Study Guide for Exam #3 - Zoo 470 – 2009

Reminder: Exam 3 will be held at **10:05 am, Monday, May 11,** in **room 105 Psychology (Brogden Hall).**

General comments: **This exam is non-cumulative**. However, if we reviewed a concept from earlier parts of the course, you will be responsible for it as it relates to topics in the last part of the course. Several tips:
(1) The nature of the material on this exam means there is more terminology and that there are more terms that need to be learned that are pure vocabulary. For some students this is actually easier; for others with a lot of molecular/genetic/cell biological background, this aspect of Unit 3 is more difficult.
(2) You may be asked to draw comparisons or cite more than one example where a pathway/process is used. One good example is the role β-catenin plays in diverse organisms; another might be similarities and differences between the Organizer equivalent in various vertebrates, etc.

Important note: Remember that there is one thing you are expected to know that we covered in class but are primarily based on your reading: Brain vesicles and what they form.

A. Axis Specification and Gastrulation in Amphibians
Note: Two resources are especially useful for the section on amphibians:
(1) Please check out the old (but still useful) frog materials at:
http://worms.zoology.wisc.edu/frogs/welcome.html
In particular, the sections on *Cortical Rotation*, the *Blastula*, and *Gastrulation* are really useful.
(2) The step-by-step guide to axis specification in amphibians, available on Learn@UW and the off-site course web page.

1. Classic experiments on axis formation in amphibians: what is the *gray crescent*, and how can *hair loop experiments* be used to show the importance of something in this region of the fertilized egg? On which side of the animal hemisphere does the *sperm entry point* lie, in relation to the future dorsal side of the embryo? What *cytoskeletal system* drives cortical rotation in frogs? Can you cite experiments that show that cortical rotation, however it is induced, is *sufficient* for axis specification in frogs/ How can blastomere transplantation be used to show that *dorsal, vegetal blastomeres* have special properties with respect to axis formation (i.e., rescue of UV-treated eggs, production of double axes, etc.)? What is the *"Nieuwkoop Center"*, and what experiments suggest that it exists? What is the *organizer experiment*?
2. Molecular basis of mesoderm induction in amphibians: What role is *β-catenin* thought to play in mesoderm induction? How can we show this using mRNA overexpression? How is localized Dishevelled protein thought to contribute to elevation of β-catenin dorsally after fertilization? What gene's expression is upregulated by dorsal elevation of β-catenin? What experiments indicate that *siamois* leads vegetal cells to behave like a Nieuwkoop Center? What role is *VegT* thought to play in establishing the endoderm? How are *Xenopus Nodal-related proteins* thought to pattern the vegetal region, and, later, the mesoderm of the marginal zone?

3. The molecular basis of the organizer: What is the role of *BMPs*, such as *BMP-4*, in establishing ventral fates in the mesoderm? You should be able to predict what would happen if the function of receptors for BMPs are disrupted, or BMPs are overexpressed. What role do *noggin and chordin* play once the organizer is established? How are they thought to interact with BMP-4? In a similar manner, what can you say about *Xwnt8, FrzB and Dickkopf*?

4. Gastrulation: What *major things does gastrulation accomplish* in amphibians? What are the major important tissues in a *Xenopus* gastrula? What basic sorts of *morphogenetic movements* do each of these regions perform? What are *deep cells* vs. *superficial cells*? You should know what superficial cells of the dorsal involuting marginal zone make, as opposed to the deep cells. How is *blastopore closure* accomplished? What do *Keller "sandwich" explants* allow us to do? How can we use cells explanted in vitro to study gastrulation movements in more detail? How can we show that the *planar cell polarity (PCP) pathway* is involved in allowing cells to display oriented protrusive behavior during *convergent extension*? What role(s) does *fibronectin* in the extracellular matrix probably play during *leading edge mesoderm migration*? What experiments indicate this?

5. Neural Induction: What is meant by *"planar" vs. "vertical" signals* in neural induction? [hint: neural induction could be occurring at two times, or a combination of both!: (1) before the involution of mesoderm during gastrulation (planar signals) or (2) after involution, when mesoderm lies underneath the neural ectoderm (vertical signals).] You should understand the following experiments: (i) *Keller sandwiches*: what happens to the ectoderm in a sandwich? (ii) the *Einsteck experiment* of Otto Mangold: why is neural induction thought to involve region-specific vertical signals from the mesoderm? What role do *BMPs* play in ectoderm differentiation? The organizer produces signals that override the epidermalizing influence of BMP-4; what are those signals? How do signals from the mesendoderm influence formation of structures in the head (e.g., what is *cerberus*?)? How are basic *anterior-posterior differences* thought to arise along the neural ectoderm?

C. Axis Specification in Other Vertebrates

1. Zebrafish: How are zebrafish and amphibian early development similar? Different? How is the *embryonic shield* similar to the dorsal involuting marginal zone of *Xenopus*? What morphogenetic movement does it undergo? What is the *hypoblast*? How is it formed? What *molecular components* (hint: what is *bozozok*?) appear to be similar in establishing a dorso-vegetal signaling center, and how is *β-catenin* involved? What can you say about *BMPs and BMP antagonists* in early fish development? How is *PCP signaling* involved in zebrafish gastrulation? Can you think of specific examples (e.g. involving a *Wnt*?)?

2. Chicks: Chicks are *amniotes*. What does this term mean? What is the *posterior marginal zone*? How does the *hypoblast* arise in chicks? How does the formation of the *embryonic endoderm* differ from amphibians? How is *Henson's node* similar to the dorsal blastopore lip in an amphibian, based on experimental embryology? How is it similar at the molecular level? How do cells move through the *primitive groove* during chick gastrulation? How is *regression of Henson's node* coupled with formation of the *notochord*? What can you say about the *timing of gastrulation* and neurulation in the anterior vs. the posterior in amniotes?

3. Mammalian early embryos (a) *Human embryo manipulation*: You should be able to answer basic questions about *"pre-embryo" biopsy (preimplantation genetic diagnosis)*, including how the procedure is performed. (b) *Compaction and blastocyst formation*: Recall what *totipotency* and *pluripotency* mean. What is *compaction*? What *cell adhesion molecule* is thought to mediate compaction? What are the *two basic types of cells* in early mammalian embryos at the 16-cell stage and beyond? What do *trophoblast* cells make? What are *inner cell mass* cells? A *blastocyst*? What does analysis of *monozygotic twins* tell us about the plasticity of early mammalian development? (c) *ES cells*: What role is *oct-4* thought to play in maintaining pluripotency in stem cells? *Nanog*? What signaling pathways contribute to maintaining pluripotency in ES cells (hint: β-catenin accumulates as a result of one of these!)? (d) *The node and anterior visceral endoderm (AVE)*: What molecules are expressed in the mouse node that are similar to those in the amphibian organizer and Henson's node? How do we know *lim-1* contributes to formation of anterior (head) mesoderm? (e) *Gastrulation*: How do chick and human gastrulation compare at a very basic level? (f) Blastocyst structure: What is the *cytotrophoblast*? *Syncytiotrophoblast*?

4. Left/right axis specification: What are *nodal cilia* [note: "nodal" here refers to association with the Node, not the molecule called Nodal…]? What is *Kartagener's triad*? What evidence shows nodal cilia are required for proper left/right specification? What experiments indicate that the *direction of nodal flow* can determine where the left side of the embryo forms? What are *nodal vesicular parcels*, and what are they thought to transport across the Node? What role is *Pitx2* thought to play after these events occur?

D. Further Development of Vertebrates

1. Neurulation and CNS development (a) *Neurulation*: What are the basic events of *neurulation*? What is a *phylotypic stage*? What is the phylotypic stage for vertebrates, and what characterizes it? What are the common features of this stage in all vertebrates? How do *neural crest cells* arise during neurulation? What *morphogenetic processes* contribute to neurulation? What role does *N-cadherin* play during neurulation? What are *hinge points*? How does the neural plate region change its overall shape (i.e., it rolls up, but what else happens?)? What experiments show that *convergent extension* in the *notoplate* is regulated by the planar cell polarity pathway? (b) *Central nervous system differentiatio*n: What *major brain structures* form from the various brain vesicles found at various stages of human development? What structures give rise to the *retina* of the eye? The *lenses*? What is a *rhombomere*? What is *metamerism*, and how are rhombomeres an example of it? How does the expression of *Ephs and Ephrins* correlate with rhombomere boundaries? (c) *Dorsoventral patterning*: How do *sonic hedgehog* and *BMPs* regulate the dorsoventral patterning of the spinal cord? What role does the *notochord* play in induction of the *floor plate*?

2. Mesoderm and Endoderm (a) *Notochord (choramesoderm) and somites (paraxial mesoderm)*: How does the *notochord* form? What do *Keller explants* tell us about morphogenesis of the notochord? What changes take place in *somites* as they form? What are *three basic tissues* that derive from somitic mesoderm (hint: each ends in "-ome")? What do they make? (b) *Lateral mesoderm*: What are the *two basic types* of lateral mesoderm? What are the *somatopleure* and the *splanchnopleure*, and what tissue layers are they made from (i.e., they're double-layered!)? What is the *central body cavity* between these layers called? (c) *Extraembryonic membranes*: What is an *amniote*? What

animals have amniotic eggs, and how do these eggs allow them to survive on land? How are the splanchnopleure and the somatopleure modified in amniotes, and what extraembryonic tissues do they give rise to? What are the *four major extraembryonic membranes*, and what are their functions? How is the *chorion* modified in mammals? The *allantois*? (e) *Blood and blood vessels*: What are *blood islands* (also known as *angiogenetic cell clusters*)? Where do they form, i.e., what extraembryonic membrane is involved? How does the *heart* in amniotes form? What modifications are found in the *fetal circulatory system* to support gas and nutrient exchange with the placenta at various times during mammalian development? (f) *Gut formation*: What major structures are formed by budding from the *primary endodermal tube* formed by ventral bending of the embryo (e.g., lungs, pancreas, liver, endocrine portion of pituitary, etc.)?

3. Neural Crest: How do we know that neural crest cells are *pluripotent*? What are the *two major migration routes* of trunk neural crest? How are *Ephs/Ephrins* expressed by the somites thought to repulse neural crest cells? What experiments indicate that trunk neural crest cells rely on cues in their environment for their differentiation? What is the evidence that neural crest cells are *pluripotent*? What structures of the head are derived from *cranial neural crest*? What are *pharyngeal arches*? *Pharyngeal pouches*? What in vitro evidence is there for effects of growth factors on trunk neural crest cell differentiation? What role does the *c-kit/steel factor* system seem to play in maintaining melanocyte viability/differentiation?

5. Axon guidance: What is a *growth cone*? What are the *major types of cues* for axon guidance? What evidence is there for guidance of chick motor neuron growth cones? What is *chemoattraction*? *Chemorepulsion*? *Contact attraction*? *Contact repulsion*? How do *semaphorins*, such as collapsins, act as repulsive cues? Can you cite examples? How do *netrins* function in axon guidance/repulsion, and what evidence is there that the *floor plate* produces an attractive signal for certain neurons in the spinal cord? What experiments indicate that netrins have a role in attractive guidance? How are *Eph receptors and ephrin ligands* thought to be involved in *retinal/tectal mapping*? What studies in vitro show that ephrins act as repulsive guidance cues? Are cues that are repulsive for some neurons repulsive for all neurons?

5. *Hox* Genes: What are the similarities between the homeotic complex in flies and *Hox* genes in mice? How are they different? How do the positions of segmentally repeated structures relate to the patterns of expression of Hox genes? How can the function of Hox genes in vertebrates be studied? Are major structures affected in Hox gene single knockouts? Why do single knockouts have little effect? What about the organization of the Hox genes in vertebrates suggests that single knockouts would have relatively small effects? How does *retinoic acid* affect Hox gene expression? What is a *teratogen*?

6. Limb Development: Note: the text is <u>very</u> good on this stuff, but only focus on what we covered in class. (a) *The proximal-distal axis*: What is the role of the *apical ectodermal ridge*? What happens when the ridge is removed early? What happens if it is removed later? What are the consequences of ridge removal for the underlying mesoderm? (b) *Molecular events in the early limb bud*: What role do *FGF-10 and FGF-8* play in establishing a limb bud and the early AER? What role are *FGFs* thought to play in mediating AER signals? Are these signals specific to forelimbs vs. hindlimbs? (c) *Limb identity*: what evidence is there that the identity of the mesoderm determines limb type? What is the role of *Tbx-5 and Tbx-4* in specifying limb identity? (d) *The anterior-posterior axis*: What is the *zone of polarizing activity (ZPA)*? How are limbs altered

when a ZPA is grafted to a new site? What role is *sonic hedgehog* thought to play in ZPA signaling? How does it mimic a ZPA? How can we test this using *shh* knockout mice? What evidence is there that *shh* regulates *Hox* genes (e.g., *Hoxd13*)? (e) How are *Hox genes* thought to provide positional identity to cells in the developing limbs, especially the arm (or forelimb)? What do multiple knockouts of Hox genes tell us about their role in specifying bones along the proximal-distal axis of the limb? What is *synpolydactyly*? What *Hox gene*, when mutated, is responsible for this condition?

Remember that you can check on your grades electronically after the semester is over.

GOOD LUCK!!! IT'S BEEN A REAL PLEASURE TEACHING YOU THIS SEMESTER!!!

Please stop by my office to visit if you get a chance this summer or in the fall!