SPECIAL ISSUE REVIEWS–A PEER REVIEWED FORUM

Non-Apoptotic Cell Death in *Caenorhabditis elegans*

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The simple nematode worm *Caenorhabditis elegans* has been instrumental in deciphering the molecular mechanisms underlying apoptosis. Beyond apoptosis, several paradigms of non-apoptotic cell death, either genetically or extrinsically triggered, have also been described in *C. elegans*. Remarkably, non-apoptotic cell death in worms and pathological cell death in humans share numerous key features and mechanistic aspects. Such commonalities suggest that similarly to apoptosis, non-apoptotic cell death mechanisms are also conserved, and render the worm a useful organism, in which to model and dissect human pathologies. Indeed, the genetic malleability and the sophisticated molecular tools available for *C. elegans* have contributed decisively to advance our understanding of non-apoptotic cell death. Here, we review the literature on the various types of non-apoptotic cell death in *C. elegans* and discuss the implications, relevant to pathological conditions in humans. *Developmental Dynamics 239:1337–1351, 2010.* © 2010 Wiley-Liss, Inc.

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INTRODUCTION

Since its introduction as an experimental model organism by Sydney Brenner (Brenner, 1974), *Caenorhabditis elegans* has played a pivotal role in the study of fundamental biological processes such as cell death. The transparency of the animal allows individual nuclei to be readily visualized using differential interference contrast optics. *C. elegans* development is invariant and a complete lineage map (the sequence of somatic cell divisions from the fertilized embryo to the 959-cell adult hermaphrodite organism) has been constructed. The lineage map provides information about which cells die, when and where they die. During development, 131 somatic cells undergo programmed cell death: 113 cells die during embryonic, and 18 during early postembryonic stages (Sulston and Horvitz, 1977; Sulston et al., 1983). In addition to these two waves of programmed, developmental cell death, there is also a third wave of cell death which occurs within the adult hermaphrodite germline (Gumienny et al., 1999). With the exception of the linker cell death, which will be discussed later, all of these death events are apoptotic (Lettre and Hengartner, 2006; Blum et al., 2008). The most prominent morphological features of apoptotic cells include a high-refractile button-like appearance, nuclear condensation, chromatin clumping and a normal appearance of cytosolic organelles (Sulston and Horvitz, 1977).

Many paradigms of cell death, distinct from apoptosis on the basis of morphological characteristics of dying cells and the molecular mechanisms involved, have been observed in *C. elegans*. Of interest, certain features of non-apoptotic cell death in worms are highly similar to developmental and pathological cell death in humans, suggesting that the molecular mechanisms responsible for cellular destruction in both organisms are evolutionarily conserved. Non-apoptotic cell death in *C. elegans* may either be programmed (as in the case of the

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linker cell) or accidental. Similarly to humans, accidental cell death in worms can be triggered by exposure to harsh environmental conditions, or by mutations that lead to extreme changes in physiological conditions. Furthermore, heterologous expression of human disease-related genes, which may not be present in the worm genome, can trigger non-apoptotic cell death in *C. elegans*. In the following sections, we present the paradigms and mechanisms of non-apoptotic cell death in *C. elegans* discovered thus far, and discuss their relevance to human pathological conditions and normal development.

NECROTIC CELL DEATH

Necrosis is a type of cell death that occurs when cells are exposed to extreme environmental or genetically encoded insults (Walker et al., 1988). In humans, changes in physiological conditions such as acute energy depletion, hypoxia, ischemia, exposure to toxins, reactive oxygen metabolites, or extreme temperature changes can ultimately cause necrotic cell death (Walker et al., 1988; Nicotera et al., 1999). Necrotic cell death is a significant contributor to many human pathological conditions and neurodegenerative diseases (Syntichaki and Tavernarakis, 2003).

In addition, necrotic cell death has been found to occur during normal mammalian development as a programmed event. For example, during normal renewal of the small intestine, enterocytes (intestinal epithelial cells) die either by apoptosis or necrosis in a regular manner (Mayhew et al., 1999). Similarly, examination of a large number of human biopsies (collected during routine colonoscopies), has shown that the lower regions of the large intestine crypts commonly contain isolated necrotic colonocytes (Barkla and Gibson, 1999) supporting a role of necrosis in normal cell loss. Ovine follicular maturation during oogenesis has been shown to entail both apoptosis and necrosis (Murdoch et al., 1999). Furthermore, to facilitate longitudinal growth of bones, chondrocytes at the lower zone of the growth plate undergo necrotic death during development (Roach and Clarke, 2000).

Although it was believed that necrosis is a chaotic and unregulated process, recent studies suggest that conserved mechanisms underlie cellular destruction during necrotic cell death (Syntichaki and Tavernarakis, 2002). Several paradigms of necrotic cell death have been identified and characterized in *C. elegans* and are presented in the sections following below.

Ionic Imbalance-Induced Necrosis

In *C. elegans*, gain-of-function mutations in genes encoding ion channels of the DEG/ENaC superfamily (named after the *C. elegans* degenerins and the mammalian epithelial sodium ion channels) lead to necrotic death of the cells that express these aberrant ion channels (Syntichaki and Tavernarakis, 2003). For example, dominant (d) alleles of two degenerin genes, *deg-1(d)* and *mec-4(d)*, induce degeneration of specific interneurons of the posterior touch sensory circuit and the six touch receptor neurons of *C. elegans*, respectively (Chalfie and Wolinsky, 1990; Driscoll and Chalfie, 1991). The time of degeneration onset correlates with the onset of degenerin expression and the severity of the effects is expression level-dependent (Hall et al., 1997). Other degenerin genes that can induce necrotic cell death when carrying gain-of-function mutations are *mec-10*, *unc-8*, and *unc-105* (Table1; Syntichaki and Tavernarakis, 2004). Necrotic cell death initiated by hyperactive degenerins in worms shows mechanistic and morphological similarities to excitotoxic cell death in mammals (see below; Choi, 1992).

At the cellular level, a main morphological characteristic of necrotic cell death is the infolding of regions of the plasma membrane that are internalized and appear to coalesce into electron-dense membranous whorls (Hall et al., 1997). These whorls are similar to analogous membranous structures observed in mouse models of amyotrophic lateral sclerosis (Blondet et al., 2002) and neuronal ceroid lipofuscinosis (Cooper et al., 1999). Nucleus distortion and chromatin clumping are also observed. During the death process, cell swells to several times its normal diameter (Fig. 1). After degradation of internal contents, the cell corpse is removed through engulfment by the neighboring cells. This process is regulated by genes (*ced-2*, *ced-5*, and *ced-10*) that also control the engulfment of apoptotic corpses (Chung et al., 2000).

Gain-of-function mutations in genes other than degenerins can also cause cell death similar to necrosis. For example, dominant alleles of the *deg-3* gene, encoding the nicotinic acetylcholine receptor alpha subunit, and hyperactivated G-protein alpha-subunit variants can cause degeneration of specific neurons (Treinin and Chalfie, 1995; Korswagen et al., 1997, 1998; Berger et al., 1998).

Genetic screens and pharmacological treatments have identified several genes required for necrotic cell death triggered by hyperactive ion channels. These studies converge to highlight the critical role of Ca^{2+} homeostasis in necrosis. Mutations in genes encoding regulators of intracellular calcium levels and pharmacological treatments that regulate the release of calcium from intracellular stores dramatically influence both the initiation and the progress of cell death (Xu et al., 2001). After cytoplasmic Ca^{2+} overload, inappropriate activation of calcium-dependent calpain proteases mediates lysosomal membrane damage or rupture and leads to the release of their acidic contents, including lysosomal cathepsin proteases. Two specific calpain proteases (TRA-3 and CLP-1) and two cathepsins (ASP-3 and ASP-4) are required for necrotic cell death triggered by various stimuli in *C. elegans* (Syntichaki et al., 2002). Intracellular pH homeostasis together with lysosomal biogenesis and function also play important roles in necrotic cell death (Syntichaki et al., 2005; Artal-Sanz et al., 2006). Mutations or pharmacological treatments that impair lysosomal biogenesis or acidification suppress necrosis while mutations that increase the number of lysosomes enhance cellular destruction. These findings are consistent with studies in primates implicating an analogous calpain–cathepsin axis in the execution of necrotic cell death following ischemic episodes and stroke (Yamashima, 2000, 2004).

TABLE 1. Triggers and Paradigms of Non-apoptotic Cell Death in *C. elegans*

Death initiator	Type of insult	Dying cells	Reference
mec-4(*u231*), referred to as *mec-4*(*d*)	Hyperactive degenerin ion channel	Touch receptor neurons	(Driscoll and Chalfie, 1991)
mec-10(A673V), referred to as *mec-10*(*d*)	Hyperactive degenerin ion channel	Touch receptor neurons	(Huang and Chalfie, 1994)
deg-1(*u38*), referred to as *deg-1*(*d*)	Hyperactive degenerin ion channel	Some polymodal neurons and specific interneurons	(Chalfie and Wolinsky, 1990)
unc-8(*n491*)	Hyperactive degenerin ion channel	Motorneurons	(Shreffler et al., 1995; Tavernarakis et al., 1997)
pnc-1(*ku212*) or *cog-3*(*ku212*) (as was initially named)	Excess nicotinamide levels	Uterine vulval 1 cells	(Huang and Hanna-Rose, 2006; Vrablik et al., 2009)
Nicotinamide	Excess nicotinamide levels	Uterine vulval 1 cells	(Vrablik et al., 2009)
deg-3(*u662*), referred to as *deg-3*(*d*)	Hyperactive nicotinic acetylcholine receptor	Subset of sensory neurons and interneurons	(Treinin and Chalfie, 1995)
gsa-1(Q208L) and $G\alpha_s$(Q227L), referred to as α_s(*gf*)	Constitutively active GTP-binding protein $G\alpha_s$	Motorneurons, interneurons, head and tail ganglia neurons, and pharyngeal neurons or epithelial cells (unidentified)	(Korswagen et al., 1997; Berger et al., 1998)
Thapsigargin	Elevation of intracellular Ca^{2+} levels	Random cells (including neuronal)	(Xu et al., 2001)
Δ*glt-3*;α_s(*gf*)	Glutamate-dependent toxicity	Head neurons	(Mano and Driscoll, 2009)
Erwinia carotovora Photorabdus luminescens	Pathogen infection	Intestinal, epidermal, and gonadal cells	(Wong et al., 2007)
Hypoxic treatment	Oxygen/energy limitation	Pharynx, gonad primordium, body wall muscles, unidentified cells	(Scott et al., 2002)
Anoxic treatment	Oxygen/energy limitation	Cells throughout the whole body	(Menuz et al., 2009)
Chemical inhibitors of the respiratory chain (sodium azide)	Oxygen limitation	Pharynx, gonad primordium, body wall muscles, unidentified cells	(Scott et al., 2002)
MPP^+ (1-methyl-4-phenylpyridinium)	Toxin exposure	Dopaminergic neurons	(Braungart et al., 2004; Pu and Le, 2008)
6-OHDA (6-hydroxydopamine)	Toxin exposure	Dopaminergic neurons	(Nass et al., 2002)
α-synuclein	Stress induction	Dopaminergic neurons	(Lakso et al., 2003; Cao et al., 2005; Cooper et al., 2006; Kuwahara et al., 2006; Qiao et al., 2008)
LRRK2 (leucine-rich repeat kinase 2)	Stress induction	Dopaminergic neurons	(Saha et al., 2009)
Tau protein	Stress induction	Several neurons (including motor neurons)	(Kraemer et al., 2003)
polyQ proteins (sensitized background)	Stress induction	Sensory neurons	(Faber et al., 1999)
dys-1 dystrophin-like gene mutations (sensitized background)	Stress induction	Muscle cells	(Gieseler et al., 2000)
egl-19(*ad965*; sensitized background)	Hyperactive voltage-gated calcium channel	Muscle cells	(Mariol and Segalat, 2001)
tph-1(*mg280*; sensitized background)	Reduction of serotonin levels	Muscle cells	(Carre-Pierrat et al., 2006b)
lin-24(*n432*)	Cytotoxicity	Pn.p hypodermal blast cells	(Galvin et al., 2008)
lin-33(*n1043*)	Cytotoxicity	Pn.p hypodermal blast cells	(Galvin et al., 2008)
Radiation	DNA damage	Clonogens (multipotent vulva precursor cells)	(Weidhaas et al., 2006a)

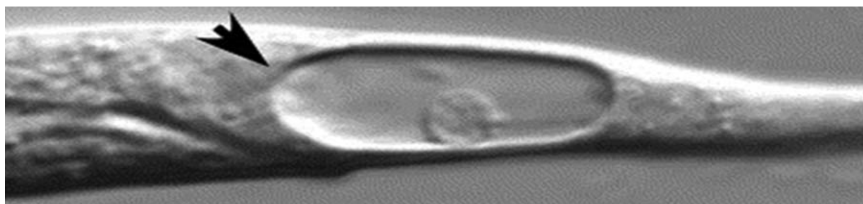

Fig. 1. Necrotic death of *C. elegans* neurons. Differential interference contrast (DIC) image of a degenerating touch receptor neuron (arrow) expressing a hyperactive degenerin ion channel. The degenerating cell swells to several times its normal diameter, while the nucleus appears distended with distorted morphology.

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Recent findings implicate autophagy (the main cellular process for bulk protein and organelle recycling) in necrotic cell death in *C. elegans* (Toth et al., 2007; Vellai et al., 2007; Samara et al., 2008). Excessive autophagosome formation is observed early during necrotic cell death and is reduced at later stages of cellular destruction. Inactivation of autophagy genes or pharmacological inhibition of autophagy partially suppresses necrotic cell death triggered by hyperactive ion channels or a hyperactive G-protein alpha subunit variant. The autophagic process synergizes with the lysosomal proteolytic pathways to facilitate necrotic cell death (Fig. 2). Necrosis is enhanced under conditions of autophagy up-regulation (inhibition of the negative regulator TOR-kinase, or under nutrient deprivation conditions).

Alteration of the nuclear pore complex (NPC), which leads to loss of nuclear permeability and aberrant nucleocytoplasmic transport has been associated with virus-mediated cell death and caspase-dependent apoptosis (Faleiro and Lazebnik, 2000; Gustin and Sarnow, 2001). Degradation of NPC components and alterations in nuclear permeability have been observed in two paradigms of Ca^{2+}-associated cell death (glutamate excitotoxicity in mammalian neurons and necrotic cell death triggered by *deg-3(d)* in *C. elegans*; Bano et al., 2009). Nuclear permeabilization and increase in NPC leakiness, allow proteins normally localized in the cytoplasm to diffuse through in the nuclei of dying neurons of *deg-3(d)* mutants. Altered nuclear membrane permeability is mediated by Ca^{2+} overload during the onset of cell death and Ca^{2+}-activated calpains in both mammalian and *C. elegans* dying neurons. Such alterations in the NPC and the nucleocytoplasmic transport may serve as a death-signal amplification mechanism, by contributing to cell disassembly.

In mammals, massive release of the excitatory neurotransmitter glutamate (Glu) leads to hyperexcitation and necrotic death of postsynaptic cells, a phenomenon known as excitotoxicity (Kauppinen et al., 1988a,b; Choi, 1992). A paradigm of glutamate-related neuronal necrosis has recently been described in *C. elegans* (Mano and Driscoll, 2009). Mutations in *glt-3*, a gene encoding a Glu transporter, when combined with expression of a constitutively active form of the G-protein alpha subunit cause extensive necrotic neuronal death of head interneurons. The necrotic effects are mediated by the Ca^{2+}-permeable AMPA-type Glu receptor. AMPA receptor hyperactivation by excess Glu and elevated G-protein signaling may cause enhanced Ca^{2+} influx which in turn initiates neurodegeneration.

Vulva Cell Necrosis

In wild-type *C. elegans* animals, synchronization of vulva and uterus development ensures a proper connection between these two components of the reproductive system. As a result of this connection, vulval LIN-3/EGF signals emanating from the anchor cell in the somatic gonad are recognized by specific cells of the uterine π lineage, which become differentiated to uterine vulval 1 (uv1) cells.

In a screen for egg-laying defective mutants, an allele (*ku212*) of a gene originally named *cog-3* (connection-of-gonad defective) was isolated. This allele causes loss of temporal synchronization between the vulva and the uterus during development, because of delayed uterine formation (Huang and Hanna-Rose, 2006). This results in connection defects between the uterus and the vulva that compromise EGF signaling. As a consequence, differentiation of the four uv1 cells fails and these cells die by necrosis. Dying cells display necrotic vacuolar morphology. Cell death requires mediators of necrosis CLP-1, ASP-3, and ASP-4, while it is CED-4 independent.

Compromised EGF signaling between the uterus and the vulva (normally required for uv1 cell specification) was initially thought to be responsible for uv1 cell necrosis because ectopic expression of LIN-3/EGF or constitutively active LET-23/EGF receptor blocked uv1 cell necrosis.

However, a recent study revealed that the *ku212* allele maps to the *pnc-1* gene locus, which encodes for 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.

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

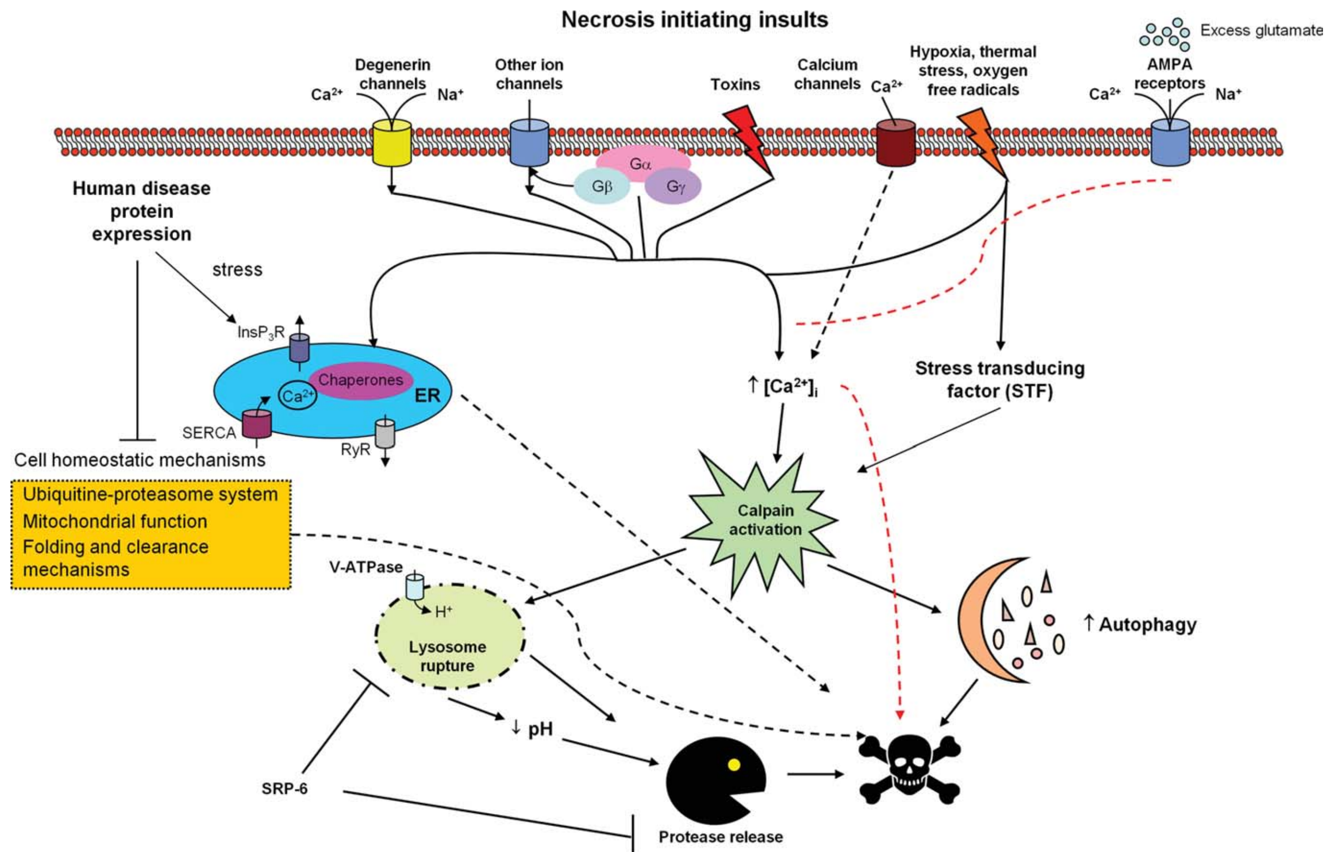

Fig. 2. Mechanisms of necrotic cell death in *C. elegans*. Diverse insults (either genetic or environmental) lead to elevated cytoplasmic calcium concentration. Subsequent activation of calpain proteases causes lysosomal membrane rupture and the release of lysosomal contents in the cytoplasm. The concomitant release of lysosomal proteolytic enzymes, combined with cytoplasmic acidification mediates cellular destruction. Autophagy up-regulation (either directly upon necrosis induction and/or through calpain activation) can synergize with lysosomal proteolytic pathways to facilitate cell demise. A core stress response pathway regulated by the SRP-6 peptidase inhibitor influences necrotic cell death induced by various stressors. SRP-6 protects from cellular injury by ameliorating lysosome rupture and its consequences. Glutamate-induced excitotoxicity in *C. elegans* may involve related mechanisms. Expression of human disease proteins in worms also disturbs cellular homeostatic mechanisms and induces stress, which, beyond a certain threshold, becomes detrimental for the cell. $[Ca^{2+}]$i, cytoplasmic calcium concentration; ER, endoplasmic reticulum; InsP3R, inositol triphosphate receptor; RyR, ryanodine receptor; SERCA, sarco-endoplasmic reticulum Ca^{2+}-ATPase; V-ATPase, vacuolar H^+-ATPase; SRP-6, serine protease inhibitor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor.

integral part of host-pathogen interaction that contributes to the pathology associated with infection in *C. elegans*.

Hypoosmotic Shock-Induced Cell Death

As noted above, 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

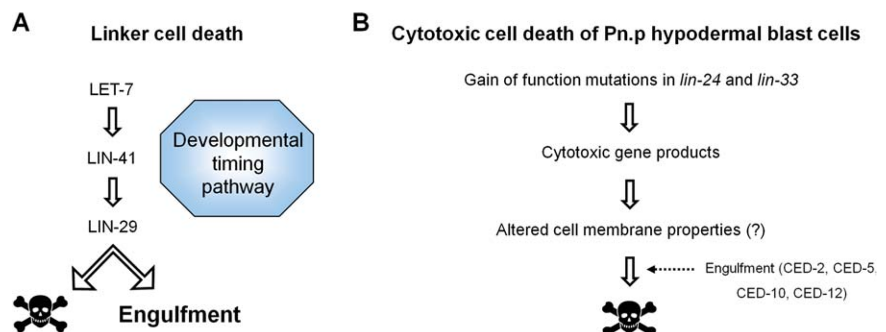

Fig. 3. **A:** Linker cell death in *C. elegans*. The *let-7* microRNA (as part of the developmental timing pathway) activates the LIN-29 Zn-finger transcription factor, which acts cell autonomously to regulate death and engulfment of the linker cell. Engulfment is mediated by an unknown mechanism. B) Cytotoxic cell death in *C. elegans*. Cytotoxic gene products encoded by gain-of-function mutations in *lin-24* and *lin-33* genes cause the inappropriate death of Pn.p hypodermal blast cells, probably by altering cell membrane properties. The canonical engulfment process involving CED-2, CED-5, CED-10, and CED-12 is required for cytotoxic cell death.

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

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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.

HYPOXIC DEATH

Hypoxic cell death is common in human pathological conditions, such as stroke and heart disease (Lee et al., 1999). In *C. elegans*, hypoxia or exposure to sodium azide (which mimics hypoxic conditions) leads to hypoxic cell death (Scott et al., 2002). Specific mutations in the insulin/IGF receptor DAF-2 render animals hypoxia resistant. Hypoxia resistance is temperature-sensitive but not stage- or age-specific. It is also independent of dauer formation, stress resistance, and effects on ageing associated with *daf-2* mutations. At the cellular level, hypoxia causes the appearance of necrotic-like cells in the pharynx, the body muscles, the gonad, and other body parts. The number of necrotic-like cells is reduced in hypoxia-resistant *daf-2* mutants. Neuronal axon beading morphology and muscle nuclear fragmentation induced by hypoxia are also rescued in these mutants. Pan-neuronal or muscle, but not intestinal, *daf-2(+)* expression increases hypoxic death, both at the organismal and the cellular level.

Several studies have identified modulators and suppressors of hypoxic death in *C. elegans*. Na^+-activated potassium channels (K_{Na}) may provide protection against ischemia in cardiomyocytes and neurons (Kameyama et al., 1984). In *C. elegans*, *slo-2* encodes a mammalian ortholog of the Na^+-activated potassium channel (rSLO-2). SLO-2 is activated by Ca^{2+}/Cl^- (Yuan et al., 2000). *slo-2* mutants are hypersensitive to the death effects of hypoxia, suggesting a protective role for the SLO-2 channel in hypoxia (Yuan et al., 2003). An association between global translational rates, unfolded protein response and hypoxia has also been reported (Anderson et al., 2009). Inactivation of *rrt-1* and other genes encoding aminoacyl-tRNA synthetases, which catalyze loading of cognate tRNA(s) with specific amino acids, confer resistance to hypoxia. RRT-1 activity is required both in somatic and germ cells to mediate hypoxia sensitivity. Features of hypoxic cell death in muscles and neurons are abolished in *rrt-1(lf)* mutants. Hypoxia resistance is not dependent on the developmental stage and is inversely correlated to relative translational rate. The inhibitory effects on translation, mediated by the inactivation of *rrt-1* and the unfolded protein response induced by hypoxia (Koumenis et al., 2007; Anderson et al., 2009) appear to synergistically promote hypoxia resistance.

Hypoxic preconditioning refers to pre-exposure to a mild, sublethal hypoxic insult that confers protection of mammalian neurons and cardiac myocytes against a subsequent hypoxic insult (Gidday, 2006). This response is adaptive and reversible. Hypoxic preconditioning also confers resistance to hypoxia in neurons and myocytes of *C. elegans* (Dasgupta et al., 2007). Hypoxic preconditioning is dependent on CED-4, while other molecules of the core apoptotic machinery (EGL-1, CED-3, and CED-9) are not required. Genome-wide RNAi screens have identified many genes conferring hypoxia sensitivity in the nematode (Mabon et al., 2009). These genes are grouped in diverse functional classes, encoding proteasomal components and molecules regulating transcription and translation, chromatin remodeling, and nucleosomal histone modifications, among others. Genes influencing aging have also been identified.

The autophagic process (macroautophagy) is up-regulated during hypoxia and ischemia in mammalian neurons and cardiac myocytes (Chu, 2008; Gustafsson and Gottlieb, 2008). Inhibition of autophagy results in increased sensitivity to hypoxia, in *C. elegans* (Samokhvalov et al., 2008). Hypoxia-induced necrotic cell death and damage of neurons and myocytes are also increased by inhibition of autophagy. Blocking of apoptotic and necrotic cell death pathways abolished increased hypoxic sensitivity caused by autophagy inhibition. Of interest, sodium azide-induced hypoxic animal death is partially suppressed by inhibition of autophagy, suggesting that autophagy contributes to pathology in this hypoxia paradigm (Samara et al., 2008).

C. elegans has been shown to survive for at least 48 hr under anoxic conditions. Prolonged anoxia causes necrosis in all tissues of the worm, evident by the accumulation of propidium iodide-stained necrotic cells (Menuz et al., 2009). Specific *daf-2* mutants are highly resistant to long-term anoxia, and this resistance is mediated by two glycolytic enzymes (glyceraldehyde-3-phosphate dehydrogenases) encoded by the genes *gpd-2* and *gpd-3* (Mendenhall et al., 2006).

Screens for mutants with altered anoxic sensitivity have led to the identification of two genes encoding for ceramide synthases (*hyl-1* and *hyl-2*). Ceramide synthases produce ceramide molecules of 14-to-26 carbons, which are involved in various cellular processes. The 16-carbon ceramide promotes germ-line apoptosis in the worm (Pewzner-Jung et al., 2006; Deng et al., 2008). Ceramides have also been shown to function as proapoptotic molecules following hypoxia or ischemia (Basnakian et al., 2005; Novgorodov and Gudz, 2009). Loss-of-function mutations in the *hyl-1* and *hyl-2* genes have opposite effects on anoxia sensitivity (Menuz et al., 2009). While *hyl-2(lf)* mutants are anoxia sensitive, *hyl-1(lf)* mutants are anoxia resistant. Given the different specificity of the two synthases (HYL-1: 24–26C ceramide, HYL-2: 20–22C ceramide), these observations suggest that ceramide molecules of specific carbon content protect against anoxia, while others do not. Ceramide synthesis by HYL-2 may control anoxia sensitivity in parallel with the insulin/IGF signaling pathway.

MODELING PARKINSON'S DISEASE IN *C. ELEGANS*

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of dopamine neurons in an area of the brain called substantia nigra pars compacta. Main behavioral features of patients suffering from PD include resting tremor, spasticity and an inability to initiate movement. Dopamine neuron degeneration is characterized by accumulation of proteins into inclusions termed Lewy bodies, observed in both sporadic and in familial cases of PD. The genetic forms of the disease account for 5–10% of all known cases. While in sporadic cases environmental factors seem to play a causative role, in familial cases of the disease several genes linked to the regulation of protein degradation and oxidative stress have been implicated (Dawson and Dawson, 2003).

Neurotoxin-Induced Degeneration of *C. elegans* Dopamine Neurons

In primate and rodent models of Parkinson's disease, dopamine neuron degeneration is mediated by the neurotoxic effects of 6-OHDA (6-hydroxydopamine), MPP^+ (1-methyl-4-phenylpyridinium), or other chemical compounds such as the pesticide rotenone (Choi et al., 1999). The worm has eight ciliated dopamine neurons grouped in four bilaterally symmetrical pairs: two pairs of CEP and one pair of ADE neurons in the head, and one pair of PDE neurons located posteriorly. A model of dopamine neuron degeneration induced by exposure to 6-OHDA has been established and characterized in *C. elegans* (Nass et al., 2002). Upon exposure of nematodes to 6-OHDA, degenerating dopamine neurons (mainly the CEPs and ADEs) display blebbed processes, rounded somas and many are even completely lost after prolonged treatment. Electron microscopy showed absence of features typical of necrotic cell death such as membranous whorls, swollen organelles or cell bodies but revealed dark and rounded cell bodies, dark nuclei, chromatin condensation and vacuolated or absent dendritic endings. Cell death is independent of CED-3 and CED-4 but dependent on the presynaptic dopamine transporter DAT-1, which suggests an essential role of it for the accumulation of 6-OHDA to dopamine neurons and consequent neurotoxicity. Torsin A is a protein that belongs to the functionally diverse family of AAA+ ATPases. Torsin A is abundant in dopamine neurons, localizes at Lewy bodies and has been shown to protect against 6-OHDA–induced dopamine neuron degeneration in *C. elegans* (Neuwald et al., 1999; Shashidharan et al., 2000; Cao et al., 2005). Protective effects are observed upon overexpression of human torsinA or the worm homologue TOR-2 in dopamine neurons, and appear to be mediated by down-regulation of DAT-1.

The toxin-based nematode models of Parkinson's disease can also be used to study the mechanisms underlying the neuroprotective effects of chemical compounds such as acetaminophen, which has been shown to protect against dopamine neuron degeneration induced by 6-OHDA in *C. elegans* and other models of Parkinson's disease (Maharaj et al., 2004; Locke et al., 2008). Moreover, the *C. elegans* model can be easily adapted to screen for new compounds against 6-OHDA–induced neurotoxicity. Indeed, two dopamine D2-type receptor agonists were found to protect against 6-OHDA–induced toxicity in a dose-dependent manner (Marvanova and Nichols, 2007).

A model of MPP^+-induced dopamine neuronal loss has also been established in *C. elegans* (Braungart et al., 2004). MPP^+ is a metabolic product of MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine). Exposure of *C. elegans* to MPTP or MPP^+ causes reduced mobility, increased lethality and the specific degeneration of dopamine neurons (dendritic loss, process blebbing, cell body loss). As in the case of 6-OHDA, MPP^+-mediated dopamine neuron degeneration is dependent on DAT-1 activity but independent of the CED-9 interactors CED-4 and CED-13. This indicates that caspase-independent cell death mechanisms are responsible for neurodegeneration (Pu and Le, 2008). Of interest, *vha-12* loss-of-function mutants, with impaired V-ATPase function, display reduced neurodegeneration, suggesting that necrotic cell death mechanisms may be involved in MPP^+-induced neurotoxicity.

α-Synuclein–Induced Dopamine Neuron Loss

Inclusions of α-synuclein are a common pathological feature of 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, either pan-neuronally or specifically in dopaminergic neurons have been established (Lakso et al., 2003; Cao et al., 2005; Cooper et al., 2006; Kuwahara et al., 2006; Qiao et al., 2008).

Overexpression of wild-type or mutant A53T, human α-synuclein either pan-neuronally or specifically in worm motor neurons causes motor deficits (Lakso et al., 2003). 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 animal age (Cao et al., 2005; Cooper et al., 2006; Kuwahara et al., 2006).

Mitochondrial dysfunction, impairment of the ubiquitine-proteasome system, production of reactive oxygen species, and ER stress have been implicated in the pathogenesis of Parkinson's disease (Dauer and Przedborski, 2003). Accumulation of α-synuclein blocks ER to Golgi vesicle trafficking in yeast. Overexpression of specific Rab GTPases that overcome blockage and promote ER to Golgi trafficking, have been shown to ameliorate dopamine neuron loss induced by α-synuclein in several animal models of Parkinson's disease, including *C. elegans* (Cooper et al., 2006; Gitler et al., 2008).

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C. elegans models of α-synuclein–induced dopaminergic neurodegeneration have been used to identify suppressors of dopaminergic neuron loss. 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).

LRRK2 and Parkinson's Disease

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. One such mutation is G2019S. Transgenic nematodes expressing the human LRRK2(G2019S) mutant form show extensive loss of dopaminergic neurons compared with control animals (Saha et al., 2009). Expression of the wild-type LRRK2 has a milder effect on neuron loss. The mutant LRRK2 increases vulnerability of dopaminergic neurons to mitochondrial stress. Loss-of-function mutations in the *lrk-1* gene, encoding the worm orthologue of LRRK2, also sensitize dopaminergic neurons to mitochondrial stress.

POLYGLUTAMINE-MEDIATED CELL DEATH

Several neurodegenerative disorders are associated with the expression and intracellular accumulation of expanded polyglutamine repeat proteins (Zoghbi and Orr, 2000). Huntington's disease is such a disorder, characterized by progressive and severe motor and cognitive impairment, and neuronal loss in the striatal and cortical brain areas (Imarisio et al., 2008). Huntington's disease is caused by abnormal expansion of a CAG trinucleotide found in the first exon of the huntingtin gene (Group, 1993). Expansion of this polyglutamine tract above 35 repeats causes disease, and the number of the CAG repeats in this tract correlates with the onset age of the disease.

In *C. elegans*, expression of an N-terminal huntingtin fragment with a polyQ expansion of 150 repeats in ASH sensory neurons (driven by the *osm-10* promoter) leads to progressive dysfunction of these neurons, evident by their dye-filling defects (Faber et al., 1999). When a fragment with a lower number of polyQ repeats is expressed, the abnormal effects are not observed. Under sensitized conditions, when Htn-Q150 fragment is coexpressed with subthreshold levels of the toxic protein fusion OSM-10::GFP (which may interfere with endogenous OSM-10), a small percentage of ASH neurons progressively die as animals age (enhanced dye-filling defects are also observed). The affected ASH neurons display swelling to 2–3 times of their original size. The CED-3 caspase is required for the death of ASH neurons in the sensitized background, although no morphological changes indicative of apoptosis are observed. However, while Htn-Q150–mediated neuronal dysfunction is CED-3–dependent, neuronal dysfunction caused by OSM-10::GFP alone is independent of CED-3. Polyglutamine protein aggregates are also observed in the sensitized background. Neuronal dysfunction precedes aggregate formation, degeneration and cell death.

Genetic screens have been performed, using this model, to identify modifiers of neurodegeneration and cell death caused by expanded polyQ huntingtin fragments. In such a screen, a glutamine/proline rich protein, named PQE-1 (polyQ enhancer-1) was identified (Faber et al., 2002). While loss-of-function mutations in the *pqe-1* gene, accelerate degeneration and death of the ASH neurons, overexpression of *pqe-1* cDNA has protective effects. These protective effects are likely mediated by binding of PQE-1 to specific Q-rich cellular proteins and preventing inappropriate interactions with expanded polyQ huntingtin fragments.

Several studies indicate that polyQ proteins interfere with transcriptional regulation. The acetyltransferase CBP (cAMP response element-binding protein) was found to be sequestered in expanded polyQ huntingtin aggregates resulting in reduced acetyltransferase activity (Nucifora et al., 2001). The contribution of specific histone deacetylases and CBP were examined in the *C. elegans* model of expanded Q150 huntingtin toxicity (Bates et al., 2006). Deletion of CREB or CBP enhanced polyQ-mediated neurodegeneration. Knockdown of the *hda-1*, *hda-4*, and *sir-2.1* genes encoding histone deacetylases enhanced Htn-Q150 degeneration, while *hda-3* knockdown reduced degeneration. These effects were specific for polyglutamine-induced degeneration, and were not observed for necrotic degeneration. It is hypothesized that HDA-3 may interfere with CREB-regulated transcription of genes protective against polyQ toxicity and degeneration.

The role of autophagy in polyQ-mediated neurodegeneration has also been studied (Jia et al., 2007; Jeong et al., 2009). RNAi knockdown of autophagy genes *bec-1*, *Ce-atg7*, and *Ce-atg18* enhances aggregation of Htn-Q150 and ASH neuronal degeneration (Jia et al., 2007). Increased acetylation of Htn-Q150 mediated by the CBP acetyltransferase protects from degeneration of ASH neurons (Jeong et al., 2009). Increased acetylation appears to facilitate trafficking and degradation of polyQ huntingtin by the autophagic degradation system, resulting in neuroprotection.

Compounds with therapeutic effects in culture or animal models of polyQ toxicity have been screened to assess their efficacy against neurotoxicity caused by Htn-Q150 expression in sensitized *C. elegans* animals carrying *pqe-1* loss-of-function alleles (Voisine et al., 2007). These screens have led to the identification of several compounds that suppress neuronal cell death in a dose-dependent manner.

TAU-INDUCED DEGENERATION

In a significant number of neurodegenerative diseases (e.g., Alzheimer's disease; frontotemporal dementia and Parkinsonism linked to chromosome 17, FTDP-17), neurofibrillary tangles

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encompassing the hyperphosphorylated microtubule-associated protein Tau have been observed (Lee et al., 2001). Although the exact role of tau in the pathogenesis of these neurodegenerative diseases is not known, the identification of autosomal dominant mutations in the MAPT gene (encoding Tau), has supported 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.

A genome-wide RNAi screen has been performed, based on the model of pan-neuronal expression of FTDP-17 mutant tau, to identify genes that enhance tau-mediated neurodegeneration, by screening for specific enhancement of the tau-mediated uncoordinated phenotype (Kraemer et al., 2006). Among the genes identified, are several kinases, phosphatases, chaperones, transcriptional regulators, proteases, enzymes, and genes of unknown function that have not been previously implicated in tau-mediated neurodegeneration. Forward genetic screens have also been performed to identify mutations that suppress tau-mediated uncoordinated phenotype. In two such screens, loss-of-function mutations in the genes, *sut-1* and *sut-2*, have been isolated as suppressors of tau-induced uncoordination (Kraemer and Schellenberg, 2007; Guthrie et al., 2009). Yeast two hybrid screens reveal that SUT-1 interacts with UNC-34 (member of the Ena/VASP protein family), suggesting the involvement of actin-dependent processes in tau-induced neurotoxicity. SUT-2 interacts with ZYG-12 (an aggresome component), suggesting the involvement of aggresomes in tau-induced neurotoxicity.

In the second model of tau-induced pathology, where wild-type or mutant tau is specifically expressed in the 6 touch receptor neurons of the worm, touch responses are weakened particularly in the case of mutant tau expression (Miyasaka et al., 2005). Morphological abnormalities (observed in affected neurons) include cell body swelling and distortion, as well as process abnormalities (thin and tortuous). The extent of morphological abnormalities correlates with the extent of dysfunction. Mutant tau-induced degeneration of touch receptor neurons is independent of the apoptotic pathway, because *ced-3* and *ced-4* loss-of-function mutations do not suppress tau-induced neurodegeneration.

MUSCLE DEGENERATION

One of the most common myopathies in humans is Duchenne's muscular dystrophy (DMD), caused by mutations in the dystrophin gene. Although no muscle cell fusion or regeneration events are observed in worms, the body wall muscles of *C. elegans* resemble mammalian striated muscles in their sarcomeric structure and composition (Moerman, 1996). Based on these similarities, a model of Duchenne's muscular dystrophy has been established in the nematode. Mutations in the dystrophin-like homologue, *dys-1*, when combined with a hypomorphic allele of the *hlh-1* gene (MyoD homologue) result in progressive impairment of locomotion that leads to paralysis after the onset of adulthood (Gieseler et al., 2000). Locomotion defects are correlated with progressive muscle degeneration that occurs during animal ageing. Degenerating body wall muscles exhibit disorganization of F-actin patterning, and cells in the mid-part of the body that later lose F-actin staining, show increased nucleolar size, abnormal nuclear shape or even absence of nuclei. Several muscle cells also accumulate vesicles that resemble disintegrated lysosomes seen during necrotic cell death (Nyamsuren et al., 2007). Muscle degeneration in these dystrophin mutants appears to be a calcium-dependent process, because a gain-of-function mutation in the *egl-19* gene (encoding the major voltage-gated calcium channel in muscles) dramatically enhances degeneration, while reduction of *egl-19* activity ameliorates degeneration (Mariol and Segalat, 2001).

Forward and reverse genetic screens have revealed additional genes that influence muscle degeneration. Down-regulation of CHN-1/CHIP, an E3/E4 ubiquitination enzyme with chaperone-like function, has been shown to increase muscle degeneration in dystrophin mutants, suggesting a protective role for the wild-type enzyme. Suppression may be mediated through the ubiquitin proteasome pathway: CHN-1 deficiency may prevent the ubiquitinylation and subsequent degradation of unfolded proteins in dystrophin mutant muscle cells. Further evidence suggesting a role for the proteasome in muscle degeneration and the probable mechanism, by which CHN-1/CHIP protects against muscle degeneration, came from the observation that treatment with a nonspecific proteasomal inhibitor phenocopies the effects of *chn-1* inactivation (Nyamsuren et al., 2007). Genetic screens, combined with pharmacological tests, have revealed several modifiers of muscle degeneration in dystrophin mutants. RNAi-mediated knockdown of genes regulating the excitation-contraction muscle machinery at various steps, suppresses muscle degeneration, suggesting a crucial role of the physical tension exerted on the muscle fibers, in the progression of dystrophin-dependent degeneration (Mariol et al., 2007). Inactivation of a Ca^{2+}-activated potassium channel (SLO-1) in muscles has also been shown to phenocopy the effects of *dys-1* loss-of-function. Moreover, experiments suggest an interaction between DYS-1

and SLO-1. DYS-1 depletion may cause down-regulation of SLO-1 and a concomitant increase in Ca^{2+} influx has been suggested to facilitate degeneration (Carre-Pierrat et al., 2006a).

Pharmacological screens that have been conducted using *C. elegans*, highlight the value of the worm as a platform to screen for therapeutic compounds against DMD (Gaud et al., 2004; Giacomotto et al., 2009). For example, such approaches based on direct application of serotonin, serotonin agonists or inhibitors of serotonin reuptake transporters, have revealed a critical role for serotonin in the suppression of dystrophin-dependent muscle degeneration (Carre-Pierrat et al., 2006b). Although, the effects of some therapeutic compounds may be different from nematodes to mammals, the use of *C. elegans* in parallel to mammalian models of DMD, holds promising potential to significantly accelerate discovery of novel drugs and aid the evaluation of candidate therapeutic compounds for the treatment of muscular degeneration.

LINKER CELL DEATH

The linker cell is born during the L2 stage and is essential for the proper extension and development of the *C. elegans* male gonad. The linker cell is normally programmed to die upon completion of male gonad development (Kimble and Hirsh, 1979; Sulston et al., 1980). Its death during the L4 to adult developmental transition promotes fusion of the vas deference with the cloaca and connects the reproductive system to the exterior, both of which are required for male fertility. Linker cell death has been reported to be non-apoptotic (Abraham et al., 2007; Kumar and Rothman, 2007). Part of the linker cell death program is cell autonomous and independent of genes participating in the core apoptotic pathway (*ced-3*, *ced-4*, *ced-9*, and *egl-1*). Moreover, inactivation of caspases CPS-1, CPS-2, or CPS-3 does not have any effect on the death process. Spatial cues (signals from the cloaca) and molecules controlling the developmental timing pathway (the LIN-29 Zn finger protein and the *let-7* microRNA) partially control linker cell death (Fig. 3A). The engulfment of the dying cell is accomplished by the U.I/rp epithelial cells through a mechanism that does not require known engulfment genes. Engulfment is not necessary for the death, per se. Dying linker cells display distinct morphological features, including the appearance of single-membrane cytoplasmic vesicles thought to be swollen mitochondria, and vacuoles reminiscent of necrotic cells during later stages. No chromatin compaction in the nucleus is observed. Knockdown of genes encoding aspartyl (*asp-3* and *asp-4*) and calpain (*clp-1* and *tra-3*) proteases involved in necrotic cell death do not suppress linker cell death. Furthermore, linker cell death is independent of autophagy and, despite some common morphological features, distinct from Wallerian axonal degeneration. Therefore, a different, unknown mechanism appears to mediate linker cell death.

CYTOTOXIC CELL DEATH

A model of cytotoxic cell death has been proposed for the Pn.p hypodermal blast cells of *C. elegans* (Galvin et al., 2008). Some of the Pn.p cells divide to give rise to vulva precursors (Sulston and Horvitz, 1977). Gain-of-function mutations in two genes, *lin-24* and *lin-33*, cause the inappropriate death of Pn.p cells (Galvin et al., 2008). DIC microscopy observations show that, during late L1 and early L2 stage, these mutants accumulate persistent refractile corpses, with increased nuclear refractility, that morphologically differ from apoptotic and necrotic cells. Refractility decreases progressively and cells may either die or survive. Surviving cells may regain a nucleus with normal appearance or they may contain an abnormally small one. The main characteristics of Pn.p cell death caused by these mutations are mitochondrial distortion, the existence of electron-dense membraneous whorls in cytoplasm, electron dense puncta in the nuclei and the dilation of the lumen of the nuclear envelope. No chromatin condensation, cell volume shrinkage, necrotic vacuoles, or single membrane-bound vacuoles indicative of other types of cell death in *C. elegans* are observed. Mutations in three genes of the core apoptotic machinery (*egl-1*, *ced-4*, and *ced-9*) that block apoptotic cell death, only mildly suppress the Vulvaless phenotype of *lin-24* and *lin-33* mutants and *ced-3* loss-of-function has no effect.

The engulfment process appears to be required for the efficient death of Pn.p cells caused by *lin-24* and *lin-33* mutations, as elimination of genes involved in the engulfment of both apoptotic and necrotic corpses suppresses cell death (Fig. 3B). Molecular characterization revealed that LIN-24 contains a domain with similarity to bacterial toxins, while LIN-33 is a novel protein. Of interest, the death-inducing mutations in *lin-24* are located in the region encoding the toxin domain. Death-inducing *lin-24* and *lin-33* alleles show gene-dosage and genetic interaction effects. By contrast, deletion of either *lin-24* or *lin-33* does not cause vulva ablation. These findings, when combined, suggest that death-inducing *lin-24* and *lin-33* mutations are probably neomorphs, generating novel cytotoxic functions for these proteins. It is likely that the cytotoxic products encoded by death-inducing *lin-24* and *lin-33* alleles alter the membrane properties of the cell to promote its death.

RADIATION-INDUCED CELL DEATH

A model of radiation-induced reproductive cell death has been established in *C. elegans* (Weidhaas et al., 2006a). Reproductive cell death is the main type of cell death that tissue multipotent precursor cells (termed "clonogens") follow after cytotoxic tumor therapy. This type of cell death seems to arise from DNA damage (double-strand breaks), that when misrepaired or not repaired at all, accumulate through several cell divisions and lead to cell death. It is a long held belief that the depletion of these cells is a crucial prerequisite for either permanent tissue damage or tumor eradication. In *C. elegans*, the vulva precursor cells (P3.p-P8.p), are considered multipotent precursor cells that resemble mammalian clonogens.

Whole body irradiation of *C. elegans* during the developmental period between the L1 and L4 stage results

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in a dose- and cell cycle-dependent effect on vulval precursor cells, which survive irradiation and divide normally to produce a normal wild-type vulva at L4 stage. However, during late L4 and early adulthood, these cells die in a stochastic manner. The main features of dying cells (vacuolation, no chromatin condensation, no apoptotic staining of the vulva, independence of core apoptotic factors such as CED-9 and CED-3) are indicative of non-apoptotic cell death. Loss-of-function mutations in genes involved in the DNA damage response render animals highly sensitive to radiation-induced cell death, suggesting a protective role for this pathway against reproductive cell death. An additional pathway shown to play a protective role is the EGFR/RAS/MAPK signaling pathway (Weidhaas et al., 2006b). Loss-of-function mutations that impair this pathway increase radiosensitivity. Epistatic analysis shows that the EGFR/RAS/MAPK pathway acts linearly with the DNA damage response pathway to protect from radiation-induced reproductive cell death.

CONCLUSIONS AND PERSPECTIVES

In this review article, we have made an attempt to provide a comprehensive survey of the non-apoptotic cell death paradigms that have been studied in *C. elegans* and to also convey our current understanding of the molecular mechanisms involved in each case. The rich repertoire of non-apoptotic types of cell death that exists in *C. elegans* renders the nematode a particularly attractive platform for dissecting the mechanisms of pathological cell death in humans. For example, the similarity of necrotic cell death triggered by hyperactive ion channels in *C. elegans* to excitotoxic cell death and neurodegeneration in higher organisms, both in morphological characteristics and mechanistic aspects, can be exploited to develop effective therapeutic strategies aiming to battle numerous devastating pathologies in humans. This notion is further supported by the extensive evolutionary conservation of genes that protect *C. elegans* and human cells from hypoxic death and the identification of adaptive responses to hypoxic injury, such as hypoxic preconditioning (*C. elegans* is the only invertebrate so far where hypoxic preconditioning has been observed).

In addition, modeling of human degenerative disorders in *C. elegans*, such as Parkinson's disease and others, has already accelerated the pace of the molecular dissection of underlying mechanisms and holds promise for the development and testing of innovative intervention strategies. Given the late onset of many neurodegenerative diseases, it is likely that factors influencing or linked to the aging process itself play a central role in the pathogenesis of these disorders. *C. elegans* has contributed decisively in deciphering the key pathways modulating ageing and thus, provides an ideal setting in which to study the molecular interplay between the ageing process and the initiation and progression of neurodegenerative disorders.

Furthermore, the development of in vivo tissue models of reproductive clonogenic cell death after cytotoxic treatment in *C. elegans* may lead to a better understanding of the mechanisms that render tumors resistant to cytotoxic therapy. This would in turn allow the identification of novel strategies to manipulate the resistance of normal and tumor cells to cytotoxic treatment.

Beyond the paradigms, where non-apoptotic death is triggered by specific genetic mutations, exogenous treatments or by expressing human disease-related proteins, one example of programmed non-apoptotic cell death during nematode development (that of linker cells) has also been identified. Dying linker cells show morphological features reminiscent of a rare type of developmental cell death, referred to as type III death (or programmed necrosis; Clarke, 1990). These morphological features are also seen during normal developmental death of chick ciliary ganglia (Pilar and Landmesser, 1976) and chick spinal cord motor neurons (Chu-Wang and Oppenheim, 1978). Type III cell death (together with type II or autophagic cell death as is usually referred) may be more prominent in cells that have differentiated and executed a specific function during development. The linker cell is such an example. After leading gonadal migration to the appropriate position, the linker cell finally dies to ensure male fertility. The observation of linker cell death morphological features in cases of dying cells in vertebrates suggests the evolutionary conservation of this developmental cell death program. Identification of the genes involved in *C. elegans* linker cell death and functional characterization of their mammalian counterparts holds promise for exposing the molecular mechanisms underlying this likely conserved cell death program.

As in the case of apoptotic cell death, where *C. elegans* has been instrumental in the identification of the mechanisms involved, the worm is now also been increasingly appreciated for its contribution toward understanding the intricacies of non-apoptotic cell death. Because of the extensive involvement of non-apoptotic cell death in numerous human pathologies, *C. elegans* is becoming a valuable tool in our efforts to understand and counter human disease. The exceptional genetic malleability and the unique molecular and cellular methodologies available for the nematode, coupled with a detailed knowledge of its development, anatomy, and physiology forecast exciting new discoveries toward this direction in the near future.

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