# 18. Determine if there are maternal effects or cytoplasmic inheritance


The egg and sperm have different compositions.

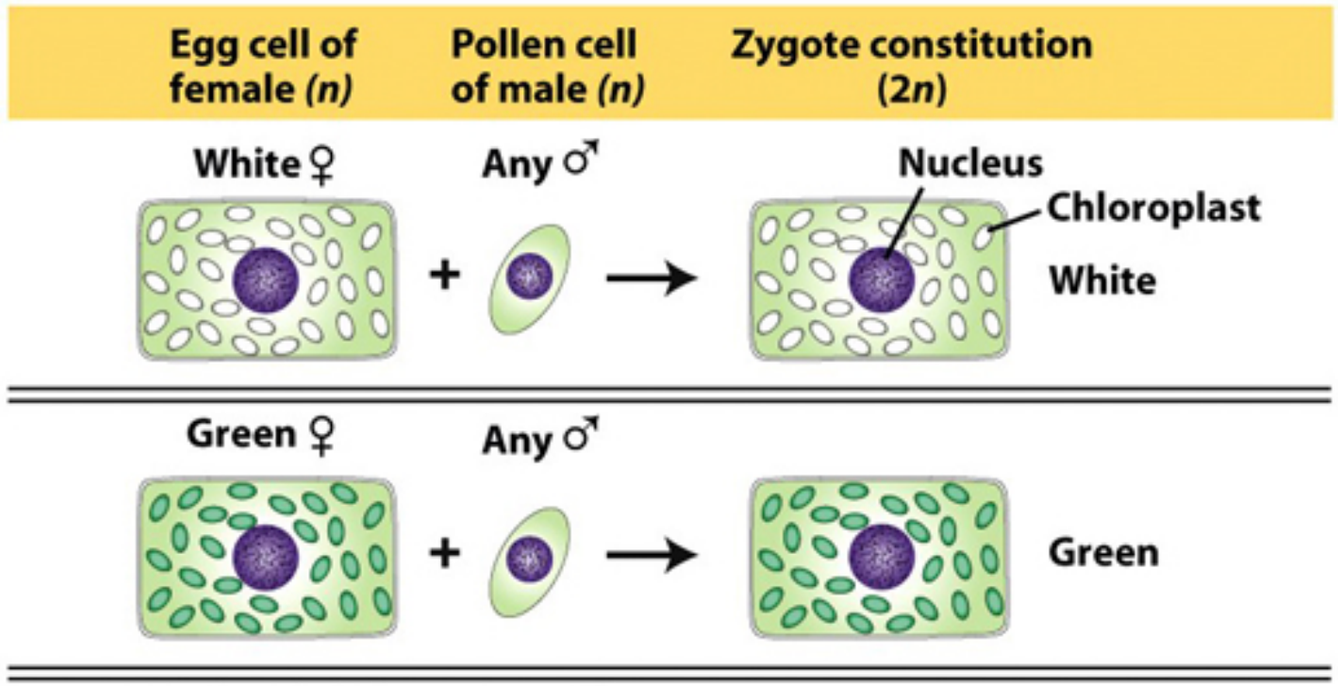
# 18. Determine if there are maternal effects or cytoplasmic inheritance

Plastid inheritance in *Mirabilis jalapa*

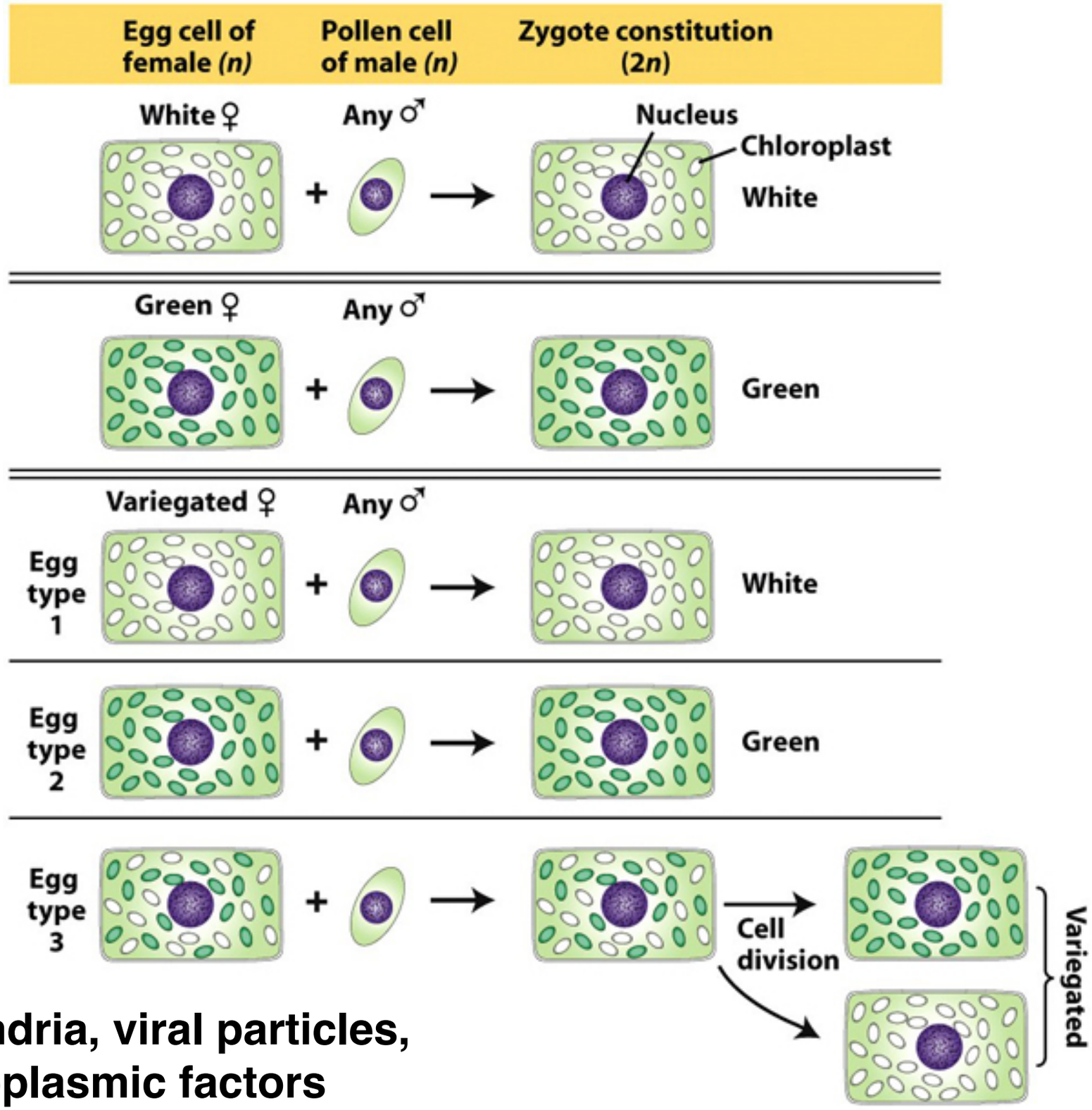
Only the color of the stem matters

White, green, or variegated

# 18. Determine if there are maternal effects or cytoplasmic inheritance



# 18. Determine if there are maternal effects or cytoplasmic inheritance




**Plastids, mitochondria, viral particles, and other cytoplasmic factors**

# 19. Determine the overexpression phenotype

What happens when the wild-type individual
has too much of gene X?

Overexpression *might* be useful
for investigating genetic interactions

# 20. Perform an overexpression screen for additional modifiers

1. Screen for dominant phenotypes similar to your mutant phenotype

2. Inducible overexpression of specific genes

3. Transposon-mediated overexpression screens

Find more genes by making hypermorphs

# 21. Isolate enhancers and suppressors of your mutant phenotype

# 22. Investigate pathways (measure genetic interactions or epistasis)

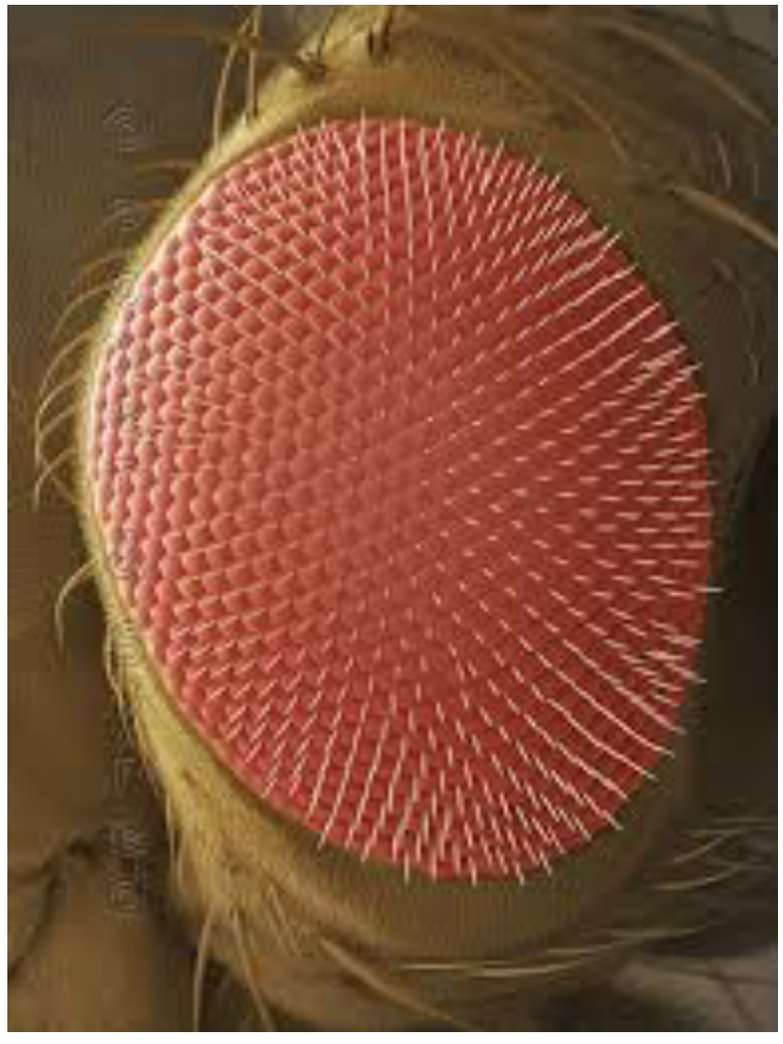
# Step-wise genetic analysis

1. Define the problem
2. Choose an organism
3. Perform a mutant hunt
4. Screen until saturation?
5. Establish a strain
6. Backcross and/or outcross
7. Test for dominance
8. Single-gene phenotype?
9. Mapping and complementation
10. Characterize the phenotype
11. Define the nature of the mutant allele(s): gene dosage
12. Perform non-complementation screens
13. Define the null phenotype
14. Clone the gene
15. Determine where gene is expressed
16. Determine site of gene action
17. Determine time of gene action
18. Determine if there are maternal effects or cytoplasmic inheritance
19. Determine the overexpression phenotype
20. Perform an overexpression screen for additional modifiers
21. Isolate enhancers and suppressors of your mutant phenotype
22. Investigate pathways (measure genetic interactions or epistasis)

# Bio393: Genetic Analysis

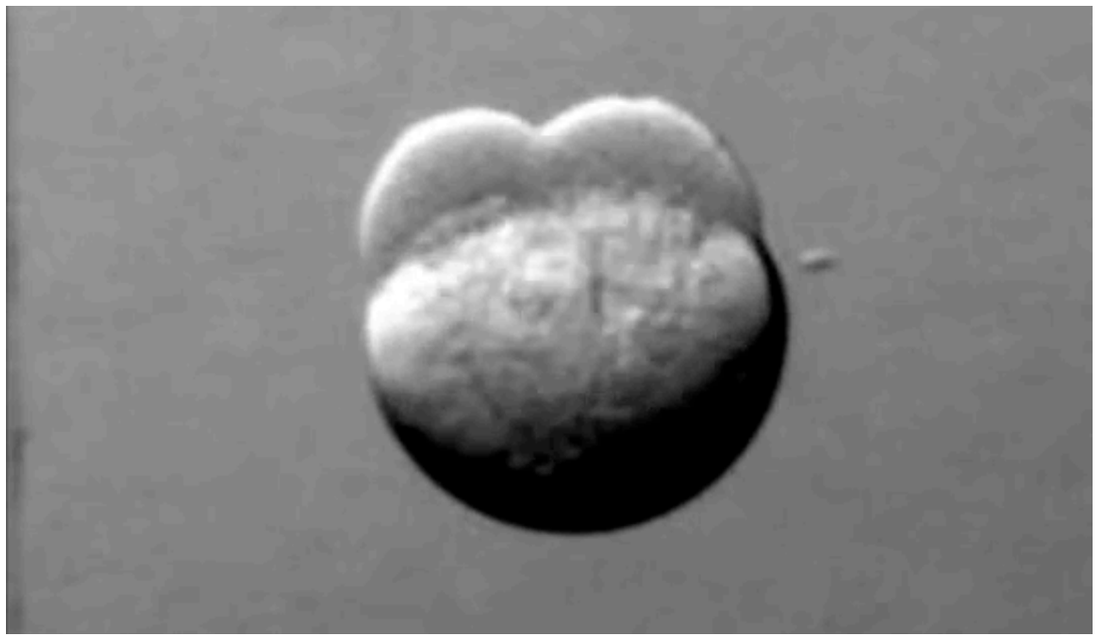
Developmental genetics

***C. elegans***



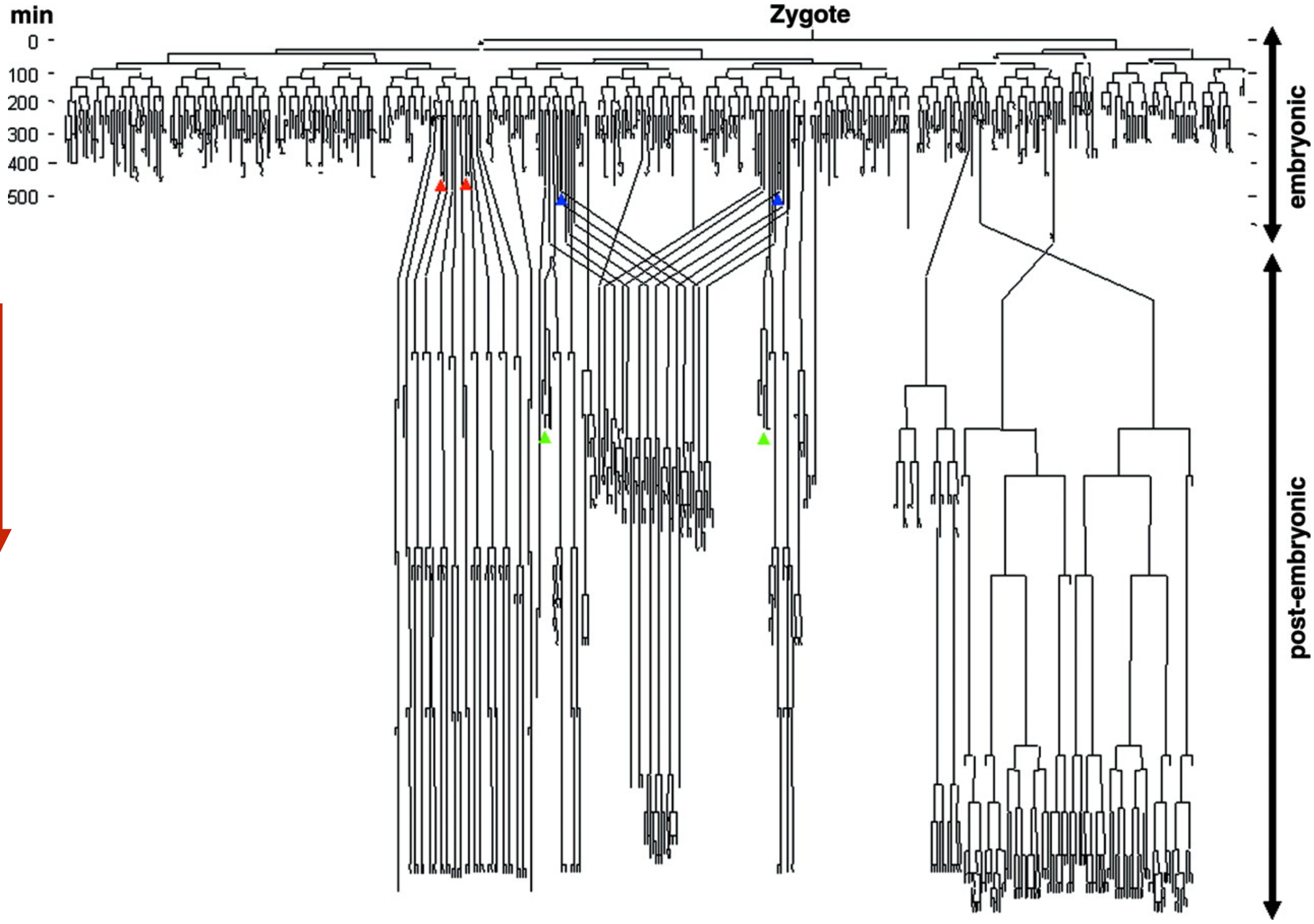
***D. melanogaster***

# 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

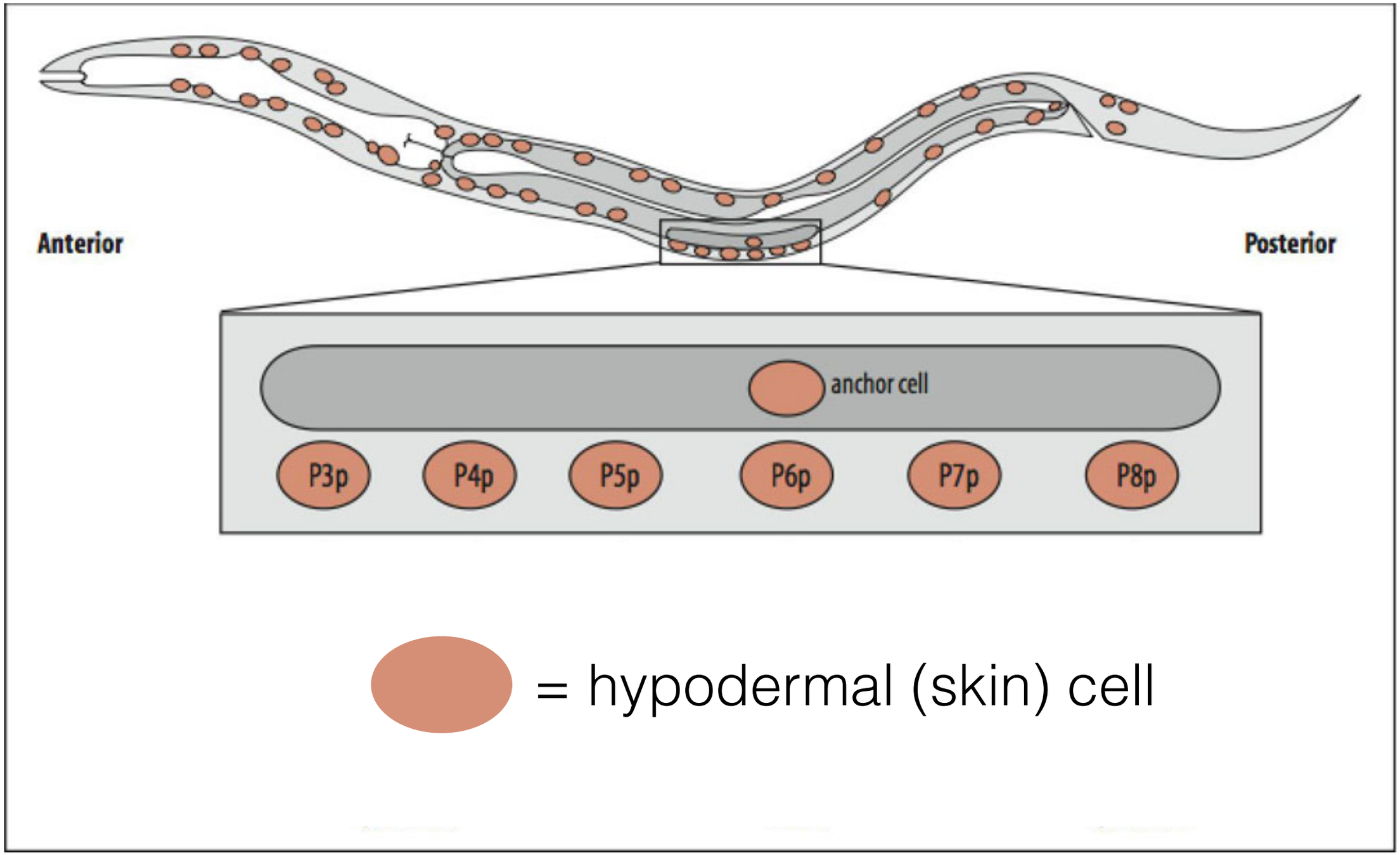
# The cell lineage of *C. elegans* is known and invariant



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


**John Sulston**

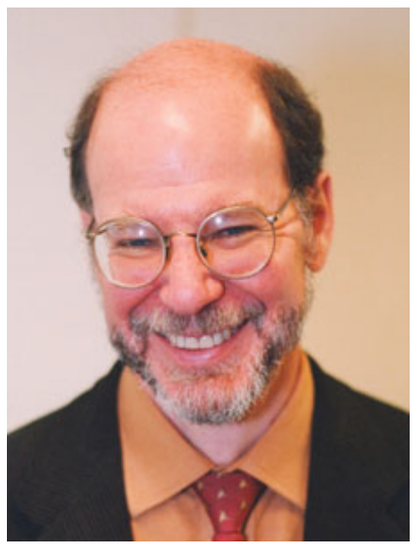
# *C. elegans* vulval development



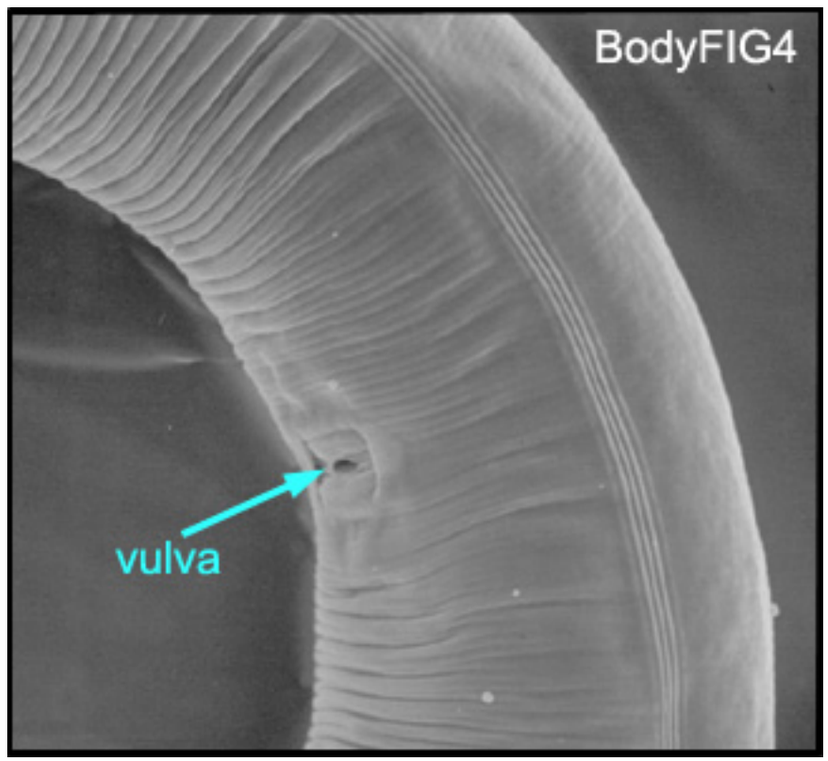
# One of the best genetic screens ever

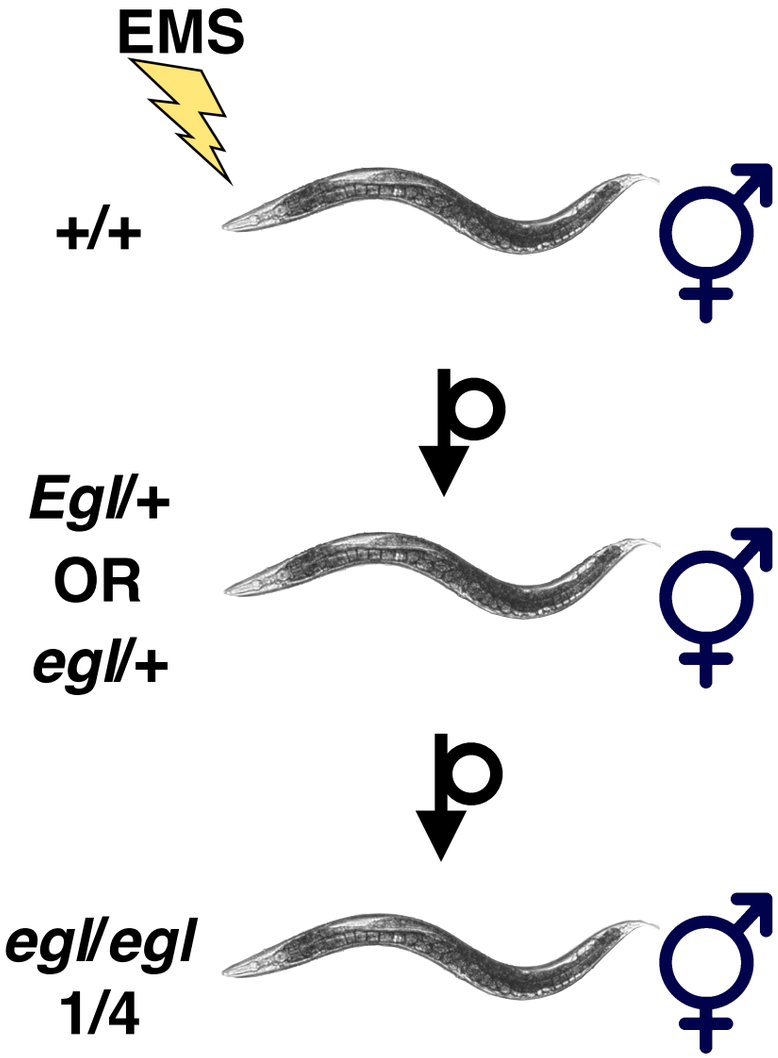
Let's say you screened for mutants that failed to lay eggs

Called Egl for egg-laying defective

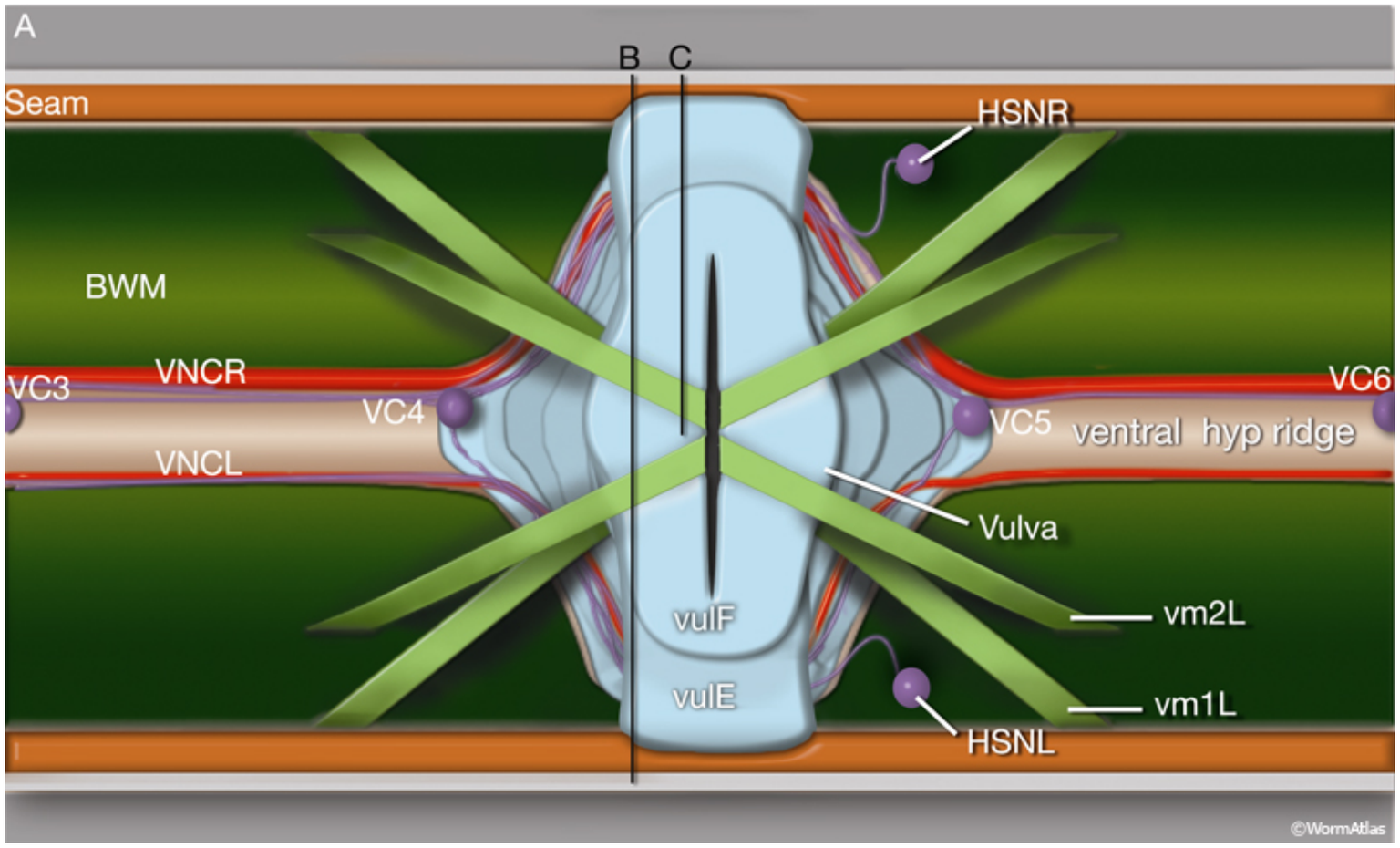


**Bob Horvitz**



# One of the best genetic screens ever

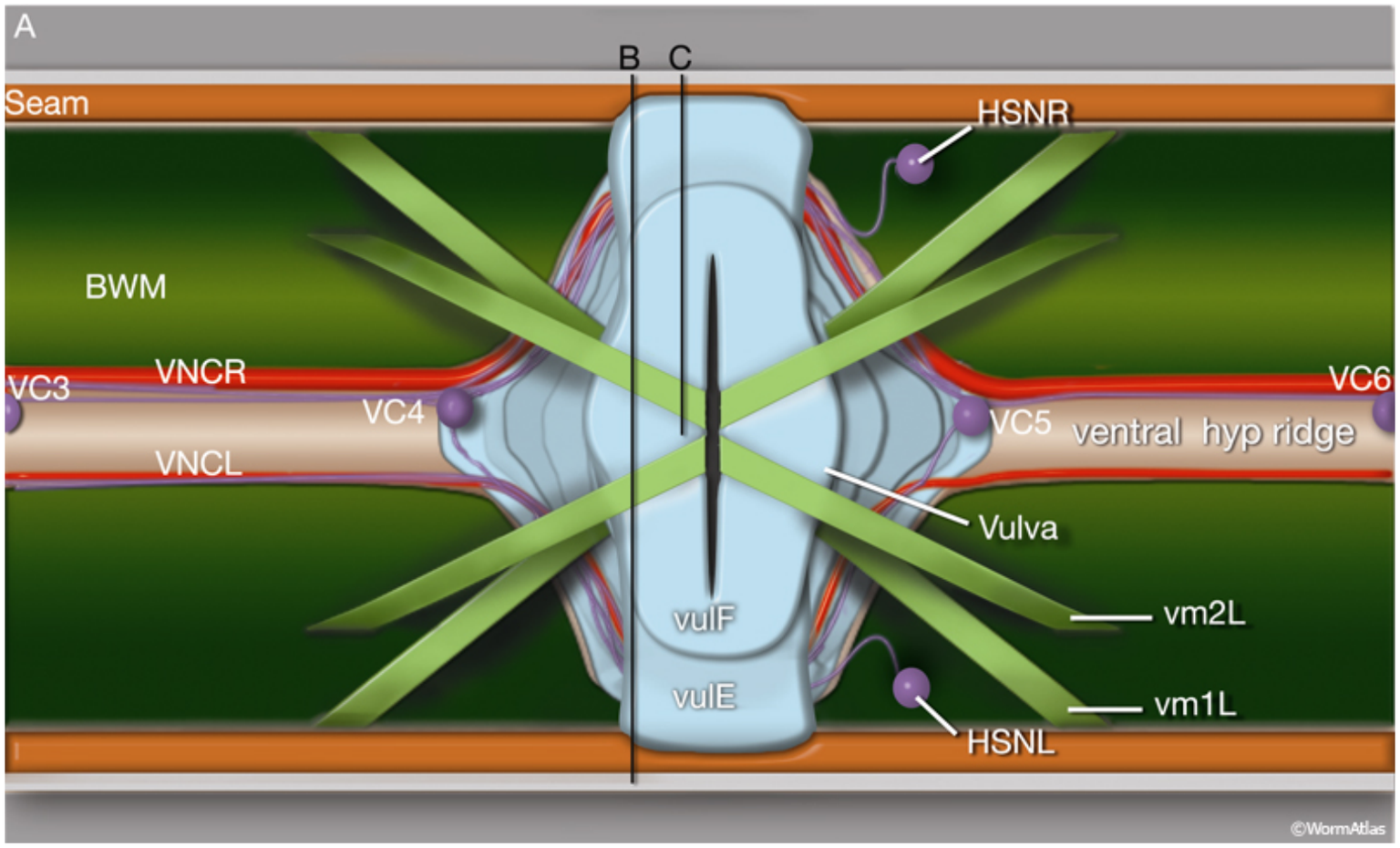



**No neuron**

*egl-1* = inducer of programmed cell death

Hermaphrodite-Specific Neuron (HSN) inappropriately dies

# One of the best genetic screens ever

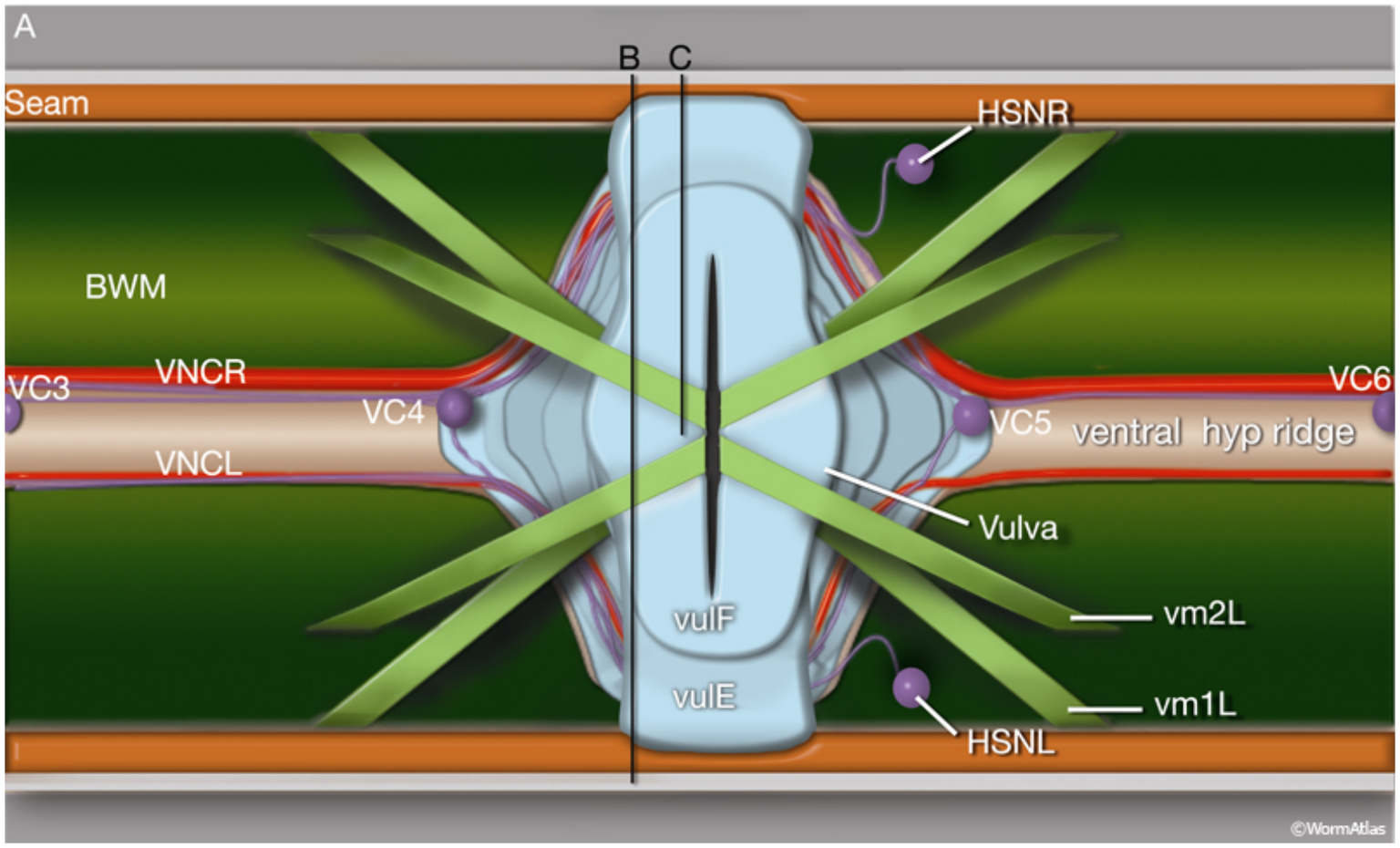



**No neuron**

*tra-1* = inducer of sex determination

Mutants are partially male so HSNs die

# One of the best genetic screens ever

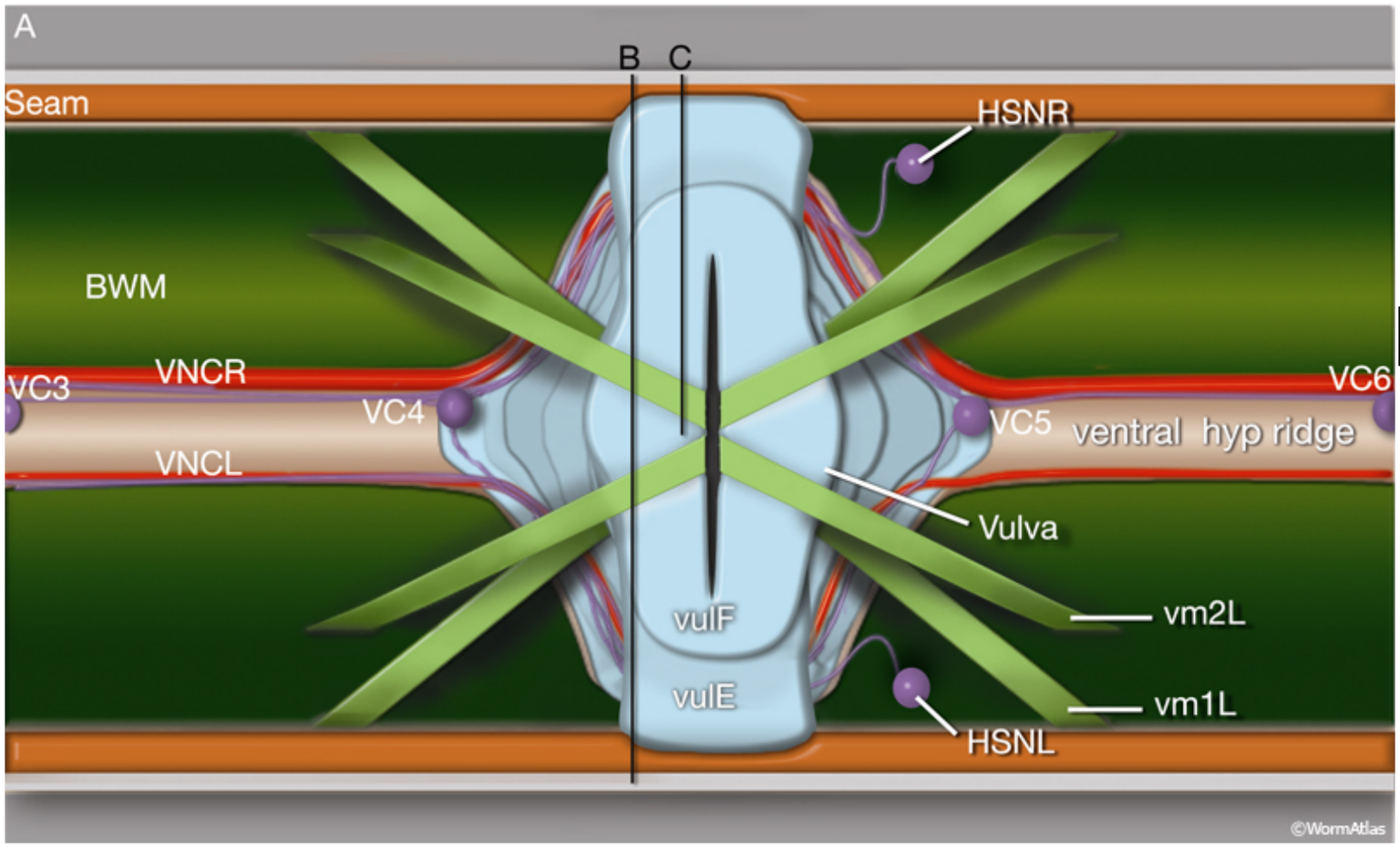



**No neuron**

**No neuronal signaling**

*egl-6* = seratonin signaling from HSN is defective

# One of the best genetic screens ever




**No neuron**

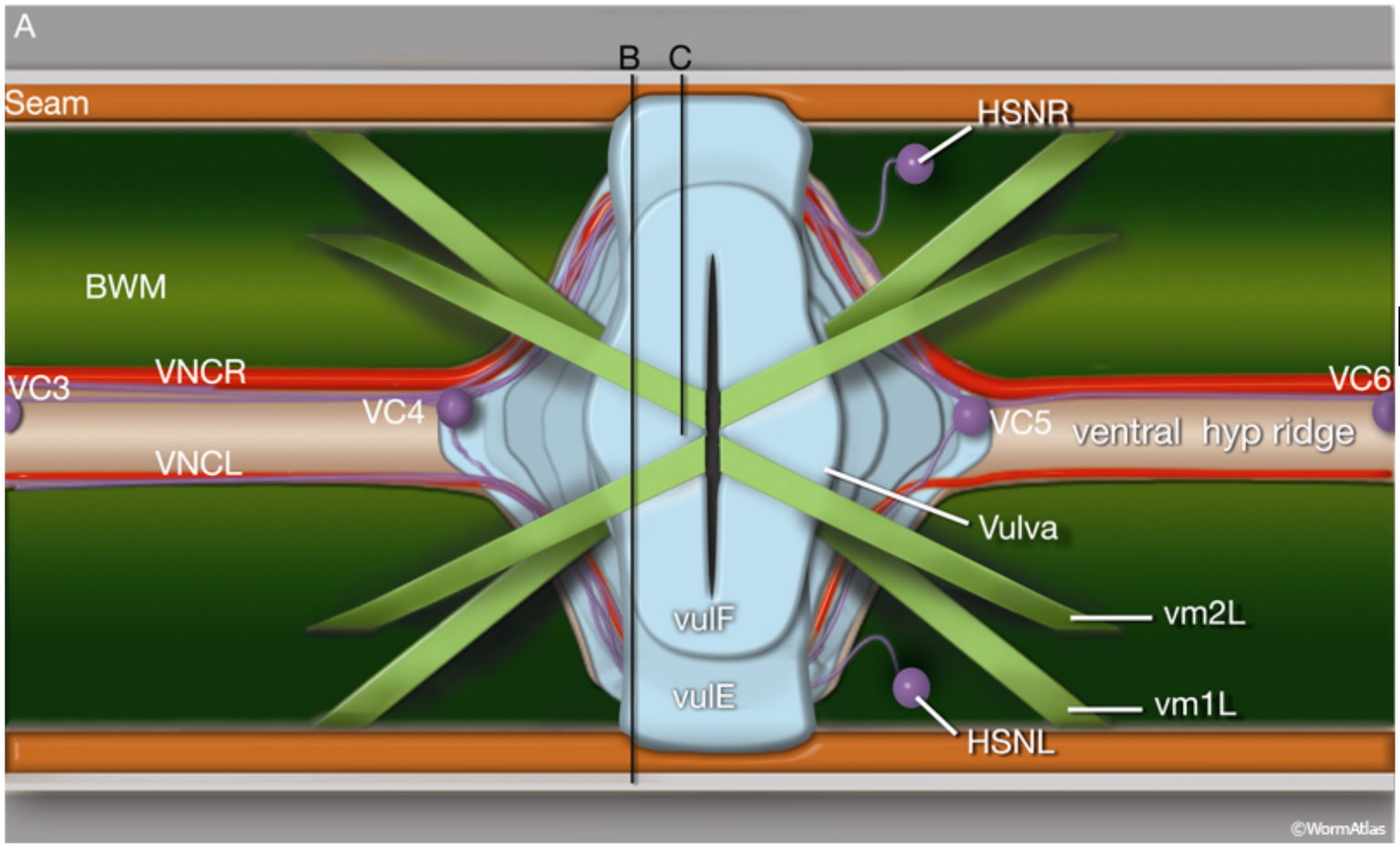
**No neuronal signaling**

**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

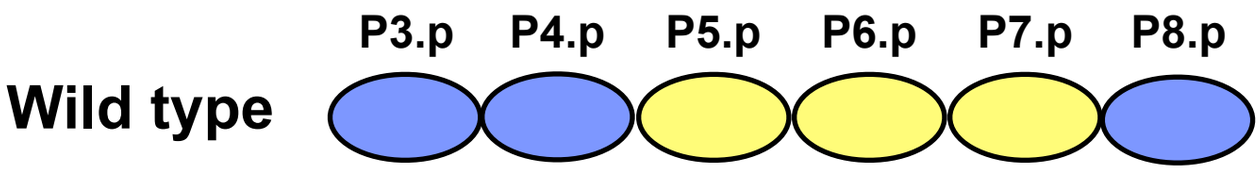
***C. elegans***

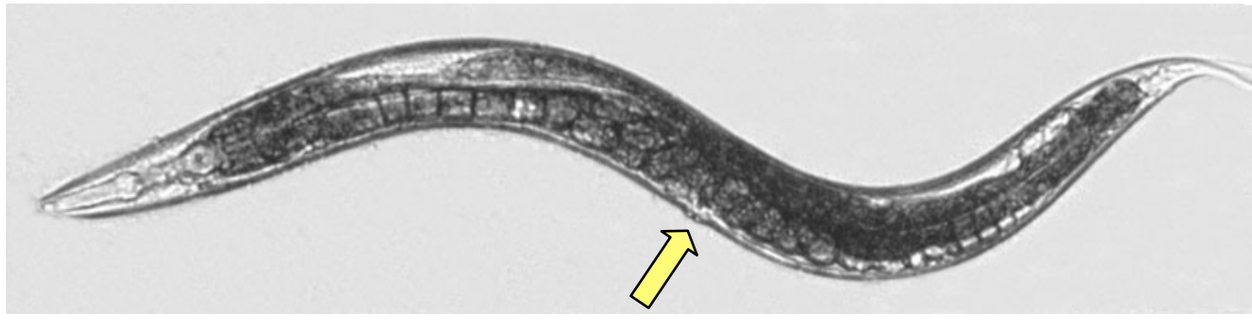
Sydney Brenner

H. Robert Horvitz

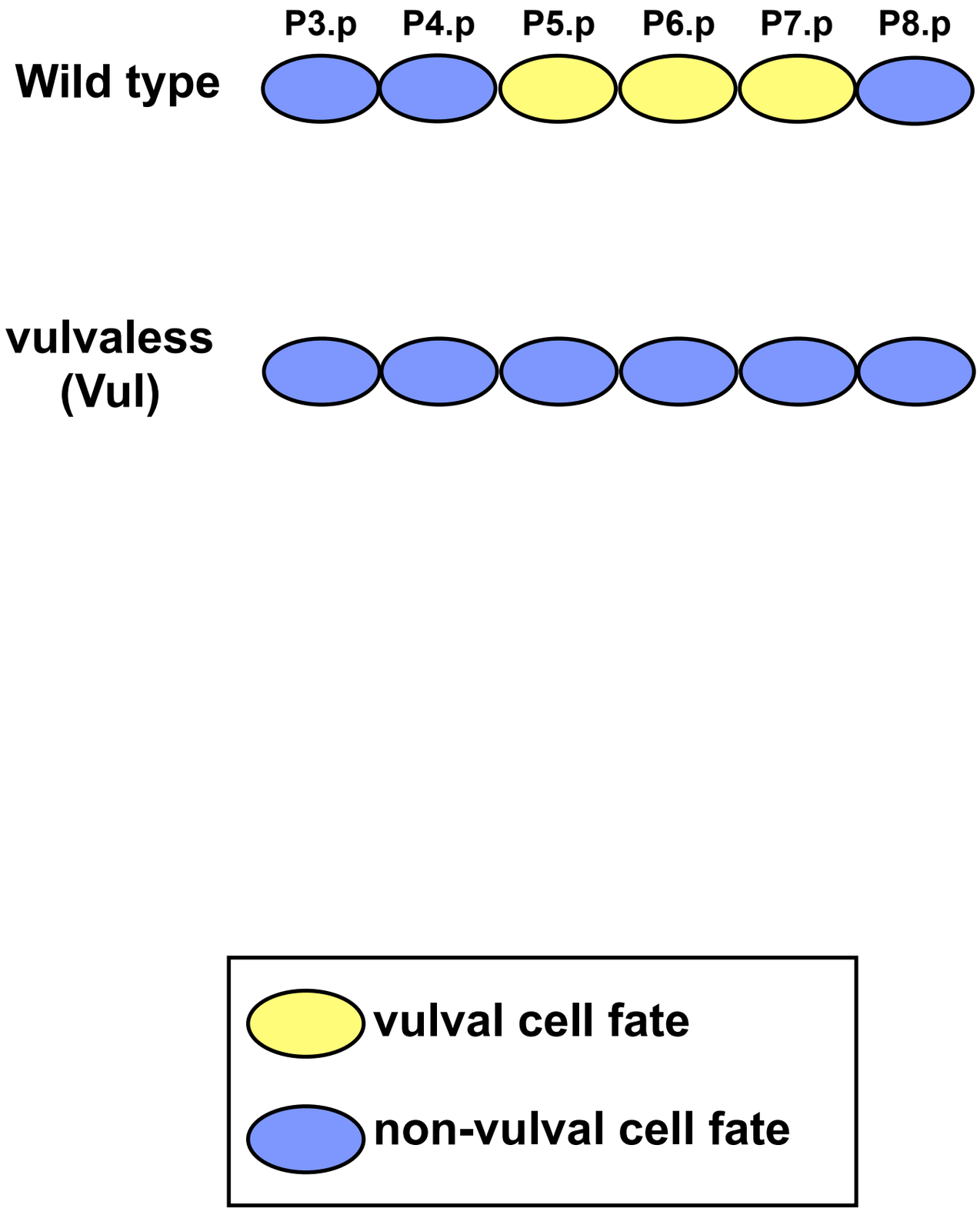
John E. Sulston

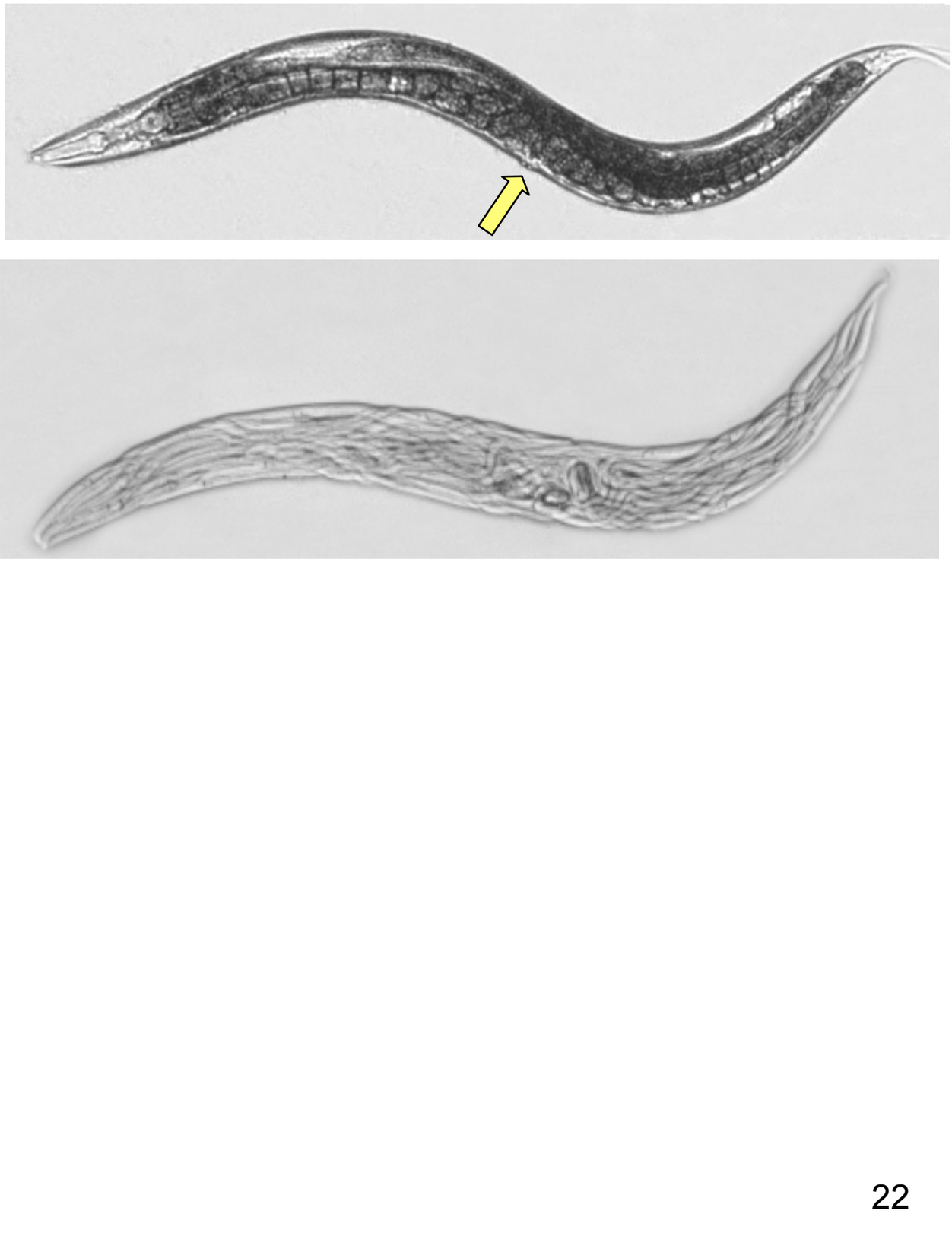
# Three cells express vulval fates in wild-type animals



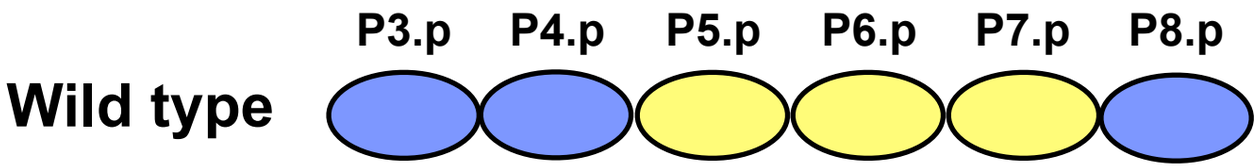



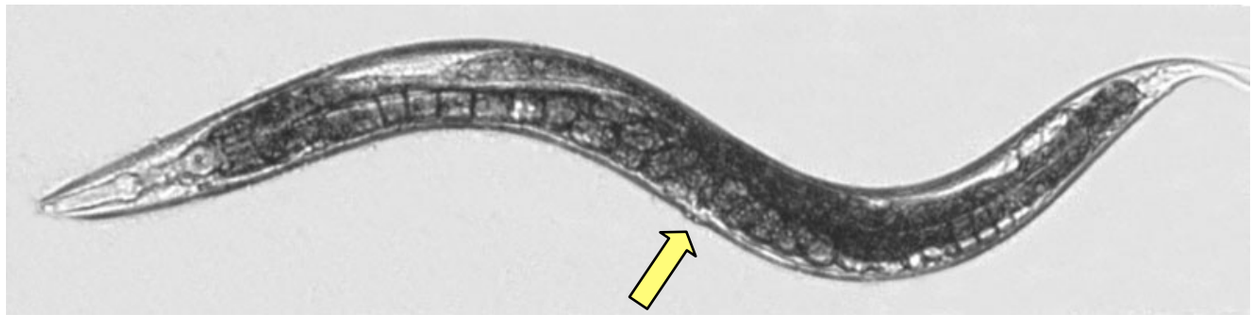
# No cells express vulval fates in vulvaless mutants

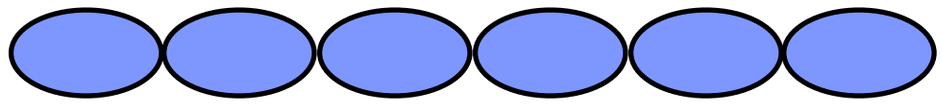


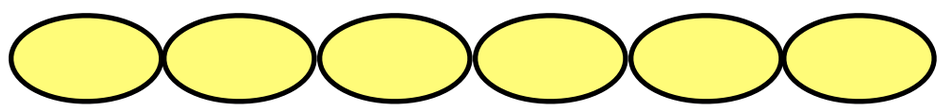



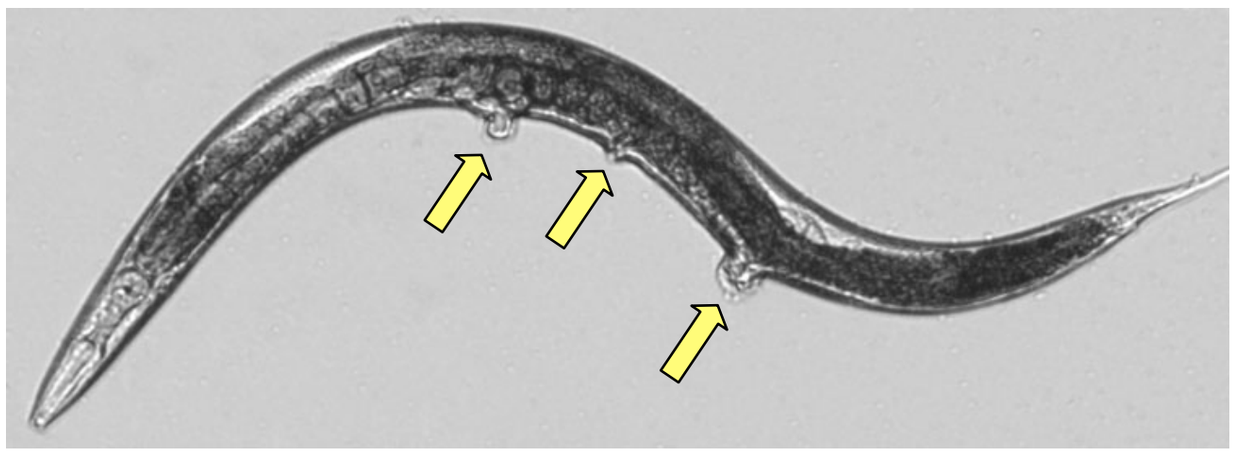
# Six cells express vulval fates in multivulva mutants

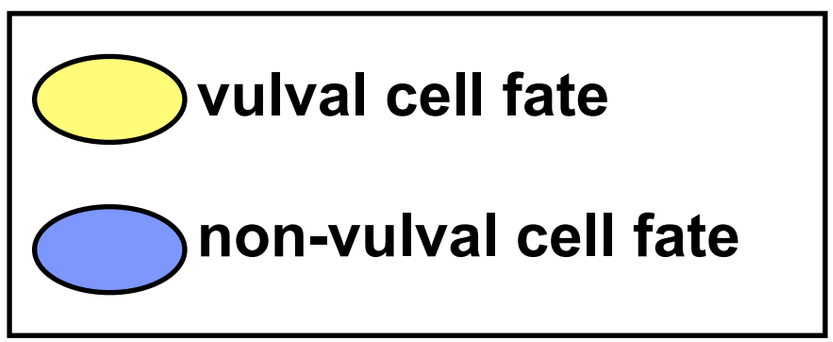






# Vulval mutants

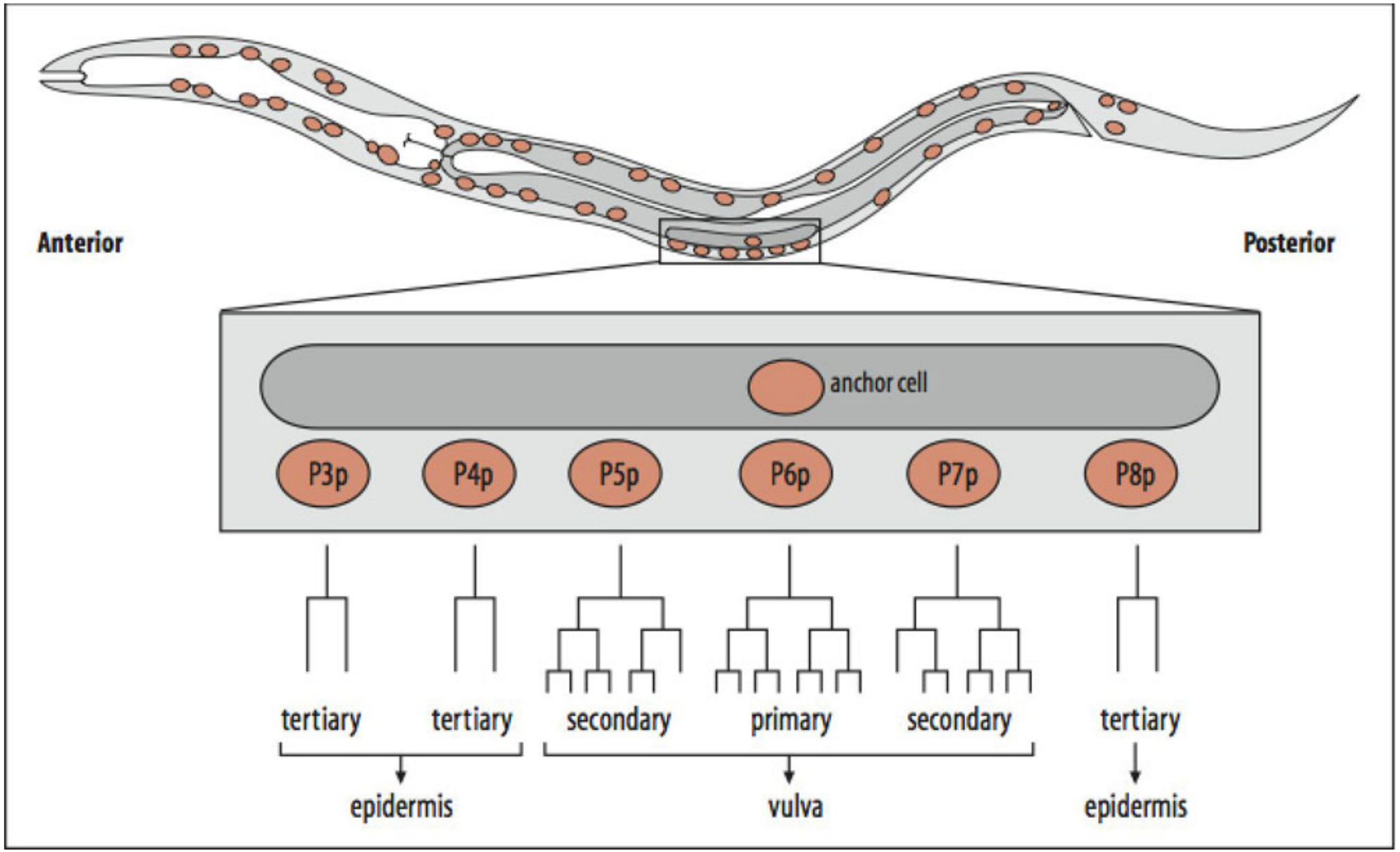
| Mutant | Phenotype |
|---|---|
| *lin-1(0)* | Muv |
| *lin-3(0)* | Vul |
| *let-60(0)* | Vul |
| *let-60(gf)* | Muv |
| *let-23(0)* | Vul |
| *let-23(gf)* | Muv |

# Double mutants defined the vulval pathway

| Mutant genotypes | Phenotype |
|---|---|
| *lin-1(0)* | Muv |
| *lin-3(0)* | Vul |
| *let-60(0)* | Vul |
| *let-60(gf)* | Muv |
| *let-23(0)* | Vul |
| *let-23(gf)* | Muv |
| *lin-3(0); let-23(gf)* | Muv |
| *lin-3(0); let-60(gf)* | Muv |
| *let-23(0); let-60(gf)* | Muv |
| *let-23(gf); let-60(0)* | Vul |
| *let-60(0); lin-1(0)* | Muv |
| *let-23(0); lin-1(0)* | Muv |

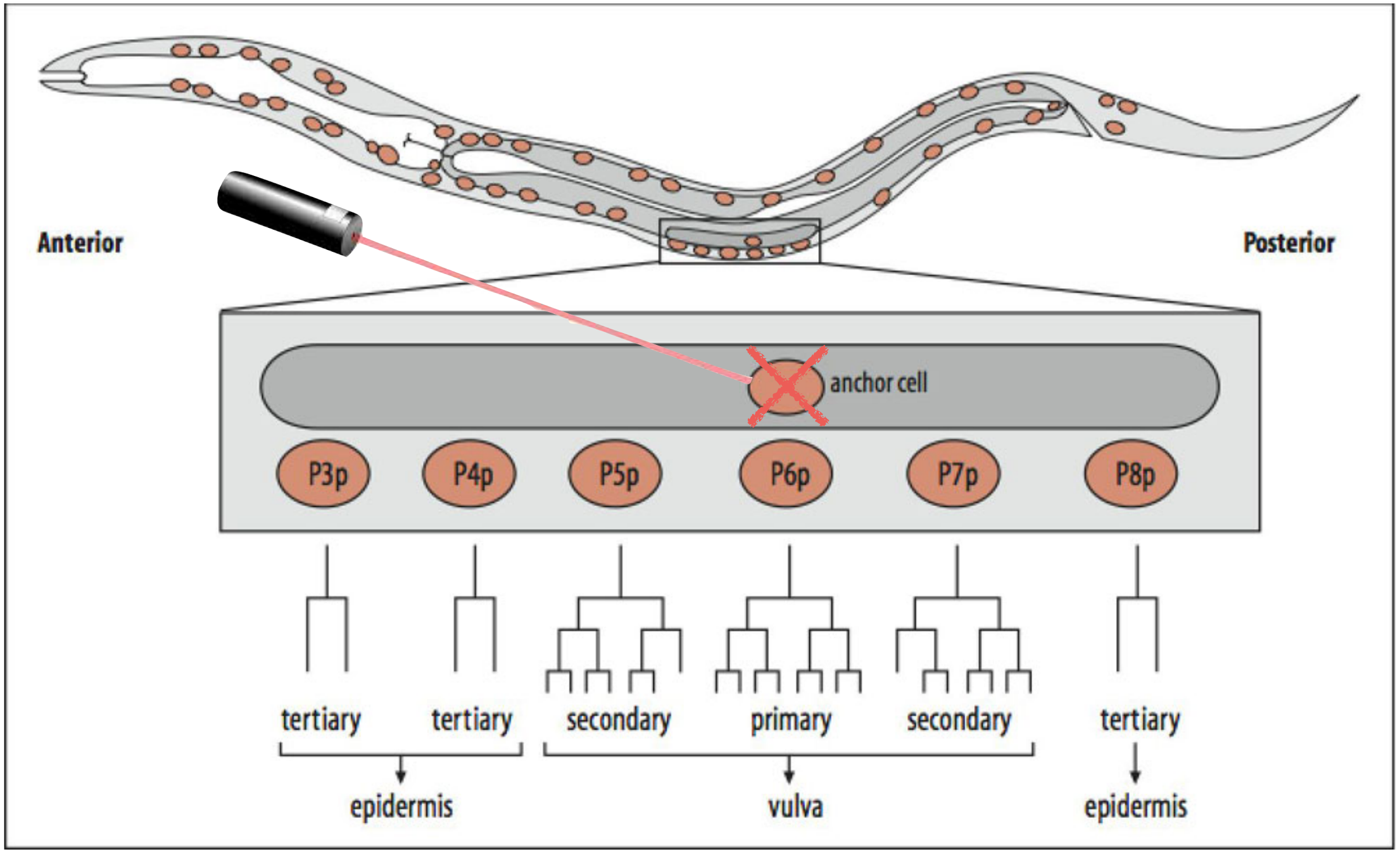
***lin-3*** → ***let-23*** → ***let-60*** ⊣ ***lin-1*** ⊣ **vulval fate**

# 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)

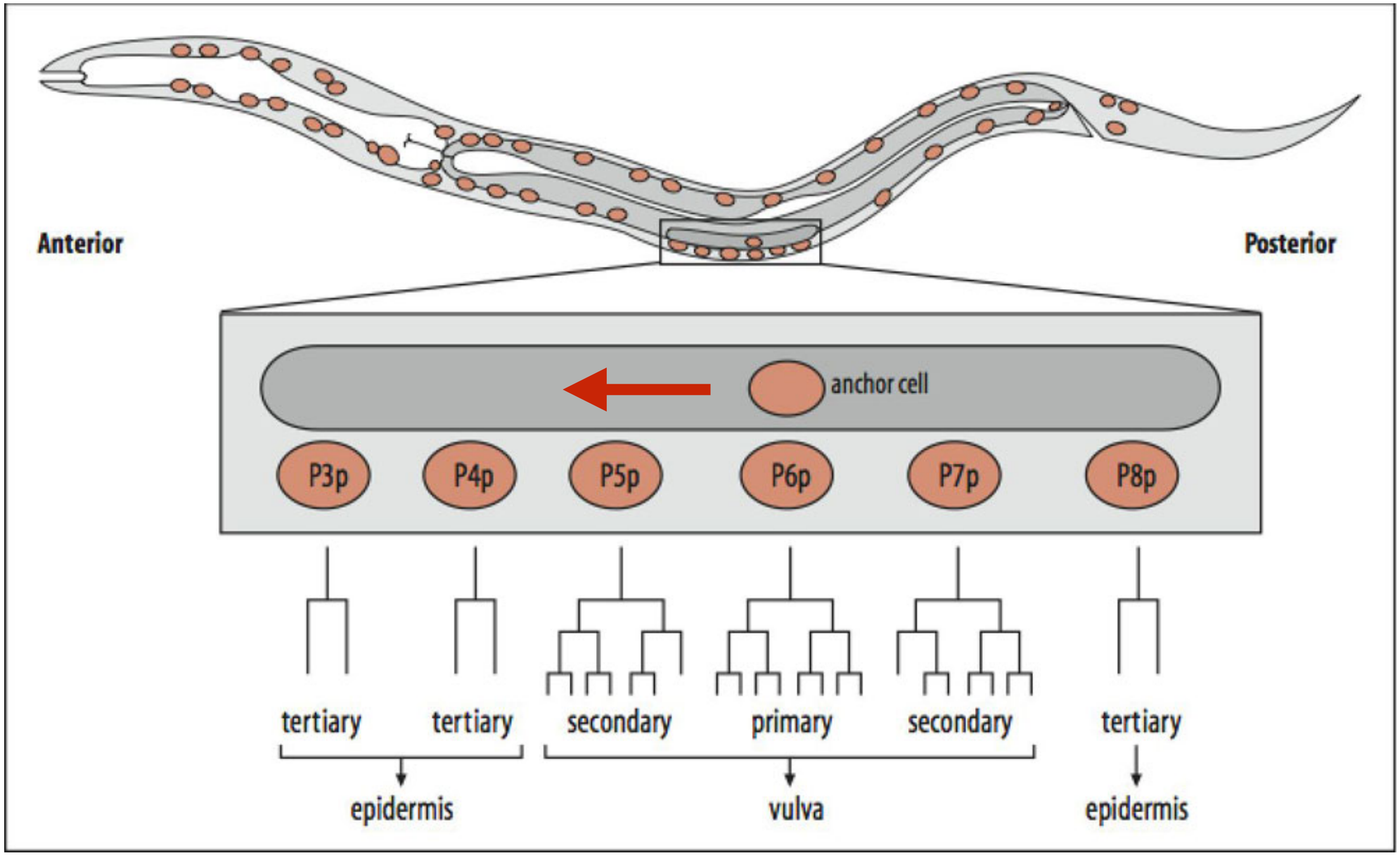



No AC leads to no vulval cell specification and a vulvaless phenotype

# All other vulval mutants are epistatic to AC ablation

| Mutant genotypes | Phenotype |
|---|---|
| AC ablation | Vul |
| *lin-1(0)* | Muv |
| *lin-3(0)* | Vul |
| *let-60(gf)* | Muv |
| *let-23(gf)* | Muv |
| AC ablation*; let-23(gf)* | Muv |
| AC ablation*; let-60(gf)* | Muv |
| AC ablation*; lin-1(0)* | Muv |

# Moving the AC moves the vulva (sufficient)

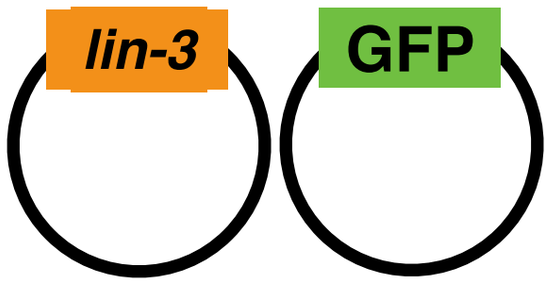


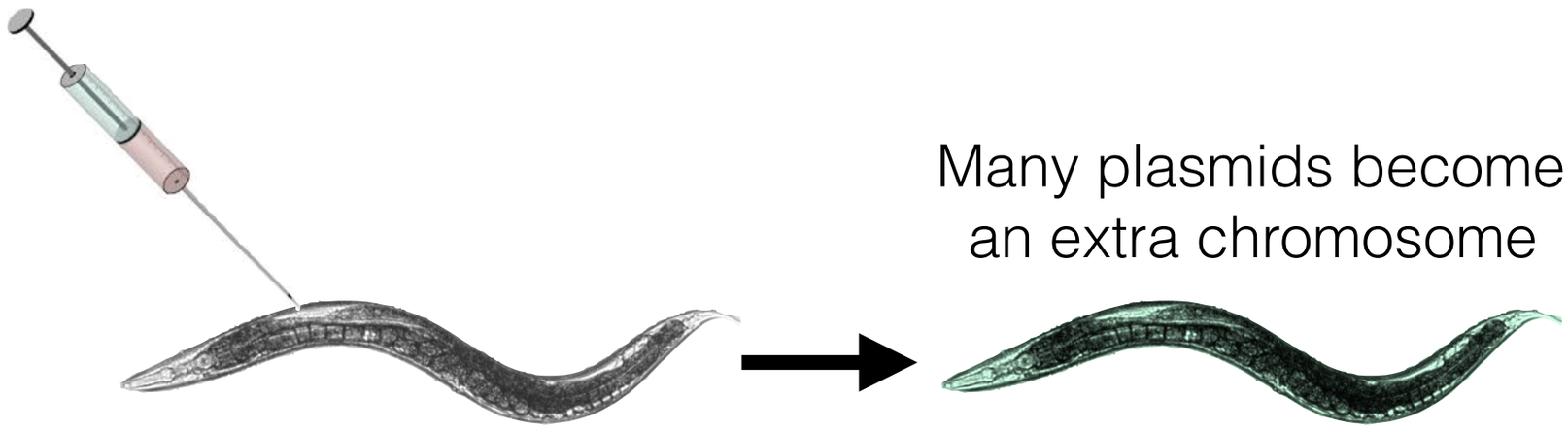

*dig-1* displaced gonad mutants

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

| Mutant genotypes | Phenotype |
|---|---|
| AC ablation | Vul |
| *lin-3(0)* | Vul |
| *lin-1(0)* | Muv |
| *let-60(gf)* | Muv |
| *let-23(gf)* | Muv |
| AC ablation*; let-23(gf)* | Muv |
| AC ablation*; let-60(gf)* | Muv |
| AC ablation*; lin-1(0)* | Muv |
| *lin-3(0); let-23(gf)* | Muv |
| *lin-3(0); let-60(gf)* | Muv |
| *lin-3(0); lin-1(0)* | Muv |

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



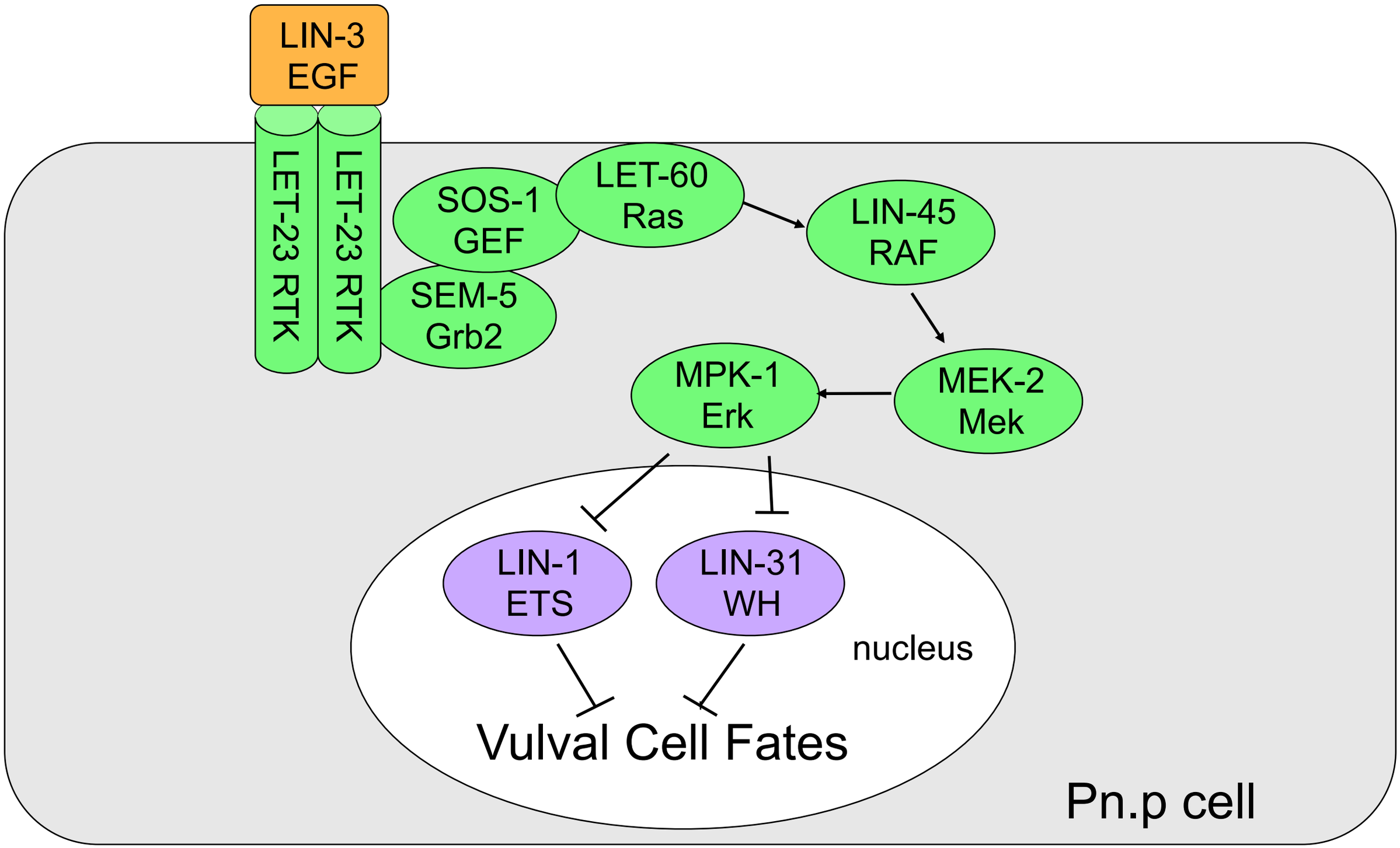



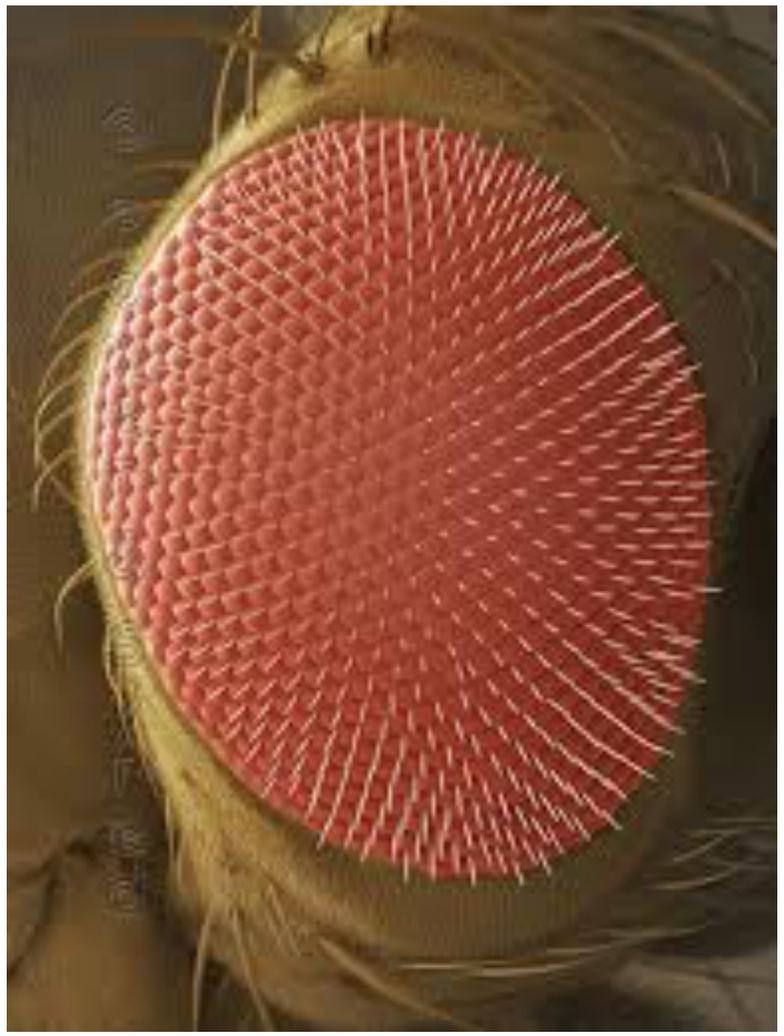

*lin-3(0)*
Vul

*lin-3(0); lin-3(+++)*
Wild-type or Muv

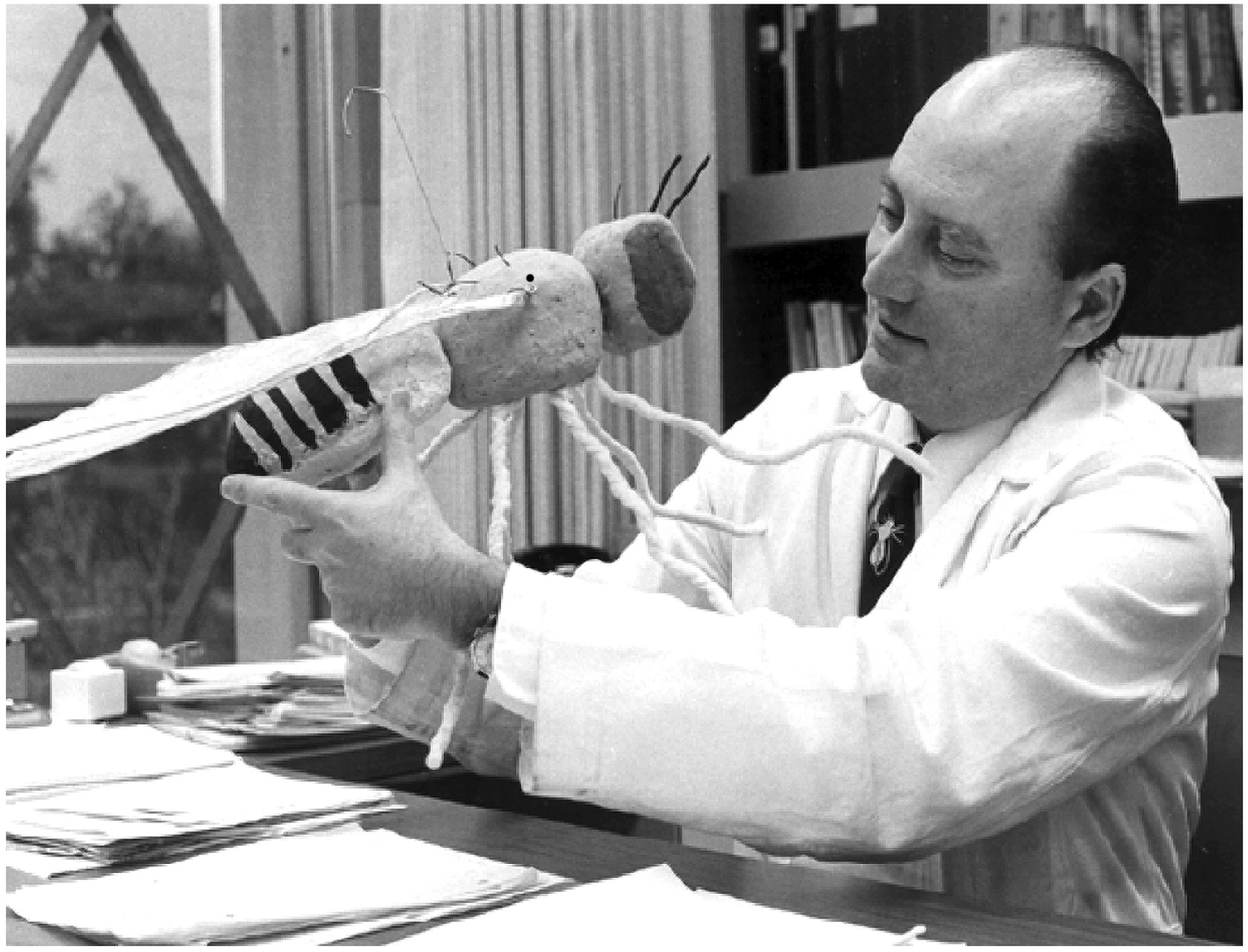
| Genotype | Phenotype | |
|---|---|---|
| *lin-3(0)* | Vul | |
| *lin-3(0); lin-3(+++)* | Wild-type | |
| *lin-3(0); vulval cell:lin-3(+++)* | Vul | Expression of *lin-3* in vulval cells |
| *lin-3(0); intestine:lin-3(+++)* | Vul | Expression of *lin-3* in the intestine |
| *lin-3(0); neurons:lin-3(+++)* | Vul | Expression of *lin-3* in the neurons |
| *lin-3(0); AC:lin-3(+++)* | Wild-type | Expression of *lin-3* in the AC |

# A Ras pathway promotes vulval fates

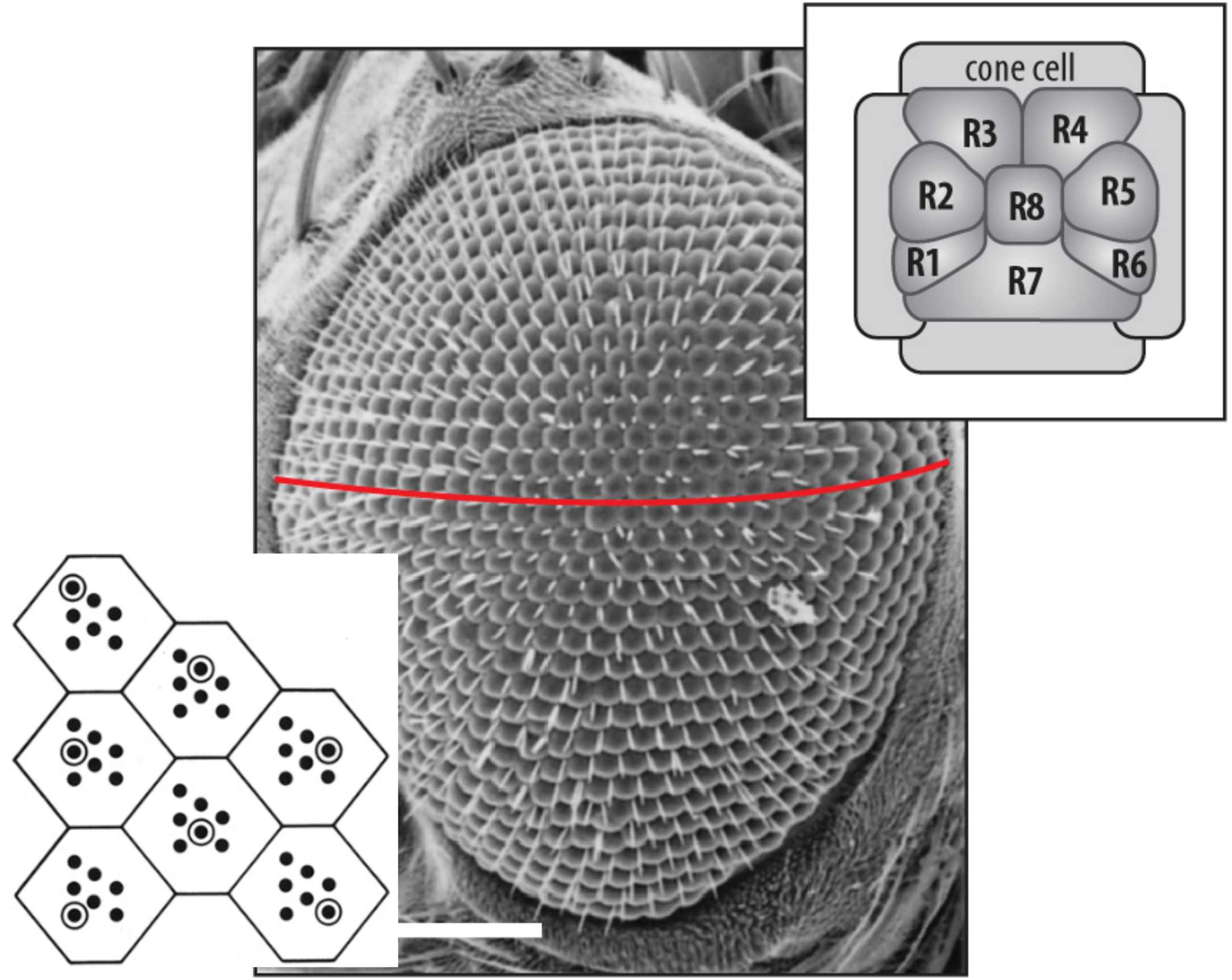




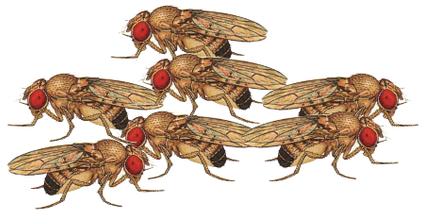
# The *Drosophila* eye



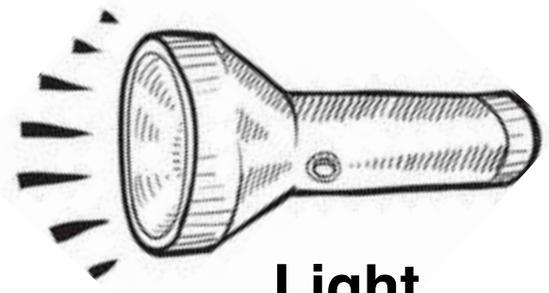
**Seymour Benzer**


cone cell
R3
R4
R2
R8
R5
R1
R7
R6

# A simple behavioral selection

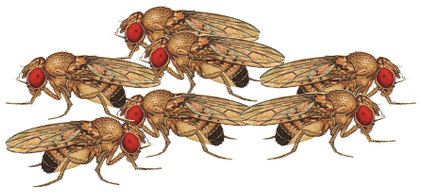


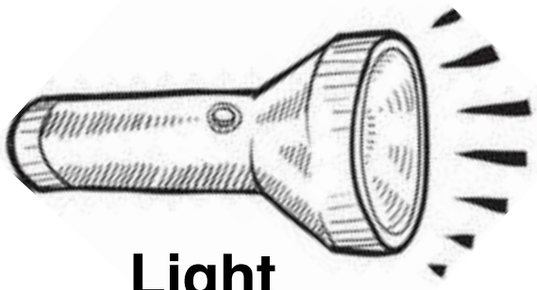
Dark



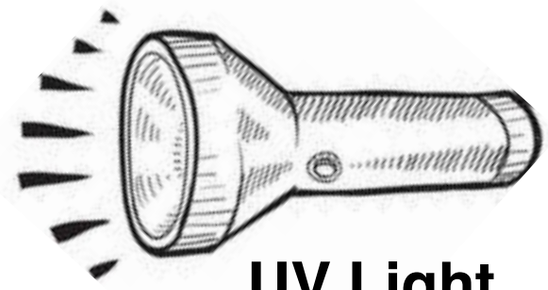
Light

# A simple behavioral selection



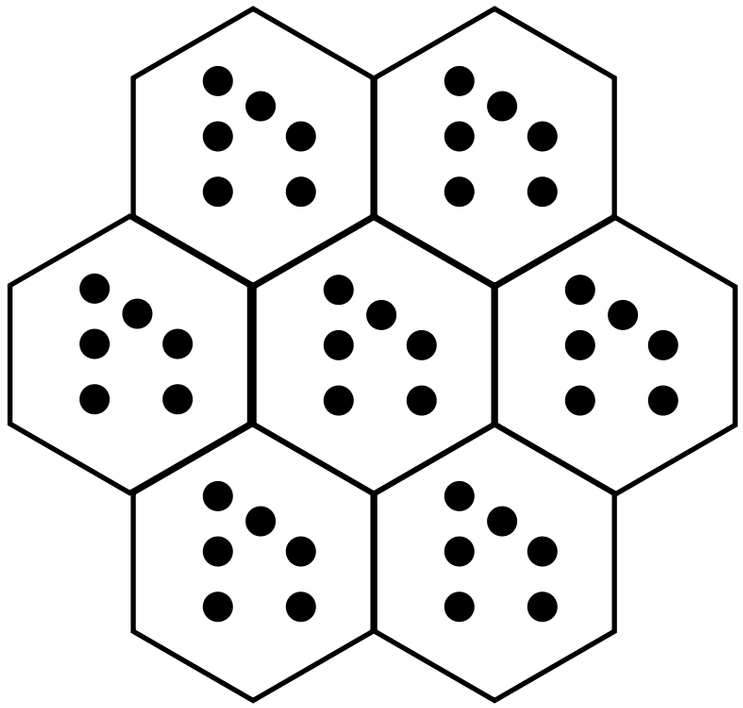


Light

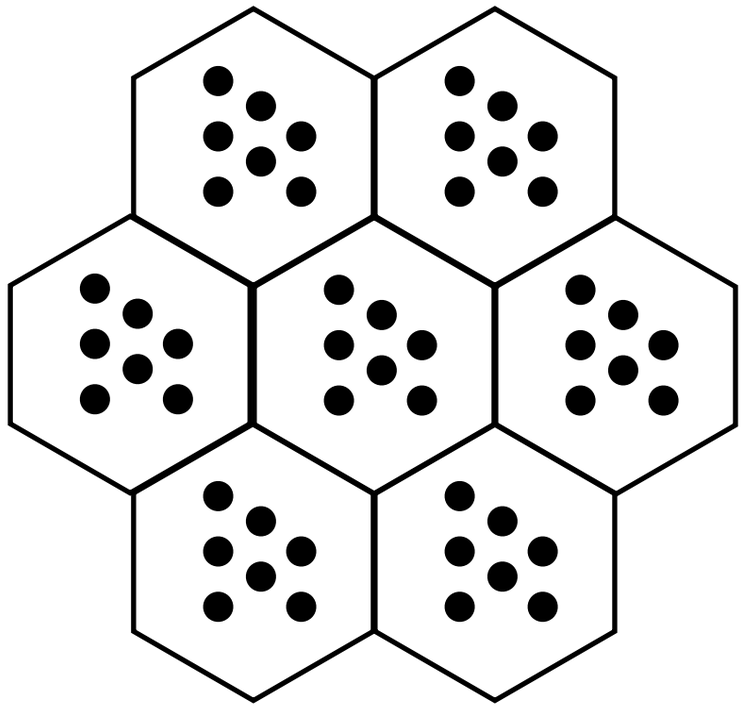


UV Light

**s*evenless* (UV blind)**



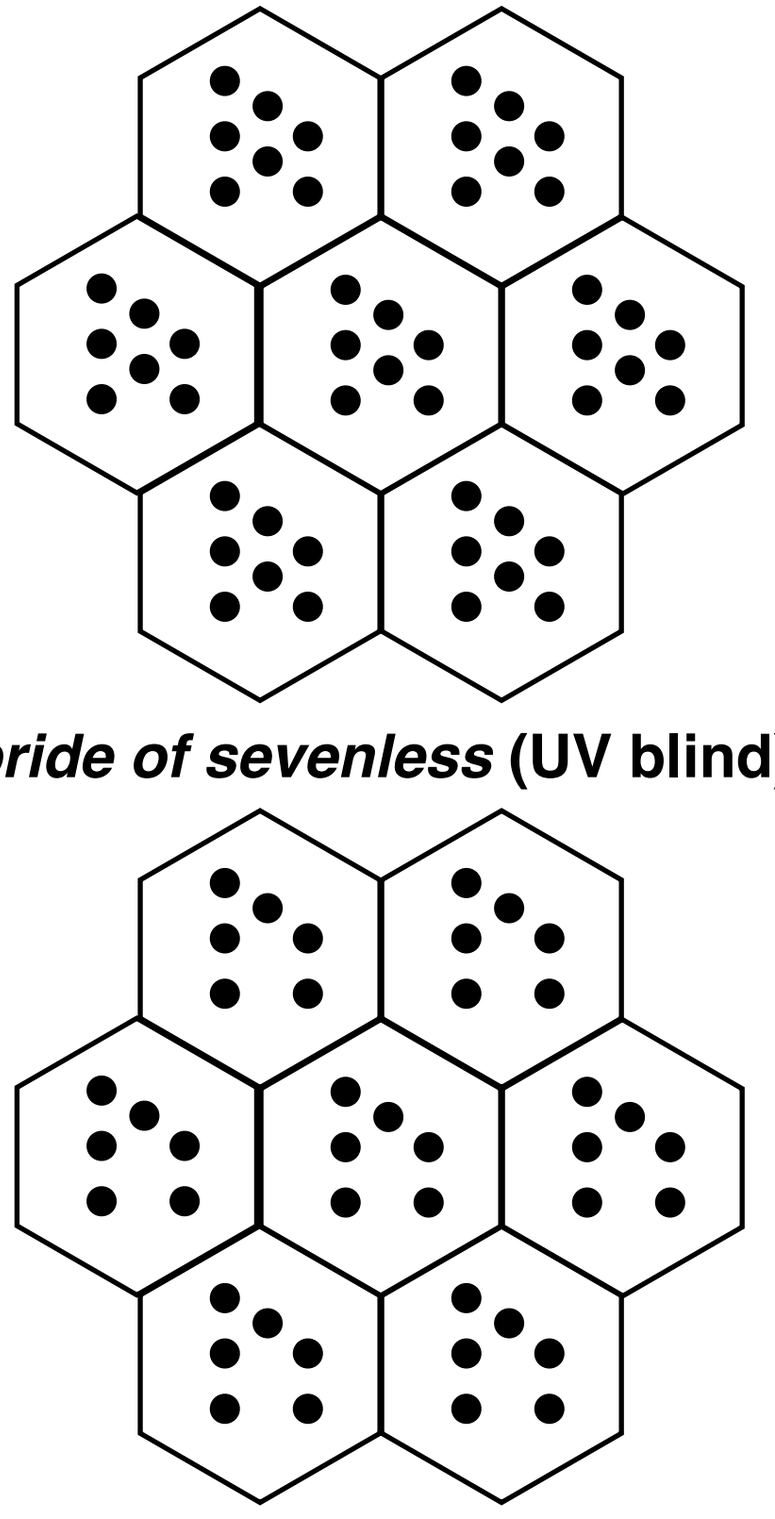
**Wild-type**



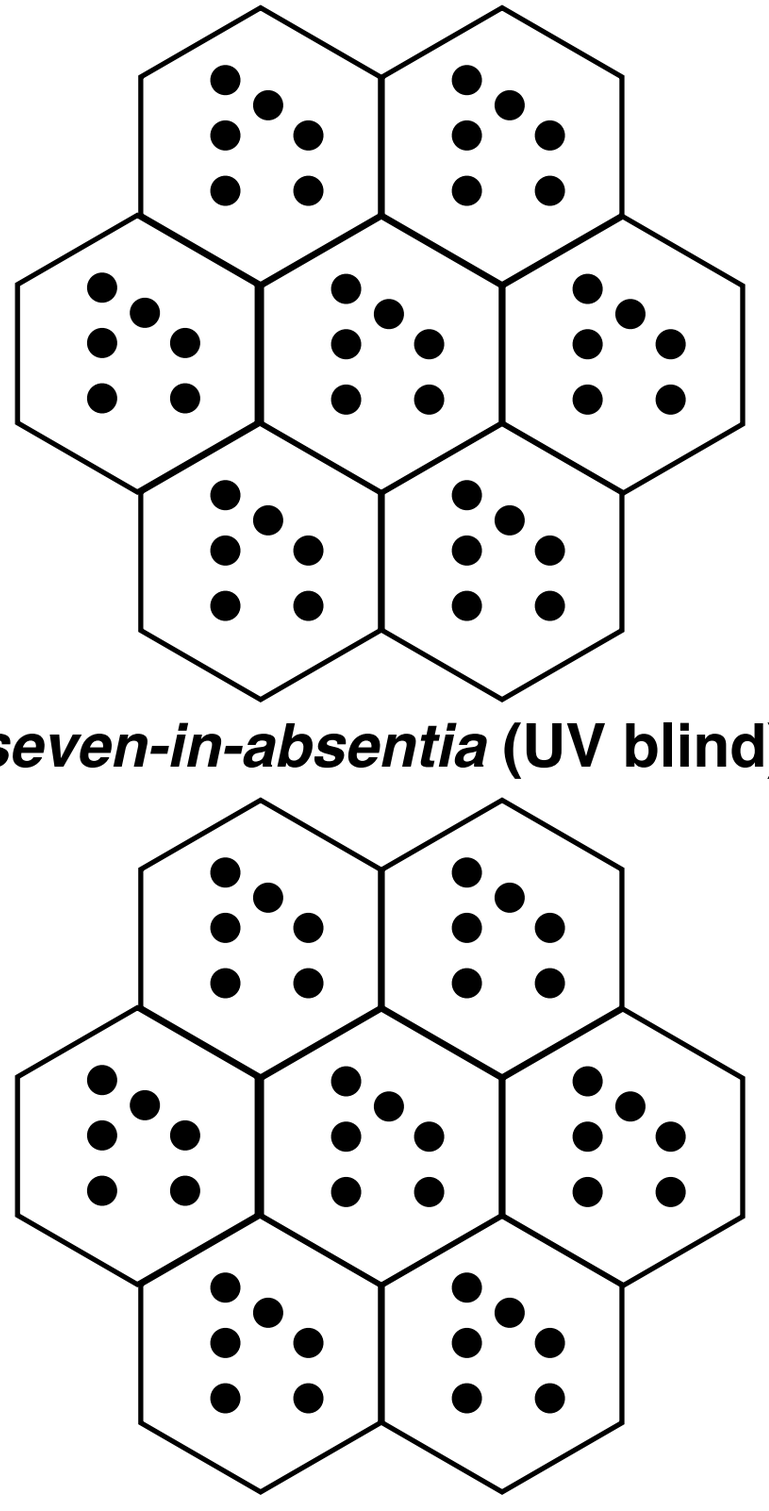
**Gerry Rubin**

# Other UV blind mutants

**Wild-type**



***sevenless* (UV blind)**

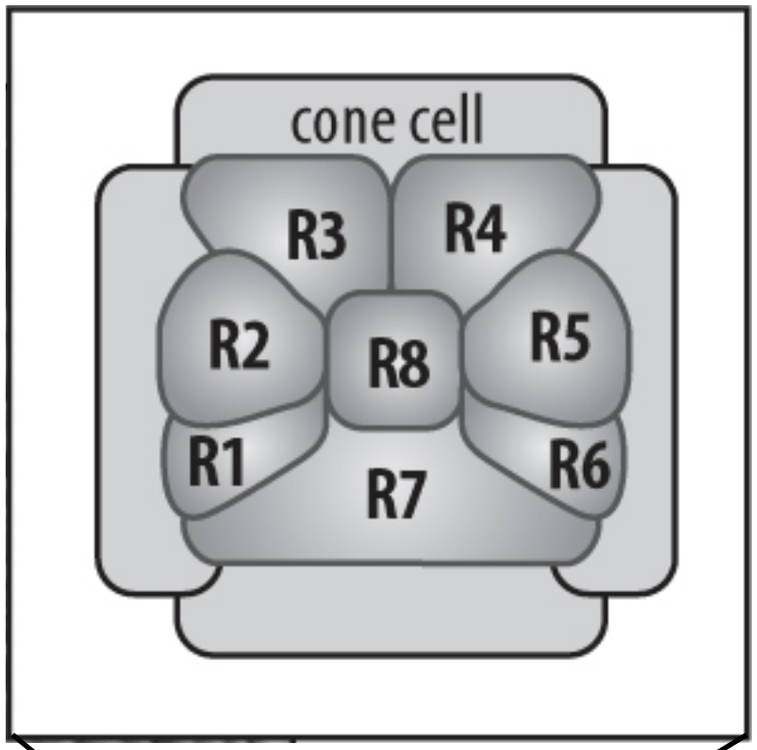


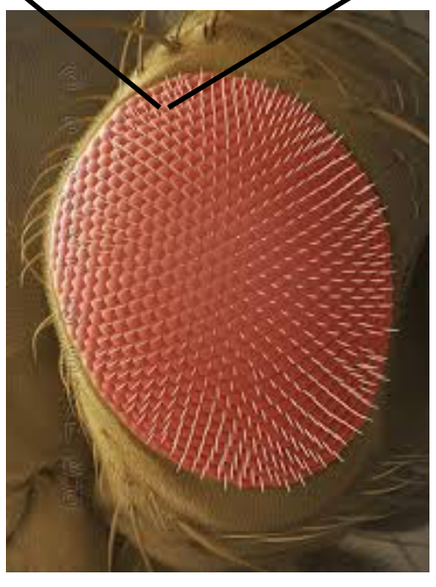
***bride of sevenless* (UV blind)**



***seven-in-absentia* (UV blind)**

# Development of an ommatidium



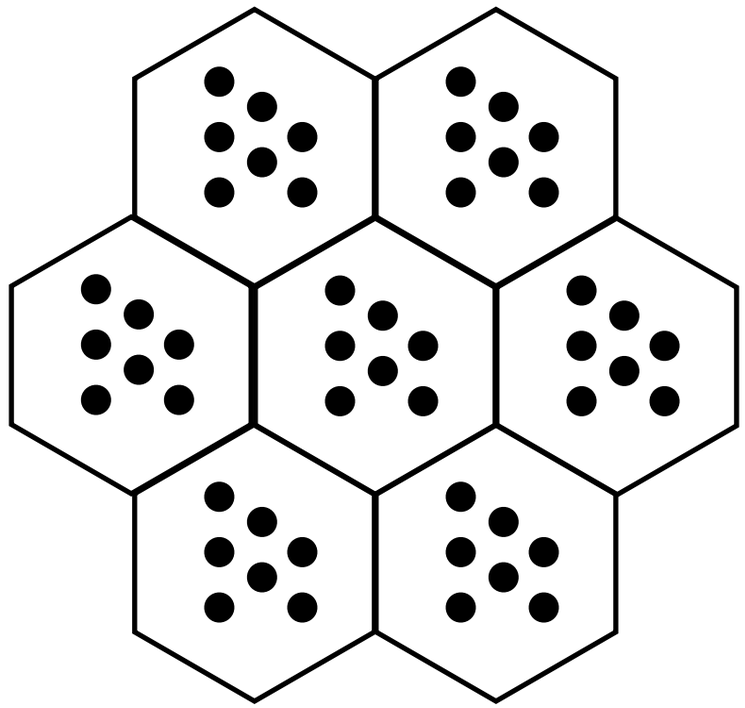
R8 born first

R1-R6 born after

R7 last to be born

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

**Wild-type**



Function in R7 is considered cell autonomous

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

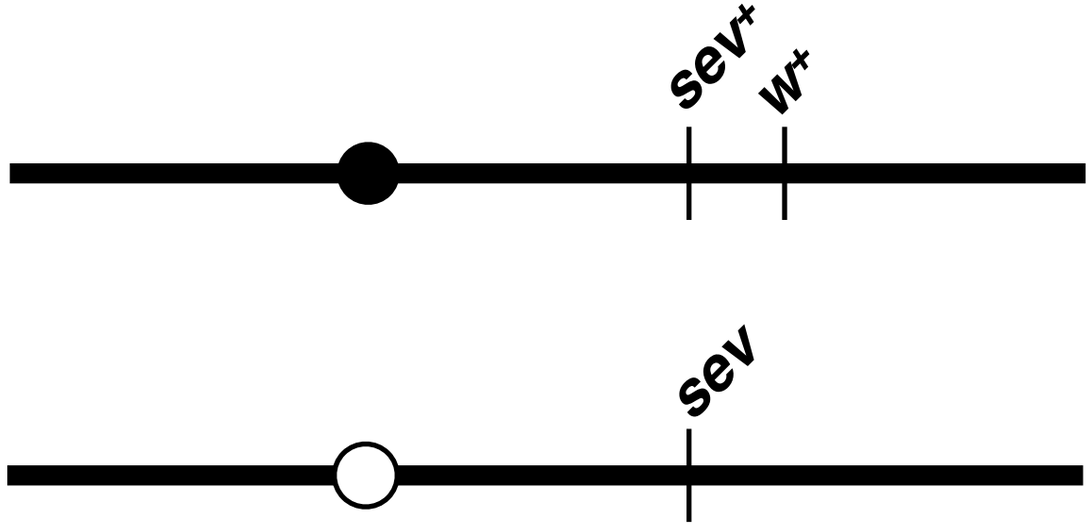
# Are genes required in ommatidia for cell viability?

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

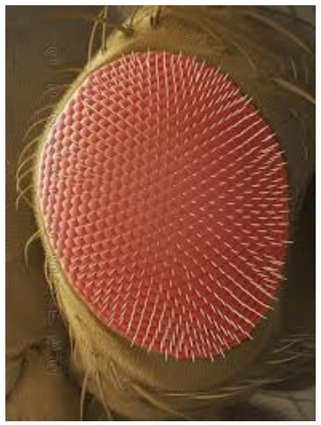
1. 

$$\frac{sev}{+} \; ; \; \frac{w}{w}$$

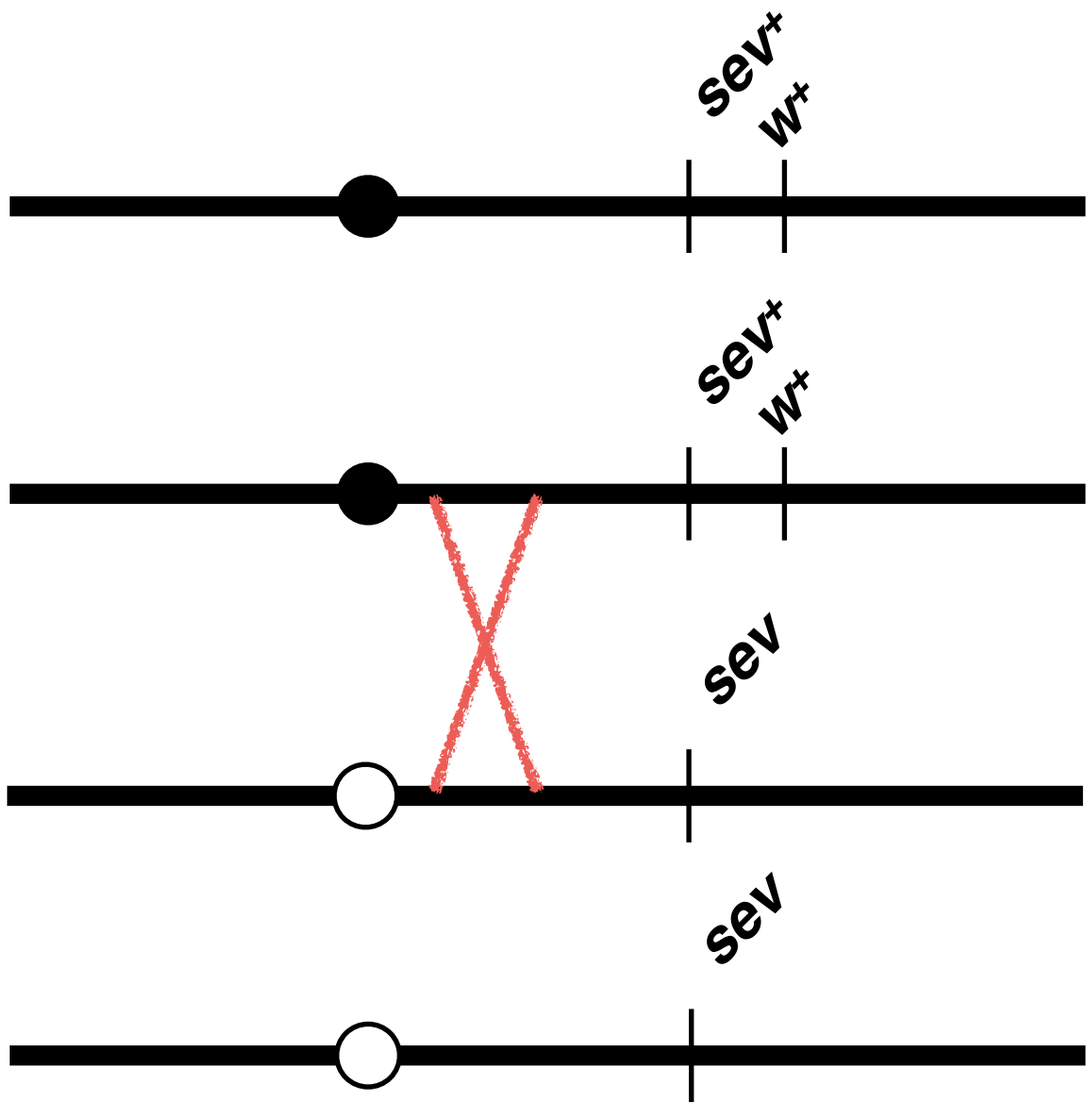
2. Use a P element with $w^+$ distal to the wild-type *sev* gene



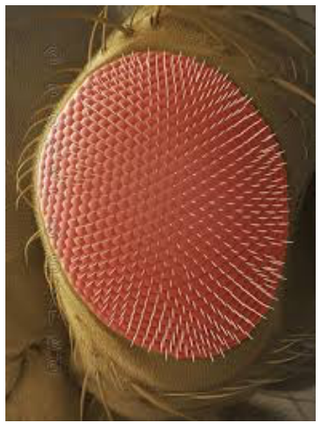
# Are genes required in ommatidia for cell viability?



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?



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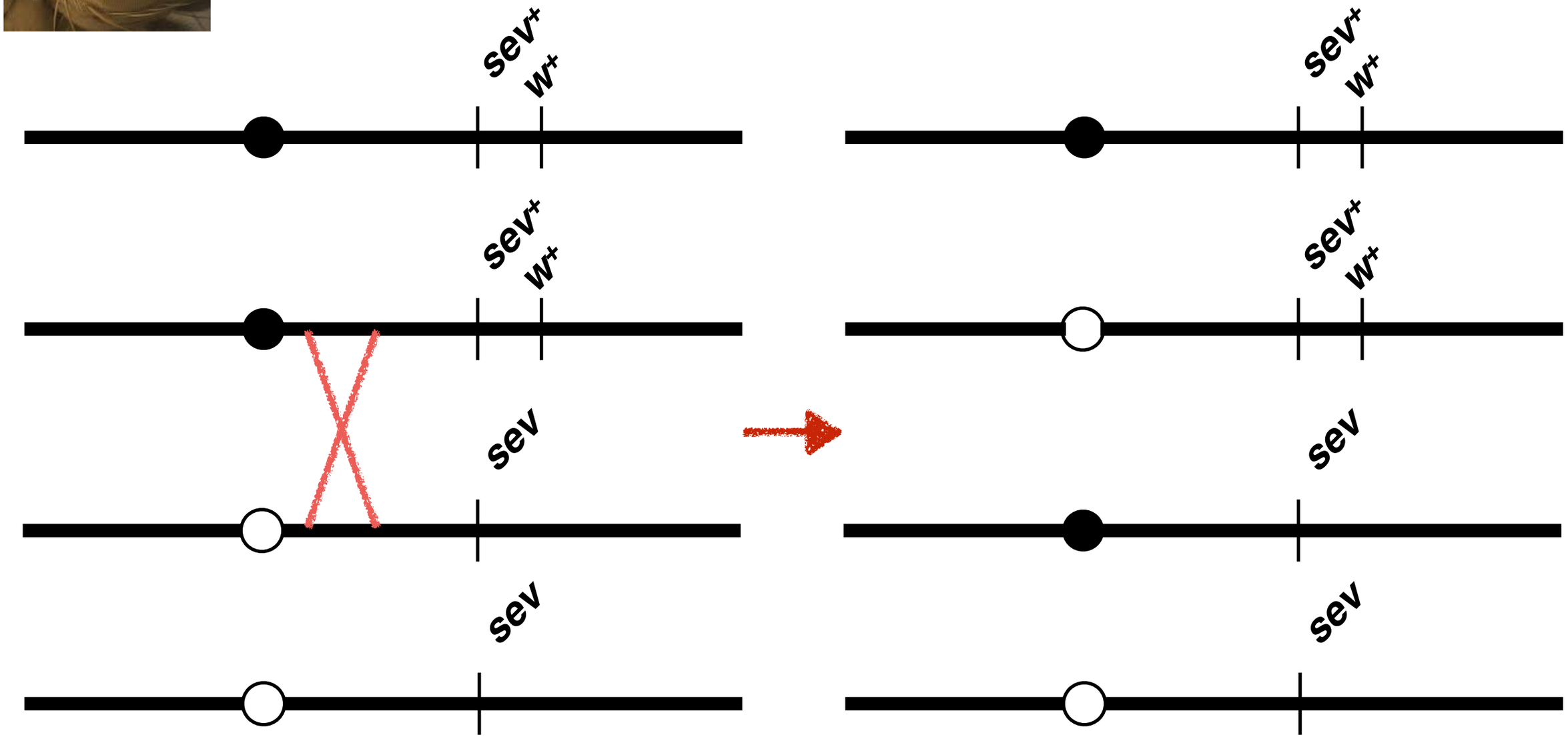
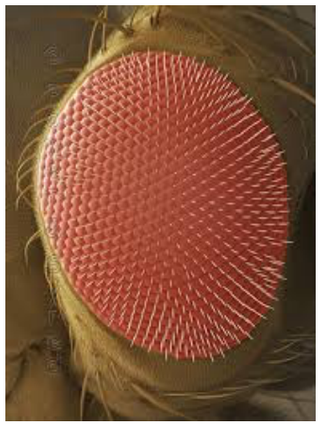
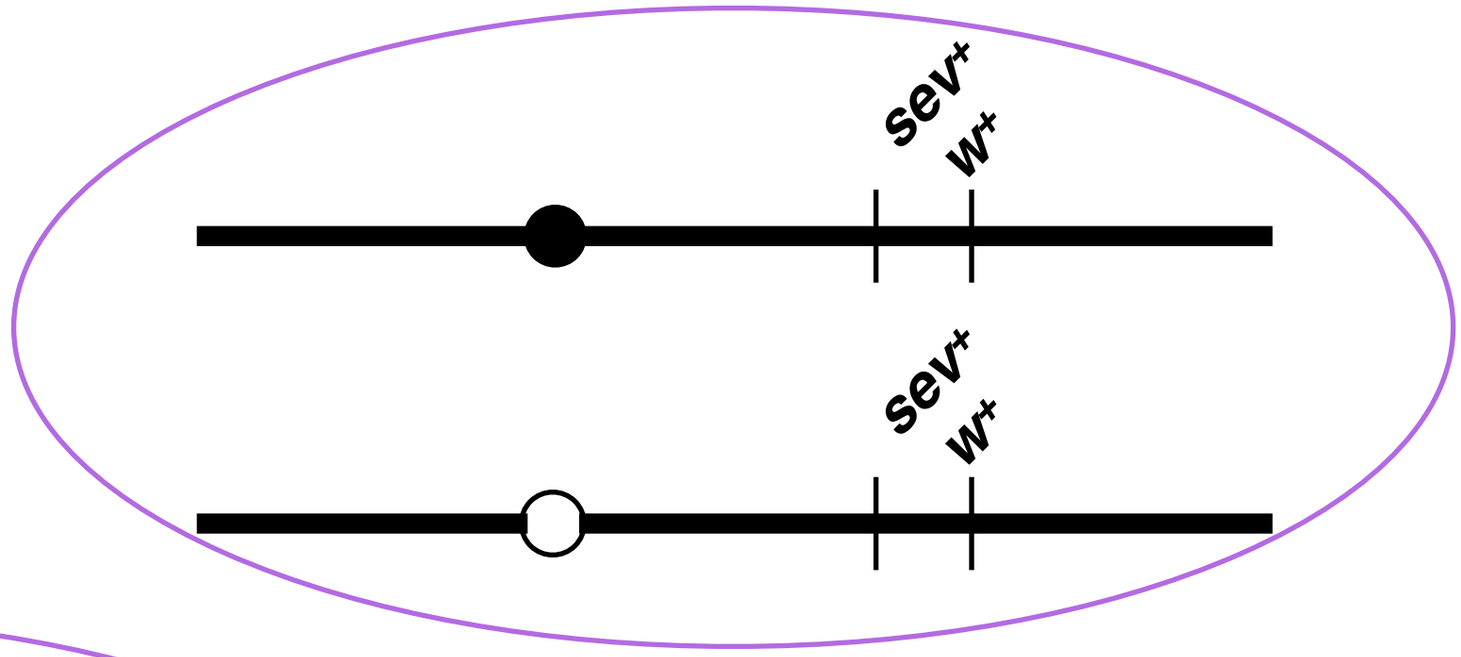


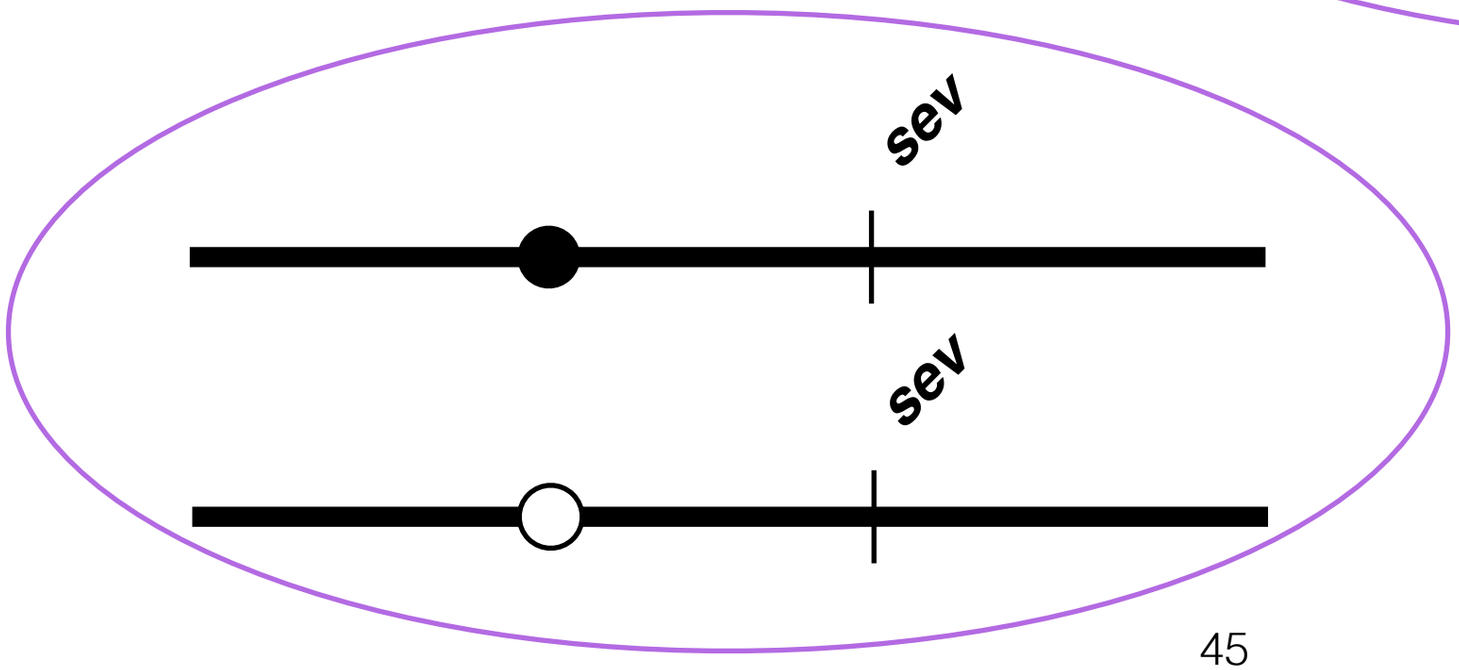
# Are genes required in ommatidia for cell viability?



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

*sev* WT and pigment

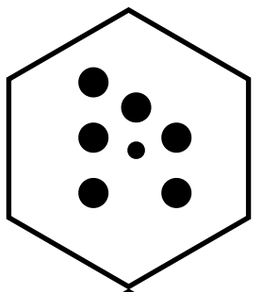


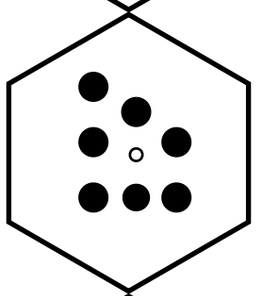

*sev* mutant
and no pigment

**Twin spots!**

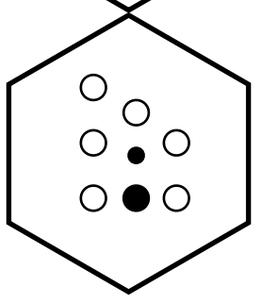
# *sev* acts cell autonomously to regulate the R7 fate



R7 cell *white* and *sev* mutant

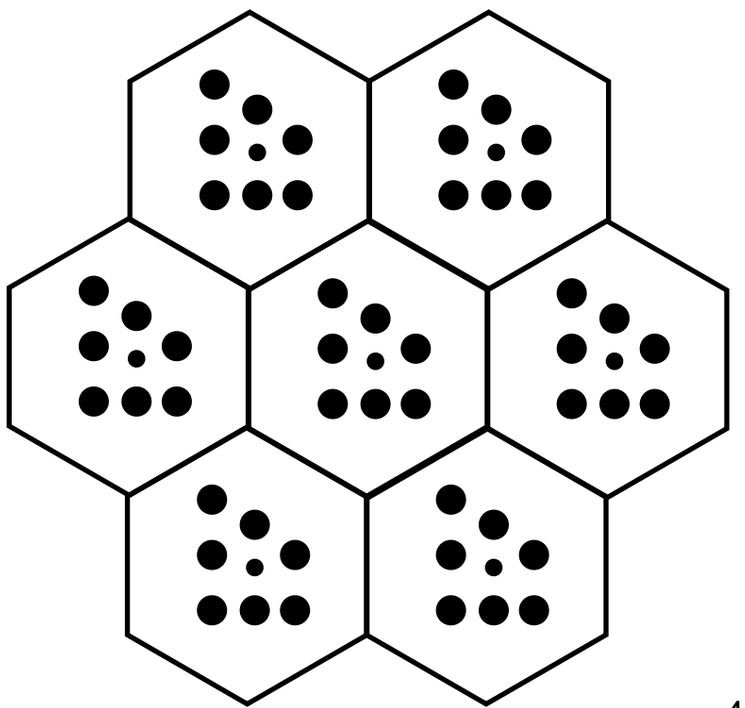


R8 cell *white* and *sev* mutant

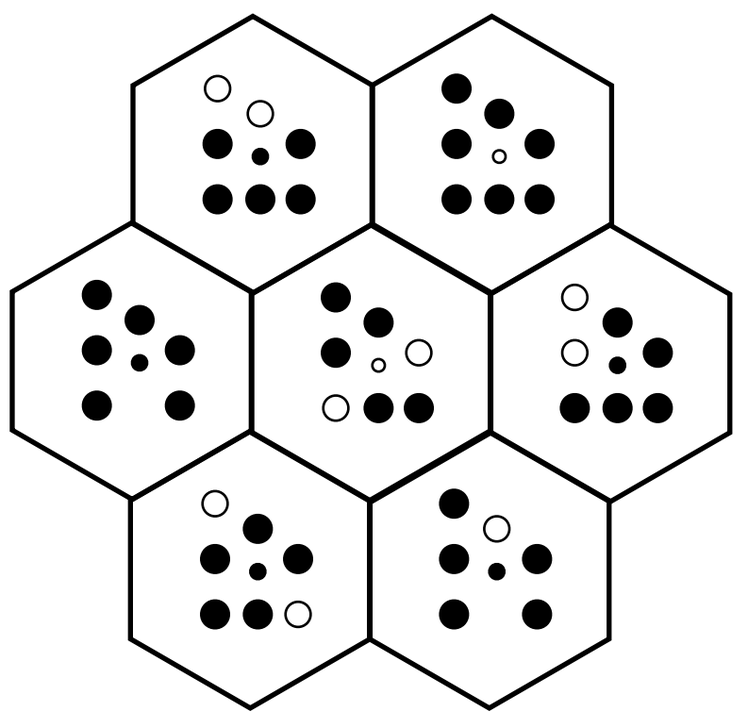


R1-R6 cells *white* and *sev* mutant

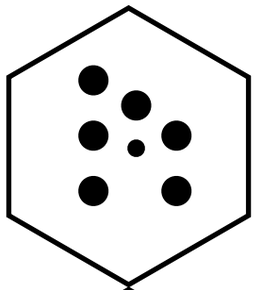
Wild-type



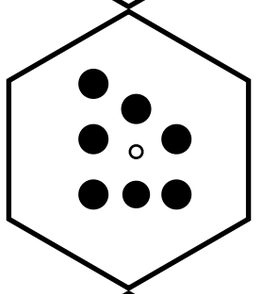
*sev* mutant



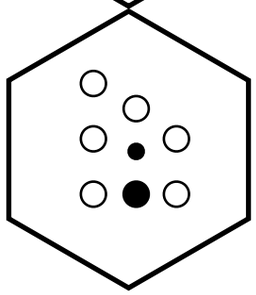
# *boss* and *sev* are required in different R cells for R7 fate



R7 cell *white* and *sev* mutant

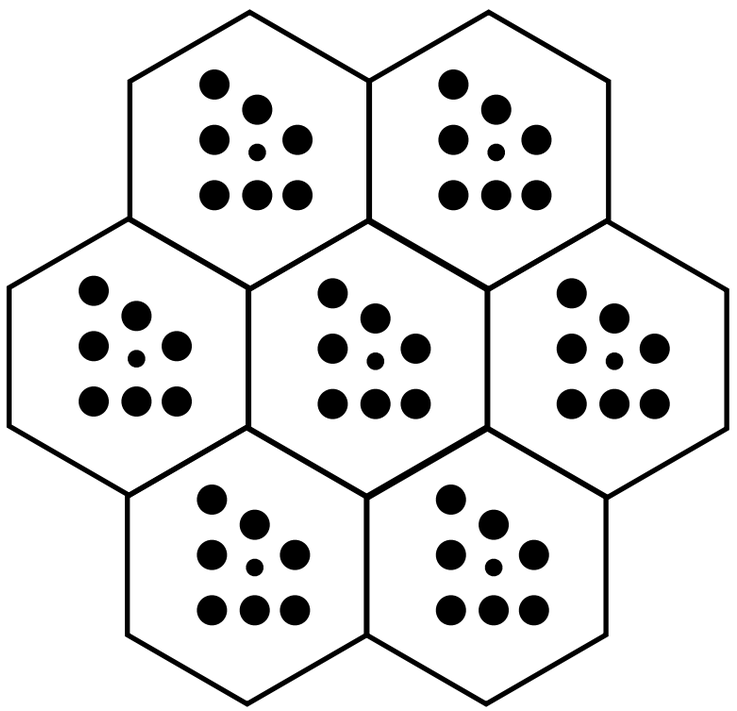


R8 cell *white* and *sev* mutant

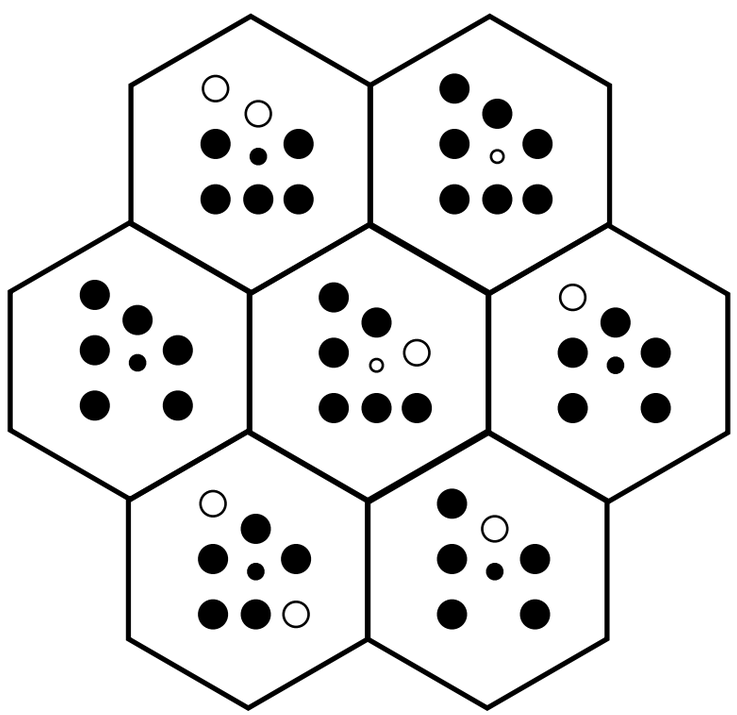


R1-R6 cells *white* and *sev* mutant

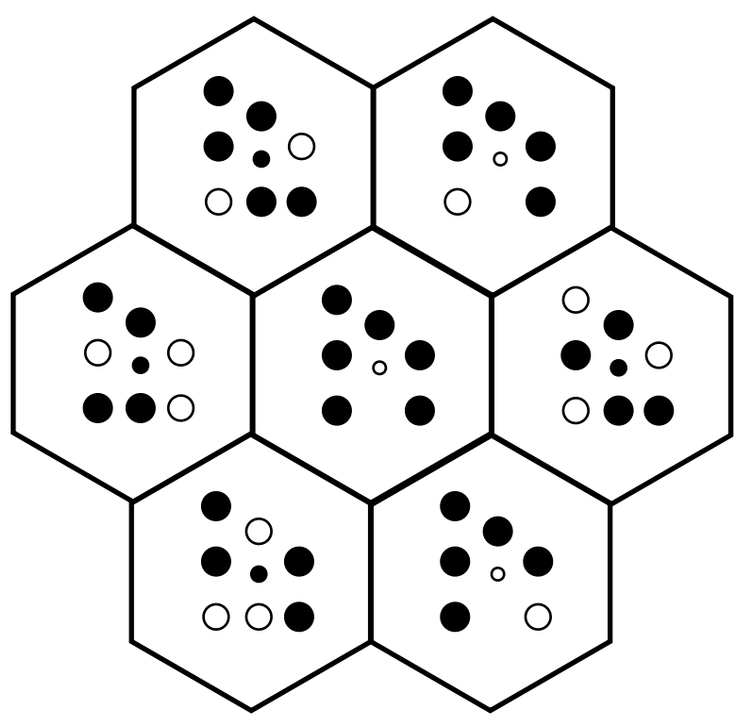
Wild-type



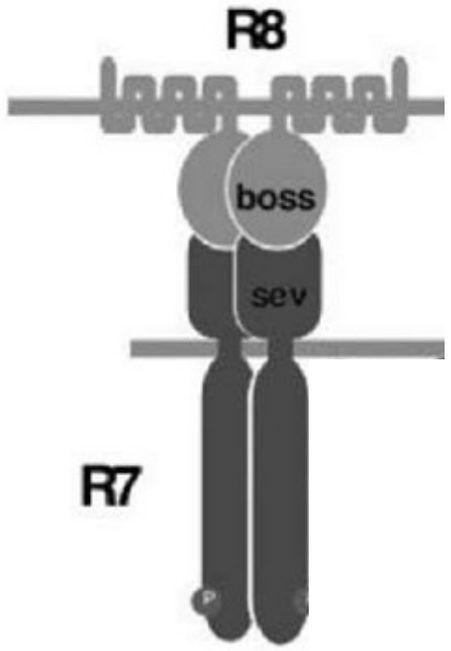
*sev* mutant



*boss* mutant

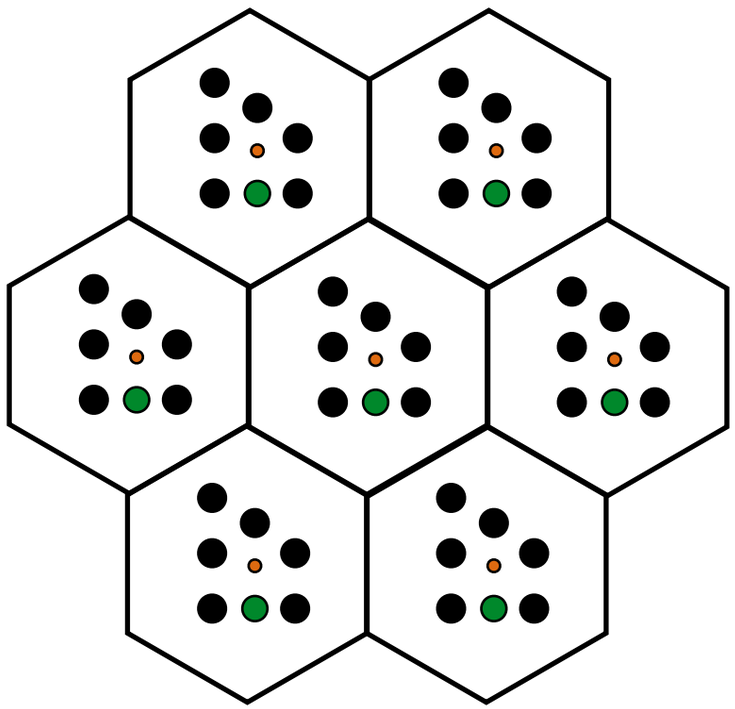


*boss* encodes
a membrane-bound protein



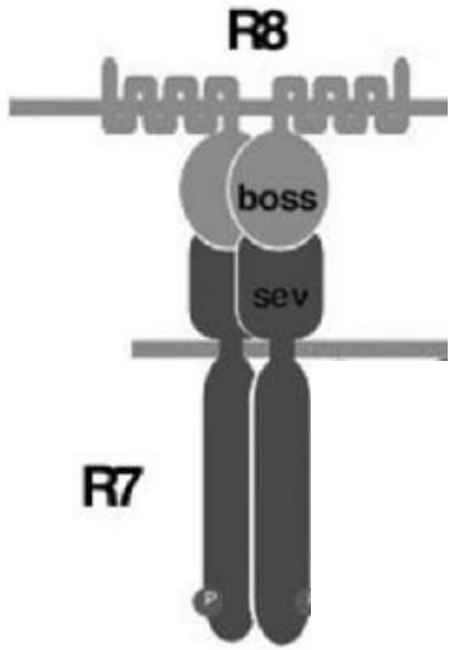

*sev* encodes
a membrane receptor tyrosine kinase

# Expression of *sev* and *boss*



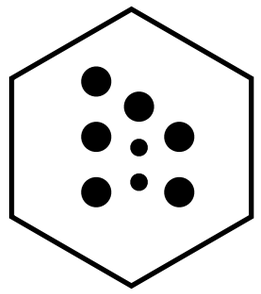
boss expressed in R8

sev expressed in R7

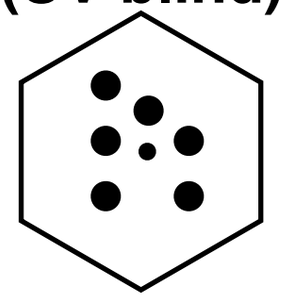


# UV blind mutants led to elements of signaling pathway

**Wild-type**



**Sevenless (UV blind)**



*bride of sevenless*
*boss*

→

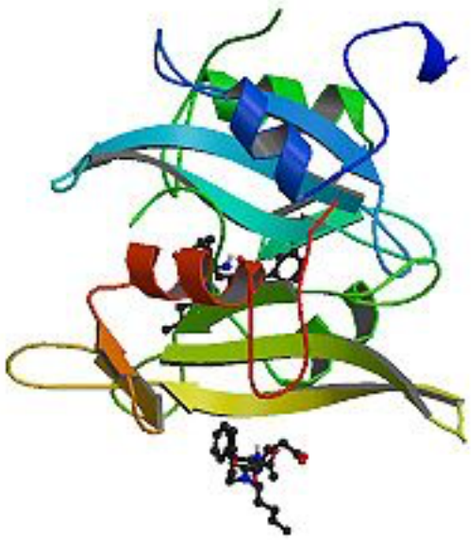
*sevenless*
*sev*

→

R7 development

# 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

**22.7ºC**

*bride of sevenless boss* → *sevenless* *sev*$^{B4}$ → 100% R7 development

**24.1ºC**

*bride of sevenless boss* → *sevenless* *sev*$^{B4}$ ⇢ 90% R7 development

# 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

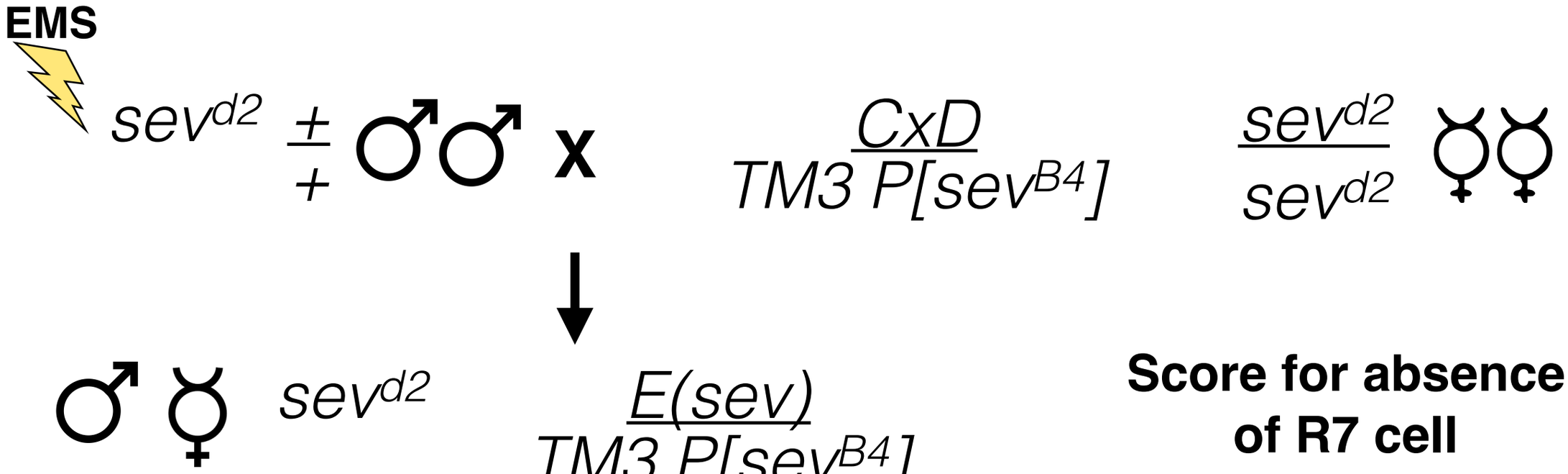
**24.1°C**

*bride of sevenless boss* → *sevenless sev*$^{B4}$ ⇢ 90% R7 development

**22.7°C**

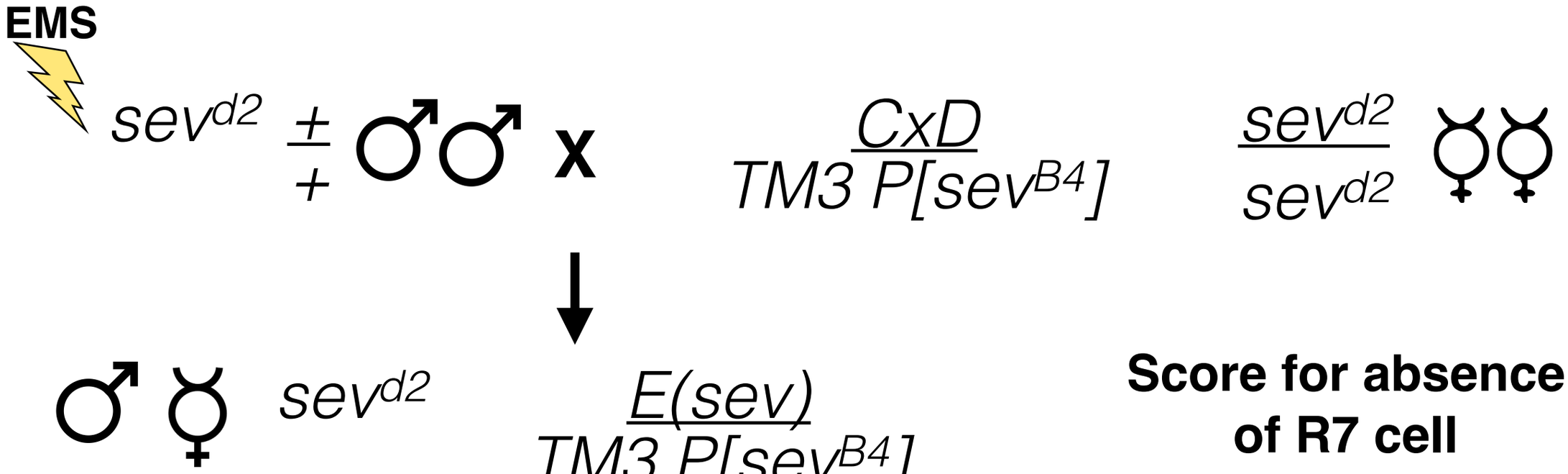
*bride of sevenless boss* → *sevenless sev*$^{B4}$ ⇢ *E(sev)* ⇢ ~10% R7 development

# 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

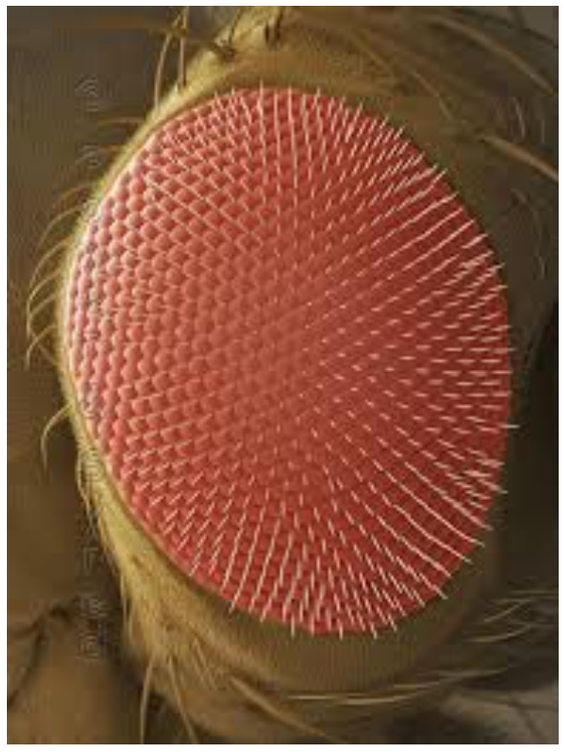
# Screen for dominant enhancers of sensitized mutant R7 phenotype




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

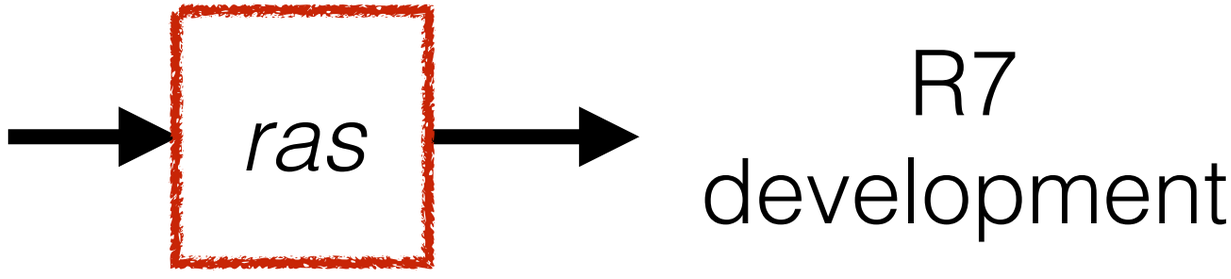
# Screen for dominant enhancers of sensitized phenotype led to the Ras pathway controlling R7 fate

*bride of sevenless*
*boss*



*sevenless*
*sev*

*son of sevenless*
*sos*



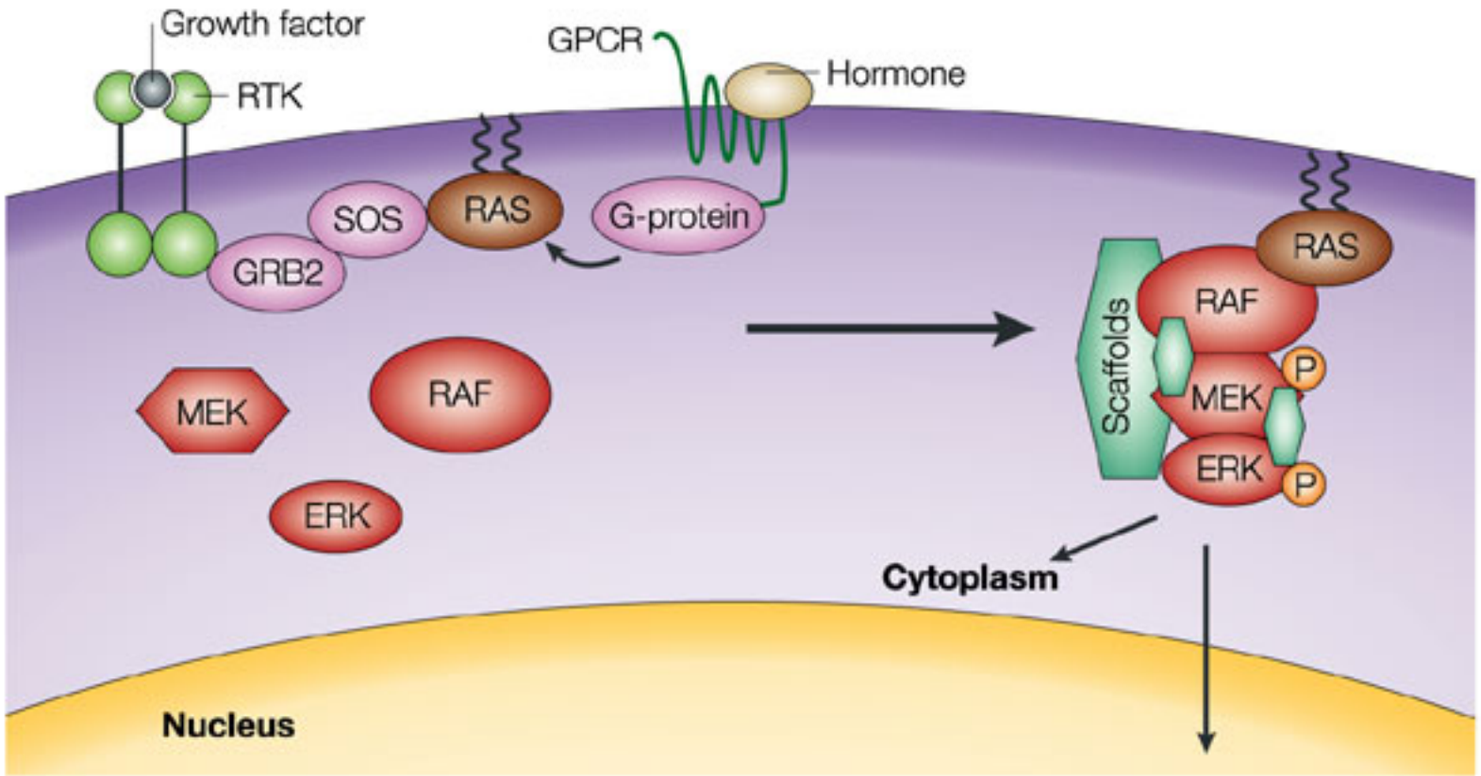
# Methods to ascertain cell autonomy

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!**