Study Guide for Exam 1 – Zoology 470 – 2009

Exam format
You can expect a good chunk of the exam to be short answer questions (i.e., 1-2 sentence response). Other types of questions, including identification, multiple choice, matching, and true/false, will also be present, but don't count on them being the largest portion of the exam. In addition, there has been an emphasis in class on **experiments** and what they tell us about mechanisms of early development. **These are really important, and you should understand them thoroughly.** The text should be used to reinforce in-class material. **Your best study hints will come from last year's exam.** Remember that it's on the course web site.

Topics for Exam 1
Introduction to development
What are the big ideas that developmental biologists study? What are some techniques that allow them to study such questions? What is the *organizer*? What is *fate mapping*? Be sure you are familiar enough with the "big ideas" to provide definitions (e.g. *differentiation*, *morphogenesis*). What is *conditional specification*? *Autonomous specification*? *Totipotency*? What is "*mosaic development*"? *Localized determinants*? How does one show *sufficiency* of a group of cells (or a molecule) for some developmental process? How does one show *necessity*? Be able to cite examples of each, and be able to describe an experiment that could used to test the state of specification of a cell or tissue using classical techniques.

Genomic equivalence
What experiments in amphibians indicate the *genomic equivalence* of nuclei? What does this tell us about how the nucleus exerts effects on differentiated cells? What do *chromosome puffs* tell us about differential gene activation in differentiated tissues? How were the experiments performed that resulted in Dolly? "Cumulina" the mouse? What do we know about how normal Dolly really was at the cellular and molecular level? Is this a universal problem for cloned mammals? What is the state of *legislation* in this country regarding human cloning? Based on your supplemental readings, what are the *major proposed uses of mammalian cloning*? What is the difference between *reproductive and therapeutic cloning*? Biologically, why is human reproductive cloning deemed unsafe? What is "*large offspring syndrome*", and what is it thought to be caused by? Be able to describe the current situation regarding success in cloning humans, including fraud by the South Korean group in recent human cloning experiments. You should also be able to explain why, in theory, therapeutic cloning is potentially superior to general use of ES cells in human patients. Also be able to state whether therapeutic cloning has been achieved in primates.

Mammalian early embryos
1. Compaction and blastocyst formation: How are *allophenic mice* produced? What is *totipotency*? What experiments demonstrate that cells from uncompacted mammalian embryos are *totipotent*? What is *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 experiments indicate that ICM cells are *pluripotent*?
2. Stem cells: How are *human embryonic stem cells* made? What is a *stem cell*? What is *pluripotency*? *Multipotency*? How might stem cells be used in clinical applications? Can you cite an example in which "therapeutic cloning" has been used to repair defects in mice? What does the standard method for harvesting human ES create *ethical problems* for many people?
3. Bioethics: You should be able to answer basic questions about the *current status of funding* for stem cell research in the U.S.. You should be able to state why there are *ethical objections* to the standard way of obtaining ES cells.
4. *Induced pluripotency*: What is *induced pluripotency*? You should be able to explain, in basic terms, how this has been achieved, but you will not be asked to produce the names of the specific molecules involved. What sorts of proteins must be expressed by a cell to induced it to become pluripotent? How can the pluripotency of such cells be assessed? (Hint: what is a *teratoma*? In mice, tests similar to those for ICM cells can be used…] As with therapeutic cloning, you should also be able to explain why such techniques are potentially superior to general use of ES cells in human patients.
5. *Transdifferentiation*: What is *transdifferentiation?* How is it different from using iPS cells? Can you cite a recent example?

Molecular biology techniques
Focus on techniques that are commonly used in developmental biology. A good guide for what I expect is the previous exam. Recall that all you will be expected to know here is the "*big idea*", as presented in the handouts, and then only the techniques I explicitly discussed in class. You should know the basic idea underlying each of the following - *(a) DNA cloning (b) cDNA (c) genomic DNA (d) PCR (e) Northern blots (f) in situ hybridization (g) immunostaining (h) transgenics (i) targeted mutations (j) RNA overexpression (k) RNA interference and morpholinos*. How are RNAi and morpholinos different? How can each of these be used? **Key: Be able to state which technique(s) would be appropriate for answering specific questions about gene expression.** What are polyclonal and monoclonal *antibodies* used for? **Note: you are not responsible for the details of how they are produced; focus on the "big idea".** What is *immunostaining*? *Immunoblotting?* What is the difference between *maternal and zygotic mutants* as tools for studying developmental processes?

The Cell Surface and Cell Biology
1. Cell adhesion: What is a *cell adhesion molecule*? What are *cadherins*? *Catenins*? Be able to explain the classical experiments and molecular basis of *differential affinity ("sorting out")*. What evidence suggests that cadherins are involved? What is the *extracellular matrix*? What are major ECM molecules? How do *integrins* function in promoting attachment of cells to the extracellular matrix? How does a *confocal microscope* help with this technique?
2. Cytoskeleton: What major *cytoskeletal systems* are there in cells?
What *drugs* are typically used to depolymerize microfilaments? Microtubules? What treatments stabilize microtubules? Microfilaments? What major *motor proteins* interact with microtubules? Microfilaments? What is the major cytoskeletal system involved in

cell migration? What is the function of a *lamellipodium/filopodium*? Remember, you are only responsible for this at the level we covered it in class.
3. Cell signaling: How do signals at the cell surface get transferred from a transmembrane receptor via signal transduction to the interior of the cell? What effects does *inositol triphosphate (IP3)* have on the endoplasmic reticulum? What is the importance of *intracellular calcium* for signal transduction? How can *calcium ionophores* be used to show the calcium-dependence of a developmental process? What are *growth factors*? What is the basic logic by which growth factors transduce signals to the interior of a cell? What is the basic importance of phosphorylation of growth factor receptors to signal transduction? We discussed several different signal transduction pathways that we'll use later in the semester. **For each, you are only responsible for the pathway at the level at which I discussed it in class.** What are *Wnts*? How do they transduce signals? What does the "destruction complex" do, and what molecule does it regulate that is important for Wnt signaling? Be able to describe how the *Wnt pathway* works, beginning with a *Wnt*, through it receptor (*Frizzled*), and ultimately leading to accumulation of *β-catenin* and its association with a *Tcf/Lef factor* in the nucleus. What *other pathway* can Wnts activate (hint: what is the *Planar Cell Polarity* pathway? How do *BMPs* transduce their signals to the nucleus in the responding cell? What about *hedgehogs*? What experiments show that *different concentrations of growth factors can elicit different responses* from responding cells? What is the fundamental difference between how *steroid and retinoid receptor* function compared to growth factor receptors?

Gametogenesis
1. Meiosis: What does meiosis accomplish? What are the differences between meiosis in *spermatogonia* and *oogonia*? What is a *polar body*?
2. Spermatogenesis: What is the basic structure of the *seminiferous tubule* in mammals? What are *Sertoli cells* and what is their function? What specializations are present in sperm that relate to their function? For example, what is the *acrosome*, *midbody*, etc.? What *molecular motor* is responsible for microtubule sliding in sperm *flagella*?
3. Oogenesis: What is the structure of the ovary? What is the structure of a *follicle*?
4. Hormonal control of ovulation in mammals: Be able to explain what role various *hormones* play in this process, where these hormones come from, what target tissue(s) they act on, and what the resulting effect(s) is on the target tissue. These include: *progesterone, estrogen, FSH, LH*. Be able to explain what *gonadotropin hormone releasing-hormones* are. Where are they produced? Where do they act? What is the *corpus luteum*, and what is its function? At *what stage of meiosis* are mammalian, amphibian, and sea urchin oocytes/eggs arrested? What is the role of *human chorionic gonadotropin* in maintaining pregnancy? Where is it produced? What is the molecular mode of action of the standard *birth control pill*? *RU486* (mifepristone)?
5. CSF and MPF: What is the effect of *CSF* on meiotic or mitotic cells when it is injected into them? Generally, how does CSF affect MPF? What is the state of *MPF* when it is active? What are its *two major protein subunits*? What is MPF required for in dividing cells, and what is the consequence when MPF is not inactivated? Very generally, how does calcium release at fertilization result (indirectly) in release from metaphase arrest?
6. *Infertility:* What have some suggested *ES cells* be used for in treating infertility?

Fertilization [Note: You'll only be responsible for material we cover by the end of class Monday, Feb. 23]

General: be able to state the *major things that fertilization accomplishes*

1. Sperm maturation/activation: What activates swimming in sea urchin sperm (hint: what is *RESACT*?)? What *ion* is responsible? What steps are required for a normal mammalian sperm to be competent to fertilize and oocyte (hint: where does *maturation* occur? What is *capacitation*, and where does it occur?)?

2. Acrosome reaction: What changes take place in the sperm? What triggers the *acrosome reaction* in sea urchins? How can we show this using *calcium ionophores*? What is an *acrosomal process*?

3. Sperm-egg binding: What molecules are involved in *sperm-egg attachment* in sea urchins? What experiments support such a role in each model system we examined? What is *bindin*? What experiments show that it mediates species-specific sperm-egg binding?

4. Cortical granule exocytosis: What triggers release of *cortical granules*? What is contained in the cortical granules? How does the *fertilization envelope* arise in sea urchins? How is *calcium release* coupled to *cortical granule exocytosis*?

5. Blocks to polyspermy: How does the *fast block to polyspermy* differ from the *permanent (slow) block to polyspermy*? What is the *ionic basi*s for the fast block? The permanent block? How could we show the calcium dependence of cortical granule exocytosis/permanent block? You should be able to work with experimental situations in which the fast or slow block are perturbed, based on what you know.

<u>Reminder</u>: The Review session for Exam 1 is 4:30-6 pm Tuesday, February 24, 2009 in room 132 Noland Hall. Exam 1 is 7:15-8:45 pm, Wednesday, February 25, 2009 in room 105 Psychology. Good luck!