Three-dimensional Structure of Myeloid Bodies in the Retinal Pigment Epithelium of the Frog, *Rana pipiens*

By

Zi Juan LIU*, Tanenori HATAE, Takao ICHIMURA, Tetsuya ISHIDA and Takanobu SAKURAI

Department of Anatomy, Kagawa Medical School, Miki, Kagawa 761-07, Japan

—Received for Publication, April 9, 1996—

Key Words: Retina, Pigment epithelium, Myeloid body, Ultrastructure, Frog (Rana pipiens)

Summary: The three-dimensional structures of myeloid bodies (MBs) in the retinal pigment epithelium (RPE) of the frog (*Rana pipiens*) were studied by transmission and scanning electron microscopy. MBs were observed to be an assemblies of numerous membranous lamellae that were formed by a flattened saecule of the paired membranes in the RPE. On thin-section, they appeared as lenticular, crescentic, circular and spherical forms and were associated with adjacent smooth endoplasmic reticulum (SER) and nuclear membranes. The lenticular MBs were observed to be in connection with the crescentic, circular and spherical forms. Under scanning electron microscopy, the lenticular and crescentic MBs showed a pile of disc-liked lamellae, while the circular and spherical MBs displayed an onion-liked appearance. It was noted that some tubular SER remained in the center of the circular MBs, while the lamellae were compactly arranged and filled the central region in the spherical MBs. All crescentic and spherical MBs were continuous with the lenticular and crescentic MBs. These results suggest that the lenticular MBs may be a precursor form of another variety of MBs which may be transformed into the crescentic, circular and spherical forms by the curving and fusion of the lamellar membranes at the MB's free ends.

Myeloid bodies (MBs) are a morphologically specialized region of the smooth endoplasmic reticulum (SER), and are found to occur in the retinal pigment epithelium (RPE) of many lower vertebrates, including birds, reptiles, and amphibians. MBs have also been observed in one mammalian species but only after exposure of the animal to extended periods of light deprivation. The function of MBs has been a subject of considerable speculation, although some studies have suggested the involvement of these organelles in the lipid metabolism of photoreceptor outer segments. In addition, a recent developmental study on regenerating newt RPE indicated that the occurrence of MBs in this species was related to the daily shedding and phagocytosis of the photoreceptor outer segment.

The basic morphological structures of MBs and the inter-relationship between MBs and surrounding organelles have been studied in the RPE of several different animals. At the same time, it is also clear that MBs can assume a variety of conformations and are capable of striking alterations in shape, size and number in response to cyclic lighting conditions. Examinations focusing on three-dimensional structures have been relatively few to date. York and Dickson investigated the three-dimensional structure of MBs cultured in vitro, using thin-section and computer-aided reconstruction techniques. To our knowledge, the three-dimensional structures of myeloid bodies have not yet been reported using scanning electron microscopy.

The three-dimensional structures of MBs in the normal frog RPE were studied with transmission and scanning electron microscopy, and the interrelations among the various forms of MBs are discussed in this report.

Materials and Methods

Adult frogs (*Rana pipiens*), without any special administration of light, were used throughout this study. The animals were anesthetized for sacrifice, the eye balls were removed, and subsequently
fixed according to the requirements for individual examination methods.

**Transmission electron microscopy**

The eye balls were placed in a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4). They were then opened by being cut into four sections with a razor blade along the equator. Following brief fixation, the four sections were further cut into small pieces and equilibrated in the same fixative for 2 hr at 4°C. After an overnight rinse in 0.1M cacodylate buffer containing 7% sucrose, the specimens were postfixed for 2 hr at 4°C in a mixture of 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1M cacodylate buffer, followed by block-staining in 1% aqueous uranyl acetate (pH 5.0) for 2 hr at 4°C. After a brief rinse with distilled water, the small pieces were dehydrated through a graded series of ethanol concentrations. The specimens were transferred to propylene oxide and then embedded in Epon 812. Thin-sections were cut on a Porter-Blum MT-2 ultramicrotome with a diamond knife and stained with saturated uranyl acetate (in 50% ethanol) and lead citrate. Micrographs were taken on a JEOL 1200EX electron microscope operating at 80 or 100 kV.

**Scanning electron microscopy**

The eye balls were sliced into several long pieces with a razor blade and immersed in a fixative containing 1% osmium tetroxide in 1/15 M phosphate buffer (pH 7.4) for 1.5 hr at 4°C. Following an overnight rinse in 1/15 M phosphate buffer at 4°C, the specimens were thoroughly washed with distilled water for 1 hr and then embedded with 1% Chitosan in an aqueous 0.5% acetic acid solution. After brief fixation in a mixture of 0.5% paraformaldehyde and 0.5% glutaraldehyde, the samples were rinsed with distilled water and transferred to 25% and then 50% dimethyl sulfoxide dissolved in distilled water for 30 min each. These small blocks were frozen on a metal plate chilled with liquid nitrogen and fractured using a precooled razor blade and a hammer. Following a complete rise with distilled water, they were treated with 1% osmium tetroxide in 1/15 M phosphate buffer for 1 hr at 4°C. Maceration was achieved by keeping the specimens in the 0.1% osmium tetroxide for 4.5 days at 20°C.

The specimens were subsequently stained with 1% tannic acid and 1% osmium tetroxide. Dehydration was accomplished in a graded series of ethanol concentrations and the specimens were dried using the critical-point method employing liquid carbon dioxide. The dried samples were coated with platinum (2 nm in thickness) and examined with a Hitachi S-900 field emission scanning electron microscope operating at 10 kV.

**Results**

**Transmission electron microscopy**

The RPE of the frog (Rana pipiens) consisted of a monolayer of large rectangular cells, which characteristically contained various types of MBs and abundant pigment granules located almost entirely in the apical portion of the cytoplasm (Fig. 1). Furthermore, each cell contained an oval nucleus situated in the basal portion of the cell, some large lipid droplets, and a dense accumulation of tubular SER.

Myeloid bodies were distributed throughout the cytoplasm of the RPE, especially in the vicinity of the nucleus and cell base. These bodies appeared in a variety of morphological conformations. In thin-sections, the MBs were of four significant types: lenticular, crescentic, circular, and spherical forms (Fig. 2-5). The central region of the circular MBs contained some tubular SER (Fig. 4), while in the spherical forms the lamellae were compactly arranged thus completely filling the central region (Fig. 5). The lenticular MBs were more widely distributed than other forms, but the circular and spherical MBs were larger. The lenticular MBs were frequently seen to be connected to the circular and spherical MBs (Fig. 4 and 5). The MBs were composed of a stack of multi-layered lamellae. Each lamellar unit was formed by a flattened saccule of paired-membranes that appeared closely apposed. The lamellar membranes of the flattened saccules were observed to be slightly dilated and continuous with the adjacent SER at the extreme periphery (Fig. 2). The outermost membranes of myeloid bodies were often seen to be associated with the nuclear membrane (Fig. 6). No free ribosomes were apparent on the peripheral cisternal membranes of the MBs observed in the present study (Fig. 2). In cross-section, the lenticular MBs initially appeared to be curved at their two free ends and became progressively more concave. The arched lamellae encircled the circular and spherical MBs from their periphery (Fig. 4 and 5).

**Scanning electron microscopy**

The osmic maceration technique allowed observation of the three-dimensional architecture of the intracellular membranous system by dissolving the cytoplasmic matrix and filamentous elements of the cells. On the fracture surface, consistent with the observations made on transmission electron microscopy, the lenticular MBs consisted of a compact assembly of many layers of lamellae. These lamellae
were formed by flattened sacculles of the paired-membranes (Fig. 7). The two free ends of the crescentic MB appeared to be especially close to each other and to be continuous with the adjacent tubular SER (Fig. 8). The disc-liked lamellae were regularly arranged to form the lenticular MB (Fig. 9).

The circular and spherical MBs displayed an onion-like appearance and were continuous with the surrounding tubular SER at their lamellar periphery (Fig. 10). Some SER were found to be present in the center of the circular MBs (Fig. 11). Many lamellae were concentrically arranged and compactly connected to the lenticular MBs on one side to form the spherical MB. Overall, the spherical MBs displayed a ball-like appearance (Fig. 12).

Discussion

The ultrastructure of the myeloid body has previously been described in the RPE of many lower vertebrates. The MBs are basically a stack of multi-layer membranous lamellae whose occurrence in the RPE has been associated with the initiation of phagocytosis of the outer segments of the cone and rod cells. It has been assumed by several investigators that the phagocytosed lipids are initially processed by the retinal pigment cell prior to transfer to the SER. The alteration of the lipodic composition of the SER leads to the formation of MBs in the pigment epithelial cell.

In the present study, we found that the MB of the frog RPE appeared as the lenticular, crescentic, circular and spherical forms. The center of the circular MB contained some tubular SER, while the lamellae in the spherical form were compactly arranged along a concentric axis in the central region. Under scanning electron microscopy, the lenticular and crescentic MBs were composed of stacked disc-like lamellae that differed in size and shape. Alternately, the circular and spherical bodies displayed an overall onion-like appearance. It was noted that the lenticular and crescentic MBs were in close association with the circular and spherical myeloid bodies.

The present scanning electron microscopic observation shows the three-dimensional structure of the MBs and distinctly reveals their interrelation with the adjacent tubular SER. These extensive features of MBs have not, to date, been visualized with the freeze-fracture and transmission electron microscopic techniques utilized in previous investigations. The results obtained in this study clearly suggest that the lenticular MB is the precursor form of the various other MBs and that they may undergo a morphological change as they participate in the metabolism of lipids during the shedding of outer segments in the pigment epithelial cell. It has also been established that the MBs first appear in the small lenticular form and then change structure dramatically, depending upon the light-dark cycle. Therefore, we can speculate that some lenticular lamellae may become curved and thereby transform into the crescentic form of MBs. These crescentic MBs may further increase their curvature promoting fusion of their free edges to eventually form an onion-like assembly by curved lamellae. The SER within the center of the circular MBs and around the assembly are probably transformed into such lamellae when the MBs incorporate more lipid, resulting in the formation of a large compact spherical MB (Fig. 13). It is possible that the lamellae of the circular and spherical MBs eventually breakdown at their site of fusion or in other regions, thereby transforming back into the crescentic or lenticular forms.

In summary, this study has demonstrated the three-dimensional structure of myeloid bodies and the results suggest that lenticular MBs are the precursor structure of the various other types of MBs. Lenticular myeloid bodies may transform into the crescentic, circular and spherical forms by curving and fusing their lamellar membranes.

References

11) Karnovsky MJ. Use of ferrocyanide-reduced osmium tetroxide


Explanation of Figures

Plate I

Fig. 1. Low magnification electron micrograph showing the frog RPE. Numerous MBs (M) are visible in the cytoplasm, while pigment granules (arrow heads) are present in the apical portion of the cell (N: nucleus). ×4,500. Bar = 5 μm.

Fig. 2. Electron micrograph showing an accumulation of several lenticular MBs in the frog RPE. The two ends of the MBs are continuous with the SER (arrows). No distinct ribosomes are observed at the outermost membranes of the lamellae. ×18,000. Bar = 0.5 μm.

Fig. 3–4. Electron micrograph showing a crescentic and a circular MB. The crescentic lamellae become increasingly concave and the free ends connect to another MB (Fig. 3). ×19,000. Bar = 0.5 μm. The circular MB appears to be connected with a crescentic MB on one side (arrow), while some cytoplasm (asterisk) remains in the center (Fig. 4). ×16,000. Bar = 0.5 μm.
Plate II

Fig. 5. Electron micrograph showing a spherical MB of the frog RPE. Some lenticular and crescentic lamellae are present in the surrounding area. \( \times 25,000 \). Bar = 0.5 \( \mu \text{m} \).

Fig. 6. Electron micrograph showing the association of the MB with the nuclear membrane (N: nucleus). \( \times 23,000 \). Bar = 0.5 \( \mu \text{m} \).

Fig. 7–8. A scanning electron micrograph showing the lenticular and crescentic MBs of the frog RPE. A mitochondrion (M) is close to the MBs (Fig. 7). \( \times 36,000 \). Bar = 0.5 \( \mu \text{m} \). The flattened lamellae are crescentic and are connected to the tubular SER at the lamellar terminals (arrow heads). Fig. 8. \( \times 30,000 \). Bar = 0.5 \( \mu \text{m} \).
Plate III

Fig. 9. A scanning electron micrograph showing a MB composed of multiple layers of disc-like lamellae. The periphery is continuous with the tubular SER (arrows). ×43,000. Bar = 0.5 μm.

Fig. 10. A scanning electron micrograph showing a fractured surface of the frog RPE. An onion-like MB appears to be continuous with the adjacent tubular SER at the extreme periphery of the lamellae (arrow). ×39,000. Bar = 0.5 μm.

Fig. 11–12. A scanning electron micrograph showing a circular MB and a spherical MB of the frog RPE. A portion of the tubular SER (asterisk) is observed in the center of the circular MB (Fig. 11). ×32,000. Bar = 0.5 μm. The lamellae of the spherical MB (SMB) are compactly arranged along a central axis and completely fill its center (Fig. 12). A lenticular MB is continuous with its lamellae (arrow). ×26,000. Bar = 0.5 μm.
Plate IV

Fig. 13. A schematic representation of the interrelations among various forms of myeloid bodies. The lenticular MB (LMB) appears to bend forming a crescentic MB (CRMB). It becomes increasingly concave creating a circular MB (CIMB) upon fusion of the free ends. Finally, the SER are replaced by lamellae, resulting in the formation of a spherical MB (SMB).