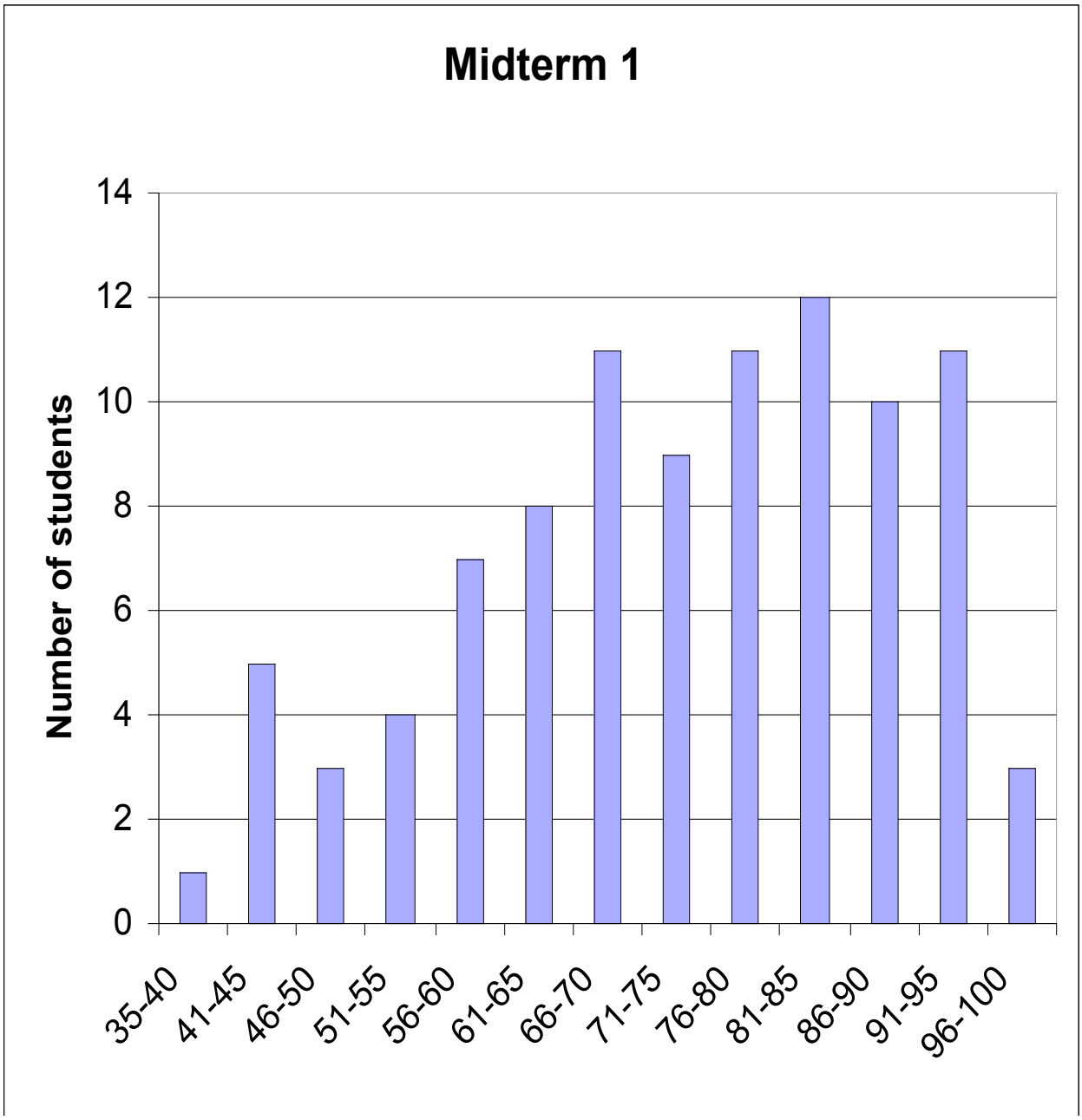


Mean: 73
Median: 75
Top Score: 98

Your name:_____Answer Key_____ Student ID#:_____

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

MCB 141 Midterm I Feb. 19, 2009

Circle the name of your TA

Jessica Lyons **Alberto Stolfi**

Question #1 _____ **/ 30 pts**

Question #2 _____ **/ 30 pts**

Question #3 _____ **/ 25 pts**

Question #4 _____ **/ 15 pts**

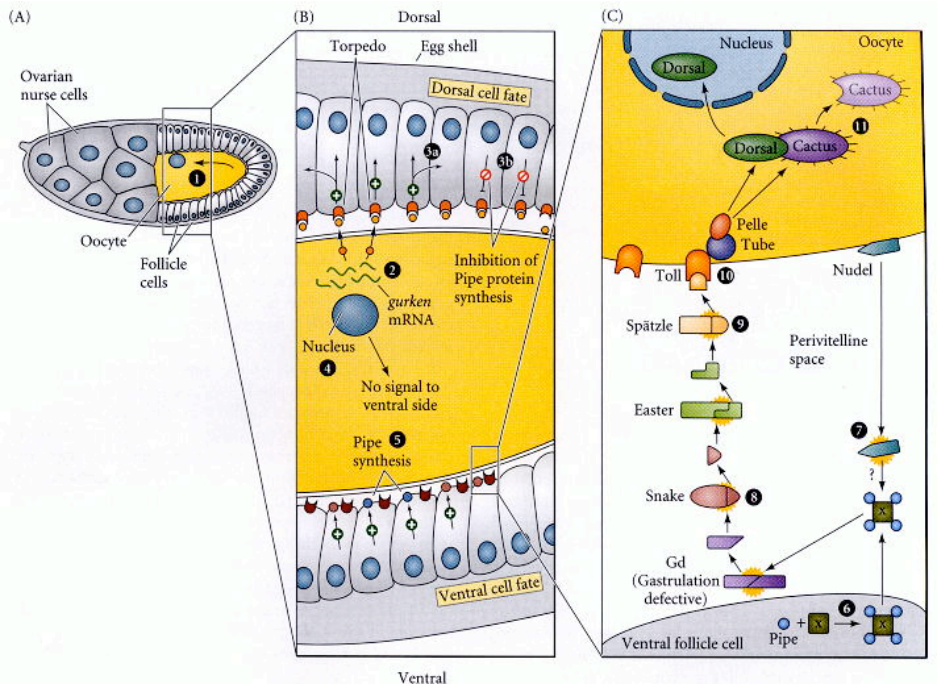
TOTAL _____ **/ 100 pts**

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.

Your name:_____Answer Key_____ Student ID#:_____

Question #1

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



It turns out that the eggshell, which is made by the follicle cells, also shows dorsal/ventral patterning. The dorsal side of the eggshell has projections, called dorsal organs, while the ventral side is smooth. You examine the eggs laid by mothers homozygous for null (complete lack-of-function) alleles of gurken, torpedo, snake, easter, Toll, cactus, and dorsal and find the following:

Female genotype	Eggshell phenotype	Embryo phenotype
gurken$^-$/gurken$^-$	**ventralized**	**ventralized**
torpedo$^-$/torpedo$^-$	**ventralized**	**ventralized**
snake$^-$/snake$^-$	**normal**	**dorsalized**
Toll$^-$/Toll$^-$	**normal**	**dorsalized**
cactus$^-$/cactus$^-$	**normal**	**ventralized**
dorsal$^-$/dorsal$^-$	**normal**	**dorsalized**

(ventralized egg shells lack dorsal organs and are smooth all around)

Your name:_____Answer Key_____ Student ID#:_____

1A (9 pts). Explain why some of these genes are required for both eggshell and embryo patterning, while others only affect the embryo.

This is because the pathway that controls D/V patterning of the eggshell and the embryo are initially the same, but then diverge. Having gurken signal to torpedo makes the dorsal follicle cells different from the ventral follicle cells (signal actively received by the dorsal follicle cells). The difference in the follicle cells is manifested in the morphology of the eggshell they make (smooth ventrally but with dorsal organs dorsally). The difference in dorsal versus ventral follicle cells also sets off the enzymatic cascade of Gd, snake, easter, and Spätzle in the space surrounding the future ventral side of the embryo, which leads to activation of Toll, release of dorsal by cactus, and movement of dorsal into the nucleus, all of which determine ventral fate for the embryo. But this whole cascade (Gd to dorsal) is specific for the embryo and the cascade has no effect on the follicle cells. Thus gurken and torpedo control D/V pattern of both the eggshell and embryo, but once the pathway splits, Gd to dorsal only influence D/V patterning of the embryo [9 pts]

[It is incorrect to say that the difference has to do with genes required in the oocyte/nurse cells versus follicle cells. While torpedo is only required in the follicle cells, gurken is coming from oocyte/nurse cells.]

1B (9 pts). Predict both the eggshell and embryo phenotypes that are produced by females homozygous for:

1) a null mutation of easter

embryo: dorsalized [1pt]
eggshell: normal [2pts]

2) a dominant allele of Toll that produces a Toll protein that acts as though it is always bound to Spätzle protein

embryo: ventralized [1pt]
eggshell: normal [2pts]

3) a dominant allele of torpedo that produces a Torpedo protein that acts as though it is always bound by Gurken protein

embryo: dorsalized [1pt]
eggshell: dorsalized [2pts]

1C (12 pts). You find a new gene x, where female flies homozygous for a null mutation of x produce eggs where the eggshell is dorsalized (has dorsal organs all around the egg shell circumference), but the embryo inside is normal. You suspect that gene x is expressed in (and functions in) the follicle cells and not in the oocyte/nurse cells. Design pole cell transplant experiments to test your hypothesis. Describe (use drawings if necessary) how you will carry out your experiments and interpret your results. Assume that you already have available wild-type embryos as well as embryos that are homozygous mutant for a null allele of x that were produced by a heterozygous mother (so no need to tell us how you produced these starting embryos).

Take pole cells from x-/x- embryo, transplant them to x+/x+ recipient embryo (having removed its pole cells). Grow this up as a female that has x-/x- germline (oocyte+nurse cells) and x+/x+ follicle cells. [3pts]

Take pole cells from x+/x+ embryo, transplant them to x-/x- recipient embryo (having removed its pole cells). Grow this up as a female that has x+/x+ germline (oocyte+nurse cells) and x-/x- follicle cells. [3pts]

Now assay the phenotype of the eggshells that these female produce.

If x-/x- germline; x+/x+ follicle cell female produces normal eggshells and
x+/x+ germline; x-/x- follicle cell female produces dorsalized eggshells
Then gene x is required in the follicle cells. [3pts]

If x-/x- germline; x+/x+ follicle cell female produces dorsalized eggshells and
x+/x+ germline; x-/x- follicle cell female produces normal eggshells
Then gene x is required in the oocyte/nurse cells. [3pts]

Your name:_____Answer Key_____ Student ID#:_____

Question #2

In a new mutant screen for maternal effect mutations, you discover five new genes (which you name n, o, p, q, and r). Female flies that are homozygous for null alleles of any one of these genes produce embryos that have no head and thorax, but have an enlarged abdomen instead. You also find that bicoid mRNA is not localized at the anterior of the oocytes produced by these females, but rather the bicoid mRNA is found uniformly (diffusely) spread out through the oocyte. Given that all your alleles produce the same phenotype, you realize that you cannot carry out a standard epistasis test by making double mutants. However, you have antibodies directed against the protein products produced by each gene n through r. You use these antibodies to look at the corresponding protein distribution of proteins N through R. You find that all five proteins are localized to the anterior end of the developing oocytes in wild-type females. You decide to use your antibodies to look at the distribution of proteins N through R in oocytes produced by females that are homozygous mutant for each of your genes n-r.

You obtain the following results:

"wt" means the same pattern as seen in oocytes in wild-type females (localized at the anterior end)

"diff" means the protein (or mRNA in the case of bicoid) is diffuse throughout the oocytes

"–" means that you see no protein (or mRNA in the case of bicoid)

Female genotype	Prot N	Prot O	Prot P	Prot Q	Prot R	bcd mRNA
n^-/n^-	–	diff	diff	wt	diff	diff
o^-/o^-	wt	–	diff	wt	wt	diff
p^-/p^-	wt	wt	–	wt	wt	diff
q^-/q^-	diff	diff	diff	–	diff	diff
r^-/r^-	wt	wt	diff	wt	–	diff
bcd^-/bcd^-	wt	wt	wt	wt	wt	–

Your name:_____Answer Key_____ Student ID#:_____

2A (15 pts). While you cannot carry out a standard genetic epistasis analysis, you can use the protein localization data to determine the order in which the proteins act to eventually localize bicoid mRNA. Draw out the order of this relationship for N, O, P, Q, and R (with bicoid mRNA at the end of your series). Briefly explain the reasoning for your answer.

Q ⇒ N ⇒ R&O ⇒ P ⇒ bcd

[9 pts; -2 pts if R and O are ordered or in parallel]

Q is first in the series as all other proteins are diffuse in q^-/q^-

N is second as Q is normal, but all other proteins are diffuse in n^-/n^-

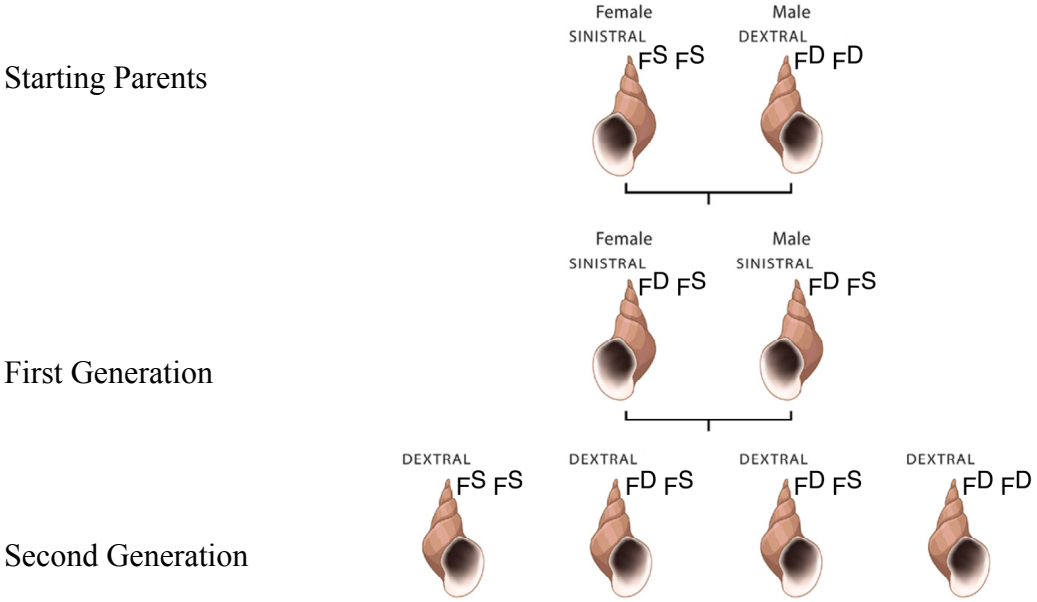
The order of R and O relative to each other cannot be resolved, but they come after N as Q and N are both normal, but P is diffuse in both o^-/o^- and r^-/r^-

P is just before bcd as all other proteins are normal in p^-/p^-

[6 pts for explanation; explanation is okay as long as the basic principle is clearly laid out. –1 point if O and R are explained as acting in parallel (which is incorrect)]

Your name:_____Answer Key_____ Student ID#:_____

2B (15 pts). In snails, the shell can coil to the left (called sinistral), or coil to the right (called dextral). The direction of coiling is controlled by a single gene, called F. There are two alleles of F, which are called $F^{DEXTRAL}$ (F^{D}) and $F^{SINISTRAL}$ (F^{S}). Below is a diagram showing a series of crosses (matings), and the shell phenotype and the genotype of each animal.



Is F acting maternally or zygotically (explain your answer)? How would you describe the interaction between the alleles F^{D} and F^{S}? [Hint: think of one as F^{+} and the other as F^{-}]

F is acting maternally. Genotype of the individual does not determine the chirality of that individual, rather the genotype of the mother determines the chirality of her offspring. This is most clear in the second generation, where all genotypes are present, but all have the same phenotype because of maternal inheritance. [9 pts; 4 pts for answer, 5 pts for explanation]

F^{D} is dominant to F^{S}. [equally correct to say that F^{S} is recessive to F^{D}] The $F^{S}F^{S}$ starting mother produces sinistral offspring, but because F^{D} is dominant to F^{S}, the $F^{D}F^{S}$ mother in the first generation produces only dextral offspring. [6 pts; 4 pts for answer, 2 pts for explanation]

Your name:_____Answer Key_____ Student ID#:_____

Question #3

You discover a fascinating insect, Dihaltere, that is closely related to flies, but it has no wings but has two pairs of halteres. In Dihaltere, T1 has legs only, T2 has halteres and legs, and T3 has halteres and legs. As in flies, the abdominal segments of Dihaltere have no legs. You expect that the pattern of Ubx expression is different in Dihaltere than in Drosophila, and you find that this is true.

3A (10 pts). What pattern do you think you would see for Ubx expression in early stage embryos (before Dll expression begins), in late stage embryos (after Dll has become autoregulatory), and in the imaginal disks of Dihaltere animals. How does this differ from what is seen in Drosophila?

In Dihaltere:

Early – Ubx in A1 on back (all abdominal segments) [2 pts]
Late – Ubx in T2 on back (T2, T3, and all abdominal segments) [2 ps]
Imaginal disks – Ubx expressed in disks (haltere disks) of T2 and T3 [2 pts]
[It is acceptable to say that expression is in T2 only at imaginal disk stage]

In Drosophila

Early – Ubx in A1 on back (all abdominal segments) [1pt]
Late – Ubx in T3 on back (T3, and all abdominal segments) [2pts]
Imaginal disks – Ubx expressed in T3 disks (haltere disks), but not T2 disks (wing) [1 pt]

3B (15 pts). Describe an experiment that would help you tell if these differences in Ubx expression between Dihaltere and Drosophila were cis changes in Ubx or were trans changes (changes in other genes). Use diagrams as needed.

First isolate Ubx enhancer from Dihaltere and Drosophila and use them to control lacZ expression (call them DhUbx-lacZ and DrUbx-lacZ respectively). [4pts]

First introduce them back into the species of origin.

DhUbx-lacZ in Dihaltere should give the following lacZ pattern:
Early embryo – A1 on back; late embryo T2 on back, disks of T2 and T3 [1.5 pts]
(can also just say that it gives the endogenous Dihaltere Ubx pattern)

DrUbx-lacZ in Drosophila should give the following pattern:
Early embryo – A1 on back; late embryo T3 on back, disks T3 [1.5 pts]
(can also just say that it gives the endogenous Drosophila Ubx pattern)

Now to test for cis versus trans:

Put DhUbx-lacZ into Drosophila
IF CIS, lacZ pattern is:
Early embryo – A1 on back; late embryo T2 on back, disks of T2 and T3
(can also just say that it gives the endogenous Dihaltere Ubx pattern) [2 pts]

IF TRANS, lacZ pattern is:
Early embryo – A1 on back; late embryo T3 on back, disks T3
(can also just say that it gives the endogenous Drosophila Ubx pattern) [2 pts]

AND

Put DrUbx-lacZ into Dihaltere
IF CIS, lacZ pattern is:
Early embryo – A1 on back; late embryo T3 on back, disks T3
(can also just say that it gives the endogenous Drosophila Ubx pattern) [2 pts]

IF TRANS, lacZ pattern is:
Early embryo – A1 on back; late embryo T2 on back, disks of T2 and T3
(can also just say that it gives the endogenous Dihaltere Ubx pattern) [2 pts]

[Acceptable to put both CIS results together and both TRANS results together when answering the question, also acceptable to ignore the early patterns, which are the same anyway, and just focus on T2 versus T3 patterns at either late embryo or disks]

Your name:_____Answer Key_____ Student ID#:_____

Question #4 (15 pts).

As described in class, eve stripe 2 is activated by bicoid and hunchback and repressed by giant and Krüppel.

You discover the following:

Bicoid protein binds to the sequence CGCGGGGG
Hunchback protein binds to the sequence ATATTTTT
Giant protein binds to the sequence GGGGGACACAC
Krüppel protein binds to the sequence TTTTTCAGCAG

You create a synthetic enhancer SynEveA with the sequence:

CGCGGGGGAAGGGGGACACACTTATATTTTTCCTTTTTCAGCAG

You put this in front of a lacZ reporter gene and introduce it into flies via P-element transposition. You expect the lacZ pattern to mimic eve stripe 2, but instead you get a stripe that is much broader (spread both anteriorly and posteriorly from the normal boundaries for eve stripe 2).

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

CGCGGGGGACACACTTATATTTTTCAGCAG

You put this 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 eve stripe 2.

Your name:_____Answer Key_____ Student ID#:_____

Give an explanation for your results. Why the difference in results for SynEveA and SynEveB? Give a possible explanation for how Giant and Krüppel proteins act as repressors and prevent activation by Bicoid and Hunchback proteins.

Giant and Krüppel appear to act as repressors by preventing the binding of the activators Bicoid and Hunchback. In SynEveB, the binding sites for Krüppel overlaps with the site for Hunchback and the binding site for Giant overlaps with the site for Bicoid. When (and where) Giant and Krüppel are present, their physical association with the DNA covers the Hunchback and Bicoid sites, keeping thes activators away and/or displacing them if they are already present. In this way, SynEveB acts like the normal eve stripe 2 enhancer and gives a stripe that matched the endogenous stripe 2.

SynEveA does not give the same pattern because, while all four binding sites are present, they are now physically separated. Krüppel and Giant can still bind, but now their binding is unable to prevent the activators Bicoid and Hunchback from binding as there is no overlap between sites.

This in effect means that Krüppel and Giant cannot function as repressors on the SynEveA enhancer. As Krüpple repression sets the posterior edge of eve stripe 2 and Giant sets the anterior edge of eve stripe 2, the result of the lack of repression is that the expression driven by SynEveA is a stripe that is much broader (spread both anteriorly and posteriorly from the normal boundaries for eve stripe 2).

Point breakdown:
Cause of the difference is the overlap in the binding sequences (5pts).
Blocking Hb and Bi is how Gt and Kr work in this example (2pts).
Explain why this gives normal expression in SynEveB (5pts).
Explain why expression is broadened in SynEveA, especially with respect to gradients of activators/repressors in the embryo (3pts).