Apoptosis (programmed cell death) plays many important roles in the development and survival of an organism

- *Histogenic cell death*: up to a half of the neurons normally die during development of parts of the brain.

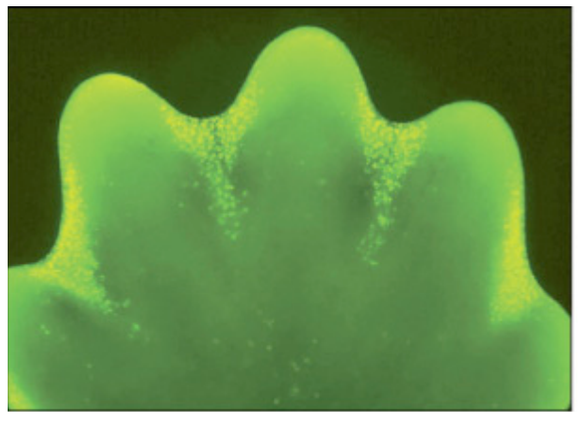
- *Phylogenic cell death*: the loss of the vertebrate tail during human fetal development.

- *Morphogenic cell death*: the loss of mesenchyme between the digits.

- *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.

Morphogenic cell death



Sidney Brenner

John Sulston

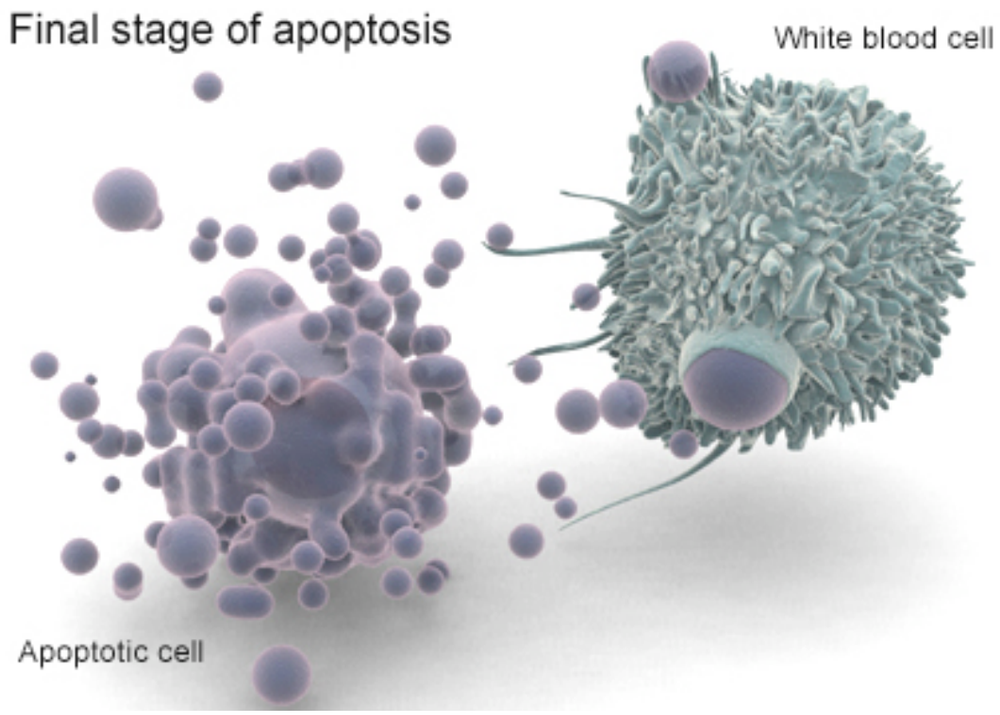
Bob Horvitz

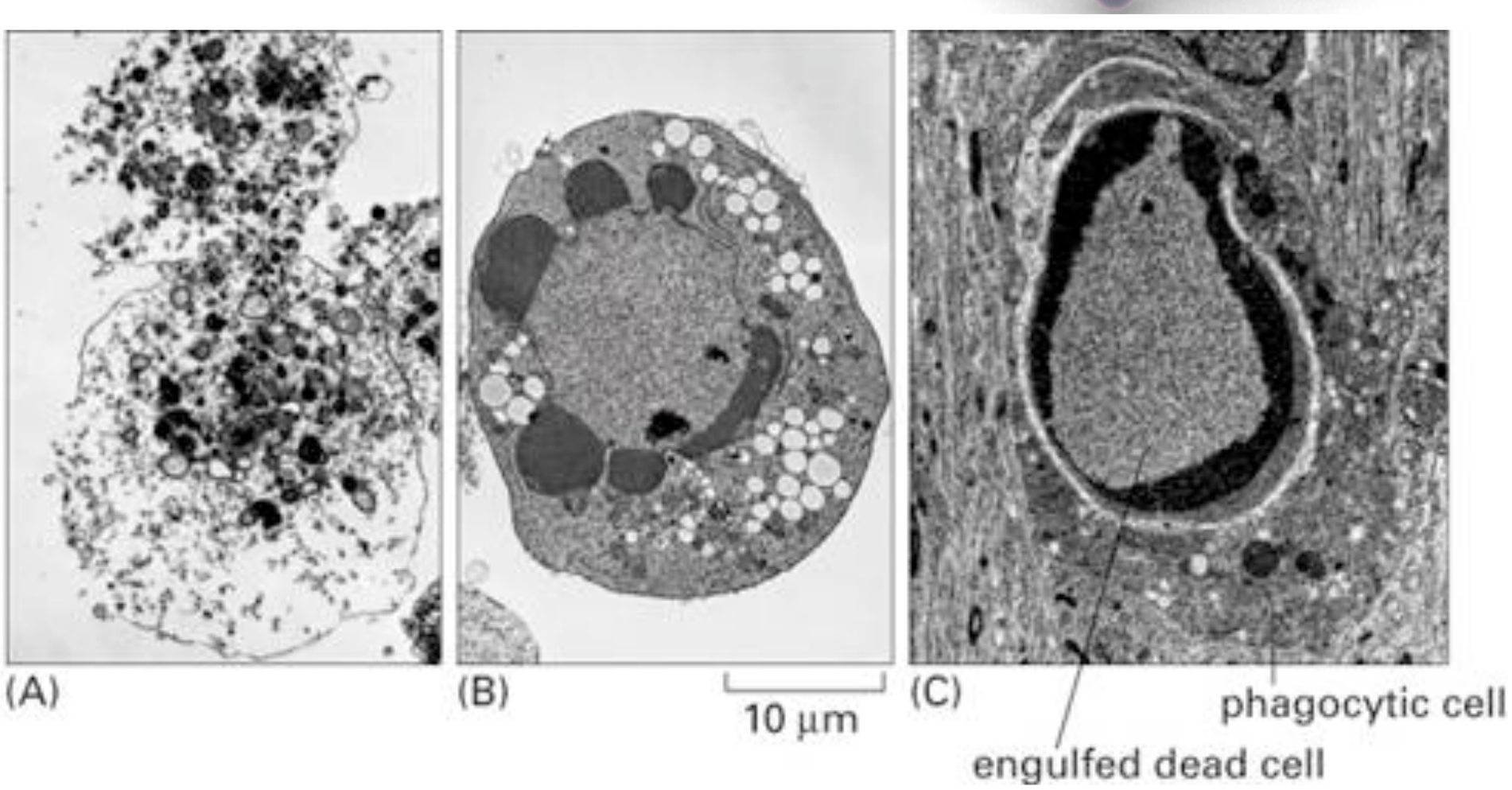
Using *C. elegans* as a genetic model system was this guy's idea

He shared the 2002 Nobel prize with these guys for working out the cell lineage and apoptosis

Hallmarks of apoptosis

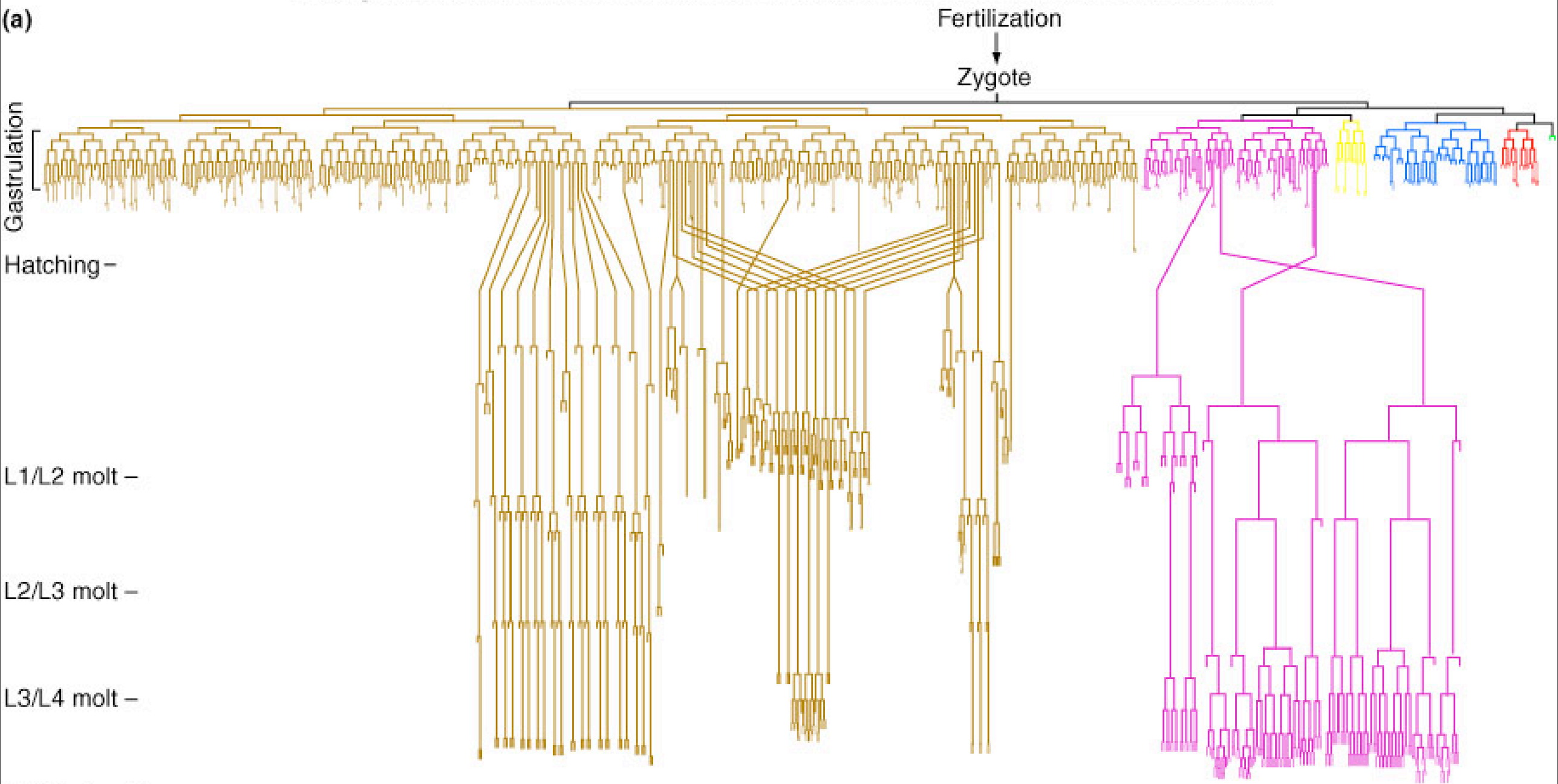
- Nuclear condensation
- DNA fragmentation
- Membrane "blebbing"
- Phagocytosis by another cell

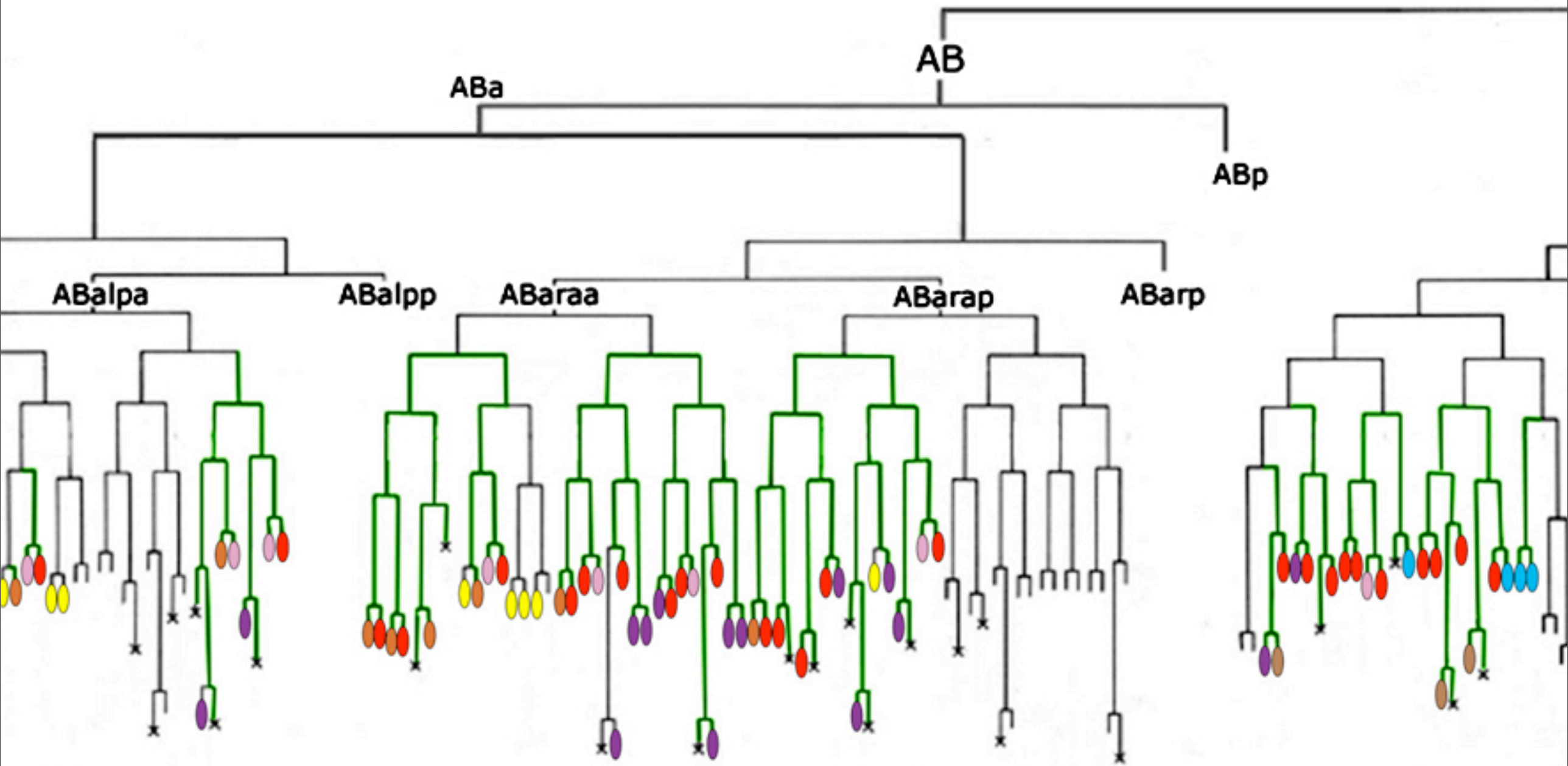




From Wednesday's lecture:

C. elegans has an "invariant" cell lineage

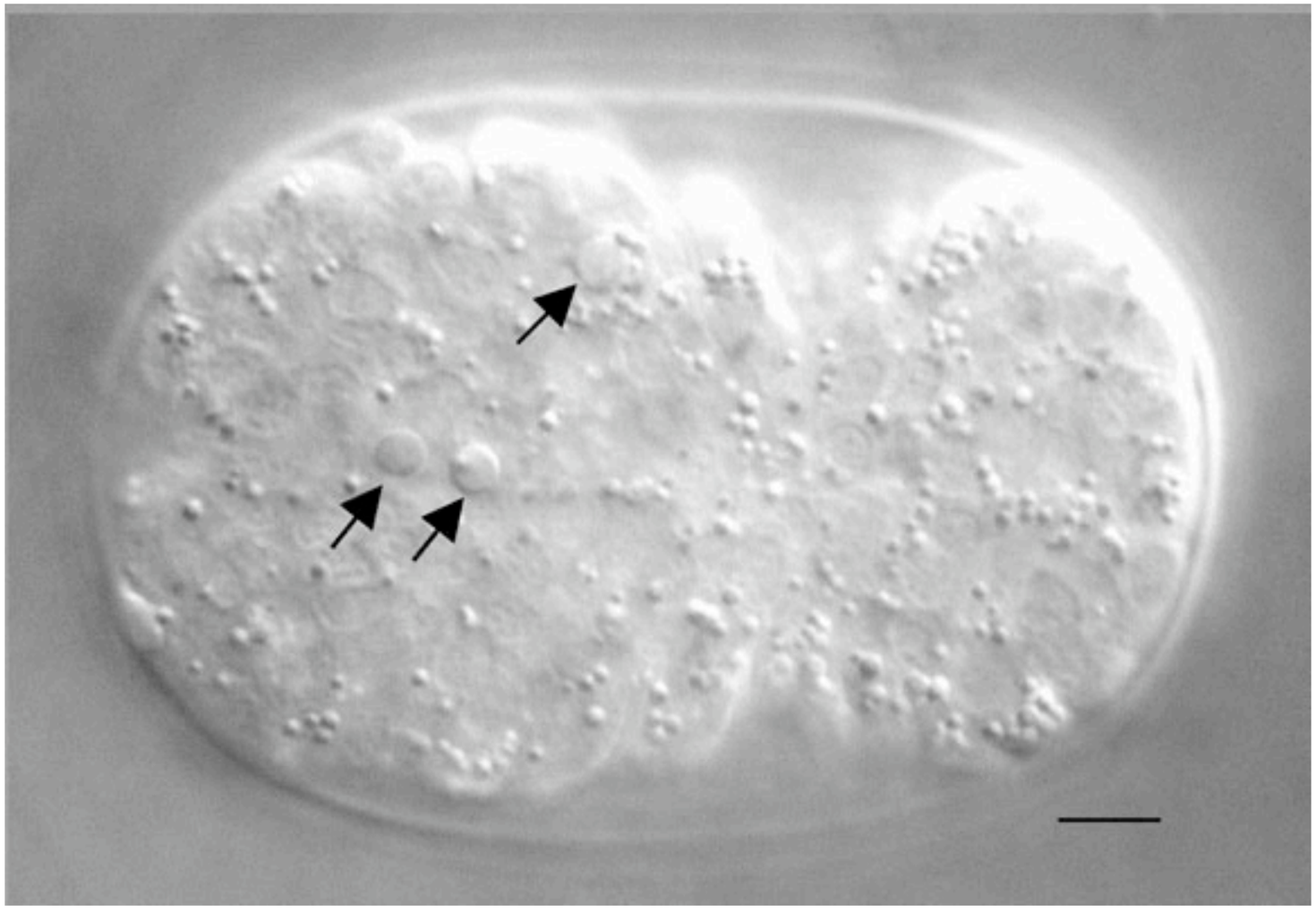




Cells that normally undergo programmed cell death (apoptosis) are marked by an X in this lineage

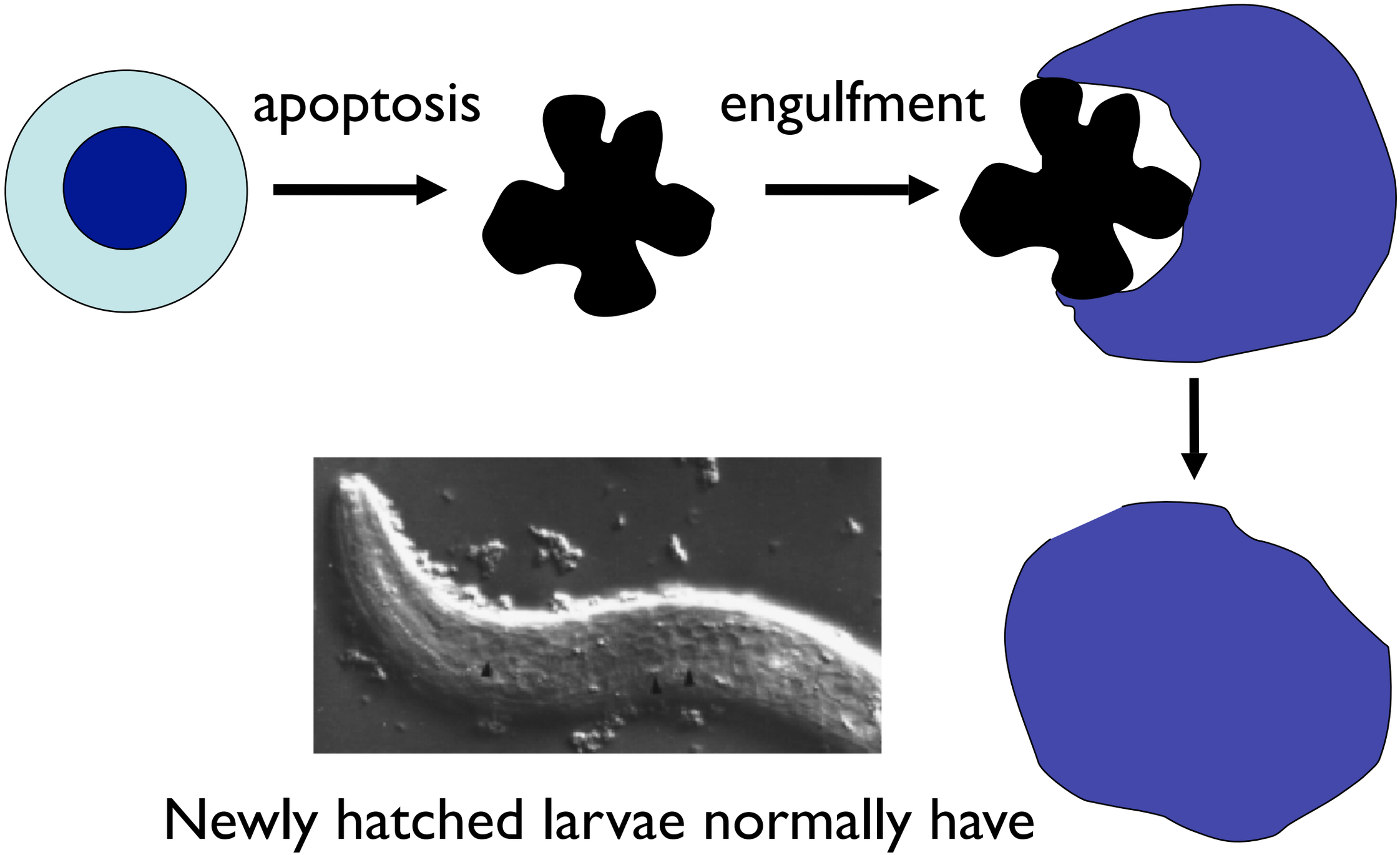
(131 out of 1090 somatic cells, leaving 959 cells)

Visualizing apoptosis (programmed cell death) in *C. elegans*



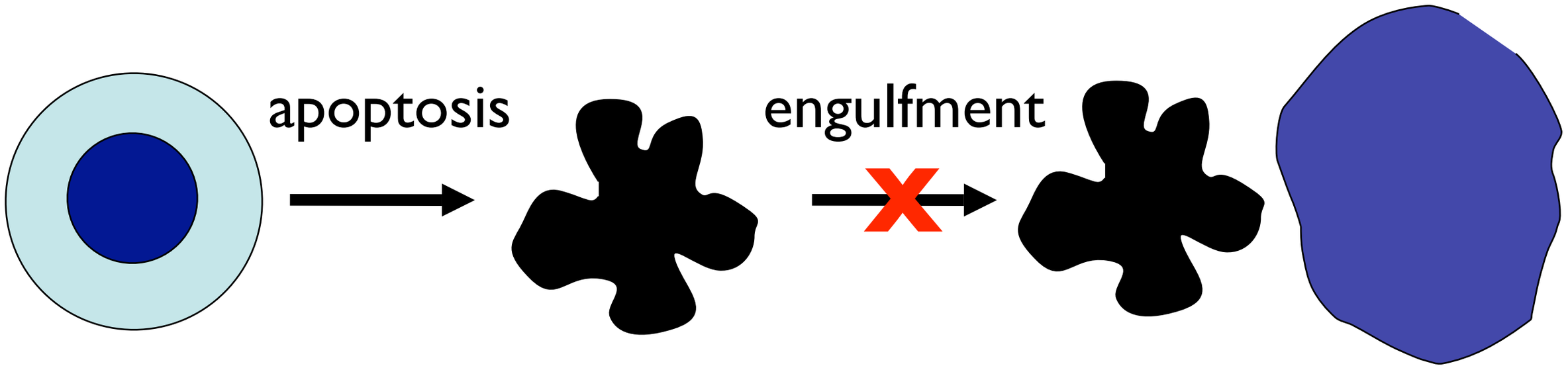
"Corpses" - dying cells in a *C. elegans* embryo

Cell death involves separate processes: apoptosis (the actual dying part) and engulfment



Newly hatched larvae normally have no corpses, due to their engulfment

Disruption of engulfment genes results in persistent corpses



Persistent corpses can be seen in newly hatched L1 larvae if there is a defect in phagocytosis.

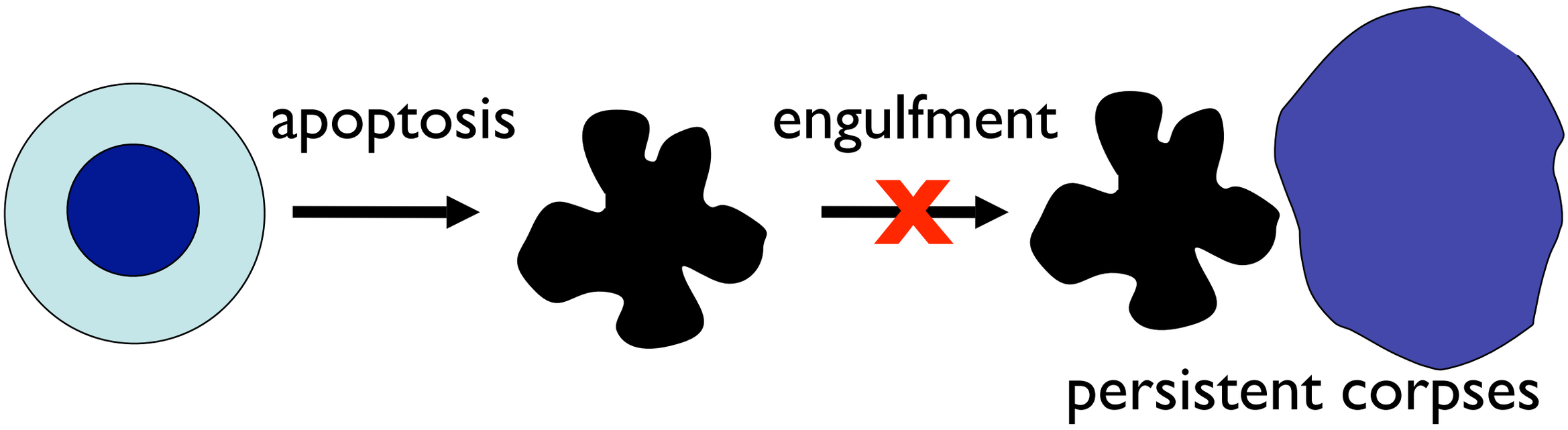
ced-1 mutants are unable to execute corpse engulfment, a.k.a. phagocytosis.

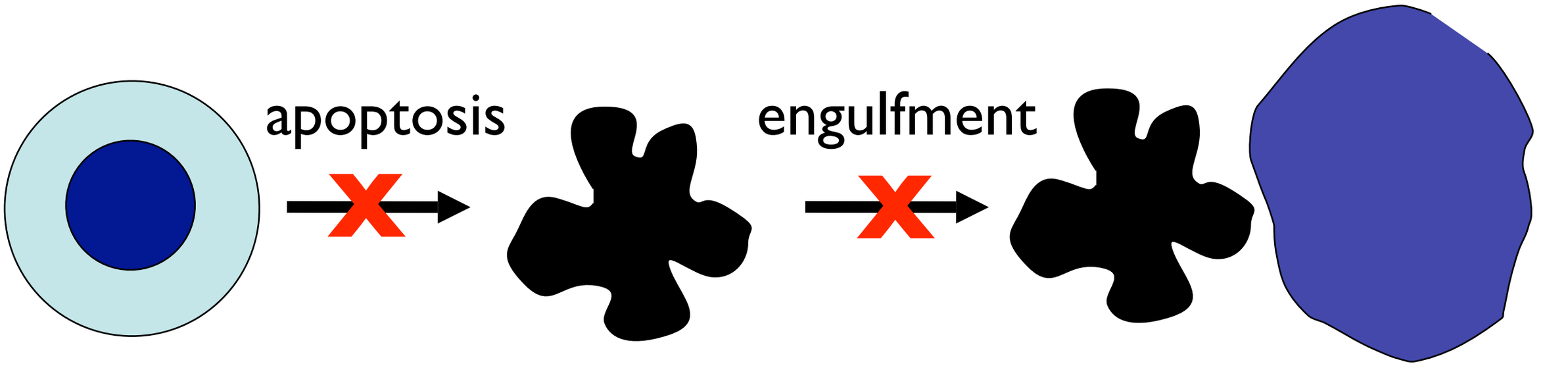
Lots of corpses are visible at the end of embryogenesis

Wild type

ced-1

Strategy to find mutations that block apoptosis: take advantage of mutations in engulfment genes, specifically, *ced-1*



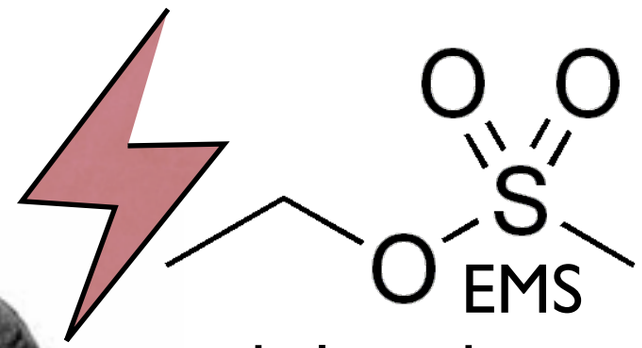


A screen for recessive cell death-defective mutants in *C. elegans*

generation

P_0

ced-1/ced-1



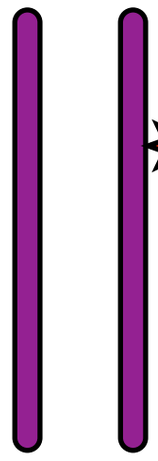
ethyl methanesulfonate

self-fertilize

F_1

ced-1/ced-1; m/+

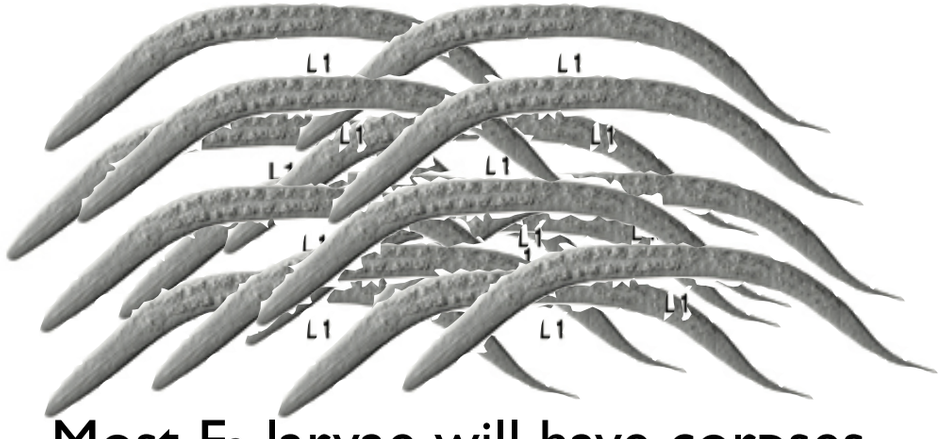
heterozygous for any new mutation



self-fertilize

F_2

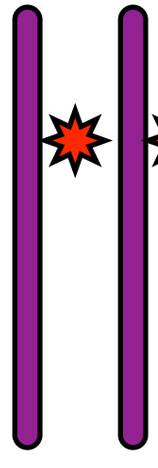
ced-1/ced-1



Most F_2 larvae will have corpses

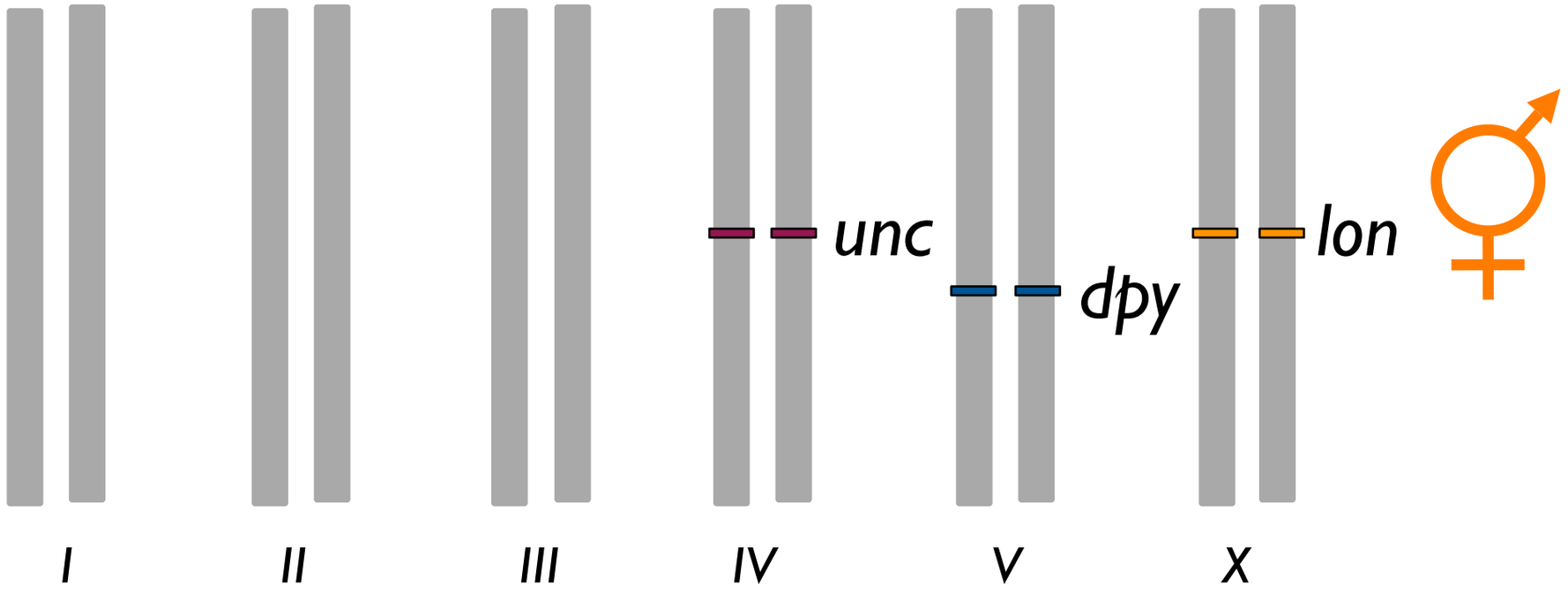
ced-1/ced-1; m/m

look for <u>absence</u> of corpses



1/4

How do you map a gene to a chromosome?
Mate your mutant animals to mapping strains:



m/m (or m/+) X *unc-5; dpy-11, lon-2*

F_1 *m/+; unc-5/+; dpy-11/+; lon-2/+*

self-fertilize

F_2

This cross allows you to test for linkage to three different chromosomes at once.

There is another mapping strain for chromosomes *I*, *II*, and *III*:

dpy-5 I; bli-2 II; unc-32 III

Pick Uncs, Dpys, and Lons separately

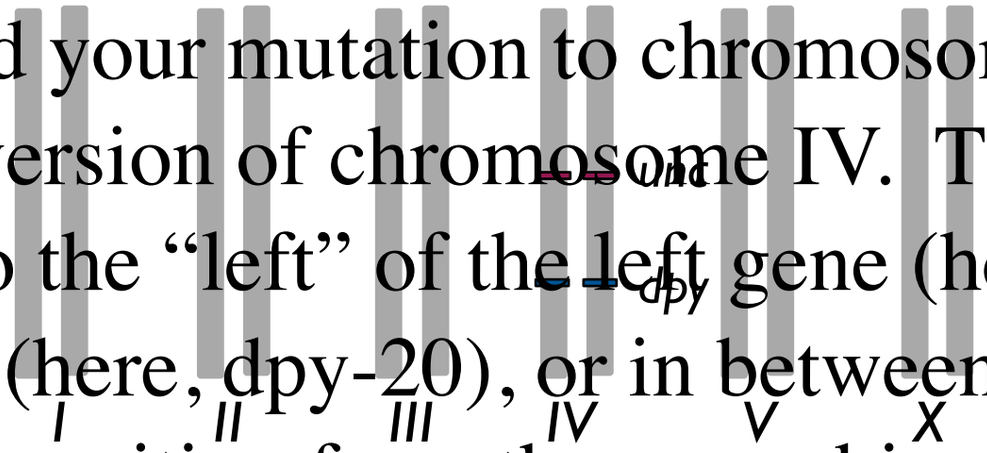
If a mutation is <u>not</u> linked to *unc-5*, 1/4 of Unc progeny are *m/m*.

If it <u>is</u> linked, fewer than 1/4 are *m/m*.

How do you map a gene to a region on a chromosome?

2- and 3-factor crosses

If you've mapped your mutation to chromosome IV, cross it to a doubly-marked version of chromosome IV. This can tell you whether the mutation is to the "left" of the left gene (here, unc-24), to the right of the right gene (here, dpy-20), or in between. If it's in between you can calculate the position from the recombination frequency between your mutation and the two markers. It's useful if you can pick markers flanking your mutation...



m/+ (IV) × *unc-24 dpy-20 (IV)*

↓

F_1 ♀ $\frac{\textit{unc-24 dpy-20}}{m}$ *(IV)*

↓ *self-fertilize*

$\frac{\textit{unc-24}\quad m?}{\textit{unc-24}\quad \textit{dpy-20}}$ + $\frac{m?\quad \textit{dpy-20}}{\textit{unc-24}\quad \textit{dpy-20}}$ + $\frac{\textit{unc-24}\quad \textit{dpy-20}}{\textit{unc-24}\quad \textit{dpy-20}}$

F_2 ♂ pick Unc nonDpy and Dpy nonUnc recombinants

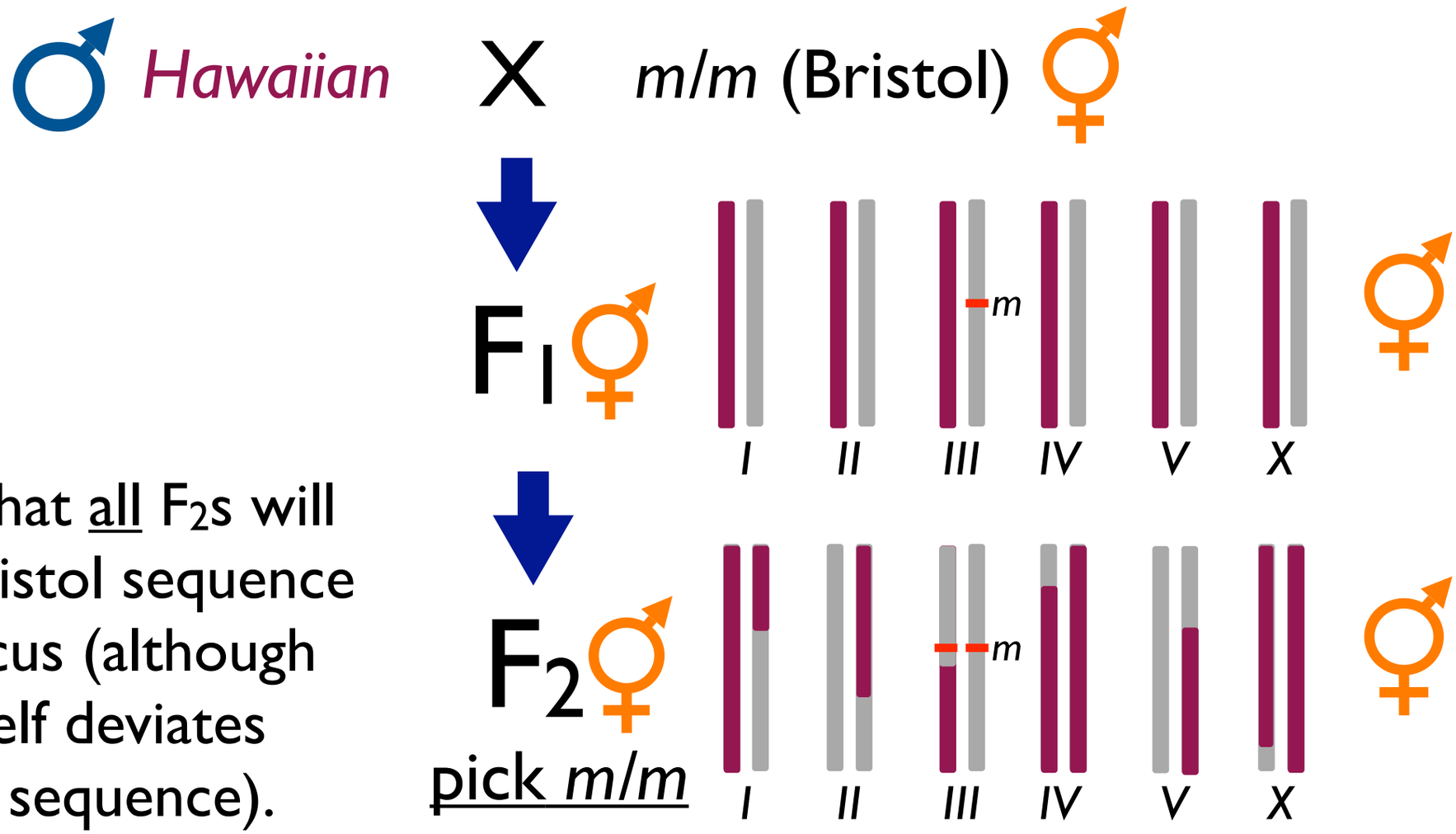
Summary of what's shown on the previous slide:

If you've mapped your mutation to chromosome *IV* (for example), a good next step is to cross it to a doubly-marked version of chromosome *IV (*e.g. *unc-24 dpy-20 (IV))*. By analyzing recombinant products (*unc-24* + and + *dpy-20*) chromosomes, you can determine whether the mutation is to the "left" of the left gene (here, *unc-24*), to the right of the right gene (here, *dpy-20*), or in between the two markers. If it's in between them, you can calculate the position from the recombination frequency between your mutation and each of the two markers. Therefore, it's most useful if you can pick markers flanking your mutation...

I recommend that you go through this exercise to make sure you understand what you would see and how you would interpret it.

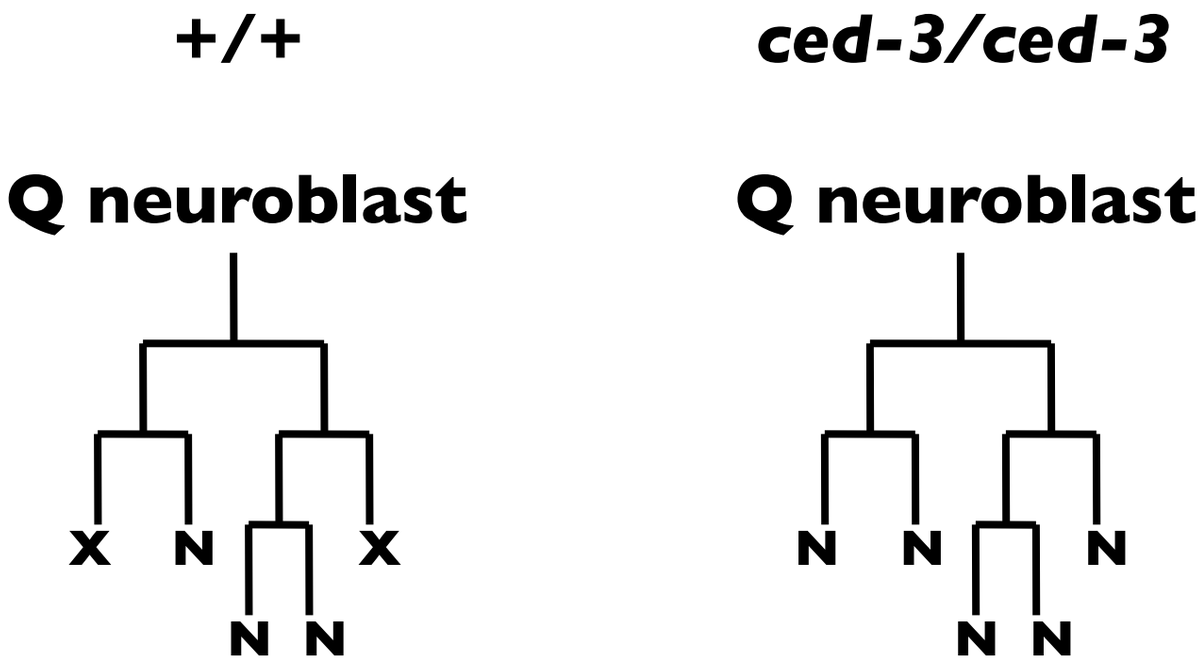
There is a more "modern" technique for mapping that uses a strain from Hawaii
(the original wild-type strain is from Bristol, England)

The Hawaiian strain and the Bristol strain have polymorphisms (base differences in their genome sequence) roughly every 1-kb.



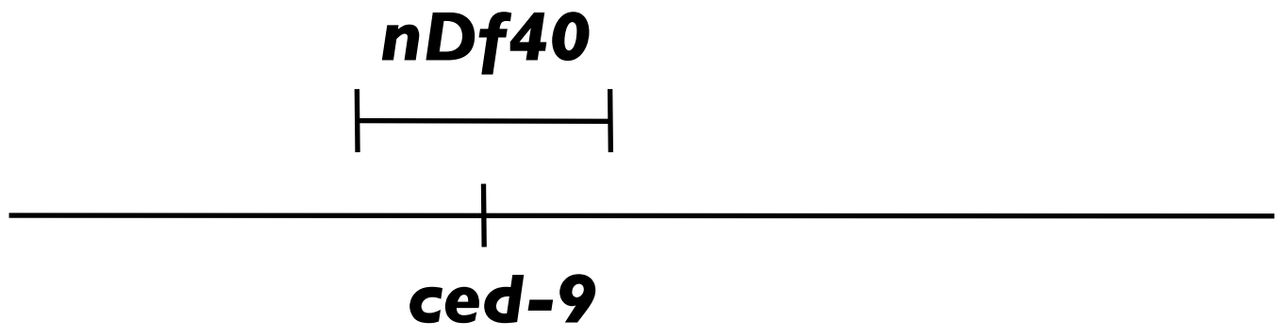
The only place that <u>all</u> F_2s will have <u>only</u> the Bristol sequence is near the *m* locus (although the mutation itself deviates from the Bristol sequence).

You find recessive mutations in *ced-3*, *ced-4*, and *egl-1* that result in the survival of all 131 cells that normally die



You also find a dominant mutation in *ced-9* that has a very similar phenotype...
why is it dominant?

ced-9(n1950) is a dominant mutant allele of *ced-9*



nDf40 is a small Deficiency (=deletion) that spans the *ced-9* locus

nDf40/+ animals are wild-type with respect to apoptosis
this indicates that loss-of-function of *ced-9*
does **not** give a dominant phenotype,
and that *ced-9(n1950)* is therefore a gain-of-function mutation

Isolation of loss-of-function alleles of *ced-9* confirmed that loss of this gene's function is recessive
and leads to hyperactivation of apoptosis (and lethality)

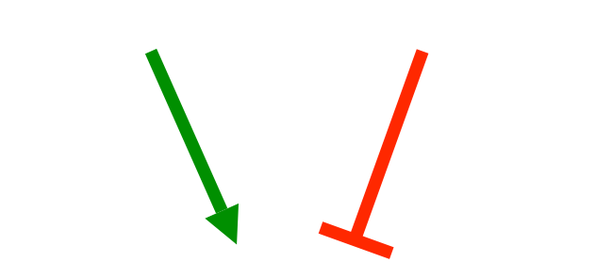
Loss of function and gain-of-function alleles of *ced-9* have opposite phenotypes.

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

ced-3 promotes apoptosis
ced-9 inhibits apoptosis

How do you put these genes (or any genes) into an ordered pathway? Make double mutants.
Note: this requires that mutations give different phenotypes!

Models



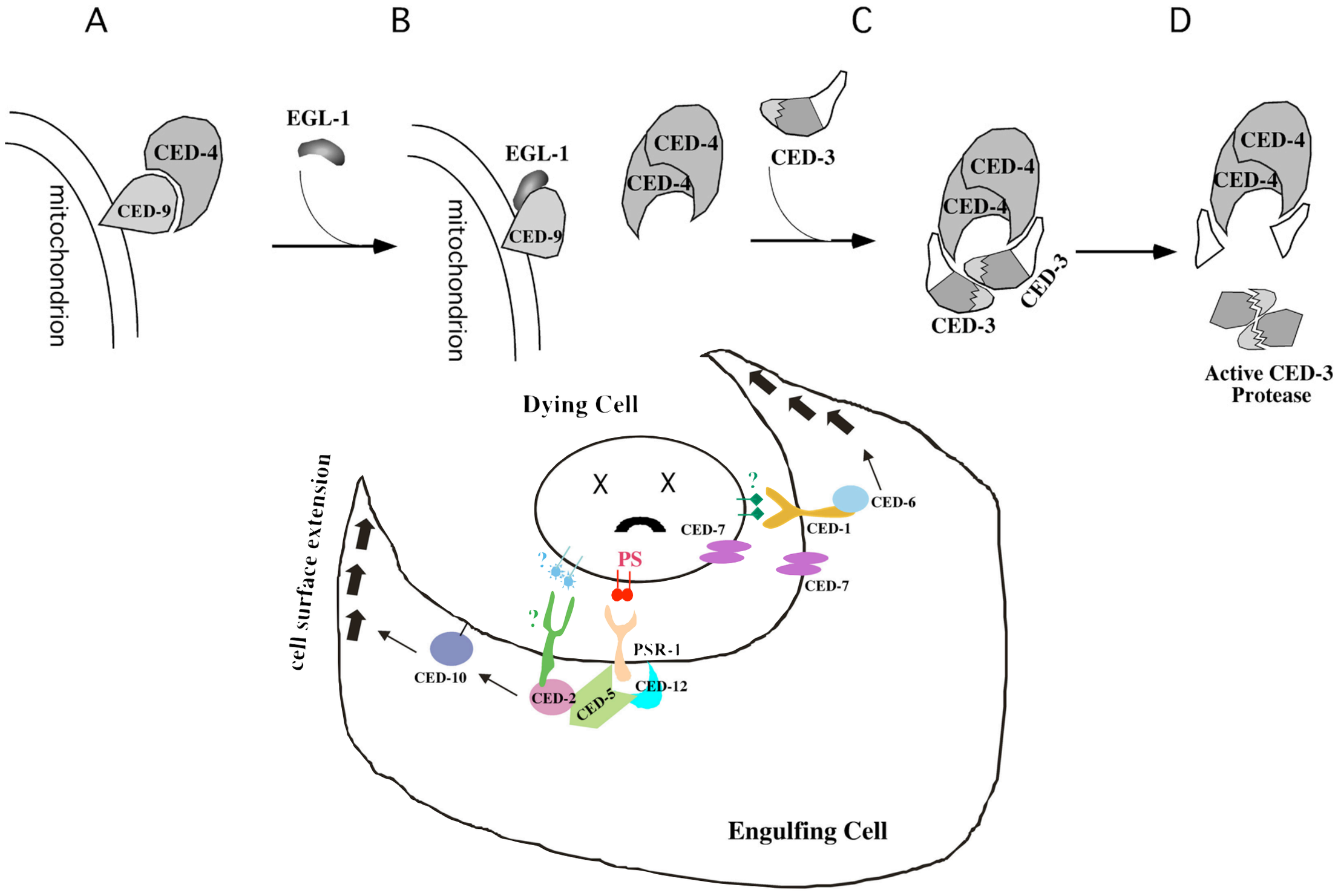
Prediction:
ced-3(lf); ced-9(lf)
intermediate phenotype

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

Combining *ced-3(lf)* and *ced-9(lf)* mutations makes it possible to put these 2 genes in an ordered pathway

Combining the genetic pathway with other information e.g., localization of the proteins encoded by the genes lets us develop a more physical view of the process

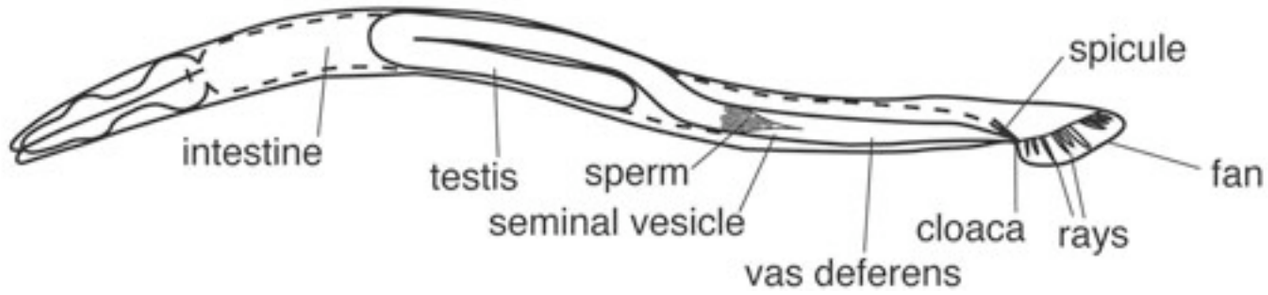


Life and Death of a Single Neuron

The hermaphrodite specific neuron (HSN),
which regulates egg laying,
lives in hermaphrodites but dies in males.

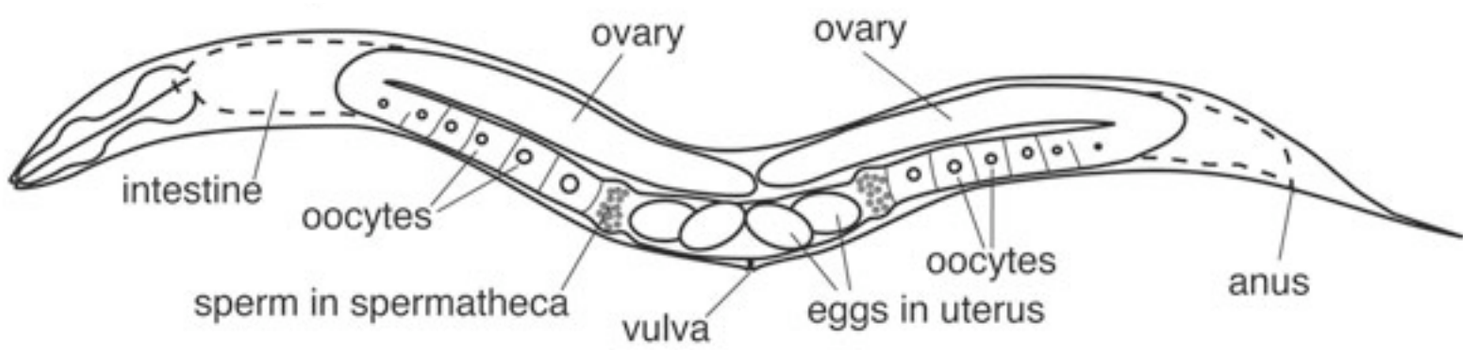
In males:

ced-9 OFF ⊣ **ced-3 ON** → **HSN dies**

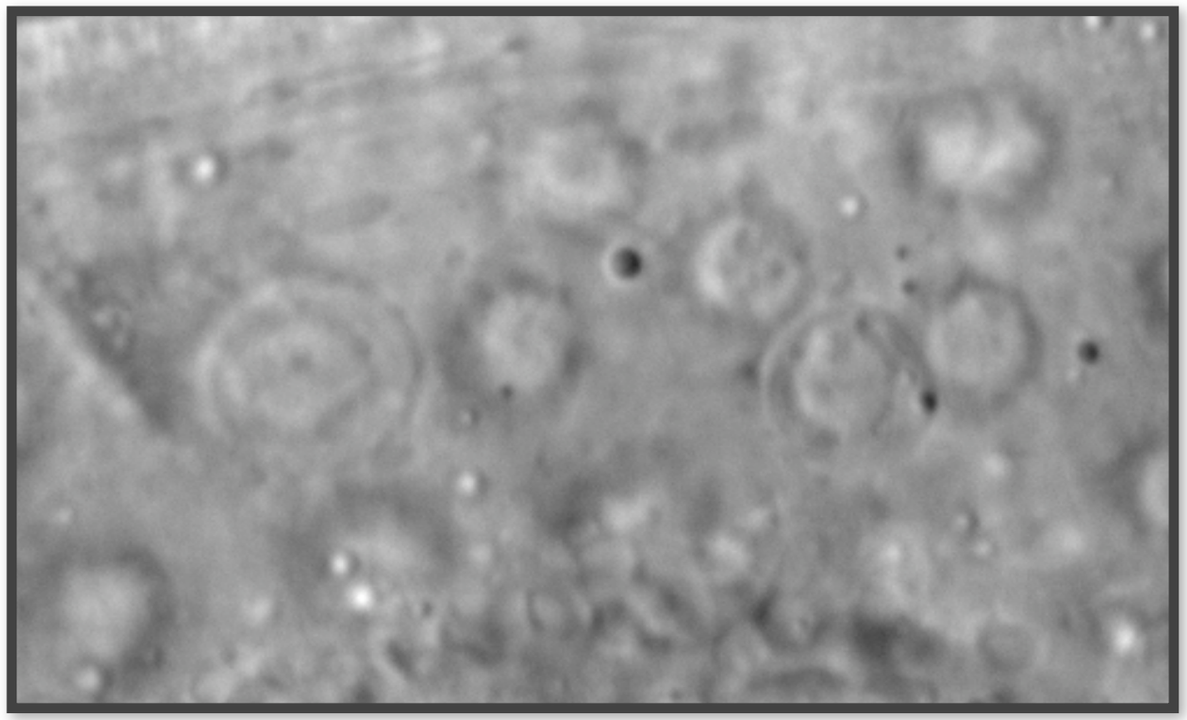


In hermaphrodites:

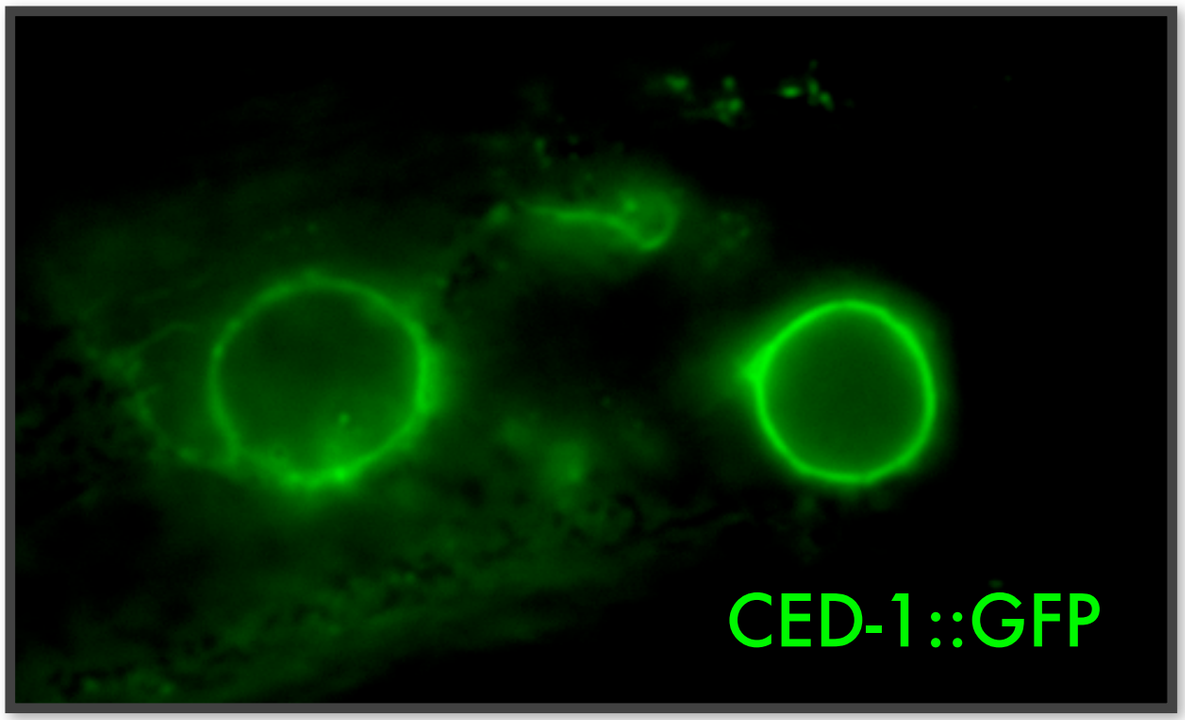
ced-9 ON ⊣ **ced-3 OFF** → **HSN survives**



An easier way to visualize apoptosis



The old way: corpses observed using Nomarski optics



The new way: fluorescent fusion protein marks cells being engulfed