A cell biology laboratory exercise to study sub-cellular organelles in *Drosophila*.

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Abstract

The fast changing scenario of undergraduate education puts emphasis on introducing students to hands-on techniques as part of their laboratory courses. In order to cater to large numbers of students and the time constraints involved with undergraduate level laboratory courses, there is a need for development of experiments that are cost effective and can be completed in a defined time frame. We have devised a laboratory exercise for teaching cell biology using the *Drosophila melanogaster* model. *Drosophila* can be reared in a short period of time in a cost effective manner. We used *Drosophila* tissue to study the sub-cellular organization of eukaryotic cells using fluorescent markers. The idea of this lab exercise is to: (a) familiarize students with the underlying principles of cell structure and function and its application to diverse areas of research, (b) allow students to sharpen their observation and quantitative microscopy skills, and (c) minimize the preparation time for the instructor.

The participation of undergraduates in research and experimental learning is an important component of several undergraduate programs. One of the important facets of a cell biology course for an undergraduate curriculum is to introduce the structural and functional aspects of the various sub-cellular organelles. Interestingly, many new text books and accompanying supplementary material provide exhaustive and detailed information, through images and movies. Although animations and videos can elucidate the complex organization of organelles in the cell, it is important that students also have the opportunity for laboratory experiences. In order to introduce undergraduate students to the sub-cellular organelles in a eukaryotic cell, we have started developing laboratory exercise(s) which can be performed in an undergraduate classroom setup.

The cell is the basic structural and functional unit of life. The sub-cellular organization of the eukaryotic cells includes different membrane bound organelles, which have specialized structures and functions. For example, the nucleus contains the genetic information in the form of DNA and RNA, the Golgi complex and the endoplasmic reticulum are specialized for packaging and folding of proteins, lysosomes contain the hydrolytic enzymes, microtubules or the microfilaments are required to provide support to the cells. Thus, different organelles are specialized for performing different functions. Any defect in their structures leads to impairment of their respective functions which eventually may lead to cell death, or have deleterious consequences on cell/organelle or organ function leading to diseases. Therefore, it is useful to observe these organelles at high resolution under the microscope, in order to understand their structures and functions.

In the majority of cell biology laboratories, students are shown the sub-cellular organelles either through images, movies, or from permanent commercial slides. However, appreciation for the different protocols/techniques used to stain cells with traditional or fluorescent dyes is lost upon the

students mostly, because they do not understand the underlying principles of organelle specific staining in cells. Therefore, we decided to develop a laboratory exercise to observe various organelles in fixed *Drosophila* tissues by using dyes in an undergraduate laboratory setup.

Different sub-cellular organelles were marked using a set of commercially available fluorescent molecules that bind specific biochemical compounds in the cells which are localized to a particular organelle (Lavis *et al*., 2006). These markers, thus, tag different organelles, which can then be observed using an epifluorescence microscope. Each marker can be visualized based on its excitation /emission properties. A variety of such molecules are commercially available (http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes.html) such as Lyso Tracker for marking lysosomes, Mito Tracker for mitochondria, Phalloidin (Small *et al.*, 1999) which marks actin filaments, and DAPI (4'-6-Diamidino-2-phenylindole) (Kapuscinski, 1995) which tags DNA in the nucleus (for details, see Table 1).

We used fluorescent dyes that act as markers for the actin filaments and the nuclei in a eukaryotic cell. The undergraduate students worked in pairs and performed this experiment as a part of cell biology laboratory course. The entire experiment lasted less than three hours which involved dissection, tissue fixation, incubation, and observation under the microscope.

Table1. List of dyes that can be used to mark the organelles in the cell.

Organelle	Dye	Molecular Probes Catalogue number	Excitation (nm)	Emission (nm)
Mitochondria	Mito Tracker Green (Vanden Berghe, 2004)	M22425	644	588
	Mito fluor Green probe	M 7502	588	622
	Mito Sox red	M36008	510	580
Lysosomes	Lysotracker	L7528	577	590
Golgi Complex	NBD C_6-Ceramide	N-1154	466	536
Endoplasmic Reticulum	Bodipy (Lavis *et al,* 2006)	D7540	589	617
Cytoskeleton	Texas Red Phalloidin (Small *et al,* 1999)	T7471	591	608
Nucleus	DAPI (Kapuscinski , 2006)	D 1306	358	461
	TOPRO	T7596	745	770
	Hoescht	H21486	350	450

(Details of dye available at http://www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook.html)

Protocol

Flies were cultured on yeast-cornmeal-agar fly medium (recipe available at http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/molassesfood.html) at room temperature and allowed to lay eggs. Third instar larvae (wandering on the wall of the culture vial) were dissected in PBS (Phosphate Buffered Saline, containing 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4; Dulbecco *et al*., 1954) using sharp Dumostar forceps (Electron Microscopy Sciences (EMS) Cat. No. #72707-01). The salivary glands and imaginal discs attached with mouth parts and brain were dissected. Imaginal discs are groups of epithelial cells housed inside the larva that will give rise to adult appendages and the cuticle after metamorphosis.

Tissue was fixed in 4% paraformaldehyde (EMS Cat. No. #15710) in PBS to preserve the morphology. The fixative was removed after fixing the tissue for twenty minutes. The tissue was

rinsed in ice cold PBS, followed by washes with PBST [PBS+ 0.2% Triton X-100 (Sigma Aldrich Cat No. T100)], to permeabilize the tissue. The tissue was then incubated with Texas Red Phalloidin- (Molecular Probes, Invitrogen, Cat. No. # T7471) and DAPI (Molecular Probes, Invitrogen, Cat. No. # D 1306) in PBST for twenty minutes at room temperature in dark. The phalloidin specifically marks the actin filament meshwork of the salivary gland cells (Figure 1a, b; red channel). The DAPI stains the nuclear material within the cell of the salivary gland (Figure 1a, b; green channel). In salivary glands, endoreduplication causes polyploid DNA that can be easily visualized in the large nuclei of salivary gland cells. The washing steps were performed using PBST and finally the tissue was mounted on glass slides in Vectashield mountant (Vector labs, Cat. No. # H-1000). Vectashield serves as an antifade agent that helps to preserve fluorescence in stained tissue.

The tissue was then observed under a Carl Zeiss epifluorescence microscope (Axio Imager. Z1). Phalloidin was observed in Alexafluor 546 channel as it has an excitation wavelength of 494nm and emission wavelength of 517nm. DAPI was observed under DAPI filter (Table 1). The digital images were obtained using CZ Focus software on the Zeiss Apotome epifluorescence microscope or Olympus Fluoview 1000 confocal microscope. Images were processed using Adobe Photoshop 5.5 software. Alternatively, images can be taken on a conventional epifluorescence microscope camera with a high speed film.

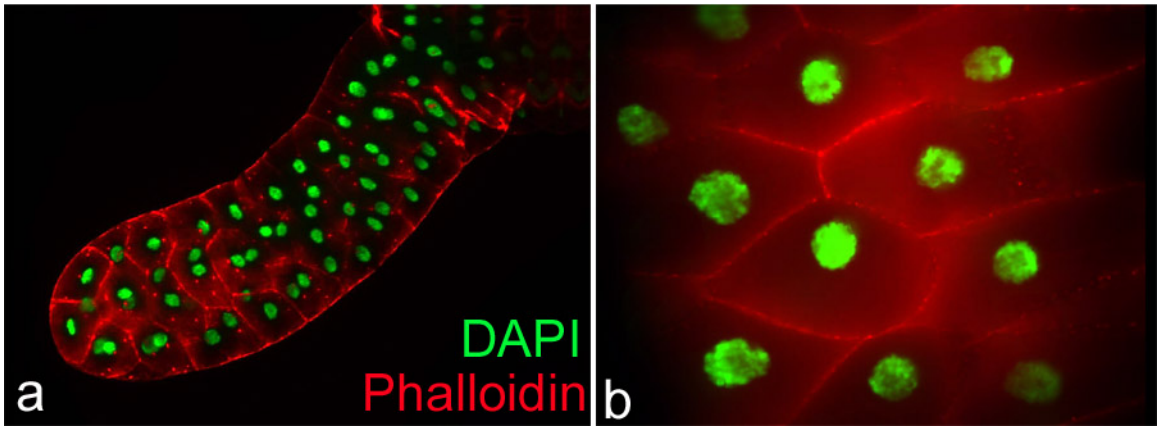

Figure1. Labeling eukaryotic cell /organelle in *Drosophila* tissue using fluorescent dyes. (a) *Drosophila* salivary gland stained for DAPI (Green) that marks the nuclei and Phalloidin (Red) which marks the actin filaments. (b) Magnified view (63×) of the salivary gland cell.

Advantages

1. The greatest challenge to teaching a cell biology laboratory is the capital investment/commitment that a university/college must make to laboratory. The use of cost-effective exercises can facilitate the execution and implementation of these laboratory programs in undergraduate academic institution setup.

2. The students were able to get hands-on experience of the technique and visualize the results in a fluorescent microscope in a single teaching laboratory credit hour of three hours per week.

3. These exercises did away with the use of tailor-made demonstration kits provided by companies.

4. This laboratory exercise helped in generating permanent slides, which can be used for final exams where students can be asked to identify the organelle. This will prevent the expense of buying commercial slides for exams.

5. It will help generate a core of trained individuals who can function in academic as well as corporate settings.

Conclusion

Cell biologists rely on images to communicate their results and to study/teach structure and function of cells. Images are powerful means of communicating scientific results. A clear high-magnification image can underscore an experimental result more effectively than any words, whereas a poor image can readily undermine a result or conclusion (Pearson, 2005; Chatterjee, 2006). Most undergraduate laboratory science courses do not actively teach students skills to communicate effectively through images (Riemeier and Gropengießer, 2007). Our laboratory exercise meets this need by teaching students to (a) develop basic laboratory skills and learn immunohistochemistry and fluorescence microscopy, (b) capture digital images using the software, (c) process the image using the Photoshop or imaging software, and (d) develop a series of image portfolios to present their results.

Additional Resources

There are several resources available at Bloomington Stock Center (http://flystocks.bio.indiana.edu/), which can be used for cell biology laboratory in undergraduate institutions. Some of the easy to work reagents are:

a. Protein-trap lines: Transgenic flies, where the proteins are tagged with the GFP reporter and these proteins localize specifically to some organelles in the cell (http://flytrap.med.yale.edu/index.html).

b. Reporter lines: There are transgenic flies available that specifically mark organelles like Golgi (BL-7193), Mitochondrion (BL-7194), and Endoplasmic reticulum (BL-7195), by the presence of EYFP reporter. These stocks can be used to demonstrate sub-cellular organelles in the cells in a cost effective and time efficient manner.

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A concise *Drosophila* laboratory module to introduce the central concepts of genetics.

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Overview

The quick generation time, ease of handling, and wide availability of striking phenotypic mutants makes *Drosophila melanogaster* a highly attractive system to expose undergraduate and advanced high school students to concepts in genetics. However, it is nevertheless very labor intensive to set up *Drosophila* for a large scale laboratory exercise in a short period of time. The previously developed P{his-hid}Y heat shock system enables a greatly optimized procedure for the collection of virgin females (Venema, 2006; Venema, 2008). Using this system allowed us to develop a short laboratory module that can be completed in only three 2-3 hour long laboratory sessions spaced two weeks apart. This format also permits the *Drosophila* genetics module to be interspersed with other modules in a single semester laboratory course.

This introductory *Drosophila* genetics laboratory module emphasizes hypothesis-driven scientific inquiry by encouraging students to form their own open-ended questions about the nature of mutations and the pattern in which they are inherited from one generation to the next. The two crosses in the module introduce several important genetics concepts including: Mendelian autosomal inheritance, sex-linked inheritance, recombination, genetic mapping, and non-disjunction. Students are also introduced to the method of using statistical tests to validate or reject biological hypotheses.

Methods

The P{hs-hid}Y stocks (Venema, 2006) greatly facilitate setting up and collecting virgin female *Drosophila* for genetic crosses. The P{hs-hid}Y line has a *P* element insertion on the Y chromosome containing a proapoptotic lethality gene, *head involution defect* (*hid*), driven by the Hsp70 heat shock promoter (Grether *et al*., 1995; Starz-Gaiano *et al*., 2001). Heat shocking flies at mid-larval stages for 2 hours activates expression of *hid*, causing the death of all male (Y chromosome-carrying) larvae (Figure 1A and B). Consequently, only the females survive to become adult flies. The P{his-hid}Y system can be applied to any mutant line by crossing P{his-hid}Y males with virgin females homozygous for the mutation of choice and then backcrossing the F1 males with the mutant phenotype to the original stock of homozygous mutant virgin females to create a stable P{hs-hid}Y line with the mutant phenotype.