

## BIOL 4554/5524: DEVELOPMENTAL BIOLOGY LAB - SPRING 2009

The lab meets on Wednesdays from 1:30 to 4:20 PM in SCEN 612.

### PROGRAM

#### **Lab No. 1 Introduction (lab protocol, instrumentation)**

Preparation<sup>1</sup>: Read the Vade Mecum<sup>2</sup> chapters “Getting Started”, “Embryological Tools” and “Using the Compound Microscope”. **Bring printouts<sup>2</sup>** to the lab to have the information you need available at the bench. Pay special attention to the section that explains how to keep a **Laboratory Notebook**. Your notebook will be one of the criteria for determining the number of points that you will get for lab performance. Another criteria will be the Formal Laboratory Report that you are required to write (see below).

Make yourself familiar with the dissecting and compound microscopes. Practice the Koehler illumination. Build darkfield stops for darkfield microscopy (we will need these in the sea urchin lab).

Craft microknives and micropipettes (you will need those in the planarian regeneration and slime mold experiments) as well as embryo spoons (for handling chicken embryos). Note: this is only necessary if these tools are not already available in sufficient numbers from previous semesters.

You should also have a pair of robust forceps (e. g. McCoy Fine Point<sup>3</sup>) and dissecting scissors<sup>3</sup> (for the chickens) as well as a fine paintbrush<sup>3</sup> (for fly work; size 0, round) available in the following labs. Purchase of a couple of robust dissection needles<sup>3</sup> (29 cents each!) is recommended as well. We will provide you with **fine forceps (Dumont #5)**, which are essential tools for dissecting fly tissues. These forceps are quite **expensive** and easily damaged. **Please, use extreme care** when using them and, after you are done with your work, put the protective plastic cap for the tips back in place!

#### **Lab No. 2 Planarian Development**

Preparation: Vade Mecum<sup>2</sup> chapter 13. Plan the experiments in advance that you would like to conduct.

Study and document the anatomy and behavior of the flatworms. Perform the surgical procedures you have planned for. Realistically, it will be difficult for you to succeed in doing complicated grafting experiments, but you are expected to at least cut planarians into halves. This isn't meant to discourage you from trying, and any success will be generously rewarded (choose between chocolate or a few extra points).

#### **Lab No. 3 Life cycle of *Dictyostelium***

Preparation: Vade Mecum<sup>2</sup> chapter 4.

Monitor the progress that your planarians have made regenerating!

Try to find the different stages of *Dictyostelium* on your plate and document what you see in your notebook. Try to stain part of a slug with a vital dye and monitor where the stained cells end up in the fruiting body. Stain a slug and then disaggregate the cells and try to monitor the fate of these cells. You may also want to bring a cup of coffee (the coffee shop in the Union Building sells a pretty strong brew) to study the effect of caffeine on the aggregation behavior (and on your attention span). We will try keeping the slime molds in the fridge (an experiment in itself) to allow you to evaluate the results of your experiments in the following week(s).

#### **Lab No. 4 Development of *Drosophila***

Preparation: Read the Vade Mecum<sup>2</sup> chapter 8, pages 1-5 and 9-12, before coming to the lab.

Continue your planarian and slime mold experiments!

Make yourself familiar with the developmental stages of *Drosophila*: Study and make sketches of eggs, larvae, prepupae/pupae, and adults. Learn to distinguish the sexes of third instar larvae and adults. Dissect third instar larvae and adult females. You are expected to find and draw the larval brain plus adhering imaginal discs, the larval salivary glands, and the ovaries of adult females (you will need the skill to dissect imaginal discs and salivary glands in upcoming labs!).

#### **Lab No. 5 Analysis of *engrailed* expression and of *Drosophila* enhancer trap lines**

Preparation: You will find no information about this section of the lab in the Vade Mecum<sup>2</sup> manual. Some basic information will be given here and more detailed information during class hours.

You will stain *Drosophila* wing imaginal discs to visualize the expression pattern of the segment polarity gene *engrailed* (*en*). To accomplish this, we will use a transgenic fly strain that carries a reporter gene construct with the *E. coli lacZ* gene. *LacZ* encodes the enzyme  $\beta$ -galactosidase, which can be easily detected by a chromogenic reaction using the substrate X-GAL. In the construct, the coding region of *lacZ* is under the control of regulatory DNA elements (enhancers) that normally control the expression of *engrailed*. The *lacZ* expression pattern therefore reflects the normal *engrailed* expression pattern.

We will hand out fly larvae carrying transposons (P elements) that are integrated at random positions in the animals' genome. The P elements carry the *E. coli lacZ* reporter gene described above, but without the *engrailed* enhancer elements. The *lacZ* gene is under the control of a so-called minimal promoter. A minimal promoter is required but not sufficient for high-level transcription of a gene. High-level transcription will only occur if the P element is integrated near an endogenous enhancer element. This element will direct expression of the *lacZ* reporter gene in the temporal and spatial pattern characteristic of the gene that is normally controlled by the endogenous enhancer. The blue dye produced by the X-GAL reaction will thus only be produced in tissues in which the "trapped" endogenous gene is normally active. Enhancer trap lines are valuable tools in determining the expression patterns of genes. However, do you think that the detected *lacZ* expression pattern will always faithfully reflect the entire expression pattern of the "trapped" gene? Think about what you know about the control of developmental genes by enhancer elements. Also consider other consequences that integration of the P element might have. Can you think of additional modifications of the P element that might be useful to manipulate/change expression of the endogenous gene?

You will carry out X-GAL staining with a number of different enhancer trap lines. Identify tissues that stain blue and make sketches of these tissues. You will see that enhancer trap lines are also excellent tools to mark specific tissues or cell populations. These may be tissues and cells you have not been aware of before. You will thus further expand your knowledge of the larval anatomy.

A detailed protocol describing the X-GAL-staining procedure will be handed out a prior to the lab.

Continue your slime mold experiments!

**Lab No. 6 Polytene chromosome squashes**

Preparation: Read pages 12-16 of Vade Mecum<sup>2</sup> chapter 8 before coming to the lab.

Make salivary gland squashes and observe and draw the polytene chromosomes found in this tissue.

Continue analysis of the enhancer-trap lines!

**Lab No. 7 Sea urchin: fertilization and early embryogenesis**

Preparation: Read Vade Mecum<sup>2</sup> chapter 6 before coming to the lab. Additional protocols will be distributed before the lab starts.

We will obtain sea urchins of the species *Arbacia punctulata* from the Gulf Specimen Marine Laboratory. We will harvest sperm and eggs and observe fertilization and early cleavage under the microscope. We will also try to induce parthenogenesis. At the end of the lab, we will transfer embryos to lower temperature (18 °C and 4 °C) to slow down development for the observation of larval stages in the following week. Students are welcome to study larval development outside the scheduled lab times.

**Lab No. 8 Sea urchin: larval development**

Preparation: Vade Mecum<sup>2</sup> chapter 6.

We will observe larval stages of sea urchin development.

**Lab No. 9 Chicken egg; early chick development**

Preparation: Vade Mecum<sup>2</sup> chapter 9.

You will study chick eggs as described in the manual. We will hand out slides that allow you to examine the early stages of chick development. Record your observations by making diagrams.

**Lab No. 10 Living chick embryos**

Preparation: Vade Mecum<sup>2</sup> chapter 11.

We will incubate fertilized chicken eggs for you. You will dissect and observe live embryos as described in the lab manual. You will finally prepare your own whole mounts of these embryos. At the end of this lab, your embryos should be in the fixative, which should be washed out 2-3 days later with 70% ethanol. This will thus be one of the occasions where you are expected to continue an experiment outside regular class hours.

**Lab No. 11 Chick 33-hour embryo**

Preparation: Vade Mecum<sup>2</sup> chapter 10.

Stain your chick embryos and keep them in 70% ethanol until the next lab.

Undertake a detailed study of chick organogenesis using whole mounts and sections of chicken embryos. Make diagrams of what you see. This will be an important preparation for recording the results of your own efforts to make whole mount preparations.

**Lab No. 12 Amphibian development; Living chick embryos (whole mounts) continued**

Preparation: Vade Mecum<sup>2</sup> chapter 14.

Dehydrate, clear, and finally mount your chick embryos.

Undertake a detailed study of amphibian development using whole mounts and sections of embryos. Make diagrams of what you see.

**Lab No. 13 Living chick embryos and amphibian development continued; Clean-up**

Evaluate your own chick embryo whole mounts. Conclude your study of amphibian development. We will have a final discussion of your lab experiences. Clean-up lab and equipment and leave everything, if possible, in a better condition as you found it at the beginning of the lab.

**Formal Laboratory Report**

One of the criteria for evaluating your performance in the lab will be a formal laboratory report about one of the topics of the lab. You can choose from 6 subjects: (1) Planarian regeneration, (2) *Dictyostelium* development, (3) *Drosophila* development (including imaginal discs and polytene chromosomes), (4) Analysis of *engrailed* expression and enhancer trap lines, (5) Sea urchin fertilization and development, (6) Chick development. The report should be structured in the following way:

1. Scientific background
2. Goals of the corresponding lab section(s)
3. Your experimental results and observations (including tables and diagrams)
4. Discussion and conclusions (you may include suggestions here for improvements in the lab program or for additional [alternative] experiments/exercises)

The **deadline** for handing in your Lab Report will be **Thursday, April 30!**

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- 1) The term "Preparation" implies that these activities are done at home and not during lab hours!
  - 2) This applies to all the following labs as well!
  - 3) These items can be obtained in the University Bookstore for a reasonable price.