Rapid immunofluorescent staining of spectrin in cultured *Drosophila* S2 cells for use in teaching undergraduate cell biology lab.

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Introduction

Immunofluorescent staining is a commonly used method in biology to study cellular structures. Immunofluorescence utilizes antibody binding to a target antigen within a cell. Indirect immunofluorescence is often used, where a primary antibody attaches to the target antigen and a fluorescently labeled secondary antibody attaches to the primary antibody. The fluorescent dye allows for the visualization of specific components of a cell using a fluorescence microscope.

Traditional immunostaining protocols usually take a minimum of 5 hours and can take more than 24 hours if an overnight antibody incubation is used. These lengthy protocols make the immunostaining procedure impractical for the undergraduate teaching laboratory that only meets a couple of hours per week. The most time-consuming portions of the procedure are the antibody incubations, so reducing these incubation times is critical. Previous studies have shown the utility of microwave irradiation to reduce the antibody incubation time in the staining of tissue sections (Leong and Milios, 1986; Hite and Huang, 1996; Hatta *et al.*, 2006). It is thought that microwaves increase molecular vibrations that speed up antibody to antigen interactions. Therefore, we sought to develop a rapid immunostaining procedure for fixed *Drosophila* S2 cells by using microwave incubation. We tested this new protocol in an undergraduate cell biology laboratory using α-spectrin antibodies and found that it produced robust staining of the spectrin cytoskeleton. The staining procedure can be performed in less than 30 minutes and is easily adapted for use in undergraduate settings.

Materials and Methods

Preparation of Drosophila S2 cells for immunofluorescence (1 hour)

S2 *Drosophila* cells were cultured in sterile petri dishes at room temperature in M3 medium supplemented with 12% fetal bovine serum and antibiotics. S2 cells adhere to the bottom of the culture dish and also create cell clumps. In order to create a homogeneous suspension, the cells were gently pipetted up and down to dislodge them from the plate and to break up cell aggregates. The cells were counted using a hemocytometer and diluted to 200,000 cells/mL with serum free medium (Thermo Scientific HyClone CCM3, Catalog #SH30065.01) before being added to each well of a PFTE printed microscope slide (Electron Microscopy Sciences, Catalog No. 63424-06). 50 µL of diluted cells were pipetted onto the microscope slide using a clean micropipette tip. The cells were allowed to attach to the treated microscope slide for 30 minutes in a humidity chamber to prevent the samples from drying out. A humidity chamber was created using a sealed pipet tip box with a damp paper towel in the bottom. After cell attachment, the medium was drawn off with a micropipette, being careful not to scrape any cells off of the slide. Using a fresh tip 30 µL of fixing solution (4% formaldehyde in PBS) was applied to each sample for 10 minutes. Next, the fixing solution was drawn off, and the cells were rinsed three times with 30 µL of PBS (phosphate buffered saline) for 2

minutes each. 30 µL of wash buffer (TBS + 0.05% Tween-20) was applied to each well for 2 minutes, three times each. 30 µL of blocking solution (wash buffer + 10% normal goat serum) was applied to each sample. For convenience the slide was placed in the refrigerator inside the humidity chamber at 4°C for one week until the next lab session. The cells are now ready for rapid immunostaining.

Immunostaining procedure (30 minutes)

A plastic tray was filled with 2 liters of cold tap water and placed inside of a microwave oven (1250W Panasonic, Model NN-T945SF). The water is used to absorb excess microwave radiation to prevent the cells from overheating. The blocking solution from the previous treatment was drawn off. A secondary antibody staining control was included by not adding any spectrin antibody to the first sample (see Figure 1A). 20 µL of diluted α-spectrin antibody (Developmental Studies Hybridoma Bank, 3A9, diluted 1:20 to 3 µg/mL in blocking solution) was applied to the cells of the second sample (See Figure 1B). The slide was then placed on a platform in the water tray and microwaved for 3 minutes at 50% power. The slide was left to stand for 2 minutes inside the microwave. Once removed, the liquid was drawn off from each sample, and the samples were washed three times with 30 µL of wash buffer, 1 minute each. The remaining washing solution was drawn off and 20 µL of the diluted secondary antibody (Alexa Fluor 488 goat anti-mouse, Invitrogen #A-11029, diluted 1:1000 to 2 µg/mL) was applied to both samples. The water in the microwave was replaced with 2 liters of fresh tap water. The slide was again placed on platform in the water tray. The samples were microwaved for 3 minutes at 50% power, then allowed to stand for 2 minutes inside the microwave. 30 µL of DAPI solution (100 ng/mL) was applied to each sample for 5 minutes. Next, the samples were washed twice in 30 µL of wash buffer for 1 minute each. Finally, two drops of Vectashield (Vector Labs, Catalog #H-1000) was added on each end of the slide and a coverslip (Electron Microscopy Sciences, Catalog #63769-01) was placed over the slide. The coverslip was sealed using clear top coat nail polish. Fluorescent staining was imaged using a fluorescence microscope (Motic BA410).

Results and Discussion

Immunofluorescence allows for the visualization of specific structures within the cell. This technique is useful in many fields of science, such as developmental biology and pathology. Immunofluorescence can be a very long and tedious process, making it prohibitive for use in the undergraduate teaching laboratory. Therefore, developing a rapid and effective method of immunostaining would be very advantageous to teaching this method to students. We used microwave irradiation to shorten the antibody incubation steps, since microwave radiation is thought to speed up the formation of antigen-antibody complexes (Hatta *et al.*, 2006). Our new procedure is much faster compared to the standard immunofluorescence protocol. Since it is fast and uses common laboratory equipment, the procedure is practical to use in any undergraduate cell biology lab.

Spectrin is an important component of the cytoskeleton. For example, spectrin gives flexible, structural support to the shape of red blood cells (Delaunay, 2007). Spectrin is located at the periphery of the cell near the plasma membrane. In our rapid antibody staining procedure, spectrin clearly localized to the outer regions of the cell, and the staining fluoresced very brightly (Figure 1B). DAPI stained DNA was used as a reference to distinguish the nuclei from the membrane-localized spectrin. In contrast, the secondary antibody control showed very little green fluorescence, demonstrating the specificity of the spectrin antibody staining (Figure 1A).

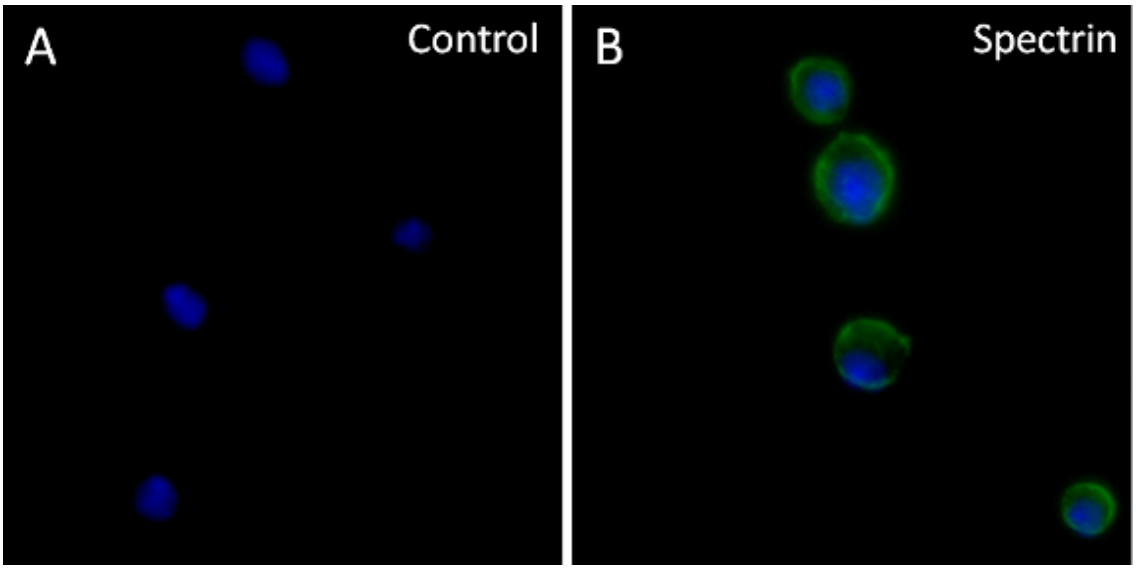

Figure 1. Immunofluorescent labeling of spectrin in *Drosophila* S2 cells. Cells were fixed with 4% formaldehyde and blocked with 10% normal goat serum before the rapid immunostaining protocol. A, To ensure that nonspecific staining of the green fluorescent secondary antibody was minimal, we performed a control stain where the primary antibody was omitted. No green fluorescence was seen in the control samples. Nuclei are in blue stained with DAPI. B, Inclusion of the α-spectrin antibody during the rapid staining procedure resulted in bright fluorescence at the periphery of the cell near to the plasma membrane.

Immunoflourescence is an excellent technique to help undergraduate cell biology students to see the location of spectrin in the cell with their own eyes and relate it to material learned in lecture. Along with being time efficient, our rapid immunofluorescence procedure gives every student useful practice and hands on learning of this important technique. Repetitive steps also allow the students to further develop micropipetting skills. Students are given the opportunity to learn about fluorescence microscopy and to further develop microscopy techniques. Importantly, students learn how to interpret their observations, which gives the student insight into what they are viewing with a fluorescence microscope. Rapid immunofluorescent staining of spectrin also gives the students exposure to DNA staining with DAPI, which is commonly used in fluorescence staining of cells to show nuclei. A practical understanding of the immunofluorescence technique will benefit students by helping them to better understand the biology of the cell. This technique is also an important aspect of scientific training, as many of these students will become future scientists and medical professionals who will be better able to interpret scientific data and perform this technique in a laboratory setting.

In conclusion, we have developed a rapid immunostaining protocol for the visualization of spectrin in cultured *Drosophila* S2 cells. This staining protocol may be used for other antibodies, although the incubation conditions may need to be optimized for each antibody. Furthermore, the protocol could also be developed to include multiple antibodies for the detection of two or more antigens. The efficiency of performing immunofluorescence in 30 minutes and the simplicity of this protocol makes our rapid immunofluorescent staining protocol ideal for any undergraduate cell biology course.

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Protein trap lines of *Drosophila* to demonstrate spatio-temporal localization of proteins in an undergraduate lab.

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Abstract

The objective of this teaching note is to generate a laboratory exercise, which allows students to get a hands-on experience of a cell biology technique. The short duration of the laboratory classes is the biggest challenge with the development of a cell biology lab for an undergraduate curriculum. Therefore, it is necessary to design a laboratory exercise that enables the students to carry out cell biological assays in the desired time. This laboratory exercise focuses on tracking protein expression levels along a spatial (space) and temporal (time) axis in developing *Drosophila melanogaster* organ primordium. Here we use the protein trap model developed in *Drosophila* to demonstrate the sub-cellular localization of proteins. The protein trap transgenic flies have Green Fluorescent Protein (GFP) reporter tags to the full-length endogenous proteins that allow observation of their cellular as well as sub-cellular distribution. Since the life cycle of *Drosophila* is short, it is easy to rear them in the lab and also use them as an excellent model for an undergraduate lab curriculum. The goal of this exercise is to train undergraduate students and teach them the use of one such powerful tool which enables the localization of proteins.

Introduction

The present day undergraduate pedagogy puts great emphases on quantitative reasoning and inquiry-based activities in a laboratory experience. It is widely accepted across the teaching community that promotion of intellectual development through habit of enquiry is an important pillar of learning in undergraduate education. We have been introducing new lab exercises in our Cell Biology (Bio-440 Lab) lab curriculum by exploiting the tools available in *Drosophila melanogaster*, a.k.a. fruit fly, model system. The short generation time, ease of handling, high reproductive ability, and wide array of genetic tools make *Drosophila* an excellent choice for demonstration of biological phenomena in the undergraduate labs (Tare and Singh, 2009; Tare *et al.*, 2010).

There are several approaches available to analyze gene expression in the tissues. The commonly used methods are visualizing gene expression by enhancer trapping, epitope tagging,