

Cell/Neuron Degeneration

N Tavernarakis and M Driscoll

Copyright © 2001 Academic Press
doi: 10.1006/rwgn.2001.0175

Tavernarakis, N

CABM, Rutgers University, 806 Hoes Lane, Picataway, NJ 08855, USA

Driscoll, M

CABM, Rutgers University, 806 Hoes Lane, Picataway, NJ 08855, USA

Introduction

Inappropriate cell death underlies the pathology of many human and animal diseases. In particular, premature neuronal cell death plays a significant role in several late onset degenerative disorders such as Alzheimer's disease and amyotrophic lateral sclerosis. Somewhat unexpectedly, genetic programs that execute and regulate cell death exist. The genetic instructions for the regulation and execution of one type of cell death, called apoptosis or programmed cell death, have been remarkably conserved. Studies in lower organisms, such as in the nematode *Caenorhabditis elegans*, have provided significant insight into the mechanism underlying this cell death process. The mechanisms of pathological or necrotic cell death are less clear, but a detailed molecular model of inherited neurodegenerative conditions, identified in the nematode, is emerging and may provide a means of identifying a conserved pathway for pathological cell death in humans.

Overview of Cell Death

Two major types of cell death were initially distinguished on the basis of morphological changes observed in the dying cell. One, termed apoptosis, is characterized by shrinkage and fragmentation of cytoplasm, compaction of chromatin and eventual destruction of cellular organelles. Frequently DNA is degraded by intranucleosomal cleavage which after electrophoretic separation generates a characteristic DNA ladder of fragments that differ in size by one nucleosome repeat length. Apoptotic cellular remains are usually removed by phagocytosis and do not invoke an inflammatory response. In several cases it has been demonstrated that apoptotic cell death is an active process, requiring RNA and protein synthesis, although this is not a universal feature of this type of cell death. Death by apoptosis often occurs as part of

normal development or homeostasis. Apoptotic death generally accounts for normal elimination of cells during development and in cell depletion due to broad range of stimuli including changes in growth factor or hormone levels, mild ischemia, cell-mediated immune attack, ionizing radiation, mild hypothermia and several chemotherapeutic agents. Genetic and biochemical studies have identified proteins that regulate and execute apoptotic death. The activities of these proteins have proved to be remarkably conserved between invertebrates and vertebrates.

A second type of cell death, termed necrosis or pathological cell death, contrasts with apoptosis in several respects. First, necrotic cell death does not appear to be part of normal development or homeostasis. Rather, this type of death generally occurs as a consequence of cellular injury or in response to extreme changes in physiological conditions. Second, the morphological changes observed during necrosis differ greatly from those observed in apoptotic cell death. Necrotic cell death is characterized by gross cellular swelling and distention of subcellular organelles such as mitochondria and endoplasmic reticulum. Clumping of chromatin is observed and DNA degradation occurs by cleavage at random sites. In general, necrosis occurs in response to severe changes of physiological conditions including hypoxia and ischemia, and exposure to toxins, reactive oxygen metabolites, or extreme temperature.

Necrotic cell death is a significant problem in human health. For example, the excitotoxic neuronal cell death that accompanies oxygen deprivation associated with stroke is a major contributor to death and disability. Ischemic diseases of the heart, kidney and brain have been cited as the primary causes of mortality and morbidity in the US and industrialized nations. Necrosis is believed to occur independently of *de novo* protein synthesis and is generally thought to reflect the chaotic breakdown of the cell. However, given that many cells of diverse origins exhibit stereotyped responses to cellular injury, it is conceivable that a conserved 'execution' program, activated in response to injury, may exist.

It should be noted that some have argued that more than just these two patterns of cell death can be distinguished. Intensive research into death mechanisms supports that the initial distinction between apoptosis and necrosis is an over-simplification. For example, alternative morphological death profiles have been described and certain dying cells are known to exhibit some, but not all, commonly distinctive features of either apoptosis or necrosis. Likewise, certain markers of death can be expressed by both apoptotic and necrotic cells. Although remarkable progress in understanding apoptosis has been accomplished,

understanding necrosis and alternative death mechanisms is more limited.

Caenorhabditis elegans as a Model System for the Study of Cell Death

C. elegans is a small (1.3 mm), free-living soil nematode that feeds on *E. coli* in the laboratory. The key strength of the *C. elegans* model system resides in the extensive genetic analyses that can be conducted with this animal. The ability of *C. elegans* to reproduce by self-fertilization renders the production and recovery of mutants easy—homozygous mutants segregate as F2 progeny of mutagenized parents without any required genetic crossing. Mutant alleles are readily transferred by male matings so that complementation analysis and construction of double mutant strains is straightforward. Positions of thousands of genes on the six *C. elegans* chromosomes have been determined. This genetic map has been aligned with the physical map of the genome (a collection of overlapping DNA clones that spans the six chromosomes). Sequence analysis of the *C. elegans* genome has been completed. Transgenic nematodes are constructed by injecting DNA into the hermaphrodite gonad where it is packaged into developing oocytes.

C. elegans is well suited for the study of both normal and aberrant cell death at the cellular, genetic and molecular levels. There is no model system in which development is better understood. The animal is essentially transparent throughout its life cycle and individual nuclei can be readily visualized using differential interference contrast optics. These attributes have enabled the complete sequence of somatic cell divisions, from the fertilized egg to the 959-celled adult hermaphrodite, to be determined. Elucidation of the lineage map has revealed that in certain lineages, particular divisions generate cells which die at specific times and locations and that the identities of these ill-fated cells is invariant from one animal to another. The ability to easily recognize dying cells within a living animal has allowed identification of mutants with aberrant patterns of both apoptotic and necrotic cell death.

A Conserved Apoptotic Death Mechanism in Caenorhabditis elegans

C. elegans development includes the programmed death of 131 identified cells. Genetic studies have identified several genes that participate in all *C. elegans* programmed cell deaths. *ced-3* (cell death abnormal) encodes a cysteine protease (caspase) that is essential for death execution. The *ced-4* product activates CED-3 activity and is also required for all programmed cell

deaths. In cells fated to live, the death program is held in check by negative regulator CED-9, which can be antagonized by EGL-1. Both activation and negative regulation may be controlled by physical association/multimerization of these proteins in the vicinity of the mitochondrial membrane. After death, cell corpses are removed by the products of two groups of genes that act in two parallel pathways (one includes *ced-1*, *ced-6*, and *ced-7*; another includes *ced-2*, *ced-5*, *ced-10*, and *ced-12*). These ‘undertaker’ genes are required for phagocytosis and degradation of dead cells.

Analysis of gene function in *C. elegans* programmed cell death has had an important influence in advancing understanding of mammalian apoptotic death mechanisms because regulators, executors, and undertakers of programmed cell death are functionally conserved from nematodes to humans. CED-3 is related to the mammalian caspases that execute apoptotic cell death, CED-4 is related to Apaf-1, CED-9 is a member of the mammalian BCL-2 family and EGL-1 is a member of the death-regulatory BH3-only family. Because apoptotic cell death is discussed elsewhere in this volume, we focus on nonapoptotic cell death in this section.

Degenerins and neurodegeneration in Caenorhabditis Elegans

Unusual gain-of-function mutations in several specific *C. elegans* ion channel genes induce necrotic-like deaths of the neurons that express these channel genes. For example, 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. (In contrast, most *mec-4* mutations are recessive loss-of-function mutations that disrupt body touch sensitivity without affecting touch receptor ultrastructure or viability). Similarly, dominant mutations in *deg-1* (degenerin; *deg-1(d)*) induce death of a group of neurons that includes the PVC interneurons of the posterior touch sensory circuit. (Loss-of-function mutations in *deg-1* appear wild-type in behavior).

mec-4 and deg-1 Encode Ion Channel Subunits of the DEG/ENaC Superfamily

mec-4 and *deg-1* encode proteins that are 51% identical. These genes were the first identified members of the *C. elegans* ‘degenerin’ family, so named because several members can mutate to forms that induce cell degeneration. Included in this 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 hypercontraction. Thus, a general feature of the degenerin gene family is that specific gain-of-function mutations have deleterious consequences for the cells in which they are expressed.

C. elegans degenerins share sequence similarity with subunits of the vertebrate amiloride-sensitive epithelial Na⁺ channel. α -, β - and γ -ENaC (for epithelial Na⁺ channel) are homologous subunits of the multimeric Na⁺ channel that mediates Na⁺ absorption in epithelia of the distal part of the kidney tubule, the urinary bladder, the distal colon and the lung. The degenerin family of *C. elegans* currently includes 23 members that have been characterized or predicted by the *C. elegans* Genome Sequencing Consortium. Given such a large *C. elegans* gene family, it is predicted that the mammalian ENaC family should likewise be large. Because many *C. elegans* degenerins can mutate to toxic forms that induce neurodegeneration, the neuronally-expressed mammalian family members are logical candidates for genes that can mutate to cause neurodegeneration in higher organisms. In this regard it is interesting that mammalian MDEG, engineered to encode an amino acid substitution analogous to the change in *mec-4(d)* (see below), induces degeneration when expressed in *Xenopus* oocytes and embryonic hamster kidney cells.

Morphology, Timing and Ultrastructure of *mec-4(d)*- and *deg-1(d)*-Induced Neurodegeneration

Although *mec-4(d)* and *deg-1(d)* mutations kill different groups of neurons, the morphological features of cell deaths they induce are the same. The time course of degeneration depends upon the dosage of the toxic allele, but on average can take approximately 8 hours. When viewed using the light microscope, the nucleus and cell body of the affected cell first appear distorted and then the cell swells to several times its normal cell diameter (**Figure 1**). Eventually the swollen cell disappears, often after shrinking but sometimes as a consequence of cell lysis. Interestingly, the swollen character of *mec-4(d)*- and *deg-1(d)*-induced deaths resembles the morphologies of mammalian cells undergoing necrotic cell death.

At the ultrastructural level, cells dying as a consequence of *mec-4(d)* and *deg-1(d)* expression exhibit some remarkable features. The first detectable abnormality apparent in an ill-fated cell is the formation of small tightly wrapped membrane whorls that seem to originate at the plasma membrane. These whorls are internalized and appear to coalesce into large electron-dense membranous structures. Large internal vacuoles form and distortion of the nucleus

by these vacuoles is associated with chromatin clumping. Finally, organelles and cytoplasmic contents are degraded, usually leaving a membrane-enclosed shell. The striking membranous inclusions suggest that intracellular trafficking may contribute to degeneration. Interestingly, in some mammalian degenerative conditions such as neuronal ceroid lipofuscinosis (Batten disease; the *mnd* mouse) and that occurring in the wobbler mouse, cells develop vacuoles and whorls (fingerprint bodies) that look similar to internalized structures in dying *C. elegans* neurons. This suggests that some degenerative processes may be similar in nematodes and mammals.

The touch receptor neurons in *mec-4(d)* mutants express terminally differentiated properties before they die and the PVC neurons in *deg-1(d)* mutants differentiate and function before they degenerate. *mec-4(d)*- and *deg-1(d)*-induced cell deaths have therefore sometimes been referred to as the nematode version of ‘late onset’ neurodegeneration. Careful studies of the timing of *mec-4* expression relative to the onset of degeneration support that onset of neurodegeneration is correlated with the initial expression of the toxic gene product.

Death-Inducing Channel Mutations and Models for Initiation of Neurodegeneration

mec-4(d) and *deg-1(d)* alleles encode substitutions for a conserved alanine that is positioned extracellularly, adjacent to pore-lining membrane-spanning domain. The size of the amino acid sidechain at this position is correlated with toxicity – substitution of a small sidechain amino acid does not induce degeneration whereas replacement of the Ala with a large sidechain amino acid is toxic. This ‘rule’ suggests that steric hindrance plays a role in the degeneration mechanism and supports the following working model for *mec-4(d)*-induced degeneration. MEC-4 is postulated to be a subunit of a channel that, like other channels, can assume alternative open and closed conformations. In adopting the closed conformation, the sidechain of the amino acid at MEC-4 position 713 is proposed to come into close proximity to another part of the channel. Steric interference conferred by a bulky amino acid sidechain prevents such an approach, causing the channel to close less effectively. Increased cation influx results, initiating neurodegeneration. That ion influx is critical for degeneration is supported by the fact that amino acid substitutions that disrupt the channel conducting pore can prevent neurodegeneration when present in *cis* to the A713 substitution. In addition, large sidechain substitutions at the analogous position in some neuronally expressed mammalian superfamily members do markedly increase channel conductance.

***mec-4(d)*- and *deg-1(d)*-Induced Neurodegeneration occur Autonomously and Independently of Programmed Cell Death Executors**

Genetic mosaic analyses first indicated that *mec-4(d)* kills as a consequence of a toxic activity within the cells that die. Ectopic expression of *mec-4(d)* can induce swelling and death of cells other than the touch receptor neurons, confirming the cell autonomy of *mec-4(d)* action. The execution of degenerative cell death occurs by a mechanism that appears distinct from that utilized in programmed cell death. At the genetic level, it has been demonstrated that *ced-3(lf)* and *ced-4(lf)* mutations do not block *mec-4(d)*- and *deg-1(d)*-induced cell degeneration. Likewise, *mec-4(d)* and *deg-1(d)* alleles do not disrupt programmed cell deaths.

Other Cellular Insults can also Induce Necrotic-Like Cell Death, Suggesting a Common Response to Cell Injury

In the case of degenerin-induced cell death, degeneration is the consequence of a highly specific stimulus. One could argue that the death process is unique to this particular ion channel family. Evidence suggests, however, that necrotic-like cell death may actually be a general response to different ‘injuries.’ At least three additional genes cause *C. elegans* cell death that is morphologically similar to that induced by degenerins:

Mutations in Other Ion Channels

Additional genes that increase channel activity cause vacuolar degeneration of *C. elegans* neurons. *deg-3* encodes a protein related to the vertebrate α -7 nicotinic acetylcholine receptor that, together with DES-2, forms a channel highly permeable to Ca^{2+} . Dominant allele *deg-3(u662)* induces swelling and degeneration of several *C. elegans* neurons. Interestingly, *deg-3(u662)* encodes a mutation similar to that of a characterized allele in the chick that decreases desensitization (thus increasing ion influx). Channel assays support that the *C. elegans* mutation causes a similar disruption. Consistent with this hypothesis, some nicotinic antagonists partially suppress *deg-3(d)*-induced defects.

Activated $\text{G}\alpha_s$

Expression of constitutively active, GTPase-defective, heterotrimeric G protein $\text{G}\alpha_s$ (either from *C. elegans* or from rat) causes swelling and degeneration of many (but not all) cells in which the mutant gene is expressed.

Human Alzheimer’s Disease Amyloid Peptide A β 1-42

Alzheimer’s disease can be caused by mutations that increase deposition of β -amyloid peptide 1-42 derived from the APP precursor protein. Expression of the toxic human fragment using the *C. elegans* bodywall muscle promoter *unc-54* causes animals to become progressively paralyzed as they develop and induces necrotic-like death of some cells around the nerve ring.

A Common Ion Channel Theme in Necrotic-Like Cell Death in *C. elegans*?

Although these genes normally are involved in distinct processes, it remains possible that they share a common death-activating mechanism: alteration of channel activity. Consistent with this possibility, G proteins are known to modulate channel activity. Likewise, some studies have linked β -amyloid toxicity with altered channel function.

Many Genes can Mutate to Cause Necrosis

New mutations that induce necrotic-like cell death can be isolated fairly readily in genetic screens for such mutations (the identities of genes affected are not yet known), consistent with the possibility that diverse insults can provoke a similar degenerative process.

Along these lines, it is interesting that necrotic-like figures (of unknown origin) are commonly noted in aged animals. Could various cell injuries, environmentally or genetically-introduced, converge to activate a degenerative death process that involves common biochemical steps?

Genetic Requirements for Degeneration

One of the key advantages of using *C. elegans* as a model organism for deciphering death mechanisms is that genetic approaches can be applied to the problem. By isolating mutations that suppress degeneration, molecular requirements for degeneration process can be identified. Since several aspects of necrotic cell death appear conserved, this strategy may reveal new targets for therapeutic intervention in humans.

Although generally acting death suppressors have been isolated, data on these has yet to be published in the scientific literature. At present, best understood death suppressors affect specific death initiating stimuli. For example, *mec-6* mutations can suppress degeneration induced by various hyperactivated degenerin channel mutations. *mec-6* is thought to be specifically required for degenerin channel function; it is not needed for $\text{G}\alpha_s$ -induced cell death.

One gene required for $G\alpha_s$ -induced cell death is *acy-1/sgs-1*, which encodes an adenylyl cyclase expressed broadly throughout the nervous system. Although *acy-1/sgs-1* is expressed in the touch receptor neurons, it is not required for *mec-4(d)*-induced touch cell degeneration. What does this say about the necrotic death process? There are two possibilities: first, distinct death mechanisms may be involved in the necrosis induced by different initiating factors. Alternatively, the initiating events may feed into a common pathway at a point downstream of *acy-1*. Characterization of broadly acting necrosis suppressors will indicate which of these possibilities applies.

Parallels Between Neurodegenerative Cell Death in *C. elegans* and Higher Organisms – a Common Degenerative Death Mechanism?

Inappropriate channel activity is known to be causative for some mammalian neurodegenerative conditions. For example, it is interesting that the working model for the initiation of degenerative cell death in *C. elegans* is remarkably similar to events that initiate excitotoxic cell death in higher organisms. In excitotoxicity, glutamate receptor ion channels are hyperstimulated by the excitatory transmitter glutamate and the resultant elevated Na^+ and Ca^{2+} transport induces death by neuronal swelling. Mammalian ion channel mutations can also induce neurodegeneration. In the weaver mutant mouse, altered gating and ion selectivity properties of the GIRK2 potassium channel are associated with vacuolar cell death in the cerebellum, dentate gyrus and olfactory bulb.

It is noteworthy, however, that mutations in channel genes are not the sole means by which vacuolar neurodegeneration can be induced in *C. elegans*. As noted above, necrotic-like death of some *C. elegans* cells can be induced by expression of human β -amyloid peptide. Mutations in transcription factor *lin-26* cause hypodermal cells to become neuroblasts which swell and die. Also, since mutations that cause swelling and death can be isolated at a relatively high frequency, multiple gene classes appear capable of mutation to induce necrotic-like death. These observations and morphological parallels between nematodes and higher organisms suggest that cell death might be induced by a variety of cellular ‘injuries’ and that a common death mechanism (rather than chaotic cellular destruction) could operate to eliminate injured cells. The peculiar internalized membranous whorls observed suggests degenerin-induced death could involve disrupted intracellular trafficking, an interesting implication given that disrupted trafficking has been implicated in Alzheimer’s disease, Huntington’s

disease, and ALS. Perhaps endocytotic responses provoked by diverse types of damage might be a common element of diverse degenerative conditions.

Future Prospects

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

The initiation of degenerative cell death in *C. elegans* and its general neuropathology are reminiscent of elements of excitotoxic cell death and other necrotic-like cell death in higher organisms. Excitotoxic neuronal death mediated via glutamate receptors (channel proteins) in cell culture or *in vivo* in response to ischemia is an example of this type of cell death. It is also interesting that there are many reported instances, in animals as diverse as flies, mice and humans, in which neurons degenerating due to genetic lesions exhibit morphological changes similar to those induced by *mec-4(d)* and other hyperactivated degenerins. Given that apoptotic death mechanisms are conserved between nematodes and humans it can be hypothesized that various cell injuries, environmentally or genetically-introduced, converge to activate a degenerative death process that involves common biochemical steps. At present the question of common mechanisms remains an intriguing but open question.

If specific genes enact different steps of the degenerative process, then such genes should be identifiable by mutation in *C. elegans*. Indeed, suppressor mutations in several genes that block *mec-4(d)*-induced degeneration have been isolated. Although some suppressor mutations affect channel function (for example mutations in *mec-6*), others are expected to be more generally involved in the death process. Analysis of such genes should result in the description of a genetic pathway for degenerative cell death. Perhaps, as has proven to be the case for the analysis of *C. elegans* programmed cell death mechanisms, elaboration of an injury-induced death pathway in *C. elegans* may provide insight into neurodegenerative death mechanisms in higher organisms.

Further Reading

Aguzzi A and Raeber AJ (1998) Transgenic models of neurodegeneration. Neurodegeneration: of (transgenic) mice and men. *Brain Pathology* 8: 695–697.

- Canessa CM, Horisberger J-D and Rossier BC (1993) Epithelial sodium channels related to proteins involved in neurodegeneration. *Nature* 361: 467–470.
- Dragunow M, MacGibbon GA, Lawlor P, et al. (1997) Apoptosis, neurotrophic factors and neurodegeneration. *Reviews in Neuroscience* 8: 223–265.
- Driscoll M (1996) Cell death in *C. elegans*: molecular insights into mechanisms conserved between nematodes and mammals. *Brain Pathology* 6: 411–425.
- Heintz N and Zoghbi HY (2000) Insights from mouse models into the molecular basis of neurodegeneration. *Annual Review of Physiology* 62: 779–802.
- Lints R and Driscoll M (1996) Programmed and pathological cell death in *C. elegans*. In: Martin GR, Holbrook N and Lockshin RA, (eds) *Cell Aging and Cell Death*. New York: Wiley-Liss.
- Min KT and Benzer S (1997) Spongecake and eggroll: two hereditary diseases in *Drosophila* resemble patterns of human brain degeneration. *Current Biology* 7: 885–888.
- Min KT and Benzer S (1999) Preventing neurodegeneration in the *Drosophila* mutant bubblegum. *Science* 284: 1985–1988.
- Nakao N and Brundin P (1998) Neurodegeneration and glutamate induced oxidative stress. *Progress in Brain Research* 116: 245–263.
- Paulson HL (2000) Toward an understanding of polyglutamine neurodegeneration. *Brain Pathology* 10: 293–299.
- Warrick JM, Paulson HL, Gray-Board GL, et al. (1998) Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. *Cell* 93: 939–949.

See also: 0152, 0065, 0892

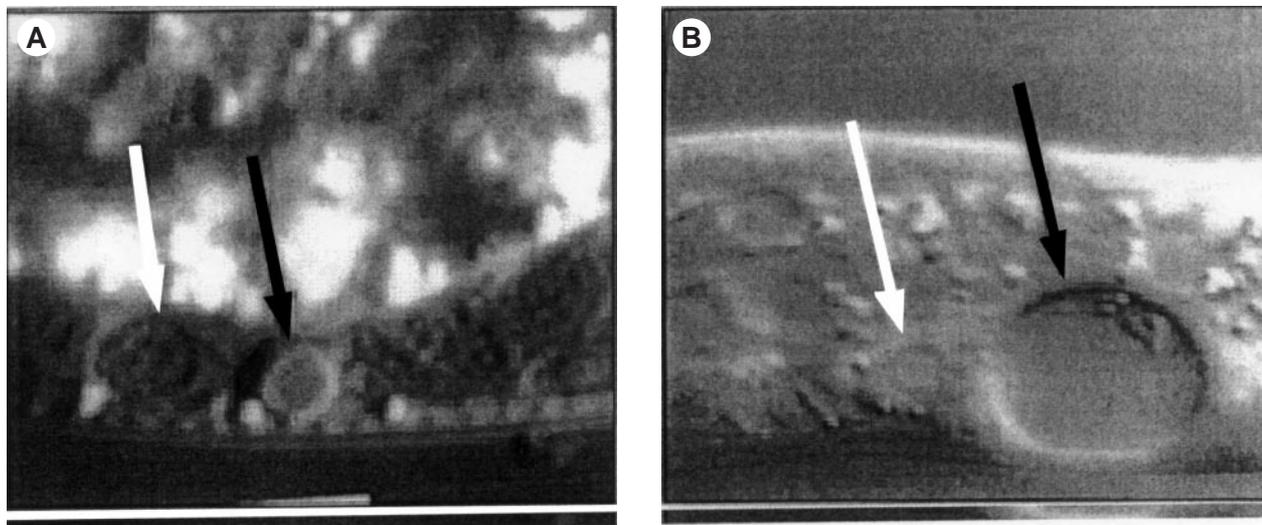


Figure 1 Apoptotic and degenerative cell death in *Caenorhabditis elegans*. White arrows indicate normal cells while black ones point to dying ones. A cell undergoing apoptotic or programmed cell death, is shown in (A). In (B), a degenerating cell has swollen to several times its diameter and adopts a vacuole-like appearance that is different from the compacted, button-like structure of the apoptotic cell.