

Question 1 (10 points):

After completing your screen for albendazole-resistant mutants on chromosome III of *C. elegans*, you get one mutant allele. This mutation causes a recessive phenotype in which *C. elegans* becomes resistant to the albendazole drug (they survive on agar plates that contain albendazole). You clone the gene and find that it encodes a beta-tubulin - the known molecular target for albendazole. Next, you are interested to know where this gene acts in *C. elegans*. You have a four different promoters (whole animal, neurons, muscles, and intestine).

(a, 5 points) Describe an experiment that allows you to determine that this beta-tubulin gene acts in the intestine for albendazole sensitivity. Please include strains, mutants, promoters, and how you will know that the experiment worked.

Normal function of the beta-tubulin gene causes lethality when animals are grown on albendazole. If the gene acts in the intestine, then resistant mutants will be rescued when the beta-tubulin gene is expressed in the intestine. Rescue in this case means lethality when grown on albendazole.

The whole-animal promoter driving the wild-type beta-tubulin gene is a good positive control to show that the resistant mutant phenotype can be rescued to cause lethality when grown on albendazole. We'll know the experiment worked if the mutant strain goes from resistant to sensitive when grown on albendazole.

The intestinal promoter driving the wild-type beta-tubulin gene will rescue the resistance mutant phenotype when grown on albendazole.

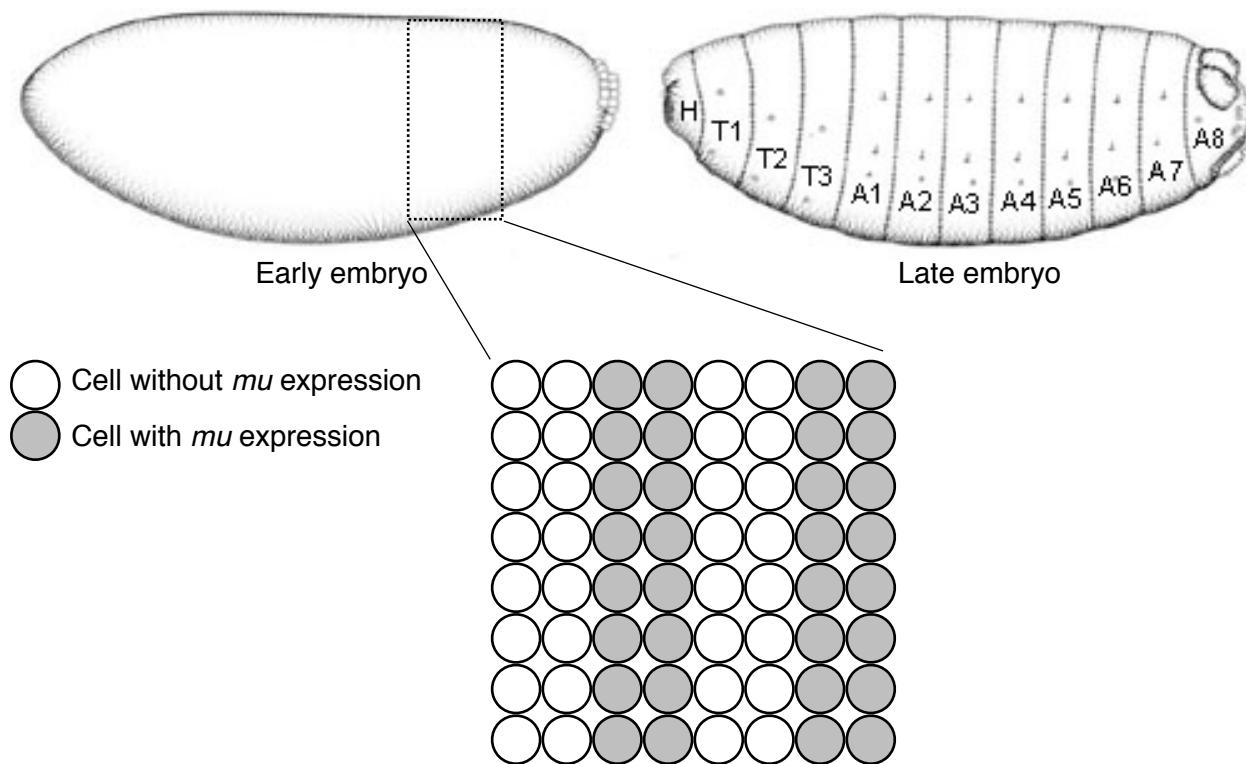
In both of the above experiments, beta-tubulin mutants will have promoters driving the wild-type beta-tubulin gene in all or some tissues of the animal.

(b, 5 points) Describe a selection to identify additional alleles of the gene mutated in part (a). Please include how you will exclude self progeny.

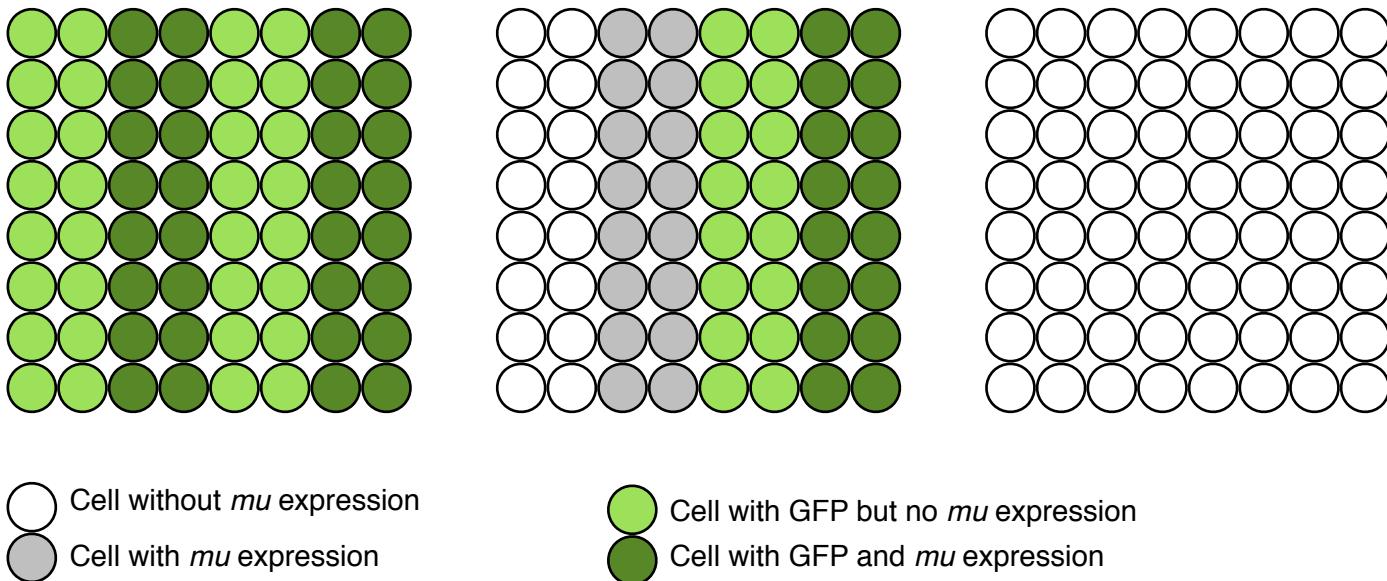
You can perform a non-complementation screen to identify additional mutant alleles of the beta-tubulin gene. It'll be a selection because you can select for resistance to albendazole. To exclude self-progeny, you can use another mutant allele (e.g. dpy) that causes a recessive phenotype. A cross of mutagenized wild-type males to doubly mutant beta-tubulin and dpy animals will yield heterozygotes for the dpy allele. If these animals survive on albendazole and are not dumpy, then we have identified a non-complementing allele of the beta-tubulin gene or a dominant resistance locus. Crosses for linkage and/or sequencing the beta-tubulin gene can help us differentiate these possibilities.

Question 2 (18 points):

You are studying pattern formation of the *Drosophila* embryo. Previous studies established that patterns can be distinguished by gene expression before they can be distinguished morphologically. One such gene is called *mixed up* (*mu*).



Mutations in the gene *disorganized* (*dis*) cause abnormal morphological patterning in the embryo. To determine whether *dis* affects the pattern of *mu* gene expression, you transplant nuclei from wild-type embryos into *dis* mutant embryos. Wild-type cells are labeled using the *GFP* gene with a constitutive promoter driving gene expression. In three representative embryos, you see the patterns of *mu* and *GFP* expression indicated below.



(a, 6 points) What two conclusions can you draw about the role of *disorganized* in controlling *mixed up* gene expression?

- (1) *disorganized promotes mixed up expression*
- (2) *disorganized acts cell non-autonomously*

(b, 6 points) Other data suggest that the *confused* (*cf*) family of genes (*cf1* and *cf2*) regulate *mu* gene expression. Previous genetic screens for embryonic patterning mutants failed to identify any *cf* mutants. Assuming the EMS screen was saturated, please suggest three other explanations for why *cf* mutants were not found.

You might not have found additional cf mutants because:

- (1) *You have saturated the sites that EMS can mutate. Other mutagens, like ENU or x-rays, might mutate additional genomic sites.*
- (2) *cf1 and cf2 are in a gene family, they might act redundantly. In that case, you would need mutations in both genes simultaneously to cause a mutant phenotype. That situation is unlikely to happen by standard mutagenesis.*
- (3) *It is possible that mutations in either or both of the genes cause a lethal phenotype. In that case you would not get mutant embryos to score for mu expression.*

(c, 6 points) Using a loss-of-function allele of *cf1*, you perform a genetic screen and identify a new mutation that causes abnormal patterning in the embryo indistinguishable from that caused by a lack of *mu* expression. Describe how you would determine if the effects of this new mutation depended upon the presence of a *cf1* mutation.

The assumption here is that the abnormal patterning phenotype is dependent on loss of cf1 and another gene. If you outcross this new mutant strain to the wild type and then intercross these heterozygous offspring, you would expect 1/16 of the offspring to have the mutant embryonic patterning phenotype if both alleles confer recessive phenotypes.

Alternatively, you could rescue the cf1 mutant in the isolated double mutant and see if the abnormal embryonic patterning is suppressed. You would have to have cloned the cf1 gene first, so the cross answer is preferred.

Question 3 (6 points):

Using a temperature-sensitive mutant, you want to determine when the gene *petal-maker* functions in flower development. You grow your flowers at permissive temperature until you see flower buds begin to develop. Then, every hour for five hours, you shift a population of flowers to the restrictive temperature. At the end of the five hours, you score for flower development. All of your shifted populations display the wild-type phenotype. Propose an explanation for these results.

The gene petal-maker acts before or after the time you shift the mutants to the restrictive temperature.

Question 4 (6 points):

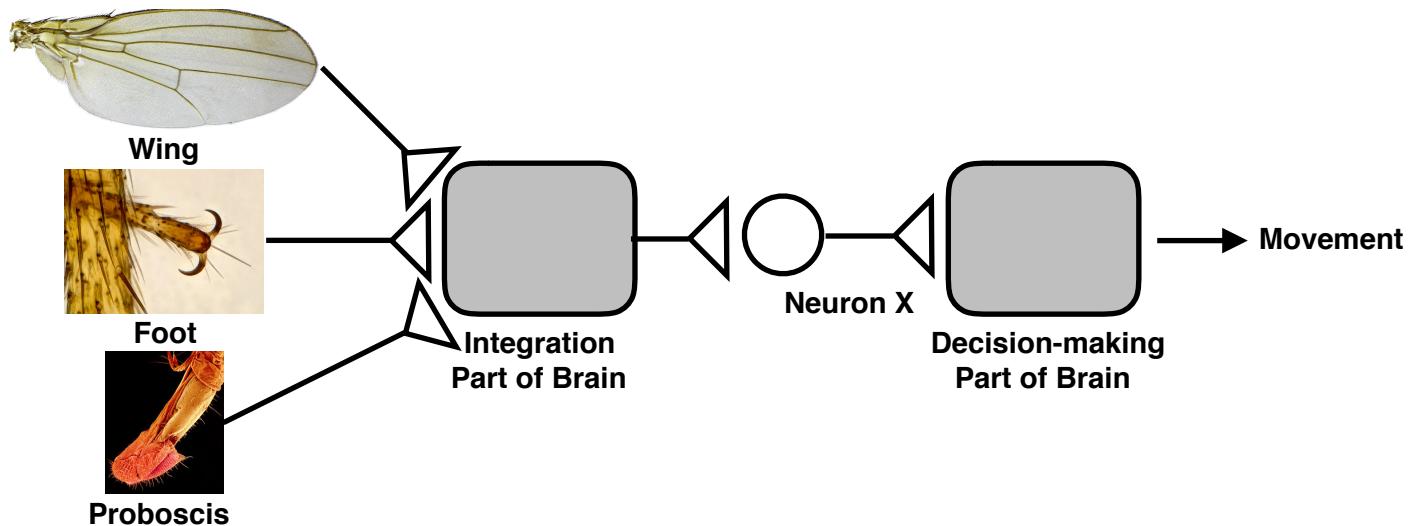
Your friend, an amateur lepidopterist or butterfly enthusiast, wants to know how butterflies generate wing spots. He grows a large colony of butterflies in his dorm room (much to his roommate's chagrin) and does a genetic screen to isolate spotless-winged animals. He gets a single mutant. Using an academic year URG, he sequences the genome of this mutant and the wild-type parent. His final project for BIO378: Functional Genomics compared these two genomes and found a variety of mutations, including one mutation in a gene similar to the pigment gene melanin. He is ecstatic! You are skeptical that he has found the gene for generating butterfly spots. Please explain why and what experiments are missing to make you less skeptical.

A single mutation in a single gene is not sufficient proof that the gene is responsible for the mutant phenotype. It is possible that the spotless phenotype is caused by some other gene besides the pigment-like gene. Mutagenized genomes are full of mutations, so you have to prove that the one you found is causally related to the gene of interest.

In order to prove that the pigment gene is involved in generating spotted butterfly wings, he will have to do two things. (1) He has to show that mutation of that gene causes the mutant phenotype. This can be accomplished using RNAi or CRISPR to knockdown or knockout the gene in normally wild-type butterflies and look for lack of spots. If those techniques are not available, he should identify additional mutant alleles using a non-complementation screen or an unbiased screen and then test that the new alleles fail to complement the original mutant allele. (2) He must show that you can rescue the spotless mutant phenotype by expression of the pigment gene. It is possible that this over expression could cause bypass suppression so both methods are required.

Question 5 (12 points):

Drosophila can taste from different regions of their body. In other words, the fly can taste a substrate when it lands on it using its feet, or taste volatile compounds that contact the edges of its wings, or taste food when its proboscis contacts it. Neurons involved in taste all use the same inducer of cell fate, so you can express channelrhodopsin in all taste neurons at the same time. Therefore, you can make a fly taste something by shining light on a certain body part. The circuit below controls taste decision making. The fly tastes with wing, foot, and/or proboscis, signals to the integration part of the brain through neurons, then signals from integration to neuron X, then signals to the decision-making part of the brain, then moves toward the tasty substrate. Two mutants, *taste-blind (tb)* and *happy meal (hm)*, fail to taste and move toward substrates. *tb* acts in the integration part of the brain; *hm* acts in the decision-making part of the brain. Fill out the table below for whether the fly will move based on the activity of neurons and genetic background with a constant food stimulus.



Channelrhodopsin stimulation	Genetic background	Movement (YES or NO)
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None	WT	YES
None	<i>tb</i>	NO
None	<i>hm</i>	NO
Wing	WT	YES
Foot	<i>tb</i>	NO
Neuron X	<i>hm</i>	NO
Neuron X	<i>tb</i>	YES

Question 6 (12 points):

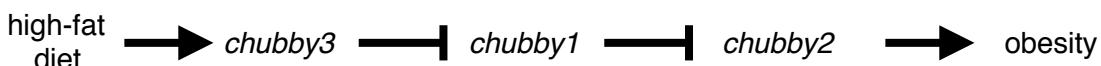
You are interested in mammalian metabolism on a high-fat diet. When wild-type gerbils are fed a high-fat diet, they become obese. Genetic screens for low and high responders to a high-fat diet identified three mutants, named *chubby1* through *chubby3*. Their phenotypes on a high-fat diet are shown in the table. You construct a series of double mutants, and the phenotypes are shown in the table.

(a, 6 points) Please write out a linear genetic pathway for how these three genes act in the process from high-fat diet intake to obesity.

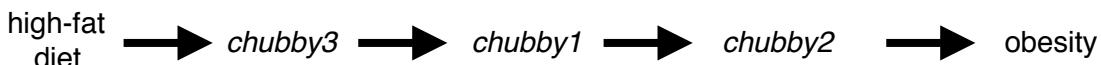
* The *chubby1* mutant has a dominant obesity phenotype even when fed a normal (not high-fat) diet.

Genetic background	Phenotype on high-fat diet
WT	Obese
<i>chubby1</i>	Obese*
<i>chubby2</i>	Skinny
<i>chubby3</i>	Skinny
<i>chubby1; chubby2</i>	Skinny
<i>chubby1; chubby3</i>	Obese
<i>chubby2; chubby3</i>	Skinny

The dominant phenotype in this problem could be caused by loss-of-function (haploinsufficiency) or gain-of-function (hypermorph). For either model, *chubby2* and *chubby3* promote obesity because both mutants have recessive skinny phenotypes. For haploinsufficiency, *chubby1* would be a negative regulator of obesity. The pathway for this model is below.



For a hypermorphic *chubby1*, *chubby1* would be a positive regulator of obesity. The pathway for this model is below.



(b, 6 points) RNAi of a fourth gene named *chubby4* causes a partial knockdown of gene function and an obesity phenotype on normal (not high-fat) and high-fat diets. You would like to put this gene in your linear pathway from part (a). Which mutants would you use? Please mention any caveats about interpreting gene order.

RNAi of *chubby4* causes obesity, so it is a negative regulator of obesity. You have to use mutants with opposing phenotypes, so you should use *chubby2* and *chubby3*. Both cause skinny phenotypes. The major caveat is that RNAi might not cause a complete loss of gene function so interpreting epistasis and gene order might be wrong. Depending on the epistatic results, *chubby4* could be epistatic to *chubby3* but not *chubby2*. In that case, we would not know the order of *chubby1* and *chubby4* with respect to each other.

Please fill out the post-midterm survey at bio393.andersenlab.org