Mutant screens I
Direct screens for cell death mutants
Nobel Prize 2002

Reading: lecture notes
Problem set 5

C. elegans development

Genetics

\[ \begin{align*}
XX \text{ h} & \quad XX \text{ h} \times XO \text{ m} \\
\downarrow & \quad \downarrow \\
\text{self progeny} & \quad \text{self progeny} \\
XX \text{ h} & \quad XX \text{ h} \\
\downarrow & \quad \downarrow \\
\text{cross progeny} & \quad \text{cross progeny} \\
1/2 XX \text{ h}; 1/2 XO \text{ m} & 
\end{align*} \]

So how do you tell the difference between self and cross progeny?

Dumpy \((dpy-5 \text{ I})\) herm \times \text{ male} \\
\downarrow \\
\text{self progeny} \\
Dpy \text{ herm} \\
\text{cross progeny} \\
1/2 \text{ nonDpy h}; 1/2 \text{ nonDpy m}

Apoptosis plays an important role in development

- Histogenic cell death: up to a half of the neurons normally die during development of parts of the brain.
- Phylogenic cell death: the loss of the vertebrate tail during human fetal development.
- Morphogenic cell death: the loss of mesenchyme between the digit.
- Cancer: damaged precancerous cells are removed by programmed cell death
- Programmed cell death in \(C. \text{ elegans}\): more than 10\% of the cells produced during development die.
Hallmarks of apoptosis

- Nucleus condensation.
- DNA fragmentation.
- Phagocytosis

C. elegans develops from an invariant lineage

131 cells undergo apoptosis or programmed cell death in the C. elegans hemaphrodite. The cell deaths are indicated by Xs in the lineage.

Cell corpses can be observed by Nomarski optics

Cell death mutants defective in different stages

Newly hatched larvae have no corpses because of phagocytosis.

Disruption of engulfment genes results in persistent corpses.

Newly hatched larvae have persistent corpses if there is a defect in phagocytosis.
In screens for engulfment mutants Ed Hedgecock identified the \textit{ced-1} and \textit{ced-2} genes. Additional screens by the Horvitz lab identified the \textit{ced-5}, \textit{ced-6}, \textit{ced-7}, \textit{ced-8}, \textit{ced-10}, and \textit{ced-12} genes.

In \textit{ced-3(f)}, \textit{ced-4(f)}, \textit{ced-9(gf)} and \textit{egl-1(f)} mutants all 131 cells that normally die survive.

The engulfment \textit{ced} genes function in phagocytosis and often cell migration. Regulate cell movement.

In \textit{ced-1} mutants identified genes involved in apoptosis.

<table>
<thead>
<tr>
<th>Engulfment mutant</th>
<th>Engulfment: apoptosis double mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoptosis</td>
<td>X</td>
</tr>
<tr>
<td>engulfment</td>
<td>X</td>
</tr>
<tr>
<td>persistent corpse</td>
<td>no persistent corpse</td>
</tr>
</tbody>
</table>

Screens for the loss of cell corpses in \textit{ced-1} mutants identified genes involved in apoptosis.

Arrowheads indicate extra cells
*n*1950 is a dominant mutation in *ced-9*

\[
\begin{array}{c}
\text{nDf40} \\
\text{ced-9} \\
\text{nDf40/+ wild type} \\
\text{n1950/+ many cells that should die survive} \\
\text{Therefore gain-of-function mutation}
\end{array}
\]

Loss of function and gain-of-function alleles of *ced-9* have opposite phenotypes.

*ced-9*(gf) disrupts apoptosis
*ced-9*(lf) is recessive lethal because of widespread cell death

**ced-3** promotes apoptosis
**ced-9** inhibits apoptosis

\[
\begin{array}{ccc}
\text{ced-3} & \text{cell death} & \text{Prediction: ced-3(lf); ced-9(lf) animals die because of extensive apoptosis} \\
\text{ced-9} & \text{cell death} & \text{Prediction: ced-3(lf); ced-9(lf) animals survive and have no apoptosis} \\
\text{Prediction: ced-3(lf); ced-9(lf)] intermediate phenotype}
\end{array}
\]

*C. elegans* is sexually dimorphic
Many differences at the cellular level
X:A ratio determines sexual fate

Life and Death of a Single Neuron

The hermaphrodite specific neuron (HSN), which regulates egg laying, lives in hermaphrodites but dies in males.
In males:
\[
\textit{ced-9} \quad \textit{ced-3} \rightarrow \text{HSN dies}
\]
OFF \quad ON

In hermaphrodites:
\[
\textit{ced-9} \rightarrow \textit{ced-3} \quad \text{HSN survives}
\]
ON \quad OFF