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Methods for Studying the Optics, Physiology, and Biochemistry of the Fly Compound Eye

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Editorial

The fly compound eye is an important biological model system for elucidating fundamental biological mechanisms. Some of the mechanisms that have been studied using this system include the following: compound eye vision with a nearly panoramic field of view[1]; the biophysics, protein assembly, and biogenesis of the light-sensitive G-protein coupled receptor[2,3,4]; the phospholipase Cβ-mediated signal transduction system[5,6]; TRP channel activation and modulation in native tissue[5,6]; the assembly and function of the multi-protein signaling complex[7,8]; the molecular structure, biochemistry, and function of inositol-phospholipid recycling[9,10]; and the signal-mediated subcellular translocation of signaling proteins[11,12]. The unique anatomical structure and optics of the fly compound eye have been extensively studied, revealing fundamental developmental mechanisms and visual capacities.

This methods collection comprises the work of leading scientists who have developed and refined methods for studying some of the above mechanisms in the fly compound eye. Muñoz et al. describe a method for quantitatively analyzing the optical arrangement of the fly eye that provides an accurate portrayal of the compound eye and its spatial organization[13]. This method is essential for studying the neuronal processing of visual information in detail. Importantly, there have been many attempts to produce bio-inspired artificial eyes, and these have relied on quantitative studies of existing compound eyes. Wagner et al. utilize the deep pseudopupil, a unique optical phenomenon of the compound eye, and high amplification microscopy with the optical neutralization of the cornea to localize eGFP-tagged proteins within *Drosophila* photoreceptor cells[14]. This method allows the detection of structural defects in the rhabdomeres due to photoreceptor degeneration and cellular localization and trafficking of important signaling proteins. Gutorov et al. utilize electrophysiological measurements from the whole eye or from single photoreceptor cells to measure the level of photopigment states *in vivo* and their modulations by specific mutations in signaling proteins[15]. This method exploits the charge displacements within a visual pigment state (R) induced by the absorption of photons, which results in the formation of a second pigment state that is stable in the dark (M). An additional method uses prolonged depolarization after light-off (PDA) which exploit the dark bi-stable properties of the visual pigment and the

differences in the absorption spectra of the fly R and M states. Since the PDA depends on the conversion of a large amount of photopigment, even the slightest defect in the biogenesis of the photopigment may lead to an easily detectable abnormal PDA. Indeed, *Drosophila* mutants displaying abnormal PDA have led to the discovery of new signaling proteins. An outstanding example of this is the discovery of the novel protein complex composed of the scaffold protein inactivation but no afterpotential D (INAD), the eye-specific protein kinase C (eyePKC), the phospholipase Cβ (PLCβ), and the transient receptor potential (TRP) channel. For the first time, Liu et al. present a technique to purify the *Drosophila* TRP channel[16]. This modified affinity purification method uses a competition strategy that is based on the assembly mechanism of the INAD signaling complex. This strategy enables the purification of the endogenous TRP channel. According to this method, first a PLCβ fragment bound to Ni-beads is tagged with histidine and used to pull down the endogenous INAD protein complex obtained from *Drosophila* head homogenates. Next, TRP fragments that are tagged with excessively purified glutathione S-transferase (GST) are added to the Ni-beads to compete with the endogenous TRP channel bound to the INAD. Finally, size-exclusion chromatography is used to separate the tagged TRP channel fragments in the supernatant from the endogenous TRP channel. Panda et al. report a sensitive technique to identify and quantify several classes of lipids in *Drosophila* photoreceptor cells using mass spectrometry[17]. The activation of PLCβ is crucial for phototransduction, as this leads to the hydrolysis of phosphatidylinositol 4,5 bisphosphate ($PI(4,5)P_2$) in the surface membrane. This reaction ultimately leads to the activation of the TRP channel. In addition, PLCβ activity results in the generation of many lipid species. It has been proposed that several of these lipid species may take part in TRP activation and play key roles in the functional organization of photoreceptors for optimizing sensory transduction. In the study by Panda et al.[17], these findings were assessed by isolating *Drosophila* mutants harboring defective enzymes that control the levels of specific lipids.

The fly compound eye is a useful experimental system for elucidating fundamental mechanisms in biology. The powerful genetic methodology developed in *Drosophila* can be used to analyze and discover the signaling proteins involved in fly phototransduction. This methods collection outlines in detail major techniques for investigating the fundamental mechanisms of fly vision, including the anatomical and optical analysis of the compound eye and the molecular components and biochemical reactions of the photoreceptor cells. The video articles also include important advice on the inherent difficulties of these methodologies. This methods collection should be also beneficial to scientists outside this field of research as these techniques could be adapted to other biological preparations.

Disclosures

The author has nothing to disclose.

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