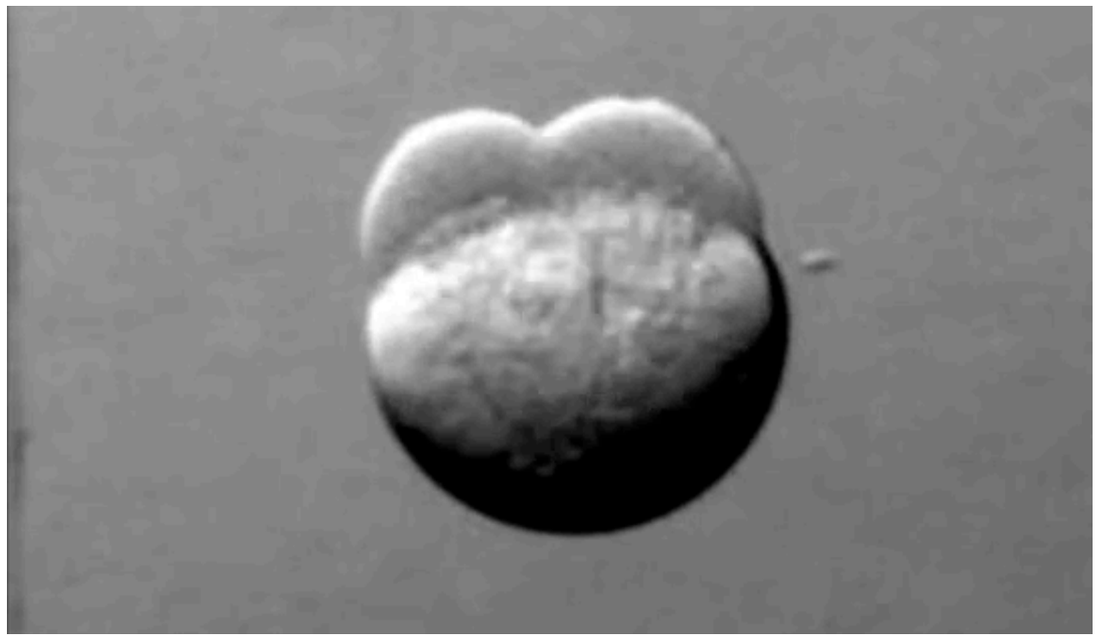
# Bio393: Genetic Analysis

## Developmental genetics I

***C. elegans***

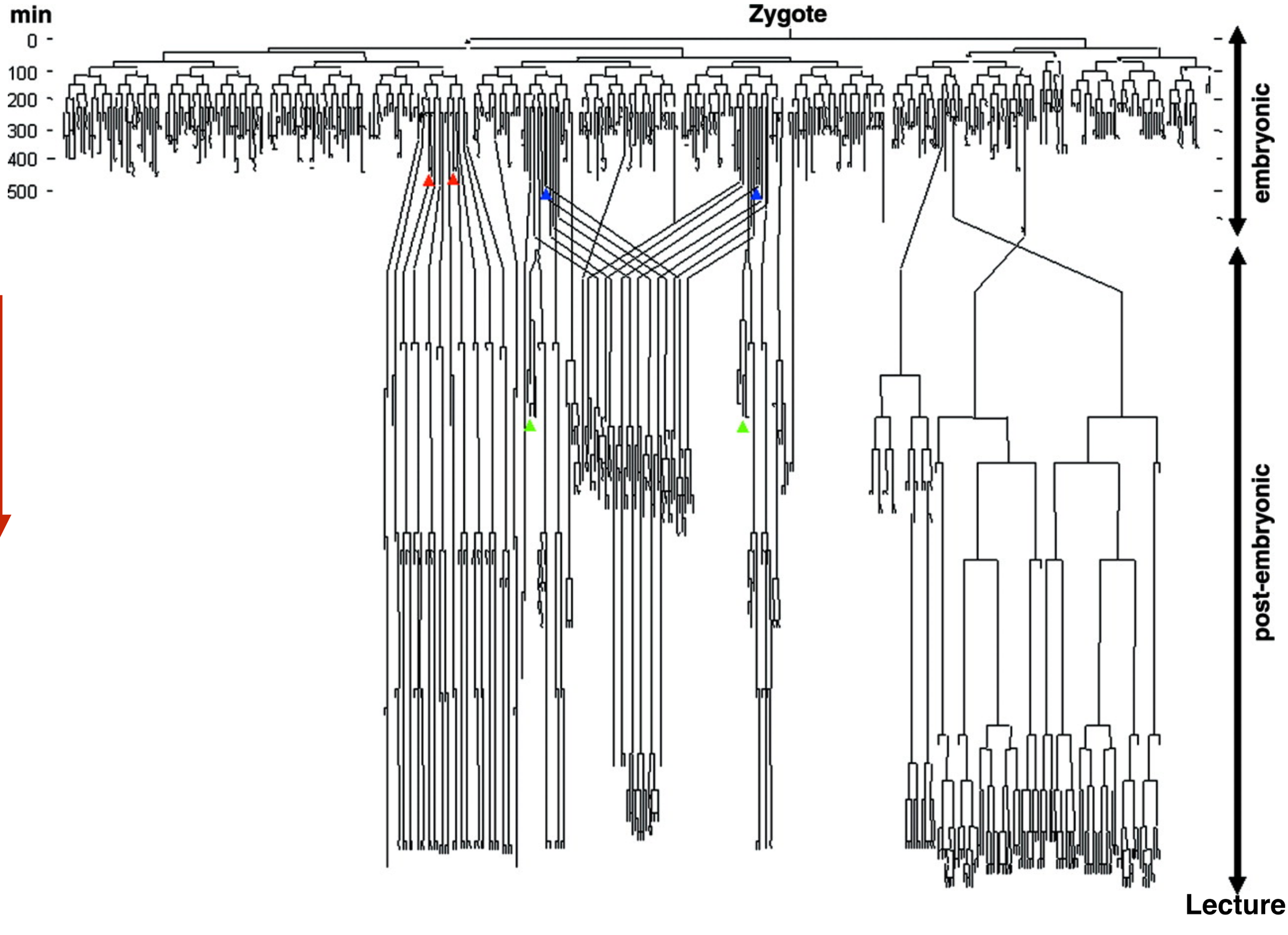
**Lecture 10**

# 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

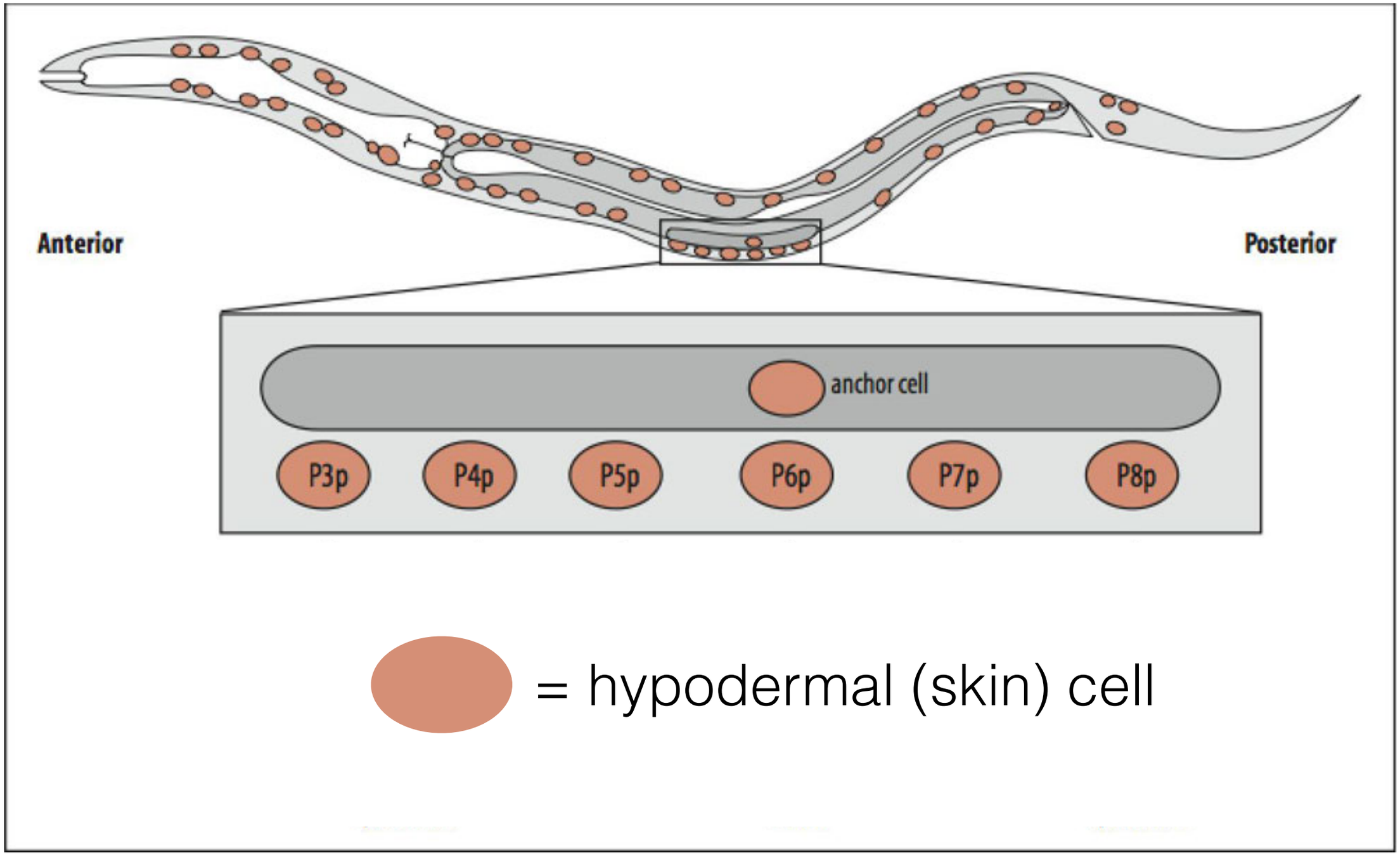
# The cell lineage of *C. elegans* is known and invariant



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


**John Sulston**

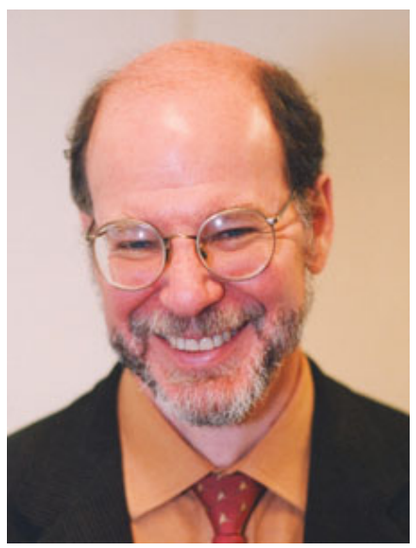
# *C. elegans* vulval development



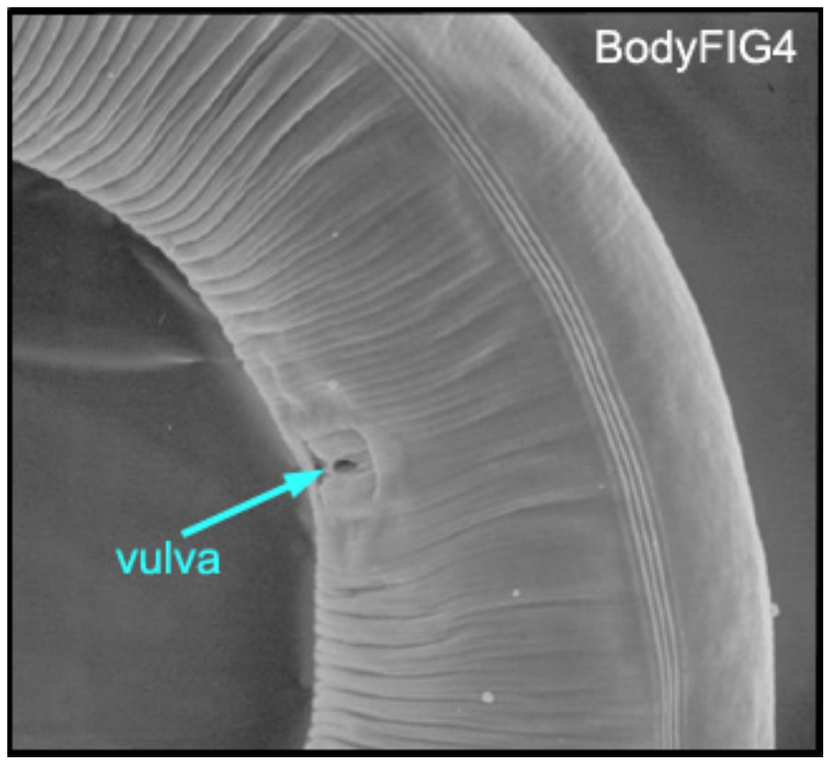
# One of the best genetic screens ever

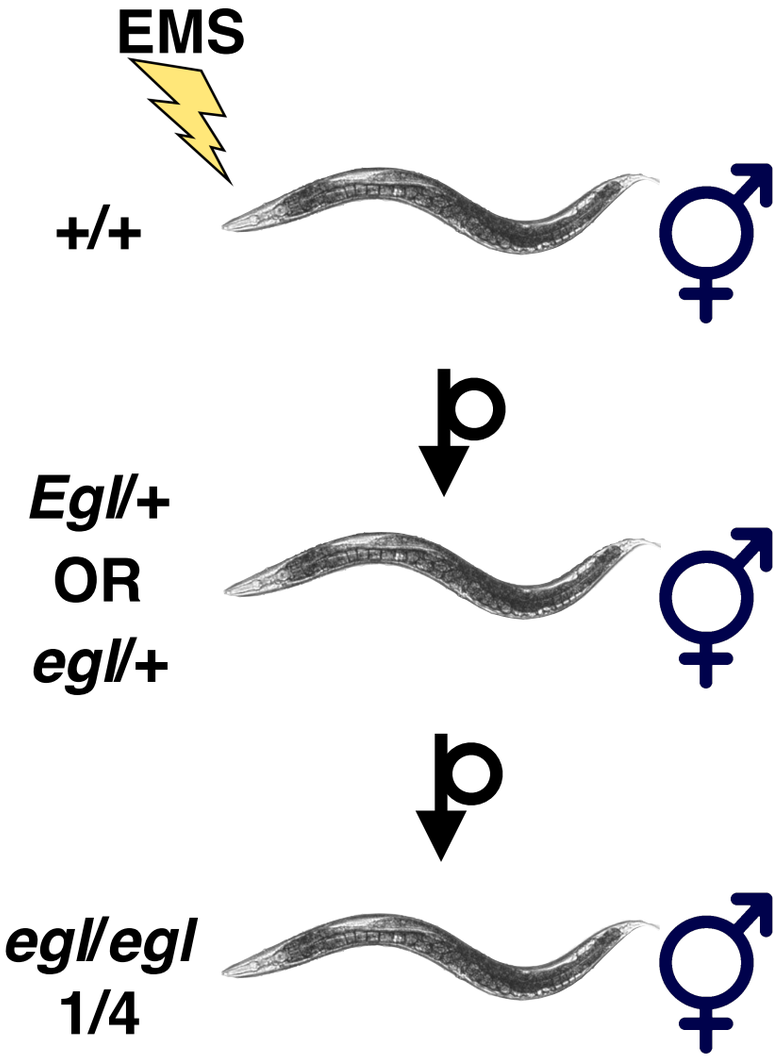
Let's say you screened for mutants that failed to lay eggs

Called Egl for egg-laying defective

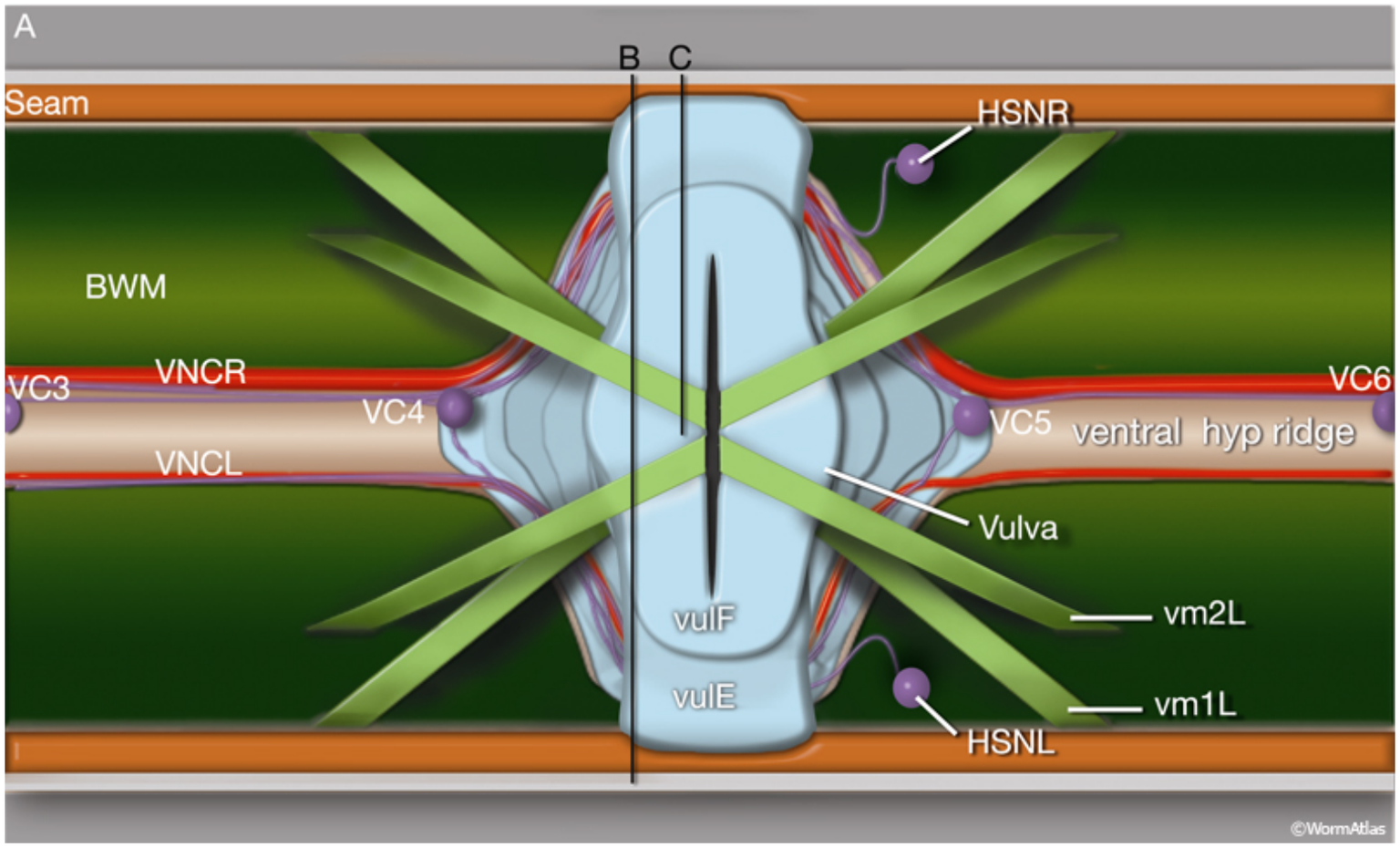


**Bob Horvitz**



# One of the best genetic screens ever

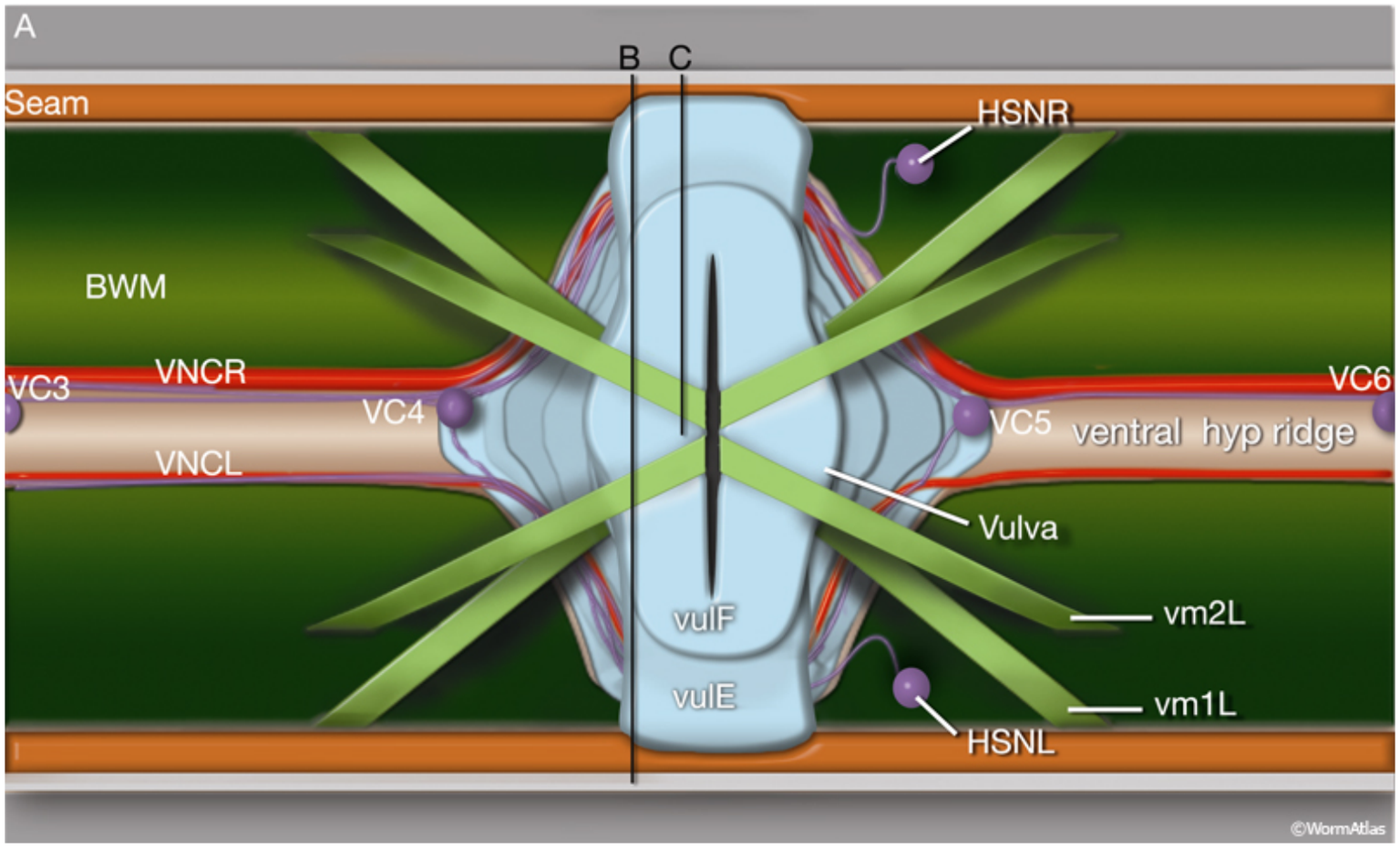



**No neuron**

*egl-1* = inducer of programmed cell death

Hermaphrodite-Specific Neuron (HSN) inappropriately dies

# One of the best genetic screens ever

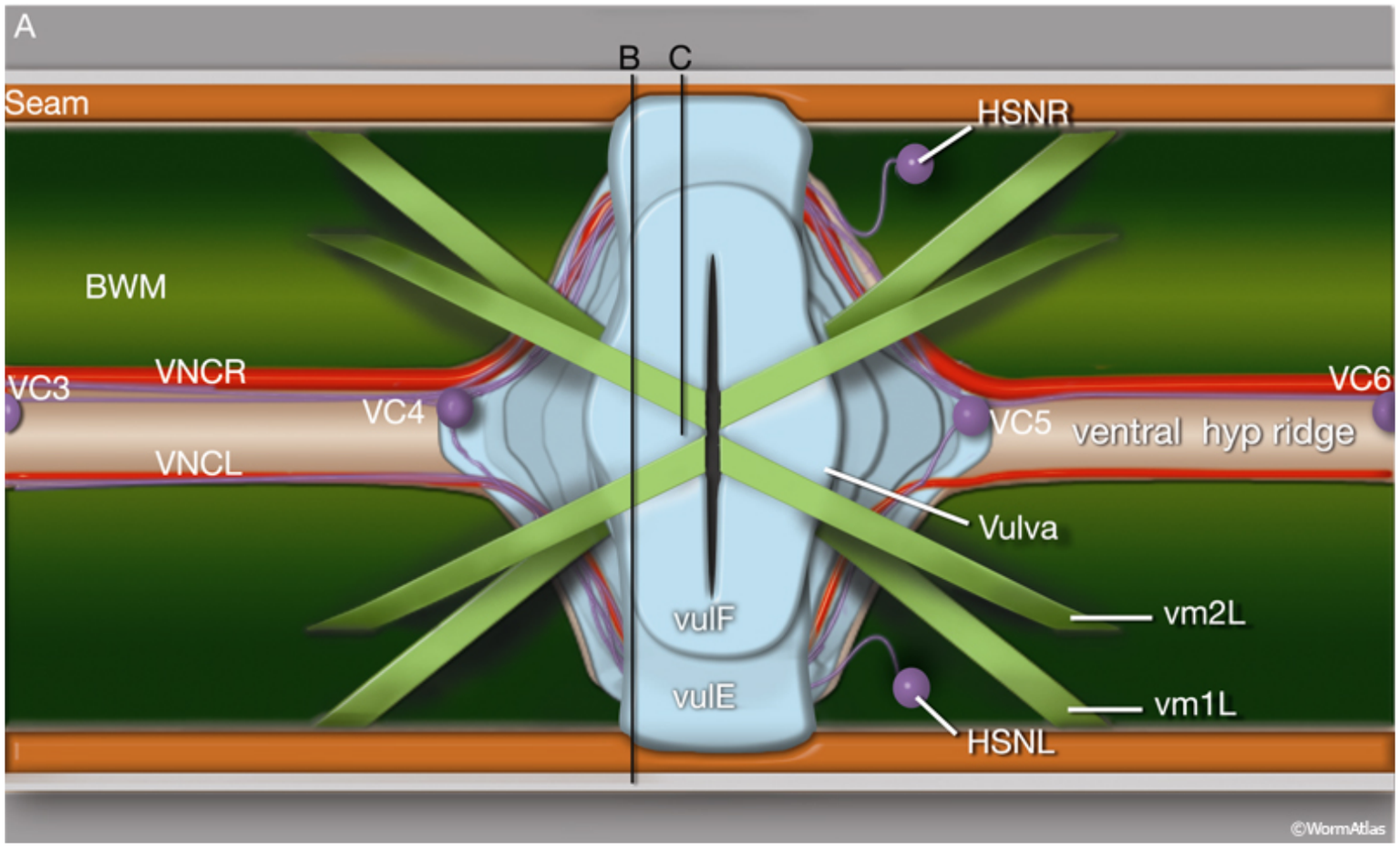



**No neuron**

*tra-1* = inducer of sex determination

Mutants are partially male so HSNs die

# One of the best genetic screens ever

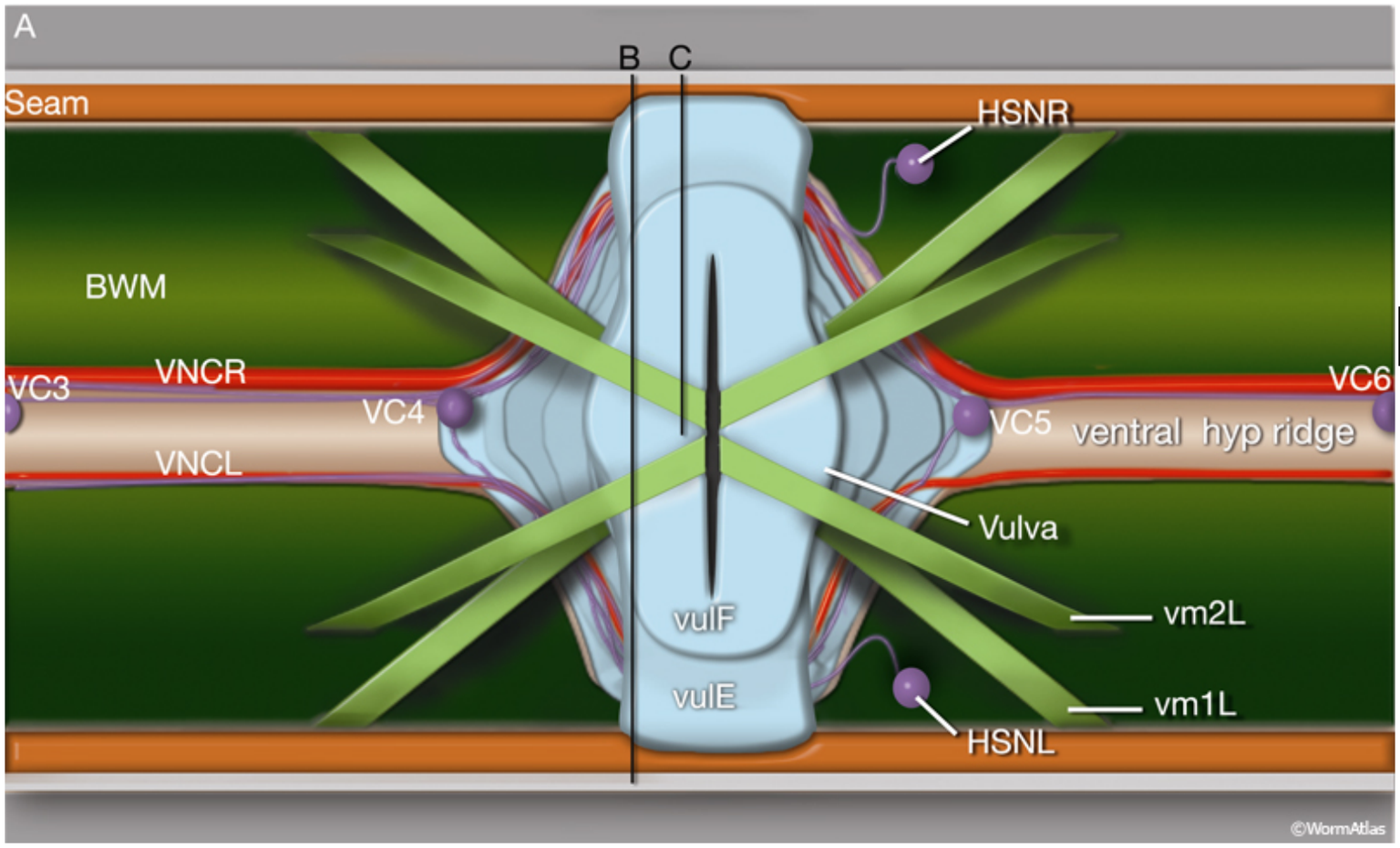



**No neuron**

**No neuronal signaling**

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

# One of the best genetic screens ever




**No neuron**

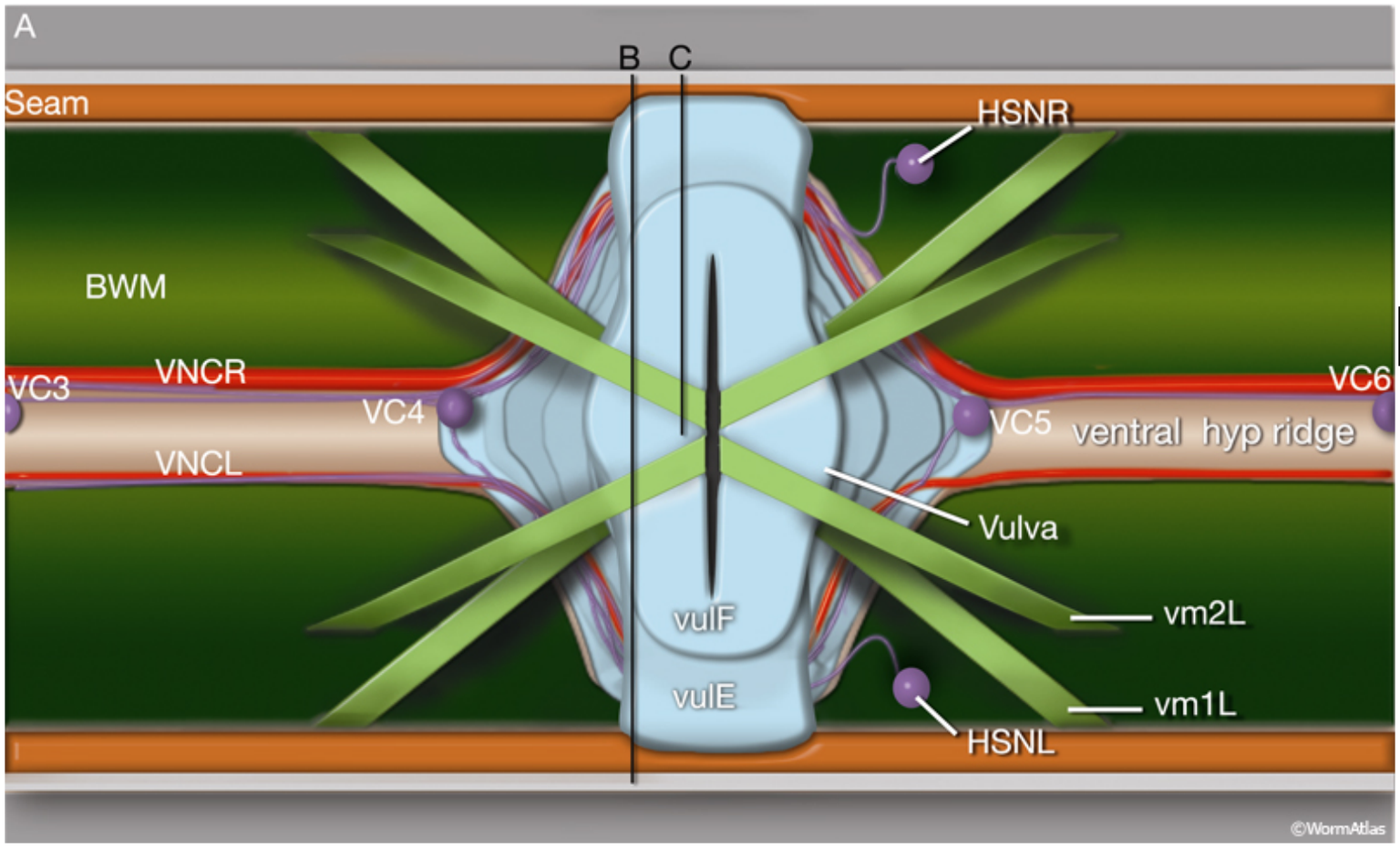
**No neuronal signaling**

**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

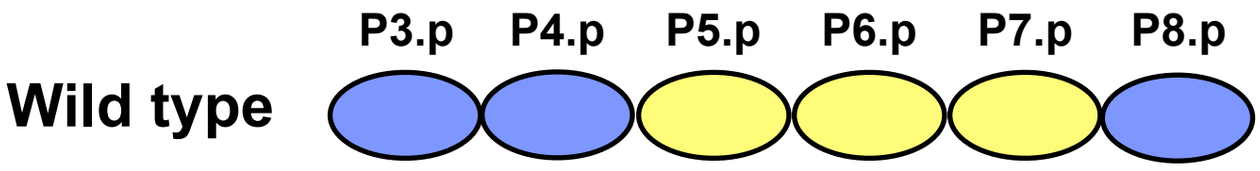
***C. elegans***

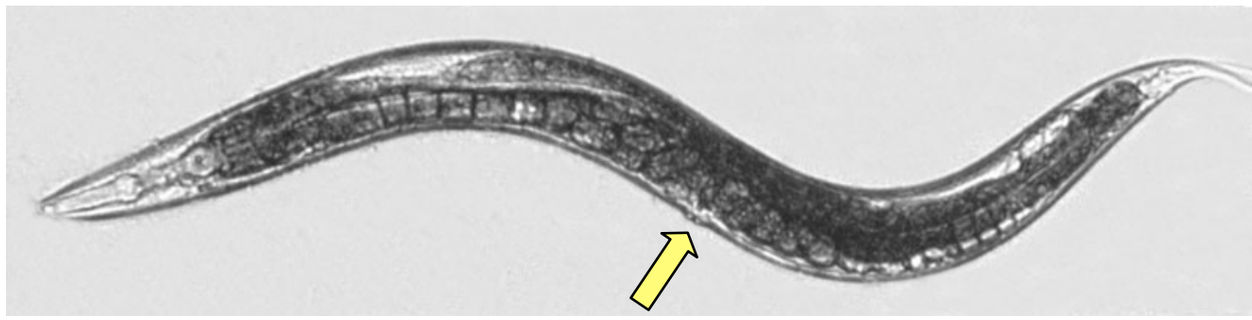
Sydney Brenner

H. Robert Horvitz

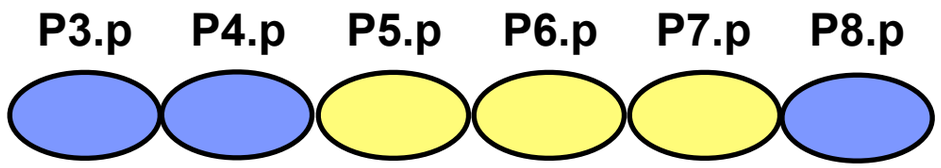
John E. Sulston

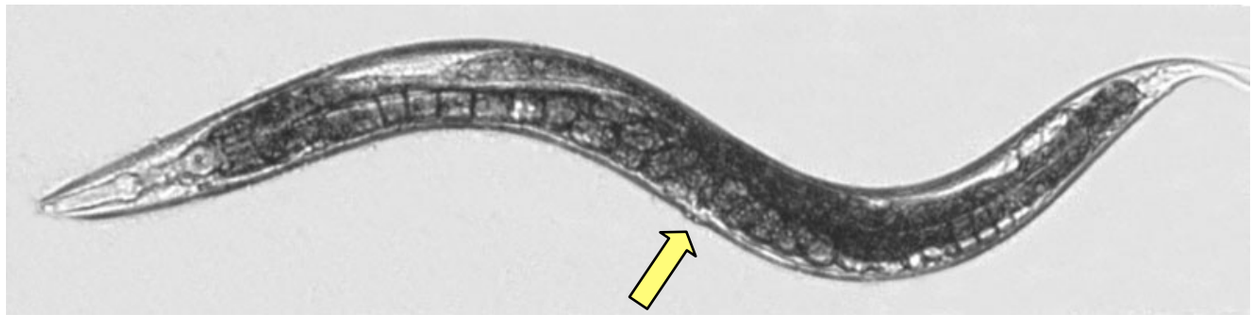
# Three cells express vulval fates in wild-type animals



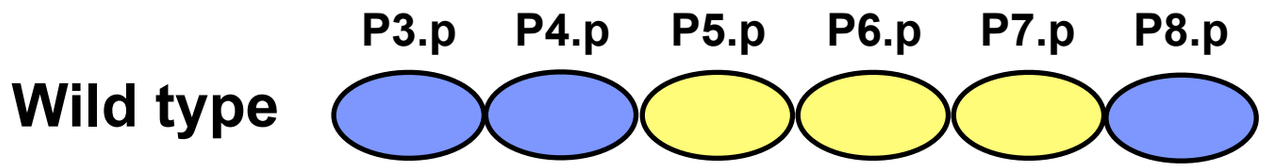



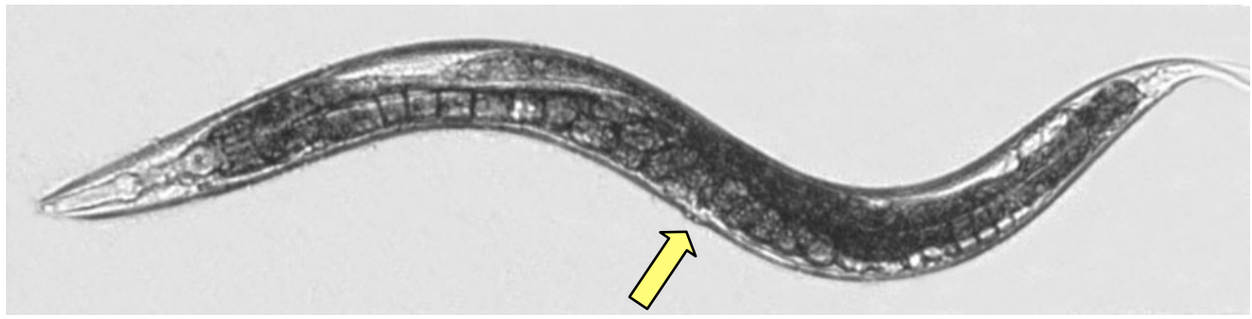
# No cells express vulval fates in vulvaless mutants

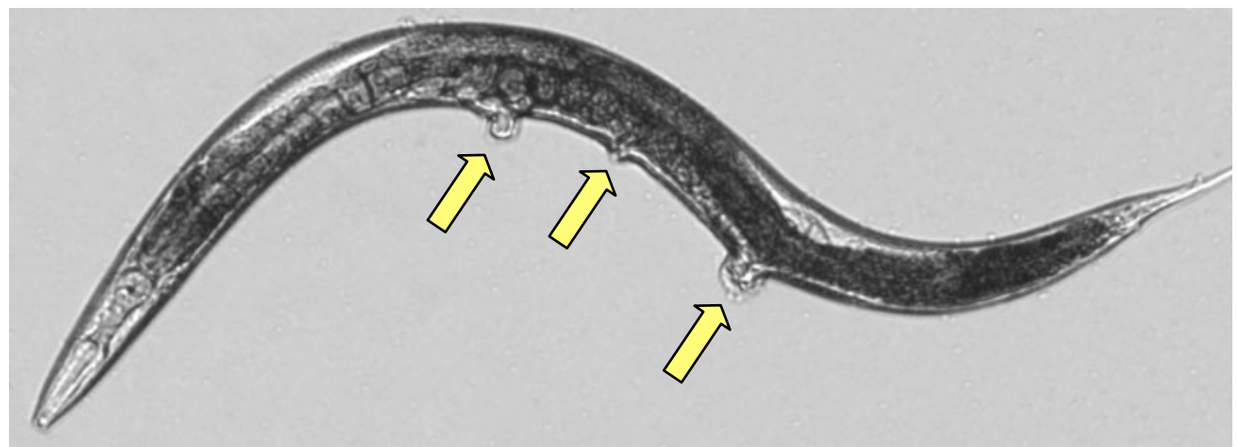


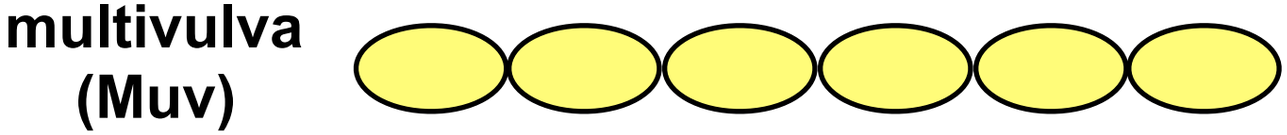



# Six cells express vulval fates in multivulva mutants






# 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 |
| *lin-39(0)* | Vul |

# 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-39(0)* | Vul |
| *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 |

***lin-3*** → **vulval fate**

***let-60*** → **vulval fate**

***let-23*** → **vulval fate**

# 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-39(0)* | Vul |
| *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 |

***lin-3*** → **vulval fate**

***let-60*** → **vulval fate**

***let-23*** → **vulval fate**

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

# 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-39(0)* | Vul |
| *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 |

***lin-3*** → **vulval fate**

***let-60*** → **vulval fate**

***let-23*** → **vulval fate**

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

# 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-39(0)* | Vul |
| *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 |

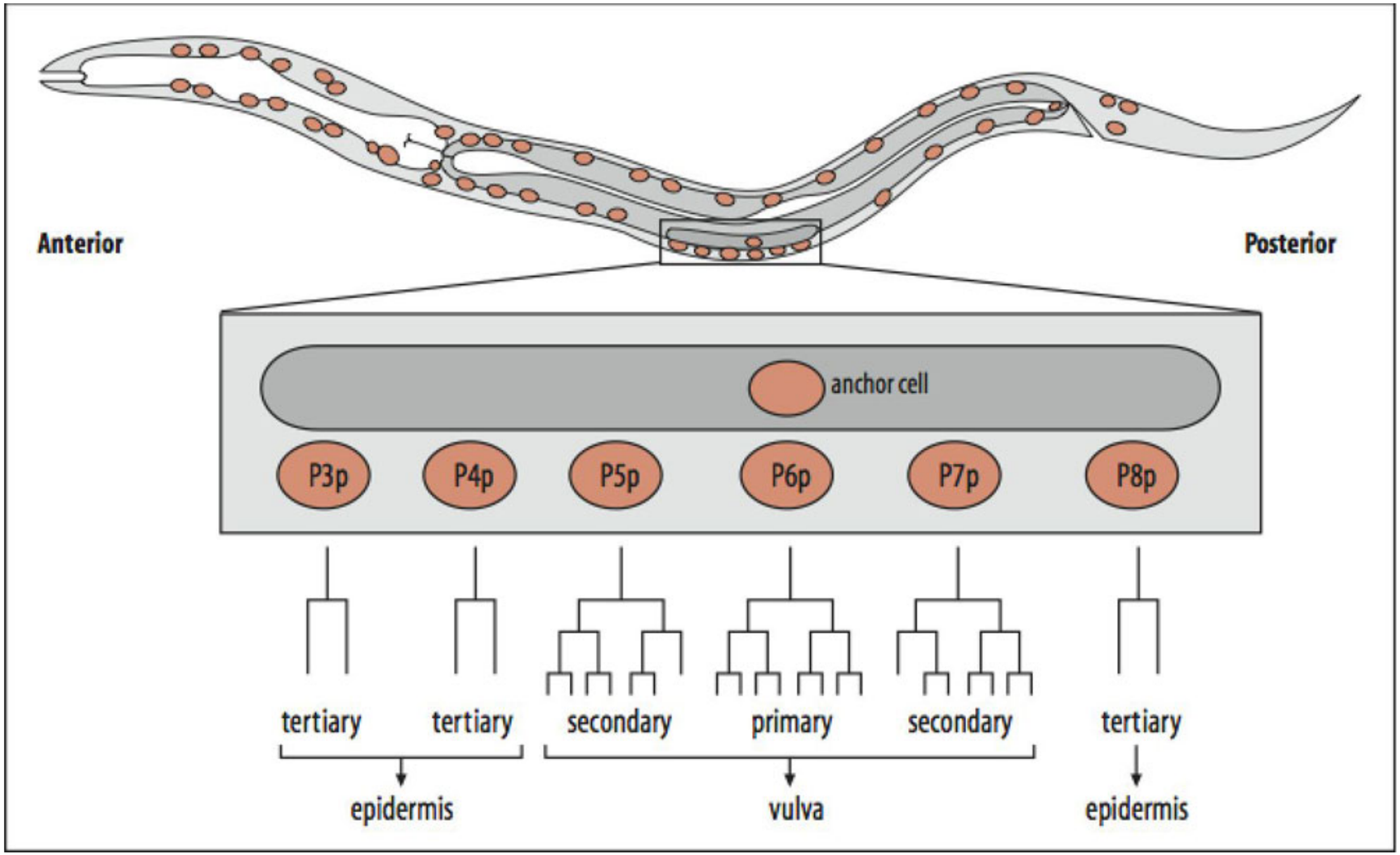
***lin-3*** → ***let-23*** → ***let-60*** ⊣ ***lin-1*** ⊣ **vulval fate**

# Double mutants defined the vulval pathway

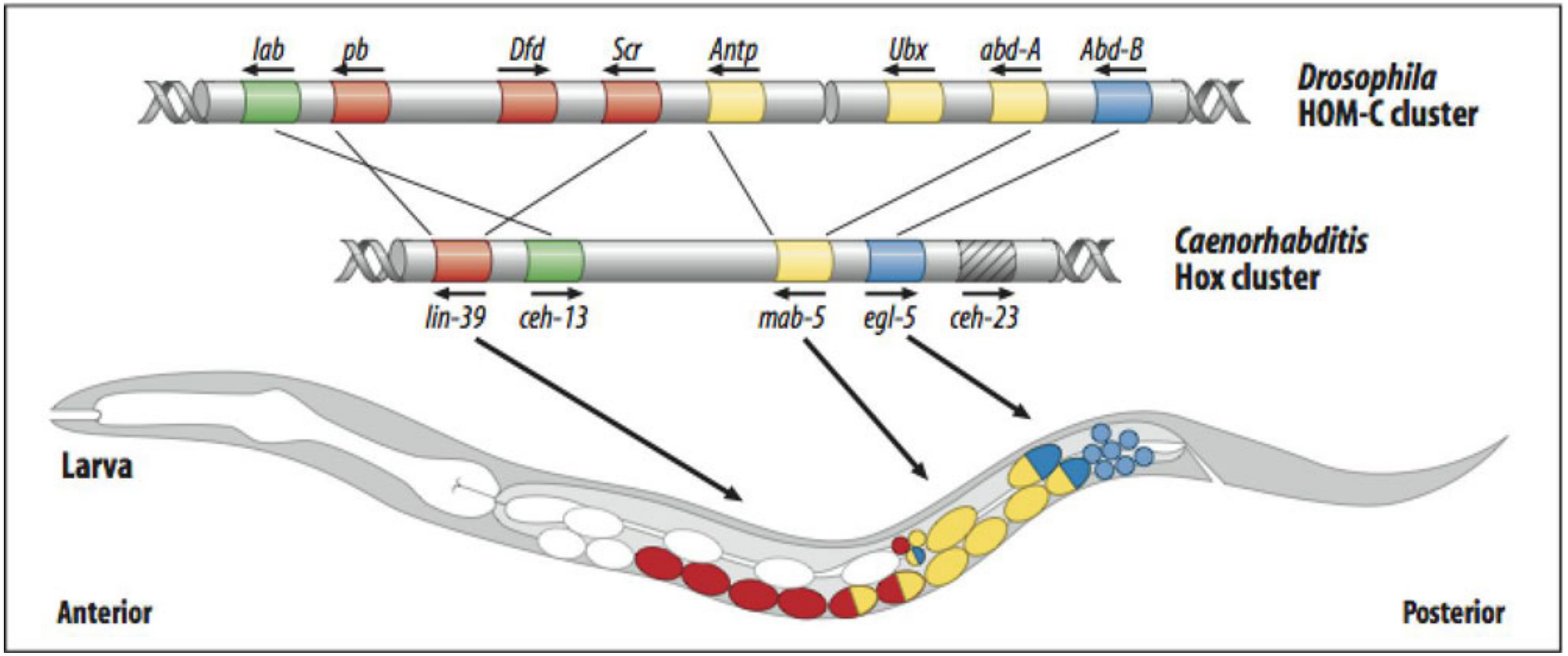
| Mutant genotypes | Phenotype |
|---|---|
| *lin-1(0)* | Muv |
| *lin-3(0)* | Vul |
| *let-60(0)* | Vul |
| *let-60(gf)* | Muv |
| *let-23(0)* | Vul |
| *let-23(gf)* | Muv |
| *lin-39(0)* | Vul |
| *lin-3(0); let-23(gf)* | Muv |
| *lin-3(0); let-60(gf)* | Muv |
| *let-23(0); let-60(gf)* | Muv |
| *let-23(gf); let-60(0)* | Vul |
| *let-60(0); lin-1(0)* | Muv |
| *let-23(0); lin-1(0)* | Muv |
| *lin-1(0); lin-39(0)* | Vul |

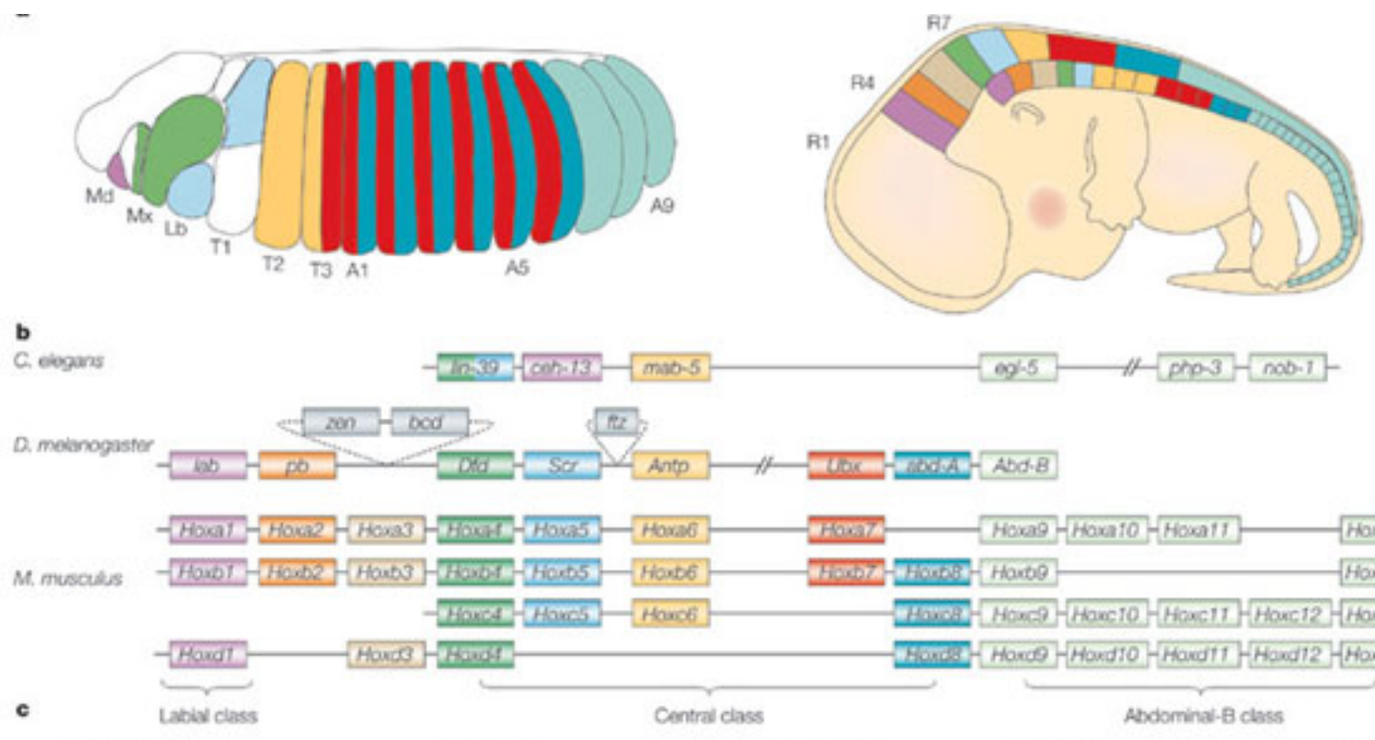
***lin-3*** → ***let-23*** → ***let-60*** ⊣ ***lin-1*** ⊣ ***lin-39*** → **vulval fate**

# Careful observation of *lin-39(0)* mutants showed that P3p through P8p were not made correctly

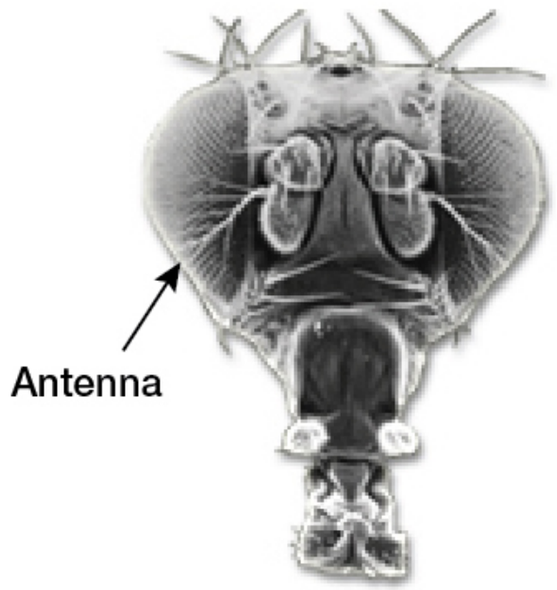


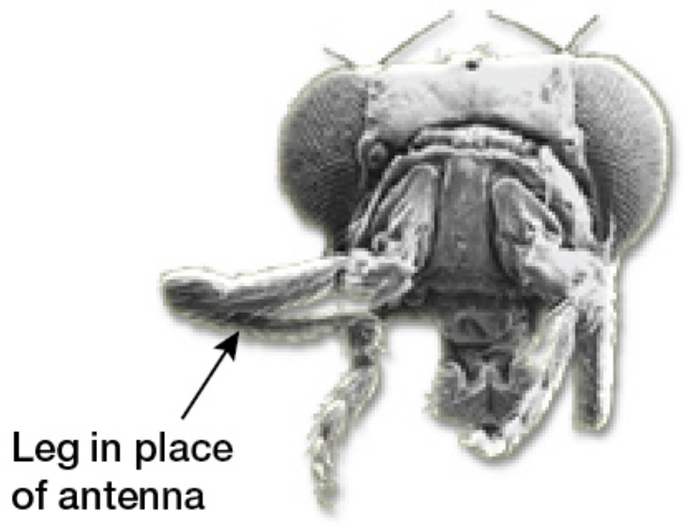
# *lin-39* is a Hox gene



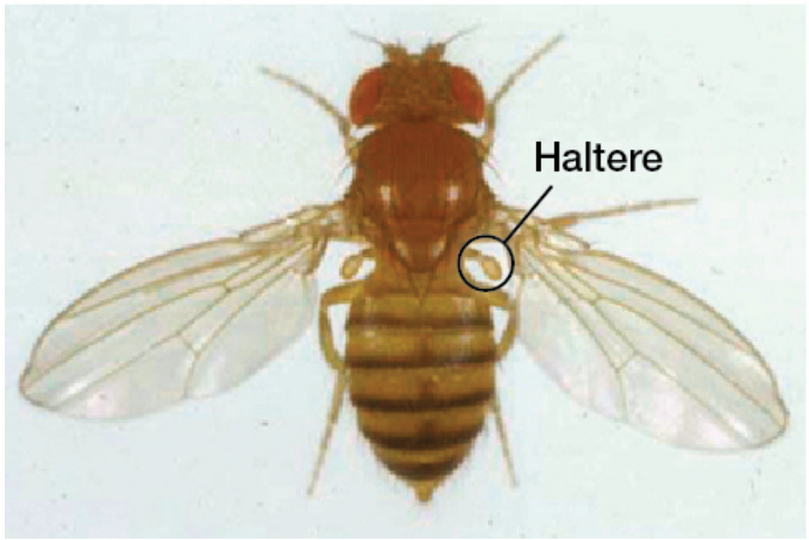



# Regional identity in the animal is deficient in Hox mutants



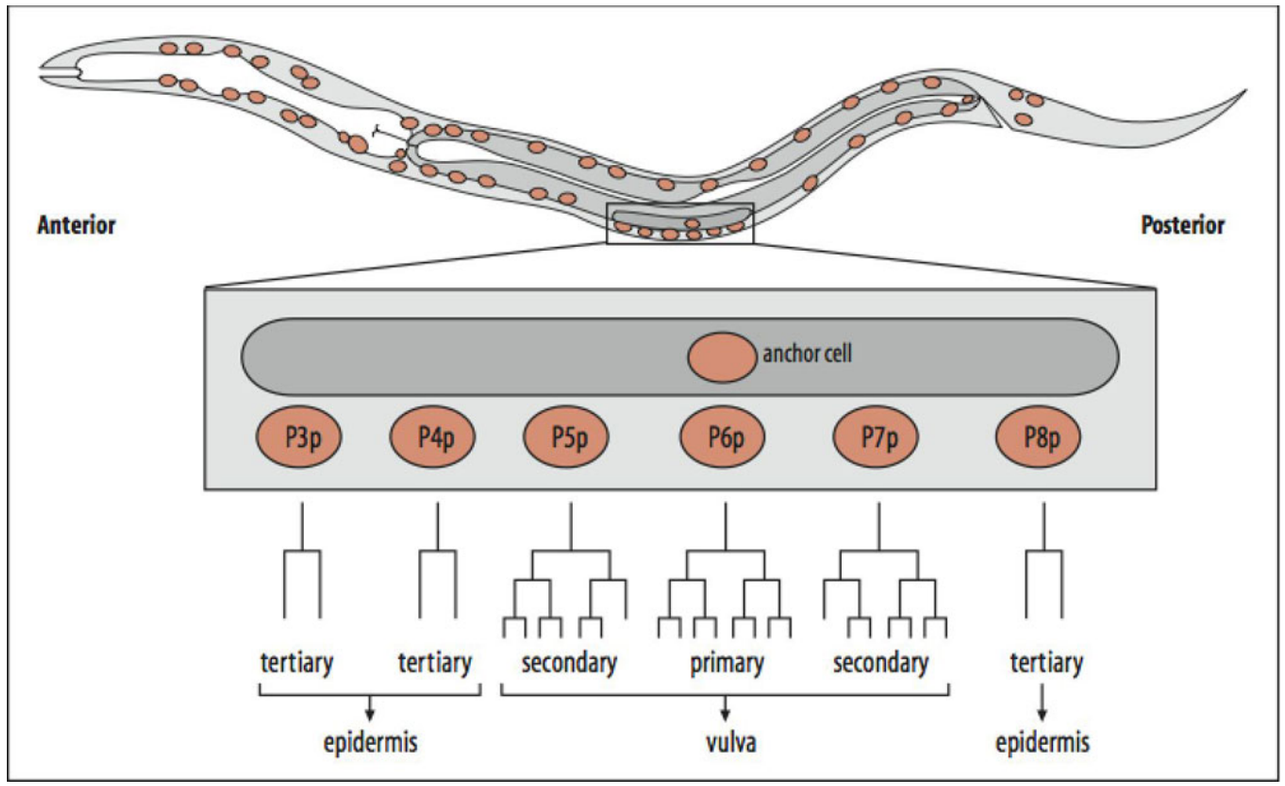




**Antenna to leg**



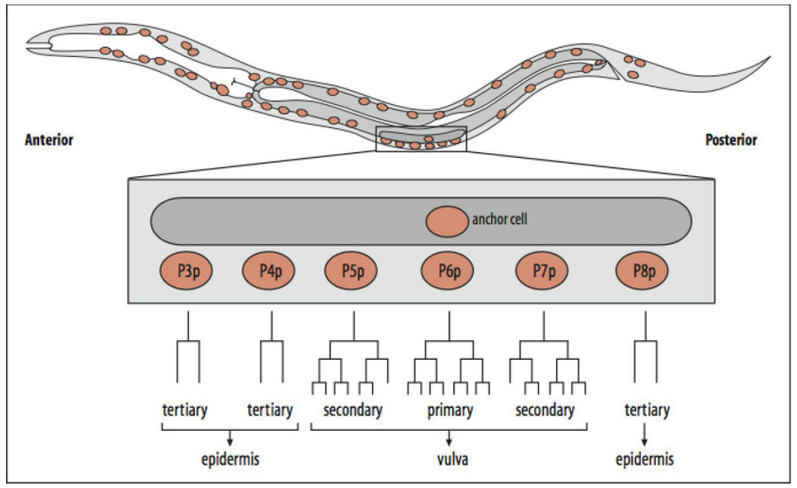

**Haltere to wing**

# *lin-39* is a Hox gene




Generation of the vulval precursor cells is epistatic to the fates of those cells

# *lin-39* is a Hox gene

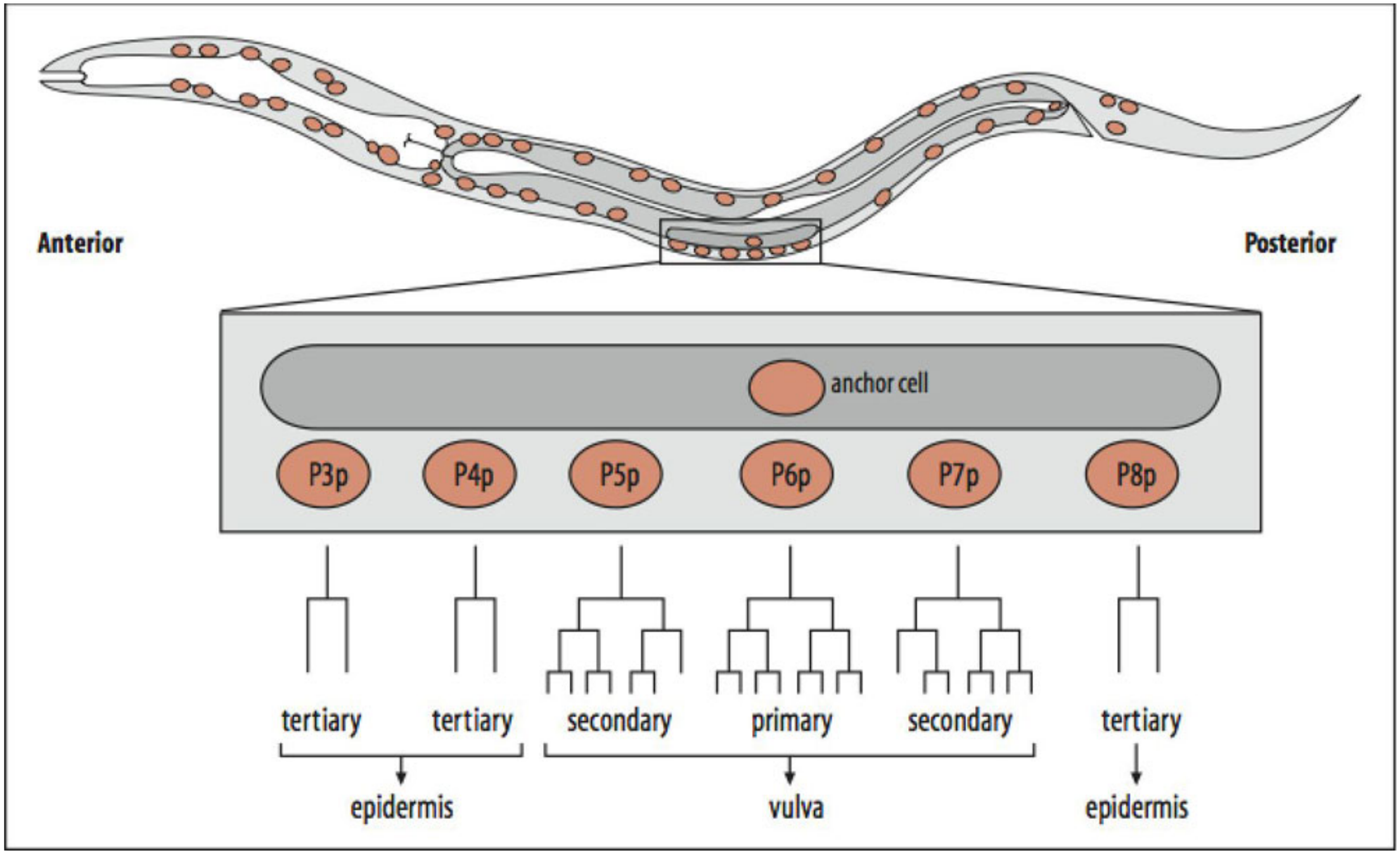



Generation of the vulval precursor cells is epistatic to the fates of those cells

***lin-39*** → ***lin-3*** → ***let-23*** → ***let-60*** ⊣ ***lin-1*** ⊣ **vulval fate**

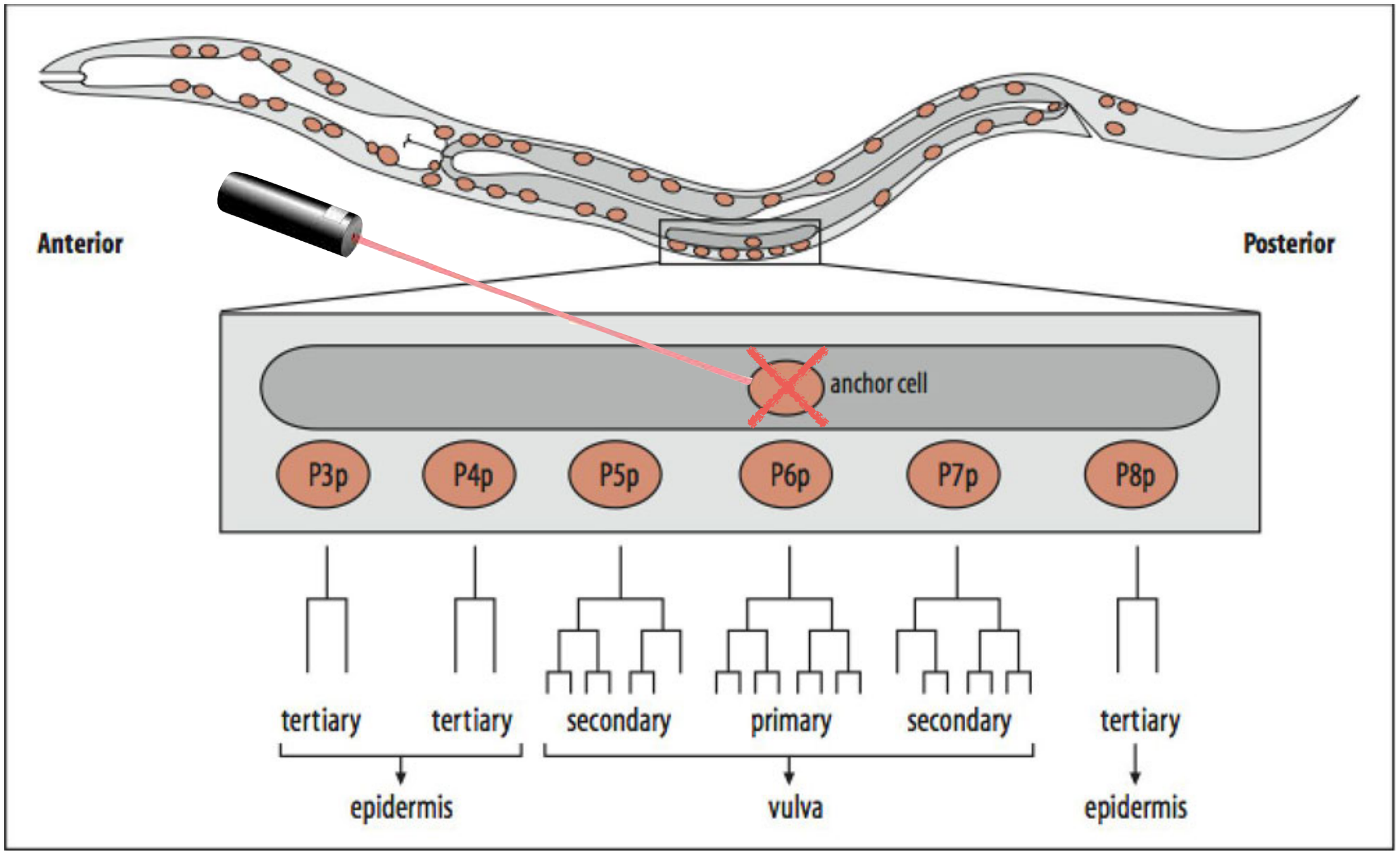
| Mutant genotypes | Phenotype |
|---|---|
| *lin-1(0)* | Muv |
| *lin-3(0)* | Vul |
| *lin-39(0)* | Vul |
| *lin-1(0); lin-3(0)* | Muv |
| *lin-1(0); lin-39(0)* | Vul |

# 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)

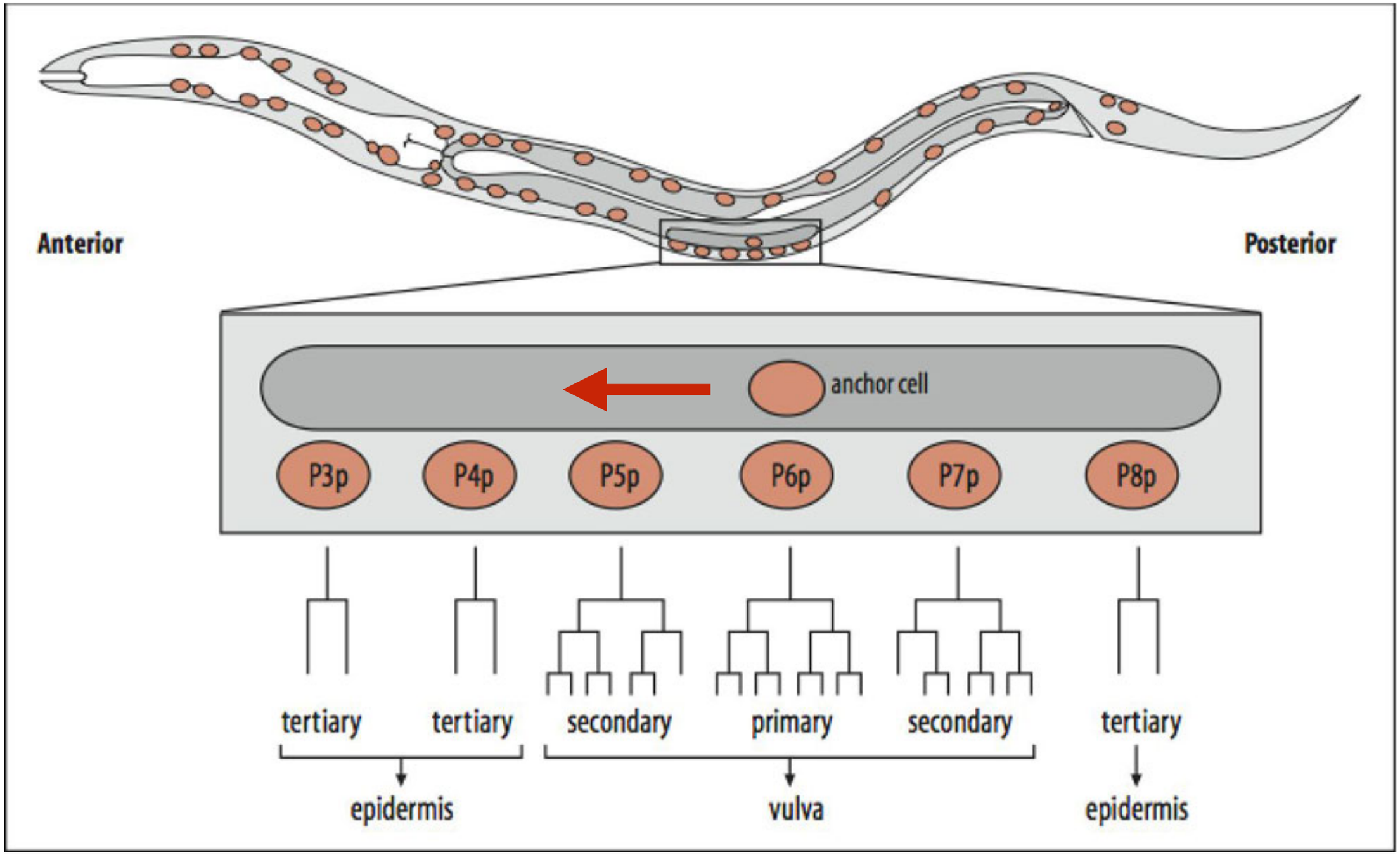



No AC leads to no vulval cell specification and a vulvaless phenotype

# 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)

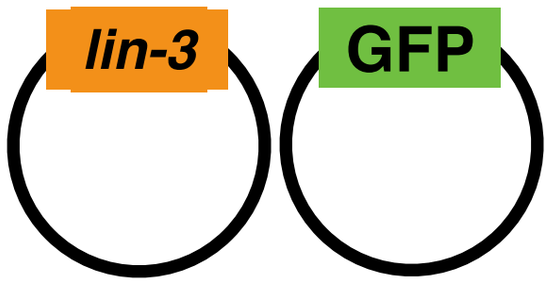


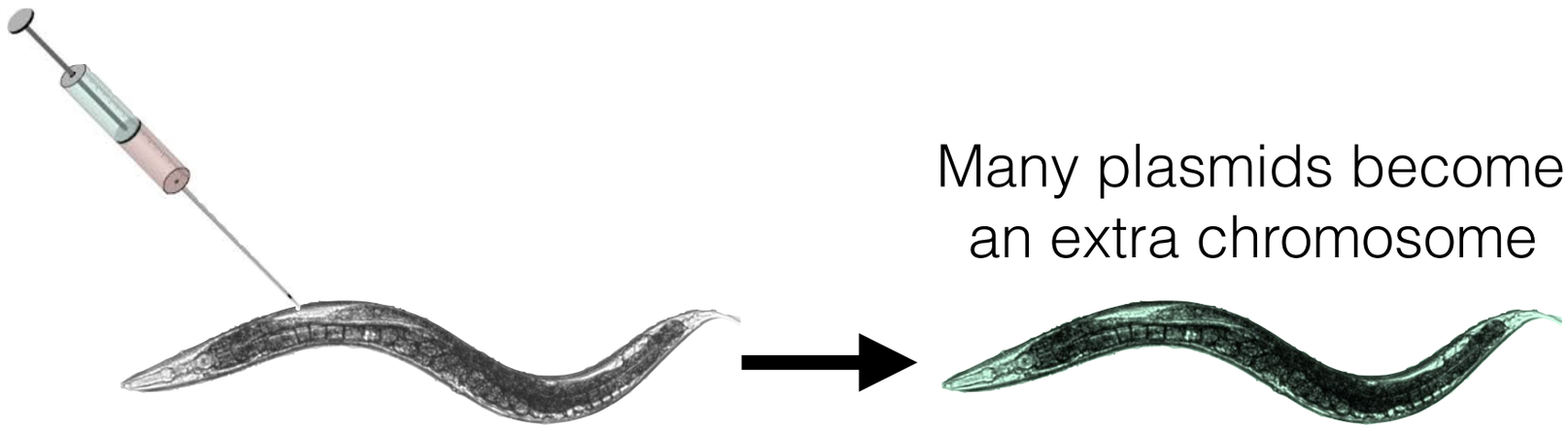

*dig-1* displaced gonad mutants

# 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?



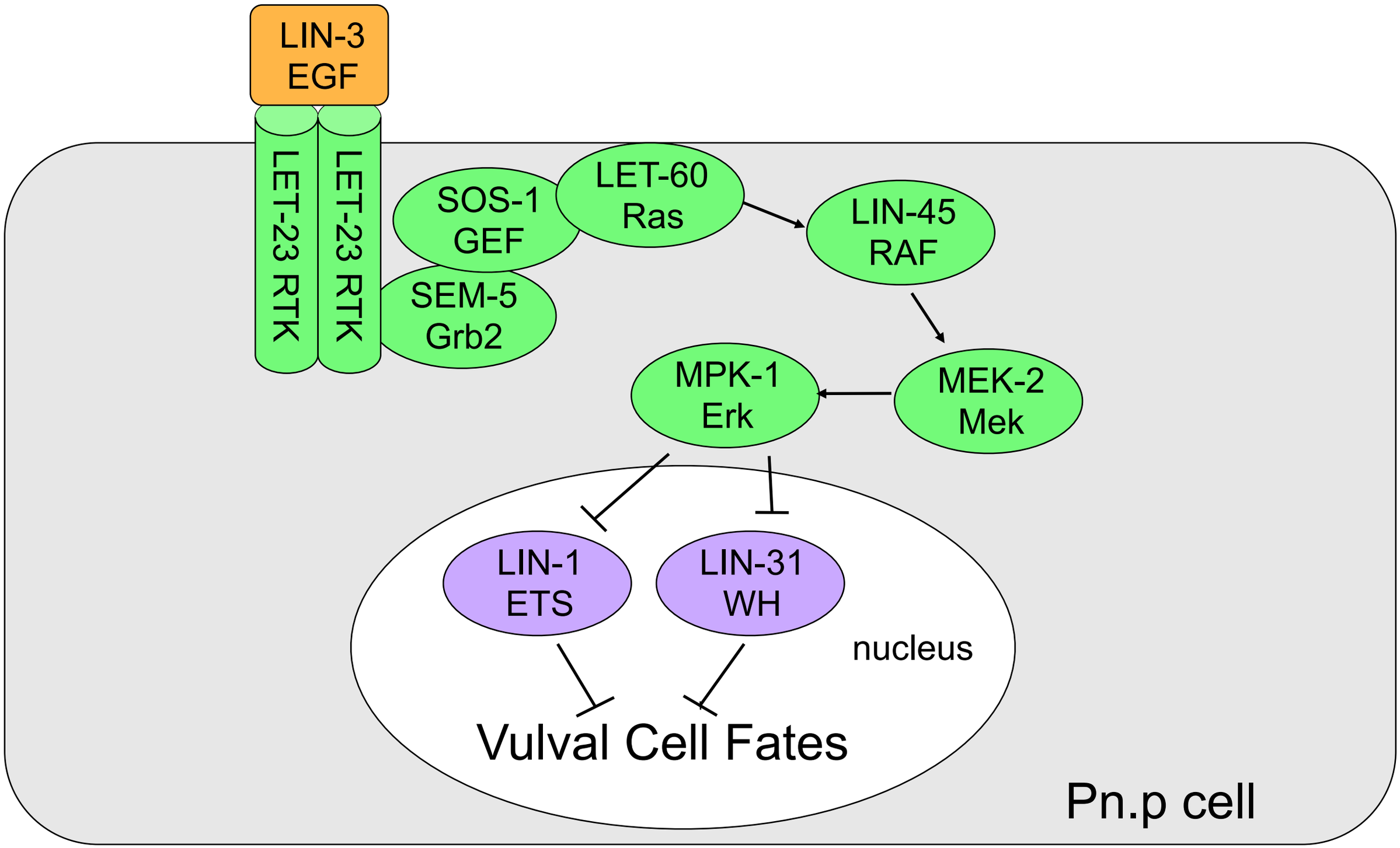




*lin-3(0)*
Vul

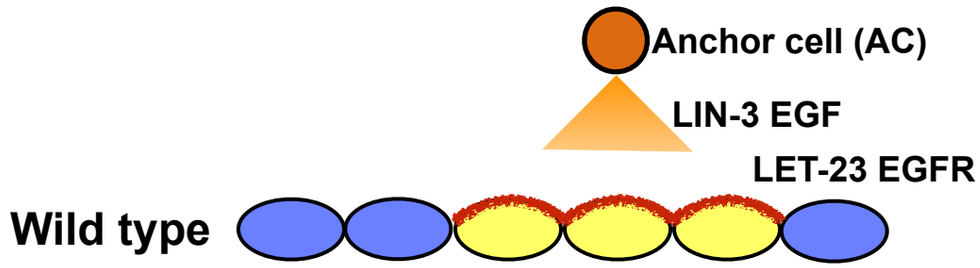
*lin-3(0); lin-3(+++)*
Wild-type or Muv

| 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 |

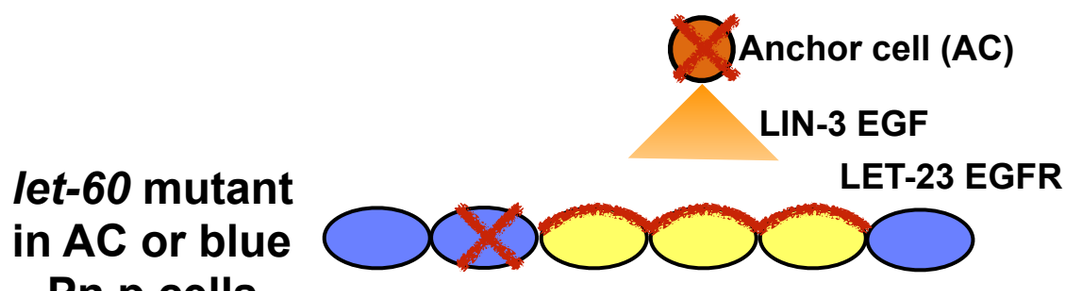
# A Ras pathway promotes vulval fates



# Cell autonomy of *let-60*

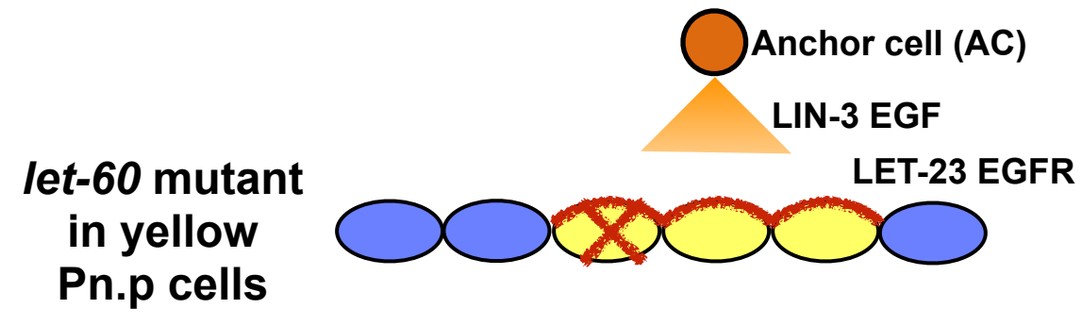



Cell autonomous action




**Wild-type vulval phenotype**

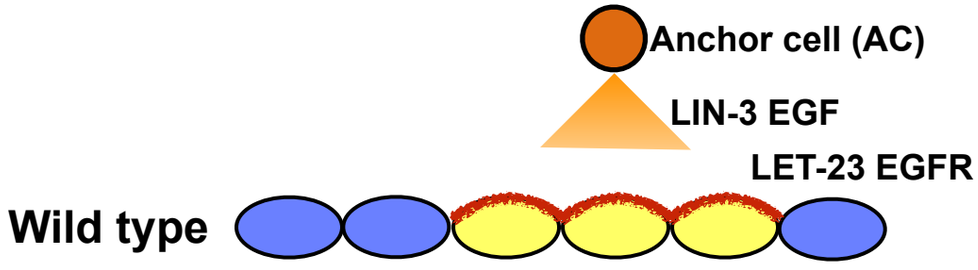
X = mutant cell



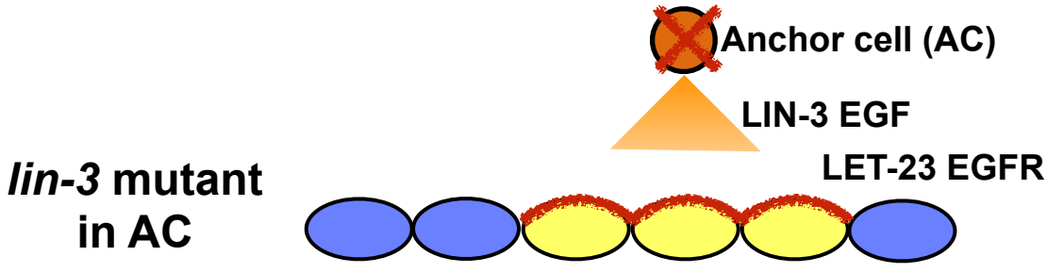

**Mutant vulval phenotype**

A cell autonomous trait is a trait in a multicellular organism in which only genotypically mutant cells have the mutant phenotype

# Cell autonomy of *lin-3*

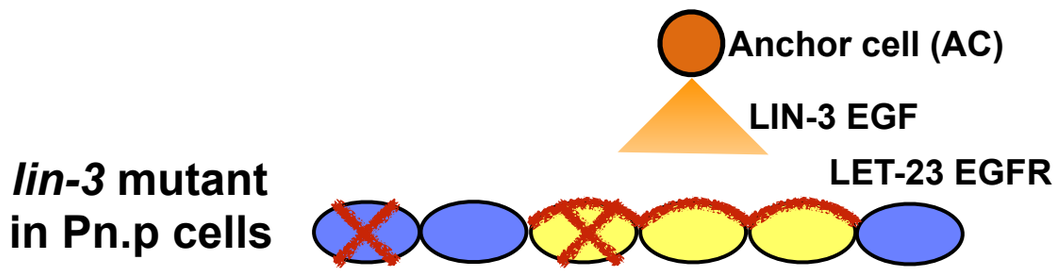



Cell non-autonomous action




**Mutant vulval phenotype**

= mutant cell




**Wild-type vulval phenotype**

A cell autonomous trait is a trait in a multicellular organism in which only genotypically mutant cells have the mutant phenotype

# 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 |
| *lin-2(0)* | a little Vul |
| *lin-7(0)* | a little Vul |
| *lin-10(0)* | a little Vul |

# 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 |
| *lin-2(0)* | a little Vul |
| *lin-7(0)* | a little Vul |
| *lin-10(0)* | a little Vul |
| *lin-2(0); lin-7(0)* | more Vul |
| *lin-2(0); lin-10(0)* | more Vul |
| *lin-7(0); lin-10(0)* | more Vul |

# 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 |
| *lin-2(0)* | a little Vul |
| *lin-7(0)* | a little Vul |
| *lin-10(0)* | a little Vul |
| *lin-2(0); lin-7(0)* | more Vul |
| *lin-2(0); lin-10(0)* | more Vul |
| *lin-7(0); lin-10(0)* | more Vul |
| *lin-2(0); lin-7(0); lin-10(0)* | Vul |

Told the geneticists that the three genes act in a redundant process, but how?

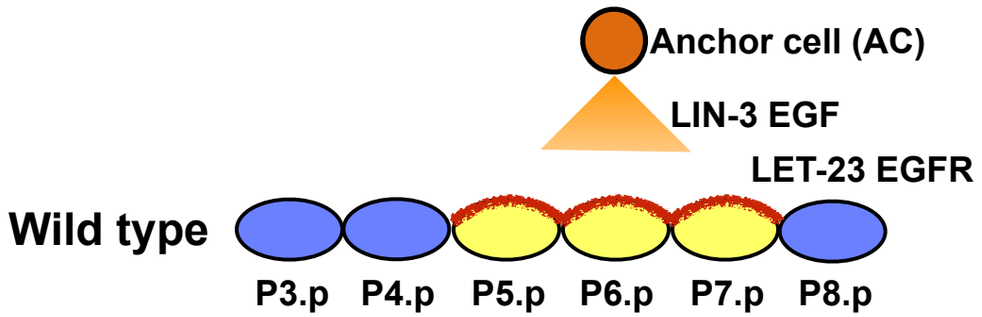
**Told the geneticists that the three genes act in a redundant process**
**But how?**

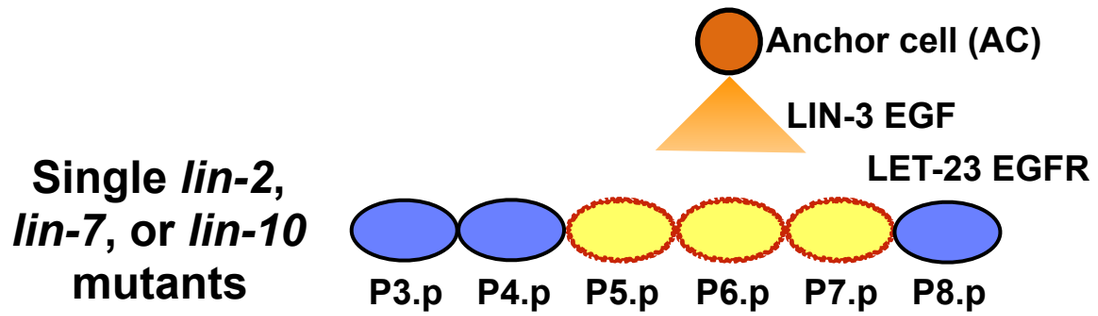
Act downstream of *lin-3*

Act upstream of *let-23*

Expressed in vulval cells

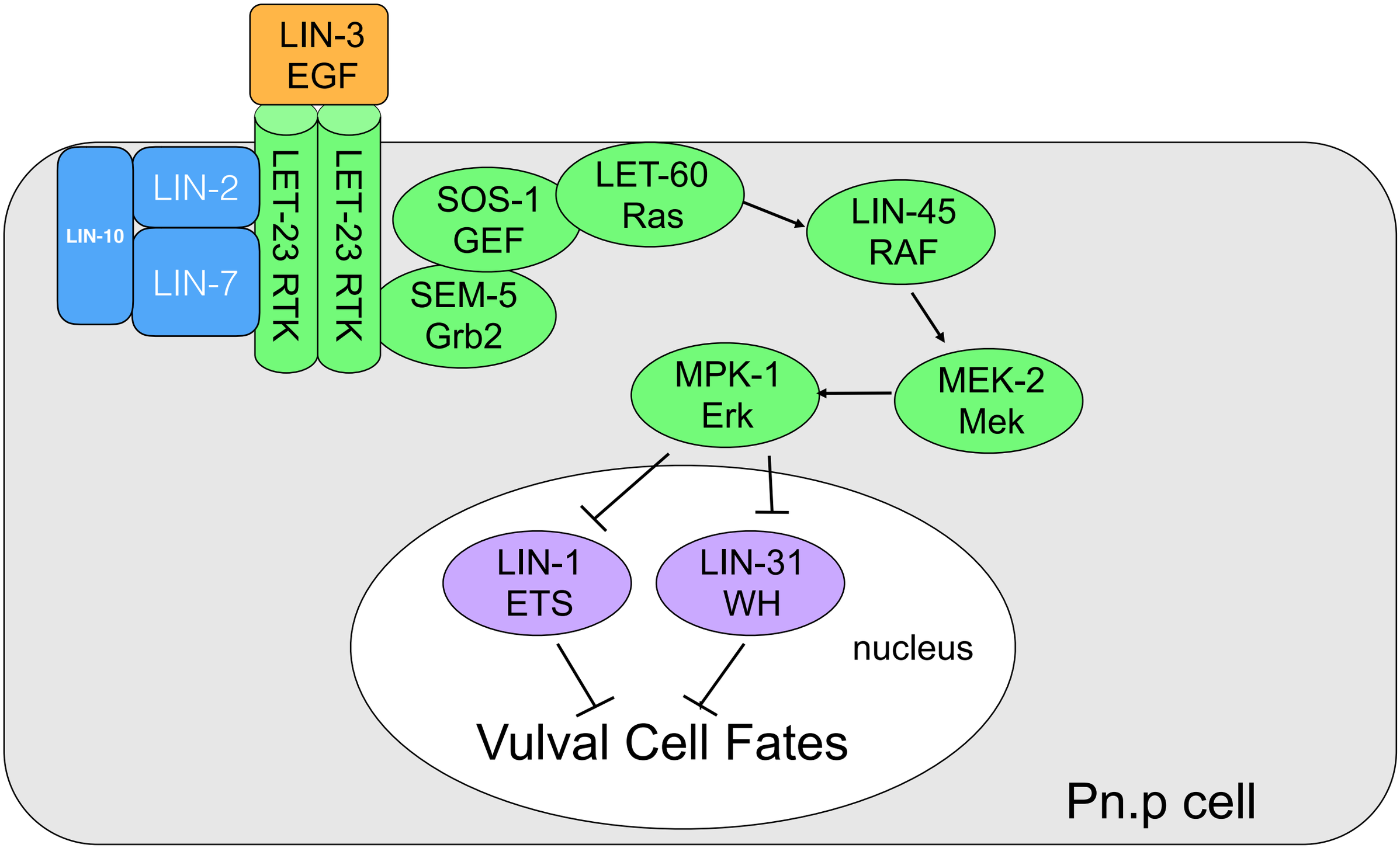
# *lin-2*, *lin-7*, and *lin-10* act redundantly to localize LET-23 RTK




***lin-3*** → ***lin-2/7/10*** → ***let-23*** → ***let-60*** ⊣ ***lin-1*** ⊣ **vulval fate**

# A Ras pathway promotes vulval fates



# Vulval mutants

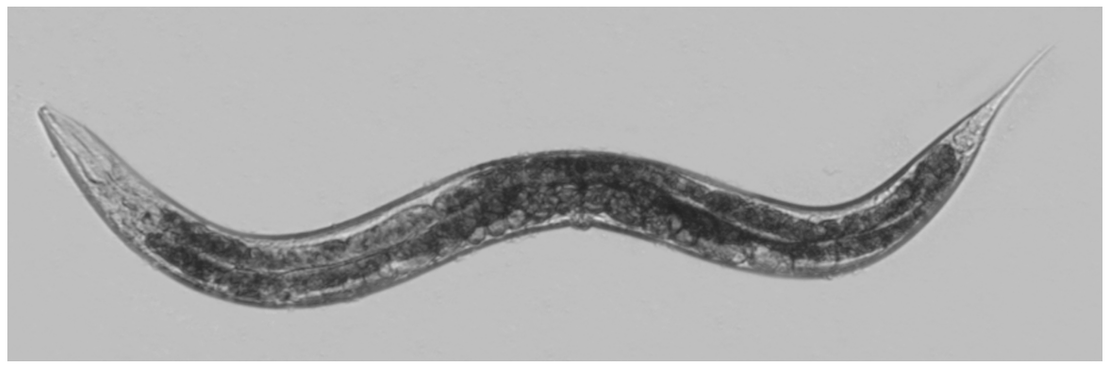
| Mutant | Phenotype |
|---|---|
| *lin-1(0)* | Muv |
| *lin-3(0)* | Vul |
| *let-60(0)* | Vul |
| *let-60(gf)* | Muv |
| *let-23(0)* | Vul |
| *let-23(gf)* | Muv |
| *lin-2(0)* | ~Vul |
| *lin-7(0)* | ~Vul |
| *lin-10(0)* | ~Vul |
| *lin-8(0); lin-9(0)* | synMuv |

**synMuv = synthetic Multivulva**

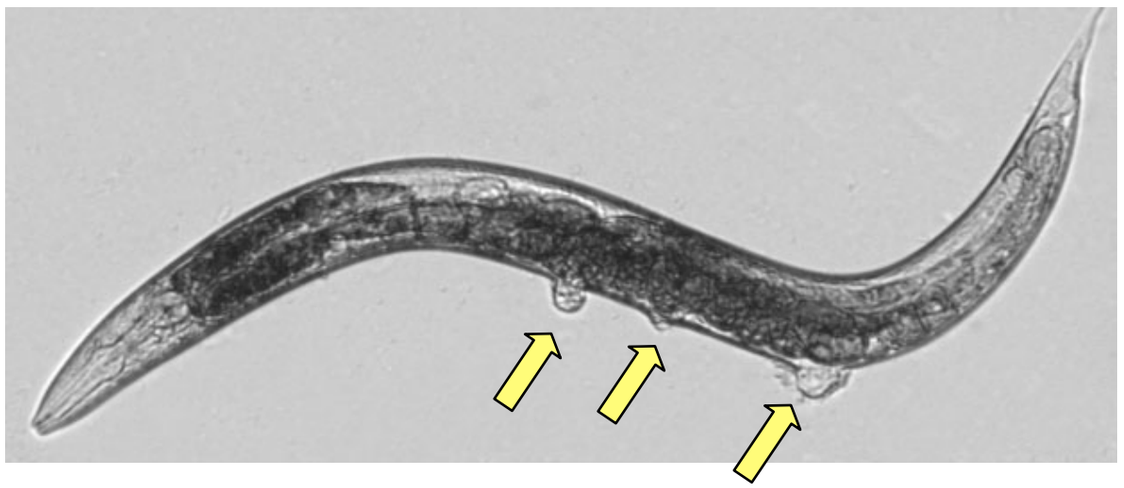
Two parallel pathways

# The synMuv phenotype is caused by mutations in both class A and B genes

class A single mutant



class B single mutant

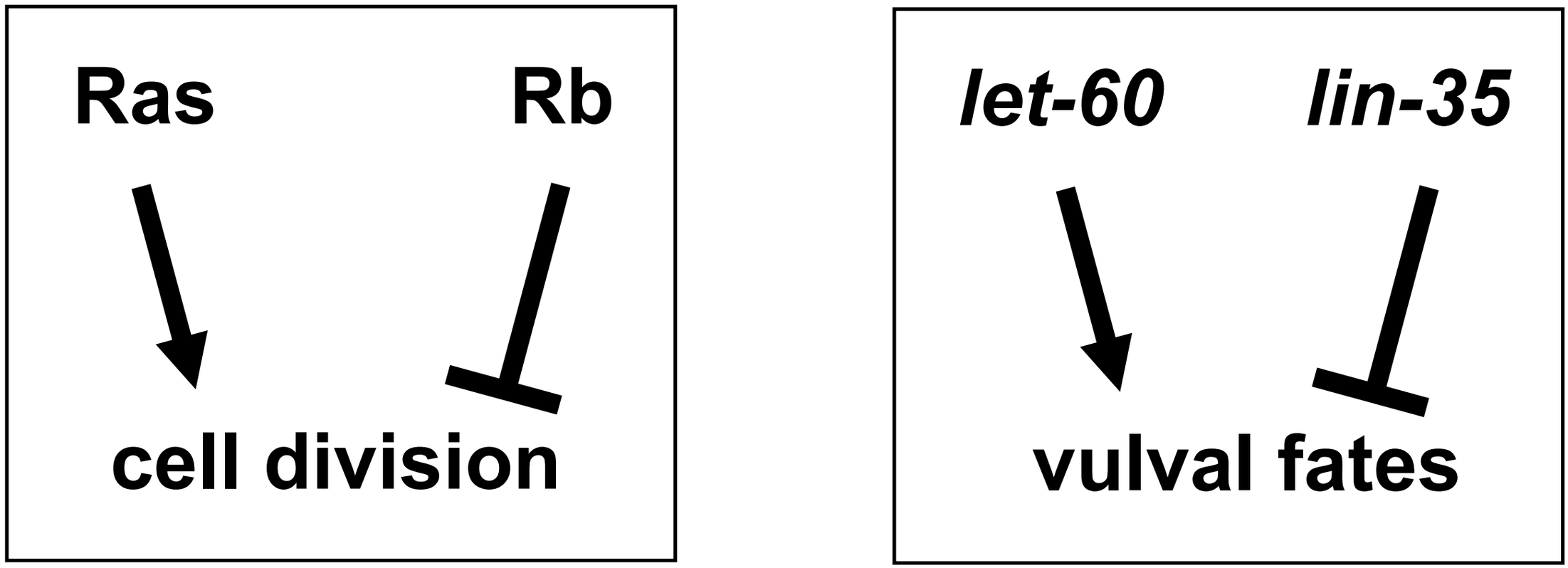


class AB double mutant

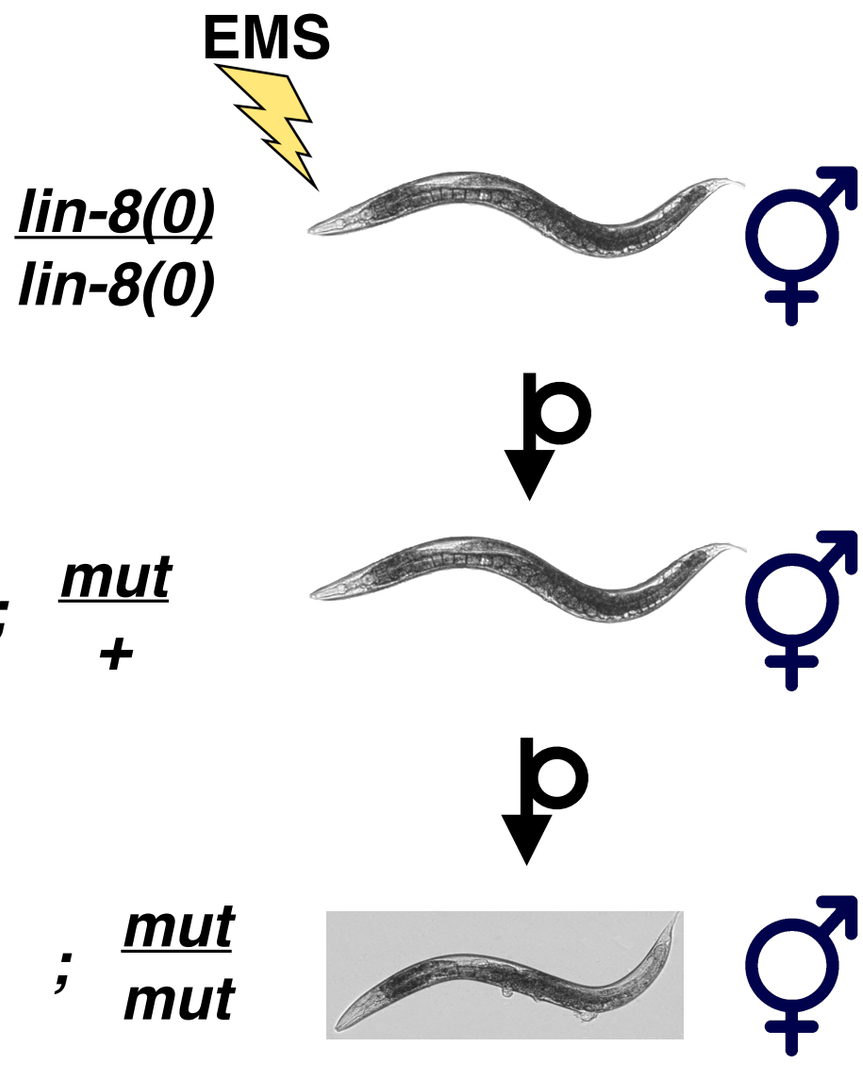
# Double mutants within the same class are not enhanced and not multivulva

| | Class A | Class B |
|---|---|---|
| Class A | non-Muv | Muv |
| Class B | | non-Muv |

# The vulval cell-fate decision models cell-fate decisions involved in carcinogenesis



# Enhancer screens to find more synthetic multivulva genes



# The synMuv genes

## class A

| | |
|---|---|
| ***lin-8*** | novel |
| ***lin-15A*** | THAP |
| ***lin-38*** | Zn finger |
| ***lin-56*** | THAP |

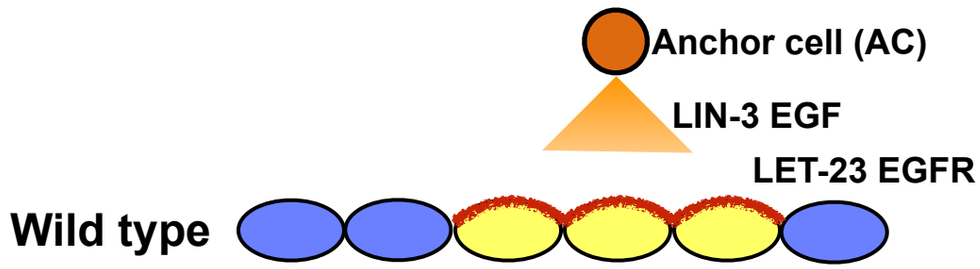
## class B

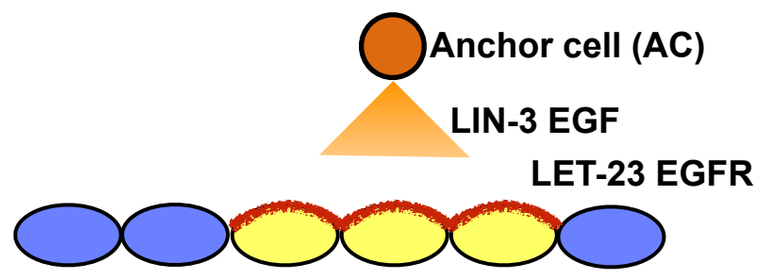
| | |
|---|---|
| ***lin-9*** | Mip130/ALY |
| ***lin-13*** | Zn fingers |
| ***lin-15B*** | THAP |
| ***lin-36*** | THAP |
| ***lin-37*** | Mip40 |
| ***lin-52*** | dLin52 |
| ***lin-54*** | Mip120 |
| ***lin-61*** | l(3)MBT |
| ***lin-65*** | novel |
| ***tam-1*** | RING finger |

| | |
|---|---|
| ***dpl-1*** | DP |
| ***efl-1*** | E2F4 |
| ***lin-35*** | Rb |
| ***lin-53*** | RbAp48 |
| ***hda-1*** | HDAC1 |
| ***let-418*** | Mi-2 |
| ***mep-1*** | Zn fingers |
| ***hpl-2*** | HP1 |
| ***gap-1*** | RasGAP |
| ***sli-1*** | c-Cbl |
| ***ark-1*** | Ack |

| | |
|---|---|
| ***trr-1*** | TRRAP |
| ***mys-1*** | HAT |
| ***epc-1*** | E(Pc) |

# Cell autonomy of synMuv genes



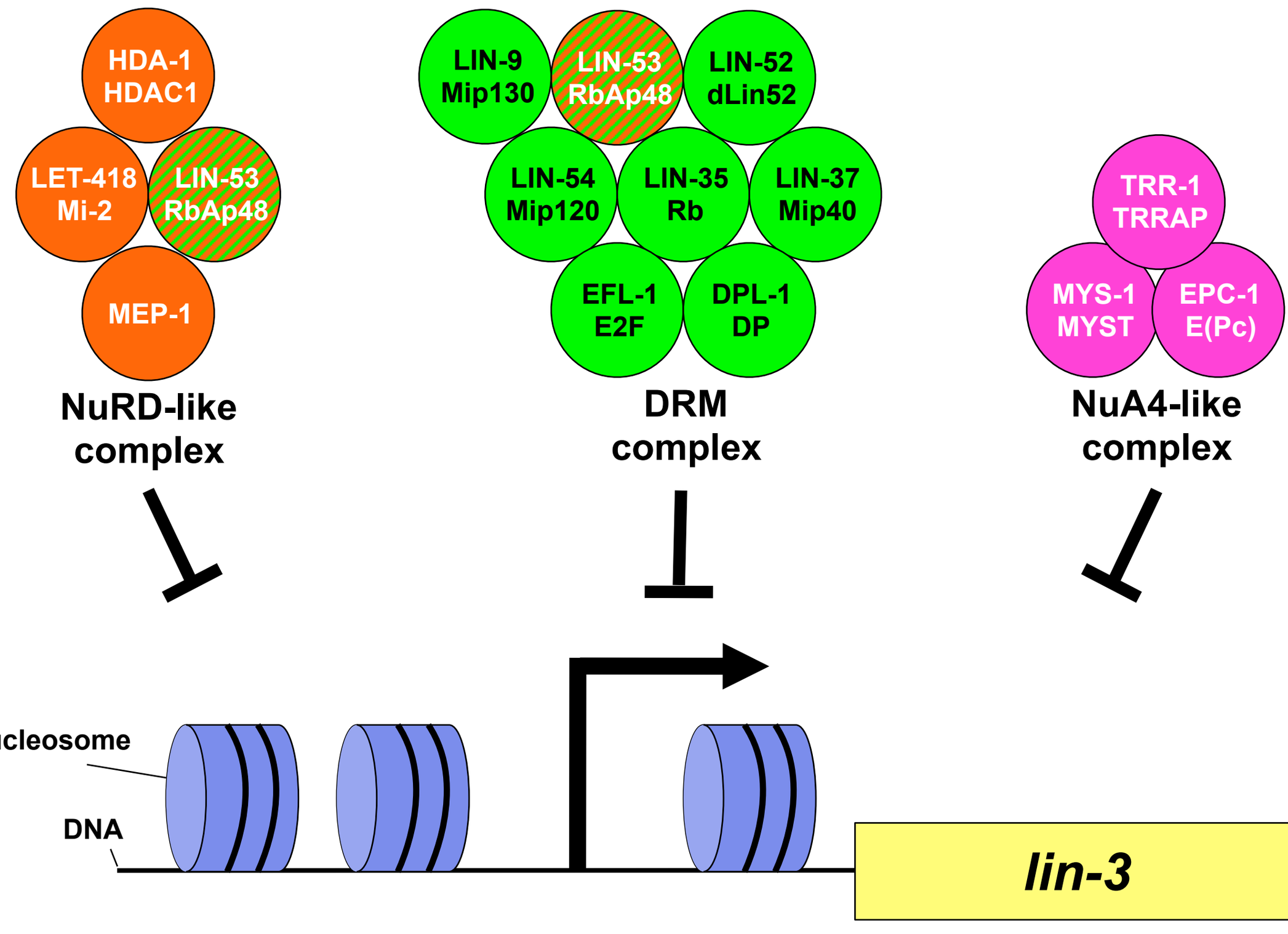




**synMuv gene rescue in AC, Pn.ps, or hypodermis**

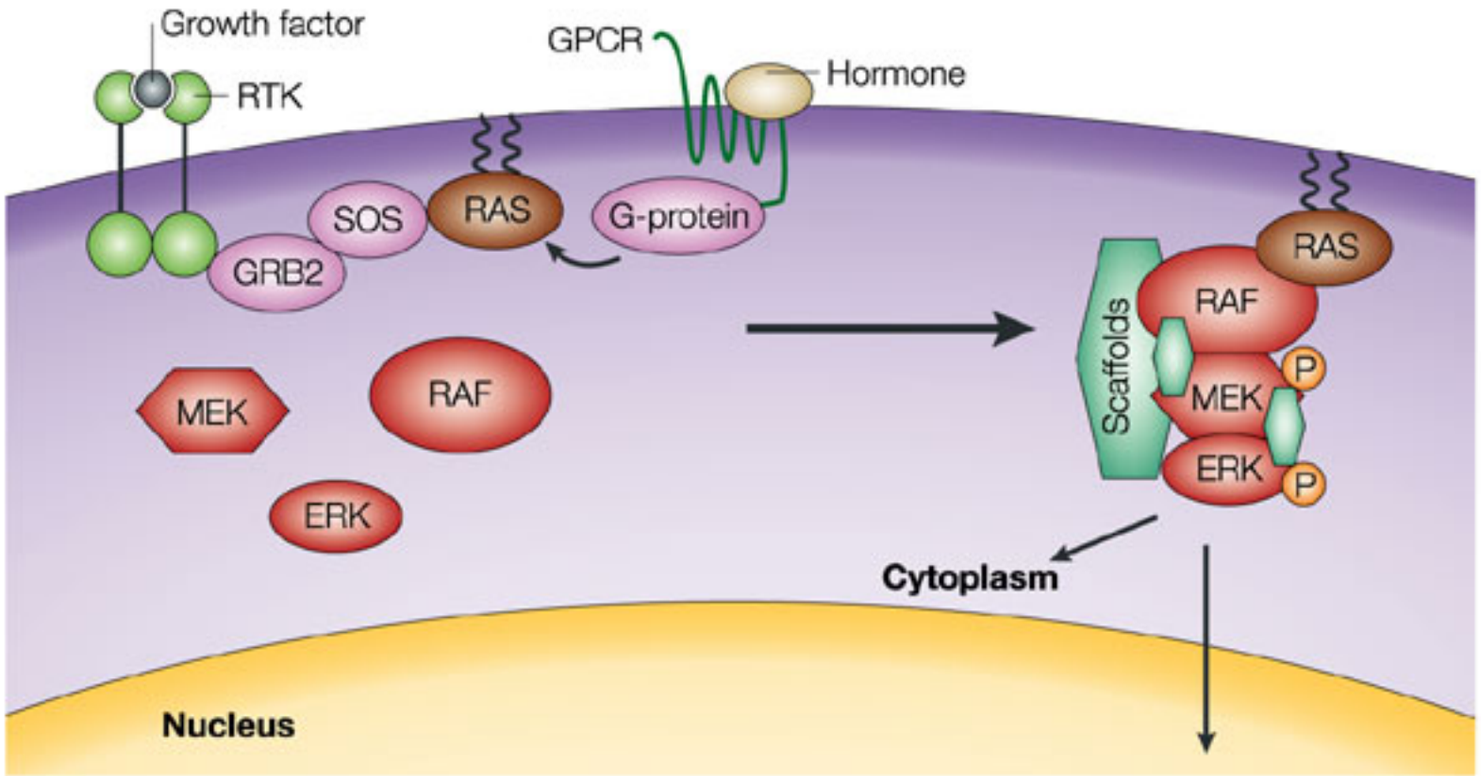
**Rescue vulval phenotype**

Cell non-autonomous action

# NuRD-like, DRM, and NuA4-like complexes affect the transcription of vulval genes



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

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**We NEED basic research for this reason!**