Apical Radial Actin Filaments (ARAFs) in the *Drosophila* eye

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1. Introduction

The *Drosophila* eye tissue is comprised of several types of epithelial cells arranged in a highly organized structure (Figure 1A and B). Each cell type has a specific shape and is arranged in a specific location to generate a functional organ. Hence the pupal retina is an optimal model to explore the processes that occur within cells during development to generate patterned organs with different cell shapes.

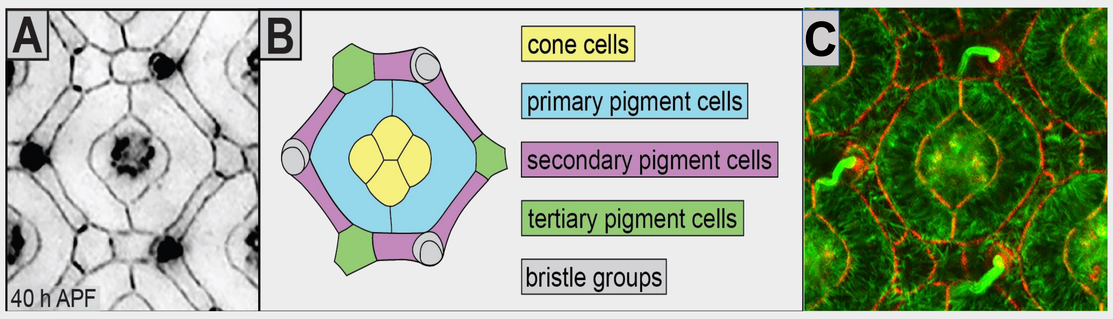

Figure 1. The *Drosophila* ommatidium: The *Drosophila* retina is comprised of individual eye units known as ommatidia. (A) An ommatidium at 40 h APF. (B) Cartoon depiction of the ommatidium shown in panel A . Cone cells are marked in yellow, primary pigment cells are marked in blue, secondary pigment cells are marked in purple, tertiary pigment cells are marked in green, and bristle groups are marked in grey. Adapted from McGhie (2021). (C) Fluorescent image of a wildtype Drosophila ommatidium at 40 h APF in fixed tissue (DeAngelis et al., 2020). E-cad is marked in red and F-actin in green.

Our lab has identified actin filaments in the apical region of 1º cells, and we have named these apical radial actin filaments (ARAFs) because of their organization (Figure 1C). We hypothesize that these actin structures behave like stress fibers (except they're apical, in contrast to stress fibers that are normally basal). We have previously observed ARAFs in fixed tissues, and here we use live imaging to more completely characterize ARAFs. To do this, we imaged the genotype *GMR-Gal4, UAS-lifeact^{GFP} / shg^{tom}.* In this genotype, Lifeact^{GFP}, a small GFP-tagged protein that binds to F-actin without disrupting its structure, is expressed in the eye. *shg^{tom}* encodes E-cadherin tagged with red fluorescent protein, so that we can see the apical outlines of cells (Brand & Perrimon, 1993). Hence, we can observe the actin cytoskeleton. We also use live imaging to conclude that ARAF development occurs at approximately 37 hours after puparium formation (APF).

2. Live Imaging of *Drosophila* Pupal Eyes

- To prepare pupae for live imaging, we removed the brown pupal case to reveal the head and thorax (Figure 2A).
- The pupa were mounted on a microscope slide at a 45º angle, supported by Scotch mounting putty, so that eye faced upward (Figure 2 A,B).
- The posterior of the pupa was glued to slide with Elmer's Glue (to secure pupa when inverted during imaging).
- A small piece of filter paper, dipped in water, was placed next to the pupa (to prevent dehydration), which was surrounded by ring of putty (Figure 2C), with a cover slip on top that was depressed to touch the eye.

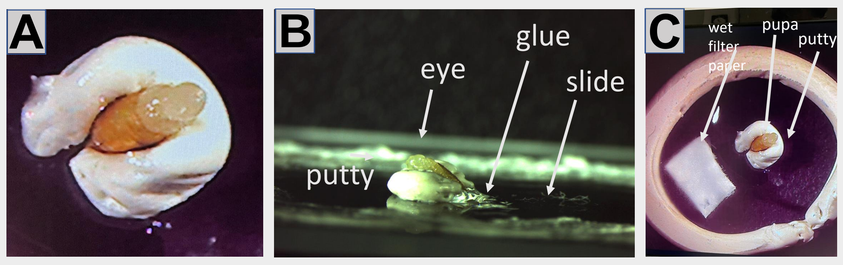

Figure 2. Live Imaging set-up: (A) Pupa nested in putty, (B) on slide at 45˚ secured with glue. (C) Setup before cover-slipping.

Imaging Protocol

- Using a Leica SP8 inverted confocal laser-scanning microscope,
- at a room temperature of 23ºC,
- we gathered Z-stacks of 32-42 'slices' through the apical region of the eye.
- Each Z-stack was gathered 5-7 mins apart and took ~5 mins to gather.
- Pupae were imaged for 3 to 9 hours (see Figure 3).

Image Processing

- Multi-focus projections were generated, using Leica LASX software, of the apical actin cytoskeleton in 3 regions of an eye. Each region was of ~3 ommatidia, the regions selected were spaced across the eye.
- Multi-focus projections were exported as TIFFs and further processed in Adobe Photoshop.
- Time-lapse videos of images were assembled in Photoshop.

Timeline of Live Imaging

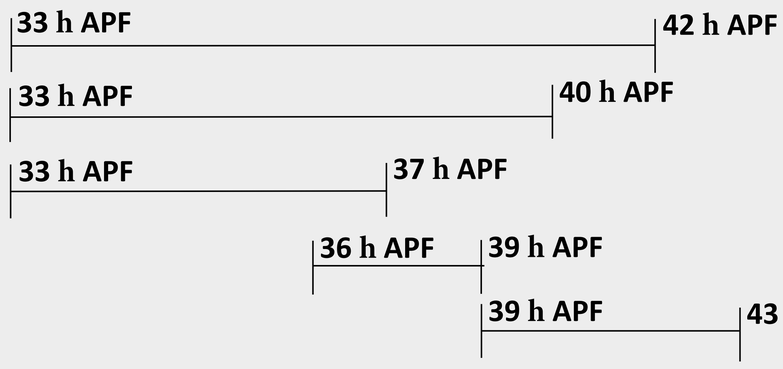

Figure 3. Live imaging sessions: *Drosophila* were imaged live a total of 5 times for the time periods as shown.

3. ARAF Development

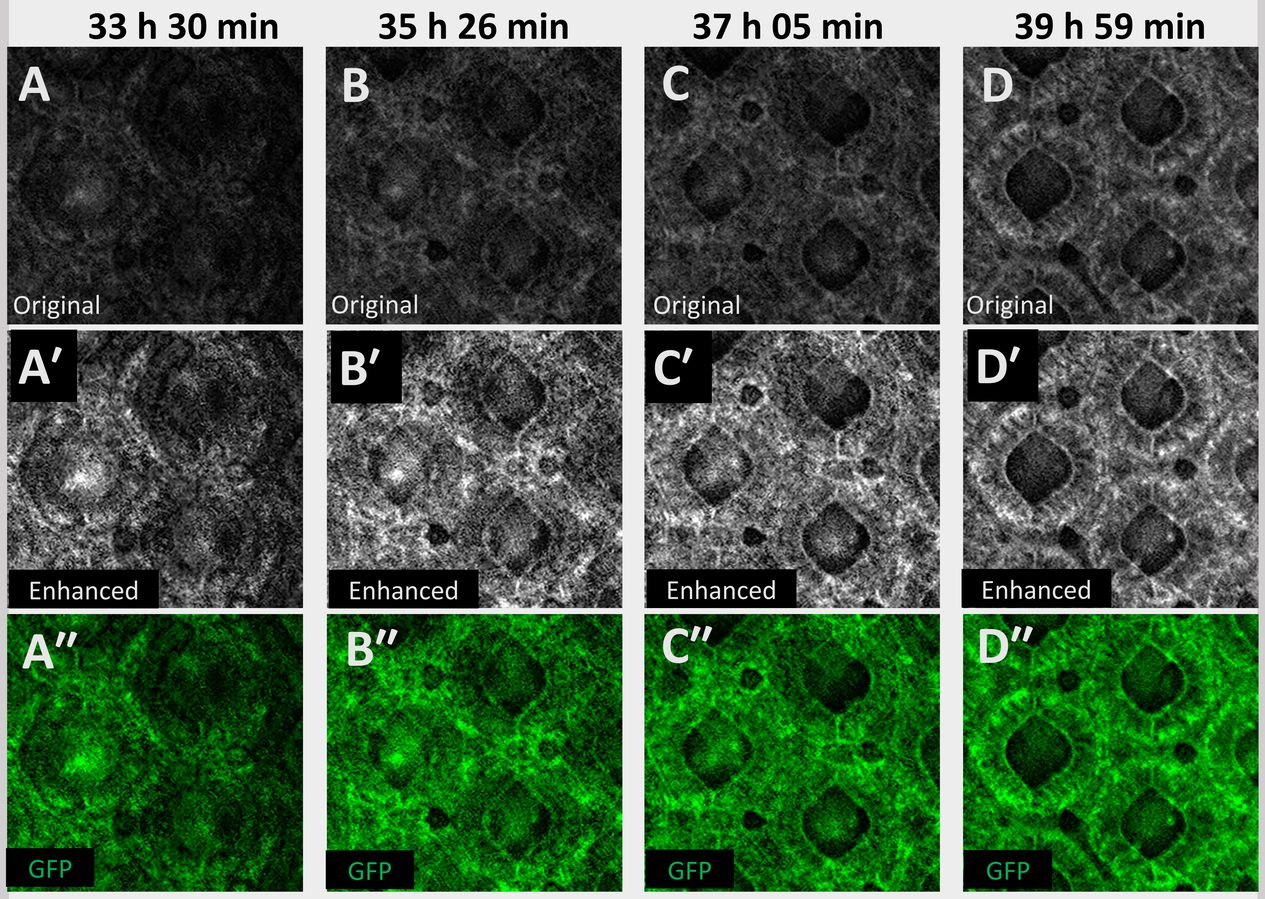

Figure 4. Images of the same ommatidia during a live imaging session: The same region of the eye, at times as indicated (A-D). Original images. (A′-D′) The same images digitally enhanced so that F-actin can be observed. (A″-D″) Enhanced images with GFP in green.

We observed that:

- The density of apical F- actin increases gradually and dramatically from 33 h APF through to 40 h APF (Figure 4 A-D).
- Whilst some apical F-actin can be observed in the 1º cells at 33 h APF, from 37 h APF we began to see clear and well-organized ARAFs. Hence we conclude that ARAFs are generated around 37 H APF.
- After 40 h APF, ARAFs became increasingly dense (Figure 4 D′, D″).
- In addition, the density of F-actin gradually decreases in the cone cells so that from about 40 h APF cone cells were mainly cleared of apical F-actin (Figure 4D).

4. Apical Expansion of the 1º Membranes

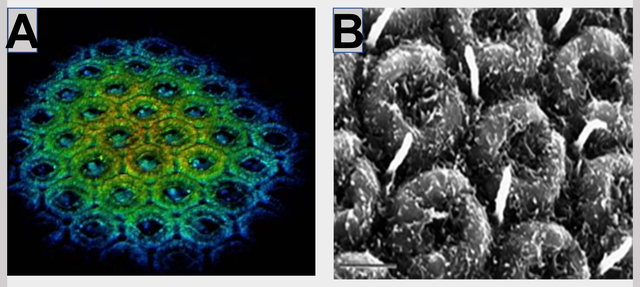

Figure 5. Apical 1º membranes "balloon" apically (A) 3D depth coding image of an eye imaged at 41 h APF. (B) SEM scan of ommatidia at 40 h APF (from Fröhlich, 2001).

In 3D depth-coding images, **we observed that** the apical membrane of 1º cells gradually expands (Figure 5A). This observation concurs with previously-published scanning electron micrographs of the *Drosophila* retina (Figure 5B).

5. Future Directions

Our goal now is to understand *how* and *why* ARAFs are generated. Hence, we will:

- Explore if ARAF generation is related to the expansion of 1º membranes. ARAFs may support this elaborated membrane.
- Investigate whether formins are required to generate the ARAFs. Formins are involved in polymerization of F-actin and actin bundles.
- Develop strategies of image analysis to quantify the ARAFs.

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