# Chlorella 細胞의 Pyrenoid 와 細胞壁構造에 關한 電子顯微鏡的 硏究

李 周 植
서울大學校 師範大學 生物學敎室

# Electron Microscopic studies on the Ultrastrucure of Pyrenoid and Cell Wall in Chlorella Cells.

Lee, Zoo Shik

Dept. of Biology, College of Education, Seoul National University

## Abstract

The author examined for observing the structures of pyrenoid and cell wall of three strains of *Chlorella ellipsoidea* and relation of pyrenoid to starch grain formation at the ultrastracture level.

1. The development of pyrenoid of *Chlorella* species from the time of its initiation and its subdetail sequent activities are described in some pictures.

2. Close correlation between the findings of light microscopy and electron microscopy is proved.

3. The pyrenoid is a dynamic organellae which continues to change its appearance throughout the development of the *Chlorella* cell.

4. The starch grains are continously formed by deposition of carbohydrate within the chloroplast with the aid of pyrenoid factors.

5. Some parental starch grains are passed on the daughter cell during cell division.

6. The Da stage cells contain only chlaroplast without pyrenoid matrix. In Da stage a pyrenoid is surrounded by starch and starch grains appear in chloroplast lamellae. In $L_1L_2$ stages, large starch grains of lens form accumulate in cell. In $L_3$ stage pyrenoid disappears for a time and starch grains are scattered. In cell division stage starch grains are divided into four groups. In $L_4$ stage, pyrenoid substance appears temporarily and disappears soon. At this stage the cell is constituted of Dn cell containing chloroplast only.

7. The cellular boundary of JE strain except Y 815 and Y 511 strain contains 250 Å intermediate layer of unknown chemical composition between the fibrillar cellulose wall and the out capsule layer.

## Introduction

JE strain isolated in Japan, Y511 and Y815 strain isolated in Korea were examined to observe the structure of pyrenoid and cell wall and relation of pyrenoid to starch grain formation at the ultrastructure level. Recently, Bisalputra & Weiter (1964) reported concerning the starch grain formation of pyrenoid factor in Scenedesmus quadricauda.

The electronmicroscopic structure of the cell of *Chlorella ellipsoidea* through the cellular life cycle of

organism was investigated by Murakami et al(1963). He showed that the statch grain are continuously formed by deposition of carbohydrate within chloroplast with the aid of pyrenoid factors and that the pyrenoid is a dynamic organellae, which continuous to change its appearance during the life cycle of chlorella cell.

Concerning the relationship between pyrenoid and starch grain formation is, however, known in completely, and on the cellular boundary of the *Chlorella* cells no report is found in the literature as far as I know.

The purpose of my investigation is for solving the problem of this unknown field.

## Materials and Methods

Pure culture of all strains were grown by synchronous culture in general algae medium for studing development sequence of their cells. The materials were stained by several usual methods to view the structure of organellae in vital. Aceto-carmin squash method and Heidenhain's iron hematoxylin stain method for nucleus. Janus graen B stain method for mitochondria, Lugol reaction for pyrenoid, Feulgen reaction for DNA is used. For the electron microscopy the materials was fixed in 2% osmium tetroxide solution at 20°C for 2:30 hours, and buffered to pH 7.2 with buffer solution of palade. Dehydration was done in ethanol and materials were finally embedded in metacrylate. Then sections for electron microscope were cut at 20—30 milimicron with ultratome (Hitachi:UM—6). And carbon-coated thin sections were examined with Hitachi HS—6.

## Results and Discussion

The structure of all cells of the three strains is morphologically same. These *Chlorella ellipsoidea* cells include pyrenoid substance and starch grains or sheath form in cells. The developmental sequent of cells of the three strain is shown in Fig 1—3. When these are cultured with non shaked broth media, Y511 and Y815 strain grow very well in upper part of medium tube and JE strain in botton of it. The cultured cells of these strains differ each other in size and structure. The JE cell is larger than other cells in size and its cell membrane is thicker then others. Tamiya et al(1961) studied on the morphology of *Chlorella* cell and found nucleus and chlorophyll substance of the cell by light microscopic measure but did not report on the pyrenoid of it. By my investigation I found that the nucleus with dense nucleolus situating near cell membrane is paralleled with pyrenoid and the nucleus has nucleo-membrane.

The structure of nucleus matrix in young cell is elemental fibril one and in old cell it is elemental vesicular type. The ultrastructure of *Chlorella* species (Y511 strain) is shown in Fig, 4—6. Drum & Pankratz (1964), Lemble, Lang (1964), & Bisalputra & Weier (1964) reported the situation of nucleus in cells and that nucleus paralleled the pyrenoid, but they did not talk about the dense nucleolus and cell nucleus membrane.

According to my study these cells contain one chloroplast with chloroplast boundary membrane and several chloroplast lamellae. But these chloroplasts lamellae do not contain lamella grana. Ueda (1958) (1960), Bex-Shal et al (1963), Stern et al (1964) observed the structure of chloroplast in euglena. Murakami & Takamiya (1961) explained that the prelamellae body is the origin of plastid formation.

I found also the substance of prelamellae body in young *Chlorella* cell but I could'nt investigate the correlation between the prelamella body and plastid formation. Many physiological studies were done by Murakami & Takamiya (1961), Shibata et al(1964), Takashima et al (1964), and Aoki & Hase (1964) but the substance of pyrenoid and its starch grain formation in chloroplast were not reported by them.

The author's study on these problems revealed that the pyrenoid exists within chloroplast and pyrenoid matrix is denser than the stroma of chloroplast and it is divided into two semilurer shape by one or two discs. Pyrenoid is a dynamic organelle which coutinues to change its appearance throughout the development of the *Chlorella* cell due to the following factors: the deposition of starch

platelets within the periphery of the expanding matrix, the seperation of starch grain into individual pockets by the intrusive growth of the chloroplast lamellae in centripetal fashion and the transition of the shape of the starch from concave-convex platelets lenticullar grains which accumulate within the chloroplast. Bisalputura & Weiter (1964) studied on the pyrenoid and starch grain in Scenedesmus and explained that the grains are formed by deposition of carbohydrate within the chloroplast by the pyrenoid factors.

Drum & Pantratz (1964) classified the 8 kinds of Diatoms according to the number of disc within pyrenoid. The author found that these *Chlorella* cells have a single pyrenoid with one or two discs, but it is difficult to observe the disces apparently. The electron microscopy showing the development of pyrenoid and starch grain formation during life cycle is shown in Fig 7—14. Dn stage cells contain only a chloroplast without pyrenoid matrix (Fig 7). In the Da and D-L stages a pyrenoid is surrounded by starch sheath and starch grain appear in chloroplast lamellae (Fig 8). At $L_1$ $L_2$ stages large starch grains of lens form accumulated in cell (Fig 9, 10). In $L_3$ stage pyrenoid disappears for a time and starch grain is scattered (Fig 11, 12). At cell division stage, the grains are divided into four group (Fig 12, 13). In $L_4$ stage pyrenoid matrix appears temporarily and disappears soon (Fig 13, 14). At this stage each part of the parent cell is constituted of Dn cell containing chloroplast only (Fig 14). The appearance of pyrenoid matrix and starch grain and chloroplast formation during each stage are, therefore, different from each other. This view was certified by Bisalputra weier on Scendesmus, but Murakami et al(1963) reported that during its life cycle pyrenoid is always in spite of *Chlorella* cell on the schematic diagrams in pyrenoid matrix not being in $L_3$ stage. In Fig. 15—18 the author showed the schematic diagram of the *Chlorella* pyrenoid formation and starch grain formation. The finding of mitochondria in my study as follow: The crista mitochondria situates near nucleus and chloroplast and the cells have about 2 to 5 mitochondrias of round form. The size of mitochondria is $0.2\text{-}0.7 \times 0.2\text{-}0.4$ micron. The mumber of mitochondria is influenced by the contents of starch grain and the size of pyrenoid. Old cells contain less mitochondria than younger cells in the number.

In the literature I found some reports about mitochondria by electron-microscopy. The author observed also other organellae such as endoplasmic reticulum, microsome, prolamellae and osmiophilic granule. Drum & Pankratz (1964) reported that the appearance of the osmiophilic granule has relation with pyrenoid. The cellular boundary is reported by Lembl & Lang (1965) on *chlamydomonas*. The author observed cellular boundary of JE strain except Y815 and Y511 strain contains Å intermediate layer of unknown chemical composition with the spike like aspect of the striation between the fibrillar cellulose wall and the out capsule layer. Fig. 19-21 show the ultrastructure of cellular boundary of *Chlorella* species.

## 摘　　要

*Chlorella ellipsoidea* (JE) 菌株및韓國에서 分離한 Y511, Y815의 菌株에 對한 各發育過程의 變化에 따르는 微細構造를 光學顯微鏡的 및 電子顯微鏡的으로 調査한 結果 pyrenoid의 構造와 starch grain과의 關係 및 細胞壁 構造에 있어서 다음과 같은 成績을 얻을 수 있었다.

1) 韓國에서 分離된 Y511, Y815는 *Chlorella ellipsoidea*와 같은 構造를 가진 細胞이고 pyrenoid 物質이 存在하여 그 周邊에 starch 形成을 이루고 있었다.

2) Pyrenoid의 作用으로 chloroplast 內에 炭化物質이 堆積되어 곧 澱粉粒子(starch grain)가 繼續的으로 形成되었다.

3) 各發育期에 따라 pyrenoid 및 澱粉粒子의 出現을 觀察한바 $L_3$ 期에 一時的으로 pyrenoid가 나타나지 않고 分裂期에 澱粉粒子의 分裂 및 通過를 觀察함으로서 pyrenoid는 細胞의 機能을 通해서 變化性이 있는

動物器官임을 觀察할 수 있었다.

4) 3 菌株의 細胞性을 比較實驗한 바 JE 株는 外部 capsule 層과 內部細胞膜 사이에 striation을 가진 中層이 觀察되었다.

## All the figures show ultrathin section through *Chlorella* cells.

Fig. 1～Fig. 3 : The development sequent of *Chlorella* species.

Fig. 4～Fig. 6 : The ultrastructure of *Chlorella* species Y511 strain only.

Fig. 7～Fig. 14 : Electronmicroscophs showing the develompent of pyrenoid and starch grain formation

Fig. 15～Fig. 18 : Schematic diagram of the *Chlorella* pyrenoid and starch grain formation described in this page paper.

Fig. 19～Fig. 21 : The ultrastructure of cellular boundary of *chlorella* cells.

Ca, capsule layer; Cb, cellular boundary; Ce, cellular layer; CH, chloroplast; CI, chloroplast lamella; Cy, cytoplasm; cym, cytoplasm membrane; Ec, empty cell; Er, endoplasmic reticulum; In, intermediate layer; M, mitochondria; N, nucleus; Nu, nucleolus; Os, osmiophilic granule Od, oil drop; Py, pyrenoid; D, pyrenoid disc; Sh, starch sheath; Sg, starch grain; St, striation; V, vacuole;

**Fig. 1～3 : The development sequent of *Chlorella* species**

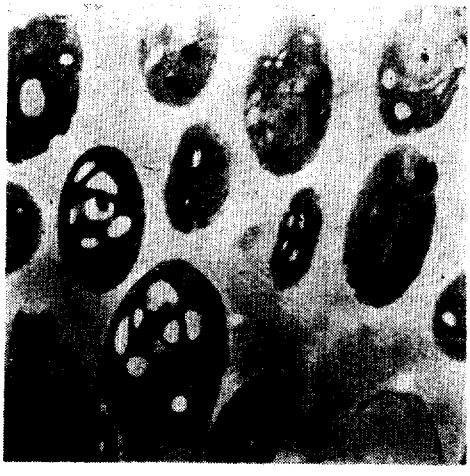

**Fig. 1** : Y 511 strain × 5,000

The development sequent of Y 511 strain cell. The most cells contain the chloroplast. The chloroplast is evident in cytoplasm.

Da stage cell: chloroplast is apparent. pyrenoid can't be recognized.

D～L, $L_1$ $L_2$ stage: : pyrenoid surounded with starch sheath can be recognized.

$L_3$, $L_4$ division stage: : We can observe the division stage cell.

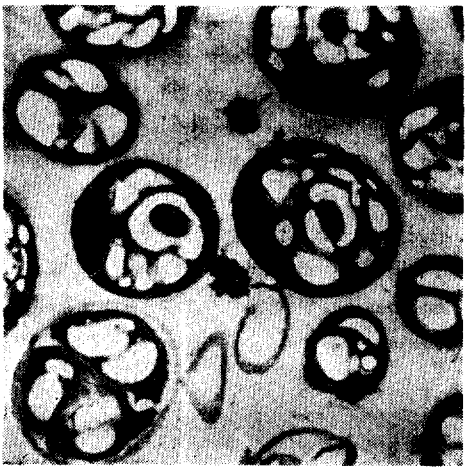

**Fig. 2** : Y 8 IS strain × 5,600

The development sequent of Y815 strain cell Da, D～L, $L_1$ $L_2$, $L_3$ and $L_4$ stage cell. pyrenoid present and nucleus evident. We can observe the division cell.

**Fig. 4~6 : The ultrastructure of *Chlorella* species (Y511 strain)**

**Fig. 3** : JE strain ×5,000
The devélopment of JE strain cell.
This picture shows $L_1$, $L_2$, $L_3$ and $L_4$ stage cell.

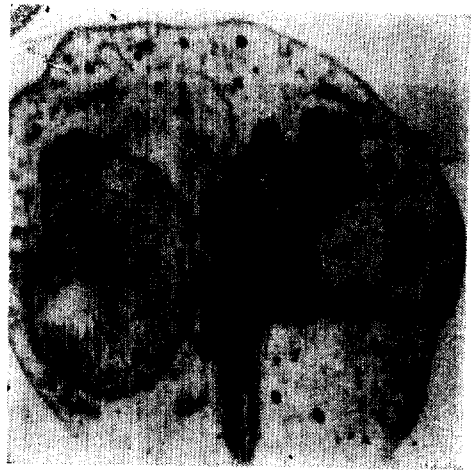

**Fig. 4** : Ultrastructure of Da stage cell. approximately ×30,000
Nucleus with dense nucleole is centerior. Long cup shaped chloroplast of *Chlorella* shows. Mitochondria locate near between chloroplast lamellae and nucleus. Osmiophilic granule and endoplasmic reticulum present. It shows the cellular boundary.

**Fig. 5** : Ultrastructure of $L_1$ or $L_2$ stage cell ×25,000
Pyrenoid with two discs which is surrounded by starch grain and sheath is centrally located.
Chloroplast lamellae present round strach grain. The mitochondria present.

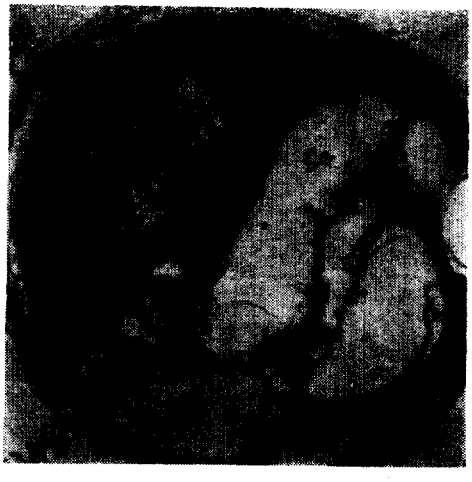

**Fig. 6** : Ultrastructure of $L_1$ stage cell approximately ×30,000
Nucleus with dense Nucleole is evident starch grain appear in reticulated chloroplast. Mitochondria with cristae present near chloroplast lamellae and nucleus.
It show thick cellular boundary.

**Fig. 7—14 Electronmicronmicroscophs showing the development of pyrenoid and starch grain formation**

**Fig. 7** : Dn stage cell ×58.000
The earliest stage at which pyrenoid can't be recognized this cell haven't pyrenoid and starch grain.

**Fig. 8** : Da stage cell ×32,000
The earliest stage at which pyrenoid can be recognized, pyrenoid to be surrounded only starch sheath is within chloroplast.

**Fig. 9** : The D-L stage cell ×23,000
It shows the chloroplast lamellae, pyrenoid with disc and starch grain and sheath.
A pyrenoid with one layer of starch platelets.

**Fig. 10** : $L_1$ stage cell ×19,000
Portion of pyrenoid showing the separation and the deposition of starch platelets by chloroplast lamellae.

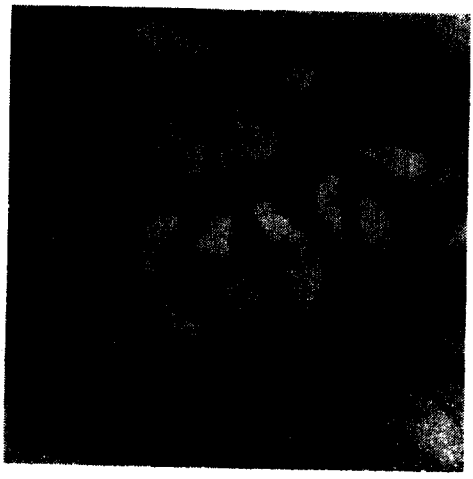

**Fig. 11** : $L_3$ stage cell $\times 25,000$
This stage (maturing cell) at which pyrenoid can't be recognized starch grain deposit the largest of all stage cells.

**Fig. 12** : $L_3$ stage cell $\times 25,000$
Some parental starch grain are passed on to daughter cell during cell division. This cell contain starch grain without pyrenoid.

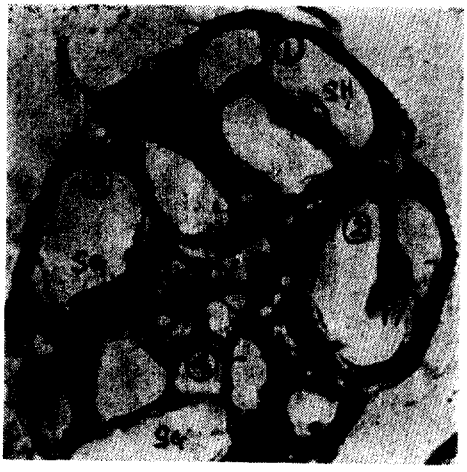

**Fig. 13** : $L_4$ stage cell, $\times 19,000$
A coenobium at various styles stages ①, ② cells shows the pyrenoid surrounding starch sheath on the division cell and ③, ④ cell only starch grain.

**Fig. 14** : $L_4$ stage cell $\times 25,000$
Dn stage cell is complete cell. One cell of division cells is Dn cell to be daughter. It contains chloroplast and a little starch grain without pyrenoid.

**Fig. 15—18 : The schematic diagram of the *Chlorella* pyrenoid formation and starch grain formation described in this page paper**

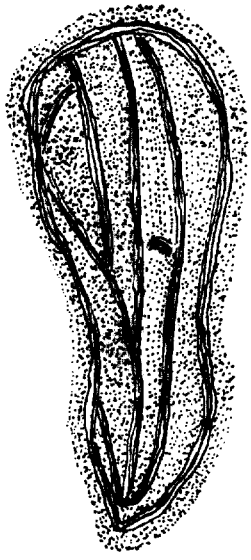

**Fig. 15—1** : Dn stage cell corresponding to Fig. 7 contain only a single chloroplast without pyrenoid.

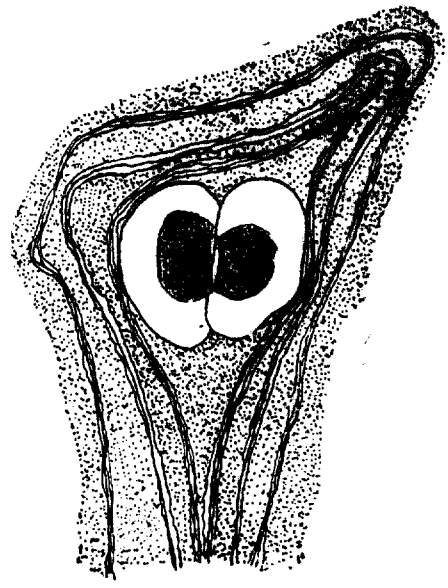

**Fig. 15—2** : In Da stage corresponding to Fig. 8 a pyrenoid is surrounded by starch sheath within chloroplasts.

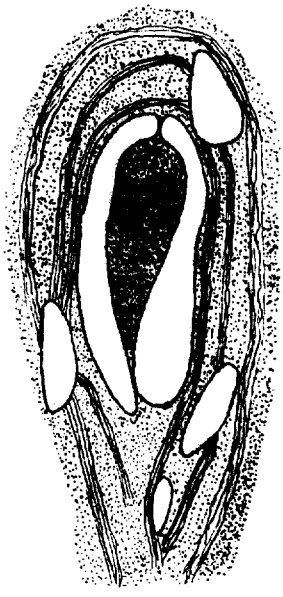

**Fig. 16—1** : In $L_1$ stage cell corresponding to Fig. 9. a pyrenoid surrounded with one or two layer of starch platelets.

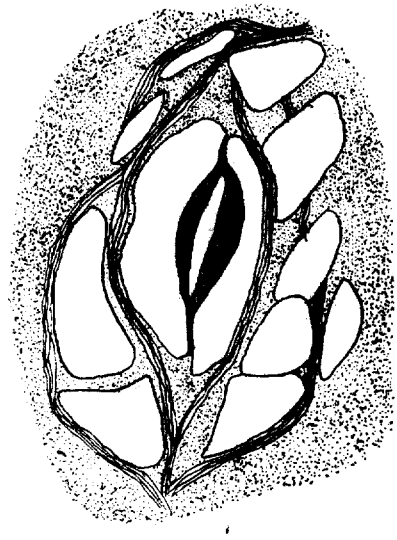

**Fig. 16—2** : In $L_2$ stage cell corresponding to Fig. 10 the largest of the starch grain during his lifecycle accumulate in it,

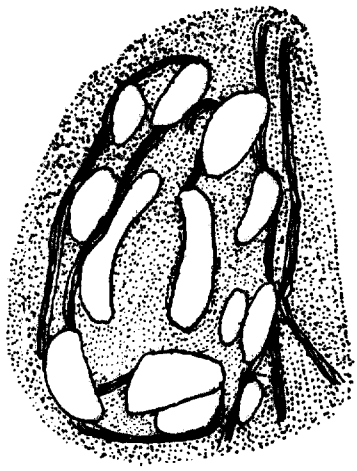

**Fig. 17—1** : $L_3$ stage starch formation corresponding to Fig. 11.
Pyrenoid disapears for a time and starch grain is scattered.

**Fig. 17—2** : In $L_3$ stage division cell corresponding to Fig. 12
Starch grain are divided into four daughter cells.

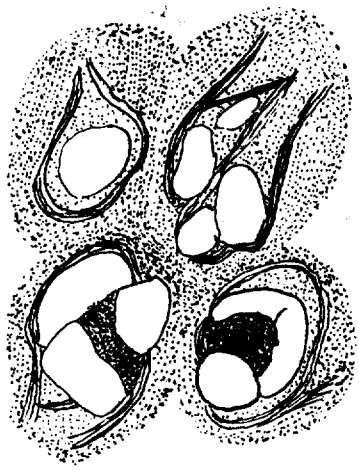

**Fig. 18—1** : In $L_4$ stage cell corresponding to Fig. 13
Pyrenoid substance appear temporally and disappear soon.

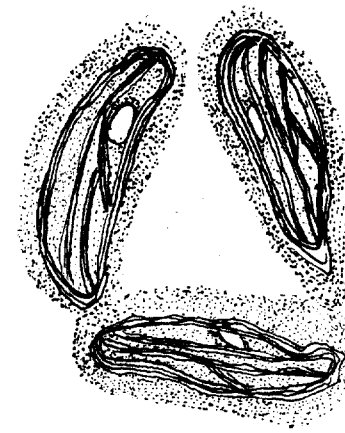

**Fig. 18—2** : $L_4$ stage cell to be Dn cell corresponding to Fiig. 14, 7
One of fourgr oup is consistituted of the earlist stage cell with single chloroplast.

**Fig. 19--21 : The Ultrastructure of Cellular Boundary of *Chlorella* species**

**Fig. 19** : Cellular boundary of the earliest Y511 strain cell shows the cellulose wall and capsule layer and cytoplasmic membr ane is evident. ×34,000

**Fig. 20** : The cellular boundary of $L_1$ stage Y815 strain shows the capsular layer, intermediate layer without striation and cellulose wall. Cytoplasmic membrane is evident. ×55,000

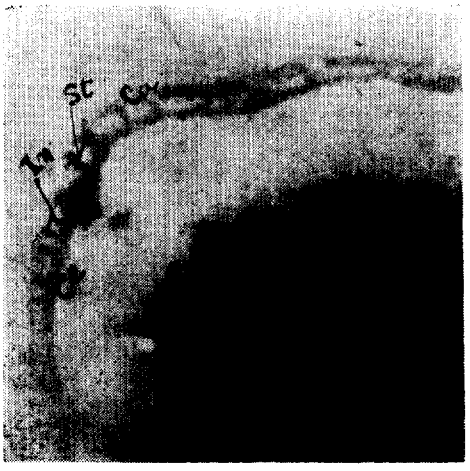


**Fig. 21** : Cellulose boundary of JE strain ce shows the cellulose wall, intermediate layer with striation (allow) and the capsular layer. cytoplasmic membrane is also evident.

Intermediate layer of this cell shows the spike like aspect of the striation. Cellulose wall is also evident. ×67,000

## References


1. AOKI,S, and HASE,E.,De and Re-generation of chloroplast in the cell of *Chlorella* protothecoides. Ⅰ. Synthesis of nucleic acid and protein in relation to the process of degeneration of chloroplast. Plant & Cell Physiol.,**5**, 473～484.
2. AOKI, S., and HASE,E.,De and Re-generation of chloroplast in the cell of *chlorella* protothecoides. Ⅱ. Effects of Actinomycin or greening of glucose-bleached "and Et olated" algal cells. Plant &Cell Physiol., **5**, 485～493
3. AOKI, S., and MIYACHI, S., 1964 Chromatographic analyses of acid- soluble polypholsphates in *Chlorella* Cells. Plant & Cell Physiol., **5**, 241～250
4. BISAPUTRA, T.,and WEIER, T. E., 1964. The pyrenoid of *Scenedesmus quathricauda*, Amer. Jour. Botany., **51**. 8, 881～892
5. BEX-SHAUL, Y., SCHIFF. J. A., and EPSTEIN, H.T., 1964. Studies of chloroplast development *Euglena* Ⅶ. Fine structure of the developing plastid. Plant Physiol. 231～240.
6. CUNNIRGHAM, W. P. 9., 1964. Oxidation of externally added NAPH by isolated corn root mitochondria. J. Plant. Physiol., **39**, 4, 699～703
7. CHIN, P. and LEE, Y.N., 1965 Studies on the organi cacids metabolism in *Chlorella* cell. Kor. Jour. Microbiol., **3**, 2, 15～21.
8. DRUM, R.W., and PANKRATZ, H.S. 1964. Pyrenoid, raphes and other fine structure in Diatoms. Amer. Jour. Botany., **54**, 4. 405～418
9. GIBER, A, and GRANICK, S., 1964. Plastids and mitochondria inheritable system. Science, **145**, 3635, 28, 860～867
10. HACKETT, D.P., 1955 Plant mitochondria. International Review. Cytology
11. HIRANO, T., and LINDEGRAN, C.C.1963. Electron microscopy of mitochondrial changes in *saccharomyces*. Jour. Ultrastructure Research **8**. 322～326.
12. IWAMURA, T., 1961. Cellular distribution and properties of Nucleic acid species in *Chlorella*. Symposia, Third Press Institute of Applied Microbiology, University of Tokyo, 259～278.
13. ISHIKAWA, I.S., and HASE, E. 1964. Nutritional control of cell pigmentation in *Chlorella protothecoides* with special reference to degeneration of chloroplast induced by glucose. Plant & Cell, Physiol, **5**, 227～240.
14. IWAMURA, T., and KUWASHIMA, S., 1964. Formation of adenosine 5-triphosphate from polyphosphate by a cell free extract from *Chlorella*. Jour. Gen. Appl. Microbiol, **10**, 83～86.
15. IWAMURA, T., and KUWASHIMA, S., 1964. Further observation on the deoxyribonucleic acid species in *chlorella* showing light-dependent metabolic turnover. Biochem. Biophys. Acta, **82**, 678～679.
16. IWAMURA, T., and Muto, N., 1964. In corporation of 5-bronouracid into the two kinds of DNA in *chlorella*. Plant. J Cell Physiol, **5**, 359～360.
17. KUSHIDA H., 1961. Artifacts occuring during embedding Sectioning and electron microscopy of thin section. Symposia Third Press, Institue of Applied Microbiology. University of Tokyo, 38～73.
18. LEMBL, C.A. and LANG, N.S., 1965. Electron microscopy of *Carteria* and *Chlamydomonas*. Amer. Jour, Botany, **52**. 5, 464～477.
19. LEHNINGER, A.L., 1964. The mitochondrion: Molecular basis of structure and function. W. A. Bentamin, INC, 16～40, 157～179, 205～253 New York.
20. LEE, H.S., 1965. Über die chlorophyll bildung von *Chlorella*

variegate beiglukose-Anzucht.
Kor. Jour. Microbiol., 3, 2, 27~43.

21. LEE, Y.N., and CHIN, P. 1964.
Action of ascorbic acid and indolacetic acid on the oxidation of succinate and coupled phosphotylation in *chlorella* mitochondria.
Kor. Jour. Microbiol., **2**, 1, 12~16.

22. LEE, Y. N., 1964
Studies on the phosphate metabolism in *chlorella* with special reference to polyphosphate.
Kor. Jour. Microbiol, **2**, 7, 1~11.

23. LEE, Y.N., and KIM, C.K., 1964.
Changes in pigment contents of *chlorella* cells during the courese of their life cycle
Dr. Lee, Fui Jae, Memorial Issue, 145~152.

24. LEE Z.S., and SHIM, J.H, 1963.
Studies on the *Chlorella* in Korea. Kor., Jour. Microbiol. **1**. 1, 38~44.

25. MURAKAMI. S., and TAKAMIYA, A., 1961.
Structure and function of chloroplast Symposia Third Press Institute of Applied Microbiology, Univ. of Tokyo 223~240

26. MURAKAMI, S., 1962.
Electronmicroscope studies on plastid development in vorigated leaves of *Liriope platyphylla.*
Ⅱ. The albicatie. plastid. Cytologica, **1**, 27, 2.

27. MURAKAMI, S., MORIMURA, Y., and TAKAMIYA, A., 1963.
Electron microscopic studies along cellular life cycle of *Chlorella ellipsoidea.* Microalgae & Photosynthetic Bacteria 66~83.

28. MORIMURA, Y, YANAGI, S.,and TAINYA, H 1964.
Synchronous mass-culture of *Chlorella.* Plant J. Cell Physiol, **5**, 281~289.

29. MORIMURA Y., and TAMIYA, H., 1964.
Anormalies of the life cycle of *Chlorella.* caused by some Antimetabolites. Jour. Indian Bot. Soc, **42**, 22, 29.

30. MIYACHI, S., KANAI, R., MIHARA, S., MIYACHI, SAYOKO, and AOKI, S., 1964.
Metabolic roles of inorganic polyphosphates in *Chlorella* cells.
Biochem, Biophys. Acta. **93**, 625~634.

31. PALAEG, L., and HYDE, 13, 1964.
Physiological effects of gèbberellicacid VII Eleclron microscopy of bareley aleurone cells Plant Physiol. **33** 4, 673~683.

32. PANGBORA, T., MARR, A.G., and ROLRISH, S.A 1962.
Localization of respiratory enzymes in intercytoplasmic membranes of *Azotobactor agilis*
Jour. Bact, **84**. 669~678.

33. STERN, A.L., SCHIFF, T,A., and EPSTEIN, H.T., 1964.
Studies of chloroplast development in *Euglena* V. Pigment biosynthesis, photosynthetic oxygen evolution and carbon dioxide fixation during chloroplast development.
Plant Physiology **1**. 39. No, 2, 220~228.

34. STERN, A.L., EPSTEIN, M.T., and SCHITT,J. A., 1964.
Studies of chloroplast development in *Euglena* Vl Light intensity as a controlling factor in development.
Plant Physiol. **39**. No.2, 226~230.

35. SHINKE, N., and UEDA, K., 1961.
An eIectronmicroscopic study of algal cells. Symposia Third Press Institude of Applied Microbiology, University of Tokyo. 23~27.

36. SATO, RGO., 1961.
Structure and functions of mitochondria and Microsome.
Symposion Third Press, Lnstitute of Applied Microbiology, University of Tokyo, 241~258

37. SHIBATA, K., MORIMURO, Y., and TAMIYA, H., 1964.
Precise measurement of the change of statistical distribution of cell size occuring during the synchronous culture of *Chlorella* plant
J. Cell Physiol. **5**. 315~320.

38. TAKASHIMA, K., ISHIKAWA, I.S., and HASE, E., 1964.
Futher notes on the growth and chlororhyll formation of *chlorella prothecoid* Plant & Cell Physiol, **5**, 321~332.

93. TAMIYA, H., MORIMURA,Y., YOKOTA.

M., and KUNIEDA, R., 1961.
Mode of nuclear division in synchronous curlture of *Chlorella* comparision of various methods of synchronization. Plant and Cell Physiol., **2**, 383~403.

40. UEDA, K., 1960.
Structure of plant cells with special reference to lower plants
IV. Structure of Trachelomonas Cytologica **25**, 8.

41. UEDA, K. 1958.
The structure of plant cells with special reference to lower plants IV. A cytological studys of *Euglena gracils* Cytology **23**, 56~

42. VENKATARAMAN, G.S., and YANAGITA, T, 1964.
Photosynthetic activity as a measure of viability in microalgae.
Jour. Gen. Appl. Microbiol. **10**. 1. 69~76.