

BIOL 369: Developmental Biology
Exam 1: In-class portion [60 pts]
12 Oct 2009

Student ID#: _____

INSTRUCTIONS: Your responses will be graded based on the correctness, appropriateness, and thoroughness of your answers, keeping in mind that for most questions there is no one right answer. Rather, you are expected to support your answers with appropriate observations, experimental evidence, and/or methodologies. You will lose credit if what you write is wrong. You will receive no credit if what you write is true but does not answer the question. You may not receive all the credit the question is worth if you leave out what I consider to be important key points. The key is to provide *concise* but *complete* answers. Each question has multiple parts. Be sure to clearly label your answers where appropriate (e.g. 1A, 1B, etc.).

1. [10 pts] Compare and contrast the acrosomal reaction in sperm and the cortical reaction ("slow block" to polyspermy) in eggs. (A) Be sure to discuss what triggers the reactions, any common key signaling molecules, the cellular origin(s) of the organelles involved, as well as the contents and function(s) of the organelles. (B) Describe one experiment that revealed some aspect of either the acrosomal or cortical reaction (e.g. role of a signaling molecule, or function of the reaction itself). Categorize your experiment as to whether it was a "find it", "block it", or "move it" experiment.

(A) Acrosomal rxn triggered by compounds in egg jelly (in sea urchins) and glycoproteins in zona pellucida (mammals) [1 pt] whereas cortical rxn triggered by binding of sperm to egg plasma membrane [1 pt]; in both cases, receptor activation triggers signal transduction pathway (e.g. iP_3) leading to release of Ca^{++} [1 pt] from endoplasmic reticulum causing exocytosis of **Golgi-derived** [1 pt] vesicles; contents of acrosomal vesicle are primarily digestive enzymes that break down egg jelly or z.p. aiding access of the sperm to the egg [1 pt], as well as bindin which is on the surface of the acrosomal process (result of polymerization of G-actin to F-actin on cytoplasmic side of acrosomal vesicle) and aids in species-specific attachment to the egg plasma membrane; contents of cortical granules include proteolytic enzymes that break bonds with bindin of other sperm, osmolytes that aid swelling of vitelline envelope and hardening agents that help it transform into the fertilization envelope, all of which contribute to the "slow block" to polyspermy [2 pts]

(B) [3 pts] Some possible examples: "Find It" experiments showing location of bindin on acrosomal process OR calcium wave in egg following fertilization

"Block It" experiments such as examining acrosomal or cortical reaction in Ca^{++} -free water, demonstrating that Ca^{++} comes from intracellular stores

"Move It" experiments in which Ca^{++} is released by a calcium ionophore in the absence of sperm resulting in lift-off of fertilization envelope.

2. [20 pts] Hilde Mangold and Hans Spemann defined the dorsal blastopore lip in the gastrulating frog embryo as “the organizer” and the functions of the organizer as the “primary inducer.” (A) What does the organizer do? (B) What does the organizer become? (C) What is the actual *primary* inductive event in the frog? (D) What is the equivalent tissue in the sea urchin, in the zebrafish, and in the chick? (E) Name one protein that is commonly expressed in all these organizers. (F) Describe one experiment that demonstrated the inductive ability of one of these organizers. Did your experiment also demonstrate whether the organizer (or equivalent tissue) was *specified* and/or *determined*? If so, explain. Did your experiment also demonstrate whether the organizer (or equivalent tissue) was *necessary* and/or *sufficient* to cause the inductive event? If so, explain.

(A) [4 pts] initiates the movements of gastrulation; dorsalizes surrounding mesoderm so that forms somites (axial mesodermal fates); dorsalizes overlying ectoderm so that becomes neural; capable of setting up a secondary axis

(B) [2 pts] primarily notochord (also pharyngeal endoderm and head mesoderm)

(C) [3 pts] fertilization: site of sperm entry determines direction of cortical rotation → activates β catenin on future dorsal side → induction of Neiukoop Center → induction of overlying cells to become dorsal mesoderm (the “organizer”)

(D) [3 pts] micromeres in sea urchins, embryonic shield in zebrafish, hensen’s node in chicks

(E) [2 pts] accepted β catenin (because of sea urchins) but was looking for noggin, chordin, and other “organizer molecules” that, admittedly, are more vertebrate-specific

(F) [6 pts] Most obvious experiments were all the transplantation experiments conducted in which micromeres were placed on top of mesomeres (animal pole) in sea urchins or vertebrate organizers were transplanted to ventral sides of embryos; all represent “move it” experiments demonstrating that organizer tissue is both *determined* (irreversibly committed) at time of transplantation and *sufficient* to induce surrounding tissue to adopt dorsal fates.

3. [10 pts] As part of this course, you have been introduced to several “model” organisms: the nematode *C. elegans*, the sea urchin *Lytechinus variegatus*, the frog *Xenopus laevis*, the zebrafish *Danio rerio*, the chick, and the mouse. These species are considered relatively ideal for *developmental* studies (as opposed to strictly *genetic* studies). (A) Choose one of these species and describe as many characteristics as you can that make it a good experimental model for developmental biology. (B) Describe one feature of your chosen model that makes it less than perfect as a model for d-bio.

(A) [8 pts] Answers will vary depending on which model chosen but most common feature is fast *embryonic* development (but not necessarily *generation* time); other answers, depending on model chosen, might include lots of progeny, transparent embryos, year-round reproduction

(B) [2 pts] Some possible answers:

For *C. elegans*: too small to isolate individual tissues and do biochemical analyses, not a vertebrate

For *L. variegatus*: only seasonally reproductive

Sea urchins, frog and chick: cannot do forward genetics (generation times too long)

Zebrafish: hmmm, not sure, it's pretty ideal—maybe we'll find out during our visit to the fish facility! Actually, maintenance of fish tanks is pretty expensive

Mouse: small litter sizes (large for a mammal, but small compared to other models on the list), internal fertilization and development (difficult to observe/manipulate)

4. [10 pts] Briefly, discuss how selective cell adhesion contributes to the process of gastrulation in the sea urchin embryo. Describe ONE example for TWO of the following stages: ingression of the primary mesenchyme, and/or first stage (invagination), second stage (convergent extension), or third stage of archenteron formation. (It is more important to describe conceptually what is happening for each example than to remember specific molecules mediating the process.)

[5 pts for each example]

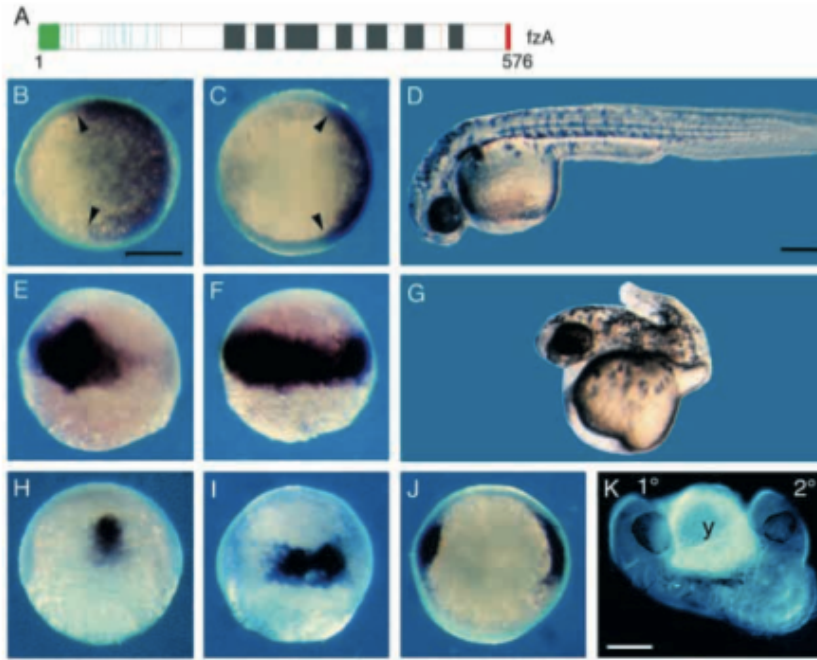
Ingression of primary mesenchyme: cells must lose affinity for their neighbors and for the hyaline layer and gain affinity for the basal lamina (lining of blastocoel) and eventually re-gain affinity for each other

First stage of archenteron formation: cells retain affinity for each other with strong cell-cell adhesion, lose affinity for hyaline, and gain affinity for basal lamina; this along with secretion of ions along apical surface causes osmotic swelling between layer of cells and hyaline and causes cell layer to “buckle” inward → invagination

Second stage: cells progressively lose and re-gain affinity for each other as they migrate toward animal pole and intercalate

Third stage: secondary mesenchyme cells retain affinity with adjacent macromere-derived cells forming the gut while extending filopodia that seek a ligand expressed on the roof of the blastocoel at the site where the mouth will invaginate; when ligand binds to receptors on filopodia, filopodia adhere to the spot and then rest of cell contracts, pulling up rest of archenteron to roof of blastocoel; once gut is complete, secondary mesenchyme cells lose affinity for their macromere-derived neighbors and migrate to either side of gut to form mesodermal-derived organs.

5. [10 pts] The following figure is from a paper on the role of *frizzled* (*fzA*), a *wnt* receptor, in determining dorsal mesoderm in the zebrafish.



Effects of misexpression of *fzA* in the zebrafish embryos. (A) Representation of *fzA* protein. B,E, and H show comparably staged control embryos. C,F,I, J, and K show *fzA* mRNA-injected embryos, stained in situ. (B,C) Animal pole view of shield stage embryos stained for GATA-2, a ventral marker. (E,F) Dorsal view of shield stage embryos stained for chordin (H,I) Dorsal view of 60% epiboly stage embryos stained for goosecoid (J) Animal pole view of the same stage injected embryo stained for goosecoid. Note two stained regions corresponding to endogenous and ectopic shields. (D) 28 hour control and (G) *fzA*-injected embryo. Note reduced trunk and tail structures. (K) Lateral view of an ectopic axis generated as a result of *fzA* misexpression.

(A) Does *frizzled* (also referred to as *fzA*) promote *dorsal* or *ventral* fates in the zebrafish?
 (B) Does the experiment above demonstrate the *necessity* and/or *sufficiency* of *frizzled* in specifying dorsal (or ventral) fates?
 (C) Describe one additional experiment you would like to see these investigators conduct that would further define *frizzled*'s role with regard to the question posed in part B. Be specific as to what technique(s) you would use in your experiment to manipulate *fzA* and to monitor the results of the manipulation.

(A) [4 pts] dorsal fates

(B) [2 pts] sufficiency

(C) [4 pts] loss-of-function, for example caused by injection of antisense morpholinos against *fzA* to determine if necessary to specificity dorsal fates; if so, would expect to generate ventralized embryo (e.g. missing head structures, notochord, dorsal nerve chord, etc.)