Video Article

Dissection and Mounting of *Drosophila* Pupal Eye Discs

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Abstract

The *Drosophila melanogaster* eye disc is a powerful system that can be used to study many different biological processes. It contains approximately 800 separate eye units, termed ommatidia[1]. Each ommatidium contains eight neuronal photoreceptors that develop from undifferentiated cells following the passage of the morphogenetic furrow in the third larval instar[2]. Following the sequential differentiation of the photoreceptors, non-neuronal cells develop, including cone and pigment cells, along with mechanosensory bristle cells[3]. Final differentiation processes, including the structured arrangement of all the ommatidial cell types, programmed cell death of undifferentiated cell types and rhodopsin expression, occurs through the pupal phase[4-7]. This technique focuses on manipulating the pupal eye disc, providing insight and instruction on how to dissect the eye disc during the pupal phase, which is inherently more difficult to perform than the commonly dissected third instar eye disc. This technique also provides details on immunostaining to allow the visualization of various proteins and other cell components.

Video Link

The video component of this article can be found at https://www.jove.com/video/52315/

Introduction

The fields of developmental and cell biology have been greatly impacted by the model organism: *Drosophila melanogaster*. Within this model, studies of the eye disc have contributed a great deal of knowledge concerning signaling, cell biology and other areas. The late third larval instar eye disc has been studied extensively and is a powerful model to utilize, as it gives a snapshot of a series of developmental periods, each with its own unique signaling molecules and processes, as the morphogenetic furrow progresses across the eye disc[8]. However, there is a need to further expand our understanding of developmental processes into the pupal phase of development. While there have been studies on the pupal eye disc[3-7], our knowledge does not approach the breadth of work that has been performed on the third instar eye disc. This is due, in part, to the greater difficulty in dissecting the pupal eye disc. Therefore, a presentation of the proper method of dissection could greatly expand research in this area.

While there are stages within pupal eye disc development that are easily dissected, particularly around the mid-pupal period, other time periods are much more challenging to dissect. This protocol represents one method for dissecting pupal eye discs that can be universally used for all pupal developmental time frames. This protocol can be used as an alternative to another protocol[9] that shows an easier and faster method to dissect eye discs from the midpupal time points. This protocol was originally filmed and developed for training advanced undergraduate students in the UCLA Undergraduate Research Consortium in Functional Genomics (URCFG)[10,11] in the technique of pupal eye dissection. Many undergraduate students were able to utilize this video and method to learn this challenging technique.

Protocol

This procedure is a 2 day procedure.

Day 1 (2 hr + dissecting time)

1. Pupal Eye Disc Dissection

1. Select a pupa for dissection.
   NOTE: The age of the pupa to be dissected will be determined by the experimental needs. However, if examining cell morphology, this is frequently done at 42 hr after puparium formation (APF) at 25 °C, which is the age of the pupae shown in the video. Collect white pupae

(considered 0 hr APF) with a wetted paintbrush and arrange them in chronological order (based on collection time) in a new fly food vial maintained at 25 °C. Dissect the aged pupae in this same chronological order to keep the time differences between pupae as close as possible.
2. Transfer the pupa into a drop of phosphate-buffered saline (PBS, pH 7.4, see materials for recipe) on a silicone dissecting plate.
3. Hold the top of the pupal case with forceps and pierce and open the lower portion of the pupal case with a pair of sharp forceps. Remove the pupa from the case in the newly formed hole.
4. Make a diagonal cut with scissors, mid-thorax, in the pupa. Remove the debris or transfer the pupa head to a new drop of PBS. Clean using the pipette blower tube (see materials and **Figure 1** for details) and add additional PBS as needed. Make sure to avoid dehydration.
5. Securely grip the top of the pupal head cuticle with a pair of forceps. Using the tip of the blower tube, gently push on the pupal case to extrude the brain and other tissues, including the eye discs, out of the pupal case. Be sure to avoid grabbing any tissue within the cuticle.
6. Using the blower tube filled with PBS, gently blow the fat body tissue and other debris out of the head case to clearly separate and identify the brain and eye discs.
NOTE: Keeping the brain attached to the pupal head case will allow the researcher to use the head case as a "handle" for future manipulation of the tissue without damaging the eye discs or brain. However, it is common to dislodge the brain and eye discs from the head case and this is not detrimental to the overall procedure and results. It often takes multiple attempts of blowing and pushing to successfully extract the eye discs and brain from within the pupal head case and so that they are clearly separate from surrounding tissue. The cleaner and more separate one can get the eye discs from the surrounding tissue will help prevent extraneous tissue from fixing to the eye discs.
7. Following dissection, immediately transfer the tissue to a 3 well glass dish on ice with 0.5 ml of Fix solution (see materials). Float the tissue on the surface of the Fix solution for a few minutes (typically the time it takes to dissect the next set of eye discs).
NOTE: If this is not the first eye disc to be dissected in this round of dissection, completely submerge the previous eye disc into the Fix solution. If performing special staining procedures (*e.g.*, reactive oxygen species, lysosomes, etc.) this must be done prior to fixation. In order to do this, store the dissected eye discs in ice-cold PBS prior to continuing with the appropriate staining protocol.
8. Following dissection and submersion of all eye discs being dissected, move the 3 well glass dish to a rocker and rock at room temperature for 30 min.

2. Immunostaining

1. Carefully remove the Fix solution and add 0.5 ml of PBS with 0.3% Triton X-100 (0.3% PBT, see materials) and wash the tissue on a rocker for 10 min. Repeat this step three more times for a total of 4 washes (10 min each).
NOTE: Careful removal of the solution from the well can be performed under a dissecting microscope to prevent tissue damage or loss. The solution can be aspirated carefully with a pipet; we prefer a fine-tipped, disposable transfer pipet that has had the opening flattened to reduce the chance of aspirating tissue.
2. At the end of the 4th wash, remove the wash and add 0.5 ml block solution (see materials) to the well and incubate for 30 min.
NOTE: This time frame can be extended according to your needs.
3. At the end of the 30 min block time, pipet 10 µl of Primary Antibody Solution (diluted in Block solution) into the appropriate number of wells in a microwell plate.
NOTE: One well of a microwell plate will accommodate about 4 pairs of eye discs attached to the pupal case or 8 isolated pairs of eye discs with brains attached. The primary antibodies used in this protocol for the representative data are anti-phosphotyrosine (1:500 dilution) and anti-Cut (1:200 dilution).
4. Transfer the dissected tissue into the microwell plate wells filled with the Primary Antibody Solution from the 3 well glass dish.
5. Place the microwell plate on a wetted paper towel (to prevent dehydration) in an empty pipet tip box (or other similar container) and store at 4 °C overnight.

Day 2 (4 hr + mounting time)

6. Transfer the tissue from the microwell plate to a new 3 well glass dish with 0.3% PBT (≈0.5 ml) and wash the tissue for 10 min on the rocker. Repeat this step three more times for a total of 4 washes (10 min each)
7. Remove the last wash and add 0.5 ml of Block solution to the well. Block for 30 min.
NOTE: This time frame can be extended according to your needs.
8. Remove the Block solution and add 0.5 ml of the Secondary Antibody Solution (made from fluorescently labeled antibodies appropriate to your primary antibody and fluorescent wavelength, diluted in Block solution) to the well and protect the 3 well glass dish from light with a cover or aluminum foil and incubate for at least 2 hr at room temperature.
NOTE: All subsequent steps should be similarly protected from light exposure. This incubation can be extended to overnight at 4 °C, in a setup similar to the Primary Antibody incubation, but it can also be performed in a 3 well glass dish covered with laboratory film, or a microwell plate if antibody conservation is desired.
9. At end of the antibody incubation, remove the Secondary Antibody Solution and add 0.5 ml of 0.3% PBT to the well and wash on the rocker for 10 min. Repeat this step for a total of 4 washes (10 min each).
NOTE: If DAPI staining is desired, before starting the first wash, add 0.5 µl of DAPI stock solution (see materials) to the 500 µl of PBT that will be used for the first wash (1:1,000 dilution).

3. Mounting

1. At the end of the last wash, mount the antennal/eye discs on a slide using 70% glycerol or PBS as a medium to remove the brain and other unwanted tissues in a pool on one side of the slide.
2. Carefully move the tissue using hydrostatic pressure between the forceps, or very gently with a forceps tip, and line up the eye discs in a column towards the center of the slide.
3. Remove all extraneous glycerol or PBS from around the tissue (using wicking action of a laboratory wipe or the forceps work for this).

4. Place a small amount (≈10 µl) of mounting medium along one side of the eye disc column and place a coverslip on the tissue so as to spread the mounting medium onto the eye discs.
5. Let slide sit for 1 to 2 min to allow the tissue/mounting medium to spread out completely.
6. Wipe any extra mounting medium off from around the edges of the coverslip and seal it with clear nail polish.
7. Capture fluorescent images of the eye discs using confocal microscopy.

Representative Results

As an example of the use of this protocol, results illustrating midpupal (42 hrs APF at 25 °C) eye discs immunostained with different antibodies are presented in Figure 2. By using an antibody directed against phosphotyrosine residues, the membrane of the cells can be observed (**Figure 2A**). This can be used to identify the regular arrangement of ommatidial cells in the pupal eye following the final patterning processes that occur prior to the midpupal stage. Another representative image depicts the nuclei of cone cells can are identified by using an antibody against the Cut transcription factor (**Figure 2B**).

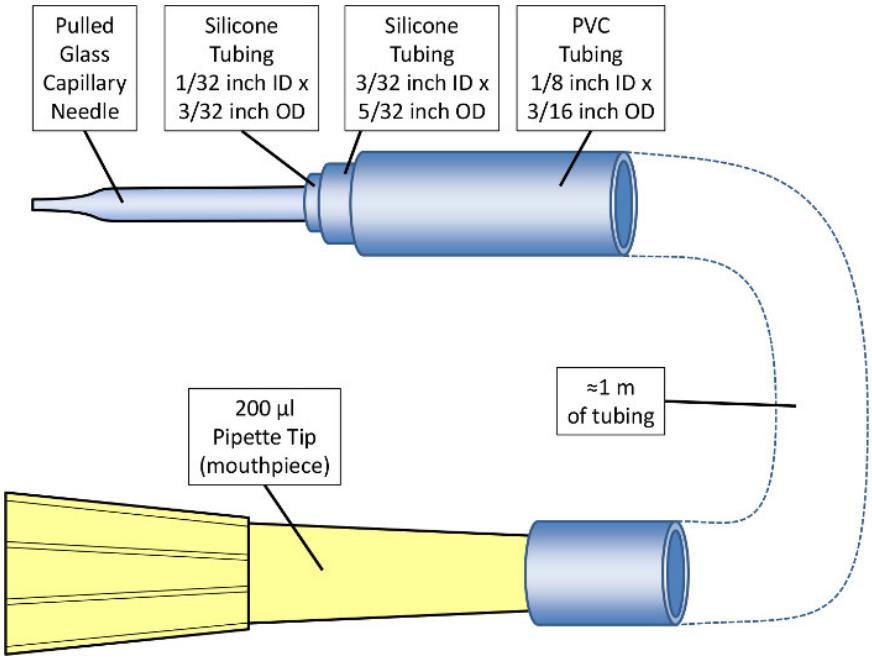

Figure 1: Assembly diagram of the blower tube used in the dissection procedure. See the materials section for details on the separate components of the blower tube.

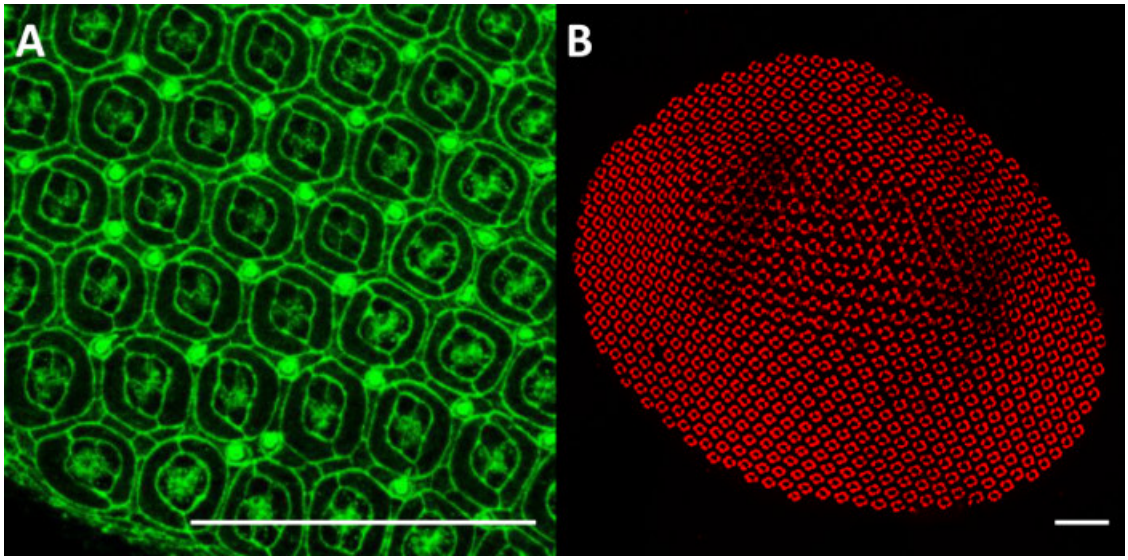

Figure 2: Immunostaining of midpupal eye discs. Representative immunostaining demonstrating the use of this protocol to visualize A) the apical morphology of ommatidia (phosphotyrosine) and B) cone cell nuclei (Cut) from pupal eye discs at 42 hrs APF. Scale bars = 50 µm.

Discussion

While it appears that the process is simple and easy to perform, in reality, this technique requires a great deal of practice to master. Routinely, we start students off by learning to dissect and mount third instar eye discs[12], which are much easier to work with. This practice helps to develop an appropriate dissection position of the arms, hands and fingers[13] so that manipulation of the forceps under the dissecting microscope is stable, easy and experienced. In essence, the practice period should achieve the goal of making the forceps into a natural extension of the fingers and thumb, allowing for the discrete and subtle movements required in the dissection.

If using PBS to mount in, always have extra PBS available, as the small amount of PBS quickly evaporates in the mounting process. Another common problem is the folding of the eye discs in the Fix solution, thus fixing the eye disc in that orientation. This can be avoided by floating the eye disc on the surface of the Fix solution for some time (typically the length of the next eye disc dissection) before submerging it completely in the Fix solution.

While other published techniques focus on the dissection of the pupal eye disc at the midpupal stage[9], when dissection is easily accomplished, this technique provides a method that can be applied to any pupal developmental time, except in the case of the pharate adult or early white pupa. We feel our technique provides an alternative and complementary approach to already published methods for pupal eye disc dissection.

This technique, accompanied with common and useful genetic manipulations like FLP-FRT analysis[14], UAS-GAL expression of transgenes[15] and others, will allow researchers to study the eye disc in further detail during any pupal time frame.

Disclosures

The authors have nothing to disclose.

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