

Video Article

Live-cell Imaging of Sensory Organ Precursor Cells in Intact *Drosophila* Pupae

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Abstract

Since the discovery of Green Fluorescent Protein (GFP), there has been a revolutionary change in the use of live-cell imaging as a tool for understanding fundamental biological mechanisms. Striking progress has been particularly evident in *Drosophila*, whose extensive toolkit of mutants and transgenic lines provides a convenient model to study evolutionarily-conserved developmental and cell biological mechanisms. We are interested in understanding the mechanisms that control cell fate specification in the adult peripheral nervous system (PNS) in *Drosophila*. Bristles that cover the head, thorax, abdomen, legs and wings of the adult fly are individual mechanosensory organs, and have been studied as a model system for understanding mechanisms of Notch-dependent cell fate decisions. Sensory organ precursor (SOP) cells of the microchaetes (or small bristles), are distributed throughout the epithelium of the pupal thorax, and are specified during the first 12 hours after the onset of pupariation. After specification, the SOP cells begin to divide, segregating the cell fate determinant Numb to one daughter cell during mitosis. Numb functions as a cell-autonomous inhibitor of the Notch signaling pathway.

Here, we show a method to follow protein dynamics in SOP cell and its progeny within the intact pupal thorax using a combination of tissue-specific Gal4 drivers and GFP-tagged fusion proteins^{1,2}. This technique has the advantage over fixed tissue or cultured explants because it allows us to follow the entire development of an organ from specification of the neural precursor to growth and terminal differentiation of the organ. We can therefore directly correlate changes in cell behavior to changes in terminal differentiation. Moreover, we can combine the live imaging technique with mosaic analysis with a repressible cell marker (MARCM) system to assess the dynamics of tagged proteins in mitotic SOPs under mutant or wildtype conditions. Using this technique, we and others have revealed novel insights into regulation of asymmetric cell division and the control of Notch signaling activation in SOP cells (examples include references^{1-6,7,8}).

Video Link

The video component of this article can be found at <http://www.jove.com/video/2706/>

Protocol

Required Materials: Dissection stereo-microscope, Double-sided tape, standard microscope slide and coverslip, dissection forceps (size 5 or 5.5), soft-bristled brush, silicone vacuum grease, 5cc syringe, Whatman paper, confocal or epifluorescence microscope with digital camera and image acquisition software.

1. Pupal Dissection

1. Set up a cross (using the appropriate combination of Gal4 line and a GFP tagged fusion protein under UAS control) or place flies from a stock you wish to image in several fresh vials at 25°C.
2. SOP cells generally begin to proliferate on the pupal thorax at eighteen hours after the onset of pupariation, we therefore select "white" pupae from the appropriate fly stock or cross. White pupae have the pupal morphology, but have an unpigmented pupal case, indicating that they have pupariated within the hour.
3. Wait approximately 18 hours.
4. Place a piece of double-sided tape onto the slide. Collect pupae and adhere the pupal case to the double-sided tape with the ventral side down. Grasp the edge of the operculum (the circular hatch on the anterior dorsal tip of the pupal case) with the forceps
5. Gently lift, remove, and discard the operculum, revealing the head of the immature fly.
6. Use the forceps to begin tearing along the side of the pupal case. Lift the midsection of the pupal case from the torn side and bring it over to the opposite side, either remove it completely or attach it to the tape, revealing the thorax and anterior portion of the abdomen. (**Note:** There is a gap between the developing fly and the pupal case. Be careful not to puncture the fly as the wall of the case is torn.)
7. Begin cutting along the same side of the pupal case towards the posterior end. Once again, be careful not to puncture the fly. Pull the pupal case to the opposite side of the fly and press it onto the double-sided tape. The abdomen should now be fully exposed. Place the soft-bristled brush directly beneath the head of the fly. Once the fly is stuck to the brush and free of the pupal case, use the brush to gently lift the fly off the slide.

2. Pupal Mounting

After dissection, pupae are then mounted between slide and coverslip:

8. Isolated pupae removed from case can then be placed on the center of a glass slide dorsal side up.
9. Make a square frame of Whatman paper 18X18mm leaving a 10X10 mm opening in the middle. Immerse paper in water until saturated. Place around the pupae.
10. Using a 5 cc syringe filled with silicone vacuum grease, apply a uniform layer of grease around the Whatman frame. The grease layer should fit within the coverslip (Figure 1) and the thickness should be only a bit greater than the pupal diameter.
11. Place a small drop of water (1 μ l) on the center of a 22X22 mm coverslip and place the coverslip on the above preparation such that the small water droplet contacts the surface you want to image, notum in this case). Compress gently to form a complete seal of vacuum grease and flat contact surface between the coverslip and pupal cuticle.
12. Prep can then be imaged on inverted or upright microscopes, using either epifluorescence, confocal (laser scanning or spinning disk type) or two-photon confocal. If you're careful, adult flies can be recovered after several days.

3. Representative Results:

Using an SOP-specific Gal4 line (*neuralized-Gal4*) crossed to GFP tagged proteins to label the mitotic spindle (tubulin or Tau-GFP) or histone proteins (H2B-GFP), one can observe division plane of SOP cell division, length of the cell cycle, or number of mitotic divisions by using time lapse imaging⁹. Other fusion proteins that target cellular organelles such as Rab-GTPases to mark different endocytic compartments (Rab5-GFP for early endosomes or Rab11 GFP for recycling endosomes) or proteins important in Notch signaling regulation (Numb-, Partner of Numb -GFP, or Sanpodo-GFP) can yield important insights into the mechanisms of asymmetric cell division and membrane protein trafficking^{2,3,7,10,11}.

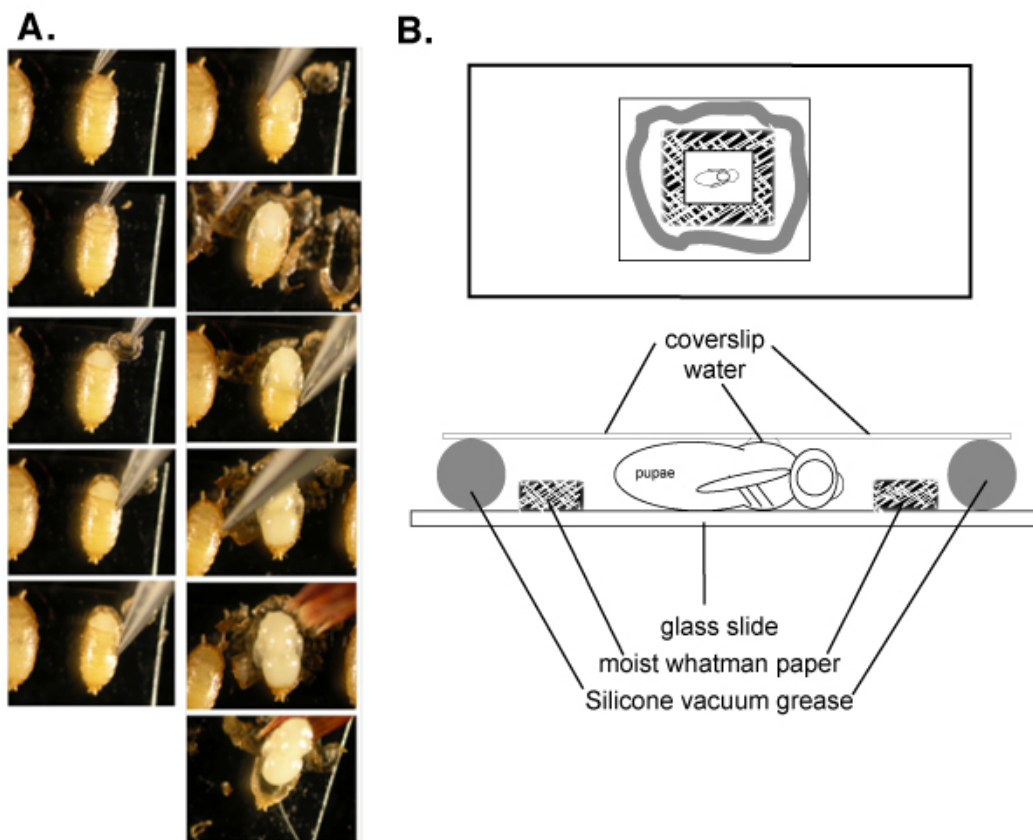


Figure 1: Pupal dissection and mounting. **A.** Step-by-step images show the procedure to remove the pupal case and prepare intact pupae for mounting and live cell imaging. Pupa are removed from the vial and placed, dorsal side up, on double stick tape attached to a glass slide. First column, removal of the operculum and tearing along the side of the pupal case. Second column, removal of pupal case from thoracic and abdominal region. The free pupa is then lifted from the pupal case using a soft bristled brush. **B.** Mounting pupae between slide and coverslip. Pupa is placed dorsal-side up on the glass slide, surrounded by a moistened frame of Whatman paper. A continuous bead of silicone vacuum grease extruded from a 5cc syringe functions to seal the slide-coverslip combination, protecting the pupa from dessication and elevating the coverslip so it rests gently on the pupal thorax. A small drop of water (1 μ l) at the interface between the coverslip and thorax cuticle improves image quality significantly when using immersion objectives (water or oil). [Click here to view a larger image.](#)

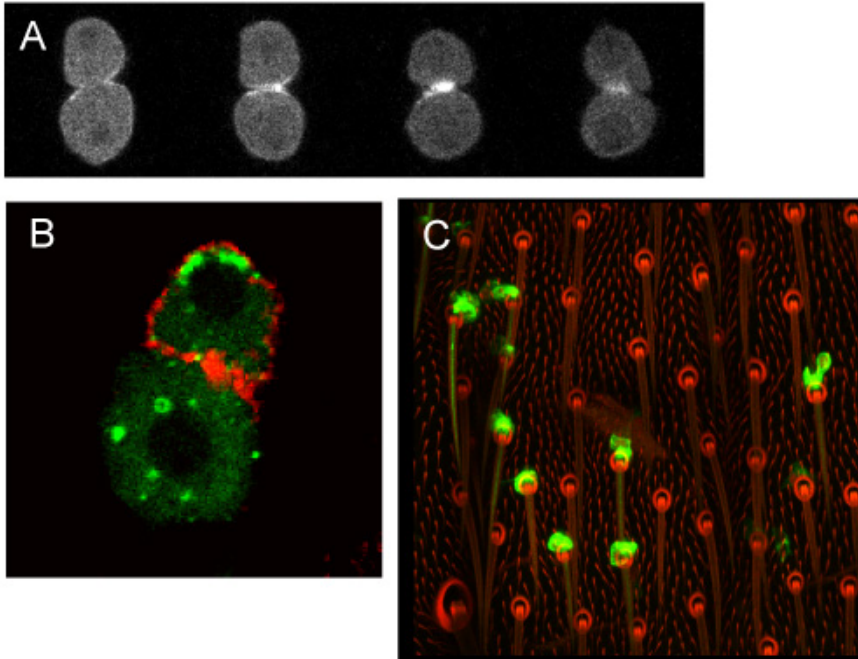


Figure 2: Representative images of live SOP cells and differentiated external sensory organs taken using our pupal dissection and mounting procedure. **A.** Images extracted from a time series of a mitotic SOP cell expressing actin-GFP fusion protein under control of *neuralized-Gal4* (*neur-Gal4/UAS-actin-GFP*), note cortical accumulation of actin at the cleavage furrow (1 image/every 2 minutes). **B.** SOP daughter cells co-expressing Partner of Numb-RFP (red) and Rab5-GFP driven by *neuralized-Gal4*, note the asymmetric distribution of Pon-RFP in one daughter cell (pIIb) and distribution of early endosomes in both daughter cells. **C.** Volume rendering of a z-series taken of differentiated external sensory organs at a late pupal stage. Cuticle structures, such as mechanosensory bristles and epidermal hairs are revealed by cuticle autofluorescence (red). In addition, we've used the MARCM system to express Lgl-GFP (driven by *neuralized-Gal4*) in a subset of sensory organ precursor cells (green). (These images were all acquired using a Nikon C1 scanning confocal microscope using a 60X 1.45 N.A. objective)

Discussion

In this video, we illustrate a technique for isolating and mounting *Drosophila* pupae for live cell imaging of SOP cells on the pupal notum. Removal of pupae from the pupal case requires a steady hand, appropriate tools, and some practice, but is easy to learn. It is important that staging of pupae be done accurately, in order to ensure relative ease of dissection and catching the proliferation phase of SOP development. Once mounted, pupae will continue to develop between slide and coverslip, and in most cases, will reach the pharate adult stage, and eventually eclose. In our experience, cell can be imaged over the course of several hours. This mounting technique is not limited to observation of PNS precursor cells on the pupal thorax, but can be used to visualize any cells that are close to the cuticle surface and accessible to the microscope objective, (please note that observations are done at a stage when the pupal case can be removed without killing the pupa, usually about 10 to 14h after puparium formation). We have successfully visualized junctional proteins, cytoskeletal proteins and vesicle trafficking regulators in pupal epithelial cells (unpublished data).

The pupal mounting technique has also allowed us to develop a method to image cuticular structures in late stage pupae using the scanning confocal microscope, in order to visualize the morphology of mechanosensory bristles and epidermal hairs using the intrinsic autofluorescence of the cuticle. These images resemble scanning electron micrographs of fly cuticle, but can be acquired from live animals, and allow us to simultaneously visualize cuticular morphology and expression of GFP fluorescence^{6,7,12}.

Disclosures

No conflicts of interest declared.

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