Name: _____

MCB 141 **Midterm 2** **March 22, 2012**

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

Question	Points	Score
1.	4 (Fate map)	_____
2.	5 (General)	_____
3.	8 (Cortical rotation)	_____
4.	7 (Wnt pathway)	_____
5.	12 (3 kinds of evidence)	_____
6.	6 (endomesoderm induction)	_____
7.	5 (organizer formation)	_____
8.	15 (gastrulation, morphogenesis)	_____
9.	12 (default model)	_____
10.	6 (posteriorization)	_____
11.	8 (chickendoblast/hypoblast)	_____
12.	8 (chick gastrulation/fate map)	_____
13.	4 (chick extraembryonic tissues)	_____
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: _____

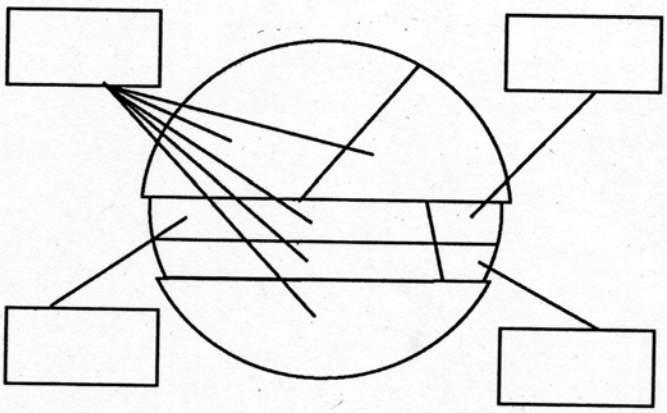
Question 1 (4 points)
The developmental fates of early gastrula cells depend on:
1) their membership in a particular competence group of the early gastrula, and
2) their distance from the organizer.

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

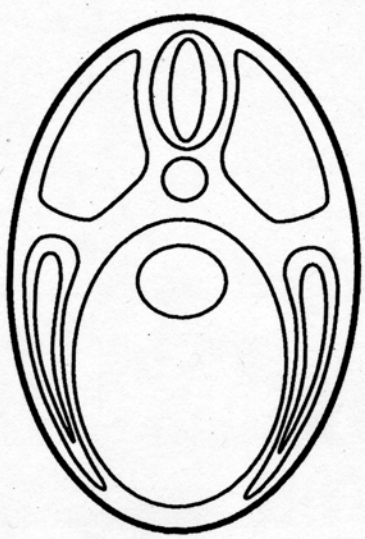
Territory of the Fate Map	Cells are exposed to Nodal signals (write yes or no in each box)	Cells are exposed to Bmp antagonists (write yes or no in each box)
Neural		
Epidermal		
Somite		
Lateral plate		

Question 2 (5 pts):
Part 2A. At the early gastrula stage, the Xenopus embryo expresses genes for the important signaling proteins listed below. These are used for patterning and morphogenesis at the gastrula/neurula stages. On early gastrula fate map shown to the right, identify the location(s) at which the genes for these signaling proteins are expressed, by writing the appropriate letter in one or more boxs:

A. Chordin, Noggin, Follistatin
B. Dkk, Crescent, Frzb
C. Wnt8 (not wnt11)
D. Bmp2 and 4



Part2B. On the cross section of the trunk of a tailbud stage embryo, shown to the right, indicate with arrows and writing:
i) the inducing and responding tissues involved in **neural induction**,
ii) the inducing and responding tissues involved in **dorsalization of the mesoderm** (somite induction), and
iii) the inducing and responding tissues involved in the **posteriorization of neural tissue**.



Name: _____

Question 3 (8 points):
Cortical rotation is required for organizer formation in the Xenopus embryo:

i. Which maternal components are moved during cortical rotation?

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

iii. Briefly describe the **microtubule array** along which they are moved, as follows:
a) its location in the egg

b) the alignment of microtubules

c) the direction the microtubules point on the grey crescent side (i.e. plus ends toward the animal or vegetal pole)

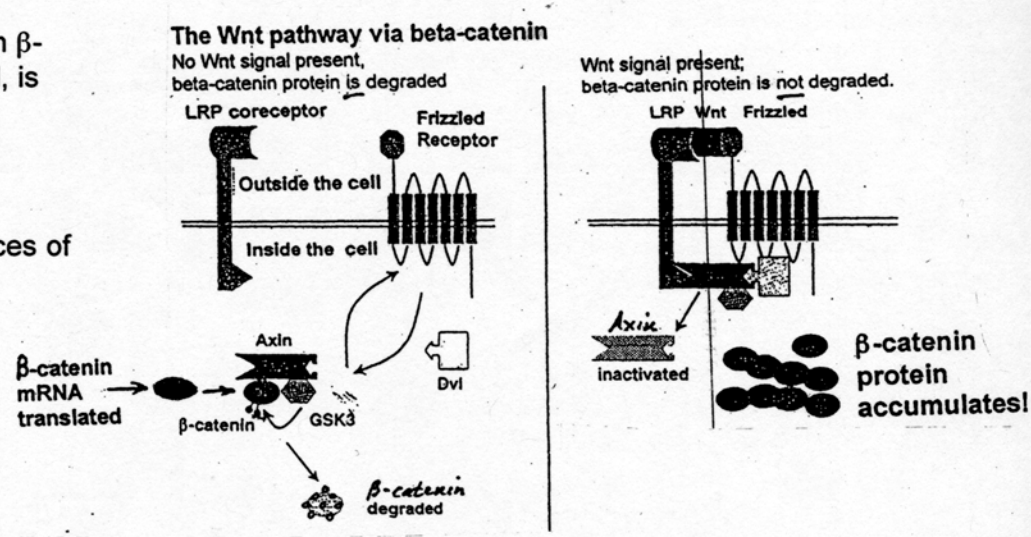
d) the direction in which kinesin motor proteins move as they tow their cargo (mRNAs, vesicles, or cortex).

iv. When microtubule polymerization is blocked in a Xenopus egg (e.g. by inhibitors, low temperature, or high pressure), cortical rotation does not occur, the organizer does not form, and a ventralized embryo eventually develops. Suggest **two experimental interventions** (done at any stage up to mid-gastrula) by which you could rescue such an embryo to develop normally.

Name: _____

Question 4 (7 points)

The Wnt pathway, by which β-catenin protein is stabilized, is shown to the right. Its importance for organizer formation has been demonstrated by the developmental consequences of "knocking down" (reducing, eliminating) and over-expressing individual components of the pathway.



For each of the following Wnt-pathway components, fill in the boxes to predict the effect of the knockdown or over-expression of the particular component on the embryo's development:

Component affected by knockdown or over-expression in the oocyte or egg	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, multiple, or larger? (write one per box)	Eventual phenotype is: ventralized, dorsalized, twinned, or normal) (write one per box)
1.Maternal β-catenin mRNA is depleted			
2. Maternal axin mRNA is depleted			
3. Maternal GSK3 mRNA is depleted			
4. Maternal mRNA for the Frizzled receptor or LRP5/6 co-receptor is depleted			
5. The fertilized egg is soaked in LiCl, a GSK3 inhibitor.			
6. At the 4 cell stage, wnt11 mRNA is injected into the ventral blastomeres			
7. At the 4 cell stage, axin mRNA is injected into the dorsal blastomeres. **			

**Explain your answer to item 7 in this space, if you think it needs an explanation:

Name: _____

Question 5 (12 points)

Various kinds of experimental evidence, when taken together, implicate **vegT mRNA** as a key maternal localized factor for endomesoderm formation in *Xenopus*. Provide that evidence in the following 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 (you may want to use a Nieuwkoop-type experiment for this section):

D. An experiment and its result demonstrating that animal cap cells directly respond to Nodal proteins when they undergo endomesoderm induction (as opposed to their responding to some unknown protein that the VegT transcription factor might stimulate to form)?

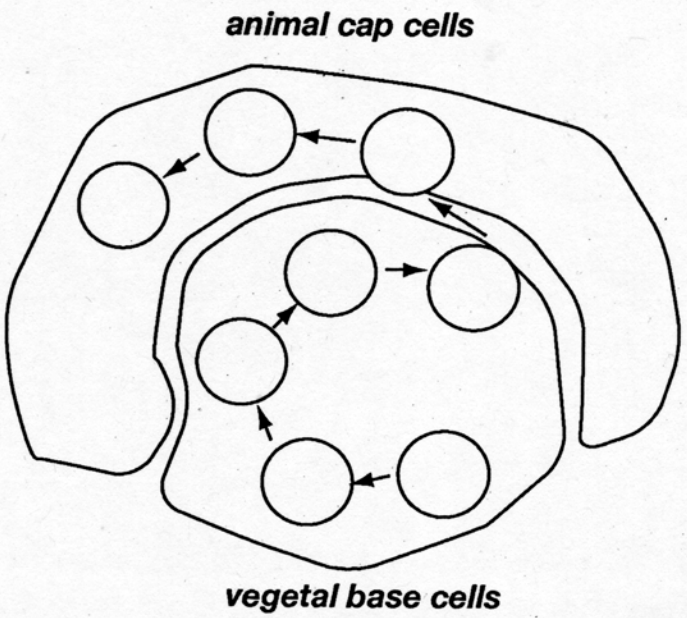
Name: _____

Question 6 (6 points):
Pieter Nieuwkoop combined animal cap cells of the midblastula *Xenopus* embryo with either vegetal cells of the same age. He discovered that after 2 days of culturing, the recombinate had formed an abundance of endo-mesoderm whereas the two pieces, if cultured separately, did not form endomesoderm.

6A (2 pts). How would you determine that an induction took place between the animal cap cells and vegetal base cells?

6B. (4 pts) Write the most appropriate letter from the list below into each circle (one letter per circle) to indicate in which piece and in which order the steps of endo-mesoderm induction occur within the recombinate (so your letters indicate the correct sequence of steps for the induction):

A. Receptors for Xnr1,2,4,5,6 and Derriere proteins are present here.
B. The transcription of *xnr1,2,4,5,6* and *derriere* genes is activated 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. Smad2/3 proteins of the Nodal signal transduction pathway are phosphorylated and activated here.
F. *VegT* mRNA, which was deposited during oogenesis, is present here.
G. Genes of mesoderm development, such as *brachyury* and *wnt8* are transcribed here.
H. *VegT* mRNA is translated to VegT protein here.



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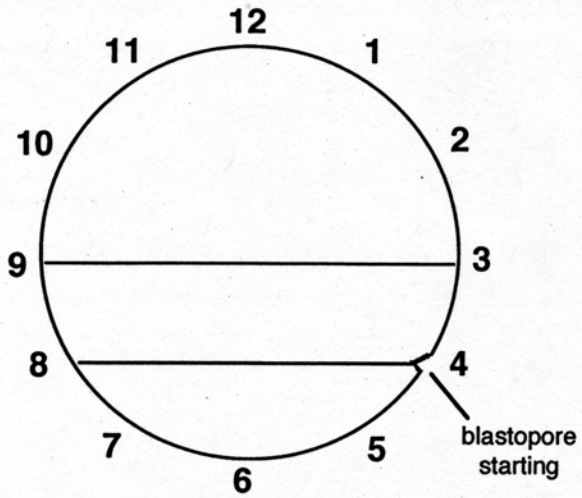
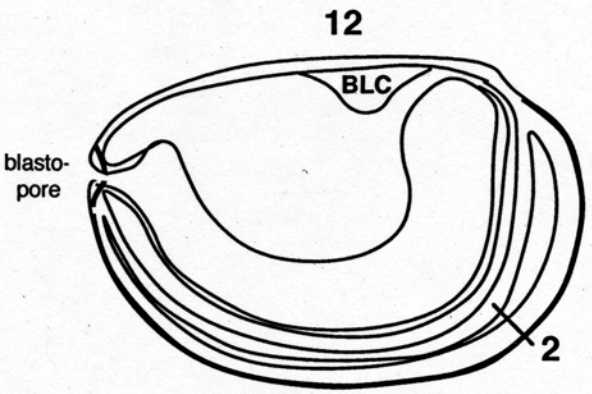
7. Question 6 (5 pts):
As you know, the organizer of the *Xenopus* early gastrula is formed by cells that receive two kinds of signals, namely, 1) from the vegTmRNA-Nodal pathway and 2) from the wnt11mRNA-beta-catenin pathway. Describe briefly the activating and derepressive effects of the wnt11-beta-catenin pathway on organizer formation (words that may be useful to include--Tcf transcription factor, Siamois/Twin transcription factors, Iroquois transcription factor, Bmp levels, Bmp antagonists such as Xnr3 and Chordin, pSmad2 synergism, Nodal levels).

Name: _____

Question 8 (15 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.
8A (3 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)

8B (6 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 notochord	h. the trunk-tail organizer
b. the dorsal side	i. anterior endomesoderm (AEM)
c. the archenteron	j. approximate site of the mouth
d. the approximate site of the heart	l. the site(s) of respread bottle cells
e. the prechordal plate (head mesoderm)	j. cells secreting Noggin, Chordin, and Follistatin
f. the head organizer	k. the location of forebrain and midbrain

8C (2 pts). Describe the morphogenetic activity of the head organizer during gastrulation (mentioning the kind of cell locomotion, the surface on which the cells move, and the shape of the cell population before and after movement):

Name: _____

Question 6 continued

8D (2 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):

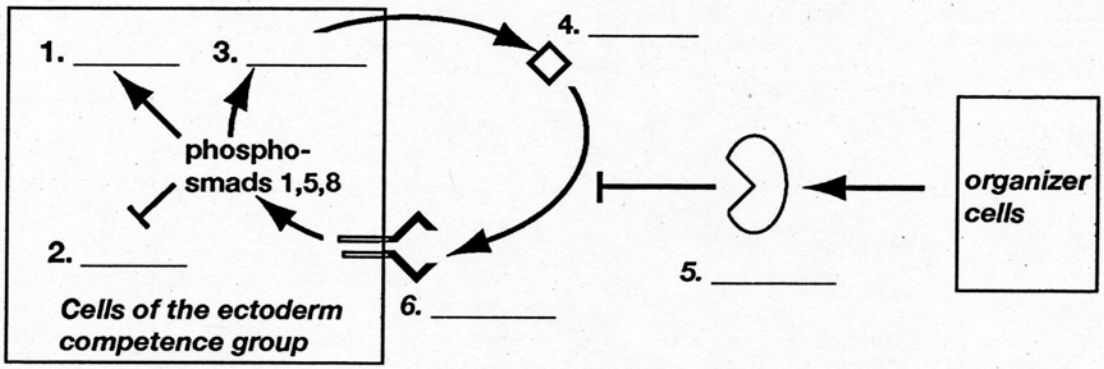
8E (2 pts). Describe briefly the morphogenetic activities of the prospective gut wall endoderm cells at the gastrula stage as they form bottle cells, undergo invagination, and direct involution of the endomesoderm (mentioning initial and final cell shapes, cell movements, and invasiveness):

Name: _____

Question 9: (12 points)

9A. (6 pts) Below is an incomplete diagram of the circuitry of neural induction 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	G. The neural development option
B. The lateral plate/coelom option	H. Bmp antagonists such as Chordin, Noggin, and Follistatin
C. The Bmp receptor	I. The receptor of Bmp antagonists
D. Nodal protein (xnr1,2,4,5,6, derriere)	J. The somite development option
E. The epidermal development option	K. Bmp gene expression
F. Wnt antagonists such as Dkk, Frzb, and Crescent.	L. The Nodal receptor



9B. ***(3pts)*** Introduce into the fertilized egg an mRNA for a truncated type II subunit of component 6, one that is unable to phosphorylate and activate the type I subunit. Predict <u>epidermis or neural tissue</u> as the outcome of the embryo developing from that egg and briefly explain your prediction in terms of the default model shown above

9C. ***(3pts)***. Introduce into the fertilized egg an antisense morpholino(s) that blocks translation of the mRNA(s) of component 5. Predict <u>epidermis or neural tissue</u> as the developmental outcome of that egg and briefly explain your prediction in terms of the default model shown above.

Name: _____

Question 10: (6 points)

Consider cells that develop to <u>posterior neural tissue</u> (hindbrain and spinal cord) in the *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 (not containing *vegT* mRNA)
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.

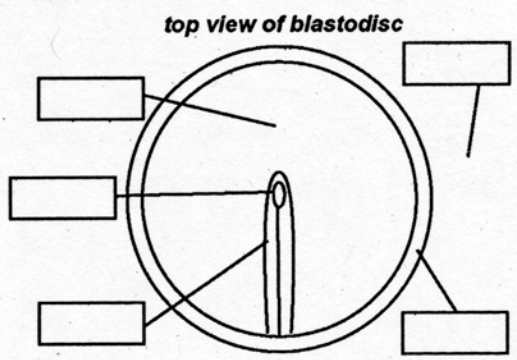
e1. were close enough to developing somites to receive Wnt signals.
e2. were too far from developing somites to receive Wnt signals.

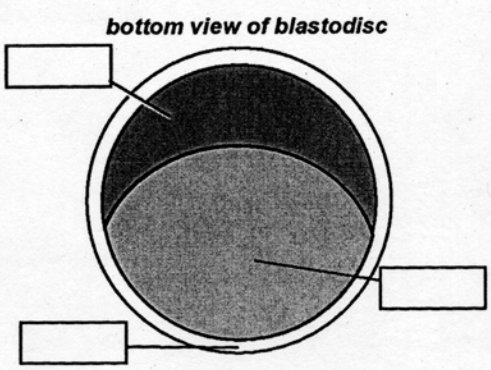
f1. were close enough to the head organizer to be exposed to Wnt antagonists.
f2. were too far from the head organizer to be exposed to Wnt antagonists.

***Question 11* (8 points).**

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. Primitive streak
B. Hensen's node
C. Vg1 protein first produced here
D. Epiblast
E. Posterior marginal zone (PMZ)
F. Endoblast
G. Marginal zone
H. Hypoblast
I. Cerberus-producing cells
J. Endo-mesoderm cells ingress here.
K. Become extra-embryonic endoderm.
L. Uncleaved yolk mass.
M. Nodal producing cells
N. Was uppermost when the blastodisc was tipped to one side in the oviduct.
O. The site from which endoblast cells migrated under the epiblast.
P. Cells of the primitive streak that first underwent endomesoderm induction.



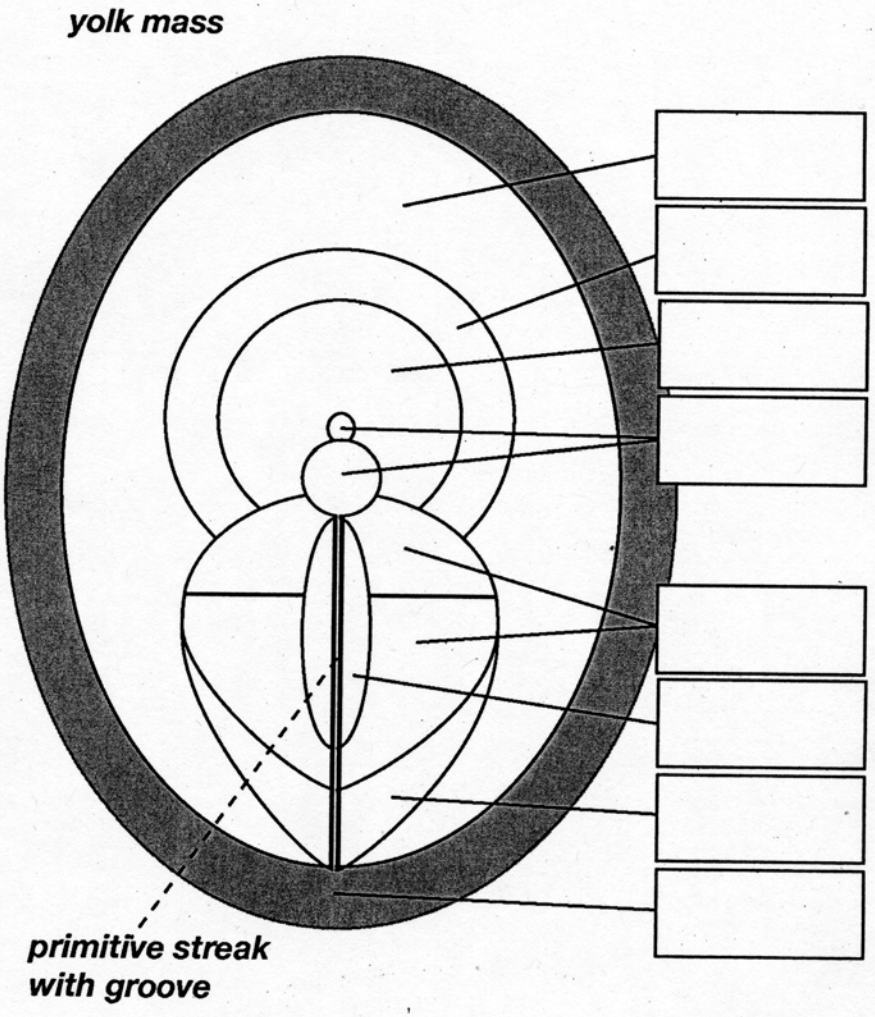


Name: _____

Question 12* (8 points): *2011

Below is shown a chick blastodisc at the time of "maximum streak extension", when gastrulation is just beginning. A fate map is drawn on the surface of the blastodisc (from the results of dye marking experiments). Fill in the eight boxes with the letters from the list (a letter may be used in more than one box) that best identify each region and its activities in gastrulation and subsequent development.

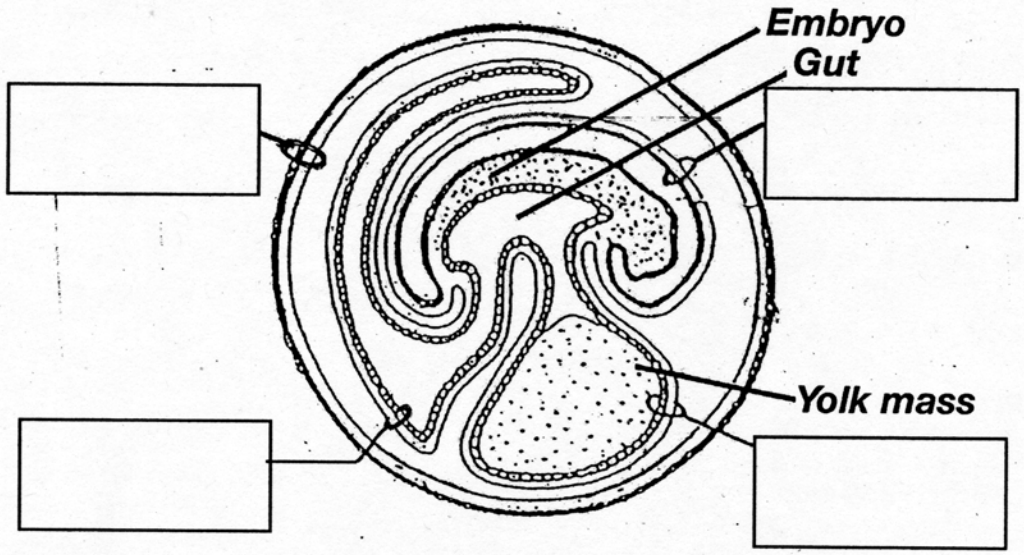
a. Prospective notochord and head mesoderm
b. A region still underlain by hypoblast
c. Prospective somites and lateral plate/coelom
d. Prospective extraembryonic mesoderm
e. Prospective neural ectoderm
f. Prospective embryonic gut endoderm
g. Will invaginate through the primitive streak first and merge into the endoblast/hypoblast layer
h. Will invaginate through the streak second and form a middle (mesodermal) tissue layer
i. Prospective embryonic epidermis
j. Site of the posterior marginal zone (PMZ)
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 endo-mesoderm 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
r. Will not undergo endo-mesoderm induction.
s. Will release neural inducers.



Name: _____

Question 13 (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. Some letters may not be appropriate to use. (Note: the orientation of the figure has been changed this year! Anterior is to the right.)



a. amnion
b. chorion
c. allantois
d. yolk sac
e. a lining composed of ectoderm and somatic (or parietal) mesoderm.
f. a lining composed of endoderm and splanchnic (or visceral) 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 ectoderm that passed through the primitive streak.
k. contains endoderm derived from the hypoblast and endoblast.
l. contains mesoderm that passed through the primitive streak.