

MCB 131 Midterm 2**April 6, 2006****100 points in 80 minutes (we need to stop at 12:30 exactly).**

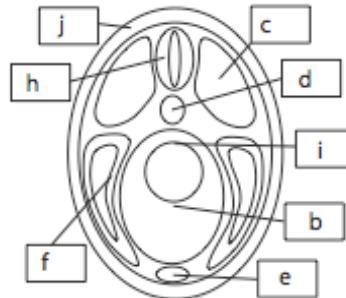
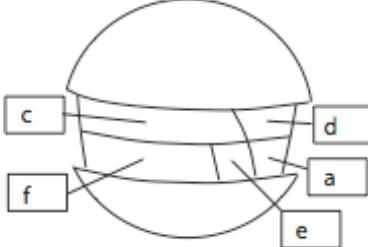
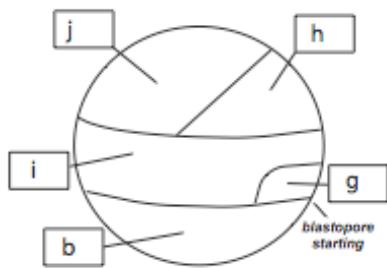
Midterm Question	Points	Score
1.	9	_____
2.	4	_____
3.	8	_____
4.	12	_____
5.	8	_____
6.	4	_____
7.	10	_____
8.	5	_____
9.	10	_____
10.	5	_____
11.	6	_____
12.	7	_____
13.	6	_____
14.	6	_____
Total for Midterm 2	100	_____

Note: Please use a pen. If you draw a picture as part of a short answer, please draw clearly and label the parts!

Number of pages you should have, including this one: 9

Question 1 (9 points):

Below are three diagrams of *Xenopus* embryos with regions marked by lines and boxes. In each box put one of more of the letters from the list to identify the designated region. (Points will be deducted for excess wrong answers).



Early gastrula (surface view)

- a. prechordal (head) mesoderm
- b. gut floor
- c. somites
- d. notochord
- e. heart

Early gastrula, deep equatorial cells shown

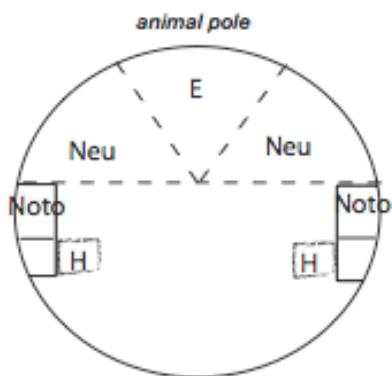
- f. lateral plate mesoderm (coelom)
- g. pharyngeal endoderm/gill slits
- h. neural plate or tube
- i. gut roof and/or wall
- j. epidermis

**Tailbud embryo
trunk cross section**

Question 2 (4 points)

Below is drawn an early gastrula embryo into which a second organizer has been grafted, as in the Spemann-Mangold experiment. The grafted organizer is dotted, whereas the host's organizer is not. Without bothering to distinguish superficial and deep layers of cells, raw a rough fate map of this grafted embryo, showing the following territory or territories:

Neural (label **Neu**) Heart (label **H**) Epidermis (label **E**) Notochord (label **Noto**)



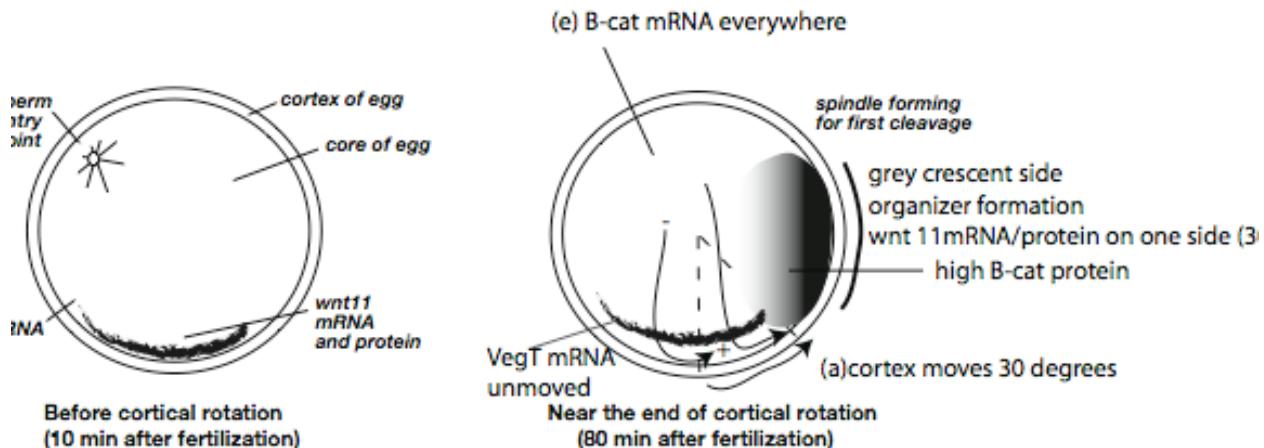
Question 3 (8 points):

Below on the left is a cross section of a *Xenopus* egg before cortical rotation. The locations of VegT mRNA and wnt11 mRNA (and protein) are indicated.

On the right is the same *Xenopus* egg just after cortical rotation.

On the figure to the right, draw and label with the appropriate letter or words:

- a. the displacement of the cortex relative to the core (assuming the core remains unmoved).
- b. the location of the parallel microtubule array during rotation. Add an arrow to indicate the plus-minus polarity of the microtubules (with the arrow point indicating the plus end of the microtubules).
- c. the location of wnt11 mRNA and protein after rotation.
- d. the location of high levels of beta-catenin protein after cortical rotation.
- e. the location of beta-catenin mRNA in the egg after rotation.
- f. the location of VegT mRNA after rotation.
- g. the approximate location of the grey crescent
- h. the approximate position at which the organizer will form in the mid- to late blastula.



Question 4 (12 points)

Give three different kinds of experimental evidence that, when taken together, implicate beta-catenin protein as a key component in the chain of events leading to organizer formation.

A. One kind of experiment and result:

Time-place criterion:

Do antibody staining for the beta-catenin protein.

Place: find it on one side, the grey crescent side, on which the future dorsal side will be, and which the organizer will later form.

Time: protein builds up in the first cell cycle, remains high until late gastrula stages, when the actual organizer is forming.

Question 4 continued

- B. A second kind of experiment and result

Eliminate beta-catenin:

using either antisense oligonucleotides to degrade the mRNA (and therefore no protein), or antisense morpholinos to block protein translation, results in a failure of organizer formation. A ventralized embryo or belly piece is the resulting phenotype. The addition of β -catenin mRNA can rescue dorsal development in these embryos. This shows that β -catenin is necessary for organizer formation, and dorsal structures.

- C. A third kind of experiment and result

Ectopic addition of β -catenin:

if you inject β -catenin in excess on ventral side (or something that causes beta-catenin protein stabilization on that side—Wnt11mRNA, dom.neg GSK3 or LiCl applied locally), you get a second organizer and a second body axis or a “twinned embryo” If you inject it all over, you get a hyperdorsalized embryo, in which organizer has formed around the equator. This demonstrates that β -catenin is sufficient to induce dorsal and organizer structures.

Question 5 (8 points): The figure below shows vegetal cells of a *Xenopus* mid-blastula embryo combined with animal cap cells of the same age. Write the most appropriate letter from the list below into each circle to indicate where each step of endo-mesoderm induction occurs within the recombinant (and thereby put the letters in the correct sequence for induction to occur):

Order: F, A, H, D, C, B, E, G

- A. *VegT* mRNA is translated to VegT protein here.
- B. Receptors for *Xnr1,2,4,5,6* and *Derriere* proteins are present here.
- C. *Xnr1,2,4,5,6* and *Derriere* proteins are secreted into the extracellular space here.
- D. *Xnr1,2,4,5,6* and *Derriere* mRNAs are translated into proteins here.
- E. Smad proteins of the TGF- β signal transduction pathway (of the nodal specific kind) are phosphorylated and activated here.
- F. *VegT* mRNA, which was deposited during oogenesis, is present here.
- G. Genes of mesoderm development are transcribed here.
- H. The transcription of *xnr1,2,4,5,6* and *derriere* genes is activated here.

Question 6 (4 points)

When an egg is soaked in pondwater containing dilute lithium chloride, beta-catenin protein builds up to high levels everywhere in the egg (because the GSK3 kinase is inhibited). At later stages, the organizer is formed around the entire circumference of the late blastula, but only at the equatorial level. Briefly explain why.

Organizer formation requires two inputs that work synergistically, both beta-catenin (normally via Wnt 11) and Nodals (normally via VegT) which specify the region to be mesoderm and must occur in animal hemisphere cells, those lacking VegT itself. Although the LiCl treatment allows beta-catenin to be stabilized everywhere in the embryo, only at the equator are these conditions met. At the animal pole, cells don't receive Nodals. At the vegetal pole, VegT blocks response to mesoderm induction (only endoderm is induced here). These animals are dorsalized.

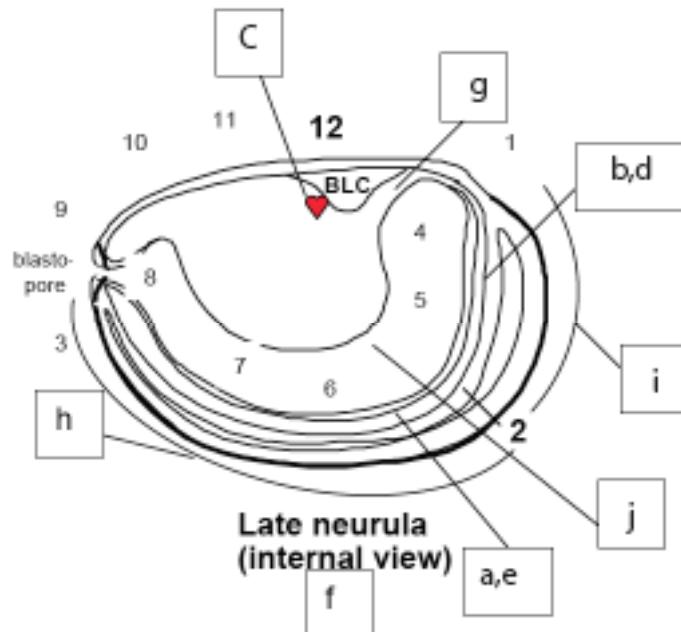
Question 7 (10 points):

In the left figure below, a *Xenopus* early gastrula embryo is drawn in surface view. Points on the bilateral plane are numbered 1 through 12.

6A. On the figure of the late neurula to the right, please locate those points after gastrulation and neurulation by marking the figure with lines and numbers. (Points 12 and 2 have been placed for you; BLC means “blastocoel”).

6B. On the late neurula diagram, draw in and label with the appropriate letter or words:

- a. the notochord
 - b. the head organizer
 - c. the approximate site of the heart
 - d. the prechordal plate (head mesoderm)
 - e. the trunk-tail organizer
 - f. the dorsal side
 - g. the anterior endomesoderm (AEM)
 - h. the site of *hox* gene expression in the neural tube
 - i. the location of forebrain and midbrain
 - j. the archenteron



Question 8 (5 points):

The *Xenopus* organizer has three parts: the head organizer (HO), the trunk-tail organizer (TTO), and the anterior endomesoderm (AEM). These differ with regard to their morphogenetic activities during gastrulation, the inductive proteins they secrete, and the tissues into which they eventually differentiate. In the space before each statement below, write HO, TTO, AEM, or some combination of these, so that each statement is true:

TTO eventually differentiate(s) into notochord

AEM eventually differentiates to liver and anterior gut.

HO eventually differentiate(s) into mesoderm of the head.

TTO engage(s) in convergent extension during gastrulation.

HO clusters of its cells migrate along the blastocoel wall during gastrulation.

HO & TTO (AEM) secrete(s) Bmps antagonists such as Noggin, Chordin, and Follistatin proteins.

HO secrete(s) Wnt antagonists such as Dkk and Frzb proteins.

AEM secrete(s) Cerberus protein, a Nodal antagonist.

HO(AEM) eventually come to underlie the forebrain and midbrain.

TTO eventually comes to underlie the hindbrain and spinal cord.

Question 9 (10 points):

Based on the Default Model for neural/epidermal development, explain each of the following experimental interventions.

9A. Introduce the mRNA for a constitutively active Bmp receptor into ectodermal animal cap cells, that is, for a receptor active even when Bmp is absent. Add high levels of Noggin, Chordin, and Follistatin. Predict epidermis or neural tissue as the outcome and briefly explain your prediction.

EPIDERMIS: Because constitutively active receptors keep the Bmp signal transduction going even if all Bmps, external to the cells, is bound up with Noggin, Follistatin, and Chordin and can't bind the receptor. Epidermal genes are activated, and neural genes repressed.

9B. Introduce into animal caps three anti-sense morpholinos, each about 25 bases long, that together block the translation of the mRNAs for Bmp2, Bmp4, and Bmp7 proteins. Omit Noggin, Chordin, and Follistatin. Predict epidermis or neural tissue as the outcome and briefly explain your prediction.

NEURAL: Because if the ectoderm fails, for whatever reason, to receive and transduce Bmp signals, it depresses neural option and can't sustain the epidermal option.

Question 10 (5 points)

Consider cells that have developed to anterior neural tissue (forebrain and midbrain) in a *Xenopus* embryo.

From the list below, choose option 1 or 2 from each of the conditions **a, b, c, d, e** to put into the designated blanks, thereby arriving at a sequence of five steps for the development of anterior neural tissue:

a 1 b 2 c 2 d 1 e 1, then become anterior neural tissue

a1. were cells of the animal cap

a2. were cells of the vegetal base (containing *vegT* mRNA)

b1. received and responded to Xnr1,2,4,5,6 and derriere (Nodal signals).

b2. did not receive Xnr1,2,4,5,6 and Derriere (Nodal signals).

c1. did not briefly make and respond to Bmp signals.

c2. did briefly make and respond to Bmp signals.

d1. were close enough to the organizer to be exposed to Bmp antagonists such as Chordin, Noggin, and Follistatin

d2. were too far from the organizer to be exposed to Bmp antagonists such as Chordin, Noggin, and Follistatin

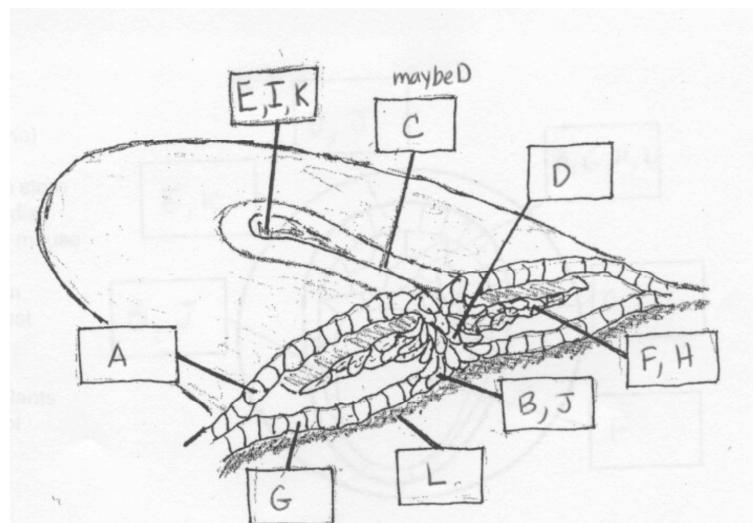
e1. were close enough to the head organizer to be exposed to Wnt antagonists such as Dickkopf and Frzb.

e2. were too far from the head organizer to be exposed to Wnt antagonists such as Dickkopf and Frzb.

Question 11 (6 points)

In the figure below, a chick gastrula embryo has been cut across the primitive streak and tipped toward you so you can see the ingressing cells. Regions are marked by lines and boxes. Select letters from the list below and enter them in the boxes to indicate the identity of each marked region. (Some boxes may contain more than one letter).

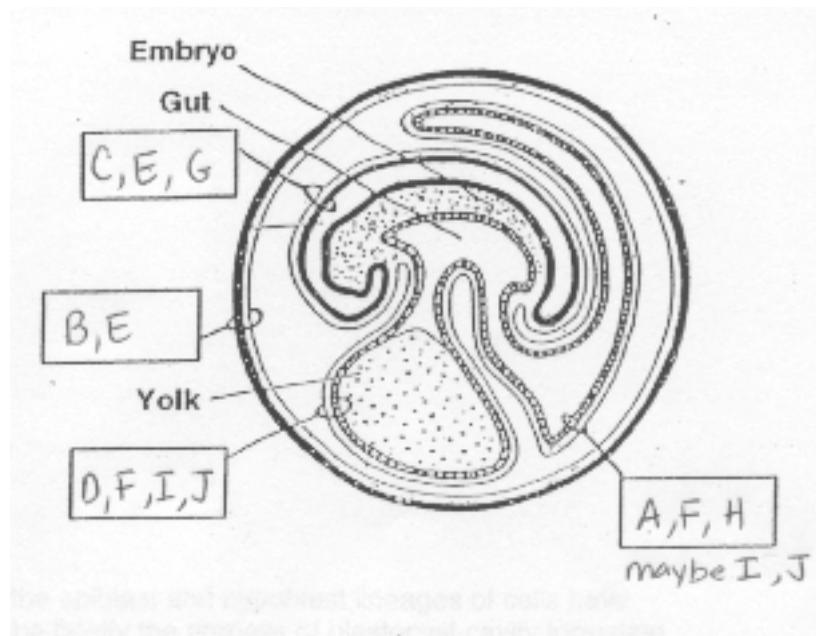
- A. ectoderm
- B. embryonic endoderm
- C. primitive streak
- D. ingressing cells
- E. Hensen's node
- F. embryonic and extraembryonic mesoderm
- G. hypoblast/endoblast
- H. later forms lateral plate and somites
- I. site at which the prechordal mesoderm has involuted and moved anteriorly
- J. comes through the primitive streak first
- K. later forms notochord and floorplate
- L. uncleaved yolk mass



Question 12 (7 points):

Below is a diagram of a chick embryo 9 days after egg laying. The **extraembryonic** parts are indicated by lines and boxes. Into each box, put appropriate letters from the list below to identify and describe the parts. A box may contain more than one letter.

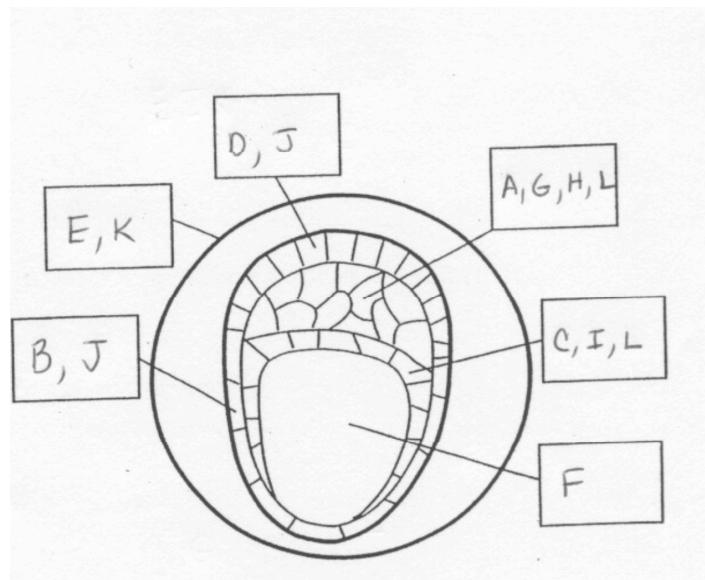
- a. allantois
- b. chorion
- c. amnion
- d. yolk sac
- e. a lining composed of ectoderm and mesoderm.
- f. a lining composed of endoderm and mesoderm.
- g. lines a cavity that surrounds the embryo in a controlled aqueous environment.
- h. lines a cavity in which metabolic wastes are stored.
- i. is involved in mobilizing nutrients for the embryo
- j. contains hypoblast and endoblast cells



Question 13 (6 points)

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

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



Question 14 (6 points)

A. By the 32 cell stage, the mouse embryo contains two irreversibly different lineages of cells-- one that will develop to trophoblast cells and one that will develop to epiblast and hypoblast cells. Describe briefly the process of compaction and cleavage by which the two lineages become irreversibly different:

At the 8 cell stage, during compaction, cells polarize in response to contact with neighbors (basal) and exposure to external medium (apical). The next cleavages are vertical or horizontal for cells, and horizontal cleavages release daughter cells internally. They don't polarize. Vertically cleaving cells polarize—they are responding to the outside medium and form tight junctions with neighbors. These cells become the trophoblast cells. Inner cells don't form tight junctions and they become the epiblast and hypoblast. By 64 cell stage, outer cells don't release more inner cells. If tested, inner cells can't polarize as outer cells.

B. By the 128 cell blastocyst stage, the epiblast and hypoblast lineages of cells have become irreversibly different. Describe briefly the process of blastocyst cavity formation by which the conditions are established for this differentiation to occur.

Outer cells polarized and connected by tight junctions, pump Na^+ across basal membrane into intercellular space. Ions are kept in by tight junctions. Cl^- comes in by passive diffusion. H_2O comes in by osmosis and swells the intercellular space, expanding the outer cell layers. Inner cell mass cells stick together but lose adherence to some of the expanding surface, so the clump is asymmetrically located. Blastocyst cavity occupies rest of interior. ICM cells exposed to cavity fluid make hypoblast. ICM cells surrounded by other cells, either hypoblast or trophoblast, make epiblast.