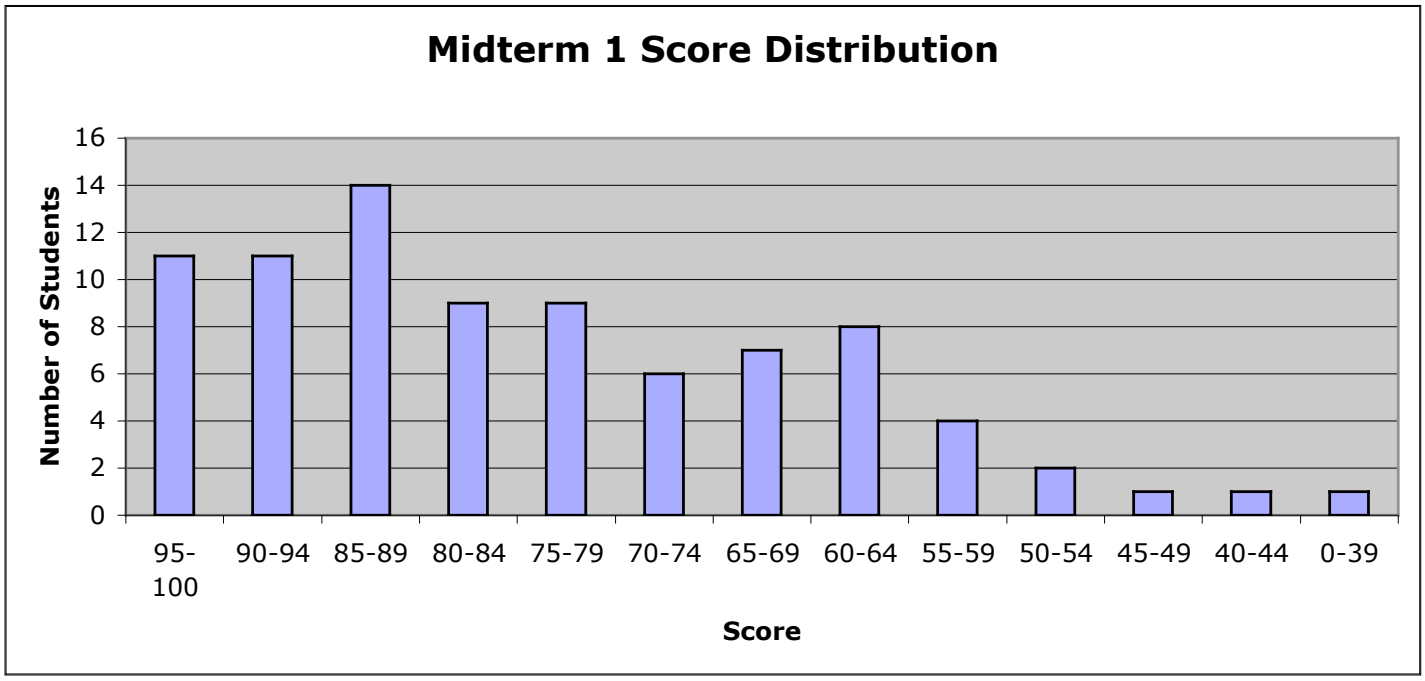
Your name:_____ Student ID#:_____

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



Mean: 78
Median: 81

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MCB 141 Midterm I Feb. 16, 2010

ANSWERS and POINT BREAKDOWN

Circle the name of your TA

Teresa Lee **John Young**

Question #1 _____ **/ 34 pts**

Question #2 _____ **/ 33 pts**

Question #3 _____ **/ 33 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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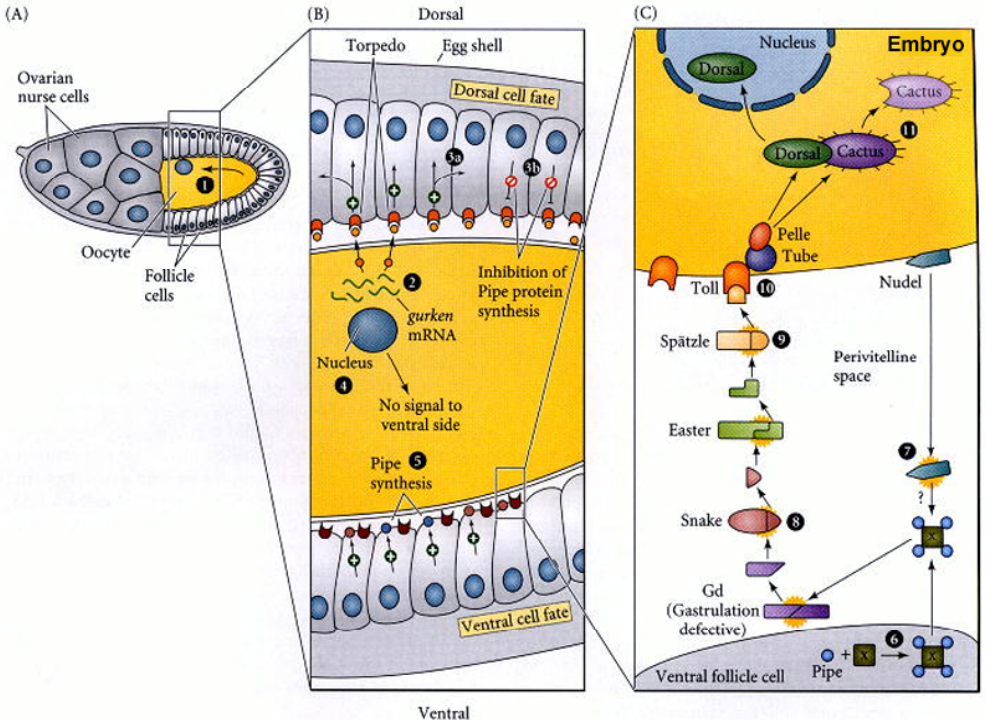
Question #1

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



You discover a new, dominant allele of pipe, which you call $pipe^{D}$. Females that are $pipe^{D}$ / $pipe^{+}$ produce embryos that are ventralized. When you examine the localization of pipe protein in the ovaries of $pipe^{D}$ / $pipe^{+}$ females, you discover that pipe protein is expressed by all follicle cells.

You also discover a new, dominant allele of torpedo, which you call $torpedo^{D}$. Females that are $torpedo^{D}$ / $torpedo^{+}$ produce embryos that are dorsalized. Your biochemistry colleague tells you that the protein produced by the $torpedo^{D}$ allele behaves as though it is always bound by gurken protein (even when there is no gurken present).

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1A [12 points] Predicted the phenotype of embryos laid by females of the following genotypes (assume all "–" alleles are complete lack of function alleles.). No explanations needed 2 points each answer

easter^{-}, easter^{-} ; pipe$^{\mathbf{D}}$ / pipe^{+}

Dorsalized

torpedo$^{\mathbf{D}}$ / torpedo^{+} ; cactus^{-}, cactus^{-}

Ventralized

torpedo$^{\mathbf{D}}$ / torpedo^{+} ; pipe$^{\mathbf{D}}$ / pipe^{+}

Ventralized

torpedo$^{\mathbf{D}}$ / torpedo^{+}, pipe^{-}/ pipe^{-}

Dorsalized

pipe$^{\mathbf{D}}$ / pipe^{+} ; torpedo^{-}/ torpedo^{-}

Ventralized

gurken^{-}/ gurken^{-} ; torpedo$^{\mathbf{D}}$ / torpedo^{+}

Dorsalized

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1B [5 points] As stated previously, $pipe^{D}$ / $pipe^{+}$ females produce embryos that are ventralized, and $torpedo^{D}$ / $torpedo^{+}$ females produce embryos that are dorsalized. As you know, the eggshell, which is made by the follicle cells, also shows dorsal-ventral patterning. Both the embryo and eggshell are dorsalized when the female is $torpedo^{D}$ / $torpedo^{+}$, but only the embryo is ventralized (eggshell is normal) when the female is $pipe^{D}$ / $pipe^{+}$. You take pole cells that are $torpedo^{D}$ / $torpedo^{+}$; $cactus^{-}$/ $cactus^{-}$ and add them to the posterior end of an embryo that is wild-type (i.e. $torpedo^{+}$/ $torpedo^{+}$; $cactus^{+}$/ $cactus^{+}$), but missing its own pole cells. You raise the resulting embryo up to be an adult female fly and mate her to a wild-type male. Predict the phenotype of the eggshells and of the embryos that are produced. No explanation needed.

Eggshell: Normal (or wild-type)
Embryo: Ventralized

2 points off if eggshell or embryo phenotype alone was wrong
5 points off if both were wrong

1C [5 points] You take pole cells that are $torpedo^{+}$/ $torpedo^{+}$; $cactus^{+}$/ $cactus^{+}$ and add them to the posterior end of an embryo that is $torpedo^{D}$ / $torpedo^{+}$; $cactus^{-}$/ $cactus^{-}$ (and missing its own pole cells). You raise the resulting embryo up to be an adult female fly and mate her to a wild-type male. Predict the phenotype of the eggshells and of the embryos that are produced. No explanation needed.

Eggshell: Dorsalized
Embryo: Dorsalized

2 points off if eggshell or embryo phenotype alone was wrong
5 points off if both were wrong

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1D [5 points] You take pole cells that are $torpedo^{+}$/ $torpedo^{+}$; $cactus^{-}$/ $cactus^{-}$ and add them to an embryo that is $torpedo^{D}$ / $torpedo^{+}$; $cactus^{+}$/ $cactus^{+}$ (and missing its own pole cells). You raise the resulting embryo up to be an adult female fly and mate her to a wild-type male. Predict the phenotype of the eggshells and of the embryos that are produced. (No explanation needed here, but you will be asked to explain in 1E on the next page).

Eggshell: Dorsalized
Embryo: Ventralized

2 points off if eggshell or embryo phenotype alone was wrong
5 points off if both were wrong

1E [7 points] Provide an explanation for the final result seen in 1D from the previous page.

The follicle cells are all derived from somatic cells, and thus all contain the protein encoded by the $torpedo^{D}$ allele. This causes all the follicle cells to behave as though they have received the gurken signal, making all the follicle cells develop as dorsal follicle cells and giving rise to a dorsalized eggshell. This also leads to the inhibition of pipe synthesis everywhere, and stops the enzymatic cascade in the perivitelline space. But, because the oocyte and nurse cells are derived from the pole cells, they are missing cactus protein, which means that dorsal protein always goes to the nucleus everywhere (cactus is epistatic to torpedo). This means that all embryonic cells behave as though they are ventral, leading to a ventralized embryo.

2 points for eggshell phenotype and explanation
2 points for embryo phenotype and explanation
3 points for stating that cactus is epistatic to torpedo

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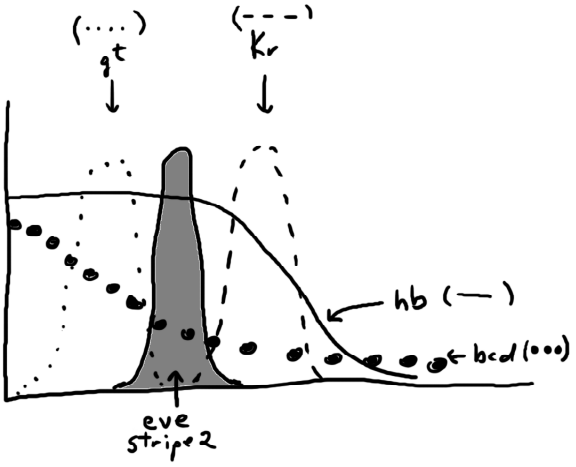
Question #2

As described in class, eve stripe 2 is activated by bicoid and hunchback and repressed by giant and Krüppel. A normal eve stripe 2 enhancer placed in front of a lacZ reporter gene gives the following pattern:



Anterior Posterior

You sequence the enhancer for eve stripe 2 and notice that four 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 four sequences that are repeated are:
ATATCCATAT
GCGCCCGCGC
GTGTCCGTGT
CACACCCACA

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 (SynA) – all the ATATCCATAT and CACACCCACA repeats are specifically eliminated. Instead of a normal eve stripe 2 pattern, you see virtually no expression of the lacZ reporter in an otherwise wild-type embryo.

Synthetic enhancer B (SynB) – all the GCGCCCGCGC repeats are specifically eliminated. Instead of a normal eve stripe 2 pattern, you see a broadened stripe of lacZ expression (broadened in the anterior direction) in an otherwise wild-type embryo.

Synthetic enhancer C (SynC) – all the GTGTCCGTGT repeats are specifically eliminated. Instead of a normal eve stripe 2 pattern, you see a broadened stripe of lacZ expression (broadened in the posterior direction) in an otherwise wild-type embryo.

2A [9 points] Based on these data, predict the sequence that is bound by giant protein (circle the correct answer below). Give a brief explanation for your answer. [Note: this is not the actual binding sequence of giant, just something we have made up for the exam.]

ATATCCATAT

GCGCCCGCGC

GTGTCCGTGT

CACACCCACA

Since giant is the repressor of eve stripe 2, deletion of giant binding sites should cause a broadening of the eve stripe 2 expression domain, and this shift should be to the anterior since giant is normally expressed just anterior to the position of eve stripe 2 in a wild-type embryo.

3 points for correct sequence
3 points for gt represses eve stripe 2 in the anterior; or gt represses eve stripe 2 and is expressed in anterior.
3 points for deletion of site in reporter construct causing broadening of LacZ expression in the anterior direction

2B [9 points] Based on these data, predict the sequence that is bound by Krüppel protein (circle the correct answer below). Give a brief explanation for your answer. [Note: this is not the actual binding sequence of Krüppel, just something we have made up for the exam.]

ATATCCATAT

GCGCCCGCGC

GTGTCCGTGT

CACACCCACA

Since Krüppel is the repressor of eve stripe 2, deletion of Krüppel binding sites should cause a broadening of the eve stripe 2 expression domain, and this shift should be to the posterior since Krüppel is normally expressed just posterior to the position of eve stripe 2 in a wild-type embryo.

3 points for correct sequence
3 points for Kr represses eve stripe 2 in the posterior or gt represses eve stripe 2 and is expressed in posterior.
3 points for deletion of site in reporter construct causing broadening of LacZ expression in the posterior direction

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2C [15 points] Imagine that you create embryos in which Krüppel protein is present in all blastoderm nuclei (without altering the expression pattern of any other gap gene). Predict what you would see for the expression of the SynB eve stripe 2 reporter in these embryos and for the SynC eve stripe 2 reporter in the these embryos. Provide a brief explanation for your answers.

For SynB, the eve stripe 2 reporter should show little or no expression. The giant binding sites are missing in this construct, but the Krüppel sites are still present. Having Krüppel expressed everywhere will now cause the reporter to be repressed everywhere and result in the lack of expression.

For SynC, the expression will be unchanged from before (broad stripe, broadened in the posterior direction relative to wild-type stripe 2 reporter in a wild-type embryo). This construct lacks Krüppel binding sites, so the additional Krüppel expression will have no effect (but still broadened in the posterior direction because of the lack of Krüppel binding sites).

2 pts: Syn B has Kr site/will bind Kr
2 pts: No LacZ expression from SynB
4 pts: explanation for SynB expression pattern
2 pts: Syn C has no Kr site/won't be bound by Kr
2 pts: Syn C will be expressed in a stripe broadened posteriorly relative to wt eve str 2
3 pts: Kr can't bind SynC so it won't affect SynC expression, even when Kr is in all nuclei

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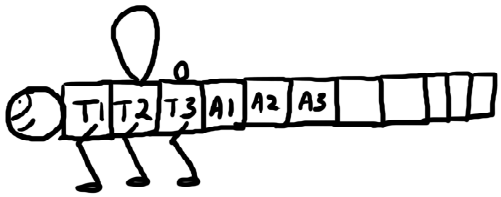
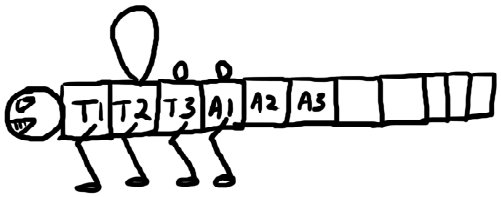
Question #3

You discover a fascinating insect, Octopod, that is closely related to flies, but it has four pairs of legs (instead of the three pairs seen for flies). In Octopod, T1 has a pair of legs, T2 has a pair of legs and a pair of wings, T3 has a pair of legs and a pair of halteres, and A1 has a pair of legs and a pair of halteres. In flies, it is Ubx that represses legs from developing in A1 (abdA represses legs in the rest of the abdominal segements). You expect that the pattern of Ubx expression is different in Octopod than in Drosophila, and you find that this is true.



Drosophila



Octopod

3A [15 points] What pattern do you think you would see for Ubx expression in (1) early stage embryos (before Distal-less expression begins), in (2) late stage embryos (after Distal-less has become autoregulatory), and in (3) the imaginal disks of Octopod animals. How does this differ from what is seen in Drosophila?

Octopod Ubx expression: 2.5 points for each stage

Early embryo: **Expressed in A2 on back**

Late Embryo: **Expressed in T3 on back**

Imaginal disks: **In imaginal disks (halteres) of T3 and A1, absent from T2 (wing) imaginal disks.**

Drosophila Ubx expression: 2.5 points for each stage

Early embryo: **Expressed in A1 on back**

Late Embryo: **Expressed in T3 on back**

Imaginal disks: **In imaginal disks (halteres) of T3, absent from T2 (wing) imaginal disks.**

3B [18 points] Describe an experiment that would help you tell if these differences in Ubx expression between Octopod and Drosophila were cis changes in Ubx or were trans changes (changes in other genes). Use diagrams as needed.
Isolate enhancers for Octopod and Drosophila Ubx and create transgenes where each is fused to a reporter (lacZ, GFP, etc.).

1) First, make sure that when these are placed into the species of origin , they give the appropriate pattern (could just refer to answer in 3A for full credit)

Drosophila Ubx-lacZ in Drosophila
Early embryo: Expressed in A1 on back; Late Embryo: Expressed in T3 on back
Imaginal disks: In imaginal disks (halteres) of T3, absent from T2 (wing) imaginal disks.

Octopod Ubx-lacZ in Octopod
Early embryo: Expressed in A2 on back; Late Embryo: Expressed in T3 on back
Imaginal disks: In imaginal disks (halteres) of T3 and A1, absent from T2 (wing) imaginal disks.

2) Then switch so that each construct goes into the opposite species and observe the reporter expression patterns.

IF CIS EVOLUTION:
Drosophila Ubx-lacZ in Octopod
Early embryo: Expressed in A1 on back; Late Embryo: Expressed in T3 on back
Imaginal disks: In imaginal disks (halteres) of T3 and A1, absent from T2 (wing) imaginal disks.

Octopod Ubx-lacZ in Drosophila
Early embryo: Expressed in A2 on back; Late Embryo: Expressed in T3 on back
Imaginal disks: In imaginal disks (halteres) of T3, absent from T2 (wing) imaginal disks.

IF TRANS EVOLUTION:
Drosophila Ubx-lacZ in Octopod
Early embryo: Expressed in A2 on back; Late Embryo: Expressed in T3 on back
Imaginal disks: In imaginal disks (halteres) of T3 and A1, absent from T2 (wing) imaginal disks.

Octopod Ubx-lacZ in Drosophila
Early embryo: Expressed in A1 on back; Late Embryo: Expressed in T3 on back
Imaginal disks: In imaginal disks (halteres) of T3, absent from T2 (wing) imaginal disks.

2 points for isolating the enhancers of Ubx from both species

4 points for testing the Ubx enhancer-reporter constructs in the species of origin, providing detailed description of expected lacZ patterns, or referring to 3A

12 points for the opposite species experiments, and detailed description of expected lacZ patterns for each organism, in cis and trans evolution scenarios (3 points for each of the four possible experimental results)