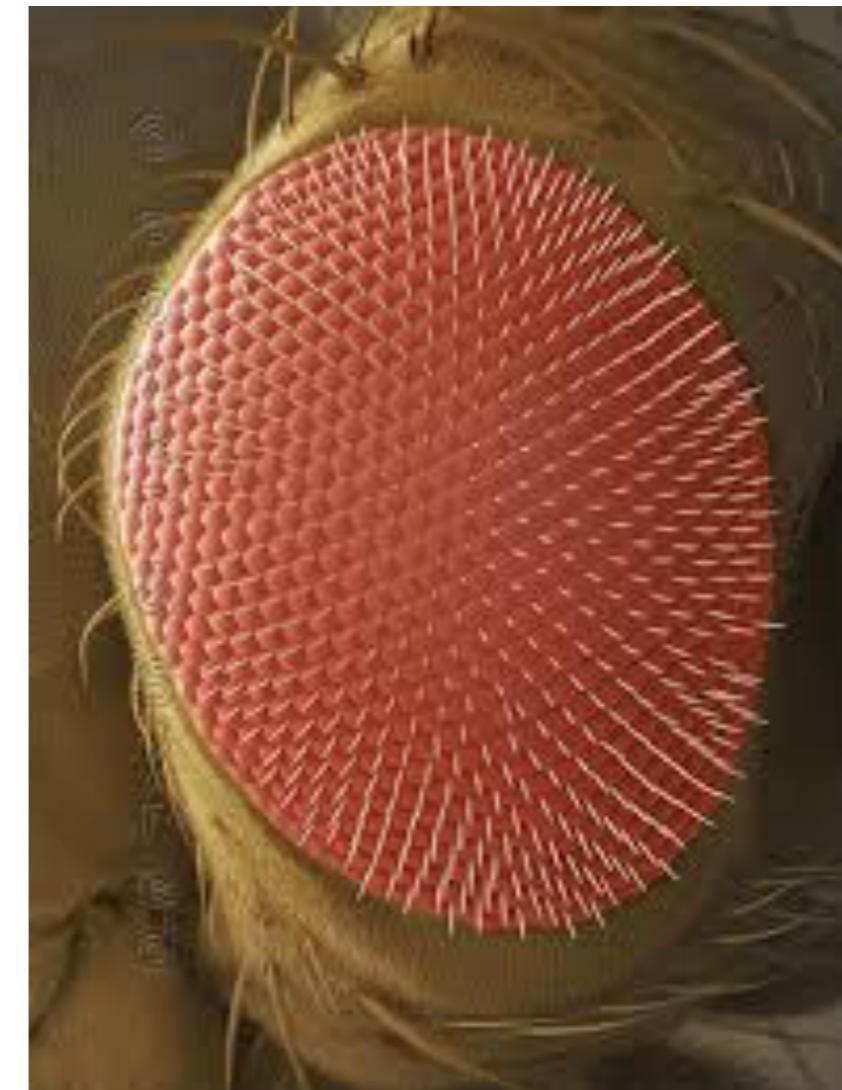


Developmental genetics



C. elegans



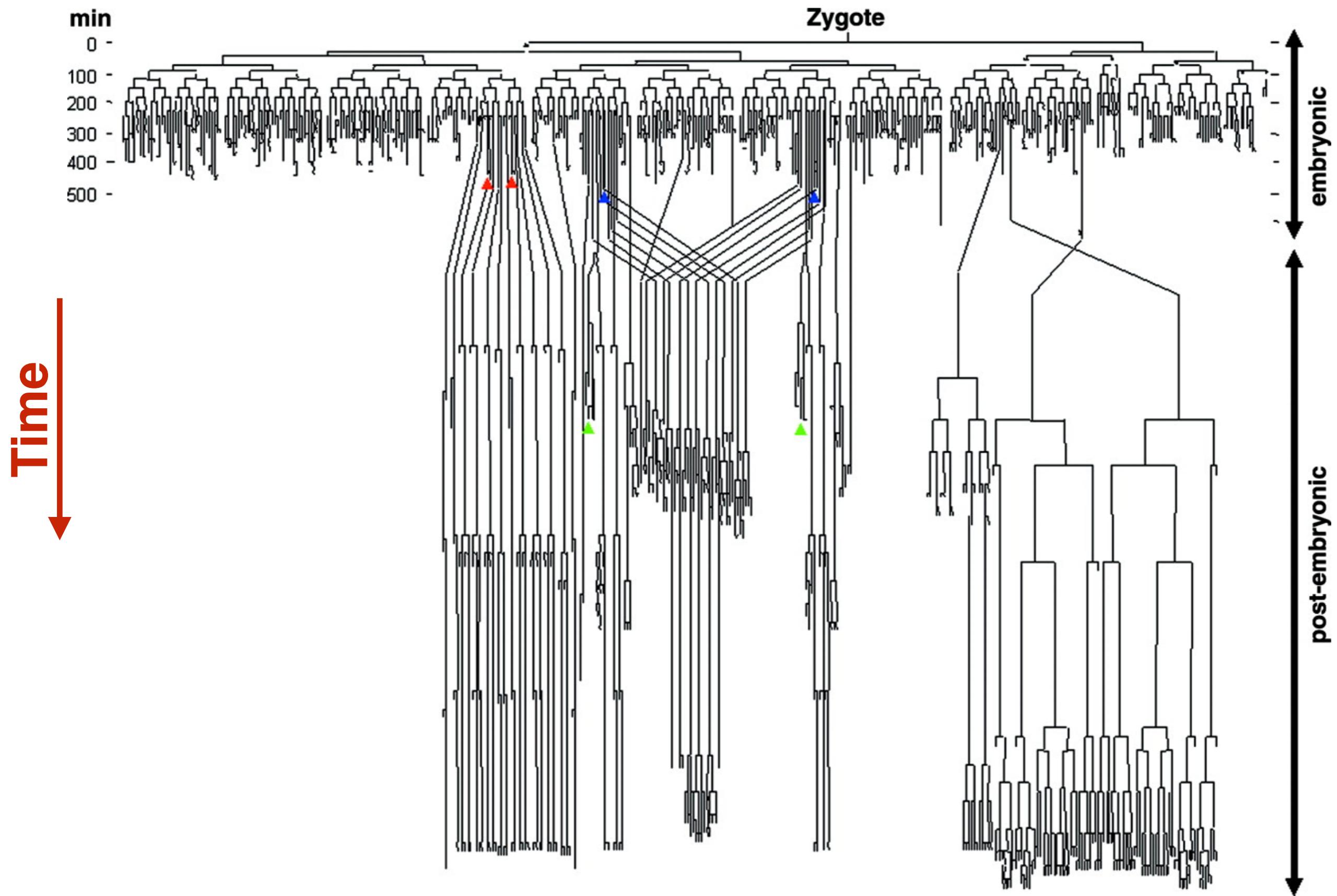
D. melanogaster

Developmental genetics is the study of how genes regulate the growth and development of an organism.



Cell location
Cell fate
Cell-cell communication
Maternal effects
Cell autonomy
Epistasis

The cell lineage of *C. elegans* is known and invariant

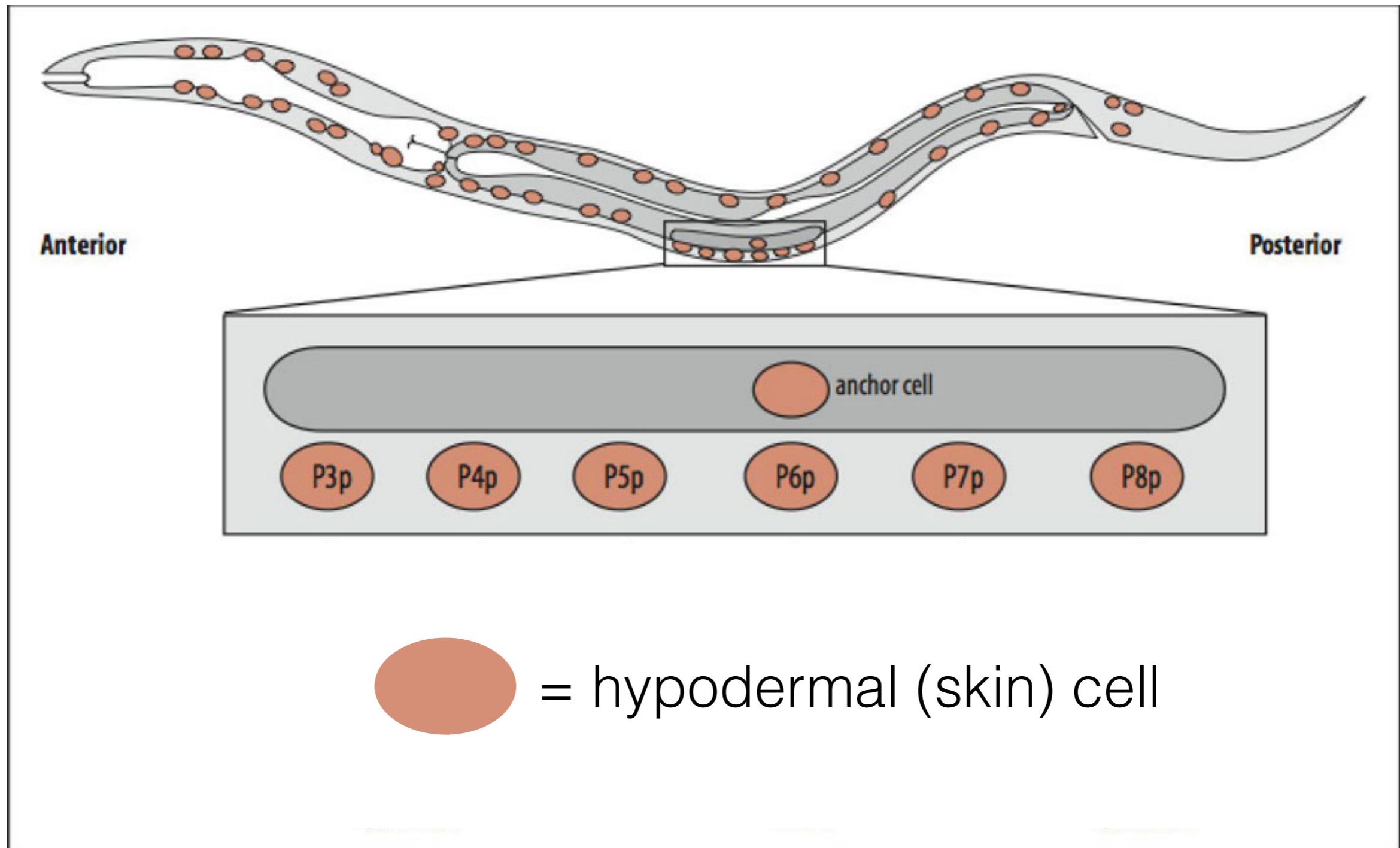


The cell lineage of *C. elegans* is known and invariant



John Sulston

C. elegans vulval development

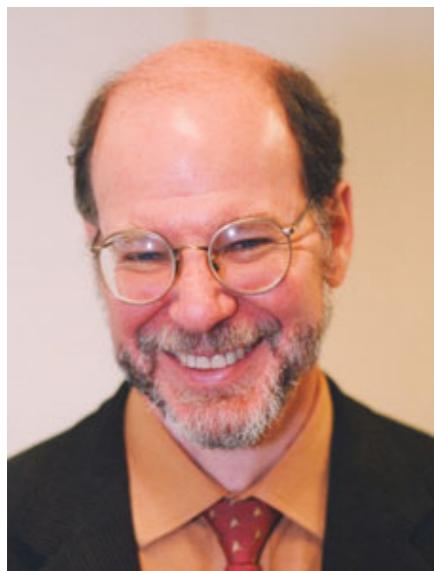
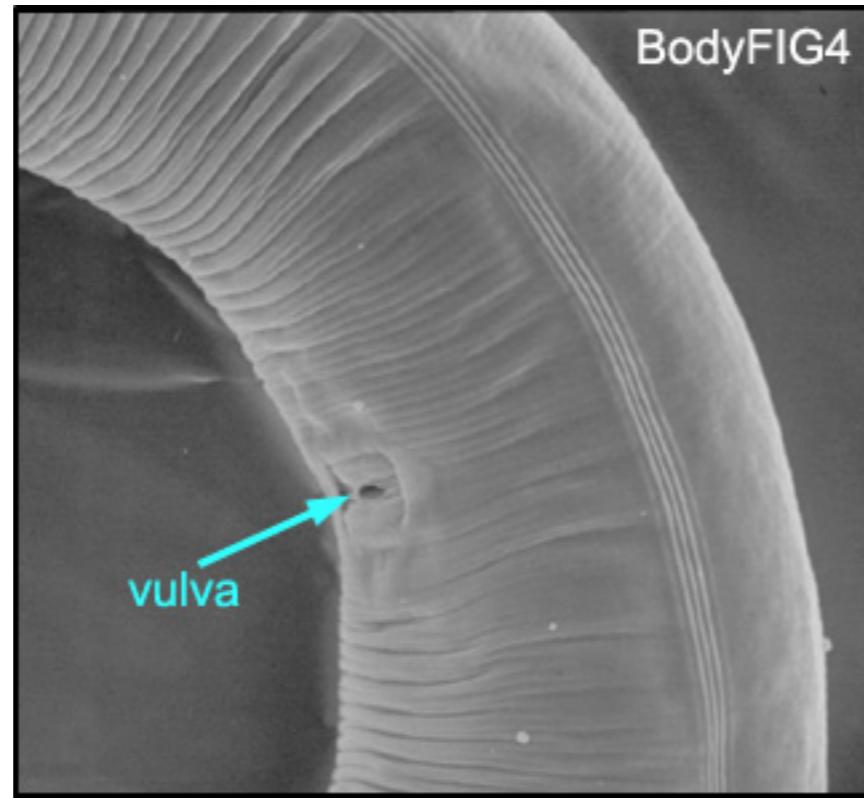


One of the best genetic screens ever

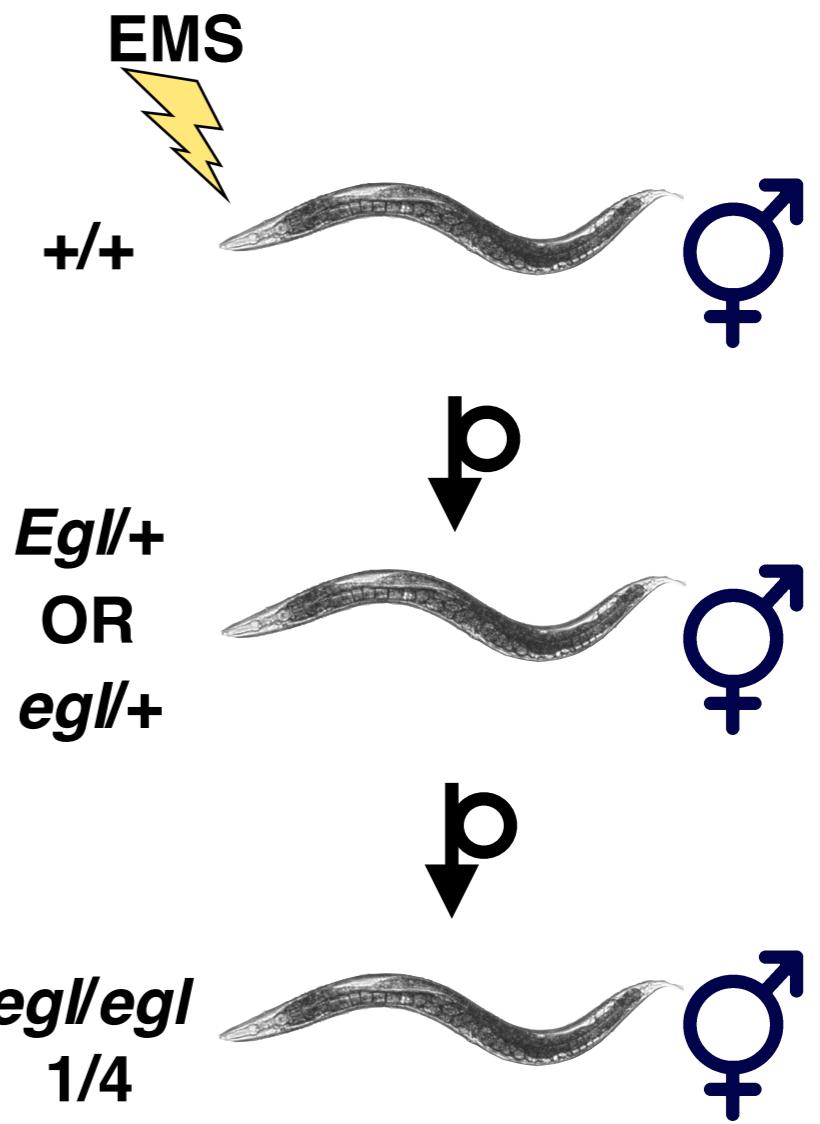


Let's say you screened for mutants that failed to lay eggs

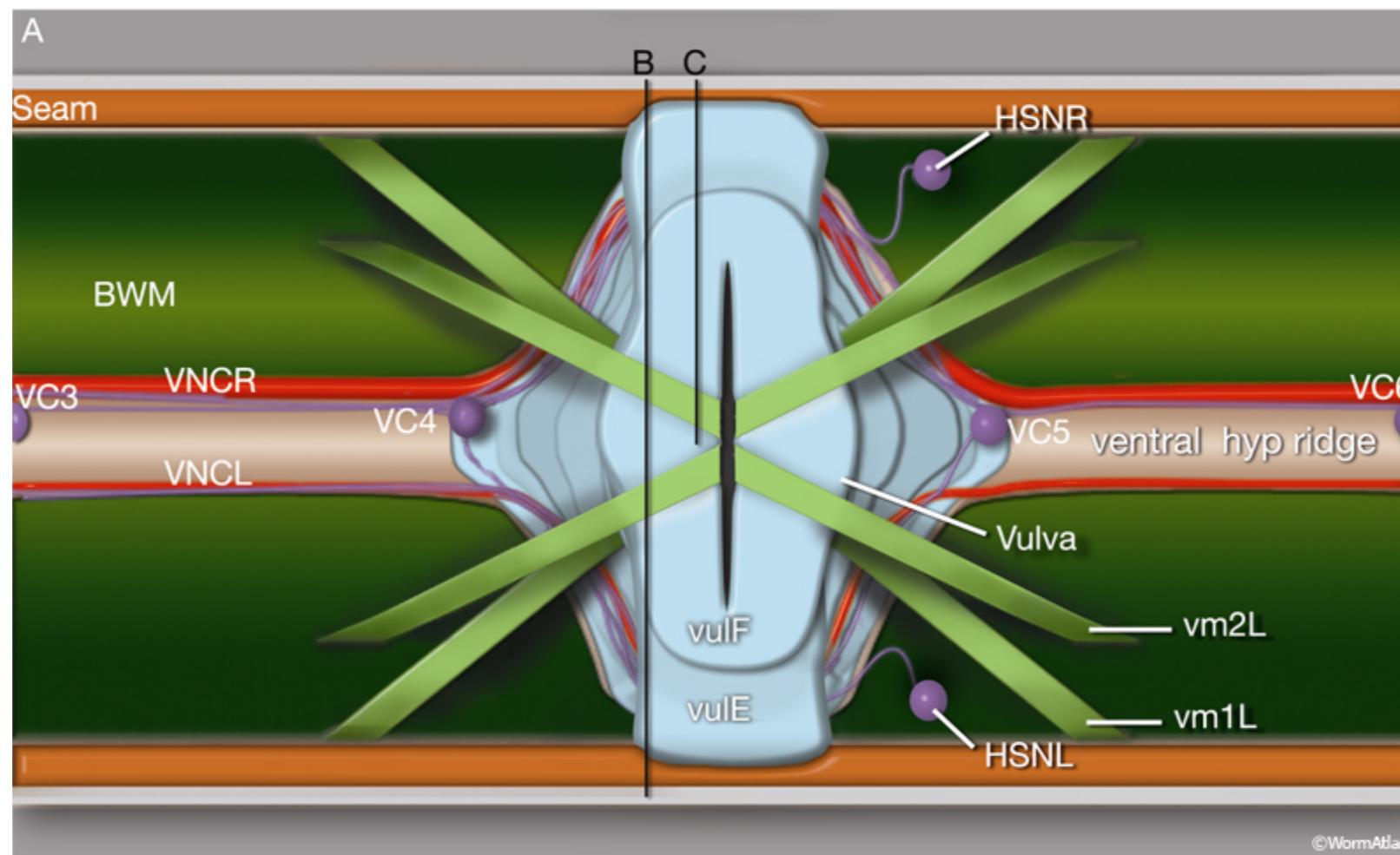
Called Egl for egg-laying defective



Bob Horvitz



One of the best genetic screens ever

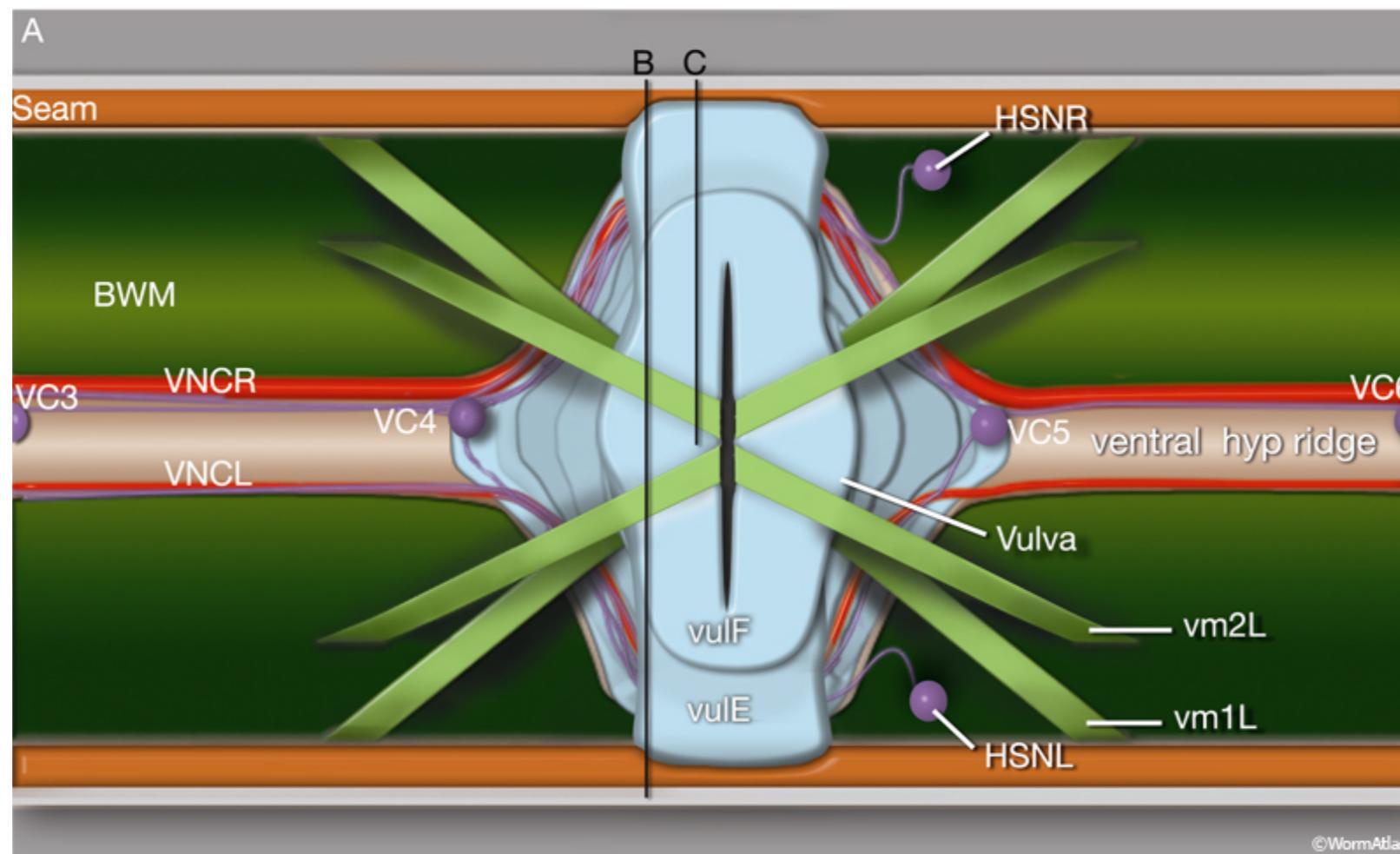


No neuron

egl-1 = inducer of programmed cell death

Hermaphrodite-Specific Neuron (HSN)
inappropriately dies

One of the best genetic screens ever

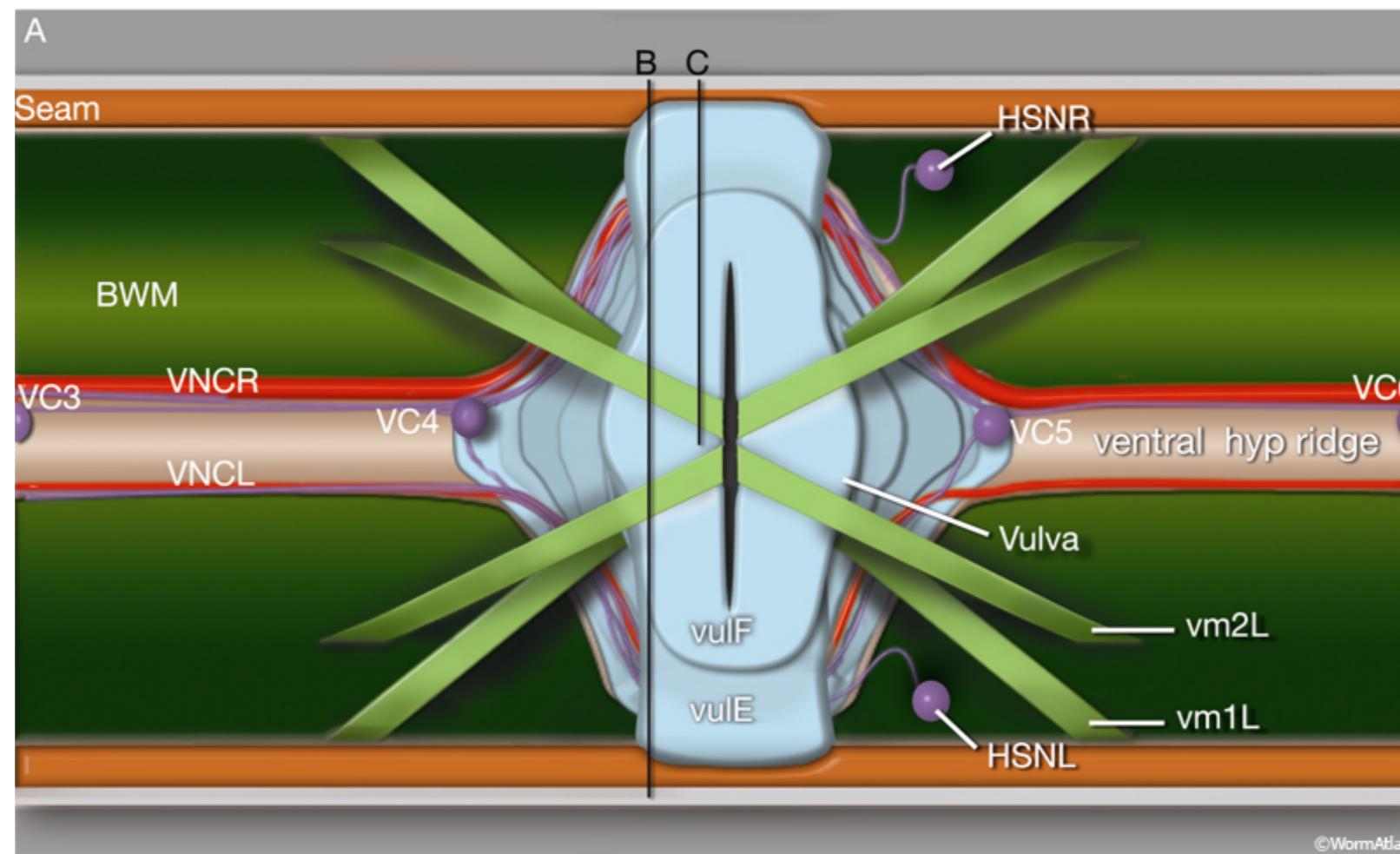


No neuron

tra-1 = inducer of sex determination

Mutants are partially male so HSNs die

One of the best genetic screens ever

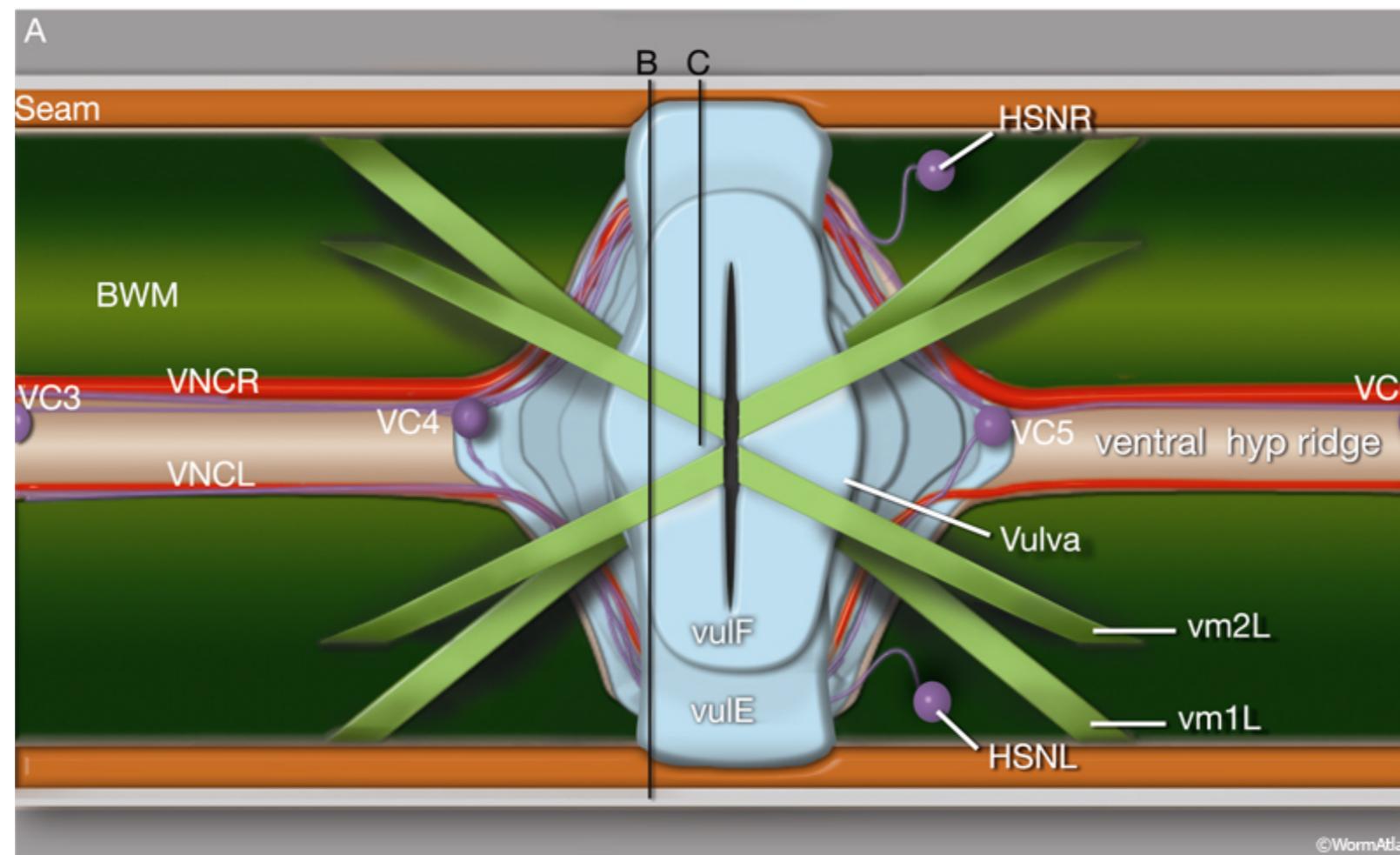


No neuron

No neuronal
signaling

egl-6 = serotonin signaling from HSN is defective

One of the best genetic screens ever



No neuron

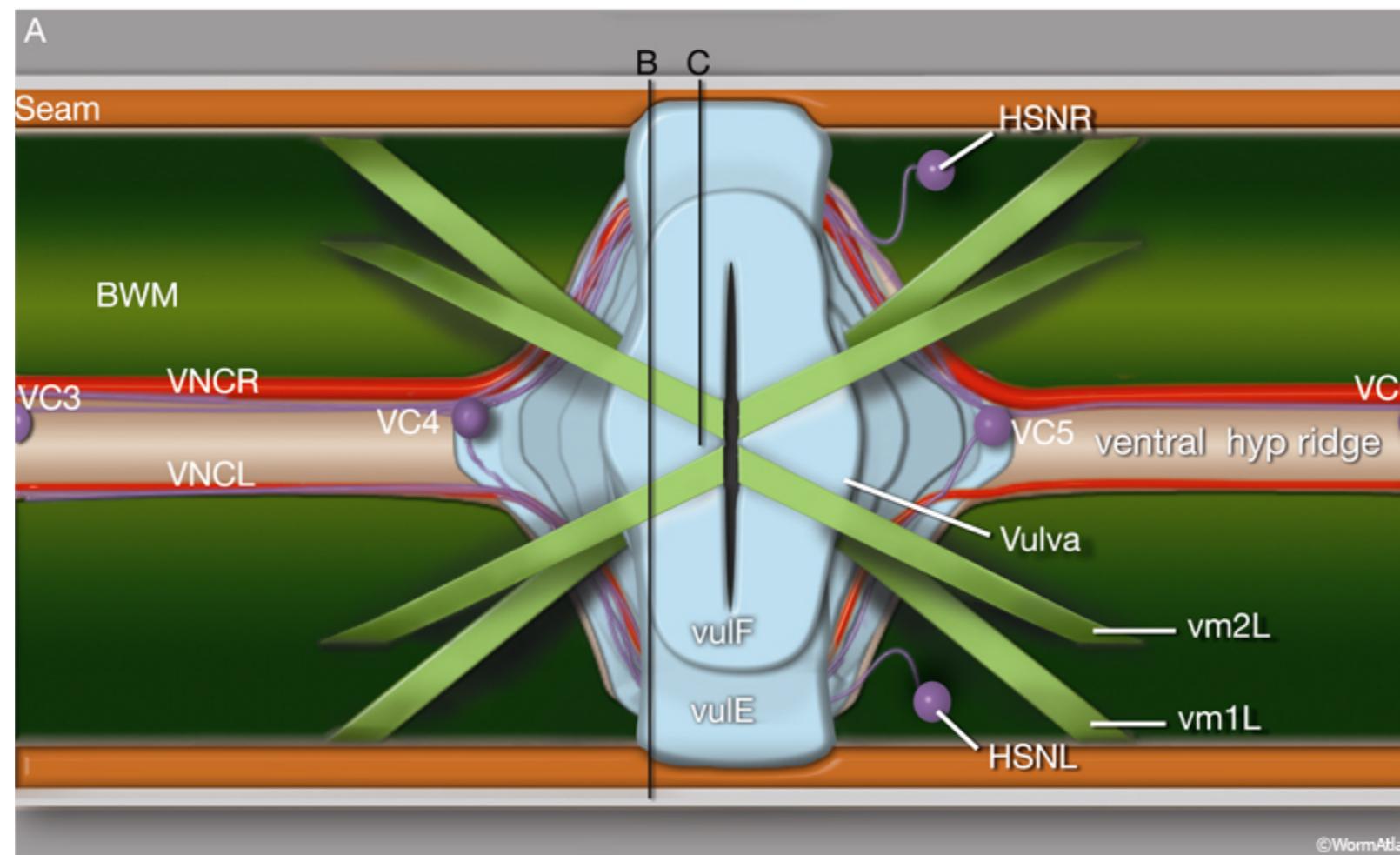
No neuronal
signaling

No vulva

lin-3 = lineage defective gene 3

Vulval cells are not specified

One of the best genetic screens ever



No neuron

No neuronal
signaling

No vulva

Vulval muscle fails to function

Vulval opening fails to form

Uterus doesn't connect to vulva

One of the best genetic screens ever



C. elegans



Sydney Brenner



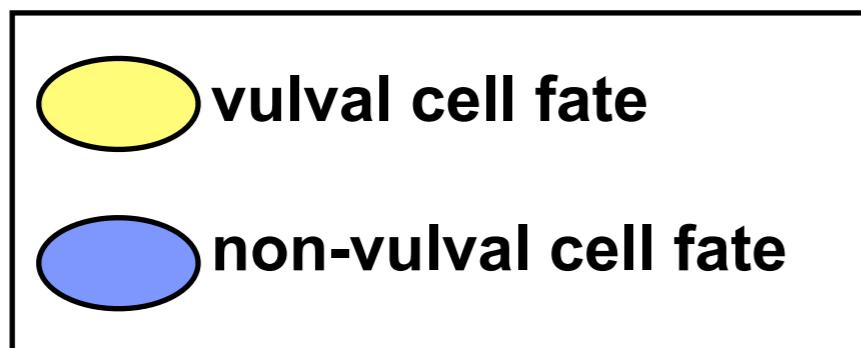
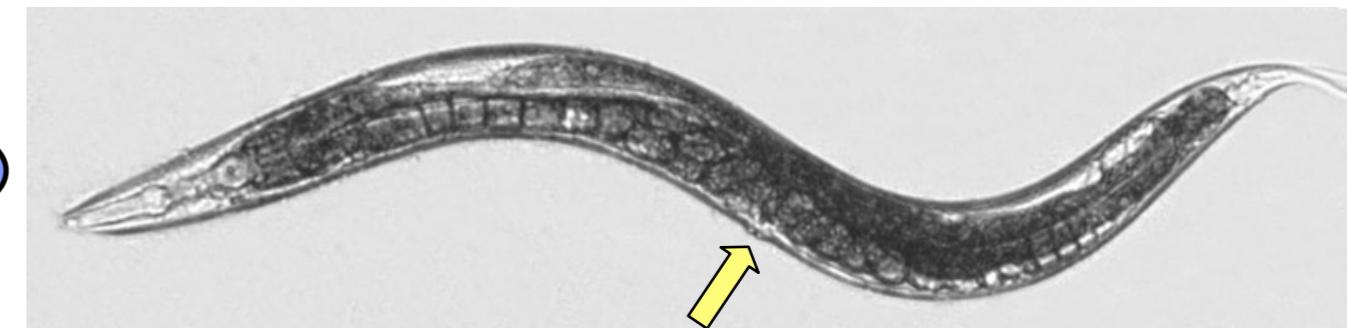
H. Robert Horvitz



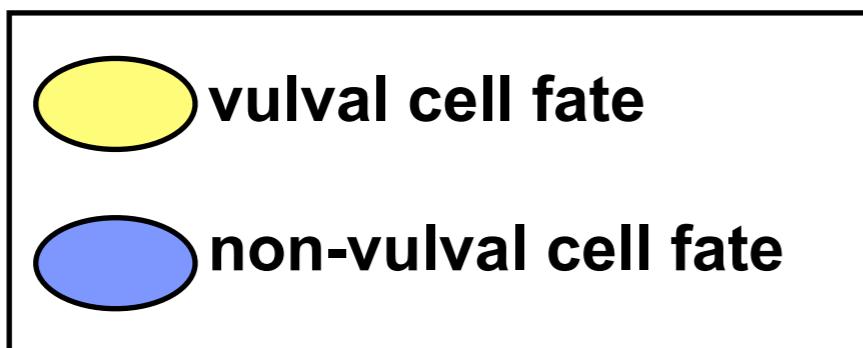
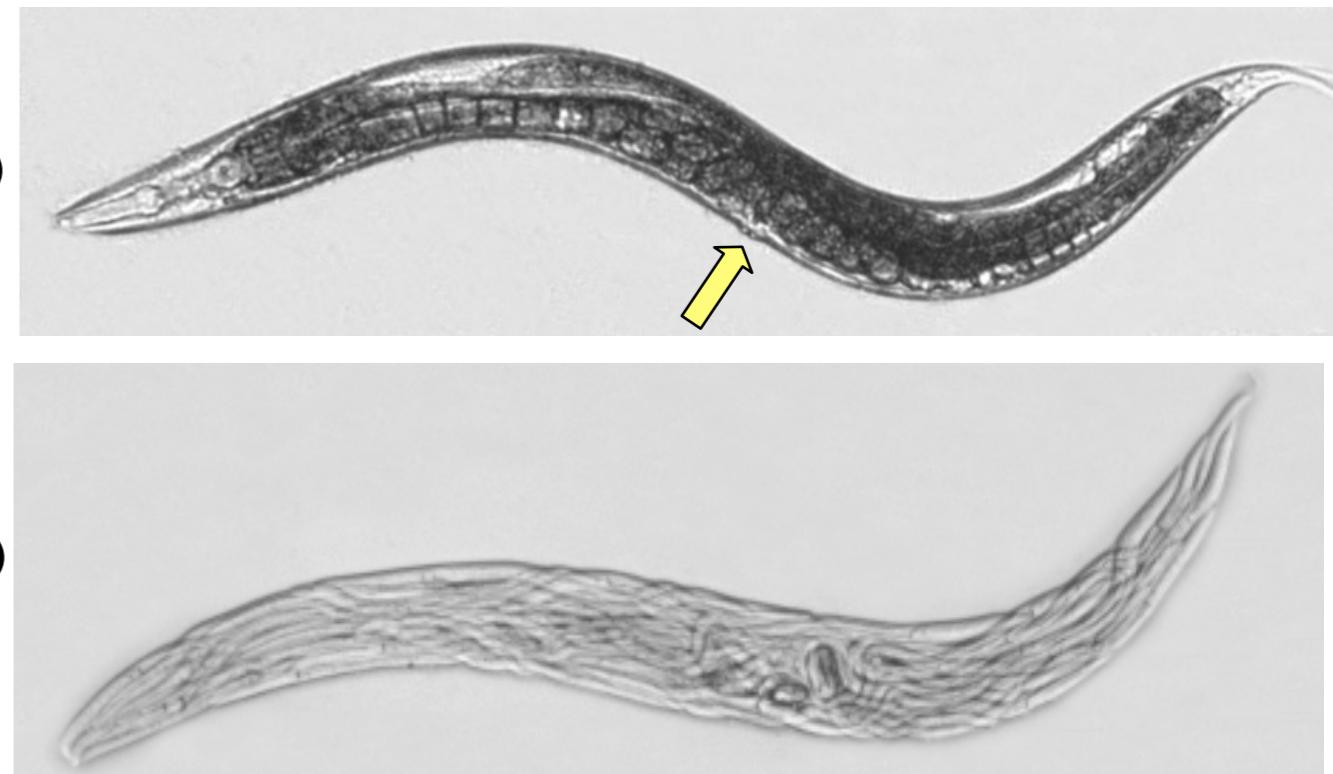
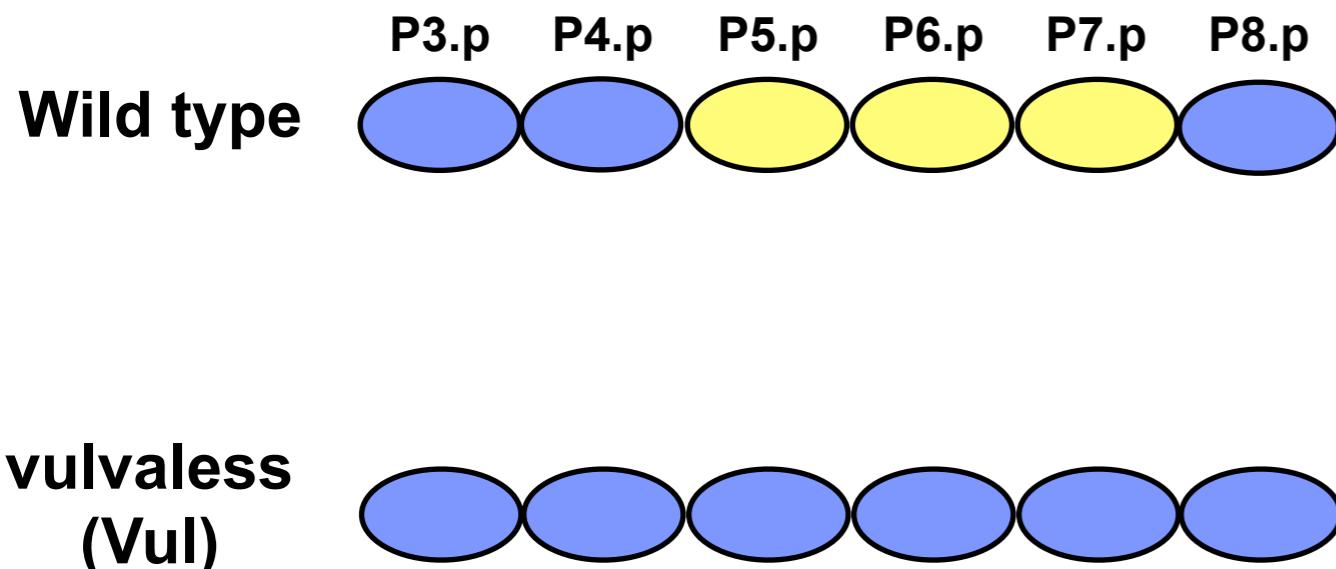
John E. Sulston



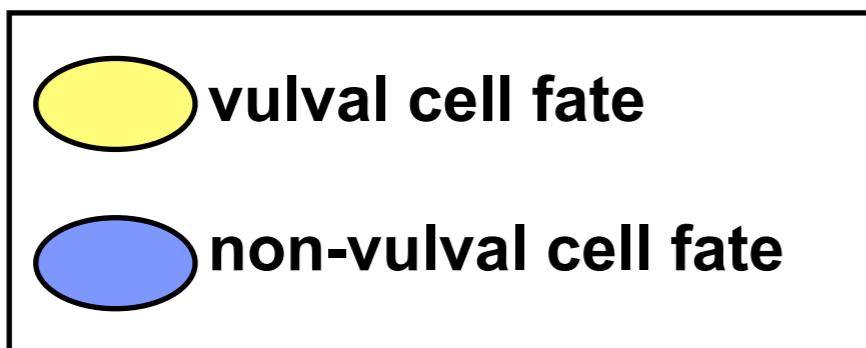
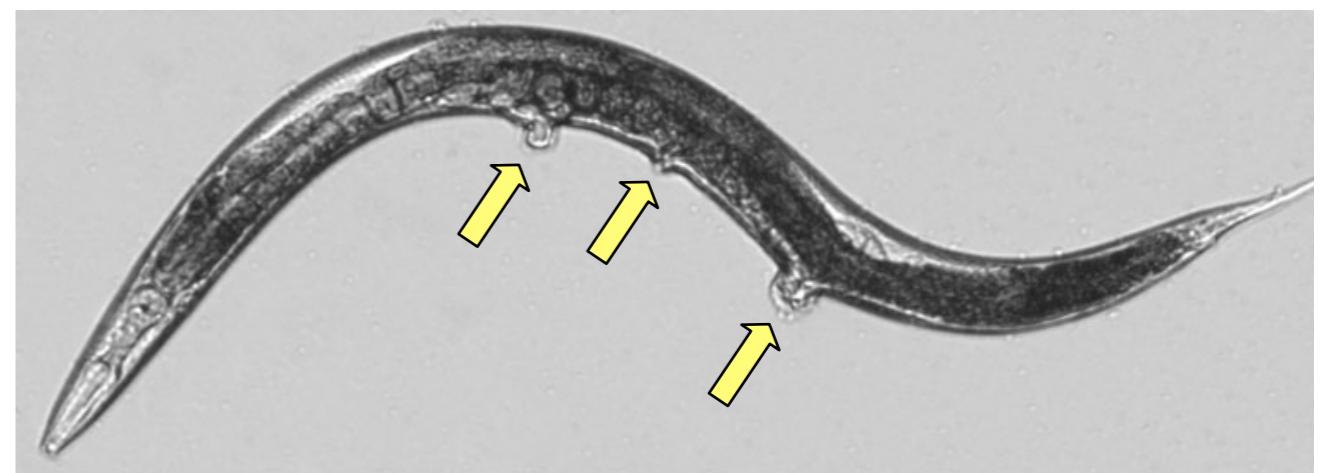
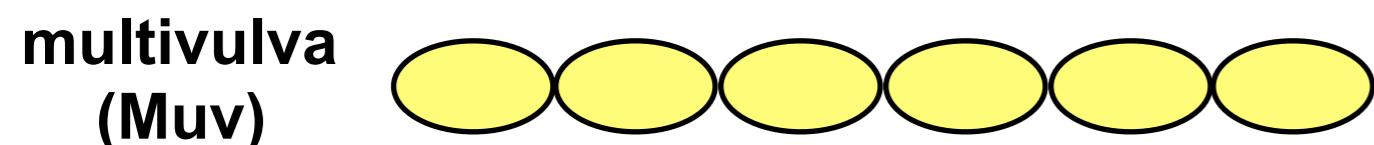
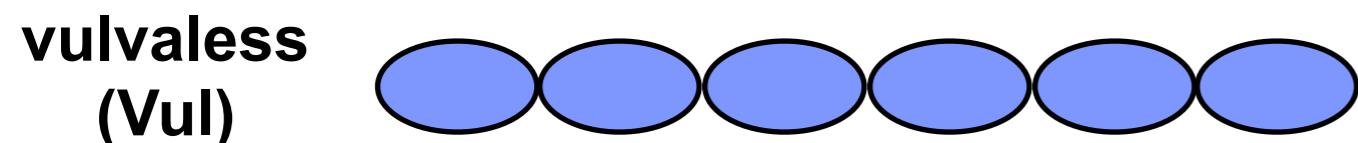
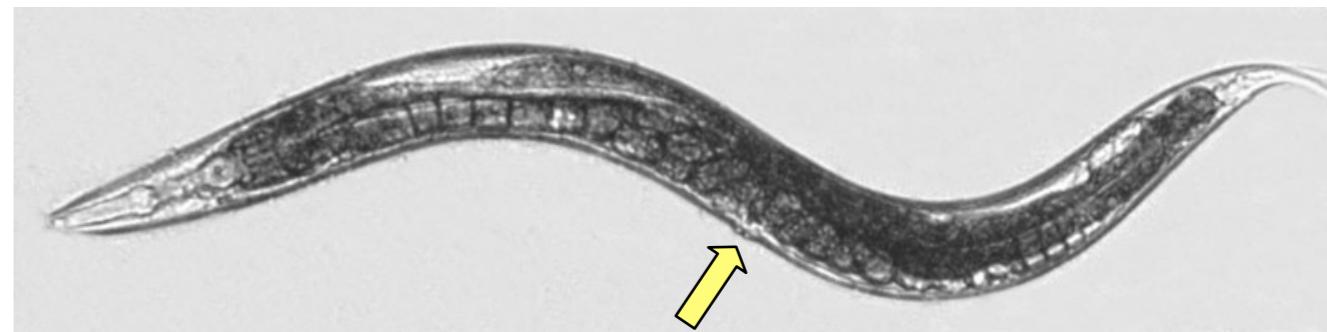
Three cells express vulval fates in wild-type animals



No cells express vulval fates in vulvaless mutants



Six cells express vulval fates in multivulva mutants



Vulval mutants

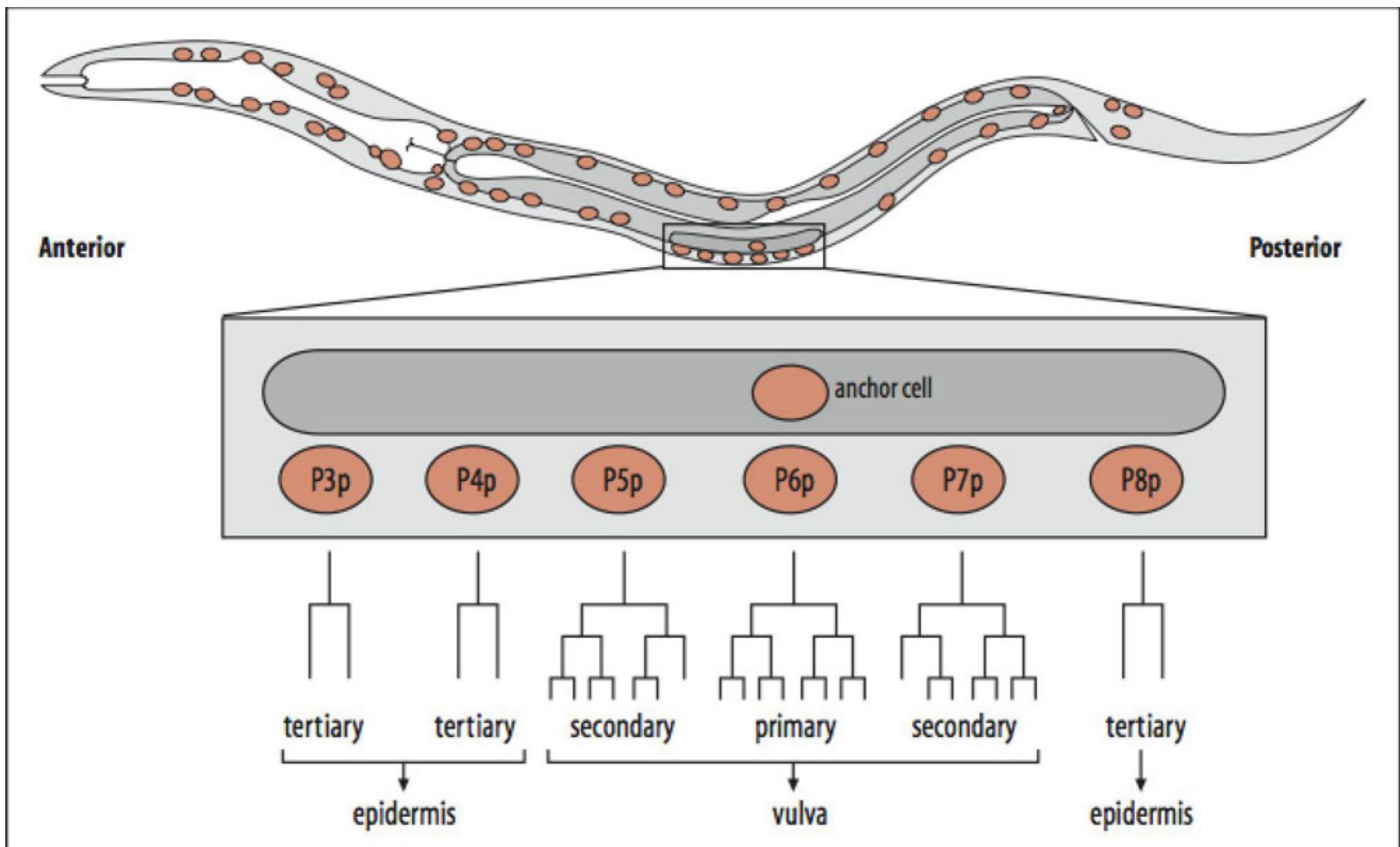
Mutant	Phenotype
<i>lin-1(0)</i>	Muv
<i>lin-3(0)</i>	Vul
<i>let-60(0)</i>	Vul
<i>let-60(gf)</i>	Muv
<i>let-23(0)</i>	Vul
<i>let-23(gf)</i>	Muv

Double mutants defined the vulval pathway

Mutant genotypes	Phenotype
<i>lin-1(0)</i>	Muv
<i>lin-3(0)</i>	Vul
<i>let-60(0)</i>	Vul
<i>let-60(gf)</i>	Muv
<i>let-23(0)</i>	Vul
<i>let-23(gf)</i>	Muv
<i>lin-3(0); let-23(gf)</i>	Muv
<i>lin-3(0); let-60(gf)</i>	Muv
<i>let-23(0); let-60(gf)</i>	Muv
<i>let-23(gf); let-60(0)</i>	Vul
<i>let-60(0); lin-1(0)</i>	Muv
<i>let-23(0); lin-1(0)</i>	Muv

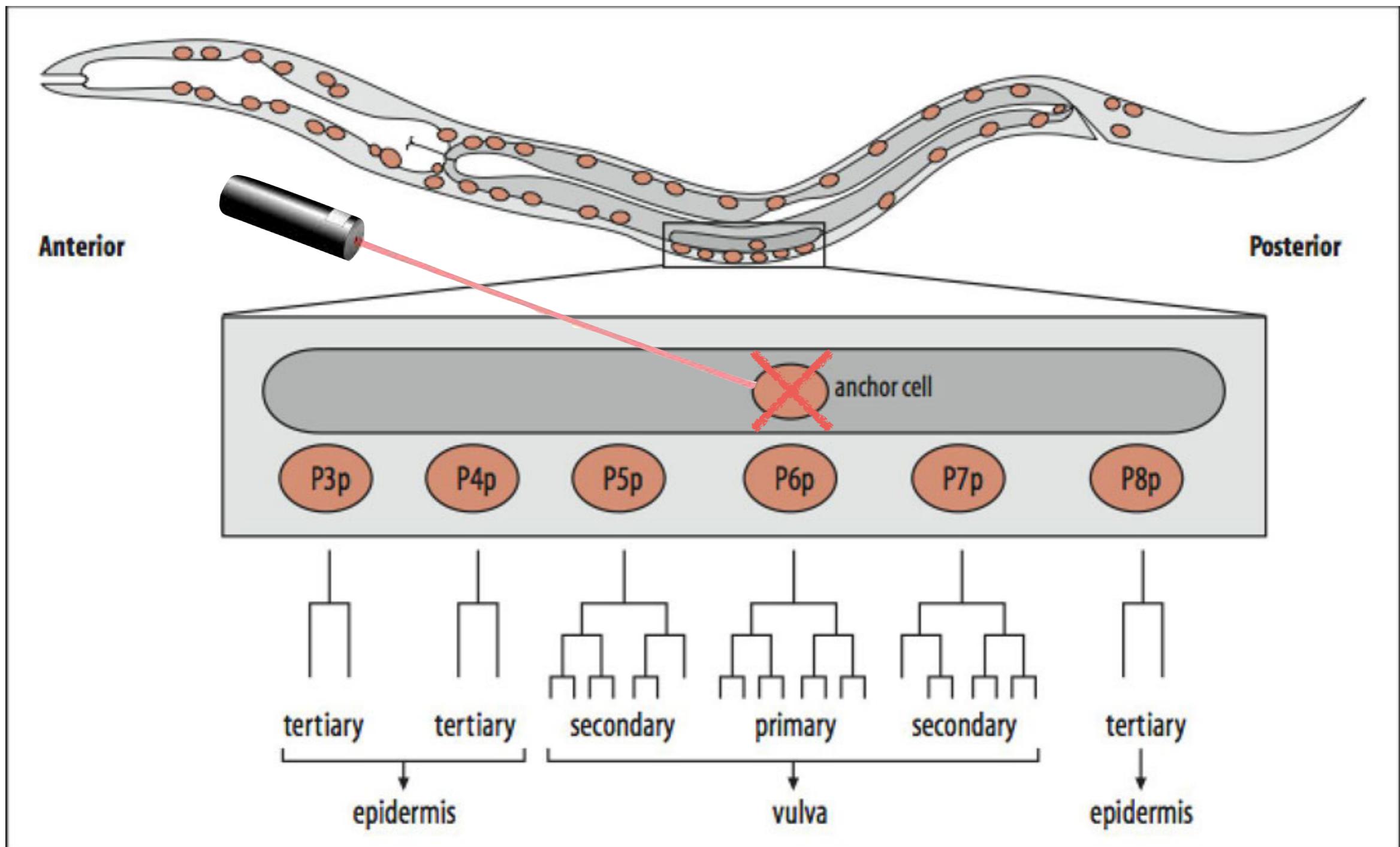
***lin-3* → *let-23* → *let-60* → *lin-1* → vulval fate**

What is the source of the inductive signal?



How do we determine that the AC is necessary and sufficient for vulval development (primary or secondary cells)?

Ablation removes cells (necessary)

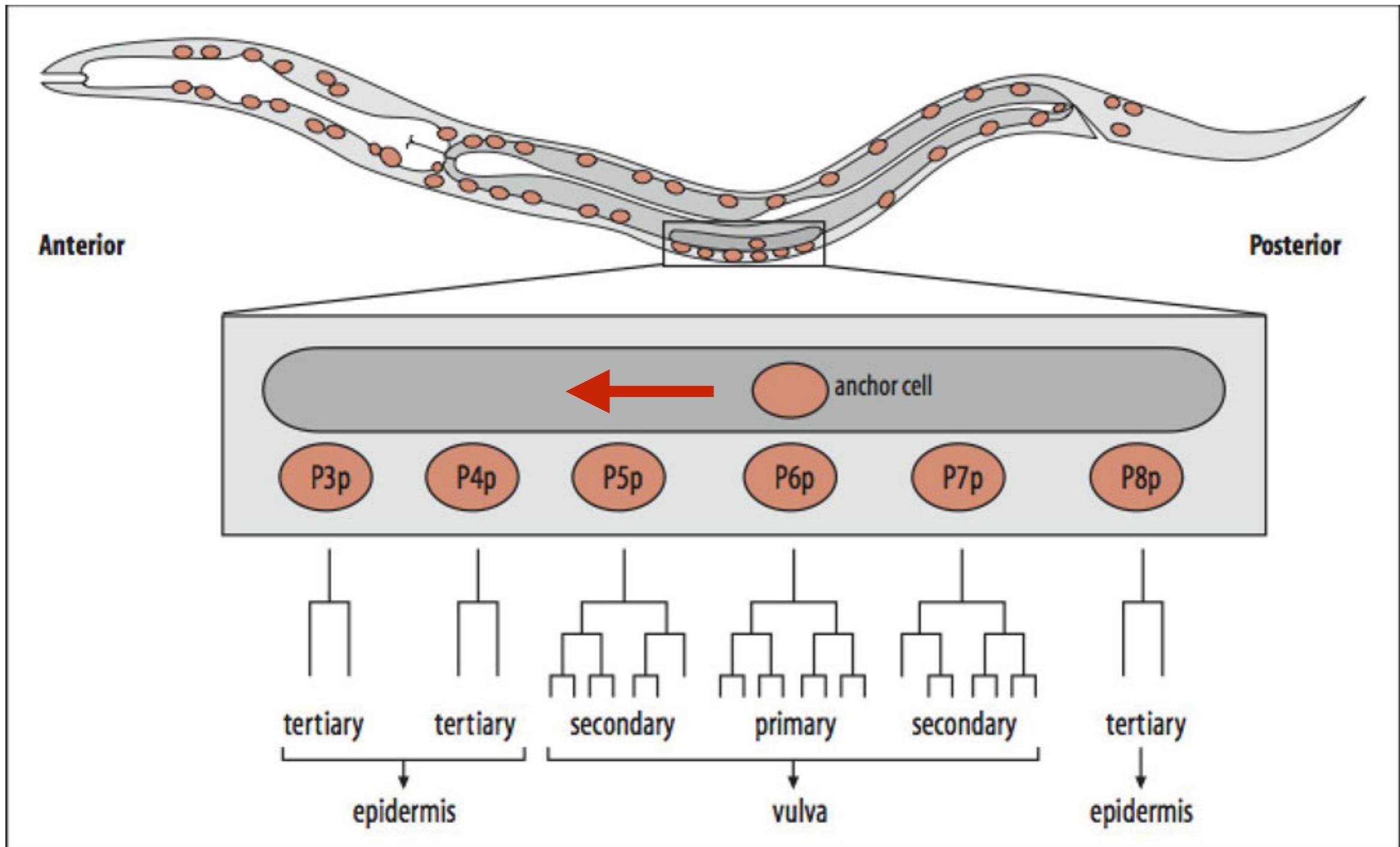


No AC leads to no vulval cell specification
and a vulvaless phenotype

All other vulval mutants are epistatic to AC ablation

Mutant genotypes	Phenotype
AC ablation	Vul
<i>lin-1(0)</i>	Muv
<i>lin-3(0)</i>	Vul
<i>let-60(gf)</i>	Muv
<i>let-23(gf)</i>	Muv
AC ablation; <i>let-23(gf)</i>	Muv
AC ablation; <i>let-60(gf)</i>	Muv
AC ablation; <i>lin-1(0)</i>	Muv

Moving the AC moves the vulva (sufficient)

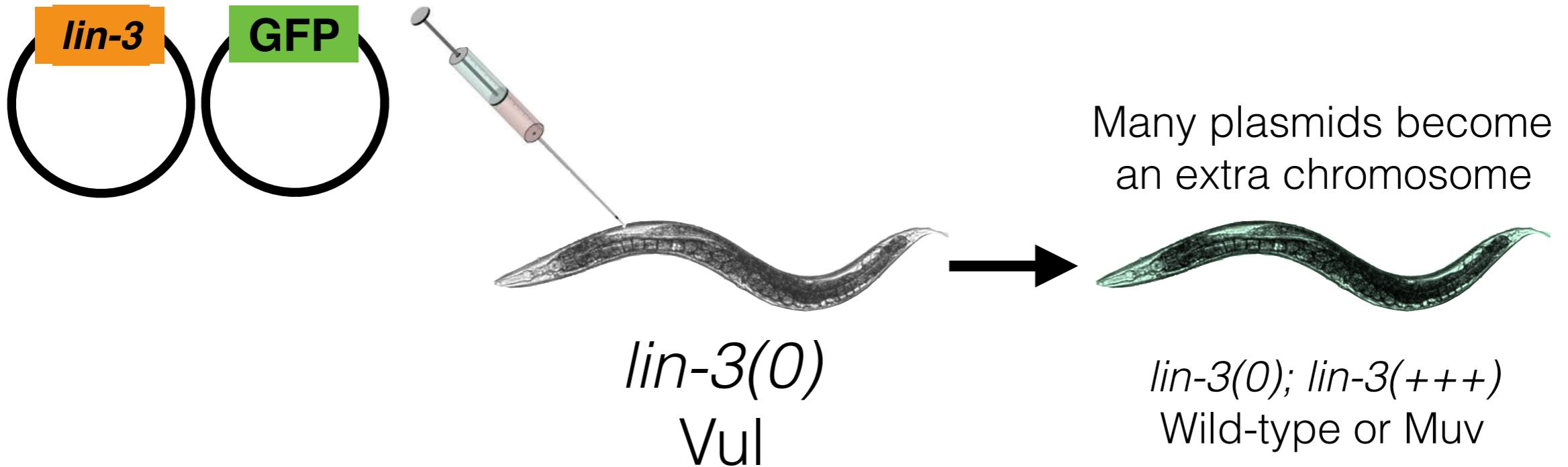


dig-1 displaced gonad mutants

AC ablation and loss of *lin-3* have the same phenotype and epistatic relationships

Mutant genotypes	Phenotype
AC ablation	Vul
<i>lin-3(0)</i>	Vul
<i>lin-1(0)</i>	Muv
<i>let-60(gf)</i>	Muv
<i>let-23(gf)</i>	Muv
AC ablation; <i>let-23(gf)</i>	Muv
AC ablation; <i>let-60(gf)</i>	Muv
AC ablation; <i>lin-1(0)</i>	Muv
<i>lin-3(0); let-23(gf)</i>	Muv
<i>lin-3(0); let-60(gf)</i>	Muv
<i>lin-3(0); lin-1(0)</i>	Muv

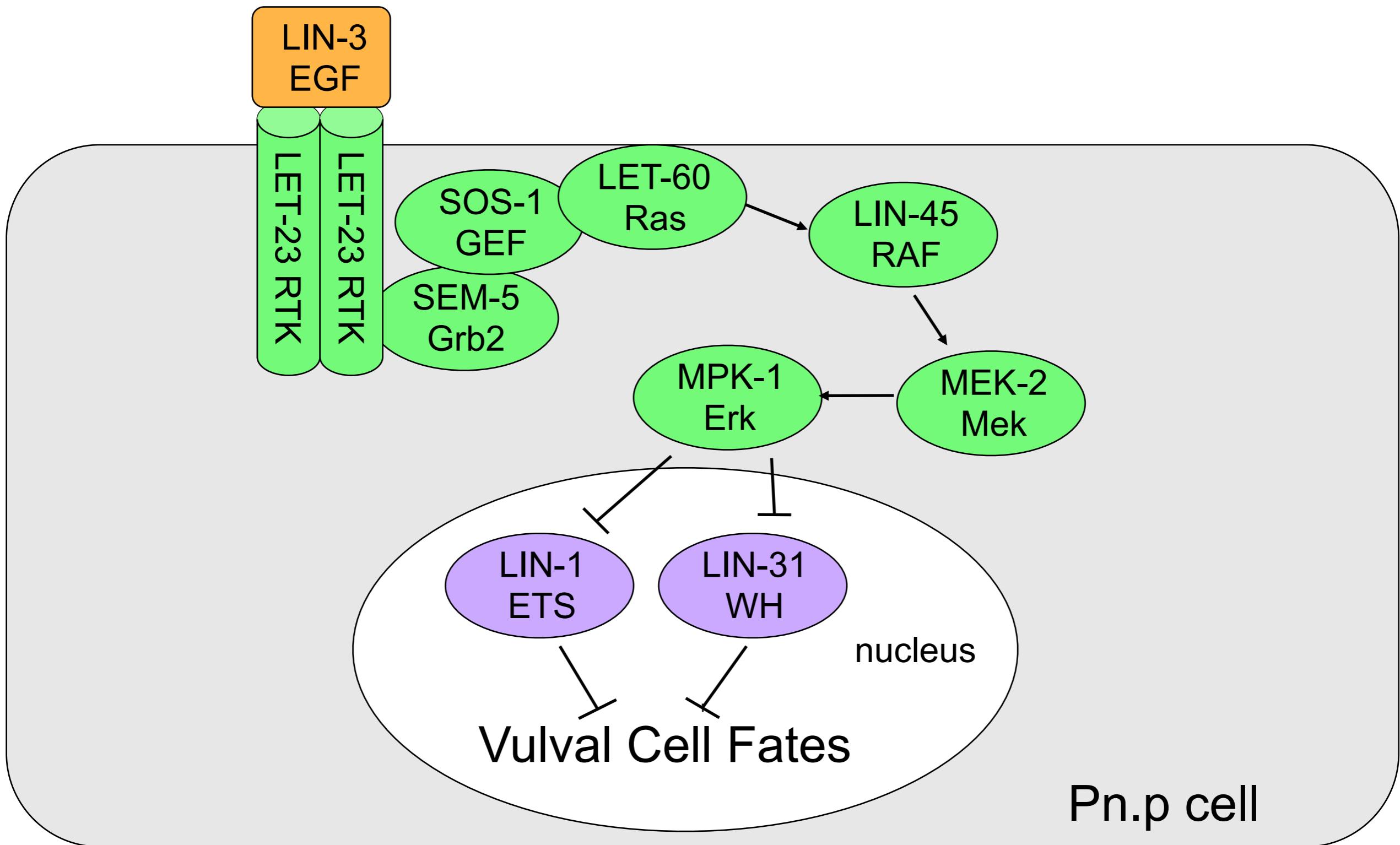
LIN-3 is expressed in the AC; does it function there?

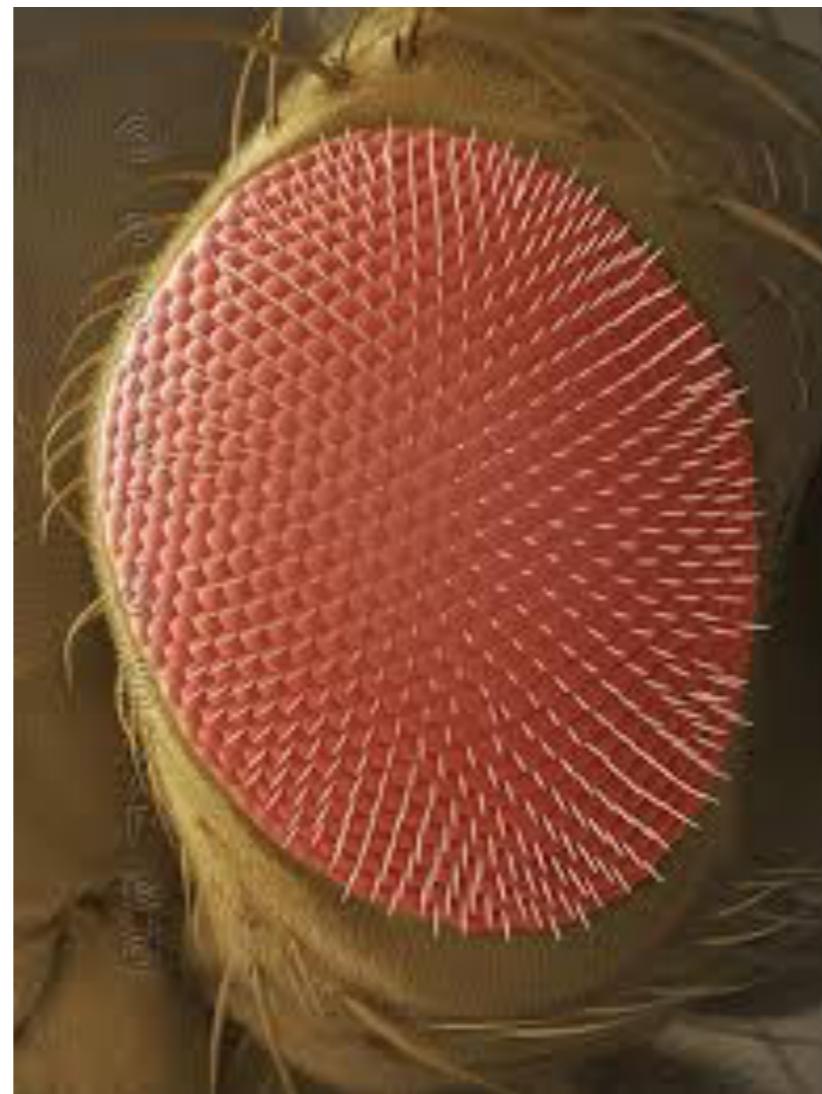


Genotype	Phenotype
<i>lin-3(0)</i>	Vul
<i>lin-3(0); lin-3(++)</i>	Wild-type
<i>lin-3(0); vulval cell:lin-3(++)</i>	Vul
<i>lin-3(0); intestine:lin-3(++)</i>	Vul
<i>lin-3(0); neurons:lin-3(++)</i>	Vul
<i>lin-3(0); AC:lin-3(++)</i>	Wild-type

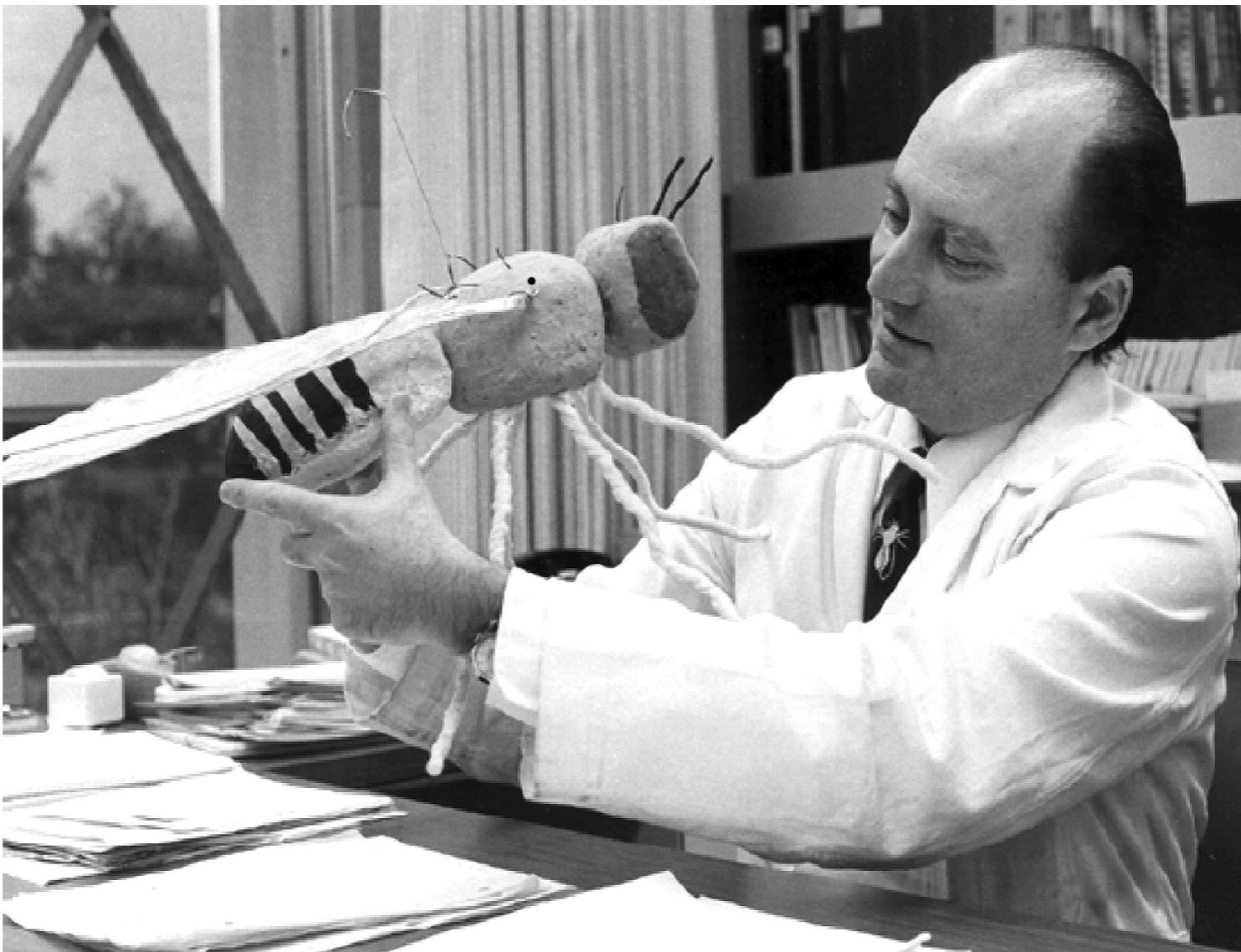
Expression of *lin-3* in vulval cells
Expression of *lin-3* in the intestine
Expression of *lin-3* in the neurons
Expression of *lin-3* in the AC

A Ras pathway promotes vulval fates

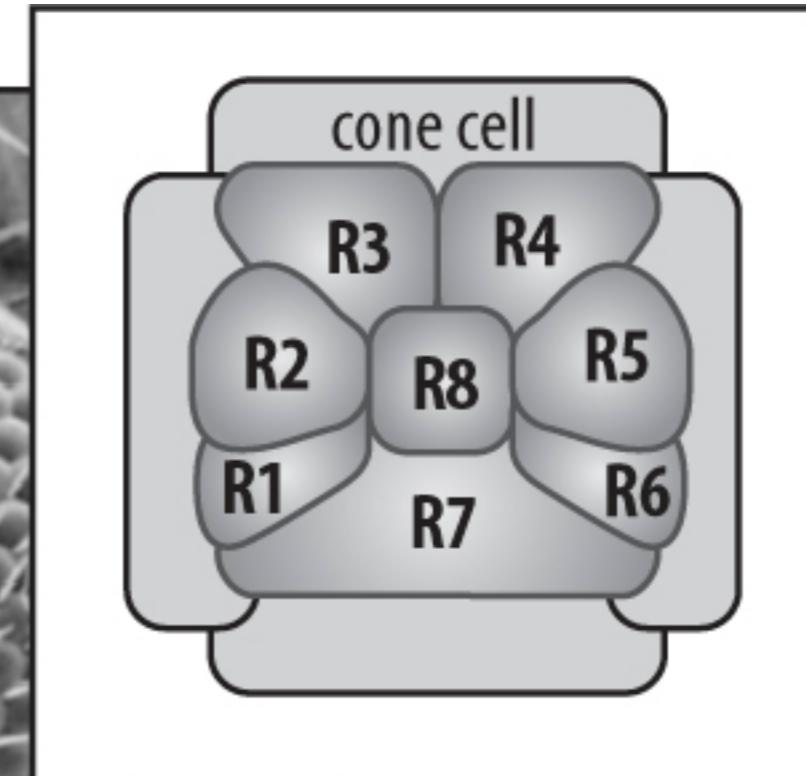
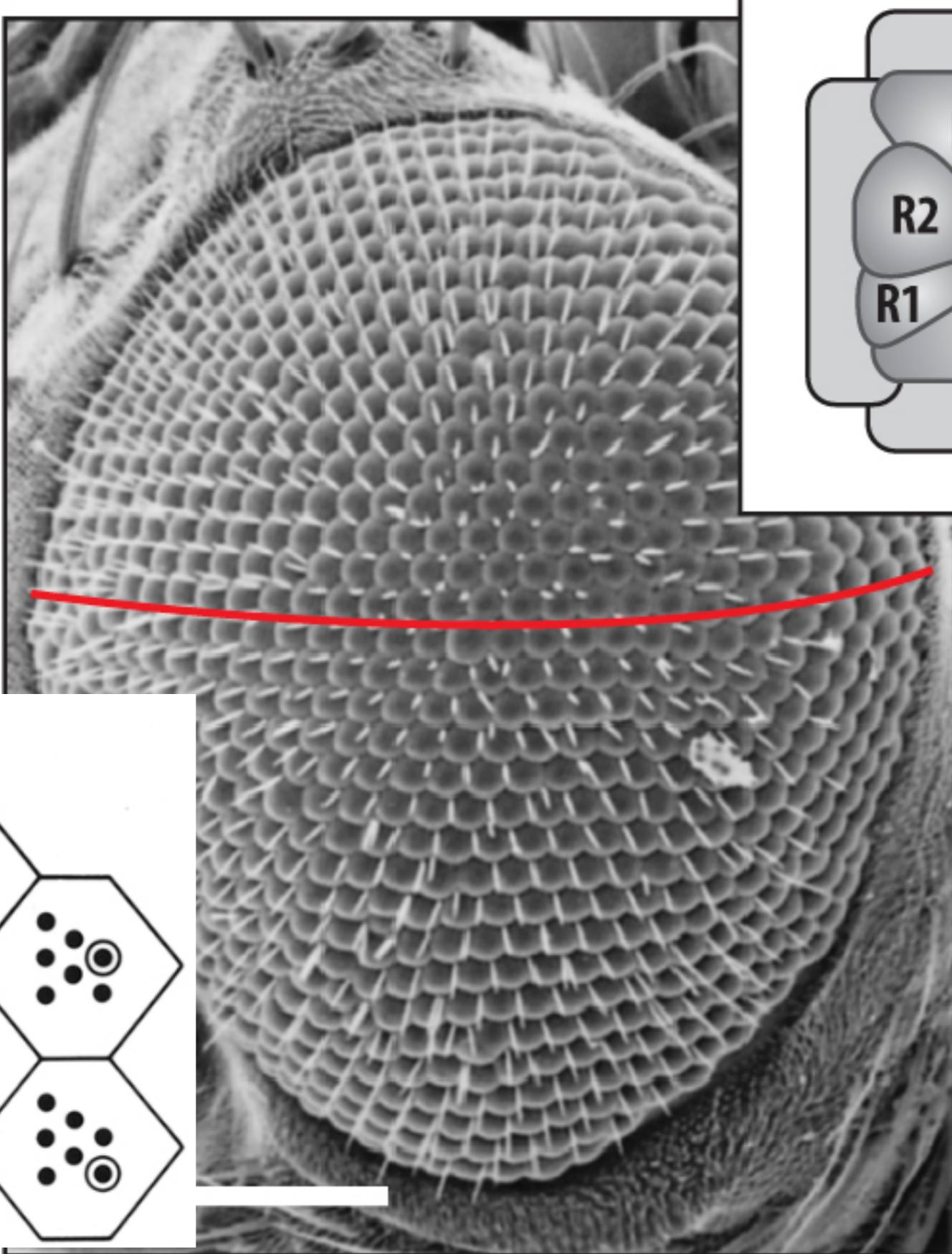
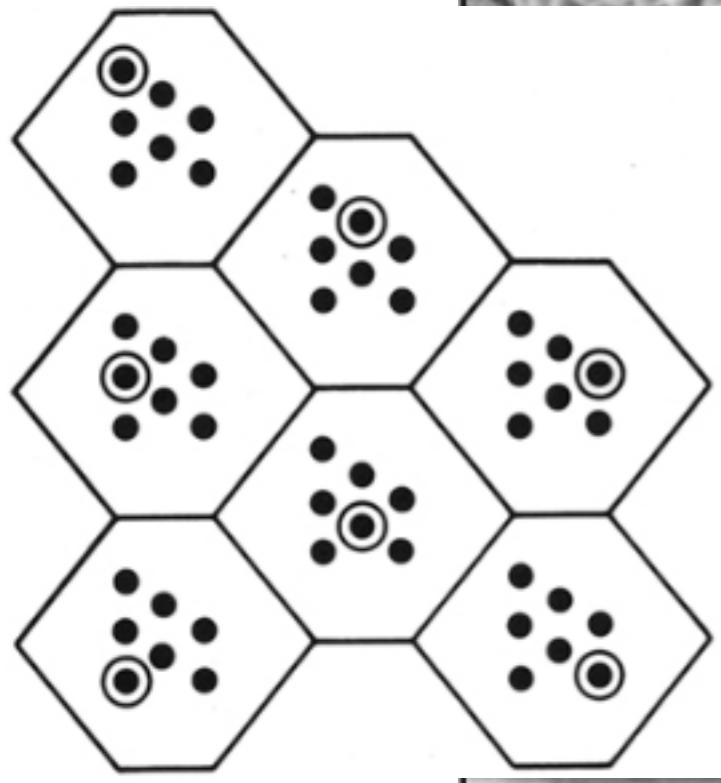




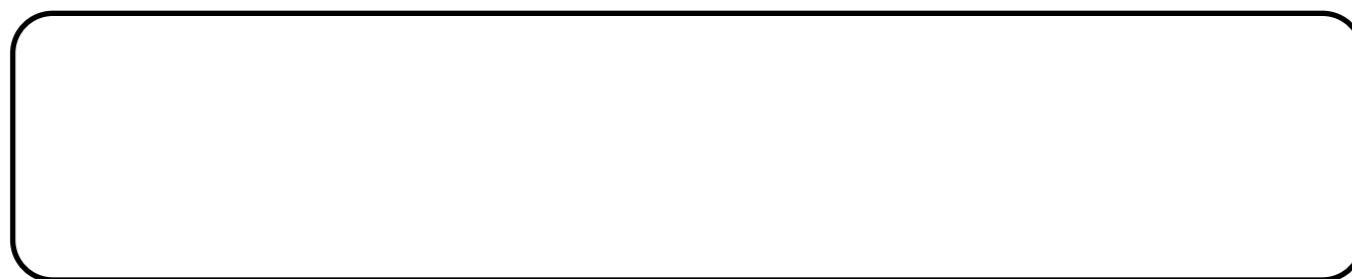
The *Drosophila* eye



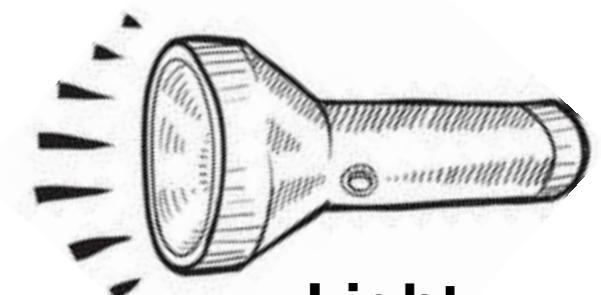
Seymour Benzer



A simple behavioral selection

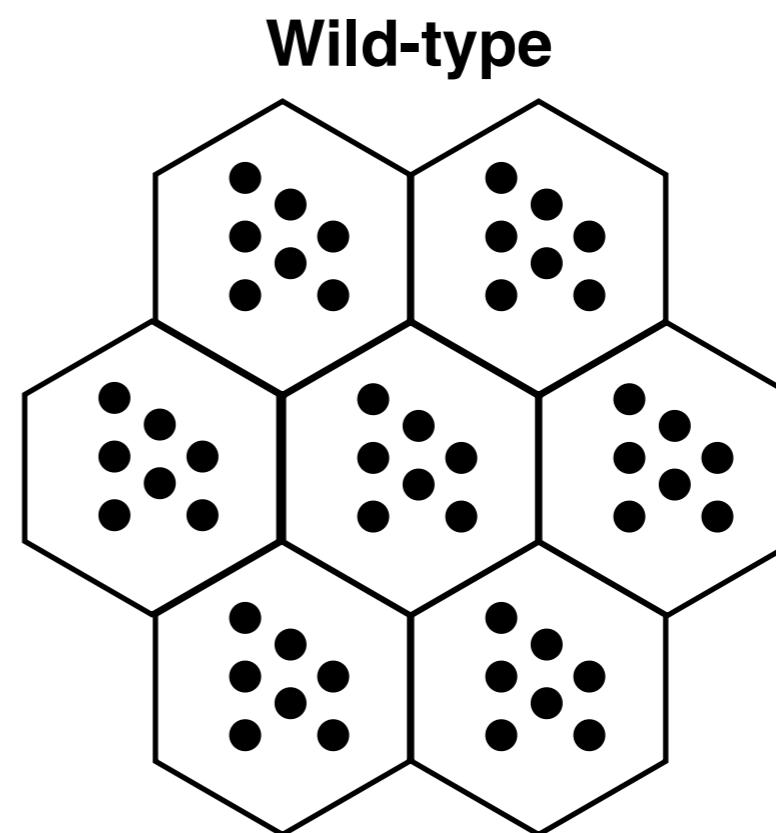
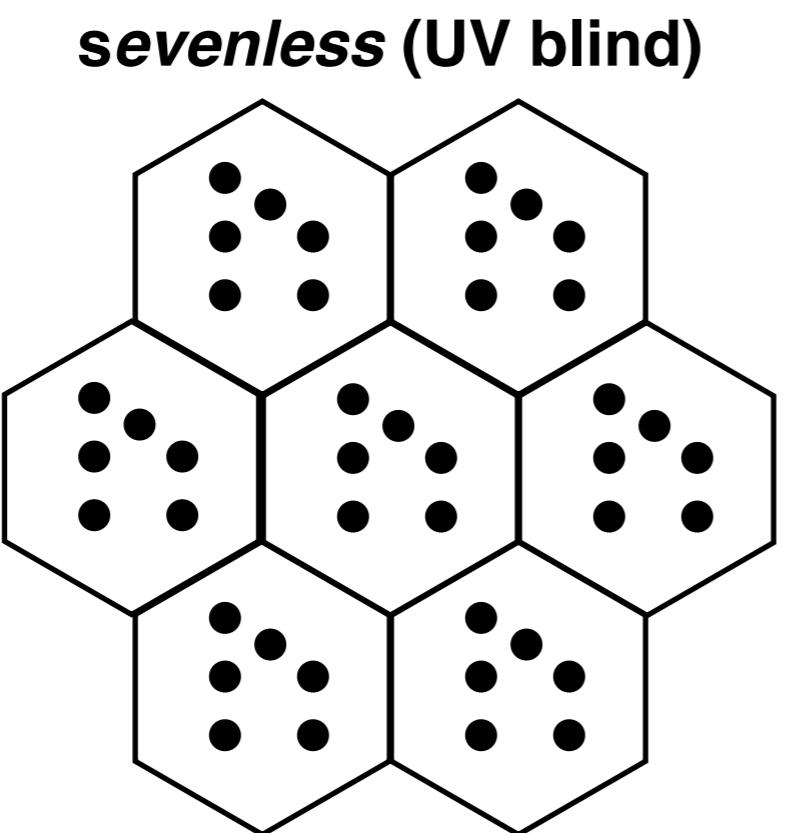
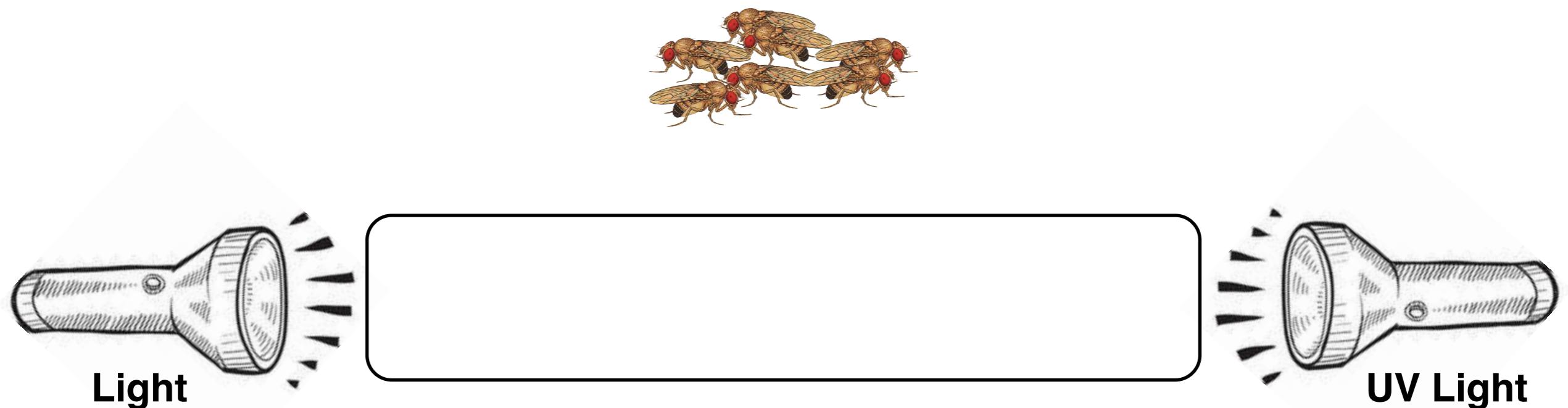


Dark



Light

A simple behavioral selection

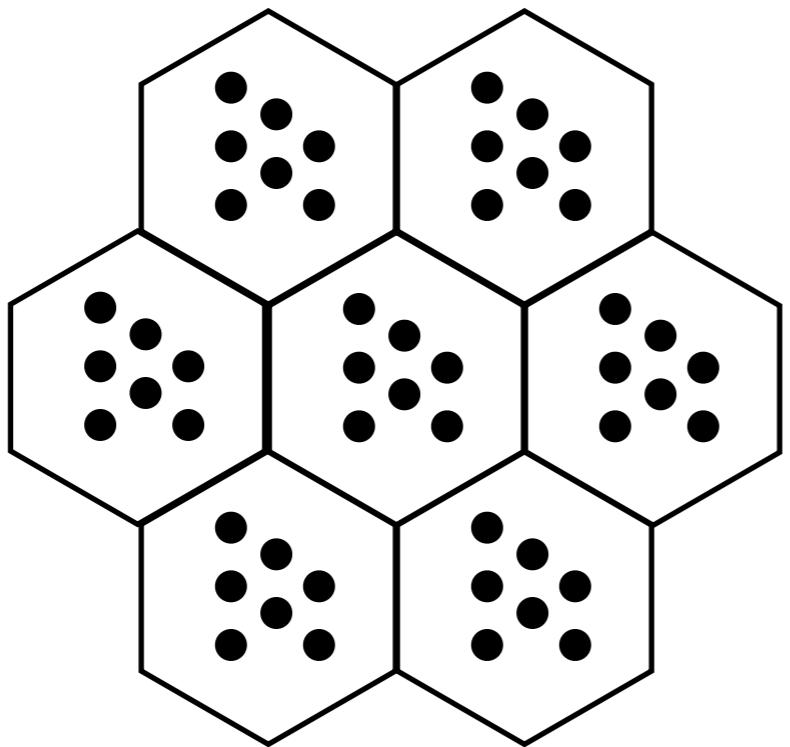




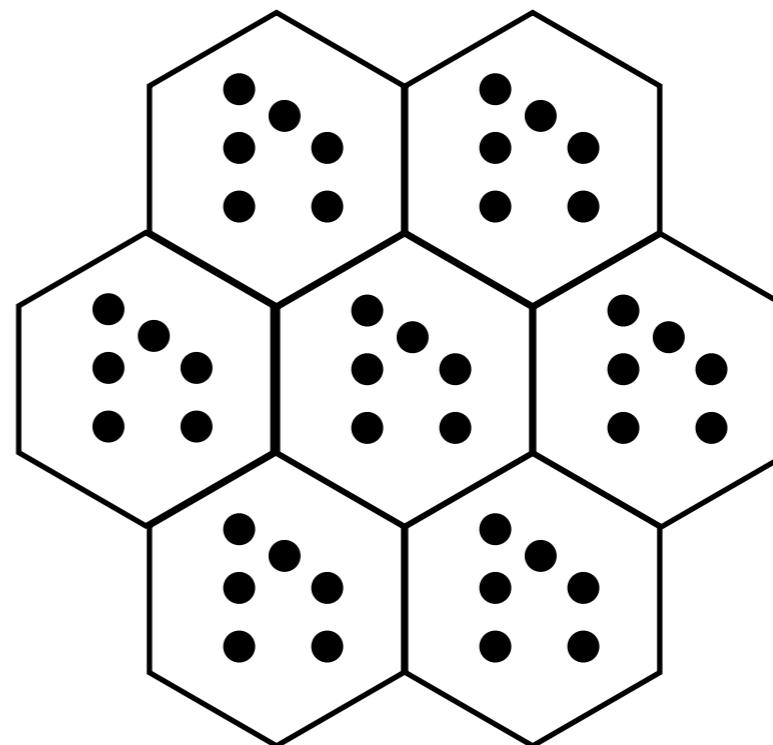
Gerry Rubin

Other UV blind mutants

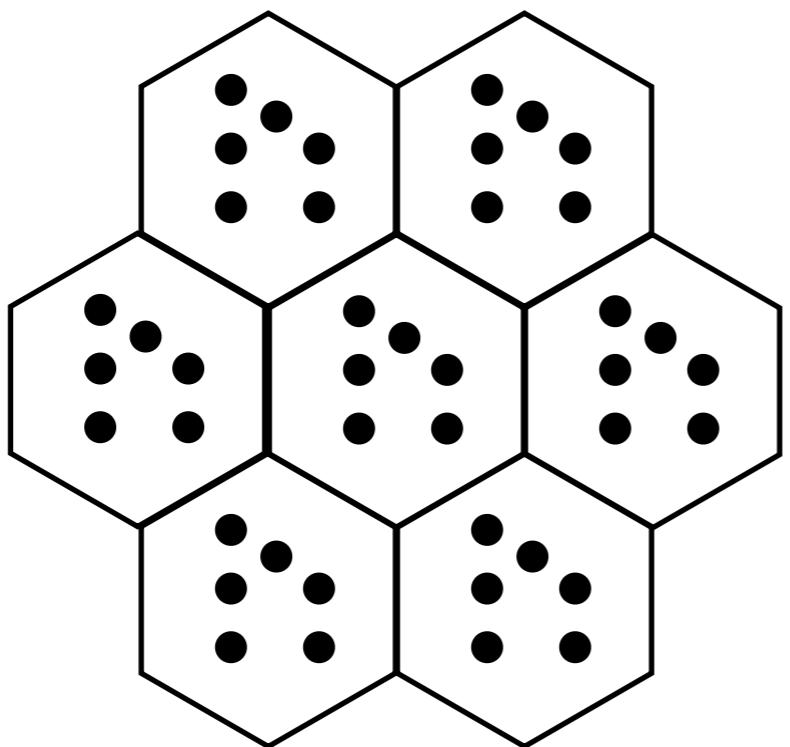
Wild-type



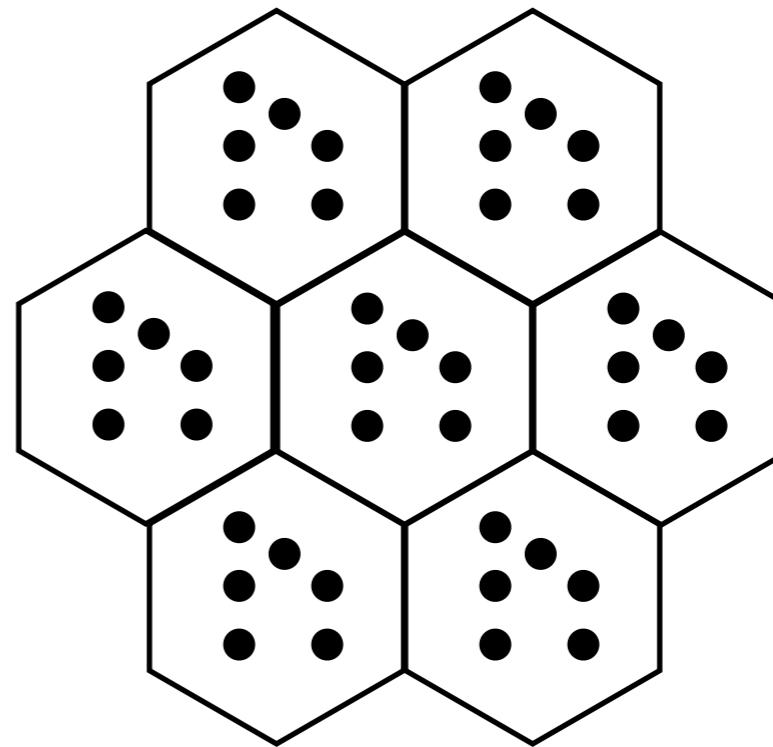
sevenless (UV blind)



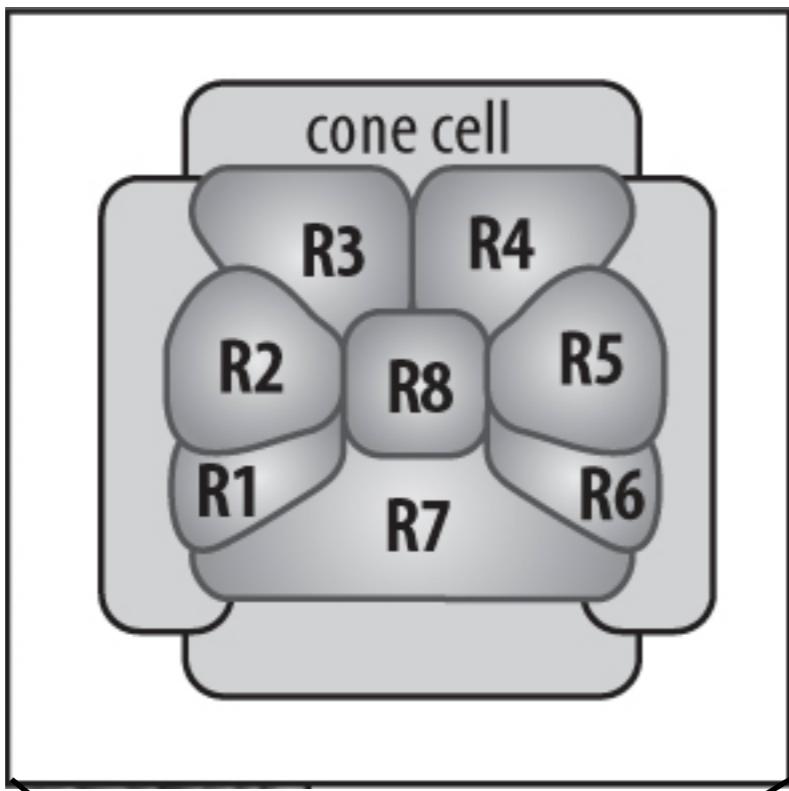
bride of sevenless (UV blind)



seven-in-absentia (UV blind)



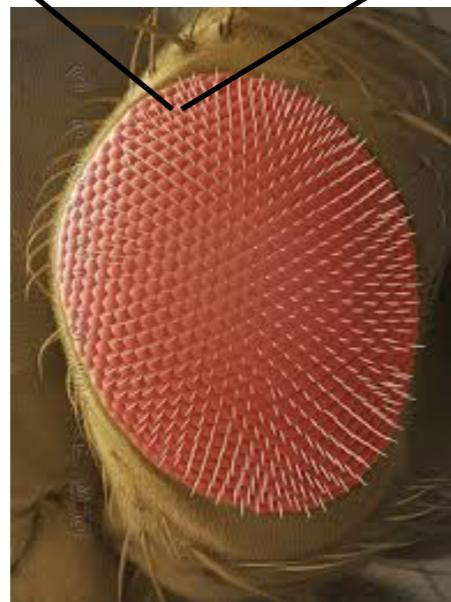
Development of an ommatidium



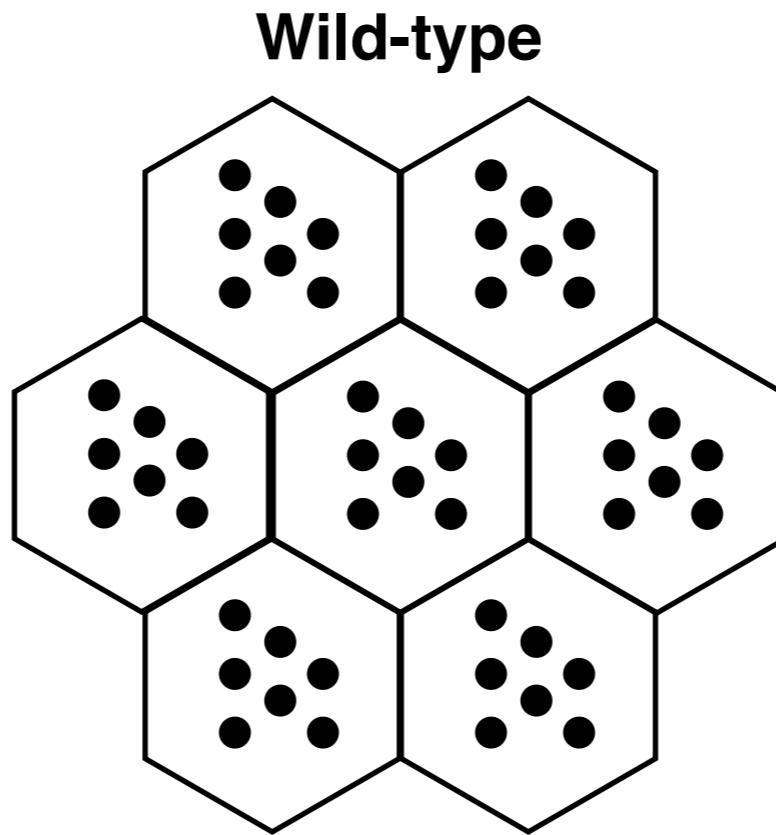
R8 born first

R1-R6 born after

R7 last to be born



Is the function of *sev*, *sina*, or *boss* required in the R7 cell?



Function in R7 is considered cell autonomous

Function in any cell besides R7 is considered cell non-autonomous

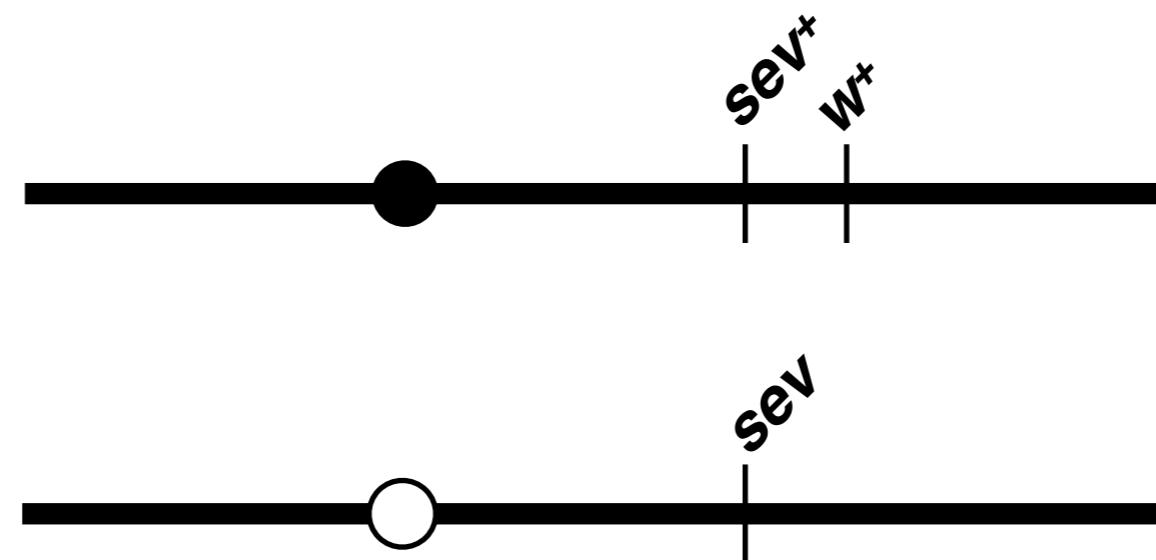
Are genes required in ommatidia for cell viability?

We want to make flies that lack the *sev* gene in certain cells

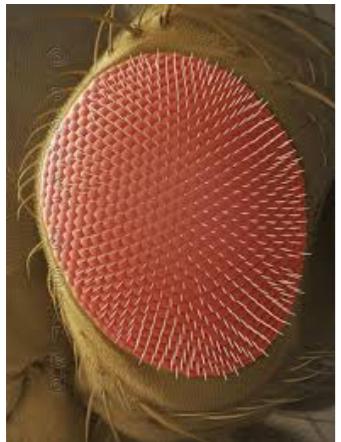


1. $\frac{\underline{sev}}{+} ; \frac{w}{W}$

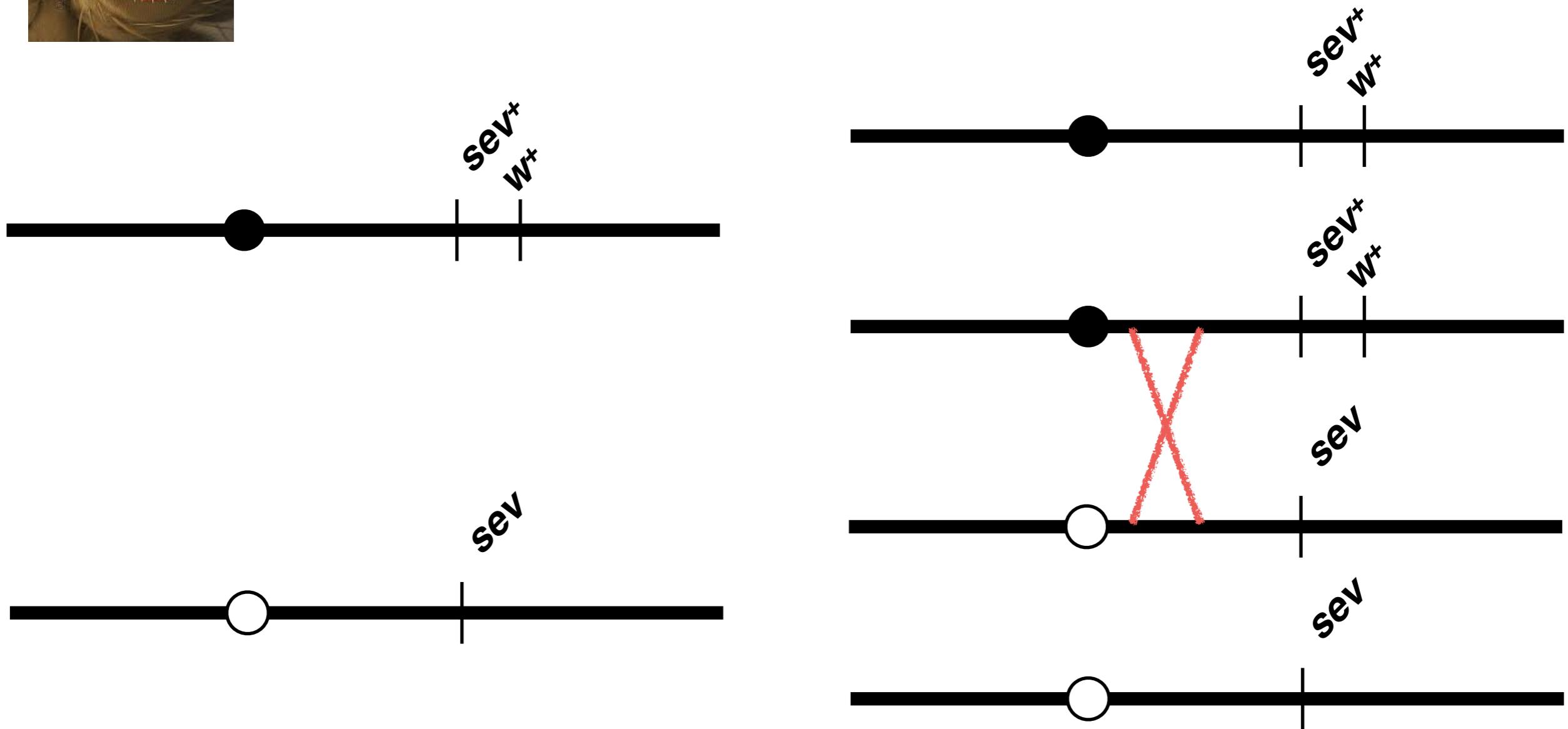
2. Use a P element with w^+ distal
to the wild-type *sev* gene



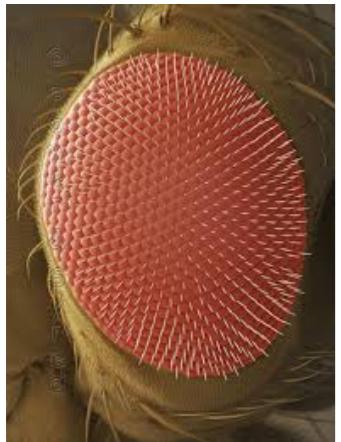
Are genes required in ommatidia for cell viability?



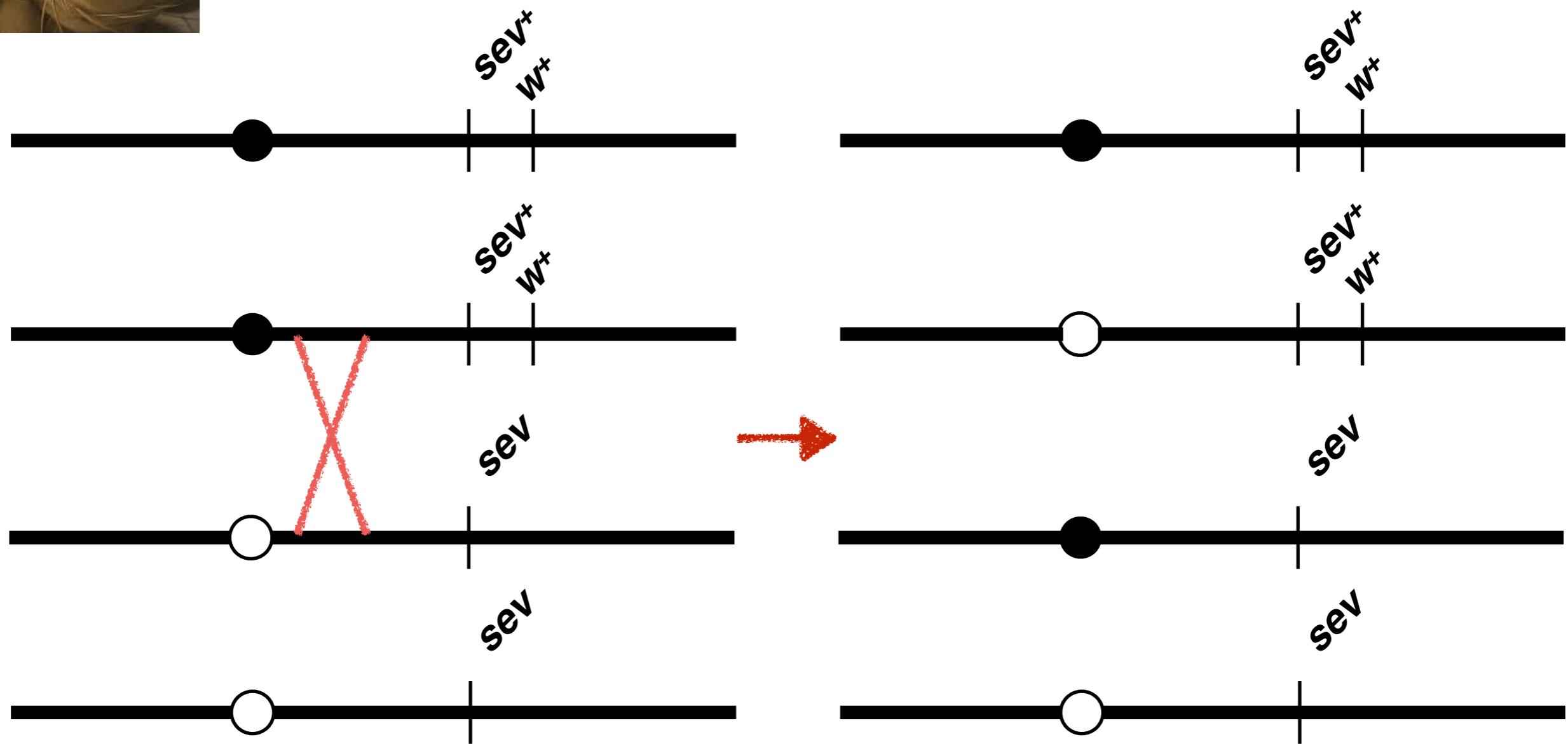
During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.



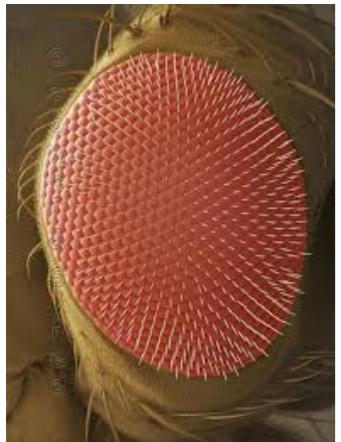
Are genes required in ommatidia for cell viability?



During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.

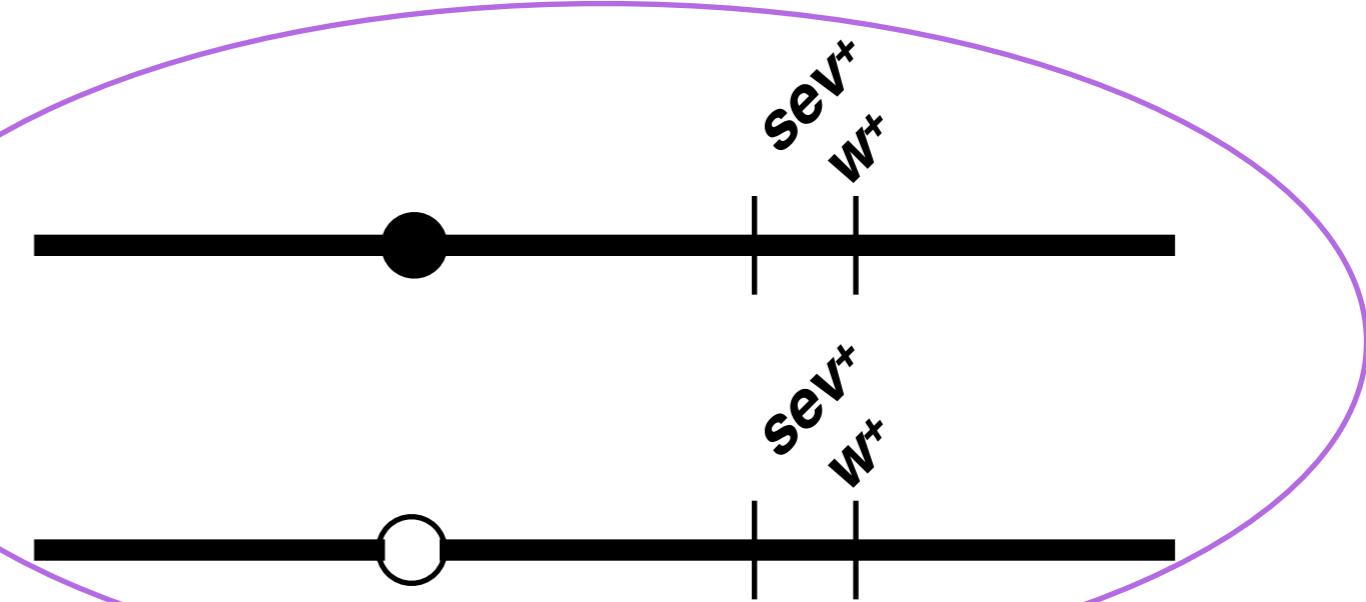


Are genes required in ommatidia for cell viability?



During mitosis for the cells that make up the eye, recombination can occur to repair double-strand breaks.

sev WT and pigment



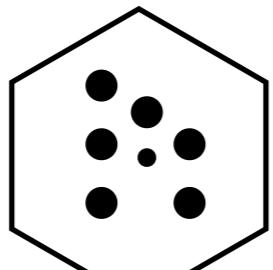
sev

sev

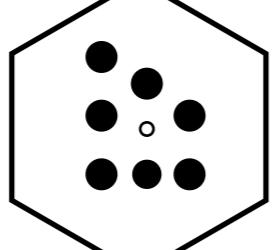
sev mutant
and no pigment

Twin spots!

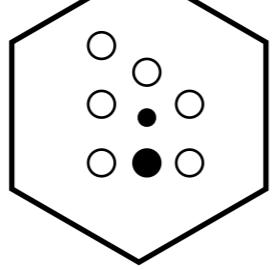
***sev* acts cell autonomously to regulate the R7 fate**



R7 cell *white* and *sev* mutant

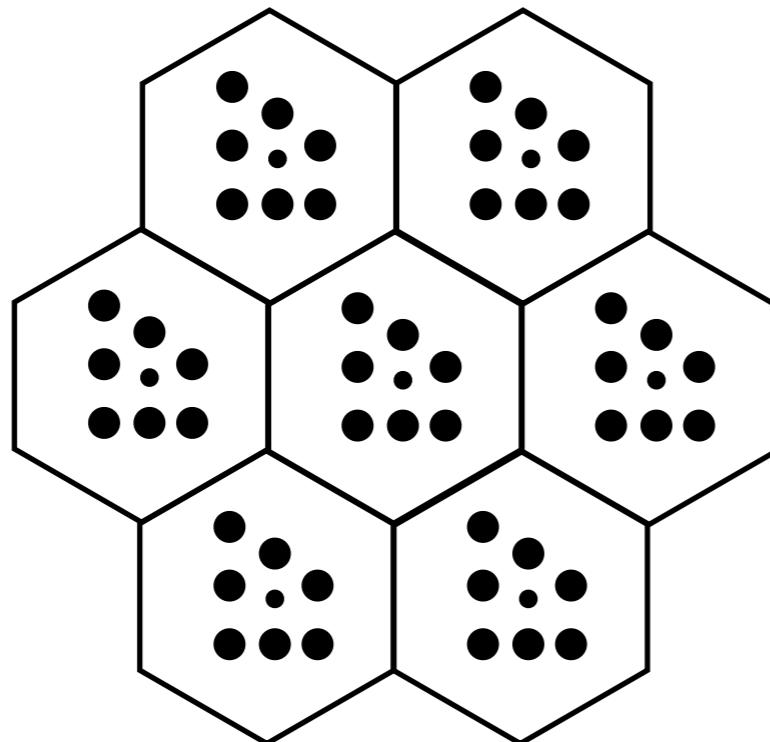


R8 cell *white* and *sev* mutant

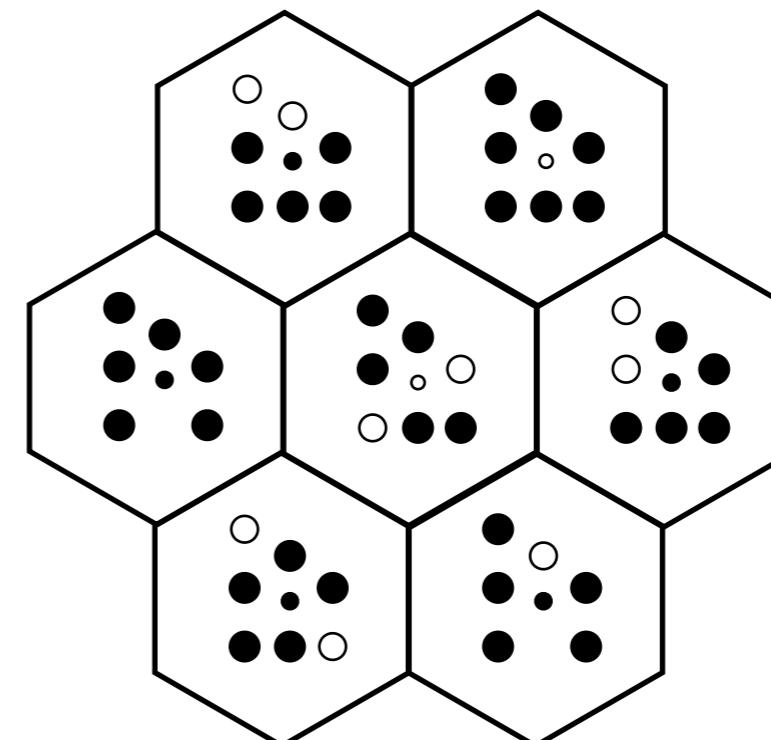


R1-R6 cells *white* and *sev* mutant

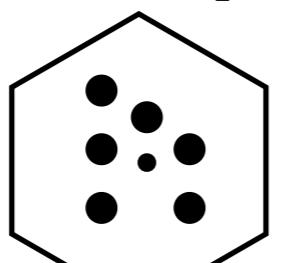
Wild-type



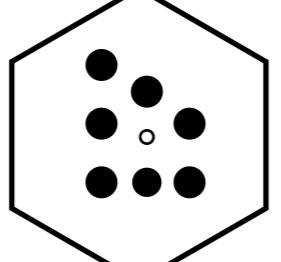
sev mutant



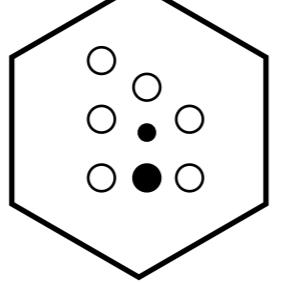
***boss* and *sev* are required in different R cells for R7 fate**



R7 cell *white* and *sev* mutant

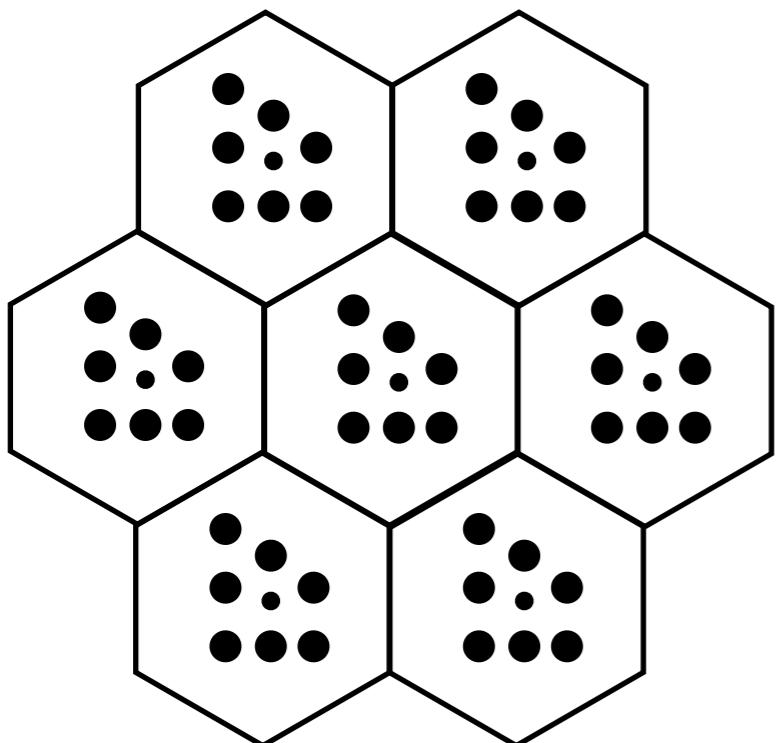


R8 cell *white* and *sev* mutant

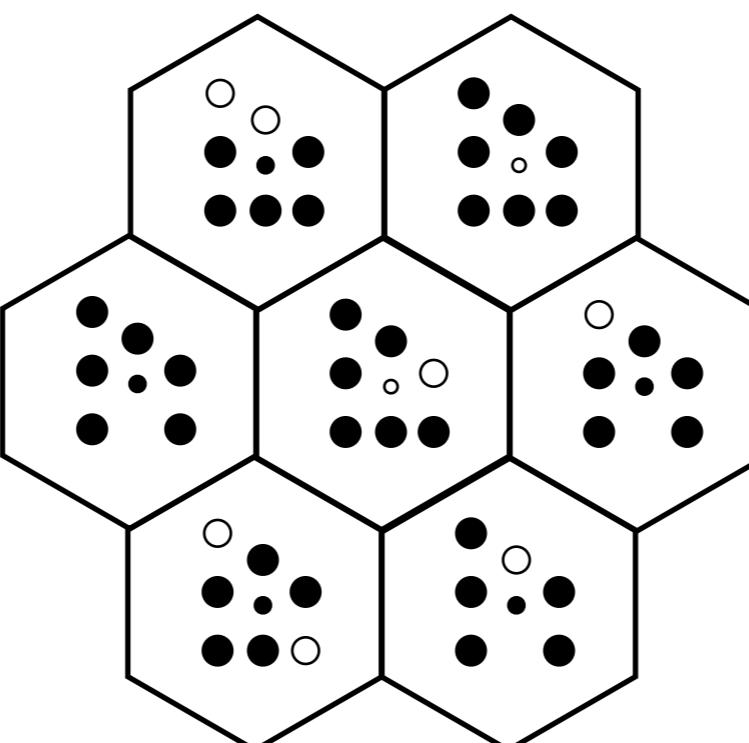


R1-R6 cells *white* and *sev* mutant

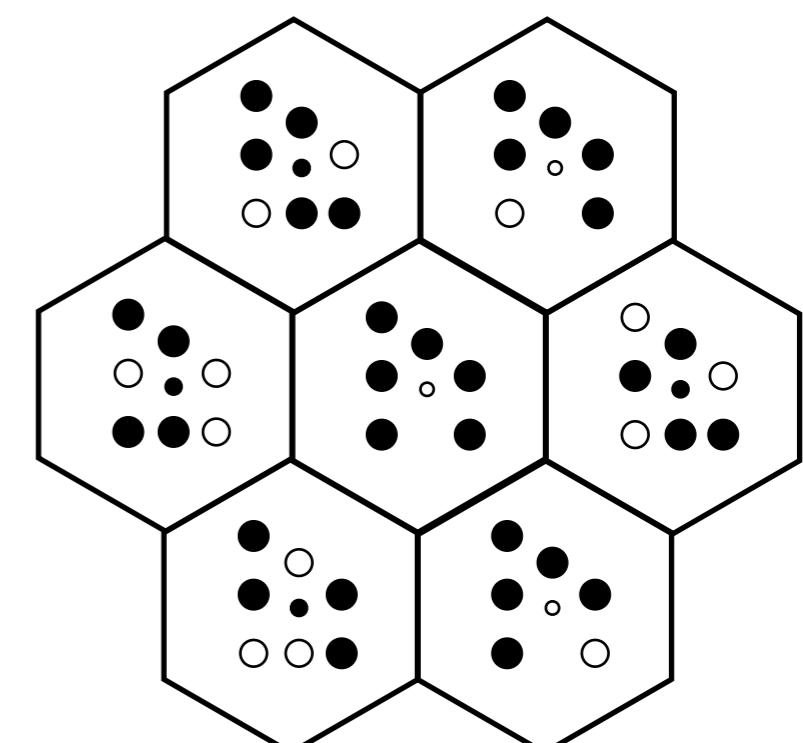
Wild-type



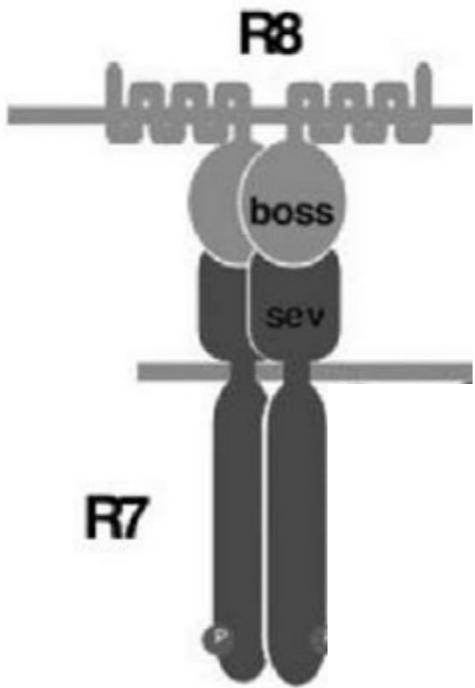
sev mutant



boss mutant

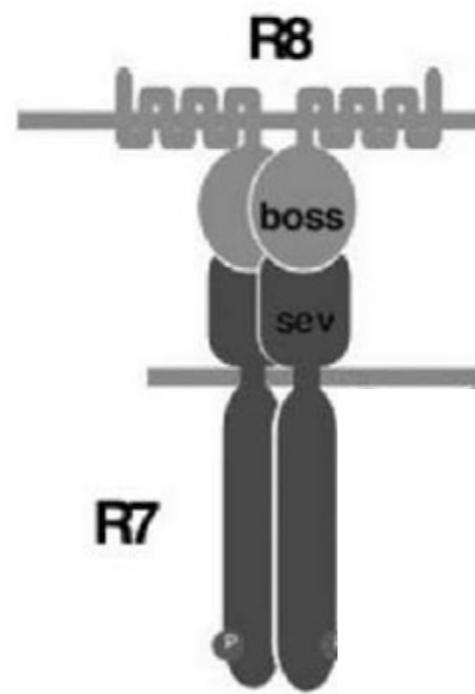
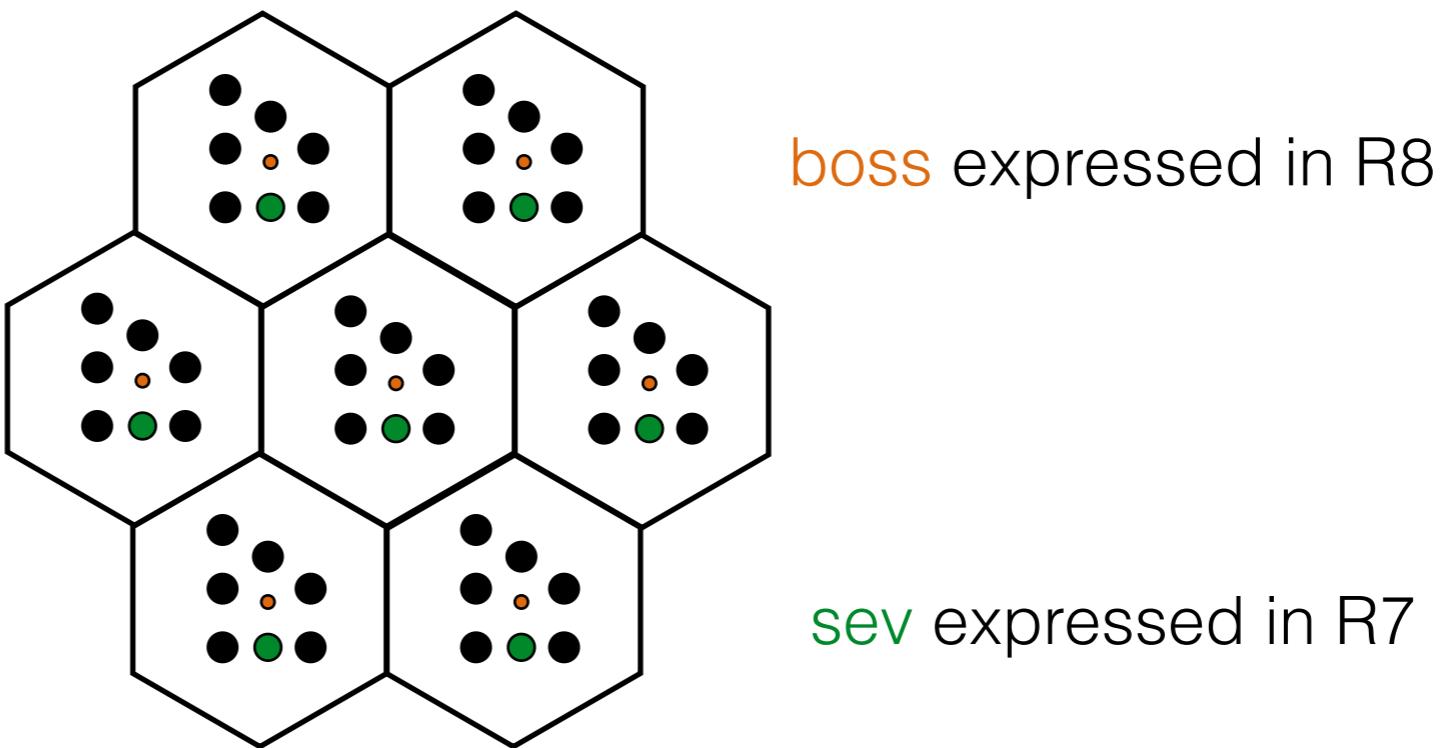


boss encodes
a membrane-bound protein

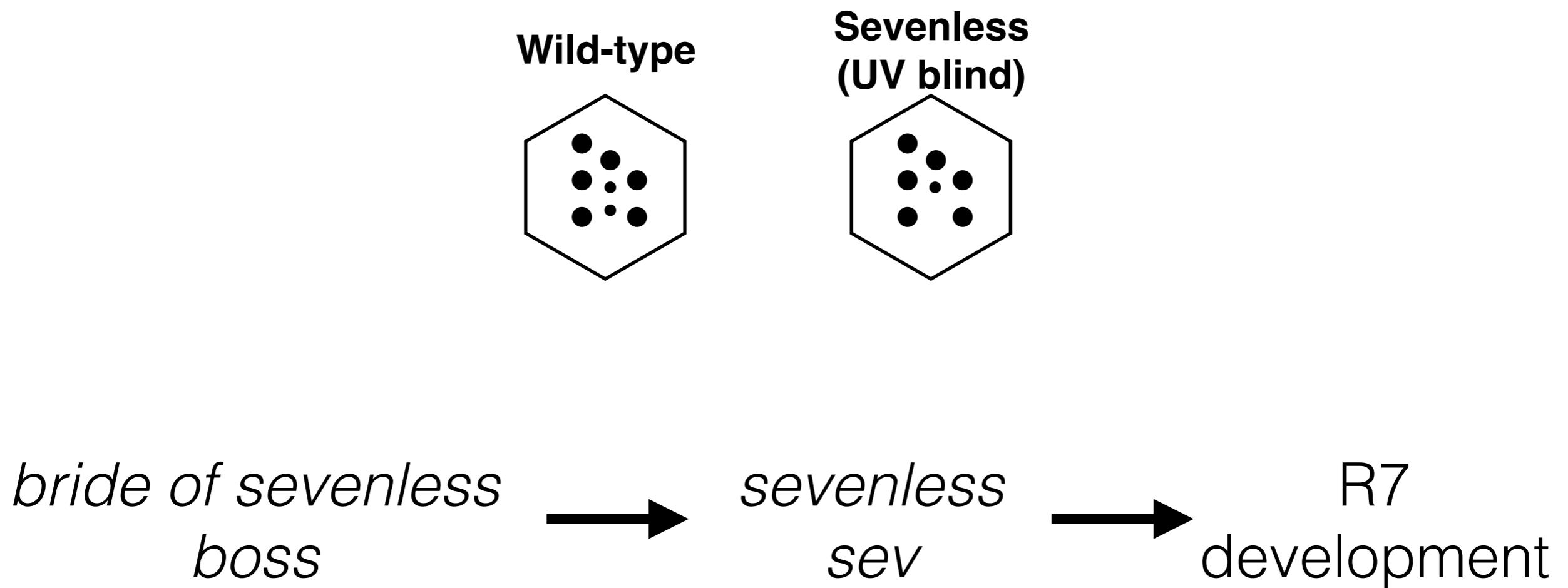


sev encodes
a membrane receptor tyrosine kinase

Expression of *sev* and *boss*



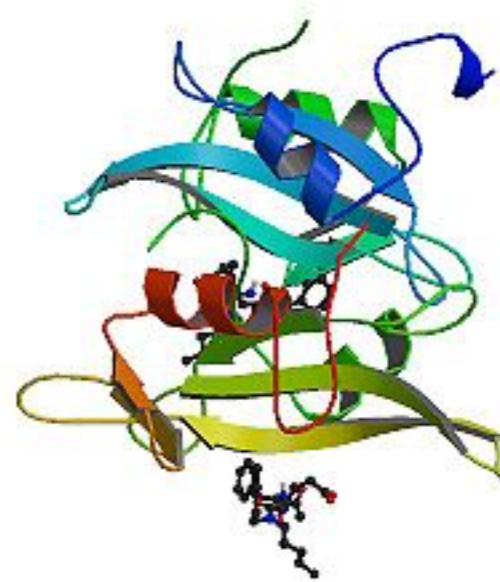
UV blind mutants led to elements of signaling pathway



Virus gene mutant from chicken to *Drosophila*



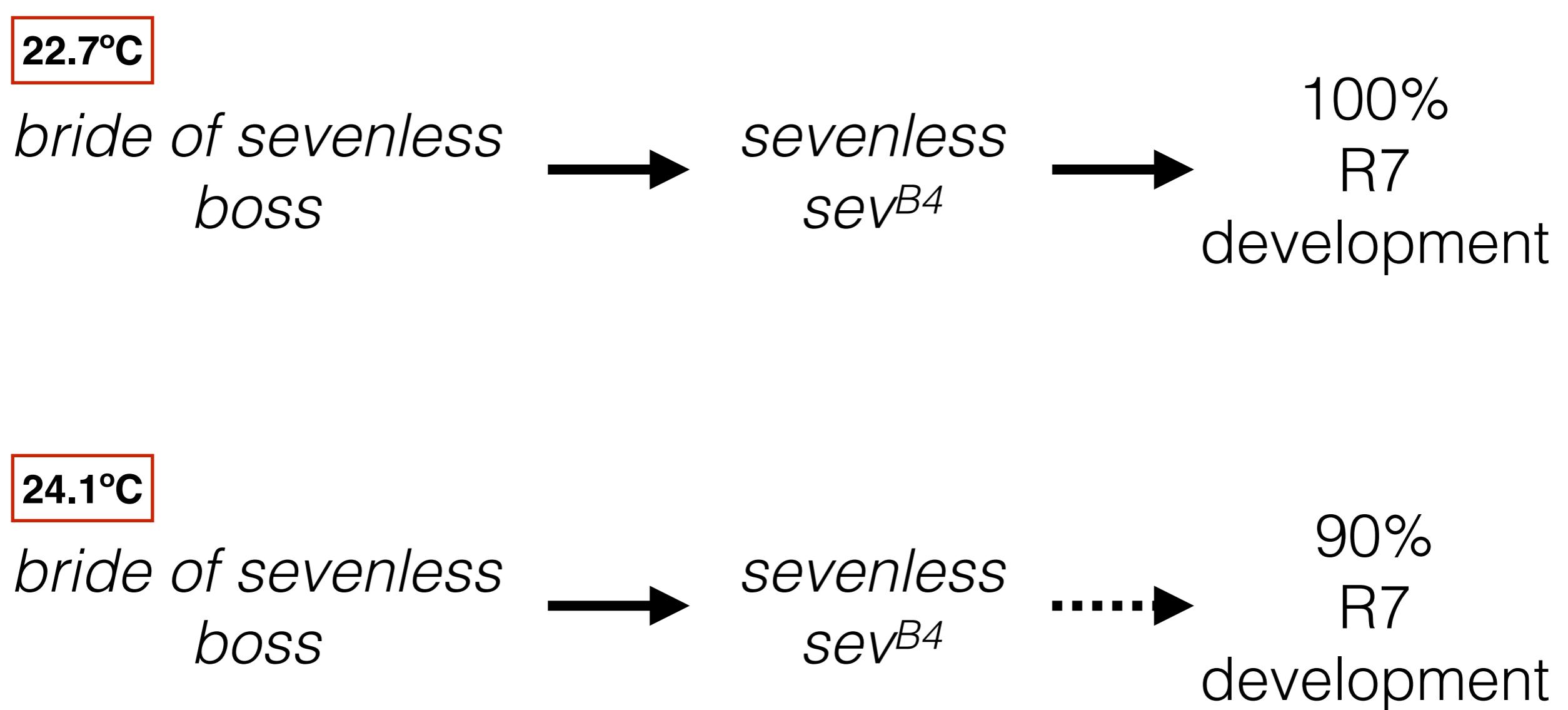
Peyton Rous discovered the first oncogenic virus in chicken



The virus expressed v-src (a tyrosine kinase) to control cell cycle

Mutagenesis of v-src led to temperature-sensitive alleles

A sensitized enhancer screen for the *sevenless* pathway



Strains used for the sensitized screen

sev^{d2} ; TM3 / CxD

sev^{d2} = complete loss of *sev*

TM3 = third chromosome balancer

CxD = third chromosome balancer

P[*sev^{B4}*]

sev^{B4} = temperature-sensitive *sev* hypomorph

Screen for dominant enhancers of sensitized phenotype

sev^{d2} ; TM3 Sb P[sev^{B4}]/ CxD

22.7°C

bride of sevenless
boss



sevenless
 sev^{B4}



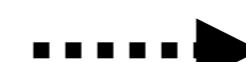
100%
R7
development

24.1°C

bride of sevenless
boss



sevenless
 sev^{B4}



90%
R7
development

22.7°C

bride of sevenless
boss



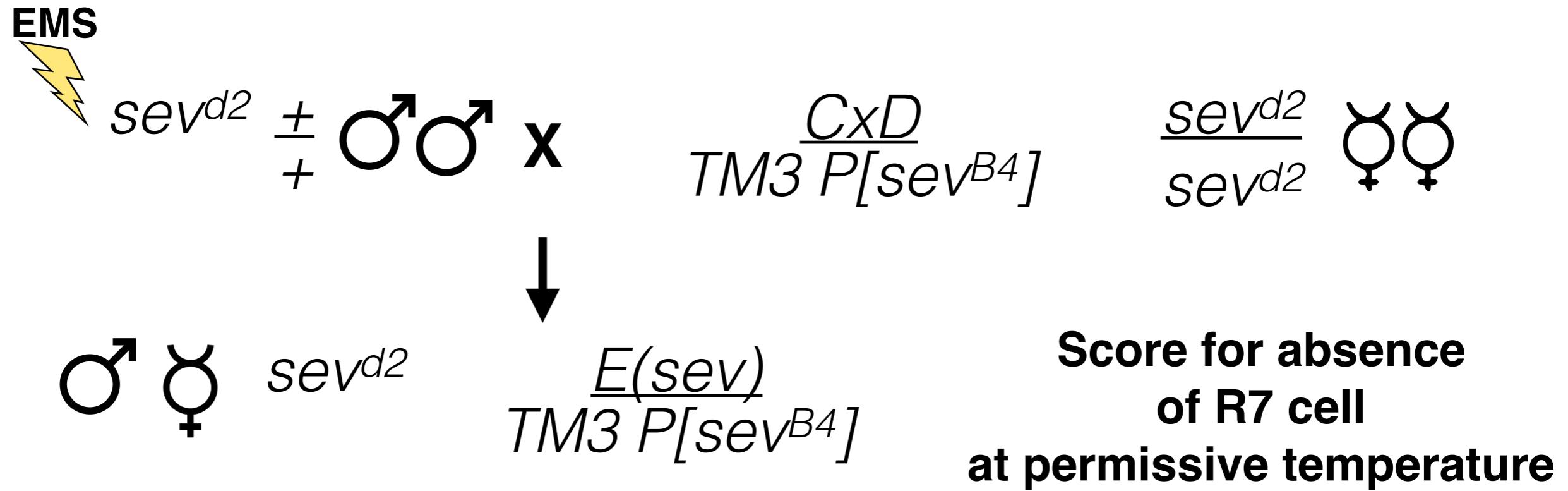
sevenless
 sev^{B4}



$E(sev)$

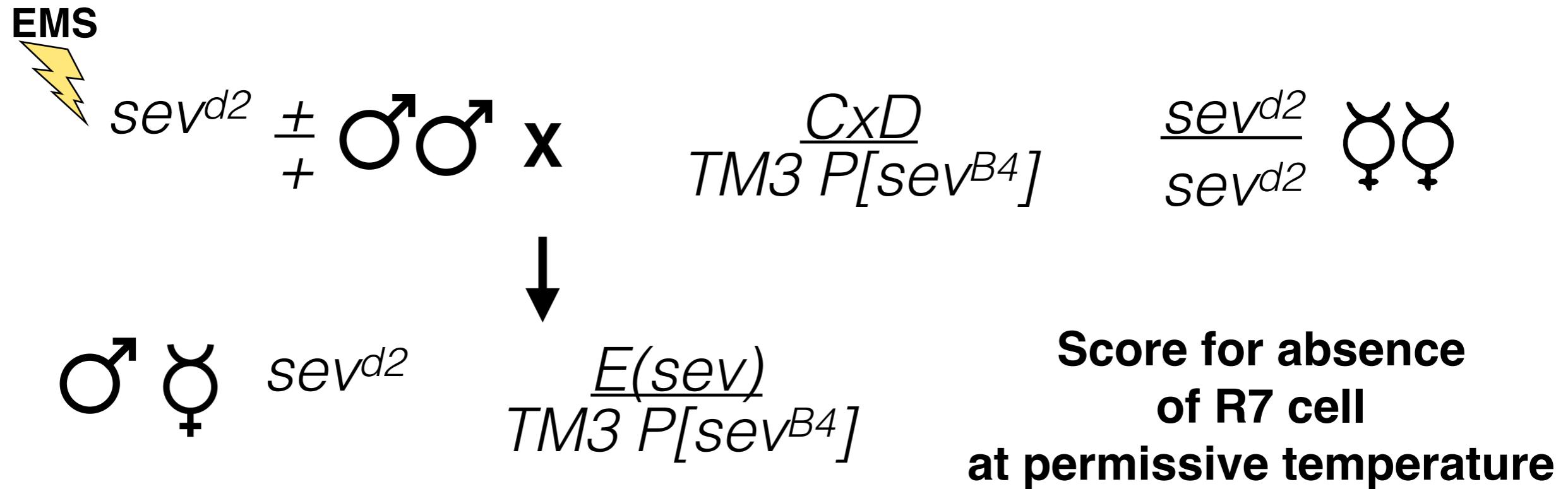
~10%
R7
development

Screen for dominant enhancers of sensitized mutant R7 phenotype



Assumptions: (1) Mutations in downstream genes required for viability and R7 fate
(2) Most genes are not haploinsufficient

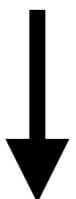
Screen for dominant enhancers of sensitized mutant R7 phenotype



Screened 30,000 flies using pseudopupil technique
Got 20 $E(sev)$ in seven complementation groups

Screen for dominant enhancers of sensitized phenotype led to the Ras pathway controlling R7 fate

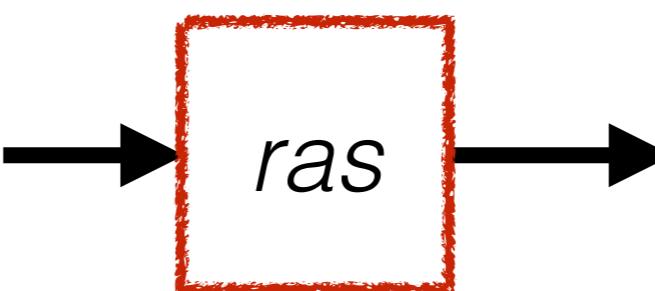
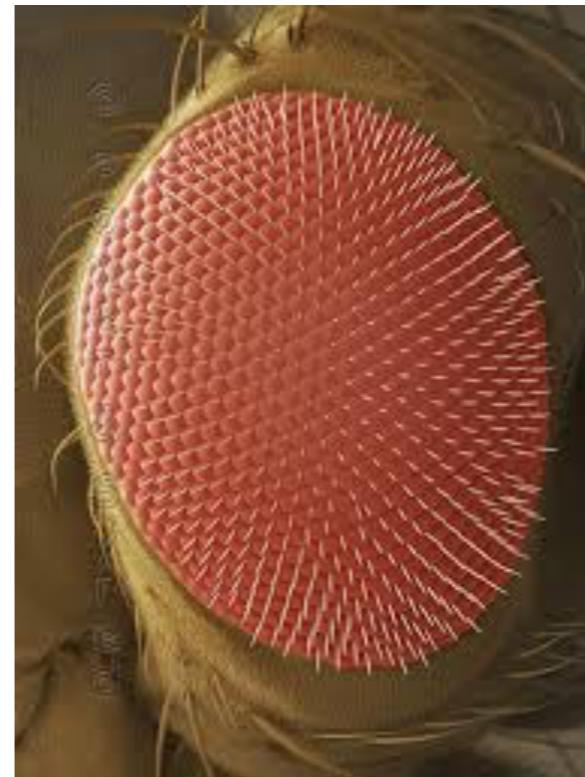
bride of sevenless
boss



sevenless
sev



son of sevenless
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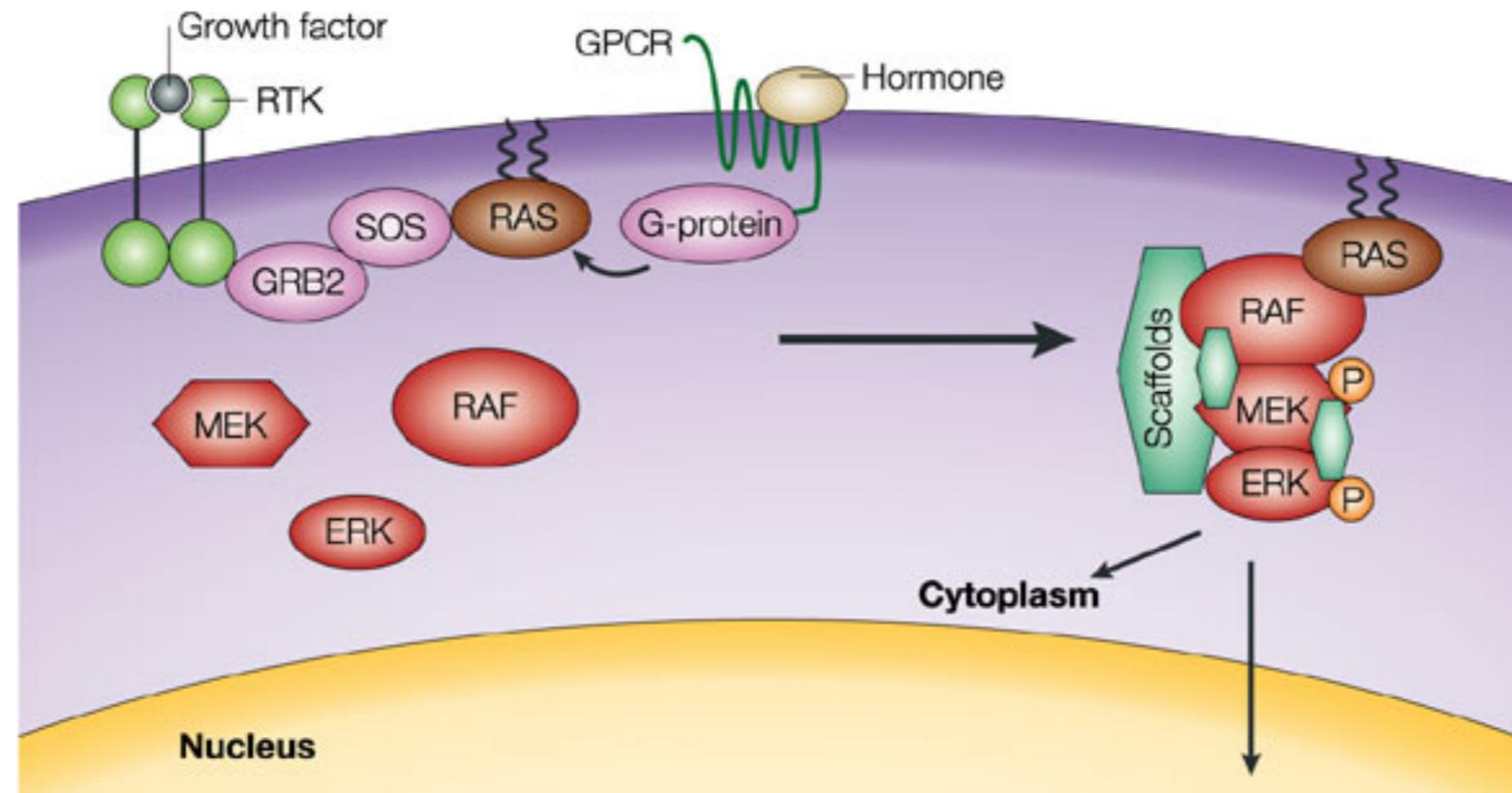


R7
development

Methods to ascertain cell autonomy

1. Expression of gene product in specific cell or tissue and look for rescue of a mutant phenotype
2. Lineage loss of unstable DNA that expresses the gene product and rescues a mutant phenotype (*C. elegans*)
3. Mitotic recombination to create clones of cells that express the gene product and rescue the mutant phenotype (*Drosophila*)

Two decades of research in *Drosophila* and *C. elegans* led to these pathways



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We NEED basic research for this reason!