Autophagy in \textit{Caenorhabditis elegans}

Evgenia V. Megalou, Nektarios Tavernarakis *

Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, N. Plastira 100, Vassiliki Vouton, PO Box 1385, Heraklion 70013, Crete, Greece

\textbf{A R T I C L E \ I N F O}

Article history:
Received 9 December 2008
Received in revised form 11 December 2008
Accepted 12 December 2008
Available online 25 December 2008

Keywords:
Ageing
Apoptosis
Autophagy
\textit{Caenorhabditis elegans}
Cell death
Hypoxia
Longevity
Necrosis
Neurodegeneration

\textbf{A B S T R A C T}

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 \textit{Caenorhabditis elegans}.

© 2008 Elsevier B.V. All rights reserved.

\section*{1. Introduction}

\textit{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 sub-cellular 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 \textit{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
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 autophagy 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 larger 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 eat-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-isopropyl 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/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 lower in pcm-1 mutants than wild type.
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 GABAA 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 GABAA 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-
upon starvation in the worm and that this effect is mediated by Genes are listed according to their function in each autophagic step.

<table>
<thead>
<tr>
<th>Autophagy-related genes</th>
<th>Yeast gene</th>
<th>Mammalian gene</th>
<th>Autophagy-related function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulation of autophagy induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOR1/TOR2</td>
<td>Hs FRAP1</td>
<td>Rapamycin-sensitive Ser/Thr protein kinase</td>
<td></td>
</tr>
<tr>
<td>ATG1</td>
<td>Hs ULK2/Mm Unc51.2</td>
<td>Ser/Thr protein kinase</td>
<td></td>
</tr>
<tr>
<td>Autophagosome nucleation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG6</td>
<td>Mm beclin 1</td>
<td>Component of class III PI3-kinase complex</td>
<td></td>
</tr>
<tr>
<td>VPS34</td>
<td>Hs PI3-kinase, class III</td>
<td>Class III PI3-kinase</td>
<td></td>
</tr>
<tr>
<td>Autophagosome expansion and completion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG3</td>
<td>Hs ATG3/Mm Apg3</td>
<td>EZ-like enzyme; conjugates Atg8 to PE</td>
<td></td>
</tr>
<tr>
<td>ATG4</td>
<td>Hs ATG4/Mammalian autophagin 1</td>
<td>Cys protease; leaves C-terminal extension of PE from Atg8</td>
<td></td>
</tr>
<tr>
<td>ATG5</td>
<td>Hs ATG5/Autophagy 5-like</td>
<td>Conjugated to Atg12 through internal lysine</td>
<td></td>
</tr>
<tr>
<td>ATG7</td>
<td>Hs ATG7/Mm APG7</td>
<td>E1-like enzyme; activates Atg8 and Atg12</td>
<td></td>
</tr>
<tr>
<td>ATG8</td>
<td>Hs GABARAP/Mm GABARAP-like 1</td>
<td>Ubiquitin-like protein conjugated to PE</td>
<td></td>
</tr>
<tr>
<td>ATG10</td>
<td>Hs/Mm ATG10</td>
<td>E2-like enzyme; conjugates Atg12 to Atg5</td>
<td></td>
</tr>
<tr>
<td>ATG12</td>
<td>Hs ATG12-like</td>
<td>Ubiquitin-like protein conjugated to Atg5</td>
<td></td>
</tr>
<tr>
<td>ATG16</td>
<td>Hs APG16-like isoform 2</td>
<td>Component of Atg12-Atg5 complex</td>
<td></td>
</tr>
<tr>
<td>Retrieval of autophagic proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG9</td>
<td>Mm APG9-like 1</td>
<td>Integral membrane protein</td>
<td></td>
</tr>
<tr>
<td>ATG18</td>
<td>Sc ATG18/Hs AAQ96867</td>
<td>Localization of Atg2</td>
<td></td>
</tr>
</tbody>
</table>

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

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

muscimurin 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

muscimurin 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 proteins 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 parts [82]. A majority of these genes are involved in protein quality control, the activation of autophagy and axon elongation [80]. Some of the genes involved in autophagy and axon elongation [80].

Lewy bodies, which are composed 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 accumu-
lation 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 autophago-
some formation is induced early during necrotic cell death. In addition,
autophagy synergizes with lysosomal catabolic mechanisms to facilitate
cell death [43,53,94]. 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 phosphatidylinositol-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.

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 fulfills 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**

<table>
<thead>
<tr>
<th>Autophagy-related genes</th>
<th>Yeast gene</th>
<th>Mammalian gene</th>
<th>Autophagy-related function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>unc-51</td>
<td>ATG1</td>
<td>hs UJK2/Mm Unc51.2</td>
<td>Required for axonal outgrowth along the antero-posterior axis</td>
<td>[21]</td>
</tr>
<tr>
<td>bec-1</td>
<td>ATG6</td>
<td>Mm beclin 1</td>
<td>Associates the development of C. elegans at different stages</td>
<td>[21,25–27,52,54]</td>
</tr>
<tr>
<td>lgg-1</td>
<td>ATG8</td>
<td>hs GABARAP/Mm GABARAP-like 1</td>
<td>Associates with preautophagosomes and autagogsomes</td>
<td>[18,21–26,43,47,48,54,56,99]</td>
</tr>
<tr>
<td>pcm-1</td>
<td>ATG3</td>
<td>Orthologue of the insulin/IGF-1 receptor.</td>
<td>Inhibits autophagy during dauer entry</td>
<td>[23,24]</td>
</tr>
<tr>
<td>daf-2</td>
<td>ATG3</td>
<td>Orthologue of the insulin/IGF-1 receptor.</td>
<td>Inhibits autophagy during dauer entry</td>
<td>[23,24]</td>
</tr>
<tr>
<td>smn-1</td>
<td>ATG3</td>
<td>Orthologue of the insulin/IGF-1 receptor.</td>
<td>Inhibits autophagy during dauer entry</td>
<td>[23,24]</td>
</tr>
<tr>
<td>ccr-1</td>
<td>ATG3</td>
<td>Orthologue of the insulin/IGF-1 receptor.</td>
<td>Inhibits autophagy during dauer entry</td>
<td>[23,24]</td>
</tr>
<tr>
<td>let-363</td>
<td>ATG3</td>
<td>Orthologue of the insulin/IGF-1 receptor.</td>
<td>Inhibits autophagy during dauer entry</td>
<td>[23,24]</td>
</tr>
</tbody>
</table>

The table contains autophagy genes involved with the processes described in this review.
trigger autophagic cell death will likely have significant implications in helping 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 a 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.

Acknowledgements

Work in the authors’ laboratory is supported by grants from EMBO and the EU Framework Programmes.

References


