

# Molecular mechanisms of necrotic cell death in *C. elegans*

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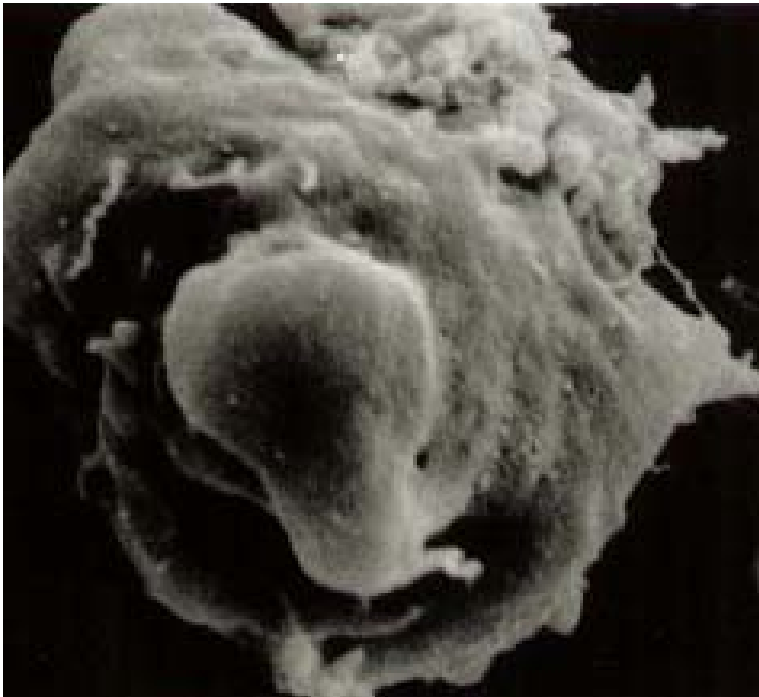
# Necrotic cell death/Necrosis: a different type of cell death

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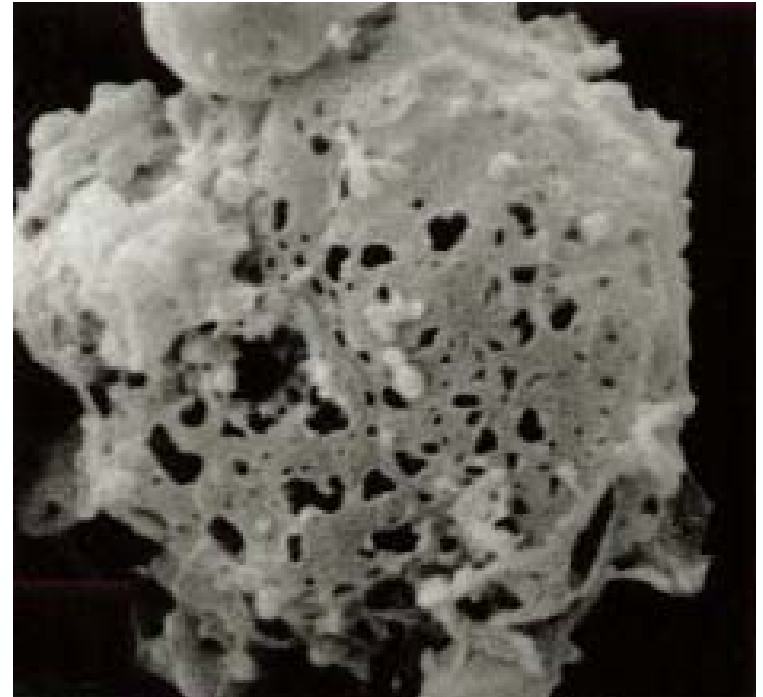
Merriam-Webster Online Dictionary:

...from the Greek ***nekros***, meaning *dead*

**Apoptosis**



**Necrosis**



# Distinct morphological characteristics of dying cells

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## Apoptosis

- nuclear compaction
- chromatin condensation
- Inter-nucleosomal cleavage of DNA
- plasma membrane blebbing
  - formation of membrane-enclosed vesicles
- no inflammatory responses
- apoptotic cells are usually scattered throughout tissues

## Necrosis

- karyolysis
  - chromatin clumping
  - DNA degradation
  - no/little plasma membrane blebbing
    - no formation of membrane-enclosed vesicles
  - inflammatory responses
  - necrotic cells are commonly found in contiguous sheets, within tissues
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- mitochondrial swelling
  - endoplasmic reticulum dilatation
  - ill defined cytoplasm
    - extensive cytoplasmic vacuolation

# Necrosis can be triggered by numerous insults

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- **Genetic factors**

Neurodegenerative disorders

OMIM: Online Mendelian Inheritance in Man (<http://www.ncbi.nlm.nih.gov/Omim/>)

- **Acute energy depletion**

- ischemia
- hypoxia
- hypoglycemia

- **Excitotoxicity**

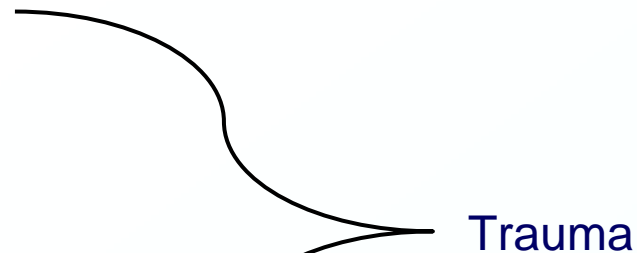
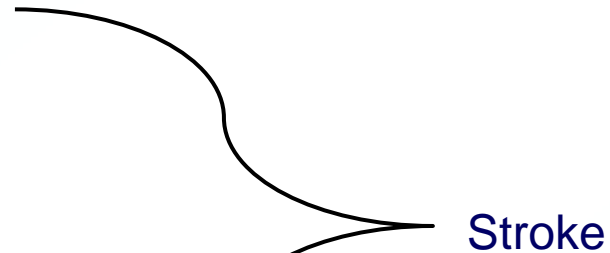
- excessive glutamate release

- **Exposure to toxic substances**

- strong detergents
- acids
- oxidants

- **Harsh environment**

- extreme heat or cold
- excessive mechanical strain



**Is necrosis simply the chaotic breakdown of cells?**

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**...or is there order in chaos?**

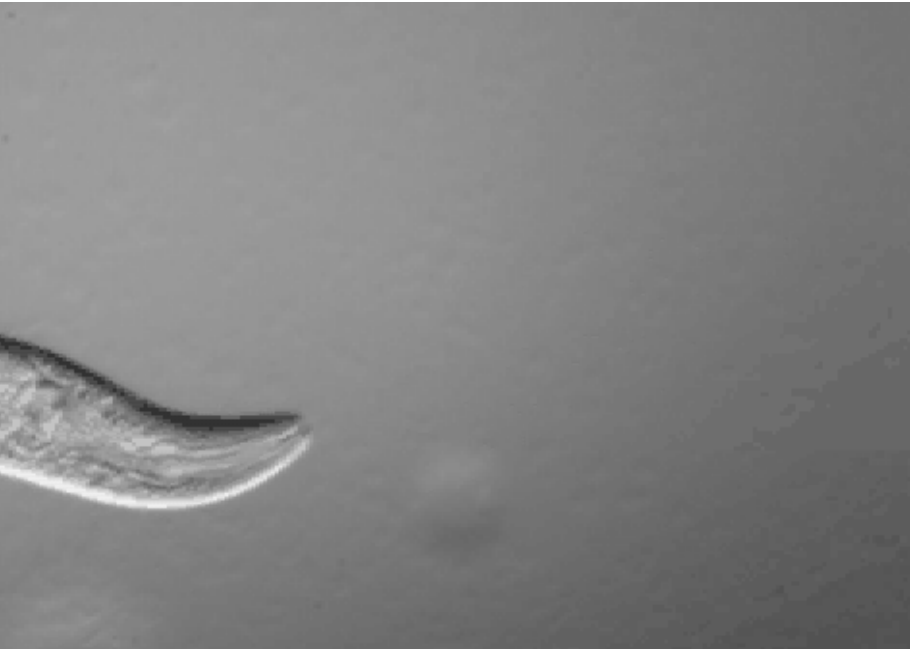
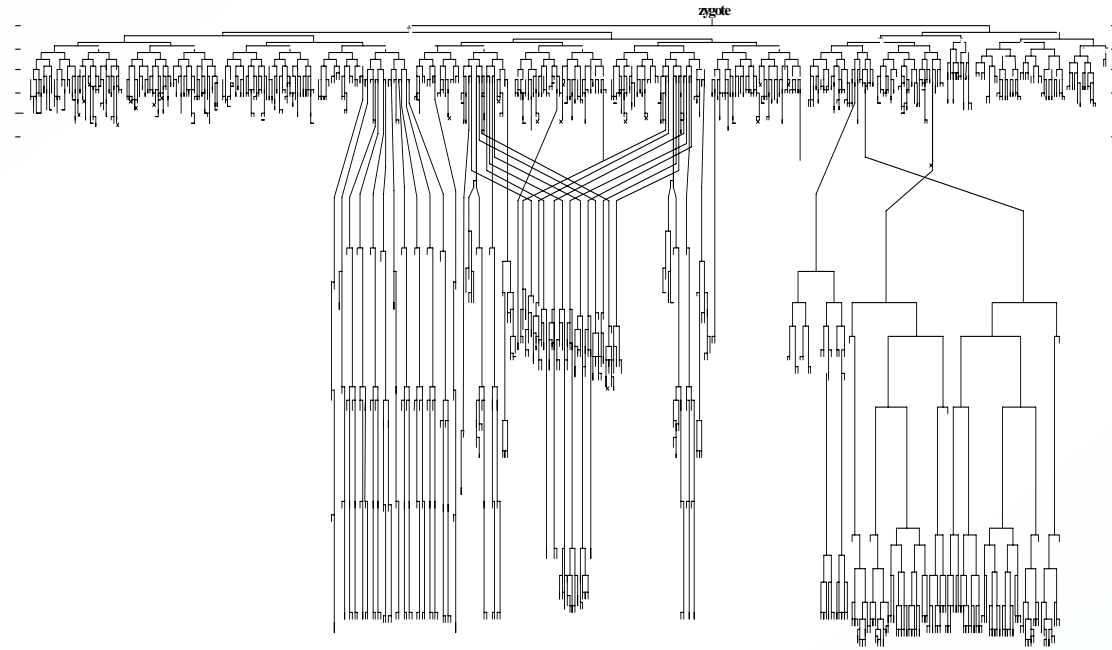
**What are the molecular events that transpire during necrotic cell death?**

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**Strategy:**  
**Identify mediators of necrotic cell death in**  
***Caenorhabditis elegans***

# The nematode *Caenorhabditis elegans*

- A hermaphroditic soil nematode
- Small, about 1mm in length
- Feeds on bacteria
- Sequenced genome
- 959 cells
- Invariable lineage

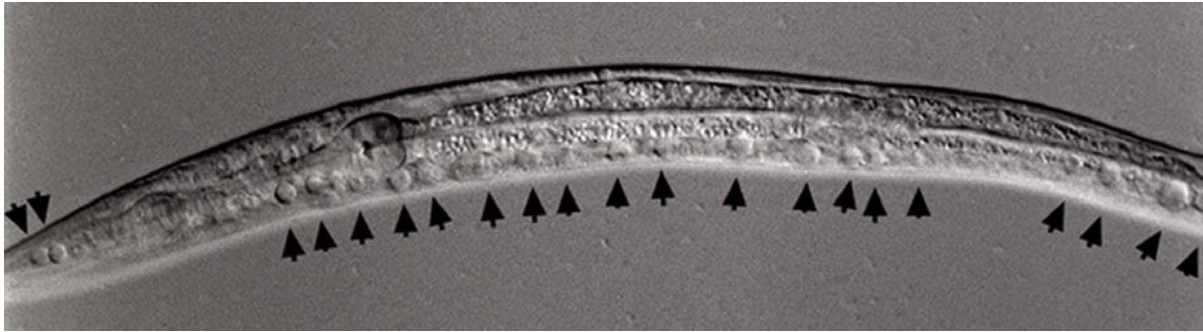
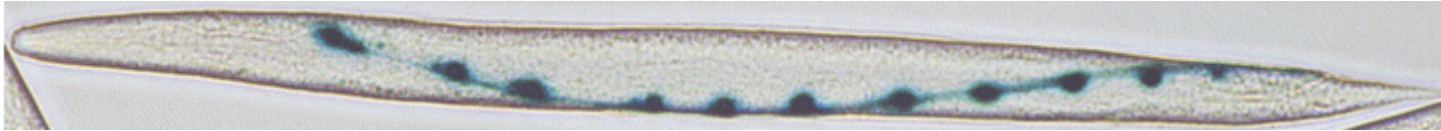




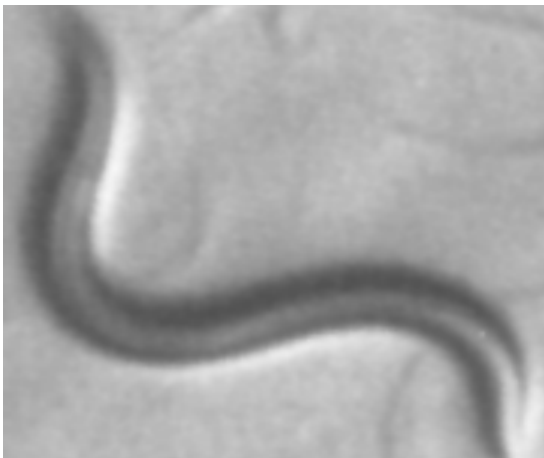


# Viable nervous system mutants

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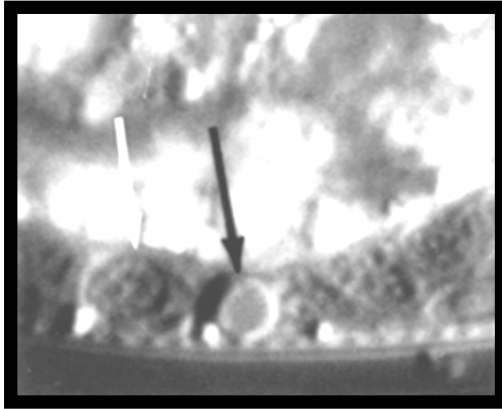
**Motorneuron degeneration and paralysis**



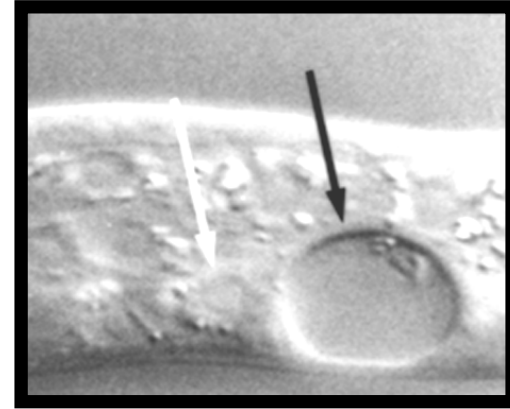
# Two distinct types of cell death in *C. elegans*

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## Apoptotic



## Necrotic

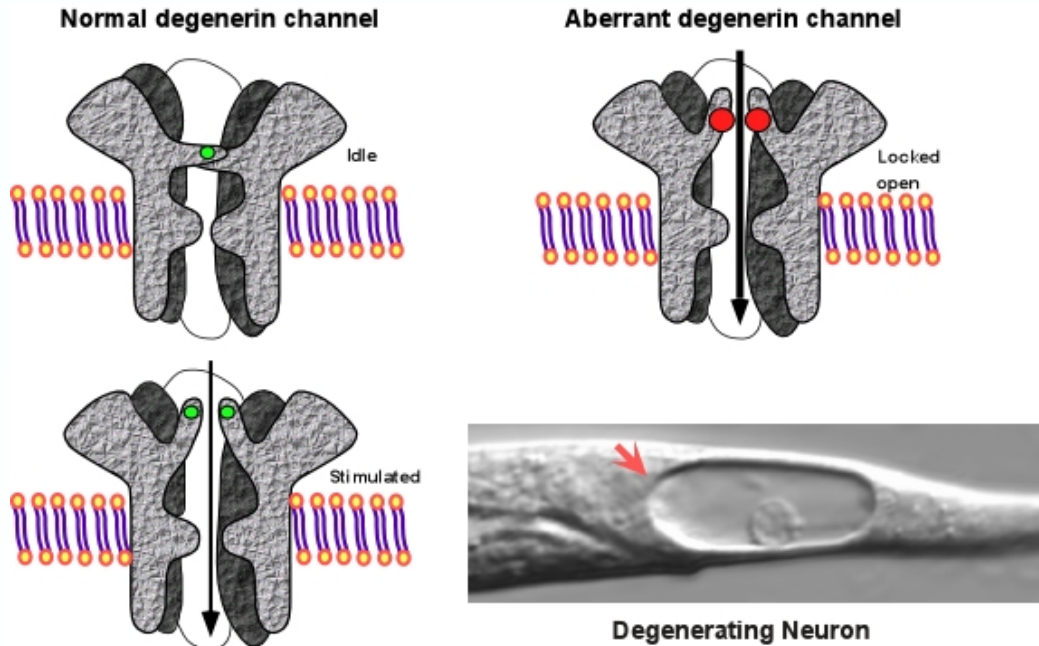


### Morphological and mechanistic differences:

Caspase proteases and other mediators of apoptosis are not required for necrotic cell death

# What triggers necrotic cell death in the worm?

- Hyperactive Degenerin ion channels (DEG-1, MEC-4, UNC-8)



(Chalfie and Wolinsky, 1990)

- Hyperactive acetylcholine receptor (DEG-3)

(Treinin and Chalfie, 1995)

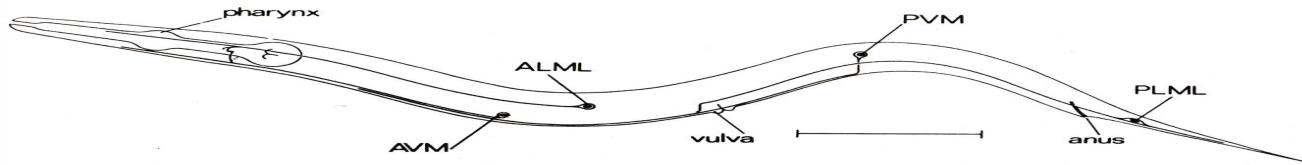
- Constitutively active  $G\alpha_s$

(Korswagen et al., 1997)

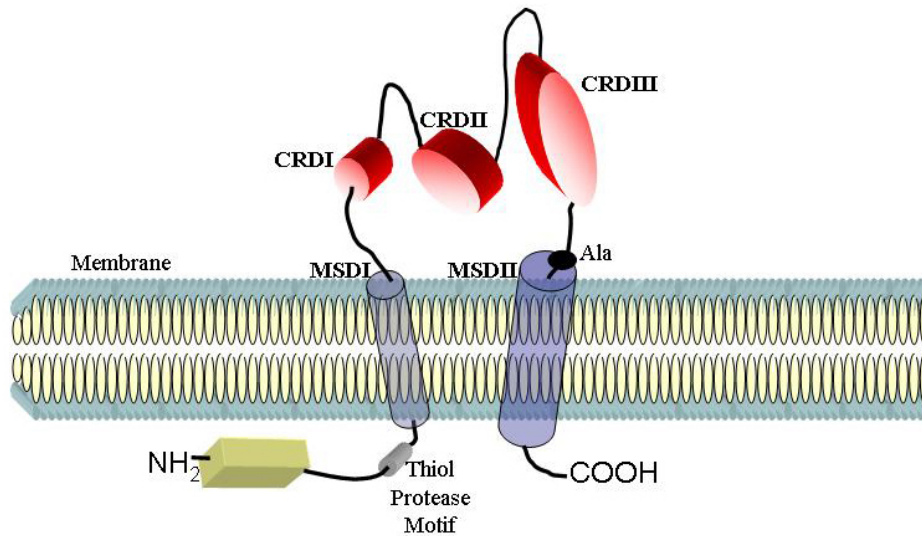
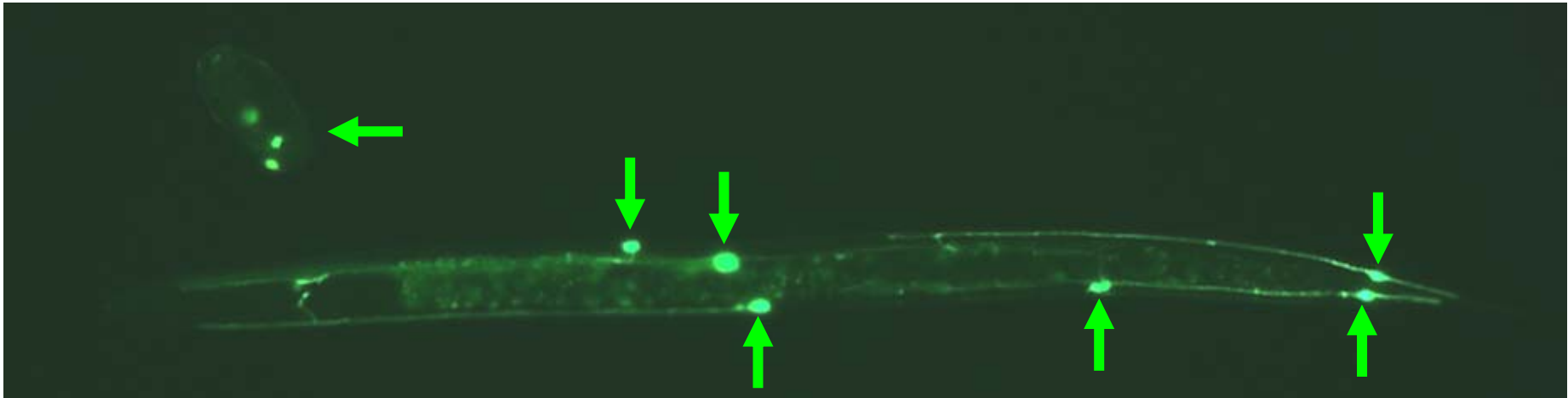
- Hypoxia

(Scott, 2002)

# The *mec-4* case



*mec-4* is exclusively expressed in the 6 touch sensory neurons



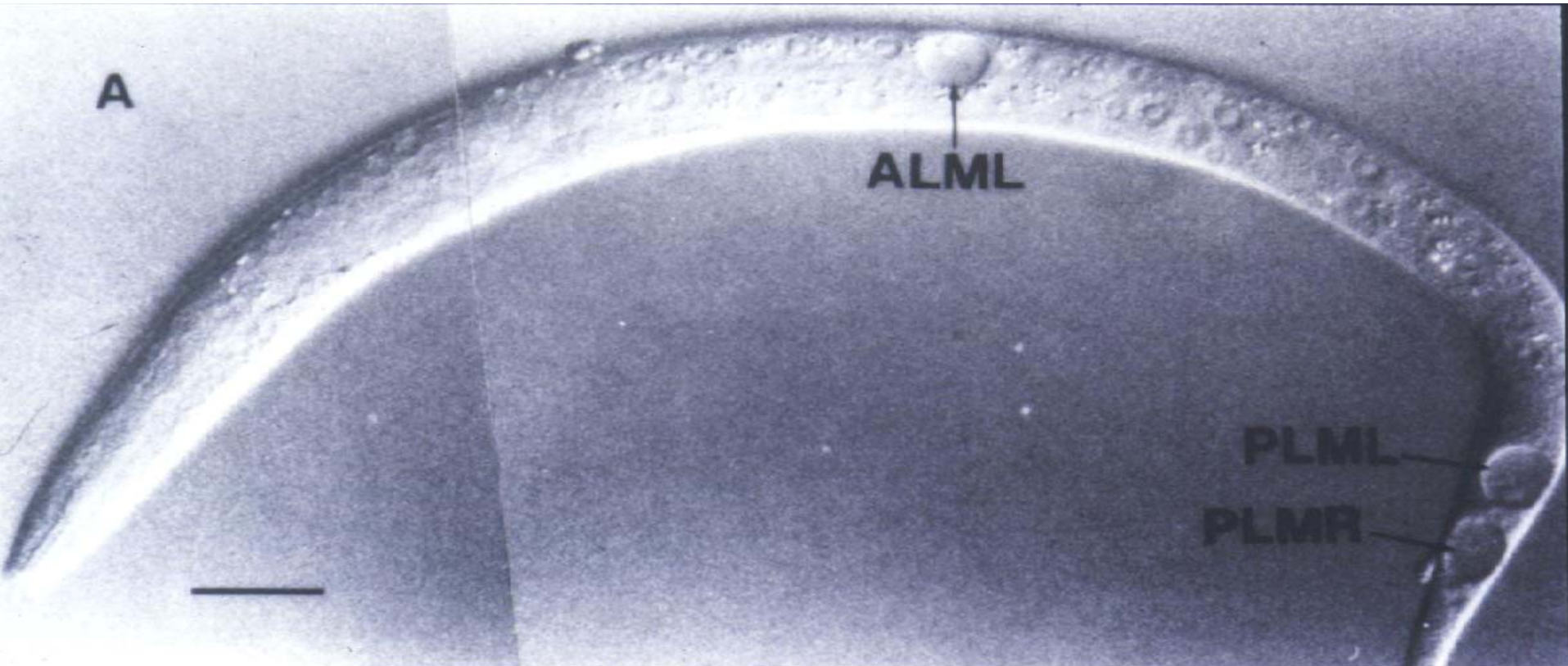
Membrane topology of MEC-4

# Pathological conditions in *C. elegans*

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A *mec-4(d)* mutant:

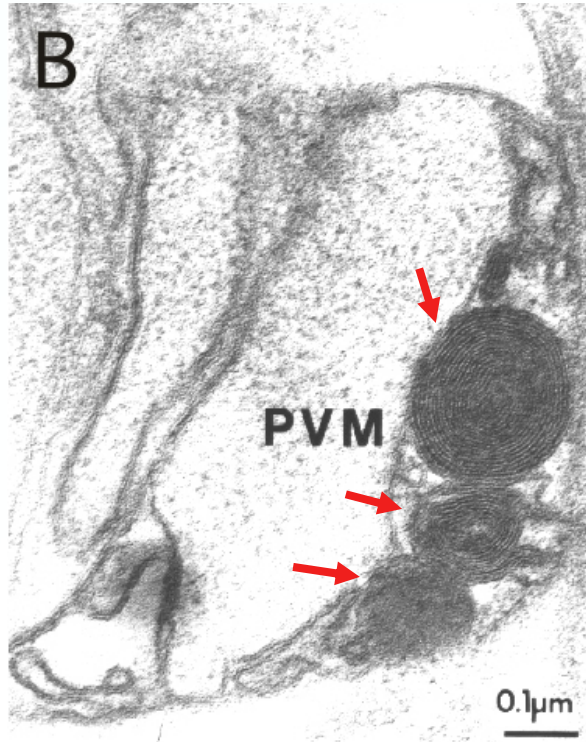
A worm with a neurodegenerative disease...



# Ultrastructural similarities between necrotic cell death in *C. elegans* and neurodegeneration in rats

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**Worms:**  
*mec-4(d)*-induced degeneration



(Hall et al., 1997)

**Rats:**  
Glu excitotoxicity



(Rothstein et al., 1996)

# What we have...

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- Necrosis does occur in *C. elegans*
- Many ways are available to trigger necrosis in *C. elegans*
- At the ultrastructural level, necrotic cell death in worms resembles the mammalian situation

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**What can we do?**

# Tango Genetica

(...one step back, two steps forward...)



## 1<sup>st</sup> step:

Model neurodegeneration in a simple organism

## 2<sup>nd</sup> step:

Characterize neurodegeneration in the simple organism

## 3<sup>rd</sup> step:

Advance understanding of neurodegeneration by genetically identifying the molecular players

insult

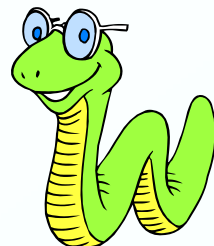
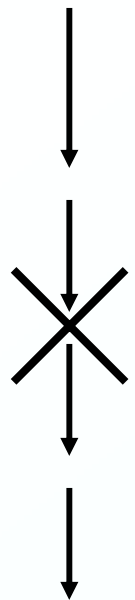


Forward/  
Reverse



Genetics

insult

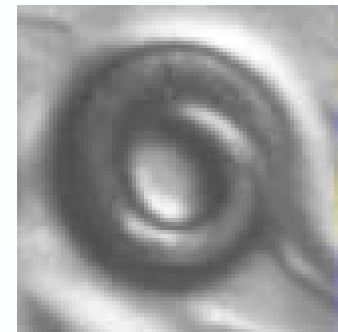
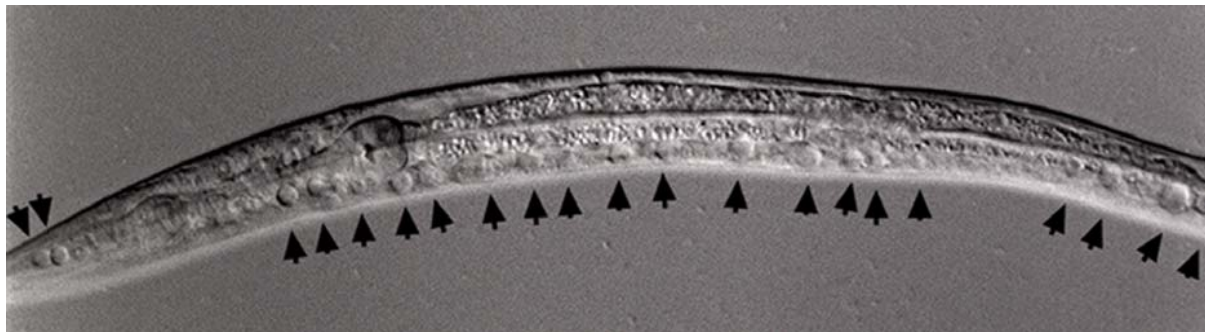
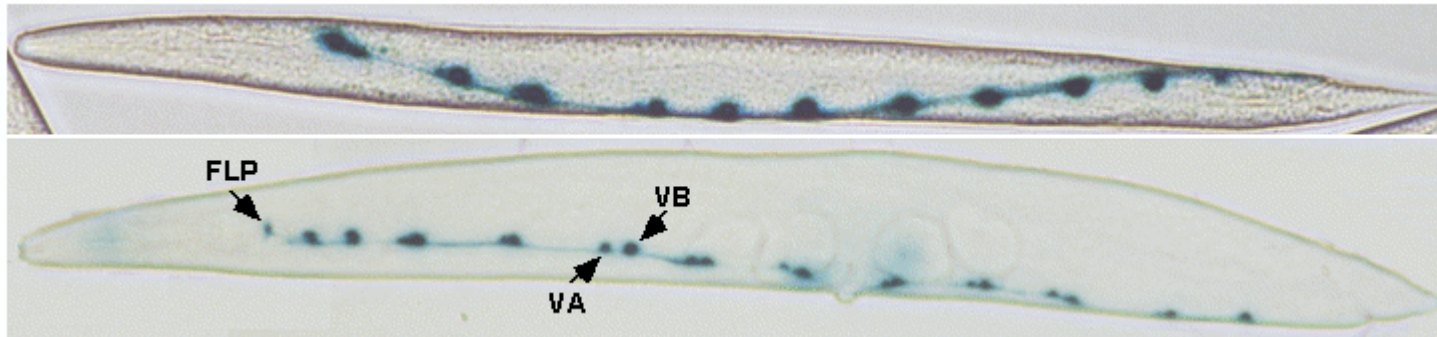




# The practical aspect: Motorneuron neurodegeneration and paralysis

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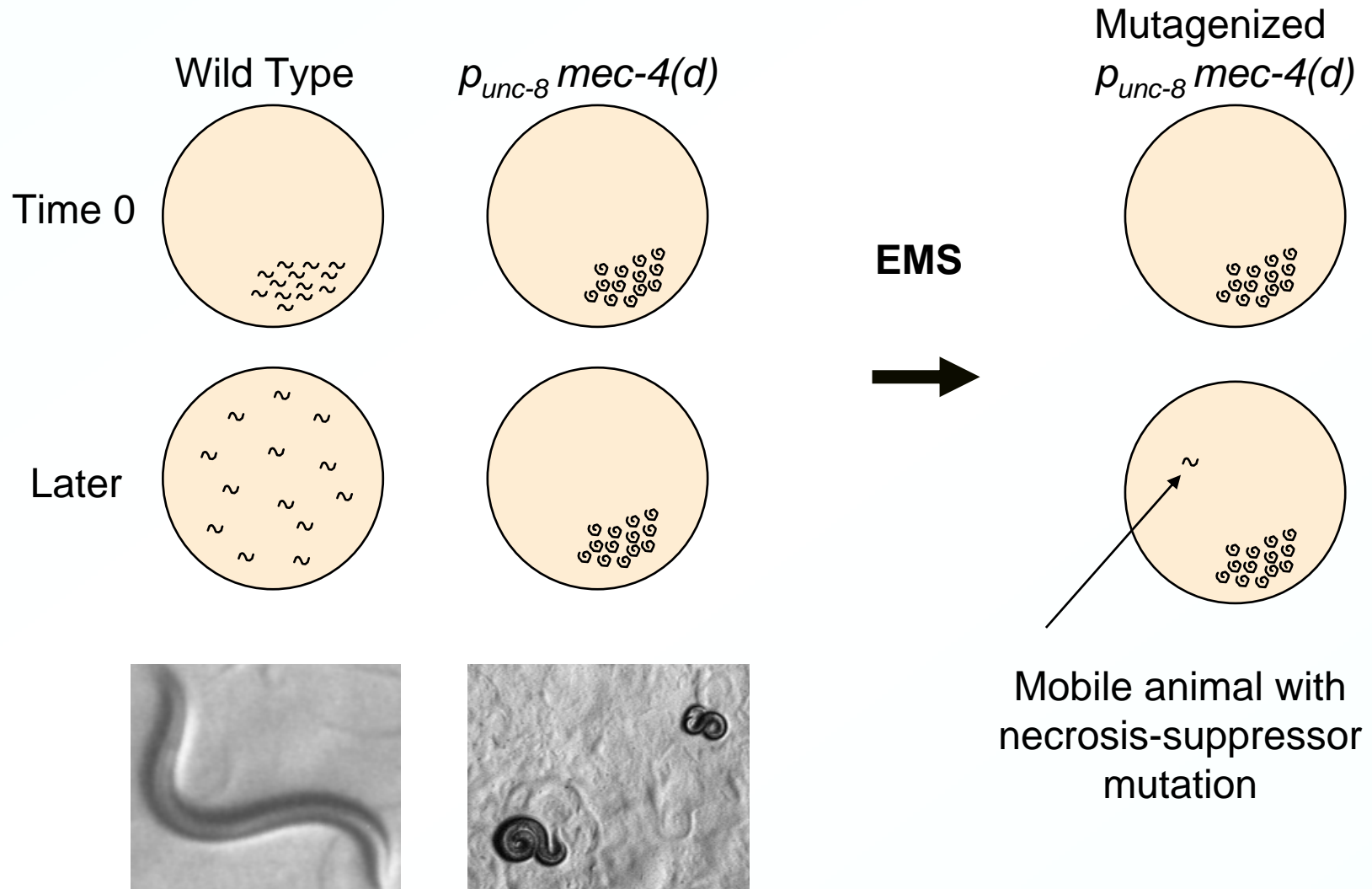
*unc-8* is a gene expressed specifically in motorneurons



Ectopic expression of *mec-4(d)* in motorneurons under the control of the *unc-8* promoter

# Screen for suppressors of necrotic cell death

## Mutagenesis strategy



# The significance of genetic suppressors

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- ✦ **Suppressor genes will shed light in the biochemistry of necrosis**
  - What enzymatic activities are required for degenerative cell death?
- ✦ **Suppressor genes may provide new targets for drug development in the effort to battle degenerative diseases**