Protocol

Drosophila Macrophage Preparation and Screening

Richard M. Parton, Ana Maria Vallés, Ian M. Dobbie, and Ilan Davis

INTRODUCTION

Drosophila plasmatocytes, also known as macrophages, are part of the *Drosophila* innate immune system and also have roles during development. In late-stage embryos, it is possible to image macrophage migration in situ during development and when they converge at sites of wounding. This protocol describes the isolation of macrophages from third instar *Drosophila* larvae. The macrophages can be cultured for several hours, and fluorescently labeled macrophages can be screened using a fluorescence-imaging system.

RELATED INFORMATION

The isolation of macrophages from third instar *Drosophila* larvae is shown in Figure 1. General information is available about **Live Cell Imaging in *Drosophila melanogaster*** (Parton et al. 2010). Information is also available on **Maintenance of a *Drosophila* Laboratory: General Procedures** (Ashburner and Roote 2007).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Concanavalin A (ConA; 300 mg/mL; Calbiochem)

Drosophila melanogaster

For culture of Drosophila*, see* ***Maintenance of a* Drosophila *Laboratory: General Procedures*** *(Ashburner and Roote 2007).*

D. melanogaster, expressing the fluorescently labeled protein of interest (optional; for fluorescent screening only)

D. melanogaster, Oregon-R strain (optional; for fluorescent screening only)

Fly food in a bottle or vial (see, e.g., http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/media-recipes.htm)

Grape agar powder (Genesee Scientific)

Prepare according to the manufacturer's instructions in 9-cm plates.

<**R**>Phosphate-buffered saline (PBS) (optional; see Steps 7 and 9)

Schneider's insect medium (Sigma-Aldrich)

Sylgard 184 silicone elastomer (Dow Corning)

Prepare Sylgard in advance by mixing the two components according to the manufacturer's instructions. Aliquot into microcentrifuge tubes. Store at -20°C until needed.

Vacuum grease may be used as an alternative to Sylgard (see Step 4).

Adapted from *Live Cell Imaging*, 2nd edition (ed. Goldman et al.).
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Equipment

Coverslips, #1.5, 22- × 22-mm
Filter paper
Fluorescence-imaging system, upright (e.g., Imsol custom-modified DeltaVision Core on an Olympus BX51 stand, equipped with a 60X 0.9-numerical aperture [NA] dipping objective) (optional; for fluorescent screening only)
Forceps or tweezers, fine, two pairs
Hood, laminar flow
Incubator preset to 21°C-25°C
Microscope, dissecting
Microscope, inverted
Microscope slide, aluminum, 2-mm thick
These slides must be custom-made from aluminum sheeting of the appropriate thickness.
Petri dish, 10-cm square
Plates, tissue culture, six-well (Iwaki 3810-006) (optional; see Step 17)
Coverglass-bottomed multiwell culture chambers are also available for use with inverted microscopes, but these tend to be expensive.
Rods, glass or wooden
Slide rack
Teflon membrane (e.g., standard membrane kit; YSI Life Sciences 5793)
Tissue paper
Watch glass

METHOD

Preparation of Slide Culture Chambers

Alternatively, use a chambered cover glass (e.g., Lab-Tek II, NUNC) that can be coated with ConA.

1. Load a modified slide rack with #1.5 coverslips. Immerse the rack in a 300-mg/mL solution of ConA for a few minutes.
2. Remove the slide rack. Blot excess solution.
3. Place individual coverslips flat on filter papers in a laminar flow hood. Allow the coverslips to dry.
4. Secure the coated coverslips to aluminum slides using either vacuum grease or Sylgard (see Fig. 2).

Collection and Preparation of Larvae

5. Place newly emerged adult *Drosophila* in a bottle or vial containing fresh fly food. Allow them to lay eggs for ~3 d before tipping them onto fresh medium.
At 25°C, third instar larvae should develop 3-5 d after eggs were laid.

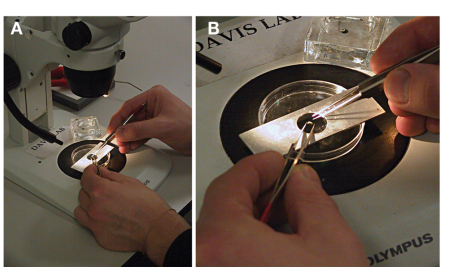

FIGURE 1. Isolation of macrophages from larvae. (*A*) Handling a larva under the dissecting microscope. (*B*) Close-up view of the slide chamber resting on a 5-cm Petri dish lid during a dissection.

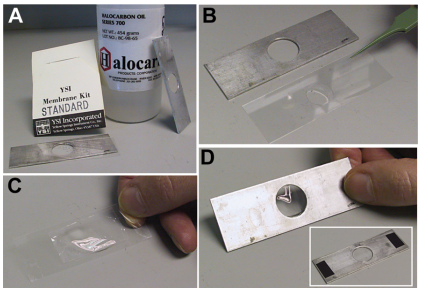

FIGURE 2. Mounting material in Halocarbon oil. (*A*) Halocarbon oil, Teflon membranes, and aluminum coverslip mounts. (*B*) Laying a piece of membrane over the mounting medium. (*C*) Embryos on a coverslip, held by heptane glue, mounted in Halocarbon oil (series 700), and covered with a Teflon membrane. (*D*) Mounting the coverslip on an aluminum coverslip mount, forming an aluminum "slide," which can fit certain stage plates or be used on an upright microscope. (*Inset*) View of the reverse side.

6. Collect several late third instar larvae, which can be found wandering up the sides of the vial or bottle. Transfer them to a 9-cm plate of grape juice agar.
7. Just before dissection, remove three larvae to a watch glass containing water or PBS.
8. While viewing under a dissecting microscope, gently manipulate with a pair of fine forceps to remove any food debris covering the larval cuticle.
9. Transfer the larvae to a second clean watch glass with water or PBS. Repeat Step 8.
10. Roll the larvae gently on a piece of dry tissue paper.

    Proceed to Step 11 or Step 17, as desired.

Isolation of Macrophages

11. Select a larva, and place in a 250-µL drop of Schneider's insect medium in the culture chamber prepared in Steps 1-4 (Fig. 1).
12. Using two pairs of fine tweezers, pinch the cuticle at two adjacent sites. Tear very slightly to release the hemolymph into the medium, avoiding breaking the gut. Repeat for three larvae.
13. Cover the medium surface with a strip of breathable Teflon membrane (Fig. 2).
14. Place the culture chamber in a humidified container made from a 10-cm-square Petri dish lined with moistened filter paper.

    Support the culture chamber above the wet filter paper on glass or wooden rods.
15. Culture at 21°C-25°C.

    To prevent macrophages being displaced to the periphery of the field, avoid moving the chamber for the first 30 min. After ~30 min on ConA, macrophages should have adhered to the coverslip surface. Cultured in this way, macrophages remain viable for at least 8 h.
16. Using an inverted microscope, image the macrophages in the slide culture chamber at high resolution (Fig. 3A-C).

Screening Macrophages by Fluorescence Imaging

Use Oregon-R larvae for the first well as a negative control. It is also useful to have a fluorescent-protein-expressing positive control available.

17. Place a 250-µL drop of Schneider's insect medium at the center of each well of a six-well plate. Place a larva in the drop.

    This prevents the macrophages from being dispersed to the edge of the well.
18. Using two pairs of fine tweezers, pinch the cuticle at two adjacent sites. Tear very slightly to release the hemolymph into the medium, avoiding breaking the gut.
19. After 30-60 min, screen the plates on an upright fluorescence-imaging system.
20. Collect images systematically at three different exposures to distinguish different levels of fluorescence expression (Fig. 3D).

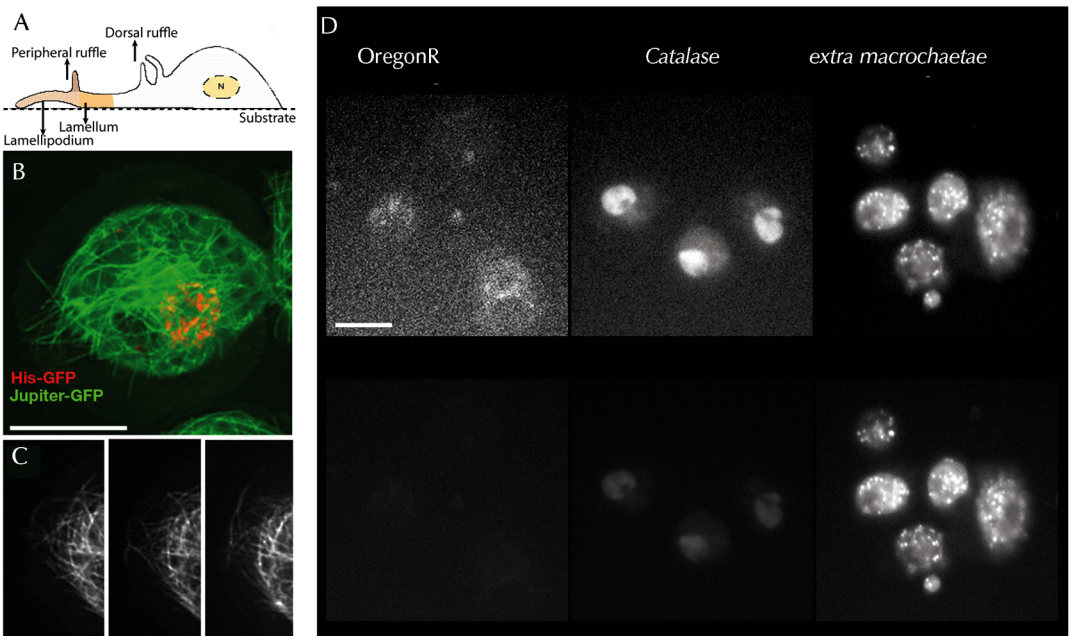

FIGURE 3. Fluorescence imaging of macrophages. (*A*) Diagram of a typical macrophage (see Chhabra and Higgs 2007). (*B*) Wide-field deconvolution live imaging of a spread macrophage labeled with Jupiter-green fluorescent protein (GFP) (microtubules in green) and histone-red fluorescent protein (RFP) (chromosomes in red). (*C*) Time series of microtubule dynamics at the lamellipodia of a spread macrophage labeled with Jupiter-GFP. (*D*) Yellow fluorescent protein (YFP) gene-trap screen in macrophages. Images shown are Oregon-R control line and two positives lines. (*Upper* row) Optimally contrasted images; (*lower* row) images contrasted relative to a positive YFP-expressing control line. Note the high and low levels of expression. Scale bar, 10 µm. (For color figure, see doi: 10.1101/pdb.prot5404 online at www.cshprotocols.org.)

DISCUSSION

Drosophila plasmatocytes (macrophages) are part of the *Drosophila* innate immune system (Lemaitre and Hoffman 2007) and also have roles during development (Wood and Jacinto 2007). In late-stage embryos, it is possible to image macrophage migration in situ during development and when they converge at sites of wounding (Stramer et al. 2005). Macrophage-specific UAS/Gal4 drivers facilitate targeted expression of tagged proteins in fly lines. The S2 insect cell lines that have previously been used in high-throughput screening projects by Perimon and Baum (Cherry 2008) share many of the properties of macrophages. In a large-scale genetic screen, macrophages taken from yellow fluorescent protein (YFP)-tagged protein trap lines were imaged to identify the subcellular localization of the tagged proteins (AM Vallés, I Davis, D St. Johnston, E Ryder, J Roote, H Spriggs, unpubl.; see also http://www.flyprot.org/).

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