Name: Key

MCB 141 **Midterm 2** **April 4, 2013**

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

Question	Points	Score
1.	8 (Cortical rotation)	
2.	12 (3 kinds of evidence)	
3.	5 (Wnt pathway)	
4.	10 (endomesoderm induction)	
5.	6 (organizer formation)	
~~6.~~	16 (fate maps, gastrulation)	
7.	7 morphogenesis	
8.	13 (default model)	
9.	4 (posteriorization)	
10.	5 (chickendoblast/hypoblast)	
11.	6 (chick extraembryonic tissues)	
12.	8 (chick gastrulation/fate map)	
13.		
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: 12

Name: Key

Question 1 (8 points):
Cortical rotation is required for organizer formation in the Xenopus embryo:

(1) i. Which maternal components are moved during cortical rotation?
Wnt11 mRNA (OK to say "and protein")
Vg1 mRNA (OK to say "and protein")

(1) ii. From where, and to where, are they moved?
From the vegetal pole region to one side of the egg (the grey crescent side, the dorsal side), a displacement of 30-120 degrees

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

At the interface of the cortex and cytoplasmic core, or just under the cortex

(1) - b) the alignment of microtubules

Parallel array, planar (thin sheet)

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

Plus ends toward the animal pole

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

Toward the plus ends, i.e., up on the dorsal side

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.

(2) **Several possibilities:**
1. **Before first cleavage, tip egg off axis to force artificial rotation of the core by 90 degrees.**
2. **At the 4 cell stage, inject wnt11 mRNA into a blastomere or two, at the equatorial level.**
3. **At the early gastrula stage, graft in an organizer from a normal embryo.**

Name: Key

Question 2 (12 points)

Various kinds of experimental evidence, when taken together, implicate ***wnt11* mRNA** as a key maternal localized factor for organizer formation in *Xenopus*. Provide that evidence in the following sections:

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

Do in situ hybridization, showing that wnt11 mRNA is present in fertilized egg and embryo at times from fertilization through the mid- and late blastula stages, that is, before and during the time of organizer formation,
and is present on the prospective dorsal side, that is, in the place of organizer formation.

B. A depletion experiment (also called a "loss of function" experiment) and its result.

Inject anti-sense deoxyoligonuucleotide to wnt11 mRNA into the oocyte, leading to RNAseH degradation of the mRNA. Mature the oocyte, fertilize the egg, let it develop. A ventralized embryo is produced—with no organizer and no-organizer induced tissues such as dorsal hollow nerve cord, somites, heart, and no organizer-derived tissues such as the notochord.

(OK to suggest the use of anti-sense morpholinos injected in the fertilized egg to block wnt11 mRNA translation)

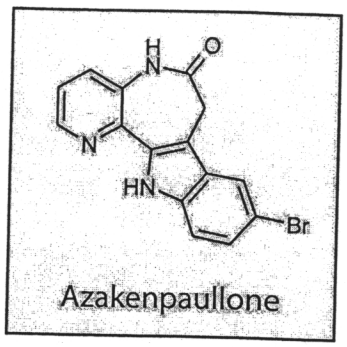
C. An ectopic expression experiment (also called a "gain of function" experiment), and its result:

Inject wnt11 mRNA in the ventral equatorial region of a fertilized egg or 2- or 4-cell stage embryo, and let develop. A twin is produced with a secondary

OK to inject in the animal pole and get an expanded organizer leading to dorsalized embryo.

Name: Key

Question 3. (5 points) Pharmaceutical chemists have recently synthesized azakenpaullone (structure to the right), a strong and specific inhibitor of GSK3, the kinase component of the Wnt pathway that phosphorylates beta-catenin protein. The agent penetrates cells quickly, imposing inhibition, and can be washed out quickly to relieve inhibition.



Azakenpaullone

A *Xenopus* embryo is exposed to azakenpaullone (dissolved in the pond water) in the period from the 2-cell stage until the late blastula stage, at which time the inhibitor is washed out.

A. during the exposure, the level of beta-catenin protein in this embryo does what? Circle one—increases decreases stays the same. Explain your answer briefly

When GSK3 is inhibited, beta-catenin protein is not phosphorylated and doesn't get degraded. It accumulates throughout the embryo.

B. Predict the location and size of the organizer formed in the subsequent gastrula, and of the eventual phenotype of the hatching embryo. Briefly explain your predictions.

The organizer forms around the entire equatorial zone where beta-catenin-Tcf and Nodal signaling intersect (i.e. not at animal pole). It's much larger, and non-organizer mesoderm is reduced or gone. The hatching embryo is dorsalized (also called dorsalized-anteriorized) with notochord, heart, neural tissue, eye-pigment cells, sucker.
Posterior-ventral parts are reduced.

OK to suggest twinning.

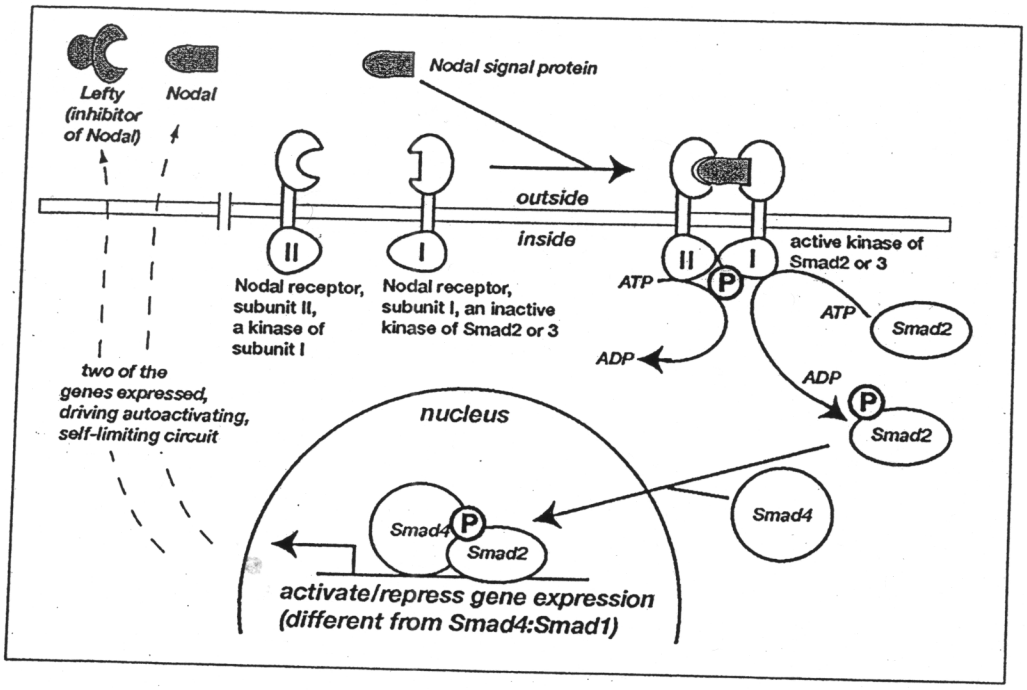
Question 4 (10 points):
Pieter Nieuwkoop combined animal cap cells of the midblastula *Xenopus* embryo with vegetal cells of the same age. He discovered that after 2 days of culturing, the recombinate had formed abundant endomesoderm whereas the two pieces, if cultured separately, did not form endomesoderm.

4A (2 pts). How would you determine that <u>an induction</u> took place between the animal cap cells and vegetal base cells?

Label an egg with a fluorescent dextran lineage tracer (OK if they say "vital dye"), remove the cap at the midblastula stage. Combine it with an unlabeled vegetal base, and show that all the endomesoderm formed by induction is derived from fluorescent animal cap cells. The control is to show that the base is needed for the animal cap response, that is, if the base is omitted, the cap doesn't form endomesoderm.

Name: Key

4B. (8 pts) The Nodal signaling pathway is shown below.



In the Table, predict the effect of each treatment on the formation of endomesoderm by the animal cap of the recombinate.

Treatment done to the animal cap or vegetal base before preparing the recombinate:	Endomesoderm formation in the animal cap cells of the recombinate**? Write "yes" or "no" in each box.
A. The vegetal base comes from a fertilized egg depleted for vegTmRNA	no
B. The vegetal base comes from a fertilized egg depleted for vegTmRNA and injected with mRNAs encoding Nodal proteins Xnr1,2,4,5,6, derriere	yes
C. The vegetal base comes from a fertilized egg depleted for the maternal mRNA encoding wnt11.	yes
D. The animal cap comes from a fertilized egg injected with mRNA encoding a truncated (kinase deficient) Nodal Receptor 1 subunit	no
E. The animal cap comes from a fertilized egg injected with high levels of the mRNA encoding the Lefty protein	no
F. The animal cap comes from a fertilized egg injected with anti-sense morpholinos to the Lefty-encoding mRNA	yes
G. The animal cap comes from a fertilized egg injected with anti-sense morpholinos to mRNA encoding Smad4.	no
H. The animal cap comes from a fertilized egg injected with mRNA encoding Smad7, a protein which prevents Smad2/3 from binding to the kinase region of Nodal Receptor subunit 1	no

**We are not distinguishing the kind of endomesoderm here—any kind is considered a positive inductive response.

Name: Key

Question 5 (6 pts):
As you know, the organizer of the *Xenopus* early gastrula is formed by cells that don't contain *vegT* mRNA but do receive two kinds of signals, namely,
1) signals from the *vegT*mRNA-Nodal pathway and
2) signals from the *wnt11*mRNA-beta-catenin pathway.
The combination of signals leads to higher levels of activation of transcription of pSmad2/3-dependent target genes on the dorsal side, compared to the lateral and ventral regions of endomesoderm.

(1) What is the contribution of each of the following toward producing, together, the higher levels of Smad2/3 mediated gene activation on the dorsal side:
A. Vg1 protein
It adds more Nodal-like signal on dorsal side (OK to adds that it forms heterodimers with Nodals and these are more effective signals than are the homodimers).

(2) B. beta-catenin:Tcf derepression of genes and the local inhibition/repression of Bmp signaling
Beta-catenin:Tcf derepresses at least two genes (as beta-catenin displaces Groucho from the repressive Groucho:Tcf):
Siamois TF which then activates the chordin gene, increasing local Bmp antagonist levels.
Iroquois TF which then represses Bmp genes, blocking further Bmp production locally.
Together these make the dorsal side a Bmp-free zone. Since Bmp signaling is repressive to the expression of some organizer genes, this free-zone favors organizer formation.

(2) C. beta-catenin:Tcf repression of the miR15,16 genes

When the miR15,16 genes are repressed, the miR15,16 RNAs don't inhibit the translation of the Nodal Receptor type1 mRNA. Hence more Receptor is formed on the dorsal side. Hence more signal reception.

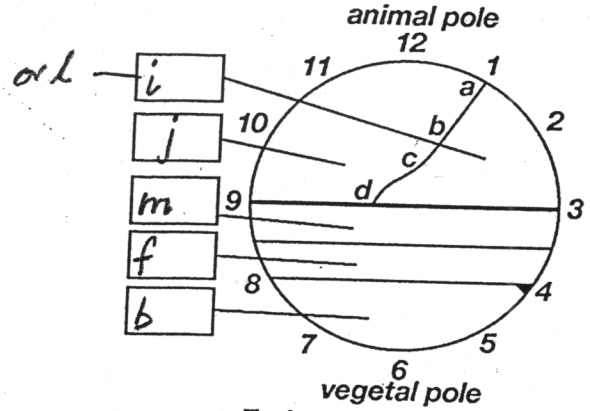
(1) D. beta-catenin:Tcf synergism with pSmad2/3 and Siamois in the activation of genes

Genes that require synergism with pSmad2/3 and beta-catenin or with pSmad2/3 and Siamois (which itself was deprepressed in the beta-catenin:Tcf region) will be expressed at much higher levels on the dorsal side.

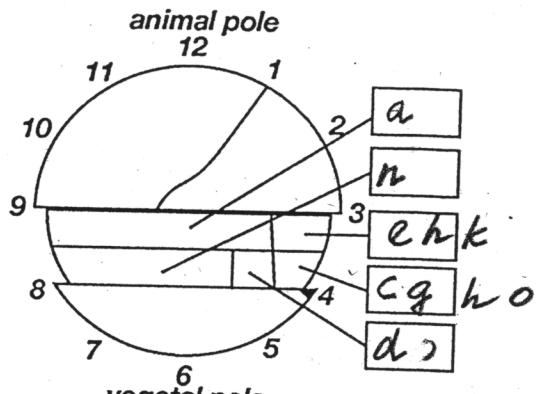
Name: Key

Question 7(16pts)

7A. Below are two diagrams for the fate map of the *Xenopus* early gastrula with regions marked by lines and boxes. ***The clockface numbers and the letters "a,b,c,d" have been added for use in the part B of this Question.*** In each box of part A, put one of more of the letters from the list to identify the fate of the region. Not all letters will be used here in part A.



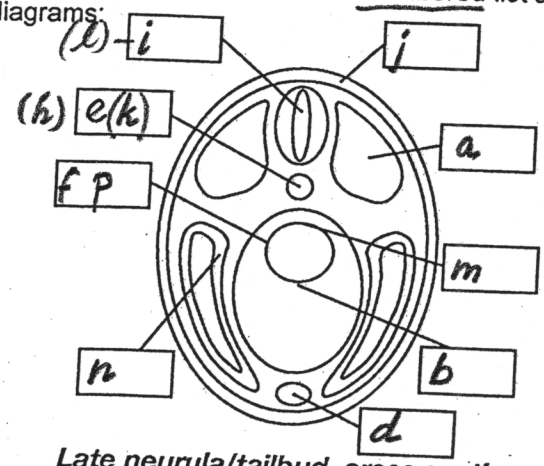
Early gastrula, surface



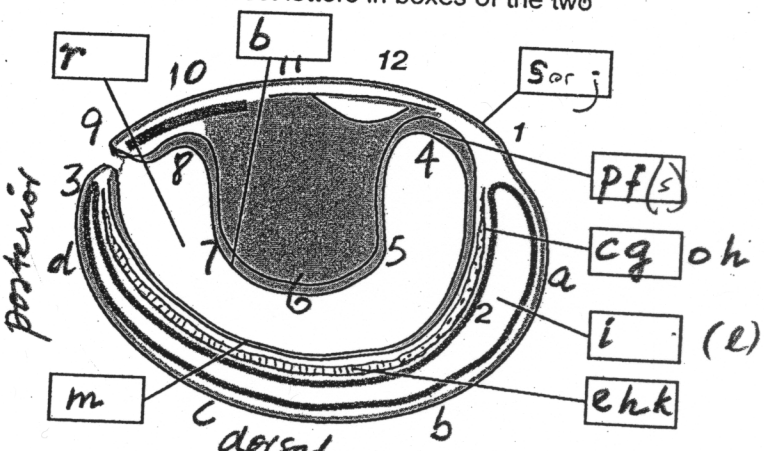
Early gastrula, deep cells

a. somites
b. gut floor
c. prechordal (head) mesoderm
d. heart
e. notochord
f. gut wall
g. the head organizer
h. cells secreting Noggin, Chordin, Follistatin
j. epidermis
k. the trunk-tail organizer
l. (i.) neural tissue, neural plate or tube
m. gut roof
n. lateral plate mesoderm (coelom)
o. cells secreting Dkk, Frzb, Crescent
p. respread bottle cells.
r. archenteron
s. site where the mouth will form

7B. Below are two diagrams of the late neurula/tailbud embryo in cross section (left) and cut sagitally (on the right). Use the numbered list above to write the correct letters in boxes of the two diagrams:



Late neurula/tailbud, cross section



Late neurula/tailbud, sagittal section

7C. On the sagittal section above, right:
Write the clockface numbers of the early gastrulae of part 7A to indicate the final location of those positions by the end of gastrulation and neurulation. Also,
Write "dorsal" to indicate the dorsal side of the sagittal section;
Write "posterior" to indicate the posterior end of the sagittal section;
Write the letters a,b,c,d from Figure 7A left on the sagittal section to indicate the site of closure of the neural tube.

Name: Key

Question 8. (7 pts) Morphogenesis

***8A.* (1 pt)** What pair of numbers in the early gastrula diagrams of Question 7A indicates the "limit of involution"?

9 and 3

8B (2 pts).
Describe briefly 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):

After the head organizer cells involute, they migrate as a group on the blastocoel wall (along fibronectin cables [not required as part of the answer]) oriented toward the animal pole. Initially the cells are packed in a multilayered cube; as they migrate their arrangement thins to one layer and spreads out. Cells are unipolar as they move (each with one tip of motile activity, i.e., one lamellapodium). This morphogenetic activity is called "spreading migration".

8C (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):

After involution, cells of the trunk-tail organizer become spindle-shaped and bipolar (with opposing tips of motile activity, i.e., two lamellapodia), aligned roughly in parallel. Cells at the <u>boundary</u> with somites inactivate the exposed tip and become unipolar in their movement, pulling in from the boundary by way of the inward-facing active tip, <u>intercalating</u> or interdigitating (inserting) between deeper cells. This exposes more cells to the boundary, and more cells become unipolar and move inward. The initial multilayered block of cells narrows and thins as cells intercalate. The population as a whole converges and extends. Eventually all tips are on the surface, no active tip remains, and a rod of cells is formed, one cell wide, all tips at the boundary. This morphogenetic activity is called "convergent extension".

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):

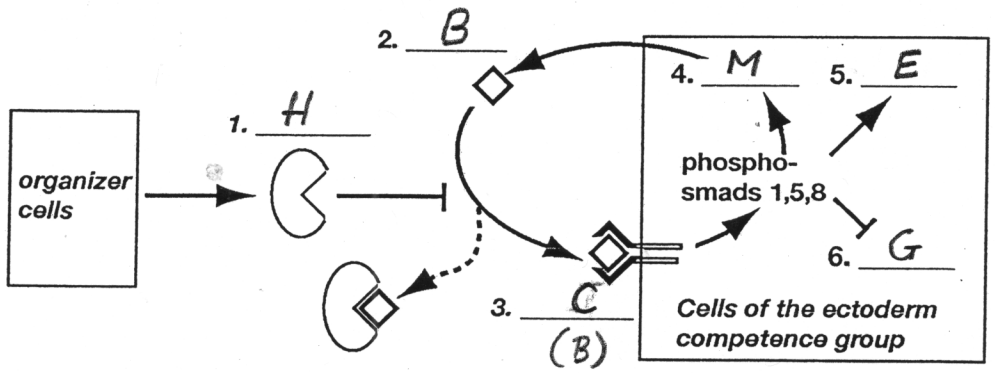
Bottle cells, apical constriction, invagination, involution:
Gut wall endoderm cells undergo apical constriction, reducing their apical surface area greatly and protruding their cell volume inward, each in a bottle shape. They ingress slightly between deeper cells and generate an invagination, a small pocket in the gastrula surface, the first step of blastopore formation. This serves as a barrier to endomesoderm cells moving toward them; the endomesoderm cells are turned in the reverse direction at the barrier. They involute and move toward the animal pole.

Name: Key

Question 9: (13 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. The lateral plate/coelom option	G. The neural development option
B. Bmp protein	H. Bmp antagonists such as Chordin, Noggin, and Follistatin
C. The Bmp receptor	J. The receptor for Bmp antagonists
D. Nodal protein (xnr1,2,4,5,6, derriere)	K. The somite development option
E. The epidermal development option	M. Bmp gene expression
F. Wnt antagonists such as Dkk, Frzb, and Crescent.	N. The Nodal receptor

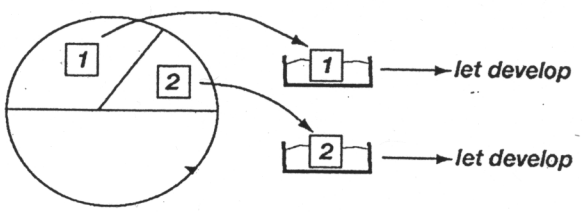


9B. ***(3pts)*** Into a fertilized egg, a mix of anti-sense morpholinos is injected against maternal beta-catenin mRNA and also against Bmp2, Bmp4, and Bmp7. Predict epidermis or neural tissue as the developmental outcome of that egg and briefly explain your prediction in terms of the default model shown above.

Prediction: the ectoderm will undergo neural development.
The anti-sense morpholinos would block organizer formation, and hence the production of Bmp antagonists. This would favor epidermis development. But the anti-sense morpholinos against Bmp2,4,7 will block their formation, and hence the ectoderm can't enforce repression of the neural option. The neural option is derepressed.

9C. Based on the Default Model, explain the century-old results of specification and competence tests done on the amphibian early gastrula:

1) The specification state of early gastrula ectoderm:
Tissue fragments 1 and 2 are removed from the early gastrula and cultured in pond water saline for 3 days.



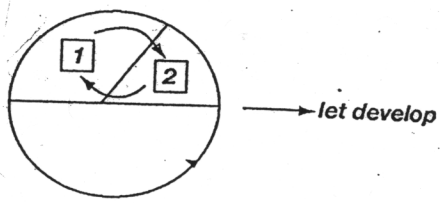
What do fragments 1 and 2 develop into?
Fragment 1 epidermis

Fragment 2 epidermis

Name: Key

(Question 9C continued)
Using the default model, explain why:
At the early gastrula stage, both explants express Bmp genes and receive Bmp, keeping the epidermis option affirmed and the neural option repressed. In the culture dish, no Bmp antagonists are present (no organizer, for example) to block Bmp signaling, so the circuits remain active and the neural option in never derepressed.

2) The competence state of the early gastrula ectoderm; Tissue fragments 1 and 2 (marked with colored vital dyes so their cell can be later recognized) are cut out and moved to replace each other in an early gastrula, which is then allowed to develop for 3 days.



Into what do fragments 1 and 2 develop at their new locations?
Fragment 1____*neural*_____

Fragment 2___*epidermis*_____
Using the default model, explain why:

See part 1) for background on the two fragments as they are removed from the early gastrula. In their new locations, fragment 1 is now close enough to the organizer to receive Bmp antagonists, to disrupt the Bmp circuit, and to derepress the neural option. Fragment 2 is now too far from the organizer to receive Bmp antagonists. It continues to activate the Bmp circuit, to repress the neural option, and to develop as epidermis.

Question 10: (4 points)
Based on your understanding of posterior neural development in *Xenopus*, explain why an injection of *chordin* mRNA into the ventral equatorial region of a 4-cell embryo leads later to the development of a secondary body axis with only a trunk (containing hindbrain and spinal cord) whereas injection of both *chordin* mRNA and *dkk* mRNA into the ventral equatorial region of a 4-cell embryo leads to a secondary body axis with a head (containing forebrain and midbrain).

Chordin mRNA alone injected into the ventral equatorial region: there, Chordin protein blocks Bmp and promotes neutral development in the ectoderm, a development that is initially anterior neural (forebrain/hindbrain). However, in the ventral equator, the somite mesoderm produces wnt8 signal that posteriorizes the neural ectoderm, to get hindbrain/spinal cord in the secondary trunk.

Chordin mRNA and dkk mRNA: See above for a description of the neuralization of ventral ectoderm by Chordin. Now, Dkk protein binds to Wnt8 and blocks it from posteriorizing the neural ectoderm. It contionues to develop as anterior neural tissue (forebrain/midbrain).

Name: Key

***Question 11* (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.):

switched is OK

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

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

switched is OK

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

2 Cells are shed from the underside of the cleaving epiblast and gradually adhere to form the hypoblast layer that secretes Cerberus protein onto the epiblast.

5 Induced cells near the posterior marginal zone move toward the center of the epiblast and form Hensen's node, and later cells become induced and fall in behind them to form the lengthening streak.

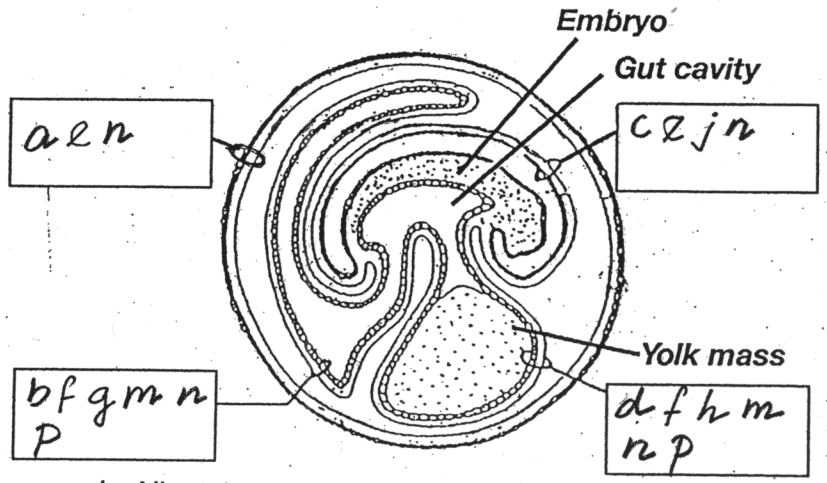
Question 12 (4 points)

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: anterior is to the right.)

-1 for incorrect

-0.5 for missing

TOTAL of 19 pts.



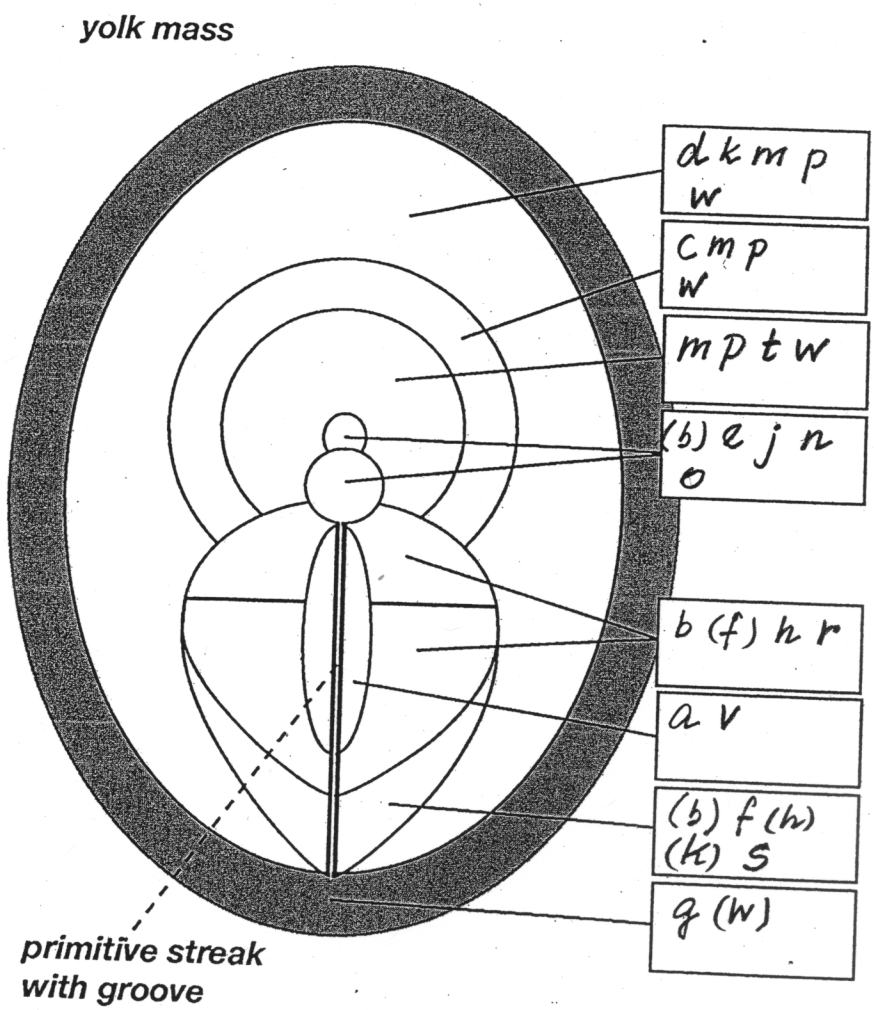
a. Chorion b. Allantois c. Amnion d. Yolk sac
e. a lining composed of ectoderm and somatic (or parietal) mesoderm.
f. a lining composed of endoderm and splanchnic (or visceral) mesoderm.
g. lines a cavity in which metabolic wastes are stored.
h. is involved in mobilizing nutrients for the embryo
j. lines a cavity that surrounds the embryo in a controlled aqueous environment.
no – (k.) contains ectoderm that passed through the primitive streak.
m. contains endoderm derived from the hypoblast and endoblast.
n. contains mesoderm that passed through the primitive streak.
p. the mesodermal layer forms many blood vessels.

Name: Key 33 42 / 75

***Question 13* (8 points):**
Below is shown a chick blastodisc at the time of "maximum streak extension", just as gastrulation is beginning. A fate map is drawn on the surface of the blastodisc (deduced 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. Will invaginate through the primitive streak first and merge into the endoblast/hypoblast layer
b. Will invaginate through the streak second and form a middle (mesodermal) tissue layer
c. Prospective embryonic epidermis
d. Prospective extraembryonic ectoderm
e. Part of it will later engage in node regression
f. The last mesoderm to pass through the streak, after all other regions have ceased
g. Site of the posterior marginal zone (PMZ)
h. At the time shown, has not yet undergone endo-mesoderm induction, but will do so later
j. Part of it will ingress and come to underlie the anterior neural plate
k. Will become part of the amnion after gastrulation
m. Will not undergo endo-mesoderm induction.
n. Will release neural inducers.
o. Prospective notochord and head mesoderm
p. A region still underlain by hypoblast
r. Prospective somites and lateral plate/coelom
s. Prospective extraembryonic mesoderm
t. Prospective neural ectoderm
v. Prospective embryonic gut endoderm
w. Will not pass through the streak

??



-1 for incorrect
-0.5 for missing
Total of 25 pts