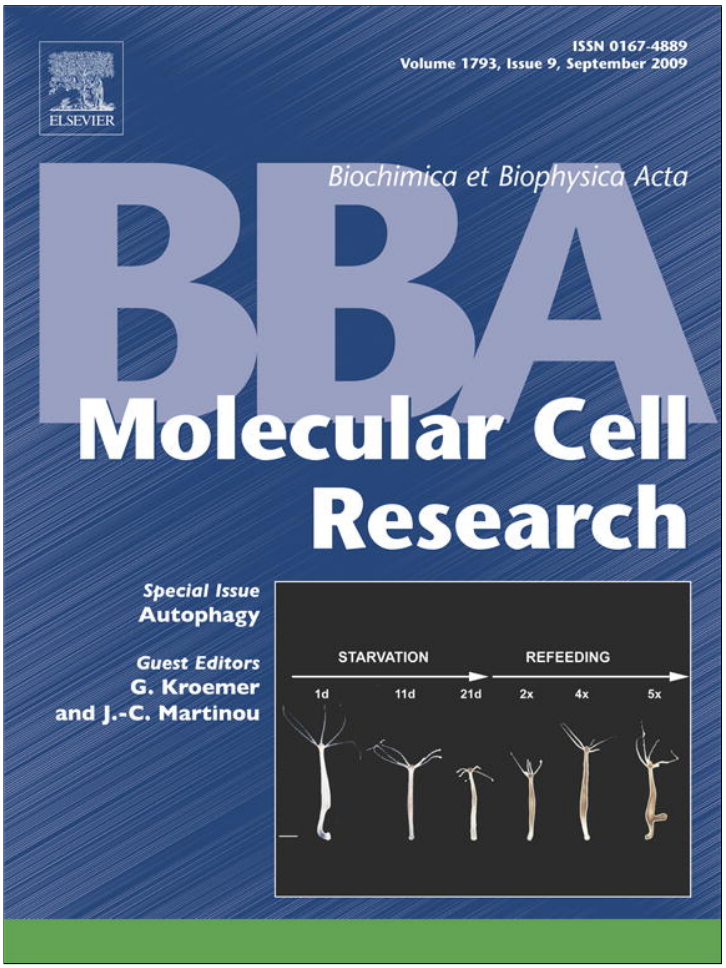

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Review

Autophagy in *Caenorhabditis elegans*

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ABSTRACT

Macroautophagy (or autophagy) is a catabolic process responsible for the degradation of long-lived proteins, molecules and organelles. Cellular stressors such as food limitation, space restriction, oxidative stress, temperature shifts, and accumulation of protein aggregates induce autophagy. Cellular material to be degraded is engulfed in autophagosomes, which fuse with the lysosome where material is degraded. Cellular components can then be recycled. Autophagy has been assigned pro-survival and pro-death functions. Here, we reviewed the roles of autophagy in cell growth and death, in ageing and longevity, as well as in neurodegeneration in the nematode *Caenorhabditis elegans*.

1. Introduction

Caenorhabditis elegans is a soil nematode, which offers particular advantages for the study of autophagic mechanisms at the whole organism level: it is a hermaphrodite species, which is easily cultivated and maintained in the lab (males are also generated at low frequency and can be used in genetic crosses [1]); it is transparent, which permits microscopic visualization of specific cells and subcellular structures; it has a short life cycle of 2.5 days at 25 °C (Fig. 1) and produces a large number of progeny (between 200 and 300 offspring). Importantly, the lineage of the 959 cells in the adult is fully described and invariable among animals [2,3]. This unique feature of *C. elegans* greatly facilitates studies of development and cell death [4–6]. In addition, detailed anatomical information is available for the entire animal and the complete nervous system wiring diagram is fully known [7,8]. Finally the availability of a completely sequenced genome [9,10] coupled with efficient forward and reverse genetics methodologies, such as random mutagenesis, transgenesis and RNA interference (RNAi), greatly facilitates the molecular characterization of genes and biochemical pathways [11–13]. These exceptional characteristics of the nematode model have allowed the detailed dissection of autophagic mechanisms in the context of animal development, nervous system function and ageing.

Three major pathways are involved in the degradation of cellular material, the ubiquitin–proteosome system, chaperone-mediated degradation and autophagy [14,15]. The first pathway is responsible for the degradation of short-lived proteins. The chaperone-mediated pathway directs for degradation cytosolic proteins that carry a particular pentapeptide consensus motif. Autophagy is a catabolic process responsible for the degradation of long-lived proteins, molecules and organelles. Cellular stressors such as nutrient limitation, amino acid deficiency, space restriction, hypoxia, oxidative stress, temperature shifts, and accumulation of protein aggregates and damaged organelles induce autophagy. Autophagic degradation occurs through three pathways, microautophagy, macroautophagy (or autophagy herein), and chaperone-mediated autophagy. In chaperone-mediated autophagy, a protein destined to degradation is tagged by a chaperone, such as hsc 70. The complex then binds to a receptor on the lysosomal membrane, and the tagged protein enters the lumen where it is degraded (Fig. 2) [16]. During microautophagy, cellular material is directly engulfed into the lysosome by invagination of its membrane, followed by fission of the formed vesicle into the lysosome lumen [14,15,17]. Microautophagy and macroautophagy differ in the manner by

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; Atg, autophagy-related gene; APP, amyloid precursor protein; BH3, Bcl-2 homology region 3; Daf, dauer formation; Deg, degeneration; FOXO, Forkhead box O family of transcription factors; GABA, γ-aminobutyric acid; GFP, green fluorescent protein; HD, Huntington's disease; IGF, insulin-like growth factor; MAPK, mitogen-activated kinase; PD, Parkinson's disease; Pha, pharynx; polyQ, polyglutamine; PTEN, phosphatase and tensin homologue; RNAi, RNA interference; TGF, transforming growth factor; TSCI, tuberous sclerosis complex I; TOR, target of rapamycin; Unc, uncoordinated; UVRAG, UV irradiation resistance-associated gene

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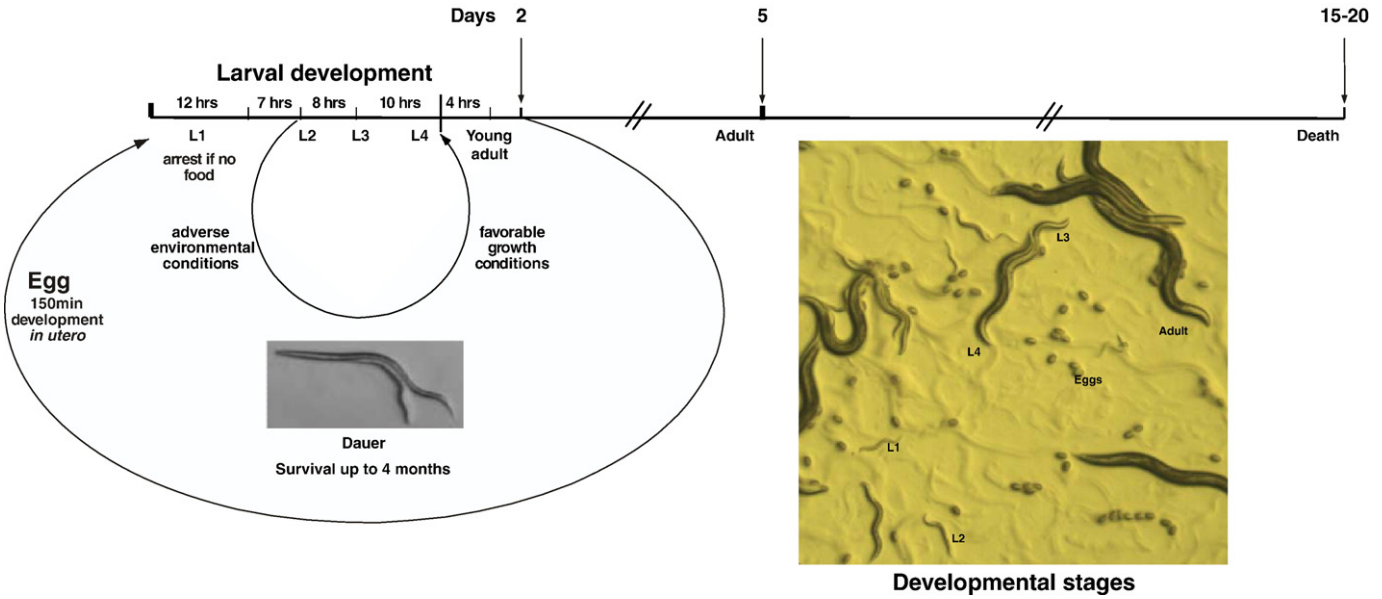

Fig. 1. The *C. elegans* lifecycle. Adult worms lay eggs that hatch into larvae, which undergo four larval stages of development (L1–L4) before maturing into an adult. Upon adverse environmental conditions, such as limited food supply, L1 larvae cease their growth, while L2 larvae develop into dauer larvae, an alternative developmental form which can survive harsh conditions. When dauer larvae encounter favourable environment, they re-enter the lifecycle at the L4 stage to resume development.

which material to be de-graded arrives to the lysosome [14]. During macroautophagy, the cellular material to be degraded is engulfed in double-membrane vesicles (300–900 nm in diameter), termed autophagosomes. These vesicles are targeted to the lysosome via a dynein-related pathway, where they bind and fuse with the lysosome membrane. The autophagic bodies that contain the material to be degraded are delivered into the lysosomal lumen, where acidic hydrolases degrade the cargo and finally, material is recycled by being re-exported back to the cytoplasm [14,18].

Autophagy is a ubiquitous conserved process implicated in normal development, ageing, disease and cell death. Autophagy research has been greatly facilitated by the discovery and characterization of autophagy genes in yeast and subsequently in other organisms, such as *Drosophila*, mice and *C. elegans* (Table 1) [14,18–20]. These studies have also elucidated the involvement of autophagy in human health and disease. Here, we review current research on the role of autophagic pathways in *C. elegans* physiology and pathophysiology, and also discuss the implications of these findings for similar studies in other organisms.

2. Cell growth

Autophagy is required for normal cell size and thus body size in the worms [21]. Two autophagy genes, *unc-51* (the yeast orthologue of *atg1*, encoding a serine–threonine kinase) and *bec-1* (the yeast orthologue of *atg6*, and the human orthologue of beclin 1) have been implicated in this process. *unc-51* mutant worms show defects in autophagy, and *unc-51* loss-of-function mutations result in worms with significantly shorter mean body size. Interestingly, wild type and small mutant worms had the same number of cells. Loss-of-function *bec-1* mutations arrest development at different stages. The insulin/insulin-like growth factor 1 (IGF-1) and the transforming growth factor beta (TGF-β) signalling pathways have also been shown to affect cell size and thus body length [21]. Animals with defective insulin/IGF-1 or TGF-β signalling have long body size. Interestingly, knock down of *unc-51* or *bec-1* in these animals results in either small or normal-sized worms. This epistatic effect indicates that these two pathways exert their effects on body size via autophagic mechanisms.

Nematodes with feeding defects are smaller in size, have smaller cells and their fat deposits are depleted. These characteristics are due to activation of autophagy [22]. For example, *eat-1*, *eat-2* and *eat-3*, or *pha-2* and *pha-3* feeding mutants, which have reduced pharyngeal pumping rates, or abnormal pharyngeal anatomy, are shorter and thinner than wild type worms. Expression of *lgg-1*, a homologue to yeast *atg8* and mammalian MAP-*LC3* [18], is increased in hypodermal seam cells of L3 *eat-3*, *pha-2* and *pha-3* worms, indicating induction of autophagy.

3. Cell survival

Autophagy is paramount for survival of mammals at birth, when the nutrient supply provided by the placenta is interrupted. At that time, autophagy is up-regulated providing the nutrients necessary to maintain cellular metabolism essential for survival by recycling cytoplasmic components. [19]. Upon food limitation, overcrowding, and temperature elevation *C. elegans* enters an alternative developmental program, the dauer larval stage. During this transition, autophagy is up-regulated to facilitate nutrient recycling [23]. Nutrient deprivation also induces L1 diapause [23]. Starved L1 larvae also depend on autophagy to provide the energy requirements for survival. In *C. elegans*, *pcm-1* mutations inhibit autophagy during dauer entry and decrease the survival of L1 arrested larvae [24]. *pcm-1* encodes a methyltransferase that repairs the formation of L-isopartyl residues on proteins, a common form of age-related protein damage. *pcm-1* mutants show defects in dauer formation and their lifespan is reduced.

Monitoring of autophagy in *pcm-1* mutants by means of LGG-1/Atg8/LC3 sub-cellular localization, which associates with pre-autophagosomes and autophagosomes, shows that the function of PCM-1 is required for proper induction of autophagy in dauer larvae [23]. Under non-inducing conditions for autophagy, the LGG-1 protein is localized diffusely in the cytoplasm of multiple tissues in *C. elegans*. During dauer formation and in long-lived animals LGG-1 localization in hypodermal seam cells shows a characteristic punctate staining that is attributed to an increase in the number of pre-autophagosomes and autophagosomes. However, the number of LGG-1-labelled puncta is

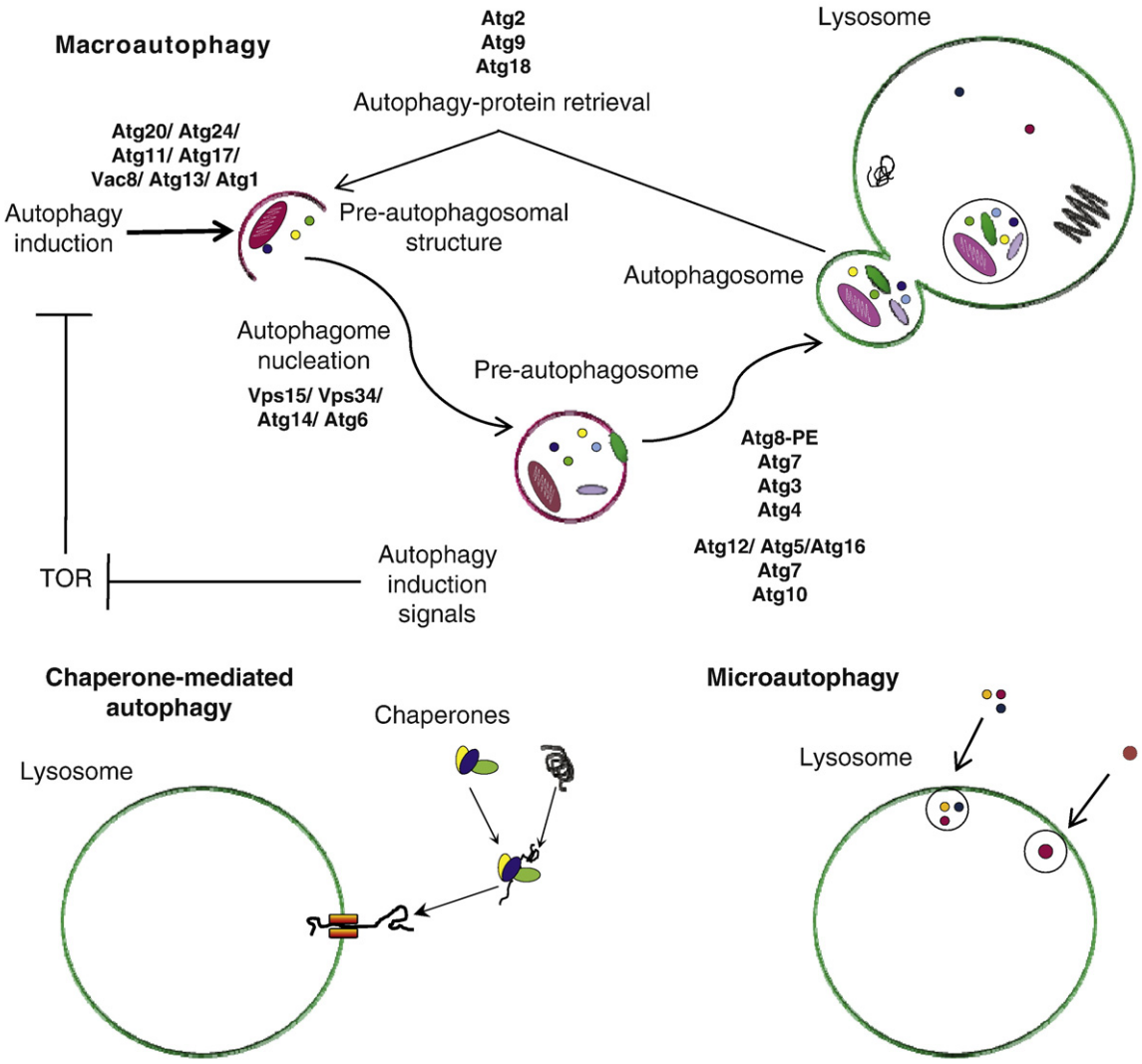

Fig. 2. Schematic diagram of the three types of autophagy in eukaryotes. In macroautophagy (or autophagy) regions of the cytoplasm containing molecules and organelles are engulfed into autophagosomes and are subsequently delivered to the lysosome for degradation. In microautophagy, molecules for degradation are directly engulfed by the lysosome membrane. In chaperone-mediated autophagy, chaperone proteins deliver material for degradation to the lysosome.

significantly reduced in *pcm-1* mutant dauer larvae, indicating that the autophagic process is impaired [25].

4. Development

Autophagy is also involved in cellular remodelling during development. Specifically in the nervous system, there is evidence that it is involved in synapse formation and remodelling. Upon de-innervation of both cholinergic and GABAergic presynaptic inputs to worm body-wall muscles, GABA, but not acetylcholine, receptors are internalized and transported to autophagosomes for degradation [26]. Such directed modification greatly influences the balance between excitation and inhibition. The involvement of autophagy in this selective process was verified by testing for autophagosome formation in *unc-51* mutants. *unc-51* is required for axonal outgrowth along the antero-posterior axis. An increased number of organelles containing tagged $GABA_A$ receptors in non-innerved muscle cells were observed. Receptor internalization and degradation is associated with autophagy since receptors co-localized both with LGG-1 and BEC-1 in autophagosomes of non-innervated muscles. Trafficking of receptors to autophagosomes is most likely occurring via the endocytic pathway since blocking endocytosis prevented receptors from aggregating in autophagosomes [27].

The presynaptic terminal is playing a critical role in keeping GABA receptors inserted in the membrane as opposed to accumulating in autophagosomes [27]. Indeed, the localization of $GABA_A$ receptors in autophagosomes is increased in *unc-3* mutants that display defects in nerve-muscle contacts. *unc-3* is involved in axonal outgrowth of motor neurons [28]. Importantly, non-innervated muscles were otherwise healthy, as they did not show any signs of degeneration [27].

5. Ageing

Reducing calorie intake has been shown to extend lifespan in mice, *Drosophila* and *C. elegans* [29–33]. Reduced insulin/IGF-1 signalling also promotes longevity. [34]. In addition, mitochondrial activity has been implicated in ageing [35]. Worms with mutations in the dauer formation (*daf*) pathway, such as *daf-2*, live longer [36,37]. *daf-2* encodes the *C. elegans* orthologue of the insulin/IGF-1 receptor tyrosine kinase. DAF-2, through a phosphorylation cascade, which involves the kinases AGE-1, AKT-1, AKT-2 and SKG-1, triggers phosphorylation of the FOXO transcription factor DAF-16, blocking its translocation to the nucleus and transcription of target genes [38,39].

The target of rapamycin (TOR) kinase (encoded by *let-363* in *C. elegans*; [5,20]) inhibits autophagy in the presence of sufficient nu-

Table 1
Autophagy-related genes in *Caenorhabditis elegans*.

Autophagy-related genes		Autophagy-related function	*C. elegans* homologue
Yeast gene	Mammalian gene		
Regulation of autophagy induction			
TOR1/TOR2	*Hs FRAP1*	Rapamycin-sensitive Ser/Thr protein kinase	*let-363*
ATG1	*Hs ULK2/Mm Unc51.2*	Ser/Thr protein kinase	*unc-51*
Autophagosome nucleation			
ATG6	*Mm beclin 1*	Component of class III PI3-kinase complex	*bec-1*
VPS34	*Hs* PI3-kinase, class III	Class III PI3-kinase	*vps-34*
Autophagosome expansion and completion			
ATG3	*Hs ATG3/Mm Apg3*	E2-like enzyme; conjugates Atg8 to PE	Y55F3AM.4
ATG4	*Hs ATG4/Mm autophagin 1*	Cys protease; leaves C-terminal extension of PE from Atg8	Y87G2A.3
ATG5	*Hs ATG5/Autophagy 5*-like	Conjugated to Atg12 through internal lysine	*atgr-5*
ATG7	*Hs ATG7/Mm APG7*	E1-like enzyme; activates Atg8 and Atg12	*atgr-7*
ATG8	*Hs GABARAP/Mm GABARAP*-like 1	Ubiquitin-like protein conjugated to PE	*lgg-1*
ATG10	*Hs/Mm ATG10*	E2-like enzyme; conjugates Atg12 to Atg5	D2085.2
ATG12	*Hs APG12*-like	Ubiquitin-like protein conjugated to Atg5	*lgg-3*
ATG16	*Hs APG16*-like isoform 2	Component of Atg12-Atg5 complex	K06A1.5
Retrieval of autophagic proteins			
ATG9	*Hs/Mm APG9*-like 1	Integral membrane protein	*atgr-9*
ATG18	*Sc* ATG18// Hs AAQ96867	Localization of Atg2	*atgr-18*

Genes are listed according to their function in each autophagic step.

trients. TOR activation promotes translation of genes involved in amino acid availability [40]. By contrast, under food deprivation TOR is inhibited and autophagy is activated [40]. The insulin/IGF-1 pathway activates TOR and both pathways cooperate to ensure the sufficient presence of nutrients in cells, regulate cell growth and influence natural lifespan [41,42].

Downregulation of the autophagy gene *bec-1* reduces the longevity of *daf-2(e1370)* mutant worms as compared to control animals indicating that autophagy is required for lifespan extension by low insulin/IGF-1 signalling [25]. In addition, knock down of *atg-7* and *atg-12*, which are orthologues of the yeast autophagy genes *atg7* and *atg12* respectively, diminishes lifespan extension induced by *daf-2* mutations and shortens the lifespan of otherwise wild type worms [37]. Similarly, inhibition of autophagy reverses the beneficial effects of dietary restriction on longevity of *eat-2(ad1116)* feeding defective mutants [43]. This effect requires the PHA-4/FOXA transcription factor, which is also shown to play a role in longevity, similar to DAF-16/FOXO [43,44]. Signalling through the insulin/IGF-1 pathway also directly regulates SKN-1, a transcription factor which is involved in the response to oxidative stress through the p38 MAPK pathway and also modulates ageing. *skn-1* mutations counteract lifespan extension conferred by *daf-2* mutations as well as by downregulation of *akt-1* and *akt-2* [45].

An additional longevity determinant in *C. elegans* is the tumour suppressor protein p53 [46]. Besides regulating DNA damage responses, senescence and apoptosis, p53 also plays a major role in the control of autophagy [47,48]. Depletion of p53 induces autophagy in human, mouse and nematode cells. Studies in *C. elegans* suggest that the life span-extending effect of mutations in the *cep-1* gene, encoding the functional homologue of p53, is mediated by autophagy, which promotes longevity in *cep-1* mutant worms [49].

6. Cell death

Autophagic cell death or type II cell death, together with apoptosis and necrosis, comprise the three main types of cell death originally identified. Autophagic cell death is characterized by dramatic increase in the number of autophagic vacuoles in the cytoplasm [50]. It is uncertain whether these accumulated vacuoles reflect a cell death or a pro-survival mechanism [51]. Genetic studies in the nematode have shown that excessive activation of autophagy has adverse effects for survival [52]. These studies revealed that *bec-1* is required for survival upon starvation in the worm and that this effect is mediated by muscurinic acetylcholine signalling, since over activation of cholinergic signalling induced excessive autophagy, whereas blocking it decreased autophagy during starvation [53]. These findings suggest that, at least in specific tissues of *C. elegans* (in this case pharyngeal muscles), autophagy is promoting survival when induced at physiologic levels, while it may contribute to cell death when induced excessively.

Three autophagy genes, *unc-51*, *bec-1* and *lgg-1*, have been directly implicated in mediating neural necrosis due to plasma membrane ion channel hyperactivity, since inactivation of these genes suppressed necrotic cell death [54]. The anti-apoptotic proteins Bcl-2 and Bcl-XL interact with Beclin1 via their BH3 domain to inhibit autophagy. Pharmacological interference with this interaction by mimetics that bind BH3 receptors, as well as by overexpression of Bad, a BH3-only protein, induces autophagy [55,56] Analogous results are obtained in *C. elegans*: deletion of the BH3-only protein EGL-1 impairs starvation-induced autophagy, whereas gain-of-function mutation increases autophagic activity [45,56].

7. Tumour suppression

Tumour suppressor genes such as PTEN, TSC1, TSC2 and AMPK regulate autophagy. Oncogenes have been reported to suppress autophagy, while tumour suppressors stimulate autophagy [57–62]. Beclin 1 function is lost in certain forms of human cancer [58,63–65]. A newly identified molecule with tumour suppressive function, UVRAG, in turn associates with Beclin 1 to activate autophagy [58,65]. The endophilin protein Bif-1 also associates with UVRAG and Beclin 1 to regulate autophagosome formation. Upon induction of autophagy, Bif-1, together with *atg5* and LC3 co-localize on autophagosomes [58]. These findings provide a direct link between autophagy and cancer [40,66,67].

Moreover, autophagy has recently been assigned tumour suppressive functions following p53 activation [57]. The transcription factor p53 arrests cell cycle upon DNA damage and activates DNA repair or initiates apoptosis if the damage is beyond repair. Interestingly, the damage-regulated autophagy modulator (DRAM) gene that encodes a lysosomal protein, which induces autophagy, is a target of p53 [68]. Activation of DRAM or p53 increases the number of autophagic vesicles in human cancer cell lines. This increase is not merely due to impaired autophagosome turnover, since induction of autophagy by p53 was suppressed by knockdown of the autophagy gene *atg5* and

DRAM. Orthologues of DRAM have been found in humans, mouse, *Xenopus*, *Drosophila*, zebrafish and *C. elegans* [68]. An alternate pathway for activating autophagy via p53 was recently identified in mouse embryonic fibroblasts. Specifically, p53 activates the AMP-activated kinase, which in turn inhibits mTOR via tuberous sclerosis complexes 1 and 2 (TSC1/TSC2) and thus induces autophagy [57,69]. Therefore, autophagy, at least in part cooperates with p53 to exert tumour suppressive functions [69].

Intriguingly, recent studies have shown that interfering with p53 function also induces autophagy in normal and cancerous human cell lines as well as in *C. elegans* [48,49]. Suppression of *cep-1*, the *C. elegans* p53 orthologue, induces autophagy as evidenced by the induction of expression and punctuate redistribution of fluorescently tagged LGG-1 in the cytoplasm of embryos and adult pharyngeal cells [47]. This apparent paradox can be explained if one considers early and late stage tumour development. In this context, autophagy may exert tumour suppressive functions early in tumour development by decreasing cell proliferation, while later may facilitate tumour development by providing nutrients to areas with in the tumours that cannot be reached due to lack of vascular supply [47,65]. The totality of these findings provide incentive for novel anti-cancer approaches that target autophagy as a means to impair survival of tumour cells [66].

8. Neurodegeneration

Correct protein folding is essential for normal cellular function and the survival of organisms. Abnormally folded or damaged proteins are associated with ageing and neurodegenerative disorders. Neurons are especially vulnerable to faulty proteome due to their post-mitotic state. Therefore, they rely on degradative pathways for proper cellular maintenance and function. Inability to degrade misfolded or aggregated proteins leads to gradual loss of neurons, that in turn results in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and polyglutamine diseases like Huntington's disease. Autophagy ameliorates cellular stress induced by accumulation of protein aggregates [70–72]. With its short lifespan and its transparent body that allows for easy visualization of protein aggregates, *C. elegans* provides an attractive platform for such studies. These virtues greatly facilitate the determination of the genes and pathways involved in the heritability, pathophysiology, and molecular mechanisms of neurodegenerative diseases.

Accumulation of polyglutamine (polyQ) expanded proteins in the nucleus or the cytoplasm caused by expansion of a trinucleotide CAG repeat, has been associated with neurodegenerative disorders such as Huntington's disease, spinal and bulbar muscular dystrophy certain forms of spinocerebellar ataxia [73]. These abnormal proteins form aggregates that can only be degraded by autophagy [71,74]. For example, in Huntington's disease, the polyglutamine repeats at the N-terminal of huntingtin are greater than 35, while the normal number of residue repeats is 10–30. These derivatives form intranuclear inclusions and cytoplasmic aggregates in the striatum, the cerebral cortex and the thalamic nuclei. To directly examine whether autophagy serves a protective role against aggregates of expanded polyQ proteins in *C. elegans*, the accumulation of such aggregates in muscle cells or sensory neurons expressing polyQ proteins was assayed in animals with autophagy defects [71]. These experiments demonstrated that reduction of autophagy exacerbates the effects of expanded polyQ protein expression resulting in elevated formation of aggregates, consequent defects in locomotion and degeneration of neurons expressing the abnormal proteins. These findings implicate autophagy in the suppression of the formation of polyQ aggregates and the protection of cells from degeneration.

Patients with Alzheimer's disease (AD) suffer from dementia, mainly due to neurodegeneration in the neocortex. The accumulation of amyloid plaques and neurofibrillary tangles in the cerebral cortex and certain sub-cortical areas is associated with the neuropathology of the disease. The plaques consist of aggregates of mostly insoluble amyloid-beta protein and cellular material in the extracellular environment of neurons. Beta-amyloid derives from abnormal proteolysis of the amyloid precursor protein (APP), a transmembrane protein involved in neuronal growth, cell signalling and adhesion, and trafficking [75]. The neurofibrillary tangles consist of aggregates of tau. Tau, a microtubule-associated protein that stabilizes microtubules when phosphorylated, becomes overly phosphorylated in Alzheimer's disease creating paired helical filaments, interfering with the neuron's transport system [75–77]. Pathologic neurons are characterized by accumulation of autophagic vacuoles. Human beta amyloid induces accumulation of autophagosomes in *C. elegans* muscle cells. Autophagosome accumulation as assayed by formation of LGG-1::GFP puncta in muscle cells was less in worms harbouring the *daf-2(e1370)* mutation which has been shown to decrease insulin receptor signalling and increase lifespan in the worm [25]. By contrast, wild type animals showed significantly greater accumulation of autophagosomes and were paralyzed much sooner compared to *daf-2* mutants (within 24–28 h as opposed to 36 h for the mutant animals). *bec-1* knock down diminishes autophagosome formation in muscles cells and increases beta-amyloid toxicity. A similar effect is observed upon inhibition of lysosomal function. In addition, decreasing insulin-receptor signalling facilitates beta-amyloid degradation via autophagy. Consistent with findings in the nematode, studies in a mouse model of Alzheimer's disease implicate beclin 1 in the regulation of autophagic clearance of intraneuronal beta-amyloid [78].

Motor dysfunctions, such as muscle stiffness, tremor, bradykinesia and postural instability are common characteristics of Parkinson's disease (PD). Dopaminergic neurons in the substantia nigra pars compacta undergo neurodegeneration around the middle stages of the disease. These neurons form cytoplasmic protein inclusions called Lewy bodies, which are composed of aggregates of the polypeptide α-synuclein, ubiquitin and 3-nitrotyrosine [79]. Lewy bodies are also a common denominator of other diseases, such as dementia with Lewy bodies and in some cases of dystonia [79]. Human α-synuclein overexpression in *C. elegans* cells induces misfolded α-synuclein inclusions that increase with age. Formation of these inclusions is ameliorated by torsinA co-expression [80]. TorsinA associates with α-synuclein in Lewy bodies [79]. Proteins of the torsin family perform chaperone-like functions and associate with misfolded or aberrant proteins [81]. TOR-2 is one of the three *C. elegans* homologues of human torsinA. TOR-2 overexpression can suppress polyglutamine protein aggregates [80].

To further investigate the molecular basis of Parkinson's disease pathogenesis, nematode genes were sought that when knocked down favour age-related aggregation of α-synuclein and thus the occurrence of the disease [80]. 80 *C. elegans* genes have been identified that influence formation of inclusions, 49 of which have human counterparts [82]. Most of these genes are involved in protein quality control, vesicle trafficking between the ER and Golgi as well as other vesicular compartments. Other genes are specifically involved in the management or clearance of misfolded α-synuclein early in *C. elegans* life. Among these genes are orthologues of known PD genes like *DJ-1* and *PINK1*, or the torsinA gene, T07F12.4, which is homologous to UNC-51, involved in autophagy and axon elongation [80]. Some of the genes identified are also related to lysosomal biogenesis, G protein signalling and ageing [80,83]. The *C. elegans* homologue of the human parkin gene has also been identified [82,84].

Amyotrophic lateral sclerosis (ALS) is another neurodegenerative disease that affects motor neurons. Patients face problems in dexterity or gait, have difficulty speaking or swallowing, and eventually become paralyzed and lose the ability to breathe. In approximately 20% of patients with familial ALS (1–2% of total cases), the disease is associated with mutations in the gene encoding for the antioxidant enzyme Cu/Zn superoxide dismutase, which removes the toxic free radical superoxide [85]. Even though, a small percent of patients are

affected by this mutation, changes in 120 out of the 153 amino acids in the protein have been linked to ALS [85]. It is proposed that accumulation of mutant SOD1 upon oxidative stress leads to motor neuron degeneration in *C. elegans* [86]. Interestingly, the number of mitochondria in motor neurons is altered in patients with ALS. This number increases significantly in mouse motor neurons upon administration of lithium, which retards the progression of the disease [87,88]. Lithium has also been shown to induce autophagy [89]. The same effect was observed with administration of rapamycin, another autophagy inducer. These results collectively point to a protective role for autophagy in ALS.

9. Excitotoxicity

Hyper activating, gain-of-function mutations in specific neuronal ion channels can trigger neuronal degeneration. In *C. elegans*, such mutations in the genes *mec-4* and *deg-1* that encode ion channels of the degenerin family, as well as dominant mutations in *deg-3*, which encodes the alpha subunit of the nicotinic acetylcholine receptor, cause the degeneration of neurons expressing the mutant proteins [90,91]. Increased Na+ and Ca2+ influx associated with such mutant ion channels leads to neuronal swelling and ultimately to necrotic cell death [92]. Autophagy is required for necrotic cell death in this paradigm. Impairment of autophagy by genetic inactivation of autophagy genes or by pharmacological treatment suppresses necrosis. These studies in *C. elegans* have shown that knock down of *unc-51*, *bec-1* and *lgg-1* among other autophagy related genes, ameliorates neuronal degeneration induced by abnormal ion channel activity [20,54,93]. Consistent with these observations, reduced TOR activity, which stimulates autophagy, promotes neuronal necrosis [54]. Furthermore, excessive autophagosome formation is induced early during necrotic cell death. In addition, autophagy synergizes with lysosomal catabolic mechanisms to facilitate cell death [4,5,93,94]. These findings demonstrate that autophagy contributes to cellular destruction during excitotoxic cell death. Thus, interfering with the autophagic process may protect neurons against necrotic damage during excitotoxicity in humans.

10. Hypoxia

Hypoxia/ischemia are pathological conditions that can result in cell demise [95]. Autophagic processes are up-regulated following hypoxic insult in mammals and *C. elegans* [95,96]. The role of autophagy following hypoxia-induced cell death, was studied by disrupting the autophagic pathway in *C. elegans* [96]. Blocking autophagy by knocking down *bec-1*, *lgg-1* and *lgg-2*, *unc-51* or inhibiting BEC-1 using a phospatidylinositol-3-kinase inhibitor increased hypoxic death compared to normal control animals. Consistently, BEC-1 deficiency reduced the number of fluorescently tagged LGG-1 puncta, a marker of autophagosomes. However, inhibiting apoptosis or suppressing necrosis prevented increased hypoxia-induced cell death in autophagy mutants. These findings argue for a cytoprotective role of autophagy following a hypoxic insult.

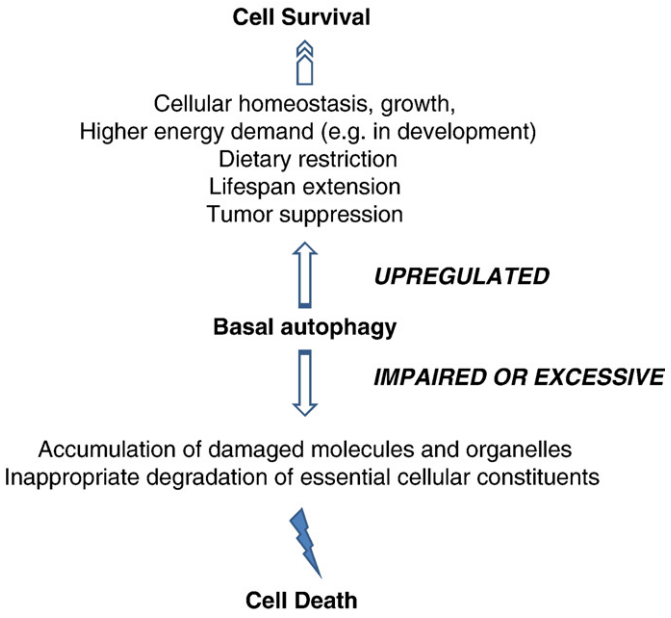

Fig. 3. Autophagy plays a role in cell survival and death. Basal autophagy maintains cellular homeostasis under normal conditions, higher energy demand (e.g. in development), and dietary restriction thus promoting survival. Impaired or excessive autophagy leads to cell death due to defective degradation of damaged components or misfolded proteins, which leads to their accumulation or inappropriate recycling of essential cellular constituents.

11. Conclusions

Autophagy, a process for degradation of cellular material, operates both to promote survival (e.g. following starvation) and to contribute to cell demise. In *C. elegans*, autophagy has been implicated in various processes such as cell growth and death, in ageing and longevity, as well as in neurodegeneration (Table 2) [5,20,93]. Most studies assign a cytoprotective role to autophagy. However, evidence that autophagy also mediates cell death also exist (Fig. 3). The hallmark of the role of autophagy in cell death is the significant increase in the formation of autophagic vacuoles. However, this observation alone is insufficient to unequivocally show that autophagy induces cell death: this increase could reflect a failed survival attempt. The most pertinent evidence has shown that autophagy fulfils the requirement for cell death following inhibition or blockage of apoptosis. Nevertheless, one cannot rule out the possibility that autophagy has indeed this dual function [51,77,97]. Further understanding of the mechanisms that

Table 2
Phenotypes of autophagy-related gene mutations cited in this review.

Autophagy-related genes				
C. elegans gene	Yeast gene	Mammalian gene	Autophagy-related function	Reference
unc-51	*ATG1*	*Hs ULK2/Mm Unc51.2*	Required for axonal outgrowth along the antero-posterior axis	[21]
bec-1	*ATG6*	*Mm beclin 1*	Arrests the development of *C. elegans* at different stages	[21,25–27,52,54]
lgg-1	*ATG8*	*Hs GABARAP/Mm GABARAP*-like 1	Associates with preautophagosomes and autophagosomes	[18,21–26,43,47,48,54,56,99]
pcm-1			Inhibits autophagy during dauer entry	[23,24]
daf-2	*ATG3*	Orthologue of the insulin/IGF-1 receptor.	Orthologue of the insulin/IGF-1 receptor	[25,36,37]
skn-1			Counteracts the lifespan extension effects of *daf-2* mutations	[45]
cep-1		*p53*	Cooperates with autophagy to exert tumour suppressive functions and increase lifespan	[47–49]
let-363	*TOR*	*HsTor/MmTor*	Promotes lifespan extension	[41,43]

The table contains autophagy genes involved with the processes described in this review.

trigger autophagic cell death will likely have significant implications in battling certain human diseases that appear to involve autophagy and will contribute to the development of novel therapies. For example, autophagy is considered as a potential target in cancer therapy [66]. Controlled induction of autophagy has also been proposed as treatment for neurodegenerative diseases characterized by toxic accumulations of material normally degraded by autophagy, as well as for the treatment of mood disorders [74,98]. *C. elegans* offers an attractive and versatile platform for testing the validity and optimizing the effectiveness of such approaches.

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