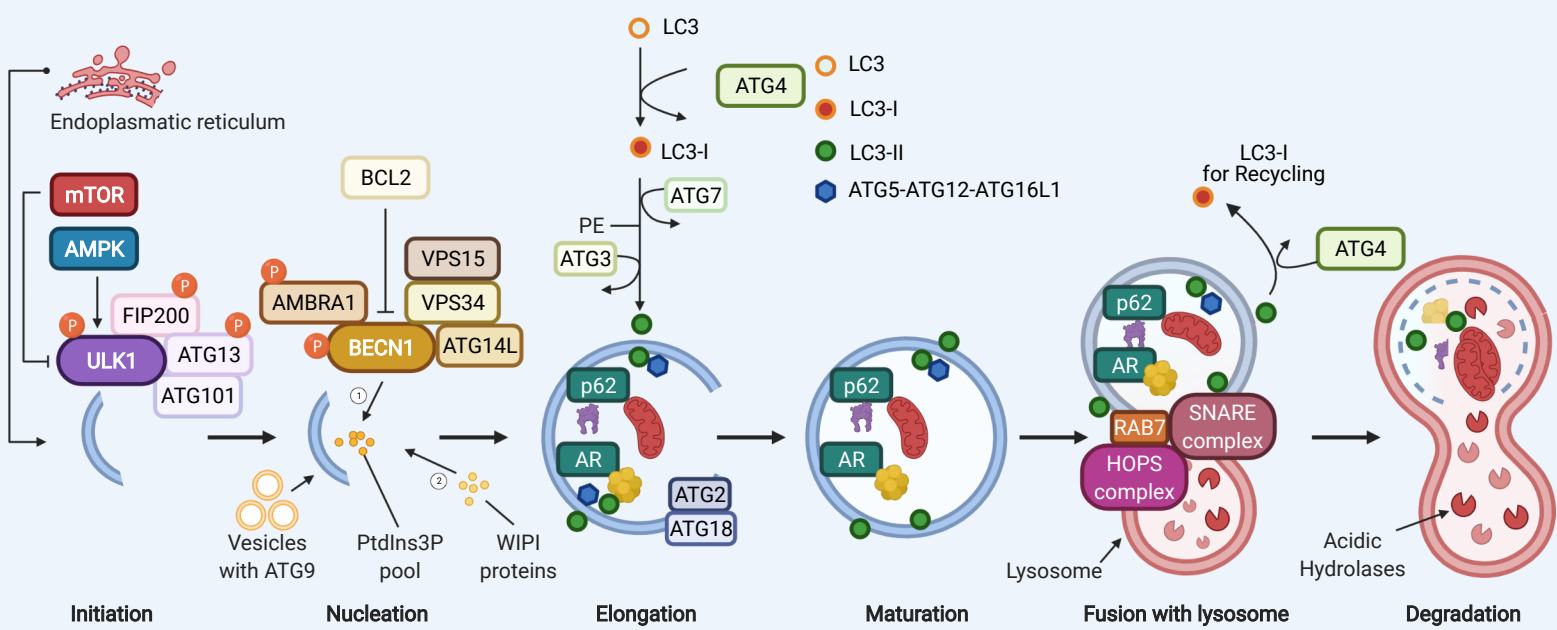| Categories	Disease	Relations to autophagy
Accelerated ageing	Ataxia Telangiectasia	ATM participates in both autophagy and mitophagy; *Atm* mutation leads to defective mitophagy[1,2].
	Cockayne syndrome	CSB participates in autophagy/mitophagy[1,3].
	Fanconi Anemia	Dysfunction of different Fanconi Anemia protein members lead to dysfunctional xenophagy and mitophagy[4].
	Hutchinson-Gilford syndrome	p62 interacts with progerin and boosting autophagy with rapamycin improves clearance of progerin[5].
	Werner syndrome	Werner participates in mitophagy[6].
	Xeroderma Pigmentosum	XPA participates in both autophagy and mitophagy; *XPA* mutation leads to defective mitophagy[1].
Neurodegeneration	Alzheimer's Disease (AD)	The familial AD gene *PS1* mutant disrupts autophagy and lysosomal proteolysis[7]. Impaired mitophagy in postmortem brain tissues from AD patients and in both Aβ and Tau animal models of AD[8]. Proteins involved in autophagy or mitophagy, including PINK1, TBK1 (p-), ULK1 (p-), OPTN, BECN1/Beclin 1, AMBRA1, Bcl2L13, FUNDC1, MUL1, BNIP3L/NIX, were reduced in some AD patient samples (postmortem brain tissue or IPSC-derived cortical neurons)[8]. Reduced BAG-3 in the entorhinal cortex of AD[9].
	Parkinson's Disease (PD)	(M) α-Synuclein, from Lewy bodies, affect the localization of ATG9[10]; α-Synuclein is also capable of impairing the chaperone-mediated autophagy pathway[11]; Overexpression of ATG6 ameliorates the aggregation of α-syn[12]; *PINK1* and *PARK2* mutations lead to familial PD (reviewed in[13]) .
	Amyotrophic Lateral Sclerosis (ALS)	(H) Mutations of many autophagy genes, such as *TBK1* and *SQSTM1*, link to ALS (reviewed in[13])
	Frontotemporal Dementia (FTD)	(H) Mutations of many autophagy genes, such *TBK1* and *SQSTM1*, link to ALS (reviewed in[13])
	Huntington's Disease (HD)	*ATG7* variants may pay a role in the age of onset[14,15]. Cargo recognition is disrupted[16]. Huntingtin mutant may be sequestering ATG6[17]. Absence of Alfy disrupts autophagy and accelerates aggregation[18].
	Autosomal Recessive Juvenile Parkinsonism (ARJP)	(H) *PARK2* mutation plays a causative role[19].
	Ataxia	(H) ATG5 mutation leads to ataxia[20].
	Hereditary Spastic Paraparesis (HSP)	(H) TECPR2 mutation causes some types of HSP[21]. SPG15 mutation may cause some types of HSP[22].
	Lafora Disease	There are correlations between lack of laforin and an increase in TOR activity, inhibiting autophagy[23].
	SENDA & BPAN	(H) WDR45 mutations have been suggested to cause it[24,25].

Abbreviations: Ataxia Telangiectasia mutated (ATM), Mice (M), Cockayne Syndrome group B (CSB), Xeroderma Pigmentosum group A (XPA), Humans (H), ↑ (Overexpression), ↓ (Reduced expression), Static encephalopathy of childhood with neurodegeneration in adulthood (SENDA), Beta-propeller protein-associated neurodegeneration (BPAN).

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Glucose

Lipase A

Lysosome/endolysosome

GAA

a. Macroautophagy

LC3 · Phagophore · STBD1 · GABARAP · LC3 · LC3 · Cdc48/VCP · Stress Granule (including RNA)

p62 · Ubiquitin · Glycogen · Lipid droplet

mReceptor · LC3

p62 · LC3C · MTX1

LC3

Fragments · Receptors such as NBR1 · LC3 · Smurf1 · Ubiquitin · c-GAS · Bacterium

Nucleus · Nuclear envelope · Chromatin · LC3 · Lamin B1

Bulk (I) · Agrephagy (II) · Glycophagy (III) · Lipophagy (IV) · Granulophagy (V)

Macromolecules

Mitophagy (VI) · ER-phagy (VII) · Nucleophagy (VIII) · Xenophagy A (IX)

LC3 · p62 · Optineurin · TBK1 · Nap & Sintbad · LC3C · NDP52 · Bacterium · Gal-8 · Glycan

LC3 · Receptor · Receptor · TRIM16 · Gal-3 · Gal-8

Xenophagy B (X) (continuation of a.) · Lysophagy (XI) (continuation of a.)

Lysosome/endolysosome

HSC70 · KFERQ motif

Bulk (XII) · Micro-ER-phagy (XIII) · Substrate (XIV)

b. Microautophagy

GFAP · LAMP2A · LAMP2C · SIDT2 · Co-chap. · HSC70 · KFERQ motif · Nucleic acid

Substrate (XV) · DNA or RNA (XVI)

c. CMA

a. Core machinery of Macroautophagy

a.

Pro-longevity

- Neural protection
- Cancer blockage
- Anti-inflammaging
- Genomic integrity
- Stem-cell rejuvenation
- Cellular/Tissue homeostasis

b.

Initiation & Nucleation

Maturation

Fusion & Degradation

NAD$^+$

SIRT1

Ca^{++}

Calcineurin

AMP

mTORC1

AMPK

FOXO1

TFEB

c.

Premature ageing diseases

AT → Mitophagy↓ → Mit. Dysf.↑ → LS/HS↓
CS → Mitophagy↓ → Mit. Dysf.↑ → LS/HS↓
FA → Fancc↓ → Virophagy↓
HG → ?
WS → Mitophagy↓ → Mit. Dysf.↑ → LS/HS↓
XP → XPA↓ → Mitophagy↓ → LS/HS↓

d.

Neurodegenerative diseases

AD
ULK1↓
p62↓
PINK1↓
Autophagy↓
Mitophagy↓
Aβ↑
Tau↑
Inflammation↑
Neural death (Pyramidal)
Memory loss

Other Factors

PD
PINK1↓
PARKIN↓
?
Mitophagy↓
CMA↓
α-synuclein↑
Lewy bodies↑
?
Neural death (Dopaminergic)
Loss muscle cont.

HD
Cargo recog.↓
PGC-1α↓
?
Autophagy↓
Mitophagy↓
mHTT↑
Inflammation↑
?
Neural death (GABAergic)
Loss muscle cont.

Other Factors

ALS
BECN1↓
p62↓
OPTN↓
Autophagy↓
Mitophagy↓
TDP-43↑
?
Neural death (Motor neurons)
Loss muscle cont.

Other Factors

FTD
TBK1↓
p62↓
OPTN↓
?
?
TDP-43↑
?
Neural death (VEN neurons)
Cognitive changes

a.

Autophagy inducers

Caloric restriction

Genetic approaches

Exercise

Potential interventions

b.

Memory improvement

Neuroprotection

Muscle maintenance

Immunity

Genomic integrity

Stem-cell rejuvenation

cellular homeostasis

Cancer inhibition

Inflammation balance

Potential benefits