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Analysis of cellulose microfibril arrangement patterns in the cell wall of new spherical cells regenerated from *Boodlea coacta* (Chlorophyceae)

Kazuo OKUDA and Shun MIZUTA

Department of Biology, Faculty of Science, Kochi University, Kochi, 780 Japan

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The three microfibrillar orientation patterns, meridionally-oriented, counterclockwise-oriented spiral and clockwise-oriented spiral patterns were found in the spherical cell walls of *Boodlea coacta*, by analyzing the replicated microfibril arrangements of cell walls. These patterns changed successively with the formation of microfibrils from each pole toward the cell equator indicating high polar dependency. In regard to change in the orientation of the microfibrils, a specialized fibril arrangement as a fountain-like structure was occasionally observed in the polar region and other parts of the spherical cells. This structure at each pole was small and fibrils dispersed widely as a curve to form a new layer. In other parts of spherical cells, this structure occupied a larger area and new fibrils extending from it were noted to change their orientation almost unidirectionally so as to form a multifibrillar layer. Microfibril changes in *Boodlea* generally recognizable by the presence of this fountain-like structures were not related to the direction of peripheral microtubule arrays and not affected by colchicine treatment, and it was considered that this structure might possibly provide some clue as to clarification of the mechanisms of polar-dependent changes in microfibril orientation.

Key Index Words: Boodlea coacta; *cell wall deposition*; *microfibril arrangement pattern*; *microfibril orientation.*

As described in the previous paper (MIZUTA *et al.* 1985), spherical cells from the protoplasm of the coenocytic green alga, *Boergesenia forbesii*, generate a cross-polylamellate cell wall beneath the initially regenerated matrix-rich layer. Microfibrils constituting the adjacent multifibrillar layers cross each other at almost right angles, as was also noted in the thallus wall (MIZUTA and WADA 1981) and show high polar dependency.

Similar types of polar-dependent microfibril arrangements in spherical or spherical-like cells have been demonstrated in *Valonia* (PRESTON and KUYPER 1951) and some other green algae (WILSON 1951, ROBINSON and PRESTON 1972). In *Valonia*, microfibrils are arranged longitudinally and transversely with the cell axis and a obliquely-arranged fibril group lies between the two adjacent microfibril layers. Cells in these algae usually have two poles, one on each side and microfibrils regenerate from both poles toward the equator (MIZUTA *et al.* 1985).

The microfibril arrangement at the polar region may induce orientation of polylamellated microfibrils. In *Glaucocystis*, fibril arrangement throughout the entire cell is determined by fibril orientation at the fibril formation center at each pole (WILLISON and BROWN 1978). In newly-formed spherical cells from *Boergesenia*, fountain-like fibril structures appear at each pole and microfibrils are deposited alongside these structures (MIZUTA *et al.* 1985). Microfibril orientation in polar areas may provide important information as to the regulatory mechanisms of microfibril orientation throughout an entire cell.

Preliminary experiments showed that the cell walls of newly-developed spherical cells

from the protoplasm of *Boodlea coacta* (DICKIE) MURRAY et DE TONI were composed of matrix-rich and thick fibril-rich layers. Although the microfibrils in the fibril-rich layers crossed each other, the fibrils in the polar area showed a wide variety of patterns. For an understanding of the morphological sequence of pattern changes in microfibril orientation for elucidation of the control mechanisms of the microfibrils, microfibril arragement patterns in the spherical cell walls in *Boodlea* were examined and change in a microfibril orientation was discussed.

Materials and Methods

Formation and culture of new cells: Boodlea coacta was cultured for about three months at 25°C under a 14 hr light and 10 hr dark regime. The culture medium was the same as that used in *Boergesenia* (MIZUTA and WADA 1981). When vegetative thalli were cut into small pieces on a stainless steel sieve (125 mesh) previously submerged in the culture medium, protoplasm flowed down through the mesh and became many spherical protoplasm bodies after about 2 hr at 25°C. The protoplasts were refiltered through sieves (125 and 170 meshes) and cells 88 to 125 μm in diameter were cultured for one to two days in a petri dish (110×25 mm) containing 100 m*l* of culture medium under light (4 W/m^2, white fluorescent tubes of Toshiba FL 40 SD were used) or in the dark at 25°C.

Fluorescence microscopy: To confirm the presence of a cell wall regenerated about the protoplasts, a fluorescence microscope (BH2-RKF, Olympus, Tokyo) was used. Cells cultured for various periods of time were incubated in 0.1% Kayaphor A. Conc (Nippon Kayaku, Co. Ltd, Japan), a bistriazinyl-aminostilbene derivative, a fluorescent brightener for β-hexapyranosyl polysaccharides which is excited by 365 nm UV. After about 5 minutes incubation, the cells were placed on a glass slide and observed under a microscope.

Electron microscopy: Preparation of cell wall materials for replica is shown in Fig. 1.

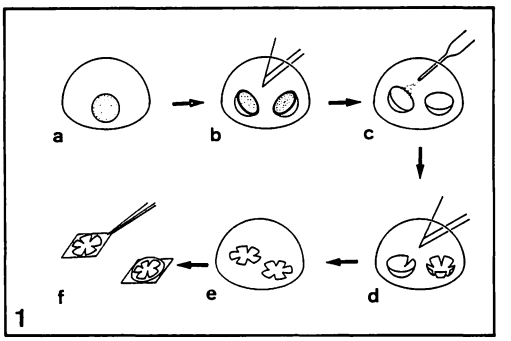


Fig. 1. Diagram of the preparation of cell wall materials for replicated specimens. The new *Boodlea* cell cultured for 1-2 days was placed in a drop of culture medium (a) and cut with a fine razor (b). After the contents were removed with a small syringe (c), both wall pieces were cut open (d, e) and mounted on acetylcellulose film (f).

First, a cell contained in a drop of culture medium was placed in a plastic petri dish and cut into two pieces with a fine razor. The contents were removed with a small syringe and both small pieces were cut open, mounted on acetylcellulose film with the inner surface of the wall facing upward. The walls were washed three times with distilled water, dried, shadowed with Pt and coated with carbon at 2×10^{-4} Pa. The samples thus obtained were put into 70% sulfuric acid for 3 days at 60°C, washed with distilled water and placed on Formvar-coated grids.

For freeze replication, EE-FED B2 freeze etching apparatus was used, as described previously (MIZUTA *et al.* 1985). All samples were observed with a JEM 100U electron microscope (JEOL Ltd. Tokyo).

Results

Cell wall development and growth of new Boodlea *cells:* Fig. 2 shows protoplasts from the protoplasm of *Boodlea*. Cell walls regenerated about the cell surface within 3 hr of culture at 25°C. The walls gradually thickened and could be clearly seen by the fluorescence of Kayaphor A Conc excited by ultraviolet light at 365 nm (Fig. 3). After

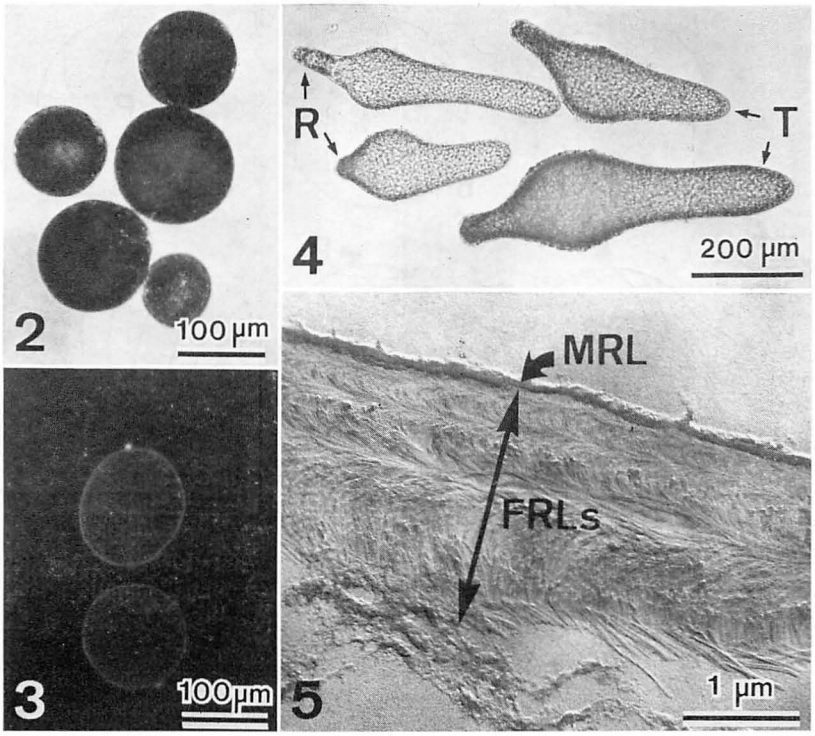


Fig. 2. Protoplasts regenerated from the protoplasm of *Boodlea*.

Fig. 3. New cells of *Boodlea* cultured for 1 day at 25°C following formation of protoplasts. After Kayaphor A. Conc (0.1%) treatment, the cells were observed under a fluorescence microscope.

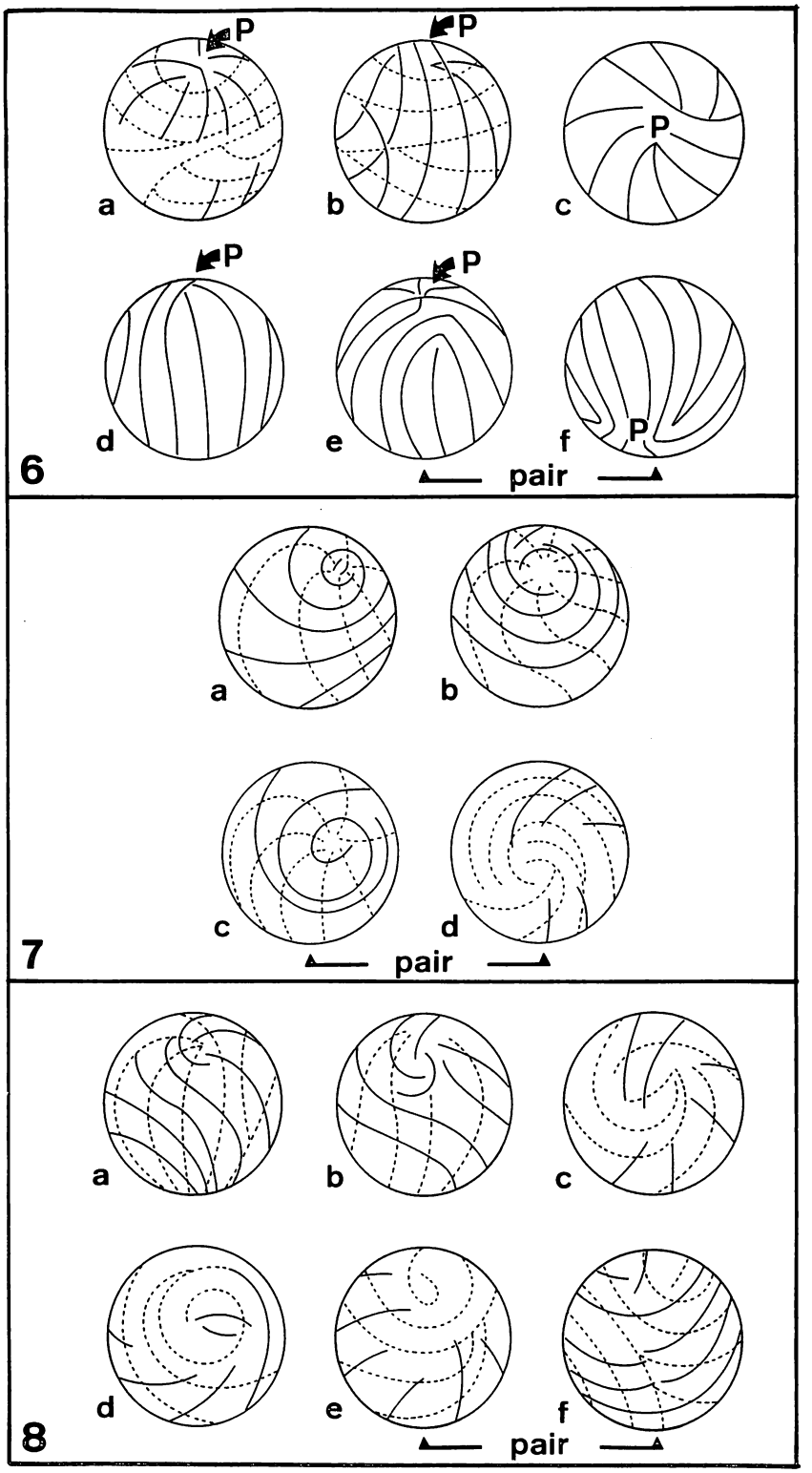
Fig. 4. New cells of *Boodlea* cultured for 3 days under continuous light at 25°C. Both rhizoid (R) and thallus (T) developed on opposite side of the cell.

Fig. 5. Cross sectional view of a new *Boodlea* cell wall cultured for 24 hr at 25°C. MRL, matrix-rich layer consisting of randomly-oriented microfibrils and an amorphous matrix. FRLs, fibril-rich layers consisting of regularly-oriented microfibril layers. The wall was shadowed with Pt-carbon following removal of the embedded resin.

about 3 days of culture under continuous light, a thallus and rhizoid protruded from each side of the cells and grew continuously (Fig. 4). A cross sectional view of the new cell wall was similar to that of *Boergesenia* and consisted of an outer surface matrix-rich layer (MRL) and inner fibril-rich layers (FRLs) (Fig. 5). The microfibrils in the FRLs were assumed perpendicular and parallel to the cut surface of the cell, but the arrangement of microfibrils throughout the entire cell could not be clearly seen. The microfibril arrangement of a replica taken from the inner surface of the wall was then analyzed. Microfibrils constituting the uppermost and underlying layers were traced in a whole replica and the fibril arrangement of the inside surface of a wall could thus be reproduced.

Microfibril orientation patterns in the spherical cells: The microfibril orientation patterns in the new round cell were generally of three types: (1) meridionally-arranged microfibrils (Fig. 6), (2) counterclockwise-oriented spiral (dotted lines in Fig. 7) and (3) clockwise-oriented spiral patterns (solid lines in Fig. 7).

In type 1, radially-arranged microfibrils from each polar region (P in Fig. 6) toward the equator densely covered the underlying helically-arranged fibril layer (**a** in Fig. 6). At each pole, there were several fountain-



Figs 6-8. Microfibril arrangement of new *Boodlea* cell wall replicated from the inner surface of walls. Solid lines show the major fibril orientation on the inner surface of wall layers covering older layers (dotted lines). 6. Typical patterns of microfibril orientation of meridionally-arranged microfibril layers; 7. Typical patterns of helical arrangement of striations; 8. Complex fibril arrangement showing transitional steps in layer formation.

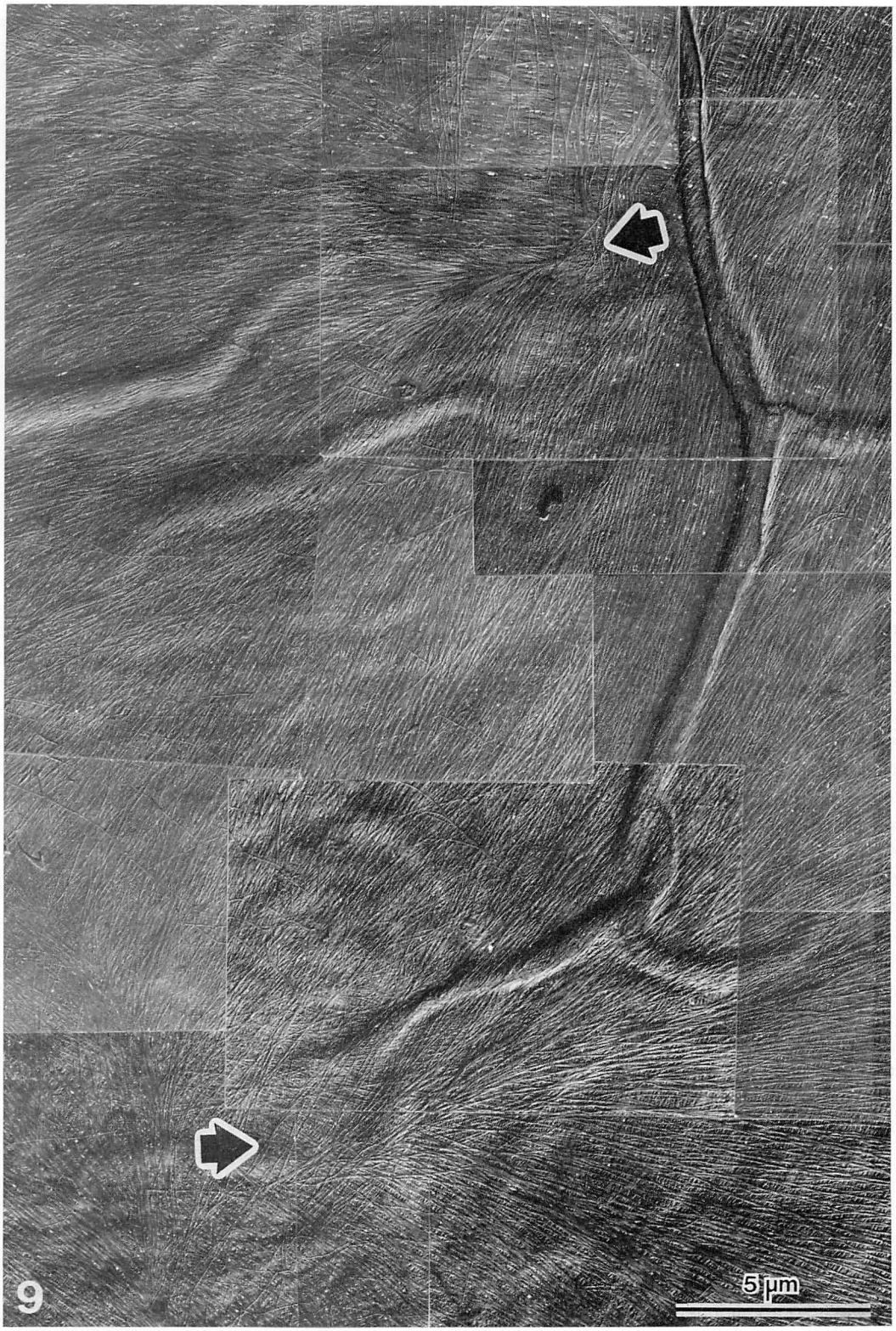


Fig. 9. Fountain-like structures (arrows) in a polar area. Replicas taken from the inner surface of the wall were shadowed with Pt-carbon.

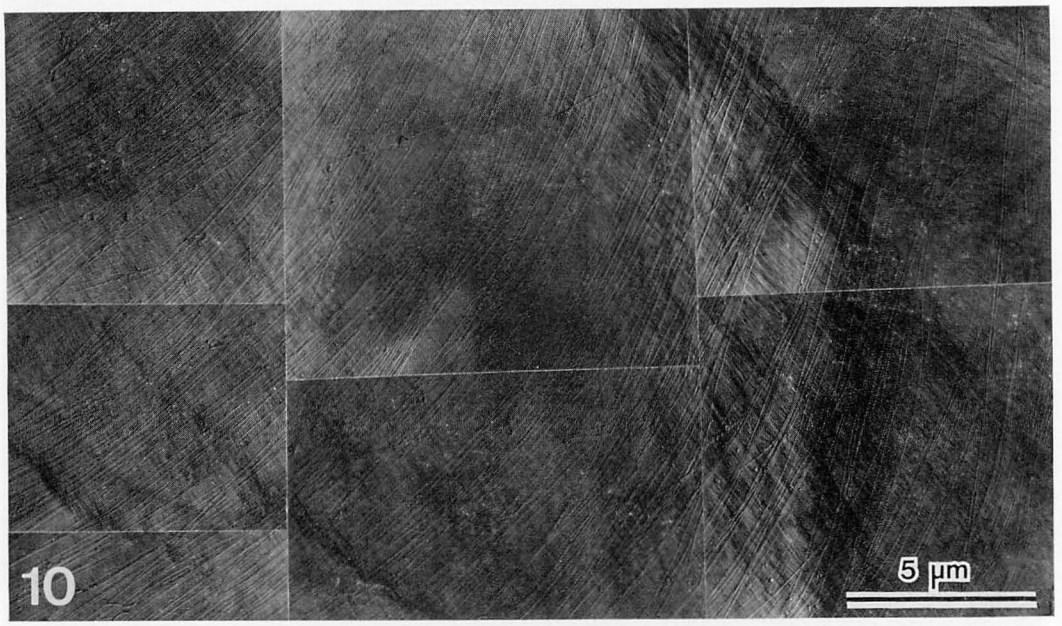
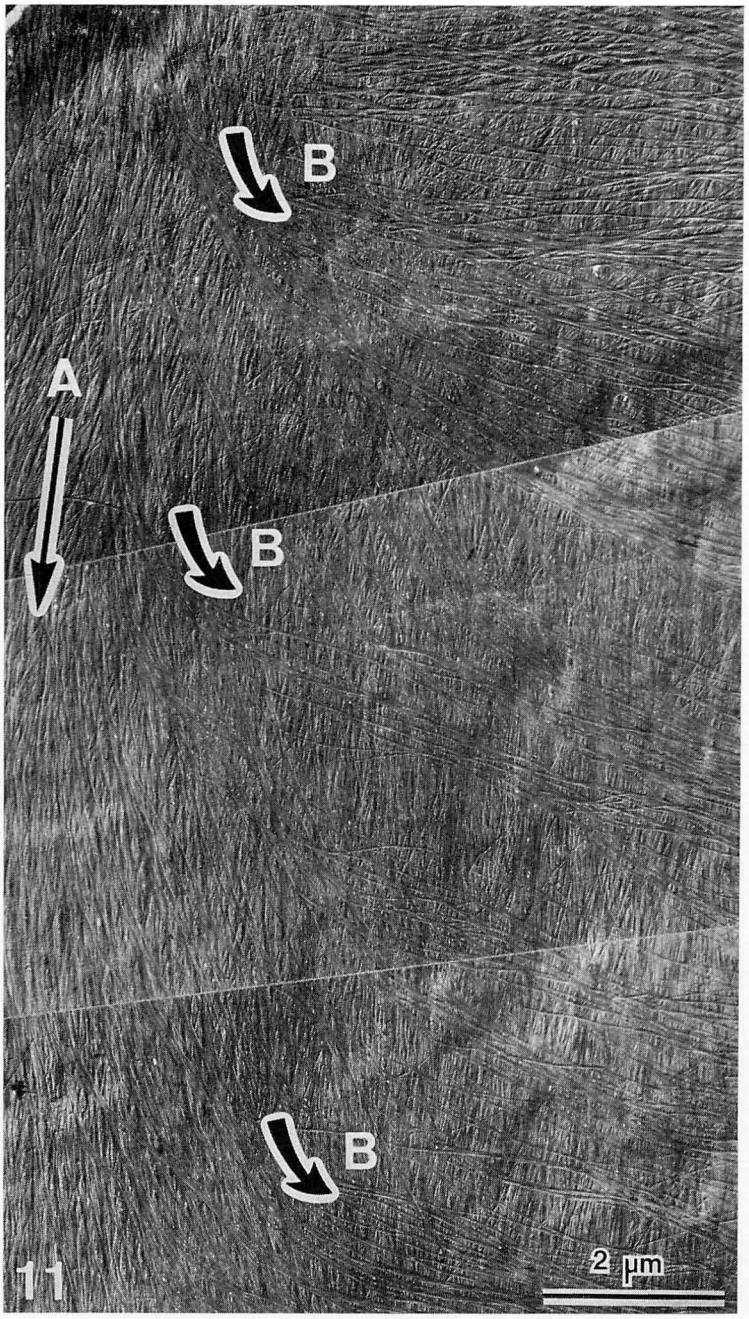


Fig. 10. A replica showing a typical microfibril layer cross pattern.

like structures (arrows in Fig. 9) quite similar to those of *Boergesenia* (MIZUTA *et al.* 1985). Microfibrils, dispersed in the fountains, changed their orientation and generated toward the equator to form a layer such as that in the case of fibril deposition in *Boergesenia* (MIZUTA *et al.* 1985). Meridionally-arranged fibrils with completed deposition were shown in **b**–**f** of Fig. 6. The fibrils were arranged quite orderly from pole to pole (P in Fig. 6) as indicated in the diagrams of replicas taken from paired hemispheres (**e** and **f** in Fig. 6). In cells which generated thick layers by the presumed high activity for wall deposition, meridional lines passing over and covering the poles could sometimes be seen (**c** and **e** in Fig. 6) and in this case, poles with radially-arranged microfibrils were not clearly visible.

Typical microfibril arrangement patterns of types 2 and 3 were shown in **a** and **b** of Fig. 7. The microfibrils were deposited so as to form either a counterclockwise (dotted lines) or clockwise helix (solid lines) layer extending from each polar region toward the equator. The mode of formation was always the same, the counterclockwise helix being generated first and then the clockwise helix to give rise to a typical crossed pattern of polylamellate fibrils (Fig. 10). The slope of each helix varied, always steep for the counterclockwise helix but relatively gentle for the clockwise helix. These layers were more predominant in one polar area than the other, as shown in the fibril tracings of paired hemispheres (**c** and **d** in Fig. 7). Thus fibril formation proved to occur more at one pole than another.

There were many complex patterns consisting of helix and straight microfibrils in addition to the three mentioned above. These may possibly represent transitional steps in microfibril configurations such as the following: orientation change from a meridional to counterclockwise helix arrangement (**a** and **b** in Fig. 8), change from a clockwise helix to meridional arrangement (**c**, **e** and **f** in Fig. 8) and change from a counterclockwise to clockwise helix (**d** in Fig. 8). In these steps, fountain-like structures could be seen in both the polar and other regions. Those situated far from the polar areas were long and fibril orientation change generally occurred on the part of orderly arranged multifibrils (Fig. 11);



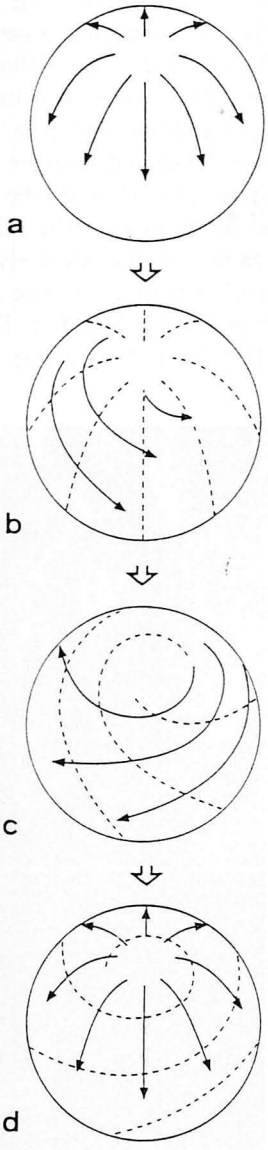


Fig. 11. Change in microfibril orientation in a multifibrillar layer with reversed fountain-like structure. Arrow A, orientation direction of microfibrils in a multifibrillar layer. Arrow B, orientation direction of a new layer with curved configuration.

Fig. 12. Diagramatical representation of pattern change in the microfibril orientation of a new *Boodlea* cell wall. **a**, formation of meridionally-oriented fibril layer; **b**, formation of counterclockwise-oriented spiral layer; **c**, formation of clockwise-oriented spiral layer; **d**, meridional-oriented layer formation, the same as in **a**.

new fibrils of the unidirectionally-arranged fibril layer (direction of formation shown by arrow A) changed their orientation almost uniformly, curving essentially in the same direction (arrow B). Following these changes, new fibrils were deposited along a line extending from one pole to the equator in a polar dependent manner and in rare cases, fibrils passed through the equator and covered the polar area on the other side of the cell because of the excessive amount of wall-forming activity in one polar area (paired diagram of **e** and **f** in Fig. 8).

Based on the tracing patterns of microfibrils in the new *Boodlea* cell, microfibril orientation change and the process of cell wall lamellation were represented in Fig. 12. **a**–**d** showed the circulation route in the wall lamellation. **a** showed the meridionally-arranged multifibrillar layer; **b**, multifibrils making up the counterclockwise helix; **c** those comprising the clockwise helix and **d**, the meridionally-arranged multifibrillar layer identical to that of **a**. Although each layer was generated from two poles, the microfibrils on one polar side predominantly converged toward the other pole.

Relationship between microfibril arrange-

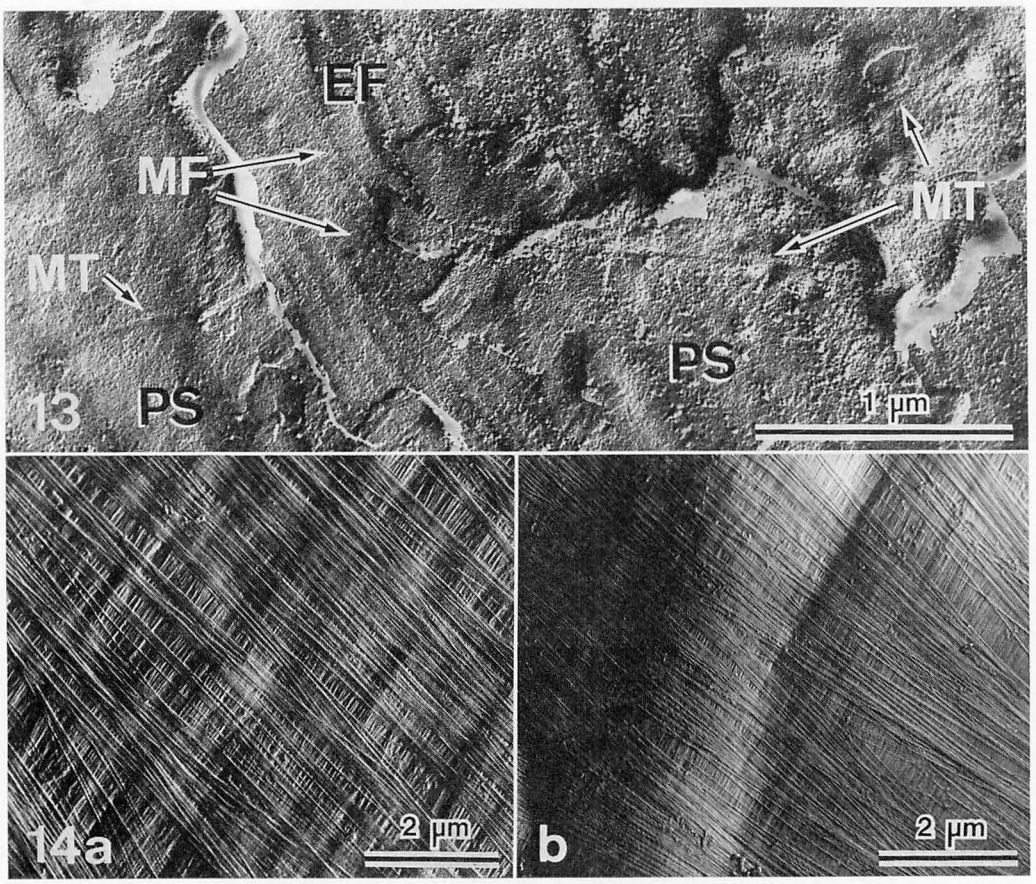


Fig. 13. Freeze-fractured plasma membrane of a new *Boodlea* cell showing both microfibril (MF) and microtubule (MT) impressions. Cells cultured for 1 day at 25°C were freeze-fractured and shadowed with Pt-carbon. EF, (outer leaflet) fracture face of the plasma membrane; PS, protoplasmic surface of the membrane.

Fig. 14. Replica of inner surface wall of a new *Boodlea* cell treated and not treated with colchicine. Cells cultured for 1 day at 25°C were recultured in the presence or absence of colchicine (2×10^{-3} M) for 1 day under continuous light. **a**, colchicine treatment; **b**, control.

ment and microtubule array and effects of colchicine on microfibril arrangement: The correlation between cell wall microfibrils and peripheral microtubule arrangement was examined. In Fig. 13, microtubule impressions (MT) which were quite similar to those of *Valonia macrophysa* (ITOH and BROWN 1984) were visible on the PS face of the plasma membrane and microfibril impressions (MF) were also evident on the EF face of the membrane. In the figure, the microfibril impressions were essentially parallel to each other, but they were different from the microtubule impressions in the manner of their arrangement.

Fig. 14 showed the effects of colchicine on the microfibril arrangement of a new *Boodlea* cell wall. The lamellation of fibrils crossing each other was not effected by 2×10^{-3} M colchicine treatment (Fig. 14, **a**). The microfibrils in each layer were generated in an orderly pattern similar to that of control (Fig. 14, **b**); no disorderly patterns could be detected.

Discussion

In the present experiment, the arrangement patterns of cellulose microfibrils in new *Boodlea* cell walls were clarified by careful observation and analysis of replicated microfibril arrangements. A meridionally-oriented, a counterclockwise-oriented spiral and a clockwise-oriented spiral were the three patterns found. These patterns successively changed and fibrils generated from each pole toward the equator in a highly polar dependent manner (Figs 6-8). The direction of microfibril formation may thus possibly be regulated by cell polarity. Similar patterns in the spherical-like cells of green algae have also been reported in *Boergesenia* (MIZUTA and WADA 1981, MIZUTA *et al.* 1985), *Valonia* (PRESTON and ASTBURY 1937) and *Dictyosphaeria* (WILSON 1951). In this case, though the patterns differed somewhat from each other, fibril layer generation was under the control of cell polarity.

The polarity of the new spherical cells of *Boergesenia* has been reported to be induced by blue light irradiation, causing a rhizoid and thallus to develop (ISHIZAWA *et al.* 1979). The data of our previous work (MIZUTA *et al.* 1985) indicated the cell polarity to be quite likely induced prior to FRL generation, possibly during MRL formation, since the microfibril arrangement in FRLs was polar dependent and, therefore inducement of rhizoid and thallus protrusion by blue light may probably be due to changes in polarity. In *Boodlea* protoplasts, prior to the formation of cross-polylamellated layers (FRLs), cells developed a randomly-oriented fibril layer and amorphous matrix (Fig. 5). Thus, the cell polarity seems to develop before the appearance of FRLs as noted in the case for *Boergesenia*.

It was pointed out a considerable time ago that there existed a correlation between microfibril arrangement and the array of peripheral microtubules (NEWCOMB 1969, HEPLER and PALEVITZ 1974). However, the data of many reports contradicted such a correlation (MILLINGTON and GAWLIK 1970, MARCHANT and PICKETT-HEAPS 1972, ROBINSON *et al.* 1972). In our previous paper on *Boergesenia* (MIZUTA and WADA 1982), change in microfibril orientation was not effected by antimicrotubule agents. In *Valonia* (ITOH and BROWN 1984), no correlation has been found between microfibril and microtubule arrangement and this tendency was almost the same in *Boodlea* (Fig. 13). Morphologically, microfibril arrangement patterns appeared essentially the same in both the presence and absence of colchicine (Fig. 14). Thus polar-dependent fibril arrangement and changes in orientation may be independent of peripheral microtubules in both *Boodlea* and other siphonocladalean algae.

Changes in microfibril orientation in *Boergesenia* have been recently reported to possibly depend on membrane fluidity (MIZUTA 1985). This report assumed that microfibrils formed adjacent to TCs (cellulose synthesizing terminal complexes) were floated partly by membrane fluidity and that microfibril orientation was controlled by the vectors of

TC movement and membrane fluidity. This was based on the fact that TCs at the time of orientation change, always curved in essentially the same direction and that intramembranous particle density on the outside of the curve was always greater than that on the inside. Multifibrils in *Boergesenia* always changed gradually as a curve, as was noted for fountain-like structures (MIZUTA *et al.* 1985) and this mode of orientation change was almost similar to that of *Boodlea* (Fig. 11). The curve of the fountain-like structure may possibly arise from TCs whose orientation changes gradually in the direction of the resultant force of the TC movement and membrane fluidity vectors. For an understanding of the regulatory mechanism for changes in microfibril orientation due to membrane fluidity, controlling of changes in the direction of membrane fluidity itself must be investigated.

Acknowledgements

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奥田一雄・水田　俊：緑藻アオモグサの原形質から分化した球形細胞における細胞壁ミクロフィブリルの配向パターンの解析

アオモグサの原形質から分化した球形細胞の細胞壁の構造及び細胞壁ミクロフィブリルの配向パターンをレプリカ法により調べ明らかにした。細胞壁は，マガタマモと同様，壁マトリックスとランダムに配向するセルロースミクロフィブリルより成る表層部と，その内側を構成する，ミクロフィブリルの規則正しく配向交変する主層部より成る。主層部におけるミクロフィブリルは次の3つの配向パターン，すなわち(1)経線方向への配向，(2)左巻らせんの方向への配向，(3)右巻らせんの方向への配向，を示し，形成順序は(1)→(2)→(3)→(1)で常に一定であった。各層におけるミクロフィブリルの形成は，両極部から赤道方向へ向う強い極性依存性を示した。形成の終了した層においては，ミクロフィブリルが扇形，或は弧状構造を描いて配向交変するのがしばしば見られた。極部におけるこの構造は，比較的小さく複数出現し，扇形に広がったミクロフィブリルは，湾曲を伴い新層を形成していた。極部以外の構造は，比較的長く，ミクロフィブリルは大きく弧を描きながら新層を形成していた。これらのフィブリル配向の形態をもとに，アオモグサの極性依存的細胞壁ミクロフィブリルの配向変換の機構について考察した。(780 高知市曙町 2-5-1 高知大学理学部生物学科)

新刊紹介

山岸高旺・秋山　優（編）：**淡水藻類写真集 1，2，3巻**，内田老鶴圃，各巻100図版，1巻4000円，2巻4000円，3巻 5000円．1959-60.

1977年に同じ出版社から，日本淡水藻類図鑑が出版され話題を集めたが，最近では線描きを用いる人が次第に少くなり，ほとんどの人は写真で調べごとをするようになった。写真はスケッチよりもずっと時間が少くてすみ，いろいろのものを数多く写して比較でき，また，何よりも顕微鏡でみる実物のイメージが現れるという利点があるので，今後もますます写真化の方向へと進むものと思われる。この図集は，このような写真化時代の要求に答えるものとして出版されたもので，1図版に1分類群を取り上げ，普通5～9枚ぐらいの写真と必要に応じてスケッチもつけるという形式が取られており，これに和文と英文の記載がつけられている。

それぞれの分野の専門家が自慢の写真を載せているので，ポイントをつかんだ写真ばかりであり，しかも，全体像，部分拡大像，生活史の各ステージと，必要なカットが揃えられているので同定には非常に役立つものである。ただ難を言えば，ルーズリーフ式で取扱いにくいこと，一つの属のものが，各巻に分散していて見にくいこと，ある属のものが全部揃っていないので，比較しにくいことなどが挙げられる。しかし，ルーズリーフ式で，それぞれの図版に番号が付いているので，ばらしても，引用に不便はないし，今3巻が出ていて，やがて4巻も出るという話であるから，その時点で400分類群がそろうことになる。出版されるごとに，次々と分類順，または属名のアルファベット順に整理していけば，それぞれの時点でかなり利用できるものと思われる。この種の出版物は長い年月をかけて延々と続くため，全部出揃ったら手に入れようなどと考えていると，その時点では歯抜けができてしまって臍をかむ場合が多い。私も，HELMCKE & KRIEGER の珪藻写真集のある巻が手に入らず難儀したので，必要と思われる方は集めておかれることをお勧めしたい。

（東京学芸大学生物学教室　小林　弘）