BIOL 369 Developmental Biology

Laboratory Schedule and Syllabus, Fall 2009

Thursdays 1:20-4:30

Instructor: Mary K. Montgomery, Associate Professor

Sept 10 **Mitosis and Meiosis: *C. elegans* Histone-GFP lines**
Concepts: mitosis, meiosis, epifluorescence microscopy, *gfp* and transgene markers
Techniques: " **Find It**": epifluorescence microscopy to view *C. elegans* adults and embryos carrying gfp-tagged histones that reveal nuclei in various stages of mitosis and meiosis
Organism(s): nematode *Caenorhabditis elegans*

Sept 17 **Echinoid Development: Fertilization and Early Development**
Concepts: fertilization, radial holoblastic cleavage, gastrulation, tissue markers
Techniques: " **Find It**": dark-field and polarizing techniques in microscopy to view cilia and spicule skeleton; alkaline-phosphatase staining of gut
Organism(s): sea urchin *Lytechinus variegatus*
(continue observations over next few days)

Sept 21 (Lecture class time)
Echinoid Development: Experimental Design
Part One: Pair up with partner & design experiment to test effects of some factor on development (e.g. UV radiation, temperature, microtubule and/or microfilament function, sperm activation, zygotic transcription, ß-catenin signaling, etc.); Consider controls, feasibility, significant numbers for stats/analysis, phenotypic markers
Part Two: Lab group meeting: present experimental design to rest of class
Part Three: Modify experimental design based on feedback
Part Four: Draw up detailed experimental plans, including list of supplies and reagents (including calculations for making reagents)

Sept 24 **Echinoid Development: Perform Experiment**

Oct 1 **Zebrafish (Visit zebrafish facility at U MN)**
Concepts: meroblastic cleavage, vertebrate development

Techniques: " **Find It**": GFP and DsRed transgene reporters
Organism(s): teleost fish *Danio rerio*
(continue observations over next few days)

Oct 8 **Chick Development**
Concepts: meroblastic cleavage, vertebrate development
Techniques: microdissection
Organism(s): chicken *Gallus gallus*
DUE by MON, OCT 5: FIRST DRAFT OF PAPER ON SEA URCHINS
DUE by OCT 8: COMMENTS ON TWO CLASSMATES' DRAFTS

Oct 15 **More Chick Development and Cardia bifida**
Concepts: organogenesis, developmental "windows", mosaic vs regulative development, *in vitro* culture
Techniques: " **Block It**": microsurgery
Organism(s): chicken *Gallus gallus*
(view results of cardia bifida experiment over next 24-48 hrs)
FINAL DRAFT: PAPER ON SEA URCHINS (+ LAB NOTES)

Oct 22 **Fruitfly Development**
Concepts: axis specification; analysis of gene function (loss-of-function vs gain-of-function)
Techniques: " **Find It**": lacZ and gfp transgenic lines ; " **Block It**": loss-of-function genetic mutants; " **Move It**": misexpression using heat shock-driven transgenes
Organism(s): fruitfly *Drosophila melanogaster*
SUMMARY ABSTRACT ON CARDIA BIFIDA DUE

Nov 5 - Dec 10 **RNAi Experiment**
Concepts: reverse genetics; double knockdown
Techniques: " **Block It**": RNA interference (RNAi), feeding delivery method
Organism(s): *C. elegans*

Tentative Schedule:

Nov 5	Review of RNAi literature; choose genes; order bacterial feeding strains
Nov 12	Prep/culture of bacterial feeding strains
Nov 19	Exposure to dsRNA/appropriate feeding strains and analysis of phenotype(s)
Nov 26	Thanksgiving Break
Dec 3	Feeding/analysis continued
Dec 10	**Clean Up**

These labs have been designed to introduce you to many of the **concepts** of developmental biology and the various **techniques** and **model organisms** used to address questions in this currently exploding field. Each week we will cover

methods that fall under one of three broad experimental approaches (referred to here as "Find It, Block It, Move It" experiments-- coined by Professor Dany Adams when she worked at Smith College). **Find It** experiments typically involve labeling of specific gene products, cell types or tissues. Development is a process by which the totipotent fertilized egg undergoes rounds of cell division followed by differentiation (i.e., cells become specialized to perform specific functions). This process involves differential gene expression (i.e., muscle cells are different from neurons because the former express muscle-specific proteins that mediate contraction whereas neurons express neuron-specific proteins, e.g. neurotransmitters) as well as localized and global signaling events. Labeling or "find it" experiments allow the investigator to localize in the embryo where and when certain genes or cell types are expressed. This type of observational study is often used to establish a *correlation* between the presence of a gene product or cell type and a developmental event under study. To establish *cause and effect* more experimental approaches are needed. Blocking expression or activity of specific genes, cells, or tissues can show that they are *necessary* for a specific developmental process; these types of experiments are considered loss-of-function (i.e. "**Block It** ") experiments. An even more powerful approach is to show that a gene or cell's activity is *sufficient* to cause a developmental process to occur; the results of such experiments are referred to as gain-of-function evidence and are obtained by moving expression or activity to a time or place in the embryo when/where it would not normally be present ("**Move It** "). All three experimental approaches are necessary for an investigator to establish that a particular molecule, cell, or tissue is both necessary and sufficient to cause a downstream event: e.g., (1) Find It experiments might establish that a particular protein is present at the right place and time to affect the event; (2) Block It experiments might show that the event does not take place in the absence of the protein; and (3) Move It experiments might demonstrate that the protein will cause the event to occur at a time or place in which neither would normally be present/occur. Often labeling methods will be used to determine whether gene expression, cell fates, etc. have been altered by blocking or moving activity. If this hypothetical protein behaves as predicted in all three experiments, we can say that it is both *necessary and sufficient* to cause the event. It is not uncommon, however, to find that a product is necessary but not sufficient to cause a process to occur; more rarely, some products are sufficient but not necessary. Finally, a well-designed experiment includes all proper *controls*, and we will discuss and include during the course of the semester appropriate controls for all of our experiments.

Although I have done my best to design labs that can be covered within a 3 hour time period, the reality is that organisms develop on their own timetables and real science is hard to do under such time constraints. All the labs will require that you come back to make additional observations or finish an experiment at times later that day or week. As we are in Minnesota, this means there will be times when you will have to trudge back through the snow to peer down your microscope. In most cases, you will find your efforts well rewarded! The final month of the course we will carry out independent projects involving RNA interference (RNAi) in *C. elegans.*

Working in pairs, you will get to choose two genes to target, select and order genetically engineered dsRNA-producing E. coli strains that target your genes of interest, and perform an RNAi experiment with these strains to determine the double-knockdown phenotype.

LAB PAPERS : You will be asked to write two papers to be written in scientific format. Mary Tyler has a very good section on how to write a scientific paper in the 1st chapter of her *Developmental Biology: A Guide for Experimental Study* (included as an electronic supplement in the CD that accompanies your textbook). I will also have some additional handouts on writing scientific papers. The format of your papers should strictly follow that of the journal *Development*. For each lab I will post on Moodle at least one paper from the primary literature that is related to the work we are doing for that particular lab; each paper can serve as a starting point from which you can delve deeper into the subject at hand either for your own paper or simply your pleasure. Papers that miss the deadlines listed in the syllabus will be assessed a 10 point penalty for each day they are late (just like most libraries and credit card companies- unlike most granting and hiring agencies, which simply toss the proposal or job application in the trash if the deadline is missed)- so you can turn your papers in late but you will pay a price.

For the first paper, you will turn in (by uploading to Moodle) a first draft after the lab is complete; two of your classmates will offer comments. Comments can be made directly on the drafts but each of you will also need to fill out a "Comments on First Draft" sheet for two of your classmates' papers, due a few days after receiving the drafts. The first draft will not be graded but your comments on others' papers will be! You will get full credit if you do a thorough and thoughtful job of offering appropriate feedback. This is an important service that you can offer one another and is also the kind of professional service that researchers do for each other. It is integral to the peer-review process. The final draft will be due a week later and will be assessed by me for a grade. Included in this grade will also be points for experimental design and how the experiment was carried out, so pay attention to comments offered during the week of Sept 21 and be sure to address when you and your partner(s) perform your experiment. And, although you will be working with partners throughout the course of the semester, ***you must individually write your own papers***. For paper #2, you will turn in the Intro and Methods sections and a list of references midway through the project. You will get comments back from me and then you will turn in a completed paper at the end of the semester. Both drafts will be graded (see below).

SUMMARY ABSTRACT: For the cardio bifida chick lab you will write up a one-page summary abstract rather than a full blown scientific paper. This abstract will be modeled after the type of abstract you would submit to a scientific meeting. Explicit instructions on writing such abstracts will be posted on Moodle.

LAB NOTEBOOK: I will also ask that you keep a laboratory notebook. Keeping a lab notebook is an absolutely essential part of doing science. The better notes that you take, the easier it will be for you to write your papers, and the more you will get out

of the laboratory section of this course. I will periodically review your notebooks throughout the semester (see schedule below) and will then assess them near the end for a final grade. You should feel free to include illustrations, photographs, timelapse files, etc. in your notebook. I will also provide some data sheets that you should fill out and keep in your notebook; these sheets will help you to organize your notes and make writing your papers easier.

LAB PREP: You will be responsible for helping to set up for one of the labs this semester. This will involve making buffers, reagents, and media that the whole class will use. These tasks will help you to gain confidence in the lab and demonstrate the shared responsibility that comes with real labwork. Students responsible for a particular week will be required to carry out the preparation work one or more days before the lab period. I will work with students to find a mutually suitable time during the week when the lab prep can be done.

LAB CLEAN-UP: You will be responsible for cleaning up your bench area and any tools (e.g. forceps), glassware, etc. that you use during the course of the semester. You will need to do a final cleanup and turn in cleaned materials at the end of the semester. Not doing so will affect your grade (see below).

LAB PORTION OF FINAL GRADE (40% OF FINAL GRADE):

Assignment	Date(s) Due	Possible Points
Paper #1 (sea urchin development)		
First Draft	10/5	
Comments	10/8	20
Final Draft	10/15	80
Summary Abstract (chick cardio bifida)	10/22	35
Paper #2 (RNAi/ worm development)		
Intro, Methods, Literature Cited	11/21	30
Final Draft	12/17	90
Lab Notebook	10/15, 12/17	100
Lab Prep	TBA	25
Lab CleanUp	Ongoing	20

(See Lecture syllabus for more details.)

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