MCB141 - Midterm #2
Early development of vertebrates through the neurula stage.
John Gerhart

Average 77.32
St. Dev. 11.59
Median 79.75
100 points in 80 minutes (we need to stop at 12:30 exactly).

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

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
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:

<table>
<thead>
<tr>
<th>Territory of the Fate Map</th>
<th>Cells are exposed to Nodal signals (write yes or no in each box)</th>
<th>Cells are exposed to Bmp antagonists (write yes or no in each box)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Epidermal</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Somite</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Lateral plate</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

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 boxes:

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

Part 2B. 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.
Question 3 (8 points):
Cortical rotation is required for organizer formation in the Xenopus embryo:

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

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

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

    b) the alignment of microtubules

    Parallel array, planar (thin sheet)

    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

    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.

    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.
**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:

<table>
<thead>
<tr>
<th>Component affected by knockdown or over-expression in the oocyte or egg</th>
<th>Does the level of β-catenin protein increase, decrease, or stay the same? (write one per box)</th>
<th>At the gastrula stage, will the organizer be absent, normal, multiple, or larger? (write one per box)</th>
<th>Eventual phenotype is: ventralized, dorsalized, twinned, or normal) (write one per box)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Maternal β-catenin mRNA is depleted</td>
<td>Decrease</td>
<td>Absent</td>
<td>Ventralized</td>
</tr>
<tr>
<td>2. Maternal axin mRNA is depleted</td>
<td>Increase</td>
<td>Large/multiple</td>
<td>Dorsalized</td>
</tr>
<tr>
<td>3. Maternal GSK3 mRNA is depleted</td>
<td>Increase</td>
<td></td>
<td>Dorsalized</td>
</tr>
<tr>
<td>4. Maternal mRNA for the Frizzled receptor or LRP5/6 co-receptor is depleted</td>
<td>Decrease</td>
<td>Absent</td>
<td>Ventralized</td>
</tr>
<tr>
<td>5. The fertilized egg is soaked in LiCl, a GSK3 inhibitor</td>
<td>Increase throughout the egg</td>
<td>Large/multiple</td>
<td>Dorsalized</td>
</tr>
<tr>
<td>6. At the 4 cell stage, wnt11 mRNA is injected into the ventral blastomeres</td>
<td>Increase (on the ventral side)</td>
<td>Multiple (two organizers)</td>
<td>Twinned</td>
</tr>
<tr>
<td>7. At the 4 cell stage, axin mRNA is injected into the dorsal blastomeres</td>
<td>Decrease (on the dorsal side)</td>
<td>Absent (or reduced)</td>
<td>Ventralized</td>
</tr>
</tbody>
</table>

**Explain your answer to item 7 in this space, if you think it needs an explanation:**
Extra axin mRNA on the dorsal side is expected to lead to more axin protein there, overwhelming axin removal by the Frizzled-LRP5/6 receptors, hence allowing beta-catenin to bind with GSK3, get phosphorylated and broken down.
**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:

*Do in situ hybridization, showing that vegT mRNA is present in the vegetal third of the egg at times from fertilization through the mid-blastula stage when the vegetal third (the vegetal base) can be combined with animal cap cells to induce endo-mesoderm.*

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:

*Use an antisense oligodeoxynucleotide to knock down maternal vegT mRNA (best knocked down in the oocyte) or an antisense morpholino (OK to use after fertilization) to block the translation of maternal vegT mRNA. (The agents are injected into the oocyte or newly fertilized egg.) You find that the egg develops into an embryo that fails to form endo-mesoderm. That is, it develops mostly as ectoderm. (Or some may suggest a Nieuwkooop experiment using the vegT depleted vegetal portion of a mid-blastula combined with a normal animal cap, and getting no endomesoderm induction).*

C. An ectopic expression experiment, and its result (you may want to use a Nieuwkooop-type experiment for this section):

*Inject vegT mRNA into the animal hemisphere of a fertilized egg (e.g., at the 1,2, or 4 cell stage). Then remove the animal cap at the mid-blastula stage and do a Nieuwkooop experiment, combining it with the animal cap of a normal embryo. You then find that the normal cap of the recombinant undergoes endomesoderm induction, whereas if it had been combined with an un.injected cap (normal), it would not. (Some may want to pursue the initial vegT mRNA injected embryo and show that it forms endomesoderm in the animal pole region, whereas it wouldn’t, if un.injected). That’s OK.*

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)?

*Use mRNA for a dominant negative Nodal receptor (e.g. the type II subunit, truncated to remove the kinase region), and inject it in the animal hemisphere of an egg. Take the cap at the mid-blastula stage, combine it with vegetal base cells and show that the cap has a greatly reduced capacity, or failed, to undergo endomesoderm induction.*
**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 recombinant 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?

*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.*

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 recombinant (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.
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).

*In dorsal cells where beta-catenin has accumulated, it binds to the Tcf transcription factor. This complex is responsible for all the transcriptional effects contributing to organizer formation.*
1) The complex activates the siamois and twin genes encoding yet other transcription factors, and these then activate the xnr3 and chordin genes encoding Bmp antagonists. These reduce Bmp levels on the dorsal side.
2) It also activates (or derepresses) the iroquois gene encoding a transcription factor that then represses Bmp gene expression, blocking zygotic Bmp expression, further reducing Bmp on the dorsal side. Lower Bmp enhances Nodal signaling. High Nodal signaling drives organizer formation.
3) Siamois/twin transcription factors synergize with pSmad2/3 from the Nodal signaling pathway to activate certain organizer genes (such as goosecoid; name not necessary).
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 "blastocoelсколько.

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  | k. the site(s) of respread bottle cells |
| e. the prechordal plate (head mesoderm) | l. the site(s) of resspread bottle cells |
| f. the head organizer                 | m. cells secreting Noggin, Chordin, and Follistatin |
|                                      |                              |

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):

Spreading migration:
After the head organizer cells involute, they migrate 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).
Question 8 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):

**Convergent extension morphogenesis:**
After involution, cells of the trunk-tail organizer become spindle-shaped and bipolar (with opposing tips of motile activity, i.e., two lamellapodia), lying roughly in parallel. Cells at the boundary 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, 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 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.

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 is turned in the reverse direction at the barrier. It involutes and moves toward the animal pole.
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.

<table>
<thead>
<tr>
<th>A. Bmp protein</th>
<th>G. The neural development option</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. The lateral plate/coelom option</td>
<td>H. Bmp antagonists such as Chordin, Noggin, and Follistatin</td>
</tr>
<tr>
<td>C. The Bmp receptor</td>
<td>I. The receptor of Bmp antagonists</td>
</tr>
<tr>
<td>D. Nodal protein (xnr1,2,4,5,6, derriere)</td>
<td>J. The somite development option</td>
</tr>
<tr>
<td>E. The epidermal development option</td>
<td>K. Bmp gene expression</td>
</tr>
<tr>
<td>F. Wnt antagonists such as Dkk, Frzb, and Crescent.</td>
<td>L. The Nodal receptor</td>
</tr>
</tbody>
</table>

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 epidermis or neural tissue as the outcome of the embryo developing from that egg and briefly explain your prediction in terms of the default model shown above.

**Predict neural development. This dominant negative receptor blocks the phosphorylation and activation of smad1/5/8, so the Bmp autoactivation cycle is disrupted. Without pSmad1/5/8 in the cells, the neural option is derepressed and the epidermal option is no longer sustained.**

9C. (3pts). Introduce into the fertilized egg an antisense morpholino(s) that blocks translation of the mRNA(s) of component 5. 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.

**Predict epidermal development. Due to the morpholinos, the Bmp antagonists are not produced, and there is no disruption of the Bmp autoactivation cycle. Continuous high pSmad1/5/8 in the cells sustains the epidermal option and represses the neural option.**
**Question 10: (6 points)**
Consider cells that develop to posterior neural tissue (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:

\[a_1\ b_2\ c_2\ d_1\ e_1\ f_2\]

- **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.
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 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
- r. Will not undergo endomesoderm induction
- s. Will release neural inducers.
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.)

- Embryo Gut
- Yolk mass

\begin{itemize}
  \item \textbf{b}, e, l
  \item \textbf{a}, e, g, l
  \item \textbf{c}, f, h, k, l
  \item \textbf{d}, f, i, k, l
\end{itemize}

\begin{itemize}
  \item \textbf{a}. amnion
  \item \textbf{b}. chorion
  \item \textbf{c}. allantois
  \item \textbf{d}. yolk sac
  \item \textbf{e}. a lining composed of ectoderm and somatic (or parietal) mesoderm.
  \item \textbf{f}. a lining composed of endoderm and splanchnic (or visceral) mesoderm.
  \item \textbf{g}. lines a cavity that surrounds the embryo in a controlled aqueous environment.
  \item \textbf{h}. lines a cavity in which metabolic wastes are stored.
  \item \textbf{i}. is involved in mobilizing nutrients for the embryo
  \item Contains ectoderm that passed through the primitive streak. \textit{not used}
  \item \textbf{k}. contains endoderm derived from the hypoblast and endoblast.
  \item \textbf{l}. contains mesoderm that passed through the primitive streak.
\end{itemize}