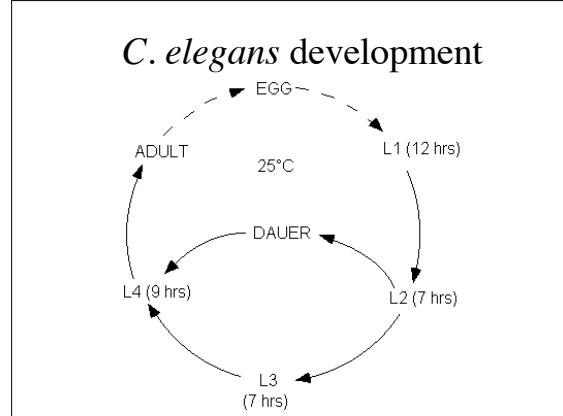




Mutant screens I
Direct screens for cell death mutants
Nobel Prize 2002

Reading: lecture notes
Problem set 5



C. elegans

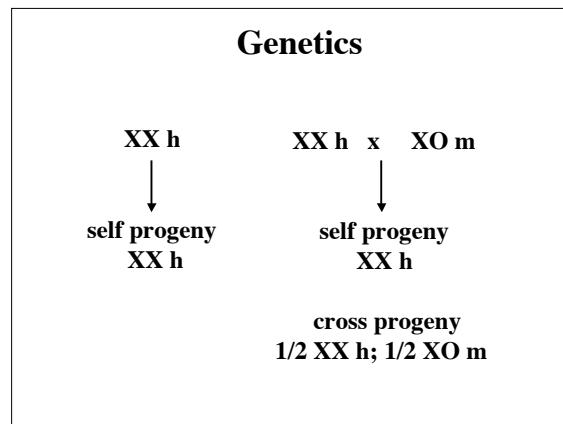
Grow on bacterial lawn

Hermaphrodites are XX

Hermaphrodites make ~300 sperm and ~1000 oocytes

Hermaphrodites produce ~300 progeny from self fertilization

Males are X0, rare (1/2000 self progeny), and result from nondisjunction



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

Dumpy (*dpy-5* I) herm x male

↓

self progeny
Dpy herm

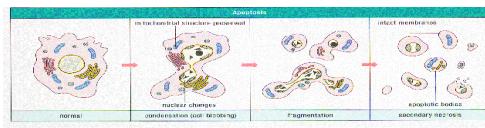
cross progeny
1/2 nonDpy h; 1/2 nonDpy m

Apoptosis plays an important role in development

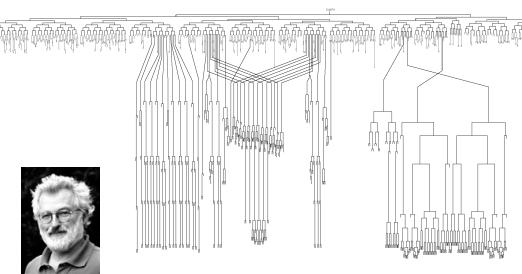
- Histogenic cell death: up to a half of the neurons normally die during development of parts of the brain.
- Phylogenetic cell death: the loss of the vertebrate tail during human fetal development.
- Morphogenetic cell death: the loss of mesenchyme between the digit.
- Cancer: damaged precancerous cells are removed by programmed cell death
- Programmed cell death in *C. elegans*: more than 10% of the cells produced during development die.

Hallmarks of apoptosis

- Nucleus condensation.
- DNA fragmentation.
- Phagocytosis

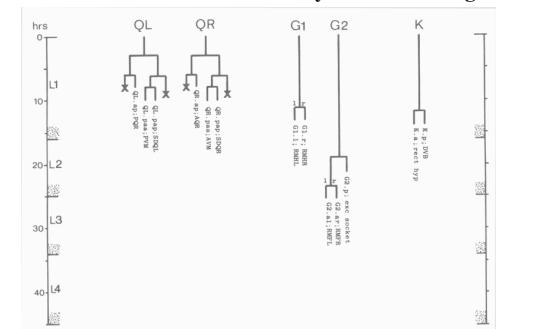


C. elegans develops from an invariant lineage

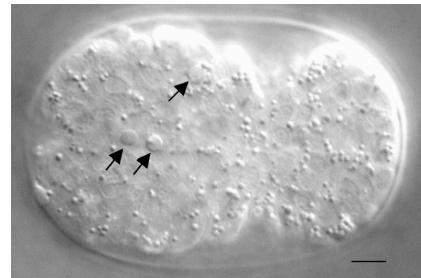


131 cells undergo apoptosis or programmed cell death in the *C. elegans* hemaphrodite.

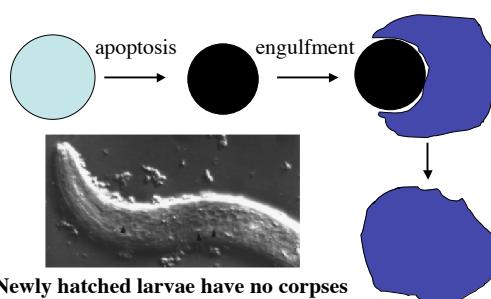
The cell deaths are indicated by Xs in the lineage.



Cell corpses can be observed by Nomarski optics

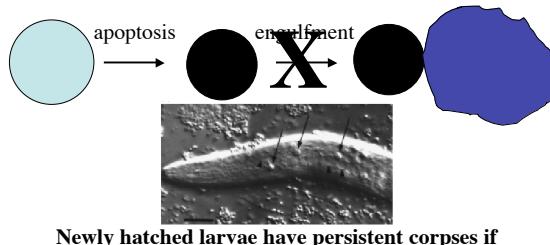


Cell death mutants defective in different stages

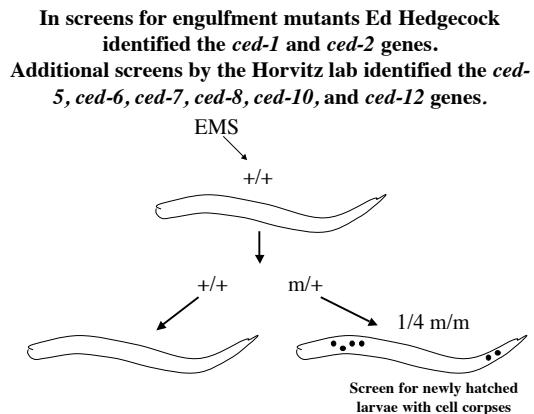


Newly hatched larvae have no corpses because of phagocytosis.

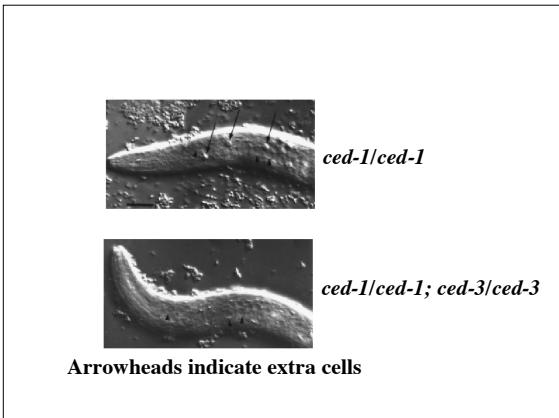
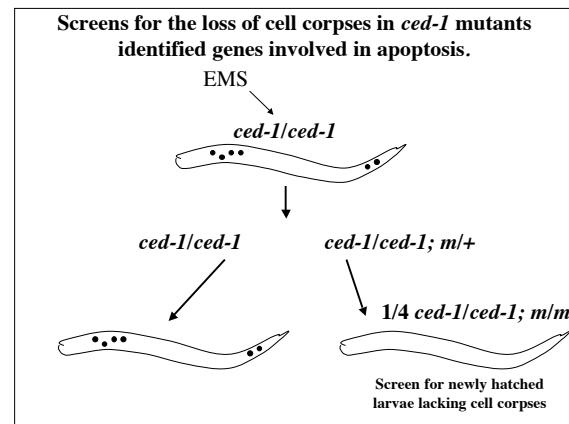
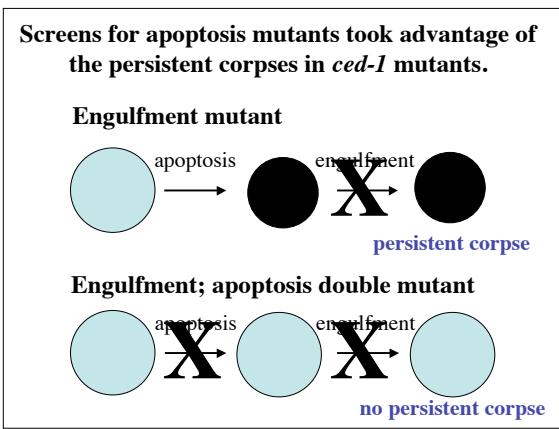
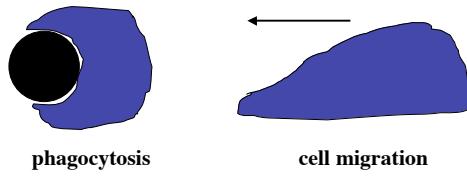
Disruption of engulfment genes results in persistent corpses.



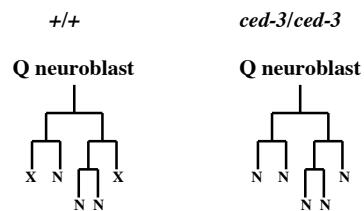
Newly hatched larvae have persistent corpses if there is a defect in phagocytosis.



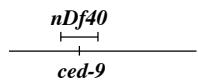
The engulfment *ced* genes function in phagocytosis and often cell migration. Regulate cell movement.



In *ced-3(lf)*, *ced-4(lf)*, *ced-9(gf)* and *egl-1(lf)* mutants all 131 cells that normally die survive.



n1950 is a dominant mutation in *ced-9*



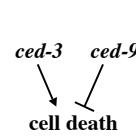
***nDf40/+* wild type**
***n1950/+* many cells that should die survive**
Therefore gain-of-function mutation

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

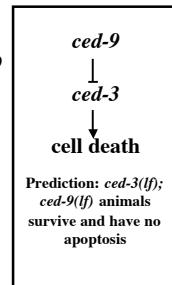
***ced-9(gf)* disrupts apoptosis**
***ced-9(lf)* is recessive lethal**
because of widespread cell death

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

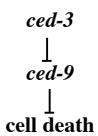
Models



Prediction: *ced-3(lf); ced-9(lf)* intermediate phenotype



Prediction: *ced-3(lf); ced-9(lf)* animals survive and have no apoptosis



Prediction: *ced-3(lf); ced-9(lf)* animals die because of extensive apoptosis

***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:

ced-9 *ced-3* —> HSN dies
OFF ON

In hermaphrodites:

ced-9 —> *ced-3* HSN survives
ON OFF