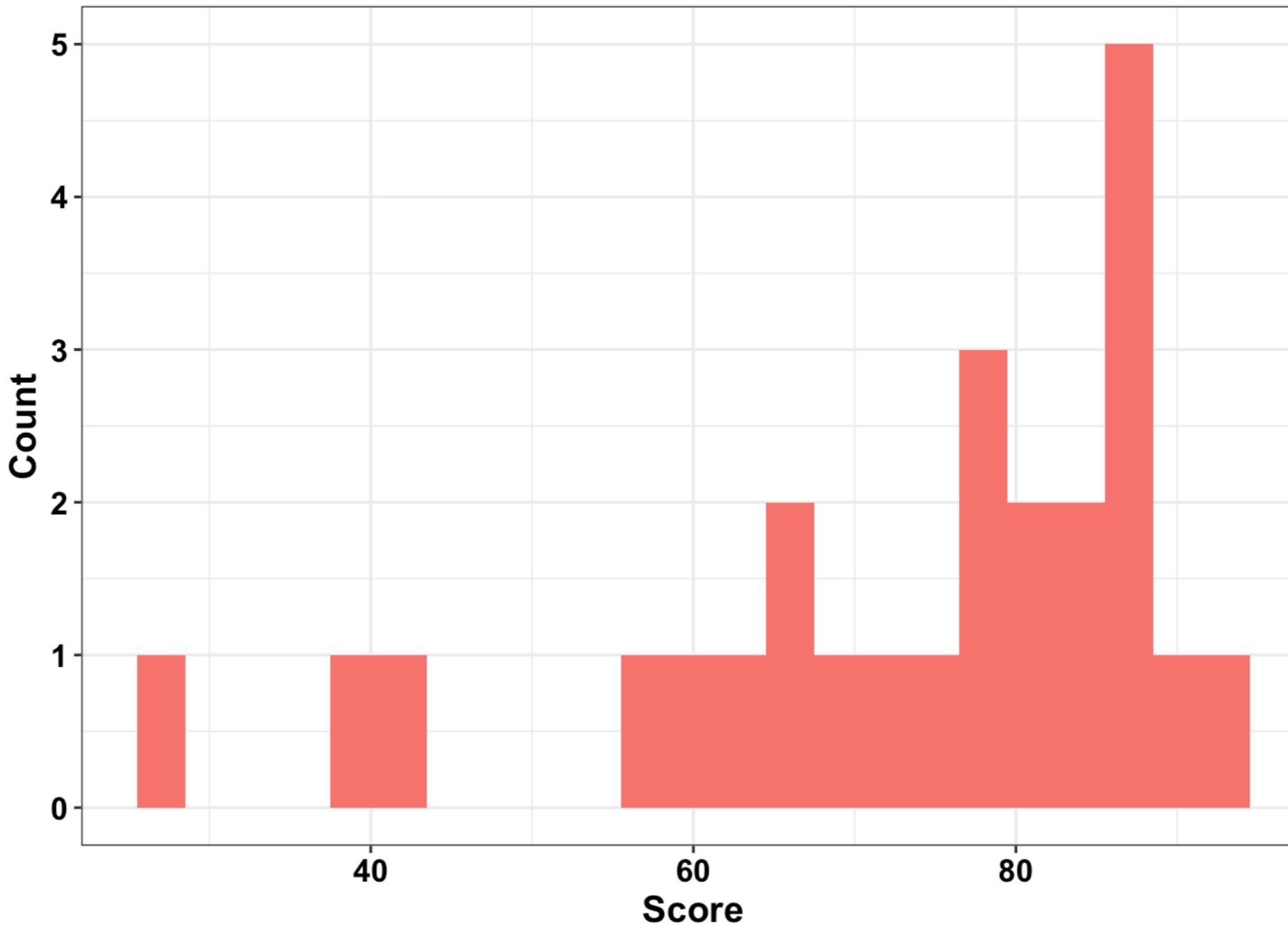
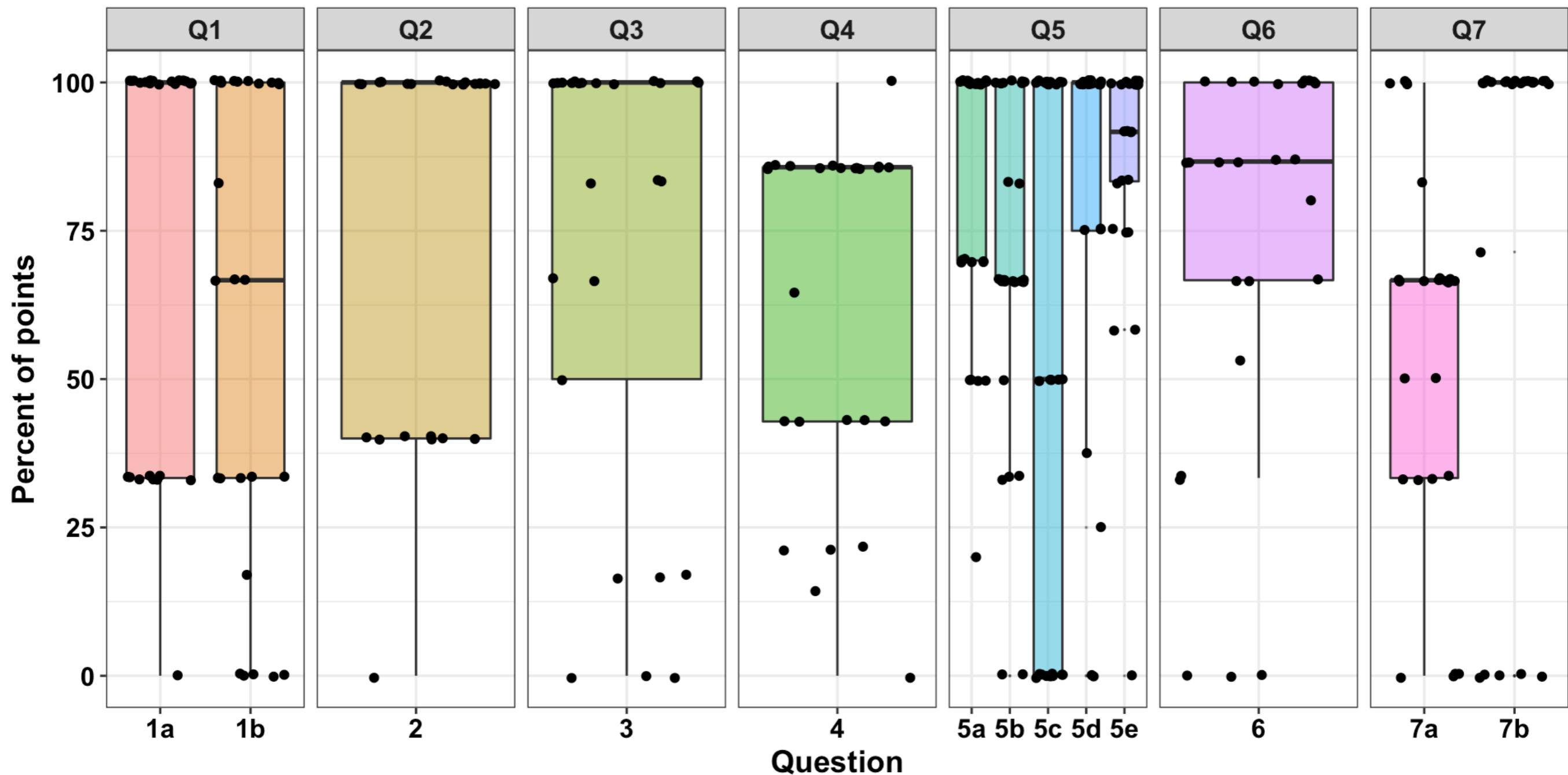


# The exam was hard.



**Mean 72.88, Median 78, SD 16.97**

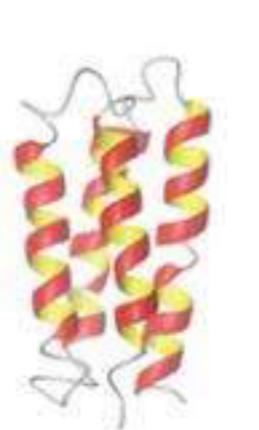


Please take the post-midterm survey at  
[bio393.andersenlab.org](http://bio393.andersenlab.org)

# 17. Determine time of gene action

When does the gene function?

1. Induce expression to rescue a mutant phenotype at a specific time
2. Use temperature-labile mutants to define the temperature-sensitive period



**WT**

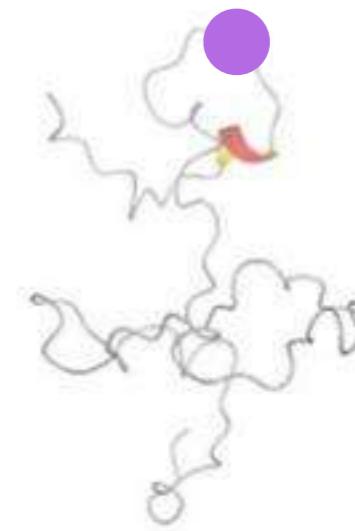


**Mutant**

Permissive temperature

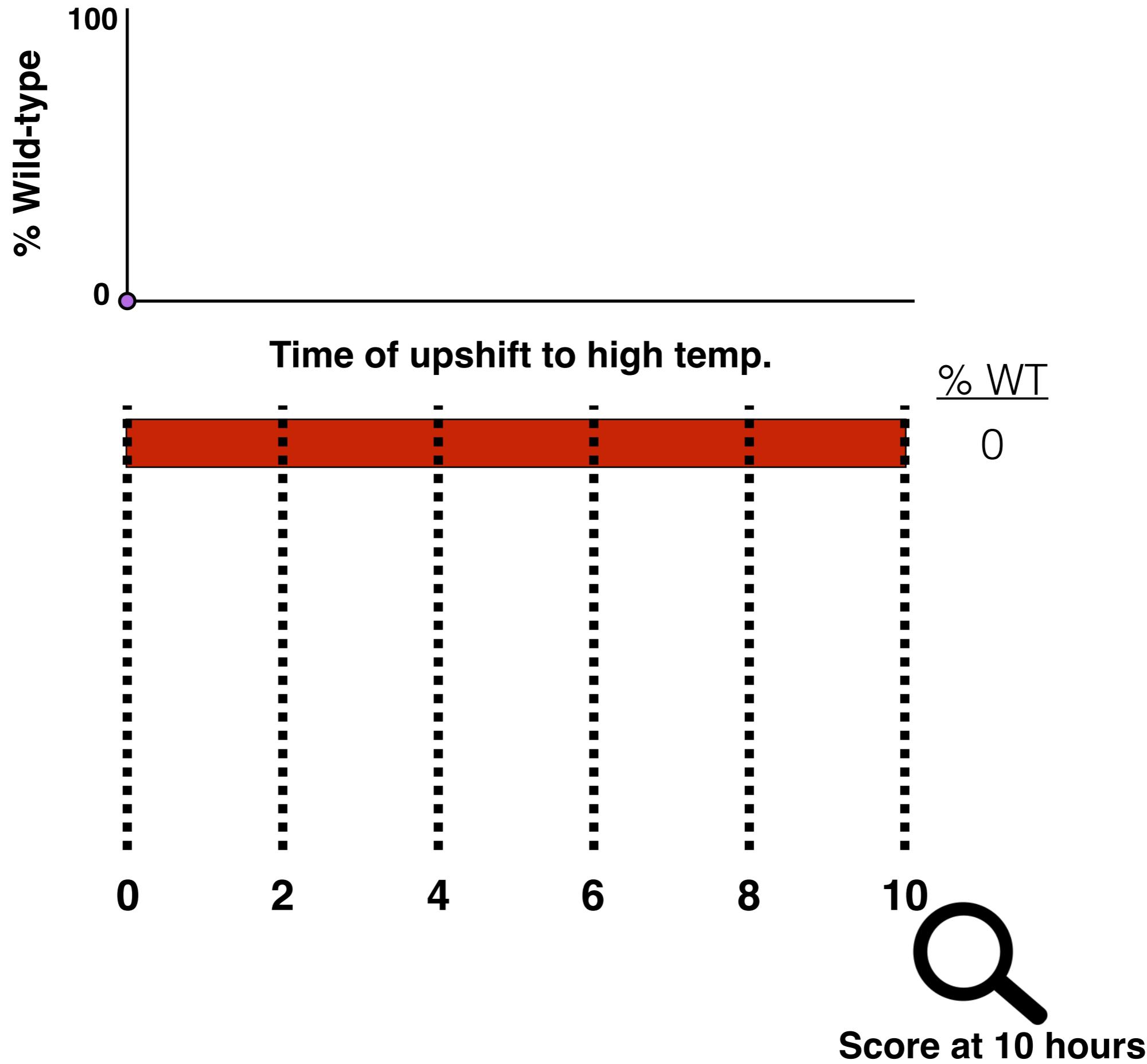


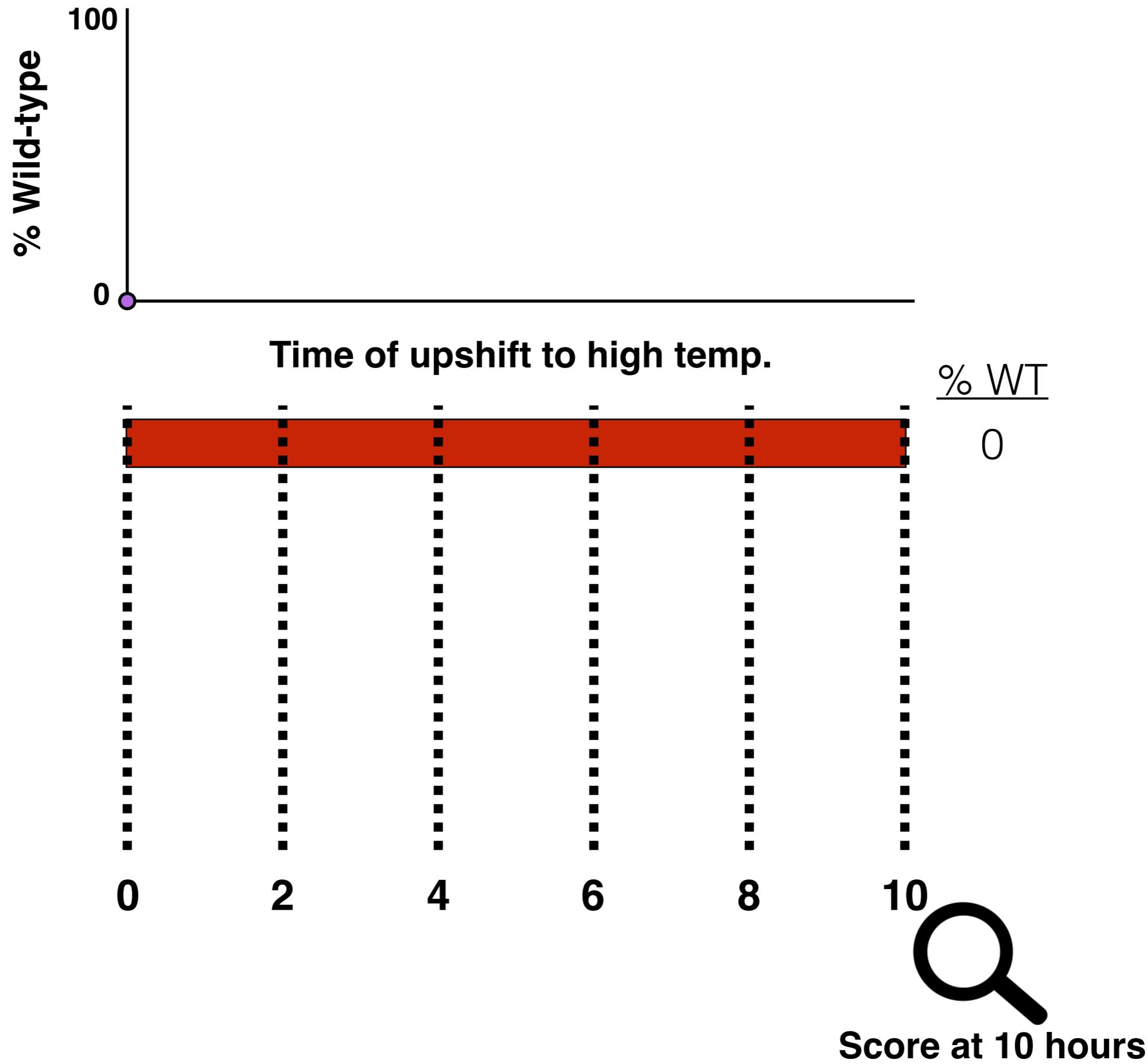
**WT**

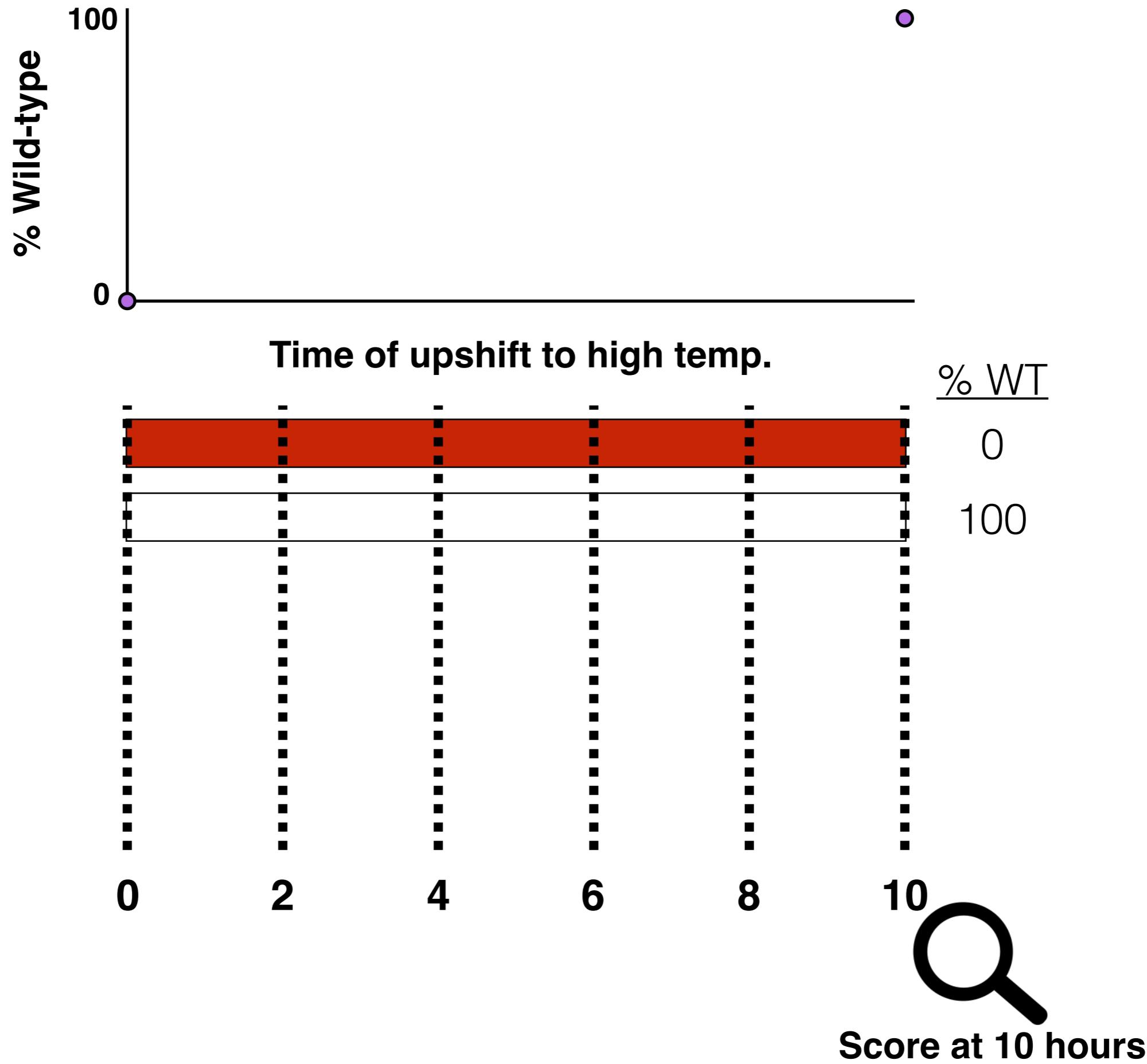


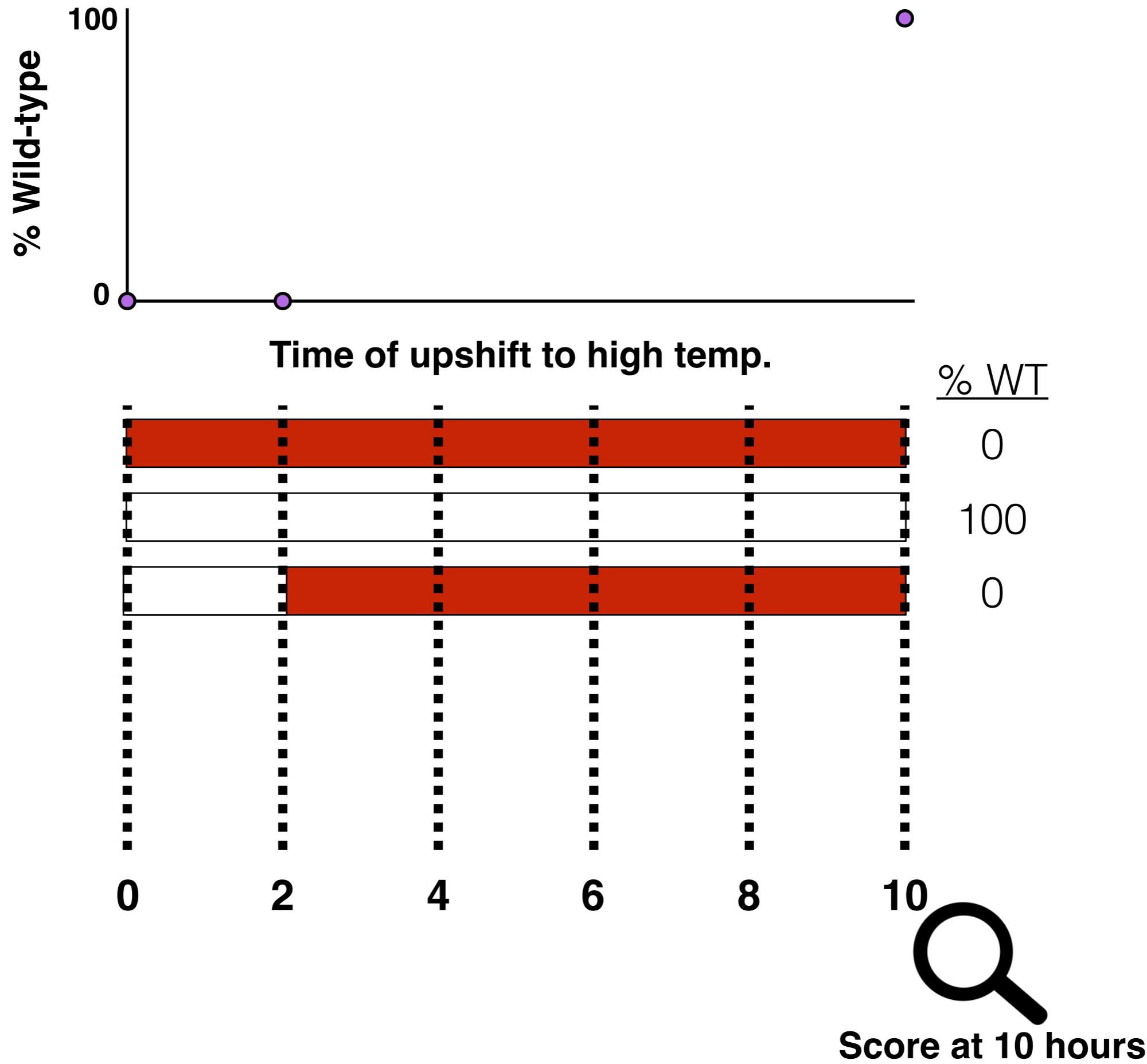
**Mutant**

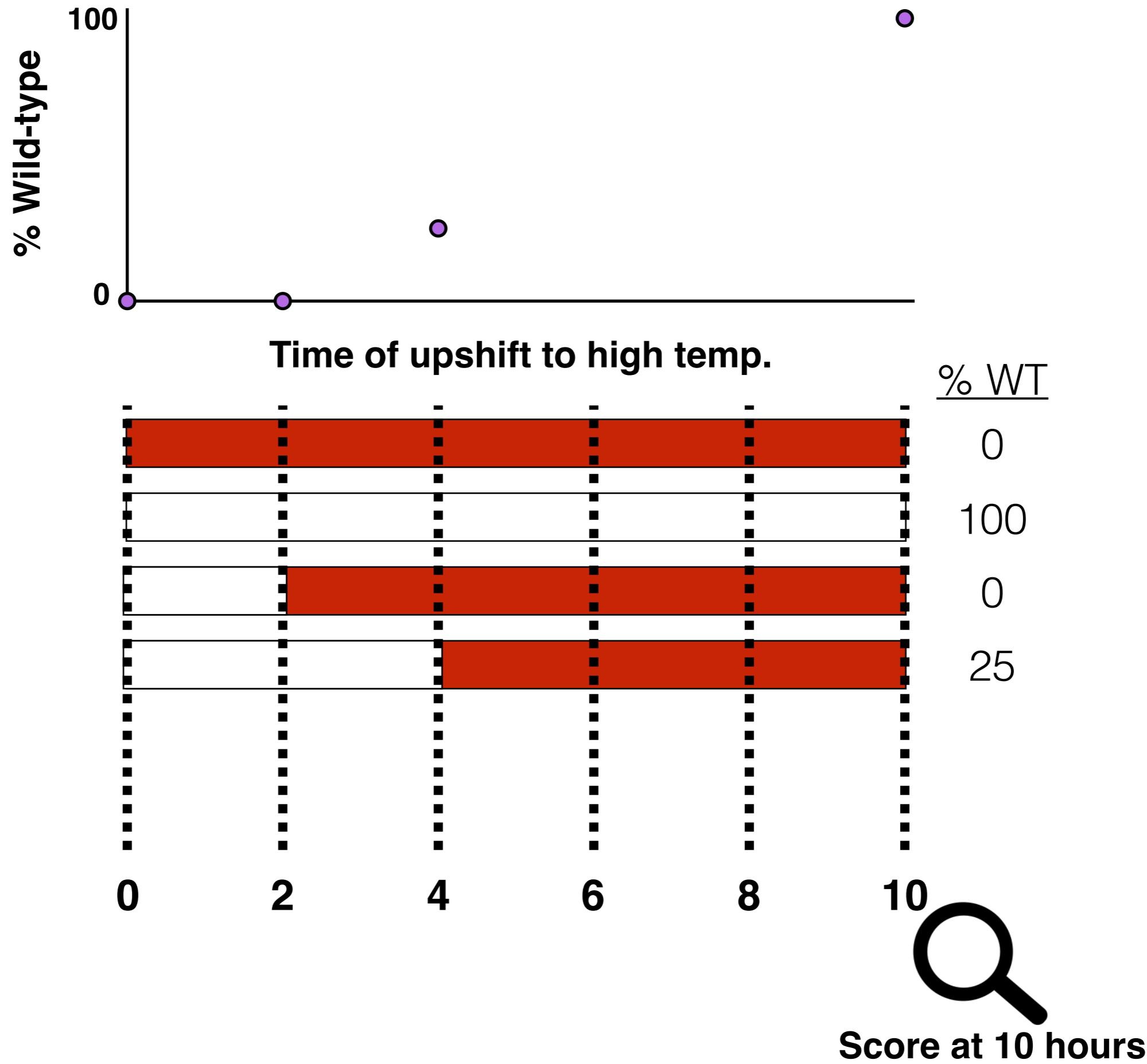
Restrictive temperature

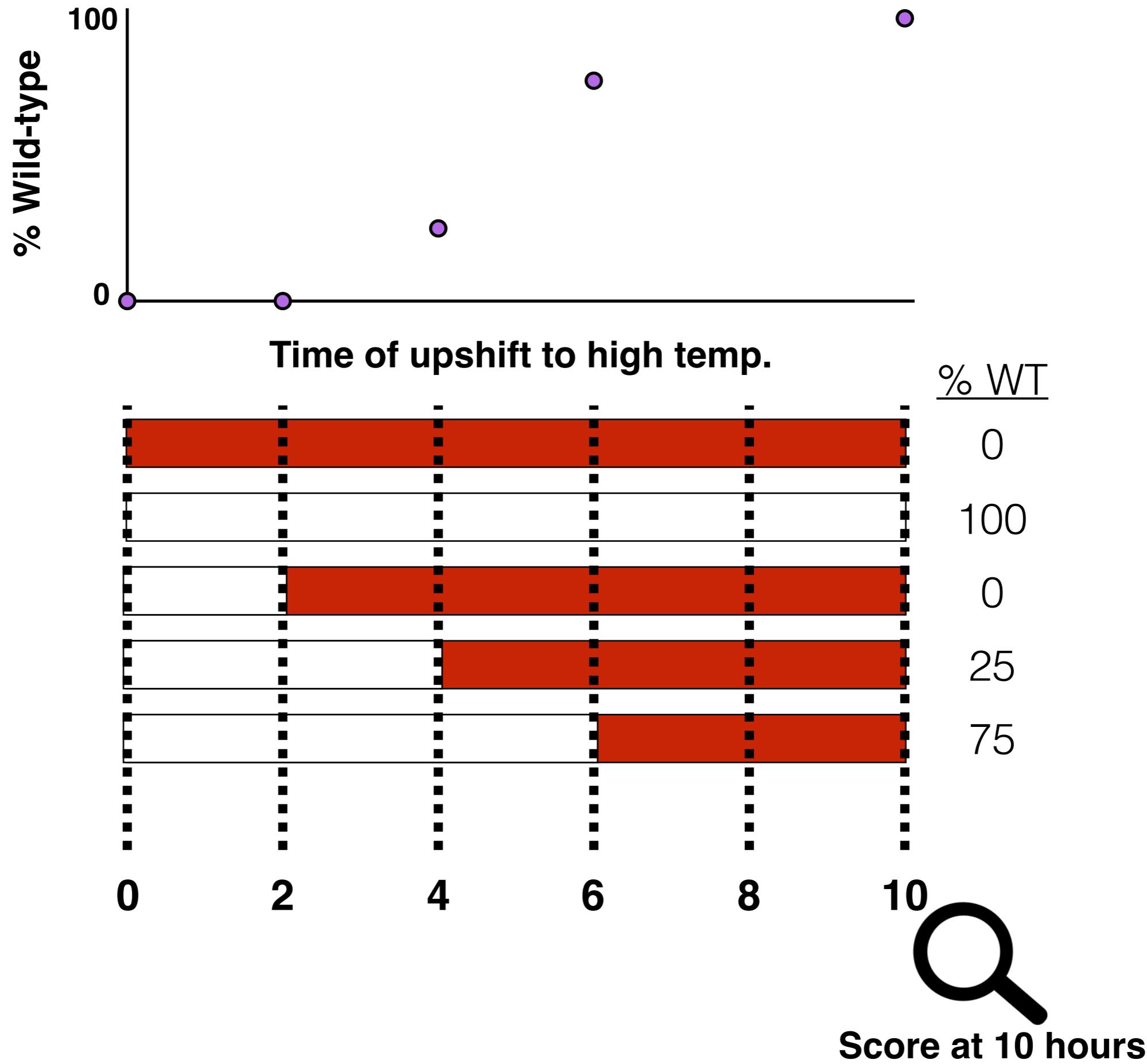


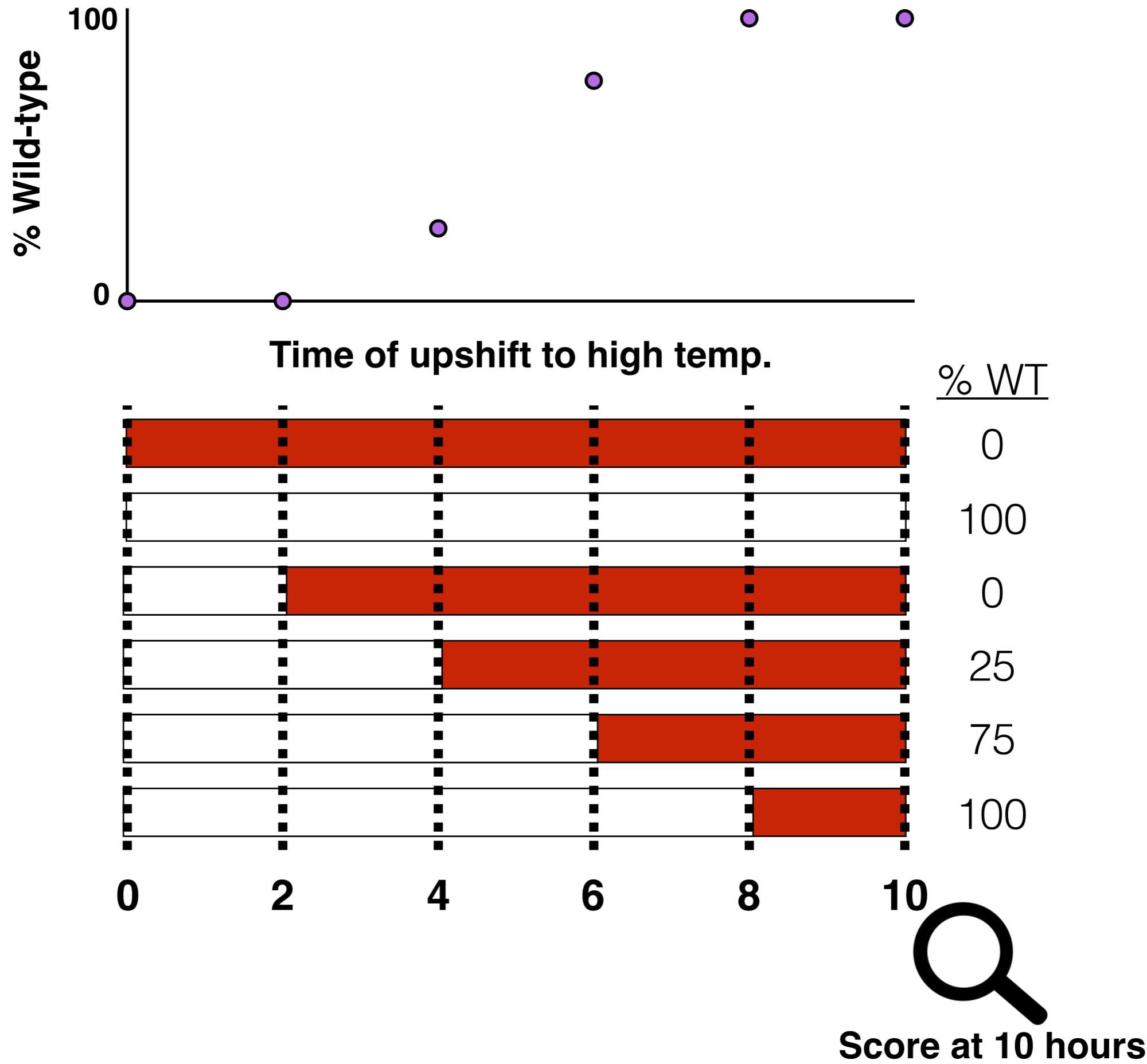


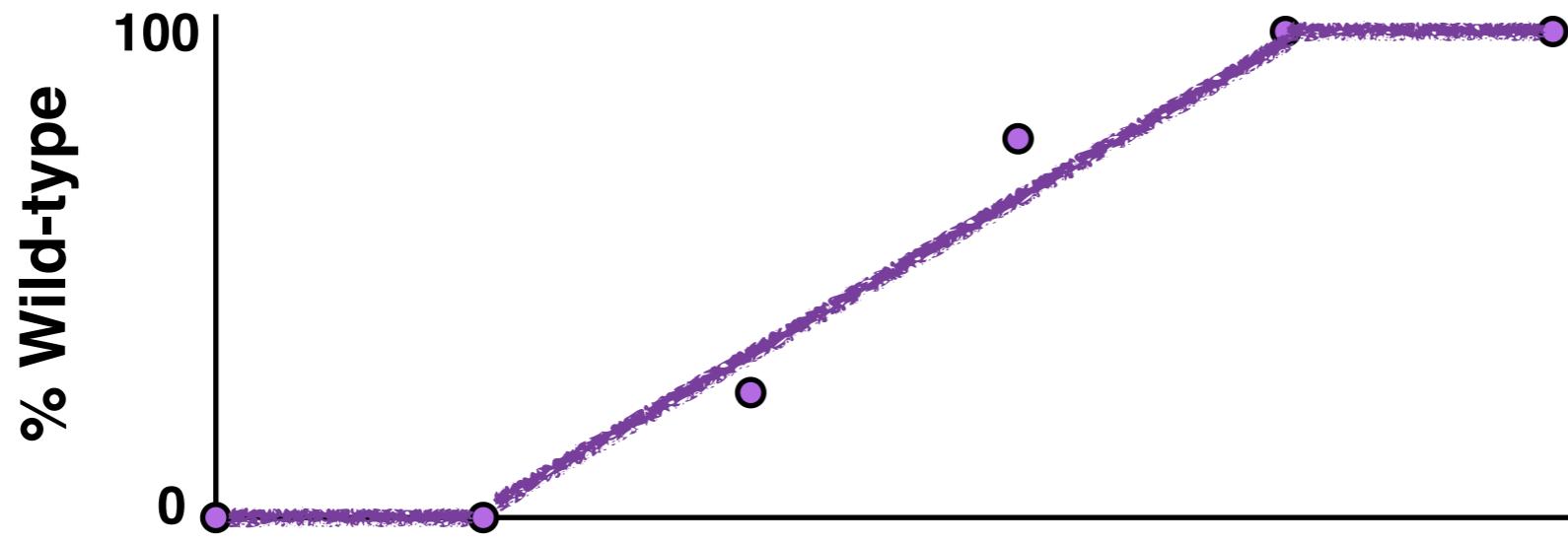






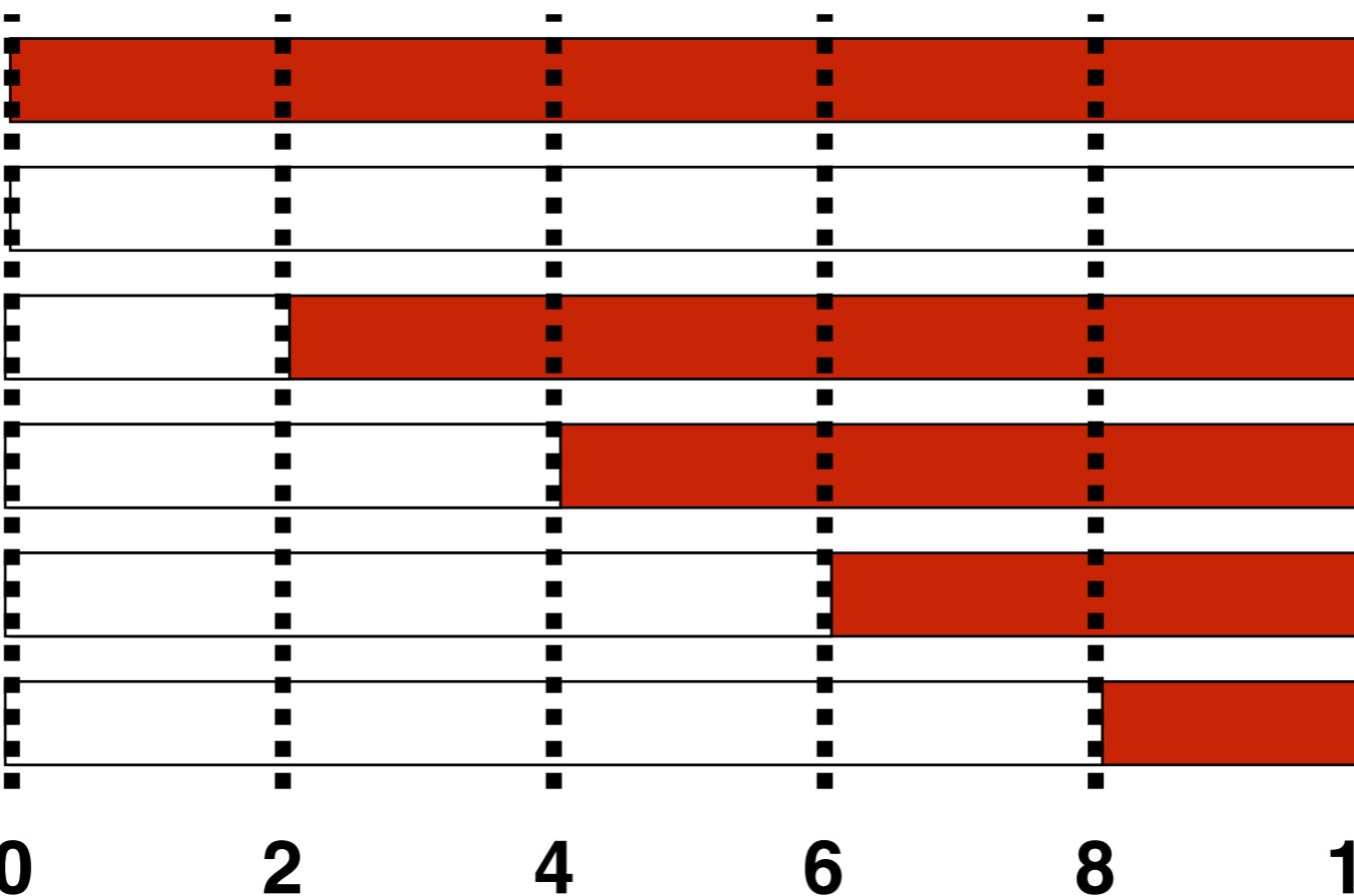




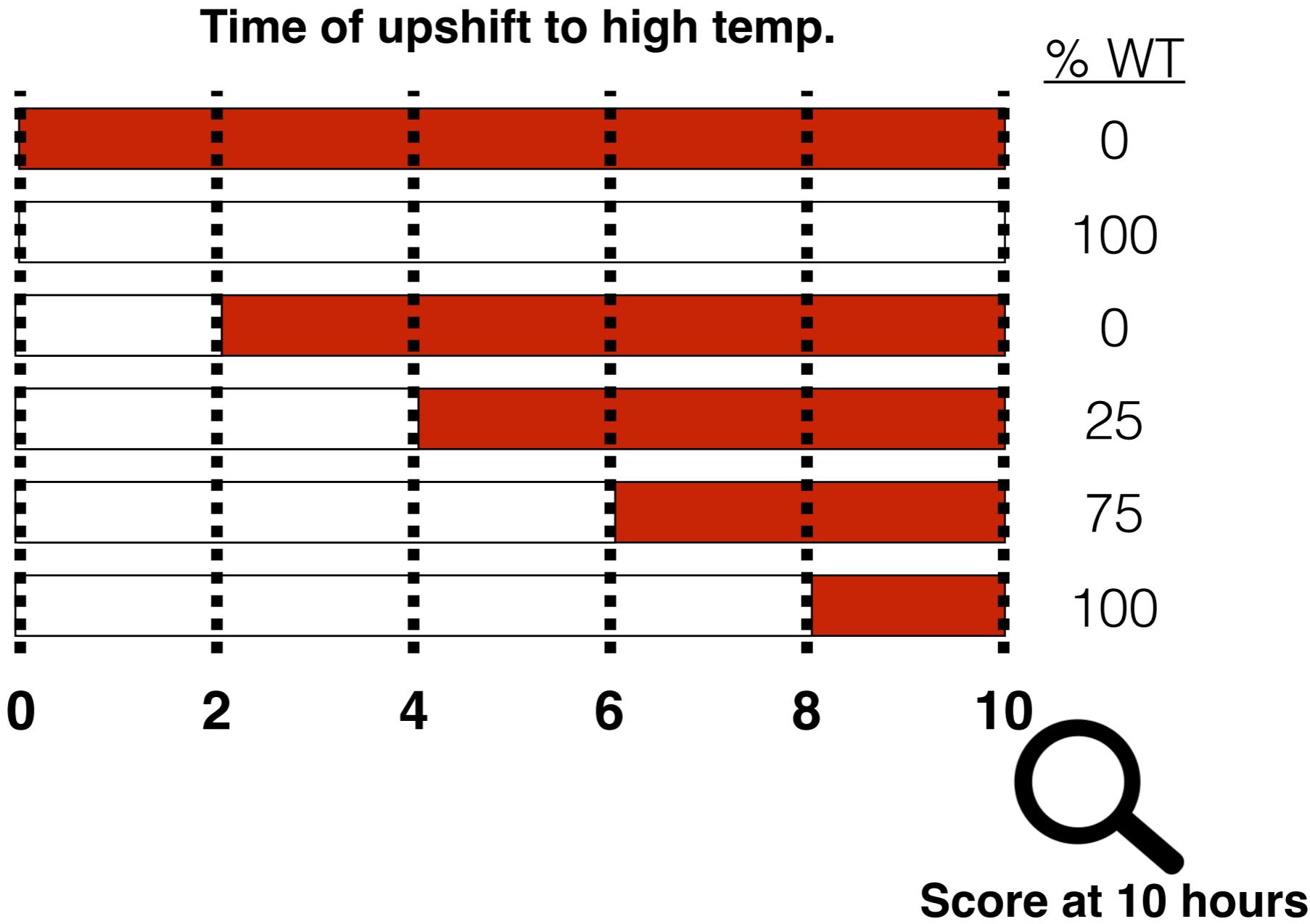
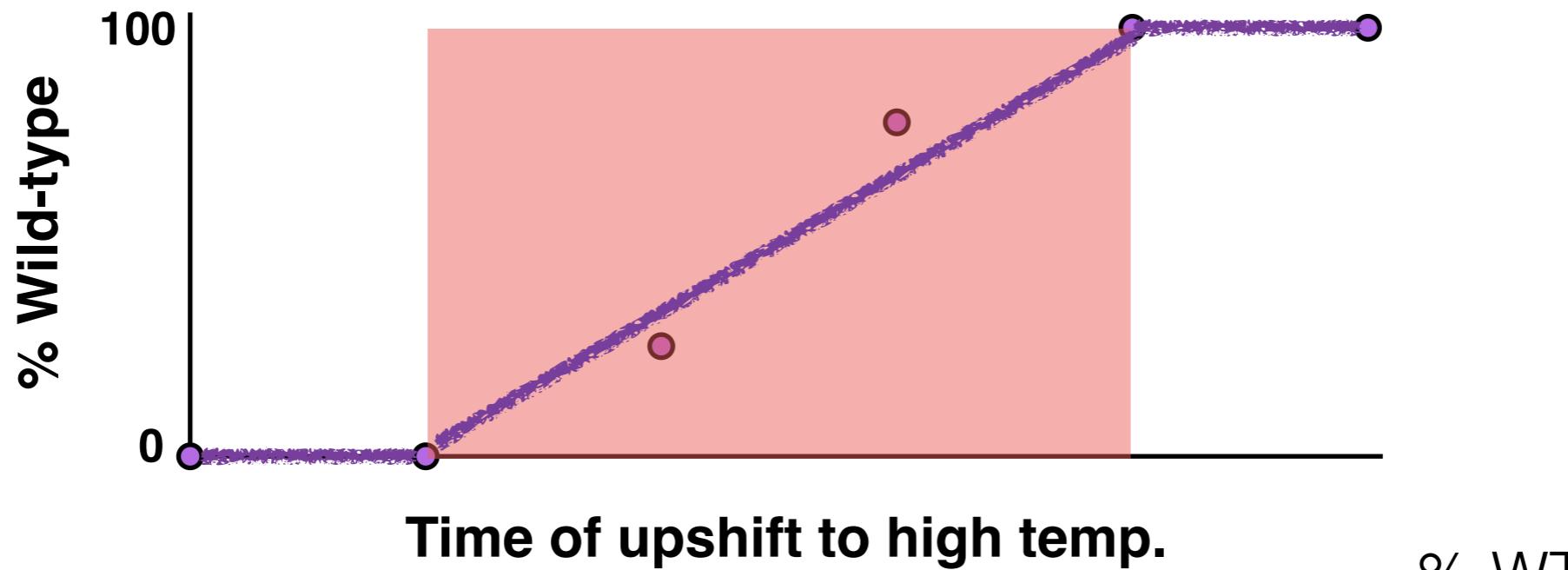


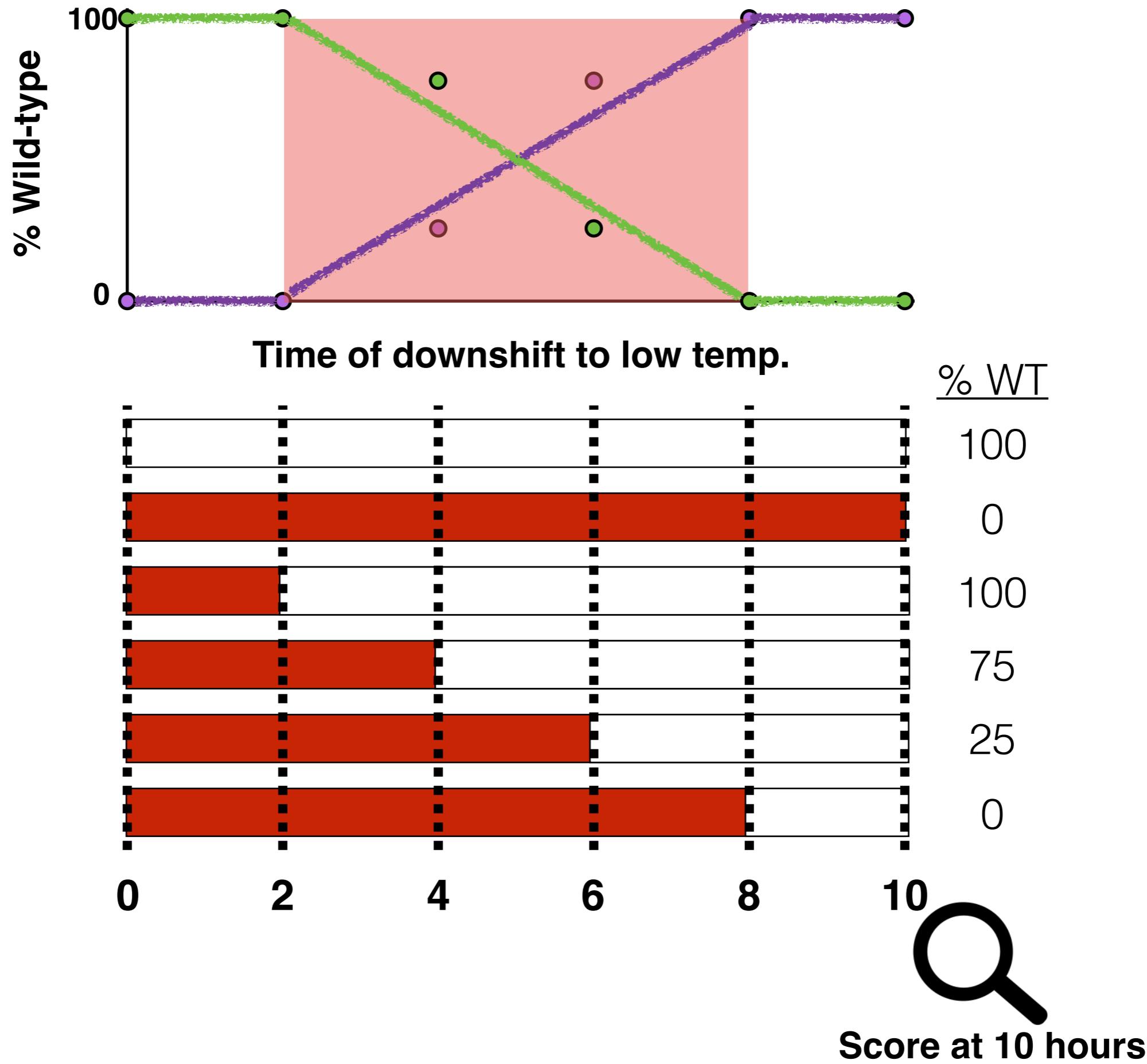
Time of upshift to high temp.

% WT

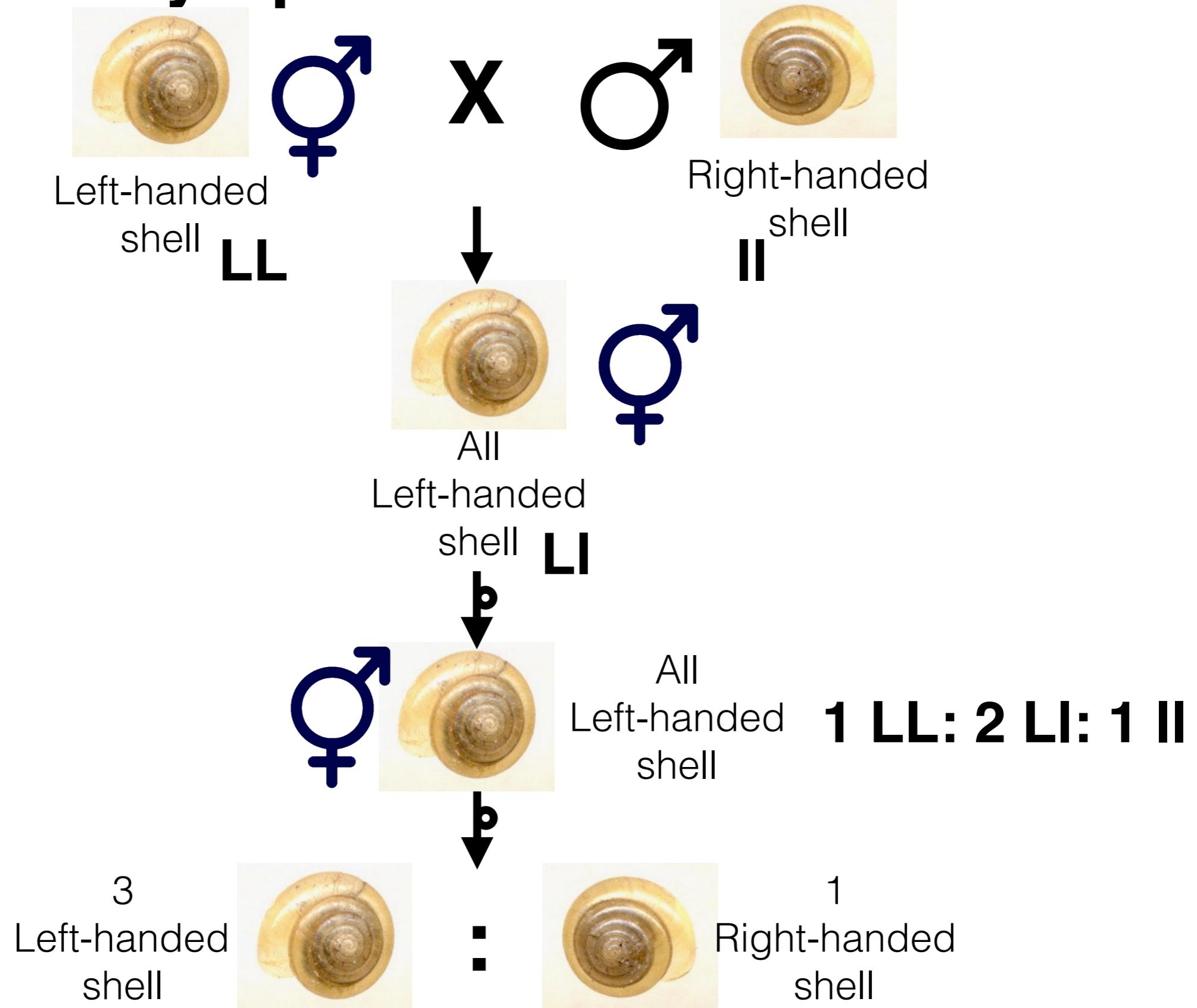


Score at 10 hours



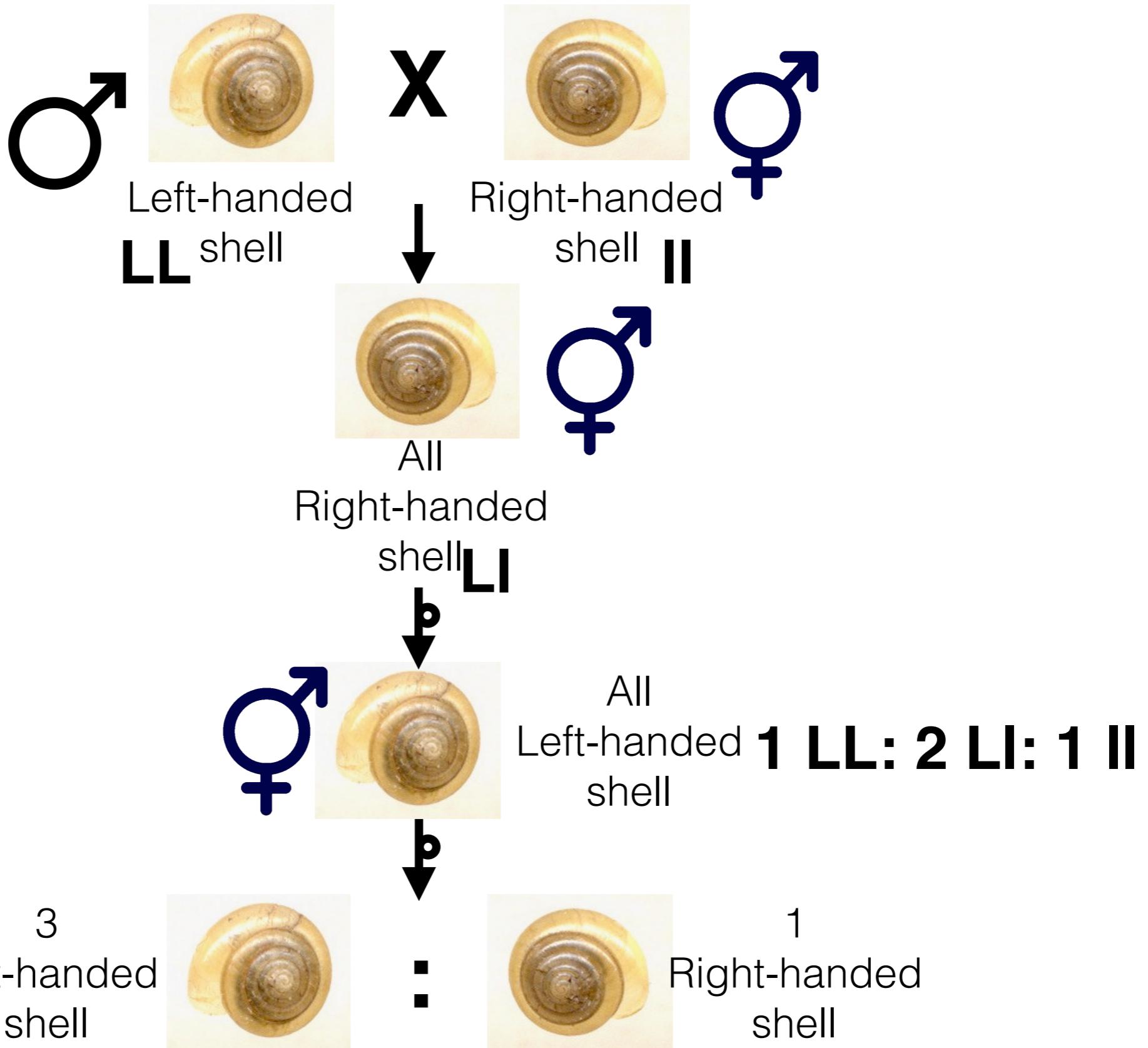


# 18. Determine if there are maternal effects or cytoplasmic inheritance



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

Reciprocal cross



# 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



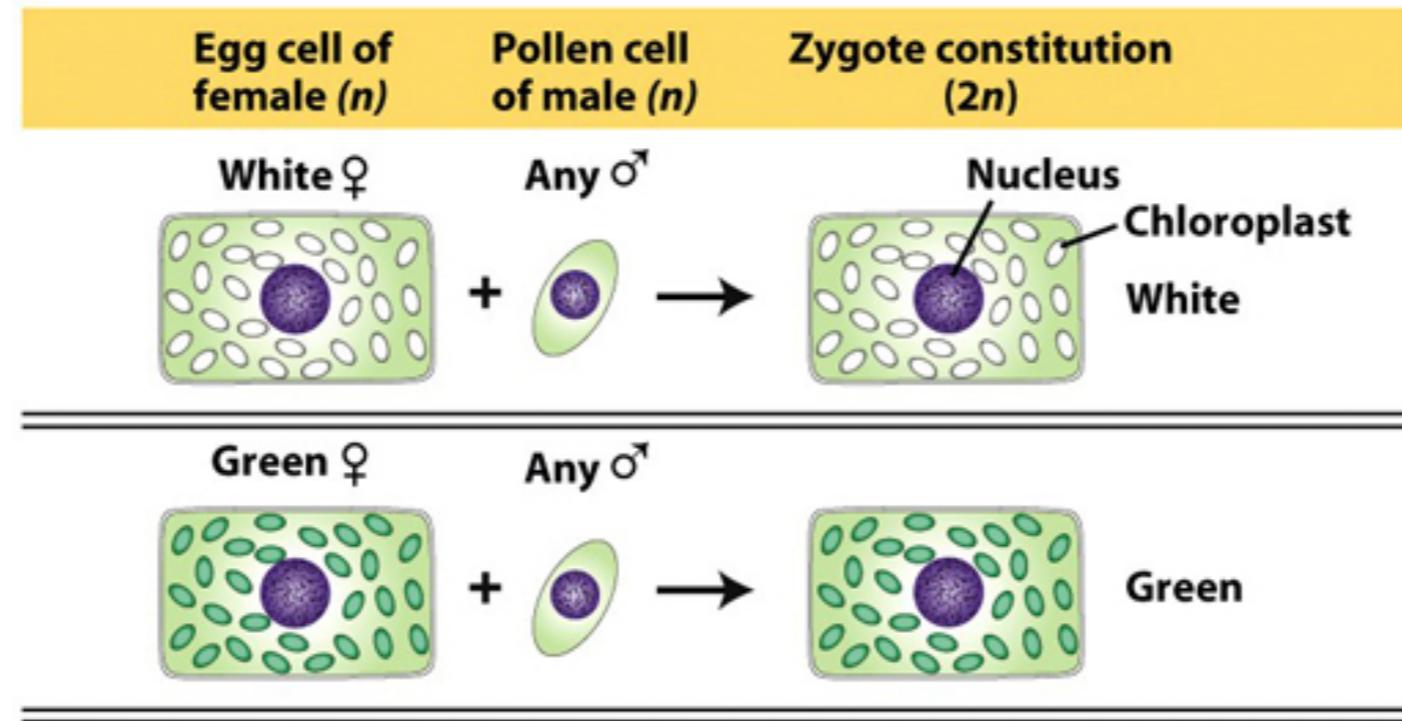
Plastid inheritance in  
*Mirabilis jalapa*

Only the color  
of the stem (mother)  
matters

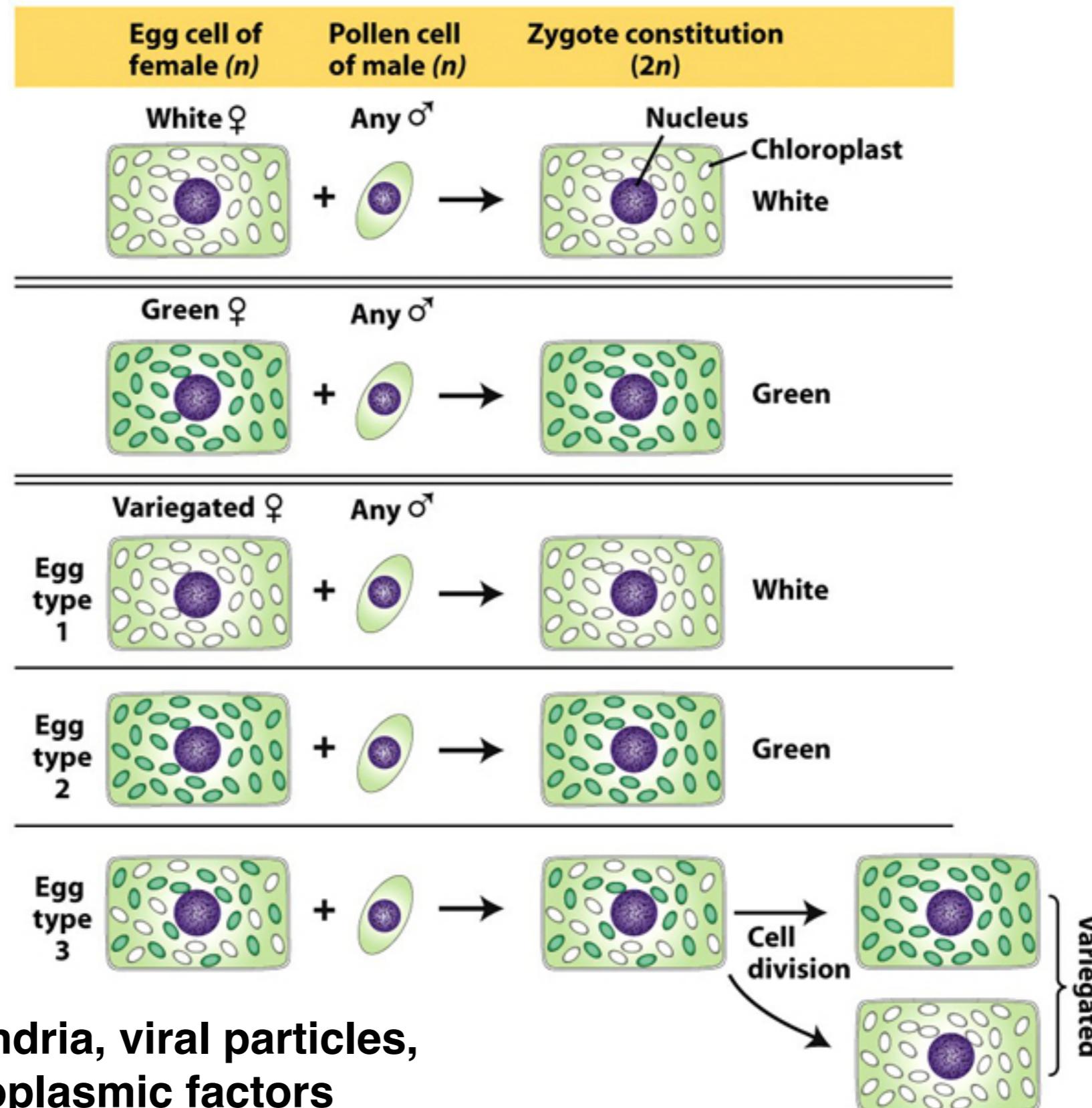
White, green, or  
variegated



# 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

# **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 I



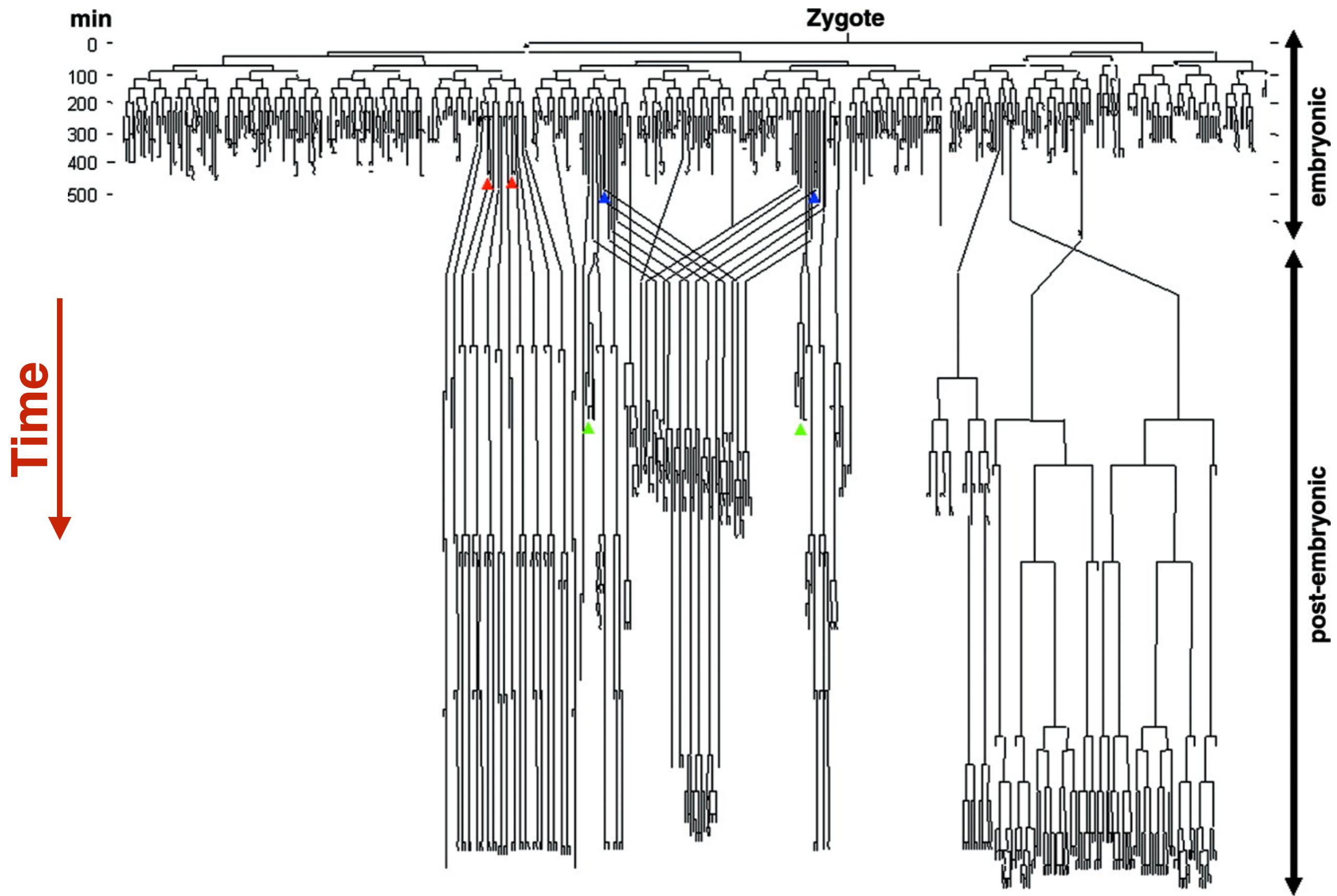
*C. elegans*

**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

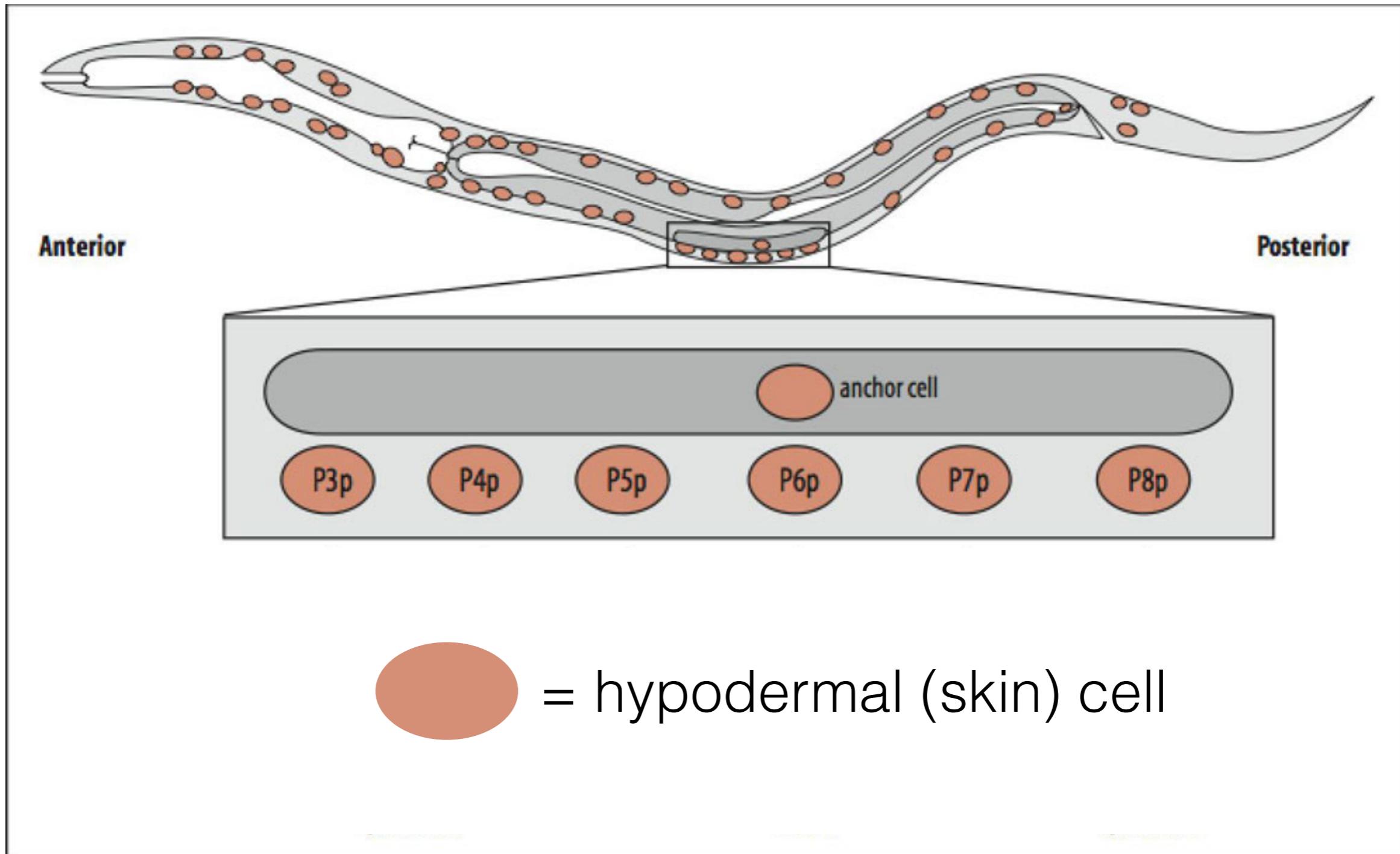


# The cell lineage of *C. elegans* is known and invariant



**John Sulston**

# *C. elegans* vulval development

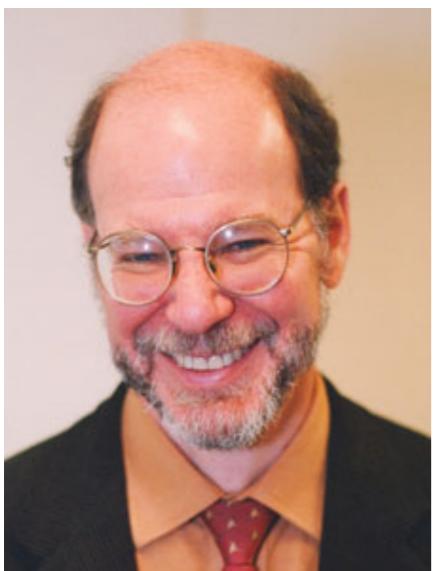
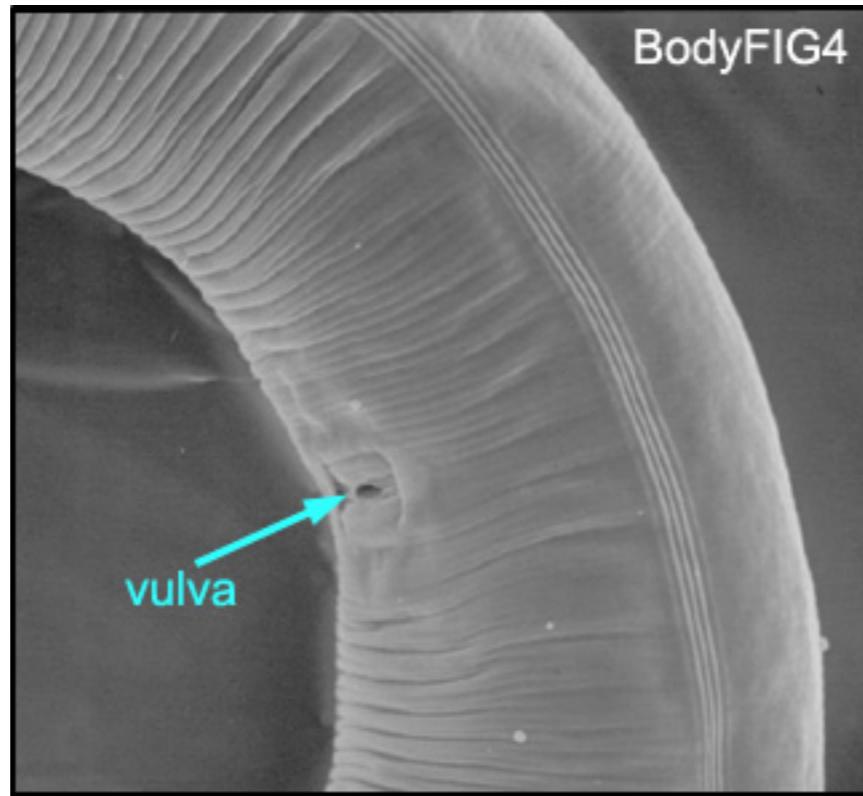


# One of the best genetic screens ever

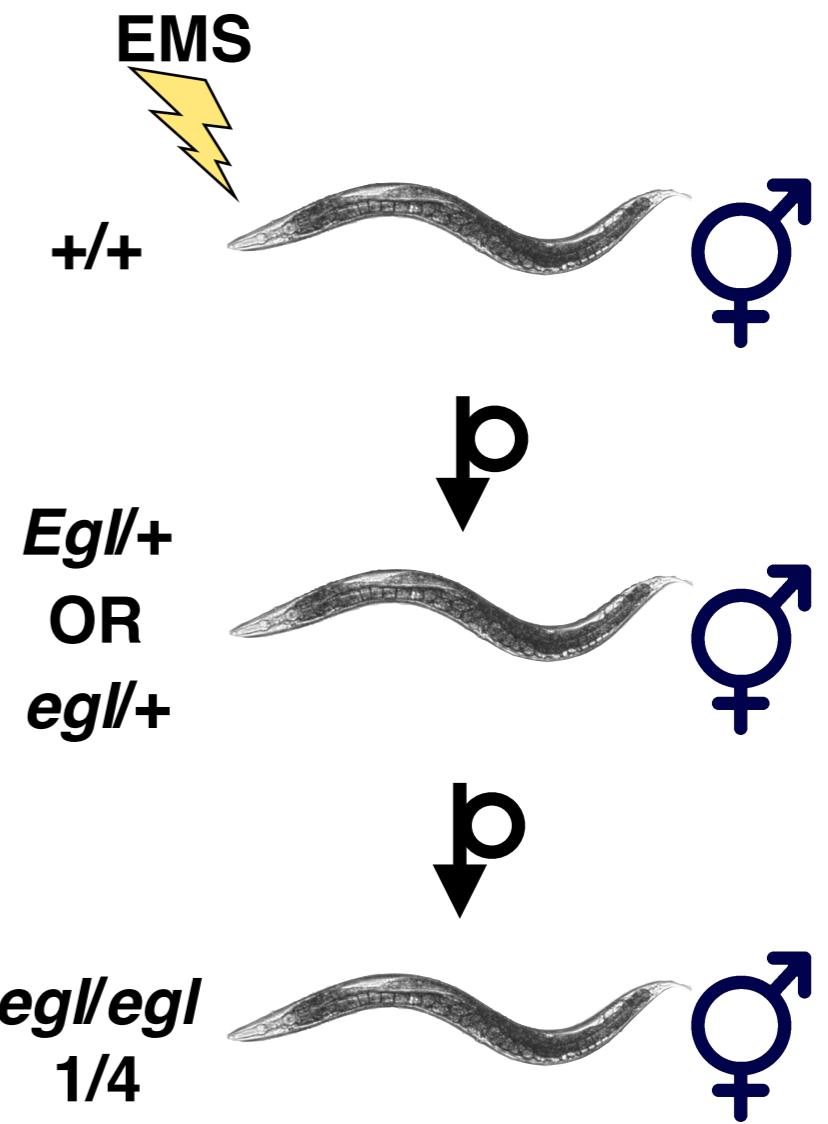


Let's say you screened for mutants that failed to lay eggs

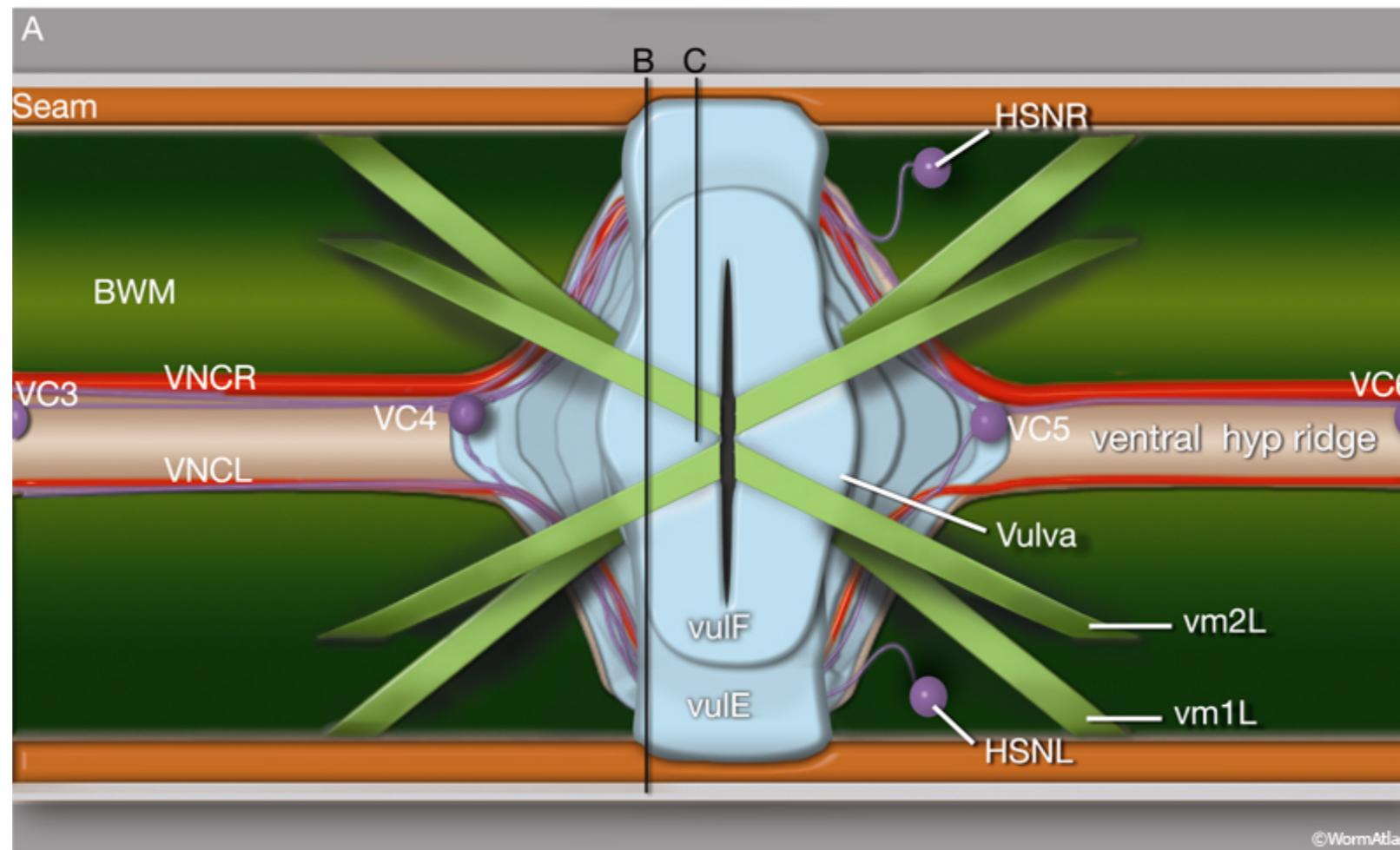
Called Egl for egg-laying defective



Bob Horvitz



# One of the best genetic screens ever

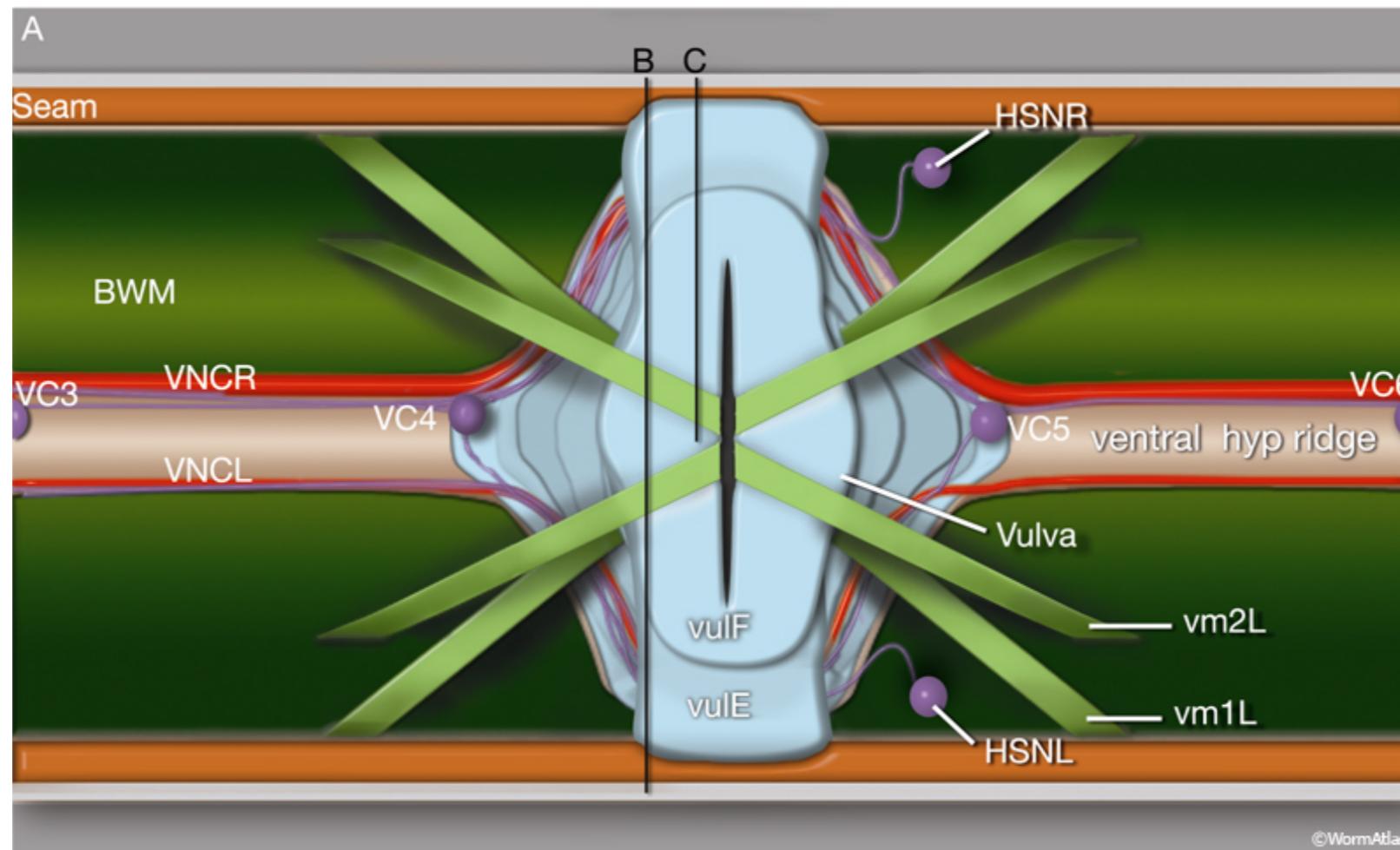


No neuron

*egl-1* = inducer of programmed cell death

Hermaphrodite-Specific Neuron (HSN)  
inappropriately dies

# One of the best genetic screens ever

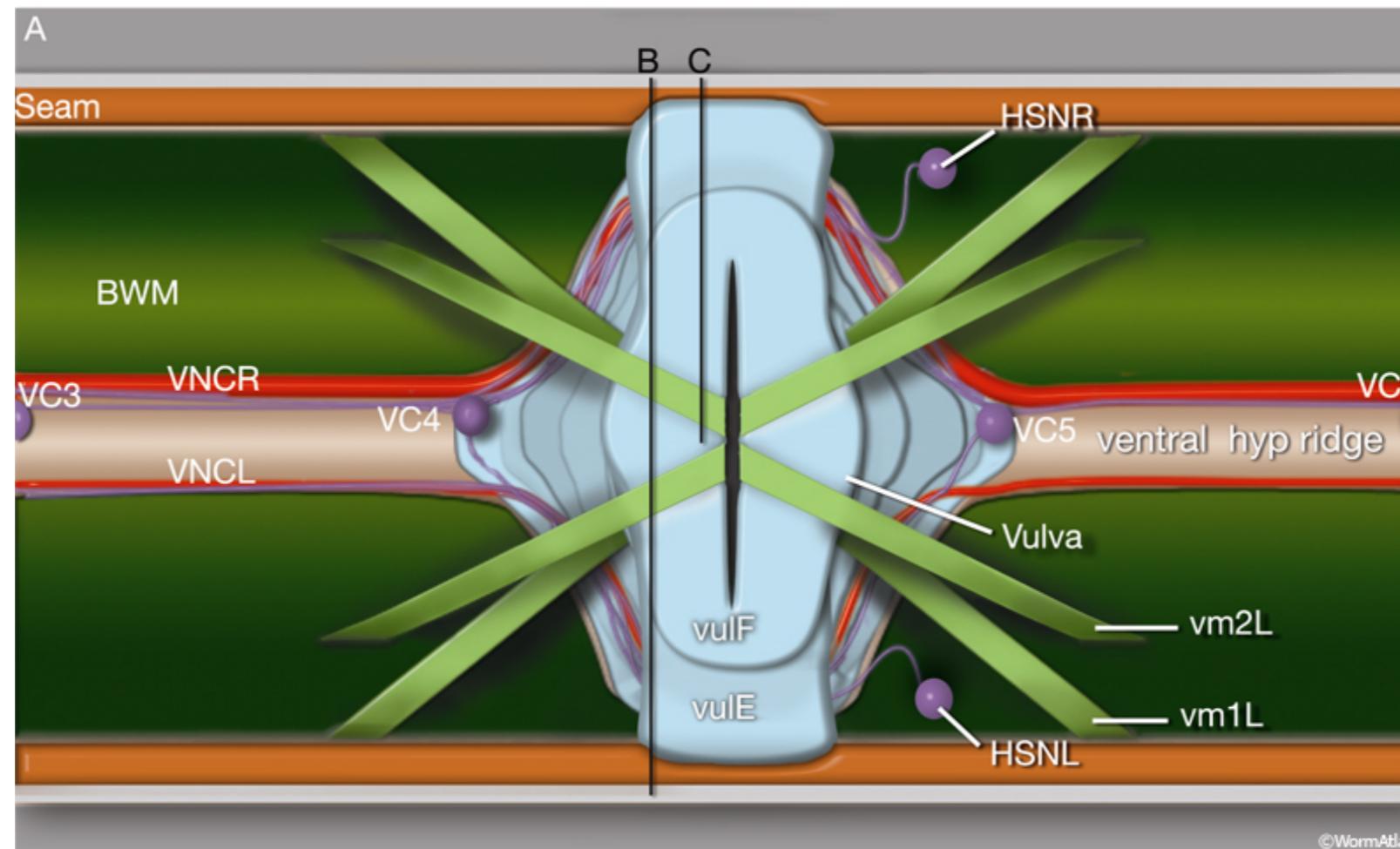


**No neuron**

*tra-1* = inducer of sex determination

Mutants are partially male so HSNs die

# One of the best genetic screens ever

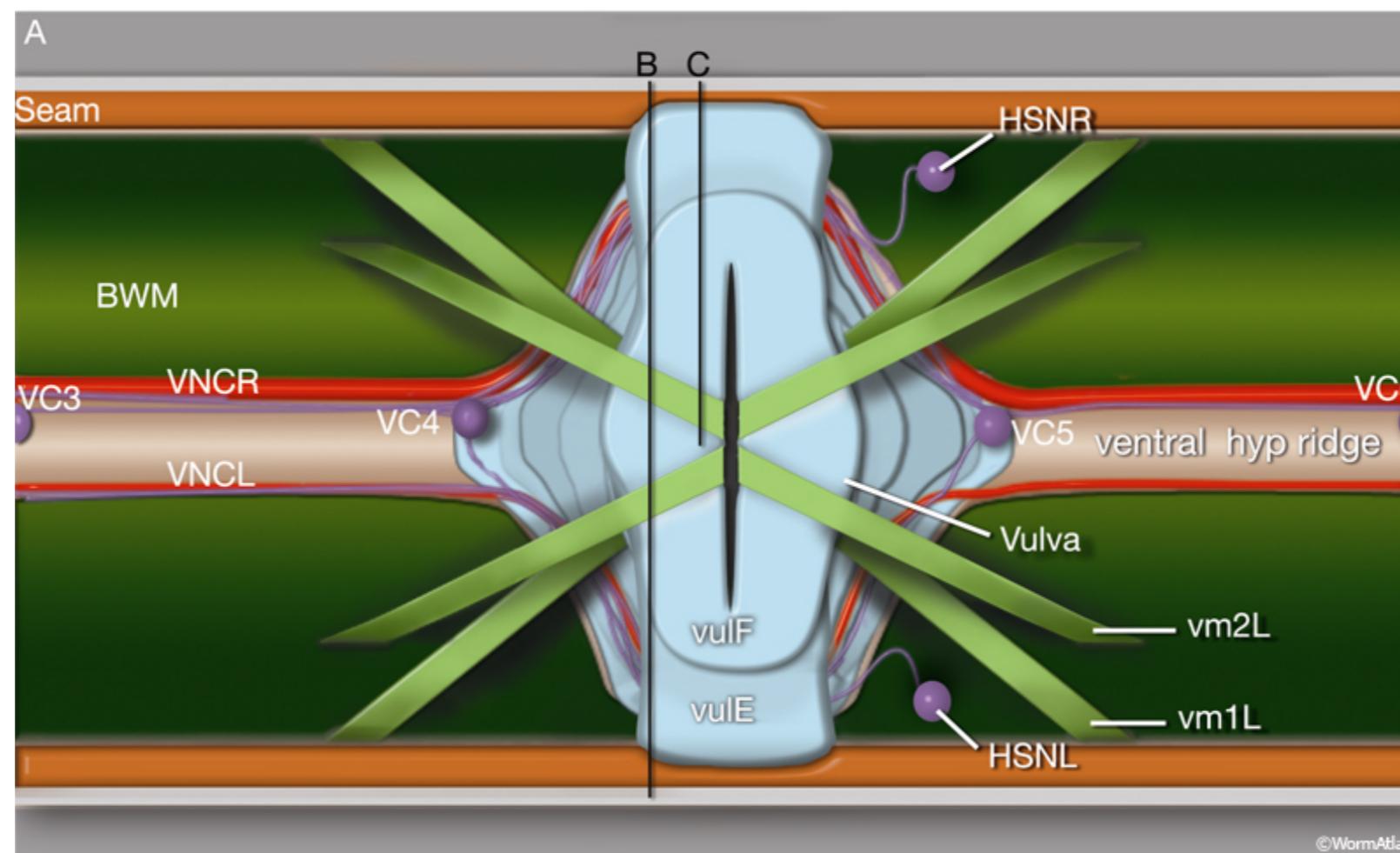


No neuron

No neuronal  
signaling

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

# One of the best genetic screens ever



No neuron

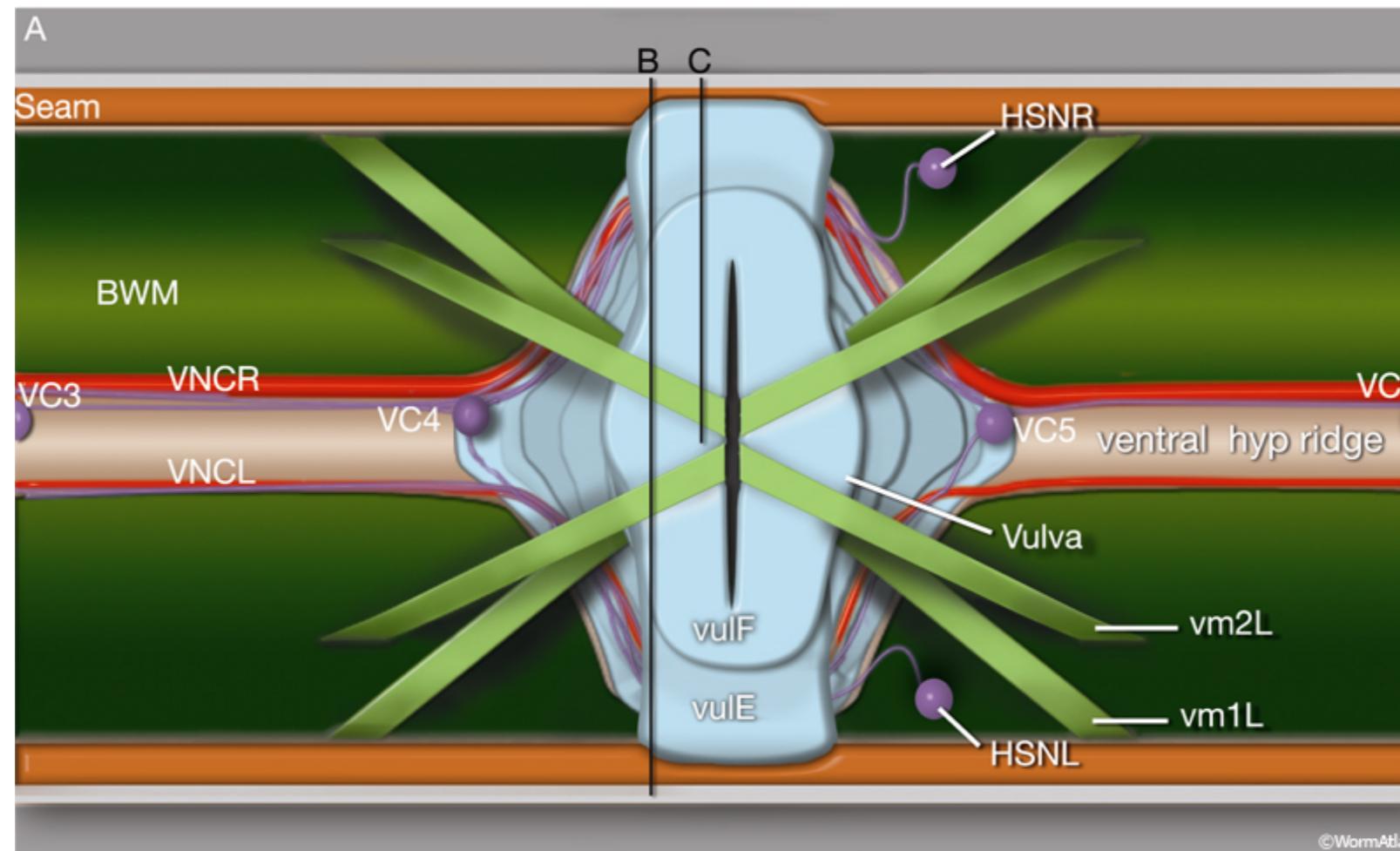
No neuronal  
signaling

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



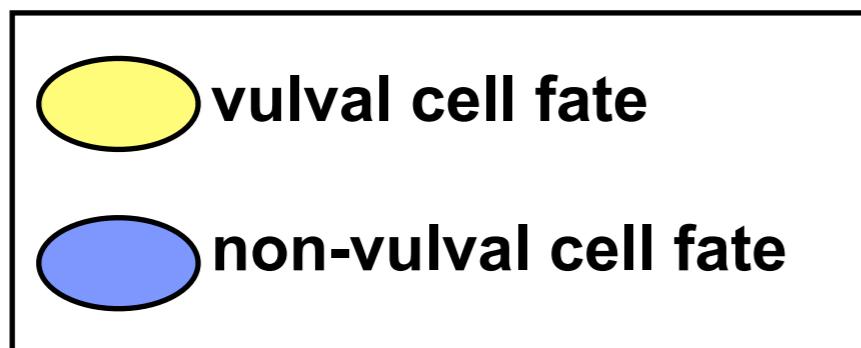
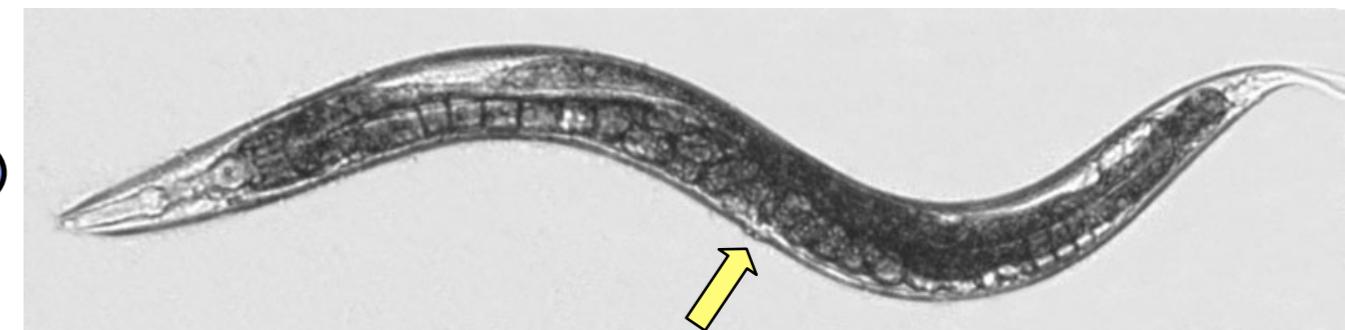
H. Robert Horvitz



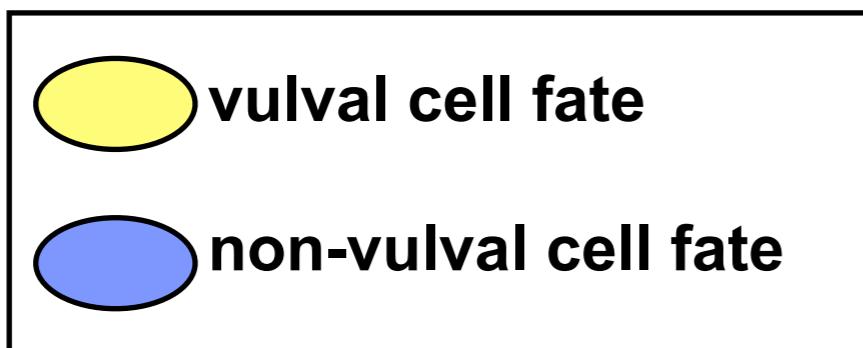
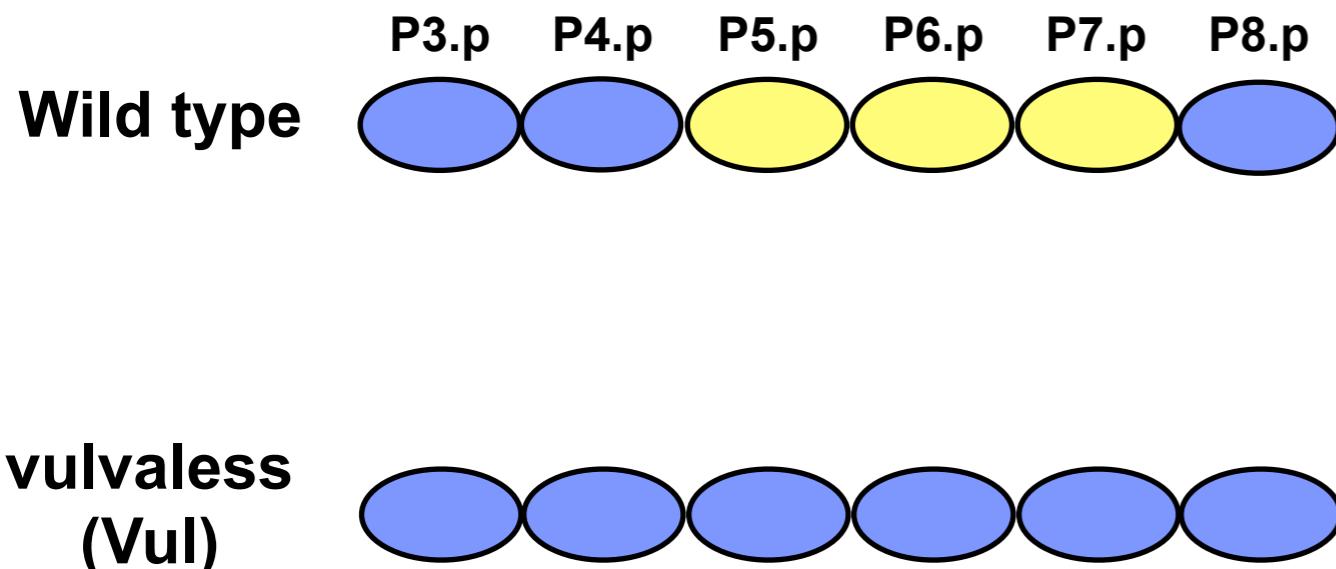
John E. Sulston



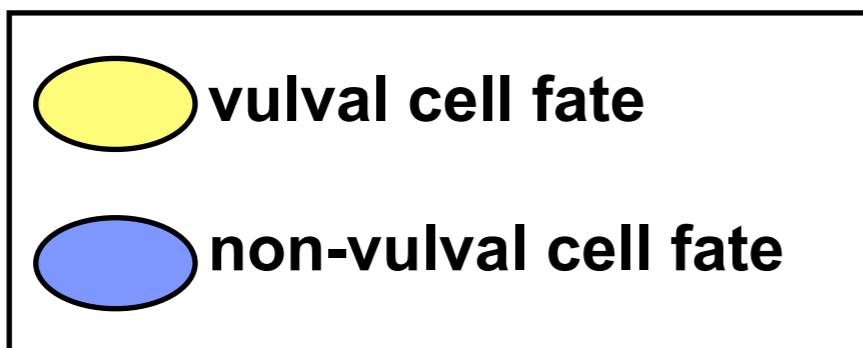
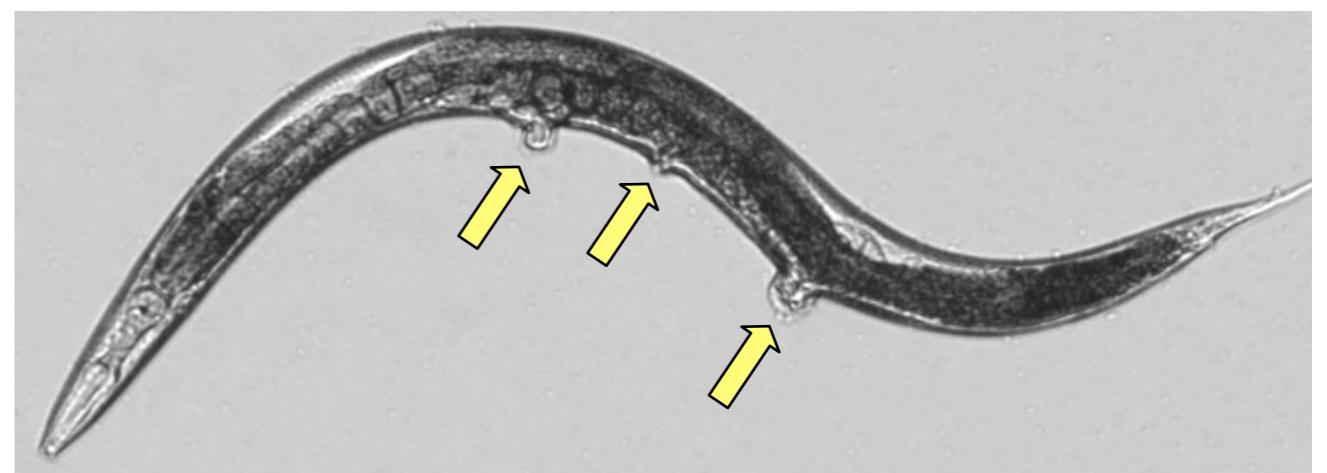
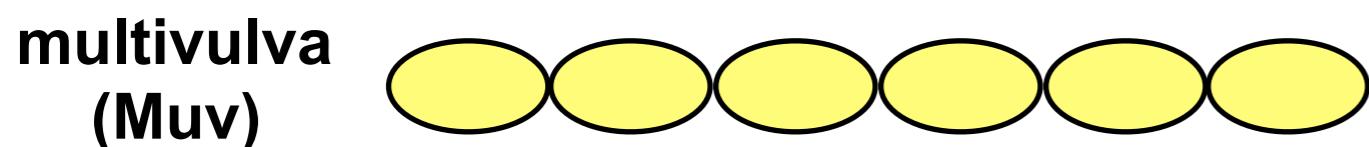
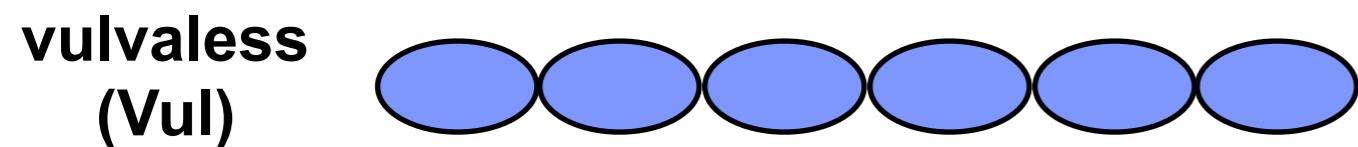
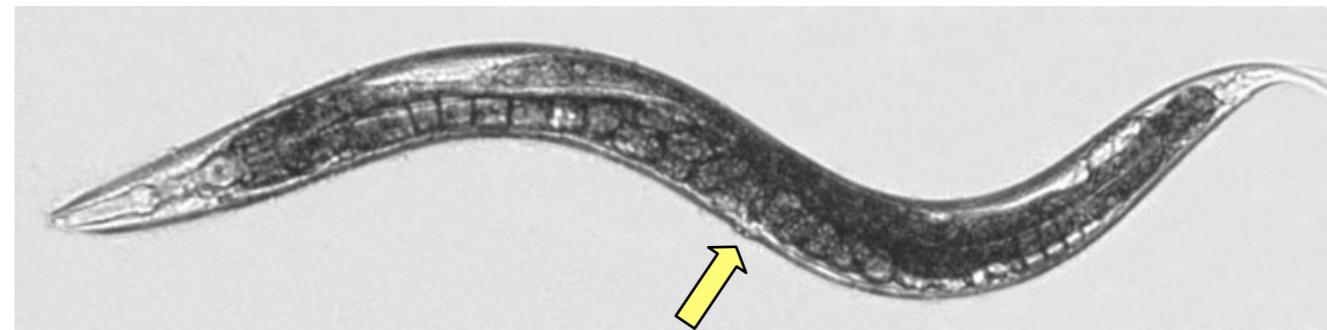
# Three cells express vulval fates in wild-type animals



# 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
<i>lin-39(0)</i>	Vul

# 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-39(0)</i>	Vul
<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

***lin-3* → vulval fate**

***let-60* → vulval fate**

***let-23* → vulval fate**

# 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-39(0)</i>	Vul
<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

***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
<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-39(0)</i>	Vul
<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

***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
<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-39(0)</i>	Vul
<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

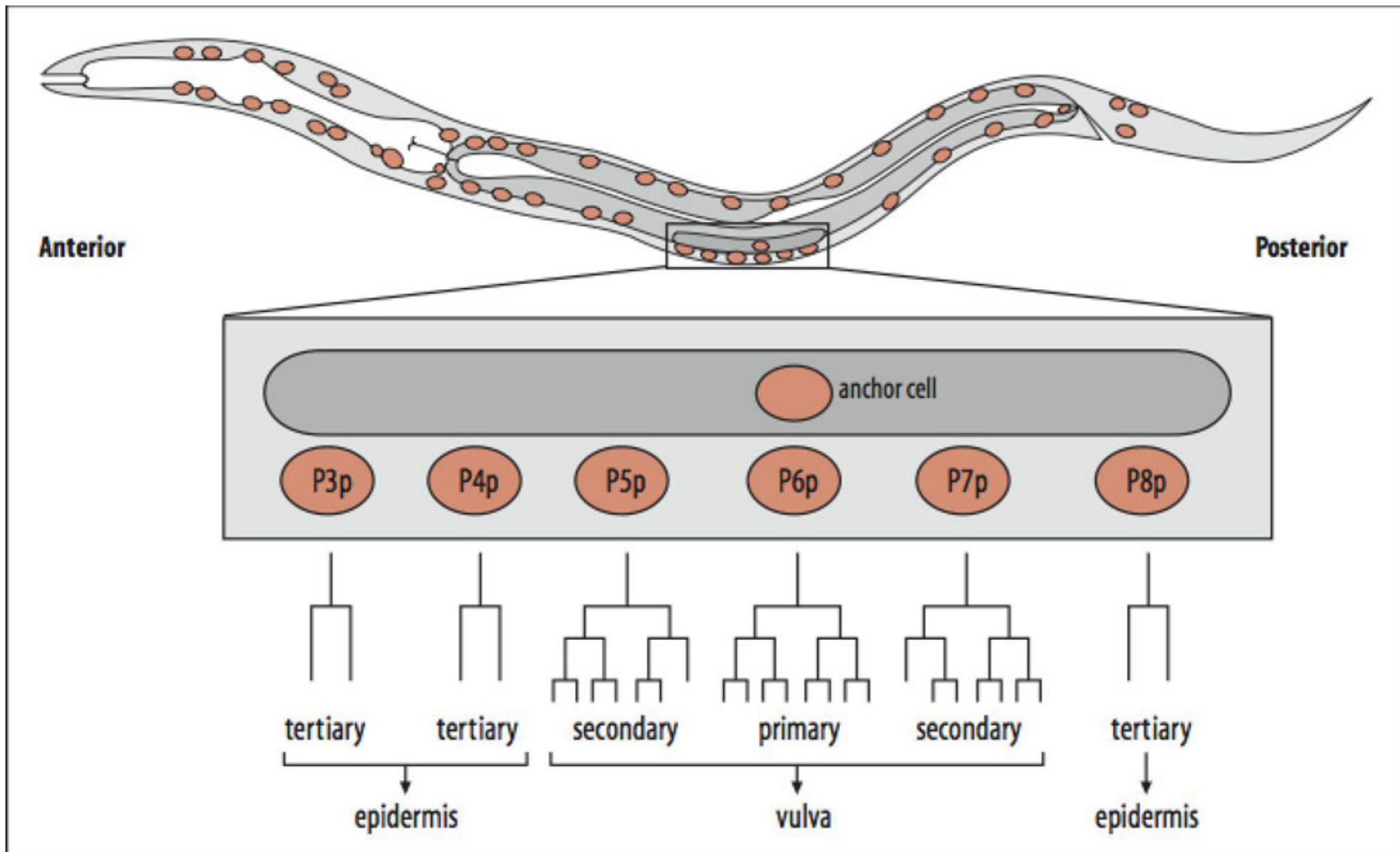
***lin-3* → *let-23* → *let-60* → *lin-1* → vulval fate**

# Double mutants defined the vulval pathway

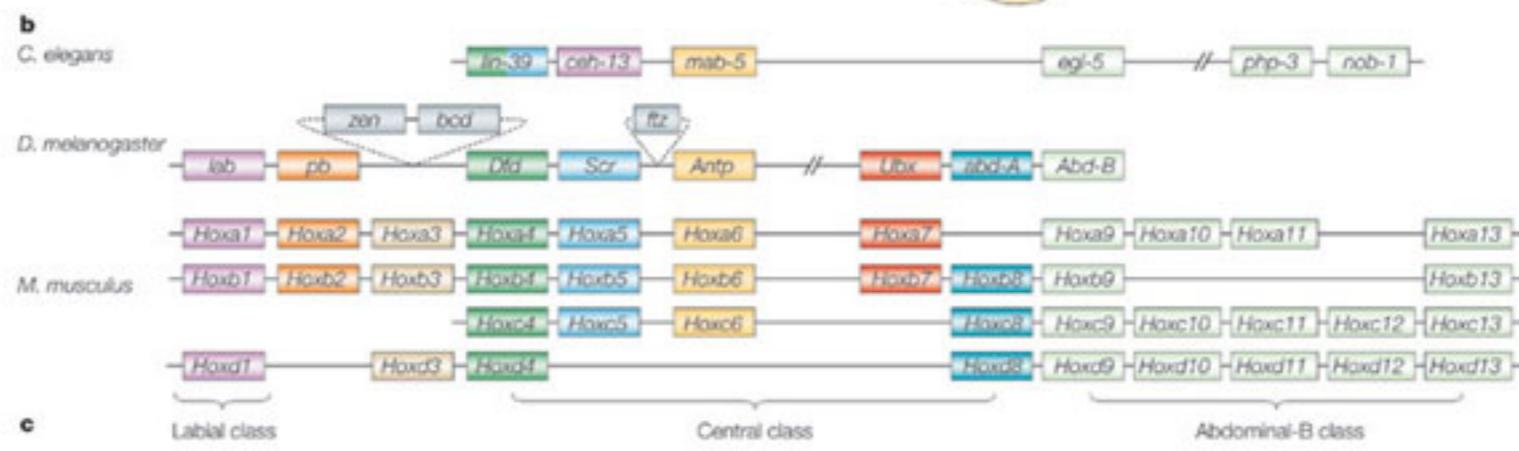
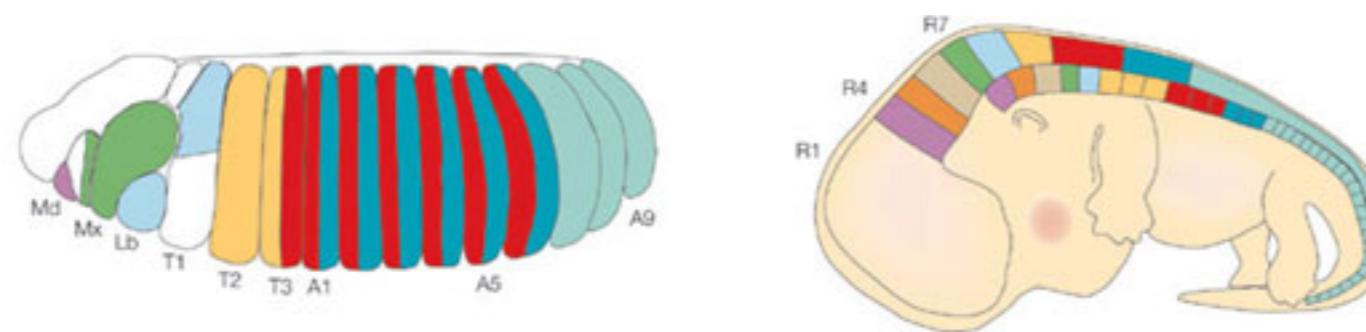
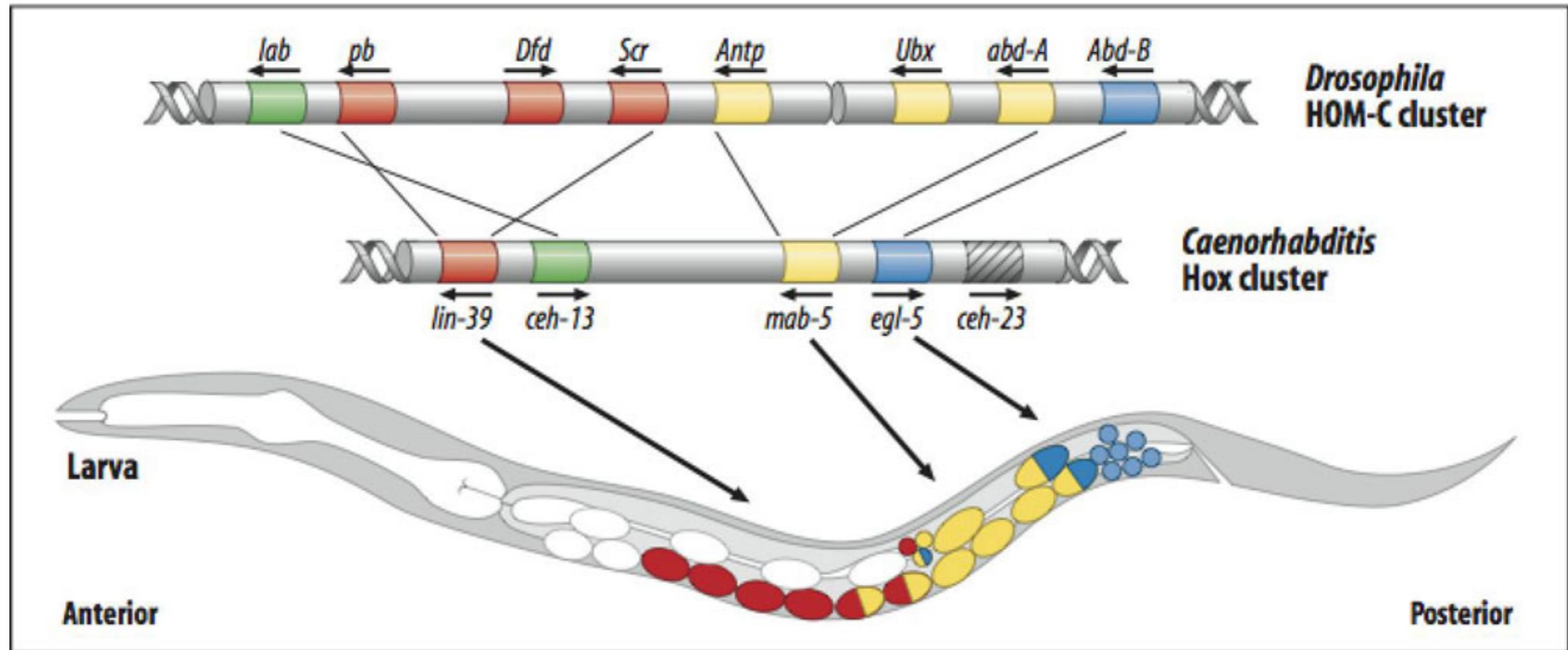
Mutant genotypes	Phenotype
<i>lin-1(0)</i>	Muv
<i>lin-3(0)</i>	Vul
<i>let-60(0)</i>	Vul
<i>let-60(gf)</i>	Muv
<i>let-23(0)</i>	Vul
<i>let-23(gf)</i>	Muv
<i>lin-39(0)</i>	Vul
<i>lin-3(0); let-23(gf)</i>	Muv
<i>lin-3(0); let-60(gf)</i>	Muv
<i>let-23(0); let-60(gf)</i>	Muv
<i>let-23(gf); let-60(0)</i>	Vul
<i>let-60(0); lin-1(0)</i>	Muv
<i>let-23(0); lin-1(0)</i>	Muv
<i>lin-1(0); lin-39(0)</i>	Vul

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

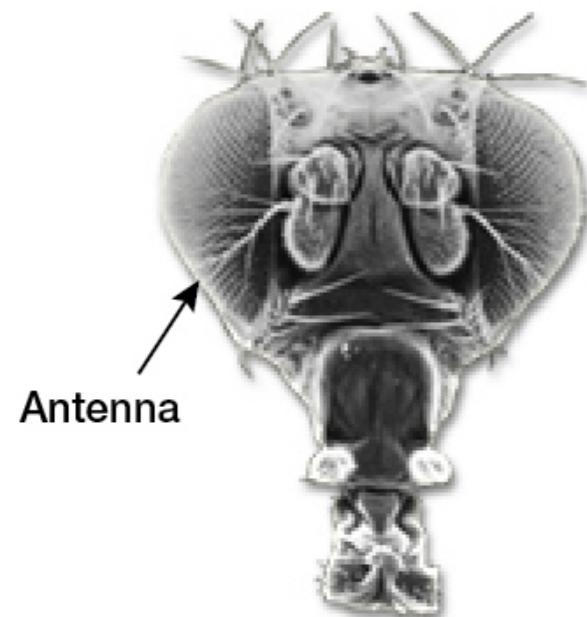
# Careful observation of *lin-39(0)* mutants showed that P3p through P8p were not made correctly



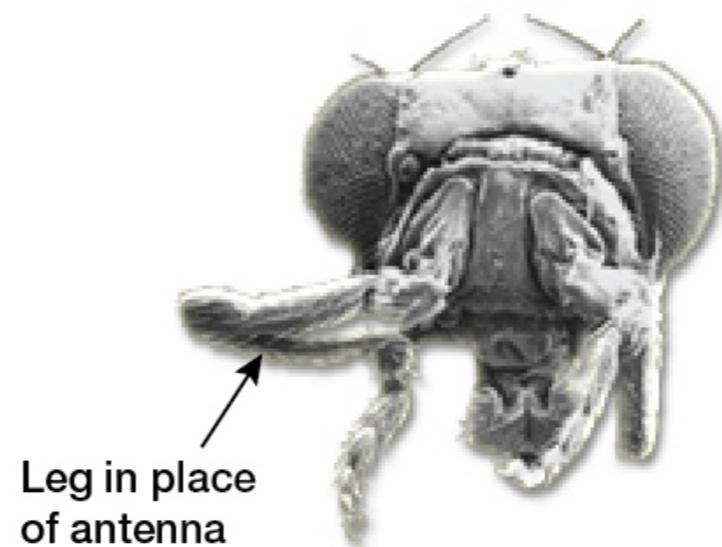
# *lin-39* is a Hox gene



# Regional identity in the animal is deficient in Hox mutants

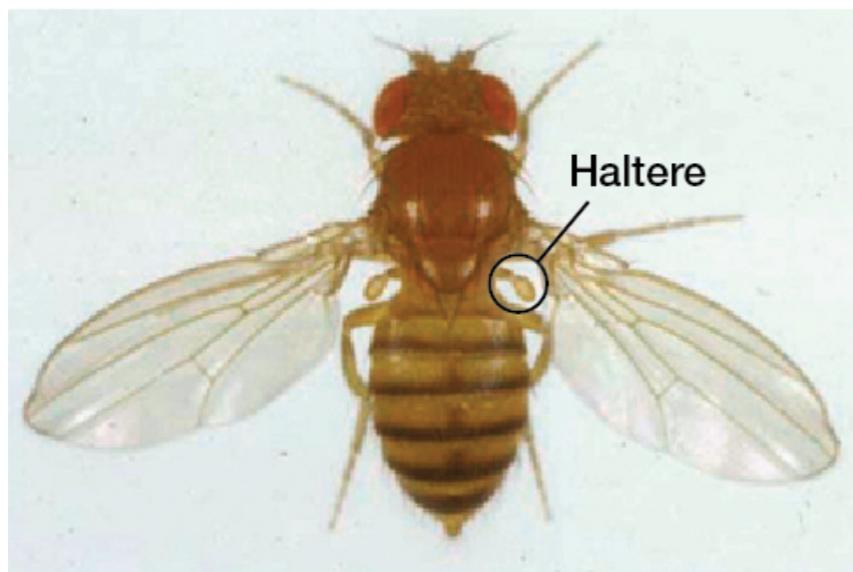


Antenna



Leg in place  
of antenna

**Antenna to leg**

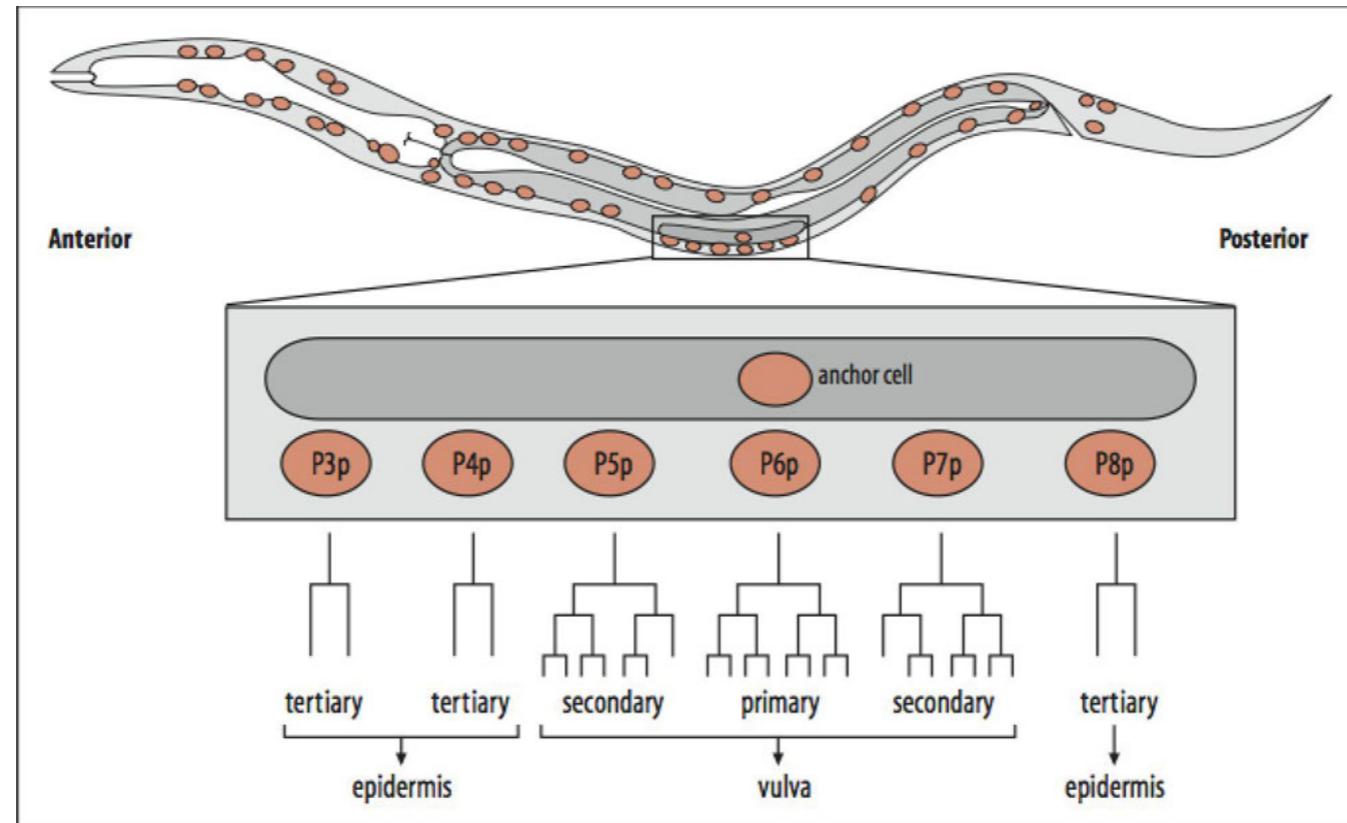


Haltere



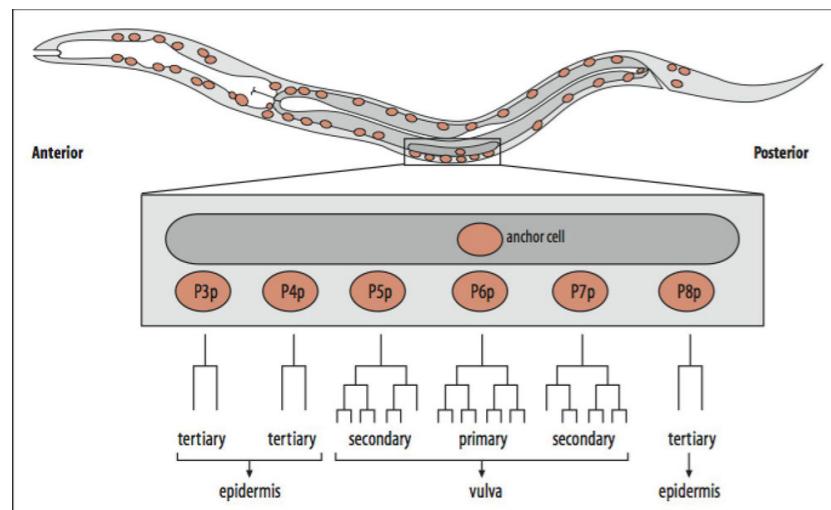
**Haltere to wing**

# *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

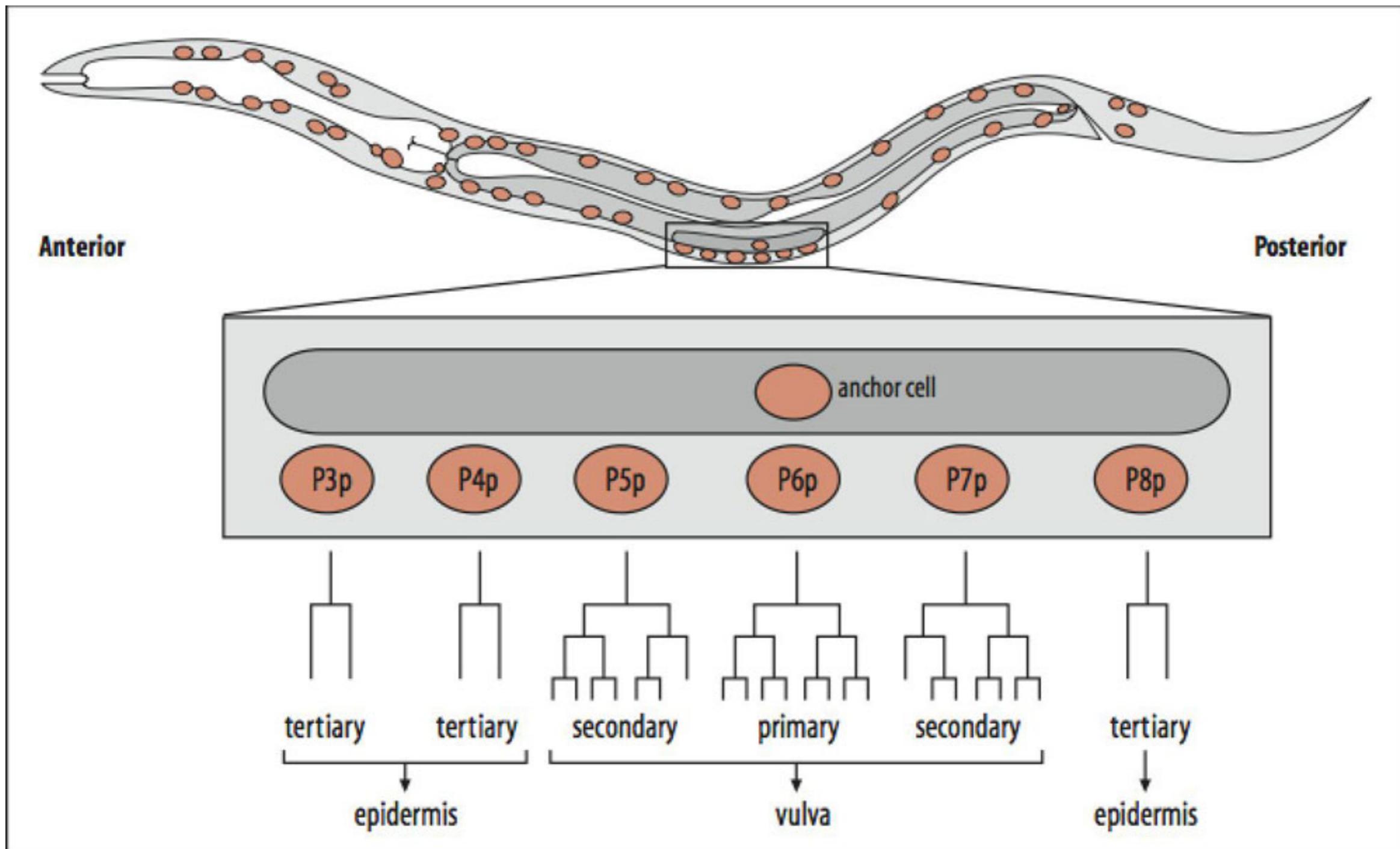


Generation of the vulval precursor cells is epistatic to the fates of those cells

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

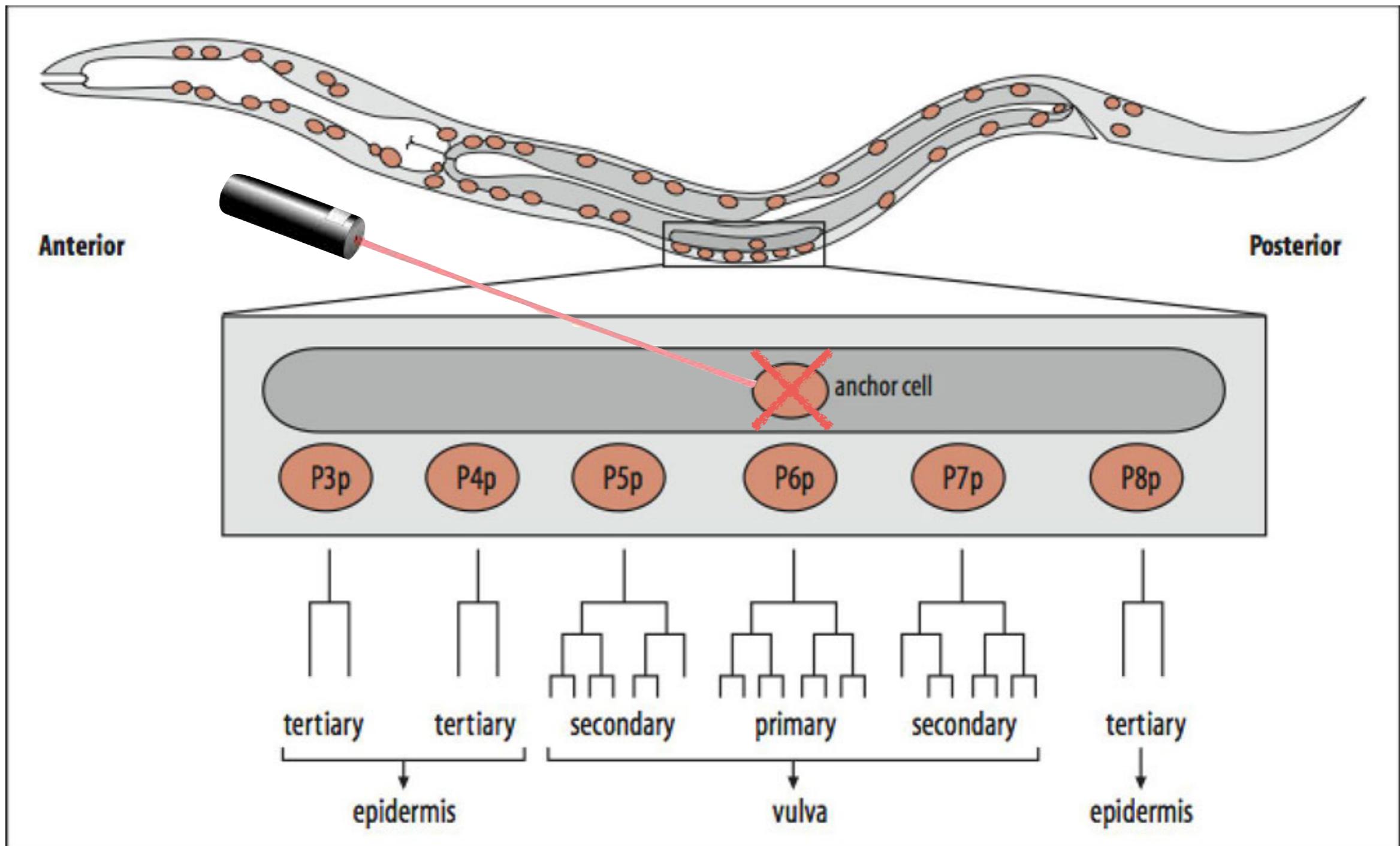
Mutant genotypes	Phenotype
<i>lin-1(0)</i>	Muv
<i>lin-3(0)</i>	Vul
<i>lin-39(0)</i>	Vul
<i>lin-1(0); lin-3(0)</i>	Muv
<i>lin-1(0); lin-39(0)</i>	Vul

# 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)

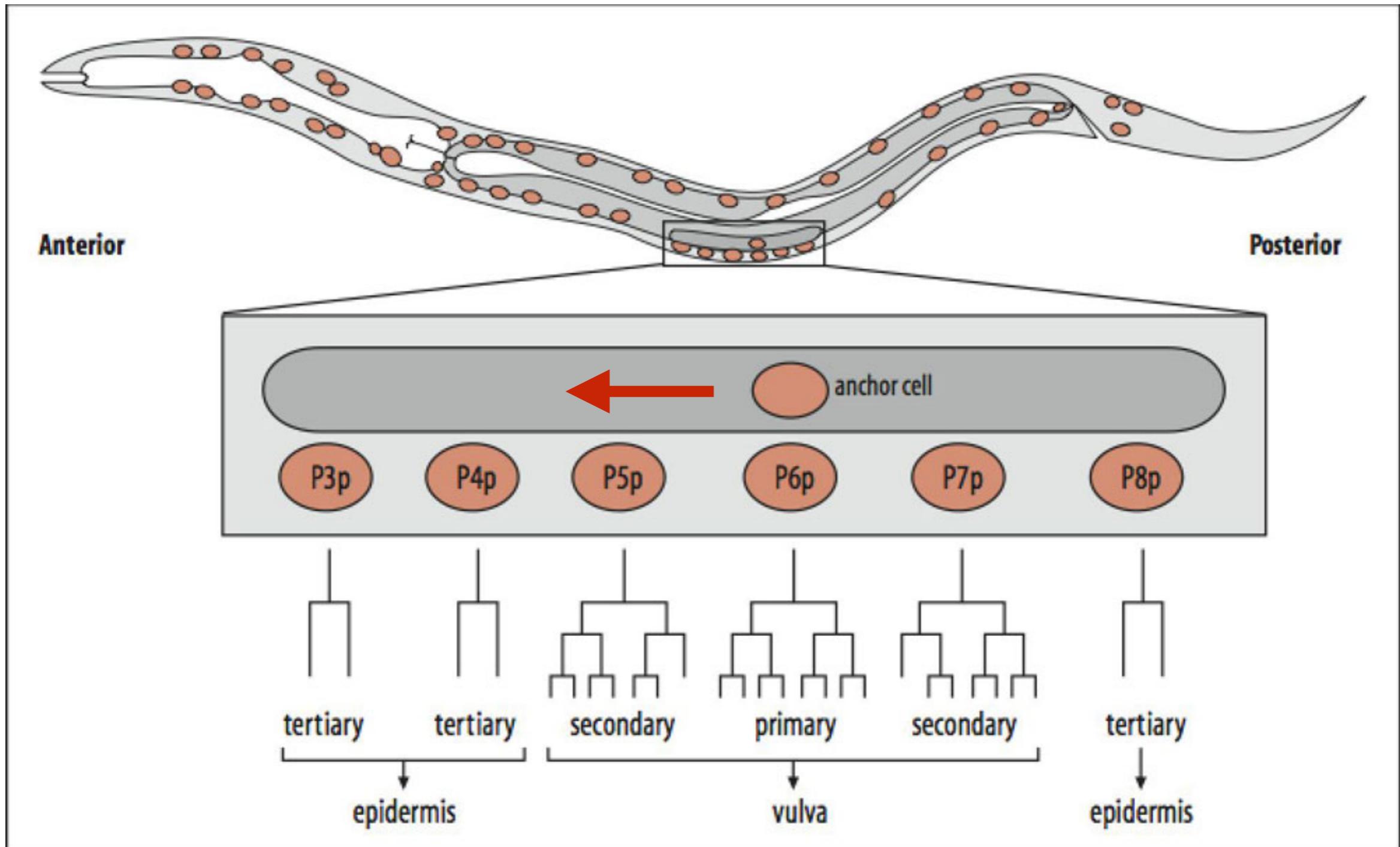


No AC leads to no vulval cell specification  
and a vulvaless phenotype

# All other vulval mutants are epistatic to AC ablation

Mutant genotypes	Phenotype
AC ablation	Vul
<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)

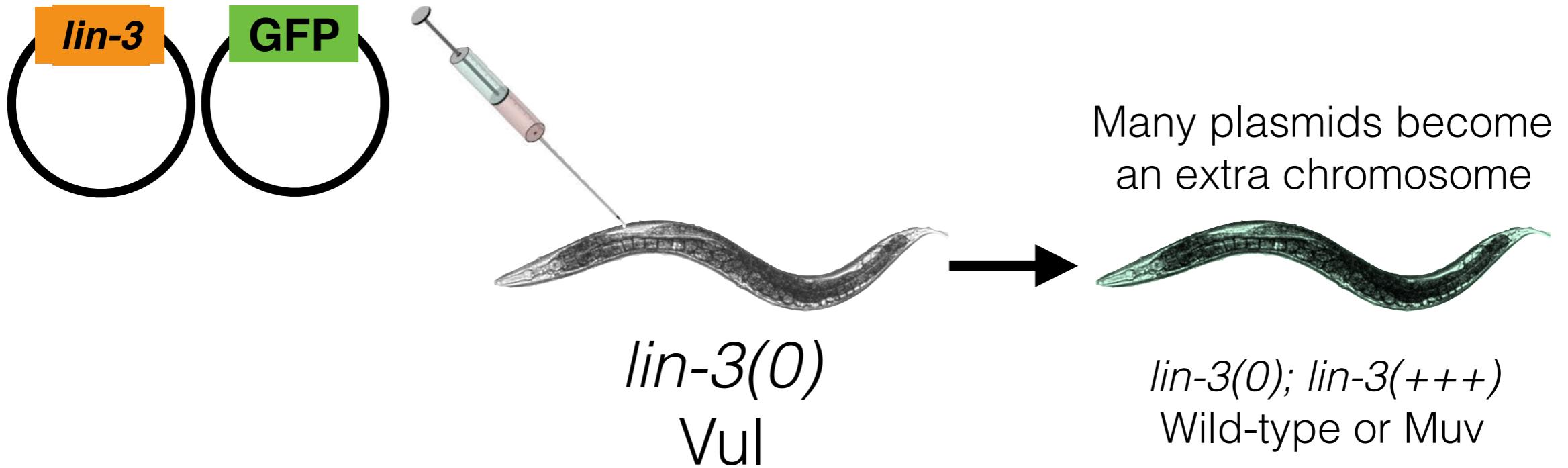


*dig-1* displaced gonad mutants

# 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?



Many plasmids become  
an extra chromosome

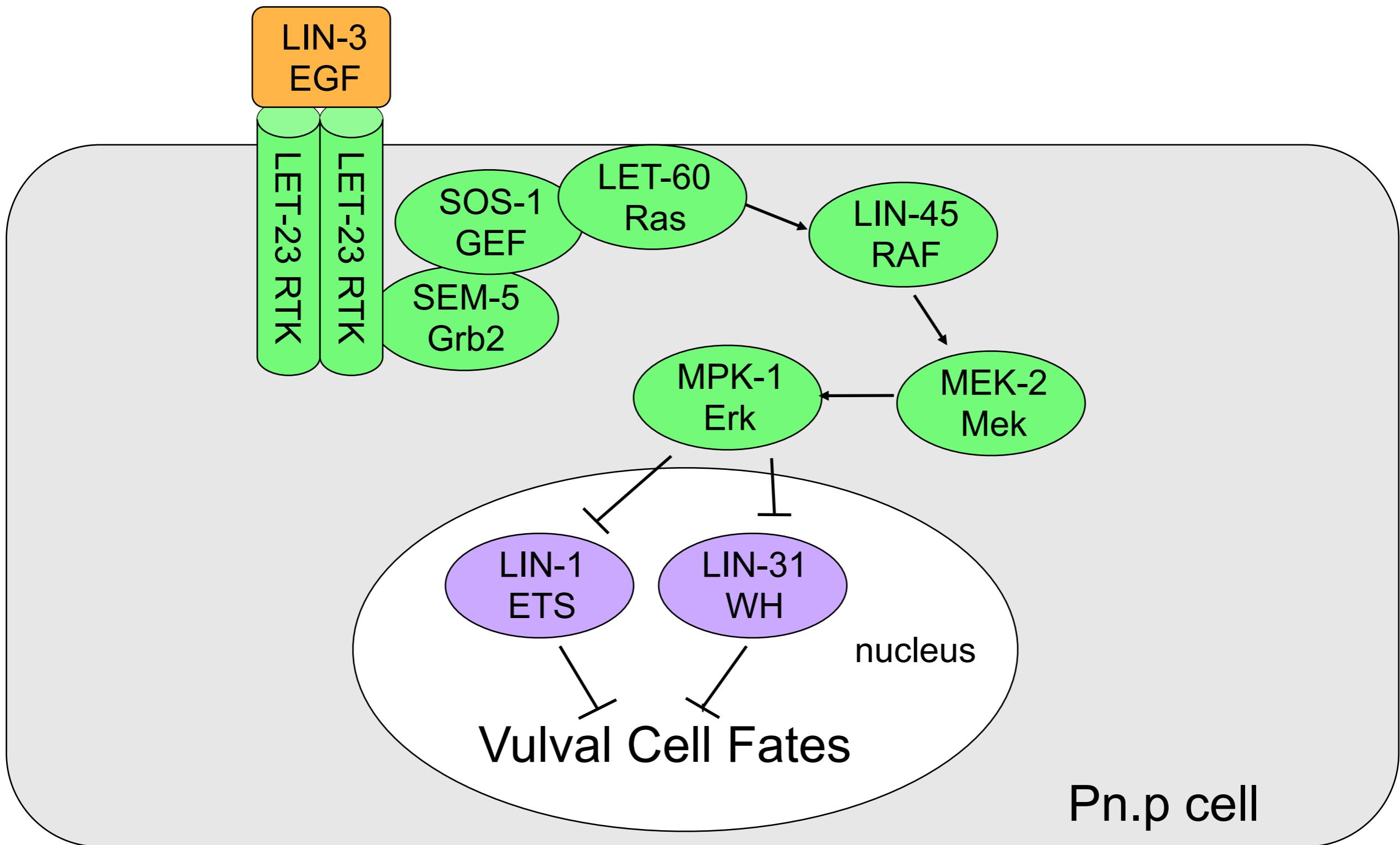
*lin-3(0)*  
Vul

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

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

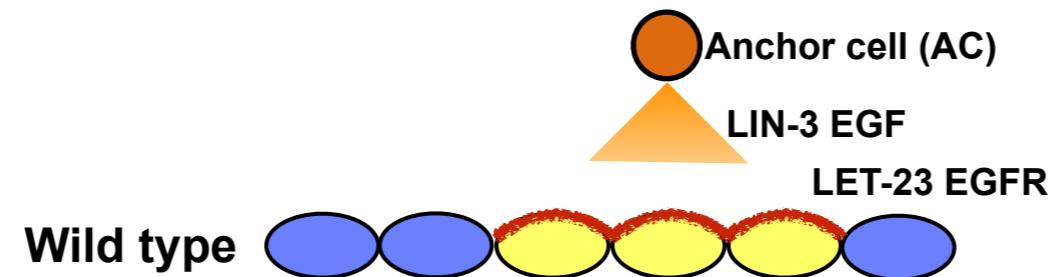
Expression of *lin-3* in vulval cells  
Expression of *lin-3* in the intestine  
Expression of *lin-3* in the neurons  
Expression of *lin-3* in the AC

# A Ras pathway promotes vulval fates

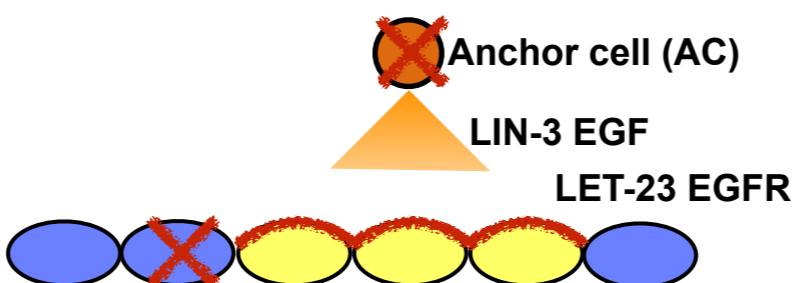


# Cell autonomy of *let-60*

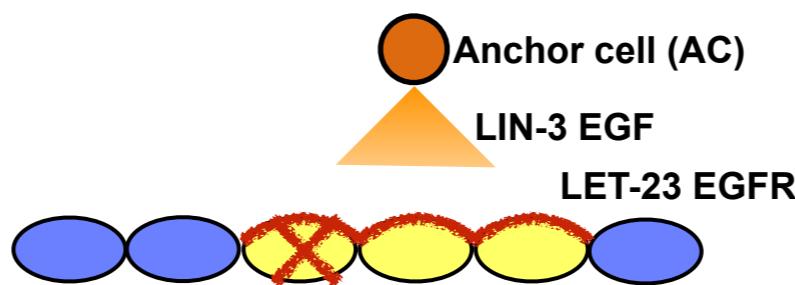
Cell autonomous action



*let-60* mutant  
in AC or blue  
Pn.p cells



*let-60* mutant  
in yellow  
Pn.p cells

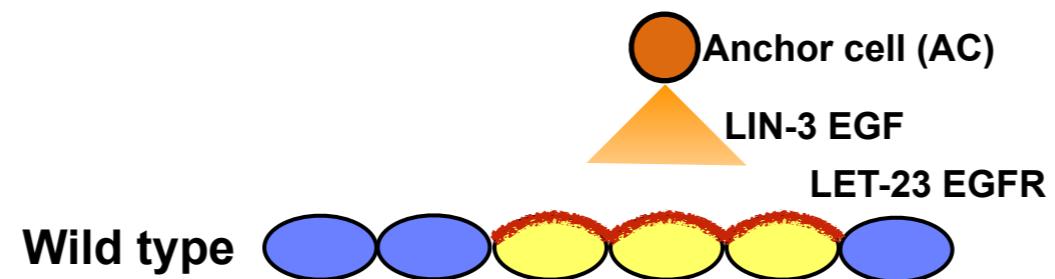


✗ = mutant cell

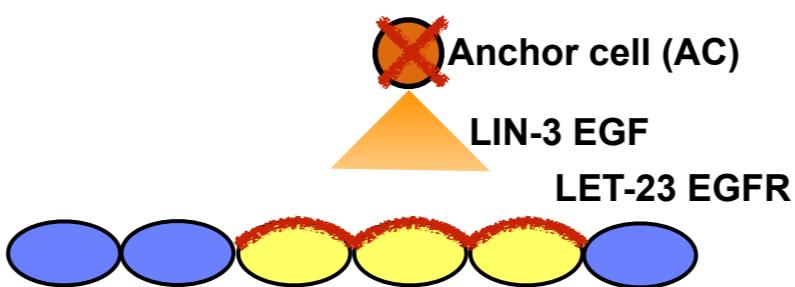
A cell autonomous trait is a trait in a multicellular organism in which only genotypically mutant cells have the mutant phenotype

# Cell autonomy of *lin-3*

Cell  
non-autonomous  
action

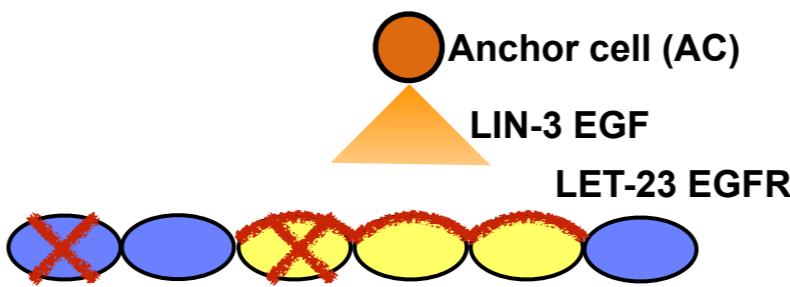


*lin-3* mutant  
in AC



Mutant vulval  
phenotype

*lin-3* mutant  
in Pn.p cells



Wild-type vulval  
phenotype

✗ = mutant cell

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
<i>lin-1(0)</i>	Muv
<i>lin-3(0)</i>	Vul
<i>let-60(0)</i>	Vul
<i>let-60(af)</i>	Muv
<i>let-23(0)</i>	Vul
<i>let-23(af)</i>	Muv
<i>lin-2(0)</i>	a little Vul
<i>lin-7(0)</i>	a little Vul
<i>lin-10(0)</i>	a little Vul

# 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(af)</i>	Muv
<i>let-23(0)</i>	Vul
<i>let-23(af)</i>	Muv
<i>lin-2(0)</i>	a little Vul
<i>lin-7(0)</i>	a little Vul
<i>lin-10(0)</i>	a little Vul
<i>lin-2(0); lin-7(0)</i>	more Vul
<i>lin-2(0); lin-10(0)</i>	more Vul
<i>lin-7(0); lin-10(0)</i>	more Vul

# 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(af)</i>	Muv
<i>let-23(0)</i>	Vul
<i>let-23(af)</i>	Muv
<i>lin-2(0)</i>	a little Vul
<i>lin-7(0)</i>	a little Vul
<i>lin-10(0)</i>	a little Vul
<i>lin-2(0); lin-7(0)</i>	more Vul
<i>lin-2(0); lin-10(0)</i>	more Vul
<i>lin-7(0); lin-10(0)</i>	more Vul
<i>lin-2(0); lin-7(0); lin-10(0)</i>	Vul

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

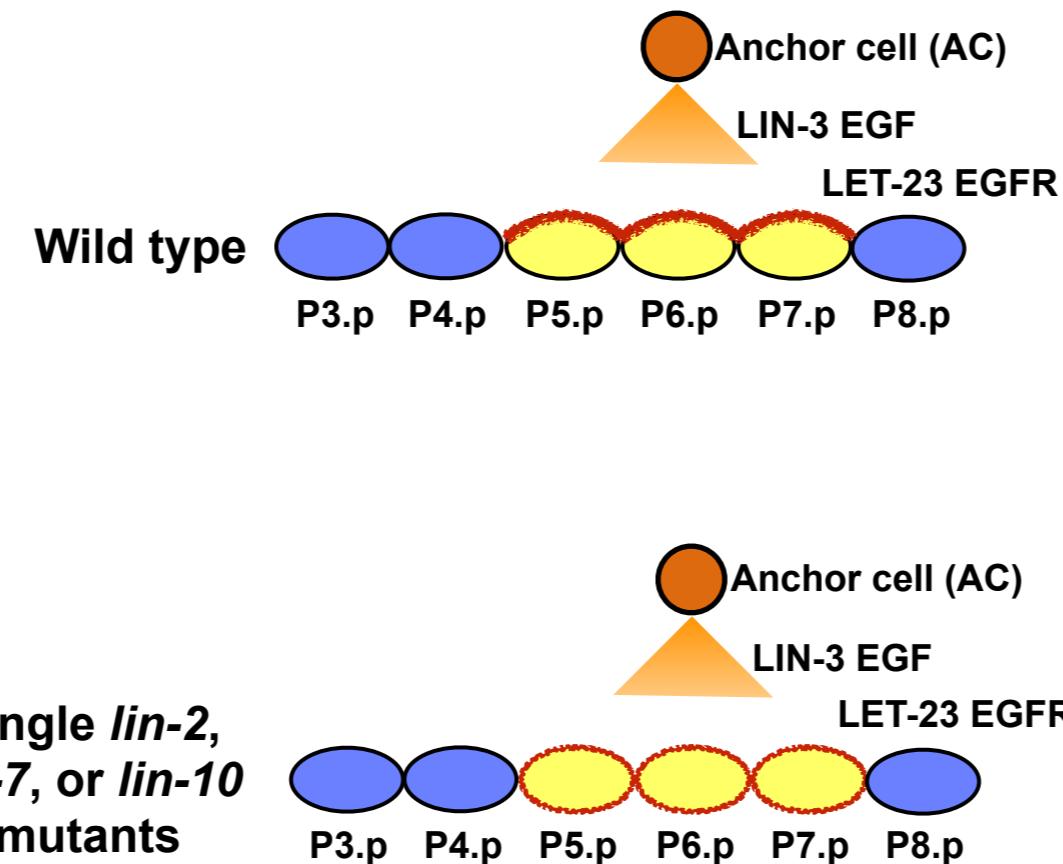
**Told the geneticists that the three genes  
act in a redundant process  
But how?**

Act downstream of *lin-3*

Act upstream of *let-23*

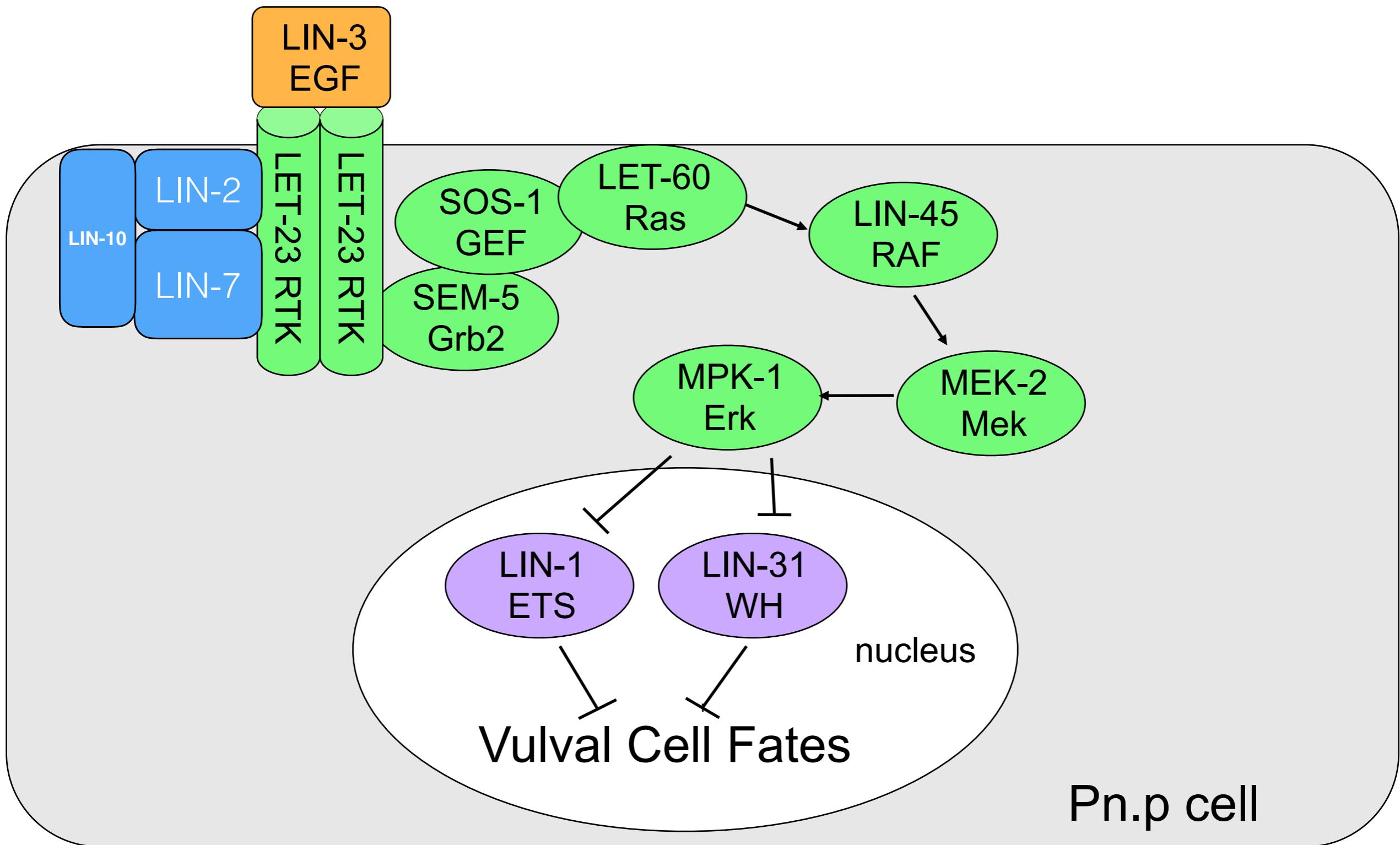
Expressed in vulval cells

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

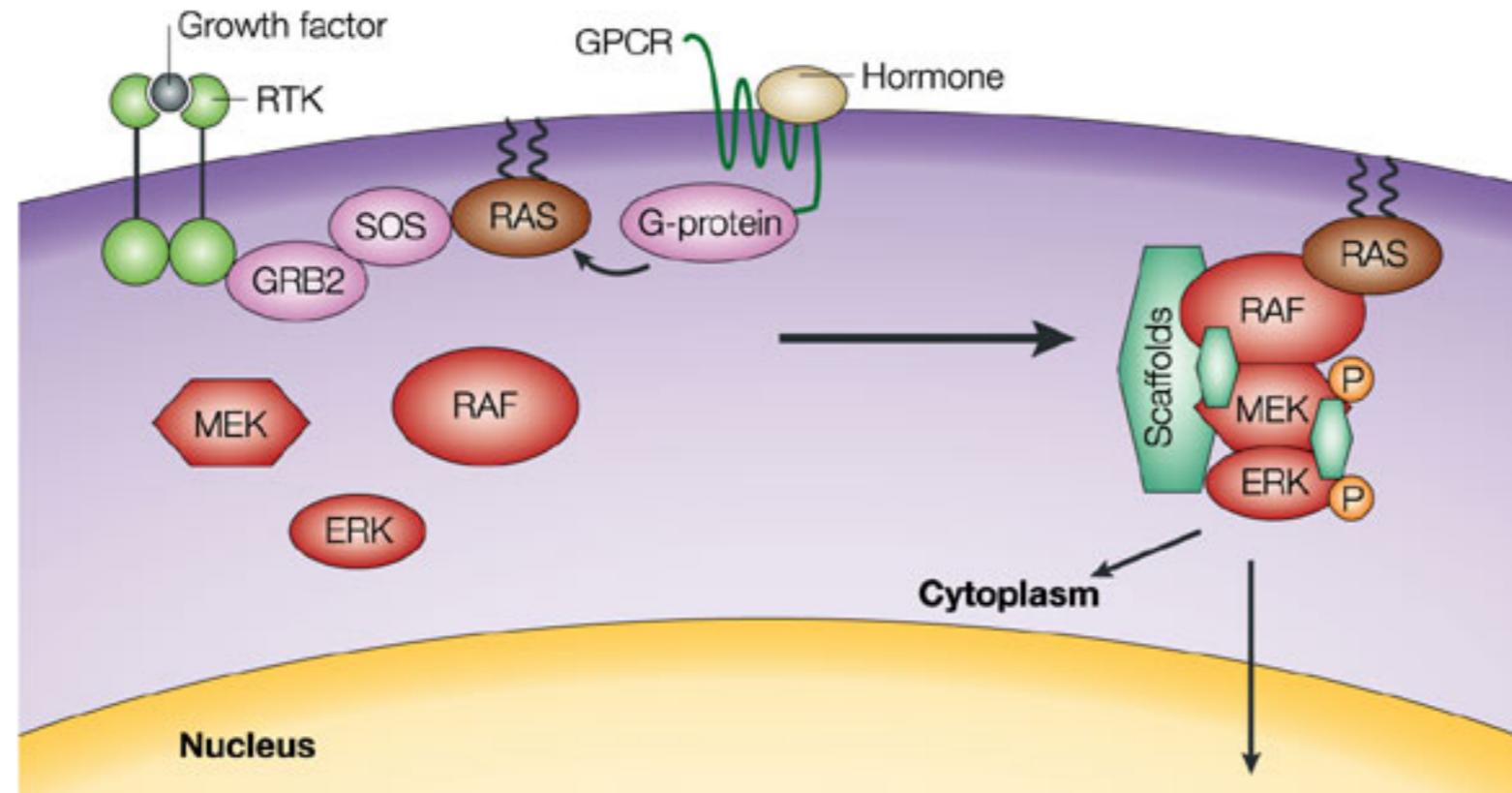


***lin-3***  
↓  
***lin-2/7/10* → *let-23* → *let-60* → *lin-1* → vulval fate**

# A Ras pathway promotes vulval fates



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



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We NEED basic research for this reason!