Additional pathways mediating death?
Autophagy

The primary intracellular catabolic mechanism for degrading and recycling proteins and organelles

Autophagy physiological roles:

- Response to stress (hypoxia, overcrowding, high temperature, accumulation of damaged cytoplasmic components)
- Growth regulation & development
- Innate immunity
- Ageing

Yoshimori, 2003
Is autophagy involved in necrosis?

- Uptregulation of autophagy in neurodegenerative diseases
  (also in cancer, cardiomyopathy, muscular disorders)

- Multivesicular structures in both autophagy and necrosis

(Yue et al. 2002) (Melendéz et al., 2003) (Rothstein et al., 1996)

Rats: Glu excitotoxicity
Upregulation of autophagy in neurodegenerative diseases:

A guardian angel or a mediator of cell death?
Molecular mechanisms of autophagy

A Regulation of autophagy

- **Tor kinase**
  - Nutrient-rich conditions: Phosphatase activates Apg13, which inhibits autophagy.
  - Starvation conditions: Inductive signal activates Tor kinase, which leads to Phosphatase activation of Apg13, promoting autophagy.

B Autophagosome formation

- Apg12
  - Apg7
  - Apg10
  - Apg12
  - Apg12
  - Apg12
  - Autophagosome formation

C Size regulation of the autophagosome

- Inductive signal activates Tor kinase, which activates Phosphatase, regulating AUT7 gene expression and autophagosome size.

(Klionsky and Emr, 2000)
<table>
<thead>
<tr>
<th>Autophagy-Related Genes</th>
<th>Protein Function</th>
<th>C. elegans Homologs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regulation of Autophagy</strong></td>
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<tr>
<td><em>Sc TOR2</em></td>
<td><em>Sc TOR2</em></td>
<td>Rapamycin-sensitive protein kinase</td>
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<tr>
<td><em>Sc ATG6</em></td>
<td><em>Mm beclin1</em></td>
<td>Component of PI3-kinase complex</td>
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<tr>
<td><em>Sc ATG1</em></td>
<td><em>Hs ULK2</em></td>
<td>Protein kinase</td>
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<tr>
<td><strong>Formation of Autophagosomes</strong></td>
<td></td>
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<tr>
<td><em>Sc ATG3</em></td>
<td><em>Mm Apg3/Aut1-like</em></td>
<td>E2-like enzyme; conjugates Atg8 to PE</td>
</tr>
<tr>
<td><em>Sc ATG4</em></td>
<td><em>Mm autophagin 1</em></td>
<td>Cleaves C-terminal extension or PE from Atg8</td>
</tr>
<tr>
<td><em>Sc ATG5</em></td>
<td><em>Mm Autophagy 5-like</em></td>
<td>Conjugated to Atg12 through internal lysine</td>
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<tr>
<td><em>Sc ATG7</em></td>
<td><em>Mm APG7</em></td>
<td>E1-like enzyme; activates Atg8 and Atg12</td>
</tr>
<tr>
<td><em>Sc ATG8</em></td>
<td><em>Mm GABARAP-like 1</em></td>
<td>Ubiquitin-like protein conjugated to PE</td>
</tr>
<tr>
<td><em>Sc ATG9</em></td>
<td><em>Hs APG9-like 1</em></td>
<td>Integral membrane protein</td>
</tr>
<tr>
<td><em>Sc ATG10</em></td>
<td><em>Mm autophagin 10-like</em></td>
<td>E2-like enzyme; conjugates Atg12 to Atg5</td>
</tr>
<tr>
<td><em>Sc ATG12</em></td>
<td><em>Hs APG12</em></td>
<td>Ubiquitin-like protein conjugated to Atg5</td>
</tr>
<tr>
<td><em>Sc ATG16</em></td>
<td><em>Hs APG16-like isoform 2</em></td>
<td>Component of Atg12-Atg5 complex</td>
</tr>
<tr>
<td><em>Sc ATG18</em></td>
<td><em>Sc ATG18</em></td>
<td>Localization of Atg2</td>
</tr>
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<td><em>Hs AAQ96867</em></td>
<td></td>
</tr>
</tbody>
</table>
unc-51 mutations generally suppress neurodegeneration

![Graph showing vacuolated cells per 100 animals for different genotypes](image)

- unc-51(e369); mec-4(d)
- unc-51(e1189); mec-4(d)
- deg-3(d)
- unc-51(e369); deg-3(d)

![Images of C. elegans fluorescently stained for αs(gf)](image)
unc-51 mutations improve survival under hypoxia

![Graph showing % survival for wild type, unc-51(e369), wild type, and unc-51(e1189)]
Specific autophagy genes are required for necrotic cell death

Induction of autophagy

Formation of autophagosomes

- let-363
- unc-51
- unc-14
- bec-1
- lgg-1
Detection of autophagosomes in degenerating neurons

\( \text{P}_{\text{mec-7}} \text{GFP::LGG-1} \)

wild type on food

wild type starved

(\text{Yoshimori, 2004})
Upregulation of the autophagosomal system precedes degeneration of *mec-4(d)* neurons

**P$_{mec-7}$GFP::LGG-1**

**mec-4(d) Mid**

**mec-4(d) Late**
Autophagy is required for, and is induced during, neurodegeneration in *C. elegans*.

- Necrosis-initiating insults
  - ↑ [Ca$^{2+}$]$_i$
  - Calpain activation
  - Lysosome rupture
    - Intracellular acidification
      - Aspartyl protease activation
      - Death
  - ↑ Autophagy
Many Thanks...

- Marta Artal
- Dafni Bazopoulou
- Angela Pasparaki
- Chrysa Samara
- Popi Syntichaki
- Kostoula Troulinaki
- Giannis Voglis
- Keli Xu (NJ)
- Monica Driscoll (NJ)
- Erik Jorgensen (Utah)
- James Rothman (NY)

- *C. elegans* gene knockout consortium
- *Caenorhabditis* Genetics Center

EU Sixth Framework Programme
Young Investigator Programme

IMBB
Heraklion, Crete, Greece