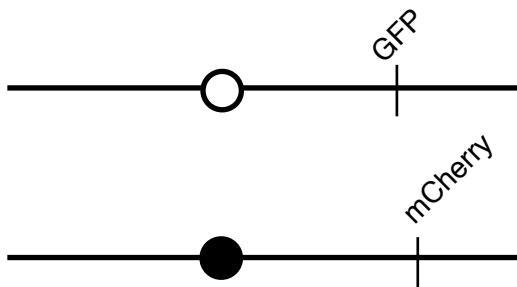


Question 1:

In *Drosophila*, you can generate twin spots using cell-specific markers. In the example below, red ommatidia are homozygous for the mCherry gene, green ommatidia are homozygous for the GFP gene, and yellow ommatidia are heterozygous for mCherry and GFP. Draw out the diploid homologous chromosomes with centromeres demarcated as open and closed circles and locations of the GFP and mCherry insertions that would lead to this mitotic recombination result.



Question 2:

A developmental geneticist at the University of Toronto identified four different promoters that drive expression of any gene in different parts of an isopod. She sends you the promoter sequences for expression in carapace, legs, antennae, and the whole animal. She also helps you to make transgenic isopods. You drive expression of the wild-type *red* gene, which when mutated makes the red color phenotype, using all four promoters.

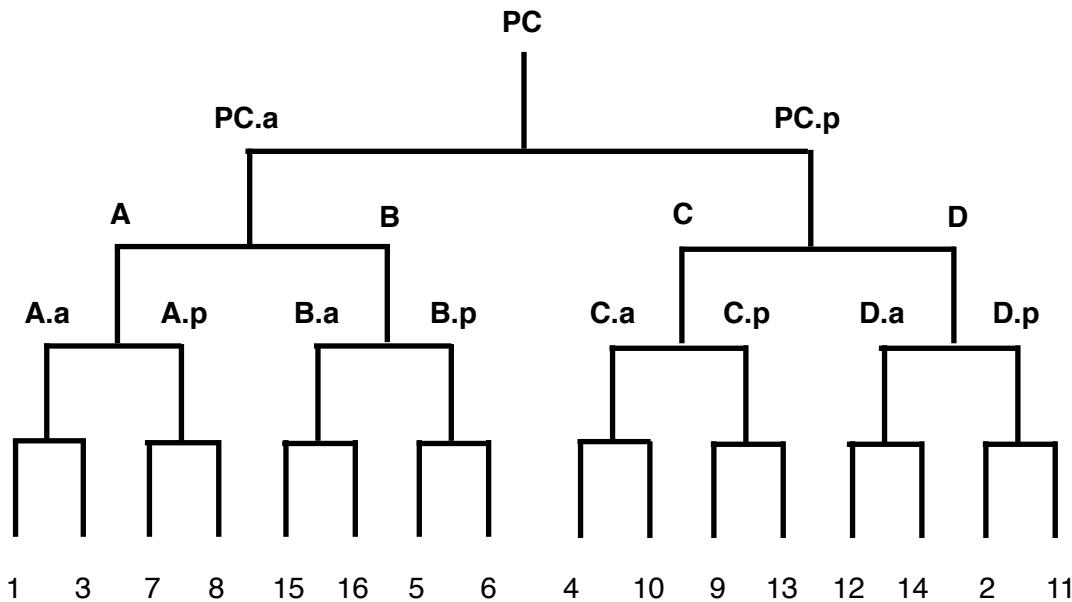
Describe the experiment (strains, promoters, etc.) that will determine where the function of the *red* gene is required in the animal using these reagents and any mutant or wild-type strains. Assume that the *red* mutant phenotype is recessive.

You want to rescue the red mutant phenotype so you will add expression constructs to red mutant isopods by transgenesis. If the gene functions in a particular cell type, then the red mutant phenotype will be rescued and the isopods will be gray.

If the red gene acts autonomously within the carapace, then you would expect that carapace promoter and whole animal promoter would drive red gene expression and rescue the red mutant phenotype. The leg and antennae promoters would not rescue the red mutant phenotype and the isopods will remain red.

Question 3:

You are studying a relative of *C. elegans* named *C. horvitzii*. Just like Bob Horvitz and John Sulston, you want to generate a lineage map of the organism from the zygote to the adult animal. Below is the lineage you have constructed so far.



You need to place cells 1-16 on the lineage. Using ablation to generate the following data:

Ablated cell	Cells that are present
PC.a	2, 4, and 9-14
A	2, 4, 5, 6, and 9-16
A.a	2, and 4-16
B.p	1,2,3,4,7,8, and 9-16
D	1,3,4,5,6,7,8,9,10,13,15, and 16
D.p	1,3,4,5,6,7,8,9,10, and 12-16
C.p	1-8, 10, 11, 12, 14-16

(a) Label the lineage above with cells 1-16.

You have several lineage defective mutants that fail to form certain cells and their direct descendants. Using these mutants, you attempt to discern which cells are needed for the development of particular components of the adult body.

In mutants for lineages A, B, C, and D.a, you always see the tail formed correctly. Mutants for lineage D never have a proper tail.

In mutants for lineages A.p, B, and PC.p, you always see a proper vulva. However, mutants for lineages A and PC.a never have a proper vulva.

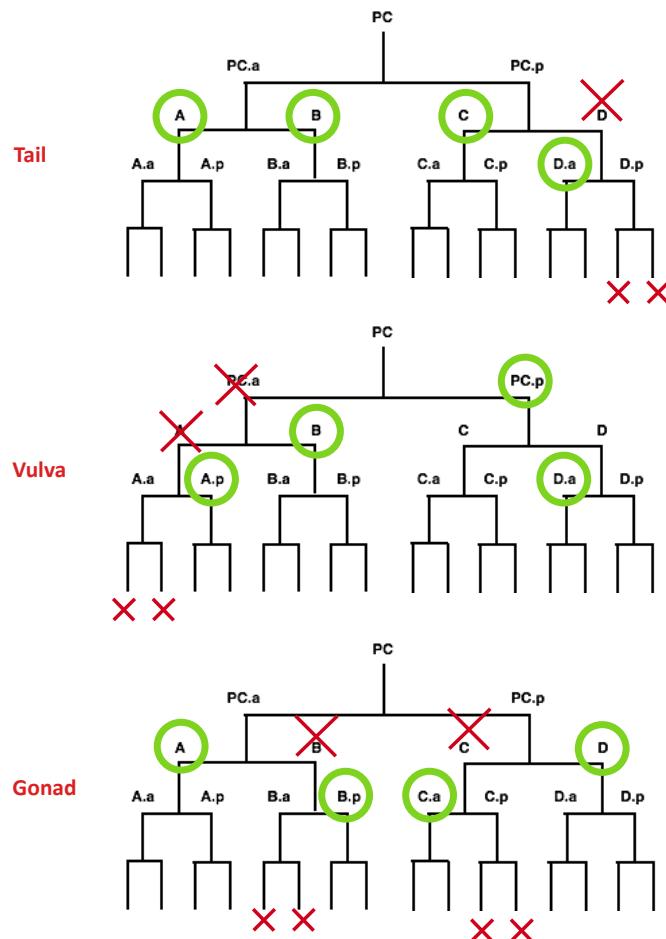
In mutants for lineages A, B.p, C.a, and D, you always see proper formation of the gonad. However, mutants for lineages B and C do not have gonads.

(b) Which cells are responsible for tail, vulva, and gonad formation?

Tail = Dp

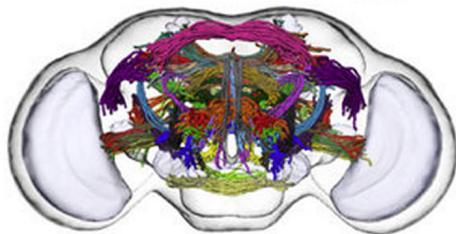
Vulva = A.a

Gonad = B.a + C.p



Question 4:

Drosophila extend their proboscis to eat or drink. This behavior is controlled by a complex system of connected neurons and muscles. The four images below show the process of proboscis extension (temporally from left to right).



- (a) You identified a mutant with a recessive phenotype of much reduced proboscis extension that you named *hungry*, and you are interested to figure out in which set of neurons shown in different colors (left) the gene regulates proboscis extension. Using a series of promoters that express gene products in these regions, please describe how you would determine in which set of neurons the *hungry* gene acts. Also, describe any caveats.

You can rescue the mutant *hungry* phenotype using the neuronal-specific promoters driving expression of the wild-type *hungry* gene. If *hungry* acts in that neuron group, then the mutant reduced proboscis extension phenotype will be rescued and flies will extend their proboscises. The major caveat is over expression or misexpression could rescue the phenotype by bypassing the requirement of *hungry* in another neuron type.

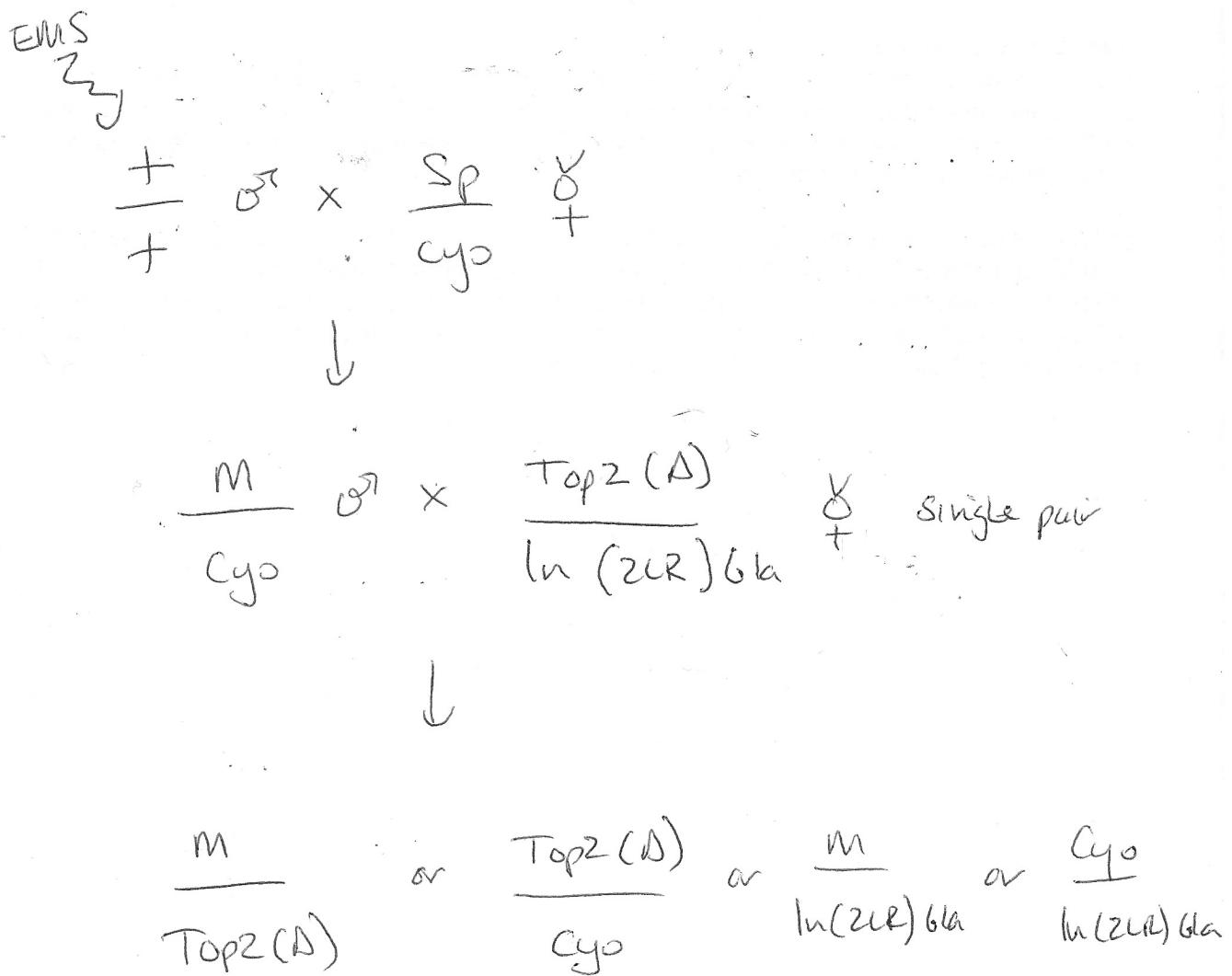
- (b) You determine that *hungry* acts in the purple set of neurons to regulate proboscis extension. Using the same promoter and optogenetic tools, describe how you would use specific tools to determine if those neurons inhibit or promote proboscis extension.

Using the same neuronal promoter that rescues the *hungry* mutant phenotype, you can express channelrhodopsin (activating) or halorhodopsin (inhibiting) in wild-type flies in those neurons. Upon stimulation with the appropriate wavelength of light, the neuron will be activated or inhibited. You can measure the proboscis extension. If the neuron is activating, then channelrhodopsin will stimulate extension and halorhodopsin will inhibit extension. If the neuron is inhibitory, then channelrhodopsin will inhibit extension and halorhodopsin will stimulate extension.

Question 5:

Topoisomerases are essential for proper DNA replication, transcription, and chromosome segregation. In *Drosophila melanogaster*, topoisomerase II (encoded by the *Top2* gene) is required for proper development to adulthood. Animals that are homozygous for a *Top2* null allele die during early larval development. You would like to get additional alleles of *Top2*.

Describe a screen to identify *Top2* but not mutations in other genes that cause larval lethality. You have a bottle of EMS and three fly stocks: the wild type, *Sp/Cyo* (Sternal plural dominant mutant with the Curly of Oster balancer chromosome), and a *Top2 Δ /In(2LR)Gla* stock (*Top2 Δ* is a null allele of *Top2* balanced by the *In(2LR)Gla* balancer chromosome that causes recessive lethality and a dominant increase in eye pigmentation). Note, *Cyo/In(2LR)Gla* flies are viable.



*In the last generation, you will see if you get any non-Cyo (non-curly winged), non-In(2LR)Gla (non-eye pigmentation) individuals. If you do not and you see larval lethality, then those mutants must have failed to complement *top2 Δ* .*

Question 6:

You are interested in understanding the genetics of congenital heart defects, so you perform an ENU-mutant screen in mice. After lots of work and heavy mutagenesis, you identify a mutant with a recessive heart defect and generate a pure-breeding stock. You notice that this stock also has a patchy hair loss phenotype, and your classmate (a self-purported mouse expert) points out that the stock is more aggressive than the parental stock.

You decide to cross wild-type parental males to your mutant stock. All of the resulting male offspring from that cross are patchy, but the mice have normal hearts and are less aggressive. After crossing siblings from that cross, you can identify males and females with heart defects and aggressiveness (but no patchiness) and use them to establish a stock that lacks patchy but has heart defects and aggressiveness.

- (a) Write out the cross and genotypes along with a brief explanation of why you performed this cross.

Let's say that the different mutant phenotypes are in three different loci (for the sake of writing the cross); *h* for heart defects, *p* for patchy hair, and *a* for aggressiveness. We are performing this cross to outcross what could be unlinked (and unrelated) mutations.

h p a mutants are crossed to *h+ p+ a+* wild-type individuals to get heterozygotes. In that generation, you see patchiness in 100% of male offspring, indicating that the mutation that confers patchiness is on the X chromosome.

h a p X h+ a+ p+

h a p males AND h a p females
 $h+a+Y$ $h+a+p+$

Once you cross siblings, you get rid of patchiness (again) showing that it is unlinked and unrelated.

- (b)** Provide a brief explanation for whether (or not) the same gene could be causing all three abnormal traits (heart defects, patchiness, and aggressiveness).

Mutations in the same gene could be causing both heart defects and aggressiveness (or they could be very closely linked and we haven't found a recombinant yet). The mutation that causes patchiness is definitely in a different gene because it is on a different chromosome (X).

Question 7:

Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric Weischaus shared the 1995 Nobel Prize in Physiology or Medicine for their work on the developmental genetics of *Drosophila*. In their screen for developmental genes, Nüsslein-Volhard and Weischaus initially identified 20 lines bearing maternal-effect mutations that produced embryos lacking anterior structures but with the posterior structures duplicated. When Nüsslein-Volhard mentioned this result to a colleague, he was astonished to hear that mutations in 20 genes could give rise to this phenotype. Explain why his astonishment was completely unfounded and showed a failure to understand genetics.

Nüsslein-Volhard never said that it was 20 mutations in 20 different genes! The 20 lines that they recovered from their screen likely have independent mutations, but those mutations could be in the same genes. Her colleague probably does not understand how mutagenesis works (random generation of mutations throughout genomes).

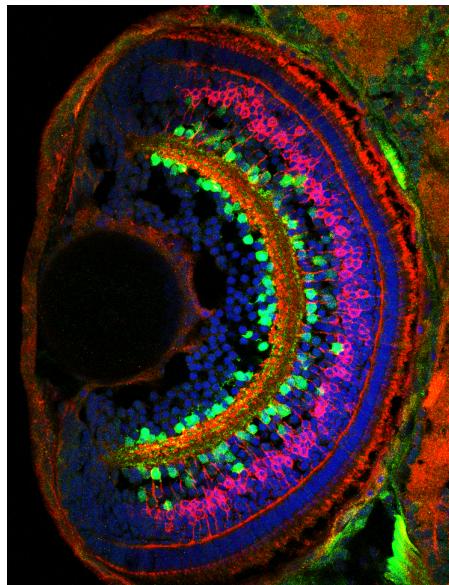
Question 8:

You are interested in vertebrate eye development and function. Therefore, like any good geneticist, you find the most tractable system to study your question – zebrafish.

- (a) You perform a mutant screen for fish that are unable to see certain colors and obtain three mutants in three separate complementation groups. Explain whether your screen is saturated and how you would know.

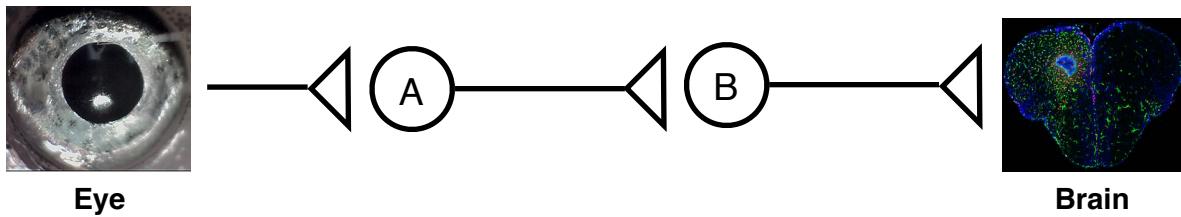
The screen is not saturated because we only have single mutations in single genes. In order to be saturated, we would expect to identify many independent mutations in the same gene.

- (b) Over the next three years, you map and clone the three genes. You chose zebrafish because of the plethora of genetic tools available. Using promoters that express your gene of interest in the different parts of the zebrafish retina, describe an experiment to test for the function of the *cb* gene in the blue, magenta, red, orange, or green neurons shown in the retina below.



In colorblind mutant fish, we use transgenesis to introduce different promoters that drive the wild-type copy of the colorblind gene in different neuron types. If the colorblind gene can rescue the mutant phenotype of the colorblind mutant when expressed in a specific neuron type, then the fish will have normal vision. The caveat of this experiment is that overexpression could ectopically bypass the colorblind mutation in certain neuron types.

(c) Using channelrhodopsin to manipulate neuronal activity in specific parts of the zebrafish, you would like to determine where and how your three genes act. You perform the following experiments and measure visual activity. Write out where *cb* and *rb* act in the rudimentary neuronal circuit below and the reasoning for your conclusions.



Channelrhodopsin stimulation	Genetic background	Visual activity
None	WT	YES
A neuron	WT	YES
B neuron	WT	YES
None	<i>colorblind</i>	NO
A neuron	<i>colorblind</i>	NO
B neuron	<i>colorblind</i>	YES
None	<i>red-blind</i>	NO
A neuron	<i>red-blind</i>	YES
B neuron	<i>red-blind</i>	YES

*Activating the B neuron bypasses the broken circuit in colorblind mutant fish, indicating that the colorblind gene (*cb*) must act previous to the B neuron in either the A neuron or in the eye.*

*Activating the A or B neurons bypasses the broken circuit in red-blind mutant fish, indicating that the red-blind gene (*rb*) must act previous to both the A and B neurons likely in the eye.*