Name: _____JG KEY_____

MCB 141 Midterm 2 April 8, 2008

100 points in 80 minutes (we need to stop at 12:30 exactly).

Midterm Question	Points	Score
1.	10	_____
2.	4	_____
3.	18	_____
4.	9	_____
5.	9	_____
6.	18	_____
7.	12	_____
8.	6	_____
9.	9	_____
10.	5	_____
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: 10

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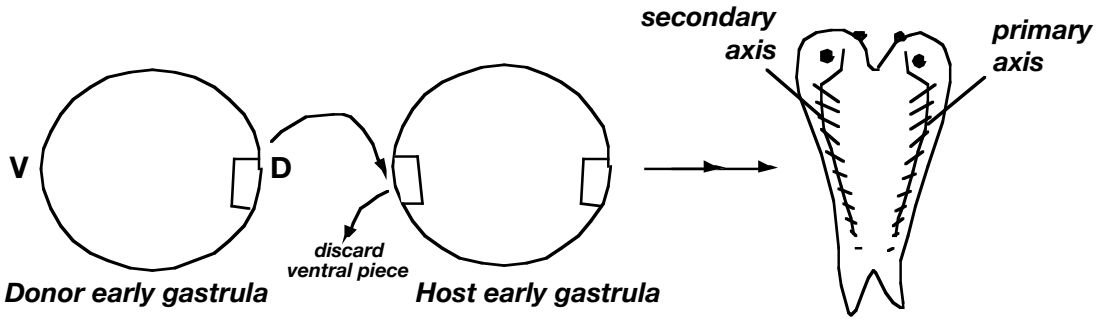
Question 1 ***(10 points):***
The Spemann and Mangold experiment of 1924 is outlined below. From their results, they concluded that the organizer graft had induced nearby ventral cells of the host to change their fates and to develop into nervous system and anterior somites of the secondary body axis.



To conclude this, they needed additional experimental results to eliminate two alternative interpretations:

A. Self-differentiation of the graft:
   i) How could you eliminate the possibility that the graft just self-differentiated into the entire secondary axis?

   (3 pts) Label the donor embryo with a lineage tracer (fluorescein-dextran) at the one cell or early cleavage stages, so all cells of the graft are labeled. Or label the graft or cells of that region with nile blue or some other vital dye, trying to get all the cells labeled. Or use a donor with different pigmentation from the host.
   Any of these will suffice.
   Section the conjoined twin and detect where the label of the graft has become located.

   ii) What did the organizer graft form in the secondary axis?

   (2 pts) The graft formed the notochord and head mesoderm, and sometimes a little somite and neural tube (floorplate) near the notochord. ("Notochord" is sufficient.)

B. Recruitment of host cells that did not change fate:
   i) How could you eliminate the possibility that the cells of the nervous system of the secondary axis did not change fate but just migrated over to the region of the graft from the host's neural territory?

   (3 pts) Label cells of the neural territory on the side near the host's organizer. Labelling could be nile blue or another vital dye, or fluorescent lineage tracer injected in the pair of dorsal cells at the 4 cell stage. The possibility of recruitment is eliminated if labeled cells don't end up in the secondary nervous system.
   (or give the reverse experiment of labeling cells of the host's epidermis territory, to show that they do go into the secondary nervous system.)

   ii) Regarding the cells that formed the nervous system of the secondary axis, what would have been their fate if the grafting had not been done?

   (2 pts) Epidermis

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Question 2 (4 points)
The developmental fates of early gastrula cells depend on 1) their membership in a particular competence group, and 2) their distance from the organizer. As you learned, these dependences reflect the exposure of cells to different protein signals at the blastula, gastrula, and neurula stages.

Explain the location of the following territories on the fate map in terms of signals the cells are exposed to:

Territory of the Fate Map	Cells exposed to Nodal signals (write yes or no in each box)	Cells exposed to Bmp antagonists (write yes or no in each box)
Neural	**NO**	**YES**
Epidermal	**NO**	**NO**
Somite	**YES**	**YES**
Lateral plate	**YES**	**NO**

Question 3 (18 points)
The β-catenin protein is important for organizer formation, as has been demonstrated by the developmental consequences of "knocking down" (reducing, eliminating) individual components of the Wnt pathway, shown to the right.

DRAWING OF WNT PATHWAY NOT INCLUDED IN THE KEY

3A (2 pts). Briefly describe (one or two sentences) <u>two</u> methods to knock down components of the pathway in the *Xenopus* oocyte, egg, or embryo:

Method 1:
Antisense deoxy-oligoniucleotides: inject these into the oocyte or egg to distribute uniformly, let them hybridize specifically with the target mRNA and recruit RNAseH (degradative enzyme), and enzyme that destroys the mRNA.

Method2:
Antisense morpholinos: inject these into the oocyte or egg to distribute uniformly, let them hybridize specifically with mRNA and block translation.

Other acceptable answers: dominant negative proteins (probably inject the mRNA); antibody to inactivate protein, specific inhibitor such as LiCl, anti-wnt proteins, etc. Or even UV irradiation or microtubule inhibitors to block formation of the parallel array of microtubules so maternal wnt11 mRNA is not translocated.

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Question 2 continued

3B (10 pts). For each of the following Wnt-pathway components, fill in the boxes to predict the consequences of the knockdown of the particular component for the embryo's development:

Component knocked down throughout the embryo	Does the level of β-catenin protein** (choose one) increase, decrease, or stay the same?	At the gastrula stage, will the organizer be (choose one): absent, normal, or larger?	Eventual phenotype (choose one): ventralized, dorsalized, twinned, or normal)
Maternal β-catenin mRNA	**decrease**	**absent**	**ventralized**
Maternal wnt11 mRNA	**decrease**	**absent**	**ventralized**
Maternal axin mRNA	**increase**	**larger**	**dorsalized**
Maternal GSK3 mRNA	**increase**	**larger**	**dorsalized**
Maternal mRNA for the Frizzled receptor or LRP5/6 coreceptor	**decrease**	**absent**	**ventralized**

***** This means, compared to the dorsal side of a normal embryo!!***

3C (6 pts). Regarding the requirement of **cortical rotation** for organizer formation:

i. Which maternal components are moved during cortical rotation?

Wnt11 mRNA (and protein), and vg1 mRNA (and protein). Students may write about the movement of vesicles carrying these; that's OK. And they may write that the cortex is moved; that's OK, though not sufficient without mention of the translocated mRNAs.

ii. From where, and to where, are they moved?

From the vegetal pole to one side (equatorial level), the grey crescent/ future dorsal/future organizer side.

iii. Briefly describe the microtubule array along which they are moved.

Microtubules are aligned in a parallel array with plus ends going the same direction. The array is only a few microtubules thick, occupying the interface between the cortex and cytoplasmic core. It appears in the middle of the first cell cycle and disappears by the end of that cycle. The microtubles are the tracks along which the wnt11 and vg1 maternal mRNAs are moved, probably in association with vesicles towed by kinesin motor proteins.

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Question 4 (9 points):
Give three different kinds of experimental evidence that, when taken together, implicate maternal **vegT mRNA** as an essential component for endo-mesoderm induction in *Xenopus* development:

A. One kind of experiment and its result:

Time and place:
Experiment: Do insitu hybridization to see when and where the vegT mRNA is present.
Result: VegT mRNA is maternal and persists at least until the midblastula period when endo-mesoderm induction occurs, and the vegT mRNA is located in the vegetal hemisphere, which is the source of endo-mesoderm inducers at the mid-blastula stage.

B. A second kind of experiment and its result:

Depletion:
Experiment: Knock down vegT mRNA with a specific antisense oligonucleotide, or block the translation of the mRNA with a specific antisense morpholino.
Result: Endoderm and mesoderm are not formed. An ectodermal ball develops.
(Some students may mention rescue by injection of vegTmRNA to show specificity of the knockdown—that's OK, though not required.)

C. A third kind of experiment and its result:

Ectopic sufficiency:
Experiment: Inject vegT mRNA into a region of the egg that normally lacks maternal vegT mRNA, namely the animal hemisphere. Then, explant the animal hemisphere before the midblastula stage and see if it can form endo-mesoderm isolation, or if it can act as an inducer when combined with an uninjected animal cap in a Nieuwkoop recombinate.
Result: Yes, the injected animal cap can form endo-mesoderm on its own, and it can act as an inducer in a Nieuwkoop recombinate.

Some students may suggest a rescue experiment: inject vegT mRNA into an egg that was previously depleted of vegT mRNA. That's acceptable.

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Question 5 (9 points):
Pieter Nieuwkoop combined animal cap cells of the midblastula *Xenopus* embryo with either (A) dorsal vegetal cells (*recombinate A*) or ventral vegetal cells (*recombinate B*) of the same age. He discovered that recombinate A formed an organizer whereas recombinate B formed lateral-ventral mesoderm such as coelom and blood cells.

***5A (2 pts)*.** Describe an experiment and the result to determine whether the organizer in *recombinate A* was formed from animal cap cells or dorsal vegetal cells:

Experiment: Inject a lineage tracer (fluorescent) into an egg. At the mid-blastula stage, combine the fluorescent animal cap with unlabelled dorsal vegetal cells. And do the reverse, with fluorescent dorsal vegetal cells combined with an unlabelled animal cap. Result: The organizer contains cells only from the fluorescent animal cap, not from fluorescent dorsal vegetal cells.
Or do the experiment with nile blue stained cells—same idea.

***5B* (*5 points of 9*).** Organizer formation in the mid- and late blastula embryo requires inputs from endo-mesoderm induction and from beta-catenin (via cortical rotation). Explain briefly, in the space below, how these inputs lead to organizer formation.

The combination of beta-catenin protein and nodal signals, including Vg1, is unique to the dorsal equatorial region where the organizer forms. Beta-catenin combines with Tcf-Lef transcription factor and activates and derepresses genes (via intermediate genes encoding transcription factors such as siamois and iroquois) that encode Xnr3 and Chordin, two Bmp antagonists, and some of these transcription factors repress Bmp gene expression. This produces a dorsal region free of maternal and zygotic Bmp. The absence of Bmp favors organizer formation. High Bmp levels can repress organizer formation.

Nodal signaling, as measured by phosphoSmad2/3 is higher on the dorsal side, due to the combined input of Nodal and Vg1 signals, and to the absence of Bmp inhibition and repression. Overall, high Nodal/Vg1 signaling and low Bmp signaling lead to organizer formation. Finally some genes of the organizer are synergistically activated by the combination of phosphosmad2/3 and beta-catenin/Tcf-Lef.

5C (2 pts). Why didn't the dorsal vegetal cells, themselves, form an organizer?

The dorsal vegetal cells contain vegT mRNA which encodes the VegT transcription factor. This transcription factor activates genes committing the vegetal cells to endoderm development, preventing mesoderm and organizer formation.

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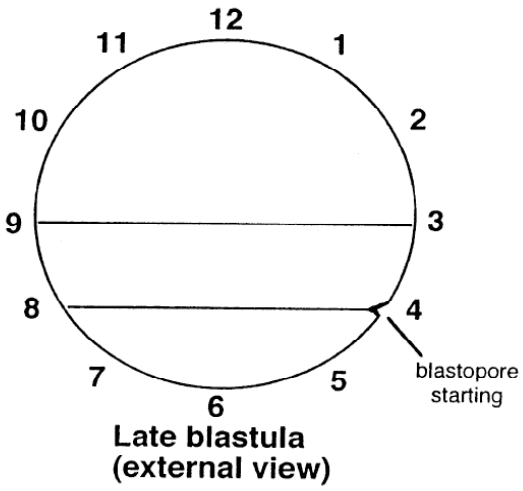
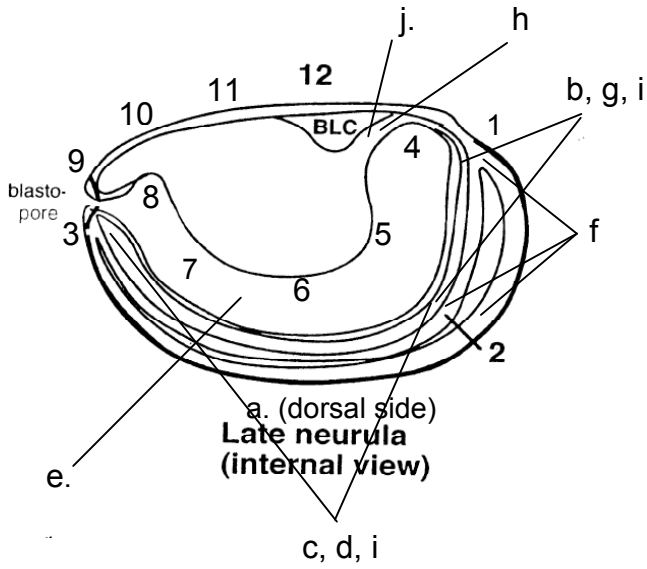
Question 6 (18 points):
In the left figure below, a *Xenopus* early gastrula embryo in drawn in surface view. Points on the bilateral plane are numbered 1 through 12.

6A (5 pts). On the late neurula diagram to the right, please locate those points after gastrulation and neurulation by marking the figure with numbers and arrows. (Points 2 and 12 have been placed for you; BLC means "blastocoel").

6B (5 pts). On the late neurula diagram, identify each of the following parts or locations by marking the diagram and labeling your marks with the appropriate letter:

a. the dorsal side
b. the head organizer
c. the notochord
d. the trunk-tail organizer
e. the archenteron
f. the location of the forebrain and midbrain
g. the prechordal plate (head mesoderm)
h. the anterior endomesoderm (AEM)
i. cells secreting noggin, chordin, and follistatin.
j. the approximate site of the heart





6C(2 pts). Identify the two points between which the greatest displacement has taken place: ____**3**_____ _____**4**_____

6D (6 pts). Identify the two kinds of morphogenetic activity that drive this greatest displacement and describe each morphogenetic activity briefly.

Morphogenetic activity 1:

Spreading migration: After they involute, cells of the head organizer region (prospective head mesoderm) migrate on the blastocoel wall, along fibronectin cables, toward the animal pole. As these cells move, the initial block of cells in which they are arranged thins to one layer and spreads out. During movement the cells are unipolar (each with one tip of motile activity, i.e., one lamellapodium).

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Question 6D continued

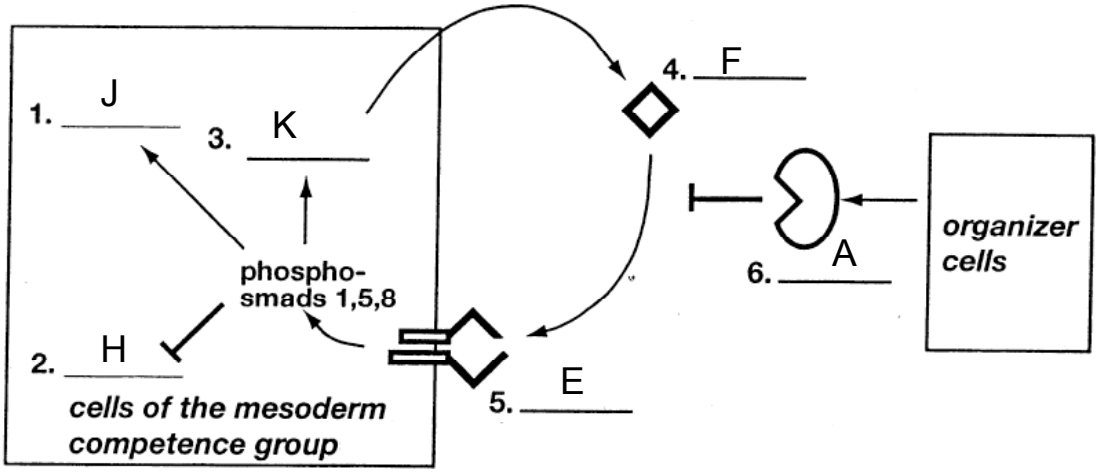
Morphogenetic activity 2:

Convergent migration: After involution (and even before), cells of the trunk-tail organizer (prospective notochord) become spindle shaped and bipolar (with opposite tips of motile activity, i.e., two lamellapodia). The initial block of cells in which they are arranged narrows and thins (converges), and the population extends as cells interdigitate (i.e., between one another). Cells with one tip at the boundary with somites become inactivated at that tip, hence unipolar, and pull in from the boundary by way of the opposite active tip. More and more cells are exposed to the boundary until no active tip remains. A rod of cells is formed.

Some students discussed bottle cells, apical constriction, invasiveness, invagination, and involution. That's acceptable for one morphogenetic activity.

Question 7: (12 points)
7A (6 pts). Below is an incomplete diagram of the circuitry of "dorsalization of the mesoderm" according to the **DEFAULT MODEL**. Complete the diagram by writing the appropriate letter from the list into each of the 6 blanks in the diagram. Not all letters will be used. Flat-headed arrows indicate inhibition or repression. Pointed arrows indicate activation or production.

A. Bmp antagonists such as chordin, noggin, and follistatin
B. Stabilized beta-catenin protein
C. The receptor of Bmp antagonists
D. Wnt antagonists such as dkk, frzb, and crescent.
E. The Bmp receptor
F. Bmp protein
G. The neural development option
H. The somite development option
I. The epidermal development option
J. The lateral plate/coelom option
K. Bmp gene expression
L. the Frizzled Wnt receptor



7B (3 pts). Introduce the mRNA for a dominant negative form of Component 5 into mesodermal cells and introduce antisense morpholinos to deplete Component(s) 6. Predict the developmental outcome, and explain your prediction briefly.
Prediction: somites will develop.
Explanation; The dominant negative (component 5) inhibits receptor function, and so the cells can't persist in Smad2/3 phosphorylation, Bmp production, and repression of the somite option. Derepression occurs even though Bmp antagonists (component 6) are absent.

Question 7 continued

7C (3 pts). Introduce the mRNA for a constitutively active form of Component 5 into the mesodermal cells and introduce excess mRNAs to increase the amount of Component(s) 6. Predict the developmental outcome, and explain your prediction briefly.

Prediction: lateral plate/coelom will develop.
Explanation: The "constitutively" active receptor (i.e., always active, even when ligand is absent) keeps phosphorylating smads2/3 and repressing the somite option, even when excess levels of Bmp antagonists (component 6) bind up all extracellular Bmp and prevent it from binding the receptor.

Question 8 (6 points):

Dkk (Dickkopf) is a Wnt-antagonist normally produced by the head organizer. When Dkk activity is knocked down, a severly “micro-cephalic” embryo results, that is, one with a greatly reduced forebrain and midbrain. The more posterior parts of the nervous system are normal. Explain this result in terms of your understanding of the induction of the anterior and posterior parts of the nervous system.

When ectoderm is neuralized by Bmp antagonists that interrupt Bmp signaling, the ectoderm begins developing as anterior neural tissue (toward forebrain and midbrain). If such neuralized ectoderm then receives Wnt signals, it develops as posterior neural tissue (hindbrain and spinal cord). Wnt signals are widely produced in the embryo, especially by the somite mesoderm (Wnt8). In the head region, Dkk from the head organizer antagonizes Wnts, so that anterior neuralized ectoderm is not posteriorized. Therefore, it continues to develop as forebrain/midbrain. Farther back in the body, where Wnt antagonists are not produced, neuralized ectoderm develops as posterior neural tissue.
When Dkk is knocked down in the head mesoderm, the anterior neuralized ectoderm becomes posteriorized by Wnts, and the forebrain and midbrain do not develop.

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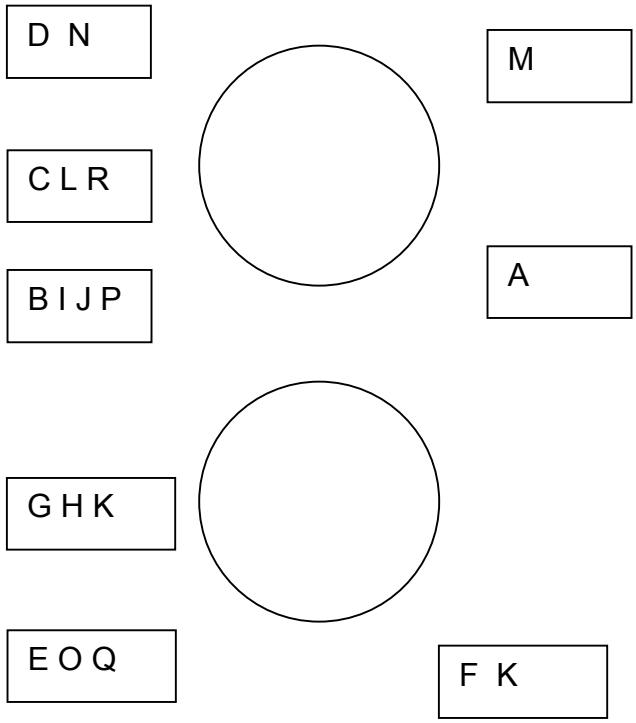
The figure below shows top and bottom views of a chick blastodisc at the "maximum streak stage", about 14 hours after egg laying. Regions are marked by lines and boxes. Select letters from the list below and enter them in the boxes to indicate the identity of each marked region. *(Some boxes may contain more than one letter, and a letter may be used in more than one box).*

A. Marginal zone B. Primitive streak C. Hensen's node D. Epiblast E. Posterior marginal zone (PMZ) F. Endoblast G. Hypoblast H. Cerberus-producing cells I. Nodal producing cells J. Endo-mesoderm cells about to engage in waterfall ingression K. Will become extra-embryonic endoderm L. Notochord precursors M. Uncleaved yolk mass N. Site at which anterior neural development will occur. O. Was uppermost when the blastodisc was tipped to one side in the oviduct. P. High level of nodal signaling Q. Vg1 protein first produced here R. Will regress the length of the primitive streak	

Question 10 (5 points):
Below is a diagram of a chick embryo 9 days after egg laying. 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. yolk sac b. allantois c. chorion d. amnion 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