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Fine Structure of Diapause Regulator Cell in the Suboesophageal Ganglion in the Silkworm, *Bombyx Mori*

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Summary

In the suboesophageal ganglion of *Bombyx mori*, the diapause regulator producing cells which may give an information to the diapause factor cells were found by means of electron microscopy.

The diapause regulator producing cells had larger granules (2000 to 5000 A° in diameter) than did the diapause factor cells which were partially surrounded by the formers. Highly electron-dense material of lysosome in the diapause regulator producing cells was observed in the diapause-egg producer but such lysosomes were not in the non-diapause egg-producer. It was found that many cytoplasmic granules fuse with lysosome, and smaller granules come out of lysosomes. Some implications of the diapause factor cell and the diapause regulator producing cell were discussed.

1. INTRODUCTION

During the course of an experiment on diapause of the silkworm, *Bombyx mori*, the author(1969, 1970a, 1970b) found the specific patterns of DNA, RNA, and protein synthetic activities concerning the incubation temperature. PARK and YOSHITAKE(1971) reported that in the diapause factor cells located in the suboesophageal ganglion, there was a difference in the rough-surfaced endoplasmic reticulum, in the process of cytoplasmic granule formation, and in the amount of cyteplasmic granules between diapause and non-diapause-egg producers. Thus they suggested that whether diapause or non-diapause eggs are laid may depend upon the quality of cytoplasmic granules formed through the different processes, or be closely related to the amount of granules in the diapause factor cells.

KOBAYASHI(1957) deomonstrated that *Bombyx mori* expected to lay diapause eggs had more neurosecretory cells than did other silkworm, but FUKUDA and TAKEUCHI (1967a, b) reported that the diapause actor responsible for the production of diapause eggs of the silkworm is produced by a pair of neurosecretory cells located in the suboesophageal ganglion. In *Leucophaea maderae*, SCHARRER(1955) suggested an endocrine link between the gonad and a pair of neurosecretory cells in the suboesophageal gangion on the basis of changes in these cells following ovariectomy. The investigators have contradicted one another.

As far as the author is aware, no one has reported the fine structure of the diapause regulator cells, and that the diapause regulator cell is directly or indirectly related to the granule formation of the diapause factor cells.

Accordingly, the author carried out electron microscopic observations on the diapause regulator cells of the suboesophageal ganglion.

2. MATERIALS AND METHODS

Of the bivoltine *Daizo* strain, the female *Bombyx mori* destined to lay diapause and non-diapause eggs was used. The brain-suboesophageal ganglion complex of *Bombyx mori* was taken out under a dissecting microscope.

The brain-suboesophageal ganglion complex was fixed for 2 hr in 5% glutaraldehyde in 0.2 M cacodylate buffer, washed with four changes of cacodylate buffer

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(0.05 M) plus sucrose (0.34M) for about 15 hr, post-fixed for 2 hr in 1% osmium tetroxide in 0.2 M cacodylate buffer, dehydrated in an ethanol series, and embedded in Epon 812. Sections were double-stained in uranyl acetate and lead acetate (SATO's (1968) modified lead staining method).

3. RESULTS

With the light microscope, cell types are distinguished by differences in the staining affinities of their secretory granules; with the electron microscope, secretory granule size is the most useful criterion for cell identification.

Besides the DF cells reported by FUKUDA and TAKEUCHI (1967a, b), the author found that there are more than four neurosecretory cells, which contain larger cytoplasmic granules than do the DF cells, and of which one is located between the DF cells and others anterior to the DF cells (Figs. 1 and 2). The author called these cells the diapause regulator producing (DR) cells. FUKUDA and TAKEUCHI(1967a) described a group of five or six neurosecretory cells in the central part toward the anterior end of suboesophageal ganglion in addition to the DF cells. They concluded that these cells are not concerned with production of diapause factor.

The DR cells like the DF, were sperical, with nuclei of irregular shape. As in the DF cells (PARK and YOSHITAKE, 1971), the Golgi areas were enlarged. Many irregular profiles of Golgi complex, of which some were filled with electron-dense material, surrounded one lysosome. The granules formed in the DR cells were oval or spherical, 2000 to 5000Å in diameter (Figs. 1 and 2). They did not seem to vary in size as the larvae grew beyond the 4th stage.

The DR cells were observed both in diapause and non-diapause suboesophageal ganglia (Figs. 1 and 2). Many granules were present in the cytoplasm, suggesting that newly synthesized secretory protein was packaged into granules and stored for a long period of time, rather than being discharged rapidly.

The cytoplasmic granules in the DR cells were concentrated peripherally (Figs. 3 and 6), occurring in groups. Between these peripheral stacks and the Golgi complex were a number of prominant rough-surfaced endoplasmic reticula (Fig. 6), oriented parallel to the cell membrane. There were lysosomes close to the cell membrane and sometimes surrounded by a number of irregular profiles of Golgi complex, some of which were part rough-and part smooth-surfaced, and contained secretory granules (Figs. 1 and 4). Such lysosomes containing electron-dense material were not recognized in the nondiapause egg producers but in the diapause.

Furthermore, it was clearly noted that in the diapause suboesophageal ganglion the process of granule formation in the DR cells is different from that in the DF cells (Fig. 5), but is similar to that in the DF cells and the DR cells of the non-diapause suboesophageal ganglion. That is, the lysosomal activity predominated in the DR cells of diapause egg produces.

LYSOSOMES: Lysosomes are now recognized as a morphologically heterogenous group of cytoplasmic particles which contain acid hydrolases and function in many ways as an intracellular digestive system (de DUVE, 1963, 1964). These enzymes are capable of breaking down all the main constituents of living matter, i.e., proteins, carbohydrates, fats, and nucleic acids. It has become apparent, primarily as a result of morphological and cytochemical studies with the electron microscope, that the relatively primitive activity of intracellular digestion has been adapted in specialized cells of multicellular organisms to subserve a variety of complex functions such as defense (HIRSCH and COHN, 1964; COHN et al, 1963), absorption(STRAUS, 1964; MILLER and PALADE, 1964), differentiation (WEBER, 1963; SCHEIB, 1963), cell involution (SLATER et al, 1963), and regulation of secretion(SMITH and FARQUHAR, 1966).

As shown in Figs. 1, 3 and 4, lysosome content of the DR cell in diapause egg producers appears to be markedly changed in its electron-dense material as the lysosome becomes active. The more the empty profiles of Golgi complex increased in number, the more the lysosome content increased. It was observed that many cytoplasmic granules fuse with lysosomes. Fig. 7 shows the active lysosomes of the DR cells in a diapause egg producer during the 5th larval stage. The cytoplasmic granules, which are smaller than those formed before entering the lysosomes, have just come out of the lysosomes. From the second day of the 5th larval stage to the 5th day after pupation, in some DR cells two-

kinds of granules were noted even in one DR cell. One kind came out of the lysosome, the other was formed before entering the lysosome. The author did not find that the cytoplasmic granules are discharged into axon or blood and fuse with the cell membrane during the larval and pupal stages, but discharging may occur.

From the above observations, the author assumed that some granules fuse with lysosomes when secretory activity is suppressed and the DR cell has an excess of stored granules.

Fig. 8 shows many electron-transparet vacuoles remaining as a result of discharging all the granules in DF cells in diapause suboesophageal ganglion. A similar condition was observed also in DR cells, and in non-diapause specimens.

4. DISCUSSION

In a previous paper (PARK and YOTHITAKE, 1971), the authors found that the cytoplasmic granules of the DF cells are discharged both in diapause and non-diapause, and that there is a difference in the process of cyoplasmic granule formation, and hence assumed that whether diapause or non-diapause eggs are laid may depend upon the quality of cytoplasmic granules formed through the different processes, or be closely related to the amount of granules in the diapause factor cells.

As described in the text, it was noted that the granules of the more than four DR cells are larger than those of the two DF cells, and many organelles of cytoplasmic inclusion are almost similar in both, except for the specific lysosomal activity of the DR cells and the granule formation process peculiar to the DF cells in diapause suboesophageal ganglion. All the DR cells were adjacent to DF cells and partially surrounded them.

The present study emphasizes the direct or indirect relation between the DF and the DR cells in the suboesophageal ganglion in *Bombyx mori*. The evidence that the fine structure of the DR cell and the DF cell in the diapause egg producer differs from that in the non-diapause egg-producer rests on the observation of the specific lysosomal activity and the myelin-like structure.

In the DR cell, the most important response to a high or low incubation temperature was the appearance of active or inactive lysosomes. These lysosomes may contribute to absorption of some cytoplasmic granules when secretion is inhibited and the DR cell must dispose of excess stored granules, and may serve as accelerators of secretion when necessary for the action of the DR cell, simultaneously with explosion of lysosoms. In this connexion, SMITH and FARQUHAR (1966) assumed that in rat anterior pituitary glands secretory products are more or less continually funneled into the lysosomal system, presumably according to fluctuations in secretory activity, and hence concluded that lysosomes operate at the catabolic end of protein turnover and constitute a regulatory mechanism to take care of overproduction of secretory products. This particular regulatory mechanism was said to be unique in that it operates very late in the secretory process on secretory products that are already concentrated into granules. Besides this, another possible explanation for the acid phosphatase results particulaly in regard to the lysosome activity around forming secretory granules, was discussed by BAINTON and FARQUHAR (1968).

It was reported by FUKUDA and TAKEUCHI (1967 a, b) that in the non-diapause egg-producer, the granules are not released from the DF cells, but in the diapause egg-producer. are rapidly released from the cells.

The electron microscopic observation which the cytoplasmic granules of the DF cells are discharged both in diapause and non-diapause may indicate that the brain has no ability to suppress the secretion of diapause hormone from the DF cells if the cytoplasmic granules serve as a carrier of diapause hormone.

From the results mentioned above, it can be said that the secretory products of the DR cells may participate in determining the quality of cytoplasmic granules of the DF cells, formed through the different processes.

The author, therefore, concludes that the silkworm eggs entering diapause or non-diapause are closely related to the quality of secretory hormone (from the DF cells) which may depend upon a certain material from the DR cells. That is, the DR cells may control either the brain or the DF cells; the DR cells may participate in determining the quality of secretory

hormone of the DF cells if the brain controls the DR cells, and the brain may determines the quality of secretory hormone of the DF cells if the DR cells control the brain as far as diapause is concerned.

Either the DF or the DR cells are controlled by the brain still remain a question for future investigation on the fine structure of neurosecretory cells of the brain.

要 約

電子顯微鏡觀察에 의하여 家蠶의 食道下神經球에서 休眠要因細胞(diapause factor cell)에 어떤 情報를 주는 것으로 생각되는 休眠調節細胞(diapause regulator producing cell)를 發見하였다.

休眠調節細胞의 顆粒은 이 세포가 部分的으로 둘러싸고 있는 休眠要因細胞의 顆粒의 直徑보다 크며(直徑 2,000~5,000Å), 休眠卵을 產卵하는 個體의 休眠調節細胞에서는 電子密度가 높은 리소좀이 觀察되었으나, 非休眠卵을 產卵하는 個體에서는 이것이 觀察되지 않았다.

多數의 細胞質顆粒이 리소좀과 結合되었다가 小形의 顆粒으로 되어 리소좀으로부터 나오는 것이 發見되었다.

休眠要因細胞와 休眠調節細胞에 대하여 論하였다.

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EXPLANATION OF FIGURES

Fig. 1. Electron-micrograph showing two kinds of neurosecretory cells located in the diapause suboesophageal ganglion. f, diapause factor cells; r, diapause regulator cell; l, lysosome. Note that the DR cell contains more and larger granules than the DF cell does, and lysosome is surrounded by a number of irregular profiles of Golgi complex. (×4600)

Fig. 2. Electron-micrograph showing two kinds of neurosecretory cells located in the non-diapause suboesophageal ganglion. Note absence of lysosome containing electron-dense material. (×8000)

Fig. 3. Electron-micrograph of DR cell. Note lysosome close to the cell membrane. (×4600)

Fig. 4. Electron-micrograph showing lysosome present in the DR cell in the diapause suboesophageal ganglion. Note that lysosome content appears markedly different in its electron-dense material. (×18000)

Fig. 5. Electron-micrograph showing granule formation in DR cell in the diapause suboesophageal ganglion. (×18000)

Fig. 6. Electron-micrograph of DR cell in the non-diapause suboesophageal ganglion.e, endoplasmic reticulum. Note that a number of prominant rough-surfaced endoplasmic reticula are oriented parallel to the cell membrane, and that many cytoplasmic granules are concentrated peripherally. (×8000)

Fig. 7. Electron-micrograph showing active lysosome of DR cell in the diapause suboesophageal ganglion. Note that two sizes of granules are present in the cytoplasm even in one DR cell. (×18000)

Fig. 8. Electron-micrograph of DF cells in diapause suboesophageal ganglion on fifth day of pupation. Note many vacuoles remain as a result of discharging all the granules. (×30000)