한국현미경학회지 제39권 제1호 2009
Korean J. Microscopy 39(2), 185~189(2009)

Three Dimensional Reconstruction of Cellular Structure in *Drosophila* Retina Using High Voltage Electron Microscopy

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초고압전자현미경을 이용한 초파리 망막 세포의 3차원 구조

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(Received May 12, 2009; Accepted June 19, 2009)

ABSTRACT

Studies about the structure of *Drosophila melanogaster* retinal cell using electron microscopy have been carried out in details since 1960s. However, these results can have limitations in functional research because of two-dimensional structure. In this study, the adult retina of *Drosophila melanogaster* was investigated by employing high pressure freezing method, serial sections, high voltage electron microscopy, and 3-dimensional reconstruction method. From there results, mitochondria, microtubules, and nuclei were reconstructed as 3-dimensional structure using IMOD program. The 3D structure of these organelles showed that mitochondira mainly located in distal region near lens, and microtubule mainly located in distal and basal region. The 3D reconstruction of these organelles can be used for a critical evaluation in the dynamic change of cellular organelles caused by functional abnormality like retinal degeneration.

Keywords : HVEM, 3D structure. Retina, *Drosophila melanogaster*

INTRODUCTION

Drosophila retina had been studied in order to investigate cell fate, development, signal transduction and so on. Also, drosophila is an animal that has most diverse mutant lines for these researches. Extensive research investigating the ultrastructure of the *Drosophila melanogaster*'s retina has begun since 1960s (Farquhar & Palade, 1963; Perry, 1968; Hess et al., 2006). However, these researches have limitations because most studies used only 2-dimensional images and conventional fixation method. High voltage electron microscopy (HVEM) has been a natural outgrowth of the desire to obtain 3-dimensional information because problems related to interpretations of 3-dimensional images from 2-dimensional electron microscopes are numerous (Hama et al., 1994). Rapid freezing method is superior to chemical fixation for ultrastructural study of *Drosophila melanogaster*'s retina (Mun et al., 2007). Therefore in this study, 3-dimensional reconstruction method using rapid freezing fixation, thick serial section, HVEM, and IMOD program were used for structural analysis of *Drosophila* retinal cells.

In this work, we used High Voltage Electron Microscope (JEM-ARM 1300S) at the Korea Basic Science Institute, Daejeon, Korea, and High Voltage Electron Microscope (Hitachi 1250M) at National Institute for Physiological Sciences, Okazaki, Japan.
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In the retina, mitochondria are located in the area demanding high energy consumption because this organelle's main function is energy production. So, mitochondria are used as a factor in functional change like abnormal signal transduction and retinal degeneration (Bravo-Nuevo et al., 2003; Gorska-Andrzejak et al., 2003; Perkins et al., 2003; Perkins et al., 2004). As universal elements of the eukaryotic cytoskeleton, microtubule in retina moves vesicles, granules and organelles like mitochondria and chromosomes (Knabe & Kuhn, 1996). The change of this cytoskeleton's distribution leads to a disturbance in the normal signaling, and due to these reports, many researchers use the change as a factor for retinal degeneration (Grayson et al., 2002; Eckmiller, 2004). Therefore, we analyzed distribution of mitochondria, microtubule, and nuclei in *Drosophila melanogaster*'s retina through 3-dimensional reconstruction method of electron microscopy.

MATERIALS AND METHODS

1. Animal

Drosophila melanogaster (Oregon-R standard strain) were raised on cornmeal-yeast agar medium at 25°C.

2. Sample preparation

1) Cryofixation method using High Pressure Freezer

After the dissected sample was put in the cavity filled with 20% bovine serum albumin (Monaghan et al., 1998; Studer et al., 2001). The cavity cover and filler rings were assembled around the specimen cavity. And this allowed rapid loading into the high pressure freezer (HPF) holder. After loading into the HPF holder, the specimen chamber was cryo-immobilized in a high-pressure freezer (HPM 010, BAL-TEC, Liechtenstein, installed KBSI in Daejeon). Immediately after HPF, the chambers were placed in liquid nitrogen for storage.

2) Freeze substitution

HPF samples were taken out of liquid nitrogen and transferred to the freeze-substitution apparatus (AFS, Leica, Vienna) where tubes filled with 2% osmium tetroxide in acetone (dried over calcium chloride) were pre-cooled. The AFS is programmed as follows: 27 h at −90°C, heating at a rate of 2 °C/h to −60°C, 8 h at −60°C, heating at a rate of 2°C/h to −30 °C, 8 h at −30°C, transfer of the samples to ice. After 1 hour, the specimens are washed three times in anhydrous acetone. Finally the samples were embedded stepwise in spurr (30%, 70%, 100% resin). Polymerization is carried out with fresh resin at 60°C for 72 h.

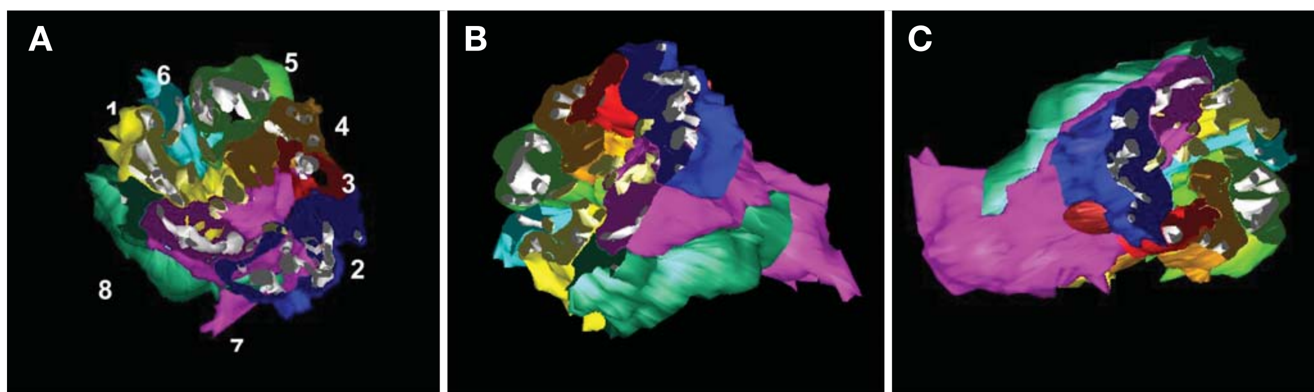

Fig. 1. 3-D model of retinal cell of *Drosophila melanogaster*. A, B, C ; Surface model of retinal cells to show the correct distribution of cells. Each organelle was assigned a separate color. R1-yellow, R2- blue, R3-red, R4-orange, R5-green, R6-sky blue, R7-pink. mitochondria (light violet), rhabdomere (ivory). 70～90 μm below distal region.

Table 1. The number of mitochondria in each area of *Drosophila* retina

	R1	R2	R3	R4	R5	R6	R7	R8
21～30 μm	18±2	17±1	14±0.3	13±1.2	16±1.5	19±2.3	7±0.2	
35～40 μm	14±1.3	11±0.5	15±1	13±0.3	18±2	22±2	5±0.1	
50～55 μm	14±1	17±2	13±0.2	11±0.2	12±0.5	17±1	13±1	
60～65 μm	16±1.5	26±2.5	11±1	18±2	17±1	15±0.5	15±1	
73～79 μm	7±0.5	8±0.2	5±1	11±1.5	8±0.2	6±0.1		2±0.1

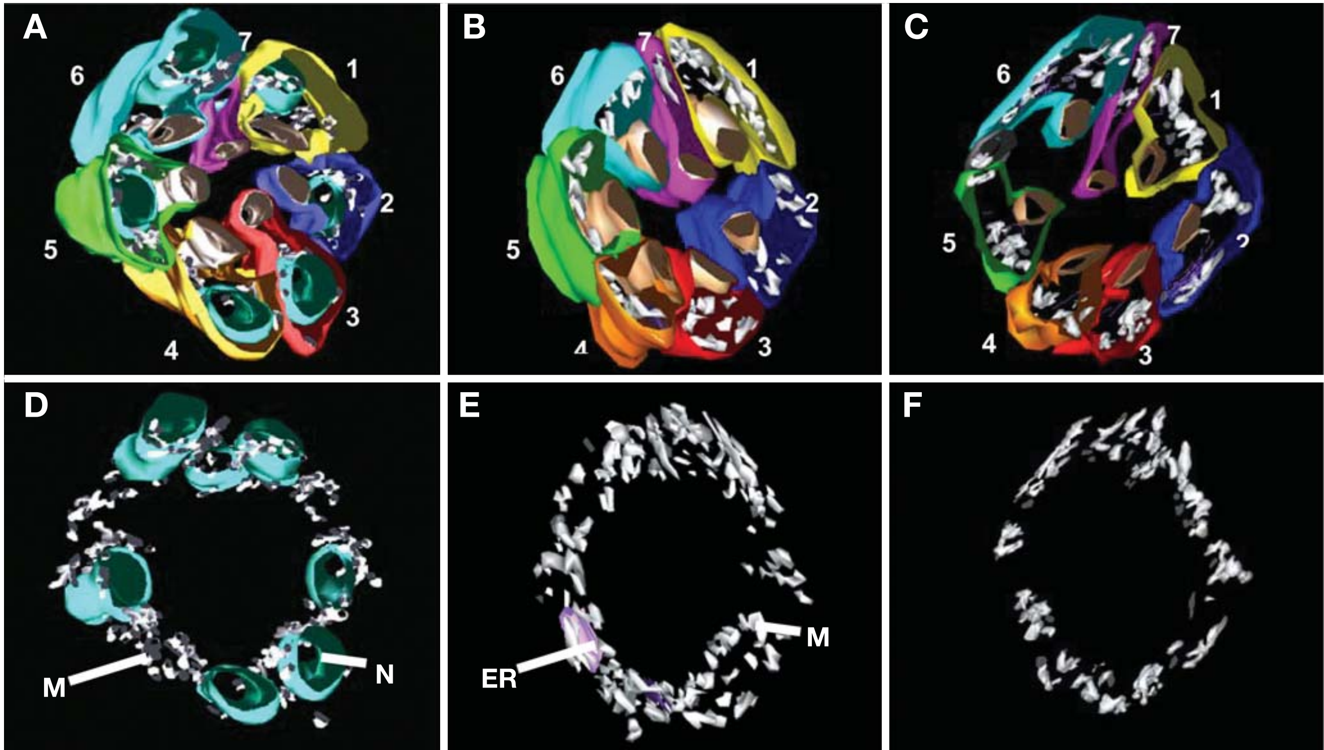

Fig. 2. 3-D model of cellular organelles in retina of *Drosophila melanogaster*. A, B, C: Surface model of retinal cells to show the distribution of nucleus, mitochondria, and rhabdomere. Each organelle was assigned a separate color. R1-yellow, R2-blue, R3- red, R4-orange, R5-green, R6-sky blue, R7-pink. D, E, F: 3-D model without cell membranes to show organelles clearly. N-nucleus (light blue), M-mitochondria (light violet), Rh-rhabdomere (ivory), ER-endoplasmic reticulum (violet) A, D: 21 ~ 30 μm below distal region, B, E: 35 ~ 40 μm below distal region, C, F: 50 ~ 55 μm below distal region).

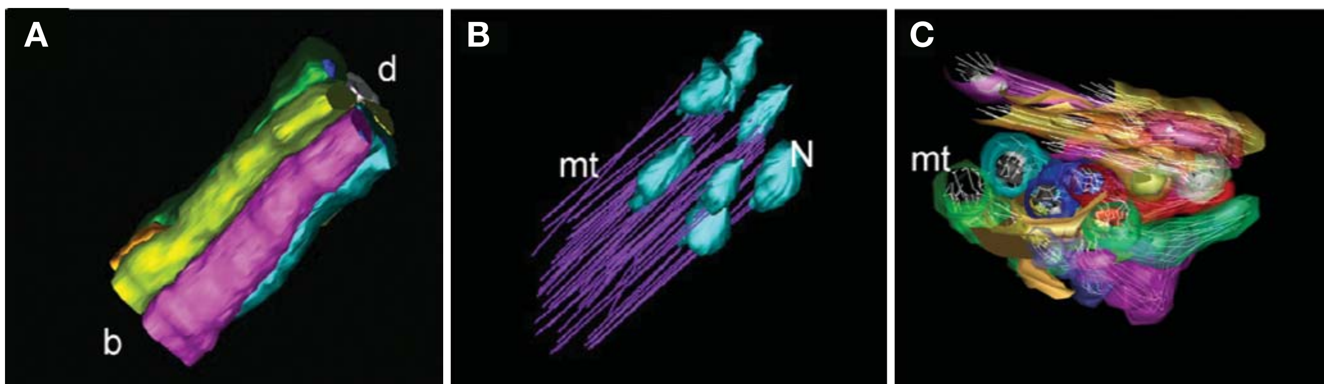

Fig. 3. 3-D model of microtubule in *Drosophila melanogaster* retina. A: Surface model of 8 retinular cells. b-basal, d-distal. B: The presence of nuclei and cross-section microtubules in ommatidium. N-nuclei, mt-microtubule. C: The presence of nuclei and cross-section microtubules in axon bundle. mt-microtubule.

3. Electron Microscopy observation

The Thickness of cross section was 250 nm with an ultra-microtome (RMC MTXL), and double-stained with 3% uranyl acetate and lead citrate. The sections were then viewed under a HVEM at 1,250 kV (JEM-ARM1300S, Jeol, Japan, installed KBSI in Daejeon) and 1,000 kV (1250M, Hitachi, Japan, installed NIPS in Okazaki).

4. 3-Dimensional reconstruction

The images of sections digitalized by using Duoscan (T2500,

Table 2. The number of microtubule in each area of *Drosophila* retina

	R1	R2	R3	R4	R5	R6	R7	R8
Upper photoreceptor	6±0.2	7±0.3	5±0.3	8±0.1	5±0.1	5±0.1	4±0.1	
Middle photoreceptor	3±0.3	3±0.2	1±0.1	3±0.2	2±0.1	3±0.4	3±0.2	
Basal photoreceptor	5±0.2	9±0.3	3±0.2	6±0.4	3±0.1	5±0.2		4±0.1
Distal Lamina	17±2	20±1	15±0.5	18±2	14±1.2	17±2.1	19±0.3	18±1
Middle of Lamina	3±0.1	2±0.1	3±0.2	2±0.3	4±0.4	3±0.4	3±0.3	3±0.5

AGFA, Japan) were captured, aligned, and rendered by the IMOD program (Sandberg, 2007).

RESULT AND DISCUSSION

1. The 3-Dimensional structure of retinal cell

We studied retinal cells in good preservation conditions through high-pressure freezing. For 3-dimensinal structure, serial sections of adult retina were obtained and aligned by IMOD (Muller-Reichert et al., 2003). The contour of serial sections was traced, and this contour was reconstructed as 3-dimensional structure using IMOD software developed by Colorado University (Sandberg, 2007). Each retinal cell is indicated in different colors (Fig. 1). 2D images of ommatidia through cross section each have 7 retinal cells. However, we could show that one ommatidium has 8 retinal cells, and among these 8 cells R8 lies proximal to R7 (Wolff & Ready, 1993).

2. 3-D distribution of mitochondria in retinal cells

We analyzed the distribution of mitochondria through 3-dimensional reconstruction of retinal cells (Fig. 2D, E, and F). The number of mitochondria is like Table 1. Each site is 21～30 μm, 35～40 μm, 50～55 μm, 60～65 μm, and 73～79 μm below distal region. This analysis on the number was carried out using 6 different ommatidia. As a result, we could show that number of mitochondria is fewer at basal region than distal region and located near the plasmamembrane. This study provided the first detailed distribution of mitochondria in *Drosophila* retina through 3-dimensional reconstruction method using serial section. Because mitochondria are dynamic organelles that can change the shape, the 3D reconstruction is a good method for quantification of this organelle (Perkins & Frey, 2000). Therefore, this 3D reconstruction method is expected to be used for detecting morphological plasticity of mitochondria depending on various changes of retinal cells.

3. The 3-Dimensional structure of microtubule and nuclei

The 3D structure of microtubule and nucleus was reconstructed by their contours in retinal cells. We observed that nucleus was situated in distal region and microtubule was projected from basal side (Fig. 3B). The number of microtubule in each retinal cell was found using 6 different ommatidia. In the photoreceptor, microtubules in the middle side are less than those of distal and proximal sides (Table 2). Microtubules mainly appeared at basal and distal sides. In the lamina, the number of microtubules in distal lamina was much larger than that in the other side. To understand this cause, we searched for the location of γ-tubulin, marker of mitcotubule organization center, through immunolabeling. As a result, we could observe there are microtubule organization centers at both basal and distal sides of retinal cells (data not shown). Therefore we could explain the cause for appearance of microtubules mainly at basal and distal sides. This study on distribution of microtubule can be of help to researches on the cause of retinal degeneration diseases like retinitis pigmentosa through various mutant lines.

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<국문초록>

초파리 망막 세포의 미세구조연구는 1960년대부터 수행되어 왔으나, 이 연구들은 2차원 구조연구에 국한되어 있었다. 본 논문은 초고압 동결법, 연속절편법, 초고압 전자현미경, 그리고 3차원 구조 구현법을 이용하여 초파리 성체의 망막 세포에 존재하는 미토콘드리아, 미세소관, 그리고 핵의 3차원 분포를 연구한 결과이다. 이를 통하여 미토콘드리아는 주로 세포막 가까이에 위치하며, 그 수는 렌즈와 가까운 말단부분에 많은 것을 알 수 있었고, 미세소관은 렌즈와 가까운 망막 말단부분과 시신경과 연결되는 기부에 특히 많은 수가 존재하는 것을 알 수 있었다. 이들은 망막 변성의 원인이 되는 세포 소기관이므로, 이들의 세포 내 3차원 분포는 병의 원인을 연구하는 데 있어 중요한 자료가 될 것으로 사료된다.