Study Guide for Exam 2 – Zoology 470 – 2009

Exam format

Remember that 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.

Important reminder: ***You are responsible for knowing the cleavage patterns presented in Gilbert, Ch. 8, and representative examples of each***.

Topics for Exam 2

Stem cells (again)

You should no something very basic about recent attempts to produce induced pluripotent stem cells (iPS cells) that do not contain viral DNA sequences, and why this will be necessary before most people think we should use iPS cells in the clinic. You should also know something about President Obama's change in federal funding policy regarding production of new human ES cell lines.

Egg activation

What is *egg activation*? What ionic events are important for full egg activation (e.g., *calcium*, *pH increase*)? How does intracellular pH change following fertilization? What experiments indicate the *pH rise* is required for the onset of protein synthesis? What experiments demonstrate that *protein synthesis* is essential immediately after fertilization for normal development, and that mRNA production is eventually required?

Pronuclear migration

What *cytoskeletal system* and *molecular motor* drive pronuclear migration, and what role is the sperm aster thought to play in this process?

Cleavage and the Blastula

1. Cleavage: What *cytoskeletal systems* and *molecular motor protein* is involved? How does the *cell cycle* during early cleavages differ from that of cells in the adult (somatic cells)? What are *cyclins*? What are *reductive cleavages*? What does this mean for the embryo? What are the basic *types of cleavage*, and what organisms are good examples of each type? What is the difference between *radial and spiral cleavage*, and what groups of organisms display these types of cleavage? How does the amount and position of *yolk* affect how cleavage occurs? What significant accomplishments take place during cleavage (e.g., *regionalization, blastocoel formation*, etc.)? How are astral microtubules thought to stimulate where the cleavage furrow forms? Can you cite an experiment that shows this? What is *Rho*, and why is it important in stimulating cleavage furrows?

2. Blastula structure: How does the *yolk content* of the egg affect blastula structure?

The Anatomy of Genes

What are the basic steps in the production and processing of mRNA? How does chromatin structure affect the possibility of transcription? What are the functions of the various regions of DNA associated with the production of a functional mRNA (e.g., *enhancers/silencers* or regulatory elements, *promoter* region)? What are the functions of various *parts* of an mRNA (e.g., *introns*, *exons*, *3'-untranslated region*, *poly-A "tail"*)?

Transcriptional regulation of specific genes-
1. DNA-binding proteins and DNA regulatory domains: How do *transcription factors* bind DNA? What effect does transfection of cultured cells with *MyoD1* have? What role are *MyoD1*, *myogenin*, and *myf-5* thought to play in myogenesis? What kind of proteins are *steroid hormone receptors*? What role does *pax-6* (*small eye*) / *eyeless* / *aniridia* play in specifying eye structures in diverse organisms? What experiments suggest this?

2. Promoters*:* What is a *promoter*? An *enhancer*? A *silencer*? How can we use *reporter constructs* to study how these pieces of DNA regulate transcriptional activation? Why are reporter constructs advantageous?

General Regulation of Gene Expression: Why is it necessary for *dosage compensation* to occur in female mammals? What is a *Barr body*? What is the putative role of the *Xist* gene? Is the pattern of X chromosome inactivation random in mammalian embryos?

RNA Processing and Translational Control: What is the difference between *maternal* and *zygotic* mRNAs? When are they made? What is the *mid-blastula transition* in *Xenopus*? What effect does the *nucleus/cytoplasm ratio* have on the onset of the mid-blastula transition? How can this ratio be altered experimentally? How can *alternative splicing* be used to regulate RNA function? What are some ways in which the translation of mRNAs might be regulated? How are *3' UTRs* thought to function in regulation of translation? How does the 3'-UTR regulate *spatial localization* of mRNAs?

Sex Determination: What differences in *Wolffian and Müllerian duct regression* are there in the male and female reproductive systems? What do they become? How do the parts of the primitive sex cords differentiate in males and females? What does *Müllerian inhibitory substance* (*hormone*) do? How is the *sry* gene thought to regulate primary sexual differentiation in mammals, and what evidence is there for its role? What role is *Dax-1* thought to play in female differentiation? Based on gene translocations, why are Sry and Dax-1 thought to act as the "male gene" and a key "female gene", respectively? What happens when *androgen receptors* are defective in humans who are genetically male (*androgen insensitivity syndrome*)? What is meant by an *indifferent gonad*? What cells produce *testosterone* in the testis? Is testosterone produced in both males and females? *Hint*: from what precursor is *estrogen* synthesized in females?

Localized Determinants
1. Basic strategies of differentiation in the early embryo*: Review:* How can *localized determinants* and *inductive interactions* be used to generate differences in early embryos, and how each contributes to *autonomous* and/or *conditional specification* in the early embryo. You should be able to work with both hypothetical situations and actual examples of each. Problem sets #1 & 2 should have helped prepare you for how to work with localized determinants, for example.

2. Germ plasm in *C. elegans*: What are *P granules*? What role do the *par* genes play in regulating early asymmetries in *C. elegans*? What is "*germ plasm*"? What are some components of P granules and other germ plasm (e.g., RNA helicases, etc.)? What role does PIE-1 protein play in transcriptional inactivation in early *C. elegans* embryos?
3. Germ plasm in *Drosophila*: What is *pole plasm* in *Drosophila*? What are *pole cells?* What experiments show that pole plasm confers special properties on the cells that receive it? What role does *oskar* play in localizing pole plasm constituents at the posterior pole of the oocyte? What experiments show that *oskar* localization determines where pole cells form?
4. Primordial germ cell migration in vertebrates: What molecular signal do primordial germ cells (PGCs) use to migrate into the gonadal ridge (hint: what is *SDF-1*)? What *receptor* do PGCs use to sense this signal (hint: what is CXCR4? Are there zebrafish mutants for this gene?) You should be able to cite experiments that show that a gradient of *SDF-1* is important for directional migration of PGCs. You should be up to speed on this already through completing Problem Set #2.

***Introduction to blastula structure and gastrulation*:** What does *gastrulation* accomplish? What are the *primary germ layers*? What basic tissues do each germ layer form? What is a *blastocoel*? What are the *basic types of cell movements* that take place in gastrulating embryos? Can you think of *specific examples* of these movements? Note: for each of the examples of gastrulation we discussed in class, be able to state which type(s) of morphogenetic movements are involved.

Blastomere Specification and Gastrulation in C. elegans (also see the C. elegans handout, available on the course web site)
1. General: How does the reproducible *cell lineage* of *C. elegans* aid the analysis of cell fate? What does the cell called *EMS* produce in the normal embryo (hint: what does its daughter, called *E*, make? The other daughter, called *MS*?)?

2. Polarity of the one-celled zygote: Why are *PAR proteins* important for establishing polarity in the zygote? What is the function of *MEX proteins*, such as MEX-5? What cytoskeletal system is implicated in causing the establishment of polarity in the zygote?

3. Specification of MS: What does the protein *SKN-1* do? What kind of protein is it? How does repression of SKN-1 activity by PIE-1 restrict the activity of SKN-1 to one cell (EMS) in the early embryo?

4. Specification of E: How does signaling from the P2 blastomere induce EMS to produce a daughter cell that makes gut? Be able to cite an experiment that shows that where P2 contacts EMS determines which of its daughters will generate gut. What is the molecular basis of this signal? You should be able to predict the effects of placing blastomeres of specific genetic constitution in contact (e.g., P2 cells lacking MOM-2/Wnt, EMS lacking MOM-5/Frizzled, etc.)

5. Gastrulation: what movement(s) characterizes gastrulation in *C. elegans?* How does the *apical constriction* of ingressing cells aid their internalization? What *cytoskeletal system* mediates constriction? What *adhesion system* is required for the epidermis to cover internalized cells during *ventral enclosure* (an epiboly movement) in *C .elegans*?

Axis specification in sea urchins

1. Cytoplasmic polarity: What classic experiments show that the four cells of the four cell zygote are *totipotent* (this is review)? What classic experiments demonstrate that there is something in the *vegetal region of the egg* that is required for mesoderm and endoderm formation? Is there a localized molecule, normally associated with the Wnt pathway, that is found in the vegetal region that might contribute to this?

2. Axis specification: What experiments show that the animal and vegetal tiers of cells in the *8-cell embryo* are not equivalent, and not totipotent? What role do *micromeres* in the 16-cell embryo appear to play in induction of endoderm in progeny of overlying macromeres? What experiments demonstrate such inductive interactions? What role does *β-catenin* play in specification of vegetal cell fates in the early embryo? What role do *Nodal-like proteins* play in specification of the dorsal-ventral (aboral-oral) axis?

3. Sea urchin gastrulation
What *changes in adhesive affinity* take place as primary mesenchyme cells ingress? How do we know that these changes take place? What are some ideas put forward to account for the *initial invagination of the archenteron*? How does *cell rearrangement* result in lengthening of the archenteron? How do we know that rearrangement takes place? What role do the *filopodia of secondary mesenchyme cells* play during sea urchin gastrulation? What experiments tell us about their role? What experiments indicate that *local pattern information* is present in the sea urchin embryo for both primary and secondary mesenchyme cells? What role does the *extracellular matrix* play during sea urchin gastrulation?

Note: Please check out the revised sea urchin materials on the course web site, at:
http://worms.zoology.wisc.edu/dd2/echino/intro.html
In particular, the section on *Gastrulation* and *Patterning* will be really useful!

Anterior-Posterior Pattern Formation in Drosophila
[**Important note #1:** Dr. Dana Byrd will deliver some of thie lecture content; depending on how far she gets, some of this information may not be on exam 2, and will be deferred until Exam 3. This will likely include the sections below on segment polarity and homeotic genes. I've included them here for completeness.

Important note #2: Several helpful review materials are available on the FlyMove web site (http://flymove.uni-muenster.de/). Under "Processes", you can find sections on maternal gradients and segmentation. In addition, there is a nice Flash animation in the Content section of the course web site that provides a nice overview of A-P patterning.]
]
1. General: How do the early mitoses in the fly embryo allow for the establishment of simple gradients of proteins that might be involved in specifying pattern? [Hint: what is a *syncytial blastoderm*? A *cellular blastoderm*?] How does analysis of the *cuticle* and its associated *denticle bands* allow genetic screens to be performed in *Drosophila* that uncover defects in segmentation? What classic experiments suggested that there were posterior and anterior *"organizing centers"* in the fly egg? What are the major systems (polarizing centers, classes of zygotically acting genes) involved in specifying anterior-posterior pattern? What are the effects of mutating important genes of these systems? What are *nurse cells*? What is a *maternal effect mutation*?

Be able to outline the levels of genetic control of anterior-posterior pattern in the fly (e.g., maternal-effect system, gap genes, pair-rule genes, segment polarity genes, homeotic genes) in an essay or short-answer format. Be able to cite specific examples of each level of control that we talked about in class.

2. Bicoid and the Anterior System: What *cytoskeletal system* is responsible for localization of maternal mRNAs in *Drosophila*? How does *bicoid* function in the anterior system? What are the distributions of *bicoid* mRNA and protein in normal fly oocytes and zygotes? How is the distribution of *bicoid* protein altered in eggs from *bicoid* mutant mothers? What are the effects of additional copies of the *bicoid* gene on protein distributions? On the location and formation of anterior structures? How does the bicoid protein gradient effect the transcription of the gap gene *hunchback*? How is bicoid thought to repress translation of *caudal mRNA*?

3. Nanos and the Posterior System: How does *nanos* function in the posterior system? What are the distributions of *nanos* mRNA and protein in normal fly oocytes and zygotes? How does the *nanos* protein gradient effect the translation of hunchback mRNA?

4. Segmentation Genes in *Drosophila*: What are the basic actions of the *gap, pair-rule*, and *segment-polarity genes*? At what approximate stage of development do these various genes act? How do these classes of genes interact with one another in a general way? How do they interact with the maternal effect systems?
(a) *Gap genes*: What is the basic *expression pattern* of a typical gap gene? What happens when one is mutated? What sorts of proteins are encoded by the gap genes? How does this provide us with clues about their function(s)?
(b) *Pair-rule genes*: What is the basic expression pattern of a typical pair-rule gene? What happens when one is mutated? How do the domains of expression of pair-rule genes relate to one another? Using *even-skipped* as an example, how are the regulatory regions of pair-rule genes constructed in terms of the control of specific stripes? How can we use *reporter constructs* to dissect the enhancer elements associated with a pair rule gene like *even-skipped?*In a general sense, how could boundaries of expression of gap proteins regulate the appearance of specific stripes? What sorts of proteins are encoded by pair-rule genes like *even-skipped* and *fushi tarazu*, and what does that suggest about their function?
(c) *Segment polarity genes*: What is the basic expression pattern of a typical *segment polarity gene*? What happens when one is mutated? In a general way, how are the levels of pair-rule gene products thought to regulate where segment polarity genes are expressed? What sorts of proteins are encoded by segment polarity genes? What does this suggest about their function(s)? How does this differ from earlier stages of development? What role is *hedgehog* thought to play in communication between *wingless* expressing cells and *engrailed* expressing cells? Be sure you know the major components of the *wingless* signal transduction pathway. What role is *β-catenin* (aka *armadillo*) thought to play in the *wingless* signaling pathway?

5. Gastrulation: what morphogenetic movement(s) characterizes gastrulation in *Drosophila?* What is the *ventral furrow?* What is *germ band extension*? What set of cells are internalized through germ band extension?

6. Homeotic Genes in Flies*:* What is a *homeotic mutation*? What DNA sequence (and therefore domain of the protein) is similar in all of these genes? In flies, what happens when many of these genes are mutated? How are the regions of expression of these genes related to their *position on the chromosome*? What other classes of genes are thought to regulate the expression of the homeotic genes? Do vertebrates have genes similar to the homeotic genes, and if so, how many? How are they similar and different from those in flies?

Reminder: The review session is April 6, 4:30-6 pm, place TBA. Exam 2 is Tuesday, April 7, 7:15-8:45 pm, place TBA. Good luck!