An Electron Microscopic Observation of Some Membrane Structures of Lens Fibers of Regenerating Lens in *Triturus pyrrhogaster*[1]

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Triturus pyrrhogaster 에서 再生되는 水晶體纖維 細胞中 膜構造에 관한 電子顯微鏡的 觀察

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摘 要

有尾兩棲類中 *Triturus pyrrhogaster* 에서 人爲的으로 水晶體를 摘出하면, 虹彩의 瞳孔周緣上部中央의 色素上皮가 水晶體로 metaplasia 하는것은 잘 알려진 事實이다. 이 過程에는 色素上皮의 脫色增殖과 水晶體原基(水晶體胞)의 形成이 보이며 그 後의 水晶體形成은 正常發生의 그것과 全혀 같은 樣相으로 進行한다. 虹彩色素上皮의 細胞가 水晶體의 細胞로 變하는 過程에 대하여서는 벌써 Eguchi, Karasaki 等에 依하여 subcellular level의 解析이 報告되었다. Eguchi(1964)의 報告에 依하면 細胞構成要素中 endoplasmic reticulum(ER) 및 mitochondria의 變化가 特히 興味를 끈다. ER는 脫色이 完了된 細胞에서 核外膜의 泡狀變形에 依하여 形成된 事實로서 觀察되었으며, mitochondria의 形態 크기 數의 變化도 顯著하였다. 本研究는 ER와 mitochondria를 問題의 焦點으로하여 活潑히 伸張되고있는 水晶體纖維細胞中 이兩者의 細胞構造가 어떤 消長을 나타내는가를 電子顯微鏡으로서 調査한 것이다.

水晶體纖維는 立方狀의 水晶體上皮細胞가 水晶體의 後壁(網膜側)에서 伸張分化한 것인데 ER는 그의 基部 特히 核의 周邊에서만이 顯著한 發達을 나타내고 있었다. 또한 核外膜은 顯著한 泡狀變化로서 近處의 ER에 連結되어 있었다. 核周邊의 ER는 一般的으로 reticular 構造를 나타내지 않고 큰 cisternae 空間을 가졌다. 그것은 規模가 큰 것이였으며 大部分은 ribosome을 그 外圍에 있는 粗面構成에 附着시키고 있었다. 一方 小型의 胞狀構造는 細胞質內에 散在하고있었는데 여기에도 ribosome의 附着이 間或觀察되는까닭에 亦是 ER의 一斷面이라고 推定되었다. 纖維細胞의 核에서 먼 伸張端(apical portion)에 있는 ER의 構造는 漸次 沈滯하여 胞狀이 보였다. mitochondria에 對하여서도 ER와 같이 基部核周邊에 發達이 顯著하였다. ER의 周邊에도 密集하여 存在하며 間或 ER의 膜構造와 mitochondria의 外膜이 密接한 것도 觀察되었다. 이와같은 境遇의 ribosome은 polysome 狀으로 되어서 緻密하게 散在하여 核의 周邊部에서 水晶體 protein의 合成이 旺盛하게 集行되고 있는 것이라고 推察될만한 微細構造上의 關係를 나타내고 있었다. 纖維의 伸張端近處의 mitochondria는 多少變形되어, 空胞化가 甚하며 때로는 mierin 狀重層膜構造를 連結시키며 大小多數의 胞狀構造와도 共存하고 있었다. 또 細胞間隙에도 이와같은 構造의 樣狀이 認定되는 것도 間或 있었다.

以上 水晶體纖維細胞의 核周邊部에 있어서 ER, mitochondria의 顯著한 發達像은, 核內 RNA

1 The present study had been conducted at the laboratory of developmental biology in the Biological Institute, Faculty of Science, Nagoya University, when the authors stayed there.

合成의 上昇에서 보이는 polysome의 增加에 뒷받침되는 硏究다. 即 水晶體蛋白質의 合成에 큰 役割을 나타내는 것이라고 생각된다. 纖維가 伸張하는데 따라서 이들 膜構造는 漸次 細胞의 伸張端에서 退化를 始作하여 消失하는 像을 보이고 있으며 細胞의 退化消失에서도 確認되었다.

INTRODUCTION

Detailed analyses on the regenerative transformation of iris cells into lens cells have been reported by Eguchi (1963 a, b and 1964 a, b). His attempts were demonstration of the subcellular differentiation during early phases of the lens fiber formation from pigmented iris cells, using adult *Triturus pyrrhogaster*. Also Karasaki (1964) reported independently the results in American newt, *T. viridescens* similar to those of Eguchi (1964 b). Some important phenomena were detected on the cytoplasmic membrane structures as endoplasmic reticulum and mitochondrion. For instance, the endoplasmic reticulum is given rise from the outer nuclear membrane as blebbing, and mitochondria change in number, size, and morphological pattern during cyto-differentiation (Eguchi, 1964 a and b). However, some differences can be recognized between the results of Eguchi (1964 b) and of Karasaki (1964), especially in changes of the endoplasmic reticulum.

The present electron microscopic study deals with the detailed observation on changes in the membrane structures during the elongation of lens fiber cells of the actively regenerating lens in adult newts. Special emphasis was focused on the endoplasmic reticulum and the mitochondrion.

MATERIALS AND METHODS

Adults of the Japanese newt, *Triturus pyrrhogaster*, were used as an experimental material. The lens was ectomized from the animal narcotized with MS 222 (Sandoz, Swiss) by a pair of fine forceps through corneal incision (after Sato, 1940). Lensectomized animals were kept in an incubator regulated at a constant temperature of 20±1° C. Around 20 days after the lens removal, when the lens regenerate attained to Sato stage 8 to 9, the lensectomized eye was carefully isolated in cold Tyrode solution. The isolated eye was fixed according to Eguchi (1964 b) and was then washed, dehydrated, and embedded in Epon 812 by the method of Luft (1961). Sections were cut on a JUM-5 ultramicrotome (Japan Electron Optics Co.) and stained with saturated aqueous solution of uranyl acetate (Watson, 1958) for an hour and with lead citrate (Reynolds, 1963) for 30 seconds at 20°C. Stained sections were observed on a JEM T-6 electron microscope of Japan Electron Optics Co. Electron micrographs were taken at instrumental magnification of 4,000 to 8,000.

RESULTS AND DISCUSSION

It was much advantageous to use the lens regenerated to Sato stage 8 to 9 as material. As shown by Eguchi (1964 b), differentiating cells of every stage from pigmented epithelial cell to elongating lens fiber cell were contained in the regenerating lens of those stages. Moreover, the lens was fairly small enough to handle for submicroscopic preparation and each cell type was easily selected in the same individual lens-regenerate.

In the basal portion of the elongating cells (correspond to EC-cell in Eguchi, 1964 b), it was generally observed that the large blebbings of the outer nuclear membrane were partially accompanied by ribosomes (Fig. 1) and they seemed to continue to the rough-surfaced endoplasmic reticulum systems around the nucleus (Figs. 1 to 4). These endoplasmic reticuli were always rough-surfaced and with complicated profiles and large space of cisternae (Figs. 1 to 4). Numbers of large and small vesicles were also observed in the cytoplasm of elongating lens fibers (Fig. 5). Majority of these vesicular membrane structures could be regarded as profiles of the endoplasmic reticulum, because these structures carried ribosomes on their outer surface (shown by arrows in Figs. 2 and 5). Ribosomal population was very dense, especially in the vicinity of the nucleus (Figs. 4, 5, and 6). Ribosomes were generally observed as clusters and small numbers of them were dispersed still in single number.

According to Eguchi (1964 b), the blebbing of the outer nuclear membrane becomes observable in the cells

Fig. 1

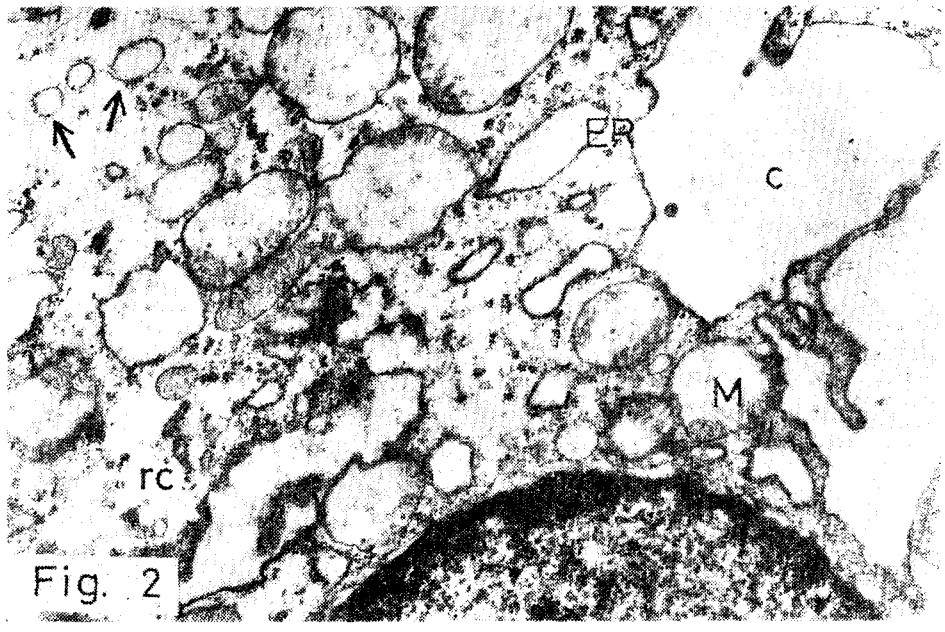

Fig. 2

Abbreviation: ER=endoplasmic reticulum, B=blebbing of outer nuclear membrane, ics=intercellular space, M=mitochondrion, N=nucleus, pm=plasma membrane, rc=ribosomal cluster, and c=cisternae.

Fig. 1. Blebbing of outer nuclear membrane and prominent rough-surfaced endoplasmic reticulum near by the nucleus in the basal portion of the young lens fiber. Mag. 18,000.

Fig. 2. Endoplasmic reticulum with large space of cisternae. Mitochondria attached closely to the endoplasmic reticulum. For further explanation see text. Mag. 18,000.

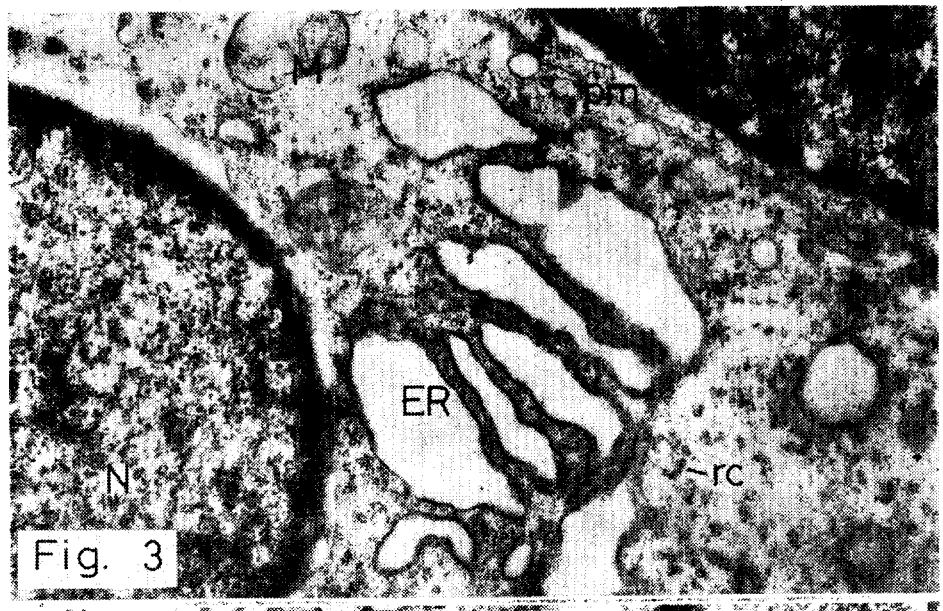

Fig. 3

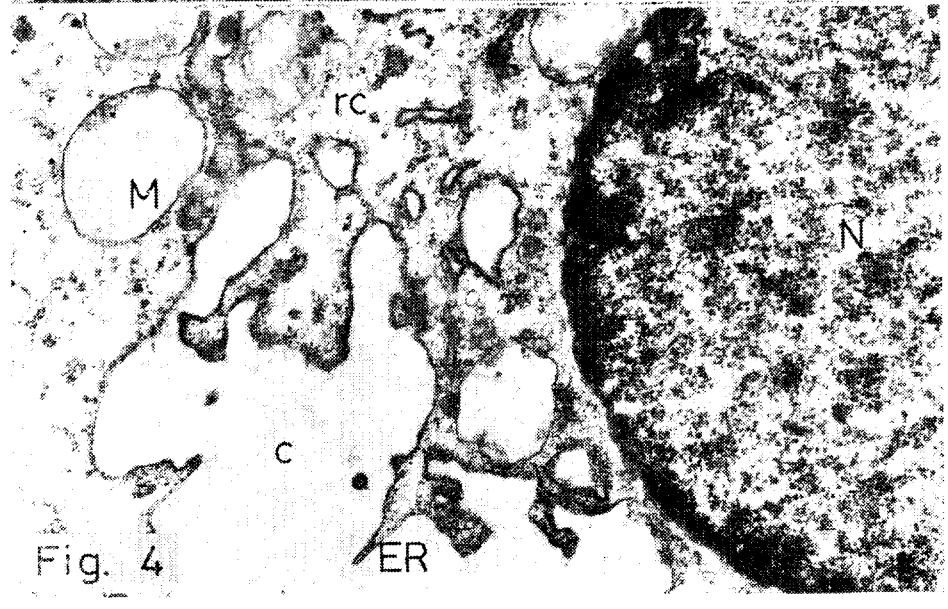

Fig. 4

Fig. 3. Prominent endoplasmic reticulum in the vicinity of the nucleus in the basal portion of elongating lens fiber. Mag. 18,000.

Fig. 4. Endoplasmic reticulum and mitochondria at the basal end (immediate inside of the lens surface) of the elongating fiber cell. The endoplasmic reticulum of the most large cisternae is partially smooth-surfaced. Mitochondria are closely attached to the surface of the endoplasmic reticulum. Mag. 18,000.

after depigmentation and vesicular profiles of smoothy and rough-surfaced endoplasmic reticulum increase in number relating closely to the bleb-formation of the outer nuclear membrane. He ascertained that these vesicular elements of the endoplasmic reticulum could be derived from the nuclear membrane. Similar phenomena were observed in other materials. Wischnitzer (1963) had elegantly demonstrated in amphibian oocytes that the cytoplasmic vesicles were derived by blebbing from the outer nuclear membrane and migrated to the periphery of the cells and then formed the reticular membrane structures. In the present observation, the large endoplasmic reticulum with large space of cisternae was observed to continue to the blebbing of the nuclear membrane in the actively elongating cells. This can be adopted as one of the evidences to support the ideas of those authors who regard the blebbing of the outer nuclear membrane as one of the origins of endoplasmic reticulum. Moreover, in the lens cells, the blebbing observable first in the undifferentiated cells seems to continue actively during cell elongation.

At the same time, when the endoplasmic reticulum developed, a large number of mitochondria were observed. Population of mitochondria was often concentrated nearby the endoplasmic reticulum in the vicinity of the nucleus (Figs. 4 and 5). Mitochondria sometimes attached to the endoplasmic reticulum. Some of the mitochondria of the elongating lens fibers were obviously changed (Fig. 6, arrow). In such cases, mitochondria were accompanied by large and small vesicular structures and they were often situated nearby the intercellular space. Recently, Jurand and Yamada (1967) reported preliminary that the mitochondria were eliminated as vesicular or myelinated structure into the intercellular

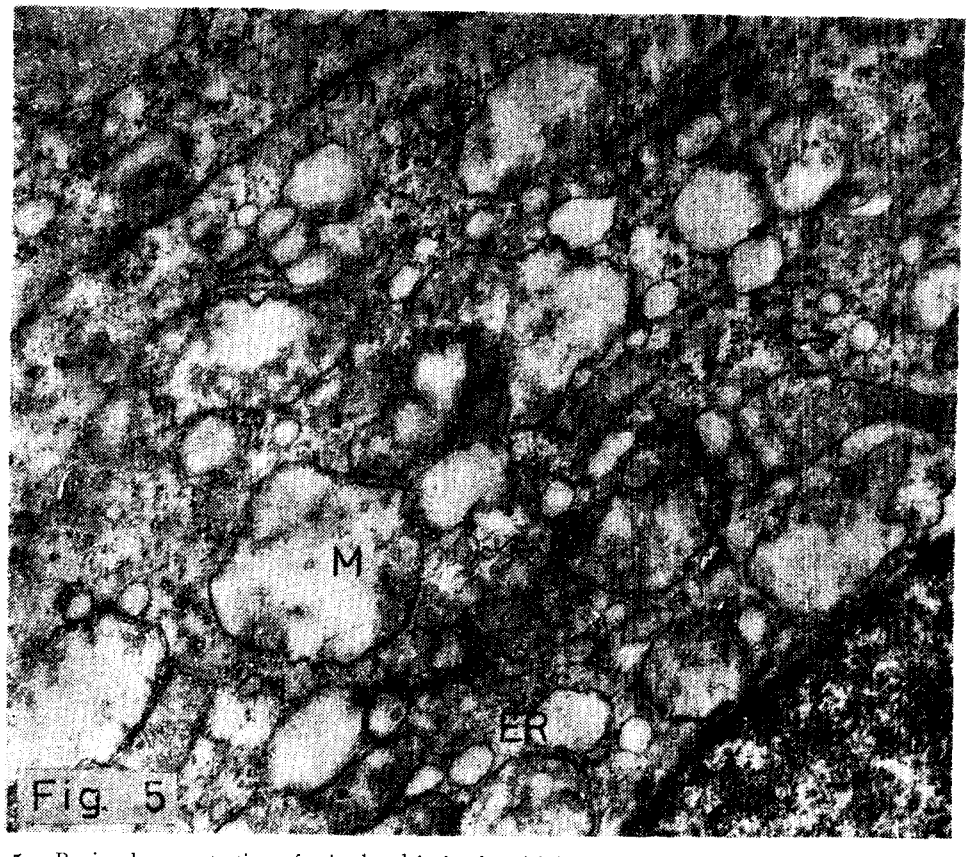

Fig. 5. Regional concentration of mitochondria in the vicinity of the nucleus of young lens fiber. Large number of vesicular profiles of the endoplasmic reticulum are seen in the figure. Some of them are bearing ribosomes (arrow). Mag. 28,000.

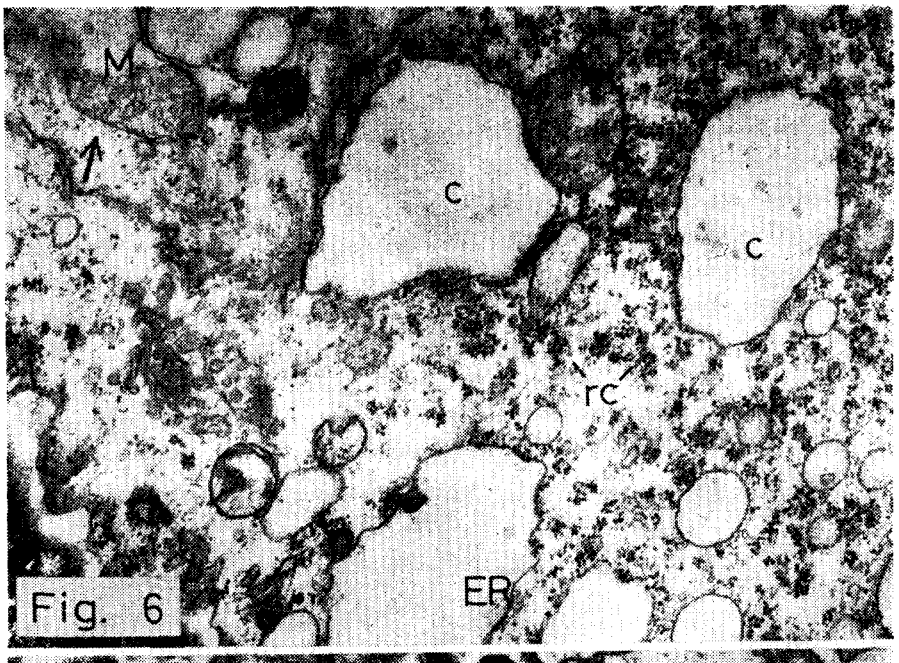

Fig. 6

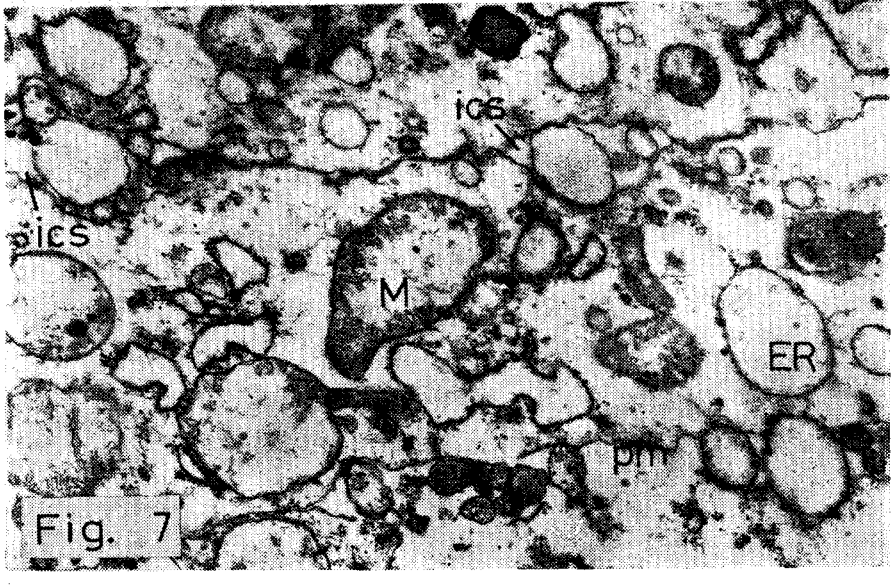

Fig. 7

Fig. 6. Concentrative ribosomal clusters in the young lens fiber cell. In the upper portion of the figure, extraordinary membrane structures are observed. Most of the endoplasmic reticuli show vesicular pattern. Mag. 28,000.

Fig. 7. Endoplasmic reticulum and mitochondria in the apical portions apart from the nucleus in the young lens fibers. Mag. 18,000.

Fig. 8. Endoplasmic reticulum and mitochondria in the apical portions apart from the nucleus in the young lens fibers. Mag. 24,000.

space during lens regeneration in *T. viridescens*.

In the apical portion apart from the nucleus (Figs. 7 and 8), the endoplasmic reticulum was not so prominent but rather disintegrating. Majority of endoplasmic reticuli showed vesicular profiles and consisted of the rough-surfaced element. Mitochondria in the apical portion were extremely swollen indicating partial disappearance of cristae mitochondriales. Vesicular structures without cristae were sometimes observed (Fig. 7). These structures seemed to be in process of disintegration. In the intercellular space, large and small vesiclular structures were observed (Figs. 7 and 8). Comparison of them with the vesicular structures observed in the intercellular space of the lens regenerate by Jurand and Yamada (1967), these structures may be given rise also through elimination of mitochondria.

The results of the present observation might lead the present authors to the following consideration: In the elongating lens fibers, the endoplasmic reticulum develops in the basal portion of fiber cell with regional concentration of the mitochondria, and plays an important role in the lens protein synthesis evoked by rapid increase of nuclear RNA synthesis (Yamada and Karasaki, 1963 and Yamada and Takata, 1963) and of ribosomal population (Eguchi, 1964 b). As the abvancement of the cell elongation, the membrane structures such as endoplasmic reticulum and mitochondrion undergo structural disintegration which comes up from the apical end to the basal portion of the lens fiber.

SUMMARY

The membrane structures were electron microscopically studied in the elongating lens fibers of the regenerating lens of adult *Triturus pyrrhogaster*. Observations were focused on the endoplasmic reticulum and mitochondria. The endoplasmic reticulum developed in the vicinity of the nucleus with active blebbing of the outer membrane. At the same time, the concentration of mitochondria around the rough-surfaced endoplasmic reticulum nearby the nucleus was always observed. Both endoplasmic reticulum and mitochondrion undergo disintegration in the apical portion apart from the nucleus. Some considerations were discussed with reference to published data.

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