Supplemental Materials

for

Long-Term Retention of Knowledge and Critical Thinking Skills in Developmental Biology

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Appendix 1. Developmental Biology Assessment Test (DBAT). The 20 multiple-choice questions used as a long-term learning metric are provided with the correct answer highlighted in yellow. The question categories are identified as indicated for Factual Recall (FR), Concept Analysis (CA), Data Analysis (DA), and Experimental Design (ED). The highest DBAT score obtained was 18 out of 20 correct. The overall performance for each question is provided as percent correct for the 2006 to 2010 participants. The questions were presented in the order shown for all students. Figures associated with the questions were generated by the authors or were modified from Gilbert and used with permission of the publisher (Sinauer Associates, Inc.).

Appendix 1: Developmental Biology Assessment Test (DBAT)

**Concept Application**
1. Which one of the following best fits the description of “differentiation” in Developmental Biology? (48.4% correct)
   A. Differentiation is the process whereby a single proliferating cell is prevented from undergoing additional rounds of cell division during its life span and the process is independent of the type of organ or tissue of which the cell is a component part.
   B. In every individual organism, differentiation is defined as one set of genes that alter the shape of a cell or tissue structure to match a particular function for that cell or tissue.
   C. Differentiation occurs when one cell converts its “fate” from one lineage to another by turning on a completely different set of genes than were previously expressed.
   D. Differentiation is the process whereby a single cell (fertilized egg) can give rise to all the cellular diversity in a given organism and it is regulated by the organism’s genomic content.
   E. Differentiation is the switch from a proliferating to a post-mitotic cell.

**Data Analysis**
2. You perform fate map studies comparing early embryonic derivatives in zebrafish, frog, mouse and chick at early gastrula stage (figure to the right). You are investigating an endoderm-specific gene, gene end. You find that it is expressed in a similar pattern restricted to the presumptive gut in all four species. You next examine a tunicate embryo and find that gene end is present in tunicate and its expression is also restricted to presumptive gut. What can you conclude from the results of your expression comparison? (91.2% correct)
   A. Gene end expression precedes specification of the three germ layers since it is expressed in all species examined.
   B. Gene end is conserved across a wide range of organisms, indicating a common ancestral expression pattern linked to endoderm fate determination or tissue-specific function.
   C. Gene end expression is found in only in the endoderm because cells that express it must have contact with the developing notochord.
   D. Gene end is required for contact-based cell fate decisions when mesoderm separates from neural ectoderm early in the gastrulation process since it is observed in all species examined.
**Factual Recall**
3. There are several ways to control gene expression during development at the level of translation. Which of the following would be most effective for that purpose? (28.6% correct)
A. Histone acetylation of RNA  
B. DNA methylation  
C. Differential mRNA longevity  
D. Hamburger/Hamilton staging  
E. Direct kinase phosphorylation and dephosphorylation of RNA

**Experimental Design**
4. You are investigating how cells interact with one another during early development, particularly in cell-cell interactions via cadherins. A cartoon of cadherin-cadherin binding on the cell surface is shown. Of the experimental choices detailed below, which one will allow you to test most directly the role of calcium in cadherin-cadherin interactions in migrating epithelial cells? (46.2% correct)
A. You compare epithelial cells grown in normal calcium-containing culture medium versus medium that has been calcium-depleted and then compare migration ability.  
B. You divide your population of epithelial cells equally into three treatment conditions (normal calcium, high calcium and cadherin-blocking antibody) and compare the migration ability among the groups.  
C. You overexpress your cadherin gene of interest in the epithelial cells and compare their migration ability relative to epithelial cells with control expression vector.  
D. You treat migrating epithelial cells in culture with increasing doses of calcium, in addition to the normal calcium levels present in the culture medium, and determine which treated populations migrate faster.

**Concept Application**
5. Of the following statements regarding specification during the differentiation process which statement is true? (83.5% correct)
A. Autonomous specification allows for flexibility in the gene expression program so that a given cell may independently change its fate if the cellular microenvironment changes during development.  
B. Conditional specification is predominantly used by members of the Insecta, particularly dipterans like *Drosophila melanogaster* and is rarely observed in higher order taxa.  
C. Autonomous specification is a mode of cellular commitment that is dependent on a set of transcription factors that direct a cell toward a particular fate, regardless of the influence of surrounding cells or factors.  
D. Conditional specification is a mode of cellular commitment that is dependent on a set of transcription factors that direct a cell toward a particular fate, regardless of the influence of surrounding cells or factors.
**Data Analysis**

6. After sperm contact with the egg during sea urchin fertilization, the membrane potential rapidly goes from negative 70 mV to close to positive 50 mV. If sea urchin eggs are fertilized in varying concentrations of sodium, there is a change in polyspermy incidence as indicated in the data table. Which of the following statements regarding the data is correct? (100% correct)

A. The 120 mM sodium concentration allows for 97% of the eggs to be normal.
B. Sodium concentration changes are not correlated with polyspermy.
C. As the sodium concentration goes down, the percentage of polyspermic eggs decreases.
D. As the sodium concentration goes down, the percentage of polyspermic eggs increases.

<table>
<thead>
<tr>
<th>Na⁺ (mM)</th>
<th>Percentage of polyspermic eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>490</td>
<td>22</td>
</tr>
<tr>
<td>360</td>
<td>26</td>
</tr>
<tr>
<td>120</td>
<td>97</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

**Factual Recall**

7. Which of the following is a FALSE statement? (28.6% correct)

A. Rotational (and isolecithal) cleavage is commonly found in fish, reptile and bird embryos.
B. Isolecithal cleavage is common in organisms with sparse and evenly distributed yolk.
C. Centrolecithal cleavage is characteristic of most insects that have yolk in the egg’s center.
D. Meroblastic cleavage occurs when the egg is not completely divided during the first cell division.

**Experimental Design**

8. You are investigating specification of the oocyte anterior-posterior (A-P) axis in *Drosophila melanogaster*. You want to determine if anterior localization of *bicoid* mRNA is required for normal A-P axis formation. Which of the following approaches is the most direct way to test this? (54.9% correct)

A. Overexpress *bicoid* mRNA after fertilization and determine if the A-P axis was affected retroactively.
B. Use a *bicoid* expression construct to distribute *bicoid* mRNA uniformly throughout the oocyte and see if the A-P axis is still formed.
C. Target *bicoid* mRNA for degradation with specific siRNA and determine if A-P axis is still formed.
D. Immunolabel for bicoid protein and see if it is localized in the anterior of the oocyte.

**Concept Application**

9. Which of the following statements about zebrafish gastrulation is TRUE? (32.9% correct)

A. The anterior portion of the embryonic shield establishes the primary dorsal-ventral axis in the zebrafish and allows gastrulation to initiate.
B. The Nieuwkoop Center is established when the cortical rotation of yolk cytoplasm occurs immediately after fertilization.
C. Active cell movement of blastoderm cells over the yolk mass surface is referred to as epiboly and is part of gastrulation.
D. The first cleavage division of a fertilized zebrafish egg completely cleaves the egg, splitting the yolk entirely and establishing a blastopore lip.
**Data Analysis**

10. You are investigating the role of Fgf8 and Nodal proteins in mesoderm induction and initiation of mesoderm cells through the primitive streak in *Gallus gallus*. You block Fgf8 and Nodal expression separately and in combination with siRNA using all the proper controls and six embryos per condition. All of the embryos survive the short assay period. You then measure mRNA expression of the early mesoderm marker, **Tbx6**, using quantitative PCR. You obtain the graph shown. One-way ANOVA indicates that the means are significantly different with an alpha value set at 0.05 confidence and a determined p-value < 0.0001. The asterisks indicate p-value < 0.001 for Tukey’s Multiple Comparison Post Test. Which of the following is a correct description of what you can conclude from the data? (81.3% correct)

A. Fgf8 siRNA has minimal effect on Tbx6 expression levels.

B. Nodal siRNA has minimal effect on Tbx6 expression levels.

C. The Tbx6 expression levels with control siRNA are statistically different from untreated.

D. The Fgf8 siRNA is nearly as effective as the combination of FGF8 and Nodal siRNA in preventing expression of Tbx6.

E. The Nodal siRNA causes a statistically significant drop in Tbx6 expression.

**Experimental Design**

11. You are investigating molecular mechanisms of neurulation during the organogenesis phase of development in the frog. You hypothesize that conversion of the neural plate to the neural tube requires direct contact between the forming notochord and the overlying ventral neural folds. Of the microdissection and explant experiments described below, which will be most helpful in allowing you to test your hypothesis (assuming all technical and control components go smoothly)? (79.1% correct)

A. Combine two neural plate explants on opposite sides of a porous membrane and determine whether or not neural tubes form on one, both, or neither side of the membrane.

B. Place a non-porous membrane between the forming neural folds and the underlying notochord and determine if a neural tube is formed.

C. Place a non-porous membrane underneath the notochord, allowing contact between the notochord and the overlying neural folds, and determine if a neural tube is formed.

D. Place beads coated with Fgf2 or BMP4 in the cup of the neural fold and determine whether or not a neural tube forms.

**Factual Recall**

12. You are investigating cellular microenvironmental influences on neural crest cell fate in mammals. You are interested in the cellular derivatives of the second pathway (dorsolateral pathway). If you were to disrupt this neural crest migration pathway, in which cell type would you most likely see an effect? (17.6% correct)

A. dorsal root ganglion neurons

B. autonomic neurons

C. adrenomedullary cells

D. Schwann cells

E. melanocytes
Data Analysis
13. BMP2 has been shown to be important in the transition between mesenchymal cells and prechondrocytes in bone formation in the mouse. However, you are investigating early regulatory elements in bone formation in alligators. You treat primary alligator mesenchymal cells with BMP2 inhibitors and see normal formation of prechondrocytes. Of the following interpretations of your preliminary results, which one best fits the observed results? (59.3% correct)
A. The BMP2 inhibitors may not be working effectively in alligators and additional experimentation needs to be done before a conclusion can be drawn from the results.
B. Primary alligator mesenchymal cells do not transition to prechondrocytes in the alligator under normal developmental conditions since they are not required for bone formation.
C. Prechondrocyte formation is not a precursor cell type in alligators since alligators require an additional transitional cell type to form the chondrocyte cell type.
D. BMP2 is not involved in any stage of bone formation in alligators, but BMP4 is a likely candidate.

Factual Recall
14. The splanchnopleure consists of the ventral layer of the lateral plate mesoderm and the underlying endoderm. The somatopleure consists of ________. (64.8% correct)
A. coelom and underlying endoderm
B. neural crest and neural tube
C. midgut cavity and overlying ventral notochord
D. dorsal lateral plate mesoderm and overlying ectoderm

Concept Application
15. Which of the following statements regarding limb formation in chickens is FALSE? (32.9% correct)
A. Posterior limb bud expression of sonic hedgehog in the zone polarizing activity is a contributing factor in digit identity during limb formation.
B. T-box transcription factors (Tbx5 and Tbx4) regulate specific identity information for forelimb and hindlimb regions, respectively.
C. The apical ectodermal ridge is a specialized area of mesoderm that outlines the perimeter of the presumptive hindlimb field.
D. Reciprocal molecular interactions between the lateral plate mesoderm and overlying ectoderm are required for specification of the limb field and formation of the limb bud.

Experimental Design
16. *Dictyostelium discoideum* have the capacity to switch between single cell myxamoebae and multicellular grex in response to nutritional stress in their environment. You hypothesize that the absence of arginine signals nutritional insufficiency and promotes the transition between myxamoebae and grex (slug). Which of the following experimental approaches will allow you to directly test your hypothesis? (91.2% correct)
A. Establish myxamoebae growth in two dishes with (1) a normal nutritional environment that includes arginine and (2) the same nutritional environment without arginine. Determine if grex form in either or both conditions.
B. Establish myxamoebae growth in two dishes with (1) low dose arginine and (2) high dose arginine. Determine if grex form in either or both conditions.
C. Establish myxamoebae growth in two dishes with (1) a normal nutritional environment containing an arginine precursor and (2) the arginine precursor alone. Determine if grex form in either or both conditions.

D. Establish myxamoebae growth in two dishes with (1) an arginine precursor and (2) the processed arginine. Determine if grex form in either or both conditions.

**Experimental Design**

17. You are interested in environmental influences on sex determination in the marine worm *Bonellia viridis*. In this species, larval contact with rock surfaces or females directs sex determination toward females or males, respectively. You want to set up an experiment that will allow you to determine if putative gene *tedxes* directs the switch to a male gender in an unspecified larval *B. viridis*. Among the choices below, which is the most direct experimental route to obtain your answer? (68.1% correct)

A. Run a complete gene array analysis on mRNA purified from male versus female *B. viridis* right after gender determination in the larva. Determine if the male expression of *tedxes* is higher than in the female.

B. Use a neutralizing antibody against the *tedxes* protein product and treat male *B. viridis* with the antibody and look for a switch to the female gender with blockade of the *tedxes* protein.

C. Express the *tedxes* gene in unspecified larval *B. viridis* and determine whether expression is able to direct the larval worm to the male gender.

D. Express the *tedxes* gene in female adult *B. viridis* and determine whether expression is able to redirect the worm to the male gender.

**Factual Recall**

18. Which of the following statements regarding holometabolous insect larva imaginal discs is most accurate? (62.6% correct)

A. The axes of leg imaginal disc cells are not established at the molecular level until the final instar transition is complete.

B. Imaginal discs are cells that are set aside in early embryogenesis to produce adult structures later in development.

C. Imaginal disc cells are fated to become the germ cell population in insects and are set aside during early cleavage stages.

D. Imaginal disc cells do not undergo proliferation in ametabolous insect pronymphs until the final stage prior to the adult form.

**Concept Application**

19. Which of the following is NOT an example of polyphenism? (78% correct)

A. Larva of some locust species can develop into either low-density or high-density morphs depending on environmental conditions.

B. Male dung beetles may develop as either a horned or a hornless adult depending on the level of juvenile hormone to which they were exposed at their last morph.

C. The leg muscle mass in humans varies with type and intensity of exercise in the adult.

D. In some colony wasp species, all larva can develop into either a worker or a queen based on the level of nutritional support received during early developmental stages.
Data Analysis
20. You are investigating the impact of heavy metal toxins on larval insect diversity in the prairie pothole region of North Dakota. You collect samples from site A that has low toxin levels in the soil and water and from site B that has extremely high levels in the soil and water. You have done three temporally paired collections from each site over the course of a three-week period. When prepping the samples for analysis, you realize that the bin containing the last time point collection from site B has a hole in the bottom and none of your original samples remain in the container. You want to determine the most accurate, ethical resolution to your research dilemma. Which of the following is the best solution?

(91.2% correct)
A. Since one time point is missing, you discard all samples from the two sites. You report a failed season to your funding agency.
B. You extrapolate the site B data from the second intact time point collection and compare this new data set to the site A material from the third time point. You decide not to mention the extrapolation in your research report since both the other site B collections were similar in content and not statistically different in the number of representative species.
C. You assess larval insect diversity in all five of your remaining sample sets (three from site A and two from site B). You analyze the data in two ways: assess changes over time points available within each site and then compare the sites at the two time points for which samples remain. You report all research results, stating that a third site B time point was collected but lost.
D. You assess larval insect diversity in all five of your remaining sample sets. You pool the diversity data from the three site A time points and then pool the data from the two site B time points. You then directly compare the larval diversity of “pooled A” and “pooled B” sample sets.
Appendix 2. Scientific Literacy Assignment for Primary Literature Critique (PLC). The in-class assignment that is the foundation for the Primary Literature Critique is provided. The students were required to choose from a pool of pre-selected, peer-reviewed primary research articles selected by the instructor to represent a broad array of developmentally relevant research published within the last year. Students were required to have read the paper prior to the in-class assignment and completed a preliminary outline for their document by the end of the class period. The components of the assignment were designed to guide students through the initial steps of delving more thoroughly into their chosen research article.

Appendix 2: Primary Literature Critique Assignment

Assignment Goal. This assignment will provide students with an opportunity to complete an in-depth study of a primary research article and then write a high quality critique of the study and its relevance to Developmental Biology. The purpose is to help students develop scientific literacy skills in understanding the scientific method, becoming familiar with different technical research approaches, learning critical thinking skills, and communicating their knowledge through the written word.

Assignment Details. The Primary Literature Critique will consist of a report based on one of 8-12 available papers in the general area of developmental biology research. The papers are available as PDF files through Blackboard. Your written critique must follow the format below.

- The BODY of the paper should be 3-5 double-spaced, typed pages using font size 12 and Times New Roman or Arial script.
- Your paper should have one-inch margins on each side. Two full pages and a partial third page will not meet the requirements for this paper!
- The paper must include 9-12 citations from peer-reviewed scientific literature that are used to support your claims, comments or criticisms.
- Full citations must be listed in a complete bibliography at the end of the paper and cited in “Author/Date” or American Psychological Association (APA) format within the text. Note: the bibliography is not part of the 3-5 page content.

Approaching/Interpreting the paper

I. Title, authors and heading information
This information is listed above the abstract. Be sure to reference the topic paper early in the review document. You should include the reference for your chosen paper in your bibliography.

II. Understanding the Abstract
If written well, the Abstract contains all the pertinent information required to put the results of the study into a larger context. The Abstract should establish the research topic, identify the primary objective of the research, indicate methodology (approach), BRIEFLY list the main finding(s) and offer the authors’ main conclusion(s) for the study.
III. Reviewing the Figures/Results
Identify key figures that you think are critical to demonstrate the most pertinent points of the paper. You may not need to describe all of them, particularly if there are extensive technical or reagent control experiments included in the manuscript.

III. Contextual assessment of Results
The key to this section is fitting the results into the context of the authors' previous work and that of others in the field. How do the results differ from previously published work? How do the results change your view of how the system works/develops? Is there a controversy in the field that these results resolve? To guide you in addressing these questions, it may be helpful to read a recent review on the topic of your paper. You will have to read and reference several papers to provide support for your commentary. Be sure the references are complete in your bibliography (Authors, year, title, full reference). Cite Author/Date within the text.

Outlining your research Primary Literature Critique

The following are suggested sections to include in your Primary Literature Critique.

- Begin with a brief overview of the general area/big idea behind the research goal of the paper.
- What is the hypothesis? What model system(s) used to test the hypothesis?
- Provide a general description of the key technical approaches used in the study with particular emphasis on the rationale for the approach.
- Identify, but do not list the most important results of the study, including their interpretation.
- Discuss the impact and relevance of the study/results on our understanding of developmental biology.
- You may wish to include a brief section describing where you would take the project next if this were your research.

Sample Outline:

I. Developmental Context of Research/Brief Description of general goals
- Research context
- Rationale for study
- Hypothesis
- Model System/general approach to test hypothesis

II. Technical Approaches and Rationale for Approach
- Specific techniques and/or experimental design
- Include rationale and advantages/disadvantages where relevant
  e.g., “In order to test their hypothesis, the authors first did....”

III. Key Results of the Study
- Critical results that came out of the study
- Glaring problems (?)
IV. Relevance of the Results/Impact on Developmental Biology

- Meaning of the results
- Alternative interpretations
- Value of the results
- How do the results/interpretation alter our understanding of developmental biology?

Formatting Requirements for Outline: You must turn in a typed outline with your name, the date, and the full reference for your Topic Review article.
Beginning the writing process

Using your outline as a guide, begin to build your topic sentences and supporting ideas into sections of text. Skip around to different sections as ideas come to you. Please do not save the writing until the last minute. Your “draft” that is due on DATE should be what you consider to be a final version, including a complete bibliography and correct formatting.

Self-critique (a.k.a. how to stay objective about your own work)
Do the topic sentences flow?
If reading only the topic sentences, do the main points come across clearly?
Do sentences within a given paragraph relate to the topic sentence of that paragraph?
Did you provide clear transitional sentences/ideas between sections?
Do you agree/disagree with the authors’ conclusions about their study?
Have you provided sufficient supporting statements (with references) indicating your view?
What did you learn from reading the paper? Is “what you learned” expressed in your review?
Did you successfully place the research project into a broader perspective that will facilitate your readers’ understanding of the importance of the topic?

Questions to help you interpret and understand your research article

What is the primary research topic as described in the abstract? (think “Big Picture”)

What is the primary objective of the research?

Why is this area of investigation important?

What are the main methodological approaches used?

At what level is there experimental integration?

What are the main findings/results as described in the abstract?

What is the main conclusion(s) of the study?

How are the results/interpretations going to affect our current knowledge of Developmental Biology?
Now that you have answered these questions and read your paper in depth, you are ready to build your outline and begin the writing process!