

MCDB 4650 Class 13

Unit 3: Using genetics to study developing organisms

We will focus on *C. elegans* and *Drosophila* and discuss mouse at the end of this unit

Learning goals:

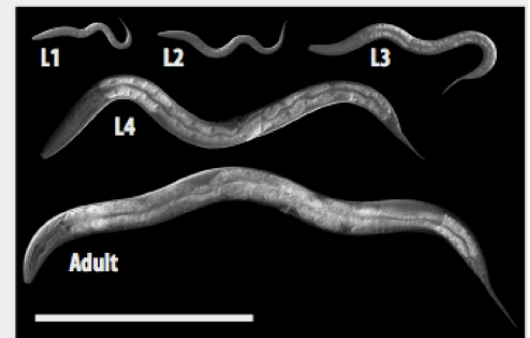
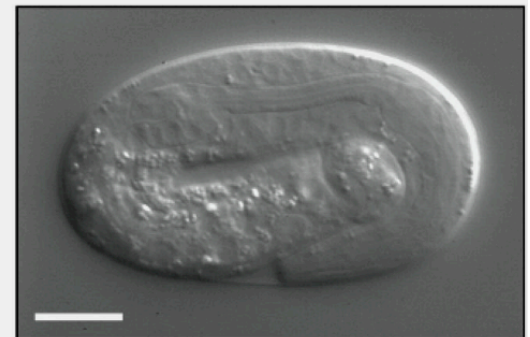
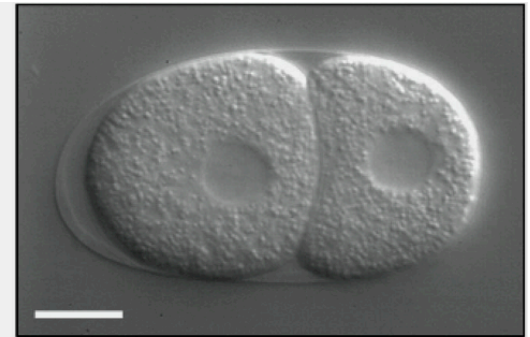
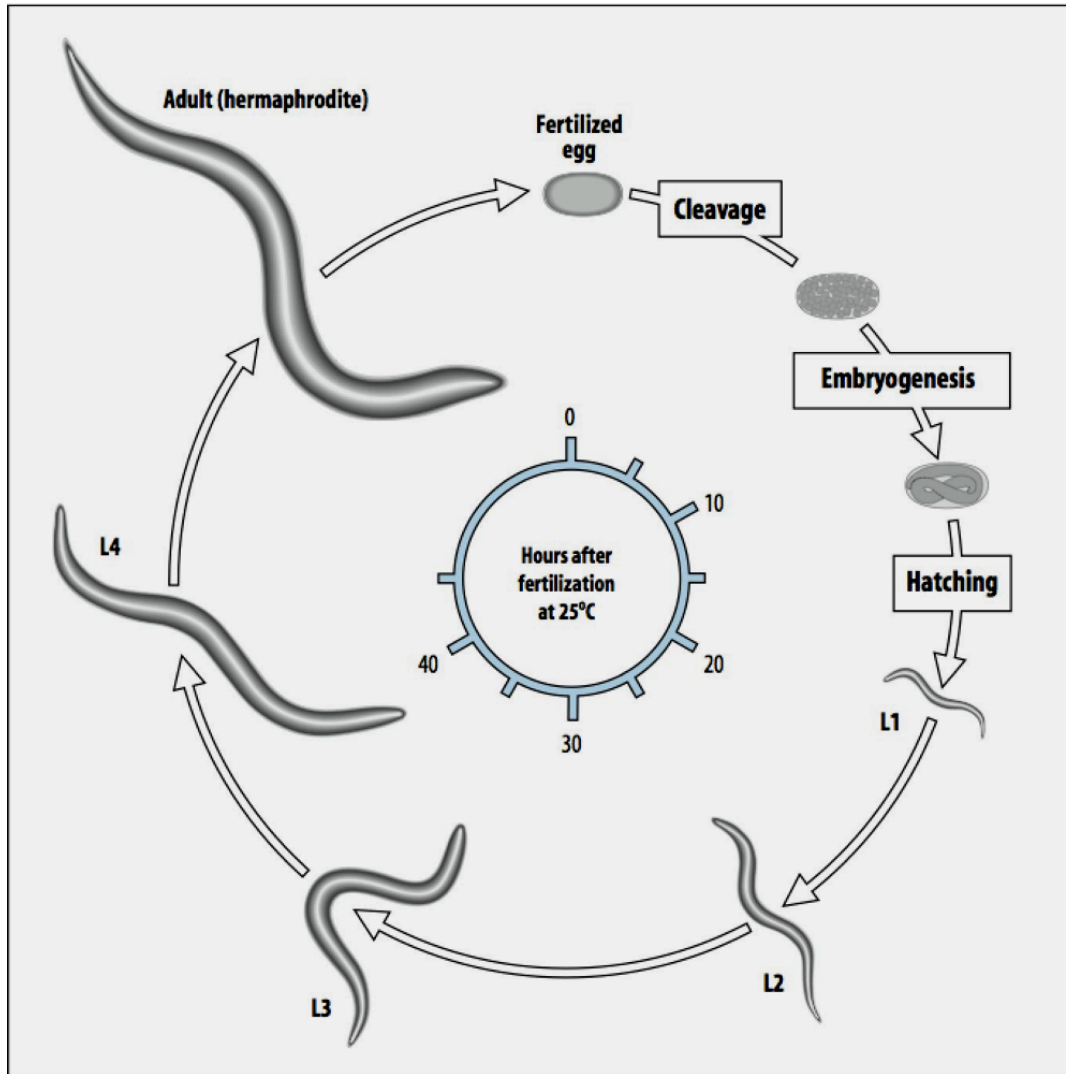
Be able to

Predict the properties of mutations based the phenotypes that result and/or information from crosses

Determine whether exploratory (forward) or manipulative (reverse genetics) should be used in different experimental settings

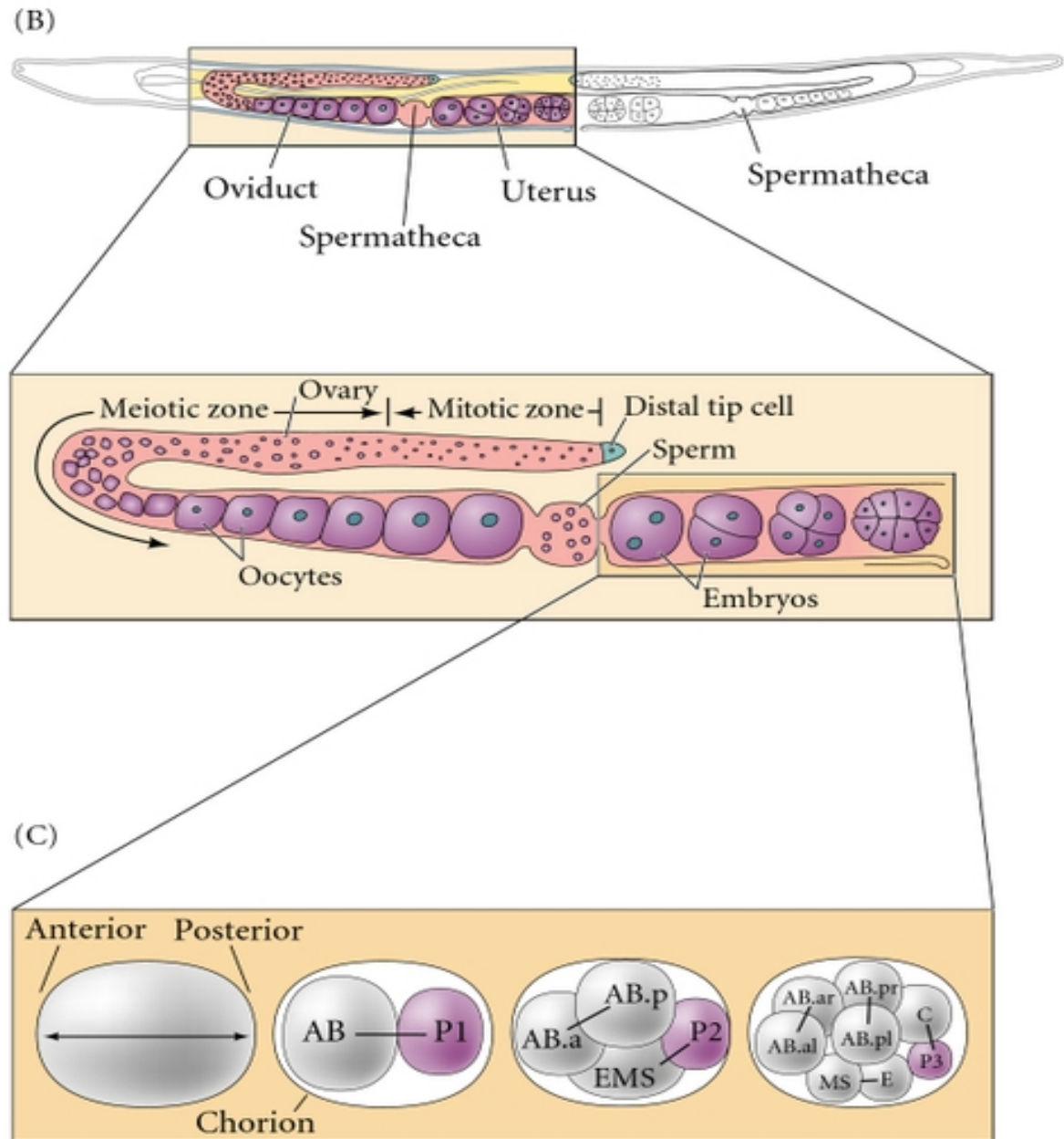
Compare different techniques used to answer different kinds of developmental genetics questions

The *C. elegans* life cycle

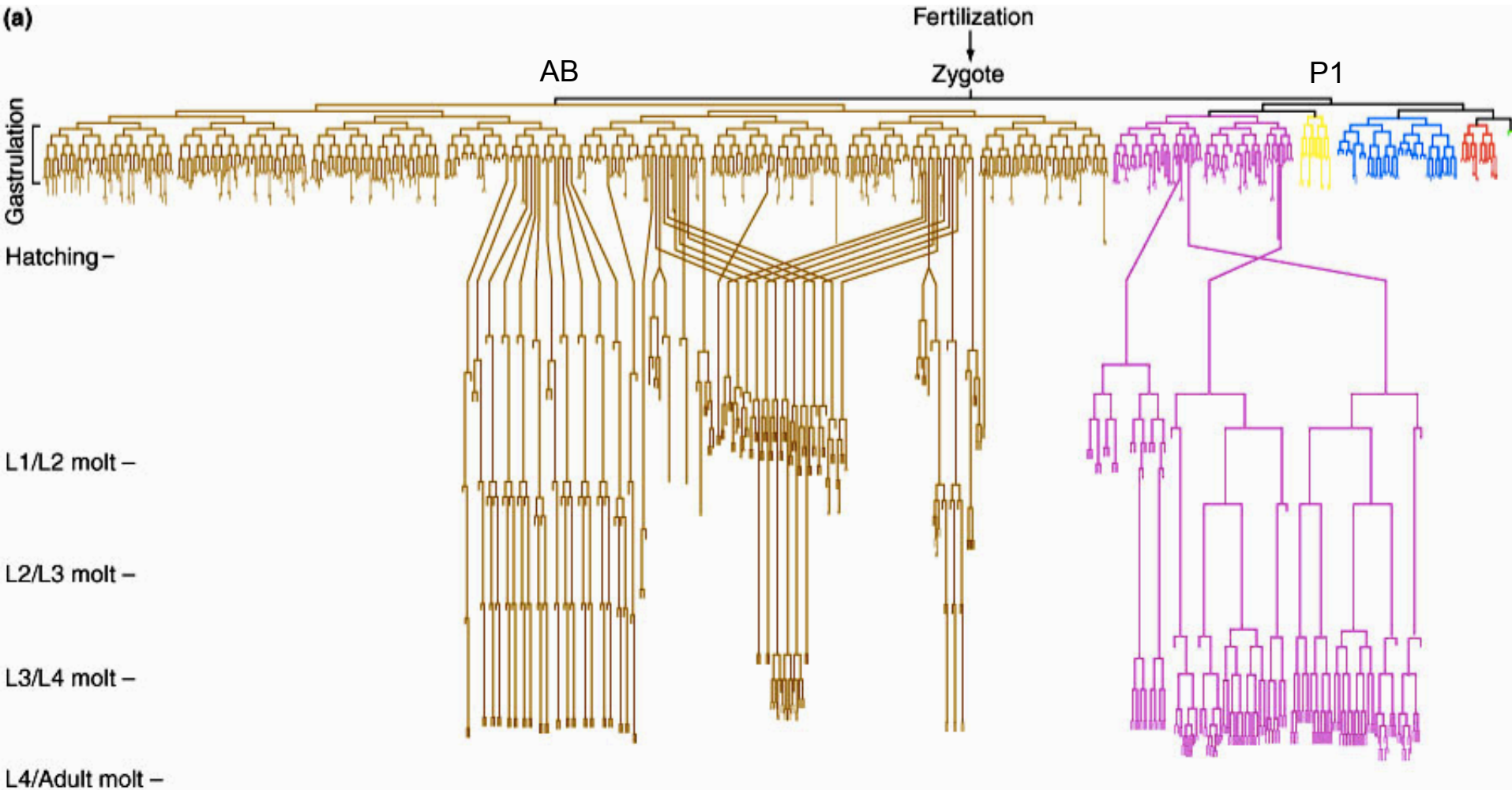


C. elegans is primarily hermaphroditic: these worms make both eggs and sperm, and “self-fertilize”

(males do exist: <1% of the population)



The complete *C. elegans* cell lineage from zygote to adult



Adult hermaphrodite has 959 cells

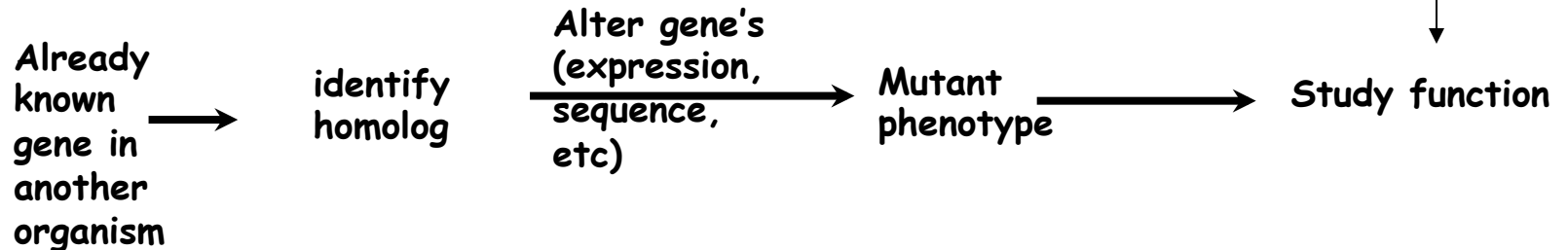
Using genetics to study development: two approaches

Exploratory genetics



vs

Manipulative genetics



Questions on handout (top half)

- a. Exploratory (forward) genetics
- b. Manipulative (reverse genetics)

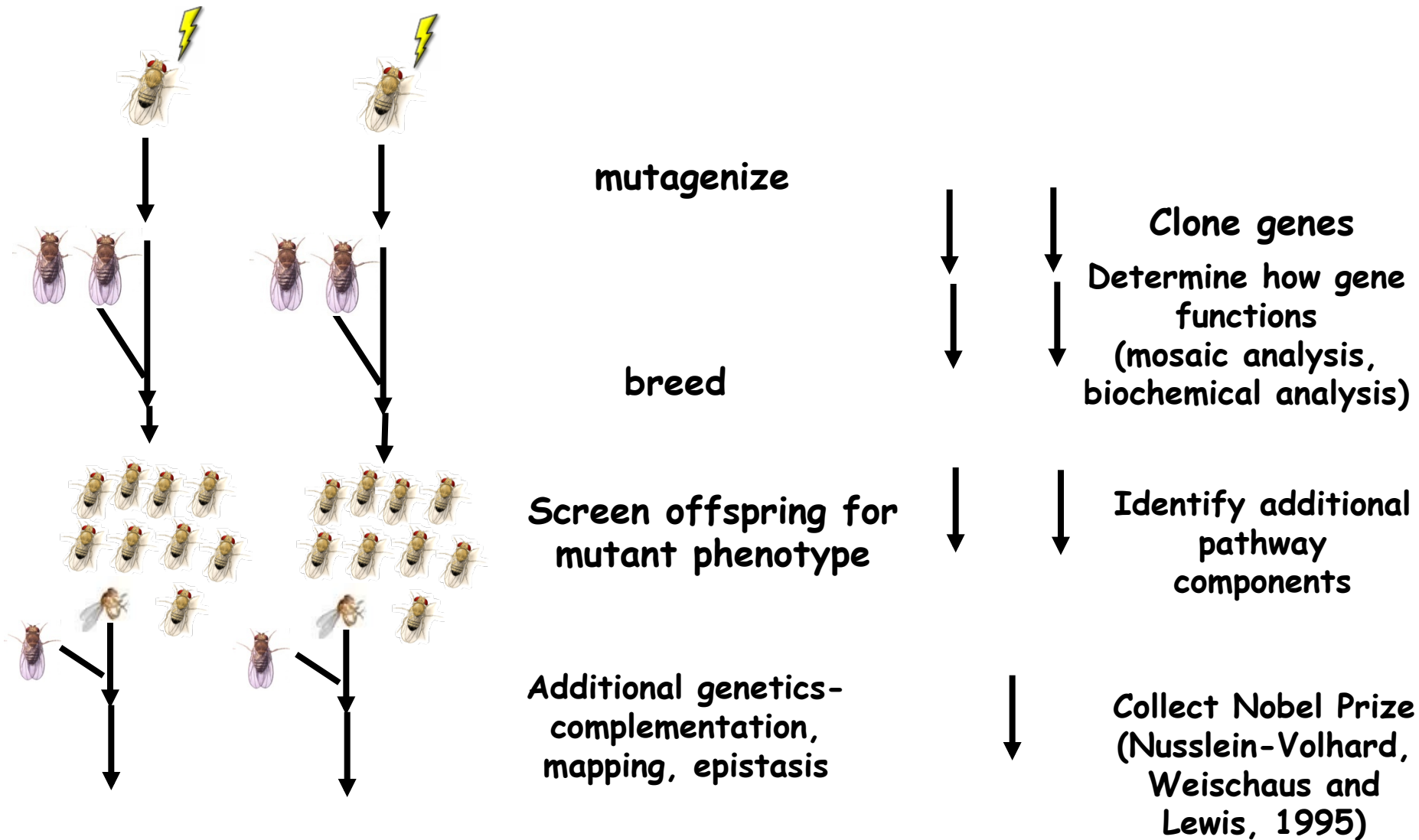
1. What are the genes that control formation of the *C. elegans* pharynx (eating apparatus) during embryogenesis?
2. Loss of function mutations in the *daf-2* gene in *C. elegans* lead to a longer lifespan. Do mutations in this gene cause a similar phenotype in mice?
3. What genes control the development of the somatic gonad during larval development in *C. elegans*?

You are trying to find out about genes that control leg formation in *Drosophila*. You have just done a mutagenesis and isolated a mutant that has no legs. That's all you have right now: a fly with no legs. What is the next step you need to take to ultimately find out what protein this gene encodes?

- a. Sequence the gene and look up the translated protein in a database.
- b. Use DNA from the gene to probe a Southern blots of DNA from mutant and wild-type to look for a difference.
- c. Genetically map the mutation to a chromosomal location.
- d. Knock out the gene using RNAi.
- e. Biochemically purify the protein.



Overview of Forward Genetics



Let's walk through this process of exploratory genetics with an example.

Say you are interested in the genes that control programmed cell death (apoptosis)

With your group, work on the questions on the handout

You have isolated three mutants that all have the same basic phenotype of excess cell survival (too few or no cell deaths).

1. What are the normal functions of the corresponding gene(s)?

- a) To promote cell death.
- b) To prevent cell death.
- c) Not yet enough information to decide.

2. Crossing of mutant strain A or mutant strain B with wild-type worms gives $A/+$ or $B/+$

Both heterozygotes have normal phenotypes (normal cell death).

Crossing of mutant strain C with wild-type gives $C/+$

This heterozygote is still cell-death defective (no cell deaths in the embryo).

What can you conclude?

a) A and B are loss of function (*lf*) mutations, C is gain of function (*gf*)

b) A and B are gain of function (*gf*) mutations, C is loss of function (*lf*)

c) not enough information yet to decide

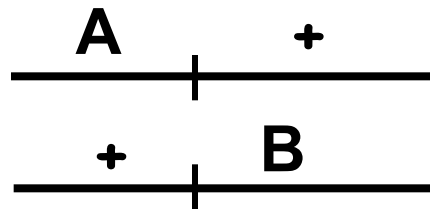
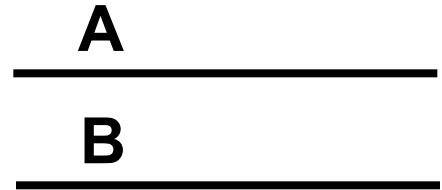
3. Mapping shows A and B are closely linked; C is on a different chromosome.

When you cross worms of genotype A/A with worms of genotype B/B, you get worms that have no cell death (like each single mutant alone)

You can conclude that A and B are

a) mutations in the same gene.

b) mutations in two different genes.



You have isolated another version of the *C* mutant, that you call *C2*. This mutant is a loss-of-function mutation. The phenotype is too many cell deaths causing embryos to die. What is the normal function of the *C* gene?

- a) To promote cell death.
- b) To prevent cell death.
- c) Not yet enough information to decide.

How about the *AB* gene? (same choices as above)

Proposed pathway?

These kinds of experiments allow the identification of the normal function of the gene

In general, **loss of function (lf) mutations are recessive**

$m/+$ has normal phenotype

(half of the normal amount of product is enough for normal function; the animal has to be lacking both copies for a phenotype to be seen)

In general, **gain of function (gf) mutation are dominant**

$m/+$ has a mutant phenotype

(half of the normal amount of product is not enough for normal function, or, presence of the altered product is enough to change the phenotype)

Exceptions to these generalized rules:

“haploinsufficient”: (incomplete dominance) loss of function mutations in which heterozygotes have an intermediate phenotype (reduced level of wild type protein is not enough for completely normal function):

e.g. Sickle cell anemia, Familial hypercholesteremia

“dominant negative”: loss of function mutation that is dominant because the mutant protein interferes with function of normal allele

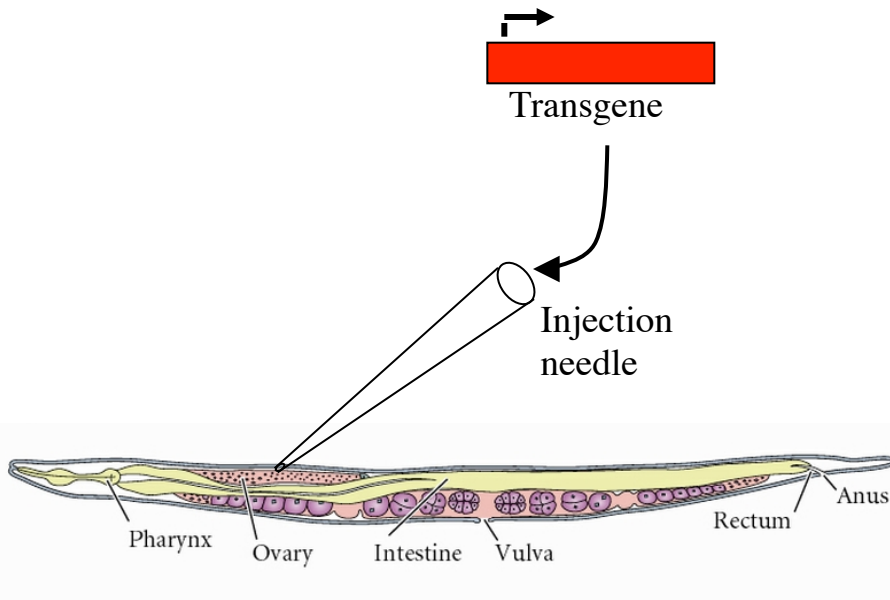
e.g. Marfan syndrome

Once you have
characterized your mutant (lf or gf)
mapped it
identified candidate genes

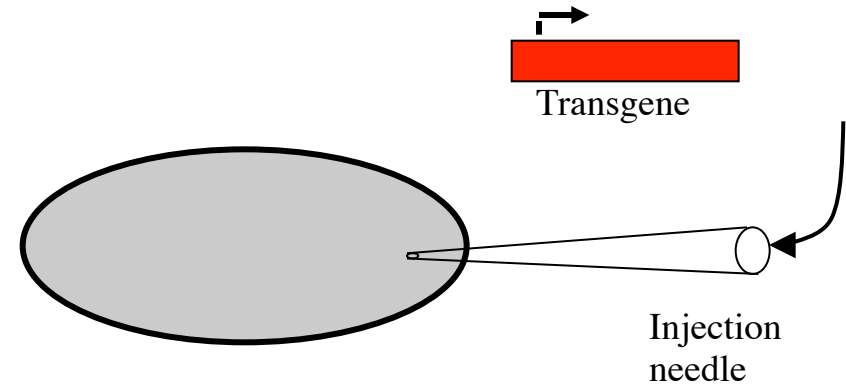
You need two more steps to collect evidence that
you have actually identified the gene in which a
mutation causes the phenotype you've been observing

To make a transgenic animal in worms and flies:

Get cloned DNA you have manipulated *in vitro* into the germline *in vivo*



Worms- inject DNA directly into the gonad of hermaphrodites: gets taken up into oocytes



Flies- inject DNA into the posterior end of the embryo, where the germ line cells will form

Next generation will express transgene

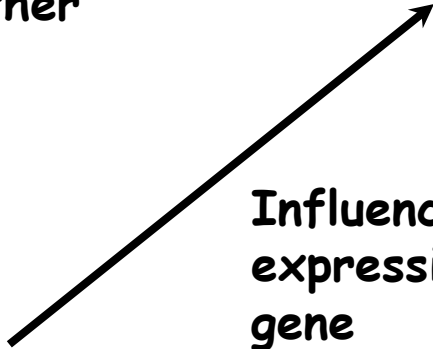
This moves us into considering manipulative genetics:
altering a known gene produce to determine effect on
phenotype

Manipulative genetics

Already known
gene in another
organism



Identify
homologue



Influence
expression of
gene

Observe
mutant
phenotype



Study function

Overview of common steps in manipulative genetics

Determine expression pattern of gene of interest for clues to function

- Make probes to the sequence you isolate (in situ hybridization)

What should we do next and what technique(s) could be used?

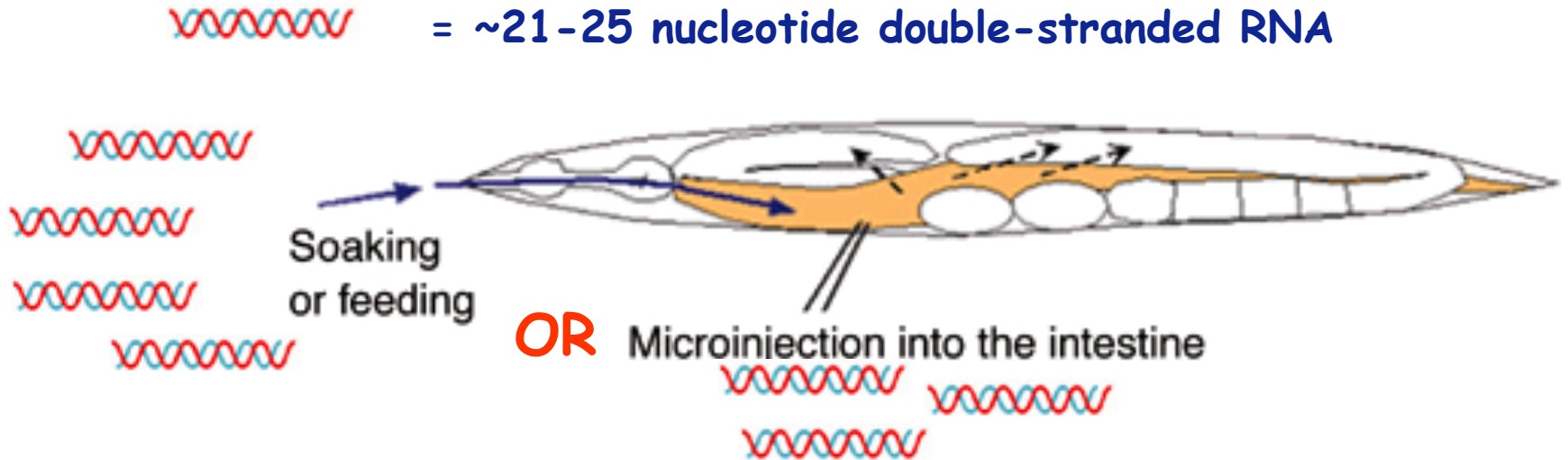
Silence the gene and observe the resulting phenotypes:

Create a mutant form of the gene in the organism's germ line

- targeted knock out in mice
- RNAi in worms and flies

RNA interference: “knock-down”

Noble prize 2006, Fire and Mello



The double stranded RNA gets from the gut into the oocytes
The progeny often display characteristics of a loss-of-function mutation
in the gene homologous to the RNA.

What else can be learned about the gene of interest?

1. What controls expression of transcript
(e.g where are the regulatory regions, how do they act, etc)

2. Upregulate transcript and study outcome

For both of these, we make a transgenic animal

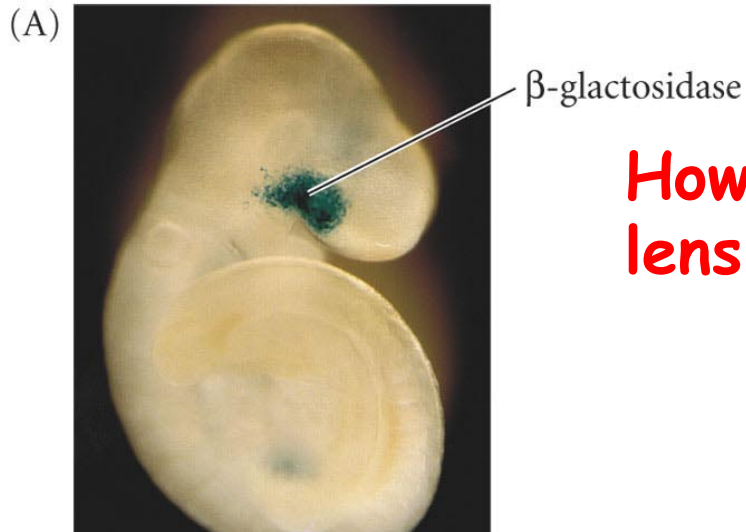
A transgenic animal has:

- a. The transgene present in every cell.
- b. The transgene present in a particular set of cells, determined by the makeup of the transgene.
- c. The transgene present only in a few random cells of the animal.
- d. The transgene present in cells targeted by the scientist.

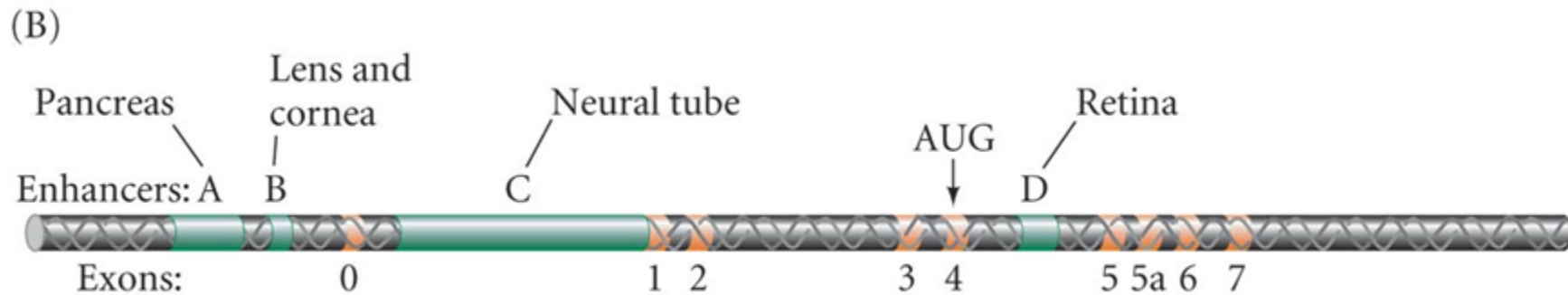
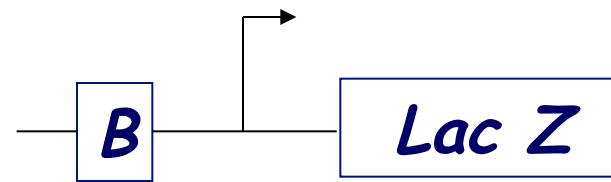
What determines where a transgene is expressed (ie, made into mRNA and ultimately into protein?)

- a. The DNA content of the cells
- b. The transcription factors present in a given cell
- c. The specific promoter/enhancer placed in front of the reporter gene
- d. A and C
- e. B and C

1. Visualizing where a gene normally acts: reporter construct



How do you get expression in the lens of this embryo?



2. Overexpressing transcript, or expressing in a new location (ectopic)

A transcript is turned on in a cell type that normally does NOT express this gene



These are TRANSGENIC flies: they have pax-6 expressed under the control of a different tissue-specific promoter

- The sets of techniques we discussed today can and are both used to determine
 - Genes that control certain processes
 - Whether homologs of these genes act in humans, and their roles in disease