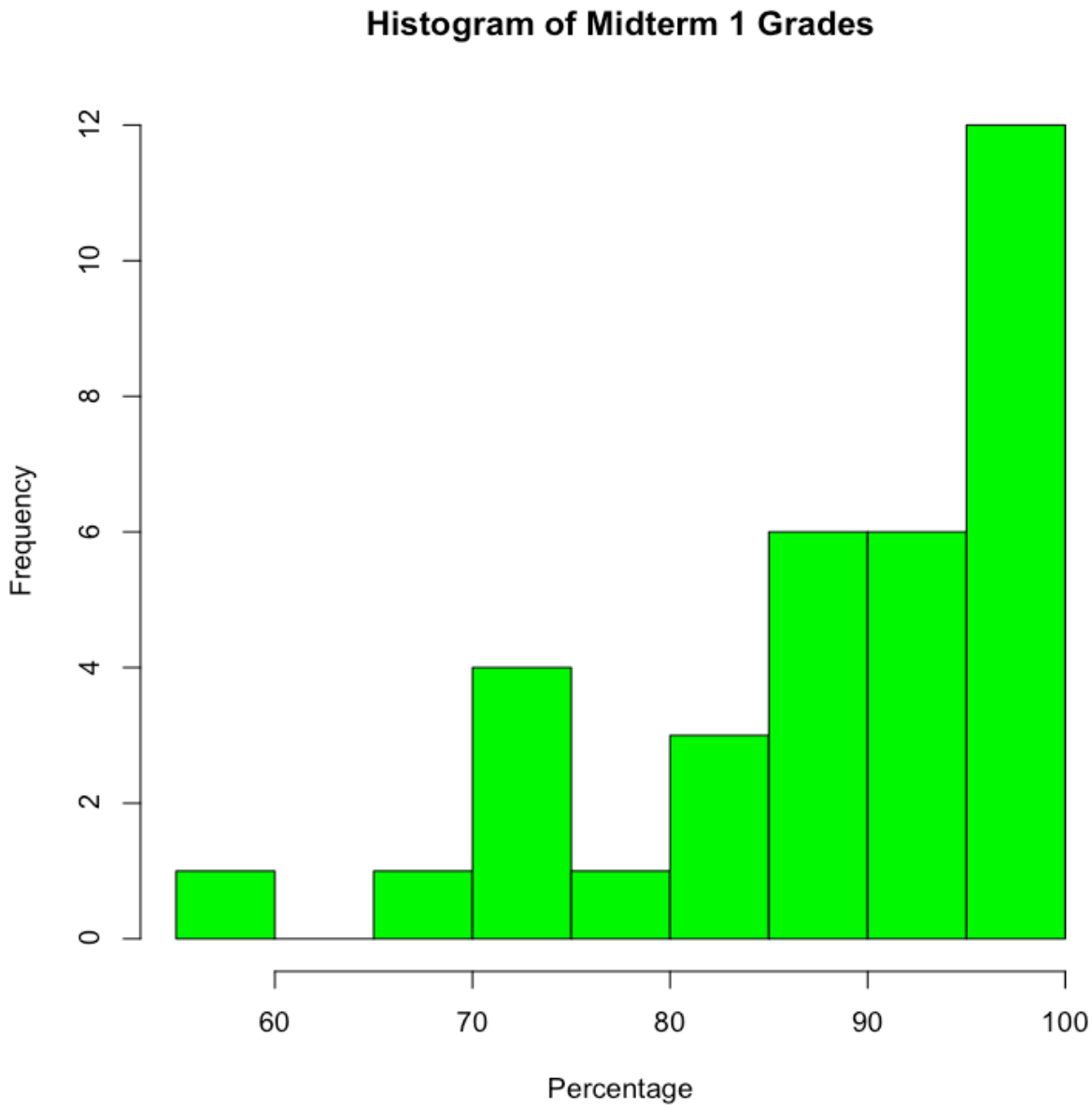
Your name: ANSWER KEY Student ID#:_____



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Write your name and student ID# on EVERY PAGE of your exam

MCB 141 Midterm I Feb. 15, 2011

Question #1 _____ **/ 32 pts**

Question #2 _____ **/ 27 pts**

Question #3 _____ **/ 24 pts**

Question #4 _____ **/ 17 pts**

TOTAL _____ **/ 100 pts**

Exam is closed book, closed notebook
NO CELL PHONES or other electronic devices
Exams must be turned in by 12:30 PM
All answers must be written in ink.
If you need extra space, write on the back of the page, but clearly indicate this on the front page of the question
Regrade policy: Turn in your entire exam to your TA and include a written explanation as to why you think you deserve additional credit.

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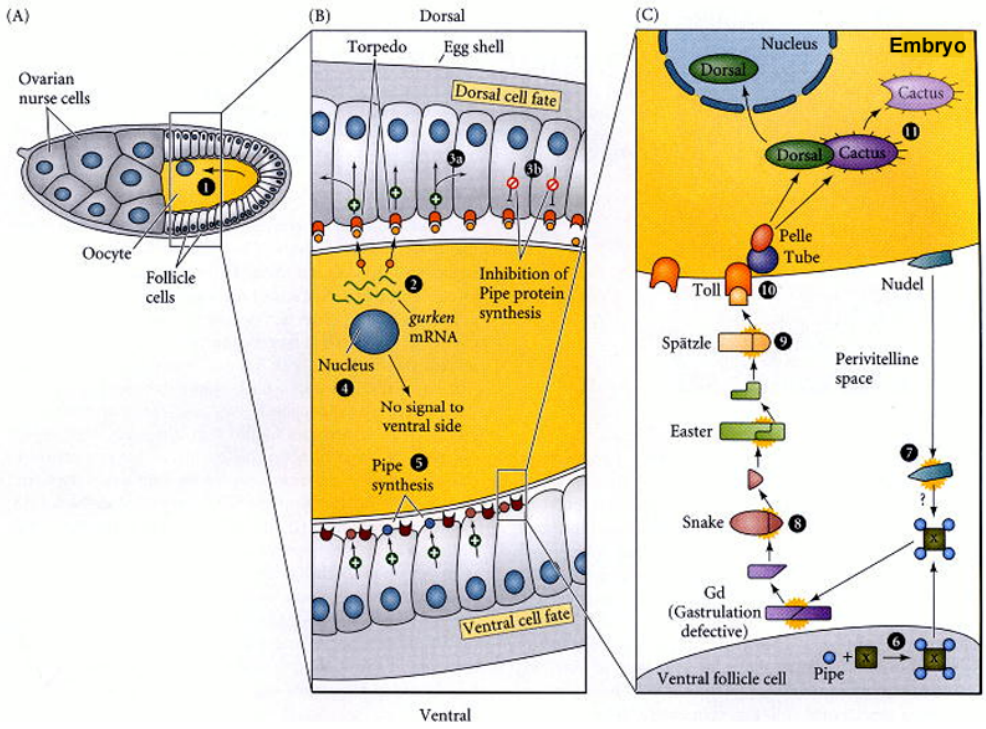
Question #1
We have discussed the pathway that establishes the initial dorsal/ventral polarity of the fly embryo (see below).



Predicted the phenotype (dorsalized, ventralized, or normal) of embryos produced by females of the following genotypes (assume all "–" alleles are complete lack of function alleles.). No explanation is needed for 1a-f, but do provide a brief explanation of your answers for 1g-i.

1a) snake^{-} / snake^{-} [1 point; no explanation needed]

Dorsalized

1b) cactus^{-} / cactus^{-} [1 point; no explanation needed]

Ventralized

1c) torpedo^{-} / torpedo^{-} [1 point; no explanation needed]

Ventralized

1d) snake^{-} / snake^{-} ; cactus^{-}, / cactus^{-} [2 points; no explanation needed]

Ventralized

1e) snake^{-} / snake^{-} ; torpedo^{-} / torpedo^{-} [2 points; no explanation needed]

Dorsalized

1f) snake^{-} / snake^{-} ; cactus^{-} / cactus^{-}; torpedo^{-} / torpedo^{-} [4 points; no explanation needed]

Ventralized

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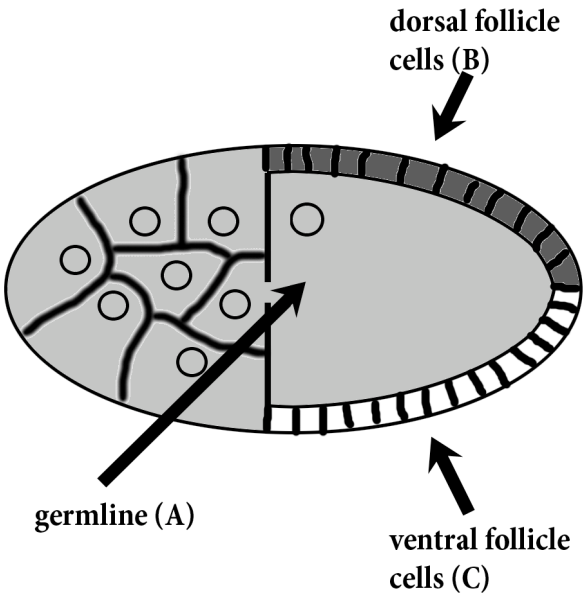


You discover a new, "magic" technique that allows you to create female flies in which you can have follicle cells that differ in their genotypes within a single female. You are able to watch oocytes developing inside a female, and as soon as you see the oocyte nucleus moving to one side (before gurken secretion begins), you can make the genotype of what should be the future dorsal follicle cells different from the genotype of what should be the ventral follicle cells. Combining this with pole cell transplants, you can thus generate females in which their are three distinct genotypes (see diagram on left)

1g) [7 points]
Assume the female has:

germline: dorsal^{-} / dorsal^{-} ; torpedo^{-} / torpedo^{-} ; cactus^{+} / cactus^{+}

dorsal follicle cells: dorsal^{+} / dorsal^{+} ; torpedo^{+} / torpedo^{+} ; cactus^{+} / cactus^{+}

ventral follicle cells: dorsal^{+} / dorsal^{+} ; torpedo^{-} / torpedo^{-} ; cactus^{-} / cactus^{-}

Predict the phenotype of the resulting embryos:

Dorsalized [3 pts]

Briefly explain your answer.

The germline is homozygous mutant for dorsal protein, which will ventralize the embryo [3 pts]. Torpedo not required in ventral follicle cells anyway, so pathway is fine all the way to dorsal [1 pt].

1h) [7 points]
Assume the female has:

germline: $dorsal^{+}$ / $dorsal^{+}$; $torpedo^{-}$ / $torpedo^{-}$; $cactus^{+}$ / $cactus^{+}$

dorsal follicle cells: $dorsal^{+}$ / $dorsal^{+}$; $torpedo^{+}$ / $torpedo^{+}$; $cactus^{+}$ / $cactus^{+}$

ventral follicle cells: $dorsal^{+}$ / $dorsal^{+}$; $torpedo^{-}$ / $torpedo^{-}$; $cactus^{-}$ / $cactus^{-}$

Predict the phenotype of the resulting embryos:

Normal (Wild-type D/V pattern) [3 pts]

Briefly explain your answer.

Torpedo protein is required in the dorsal follicle cells only. So, torpedo being homozygous mutant in the germline and the ventral follicle cells is inconsequential [2 pts]. Cactus protein is required in the germline only, so it also does not matter that the embryo is homozygous mutant for cactus in the ventral follicle cells [2 pts]. Therefore, the embryo is wildtype.

1i) [7 points]
Assume the female has:

germline: $dorsal^{+}$ / $dorsal^{+}$; $torpedo^{+}$ / $torpedo^{+}$; $cactus^{+}$ / $cactus^{+}$

dorsal follicle cells: $dorsal^{+}$ / $dorsal^{+}$; $torpedo^{-}$ / $torpedo^{-}$; $cactus^{+}$ / $cactus^{+}$

ventral follicle cells: $dorsal^{-}$ / $dorsal^{-}$; $torpedo^{+}$ / $torpedo^{+}$; $cactus^{-}$ / $cactus^{-}$

Predict the phenotype of the resulting embryos:

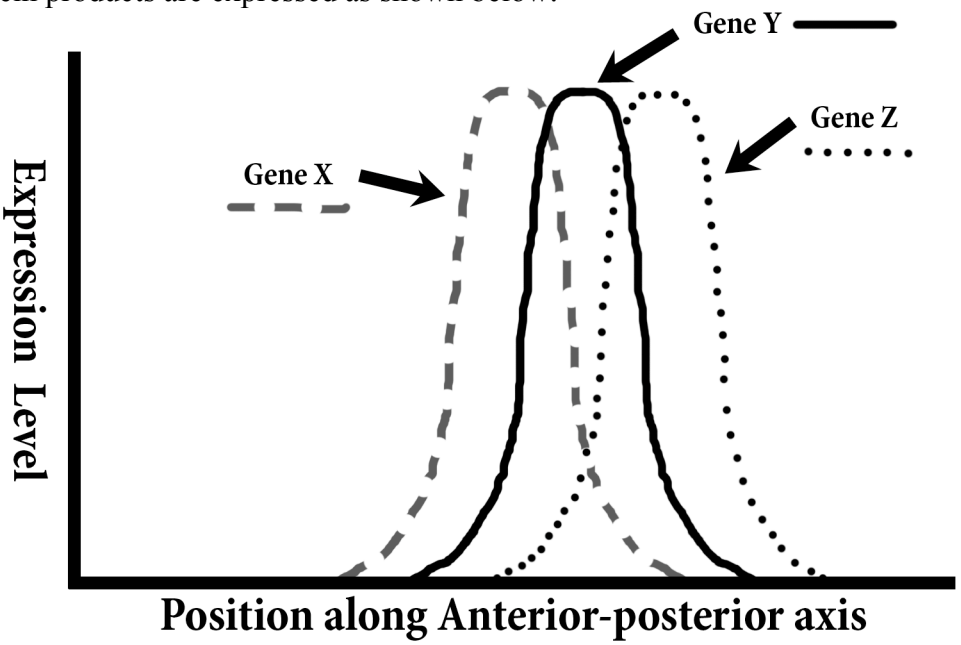
Ventralized [3 pts]

Briefly explain your answer.

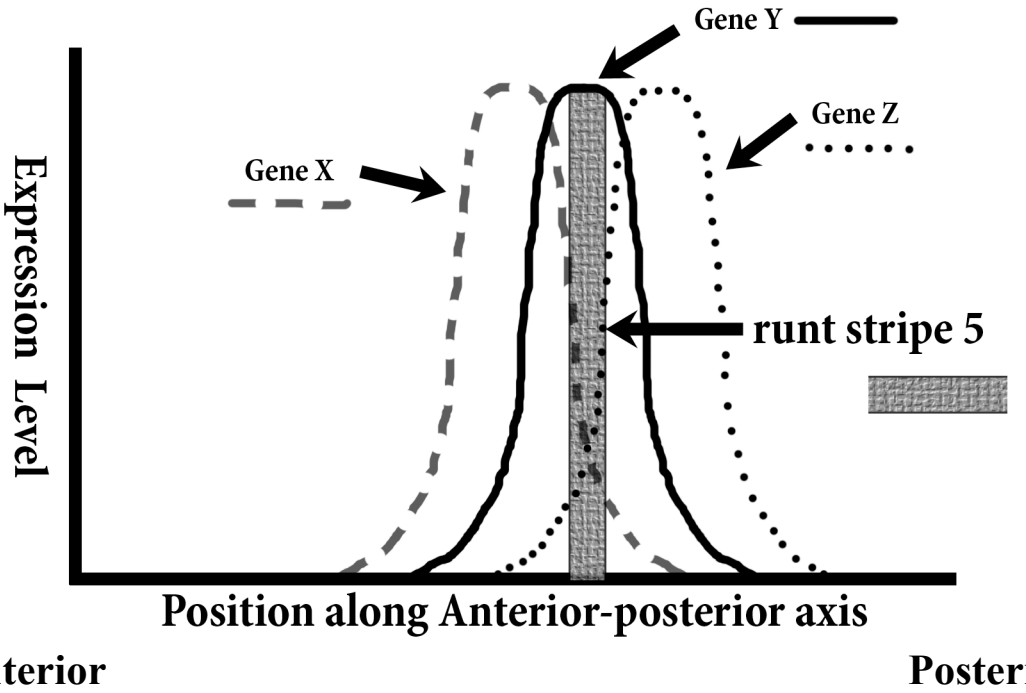
The embryo is homozygous mutant for torpedo in the dorsal follicle cells, which will ventralize the embryo [2 pts]. The fact that the embryo is homozygous null for dorsal and cactus in the ventral follicle cells does not affect D/V patterning because these proteins are only required in the germline [2 pts].

Question #2

You are studying the function of three gap genes, X, Y, and Z. The corresponding gap gene protein products are expressed as shown below:



You also investigate the regulation of stripe 5 of the primary pair-rule gene, runt. You isolate the enhancer for runt stripe 5, and observe that runt stripe 5 (indicated by grey bar below) is expressed in between the expression domains of gap genes X and Z as shown below:



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You sequence the enhancer for runt stripe 5 and notice that three different nucleotide sequences are repeated several times within the enhancer. [Note: Do not worry about the reverse strand DNA sequence – this is not meant to be a trick question.]

The three sequences that are repeated are:

CGCGATAT

TTTTTCGCG

ATATCCCCC

You create a series of synthetic enhancers (see below) and place each one in front of a lacZ reporter gene and introduce each into flies via P-element transposition. You then observe the pattern of lacZ expression in embryos of the transgenic lines that you establish.

Synthetic enhancer A (SynR5A) – all the CGCGATAT repeats are specifically eliminated from the normal enhancer. Instead of a normal runt stripe 5 pattern, you see no expression of the lacZ reporter in an otherwise wild-type embryo.

Synthetic enhancer B (SynR5B) – all the TTTTTCGCG repeats are specifically eliminated from the enhancer. Instead of a normal runt stripe 5 pattern, you see a broadened stripe of lacZ expression (broadened in the anterior direction) in an otherwise wild-type embryo.

Synthetic enhancer C (SynR5C) – all the ATATCCCCC repeats are specifically eliminated. Instead of a normal runt stripe 5 pattern, you see a broadened stripe of lacZ expression (broadened in the posterior direction) in an otherwise wild-type embryo.

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2a) Based on these data, predict the sequence that is bound by protein Y (circle the correct answer below). Give a brief explanation for your answer. [6 points]

CGCGATAT [2 pts]

TTTTTCGCG

ATATCCCCC

Protein Y is the one expressed most prominently in the region where the stripe is normally expressed and is thus likely to be the activator [1 pt]. CGCGATAT is present in both constructs that show reporter expression, but expression is missing in the one construct that is missing these sites (SynR5A), thus this is likely the site where the transcriptional activator, protein Y, binds. [3 pts]

2b) Based on these data, predict the sequence that is bound by protein Z (circle the correct answer below). Give a brief explanation for your answer. [6 points]

CGCGATAT

TTTTTCGCG

ATATCCCCC [2 pts]

The distribution of protein Z to the posterior of the endogenous runt stripe 5 supports protein Z's role as a posterior transcriptional repressor of runt expression [1 pt]. The sequence ATACCCC is likely bound by this repressor because when you loss these binding sites in the enhancer the lacZ runt stripe 5 is broadened in the posterior direction as seen for SynR5C [3 pts].

2c) [15 points]
You create a synthetic enhancer SynR5D with the sequence:

TTTTTCGCGCGCGATATATATCCCCC

You put SynR5D in front of a lacZ reporter gene and introduce it into flies via P-element transposition. You expect the lacZ pattern to mimic runt stripe 5, but instead you get a stripe that is much broader (spread both anteriorly and posteriorly from the normal boundaries for runt stripe 5).

You create a second synthetic enhancer called SynR5E with the sequence:

TTTTTCGCGATATCCCCC

You put SynR5E in front of a lacZ reporter gene and introduce it into flies via P-element transposition. You find that the lacZ pattern now perfectly mimics the pattern of runt stripe 5.

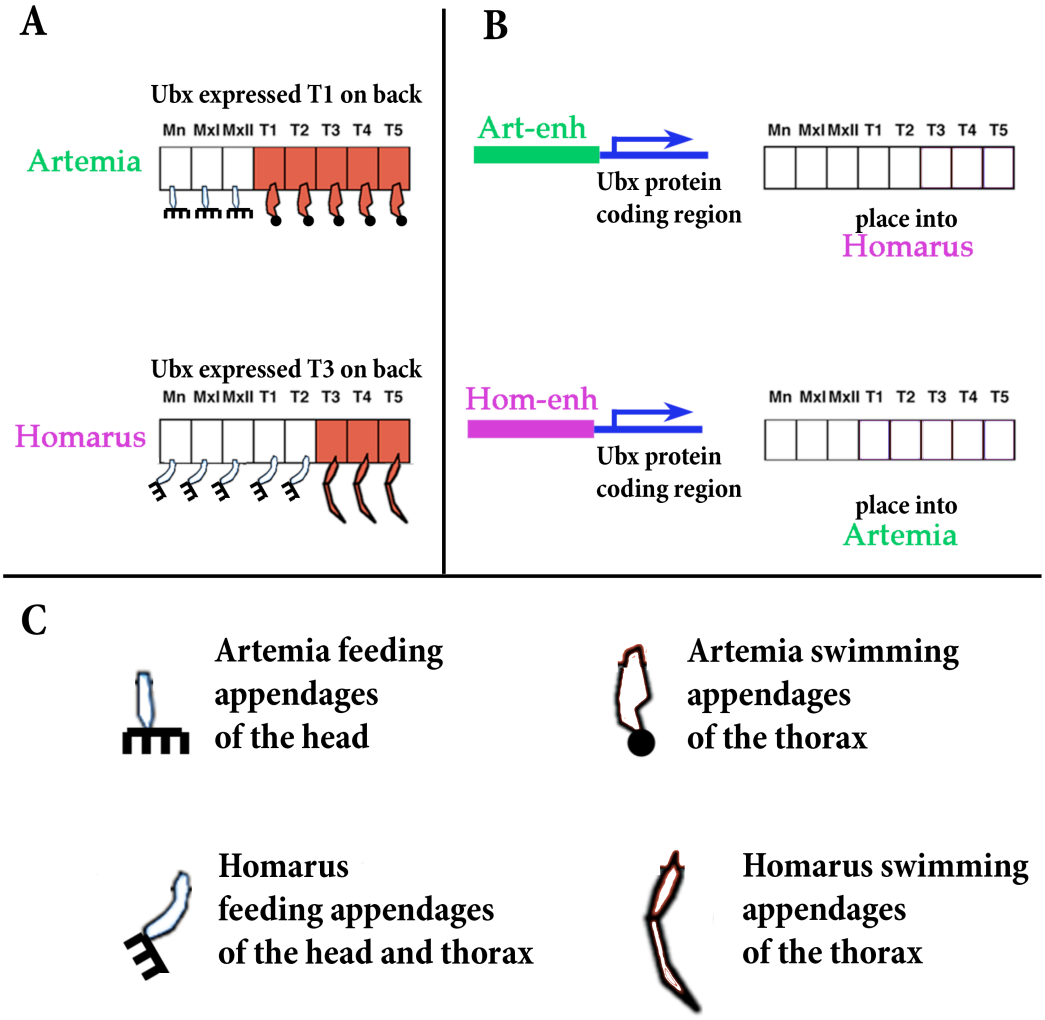
Give an explanation for your results. What does this tell you about how protein X, Y, and Z function? Why the difference in results for SynR5D and SynR5E?

Proteins X and Z are transcriptional repressors of the runt gene and Z is a transcriptional activator. Proteins X and Z appear to act as repressors by preventing the binding of the activator, Protein Y [5 pts].

In the SynR5E enhancer the repressor binding sites overlap the binding sites of Y. When the repressors are present, they hinder the binding of Y (physically get in the way, steric hindrance) [5 pts]. Thus SynR5E recapitulates the normal expression pattern of runt stripe 5. In SynR5D, the binding sites of Protein X and Z are present, but no longer overlap the binding site of protein Y, thus protein X and Z can bind, but cannot acts as repressors, and thus SynR5D gives a broader than normal stripe [5 pts].

Question #3

Ubx in Artemia (brine shrimp) is expressed from T1 on back, while Ubx in Homarus (lobster) is expressed from T3 on back (see panel A below). Recall the cis versus trans test that we designed in lecture to test why Ubx showed different expression patterns in the two species. In that case we used the Artemia and Homarus enhancers to control the expression of lacZ. Imagine instead that we replace both copies of the endogenous Ubx gene (that includes the coding region plus enhancers) with the Ubx enhancer plus Ubx coding region from the opposite species (see panel B below). Draw the expected results if the different expression patterns are from trans changes, and the expected results if the different expression patterns are due to cis changes. In your drawings show the expected expression domain of Ubx as well as the expected morphology of the appendage on each segment (see panel C below), and provide a brief explanation for your answers. (Note: Assume that there are no amino acid differences between the Ubx proteins of Artemia and Homarus, thus the two proteins function identically. Also assume that, unlike in *Drosophila*, there are no temporal differences to worry about in crustaceans - that is to say late and early patterns are the same within a given species).



3a) [12 points]

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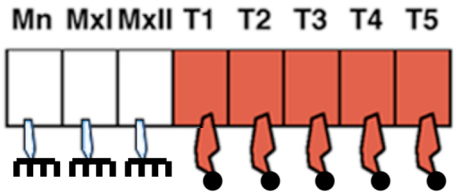
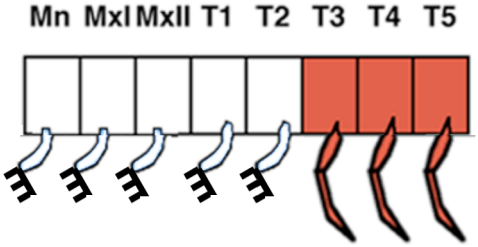
3a) [12 points]
ASSUME **TRANS** CHANGES BETWEEN ARTEMIA AND HOMARUS
(draw the expected Ubx expression domains and draw the appendage morphology you expect to see on each of the indicated segments)

Artemia now containing [Hom-enh]-Ubx



Homarus now containing [Art-enh]-Ubx



[3 pts for correct Ubx patterns; 4 pts for correct limb patterns, must not reverse Artemia and Homarus walking limb morphology]

Explain your answer:

In the trans model, the expression difference between Artemia and Homarus is due to a change that is independent ("upstream") of the cis-regulatory element responsible for regulating the UBX expression pattern. Therefore, when you inject the [Hom-enh]-Ubx construct into Artemia or the [Art-enh]-Ubx into Homarus, the UBX pattern and segment identity will be determined by the host species not the transgene. Thus the Ubx pattern will appear unchanged and the pattern and morphology of the limbs will be unchanged [5 pts].

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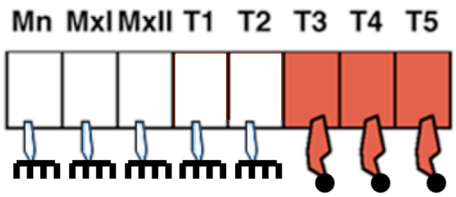
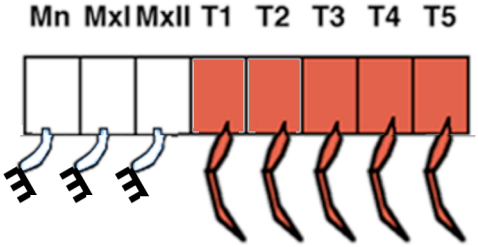
3b) [12 points]
ASSUME **CIS** CHANGES BETWEEN ARTEMIA AND HOMARUS
(draw the expected Ubx expression domains and draw the appendage morphology you expect to see on each of the indicated segments)

Artemia now containing [Hom-enh]-Ubx



Homarus now containing [Art-enh]-Ubx



[2 pts for correct Ubx patterns; 5 pts for correct limb patterns, must not reverse Artemia and Homarus walking limb morphology]

Explain your answer:

In the cis model, the expression difference between Artemia and Homarus is due to a change in the cis-regulatory elements responsible for controlling the UBX expression pattern. Therefore, when you inject the [Hom-enh]-Ubx construct into Artemia or the [Art-enh]-Ubx into Homarus, the UBX expression domain will be determined by the cis-regulatory element that you are introducing. Thus, the transformed Artemia will have the Ubx expression pattern of Homarus, and the transformed Homarus will have the Ubx expression of Artemia. This will then cause a shift of the boundary between feeding and swimming appendages (as shown in the drawing), but Artemia will still have Artemia type swimming legs and Homarus will still have Homarus type swimming legs (Ubx protein is the same in both animals, and the precise morphology is controlled by many downstream genes which have not been exchanged between the two animals) [5pts].

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Question #4 [17 points]
In the crab, Cleves, one claw is always large than the other claw. Crabs with larger right claws are known as "Righties", and those with larger left claws are known as "Lefties". This difference is controlled by a single maternal effect gene, called Claw. Gene Claw has two alleles, called Claw(R) and Claw(L).

On the island of Moorea, all Cleves are Righties and all have the genotype Claw(R)/Claw(R).
On the Island of Bora Bora, all Cleves are Lefties and all have the genotype Claw(L)/Claw(L)

You are told by a colleague that Claw(L) is dominant to Claw(R). You cross a Cleves from Moorea with a Cleves from Bora Bora to obtain animals that have the genotype of Claw(R)/Claw(L)

You then set up a cross between a female Cleves with the genotype Claw(R)/Claw(L) to a male Cleves with the genotype Claw(R)/Claw(L). Write out the different genotypes seen in the progeny of this cross, and the phenotype (Rightie or Leftie) for each of the genotypes. Provide an explanation for your answer.

The genotypes will be:

Claw(L)/Claw(L)

Claw(L)/Claw(R)

Claw(R)/Claw(R)

[5 pts; the ratio will be 1:2:1, but this is not needed for full credit]

All will be Leftie. [6 pts]

This is because Claw is a maternal effect gene, where Claw(L) is dominant to Claw(R). The mother that generated these individuals was heterozygous Claw(R)/Claw(L), so all her progeny will display the dominant phenotype; Leftie [6 pts].