Student-Directed Investigations of the Actin Cytoskeleton in Chinese Hamster Ovary Cells

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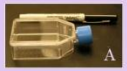
Abstract

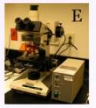
As part of an upper-level Cell Biology course, students utilize fluorescently labeled phalloidin to visualize actin in fixed Chinese hamster ovary (CHO) cells. Students are first introduced to the growth of animal cells in culture through an aseptic technique practice exercise. Next, they learn to grow and stain the cells for observation and characterization of chromosome number. The lecture portion of the class discusses the cytoskeleton in some depth, so students are asked to design their own experiment to manipulate some aspect of the CHO cell growth condition to see if changes in actin cytoskeletal structures can be observed. All groups use the same actin visualization procedure and each group gets time on the fluorescence microscope, using digital microscopy to record data. The fluorescent visualization of cortical actin is always dramatic, and students gain insights into cell culture methods, experimental design, fluorescence microscopy, and data analysis. Sample size is often limited, and variable results allow for important discussions about the importance of quantitation and reproducibility.

Introduction

Images of the cytoskeleton are among the most compelling visuals in modern cell biology. The lab exercises presented here were designed to give upper-level biology majors experience using animal cell tissue culture and the opportunity to create and analyze their own images of the cytoskeleton. Biol L-312 Cell Biology Lectures is an upper-level course required for the BS in Biology at Indiana University Southeast. The associated laboratory course (Biol L-313) is optional for most biology majors but fulfils an elective and is required for the BS in Clinical Laboratory Sciences. The class size is typically quite small (5-10), containing mainly students who enjoy laboratory courses. The laboratory curriculum is organized around several multi-week series of experiments, with each series including a student-directed component. Because we have two tissue culture hoods and a water-jacketed CO_2 incubator, one of the lab series involves the growth and analysis of animal culture cells. Chinese hamster ovary (CHO) cells were chosen for the investigation because they were commercially available and, as non-human cells, pose little risk to student researchers. The CHO cell lab series involved four main exercises: (1) an introduction to aseptic technique, (2) cell staining with crystal violet and Giemsa stains, (3) mitotic chromosome preparation and analysis, and (4) visualization of filamentous actin cytoskeleton. The student-directed aspect of the investigation focused on the actin visualization component. Students designed various treatments they believed would influence the structure of the actin cytoskeleton in the CHO cells and used rhodamine-phalloidin to fluorescently label filamentous actin in the cells.

Materials and methods





Cells lines, growth media and growth conditions. Chinese hamster (*Cricetulus griseus*) ovary (CHO-K1) cells were purchased from ATCC (#CCL-61). Cells were grown in F12-K media supplemented with 10% Fetal Clone II (a fetal bovine serum substitute optimized for CHO cells). Cells were grown in sterile Petri plates or in T25 cell culture flasks (A) in a 37°C incubator with 5% CO_2(B). Cell media was replaced approximately every 3 days and cultures were split approximately once per week. All manipulations were performed in a tissue culture hood (C). Cells will be stored for future use in a liquid nitrogen dewar (D).

Cell staining and chromosome spreads. CHO cells grown on coverslips were stained with crystal violet and Giemsa stains according to protocols described in Freshney's *Culture of Animal Cells: A Manual of Basic Technique*. Digital photos of stained cells were taken by mounting a digital camera in place of one ocular lens on a light microscope. To extend the characterization of the cells, mitotic chromosomes were prepared and spread on glass slides, again according to protocols described in Freshney. Cells were then stained with crystal violet and observed by light microscopy.

Cell manipulations. Students were encouraged to develop their own manipulation that they thought might have an effect on the overall morphology of the actin cytoskeleton in CHO cells. In the course of two years, student groups have studied various cytoskeleton disrupting drugs (e.g. cytochalasin D, colchicine), differences in media conditions, density of cells, and presence of antibiotics. Treatments likely to prevent culture growth (e.g. cytoskeleton-disrupting drugs) were applied hours before observation, while those less likely to damage the cells permanantly (e.g. varying cell densities) were applied several days before observation.

Actin visualization. The F-actin visualization Biochem Kit (Cat #BK005) from Cytoskeleton, Inc. was used for fluorescent visualization of filamentous actin in CHO cells. The kit includes rhodamine-phalloidin, a chemical with a phalloidin moiety to bind to filamentous actin linked to the fluorophore rhodamine. Steps in the visualization process included first growing cells on sterile glass cover slips, fixing the cells, permeabilizing the cells, incubating with rhodamine-phalloidin, extensive washing, mounting onto glass slides and observation by fluorescence microscopy (E).

Data acquisition. Each student group had time to observe their slides by fluorescence microscopy. Additionally, a digital camera was inserted in place of one of the ocular lenses on the microscope, allowing for acquisition of digital images of various preparations. Image files were uploaded to a classroom management site and students retrieved their photos for analysis

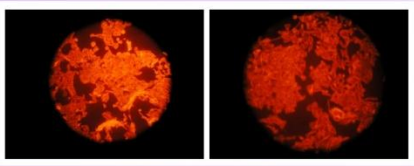


Figure 3. Fluorescence micrographs of CHO cells visualized with rhodamine-phalloidin. These images represent the view of fluorescent cells in the microscope's field of view. The digital camera could be further zoomed to capture more cellular detail. These images represent multiple student preparations using a 100x objective.

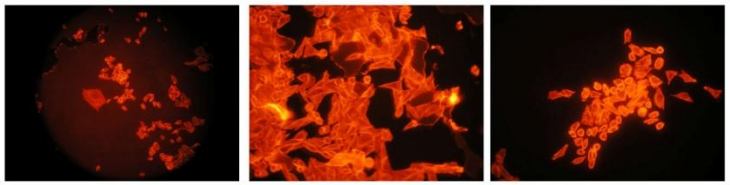


Figure 4. Fluorescence micrographs of CHO cells visualized with rhodamine-phalloidin. These are examples of images collected by students using a digital camera mounted in the ocular lens of the microscope. Each group had used various degrees of zoom on the camera to focus on the cells. The 100x objective was used for each sample.

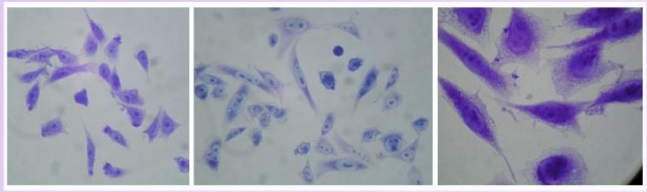


Figure 1. Micrographs of crystal violet (CV) stained CHO cells. Cells were fixed onto coverslips, stained, and mounted for observation. CV is a monochromatic stain. Darker areas within the central nucleus represent nucleoli. Images represent multiple student preparations and magnifications.

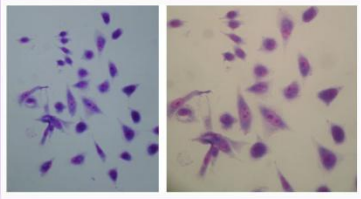


Figure 2. Micrographs of Giemsa stained CHO cells. Cells were fixed onto coverslips, stained and mounted on glass slides for observation. Giemsa is a dichromatic stain, marking the nucleus pink and both the cytoplasm and nucleoli purple. Images represent multiple student preparations and magnifications.

Results

- Students were able to successfully manipulate the CHO cells in culture. No overt examples of contamination were observed either semester the lab was run.
- Students gained experience using aseptic technique and working in the tissue culture hood.
- Students successfully stained and observed cells they had grown in culture (see Figures 1 and 2.)
- Chromosome spreads were not reproducibly successful. One semester's students produced nice slides, but the next did not.
- Students designed and carried out their own investigation into a cell manipulation that might affect the cytoskeletal structure of CHO cells.
- Students followed complex directions from a kit for visualization of a fluorescent marker, gained experience using the fluorescence microscope (see figures 3 and 4), documenting and interpreting their results.

Resources

- Alberts, B *et al.* 2007. *Molecular Biology of the Cell*, 5th edition, Garland Science.
- Freshney, IR. 2005. *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition, Wiley-Liss.
- F-actin visualization Biochem Kit (Cat #BK005), Cytoskeleton, Inc. (www.cytoskeleton.com)

- NOTE: I am happy send electronic files of my lab handouts for Aseptic Technique Practice, Cell Staining, Chromosome Spreads, Culture Splitting, and Actin Visualization via email.

Discussion

BENEFITS

- Students gained experience doing tissue culture, a fairly specialized skill that not all biology majors experience.
- The importance of group work was emphasized.
- Students designed their own experiments. They had to consider controls, replicates, time management, and other issues.
- Students gained experience with fluorescence microscopy and produced compelling digital photos of their cells.
- Students had a real introduction to the multiple steps, long time-frame, and attention to detail required for a significant cell biological experiment. They now have personal experience to relate to textbook fluorescence microscopy images.

COMPLICATIONS

- Some groups allowed the most experienced group member do all of the work in the tissue culture hood rather than taking the opportunity to get their own experience.
- Students have difficulty conceiving their own experiments. They want direction in what would be interesting to study as well in details of how to set up the experiment.
- Because of lab time constraints, we were not able to troubleshoot and determine reasons for unexpected results (particularly absence of cells.)
- Photodocumentation is time consuming and challenging.
- Students need assistance to arrive at reasonable conclusions. Although many manipulations resulted in no observed difference between treatments, many students found that conclusion to be unsatisfactory and would often overstate their results.

IDEAS FOR THE FUTURE

- Require group oral presentation of results in a lab meeting atmosphere.
- Allow more time for modification and repetition of the experiment.
- Require all students to demonstrate competency in the tissue culture hood.
- Design a practice photodocumentation exercise to help students become comfortable with the fluorescence microscope and the camera, as well as to emphasize the need for and complexity of quantitative analysis of results.

Acknowledgements

- Indiana University Southeast Cell Biology Laboratory (Biol L-313) students from Spring 2008 and Spring 2009 for their patience during development of this lab series.
- Mr. Jon Norman for assistance with cell culture ordering, equipment, and maintenance.
- Dr. Adam Hammond for my long ago and very brief training in animal cell culture and the assurance that it is actually fairly hard to kill some cells.