Molecular mechanisms of necrotic cell death in *C. elegans*
Necrotic cell death/Necrosis: a different type of cell death

Merriam-Webster Online Dictionary:

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

Source: Purdue University Cytometry Laboratories
# Distinct morphological characteristics of dying cells

## 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

- mitochondrial swelling
- endoplasmic reticulum dilatation
- ill defined cytoplasm
  
  extensive cytoplasmic vacuolation

Kerr, Wyllie, and Currie, 1972
Necrosis can be triggered by numerous insults

- **Genetic factors**

- **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

**Neurodegenerative disorders**

- Stroke

- Trauma
Is necrosis simply the chaotic breakdown of cells?

...or is there order in chaos?
What are the molecular events that transpire during necrotic cell death?

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
A simple nervous system

- Exactly 302 neurons
- All neuronal connections recorded
- The *only* animal with a completely known neuron wiring diagram
- **Viable nervous system mutants**

White et al., 1986

http://www.biologie.ens.fr/bcsgne/
Viable nervous system mutants

Motorneuron degeneration and paralysis
Two distinct types of cell death in C. elegans

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)

- Hyperactive acetylcholine receptor (DEG-3)

- Constitutively active $G_{\alpha_s}$

- Hypoxia
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*

*A mec-4(d) mutant:*

A worm with a neurodegenerative disease...
Ultrastructural similarities between necrotic cell death in \textit{C. elegans} and neurodegeneration in rats

\textbf{Worms:}
\textit{mec-4(d)}-induced degeneration

\textbf{Rats:}
Glu excitotoxicity

(Hall et al., 1997) \hspace{1cm} (Rothstein et al., 1996)
What we have:

- 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

What can we do?
Tango Genetica
(...one step back, two steps forward...)

1st step:
Model neurodegeneration in a simple organism

2nd step:
Characterize neurodegeneration in the simple organism

3rd step:
Advance understanding of neurodegeneration by genetically identifying the molecular players
The practical aspect:
Motorneuron neurodegeneration and paralysis

*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**

- Wild Type
- $p_{unc-8} mec-4(d)$
- Mutagenized $p_{unc-8} mec-4(d)$

Time 0

Later

Mobile animal with necrosis-suppressor mutation

EMS
The significance of genetic suppressors

-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