Jap. J. Phycol. (Sôrui) 30: 103-112. June 20, 1982

Culture studies of *Dictyosphaeria* (Chlorophyceae, Siphonocladales) II. Morphological analysis of segregative cell division in *Dictyosphaeria cavernosa*[1,2)]

Sachito ENOMOTO*, Terumitsu HORI**, and Kazuo OKUDA***[3)]

* *Marine Biological Station, Faculty of Science, Kobe University, Iwaya, Hyogo-ken, 656-24 Japan.* ** *Institute of Biological Sciences, University of Tsukuba, Sakura-mura, Ibaraki, 305 Japan.* *** *Institute of Algological Research, Faculty of Science, Hokkaido University, Muroran, Hokkaido, 051 Japan.*

ENOMOTO, S., HORI, T. and OKUDA, K. 1982. Culture studies of *Dictyosphaeria* (Chlorophyceae, Siphonocladales) II. Morphological analysis of segregative cell division in *Dictyosphaeria cavernosa*. Jap. J. Phycol. 30: 103-112.

The entire process of "segregative cell division" characteristic of the siphonocladalean algae was followed in the laboratory cultured material of the coenocytic marine green alga *Dictyosphaeria cavernosa*.

Cell divisions both in the unicellular vesicles and the multicellular thalli were fundamentally the same, except for the number of daughter cells formed and pattern of their distribution in the mother cell.

When the unicellular primary vesicles were exposed to conditions of 14 hr L: 10 hr D (the lights are on at 0500 and off at 1900), 1000-2500 lux and 23°C, in PES, the cell division usually took place about 6 hr after the onset of the dark period and finished by the next morning (about 0700). At first the protoplasm of each vesicle broke down into small granular pieces, which then fused with one another to construct a network. In the following stage, the network broke down again into many spherical protoplasmic masses that synthesized new cell walls, as they increased rapidly in volume. By evening (about 1700), they became nearly twice in diameter.

Under continuous light the primary vesicle continued enlarging without cell division. However, if they were transferred to a light-dark cycle within about 10 days, normal cell division was induced within a few days.

Preliminary observations on the ultrastructure of the secondary vesicles are also presented.

Key Index Words: *Chlorophyceae*; *coenocyte*; Dictyosphaeria cavernosa; *segregative cell division*; *Siphonocladales*.

In 1905 BOERGESEN recorded and illustrated for the first time the distinct process of cell division in the coenocytic marine green alga *Siphonocladus tropicus*. According to him "the whole protoplasts with nuclei and chromatophores are divided into a number of small clumps, which are soon surrounded by a membrane and grow larger filling out the whole lumen of the mother cell, which in this way becomes divided into a number of small cells". Later, he detected this mode of cell division in some allied genera *Dictyosphaeria, Struvea,*

1) This work was supported by Grant-in-Aid No. 248015 and No. 534028 from the Scientific Research Fund of the Ministry of Education, Science and Culture, Japan.

2) Contribution No. 5 from the Marine Biological Station, Kobe Univ.

3) Present address: Department of Biology, Faculty of Science, Kochi University, Kochi, 780 Japan.

Chamaedoris, *Cladophoropsis* and *Boodlea* (BOERGESEN 1912, 1913). For this mode of vegetative cell division he coined the term "segregative cell division" (BOERGESEN 1913). After BOERGESEN (1912, 1913), segregative cell division in *Dictyosphaeria* was observed by ARNOLDI (1913) and EGEROD (1952).

Since then, many phycologists have confirmed this unique process of cell division and have discussed its biological and systematic significance for a long time without further investigation of the whole process.

In the previous paper on the life history of *D. cavernosa* (ENOMOTO and OKUDA 1981), we briefly mentioned this mode of division. The present paper includes the whole process of cell division both in unicellular and multicellular germlings which originated from zoospores, zygotes and both sexes of parthenogametes of *D. cavernosa*.

Materials and Methods

Vegetative plants of *Dictyosphaeria cavernosa* collected at Amami Island, southwestern Japan, during March and June of 1976-1980, were cultured in filtered-autoclaved sea water under 23-25°C, 1000-2500 lux, 14-16 hr L : 10-8 hr D. Although this light intensity is lower than these plants received in the field, plants normally produced reproductive cells, which grew into new plants under this light condition in the laboratory. For prevention of the luxuriant growth of algal epiphytes no nutrients were added. About one month after initiation of the cultures, zoospores, male and female gametes were obtained from separate plants, and zoospores, zygotes and male and female parthenogametes were cultured under two different conditions: (1) 16 : $\bar{8}$ hr, 25°C and (2) 14 : $\bar{10}$ hr, 23°C (see ENOMOTO and OKUDA 1981). For unialgal cultures, PROVASOLI's ES-medium (PES) (prepared according to MCLACHLAN 1973) was used to facilitate the growth of the germlings. All germlings were maintained under 1000-2500 lux.

About 80-90 days after the establishment of a unialgal culture, all germlings developed into unicellular spherical vesicles (3-5 mm in diam.) (Figs. 1, 2). These vesicles were set under two different photoperiodic conditions: (A) 14 : $\bar{10}$ hr (0500-1900 light period; 1900-0500 dark period) and (B) 24 : $\bar{0}$ hr (continuous light illumination). All other conditions were as follows, temperature: 23°C; light intensity: 1000-2500 lux; and population density: 10 vesicles/150 m*l* of the fresh medium of PES in glass vessel (60 mm diam., 90 mm depth).

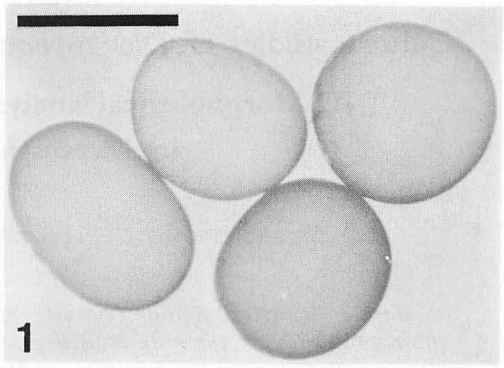


Fig. 1. *Dictyosphaeria cavernosa*. Zoospore-derived unicellular vesicles cultured for 90 days. Scale: 3 mm.

Light microscopy: For microscope observation 3-4 vesicles in which an impending division was expected were transferred into a flat vessel (24×40×8 mm, 6 m*l* of PES) just before the inset of the dark period. According to our preliminary experiments (unpubl. data), the total increase in number of vesicles which had undergone to the first segregative cell division amounted within 3-5 days to 95% of all vesicles, so we used such vesicles as not yet started division at 4th-5th day after transfer. This vessel was put on the mechanical stage of a microscope and then kept under the dark condition (23°C). For stepwise observations 12-13 times of brief illuminations (2-3 min/a observation) were given.

Electron microscopy: Procedures for electron microscopy are identical to those described earlier in detail (Hori and Enomoto 1978).

Observations

Cell division under a light-dark cycle condition: The present observation described two successive events of the segregative divisions occurring both in unicellular and multicellular plants. Since the process of both divisions is fundamentally identical, the description depends on the first division in an unicellular vesicle. The first cell division usually occurred after midnight (0100-0130 about 6 hr after the inset of the dark period) between 3-5 days after the unicellular vesicles were placed under culture condition (A).

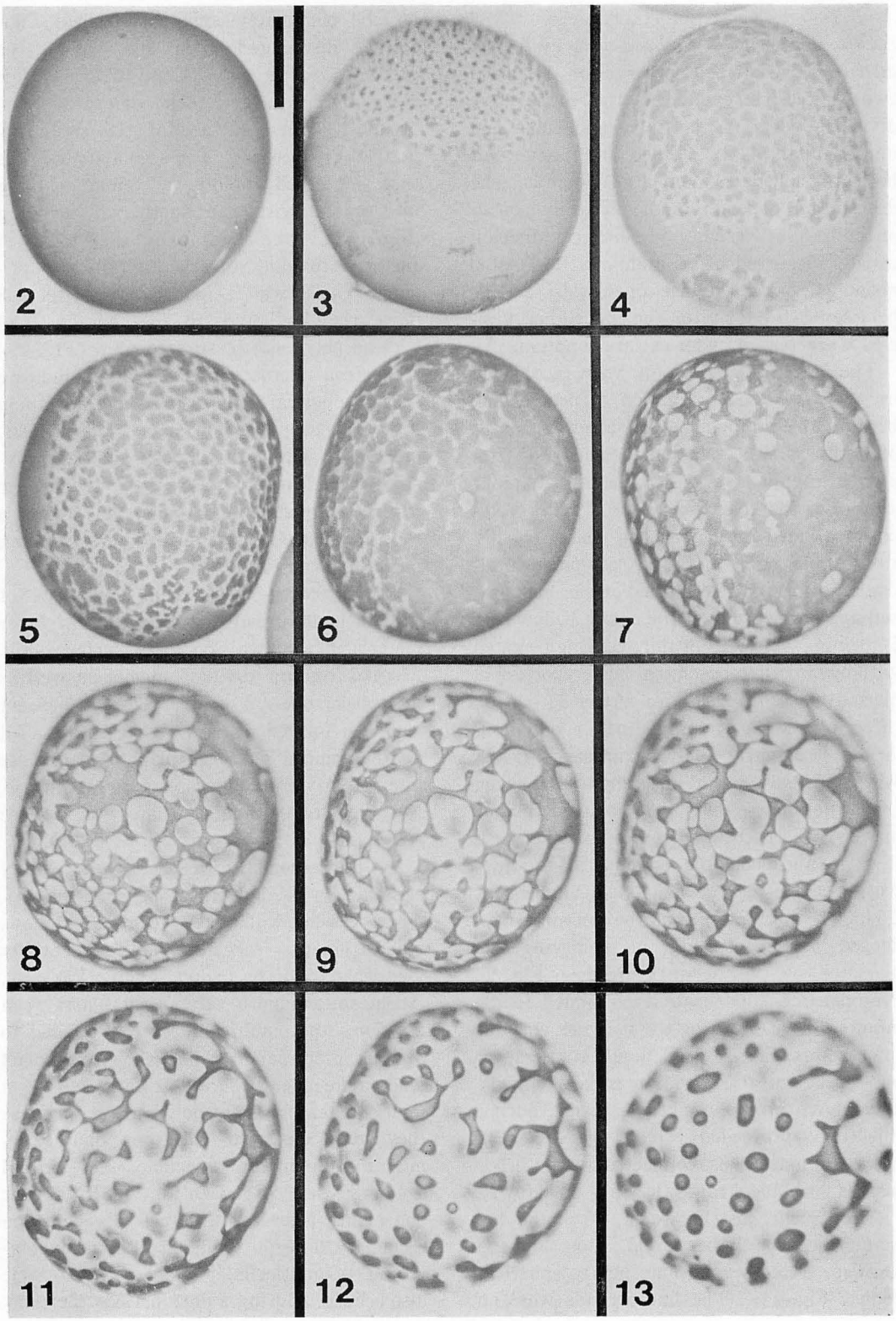
The impending division was recognized by a change in aggregation of protoplasm, because the protoplasm just after the onset of darkness was still homogenously distributed in a thin layer along the entire inner surface of the cell (Figs. 2, 26). The protoplasm divided into numerous nearly equally sized units (Fig. 3). This compartmentalization of protoplasm started at one end of a cell and expanded over the entire cell within 5-10 min. After 10-30 min, they aggregated with one another becoming larger polygonal discoid masses (Figs. 4, 5). After 30-60 min, these aggregations fused further becoming larger irregularly shaped masses (Fig. 6). About 2 hr after the initiation of division, the larger masses absorbed adjacent smaller masses, constructing a protoplasmic network (Fig. 7), and soon, the contour of the network became clearer (Fig. 8). After about 2.5 hr, the protoplasms of the network condensed more, resulting in enlarging the diameter of the network meshes (Fig. 9). Protoplasmic condensation continued for 30 minutes more breaking the network apart at some points (Fig. 10), and finally segregating into irregularly shaped masses (Fig. 11). Breakdown process of the network during this stage proceeded rapidly (Figs. 12, 13), but each mass was connected to the other masses with fine strands (Figs. 13, 20 arrows).

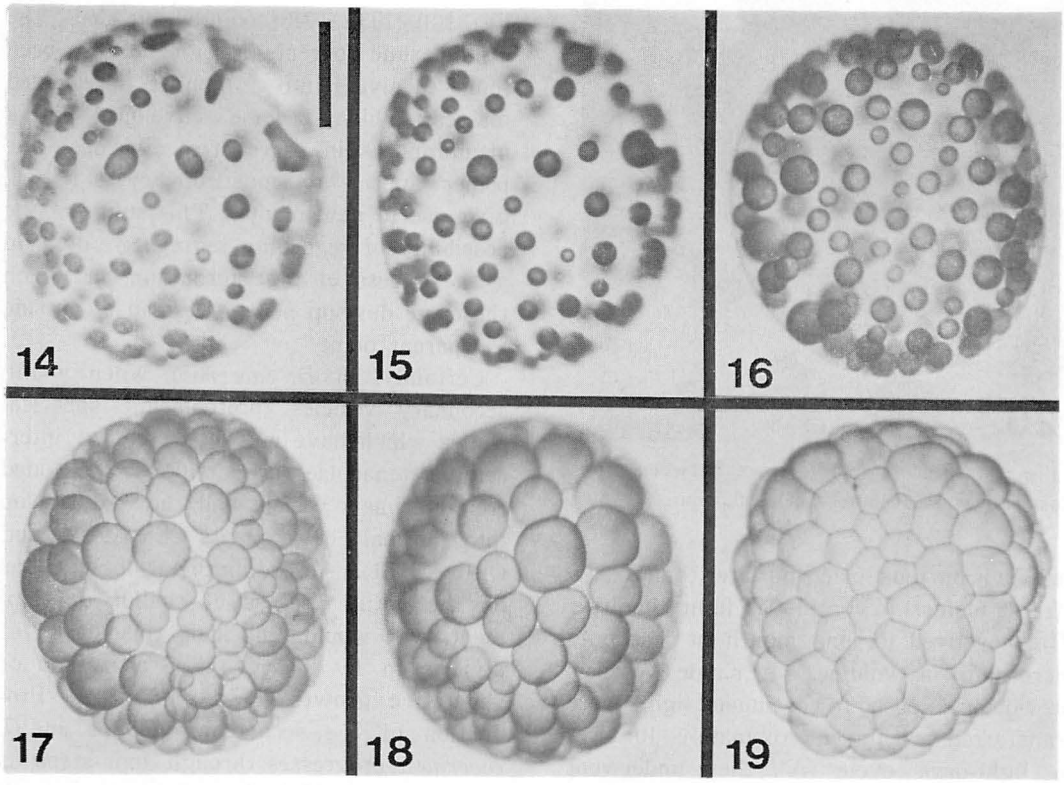
After 3.5 hr, just before or after the beginning of the light period, most of the segregated masses became nearly spherical bodies (Fig. 14). The fine strands were no longer detected. After about 5.5 hr they became completely spherical bodies (Figs. 15, 27), distributed in a layer along the primary vesicle wall (Figs. 16, 27). After about 6-8 hr, a new cell wall was seen around the cell surface of the secondary vesicle (Figs. 16, 28). The secondary vesicles then increased rapidly in volume coming into contact with one another by evening (Fig. 17). After about 20 hr, they enlarged further, crowding together within the primary vesicle wall (Figs. 18, 29). After 2-3 days they adhered to one another and became polygonal in surface view (Fig. 19).

Electron microscopic observations indicate that a spherical body at the stage shown in Fig. 14 is occupied by many chloroplasts, interphase and dividing nuclei, and variously-sized vacuoles with or without their inner contents (Figs. 21, 22). A new cell wall and a central vacuole are not formed yet. Dividing nuclei are centric.

The secondary vesicles at the stage of Fig. 15 have already secreted a thin layer material of cell wall on their surface (Fig. 23) and contain dividing nuclei as well as interphase one. A central vacuole has not yet been formed even at this stage. All dividing nuclei observed in both stages are interestingly either in prophase or metaphase. No other stages of nuclei later than these are found.

As the secondary vesicles were closely arranged in a single layer beneath the primary vesicle wall, they formed a hollow, monostromatic saccate plant essentially identical with an adult plant (Fig. 30). About one month after the first cell division, the multicellular unit grew to 5-8 mm diam. and finally the parent cell wall broke open (Fig. 30). Each cell of a multicellular plant did not disperse, because they were connected with one another by minute intercellular tenaculae (see Figs. 44—46 in ENOMOTO and OKUDA 1981). About one month after the first cell division, the secondary division occurred in all cells composing a multicellular plant (Fig. 24). It also occurred during a dark period. Tertiary


2
3
4
5
6
7
8
9
10
11
12
13



Figs. 2-19. The entire process of protoplasmic changes with the passage of time during the first segregative cell division in a primary vesicle of *D. cavernosa*. All photographs were taken fo a single vesicle, though some of them appear different from one another. 2. Unicellular vesicle just before division (0100) ; 3. Initiation of division, numerous small granular protoplasmic units formed on one side of a cell (0130) ; 4. Progression of granular unit formation (0140) ; 5. Granular unit aggregated with one another to become larger discoidal masses (0150) ; 6. Discoidal masses fused with each other (0230) ; 7. Masses fused with adjacent masses to form a network (0330) ; 8. Contour of network becoming clearer (0350) ; 9. Network condensed and the meshes enlarged (0400) ; 10. Network broken apart at various spots (0410) ; 11. Network broken down into a number of irregularly-shaped masses (0425) ; 12. Protoplasmic masses forming spheres (0430) ; 13. Segregated spherical protoplasmic masses (0440) ; 14. Protoplasmic masses nearly spherical, fine strands already disappeared (0450) ; 15. Spherical protoplasmic masses formed cell walls and became secondary vesicles (0700) ; 16. Nearly 100 secondary vesicles formed in a primary vesicle (1000) ; 17. Secondary vesicles enlarged and came into contact with one another (1730) ; 18. Secondary vesicles increased their volume, crowding together within a primary vesicle (2030) ; 19. A saccate young plant, secondary vesicles adhering to adjacent ones, becoming polygonally shaped vesicles in surface view, after 3 days. Scale : (Figs. 2-19) = 1 mm.

vesicles in a secondary vesicle were fewer in number than in the secondary vesicles in the primary vesicle (Fig. 25). Since the protoplasm of the secondary vesicles were densely distributed at the sides facing the outer surface of the secondary vesicle wall (Fig. 31), the tertiary vesicles were conspicuously located at this side in a single layer as shown Fig. 25 and Fig. 32. Therefore, as the thalli grew into adult plants, they still remained monostromatic saccate structures (Fig. 33).

Cell division under continuous light : As described above, when single vesicles were exposed to light-dark cycle condition (A),

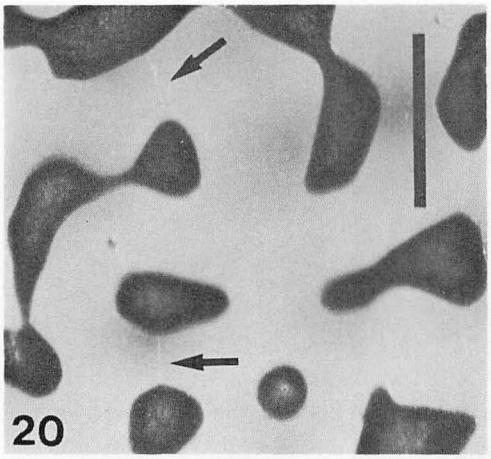


Fig. 20. Fine strands (arrows) between protoplasmic masses (0430). Scale: 0.5 mm.

cell division took place no later than 5 days after transfer. In continuous light condition (B), they lived for one month or less only increasing in volume. If single vesicles, previously exposed to continuous light, were transferred within approximately 10 days to light-dark cycle (A), they underwent normal cell division within 9 days, but abnormal divisions, that is, formation of several (usually 5–8) irregularly-shaped, variously-sized, larger masses (or clumps) of protoplasm, occurred in 50% of the vesicles. When exposed to continuous light, they grew to 8–10 mm vesicles in diameter after one month. Abnormal protoplasmic masses also synthesized new cell walls. The other vesicles remained in the unicellular state under continuous light for 45 days, but during this period their chloroplasts gradually changed from dark green to yellow-green and white-yellow-green, finally bleached white, resulting in the death of the vesicles.

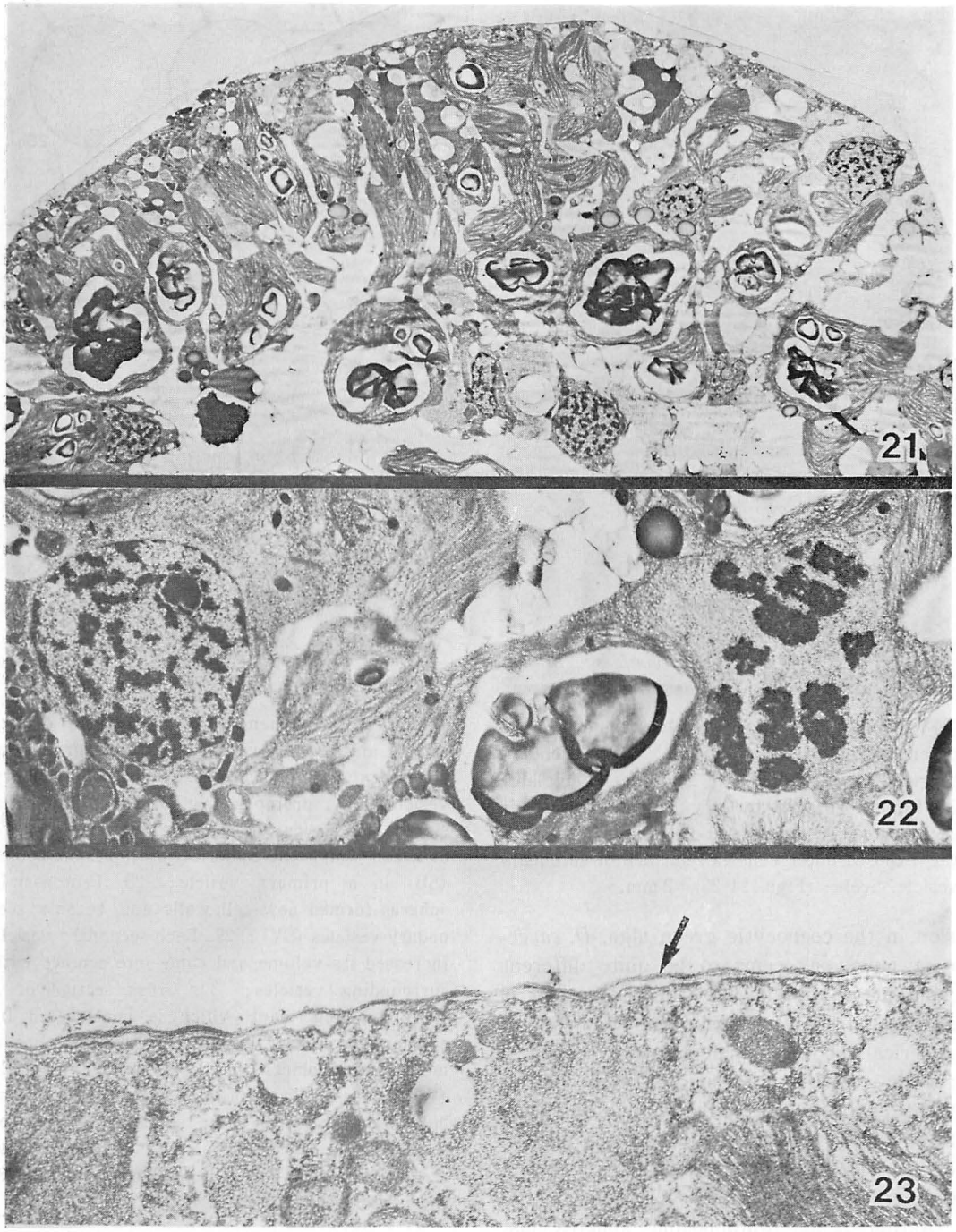
Discussion

Since segregative cell division was observed by BOERGESEN (1905), several authors (BOERGESEN 1912, 1913; ARNOLDI 1913; EGEROD 1952) have described only the final features of the division. They also discussed the biological significance and validity of this feature as a systematic criterion. FRITSCH (1935, 1946) pointed out that "the protoplasmic contents of segment have been found to divide into a number of rounded masses, which become enveloped by a membrane as in segregative division. The bodies appear to constitute cysts which give rise to new plants. This suggests the possibility of regarding segregative division as a process of cyst formation, in which the cysts develop *in situ*, instead of outside the parent plant".

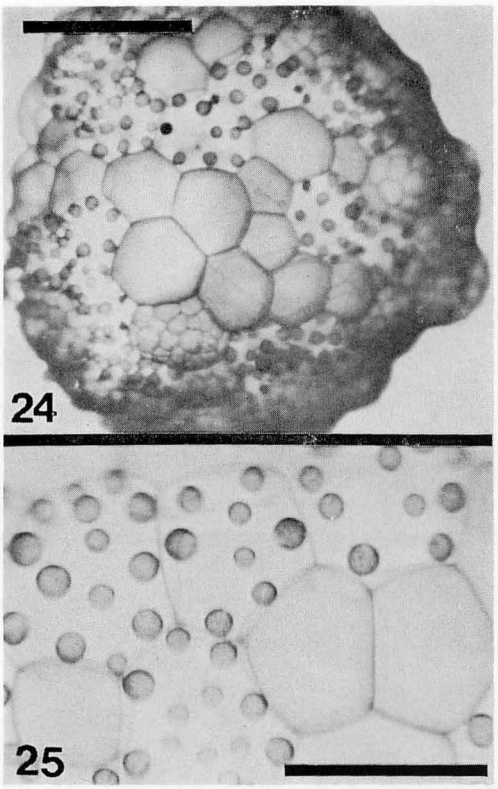
Certainly, in *D. cavernosa*, when young secondary vesicles (protoplasmic spherical bodies which have not yet developed intercellular tenaculae) are artificially excluded from a primary vesicle wall, they did develop into normal new plants (ENOMOTO and OKUDA 1981). According to our observations on the entire process of thallus development, we would consider this mode of division in *D. cavernosa* as "a method of vegetative growth" (EGEROD 1952). Production of the secondary vesicles in *D. cavernosa* progresses through four steps, 1) protoplasts divided into numerous small protoplasmic masses, 2) each fuses with another resulting in a protoplasmic network, 3) the network breaks down into many spherical bodies, and 4) each body synthesizes a new cell wall and becomes a secondary vesicle.

The protoplasmic network also appears during process of artificially induced aplanospore formation in *Boergesenia forbesii*. However, the network in this alga is formed directly by aggregation of protoplasm which was distributed in a thin layer (ENOMOTO and HIROSE 1972). The protoplasm of *D. cavernosa* does not segregate directly into the network leading to spherical bodies.

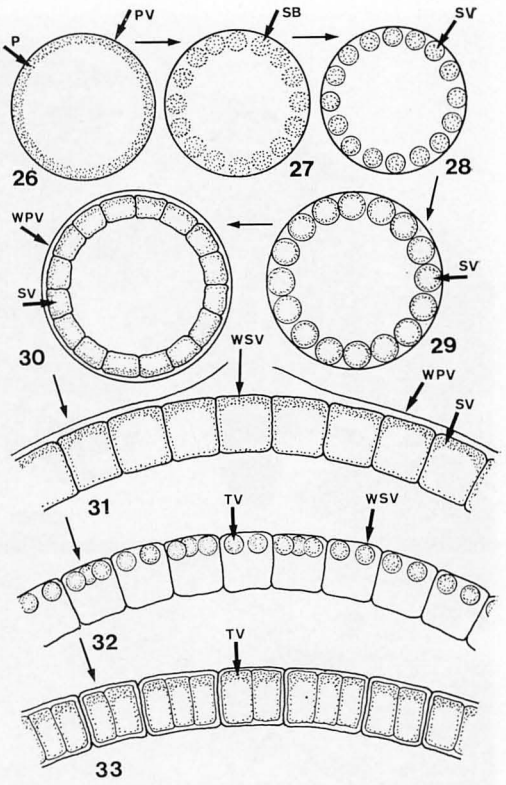
Recent electron microscopical studies revealed that nuclear division in uninucleate green algae is generally followed by cytokinesis (see PICKETT-HEAPS 1975). In some algae the cytokinetic cleavage furrows precociously appear even before prophase (LØVLIE and BRATEN 1970; MATTOX and STEWART 1974). The segregative cell divi-



Figs. 21-23. Electron micrographs showing subcellular structure of the nearly-formed secondary sphere in primary vesicles of *D. cavernosa*. 21. Part of a sphere corresponding to that of Fig. 14. ×2000; 22. Interphase (left) and metaphase (right) nuclei seen in another portion of the same sphere of Fig. 21. ×6000; 23. Part of a sphere corresponding to that of Fig. 15, showing a thin layer of wall material (arrow) secreted on the surface. ×18000.



Figs. 24-25. Tertiary vesicles formed in the parent plant of *D. cavernosa*. 24. Secondary segregative cell division in a multicellular plant; 25. Eight to fifteen tertiary vesicles formed in each secondary vesicle and distributed just below the exposed outer cell wall surface of secondary vesicle. Scale: (Figs. 24-25) = 2 mm.



Figs. 26-33. Schemata of distribution of secondary and tertiary vesicles and their growth in *D. cavernosa*. 26. Undivided unicellular primary vesicle (PV), protoplasms (P) distributed in a single thin layer along the entire inner surface of a cell wall; 27. Spherical protoplasmic bodies (SB) in a primary vesicle; 28. Protopasmic spheres formed new cell walls and became secondary vesicles (SV); 29. Each secondary vesicle increased its volume and came into contact with surrounding vesicles; 30. Cross section of a saccate young plant which is constructed by adjacent secondary vesicles; 31. A part of a multicellular plant. Wall of primary vesicle (WPV) burst. Protoplasms were distributed just below the exposed outer surfaces of wall of secondary vesicles (WSV); 32. Distribution of tertiary vesicles (TV) in secondary vesicles; 33. Growth of tertiary vesicles in a plant.

sion in the coenocytic green alga, *D. cavernosa*, however, seems to be quite different from them. In this alga the protoplasm is segregated simultaneously into a number of spherical bodies when a cell reaches a certain volume. Although these newly-formed spheres contain many dividing nuclei, there is no direct evidence that nuclear division is in concord with the progression of segregative cytoplasmic division.

Rather, that protoplasmic spheres just before or after the completion of segregative division contain interphase, prophase or metaphase nuclei probably indicates that the onset of their division might start near the end of the cytoplasmic division.

Before this unique division in the coenocytes is characterized, cytological studies will be necessary more in detail.

JÓNSSON (1965) considered that the difference between the segregative division and *Cladophora*-type division in which the septum is formed centripetally is regulated by

relative abundance of protoplasm in the cell at the moment of the division, because he observed these two types of divisions in different portions of a thallus of *Anadyomene stellata*. We can not follow his opinion, for only centripetal division was observed in all cells of *Anadyomene wrightii* (ENOMOTO and HIROSE 1970) and *Microdictyon okamurai* (ENOMOTO and HIROSE 1971). We believe that the ratio of protoplasmic quantity either to cell volume or to cell-sap volume induces the segregative cell division. In all the siphonocladalean algae undergoing the segregative cell division, cells composing a thallus are occupied by a large central vacuole, always appressing the cytoplasm in a thin layer against the cell wall. Therefore, this thin layer of protoplasm may bring about the segregative cell division.

In *D. cavernosa*, the protoplasmic network is formed both in production of secondary vesicle for vegetative growth and in formation of reproductive cells in fertile cells. Both processes are very similar, but they are easily distinguished by the following features: 1) during swarmer formation the protoplasts change their color from green to yellowish green, 2) several protoplasmic dense aggregations are formed in a fertile cell, 3) ostioles for swarmer discharge are formed in the cell wall of a fertile cell (HORI and ENOMOTO 1978; ENOMOTO and OKUDA 1981), and 4) the network during a vegetative growth division appears only during a dark period, whereas the network formation leading to reproductive cells in a fertile cell is not always restricted to a dark period (ENOMOTO and OKUDA 1981).

Present observations suggest that cell division requires a dark period, for several hours at least each day, and that continuous light or lacking of darkness inhibits cell division. However, once division has begun, several short periods of illumination do not inhibit the normal progression of division. Further studies on the relationship between cell division and light conditions (intensity, quality, photoperiod etc.) will provide a better understanding of segregative cell division. The systematic significance of the division will be discussed in a subsequent article.

Acknowledgement

The authors would like to express their sincere thanks to Professor JOHN A. WEST, Department of Botany, University of California, Berkeley, for his kind criticism and helpful advice about the manuscript.

References

ARNOLDI, W.M. 1913. Bau des Thalloms von *Dictyosphaeria*. Flora **105**: 144-161.

BOERGESEN, F. 1905. Contributions à la connaissance du genre *Siphonocladus* SCHMITZ. Overs. K. Dansk. Vidensk. Selsk. Forhandl. **1905**: 259-291.

BOERGESEN, F, 1912. Some Chlorophyceae from the Danish West Indies. II. Bot. Tidsskr. **32**: 241-273.

BOERGESEN, F. 1913. The marine algae of the Danish West Indies. Part I. Chlorophyceae. Dansk Bot. Arkiv. **1**(4): 1-158.

EGEROD, L.E. 1952. An analysis of the siphonous Chlorophycophyta with special reference to the Siphonocladales, Siphonales, and Dasycladales of Hawaii. Univ. Calif. Publ. Bot. **25**(5): 325-454.

ENOMOTO, S. and HIROSE, H. 1970. On the life-history of *Anadyomene wrightii* with special reference to the reproduction, development, and cytological sequences. Bot. Mag. Tokyo **83**: 270-280.

ENOMOTO, S. and HIROSE, H. 1971. On the septum formation of *Microdictyon okamurai* SETCHELL. Bull. Jap. Soc. Phycol. **19**: 90-93.

ENOMOTO, S. and HIROSE, H. 1972. Culture studies on artificially induced aplanospores and their development in the marine alga *Boergesenia forbesii* (HARVEY) FELDMANN (Chlorophyceae, Siphonocladales). Phycologia **11**: 119-122.

ENOMOTO, S. and OKUDA, K. 1981. Culture studies of *Dictyosphaeria* (Chlorophyceae, Siphonocladales). I. Life history and morphogenesis of *Dictyosphaeria cavernosa*. Jap. J. Phycol. **29**: 225-236.

FRITSCH, F.E. 1935. The structure and reproduction of the algae. Vol. 1. Cambridge Univ.

Press, London.

FRITSCH, F.E. 1946. The status of the Siphonocladales. J. Indian Bot. Soc. M.O.P. IYENGAR Commemoration Vol.: 29-48.

HORI, T. and ENOMOTO, S. 1978. Developmental cytology of *Dictyosphaeria cavernosa*. I. Light and electron microscope observations on cytoplasmic cleavage in zooid formation. Bot. Mar. 21: 401-408.

JÓNSSON, S. 1965. La validité et la délimitation de l'ordre des Siphonocladales. Trav. Déd. Luci. Plant. Masson & Cie, Paris. pp. 391-406.

LØVLIE, A. and BRATEN, T. 1970. On mitosis in the multicellular algae *Ulva mutabilis* FØYN. J. Cell Sci. 6: 109-129.

MATTOX, K.R. and STEWART, K.D. 1974. A comparative study of cell division in *Trichosarcina polymorpha* and *Pseudendoclonium basiliense* (Chlorophyceae). J. Phycol. 10: 447-456.

MCLACHLAN, J. 1973. Growth media-marine. p. 25-51. *In* J.R. STEIN [ed.], Handbook of phycological methods. Cambridge Univ. Press, London.

PICKETT-HEAPS, J.D. 1975. Green algae. Sinauer Associates, Inc., Publ. Massachusetts.

榎本幸人・堀　輝三*・奥田一雄: 暖海産緑藻キッコウグサ（*Dictyosphaeria cavernosa*）の分割細胞分裂（segregative cell division）について**

奄美大島産のキッコウグサから得た単藻培養体において分割細胞分裂の全過程を観察した。分裂は 14 hr L/10 hr D (05:00-19:00 明期, 19:00-05:00 暗期), 1000-2500 lux, 23°C の条件下で深夜（1時頃）に始まり朝（7時頃）までには完了する。母細胞内壁に沿って均一に単一薄層状に分布していた核，葉緑体，ミトコンドリア，その他の原形質は，まずそれらを含む無数の顆粒状の小塊に分裂し，ついで互いに融合し，より大きな板状塊となり，それらがさらに互いに融合して網状塊となる。原形質の凝集により網状分布は各所で分断され，ほぼ同大の多数の原形質塊に分離する。原形質塊は球形化し，細胞壁を新生して娘細胞となる。連続照光下では細胞は未分裂のまま生長を続ける。10日後この未分裂の細胞を上記の明暗条件に移すと数日以内に正常な分裂が起る。

（656-24 兵庫県津名郡淡路町岩屋，神戸大学理学部臨海実験所；*305 茨城県新治郡桜村天王台1-1-1，筑波大学生物科学系；**051 北海道室蘭市母恋南町，北海道大学理学部海藻研究施設）