

MCDB 4650 Class 15

Determining gene interactions, ordering, and functions in signaling pathways

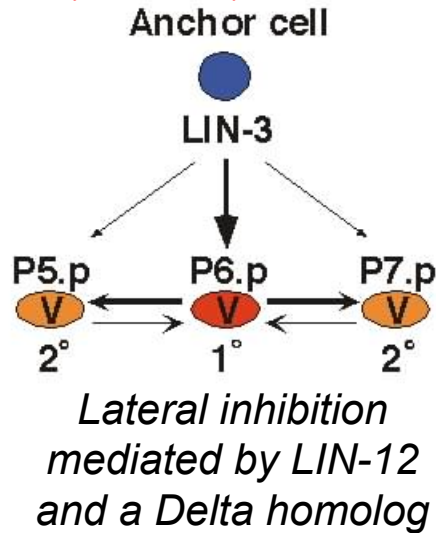
Learning goals:

- Distinguish between metabolic pathways and signaling pathways
- Predict the interactions of genes that act in a pathway, based on their phenotypes when mutant
- Interpret the results of epistasis tests in determining the order of genes in a pathway.
- [Predict the outcomes of mosaic analysis experiments, and explain how these outcomes can be used to determine how a gene product acts (within a cell, or on neighboring cells)]

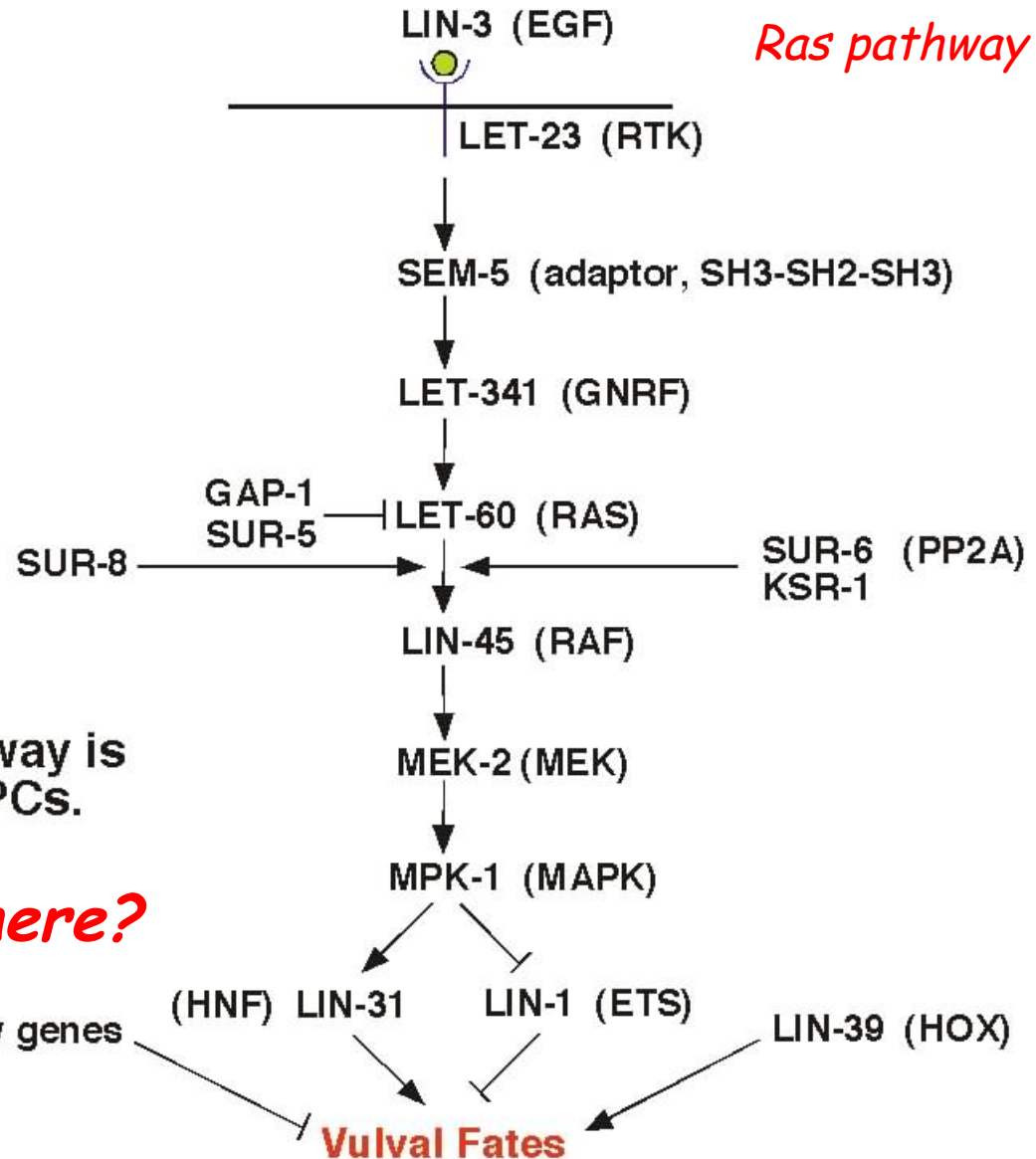
I will return the exams at the end of class: nice job!

We know the details of the genes involved in vulval determination

Notch-Delta pathway



Ras pathway



•RAS/MAP kinase pathway is activated in three VPCs.

But how did we get here?

Do the genes act in a pathway? If they do, how do they interact?

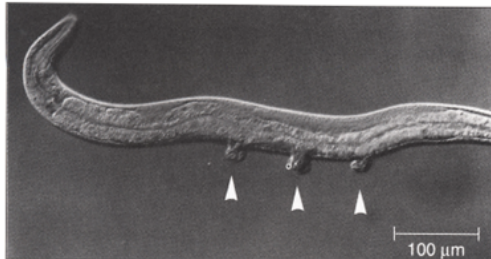
Mutation

Phenotype



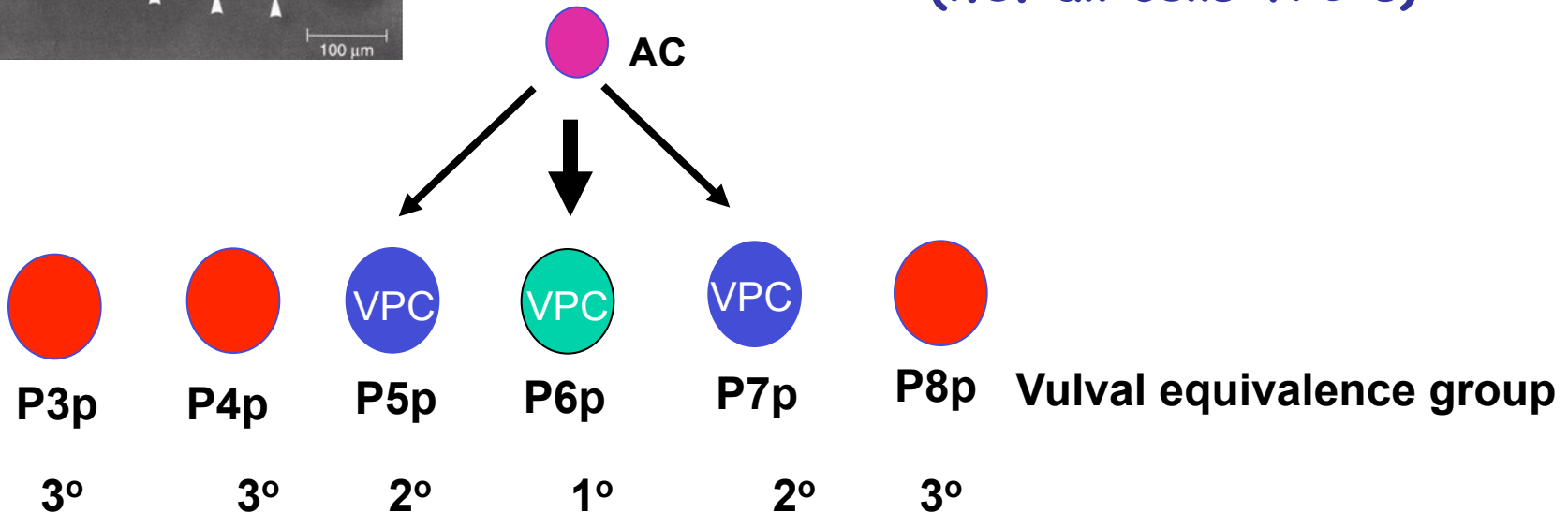
let-60(lf)

Vul; all Pnp cells 3° fate
(i.e. no VPC's)



lin-1(lf)

Muv; all Pnp cells 1° or 2° fate
(i.e. all cells VPC's)



Possible explanations for these phenotypes

(*let-60(lf)*: no VPC fates; *lin-1(lf)*: all VPC fates)

Different pathways *let-60* → VPC fates ⊥ *lin-1*

Same pathway *lin-1* and *let-60* act sequentially to specify VPC fate, and one regulates the other

Figuring out the order of genes in a pathway:
How do you do it?

5 clicker questions that examine what you already know about this topic

I am not going to give you the answers or show you the distributions of the answers right now

BUT:

--We will spend the rest of the class period working on this topic

--The answers to all questions will be available at the end of the week, as usual

1. The synthetic pathway for the neurotransmitter norepinephrine involves two enzymes, tyrosine hydroxylase (TH) and DOPA decarboxylase (DOPA-D) and can be drawn as follows:



If you are analyzing the output of a cell that has a loss of function mutation (lf) in the DOPA-D gene and an lf mutation in the TH gene, which neurotransmitter will be produced?

- a. Tyrosine
- b. Dopamine
- c. Norepinephrine

2. The synthetic pathway for the neurotransmitter norepinephrine involves two enzymes, tyrosine hydroxylase (TH) and DOPA decarboxylase (DOPA-D) and can be drawn as follows:



What is the epistatic relationship of the TH and DOPA-D genes?

- Lf* mutations in TH will be epistatic to *lf* mutations in DOPA-D
- Lf* mutations in DOPA-D will be epistatic to *lf* mutations in TH
- You cannot determine the relationship from this pathway

3.

Gene:	1	→	2	—	3	—	4	→	5	—	6	Outcome
Cell X	on		on		off		on		on		off	“A” fate
Cell Y	off		off		on		off		off		on	“B” fate

What will happen if there is a loss of function mutation in Gene 3?

Answer	Cell X will take on:	Cell Y will take on:
A.	“A” fate	“B” fate
B.	“A” fate	“A” fate
C.	“B” fate	“B” fate
D.	“B” fate	“A” fate
E.	Not enough information	Not enough information

4.

Gene:	1	→	2	—	3	—	4	→	5	—	6	Outcome
Cell X	on		on		off		on		on		off	“A” fate
Cell Y	off		off		on		off		off		on	“B” fate

What will happen if there are loss of function mutations in both Gene 5 and Gene 3?

Answer	Cell X will take on:	Cell Y will take on:
A.	“A” fate	“B” fate
B.	“A” fate	“A” fate
C.	“B” fate	“B” fate
D.	“B” fate	“A” fate
E.	Not enough information	Not enough information

5. You are studying programmed cell death in *C. elegans* and have isolated two genes *ced-3* and *ced-4*, which have the following phenotypes when mutant:

ced-4 (lf): no cell death

ced-3 (lf): no cell death

ced-3 (gf): excessive cell death

Loss of function= lf

Gain of function= gf

You make a strain of worms that is mutant for both *ced-4 (lf)* and *ced-3 (gf)*. The phenotype is no cell death. You can conclude:

a. *ced-4 (lf)* is epistatic to *ced-3 (gf)*

b. *ced-3 (gf)* is epistatic to *ced-4 (lf)*

c. You cannot make a conclusion based on this information

Figuring out the order of genes in a
pathway:
How do you do it?

One way: conduct an “epistasis test”

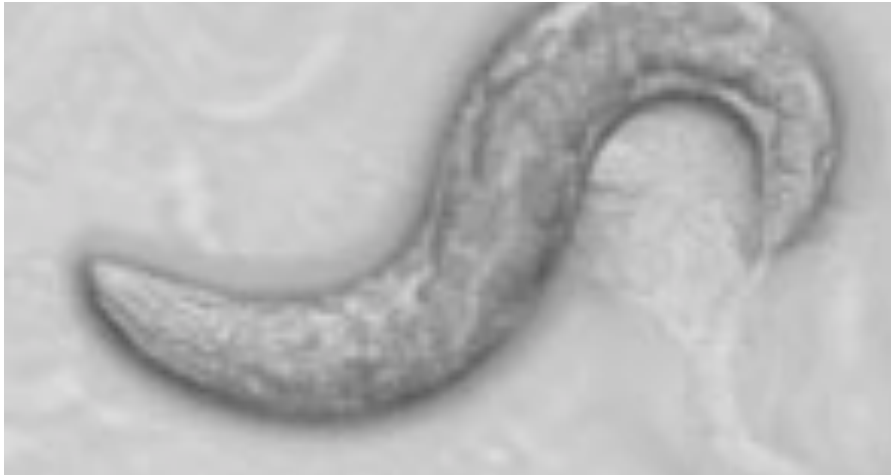
Epistasis refers to the following situation: a mutation in a single gene phenotypically “masks” or “wins” over a phenotype caused by a mutation in a different gene.

Said another way: when two mutations in different genes that result in different phenotypes are combined genetically to make a double mutant, the double mutant has the same phenotype as one of the two single mutants.

A note on creating "double mutants" in *C. elegans*

- *C. elegans* strains almost always have at least two phenotypes: the phenotype of the mutation one is trying to learn more about, and the phenotype of some kind of marker that will help you in crosses and analysis.

dpy



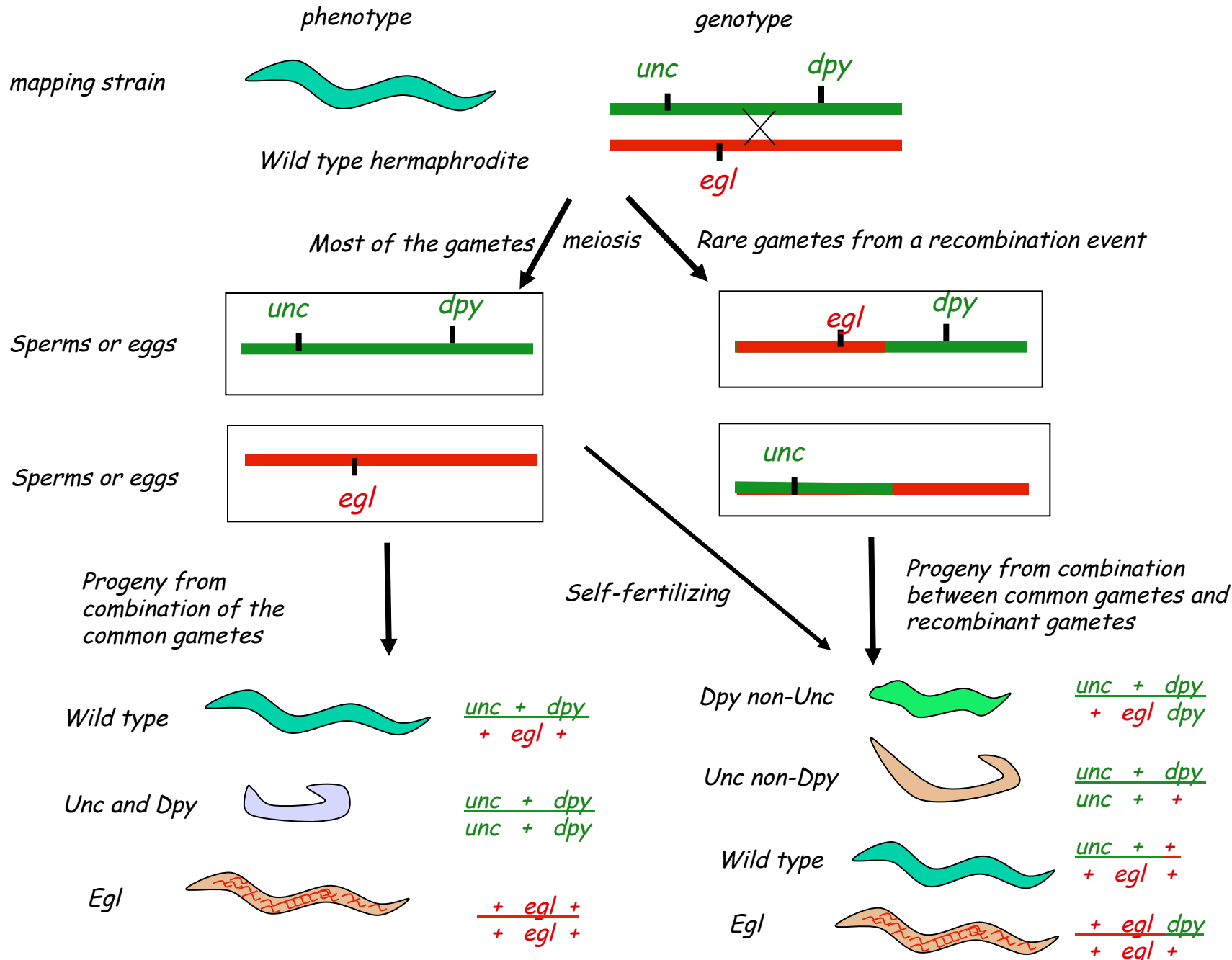
Wild type



egl



Figure 8.16. Example of genetic three point mapping



The worksheet being passed out will give you an opportunity to work through some examples on epistasis

Please work in groups

Please stop working when you come to a STOP, so that we can talk about the questions as a group

We'll start out by thinking about a metabolic pathway, the kind of pathway frequently discussed in Genetics

3. In a double-mutant strain with *lf* mutations in both Gene 1 and Gene 2, the colonies that form will be

- a. colorless (white).
- b. blue.
- c. green.

If=loss of function

For the simple metabolic pathway you just looked at,

- a) If mutations in Gene 1 will be epistatic to mutations in Gene 2.
- b) If mutations in Gene 2 will be epistatic to mutations in Gene 1.
- c) neither of these mutations will be epistatic to the other.

Return to the worksheet

6. In a strain that has an *lf* mutation in Gene 8, the colonies that form on glucose plates will be
- a. colorless (white).
 - b. blue.
 - c. green.

8. In a strain that has an *lf* mutation in Gene 8, the colonies that form on lactose plates will be
- a. colorless (white).
 - b. blue.
 - c. green.

Return to final 3 questions on
worksheet

11. In a double-mutant strain that has *lf* mutations in Genes 8 and 9, the colonies that form on glucose plates will be

- a. colorless (white).
- b. blue.
- c. green.

12. In a double-mutant strain that has *lf* mutations in Genes 7 and 9, the colonies that form on glucose plates will be

- a. colorless (white).
- b. blue.
- c. green.

If=loss of function

For the signaling pathway, If mutations in Gene 9 will be,

- a) epistatic to If mutations in Gene 7, but not in Gene 8.
- b) epistatic to If mutations in Gene 8, but not in Gene 7.
- c) epistatic to If mutations in Genes 7 and 8.

A pathway can be thought of as a stream, running in the direction of the arrows. Upstream genes or proteins are those nearer the start of the pathway, downstream nearer the end.

As a general rule, for a metabolic pathway,

- a) A If mutation in a more upstream gene will always be epistatic to a If mutation in a more downstream gene.
- b) A If mutation in a more downstream gene will always be epistatic to a If mutation in a more upstream gene.
- c) Neither generalization can be made; epistatic relationships must be determined for each metabolic pathway individually.

As a general rule, for a regulatory signaling pathway,

- a) A If mutation in a more upstream gene will always be epistatic to a If mutation in a more downstream gene.**
- b) A If mutation in a more downstream gene will always be epistatic to a If mutation in a more upstream gene.**
- c) Neither generalization can be made; epistatic relationships must be determined for each regulatory pathway individually.**

A signaling pathway can be thought of as a series of switches with a particular cell fate as a final outcome:

A metabolic pathway involves the construction of a particular PRODUCT (ie, a pigment, or some other kind of molecule), where each step forms an intermediary. Thus, it is fundamentally different from a signaling pathway of on-off switches

Even in the case of a series of transcription factors that act sequentially to determine cell fate, the epistatic relationship we have discussed for signaling pathways is still true:

$A \rightarrow B \rightarrow C \rightarrow \text{fate } X$

If C is “off” (not activated, or loss of function mutation), fate X cannot be made, no matter what happened upstream

If C is “on” (active, or gain of function mutation), fate X will always be made, no matter what happened upstream

Thus, mutations in C will be epistatic to mutations in A and B .

Applications
(back to the vulval fate determination pathway)

Vulval fate determination

let-60(lf): no VPC fates (Vul)

let-60 (gf): all Pnp cells take on fate of Vulva (Muv)

lin-1(lf): Muv

Different pathways *let-60* → VPC fates ⊥ *lin-1*

Same pathway *lin-1* and *let-60* act sequentially to specify VPC fate, and one regulates the other

If the “end result” of your pathway is “vulval fate”, how could you draw these two genes interacting in a pathway?

You know the following information about mutants in the pathway of genes that help to specify the vulva:

Mutant gene (If mutations)	Phenotype
let-60	Vul; all Pnp cells make epidermis (3° fate)
lin-1	Muv; all Pnp cells 1° or 2° fate of vulva
let-60; lin-1	Muv; all Pnp cells 1° or 2° fate of vulva

Of the pathways shown below, which best describes the above data?
(Pathways show a gene's normal function.)

- a) lin-1 —| let-60 → vulval fate
- b) lin-1 → let-60 —| vulval fate
- c) let-60 —| lin-1 —| vulval fate
- d) let-60 → lin-1 —| vulval fate
- e) a and c

You have two loss of function mutants, *let-60* (lf) and *lin-23* (lf), both of which have the Vulvaless phenotype. What can you do if you want to figure out the order of these two genes in the pathway of vulval fate?

- a. Make a double mutant strain with these two loss of function mutants
- b. Search for a gain of function mutation in one of these genes, then make double mutant
- c. Epistasis testing won't work; find another approach

Other ways to analyze gene function

The kinds of genetic analyses we have described so far allow us to interpret a gene's general role: *ie*, this gene is required for vulval cell fate

How do you find out the actual function of a gene?

- search the available genomic database to find out what the predicted protein's function is

If you don't yet know the sequence of the gene...you can take a different approach: mosaic analysis (more later in the course)