Electron Tomography of the Adult Retinal cells in *Drosophila melanogaster*

Sung Sik HAN

School of Life Sciences and Biotechnology, Korea University, Seoul, Korea

1. Introduction

The study about structure of Drosophila melanogaster's photoreceptor cell using electron microscopy were carried in detail by Waddington (1962), Farquhar and Palade (1963), and Perry (1968). But, these results can have limitation in function research because of two-dimensional structure. High voltage electron microscopy (HVEM) has been a natural outgrowth of the desire to obtain 3-dimensional information due to problems related to interpretations of 3-dimensional images from 2-dimensional electron microscophs are numerous. Also, The fine-structure preservation of cells or tissues can be achieved by using fast-freezing followed by freeze-substitution techniques. The fast freezing is so much better due to the speed of fixation, which freezing virtually means stopping all molecular movement. It is estimated that samples prepared by high pressure freezing are fixed in 20-50 msec, as compared to the seconds and/or minutes by chemical fixations. In this study, the adult retina of Drosophila Melanogaster was investigated employing HVEM, fixation by high pressure freezing followed freeze-substitution, thick serial sections, and electron tomogram. The thickness of section was 200 nm and HVEM images were obtained from about 500 serial sections. From all these images the distribution of microtubules, mitochondria, and nuclei was reconstructed as three-dimensional structure using IMOD program. The current data provide us more precise cellular information and better understanding on the animal vision mechanism in new dimension.

2. Material and methods

Animal

Drosophila melanogaster were raised on cornmeal-yeast agar medium at 25℃.

Sample Preparation using High-Pressure Freezer

After the dissected sample was put in the cavity filled with 35% w/v dextran

(nonpenentrating cryoprotectant 178 000 MW; Sigma, St Louis, MO). The cavity cover and filler rings were assembled around the specimen cavity. And this allowed rapid loading into the HPF holder. After loading into the HPF holder, the specimen chamber was cryoimmobilized in a high-pressure freezer (2100 bar atmosphere)(HPM 010; BAL-TEC Inc.). Immediately after HPF, the chambers were placed in liquid nitrogen for storage. HPF samples were taken out of liquid nitrogen and transferred in liquid nitrogen to the freeze-substitution apparatus where tubes filled with 2% osmium tetroxide in acetone (dried over calcium chloride) were precooled. The substitution is carried as follows: 8h at 80 C, 8h at 30 °C, and 8h at 0 °C. The specimens are washed three times in anhydrous acetone. Finally the samples were embedded stepwise in Epon 812 (30%, 70%, 100% resin) at 25 C. Polymerization is carried out with fresh resin at 60 C for 72h.

High-voltage transmission electron microscope

For high voltage transmission electron microscope, the thickness of section was 200nm. The sections were collected on Formvar - Carbon coated copper slot grids, and stained with 3% uranyl acetate for 60 min and lead citrate for 40 min. The electron microscope was a Hitachi 1250M operating at 1000 kV. For 3-dimensional reconstruction, serial images was obtained.

3-Dimensional Reconstruction

The images of sections were digitalized by using Duoscan T2500 (AGFA). After the structures of retina cells were captured by the IMOD program.

3. Result

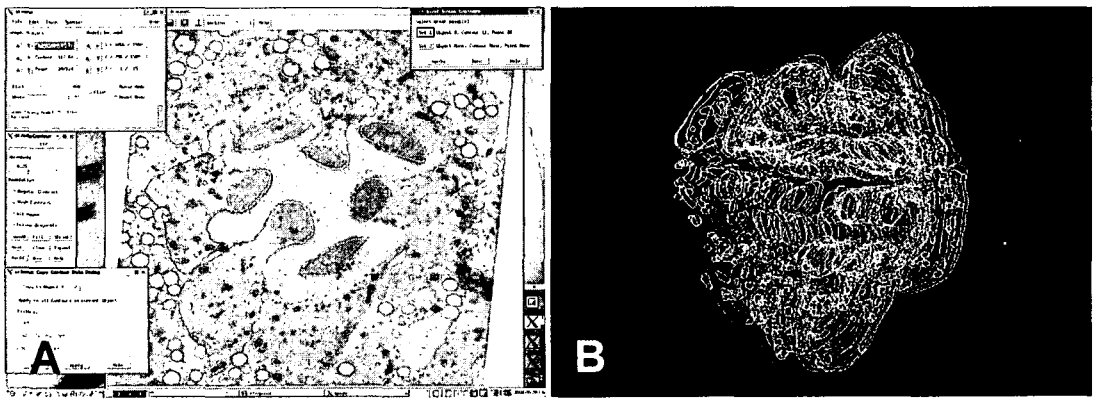

Fig. 1. Image Capture for 3-D structure using IMOD.

A; A single thin section from a series of 500 consecutive serial sections. In this section, rhabdomere, ommatidia, microtubule, and nucleus are outlined to form the set of contours defining the each organelle.

B; A series of consecutive contours (rhabdomere, mitochondria, nucleus) traced from 15 serial sections.

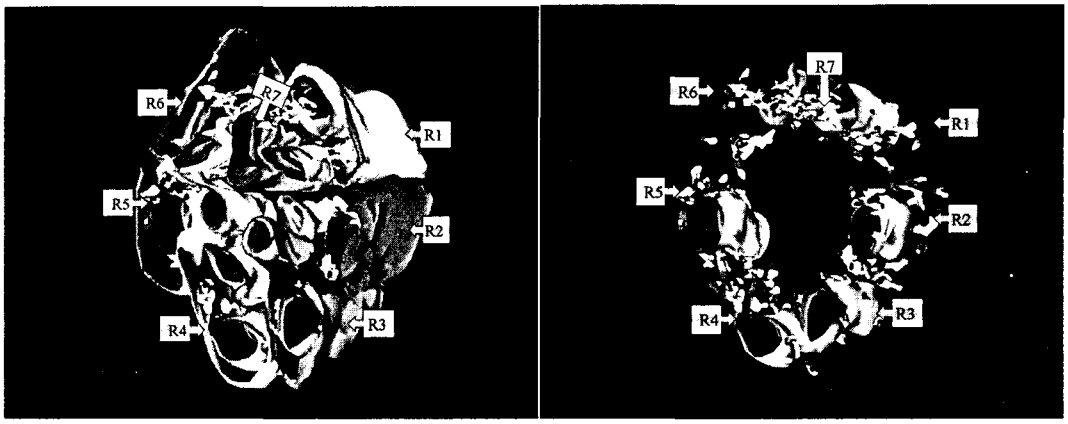

Fig. 2. 3-D model of distal side in ommatidia (21 ㎛ ~ 30 ㎛ below distal region)

In distal region, 3-D models of rhabdomere (ivory), nuclei (dark green), and mitochondria (lavender) were reconstructed in retinular cells. The number of mitochondria is 18 (R1), 17(R2), 14 (R3), 13 (R4), 16 (R5), 19 (R6), 7 (R7), in this region.

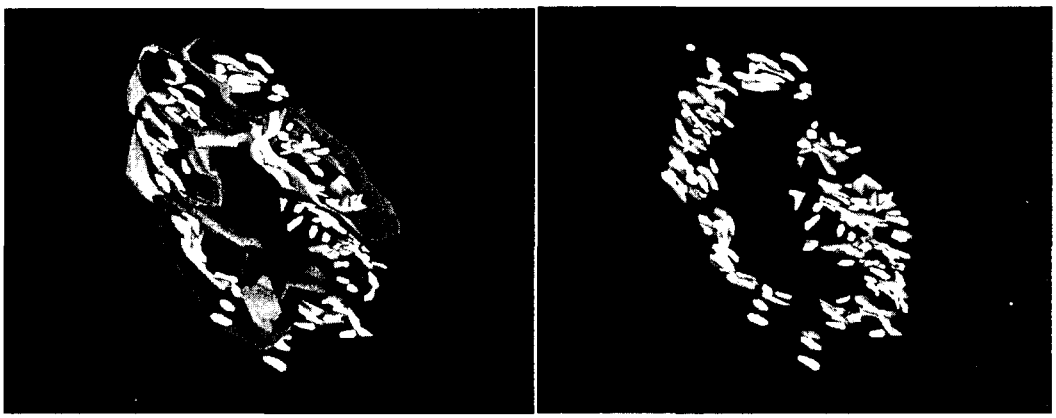

Fig. 3. 3-D model of middle side in ommatidia (60 ㎛ ~ 65 ㎛ below distal region)

This model is about 60 ㎛ ~ 65 ㎛ from distal region. 3-D model of rhabdomere (ivory), mitochondria (lavender) and ER (light violet) were reconstructed in retinular cells. The number of mitochondria is 16 (R1), 26 (R2), 11 (R3), 18 (R4), 17 (R5), 15 (R6), 15 (R7), respectively.

4. Reference

Müller-Reichert T., Hohenberg, H., O'Toole, E.T. & McDonald, M. (2003) Cryoimmunobilization and three-dimentional visualization of C. elegans ultrastructure. J. Microsc. 212, 71-80

Müller-Reichert T., Sassoon, I., O'Toole, E., Romao, M., Ashford, A.J., Hyman, A. & Antony, C.(2003) Analysis of the distribution of the kinetochore protein Ndc 10p in Saccharomyces cerevisae using 3-D modeling of mitotic spindles. Chromosoma. 111, 417-428

Perry, M. (1968) Further studies on the development of the eye of Drosophila melanogaster 1. The ommatidia. J. Morph. 124, 227-248

Studer, D., Michel, M. & Muller, M. (1989) High pressure freezing comes of age. Scann. Microsc. Suppl. 3, 253-269

Waddington, C.H. & Perry, M.M. (1960) The ultrastructure of the developing eye of Drosophila. Proc. Roy. Soc. London Ser B 153, 155-178