

Zoology 470 - Exam #3 – 2009

This exam has **6 pages** and a total of **50 points**. You will have **120 minutes** to complete it. Answer all short answer questions as briefly as possible. Make sure your name and ID number are on all pages.

1. Provide concise definitions for each of the following (**6 points**)

a. nodal vesicular parcel (NVP): *Vesicles [containing shh] propelled by nodal cilia from the right to the left side of the node in mammals (and perhaps other vertebrates). These vesicles are thought to be important for establishing left/right asymmetries in amniotes.*

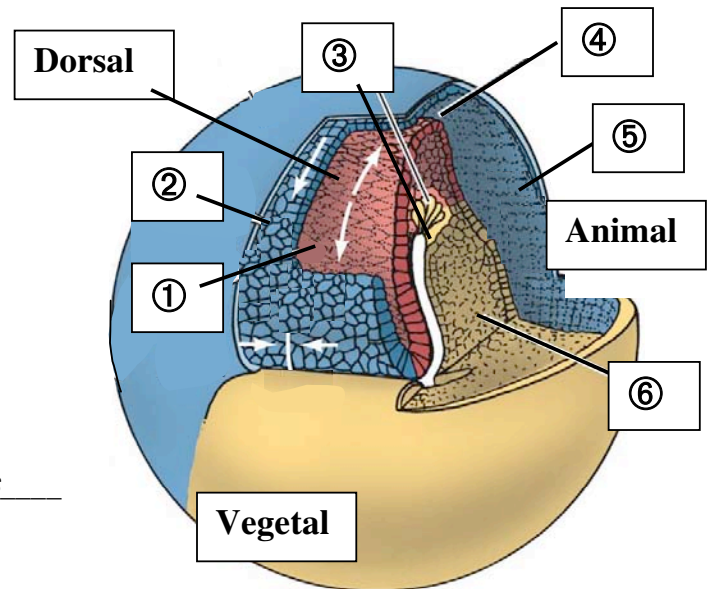
b. coelom: *Primary body cavity, within which major internal organs lie. In vertebrates, forms as a separation between the somatic and splanchnic mesoderm.*

c. regression of Henson's node: *Apparent movement toward the posterior of Henson's node during chick gastrulation. As Henson's node moves posteriorly, the notochord is laid down anterior to the node simultaneously.*

d. amniote: *Vertebrates, including reptiles, birds, and mammals, that either produce that produce embryos with amniotic membrane, either within an amniotic egg (reptiles, birds), or a placenta (most mammals).*

2. For each phrase referring to various aspects of amphibian gastrulation on the left match ALL appropriate response(s) drawn from the column on the right. Note : in some cases, the correct answer will require more than one choice from the right-hand column. If none of the answers on the right are appropriate, write "none" (**5 points**).

- a. cells from here contribute to the somites ___ 1 ___
- b. undergoes apical constriction ___ 3 ___
- c. forms central nervous system ___ 2 ___
- d. undergoes convergent extension ___ 1,2 ___
- e. uses directed migration via lamellipodia, but does **not**
undergo convergent extension ___ 4 ___
- f. some cells from here form part of the archenteron ___ 3,6 ___
- g. undergoes epiboly ___ 5 ___
- h. requires aligned fibronectin for proper migration ___ 4 ___



3. Complete the following table referring to extraembryonic membranes in **mammals**. Note: only provide mammalian functions of the indicated membranes (4 points).

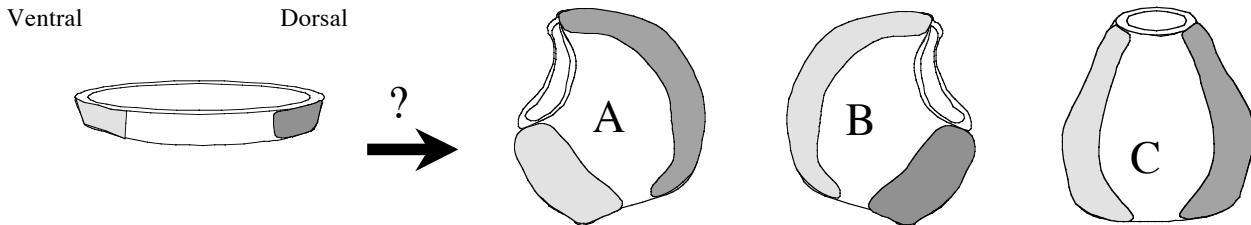
Membrane	Double-layered tissue from which it is derived	Major function(s) in <u>mammals</u>
Amnion	<i>somatopleure</i>	<i>Provides fluid-filled environment around the embryo</i>
Chorion	<i>somatopleure</i>	<i>Placenta (gas/waste exchange and nutritive functions combined)</i>
Yolk sac	<i>splanchnopleure</i>	<i>Blood cell production</i>
Allantois	<i>splanchnopleure</i>	<i>Umbilical cord (hence waste removal/gas exchange)</i>

4. Circle true (T) or false (F) for each of the following. Read each statement carefully. (8 points)

T	Researchers at the University of Wisconsin can now receive federal funding for creation of new human embryonic stem cell lines, because of changes in the Obama administration's policies
F	<i>Siamois</i> is a gene involved in differentiation of the Nieuwkoop Center, which forms on the ventral side of the early <i>Xenopus</i> embryo [<i>dorsal side!</i>]
T	Repulsion between cells expressing Ephrin ligands in the optic tectum and neurons expressing Eph receptors is more likely when then neurons have high levels of the Eph receptors on their surfaces
F	Adrenal medulla cells form from neural crest that migrate over the dorsal surface of somites [<i>under/through somites</i>]
T	The notochord induces the differentiation of the overlying floor plate of the neural tube by secreting <i>sonic hedgehog</i>
T	The umbilical vein is important in mammals because it carries oxygenated blood from the placenta back to the fetus
F	Treating amphibian zygotes with the microtubule depolymerizing agent, nocodazole, within a few minutes after fertilization would result in embryos with excess dorsal and anterior structures. [<i>ventral/posterior</i>]
F	Knockdown of <i>Cerberus</i> expression in a frog embryo would be expected to result in loss of tail structures [<i>anterior</i>]
F	In vertebrates, the lungs originate as simple outpocketings of ectodermal tissue [<i>endodermal tissue</i>]
T	The organizer equivalent in zebrafish is called the embryonic shield
F	Local cell shape changes, such as apical constriction, contribute to the invagination of the neural tube, but convergent extension does not [<i>notoplate does conv., extension!</i>]
F	Chordin is a secreted protein produced by the node in mice and the organizer in frogs that counteracts BMP signals, resulting in induction of epidermal tissue [<i>neural tissue</i>]
F	Ventral vegetal cells in the <i>Xenopus</i> blastula express the highest levels of nodal-related proteins, and the levels of nodal-related protein fall off progressively towards the dorsal side of the embryo [<i>the opposite is true</i>]
F	In mammals, the <i>embryonic</i> endoderm forms primary from the hypoblast
F	The somite derivative known as the myotome forms part of the vertebral column [muscle]
T	The phylotypic stage for chordates occurs after the structures form that arise during neurulation.

5. The morphogenesis of specific cells is crucial during gastrulation in vertebrates. Because the sturgeon is an endangered species in the upper Midwest, you are reexamining how sturgeon embryos gastrulate. Answer each of the following, stating your reasoning in each case.

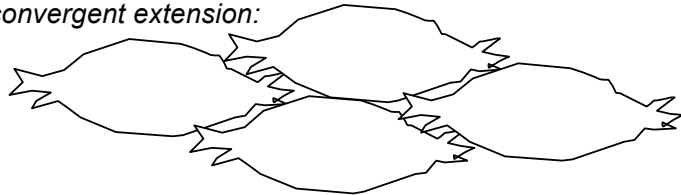
a. Even though it is a fish, previous fate mapping studies have shown that the sturgeon gastrulates in a manner very similar to amphibians. From the following diagrams, select the one that best represents the overall changes in shape of the sturgeon's involuting marginal zone, starting with the diagram at left.



The diagram that best represents the final shape of the involuting marginal zone is (1 point): A

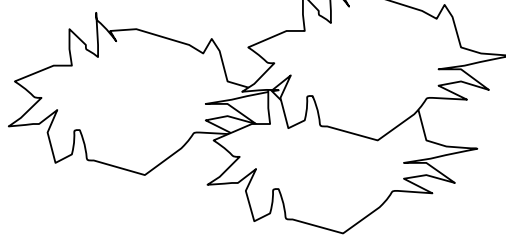
b. You believe that the cells of the dorsal involuting marginal zone in sturgeon embryos will undergo the same set of behaviors as in *Xenopus*. If your hypothesis is true, describe the detailed protrusive activity of individual cells in the explant. Assume you can visualize the cells using fluorescent markers as in *Xenopus*. Feel free to use diagrams if you feel they will aid your explanation (2 points).

They display bipolar protrusive activity, allowing convergent extension:



c. You use an inhibitory form of the sturgeon Disheveled protein to disrupt planar cell polarity (PCP) signaling and repeat the experiment in (b). Describe how such treatment would affect the detailed cell behavior of individual cells in the experimentally disrupted explant (2 points)

They display less polarized protrusive activity, inhibiting effective convergent extension:



6. Human embryos can be genotyped at an early stage of their development in several ways. Describe **two** ways in which the genetic status of a human embryo can be assessed (2 points):

Method #1: "Pre-embryo biopsy" (preimplantation genetic diagnosis) – remove blastomere from pre-compacted embryo for testing.

Method #2: Amniocentesis (remove amniotic fluid, grow cells from the fluid, test)

Other: chorionic villus sampling (remove villus from placental tissue, test)

7. Match each structure indicated to the appropriate portion of the neural tube from which it arises. If none is appropriate, please indicate this using choice (4). (2 points)

Region of the neural tube: (1) forebrain (2) midbrain (3) hindbrain (4) NONE

Structure: a. cornea of the eye ___4___ b. medulla ___3___ c. hippocampus ___1___ d. olfactory lobe ___1___

8. Neural crest cells often differentiate in response to cues within their local environment.

a. Provide **two** pieces of evidence that neural crest cells respond to local environmental cues for their differentiation (2 points).

Evidence #1:

Cultured NC will differentiate differently when different growth factors are applied

Evidence #2:

Ledouarin experiments: move cervical crest to new location, and they will form ganglia appropriate to their new position.

Other: (1) Steel/white mutants: melanocytes need local factors to survive/differentiate; (2) single injected NC generate multiple cell types, and not always the same, suggesting local cues [not as good as the others, but we'll take it!]

b. You are studying a knockout mouse in which homozygous embryos are missing rhombomeres 2-6. What **non-brain** structures would you expect to be missing? (1 point) *cartilage associated with the head (pharyngeal arches, inner ear, etc.)*

9. You work in Marc Tessier-Lavigne's lab, and you are studying axon guidance. Please answer the following questions about guidance of axons in embryos.

a. You are reviewing previous axon guidance experiments. Cite **two** pieces of evidence that indicate netrins act as attractive guidance cues for axons that migrate ventrally in animal embryos (2 points).

Evidence #1: *Culture commissural axons next to engineered cells expressing netrins, and they grow toward them*

Evidence #2: *Commissural axons in netrin KO mice have ventral migration defects*

Other: unc-6 (netrin) mutant C. elegans have neuronal migration defects

10. Suppose you go on to medical school after your undergraduate career. You have become interested in the causes of autism, a brain disorder, through your association with a family with an autistic child, and because you remember that your developmental biology professor has an autistic son.

a. You do some reading and discover that one rare type of autism may result from a mutation in a human anterior *Hox* gene, *HoxA1*. You then try to explain to the family you work with what a "homeotic gene" is. What would you say? (1 point)

Homeotic genes are gene, which when mutated, result in transformations of body parts.

b. You then go on to discuss similarities and differences between fly homeotic genes and human Hox genes. Name **two** expected **similarities** between human homeotic genes and *Drosophila* homeotic genes. (2 points)

Similarity #1: They all encode homeodomain transcription factors

Similarity #2: Their primary domain of actin along the A-P axis corresponds to their 3'-5' location on the chromosome

Other: mutation can lead to transformation of identity of segmentally repeated elements

c. Given how important they are, it is surprising to the family you work with how little phenotypic effect a mutation in a particular Hox gene has. Having paid attention in your developmental biology course as an undergraduate, you are not surprised by this. Why? **State your reasons (1 point)**

Functional redundancy, due to four different Hox gene clusters in mammals.

d. The family has now become interested in Hox genes, and asks more questions. You explain that non-genetic treatments, such as retinoic acid, can have dramatic effects on *Hox* gene expression. Such agents, that do not result in heritable changes in treated offspring but cause dramatic developmental defects are known as (1 point):

_____ teratogen _____

11. You are assaying for mesoderm differentiation in *Xenopus* embryos under a variety of circumstances, using the *loose lips* gene as a marker. *loose lips* encodes a transcription factor normally expressed in the **neural ectoderm** in *Xenopus* at the early gastrula stage. After each treatment, you allow the embryos to develop until they are equivalent in age to a normal early gastrula. In each case, state whether you expect the levels of expression of loose lips to be higher than normal (**high**), **normal**, or lower than normal (**low**). (5 points).

Treatment	<i>loose lips</i> level
You inject of β -catenin mRNA into <u>all ventral</u> cells of the four-cell embryo	high
You inject of high levels of chordin mRNA into <u>ventral</u> blastomeres at the four-cell stage	high
You inject of large quantities of BMP-4 mRNA into <u>dorsal</u> blastomeres of the early embryo	low
You combine <u>animal cap</u> cells with <u>ventral, vegetal</u> cells at the 16-cell stage	low
In those rare cases in which the gray crescent is not bisected by the first cleavage in <i>Rana pipiens</i> (another frog species), you isolate the cell that does not contain the gray crescent at the two-cell stage, and assess expression of the <i>Rana</i> version of <i>loose lips</i>	low

12. Limb bud patterning involves carefully orchestrated cell signaling events.

a. Describe the results of the following manipulations of the chick limb bud. In your answer, be sure to state what type of limb would result (wing, leg), where appropriate. (3 points)

Operation	Expected outcome
Remove the apical ectodermal ridge (AER) from a wing that has just formed and replace it with the AER from a leg bud of comparable age.	<i>Still get a wing, since the AER provides a non-specific growth signal.</i>
Remove the zone of polarizing activity (ZPA) from a normal wing bud and place a ZPA at its anterior margin	<i>Wing with inverted A-P polarity (thumb at anterior, digit IV at posterior)</i>
Implant a bead soaked in FGF-10 in the flank next to the site of normal leg bud outgrowth	<i>A third (extra) leg will grow out at the site of the implant.</i>

b. Retinoic acid also has effects on limb buds when administered at the proper time. In fact, when implanted into a limb bud, a retinoic acid-soaked bead will induce a ZPA in tissue that does not normally make one. You perform an in situ hybridization experiment to see whether RA can induce mRNA expression appropriate for a ZPA. Assuming you assay for mRNA that encodes a secreted protein, what mRNA should you assay for? (1 point)

mRNA to look for encodes the following protein: _____ *sonic hedgehog* _____