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Eye development

Nicholas E. Baker [a,b,c,*], Ke Li [a], Manon Quiquand [a], Robert Ruggiero [a,1], Lan-Hsin Wang [a]

[a] Department of Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States
[b] Department of Ophthalmology and Visual Sciences, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States
[c] Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States

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ABSTRACT

The eye has been one of the most intensively studied organs in *Drosophila*. The wealth of knowledge about its development, as well as the reagents that have been developed, and the fact that the eye is dispensable for survival, also make the eye suitable for genetic interaction studies and genetic screens. This article provides a brief overview of the methods developed to image and probe eye development at multiple developmental stages, including live imaging, immunostaining of fixed tissues, *in situ* hybridizations, and scanning electron microscopy and color photography of adult eyes. Also summarized are genetic approaches that can be performed in the eye, including mosaic analysis and conditional mutation, gene misexpression and knockdown, and forward genetic and modifier screens.

1. Introduction

Studies of the *Drosophila* eye have made multiple contributions to developmental biology. The *Drosophila* eye is one of the first parts of the adult nervous system to differentiate, and the most substantial part of the nervous system to differentiate outside the protection of a pupal case or egg shell. Furthermore, while the eye is required for vision, it is not required for life, facilitating the study of lethal genotypes. The color and detail of eye structure has made the eye appropriate for forward genetic screens.

The *Drosophila* eye differentiates from an epithelium, the eye imaginal disc, in the late third larva instar and early part of the pupa [1]. As a compound eye, the *Drosophila* eye contains ~750 ommatidia, or unit eyes, each of which contains eight photoreceptor neurons (Fig. 1A). Each ommatidium also contains four non-neuronal cone cells and two primary pigment cells, and is surrounded by a shared lattice of secondary and tertiary pigment cells and interommatidial bristle organs. The ommatidial structure is very precisely repetitive in normal individuals, so that subtle abnormalities may be recognized (Fig. 1B).

Modern study of the *Drosophila* eye may be traced to the classic paper of Ready et al. [2], which in addition to descriptive study, also demonstrated that ommatidia were not clonal units. Lawrence and Green [3] later demonstrated that almost any pair of eye cell types could be related at the final mitosis, ruling out inheritance of determination states in eye cell fate specification, thereby implying that cell interactions must specify this highly repetitive structure. Electron microscopic reconstruction of ommatidial assembly then led to a model that short range cell interactions determined the majority of eye cell fates [4]. This understanding underscored the molecular genetic studies of eye development that were instrumental for uncovering many aspects of developmental signaling by receptor tyrosine kinases, the Notch pathway, and other universal developmental regulators [5–10].

While many important questions remain in the study of eye development itself, the tools developed in the course of *Drosophila* eye studies, coupled with the readily apparent structure and dispensable function of the organ, also make the *Drosophila* eye an exemplary system for investigating general biological processes, and for unbiased genetic interaction screens with the potential to characterize new pathways, such as those associated with human disease genes. The purpose of this article is to outline some of the basic tools, both experimental and genetic, that can be used to characterize development and gene function using the *Drosophila* eye. It is not a list of protocols, nor intended as an update for the expert, but provides summaries of the main approaches that would be routine in many 'eye labs', wherever possible including citations

* Corresponding author at: Department of Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States. Fax: +1 718 430 8778.
E-mail address: nicholas.baker@einstein.yu.edu (N.E. Baker).
[1] Present address: Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO 80045, United States.

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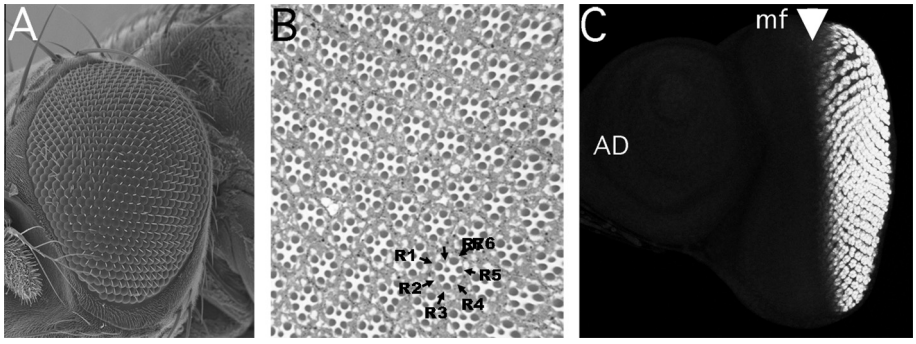

Fig. 1. Summary of *Drosophila* eye structure and development (A). Scanning Electron Micrograph of the adult eye, with anterior to the left. Each adult compound eye contains ~750 facets, or ommatidia. (B) Thick section through an eye revealing the repetitive cellular pattern. The rhabdomeres of seven of the eight photoreceptor neurons are apparent in each plane of section, and are labeled for one ommatidium. (C) Eye-antennal imaginal disc from a mid-third instar larvae (~96 h after egg laying). Clusters of differentiating photoreceptor neurons behind the morphogenetic furrow (arrowhead) are labeled with antibody against ELAV. The morphogenetic furrow moves from posterior to anterior (left or right in this image) across the eye disc. Ahead of the morphogenetic furrow, individual cell fates remain unspecified and no ELAV labeling is seen. The antennal disc (AD) is also unlabeled.

to more detailed methods. This may provide an entry point and resource for those considering exploiting *Drosophila* eye methods for their research.

2. Eye methods

2.1. Development and anatomy of the eye

General features of the *Drosophila* eye and eye-imaginal disc are shown in Fig. 1. For more detailed accounts of the development of the eye imaginal disc, see [1].

Cells that will contribute to the *Drosophila* eye, head capsule, and antenna, separate from the larval epidermis during embryogenesis [11,12]. After hatching (about 22 h after egg laying at 25 °C), imaginal discs grow suspended in the body cavity of the three successive larval instars until pupariation (about 120 h after egg laying at 25 °C). The distinction between antennal and eye portions becomes more apparent over larval life. By the third larval instar (~72 to ~120 h after egg laying), the 'eye disc' portion also contains cells that will contribute to the adult head epidermis. Specification and differentiation of individual retinal cells begins early in the third larval instar, and continues beyond pupariation. Once all the cells are specified, head eversion moves the eye and head tissues into their adult configuration before the end of pupation. Adults emerge typically from the pupae 9 days after egg laying at 25 °C. For a detailed account of the *Drosophila* lifecycle, see [13].

Specification of the individual retinal cells begins in the third larval instar, ~72 h after egg laying, and is associated with a 'morphogenetic furrow' that progresses across the eye disc epithelium [1,2]. The morphogenetic furrow is an indentation of the epithelium, associated with cell shape changes and transient cell cycle arrest. The morphogenetic furrow appears to be one of the first aspects of adult differentiation to begin after the commitment to begin adult differentiation.

Within the morphogenetic furrow, individual R8 photoreceptor precursors are specified that each found an ommatidium by recruiting adjacent cells to other photoreceptor cell fates (Fig. 1C) [4,14]. Many publications describe the cellular sequence of ommatidial assembly in the eye disc [1,4,15,16]. One new ommatidial column begins every 90–120 min (slowing with time), up to a total of ~30 columns. The progression of the morphogenetic furrow across the disc means that each eye disc contains ommatidia at a range of developmental stages, arrayed in sequence posterior to the morphogenetic furrow.

Once specified, cells begin to execute their specific differentiation programs [1]. Still before pupariation, larval photoreceptor neurons extend axons down the optic stalk towards the brain, where the find their target zones in the lamina and medulla. Glial cells migrate in the reverse direction, following photoreceptor axons from the brain back to the eye disc.

When the larva pupariates and pupation begins, the eye imaginal discs at first continues to occupy a peripheral position close to the surface of the animal. Later, tissue movements associated with head eversion rearrange the eye and brain into their adult organization.

2.2. Live imaging

2.2.1. Live imaging eye imaginal discs

Two approaches have been described to image living eye discs. One is to visualize the eye disc within the immobilized pupa, the other cultures eye discs explanted from the larva. In both methods the eye discs should express a fluorescent-tagged protein for imaging, such as Cadherin::GFP which label cell boundaries [17], or Histone 2N::GFP which labels chromatin [18].

2.2.2. Imaging the developing eye within the pupa

During the first part of pupal development, while the morphogenetic furrow is still advancing, the eye disc lies close to the surface of the animal and can be imaged through the pupal epidermis after removing part of the pupal case. More detailed protocols are available elsewhere [19,20]. Animals are immobilized at the white prepupa stage on a microscope slide coated with double-sided tape. The operculum region of the puparium is removed and a three-sided supporting enclosure fashioned out of parafilm or similar material. Within this enclosure the exposed pupa is bathed in buffer and covered with a cover-slip. The whole assemblage is sealed with paraffin on three sides with halocarbon oil at the free side to permit gas exchange. Drugs can be included in the buffer, indeed levamisole is normally included to paralyze the animals. Since the animals do not feed during the pupal stage, they can be paralyzed without affecting nutrition, an advantage of imaging at this stage.

2.2.3. Imaging explanted eye discs

For a more detailed protocol see [21]. Eye-antennal discs are removed from later third-instar larvae (see Section 2.3.2 below) in PBS and transferred immediately to a glass-bottom culture dishes (e.g. MatTek Corporation), where they adhere to the glass. The PBS is immediately replaced with Schneider culture medium