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**Midterm #3 - 2012**

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Average 122.06  
St. dev 28.44  
Max 169  
Min 53.5
Question 1 (22 points)

Mutations in Drosophila Hox genes create obvious changes in the body plan and have become of great interest to developmental and evolutionary biologists.

An animal homozygous for a null mutation of the *Drosophila* Hox gene *Ubx* dies as an embryo, and displays a phenotype in which the third thoracic segment (T3) and first abdominal segment (A1) are both transformed to the identity of the second thoracic segment (T2). However, there is an allele of *Ubx* that can lead to an adult fly in which T3 is transformed to T2, giving rise to a four-winged fly. Describe the nature of this *Ubx* allele, that is, describe whether the mutation is in the coding region or the regulatory region, and what this does to the pattern of *Ubx* expression in the embryo, larvae, and pupae that leads to this remarkable phenotype.

This allele of *Ubx* is a caused by a mutation in the regulatory region of this gene. Expression in the embryo of *Ubx* is normal - that is from A1 back early in embryogenesis, and then from T3 back during later embryogenesis. Expression is altered from wild-type after embryogenesis, either at the larval or pupal stage (either larval or pupal, or both, are correct answers). Normally *Ubx* would be expressed in the haltere disk (part of T3), but not the wing disk (part of T2). In this allele of *Ubx*, expression is lost from the haltere disk, and the haltere disk is transformed to a wing disk.
Question 2 (11 points)

Describe an actual example where changes in a Hox gene are believed to have given rise to an evolutionary change in animal morphology.

The two examples given in class were:

1) Changes in Ubx expression are associatied with changes in the number and position of crustacean maxillipeds (feeding appendages). Ubx expression starts at either T1, T2, T3, or T4 and is associated with having 0, 1, 2, or 3 pairs of maxillipeds and a corresponding reduction in locomotory appendages.

2) Changes in HoxC6 are associated with evolutionary changes in the number of cervical versus thoracic vertebrae. Thus, differences in the number of neck vertebrae are associated with shifts in the Hox gene (for example between mice and chicks the aial level of HoxC6 is altered)/

One answer that is incorrect, is an association between Hox gene expression and wing number in insects. In class we pointed out that while four-winged Drosophila could be created by altering Ubx regulation, that this was not how evolution had achieved the transformation between 4 and 2 wings.
Question 3 (16 points total)

3A. (6 points)
A schematic drawing is shown of a 128 cell mouse blastocyst, shortly before hatching and implanting in the wall of the uterus. Into each box, put the appropriate letters from the list to best identify each region. A letter may be used in one box, more than one box, or not used.

<table>
<thead>
<tr>
<th>A. cells of the epiblast</th>
<th>H. cells of the hypoblast (or visceral endoderm)</th>
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<tr>
<td>B. cells that will later develop into extraembryonic endoderm</td>
<td>I. cells that proliferate as embryonic stem cells if cultured in a Petri dish</td>
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<tr>
<td>C. cells of the mural trophoblast</td>
<td>J. cells derived from the outermost cells of the 64 cell stage embryo</td>
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<tr>
<td>D. cells of the polar trophoblast</td>
<td>K. cells derived from inner cells of the 64 cell stage embryo</td>
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<tr>
<td>E. blastocyst cavity</td>
<td>L. cells that first invade the uterine wall</td>
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<tr>
<td>F. zona pellucida (must be destroyed before implantation occurs)</td>
<td>G. cells that will later develop into the mouse</td>
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3B. (5 points)
By the 32-64 cell stages, the mouse embryo contains irreversibly different populations of trophoblast and inner cell mass cells. Briefly describe how compaction (which begins at the 8-cell stage, see figure) and continuing cleavage contribute to the formation of these two populations.

At the 8 cell stage, compacting cells undergo apical-basal polarization and form tight junctions with neighbors. Part of the surface of each cell is exposed to the outside medium, and part is exposed internally. As 8 cells divide to 16, some divide vertically (to the surface), and both daughter cells remain in the surface, are polarized, and form more tight junctions. Others divide horizontally, and one daughter cell remains in the surface layer and one is entirely inside, with no apical-basal polarization and no tight junctions (nor does it form any). At the next divisions to 32 and 64 cells, surface cells again divide vertically or horizontally, the latter divisions releasing more inner cells, unpolarized and without tight junctions. Surface cells with tight junctions stay in the surface; these become trophoblast. Inner cells don’t enter the surface; they stay as an “inner cell mass” and later become the epiblast and hypoblast.

(OK to mention: the outer and inner cells signal differently through the Hippo pathway, activating genes of trophoblast development in the outer cells, such as cdx.)

3C. (5 points) By the 128 cell stage, the mouse blastocyst contains irreversibly different epiblast cells and hypoblast cells (“visceral endoderm”). Briefly describe the cell activities leading to blastocyst cavity formation, and tell how it and cell sorting contribute to the formation of the epiblast and hypoblast cell populations:

Blastocyst cavity formation: polarized cells of the outer layer of the 16, 32, 64, and 128 cell stages pump Na+ through the basal cell surface into the internal intercellular space, and Cl− follows (OK to say inner cells also contribute some ions), creating a higher salt concentration in the intercellular fluid relative to the outside fluid. Water moves in by osmosis. As the cavity expands, the trophoblast layer expands, but the inner cell mass remains a single compact clump of cells, contacting only one part of the trophoblast inner surface. Blastocyst cavity fluid surrounds it elsewhere.

Sorting out: Inner cell mass cells become heterogeneous at the 64 and 128 cell stages, some expressing a gata gene. These sort out, toward the cavity and away from the trophoblast contact region, to form a cell layer (the hypoblast) between the remaining inner cell mass cells (the epiblast) and the cavity fluid.
Question 4 (11 points total)

4A (3 points). In the cup-shaped egg cylinder stage of the newly implanted mouse embryo, the anterior visceral endoderm (AVE) region of the hypoblast layer determines where the primitive streak and future anterior neural tissue will form, and hence the orientation of the anterior-posterior axis of the embryo. To indicate the correct spatial relationships, circle one of the letters in the list of statements below: **Answer C is correct**

a. The primitive streak and anterior neural tissue form on the same side of the cup as the AVE.
b. The primitive streak forms on the same side of the cup as the AVE, and the anterior neural tissue forms on side opposite the AVE.
c. The primitive streak forms on the side of the cup opposite the AVE, and the anterior neural tissue forms on the same side as the AVE.
d. The primitive streak and anterior neural tissue form on the side of the cup opposite the AVE.

4B (5 points). Briefly explain why these spatial relationships hold (noting the appropriate signaling ligands and antagonists).

*The AVE releases Nodal antagonists such as Cerberus and Lefty. Primitive streak formation, which requires Nodal signaling, occurs in the epiblast on the side of the cup opposite the AVE, where Nodal and Lefty (antagonist) concentrations from the AVE are lowest. Cerberus from the AVE, in addition to antagonizing Nodal, antagonizes Bmps, thereby blocking Bmp-promoted epidermis formation and favoring neuralization in the epiblast on the AVE side of the epiblast. Also the AVE releases Dkk and Cerberus that block Wnt-mediated posteriorization of neural ectoderm in the region of epiblast close to the AVE, that is, favoring anterior neural development on the same side of the cup.*

4C (3 points). The nine-banded armadillo normally gives birth to monozygotic (genetically identical) quadruplets, the only mammal to do this. The fertilized egg develops to a single blastocyst with an unusually large epiblast composed of four cup-shaped quadrants, all underlain by a continuous hypoblast layer (visceral endoderm). Four primitive streaks then develop in the epiblast, one per quadrant, followed by the development of four embryonic bodies, arranged as shown in the image on the right below. Draw on the left diagram to indicate where the AVE or AVEs would have been located in the hypoblast layer at the time the four primitive streaks were forming, and explain your answer briefly. Assume we can see through the epiblast layer to the underlying hypoblast layer.
4C Explanation:

*The AVE must be in the center, where it releases Nodal antagonists and blocks primitive streak formation. Streak formation then occurs at epiblast sites most distant from AVE, that is, at the outer edge of each quadrant. Also, the AVE released Wnt antagonists and these favor the development of anterior neural structures closest to the AVE (the four heads are pointed inward).*
Question 5 (6 points)

Glinka and Niehrs discovered in 1997 that they could provoke the development of a secondary head on a hatching tadpole if they injected a mixture of the two mRNAs into the ventral blastomeres of a 4-cell stage embryo (see figure), namely:

One mRNA encoding tBR, a dominant negative [truncated] receptor for Bmp, and the other mRNA encoding the Dkk protein (Dickkopf), an antagonist of Wnt signaling.

However, if they injected the mRNA for tBR alone or for Dkk alone, the hatching tadpole didn’t develop a secondary head.

From your knowledge of the roles of BMPs and Wnts in axis formation in *Xenopus*, explain why the mixture of mRNAs, but not the single ones, worked in provoking secondary head formation.

*The ventral side of the egg produces high levels of Bmp and Wnts during early development (OK to mention Wnt8 from the presomitic and somite mesoderm). Bmp represses neuralization of ectoderm, and Wnts cause posteriorization of neuralized ectoderm.*

*tBR alone injected in the ventral side would block Bmp signaling and derepress ectodermal cells to neuralize. However, Wnts would still be present and posteriorize that neural tissue, so no anterior head (forebrain, midbrain, eyes) would form. Dkk alone injected in the ventral side would not neuralize ectoderm (Bmps still high and repressing neuralization), so no head would form.*

*When injected as a mix, tBR would neuralize ectoderm (tBR blockage of Bmp signals) and Dkk would block its posteriorization. Anterior neural tissue (head) would form.*

*(OK to say: tBR and Dkk also affect ventral mesoderm, driving it to form prechordal plate/head organizer, which also induces anterior neural development in the ectoderm).*
**Question 6 (18 points)**

6a (4 points) Where do muscle precursor cells form? Draw a cross section through a chick embryo at a stage before neural tube closure and after neural tube closure indicating where they will be formed.

6b (3 points) Name one molecular signal that contributes to formation of the muscle precursor and indicate on the diagram above where it comes from and which population it acts upon.

Noggin, Sonic hedgehog, gremlin, wnt for primaxial/epaxial

BMP/FGF for hypaxial/abaxial

6c (6 points) How would you test whether there is a contribution of neural crest cells to any of the muscles in the mouse. Provide a specific example if you can. Give an example of an advantage of the method you propose, and a disadvantage.

Neural crest cells contribute to the cardiac outflow tract/aorta

To trace these in the mouse, one could use Dil labeling etc. Label locally in the neural crest with injection of Dil. Ask in later mouse if Dil labeled cells form muscle.

Advantage: Dil is a good tracer, long lived, cell autonomous

Disadvantage: may be diluted by growth and cell division, may “miss” a lot of neural crest muscle precursors by injecting the wrong place, etc.

Or a genetic method, such as Wnt1-Cre with a “floxed” reporter such as GFP that could be activated by the cre In such a mouse the cre is activated in neural crest precursors from the roof plate, labeling the cells with GFP. One would look for GFP labeled cells that develop as muscle- advantage sensitive, and marks most of the neural crest permanently disadvantage- may not activate in all of the neural crest if it isn’t active early enough for example
6f (6 points) Provide another six examples of neural crest derivatives.

- Dorsal root Ganglion cells
- Melanocytes
- Sympathetic Ganglion neurons
- Schwann cells
- Bones of the skull
- Endocrine cells of the adrenal medulla

Question 7 (18 points)

7a (6 points) How would you make a mouse chimera where you could test the effect of the manipulations below.

We'll inject ES cells of the correct genotype into the blastocyst of a host mouse.

Need to use a host (or donor) that is marked with GFP or lacZ expression to distinguish graft and host

Since the mutations are all lethal as homozygotes, ES cells will be derived from blastocysts after crosses of heterozygotes.

ES cells would be derived from several blastocysts, and need to be genotyped to find ones that are homozygous mutants. These will be the donors for ES cell injections into the blastocyst.

Or make a chimera by fusing two morula stages (also explain derivation, marking)
7b, c, d (4 points each) Let’s assume you can make enough specimens to find the potentially informative example (in the neural tube) of the chimera below, where the hollow shapes are wild type, and the grey zone has the genotypes below. Indicate the zones of expression of the following genes: Nkx2.2, Olig2, Irx3, Pax3

![Chimera Diagram]

for b, need to make the point that HH signaling is more effective, (smo is constitutive), expansion of ventral fates, contraction of dorsal (but still BMP signaling so not absent)

for c. clone has no HH signaling fate, cross repression still occurs with sharp boundaries

d. no effect

Question 8 (15 points)

8a (4 points) Let’s suppose you were starting to try to make induced pluripotent stem cells, back before Yamanaka showed it was possible, and before you knew what the “fantastic four” genes were that could be used to reprogram fibroblasts to become stem cells. On the other hand you do know everything about the expression of genes in the inner cell mass of the muse embryo.

How would you set up a selection for genes that could induce pluripotency?

Make a fibroblast cell line with a selectable marker, e.g. neomycin resistance under control of a stem cell promoter/enhancer- e.g. nanog oct4, Sox2, (not genes like myc, which are expressed widely)

In order to survive in the drug (G418/neomycin) the cell needs to turn on the Nanog promoter, and thereby becomes neo resistant. SO by selecting for turning on this promoter, we are selecting for manipulations that activate the stem cell program.
8b (3 points) What tool would you use to introduce genes into these cells?

A library of Retroviruses, into which cDNA from ES cells has been cloned.

8c (4 points) Let’s suppose your scheme worked, and you found that a population of induced cells contained a mixture of 36 genes that were introduced. How would you tell how many of these are important?

- Control- use all 36 to ensure that this collection works.
- To test which are essential, drop out single genes and see whether pluripotency (selectable marker gene expression) is still turned on.
- Or can drop out pools, and subsequently individuals from pools.
- Then need to test that the putative important genes are sufficient as a sub-collection to induce pluripotency.

8d (4 points) What kinds of genes would you expect to find that are crucial, name them if possible.

- Transcriptional factors/regulators of the stem cell fate- Oct4, Sox2, Nanog
- Regulators of proliferation (like c-myc)
- Regulators of Chromatin remodeling (klf4 –extra credit)
Question 9 (14 points)
Strong signals in the embryo usually evoke a feedback response, and in some cases there are both positive and negative feedback loops.

9a (6 points) Provide an example in the mouse gastrula where positive feedback ensures that mesoderm is formed effectively. Draw out how the pathway causes this positive feedback.

![Diagram of NODAL signaling pathway](image)

9b. (4 points) If the first example worked too well, then the whole embryo would become endomesoderm. What mechanism ensures that mesoderm is not formed in excess?

The Lefty antagonist is also activated by Nodal, using a similar mechanism, even down to the FoxH1 enhancer in the first intron. Lefty is more diffusible than Nodal, so moves out into a zone of low Nodal, where it prevents Nodal from initiating the positive feedback loop. So Nodal acts to activate its antagonist in a negative feedback, and using a reaction diffusion mechanism to limit the zone of Nodal activity.

9c (4 points) Provide another example of negative feedback that is important in development.

Hedgehog signaling induces expression of the Patched receptor, which binds and soaks up Hedgehog.

Etc.
**Question 10 (20 points)**

Joe Sarkozy makes a transgenic zebrafish where he hopes to have a good reporter for oscillating gene activity during somite formation. He makes a construct, driving GFP from the her1 promoter/enhancer. However, on looking at successful transgenic fish, he sees that the entire somitic and presomitic mesoderm glows brightly, and it is difficult to make out the individual cells.

10a. (4 points) How would you modify the construct to make it more useful? Describe aspects of your designed construct that would improve its effectiveness.

The GFP is too stable, and so does not turn over, it also fills the cell, making it difficult to see cell boundaries. So insert GFP into the hairy gene, such that there is a chimeric hairy/GFP, and the other regulatory elements for transcriptional activation and mRNA turnover. The hairy protein is unstable so causes destabilization of the GFP. It is also nuclear, so carries the GFP into the nucleus, so that individual nuclei can be distinguished in a field of cells.

10b (4 points) Bearing in mind that a somite is added every 20 minutes in the fish, draw out the pattern of expression of the reporter at two ten minute intervals.

Something like this! Showing dynamic expression in the tailbud/presomitic mesoderm, and non-dynamic in formed somites (though anterior somites do turn the gene off)

10c (4 points) Draw one of these time points again, but adding in where the endogenous her1 mRNA and Protein would be expressed.

2nd time point

- Protein expression same as the GFP from the transgene
- Out of phase mRNA expression
- Stable mRNA and protein expression
**Question 10d (4 points)** Suppose you place a bead soaked in the FGF inhibitor SU5402 onto one side of the notochord, in the presomitic mesoderm. The bead will release SU5402 over time to *locally* inhibit FGF signaling (i.e. it doesn't reach the other side of the notochord). Draw out the predicted consequence of this manipulation on the pattern of somites.

![Smaller somites](image)

Precocious differentiation due to loss of FGF leads to smaller somites

**Question 10e. (4 points)** Suppose you do the same experiment with a bead soaked in FGF. Draw out the predicted consequence

![Smaller somites](image)

Delayed differentiation of somite due to loss of low FGF wavefront

**Question 11 (10 points)** Provide ten examples where BMP signaling is used in development. What is the target tissue and what is the result?

- **Fly dorsal development**: DPP acts on cellular blastoderm to induce dorsal epidermis
- **Epidermal induction in Xenopus ectoderm**
- **Ventral mesoderm induction in Xenopus mesoderm**
- **Dorsalization of the spinal cord to induce dorsal fates like commissural neurons**
- **Induction of neural crest from dorsal neural tube**
- **Induction of Neurons from migrating neural crest past the BMP producing aorta**
- **Suppression of sclerotome induction in the somite**
- **Induction of lateral somite fate (including hypaxial muscle)**
- **Suppression of ventral fates in the spinal cord- opposing Shh**
- **Induction of cartilage condensation from mesenchyme in the limb bud**
**Question 12 (9 points)**
You have available two mouse strains. One is transgenic for a GFP transgene that is expressed in the Wolffian duct and ureteric bud, and another that is a ret+/- strain.

12a (4 points) Draw out the crosses you would do to examine whether Ret signaling is required for kidney formation, at each step what genotype would you select from the pups for the next cross or experiment?

Optimally need to have the GFP marker in the Ret- strain
1. cross GFP strain with Ret+/- strain.
2. Select those pups that are GFP+ and Ret+/-
3. Intercross these animals
4. dissect out pups at midgestation, or newborn, and screen for those that are Ret-/- and GFP +

Use the GFP to ask whether they have ureteric bud branching/kidneys

Controls are those that are ret +/- with GFP- presumably they should have kidneys and branched ureteric buds

12b (5 points) How would you determine what tissues require the presence of the Ret gene?

Dissect out mesenchyme and wolffian ducts from different embryos (from the cross above) and co-culture- genotype the rest of the mouse to ask what is in the co-culture

Need examples where mesenchyme is ret-/- and wolffian duct is GFP+:ret+/-
Also ret+/- or +/- in the mesenchyme and ureteric bud is GFP + and ret -/-

Others act as controls.

Outcome would be tested by presence of branched ureteric bud/kidney- expected not to be formed when mesenchyme is ret-/-
Question 13 (10 points)

13a (5 points) Spina Bifida is a fairly common birth defect in human populations. Even after supplementation with folic acid, some babies are born with such problems. If you were a human geneticist screening such babies for mutant alleles, what kinds of genes might you expect to find that are mutated? Provide an example, and explain how you would do an experiment in a frog embryo to show that the normal gene is required for efficient neural tube closure.

Expect genes like Shroom or Vangl (Van Gogh/strabismus)

In the frog embryos, first identify the sequence of the frog homologue, then design a morpholino oligonucleotide that would base pair to the 5’ end of the mRNA so as to block translation.

Inject MO alone with a lineage tracer (like Flouresceinated morphilno oligonucleotide, or GFP mRNA).

Ask whether the injected region neurulates properly - predict that interference with either one would block neurulation movements for Shroom, blocks bending of the neural plate, for Vangl blocks convergence and extension of the neural plate.

13b (5 points) How would you test if the gene is required cell-autonomously?

Use the tracer (ideally the fluor-Morpholino oligo, to ask whether cells that received the injected MO are affected, while the rest of the embryo is ok (autonomous). Or alternatively do the effects spread beyond the injected/traced cells (non-autonomous).
Question 14 (12 points)

14a. (3 points) What descriptive experiments would you do that might suggest that the vertebrate hindbrain is intrinsically segmented? (no more than three)

Observe Morphological bumps
Observe Emergence of motor neurons in a periodic fashion corresponding to morphological bumps
Observe Expression patterns (anterior boundaries) of hox genes by in situ hybridization

14b. (3 points) What lineage tracing experiment would you do to test whether the hindbrain is intrinsically segmented. Draw out the results you would expect.

Inject Dil, at a stage just as the rhombomere structure is becoming evident morphologically. Do many examples and compare the distribution of the cells. As seen (grey) in the picture, the labeled clones/polyclones respect the rhombomere boundaries. This implies an intrinsic difference between adjacent rhombomeres

14c. (3 points) Draw out and explain what you expect to occur is you grafted a piece of rhombomere 2 into rhombomere 3.

Draw out that the graft would be displaced back into rhombomere 2,
Or “popped out”/circular clone (minimizing interaction with surroundings
Or crossing the boundary with r2

14d. (3 points) Let's suppose you get a different result when you graft rhombomere 2 into rhombomere 4. Draw this and explain why you might get a different result. What further experiments might you do to see whether your prediction might be correct?
Are the rhombomeres following a “pair-rule” identity? Draw out that even numbered rhombomeres mix freely with other even numbered rhombomeres. Test by grafting into r6-same result free mixing/jagged boundary. Graft into r5 no mixing, may fuse with adjacent even rhombomere or remain circular/popped out.
Question 15 (8 points)

The hemichordate worm is in the same phylum as we are, so might be expected to share some developmental mechanisms. However, in contrast to us, the worm develops a distributed nerve net, with neurons distributed throughout the skin. In some ways this is more like neurogenesis in Drosophila than vertebrates. The hemichordate has a fairly simple genome, without the whole genome duplications that occurred on the vertebrate lineage, so let’s assume they have only one copy of any gene.

John Gerhart and his colleagues have developed a method to manipulate gene expression, by knockdown using RNA interference.

How would you test whether neurogenesis uses a similar mechanism to a conserved regulation of neurogenesis in vertebrates and invertebrates (specifically Drosophila)? Give some details on the expected mechanism, then how you would test it.

Hypothesis: Neurogenesis is regulated by Notch signaling. So knockdown of Notch is predicted to be neurogenic, while knockdown of delta is expected to suppress neurogenesis. Experiment: Use RNAi against Notch, stain neurons, compare to controls- expect many more neurons with the Notch RNAi.

Use RNAi agains Delta, expect fewer or no neurons relative to the control.