

MCB 141 final  
May 20, 2008

MCB 141 Final Spring 2008

Name: \_\_\_\_\_  
SID# \_\_\_\_\_  
Section \_\_\_\_\_

Question	Points	Score
1.	12	_____
2.	10	_____
3.	10	_____
4.	5	_____
5.	12	_____
6.	15	_____
7.	10	_____
8.	6	_____
9.	9	_____
10,	10	_____
11.	6	_____
12.	10	_____
13.	8	_____
14.	4	_____
15.	6	_____

Total for Final: 200

1. When Rosa Beddington transplanted the Node of an early mouse embryo to an ectopic site, only a partial secondary axis resulted. What is necessary in this type of experiment for a nearly complete secondary axis to form, and why is the Node alone insufficient? Include in your discussion the role of antagonists of BMP, Wnt and Nodal. (12pts)

Need AVE in addition in order to get ant. axis

Node alone doesn't sufficiently block ligands that need to be cleared out for Otx2 expression for anterior CNS development, e.g., AVE expresses lefty-1, blocking Nodal, and cerberus, a multipotent antagonists of Wnt, nodal, and BMP.

2. Nodal plays an important role in early mouse development. How is the concentration of Nodal regulated? Is Nodal a morphogen? Give specific examples to support your views. (10 pts)

Nodal is regulated, at least in part, by a negative feed-back loop. Nodal stimulates lefty, which in turn is an antagonists of Nodal. Nodal is also auto-stimulatory, serving to upregulate itself. So there are positive and negative influences on Nodal, allowing for fine control. Also, micro-RNAs have recently been discovered that may modulate the suppressive effects of lefty.

Nodal has not been shown to be a morphogen in the mouse, though it is possible. There is good evidence Nodal is a morphogen from experiments done in z-fish, not described in the course. Students need to know what is needed to show that something is a morphogen.

One would need to show gradients of this ligand, demonstrate it diffuses, and show it produces different kinds of differentiation at different concentrations. We didn't discuss any experiments of that kind, though we did for Sonic Hh, and mention of that would be relevant in a general way.

3. Draw a cross section, at the approximate level of the thorax, of the neural plate and notochord in a chicken embryo at early neural plate stage (i) and after closure (ii). Indicate the regional expression of the two major signals that pattern the neural tube, and indicate the location of the cell bodies of the major classes of neurons (sensory, motor, interneurons) (10)

(See attached drawing)

4. Retinoic acid gradients are thought to be responsible for patterning the hindbrain, yet complete elimination of RA synthesis and its replacement by uniform exposure to exogenous RA produces a regularly patterned hind brain. How can you account for this result? (5)

Exogenous RA can, indeed, rescue an RA deficient embryo at early stages. There is an oxidase that inactivates RA, and this has been found to be more active anteriorly than posteriorly. Hence, even uniform Exogenous RA, the differential inactivation anteriorly produces a posterior ( high) to anterior (low) gradient of RA.

5. Name 3 different structures/tissues/cell types derived from neural crest.

Describe the cell migrations involved for each of your choices. (12)

head mesenchyme - migrate from cranial folds - forms many structures of head ( cranial bones, placodes, etc)

adrenal medulla - migrate of trunk crest, early and ventral- form epinephrine secreting cells. glucocorticoids from adjacent adrenal cortex stimulate differentiation.

dorsal root ganglion - migrate early through anterior somite (ephrin repellents) - forms sensory neurons- Wnts influence

pigment cells - migrate late via dermatome

sympathetic neurons - migrate early, ventro medially, close to cord. Form sympathetic ganglia ( also, ant and post parasympathetic ganglia)

6. Identify the position on the enclosed diagram of:

Telecephalon, Diencephalon, Mesencephalon, Metencephalon and Myelencephalon, Nasal placode, Lens placode, Otic placode, Isthmus, and prospective cerebellum(10)

(see attached diagram)

Name a part of the adult brain that arises from each of the 5 major brain vesicles. (5)

telecephalon: cerebral hemispheres ( frontal cortex); diencephalon : optic cup, hypothalamus; mesencephalo: thalamus, optic tectum; myelencephalon: pons, cerebellum, 8th nerve ( auditory); metencephalon ( hindbrain, medulla oblongata)

7. How do efferent neurites from the ganglion cells in different regions of the retina manage to terminate in the appropriate regions of the optic tectum? In other words, briefly describe the principal molecules and cell behaviors, insofar as they are known, that involved in this pathfinding and selective connectivity. (10)

A principal system was shown by Bonhoffer and others to be the expression of ephrins and the receptors (EphR). Ganglion cell efferent neurites express different EphR. For example, neurites from nasal quadrant express Eph A3, which is incompatible with ephrin A2; consequently these neurites terminate appropriately in anterior optic tectum and are prevented from entering posterior tectum. Neurites from the temporal quadrant express eph A4/5 and can penetrate the ephrin A2 zone; they cannot go further, however, being prevented by a stripe of ephrin A5 in the posterior tectum

(Note: students don't need to know precise ephrins, etc., but should know the concept and what's different between different connections.

8. Suppose a conditional knock-out of the FGF receptor in the presomitic mesoderm has been imposed after the formation of somite pair 4. What effect does this have on gene expression in presomitic mesoderm that will form somite 5? (6)

Basically, knocking out FGF would stop the process that gets the clock ticking. So somite 5 would have its anterior border, because it formed as a consequence of somite 4 forming. But no FGF means no Wnt 3, Notch or hairy oscillations, no compaction of presomitic mesoderm, and no subsequent somite.

9. Describe the three major tissues formed from somites, and what are the principal signaling molecules that initially elicit their different developmental pathways. (9)

Dermatome: factors from neural tube (NT3), form pigment cells

Myotome: Shh + Wnt, form striated muscle

sclerotome: Shh: forms cartilage (vertebrae)

10. Describe the outcome of a transplantation of an additional Apical Ectodermal Ridge (AER) to the anterior border of an early limb bud, and contrast that with the result of transplantation of an additional ZPA(zone of polarizing activity) to the same ectopic anterior site (10).

An additional AER will induce additional proximo-distal outgrowth, resulting a limb duplication, either partial or more complete, depending on time of transplantation. The digits will be in the correct ant. post order. The ZPA transplant will also result in duplication, especially of distal structures, but digits will be a mirror image duplication, with ant-post inverted. This is because FGF from AER stimulates outgrowth, but Shh from ZPA specifies positional information in a gradient.

11. Contrast the roles of FGF10 and Tbx 4/5 in limb development. (6)

FGF 10 is the initial signal from somatopleure (lateral) mesoderm to form a limb bud, followed by establishment of AER etc. Tbx 4/5 is expressed in lateral mesoderm, probably under ultimate control by hox paralog code, that specifies the character of the outgrowth, whether it is hind limb ( Tbx 5) or forelimb ( Tbx4)

12. Suppose you could remove (either surgically or a functional removal with appropriate conditional molecular knock-out) the intermediate mesoderm of the prospective mesonephros. Predict the effects on development of the reproductive system of both males and females and explain why. (10)

Removing the mesonephros sufficiently early would lead to lack of formation of the gonad and duct area of the genital ridge, thus gonads and Mullerian duct would be lacking, regardless of sex. If not done sufficiently early, perhaps there would be some genital ridge development. In which case the Mullerian duct and gonad area might form. But the male would have no mesonephros, and hence, seminiferous tubules, vas deferens, would be missing from the male.

Possibly testosterone would be lower and no AMDH would be made to eliminate the mullerian duct - difficult to predict. The female in any case would have mullerian duct, the mesonephric duct would still degenerate, and differentiation of the reproductive system might be OK.

13. Suppose you were able to "knock-out" the GDNF gene of mice by homologous recombination, and to obtain homozygotes of this condition. What would be the effect on kidney development? Explain why. (8)

A Lack of GDNF would stop formation of a ureteric diverticulum (bud), and hence, metanephrogenic mesenchyme would not be induced to form tubules. All definitive kidney development would probably cease, though there is a strong propensity for mesoderm in this region to form tubules of some kind. Certainly no kidney as such.

14. Mesodermal cells from several different locations participate in formation of the erythrocytes that form in the developing mammalian embryo. List 4 of them; Indicate the order in which each of them becomes an important sources of erythrocytes. (4)

Here are 5, in order:

Blood islands, extraembryonic  
dorsal aorta  
liver  
spleen and bone marrow, about the same time

15. Signals emanating from cardiac mesoderm, notochord, and dorsal aorta are recognized and transduced by developing pancreas. Compare the effects of these various signals. (6)

Cardiac mesoderm tends to inhibit pancreas formation from competent endoderm ( i.e. pdx 1 expressing endoderm), and if this endoderm and heart are explanted, no pancreas forms.

On the other hand, notochord stimulates pancreas differentiation (probably via FGF), opposing the action of cardiac mesoderm. It's a balancing act. Pancreas develops close to the dorsal aortae ( as well as the vitelline veins). The close association of endoderm with these blood vessels is necessary for the endoderm to express Pdx 1 and become competent to form pancreas.

**Question 16 (18 points)**

**A. (6 points)**

A cross section is shown of a 128-cell mouse blastocyst, with lines and boxes to designate particular regions of cells. Into each box, put the appropriate letters from the list to best identify each region.

- A. cells of the hypoblast
- B. cells of the mural trophoblast
- C. cells of the epiblast
- D. cells of the polar trophoblast
- E. fertilization envelope (zona)
- F. blastocyst cavity
- G. cells that form embryonic stem cells if cultured in a Petri dish
- H. cells that will later develop into the mouse
- I. cells that will later develop into extraembryonic endoderm
- J. cells derived from the outermost cells of the 64 cell stage embryo
- K. cells derived from inner cells of the 64 cell stage embryo
- L. will be broken down before the blastocyst implants

D J

E L

C G H K

A I K

B J

F

**B. (6 points)** By the 32-64 cell stage, the mouse embryo contains irreversibly different trophoblast cells and inner cell mass cells. Describe briefly how compaction and cleavage contribute to the formation of these different cell populations.

**At the 8 cell stage, each cell forms a thin ring of tight junctions with its neighbors, so part of its surface is exposed to the outside medium and part is exposed internally. As the cells divide to 16, some divide vertically (to the surface), so that both daughter cells remain in the surface and form more tight junctions. Others divide horizontally so that one daughter cell remains in the surface layer and one is entirely inside, having 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 having no 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).**

*(Question 16 continued)*

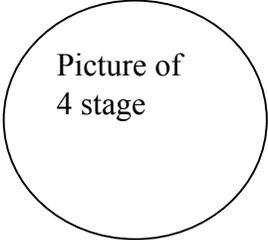
C. (6 points) By the 64-128 cell stage, the mouse embryo (the “blastocyst”) contains irreversibly different epiblast cells and hypoblast cells. Briefly describe blastocyst cavity formation, and tell how it and cell sorting contribute to the formation of these two cell populations.

**Blastocyst cavity formation:** Cells of the outer layer, in addition to forming tight junctions, undergo apical-basal polarization and pump ions, especially Na<sup>+</sup> through the basal surface and into the intercellular space. Tight junctions keep the ions from escaping to the outside medium. Counterions also move in, and the intercellular space reaches a higher osmolarity than the outside medium, that is, creating osmotic imbalance. Water moves in and inflates the intercellular space, forming the blastocyst cavity. As water moves in, the trophoblast surface layer of cells expands in surface area, and the inner cell mass (a clump of cells) remains stuck to it at only one point.

**How cavity formation and cell sorting contribute to the formation of epiblast and hypoblast:** The inner cell mass differentiates two kinds of cells, intermixed at first (students may say: some cells expressing *oct4*, *nanog*, *sox2*, and others expressing *gata6*). Some cells, the *gata6* expressing ones, sort out toward the blastocyst fluid, to form the hypoblast, leaving behind the *oct4/nanog/sox2* expressing cells, which form the epiblast, buried between hypoblast and trophoblast (which becomes polar trophoblast).

**Question 17 (15 points):**

Shortly after fertilization, a *Xenopus* egg is injected with antisense morpholinos to *Bmp2*, *4*, and *7* mRNAs. At the four cell stage, the ventral pair of blastomeres is isolated, that is, the pair lacking the grey crescent (see diagram). The pair is allowed to develop for several days, until hatching. Predict the phenotype of the half-sized hatched embryo, and explain your prediction.

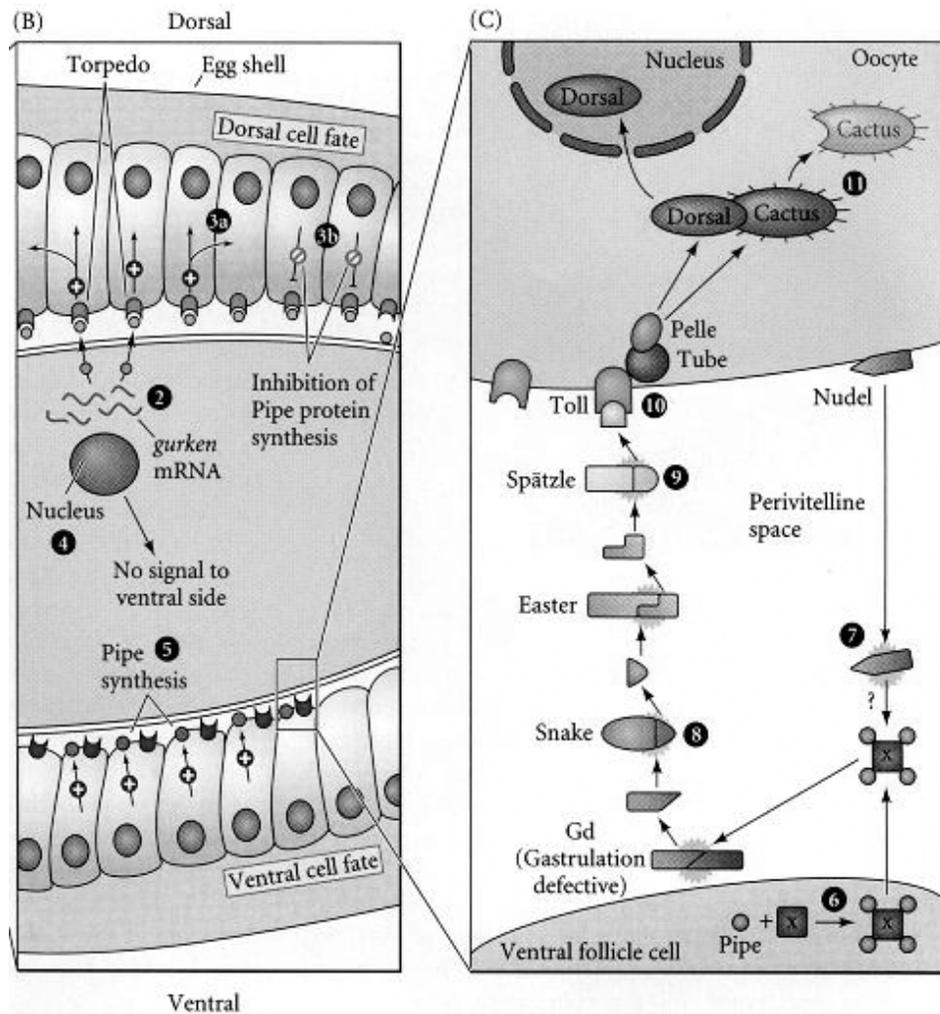


Picture of  
4 stage

**The ventral pair, without morpholinos, would develop to a ventralized embryo, with ectoderm going to ciliated epidermis, mesoderm to coelom and blood, and endoderm to posterior gut. There is no organizer present to release Bmp antagonists, hence only the Bmp-supported competence options of the germ layers are expressed.**

**With morpholinos, the ectoderm goes to neural tissue, the mesoderm to somites, and the endoderm to anterior gut, because these competence options of the germ layers are derepressed when Bmps are eliminated, by Bmp antagonists normally, or by antisense morpholinos experimentally.**

Here is a schematic of the dorsal-ventral patterning pathway in *Drosophila*:



You discover a new gene *Z*. Mothers that are homozygous mutant for a null (complete lack of function) allele of *Z* lay eggs that develop into embryos that are ventralized. You are told that gene *Z* encodes a protein involved in the cascade between *Gd* and the activation of the *Toll* receptor. Your goal is to more precisely define where in the pathway *Z* functions during development. Describe the genetic experiments (hint: think double mutants; you already have available to you individual fly strains mutant for *Z* plus each of the other D/V patterning genes) that you would carry out to answer the question, and how you would interpret your results.

Answer:

You would examine the embryos produced by females double mutant for Z and Gd, snake, easter, and spätzle and score for ventralized or dorsalized phenotypes.

If the gene is “upstream” (acts earlier in the cascade) of Z, then the phenotype of embryos from the double mutant mothers will be ventralized. If the gene is “downstream” (acts later in the cascade) of Z, then the phenotype of embryos from the double mutant mothers will be dorsalized.

So for example (and this is just one possible outcome):

Z;Gd	ventralized
Z;snake	ventralized
Z;easter	dorsalized
Z;spätzle	dorsalized

Then Z acts somewhere between snake and easter.

More complicated biochemical answers are possible for partial credit.