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Review

Non-developmentally programmed cell death in *Caenorhabditis elegans*

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Abstract

The simple nematode worm *Caenorhabditis elegans* has played a pivotal role in deciphering the molecular mechanisms of apoptosis. Precisely 131 somatic cells undergo programmed apoptotic death during development to contour the 959-cell adult organism. In addition to developmental cell death, specific genetic manipulations and extrinsic factors can trigger non-programmed cell death that is morphologically and mechanistically distinct from apoptosis. Here, we survey paradigms of cell death that is not developmentally programmed in *C. elegans* and review the molecular mechanisms involved. Furthermore, we consider the potential of the nematode as a platform to investigate pathological cell death. The striking extent of conservation between apoptotic pathways in worms and higher organisms including humans, holds promise that similarly, studies of non-programmed cell death in *C. elegans* will yield significant new insights, highly relevant to human pathology.

Keywords: *Caenorhabditis elegans*; Excitotoxicity; Ion homeostasis; Necrosis; Neurodegeneration; Proteolysis; Stress response

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1. *Caenorhabditis elegans* biology: the essentials

C. elegans is a small (about 1.3 mm in length and 100 μm in diameter), free-living, soil nematode worm. Since its introduction as a research platform by Sydney Brenner in 1974, *C. elegans* has become a valuable tool for biomedical research [1]. The key strength of the *C. elegans* model system resides in the extensive genetic analyses that can be conducted with this animal. Its small size and simple dietary demands (feeds on a lawn of *Escherichia coli* on petri dishes or liquid cultures) permit easy and cheap cultivation in the laboratory. The worm completes a reproductive life cycle in 2.5 days at 25 °C, pro-

Abbreviations: 6-OHDA, 6-hydroxydopamine; ALS, amyotrophic lateral sclerosis; $[Ca^{2+}]_i$, cytoplasmic calcium concentration; CBP, CREB binding protein; CREB, cAMP response element-binding protein; dsRNAi, double stranded RNA interference; EGF, epidermal growth factor; ENaC, epithelial sodium channel; ER, endoplasmic reticulum; GFP, green fluorescent protein; HDAC, histone deacetylase; $InsP_3R$, inositol triposphate receptor; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PCD, programmed cell death; OST, ORF sequence tag; RyR, ryanodine receptor; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; V-ATPase, vacuolar H^+-ATPase; YAC, yeast artificial chromosome

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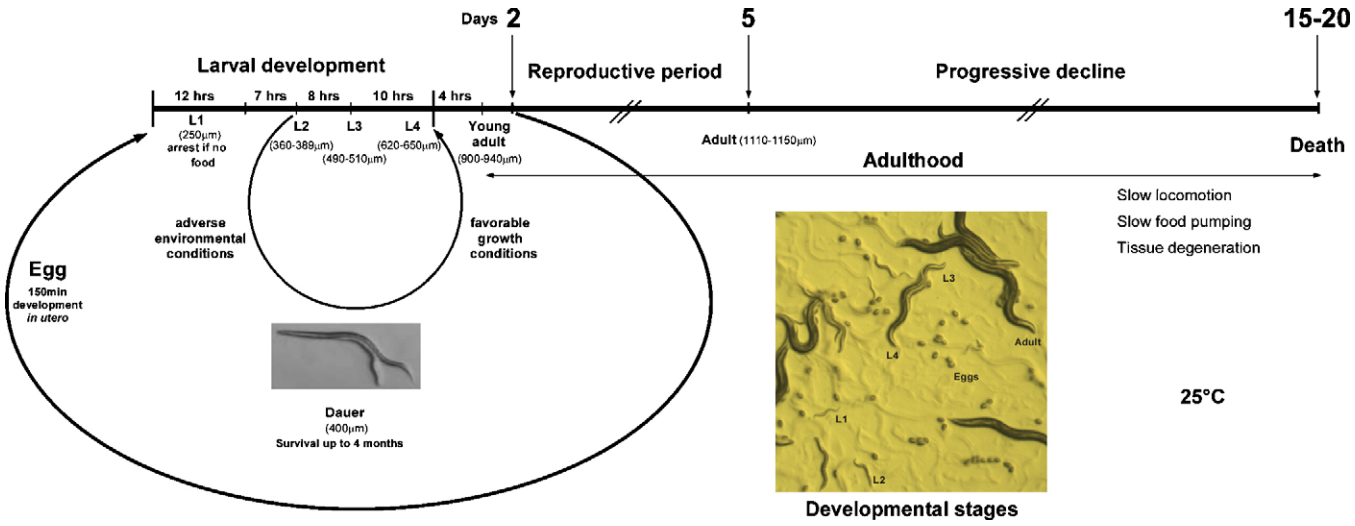

Fig. 1. Life cycle of *Caenorhabditis elegans* at 25 °C. Following hatching, worms progress through four larval stages before reaching adulthood and complete their lifecycle in about 2 days, with a lifespan of between 15 and 20 days. If L2 larvae encounter stress conditions such as high temperature, lack of food and overcrowding, they enter a different developmental stage, the dauer larva. When the conditions become favorable, worms re-enter the normal lifecycle at the L4 stage and resume the rest of their development. As worms age, metabolic rhythms are slowed down and tissues deteriorate. The duration of each stage is shown in hours. The approximate animal length at each developmental stage is given in parentheses.

gressing from a fertilized embryo through four larval stages, to become an egg-laying adult which consists of 959 cells and lives for about 2–3 weeks (Fig. 1). Under non-favorable conditions, such as starvation or stress, larvae may enter an alternative life stage called dauer larva, during which animals move but do not feed. In this resistant form, animals survive for weeks or even months [2]. When a dauer larva encounters favorable environmental conditions, it re-enters the life cycle at the fourth larval stage and completes the final week or so of its lifespan. The ability of *C. elegans* to reproduce by self-fertilization leads to genotypically homogeneous populations and renders the production and recovery of mutants straightforward. Mutagenized parents segregate homozygous mutants as F2 progeny without any required genetic crossing. Occasional males that appear at a small percentage in the population (approximately 0.1%) are able to mate with hermaphrodites, facilitating genetic manipulations. Thus, mutant alleles can be readily transferred by male mating so that complementation analysis and construction of double mutant strains is effortless. Rapid and precise genetic mapping can be achieved by taking advantage of a dense single nucleotide polymorphism map [3–5].

C. elegans molecular biology enables a considerable amount of information on *in vivo* activities of genes of interest to be determined rapidly. A physical map of the *C. elegans* genome, consisting of overlapping cosmid and YAC clones covering most of the six chromosomes, has been constructed to facilitate cloning of genes that have been positioned on the genetic map [6,7]. Sequencing and high quality annotation of the complete *C. elegans* genome organized in six chromosomes (5 autosomes and the sex chromosome X) has been accomplished (http://www.wormbase.org) [8]. In addition, ongoing efforts to obtain Expressed Sequence Tags (ESTs) and Open reading frame Sequence Tags (OSTs) for all *C. elegans* genes have provided an extensive collection of nematode cDNAs [9,10]. All approximately 20,000 predicted open reading frames (ORFs) have been subjected to expression profiling under numerous conditions using microarray technology [11,12]. Detailed gene expression profiles and protein–protein interaction maps have been developed and are publicly available [13–15].

C. elegans is also particularly amenable to reverse genetics studies. Investigators can take advantage of the wealth of genome data available to perform ‘reverse genetics’, directly knocking out genes [16]. Double-stranded RNA mediated interference (dsRNAi), a method of generating mutant phenocopies, enables probable loss-of-function phenotypes to be rapidly evaluated [17,18]. Comprehensive RNAi approaches to knock down expression of each of the 20,000 ORFs have already been published [19,20]. Transgenic nematodes for functional and genetic assays can be readily constructed by microinjecting DNA manipulated *in vitro* into the gonad of hermaphrodite adults, where it is packaged into developing oocytes [21]. Vectors are available for identification of transformants, cell-specific expression, and generation of fusions to marker genes such as *E. coli* β-galactosidase and the jellyfish Green Fluorescent Protein (GFP) so that individual cells can be visualized in stained or living animals [22–24].

Throughout its short history as a research model organism, *C. elegans* has been used successfully to study basic biological phenomena. *C. elegans* is exceptionally well-suited for the study of both normal and aberrant cell death at the cellular, genetic and molecular level. There is no other organism in which development is better understood. The simple body plan, the transparent egg and cuticle, and the nearly invariant developmental program of this nematode have facilitated the detailed developmental and anatomical characterization of the animal (Wormatlas: http://www.wormatlas.org). Individual nuclei can be readily visualized using differential interference contrast optics. These attributes have enabled the complete sequence

of somatic cell divisions, from the fertilized egg to the 959-cell adult hermaphrodite, to be determined [25,26]. Elucidation of the cell lineage map has revealed that in certain lineages, particular divisions generate cells which die at specific times and locations and that the identities of these ill-fated cells are invariant from one animal to another. Exactly 131 somatic cells undergo programmed cell death (PCD) as the fertilized egg develops into the adult animal. This detailed knowledge of normal development has allowed easy identification of mutants with aberrant patterns of cell death. The simple nervous system of the animal consists of only 302 neurons. The pattern of synaptic connections made by each of the neurons has been described, so that the full 'wiring diagram' of the nervous system is known [27–29]. Microsurgery with a laser beam can be used to specifically ablate individual cells, and whole classes of cells can be rendered non-functional or killed by cell-specific expression of toxic genes [30,31]. Primary culture methodologies are available for the analysis of specific groups of cells and neurons *ex vivo* [32].

Nematode genes and major signaling pathways show a significant conservation during evolution and more than 50% of the *C. elegans* genes have counterparts in humans. In addition to its contribution in elucidating developmental processes, the worm has also served as a platform to model many human pathological conditions such as neurodegenerative disorders, cancer, ageing and associated diseases [33–35]. Systematic mapping of gene interactions and signaling pathways implicated in human disease using *C. elegans* has provided better understanding of complex pathologies [36]. The ability to produce 'humanized' worms, which express human genes not present in the *C. elegans* genome, has further enhanced the experimental value of the nematode by allowing the dissection of molecular mechanisms relevant to human disorders. In addition, the ease of drug testing coupled with the efficiency of genetic screens in worms, has made *C. elegans* a favorable tool for the identification and validation of novel drugs and drug targets, aiming to battle human pathological conditions [37].

2. Non-programmed cell death in *C. elegans*

The genetic instructions for the regulation and execution of developmentally programmed cell death have been remarkably conserved. In *C. elegans*, programmed cell death is almost exclusively apoptotic. Elaborate genetic and molecular studies have provided significant insight into the mechanisms underlying this cell death process. In the 131 cells destined to die during development, the level of EGL-1, a BH3 domain protein, is increased. EGL-1 interacts with a protein complex composed of CED-9 (similar to the human B-cell lymphoma protein 2; BCL-2) and CED-4 (similar to the human apoptotic protease activating factor 1; Apaf-1), releasing CED-4 which in turn activates CED-3 (similar to human caspases) [38].

Not quite all programmed cell death events during *C. elegans* development follow the typical apoptotic pathway that involves CED-4 and CED-3. In the *C. elegans* male, the linker cell is required to guide the developing male gonad to the tail. Once the destination is reached, the linker cell is engulfed by a neighboring cell, in a CED-3-independent process [39]. In another case, one of two equivalent cells forms part of the male reproductive system (vas deferens), whereas the other one is engulfed by a neighboring cell [40]. Laser ablation of the engulfing cell allows survival of both cells [41]. This indicates that, instead of a hardwired program, the interaction between the two cells and the engulfing cell defines which cell will die.

Less is clear about the mechanisms of non-developmentally programmed, pathological cell death in the nematode. In certain cases, precise spatial and temporal control of cell differentiation is critical for cell survival. For example, lack of coordinated development between the vulva and the uterus in *cog-3* mutants leads to insufficient epidermal growth factor (EGF) signaling, which triggers necrosis of cells that should normally adopt the uterine vulva fate [42]. In addition, mutations in the transcription factor *lin-26* cause hypodermal cells to become neuroblasts, which swell and die [43].

Non-programmed cell death contrasts with apoptosis or programmed cell death in several respects (Table 1). By definition, non-programmed cell death is not part of normal development. Rather, this type of death generally occurs as a consequence of cellular injury or in response to extreme changes in physiological conditions and typically takes the form of necrosis. Second, the morphological changes observed during pathological cell death differ greatly from those observed in apoptotic cell death. Necrotic cell death is characterized by gross cellular swelling and distention of subcellular organelles such as mitochondria and endoplasmic reticulum. Clumping of chromatin is observed and DNA degradation occurs by cleavage at random sites. In general, necrosis occurs in response to severe changes of physiological conditions including hypoxia and ischemia, and exposure to toxins, reactive oxygen metabolites, or extreme temperature. Necrotic cell death contributes significantly in devastating pathological conditions in humans. For example, the excitotoxic neuronal cell death that accompanies oxygen deprivation associated with stroke is a major contributor to death and disability. Ischemic diseases of the heart, kidney and brain

Table 1
Differences between developmentally programmed and non-programmed cell death in *C. elegans*

Programmed cell death	Non-programmed cell death	References
Cell death transpires within an hour	Degeneration occurs over several hours	[100]
CED-3 and CED-4 are required for the execution of cell death	No requirement for CED-3 or CED-4	[101,102]
Compacted, "button-like" appearance	Swollen and enlarged cells	[25]
Nucleus becomes compacted, chromatin clumping	Nucleus becomes distorted, chromatin aggregation	[25,49]
Normal cytosolic organelles	Mitochondrial swelling	[25,49]
Maintenance of membrane integrity	Infolding of the plasma membrane, formation of electron-dense whorls	[49]

have been cited as the primary causes of mortality and morbidity in industrialized nations. Necrosis is believed to occur independently of *de novo* protein synthesis and was generally thought to reflect the chaotic breakdown of the cell [44]. However, given that many cells of diverse origins exhibit stereotyped responses to cellular injury, it is conceivable that a conserved execution program, activated in response to injury, may exist. It should be noted that more than just two patterns of cell death can be distinguished and the initial distinction between apoptosis and necrosis is an over-simplification. For example, alternative morphological death profiles have been described and certain dying cells are known to exhibit some, but not all, commonly distinctive features of either apoptosis or necrosis. Likewise, certain markers of death can be expressed by both apoptotic and necrotic cells. Although remarkable progress in understanding apoptosis has been accomplished, understanding necrosis and alternative death mechanisms is more limited. The emerging, detailed molecular models of degenerative conditions established and characterized in the nematode may provide means of identifying a conserved pathway for pathological cell death in humans. In the following sections, we present and discuss studies of non-programmed cell death in *C. elegans*, aiming to outline the molecular mechanisms involved and identify likely common denominators.

3. Necrotic cell death

Necrotic cell death can be triggered by a wide variety of both extrinsic and intrinsic signals [45]. The most extensively characterized paradigm of non-programmed cell death in *C. elegans* is the necrosis of cells expressing aberrant ion channels harboring unusual gain-of-function mutations [46]. For example, dominant mutations in *deg-1* (*deg*enerin; *deg-1*(d)) induce death of a group of interneurons of the nematode posterior touch sensory circuit [47]. Similarly, dominant mutations in the *mec-4* gene (*mec*hanosensory; *mec-4*(*d*)) induce degeneration of six touch receptor neurons required for the sensation of gentle touch to the body [48]. MEC-4 functions as the core subunit of a multimeric, mechanically gated Na^+ channel complex. Large side chain amino acid substitutions near the MEC-4 pore enhance sodium and calcium conductivity and induce necrotic cell death.

Cell demise is accompanied by characteristic morphological features [49]. During the early phase of death, the nucleus and cell body become distorted. Gradually, the cell swells to several times its normal diameter (Fig. 2). A distinguishing feature of the necrotic process is the formation of small, tightly wrapped membrane whorls, originating from the plasma membrane. These whorls are internalized and seem to coalesce into large, electron dense membranous structures [49]. It is striking that these membranous inclusions appear also in mammalian neurodegenerative disorders, such as neuronal ceroid lipofuscinosis (Batten's disease; the *mnd* mouse) and in *wobbler* mouse, the model of amyotrophic lateral sclerosis (ALS) [50,51].

deg-1 and *mec-4* encode proteins that are very similar in sequence. These genes were the first identified members of the *C. elegans* "degenerin" family, so named because several members can mutate to forms that induce cell degeneration (Table 2) [52]. Degenerins bear sequence similarity to mammalian epithelial sodium channels (ENaCs). The time of degeneration onset correlates with the initiation of degenerin gene expression and the severity of cell death is analogous to the dose of the toxic

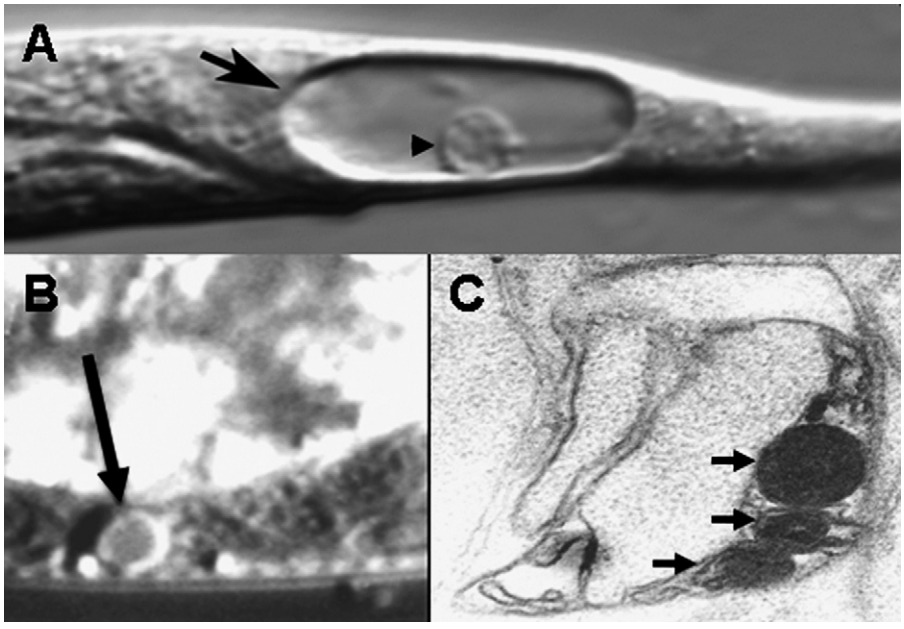

Fig. 2. Morphology of cell death in *Caenorhabditis elegans*. Nematode neurons undergoing necrosis as a result of degenerin ion channel hyperactivation show remarkable morphological differences from apoptotic cells. (A) The degenerating cell (arrow) appears extensively swollen, while the nucleus is distended and has a distorted morphology (arrowhead). (B) Apoptosis which occurs normally during development, generates refractile cell corpses compact in size with a characteristic button-like appearance (arrow). (C) Distinctive electron dense membranous circumvolutions (arrows) that accompany necrotic cellular destruction, observed under the electron microscope [49]. Similar membranous inclusions are observed in rat neurons undergoing excitotoxic cell death [107].

Table 2
Initiators of non-programmed cell death in *C. elegans*

Initiator	Type of insult	Affected cells	References
deg-1(*u38ts*)	Hyperactive degenerin ion channel	Small subset of neurons including specific interneurons	[47]
deg-3(*u662*)	Hyperactive acetylcholine receptor	Touch receptor neurons and interneurons	[53]
egl-19(*ad695*)	Hyperactive voltage-gated calcium channel	Muscles	[95]
gsa-1; $G\alpha_s$(Q208L)	Constitutively active $G\alpha_s$	Body wall muscles, motorneurons, head and tail ganglion neurons	[54,55]
mec-4(*u231*)	Hyperactive degenerin ion channel	Touch-receptor neurons	[103]
mec-10(*A673V*)	Hyperactive degenerin ion channel	Touch-receptor neurons	[104]
unc-8(*n491*)	Hyperactive degenerin ion channel	Motor neurons	[105,106]
Radiation	Macromolecular damage	Multipotent vulva precursor cells	[96]
Chemical inhibitors of the respiratory chain (NaN_3)	Energy limitation	Pharynx, body wall muscles, gonad primordium, unidentified cells	[88]
Hypoxic treatment	Oxygen/energy limitation	Pharynx, body wall muscles, gonad primordium, unidentified cells	[88]
6-Hydroxydopamine (6-OHDA)	Toxin treatment	Dopaminergic neurons	[70]
MPTP/MPP+	Toxin treatment	Dopaminergic neurons	[74]
Thapsigargin	Perturbation of calcium homeostasis	Touch receptor neurons	[62]
α-Synuclein	Stress induction	Dopaminergic neurons	[71]
Dystrophin	Stress induction	Muscles	[92]
PolyQ proteins	Stress induction	Sensory neurons	[81]
Tau protein	Stress induction	Motor neurons	[85]

allele [49]. Expression of mammalian homologous proteins, carrying amino-acid substitutions analogous to those of toxic degenerins, leads to degeneration of cells in a manner reminiscent of necrotic cell death in *C. elegans*. Included in this family are *mec-10*, which can be engineered to encode toxic degeneration-inducing substitutions, *unc-8*, which can mutate to a semi-dominant form that induces swelling and dysfunction of ventral nerve cord and *unc-105*, which appears to be expressed in muscle and can mutate to a semi-dominant form that induces muscle hypercontraction (Table 2) [48]. Thus, a general feature

Table 3
Genes implicated in non-programmed cell death in *C. elegans*

Gene	Protein (mode of action)	Expressing cells	References
acy-1	Adenylyl cyclase (required for $G\alpha_s$-induced neuronal cell death)	Muscles, neurons	[55]
asp-3	Aspartyl protease (required for the execution of necrotic cell death)	Touch receptor and motor neurons	[67]
asp-4	Aspartyl protease (required for the execution of necrotic cell death)	Touch receptor and motor neurons	[67]
cad-1	Unknown (mutants show diminished aspartyl protease activity)	Touch receptor neurons	[68]
clp-1	Calcium-activated cystein protease (required for necrotic cell death)	Touch receptor and motor neurons	[67]
cnx-1	Calnexin, ER Ca^{2+}binding chaperone (required for necrosis induced by degenerin hyperactivation)	Touch receptor neurons	[62]
crt-1	Calreticulin, ER Ca^{2+} binding-storing protein (required for necrosis induced by degenerin hyperactivation)	Many neurons, muscles, gastrointestinal cells	[62]
cup-5	Mucolipin-1 homologue (mutants enhance necrotic cell death)	Touch receptor neurons	[68]
daf-2	Receptor of insulin-like ligands (mutants are resistant to hypoxia)	Myocytes and different neurons	[88]
dat-1	Dopamine transporter (mutants are resistant to the 6-OHDA toxin)	Dopaminergic neurons	[70]
itr-1	Inositol triphosphate receptor ion channel (required for necrosis induced by degenerin hyperactivation)	Touch receptor neurons	[62]
pqe-1	Q/P-rich protein (protects neurons from polyQ protein toxicity)	Sensory neurons	[82]
sgs-1	Adenylyl cyclase (required for $G\alpha_s$-induced neuronal cell death)	Head and tail ganglion neurons, motorneurons	[56]
spe-5	Vacuolar H^+-ATPase B subunit (required for intracellular acidification during necrosis)	–	[63]
tra-3	Calcium-activated cystein protease (required for necrotic cell death)	Touch receptor and motor neurons	[67]
unc-68	Ryanodine receptor, ER Ca^{2+} release channel (required for necrosis induced by degenerin hyperactivation)	Touch receptor neurons	[62]
unc-32	Vacuolar H^+-ATPase subunit (required for intracellular acidification during necrosis)	Head neurons, ventral nerve cord neurons	[63]
vha-2	Vacuolar H^+-ATPase c subunit (required for intracellular acidification during necrosis)	Excretory canal cell	[63]
vha-10	Vacuolar H^+-ATPase G subunit (required for intracellular acidification during necrosis)	Most neurons, muscles, gastrointestinal cells, hypodermis	[63]
vha-12	Vacuolar H^+-ATPase B subunit (required for intracellular acidification during necrosis)	–	[63]

of degenerin gene family is that specific gain-of-function mutations have deleterious consequences for the cells in which they are expressed.

In addition to degenerins, gain-of-function mutations in other ion channel genes such as *deg-3*, lead to vacuolar degeneration of various types of *C. elegans* neurons (Table 2). *deg-3* encodes an acetylcholine receptor ion channel, related to the vertebrate nicotinic acetylcholine receptor (nAChr) that participates in the formation of a channel highly permeable to Ca^{2+} [53]. Moreover, expression of a constitutively active form of a heterotrimeric G-protein subunit $G\alpha_s$ results in degeneration of a specific subset of neurons [54]. Genetic suppressor analysis identified an adenyl cyclase as a downstream effector of $G\alpha_s$-induced neurodegeneration, indicating that cAMP signaling is critical for degeneration (Table 3) [55,56].

Ionic imbalance and subsequent necrotic cell death induced by aberrant ion channel function in *C. elegans* is mechanistically and morphologically similar to excitotoxicity in vertebrates. Excitotoxic cell death is prevalent during stroke, where the energy required to sustain ionic gradients and the resting potential of neurons is lost. Because membrane potential collapses, massive amounts of the excitatory neurotransmitter glutamate are released at synaptic clefts [57,58]. Energy depletion also prevents re-uptake of glutamate by dedicated transporters leading to accumulation of glutamate at synapses, hyper-excitation and eventually necrotic death of downstream synaptic target neurons. Excitotoxicity is critically dependent on Ca^{2+} influx through glutamate-gated receptor ion channels.

4. Molecular mechanisms mediating necrotic cell death

Excessive and prolonged activation of ion channels irreversibly compromises cellular ionic homeostasis. Intracellular calcium overload through different sources is considered as one of the initial steps in the necrotic pathway (Fig. 3). Calcium may enter the cell through voltage-gated channels and the Na^+/Ca^{2+} exchanger. Mutations that increase sodium influx facilitate calcium entry through these paths. The main intracellular compartment for calcium storage is the endoplasmic reticulum (ER) [59–61]. Calcium is sequestered into the ER

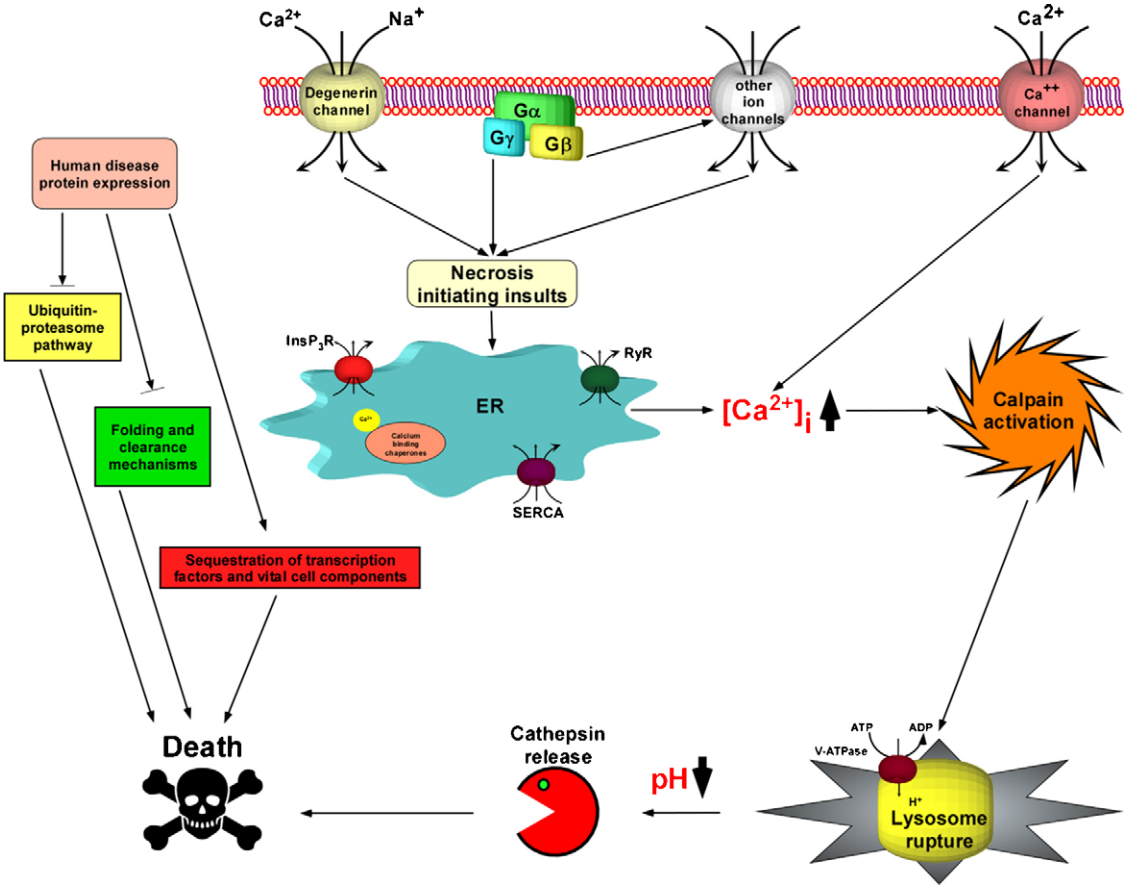

Fig. 3. Necrotic cell death pathways in *Caenorhabditis elegans*. Various necrosis-initiating insults converge to elicit a sharp increase of cytoplasmic calcium concentration ($[Ca^{2+}]_i$), which is the principal death-signaling event. Intracellular calcium stores also contribute to the elevation of calcium concentration beyond tolerable levels. The channels and molecules involved in calcium homeostasis are shown. Increased calcium concentration activates cytoplasmic calpain proteases, which facilitate lysosomal rupture and release of acidic lysosomal contents into the cytoplasm, which consequently becomes acidified. The pump responsible for lysosomal acidification is depicted (V-ATPase). Low pH conditions favor activation of cathepsin proteases and contribute to cellular destruction. Expression of human disease proteins in worms also disturbs cellular homeostasis mechanisms and induces stress, which, beyond a certain threshold, becomes detrimental for the cell ($[Ca^{2+}]_i$, cytoplasmic calcium concentration; ER, endoplasmic reticulum; G_α, G_β, G_γ, G-protein subunits; $InsP_3R$, inositol triposphate receptor; RyR, ryanodine receptor; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; V-ATPase, vacuolar H^+-ATPase).

by the sarco-endopasmic reticulum Ca^{2+}-ATPase (SERCA) and is released back to the cytoplasm by ryanodine (RyR) and inositol-1,4,5-triphosphate receptors ($Ins(1,4,5)P_3PR$). Extensive genetic screens for suppressors of *mec-4*(*d*)-induced necrosis have identified genes required for the execution of necrotic cell death. Calreticulin and calnexin, which are calcium-binding chaperones regulate intracellular calcium levels and are required for necrotic cell death [62]. Treatment of animals with thapsigargin, which induces release of calcium from the ER to the cytoplasm, triggers necrotic cell death. In contrast, pharmacological treatments or genetic mutations that inhibit calcium release from the ER have a strong protective effect against necrotic cell death.

Genetic studies in *C. elegans* have also shown that intracellular pH is an important modulator of necrotic cell death. Cytoplasmic acidification occurs during necrosis, whereas the vacuolar H^+-ATPase, which is a pump that acidifies lysosomes and other intracellular organelles, is required downstream of cytoplasmic calcium overload to promote necrotic cell death [63]. Reduced vacuolar H^+-ATPase activity or alkalization of acidic endosomal/lysosomal compartments by weak bases has a neuroprotective role against necrosis. Acidic conditions are required for full activity of cathepsins, aspartyl proteases that are primarily confined to lysosomes and other acidic endosomal compartments [64].

Lysosomal as well as cytoplasmic, proteases have been implicated in cellular destruction following the onset of necrosis. Calpains are cytoplasmic, papain-like cystein proteases that depend on calcium for their activity. Under normal conditions, calpains function to mediate essential signaling and metabolic processes. However, during the course of necrotic cell death these proteases localize onto lysosomal membranes and may compromise lysosomal integrity, thereby causing leakage of their acidic contents, including lysosomal proteases, into the cytoplasm [65]. In primates, calpains rapidly localize to lysosomal membranes after the onset of ischaemic episodes [66]. In *C. elegans*, two specific calpains – TRA-3 and CLP-1 – and two lysosomal cathepsin proteases – ASP-3 and ASP-4 – are required for neurodegeneration [67]. It is likely that ensuing cytoplasmic acidification activates the lysosomal, low-pH dependent cathepsins and hydrolases that contribute to cell demise.

Mutations that interfere with lysosomal biogenesis and function influence necrotic cell death. For example, necrosis is exacerbated in mutants that accumulate abnormally large lysosomes, whereas impairment of lysosomal biogenesis protects from cell death [68]. Interestingly, lysosomes appear to coalesce around the nucleus and dramatically enlarge during early and intermediate stages of necrosis. In advanced stages of cell death, GFP-labeled lysosomal membranes fade, indicating lysosomal rupture.

5. Modeling Parkinson's disease in *C. elegans*

Parkinson's disease is a chronic and progressive neurodegenerative disorder that impacts motor skills and speech, usually in the elderly. Patients suffer from involuntary movements including resting tremor, muscle rigidity, slowed movement and difficulty in balance, symptoms associated with loss of neurons that produce dopamine in specific parts of the brain. Characteristic of the disease is the intracytoplasmic accumulation of Lewy bodies. Familial forms of the disease can be caused by mutations in specific genes, implicating the components of the Lewy bodies and the misfolded protein degradation machinery, in the pathogenicity of the disease.

In rodent and primate models of Parkinson disease, neuroroxicity is induced by the neurotoxins 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and more recently the pesticide rotenone. The pathway through which neurons degenerate depends on the neurotoxin and the conditions used [69]. Presynaptic dopamine transporters (DATs) are necessary for the accumulation of these toxins to dopamine neurons, resulting in an increase in reactive oxygen species production and/or mitochondrial dysfunction. In *C. elegans*, dopamine neurons degenerate after exposure to 6-OHDA. Degenerating cells do not display membranous whorls, swollen organelles or swollen cell bodies, which are characteristics of necrotic cell death. Instead, chromatin condensation is evident, a feature reminiscent of apoptotic cell death. However, neither the caspase CED-3 nor its activator CED-4 appear to play a role in 6-OHDA-induced dopamine neuron degeneration [70]. Instead, the dopamine transporter DAT-1 is required for 6-OHDA toxicity (Table 3).

Parkinson's disease has also been modeled in *C. elegans* by overexpression of wild and mutant types of α-synuclein, the main component of Lewy bodies. Overexpression throughout the nervous system or specifically in motor neurons caused motor deficits in transgenic worms. In addition, loss of a number of cell bodies and dendrites of dopaminergic neurons was observed when human α-synuclein was overexpressed under the control of a dopaminergic or pan-neuronal promoter [71].

Several cellular mechanisms have been implicated in the pathogenesis of Parkinson's disease and the associated neurodegeneration. These include impairment of the ubiquitin-proteasome system, stress within the ER, mitochondrial dysfunction and accumulation of reactive oxygen species [72]. Inhibition of ER–Golgi trafficking by α-synuclein aggregates also contributes to toxicity and cell loss [73]. Impairment of trafficking would result in accumulation of proteins in the ER, inducing ER stress. Interestingly, overproduction of gene products which increase transport between ER and Golgi overcomes the α-synuclein-induced transport block. Conversely, manipulations that negatively regulate ER-Golgi transport exacerbate the α-synuclein-induced problems.

An MPTP-based nematode model of Parkinson's disease, targeting specifically the dopaminergic neurons has also been established and used as a platform for drug testing in living animals. Treatment of *C. elegans* with MPTP or its active metabolite MPP+ (1-methyl-4-phenylpyridinium) resulted in significantly reduced mobility and increased lethality, which was accompanied by specific degeneration of the dopaminergic neurons [74]. This model allows the implementation of large-scale genetic and pharmacological screenings designed to elucidate the molecular mechanisms underlying neuronal death and identify targets and drugs for therapeutic intervention [75].

6. Expanded polyglutamine protein toxicity

Several progressive neurodegenerative conditions are associated with accumulation of expanded polyglutamine (polyQ) proteins [76]. Disease severity is correlated with the extent of polyQ expansion, with increased expansion resulting in earlier onset and more severe symptoms. The pathogenesis mechanisms associated with polyQ expansion are subject to intense study and many animal models have been developed towards this end [77,78]. In *C. elegans*, expression of proteins with expanded polyQ in the nervous system results in neuronal dysfunction and degeneration. Ataxin-3 is the product of the gene MJD1 associated with the common spinocerebellar ataxia degenerative disorder also known as Machado-Joseph disease (SCA-3/MJD). Expression of MDJ1 throughout the nematode nervous system impaired synaptic transmission and the ubiquitin-proteasome system, resulting in accumulation of polyQ aggregates and morphological abnormalities, such as swelling of neuronal processes [79]. Age-dependent alterations in the branching of neuronal processes were also observed. Cellular folding and clearance mechanisms may sustain homeostasis and promote cell survival to some extend. However, accumulation of aggregation-prone proteins overwhelms these defenses, disrupting the balance and leading to cell degeneration [80].

Amino-terminal huntingtin fragments with varying polyglutamine repeat lengths have also been expressed in *C. elegans* sensory neurons. Fragments carrying the longest polyglutamine stretch (150 glutamine residues) inhibit neuronal function in an age-dependent fashion. Toxicity is characterized by the formation of protein aggregates and loss of neuronal function, which precedes physical degeneration. Actual cell death of sensory neurons requires a sensitized genetic background expressing a toxic but sub-lethal OSM-10::green fluorescent protein (GFP) fusion together with the long polyglutamine repeat transgene [81]. This indicates that a second toxic signal is required for rapid onset of the disease. OSM-10::GFP is non-functional and may interfere with the function of endogenous OSM-10, generating a state of cellular stress and neuronal dysfunction. While cell death induced by the co-expressed transgenes could be suppressed by a loss-of-function mutation in the caspase gene ced-3, the ability of OSM-10::GFP alone to cause neuronal defects was not dependent on CED-3. Nuclear inclusions were not observed in this model, although cytoplasmic aggregates were observed in older animals expressing the long polyglutamine repeat transgene.

Genetic screens for enhancers of mutant huntingtin toxicity in *C. elegans*, led to the identification of the poly-Q enhancer 1 (*pqe-1*) gene (Table 3). Loss of PQE-1 function strongly and specifically accelerates neurodegeneration, whereas overexpression protects against neurotoxicity [82]. It is hypothesized that PQE-1 may interact with cellular proteins, preventing interference by expanded huntingtin fragments. In addition, loss of the cAMP response element-binding protein (CREB) or the CREB binding protein (CBP), enhanced polyglutamine toxicity in *C. elegans*. Similarly, knockdown of specific histone deacetylases (HDACs) exacerbates toxicity [83]. These findings suggest that expanded polyglutamine repeat proteins hinder transcription regulation and that targeting these deleterious effects may be an efficient strategy to battle neurodegeneration.

7. Tau toxicity

Accumulation of hyper-phosphorylated tau, a microtubule binding protein, in the form of neurofibrillary tangles, is correlated with many neurodegenerative disorders, like Alzheimer disease and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). The mechanisms that underlie tau-induced neurodegeneration remain largely unknown. The *C. elegans* genome encodes a protein with significant sequence similarity to mammalian Tau. Expression of normal and mutant (P301L and R406W) tau in *C. elegans* neurons causes altered animal behavior, reduced lifespan, defective cholinergic transmission, accumulation of insoluble phosphorylated protein and age dependent loss of axons and neurons (Table 2). Tau accumulates in an age-dependent manner and neurodegeneration is more severe upon expression of mutant, rather than wild type tau [84]. Interestingly, there is no formation of tau filaments in transgenic animals, questioning the necessity of neurofibrillary tangles for the pathogenesis of the disease. Neurodegeneration is characterized by vacuolar axon clearing, collapsed membrane structures, concentric layers of axonal membrane and axonal swelling [85]. Specific expression of wild type and mutant tau in touch receptor neurons results in morphological abnormalities, although tau does not accumulate in cell bodies. In worms expressing mutant tau, degeneration of affected neurons does not involve the apoptotic pathway, since cell survival is not improved in *ced-3* or *ced-4* mutant background [86].

8. Hypoxic death

Oxygen deprivation induces cell death in pathological conditions such as stroke and heart attack [87]. In *C. elegans*, hypoxia can inflict necrotic death in various cell types [88]. Interestingly, mutations in the *daf-2* gene, which encodes the *C. elegans* insulin/IGF receptor tyrosine kinase, confer resistance against hypoxic cell death (Table 3). DAF-2 is a component of the insulin signaling pathway that regulates ageing and dauer formation in *C. elegans* [35]. It is remarkable that many human neurodegenerative disorders show a late-onset pathogenesis, indicating that ageing may alter the vulnerability of cells to various insults. However, while hypoxia resistance in *C. elegans* appears to be modulated by insulin signaling, other *daf-2* mutations that affect longevity and stress resistance do not affect hypoxic death. Selective expression of wild type *daf-2* in neurons and muscles restores hypoxic death in *daf-2* hypoxia-resistant mutants, demonstrating a role of the insulin/IGF receptor in the protection of myocytes and neurons from hypoxic injury.

Na^+-activated potassium channels (K_{Na}) have been identified in cardiomyocytes and neurons, where they may provide protection against ischaemia [89,90]. The *slo-2* gene encodes a K_{Na} ion channel in *C. elegans*. *slo-2* mutants are hypersensitive to hypoxic death, suggesting that SLO-2 protects against hypoxia effects. Thus, molecular characterization of K_{Na} channels may

allow the development of specific agonists and antagonists, in an effort to combat hypoxia caused pathologies [91].

9. Muscle degeneration and reproductive cell death

Degeneration of *C. elegans* muscles is observed in *dys-1* mutants. The *dys-1* gene encodes a dystrophin-like protein. In humans, mutations in the dystrophin gene cause Duchenne's muscular dystrophy, one of the most common neuromuscular diseases (Table 2). In *C. elegans*, degenerating body wall muscles show fragmentation and destruction of actin fibers [92]. The ease of scoring muscle dysfunction and degeneration, establishes *C. elegans* as an ideal model for high-throughput testing of compounds fighting dystrophy [93,94]. Muscle degeneration, induced by dystrophin mutations, dramatically increases in a sensitized genetic background carrying a gain-of-function mutation in the *egl-19* calcium channel gene [95]. This suggests that calcium levels are critical for the process of degeneration.

Reproductive cell death is considered to be the primary mechanism of radiation-induced death of clonogens, which are multipotent precursor cells, during cytotoxic tumor targeting. Recently, a model of radiation-induced reproductive cell death in the *C. elegans* vulva, a tissue with multipotent precursor cells, has been developed [96]. In this model, cell death of vulva precursor cells is sufficient to lead to tissue death, as predicted for human clonogens. Radiation-induced reproductive cell death is distinct from apoptosis and resembles necrosis. The DNA damage response pathway appears to be critical for clonogen cell survival. This system can be exploited to elucidate the cellular mechanisms that act in response to tumor irradiation to facilitate cell death.

10. Concluding remarks and perspectives

The identification of *C. elegans* mutations that trigger pathological or necrotic cell death enables us to exploit the strengths of this model system to gain novel insight into a non-apoptotic death mechanism. The intriguing observation that distinct cellular insults can induce a similar necrotic-like response suggests that *C. elegans* cells may respond to various injuries by a common process, which can lead to cell death.

Inappropriate channel activity is known to be causative for mammalian neurodegenerative conditions. For example, it is interesting that initiation of degenerative cell death by hyperactive ion channels in *C. elegans* is remarkably similar to events that initiate excitotoxic cell death in higher organisms. In excitotoxicity, glutamate receptor ion channels are hyper-stimulated by the excitatory transmitter glutamate and the resultant elevated Na^+ and Ca^{2+} influx induces neuronal swelling death. Mammalian ion channel mutations can also induce neurodegeneration. In the weaver mutant mouse, altered gating and ion selectivity properties of the GIRK2 potassium channel are associated with vacuolar cell death in the cerebellum, dentate gyrus and olfactory bulb [97,98]. It is also interesting that there are many reported instances, in animals as diverse as flies, mice and humans, in which neurons degenerating due to genetic lesions exhibit morphological changes similar to those induced by hyperactive ion channels, in the nematode. Given that apoptotic death mechanisms are conserved between worms and humans, we are prompted to ask: might various cell injuries, environmentally or genetically-introduced, converge to activate a degenerative death process that involves common biochemical steps? At present the question of common mechanisms remains an intriguing but open issue.

It is noteworthy, that mutations which cause cell swelling and death can be isolated at a relatively high frequency, suggesting that there might be multiple types of genes that can mutate to induce necrotic-like death. These observations suggest that degenerative cell death might be induced by a variety of cellular "injuries" and that a common death mechanism could operate to eliminate injured cells. Indeed, electron micrograph analyses of degenerin-induced cell death revealed a reproducible sequence of cellular changes that transpire during degeneration, suggesting that specific regulated steps (rather than chaotic cellular destruction) are involved. The peculiar internalized membranous whorls observed suggest that degenerin-induced death could involve disrupted intracellular trafficking, an interesting implication given that disrupted trafficking has been implicated in Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS). Interestingly, Vps54 a protein involved in vesicular trafficking is defective in the wobbler mouse, an animal model of ALS [99]. Perhaps, endocytotic responses provoked by diverse types of damage might be a common element of diverse degenerative conditions. Research on *C. elegans* models of human degenerative disorders holds promise for the elucidation of important but poorly understood aspects of the relevant mechanisms. Why is there an age-dependent aggravation of symptoms? What is the reason for increased vulnerability of specific cell types? Does cell degeneration associated with aggregation of diverse proteins transpire via a common pathway? What is the relationship between protein aggregation and toxicity? There is currently much debate about whether aggregation is a cause of the pathology, a mere consequence or even a protective measure.

If specific genes enact different steps of the degenerative process, then such genes should in principle, be identifiable by mutations in *C. elegans*. Indeed, suppressor mutations in several genes that block non-programmed cell death have been isolated. Although some suppressor mutations may affect the expression or efficacy of the death stimulus itself, others are expected to be more generally involved in the death process. Analysis of such genes should result in the description of a genetic pathway for degenerative cell death. Perhaps, as has proven to be the case for the analysis of programmed cell death mechanisms, elaboration of an injury-induced death pathway in *C. elegans* may provide insight into neurodegenerative death mechanisms in higher organisms.

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