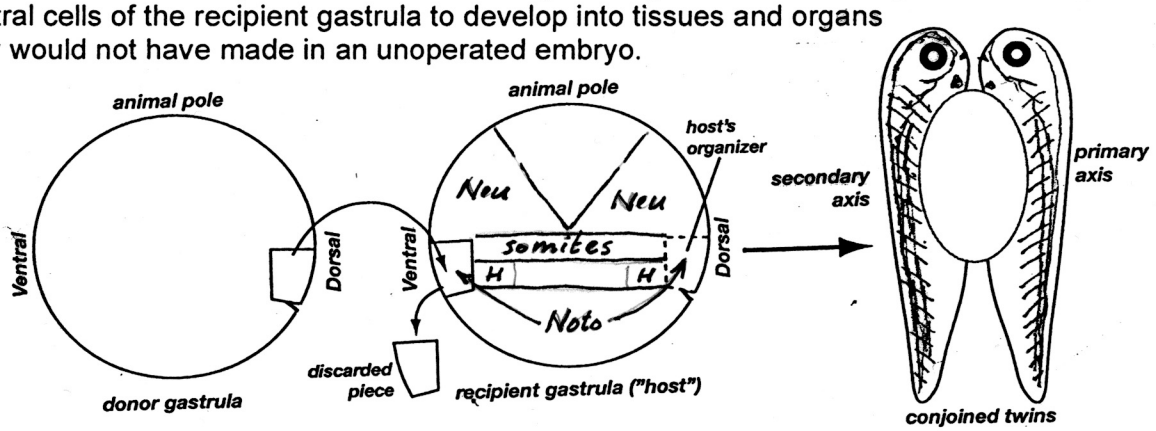
Name: KEY

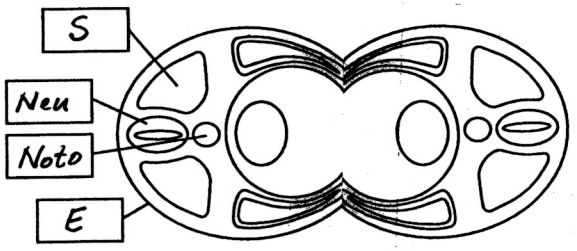
Question 1 (8 points): The Spemann-Mangold experiment of 1924 is diagrammed below. From their results, they concluded that the organizer graft from a donor gastrula induced nearby ventral cells of the recipient gastrula to develop into tissues and organs they would not have made in an unoperated embryo.



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. Self-differentiation of the graft:

i) How could you eliminate the possibility that the graft just self-differentiated into the entire secondary axis?

Prepare an organizer that is labeled with fluorescent lineage tracer by injecting a fertilized egg with such tracer and letting it develop to the early gastrula stage. (Or mark the organizer cells with a vital dye.) Transplant the organizer to the unlabeled host. When the conjoined twin develops, all progeny cells of the labeled organizer will be labeled. When the twin is inspected closely, it is found to contain none or a small fraction of labeled cells in the somites, neural tube, and heart of the secondary axis. Thus, these did not form by self differentiation of the graft.

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

The graft formed the notochord of the secondary axis. (Some may add that the graft also formed a small amount of the somites and of the floor plate of the neural tube.)

Name: __KEY_____

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)

Mark cells of the host's neural territory at the location expected from the fate map, using a vital dye or by injecting fluorescent dextran lineage tracer (some may say at the 8-cell stage, into dorsal animal blastomeres). Then inspect the neural tube of the secondary axis of the conjoined twin to see if it contains labeled cells. It doesn't; neural cells of the host's neural territory didn't migrate over. The secondary neural tube came from cells of non-neural territories of the host.

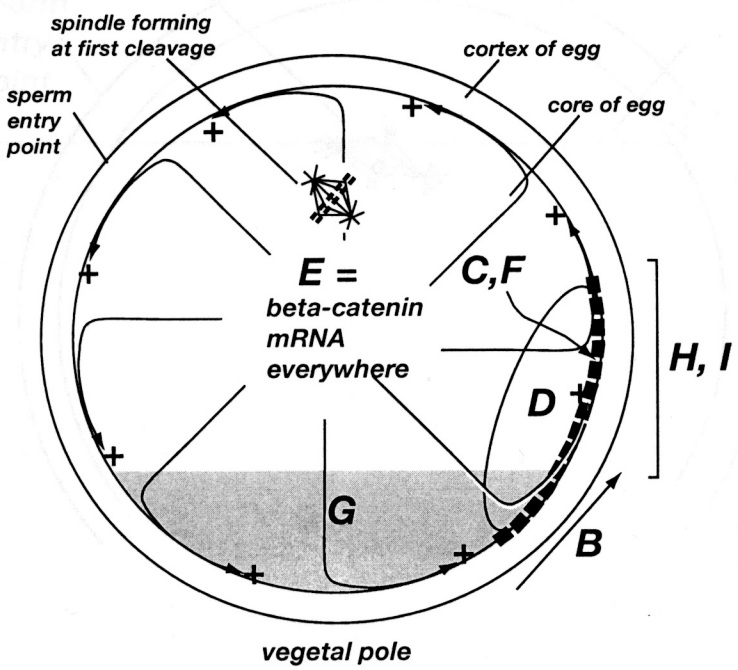
iii) Regarding the cells that formed the nervous system of the secondary axis, what would have been their fate if the grafting had not been done?

Epidermis

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:**

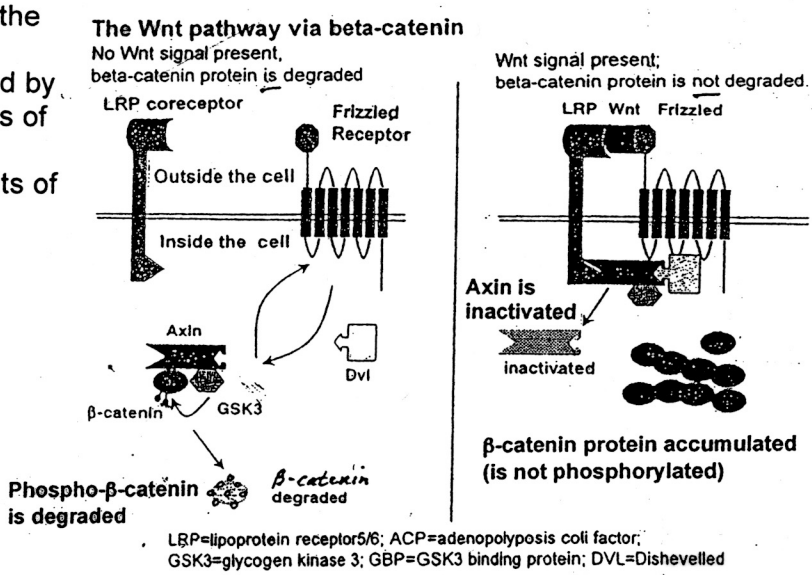
a. the location of the parallel microtubule array during rotation. Add an arrow to indicate the polarity of the microtubules (with the arrow head at the **plus end** of the microtubules).
b. the direction the cortex rotates relative to the core (assuming the core remains unmoved).
c. the location of *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.



Name: KEY

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.



LRP=lipoprotein receptor5/6; ACP=adenopolyposis coli factor; GSK3=glycogen kinase 3; GBP=GSK3 binding protein; DVL=Dishevelled

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	**decrease**	**absent**	**ventralized**
Maternal β-catenin mRNA	**decrease**	**absent**	**ventralized**
Maternal axin mRNA	**increase**	**larger**	**dorsalized (twins = partial)**
Maternal GSK3 mRNA	**increase**	**larger**	**dorsalized (twins = partial)**
Maternal mRNA for the Frizzled receptor or LRP5/6 coreceptor	**decrease**	**absent**	**ventralized**

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: __KEY_____

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:

A. Visualize nodal mRNAs by in situ hybridization and find that they are present at the mid and late blastula stages when EMI is taking place and that they are in vegetal hemisphere cells, which are thought to be the source of inducers (OK to say also that these mRNAs appear in equatorial cells as they respond).
Or visualize Nodal proteins by antibody staining, and find the same time and place—mid to late blastula, vegetal hemisphere (and into equatorial region), where EMI takes place.

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:

B. Use antisense deoxyoligonucleotides or morpholinos to deplete nodal mRNA or block their translation. The result: EMI does not occur (endomesoderm does not form).

The control: rescue the antisense treated embryo with Nodal mRNA having a modified sequence that doesn't hybridize with the antisense agent (or could say, swamp out antisense agent with excess of injected unmodified mRNA.) Result: EMI occurs.

C. An ectopic expression experiment, and its result:

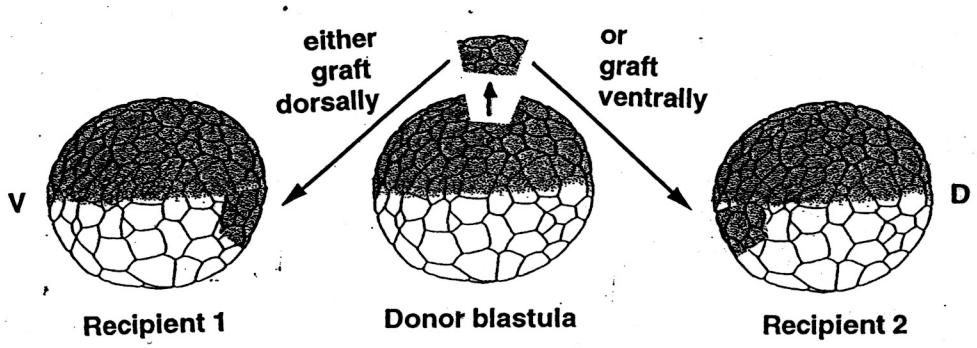
C. Introduce Nodal mRNA into an animal cap (e.g., inject it into a fertilized egg, cut off the animal cap before the mid-blastula stage when EMI starts). Result: the animal cap makes endomesoderm, whereas an uninjected control cap would not.
Or a variation of this: wrap that Nodal secreting cap with a normal cap and find that endomesoderm is induced in the normal cap (a Nieuwkoop recombinate).

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)?

D. Knock down the Nodal receptor or Smad2/3 in the animal cap (by injecting antisense deoxyoligonucleotides or morpholinos into a fertilized egg, and cutting off cap before the midblastula stage). Combine this cap with a normal vegetal piece. Find that EMI does not occur, whereas the base combined with a normal cap does undergo EMI.
Or use a dominant negative nodal receptor in the animal cap to block nodal signaling. Combine with vegetal base piece. Find that EMI fails.

Name: KEY

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?

A. The transplanted animal pole cells develop to mesoderm, namely, somites and lateral plate. (OK to say, develop to gut roof and gut wall endoderm.)

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

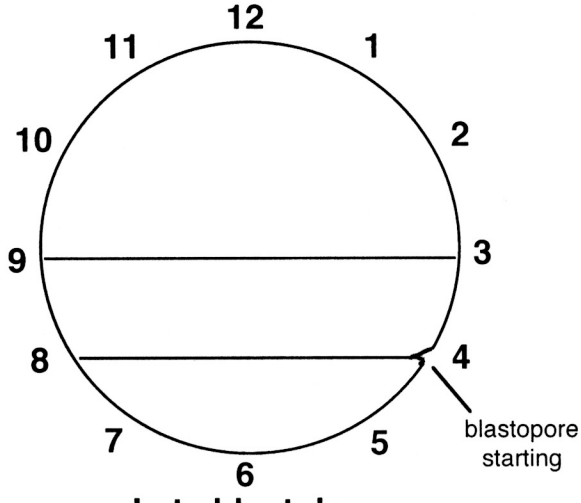
B. The transplanted animal pole cells develop to organizer (dorsal) mesoderm, namely, notochord and head mesoderm. Or can say, they develop to head organizer and trunk-tyail organizer. (OK to say, develop to gut roof and gut wall endoderm and gill slits. Also OK to mention they might develop to heart.)

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

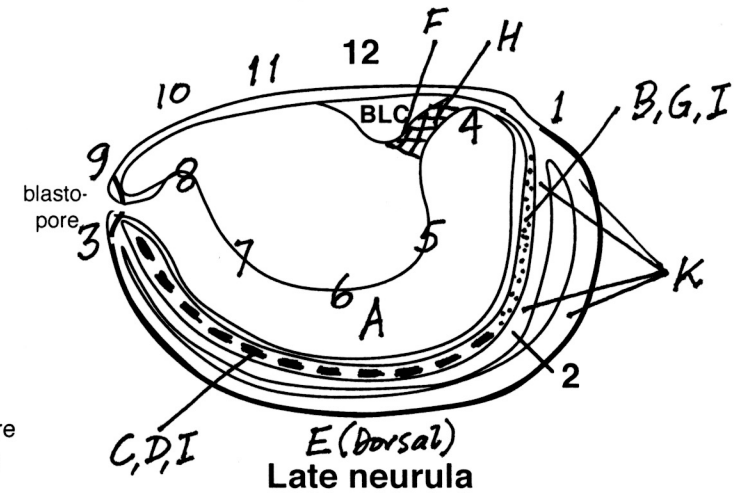
Animal pole cells moved to:	*Receive Vg1 protein (write yes or no in the box)*	*Receive Nodal signals (write more or less in the box)*	*Receive Wnt11protein (write yes or no in the box)*	*Receive Bmp protein (write more or less in the box)*
The ventral side (recipient 2)	*NO*	*LESS*	*NO*	*MORE*
The dorsal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

Name: __KEY_____

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):

C. Spreading migration:
After the head organizer cells involute, they migrate on the blastocoel wall (along fibronectin cables [this is not required as part of the answer]) toward the animal pole. Initially the cells are packed in a multilayered cube; as they migrate the population thins to one layer and the cells spread out. Cells are unipolar as they move (each with one tip of motile activity, i.e., one lamellapodium). (Some may say the cell tips underlap one another, and the cells move as groups.)

Name: __KEY_____

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):

D. Convergent extension:
After involution, cells of the trunk-tail organizer become spindle-shaped and bipolar (with opposing tips of motile activity, i.e., two lamellapodia; some may draw a picture). Cells at the boundary with somites inactivate the exposed tip and become unipolar in their movement. They pull in from the boundary by way of the inward facing active tip, intercalating 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 (some may say 10cells x 10cells x 5 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 one-cell-thick (some may say 500 cells long) is formed, all tips at the boundary.

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:__**D E F**_____

Trunk-tail organizer: _____

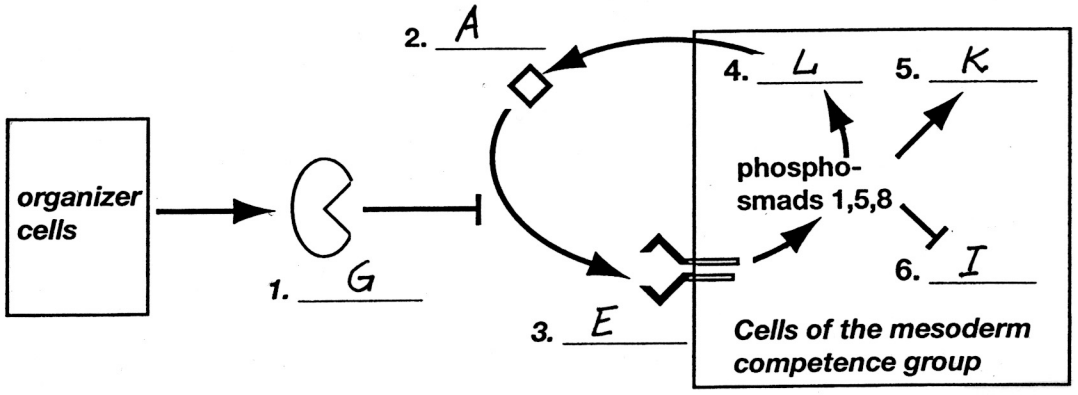
Both: _____**A B C** _____

Name: KEY

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.

7B: Somite. When 3 (Bmp receptor) is a dominant negative form, all signaling is blocked and Smad1/5/8 does not form. Hence, the somite option is derepressed.

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.

7C: Lateral plate/coelom. When 3 (Bmp receptor) is always active, pSmad1/5/8 is always present and active, so the somite option is repressed and the LP/coelom option is activated.

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.

7D: The two competence groups have different developmental options available to them, namely: epidermis/neural for the ectoderm and somite/lateral plate (coelom) for the mesoderm. These options are their possible responses to Bmp signals, including the absence of Bmp due to Bmp antagonists. The different responses were established during Nodal mediated endomesoderm induction in the mid and late blastula stages. The mesoderm competence group received Nodal signals; the ectodermal group did not.

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__**1**_ b_**2**__ c_**2**__ d_**1**__ e_**1**__ f__**2**_, 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.

Name: KEY

***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.):

__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 Nodal protein) and begin to express nodal genes at high levels, to engage in Nodal signaling, and to undergo endomesoderm induction.

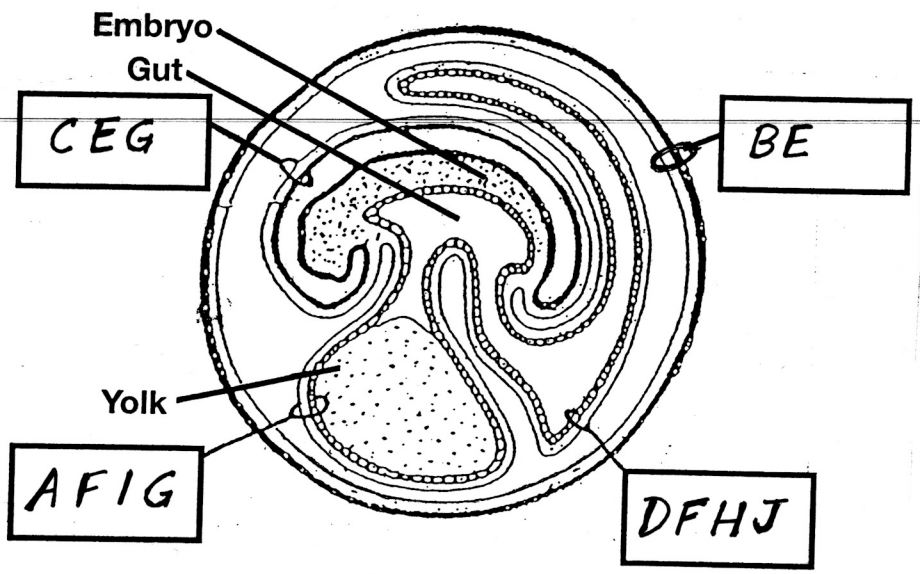
__1__ Vg1 gene is expressed in the uppermost sector of the marginal zone of the blastodisc as the egg is tipped obliquely in the oviduct.

__2__ Cells are shed from the underside of the cleaving blastodisc and gradually adhere to underside of the epiblast, forming 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, 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.

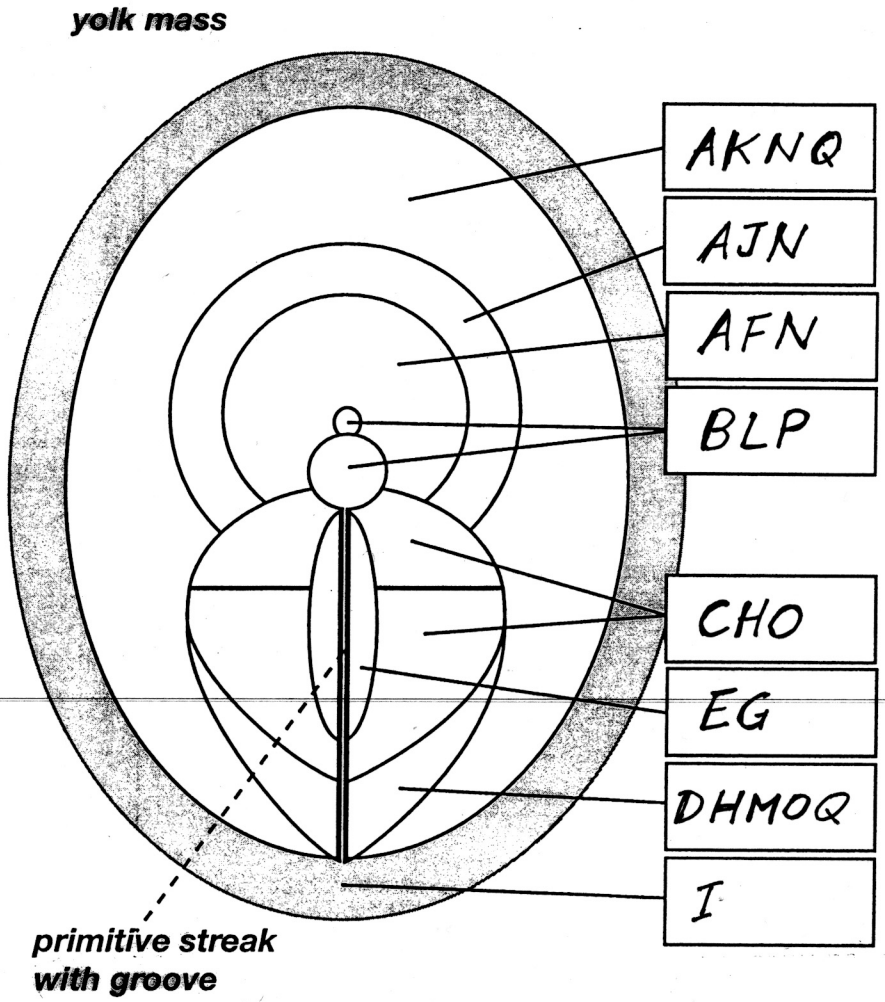
a. yolk sac
b. chorion
c. amnion
d. allantois
e. a lining composed of ectoderm and mesoderm.
f. a lining composed of endoderm and mesoderm.
g. lines a cavity that surrounds the embryo in a controlled aqueous environment.
h. lines a cavity in which metabolic wastes are stored.
i. is involved in mobilizing nutrients for the embryo
j. contains hypoblast and endoblast cells
k. contains ectoderm that passed through the primitive streak \ none



Name: KEY

***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



Name: KEY

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