Chapter 15
Necrotic Cell Death in *Caenorhabditis elegans*

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15.1 Introduction

Early studies in the field of cell death described two major forms of cellular demise, apoptosis and necrosis, and contrasted them as being diametrically different in every aspect examined (Walker et al. 1988). In 1972, Kerr, Wyllie, and Currie described apoptosis as a controlled cell death process and proposed that it functions as a tissue homeostatic mechanism that is complementary and opposite to cell division (Kerr et al. 1972). Necrosis was classically contrasted to apoptosis, also referred to as caspase-dependent programmed cell death, not only on grounds of context and mechanistic regulation or lack thereof but also based on notable morphological differences. The apoptotic cell profile is characterized by cell rounding, detachment from the basal membrane or cell culture substrate, chromatin condensation and nuclear fragmentation, blebbing of the plasma membrane, and shedding of vacuoles known as apoptotic bodies (Galluzzi et al. 2007). Necrotic cells were initially characterized in a negative fashion, exhibiting neither an apoptotic morphological profile nor an extensive vacuolization characteristic of autophagic cell death. However, specific morphological features were soon attributed to necrotic cells. These included in particular an increasingly translucent cytoplasm, osmotic swelling of most organelles, increased cell volume, and finally rupture of the plasma membrane (Fig. 15.1). Notably, unlike apoptosis, necrosis does not feature major nuclear modifications but only minor ultrastructural changes. Moreover, necrotic cells do not fragment into distinct corpses as their apoptotic counterparts do (Galluzzi et al. 2007).

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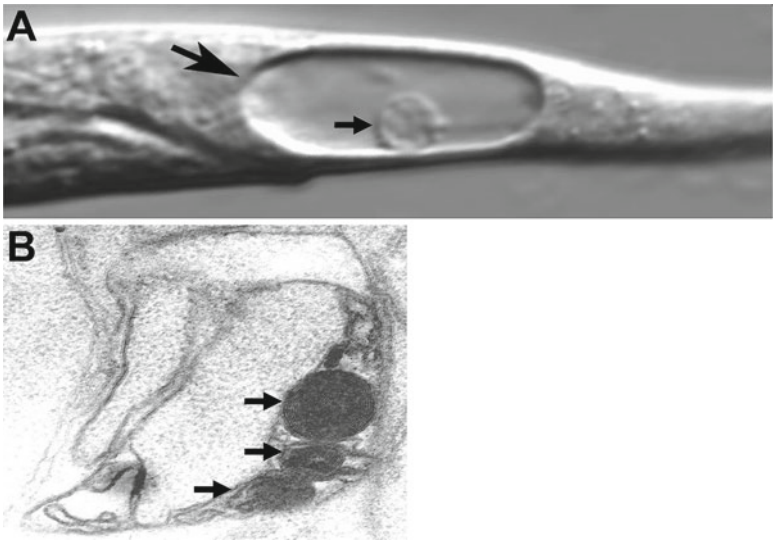

Fig. 15.1 Morphology of necrotic cell death in *Caenorhabditis elegans*. Nematode neurons undergoing necrosis as a result of degenerin ion channel hyperactivation show distinct morphological features (Kourtis and Tavernarakis 2007). (**a**) The degenerating cell (*arrow*) appears extensively swollen, while the nucleus is distended and has a distorted morphology (*arrowhead*). (**b**) Distinctive electron-dense membranous circumvolutions (*arrows*) observed under the electron microscope (Hall et al. 1997). Similar membranous inclusions are observed in rat neurons undergoing excitotoxic cell death

C. elegans has been instrumental in deciphering both apoptotic and necrotic cellular programs. This can be largely attributed to the specific characteristics and well-described developmental stages of this nematode, which make it exceptionally well suited for the study of both normal and aberrant cell death at the cellular, genetic, and molecular level. Due to its transparency, the visualization and tracking of single cells as well as of individual nuclei is readily feasible by differential interference contrast optics, enabling researchers to follow somatic cell divisions from the fertilized egg all the way to the 959 cell adult hermaphrodite (Sulston and Horvitz 1977; Sulston et al. 1983). The resulting cell lineage map indicated early on that in certain lineages, particular divisions generate cells which are destined to die at specific times and locations that remain faithfully invariant from one animal to another. Exactly 131 somatic cells die every time the fertilized egg normally develops into the adult animal, by an apoptotic programmed cell death (PCD) process.

Genetic and molecular studies performed in *C. elegans* provided a fundamental 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 mammalian B-cell lymphoma protein 2, BCL-2) and CED-4 (similar to the mammalian apoptotic protease activating factor 1, Apaf1), releasing CED-4 which in turn

activates CED-3 (similar to human caspases) (Hengartner 2000). In *C. elegans*, four caspase-related genes exist: *ced-3*, *csp-1*, *csp-2*, and *csp-3* (Yuan et al. 1993; Shaham 1998); however, only *ced-3* seems to be required for programmed cell death (Yuan et al. 1993; Abraham and Shaham 2004), and only ced-3 and *csp-1* are proteolytically active (Shaham 1998). The CSP-2 caspase lacks key active-site residues, and *csp-3* encodes only a C-terminal caspase domain, entirely lacking the active site (Shaham 1998). As it turns out, the genetic encoding for the regulation and execution of developmental apoptosis has been remarkably conserved between *C. elegans* and mammals.

15.2 Necrotic Cell Paradigms During *C. elegans* Development

The identification of the caspase CED-3 as a key regulator of apoptosis has been a key contribution of *C. elegans* to the cell death field, as caspases also play crucial roles in the execution of programmed cell death across many species. However, is all developmental cell death in *C. elegans* dependent on caspases? As it turns out, not quite all cell death events during *C. elegans* development follow the typical apoptotic pathway that involves CED-4 and CED-3.

15.2.1 Death of the Linker Cell

The *C. elegans* linker cell has been identified as such an exemption (Horvitz et al. 1983). The linker cell is born during the second larval stage (L2) in the central region of the animal and follows a stereotypical path of migration. As the cell migrates, it leads the extension of the male gonad behind it (Kimble and Hirsh 1979; Sulston et al. 1980), and upon completion of its migratory route, it is positioned between the gonad (vas deferens) and the cloacal tube, serving as an exit channel for sperm in the adult. It is generally thought that the death and removal of the linker cell around the L4/adult transition facilitates the fusion between the vas deferens and cloaca, to connect the male reproductive system to the exterior.

Following up on early observations that the programmed death of the linker cell persists even in *ced-3* mutant animals, Abraham and colleagues thoroughly studied the death of this cell by following the fate of a GFP-marked linker cell in animals harboring mutations in core genes of the apoptotic machinery, such as *ced-3* and *ced-4*, as well as in engulfment genes. They convincingly demonstrated that the linker cell dies in a cell autonomous manner that, unlike what was postulated by previous reports (Sulston et al. 1980), does not require extrinsic signals from engulfing or other cells. Moreover, they showed that this death event is independent of any known apoptotic genes, in line with the lack of apoptotic morphological features, such as chromatin condensation. Instead, there was a noted presence of swollen and

degraded mitochondria within large multilayered membrane-bound structures, as well as small electron-translucent "empty" membrane-bound cytoplasmic structures that resembled vacuoles typically seen during necrotic cell death in *C. elegans* (Hall et al. 1997) (Fig. 15.1). Although linker cell death does not satisfy all classical criteria of necrotic death, it is even further away from classical apoptotic paradigms, perhaps falling under the characteristics of more recently described programmed necrosis processes, also known as necroptosis. More experiments are however needed to further characterize the precise mode of death of the linker cell.

15.2.2 Death of Mis-specified Uterine–Vulval (uv1) Cells

Yet, a more robust example of a necrotic event during development is the demise of mis-specified uterine–vulval (uv1) cells that have an important role in egg laying. Egg laying in *C. elegans* requires a connection between the lumens of the uterus in the somatic gonad and the vulva in the extra-gonadal epithelium, facilitated by cell–cell interactions between gonadal and vulval cells. Two specialized cell types of the ventral uterine π lineage are integral components of the uterine–vulval connection. These are the syncytial uterine seam (utse) cell, which overlies the vulval lumen, and the four uterine–vulval (uv1) cells, which directly contact the most dorsal vulval cell vulF (Newman et al. 1996). The temporal and spatial specification of both these cell types largely relies on a specific signaling axis, where an inductive LIN-3 epidermal growth factor (EGF) signal derived from a single gonadal cell called the anchor cell (AC) activates the LET-23 EGF receptor on the receiving vulval precursor cells (Aroian et al. 1990; Hill and Sternberg 1992). Mutations in genes of the LIN-3/LET-23/Ras signaling pathway compromise uv1 fate specification. Work from the laboratory of Hanna-Rose (Huang and Hanna-Rose 2006) described the isolation of the *cog-3*(*ku212*) mutant, which uncouples gonadogenesis from its normal progression relative to the development of the vulva and shares phenotypes with heterochronic mutations that disturb the temporal coordination of vulval and uterine development. In *cog-3*(*ku212*) mutants, the entire uterus, including the pre-uv1 cells, is generated at a later stage of vulval development than is normal. Notably, the delayed pre-uv1 cells subsequently die by necrosis leading to the absence of uv1 cells in the adult stage. Moreover, the study investigated if a LIN-3/LET-23/Ras signaling defect underlies the necrosis of uv1 defect in *cog-3* (*ku212*) mutants, by analyzing *cog-3*(*ku212*) double mutants with a gain-of-function allele of *let-23*. The results indicated that the *let-23*(*gf*) mutation rescued the mis-specification and death phenotype of uv1 cells, suggesting that the necrotic program is recruited during development in response to uncoordinated spatiotemporal development.

A recent study revealed the involvement of the *ku212* allele in uv1 cell necrosis, which maps to the *pnc-1* gene locus, encoding a nicotinamidase (van der Horst et al. 2007; Vrablik et al. 2009). Nicotinamidases are the first enzymes of the NAD^+ salvage pathway in invertebrates, using nicotinamide (NAM) as a substrate (Magni et al. 1999). Administration of high levels of nicotinamide causes uv1 cells to die by

necrosis at high frequency in wild-type animals. Thus, instead of compromised EGF signaling, the necrotic death of uv1 cells in *pnc-1* mutants may result from accumulation of the substrate nicotinamide. In addition, the gonad-defective and uv1 cell death phenotypes are separable in *pnc-1* mutants. Constitutively active LET-23/EGF receptor prevents NAM-induced uv1 necrotic cell death, suggesting that EGF signaling may provide a survival cue that rescues uv1 cells from NAM-induced necrosis (reviewed in Vlachos and Tavernarakis 2010).

15.3 Nondevelopmental Necrotic Death

In the adult nematode, necrotic cell death can be triggered by a wide variety of both extrinsic and intrinsic signals (Walker et al. 1988). Several well-defined conditions are known to trigger necrotic cell death in *C. elegans*. The best-characterized case is that of unusual gain-of-function mutations, in several ion channel genes, which inflict a necrotic pattern of death on the neurons that express their protein products. Cell demise in these paradigms is accompanied by characteristic morphological features of necrosis, starting with the appearance of a distorted nucleus and cell body during the early phase of death. Gradually, the cell swells to several times its normal diameter, and small, tightly wrapped membrane whorls form, originating from the plasma membrane and coalescing into large, electron-dense membranous structures (Hall et al. 1997). Interestingly enough, these membranous inclusions also represent characteristic hallmarks in mammalian neurodegenerative disorders, such as in neuronal ceroid lipofuscinosis (Batten's disease, the *mnd* mouse) as well as in the wobbler mouse, a model of amyotrophic lateral sclerosis (ALS) (Cooper et al. 1999; Blondet et al. 2002).

15.3.1 Cell Death Induced by Ionic Imbalance

The most extensively characterized paradigm of nonprogrammed cell death in adult *C. elegans* animals is the necrosis of cells expressing aberrant ion channels harboring unusual gain-of-function mutations (Syntichaki and Tavernarakis 2003). For example, dominant mutations in *deg-1* [degenerin; *deg-1*(*d*)] induce death of a group of interneurons of the nematode posterior touch sensory circuit (Chalfie and Wolinsky 1990). Similarly, dominant mutations in the mec-4 gene [mechanosensory; *mec-4*(*d*)] induce degeneration of six touch receptor neurons required for the sensation of gentle touch to the body (Syntichaki and Tavernarakis 2004).

deg-1 and *mec-4* encode proteins that are very similar in sequence and were the first identified members of the *C. elegans* "degenerin" family, so named because several members can mutate to forms that induce cell degeneration (Chalfie et al. 1993). 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 allele (Hall et al. 1997). 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*. Additional members of the degenerin family are *mec-10*, which can be engineered to encode toxic degeneration-inducing substitutions; *unc-8*, which can mutate to a semidominant 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 semidominant form that induces muscle hyper-contraction (Syntichaki and Tavernarakis 2004). Thus, a unifying feature of degenerin family members is that specific gain-of-function mutations have deleterious consequences for the cells in which they are expressed, which, at least in neurons, culminate into a necrotic cell death event.

C. elegans degenerins share sequence similarity with Drosophila Ripped Pocket (RPK) and Pickpocket (PPK), with subunits of the vertebrate amiloride-sensitive epithelial sodium channel (ENaC), and with other neuronally expressed ion channels. Together, these proteins define the DEG/ENaC protein superfamily (Tavernarakis and Driscoll 2001). Although mutant degenerins can kill different groups of neurons depending on their expression patterns, the morphological features of the cell death that they induce are the same and resemble those of mammalian cells undergoing necrotic cell death. The pattern of necrotic cell death inflicted by degenerins is not a peculiarity of this gene class. For example, *C. elegans deg-3*, whose product is related to the vertebrate α-7 nicotinic acetylcholine receptor and together with the related protein DES-2 forms a very efficient calcium channel, can mutate to induce necrotic cell death similar to that induced by degenerins (Treinin et al. 1998). In addition, mutant-activated forms of the heterotrimeric G protein α-subunit ($G\alpha_s$ Q208L), from both *C. elegans* and rat, cause swelling and degeneration of many cell types when expressed in *C. elegans* (Korswagen et al. 1997; Berger et al. 1998).

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. 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+} (Treinin and Chalfie 1995). 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. Genetic suppressor analysis identified an adenylyl cyclase as a downstream effector of $G\alpha_s$-induced neurodegeneration, indicating that cAMP signaling is critical for degeneration (Berger et al. 1998; Korswagen et al. 1998).

Ionic imbalance and subsequent necrotic cell death induced by aberrant ion channel function in *C. elegans* are mechanistically and morphologically similar to excitotoxicity in vertebrates. Excitotoxic cell death is prevalent during stroke, where the energy required for sustaining 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 (Kauppinen et al. 1988a, b). Energy depletion also prevents reuptake of glutamate by dedicated

transporters leading to accumulation of glutamate at synapses, hyperexcitation, and eventually necrotic death of downstream synaptic target neurons. Excitotoxicity is critically dependent on Ca^{2+} influx through glutamate-gated receptor ion channels (reviewed in Kourtis and Tavernarakis 2007).

Malfunction of glutamate transporters and the resulting accumulation of glutamate are known to trigger excitotoxicity in several neurodegenerative diseases (Cleveland and Rothstein 2001). However, the details on the cascade of events leading to neurodegeneration remain unclear. The molecular components of glutamatergic synapses assembled in *C. elegans* are highly conserved from nematodes to humans. A recent study describes a novel paradigm for nematode excitotoxicity, by investigating the in vivo effects of multiple mediators of glutamate-induced neuronal necrosis (Mano and Driscoll 2009). Combined *Δglt-3* glutamate transporter-null mutations and expression of a constitutively active form of the alpha subunit of the G protein Gs induce extensive neurodegeneration in head interneurons. *Δglt-3-dependent* neurodegeneration acts through Ca^{2+}-permeable Glu receptors of the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype, requires calreticulin function, and is modulated by calcineurin and type-9 adenylyl cyclase (AC9). This glutamate-dependent toxicity defines a novel necrotic death paradigm in *C. elegans* that shares many basic features with excitotoxicity in mammalian neurons and may potentially be operative also in higher organisms.

15.3.2 Heat-Induced Necrotic Death

Climate change has brought about a dramatic increase in the cases of heatstroke and related pathologies in humans. With core body temperature reaching over 40 °C, heatstroke causes immediate devastating tissue damage and inflammatory response that can be fatal, as well as long-term defects. To gain insight into the molecular mechanisms of heat cytotoxicity and to circumvent the confounding influence of secondary physiological and inflammatory responses, our laboratory developed and characterized a genetically tractable model of heatstroke in *C. elegans*. Widespread cell death across several tissues could be observed in animals exposed to hyperthermia, which in the nematode was simulated by a short exposure to 39 °C (Kourtis et al. 2012). Dying cells displayed morphological features characteristic of necrosis, expressed markers of necrotic death, and became permeable to propidium iodide. Moreover, depletion of proteins required for necrosis strongly facilitated survival after heatstroke. In contrast, loss of key mediators and core components of the apoptotic or autophagic machineries did not suppress heatstroke-induced cell death. Thus, heatstroke compromises viability by triggering extensive necrotic cell death and represents a newly added necrotic cell paradigm in the nematode.

Notably, we also observed that preconditioning animals at an intermediate, nonlethal temperature markedly enhanced their capacity to withstand a subsequent heatstroke. This protective effect is in line with the previously described phenomenon of hormesis (Calabrese 2004), where preexposure to mild stress elicits increased

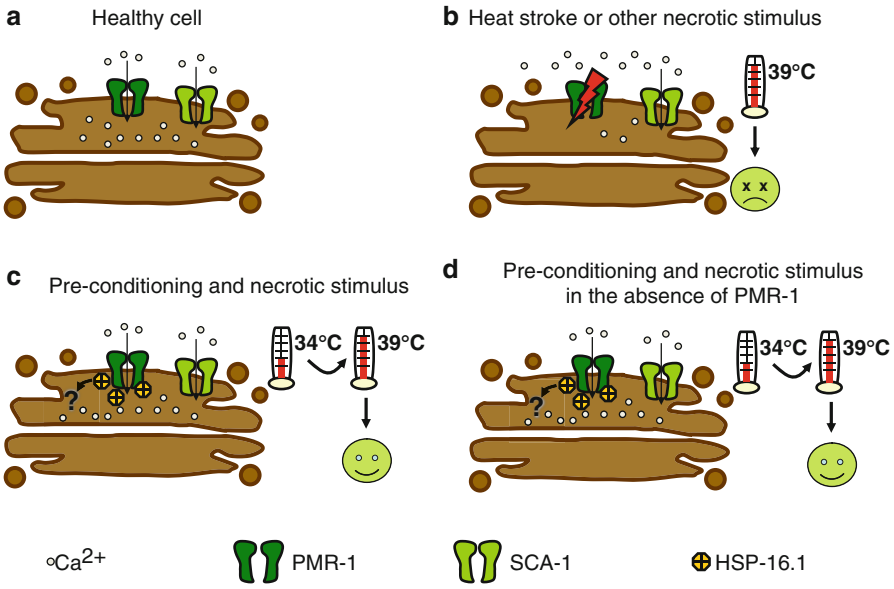

Fig. 15.2 A cytoprotective mechanism against necrotic cell death engaged by heat preconditioning. The contribution of the medial Golgi, shown here by brown cisternae, is depicted (**a**) in healthy cells under normal conditions, (**b**) upon exposure to heatstroke or other necrotic stimuli, (**c**) upon heat preconditioning and subsequent heatstroke, and (**d**) upon heat preconditioning and subsequent heatstroke in the absence of PMR-1. Under normal conditions (**a**) the Golgi P-type ATPase Ca^{2+}/Mn^{2+} pump PMR-1 and SCA-1 maintain Ca^{2+} homeostasis by sequestering Ca^{2+} inside the Golgi. (**b**) Heatstroke perturbs the normal function of PMR-1 leading to aberrant release of Ca^{2+} into the cytoplasm and causing necrosis. Heat preconditioning (**c**) increases the levels of HSP-16.1, which restores PMR-1 function upon subsequent heatstroke, therefore preventing necrosis. The effect of HSP-16.1 is entirely dependent on PMR-1; as in the absence of PMR-1 (**b**), heat preconditioning cannot confer cytoprotection against subsequent heatstroke (Adapted from Kourtis et al. 2012)

resistance to subsequent severe stress. It is also worth noting that in addition to heatstroke, heat preconditioning conferred resistance against a wide range of necrotic death insults, including in particular ionic imbalance paradigms (discussed earlier), overexpression of aggregation-prone proteins (such as α-synuclein), and hypoxic conditions. In the case of hormesis by heat preconditioning, we found that cytoprotection is orchestrated at the molecular level by the hermetic induction of a single sHSP, HSP-16.1. sHSPs assemble into oligomeric complexes and serve as molecular chaperones, efficiently binding denatured proteins and/or preventing irreversible protein aggregation and insolubilization (Van Montfort et al. 2001). HSP-16.1 localizes in the Golgi, where it functions together with the PMR-1 pump to prevent cytoplasmic Ca^{2+} overload under extreme stress. We propose that HSP-16.1 contributes to stabilize and protect the stress-labile PMR-1 pump, allowing for efficient clearance of Ca^{2+} from the cytoplasm, after necrotic insult (see Fig. 15.2).

Importantly, mammalian PMR1 is selectively impaired during ischemic or reperfusion brain injury (Lehotsky et al. 2002; Gidday 2006; Pavlikova et al. 2009). Given the strong evolutionary conservation of the proteins involved, this mechanism is probably relevant to related human pathologies. Relevant to that, we also demonstrated that heatstroke induces widespread necrotic death in mammalian neurons, which can be largely prevented by heat preconditioning. Moreover, hormesis in mammalian neurons in response to heat preconditioning also requires the function of PMR1 and is mediated by the same molecular players as in the nematode.

15.3.3 Bacterial Infection-Induced Necrosis

Infection of *C. elegans* with different bacterial pathogens has been shown to induce necrotic death of intestinal cells as part of a pathogen-shared response to infection (Wong et al. 2007). At later stages of infection, necrotic vacuoles are also observed in epidermal and gonadal cells. Mutations in genes required for necrosis ameliorate the consequences of infection, suggesting that necrosis is an integral part of host–pathogen interaction that contributes to the pathology associated with infection in *C. elegans*.

15.3.4 Hypoosmotic Shock-Induced Cell Death

Lysosomal integrity and lysosomal proteolytic mechanisms are key factors modulating necrotic cell death in the nematode. Serpins are extracellular or intracellular regulators of proteolytic pathways and inhibitors of multiple peptidases (Silverman et al. 2001). One of the functions of intracellular serpins is the inhibition of lysosomal cysteine peptidases. SRP-6 is such an intracellular serpin in *C. elegans*. *srp-6-null* mutants experiencing hypoosmotic conditions die rapidly and display marked increase of necrotic cell death of the intestinal epithelium (Luke et al. 2007). Ca^{2+} release from endoplasmic reticulum (ER) stores, together with other factors, induces calpain-mediated lysosomal rupture and massive release of lysosomal peptidases into the cytoplasm that mediate necrotic cell death. In addition to hypoosmotic conditions, *srp-6*-null mutants are susceptible to other stressors such as thermal and oxidative stress, hypoxia, and channel hyperactivity. SRP-6 appears to protect cells from lysosomal rupture and also ameliorate the deleterious consequences of lysosomal rupture triggered by various stressors. The protective function of SRP-6 may be adaptive by enhancing the degradation of misfolded proteins or by aiding cytoskeletal rearrangements through altering lysosomal membrane permeability and allowing the leakage of small amounts of peptidases. In the absence of SRP-6, the uncontrolled release of these peptidases leads to necrotic cell death.

15.4 Execution of Necrosis

Intracellular calcium overload through different sources is considered as one of the leading steps in the necrotic pathway. Calcium may enter the cell through voltage-gated channels, and the Na^+/Ca^{2+} exchanger and mutations that increase sodium influx augment calcium entry through these paths. The main intracellular compartment for calcium storage is the endoplasmic reticulum (ER) (Mattson et al. 2000; Paschen 2001; Paschen and Frandsen 2001), where calcium is sequestered by the sarco–endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and is released back to the cytoplasm by ryanodine (RyR) and inositol 1,4,5-trisphosphate receptors [Ins(1,4,5) P3PR]. In *C. elegans*, extensive genetic screens for suppressors of *mec-4*(*d*)-induced necrosis have identified genes required for the execution of necrotic cell death. Two of these genes encode the calcium-binding chaperones calreticulin and calnexin, which were found to regulate intracellular calcium levels and to be required for necrotic cell death (Xu et al. 2001). Moreover, treatment of animals with thapsigargin, a drug that induces release of calcium from the ER to the cytoplasm, triggers necrotic cell death, whereas 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 in addition to calcium homeostasis, intracellular pH is also 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 (Syntichaki et al. 2005). In line with this, 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 (Ishidoh and Kominami 2002).

Lysosomal as well as cytoplasmic proteases have been implicated as downstream effectors of cellular destruction in necrosis. Calpains are cytoplasmic, papain-like cysteine 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 (Yamashima 2004). In primates, calpains rapidly localize to lysosomal membranes after the onset of ischemic episodes (Yamashima 2000). 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 (Syntichaki et al. 2002). It is likely that ensuing cytoplasmic acidification, activation of the lysosomal, low-pH-dependent cathepsins and hydrolases contributes 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 (Artal-Sanz et al. 2006). 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, as lysosomes rupture.

In a recent study from our laboratory, we utilized well-characterized necrosis models in *C. elegans* to dissect the involvement of clathrin-mediated endocytosis and intracellular trafficking by kinesin motor proteins in cellular destruction during necrotic death (Troulinaki and Tavernarakis 2012a). Our findings revealed for the first time that both clathrin-mediated endocytosis and intracellular trafficking are required for the execution of necrosis in the nematode. Downregulation of endocytosis or kinesin-mediated trafficking by interfering with key proteins regulating these processes, including SNT-1, endophilin (UNC-57), AP180 (UNC-11), synaptojanin (UNC-26), heavy chain of kinesin 1 (UNC-116), and the monomeric kinesin UNC-104, significantly suppresses neurodegeneration induced by hyperactive ion channels without affecting the expression, the localization, or the function of the toxic insults.

Moreover, using the same well-defined necrotic cell paradigm, we assayed animals that were deficient for both autophagy and endocytosis and observed significant synergistic protection against degeneration. These results suggest that autophagy and endocytosis function in parallel to contribute to necrotic cell death (Troulinaki and Tavernarakis 2012a). A graphical representation of the crosstalk of the different mechanisms that cooperate in the execution of necrosis is depicted in Fig. 15.3.

15.5 *C. elegans* as a Model for Human Diseases Entailing Necrosis

Nematode genes and major signaling pathways show 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, aging, and associated diseases (Lee et al. 2001; Baumeister and Ge 2002; Poulin et al. 2004). Systematic mapping of gene interactions and signaling pathways implicated in human disease using *C. elegans* has provided better understanding of complex pathologies (Bussey et al. 2006). 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 (Kaletta and Hengartner 2006). Here, we overview *C. elegans* models of human diseases that entail necrosis, focusing on hypoxia, Parkinson's disease, and tauopathies.

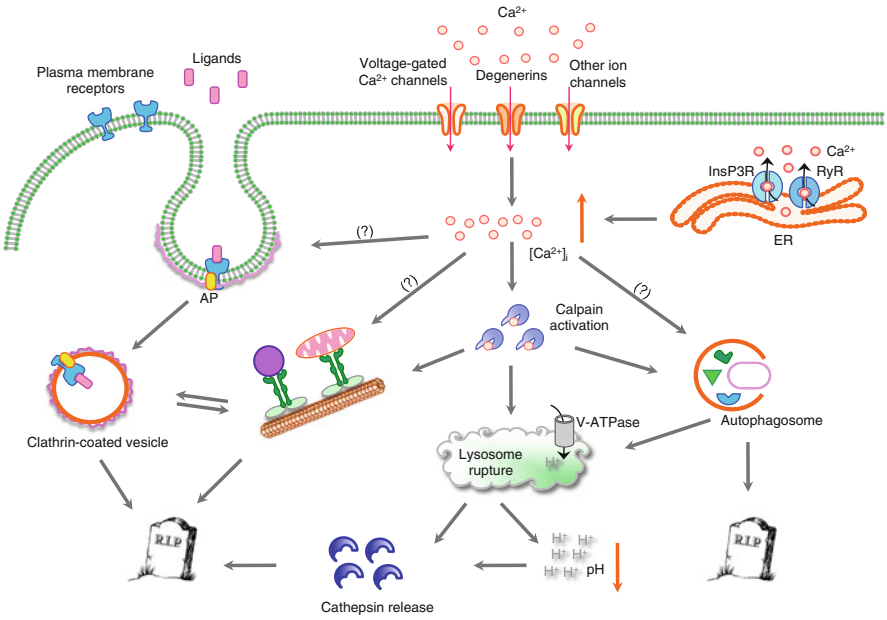

Fig. 15.3 Crosstalk between necrotic cell death mechanisms in *C. elegans*. Necrotic insults converge to increase intracellular Ca^{2+} levels by calcium influx from extracellular pools through various plasma membrane channels, such as voltage-gated receptors or sodium/calcium exchangers (NCX), or by calcium efflux from subcellular organelles with substantial Ca^{2+} stores, such as the endoplasmic reticulum via the ryanodine (RyR) and the inositol 1,4,5-trisphosphate receptors [Ins(1,4,5)P3R]. Ca^{2+} ions then activate cytoplasmic calpain proteases that attack lysosomal membrane proteins, compromising lysosomal integrity and causing the release of hydrolytic enzymes, such as cathepsin proteases. Vacuolar H^+ ATPase (V-ATPase)-mediated lysosomal acidification is important for subsequent acidification of the cytoplasm and enhancement of cathepsin activity. In addition, autophagy is induced during necrosis, either directly or through calpain activation, and synergizes with lysosomal cathepsin proteases to facilitate cellular destruction. Moreover, both clathrin-mediated endocytosis and intracellular trafficking are required for cell death and become upregulated by necrosis-triggering insults. $[Ca^{2+}]_i$ cytoplasmic calcium, *InsP3R* inositol trisphosphate receptor, *RyR* ryanodine receptor, *ER* endoplasmic reticulum, *AP* adaptor proteins for clathrin-mediated endocytosis, *V-ATPase* vacuolar H^+ ATPase (Adapted from Troulinaki and Tavernarakis 2012b)

Clearly, this list is only indicative of the applications of *C. elegans* in understanding complex human pathologies that involve necrotic death, and many more such diseases that are not mentioned here have been usefully modeled in the nematode.

15.5.1 Hypoxia

In humans oxygen deprivation induces cell death in pathological conditions such as stroke and heart attack. In *C. elegans*, hypoxia inflicts necrotic death in a variety of cell types (Scott et al. 2002). Interestingly, mutations in the *daf-2* gene, which

encodes the *C. elegans* insulin/IGF receptor tyrosine kinase, confer resistance against hypoxic cell death. DAF-2 is also known to regulate aging and dauer formation in *C. elegans* (Libina et al. 2003). Related to this, many human neurodegenerative disorders show a late-onset pathogenesis, indicating that aging 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 (KNa) have been identified in cardiomyocytes and neurons as mediators of the protective mechanisms against hypoxic death (Kameyama et al. 1984; Bader et al. 1985). In *C. elegans*, a KNa ion channel is encoded by the *slo-2* gene. *slo-2* mutants are hypersensitive to hypoxic death, suggesting that SLO-2 protects against hypoxia effects. Thus, molecular characterization of KNa channels may allow the development of specific agonists and antagonists, in an effort to combat hypoxia-caused pathologies (Yuan et al. 2003).

15.5.2 Parkinson's Disease

Inclusions of α-synuclein represent a hallmark feature of pathology in both sporadic and familial cases of Parkinson's disease. α-synuclein is the main component of Lewy bodies found in degenerating dopamine neurons (Spillantini et al. 1997). Mutations in the α-synuclein gene or multiplications of the α-synuclein locus have also been associated with some autosomal dominant familial cases of Parkinson's disease (Polymeropoulos et al. 1997; Singleton et al. 2003; Chartier-Harlin et al. 2004). *C. elegans* models of wild-type or mutated human α-synuclein overexpression have been established, either pan-neuronally or specifically in dopaminergic neurons (Lakso et al. 2003; Cao et al. 2005; Cooper et al. 2006; Kuwahara et al. 2006; Qiao et al. 2008), and result in significant motor deficits. No inclusion bodies or α-synuclein aggregation is observed, and intracellular inclusions are rarely observed in these transgenic animals. Overexpression of wild-type or mutant human α-synuclein specifically in worm dopaminergic neurons causes their degeneration, which becomes more pronounced as the animal ages (Cao et al. 2005; Cooper et al. 2006; Kuwahara et al. 2006).

One of the mechanisms implicated in the pathogenesis of Parkinson's disease is mitochondrial dysfunction (Schapira 2008). Autosomal dominant mutations in the leucine-rich repeat kinase 2 (LRRK2) have been associated with both familial and late-onset cases of PD, with G2019S being a prominent such mutation. *C. elegans* engineered to express the human LRRK2 (G2019S) mutant form show extensive loss of dopaminergic neurons (Saha et al. 2009), by increasing their vulnerability to mitochondrial stress. Expression of the wild-type LRRK2 has a milder effect on neuron loss. Similarly, loss-of-function mutations in the *lrk-1* gene, encoding the worm orthologue of LRRK2, also sensitize dopaminergic neurons to mitochondrial stress.

C. elegans models of α-synuclein-induced dopaminergic neurodegeneration have been used as a platform to identify suppressors of dopaminergic neuron loss with some success. For example, specific overexpression of human torsinA or the worm homologue TOR-2 protects dopamine neurons in these models (Cao et al. 2005). In addition, overexpression of the human lysosomal enzyme cathepsin D has a similar neuroprotective effect (Qiao et al. 2008). Several other molecules involved in autophagy, lysosomal function, trafficking, and G protein signaling have also been identified in RNAi suppressor screenings (Hamamichi et al. 2008).

15.5.3 Tau Toxicity

A significant number of neurodegenerative diseases (including in particular Alzheimer's disease, frontotemporal dementia and Parkinsonism linked to chromosome 17) is characterized by neurofibrillary tangles consisting of hyperphosphorylated forms of the microtubule-associated protein *Tau*, encoded by the *mapt* gene (Lee et al. 2001). Although the exact role of *tau* in the pathogenesis of these diseases is not clear, the identification of autosomal dominant mutations in the *mapt* gene indicates a crucial role for the altered *tau* protein in the neurodegenerative process (Hutton et al. 1998; Poorkaj et al. 1998; Spillantini et al. 1998).

Two studies have reported the expression of human *tau* (wild-type *tau* or *tau* carrying FTDP-17 mutations) either pan-neuronally, under the control of the *aex-3* promoter (Kraemer et al. 2003), or specifically in touch receptor neurons of *C. elegans*, under the control of the *mec-7* promoter (Miyasaka et al. 2005). In the first model, expression of the human *tau* results in reduced lifespan, behavioral abnormalities, progressive uncoordinated movement, and accumulation of insoluble phosphorylated *tau*, defective cholinergic neurotransmission, and age-dependent axonal and neuronal degeneration. Among the morphological features of neurodegeneration are axonal vacuolar clearing, collapsed membrane structure, and membranous infoldings and whorls (which are characteristic of necrotic cell death), with associated amorphous *tau* accumulations and abnormal *tau*-positive aggregates. Axonal degeneration and uncoordinated movement are more severe in lines expressing mutant *tau*. However, no *tau* filaments are observed.

15.6 Concluding Remarks

In this chapter, we have attempted to provide a comprehensive overview of the necrotic cell death paradigms that have been established in *C. elegans* (see Table 15.1) and to also convey our current understanding of the molecular mechanisms involved. The rich repertoire of necrotic cell death events that occur in *C. elegans* both during development and in the adult renders the nematode a particularly attractive platform for dissecting the mechanisms of pathological cell death in humans, which is typically mediated by necrotic processes.

Table 15.1 Triggers and paradigms of necrotic death in *C. elegans*. Necrotic stimuli and cell populations affected

Death initiator	Type of insult	Dying cells
mec-4(*u231*) referred to as *mec-4*(*d*)	Hyperactive degenerin ion channel	Touch receptor neurons
mec-10(A673V) referred to as *mec-10*(*d*)	Hyperactive degenerin ion channel	Touch receptor neurons
deg-1(*u38*) referred to as *deg-1*(*d*)	Hyperactive degenerin ion channel	Some polymodal neurons and specific interneurons
unc-8(*n491*)	Hyperactive degenerin ion channel	Motor neurons
pnc-1(*ku212*) or *cog-3*(*ku212*)	Excess nicotinamide levels	Uterine–vulval 1 cells
Nicotinamide	Excess nicotinamide levels	Uterine–vulval 1 cells
deg-3(*u662*) referred to as *deg-3*(*d*)	Hyperactive nicotinic acetylcholine receptor	Subset of sensory neurons and interneurons
gsa-1(Q208L), $G\alpha_s$(Q227L) referred to as α_s(*gf*)	Constitutively active GTP-binding protein $G\alpha_s$	Motor neurons, interneurons, head and tail ganglia neurons, and pharyngeal neurons or epithelial cells (unidentified)
Thapsigargin	Elevation of intracellular Ca^{2+} levels	Random cells (including neuronal)
Δ*glt-3*;α_s(*gf*)	Glutamate-dependent toxicity	Head neurons
Erwinia carotovora *Photorhabdus luminescens*	Pathogen infection	Intestinal, epidermal, and gonadal cells
Hypoxic treatment	Oxygen/energy limitation	Pharynx, gonad primordium, body wall muscles, unidentified cells
α-synuclein	Stress induction	Dopaminergic neurons
LRRK2, leucine-rich repeat kinase 2	Stress induction	Dopaminergic neurons
Tau protein	Stress induction	Several neurons (including motor neurons)

The similarity of necrotic cell death triggered by hyperactive ion channels in *C. elegans* to excitotoxic cell death and neurodegeneration in mammals, both in terms of morphological characteristics and mechanistic aspects, reflects the extensive evolutionary conservation of necrosis-relevant genes between *C. elegans* and mammals. Moreover, conservation of the mechanisms that protect *C. elegans* and mammalian cells from necrotic death inflicted by diverse stimuli, as exhibited, for example, by the hormetic induction of HSF16.1 upon heat preconditioning, provides new prospects for employing the nematode in the battle against degeneration. Concomitantly, modeling of human degenerative disorders, such as Parkinson's disease and others, in *C. elegans* has already accelerated the pace of the molecular dissection of the underlying mechanisms and holds promise for the development and testing of innovative intervention strategies.

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References

Abraham MC, Shaham S (2004) Death without caspases, caspases without death. Trends Cell Biol 14:184–193

Aroian RV, Koga M, Mendel JE, Ohshima Y, Sternberg PW (1990) The let-23 gene necessary for *Caenorhabditis elegans* vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. Nature 348:693–699

Artal-Sanz M, Samara C, Syntichaki P, Tavernarakis N (2006) Lysosomal biogenesis and function is critical for necrotic cell death in Caenorhabditis elegans. J Cell Biol 173:231–239

Bader CR, Bernheim L, Bertrand D (1985) Sodium-activated potassium current in cultured avian neurones. Nature 317:540–542

Baumeister R, Ge L (2002) The worm in us – Caenorhabditis elegans as a model of human disease. Trends Biotechnol 20:147–148

Berger AJ, Hart AC, Kaplan JM (1998) G alphas-induced neurodegeneration in Caenorhabditis elegans. J Neurosci 18:2871–2880

Blondet B, Carpentier G, Ait-Ikhlef A, Murawsky M, Rieger F (2002) Motoneuron morphological alterations before and after the onset of the disease in the wobbler mouse. Brain Res 930:53–57

Bussey H, Andrews B, Boone C (2006) From worm genetic networks to complex human diseases. Nat Genet 38:862–863

Calabrese EJ (2004) Hormesis: a revolution in toxicology, risk assessment and medicine. EMBO Rep 5(1):S37–S40

Cao S, Gelwix CC, Caldwell KA, Caldwell GA (2005) Torsin-mediated protection from cellular stress in the dopaminergic neurons of Caenorhabditis elegans. J Neurosci 25:3801–3812

Chalfie M, Driscoll M, Huang M (1993) Degenerin similarities. Nature 361:504

Chalfie M, Wolinsky E (1990) The identification and suppression of inherited neurodegeneration in Caenorhabditis elegans. Nature 345:410–416

Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, Levecque C, Larvor L, Andrieux J, Hulihan M, Waucquier N, Defebvre L, Amouyel P, Farrer M, Destee A (2004) Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 364:1167–1169

Cleveland DW, Rothstein JD (2001) From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat Rev Neurosci 2:806–819

Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu K, Strathearn KE, Liu F, Cao S, Caldwell KA, Caldwell GA, Marsischky G, Kolodner RD, Labaer J, Rochet JC, Bonini NM, Lindquist S (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 313:324–328

Cooper JD, Messer A, Feng AK, Chua-Couzens J, Mobley WC (1999) Apparent loss and hypertrophy of interneurons in a mouse model of neuronal ceroid lipofuscinosis: evidence for partial response to insulin-like growth factor-1 treatment. J Neurosci 19:2556–2567

Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L, Kroemer G (2007) Cell death modalities: classification and pathophysiological implications. Cell Death Differ 14:1237–1243

Gidday JM (2006) Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci 7:437–448

Hall DH, Gu G, Garcia-Anoveros J, Gong L, Chalfie M, Driscoll M (1997) Neuropathology of degenerative cell death in Caenorhabditis elegans. J Neurosci 17:1033–1045

Hamamichi S, Rivas RN, Knight AL, Cao S, Caldwell KA, Caldwell GA (2008) Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. Proc Natl Acad Sci U S A 105:728–733

Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770–776

Hill RJ, Sternberg PW (1992) The gene lin-3 encodes an inductive signal for vulval development in *C. elegans*. Nature 358:470–476

Horvitz HR, Sternberg PW, Greenwald IS, Fixsen W, Ellis HM (1983) Mutations that affect neural cell lineages and cell fates during the development of the nematode Caenorhabditis elegans. Cold Spring Harb Symp Quant Biol 48(Pt 2):453–463

Huang L, Hanna-Rose W (2006) EGF signaling overcomes a uterine cell death associated with temporal mis-coordination of organogenesis within the *C. elegans* egg-laying apparatus. Dev Biol 300:599–611

Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P (1998) Association of missense and 5′-splice-site mutations in *tau* with the inherited dementia FTDP-17. Nature 393:702–705

Ishidoh K, Kominami E (2002) Processing and activation of lysosomal proteinases. Biol Chem 383:1827–1831

Kaletta T, Hengartner MO (2006) Finding function in novel targets: *C. elegans* as a model organism. Nat Rev Drug Discov 5:387–398

Kameyama M, Kakei M, Sato R, Shibasaki T, Matsuda H, Irisawa H (1984) Intracellular Na+ activates a K+ channel in mammalian cardiac cells. Nature 309:354–356

Kauppinen RA, Enkvist K, Holopainen I, Akerman KE (1988a) Glucose deprivation depolarizes plasma membrane of cultured astrocytes and collapses transmembrane potassium and glutamate gradients. Neuroscience 26:283–289

Kauppinen RA, McMahon HT, Nicholls DG (1988b) Ca^{2+}-dependent and Ca^{2+}-independent glutamate release, energy status and cytosolic free Ca^{2+} concentration in isolated nerve terminals following metabolic inhibition: possible relevance to hypoglycaemia and anoxia. Neuroscience 27:175–182

Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257

Kimble J, Hirsh D (1979) The postembryonic cell lineages of the hermaphrodite and male gonads in Caenorhabditis elegans. Dev Biol 70:396–417

Korswagen HC, Park JH, Ohshima Y, Plasterk RH (1997) An activating mutation in a Caenorhabditis elegans Gs protein induces neural degeneration. Genes Dev 11:1493–1503

Korswagen HC, van der Linden AM, Plasterk RH (1998) G protein hyperactivation of the Caenorhabditis elegans adenylyl cyclase SGS-1 induces neuronal degeneration. EMBO J 17:5059–5065

Kourtis N, Nikoletopoulou V, Tavernarakis N (2012) Small heat-shock proteins protect from heat-stroke-associated neurodegeneration. Nature 490:213–218

Kourtis N, Tavernarakis N (2007) Non-developmentally programmed cell death in Caenorhabditis elegans. Semin Cancer Biol 17:122–133

Kraemer BC, Zhang B, Leverenz JB, Thomas JH, Trojanowski JQ, Schellenberg GD (2003) Neurodegeneration and defective neurotransmission in a Caenorhabditis elegans model of tauopathy. Proc Natl Acad Sci U S A 100:9980–9985

Kuwahara T, Koyama A, Gengyo-Ando K, Masuda M, Kowa H, Tsunoda M, Mitani S, Iwatsubo T (2006) Familial Parkinson mutant alpha-synuclein causes dopamine neuron dysfunction in transgenic Caenorhabditis elegans. J Biol Chem 281:334–340

Lakso M, Vartiainen S, Moilanen AM, Sirvio J, Thomas JH, Nass R, Blakely RD, Wong G (2003) Dopaminergic neuronal loss and motor deficits in Caenorhabditis elegans overexpressing human alpha-synuclein. J Neurochem 86:165–172

Lee VM, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. Annu Rev Neurosci 24:1121–1159

Lehotsky J, Kaplan P, Murin R, Raeymaekers L (2002) The role of plasma membrane Ca^{2+} pumps (PMCAs) in pathologies of mammalian cells. Front Biosci 7:d53–d84

Libina N, Berman JR, Kenyon C (2003) Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. Cell 115:489–502

Luke CJ, Pak SC, Askew YS, Naviglia TL, Askew DJ, Nobar SM, Vetica AC, Long OS, Watkins SC, Stolz DB, Barstead RJ, Moulder GL, Bromme D, Silverman GA (2007) An intracellular serpin regulates necrosis by inhibiting the induction and sequelae of lysosomal injury. Cell 130:1108–1119

Magni G, Amici A, Emanuelli M, Raffaelli N, Ruggieri S (1999) Enzymology of NAD+ synthesis. Adv Enzymol Relat Areas Mol Biol 73:135–182, xi

Mano I, Driscoll M (2009) Caenorhabditis elegans glutamate transporter deletion induces AMPA-receptor/adenylyl cyclase 9-dependent excitotoxicity. J Neurochem 108:1373–1384

Mattson MP, LaFerla FM, Chan SL, Leissring MA, Shepel PN, Geiger JD (2000) Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. Trends Neurosci 23:222–229

Miyasaka T, Ding Z, Gengyo-Ando K, Oue M, Yamaguchi H, Mitani S, Ihara Y (2005) Progressive neurodegeneration in *C. elegans* model of tauopathy. Neurobiol Dis 20:372–383

Newman AP, White JG, Sternberg PW (1996) Morphogenesis of the *C. elegans* hermaphrodite uterus. Development 122:3617–3626

Paschen W (2001) Dependence of vital cell function on endoplasmic reticulum calcium levels: implications for the mechanisms underlying neuronal cell injury in different pathological states. Cell Calcium 29:1–11

Paschen W, Frandsen A (2001) Endoplasmic reticulum dysfunction – a common denominator for cell injury in acute and degenerative diseases of the brain? J Neurochem 79:719–725

Pavlikova M, Tatarkova Z, Sivonova M, Kaplan P, Krizanova O, Lehotsky J (2009) Alterations induced by ischemic preconditioning on secretory pathways Ca^{2+}-ATPase (SPCA) gene expression and oxidative damage after global cerebral ischemia/reperfusion in rats. Cell Mol Neurobiol 29:909–916

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045–2047

Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD (1998) *Tau* is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol 43:815–825

Poulin G, Nandakumar R, Ahringer J (2004) Genome-wide RNAi screens in Caenorhabditis elegans: impact on cancer research. Oncogene 23:8340–8345

Qiao L, Hamamichi S, Caldwell KA, Caldwell GA, Yacoubian TA, Wilson S, Xie ZL, Speake LD, Parks R, Crabtree D, Liang Q, Crimmins S, Schneider L, Uchiyama Y, Iwatsubo T, Zhou Y, Peng L, Lu Y, Standaert DG, Walls KC, Shacka JJ, Roth KA, Zhang J (2008) Lysosomal enzyme cathepsin D protects against alpha-synuclein aggregation and toxicity. Mol Brain 1:17

Saha S, Guillily MD, Ferree A, Lanceta J, Chan D, Ghosh J, Hsu CH, Segal L, Raghavan K, Matsumoto K, Hisamoto N, Kuwahara T, Iwatsubo T, Moore L, Goldstein L, Cookson M, Wolozin B (2009) LRRK2 modulates vulnerability to mitochondrial dysfunction in Caenorhabditis elegans. J Neurosci 29:9210–9218

Schapira AH (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. Lancet Neurol 7:97–109

Scott BA, Avidan MS, Crowder CM (2002) Regulation of hypoxic death in *C. elegans* by the insulin/IGF receptor homolog DAF-2. Science 296:2388–2391

Shaham S (1998) Identification of multiple Caenorhabditis elegans caspases and their potential roles in proteolytic cascades. J Biol Chem 273:35109–35117

Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG, Irving JA, Lomas DA, Luke CJ, Moyer RW, Pemberton PA, Remold-O'Donnell E, Salvesen GS, Travis J, Whisstock JC (2001) The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. J Biol Chem 276:33293–33296

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) Alpha-Synuclein locus triplication causes Parkinson's disease. Science 302:841

Spillantini MG, Crowther RA, Kamphorst W, Heutink P, van Swieten JC (1998) *Tau* pathology in two Dutch families with mutations in the microtubule-binding region of tau. Am J Pathol 153:1359–1363

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388:839–840

Sulston JE, Albertson DG, Thomson JN (1980) The Caenorhabditis elegans male: postembryonic development of nongonadal structures. Dev Biol 78:542–576

Sulston JE, Horvitz HR (1977) Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Dev Biol 56:110–156

Sulston JE, Schierenberg E, White JG, Thomson JN (1983) The embryonic cell lineage of the nematode Caenorhabditis elegans. Dev Biol 100:64–119

Syntichaki P, Samara C, Tavernarakis N (2005) The vacuolar H+-ATPase mediates intracellular acidification required for neurodegeneration in *C. elegans*. Curr Biol 15:1249–1254

Syntichaki P, Tavernarakis N (2003) The biochemistry of neuronal necrosis: rogue biology? Nat Rev Neurosci 4:672–684

Syntichaki P, Tavernarakis N (2004) Genetic models of mechanotransduction: the nematode Caenorhabditis elegans. Physiol Rev 84:1097–1153

Syntichaki P, Xu K, Driscoll M, Tavernarakis N (2002) Specific aspartyl and calpain proteases are required for neurodegeneration in *C. elegans*. Nature 419:939–944

Tavernarakis N, Driscoll M (2001) Degenerins. At the core of the metazoan mechanotransducer? Ann N Y Acad Sci 940:28–41

Treinin M, Chalfie M (1995) A mutated acetylcholine receptor subunit causes neuronal degeneration in *C. elegans*. Neuron 14:871–877

Treinin M, Gillo B, Liebman L, Chalfie M (1998) Two functionally dependent acetylcholine subunits are encoded in a single Caenorhabditis elegans operon. Proc Natl Acad Sci U S A 95:15492–15495

Troulinaki K, Tavernarakis N (2012a) Endocytosis and intracellular trafficking contribute to necrotic neurodegeneration in *C. elegans*. EMBO J 31:654–666

Troulinaki K, Tavernarakis N (2012b) Necrotic cell death and neurodegeneration: the involvement of endocytosis and intracellular trafficking. Worm 1:176–181

van der Horst A, Schavemaker JM, Pellis-van Berkel W, Burgering BM (2007) The Caenorhabditis elegans nicotinamidase PNC-1 enhances survival. Mech Ageing Dev 128:346–349

Van Montfort R, Slingsby C, Vierling E (2001) Structure and function of the small heat shock protein/alpha-crystallin family of molecular chaperones. Adv Protein Chem 59:105–156

Vlachos M, Tavernarakis N (2010) Non-apoptotic cell death in Caenorhabditis elegans. Dev Dyn 239:1337–1351

Vrablik TL, Huang L, Lange SE, Hanna-Rose W (2009) Nicotinamidase modulation of NAD+ biosynthesis and nicotinamide levels separately affect reproductive development and cell survival in *C. elegans*. Development 136:3637–3646

Walker NI, Harmon BV, Gobe GC, Kerr JF (1988) Patterns of cell death. Methods Achiev Exp Pathol 13:18–54

Wong D, Bazopoulou D, Pujol N, Tavernarakis N, Ewbank JJ (2007) Genome-wide investigation reveals pathogen-specific and shared signatures in the response of Caenorhabditis elegans to infection. Genome Biol 8:R194

Xu K, Tavernarakis N, Driscoll M (2001) Necrotic cell death in *C. elegans* requires the function of calreticulin and regulators of $Ca^{(2+)}$ release from the endoplasmic reticulum. Neuron 31:957–971

Yamashima T (2000) Implication of cysteine proteases calpain, cathepsin and caspase in ischemic neuronal death of primates. Prog Neurobiol 62:273–295

Yamashima T (2004) Ca^{2+}-dependent proteases in ischemic neuronal death: a conserved 'calpain-cathepsin cascade' from nematodes to primates. Cell Calcium 36:285–293

Yuan A, Santi CM, Wei A, Wang ZW, Pollak K, Nonet M, Kaczmarek L, Crowder CM, Salkoff L (2003) The sodium-activated potassium channel is encoded by a member of the Slo gene family. Neuron 37:765–773

Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The *C. elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 75: 641–652