Name: _____

MCB 141 **Midterm 2** **March 31, 2011**

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

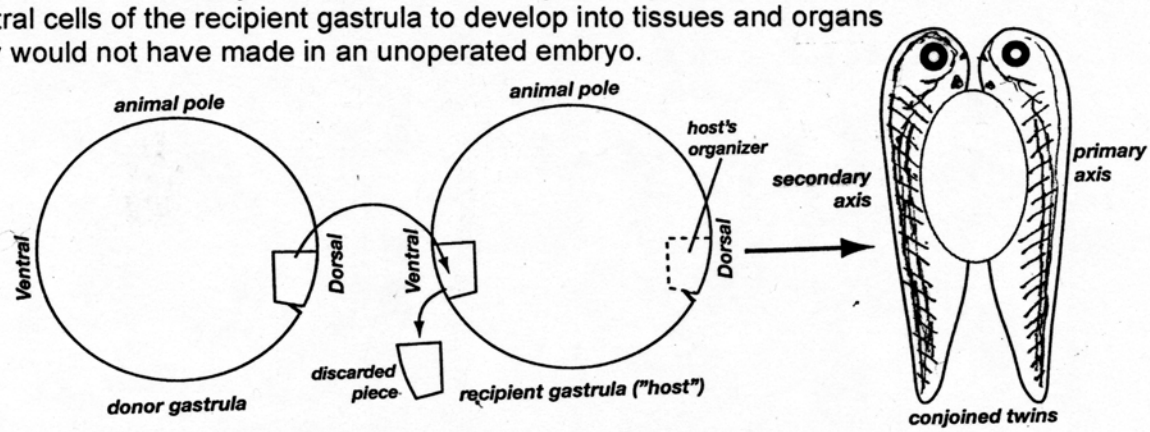
Question	Points	Score
1.	8 (Spemann Mangold)	_____
2.	8 (cortical rotation)	_____
3.	7 (Wnt pathway)	_____
4.	12 (tests of Nodal)	_____
5.	6 (dorsal/ventral mesoderm)	_____
6.	15 (morphogenesis)	_____
7.	3 (organizer signals)	_____
8.	13 (default model)	_____
9.	6 (posterior neural)	_____
10.	5 (endoblast/hypoblast)	_____
11.	4 (chick extraembryonic tissues)	_____
12.	8 (chick gastrulation/fate map)	_____
13.	5 (mouse blastocyst)	_____
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: 13

Name: _____

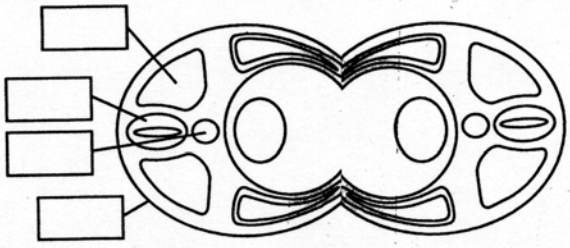
Question 1 (8 points): The Spemann-Mangold experiment of 1924 is diagrammed below. From their results, they concluded that the organizer graft from a donor gastrula induced nearby ventral cells of the recipient gastrula to develop into tissues and organs they would not have made in an unoperated embryo.



Part 1A: On the recipient gastrula ("host") above, which has two organizers, draw a rough fate map consistent with the result of a conjoined twin, labelling the following territories. You needn't bother to distinguish superficial and deep layers of cells:

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

Part 1B: To the right is a cross section of the conjoined twin that developed from the recipient gastrula. In each box, write one or more labels from the list of **part 1A** to identify the tissues and organs of the secondary axis.



Part 1C: For Spemann and Mangold to conclude that the cells of the ventral side of the early gastrula had really changed fate, they needed additional experimental results to eliminate two other interpretations:

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

   <u>ii)</u> What did the organizer graft itself form in the secondary axis?

Name: _____

Question 1 con't

B. Recruitment of host cells that did not change fate:

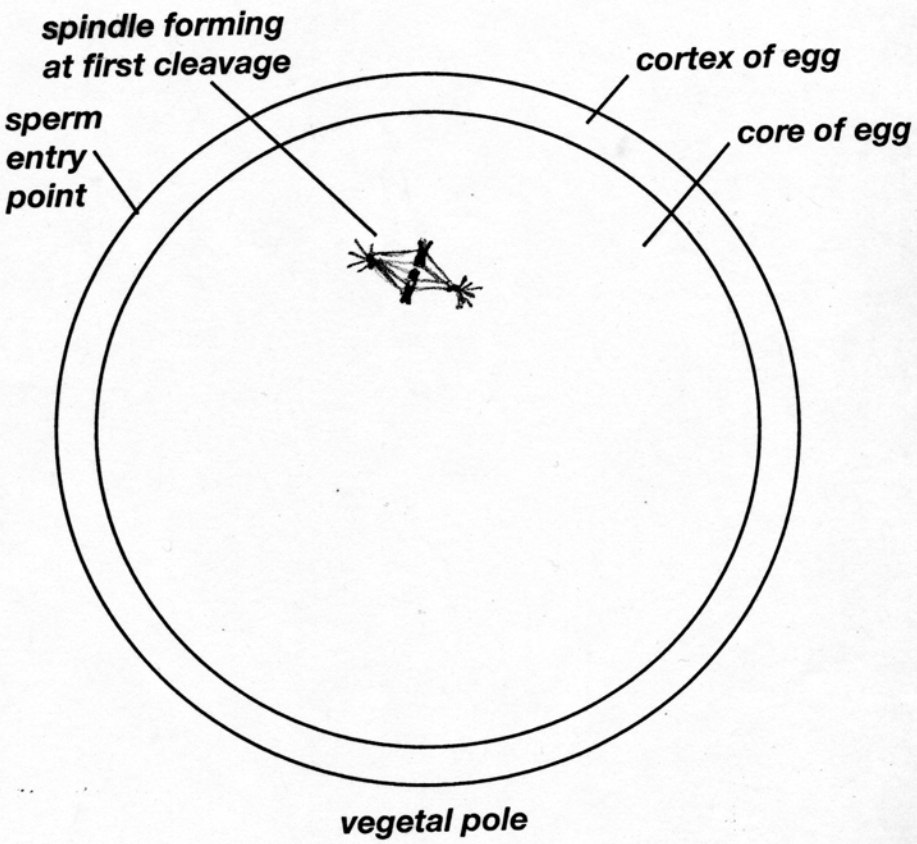
i) How could you eliminate the possibility that the cells of the nervous system of the secondary axis did not change fate but just migrated over to the region of the graft from the host's neural territory?

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?

Question 2 (8 points):

Below is a cross section of a *Xenopus* egg just after cortical rotation. The animal pole is up, and the vegetal pole down. **By drawing and labeling with the appropriate letter or words, indicate:**

a. the location of the parallel microtubule array during rotation. Add an arrow to indicate the polarity of the microtubules (with the arrow head at the **plus end** of the microtubules).
b. the direction the cortex rotates relative to the core (assuming the core remains unmoved).
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 *vg1* mRNA after rotation.
g. the location of *vegT* mRNA after rotation.
h. the approximate location of the grey crescent.
i. the approximate position at which the organizer will form in the mid- to late blastula.



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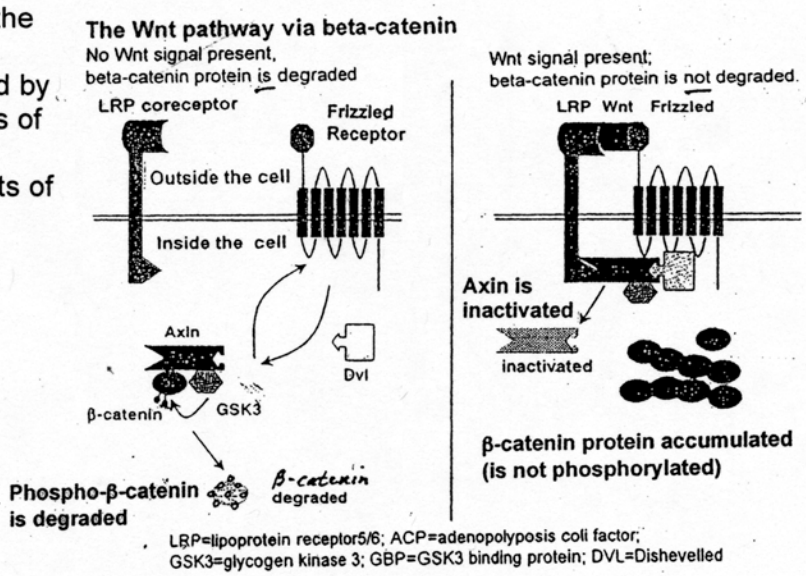
Question 3 (7 points)

The Wnt pathway, by which β-catenin protein is produced, is shown to the right. It's important for organizer formation has been demonstrated by the developmental consequences of "knocking down" (reducing, eliminating) individual components of the pathway.



3A. 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 increase, decrease, or stay the same? (write one per box)	At the gastrula stage, will the organizer be absent, normal, or larger? (write one per box)	Eventual phenotype is: ventralized, dorsalized, twinned, or normal) (write one per box)
Maternal wnt11 mRNA			
Maternal β-catenin mRNA			
Maternal axin mRNA			
Maternal GSK3 mRNA			
Maternal mRNA for the Frizzled receptor or LRP5/6 coreceptor			

3B. In the list below, circle the tissues and organs developed by a ventralized embryo:

neural tube	coelom/lateralplate	gill slits
posterior gut	blood	heart
somites	notochord	epidermis

Name: _____

Question 4 (12 points)

Various kinds of experimental evidence, when taken together, implicate Nodal proteins (Xnr1,2,4,5,6, derriere) as the key inducers of endomesoderm in *Xenopus*. Provide that evidence in the following four sections:

A. The "time/place" experiment and its result:

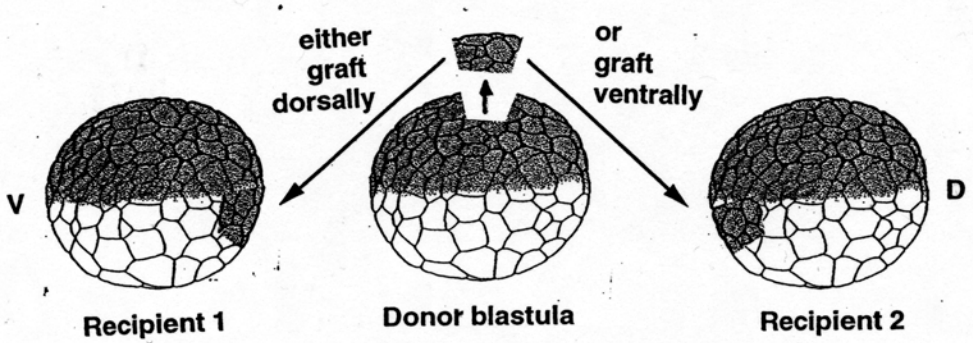
B. A depletion experiment and its result. Also include a control experiment and its result to eliminate the possibility of "off-target" effects of the depletion agent:

C. An ectopic expression experiment, and its result:

D. An experiment and its result demonstrating that animal cap cells directly respond to Nodal proteins when they undergo endomesoderm induction (as opposed to responding to some other protein that Nodals stimulate to form)?

Name: _____

Question 5 (6 points). Shortly before the midblastula stage, cells from the animal pole region of a donor embryo are transplanted to the equatorial level of a recipient embryo, either replacing cells of the dorsal [grey crescent side] (recipient1) or replacing cells the ventral [sperm entry side] (recipient 2). See the following figure:



a) What will the animal pole cells develop when they are transplanted to the ventral side?

c) What will the animal pole cells develop when they are transplanted to the dorsal side?

d) Indicate the difference in signaling agents the graft encounters on the two sides .

Animal pole cells moved to:	*Receive Vg1 protein (write yes or no in the box)*	*Receive Nodal signals (write more or less in the box)*	*Receive Wnt11protein (write yes or no in the box)*	*Receive Bmp protein (write more or less in the box)*
The ventral side (recipient 2)				
The dorsal side (recipient 1)				

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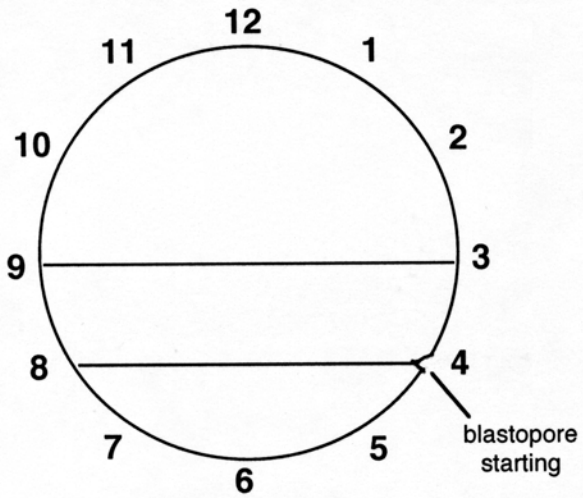
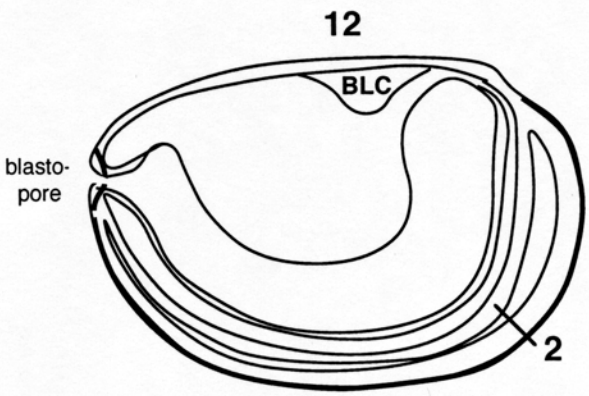
Question 6 (15 points): 2010

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.

8A (4 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").



Late blastula (external view)



Late neurula (internal view)

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

6C (3 pts). Describe the morphogenetic activity of the head organizer during gastrulation (mentioning the kind of cell locomotion, the surface on which they move, and the shape of the cell population before and after movement):

Name: _____

Question 6 continued

6D (3 pts). Describe briefly the morphogenetic activity of the trunk-tail organizer during gastrulation (mentioning the initial cell shape, the kind of cell locomotion, the role of the boundary with somite cells, and the shape of the cell population before and after movement):

Question 7 (3 pts). The organizer secretes inducers as it moves. For each of the inducers below, indicate whether it is secreted by the head organizer, or by the trunk-tail organizer, or by both, by writing the particular letter in one of the blanks below:

a. Chordin, a Bmp antagonist
b. Noggin, a Bmp antagonist
c. Follistatin, a Bmp antagonist
d. Frzb, a Wntantagonist
e. Dickkopf (Dkk), a wnt antagonist
f. Crescent, a Wnt antagonist

Head organizer:_____

Trunk-tail organizer: _____

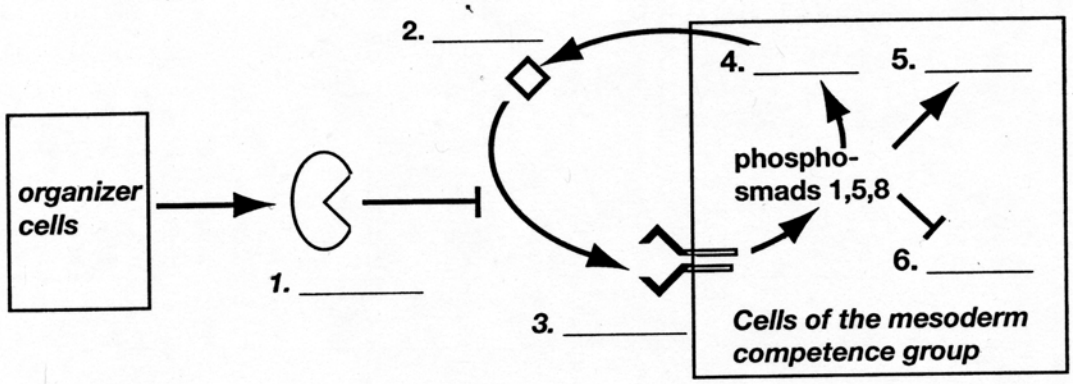
Both: _____

Name: _____

Question 8: (13 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 protein
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. the Frizzled Wnt receptor
G. Bmp antagonists such as chordin, noggin, and follistatin
H. The neural development option
I. The somite development option
J. The epidermal development option
K. The lateral plate/coelom option
L. Bmp gene expression



7B (2 pts). Introduce the mRNA for a dominant negative form of Component 3 into mesodermal cells and introduce antisense morpholinos to deplete Component(s) 1. Predict the developmental outcome, and explain your prediction briefly.

7C (2 pts). Introduce the mRNA for a constitutively active form of Component 3 into the mesodermal cells and introduce excess mRNAs to increase the amount of Component(s) 1. Predict the developmental outcome, and explain your prediction briefly.

Name: _____

Question 7 con't:

7D (3 pts) In both dorsalization of the mesoderm and neural induction of the ectoderm, the cells produce and receive the same kind of Bmp signals and are exposed to the same kinds of organizer inducers. Nonetheless, the ectoderm cells produce neural tissue and the mesoderm produces somites. Explain this difference, and trace itheir divergence in behavior back to an earlier stage when competence groups were not yet different.

Question 9: (6 points)

Consider cells that have developed to posterior neural tissue (hindbrain and spinal cord) in a *Xenopus* embryo.

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

a____ b____ c____ d____ e____ f____, then becomes posterior 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, and Derriere (Nodal signals).
b2. did not receive Xnr1,2,4, 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, follistatin, and noggin
d2. were too far from the organizer to be exposed to Bmp antagonists such as chordin, follistatin, and noggin.

e1. were close enough to developing somites to receive Wnt signals.
e2. were too far from developing somites to receive Wnt signals.

f1. were close enough to the head organizer to be exposed to Wnt antagonists.
f2. were too far from the head organizer to be exposed to Wnt antagonists.

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***Question 10* (5 points).** Next to the following statements, put numbers 1,2,3,4, or 5 to indicate the correct order in which the developmental steps occur, leading to the formation of the full length primitive streak at one place in the chick epiblast (1=earliest step; 2=next step, etc.):

_____ Endoblast cells, which do not secrete Cerberus protein, begin to migrate from the posterior marginal zone and displace hypoblast cells from the epiblast undersurface.

_____ Epiblast cells near the posterior marginal zone receive Vg1 protein (and some Nodal protein) and begin to express nodal genes at high levels, to engage in Nodal signaling, and to undergo endomesoderm induction.

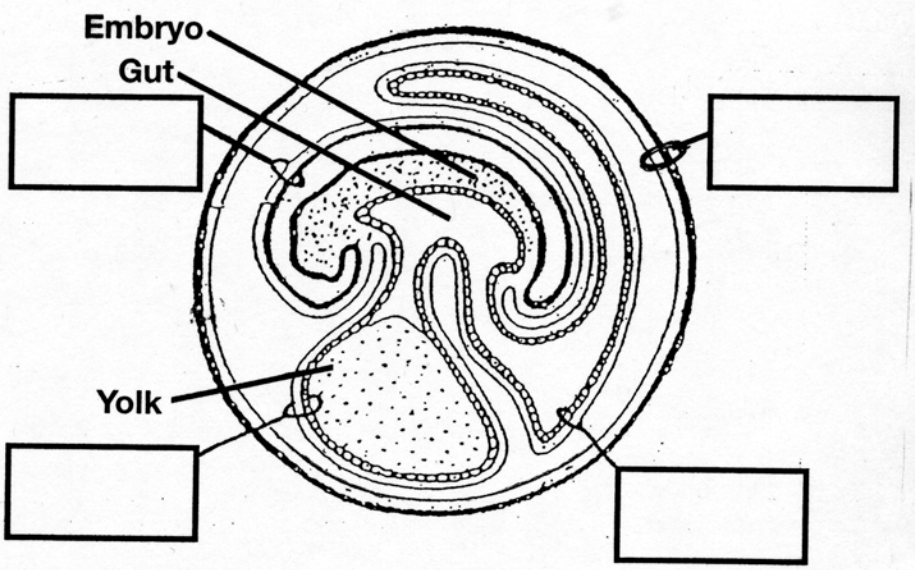
_____ Vg1 gene is expressed in the uppermost sector of the marginal zone of the blastodisc as the egg is tipped obliquely in the oviduct.

_____ Cells are shed from the underside of the cleaving blastodisc and gradually adhere to underside of the epiblast, forming the hypoblast layer that secretes Cerberus protein onto the epiblast.

_____ Induced cells near the posterior marginal zone move toward the center of the epiblast and form Hensen's node, as cells from both sides move toward the streak, get induced by Nodal signals, and join the streak as it extends.

Question 11 (4 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. chorion
c. amnion
d. allantois
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
k. contains ectoderm that passed through the primitive streak



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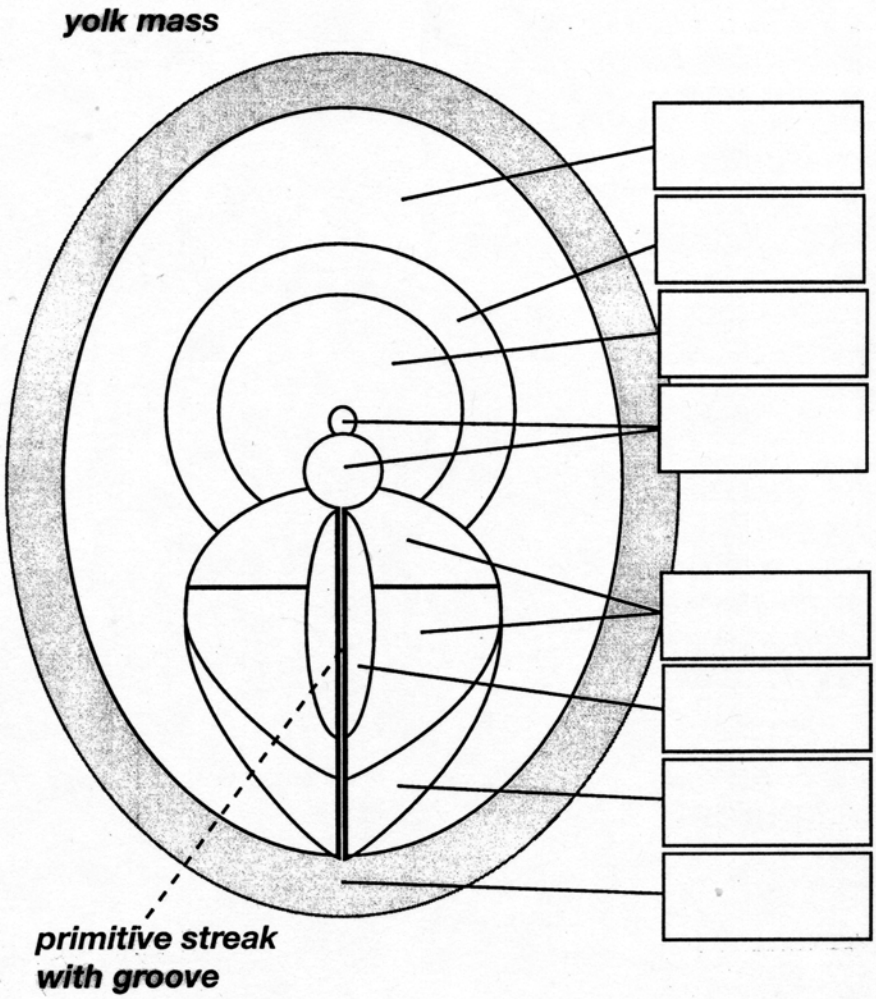
***Question 12* (8 points)**: Below is shown a chick blastodisc at the time of "maximum streak extension", when the primitive streak is longest and gastrulation is just beginning. A fate map if drawn on the surface of the blastodisc (from the results of fate mapping). Fill in the eight boxes with the letters from the list (a letter may be in more than one box) that best identify each region and its activities in gastrulation and subsequent development.

a. A region still underlain by hypoblast
b. Prospective notochord and head mesoderm
c. Prospective somites and lateral place/coelom
d. Prospective extraembryonic mesoderm
e. Prospective embryonic gut endoderm
f. Prospective neural ectoderm
g. Will invaginate through the primitive streak first and merge into the endoblast layer
h. Will invaginate through the streak second and form a middle tissue layer
i. Site of the posterior marginal zone (PMZ)
j. Prospective embryonic epidermis
k. Prospective extraembryonic ectoderm
l. Part of it will later engage in node regression
m. The last mesoderm to pass through the streak, after all other regions have ceased
n. Will not pass through the streak
o. At the time shown, has not yet undergone endomesoderm induction, but will do so later
p. Part of it will ingress and come to underlie the anterior neural plate
q. Will become part of the amnion after gastrulation



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Question 13 (5 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
M. will actively invade the uterine wall tissue