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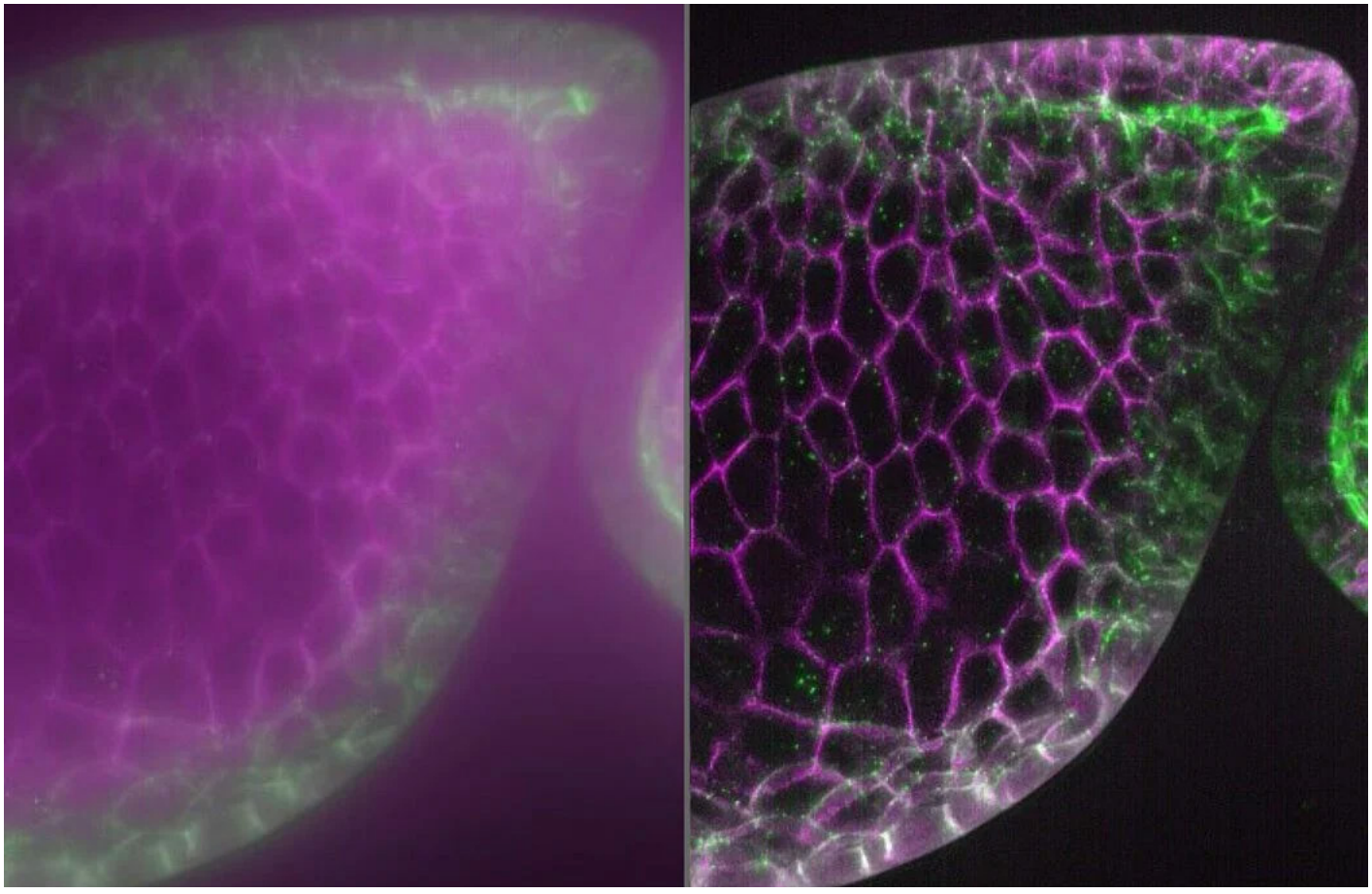
INVESTIGATING EPITHELIAL MORPHOGENESIS AND THE GROWTH OF EPITHELIA

Drosophila follicles as a model system to study the effect of genetic mutations on epithelial organization



Authors

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Abstract

The results reported here show the advantage of studying epithelial morphogenesis as it relates to cancer research with a THUNDER Imager 3D Assay. The Drosophila follicle, or egg chamber, is used as a model system to study the factors that control polarity, growth, and morphogenesis of epithelial tissue. One goal is to better understand how epithelial organization prevents tumor formation. THUNDER imaging of Drosophila follicles can enable the apicobasal-proteins distributions in mutant and wild-type follicles to be more clearly distinguished.

Epithelial morphogenesis

Cancer researchers study the molecules and mechanisms that govern the polarity, growth, and morphogenesis of epithelia, the fundamental tissue of all animals and the major constituent of human organs. They also use the follicles or egg chambers of Drosophila fruit flies as a model system for cancer studies to understand how epithelial organization prevents tumor formation and how tumors kill their hosts. The effect of various genetic mutations on the apicobasal polarity of the follicle are evaluated.

Challenges when imaging Drosophila follicles

There were 2 main challenges encountered when imaging thick follicles or egg chambers of Drosophila model organisms with conventional widefield microscopy. One was being able to quickly identify follicles in the stage of interest, especially those with such small structures. Another challenge was that widefield images often had a haze or out-of-focus blur produced by light scattering [3,4]. The haze can obscure interesting structures inside the follicle.

Methods to investigate Drosophila follicles

The LAS X Navigator software was used with a THUNDER Imager 3D Assay to quickly screen the Drosophila specimen and identify the egg chambers or follicles of interest. Specimens of whole Drosophila follicles expressing fluorescently-tagged aPKC, an apical marker, and Dlg, a basolateral marker, were imaged with the THUNDER Imager using a 63x/1.4 numerical aperture (NA) oil-immersion objective. Maximum intensity projections of z-stacks in 3D were also acquired. After imaging a follicle, large volume computational clearing (LVCC) was applied..

Results concerning apicobasal proteins

A critical and time-consuming part of the workflow is identifying the correct stage and genotype of Drosophila follicles to image when searching over the whole coverslip area of a specimen of dissected ovaries. The THUNDER Imager 3D Assay with LAS X Navigator made screening more rapid [5]. Clusters of dissected follicles were quickly located at low magnification and then the THUNDER Imager objective was switched to the 63x oil-immersion one to find the right stages of follicle development. A parfocal and parcentric imaging solution was key for a seamless transition between the lower and higher magnification scans. The images in figure 1 show 2 follicles from a single ovariole. The larger follicle seen on the left is at a later stage of development than the one on the right. The follicle epithelial cells exhibit apicobasal polarity which is revealed by the juxtaposition of the apical marker aPKC (green) and the basolateral marker Dlg (magenta). The follicle on the left is a mutant for the polarity gene Scrib [1]. It has lost polarity, as evidenced by the disorganized architecture and loss of segregation between aPKC and Dlg. The follicle on the right is wild type. The difference in distribution of apicobasal proteins between wild-type and mutant egg chambers is more clearly distinguished with THUNDER images.

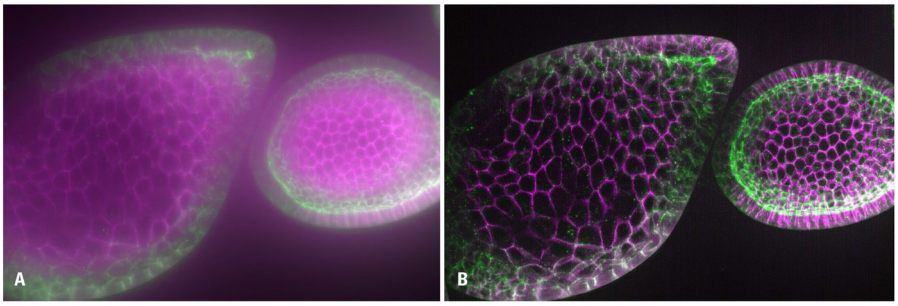

Figure 1: A) Raw widefield and B) THUNDER image representing a 3D maximum intensity projection of a 27.5 µm thick z-stack of Drosophila follicles. The THUNDER image was processed with LVCC. Green indicates aPKC and magenta Dlg. Images courtesy of Mark Khoury and Dr. David Bilder, University of California, Berkeley, USA.

Conclusions

The results demonstrate that distributions of apicobasal proteins can be more clearly distinguished between mutant and wild-type Drosophila follicles or egg chambers in THUNDER images, where large volume computational clearing (LVCC) was applied, compared to raw widefield ones. Such haze-free, rapidly acquired THUNDER images are useful for the study of epithelial morphogenesis in cancer research.

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