Jap. J. Phycol. (Sôrui) **35**: 113–120, June 20, 1987

Accumulation of cytoplasmic vesicles in cell walls during cell modification in *Chaetomorpha moniligera*

Kazuo OKUDA and Shun MIZUTA

Department of Biology, Faculty of Science, Kochi University, Akebono-cho Kochi, 780 Japan

OKUDA, K. and MIZUTA, S. 1987. Accumulation of cytoplasmic vesicles in cell walls during cell modification in *Chaetomorpha moniligera*. Jap. J. Phycol. **35**: 113–120.

Ultrastructure of the cell wall during modification of the thallus cells of *Chaetomorpha moniligera* were investigated with an electron microscope. Cylindrical thallus cells growing at 27°C showed rapid expansion on treatment with Fluostain and colchicine. The wall of expanded cells thickened markedly with considerable deposition of matrix polysaccharides in which numerous osmiophilic vesicles were embedded. The vesicles were assumed to accumulate within the wall by their release from the cytoplasm during cell modification. The relation between cell wall thickening and osmiophilic vesicles, and the action of Fluostain and colchicine in bringing about modification in the shape of cells of *Chaetomorpha moniligera* are discussed.

Key Index Words: Cell shape modification; Chaetomorpha moniligera; *colchicine; Fluostain; wall vesicle.*

Plant cell shape is considered to be regulated by newly-formed microfibril orientation which is affected indirectly by cortical microtubule arrangement. Evidence for this is the fact that when microtubules are disrupted by antimicrotubule agents, cells become spherical in shape (see review by NEWCOMB 1969, HEPLER and PALEVITZ 1974, LLOYD 1984). However, there are many cases which cannot be explained on the basis of this hypothesis (HEPLER and PALEVITZ 1974, GUNNING and HARDHAM 1982) and microfibril orientation due to cortical microtubule arrangements is considered not always to govern plant cell shape.

As reported previously (MIZUTA *et al.* 1986, OKUDA and MIZUTA 1987), the shape of growing cylindrical thallus cells of *Chaetomorpha moniligera* becomes spherical on treatment with colchicine. The direction of microfibril arrangement in cross polylamellated walls of spherical cells was found to be not random but orderly, and the same tendency was also noted in the most recently formed wall layer. Microfibril orientation does not affect the growth direction of cells and modification in cell shape of *Chaetomorpha* is considered to result exclusively from the loosening of interfibrillar substances.

In this paper, the remarkable accumulation of cytoplasmic vesicles into cell walls during cell modification in *Chaetomorpha* on treatment with colchicine and Fluostain is reported and the involvement of vesicles into cell wall loosening is discussed.

Materials and Methods

Cultures: *Chaetomorpha moniligera* KJELLMAN was collected from inshore sites in Kochi prefecture, Japan, and cultured in PES-medium (PROVASOLI 1966) at 27°C. The young growing plants were recultured in the media in the presence and absence of

Fluostain II and colchicine for 6 days. The Fluostain II (Dojin Chemical Co., Kumamoto, Japan) used was a fluorescent brightener of a bis-triazinylaminostilbene derivative and quite similar to Calcofluor White ST (American Cyanamid Co., Wayne, New Jersey, U.S.A.) which is known to have strong affinity to β-hexapyranosyl polysaccharides (MAEDA and ISHIDA 1967).

Electron microscopy: *Chaetomorpha* thalli were cut at a length of about 4 mm and fixed in 4% glutaraldehyde dissolved in sea water at 25°C for 1 hr and then at 2°C another hour. After being washed with sea water, they were postfixed with 2% OsO_4, dehydrated with acetone and embedded in Spurr resin. The specimens were then sectioned using a Reichert Om U2 ultramicrotome and then mounted on Formvar-coated grids. The sections were stained with uranyl acetate and lead cirtate solution before observation with a JEOL JEM 100U electron microscope. Some sections were treated with KOH-ethanol solution for 7 minutes to dissolve the embedded resin and shadowed with Pt before observation. Cell wall thickness of 5 individual cells was measured.

Results

Fig. 1A shows *Chaetomorpha* thalli growing at 27°C. Each cell, cylindrical in shape, grows by cellular division as was also noted for *Chaetomorpha darwinii* (KORNMANN 1969) and then sporulated. During the culture, cell shape remained almost constant, appearing cylindrical or slightly cask-like. When the thalli were cultured in media with Fluostain (0.1 mg/m*l*) or colchicine (5×10^{-3} M) for 6 days, each cell expanded remarkably (Fig. 1B and C). This expansion generally began at the lower (closer to rhizoid) portion of each cell and was affected more strongly by colchicine than Fluostain.

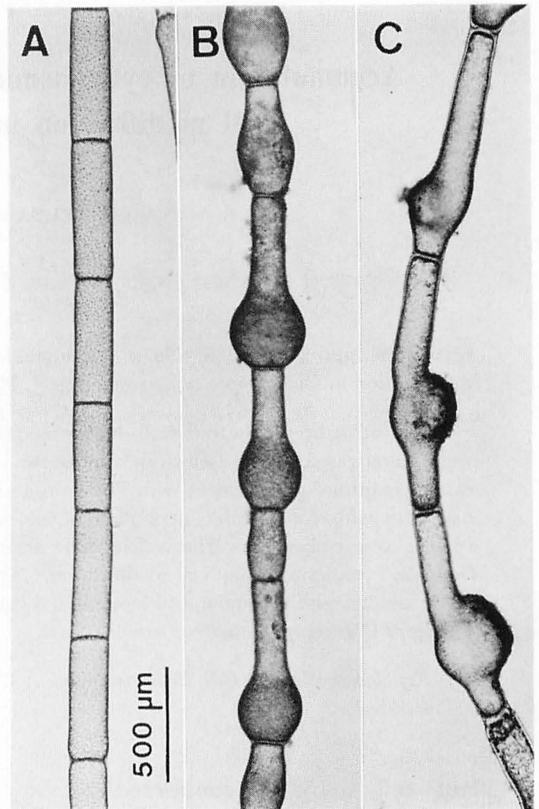

Fig. 1. Growing *Chaetomorpha* thallus cultured in the presence and absence of Fluostain and colchicine. A, control; B, colchicine (5×10^{-3} M) treatment; C, Fluostain (0.1 mg/m*l*) treatment. Scale in A applies also to B and C.

Fig. 2 shows cell wall sections of both cylindrical and expanded cells cut longitudinally along the longer cell axis. It was quite evident that the wall of a control cylindrical cell (Fig. 2A) was thinner than that of any expanded cell, being 3.9 ±0.4 μm in thickness. Microfibrils in the wall were oriented alternately in longitudinal and transverse directions to the longer cell axis (long arrows), a pattern appearing consistently throughout the cell wall. Remarkable thickening was noted in the cell wall of those cells which expanded by treatment with 0.1 mg/m*l* of Fluo-

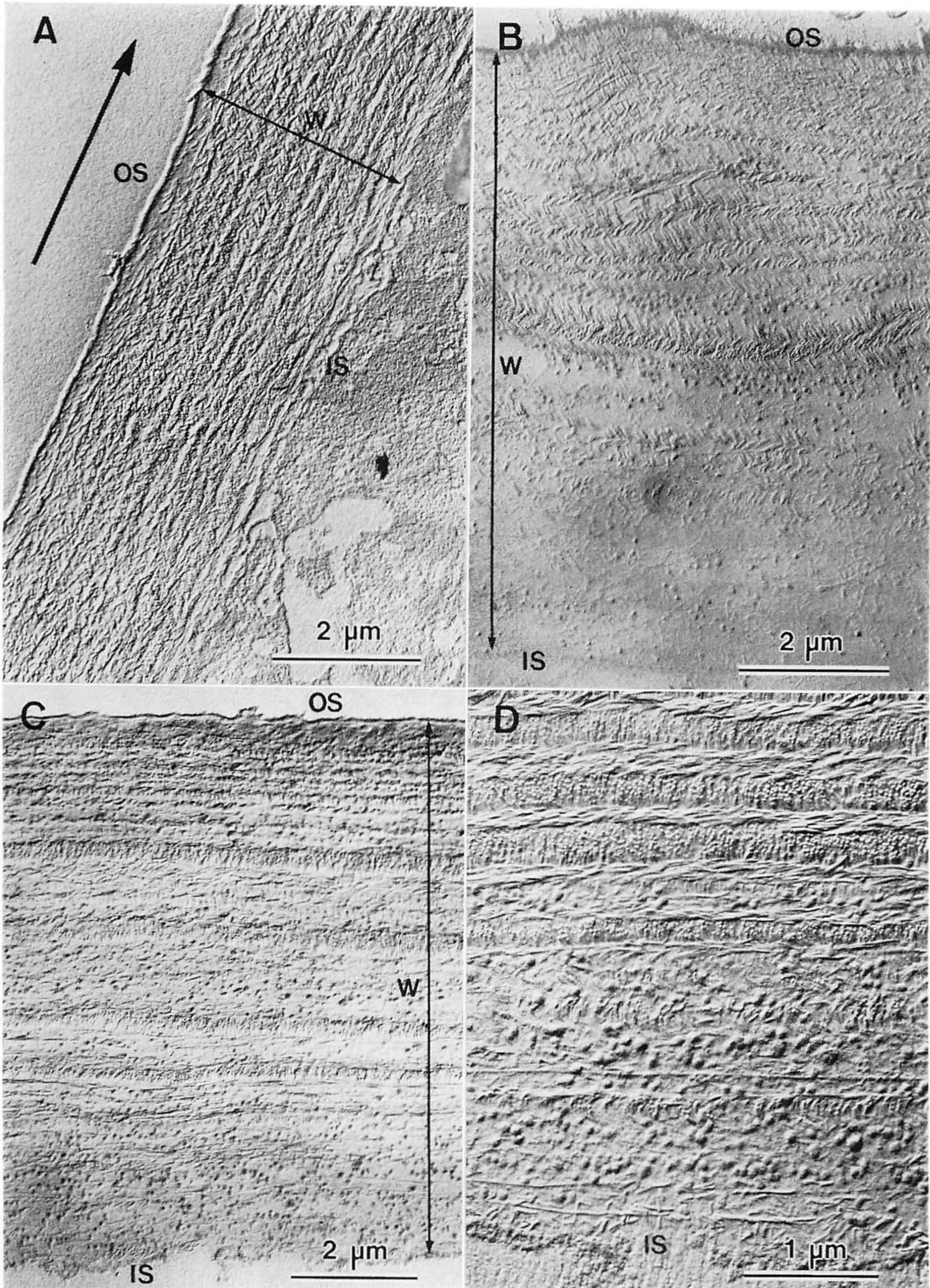

Fig. 2. Cross sectional view of the walls of *Chaetomorpha* cells cultured in the presence and absence of Fluostain and colchicine. Thin sections were shadowed with Pt following treatment with KOH-ethanol for 7 min. A, control; B, Fluostain (0.1 mg/m*l*) treatment for 6 days; C and D, colchicine (5×10^{-3} M) treatment for 6 days. W, cell wall; IS, inner surface of the wall; OS, outer surface of the wall; large arrow, longer cell axis.

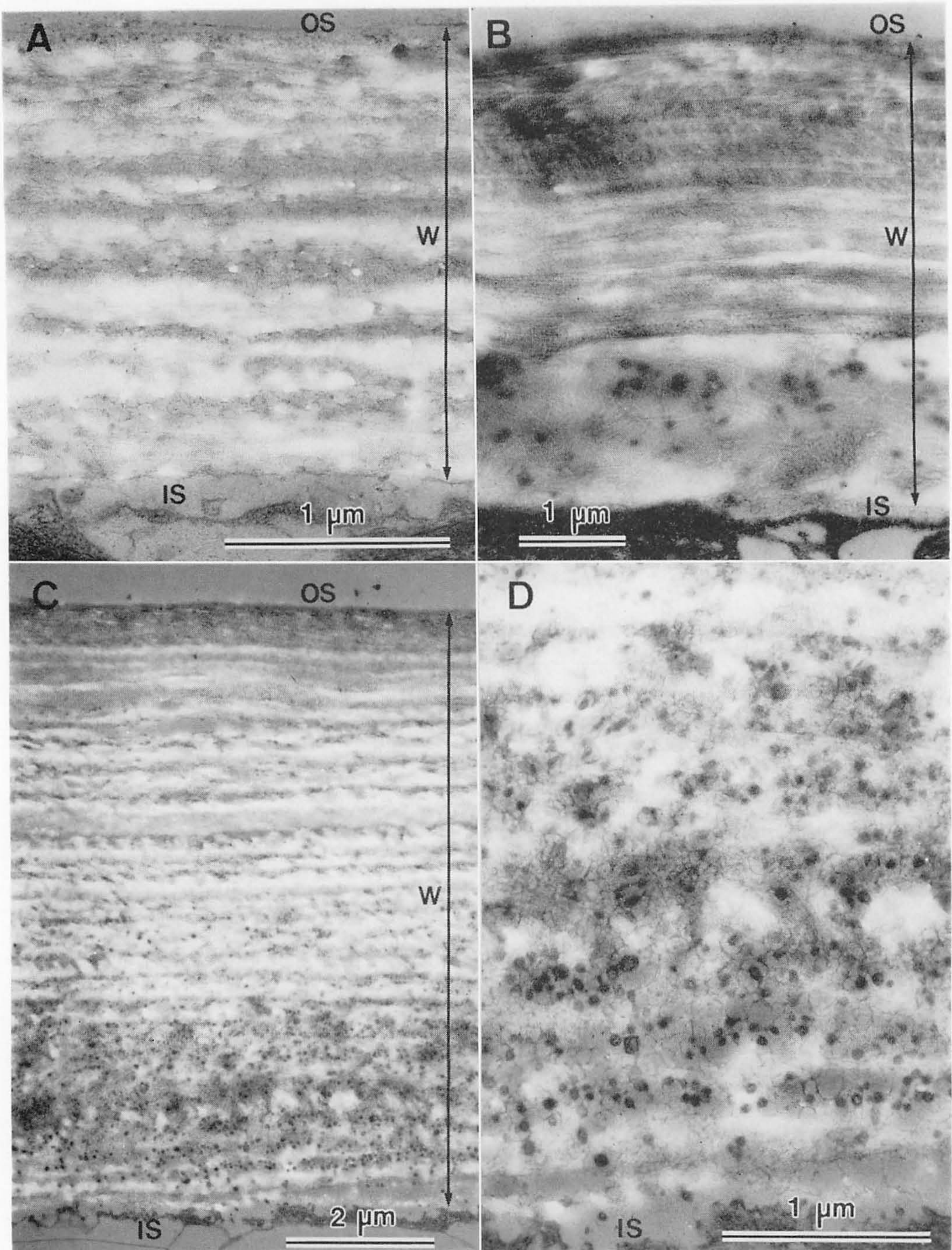

Fig. 3. Cross sectional view of the walls of *Chaetomorpha* cells cultured in the presence and absence of Fluostain and colchicine. A, control; B, Fluostain (0.1 mg/m*l*) treatment for 3 days; C and D, colchicine (5×10^{-3} M) treatment for 6 days. W, cell wall; IS, inner surface of the wall; OS, outer surface of the wall.

stain (Fig. 2B) and 5×10^{-3} M colchicine (Fig. 2C and D). The average wall thickness of expanded cells caused by Fluostain and colchicine treatment was 7 ± 0.9 μm and 6.7 ± 0.5 μm, respectively. This tendency was almost the same by treatments with 0.01 mg/m*l* of Fluostain and 10^{-3} M colchicine. The inside part of the thickened walls of Fluostain treated cells was assumed to be composed of a considerable amount of matrix substances and some microfibrils were also noted to be

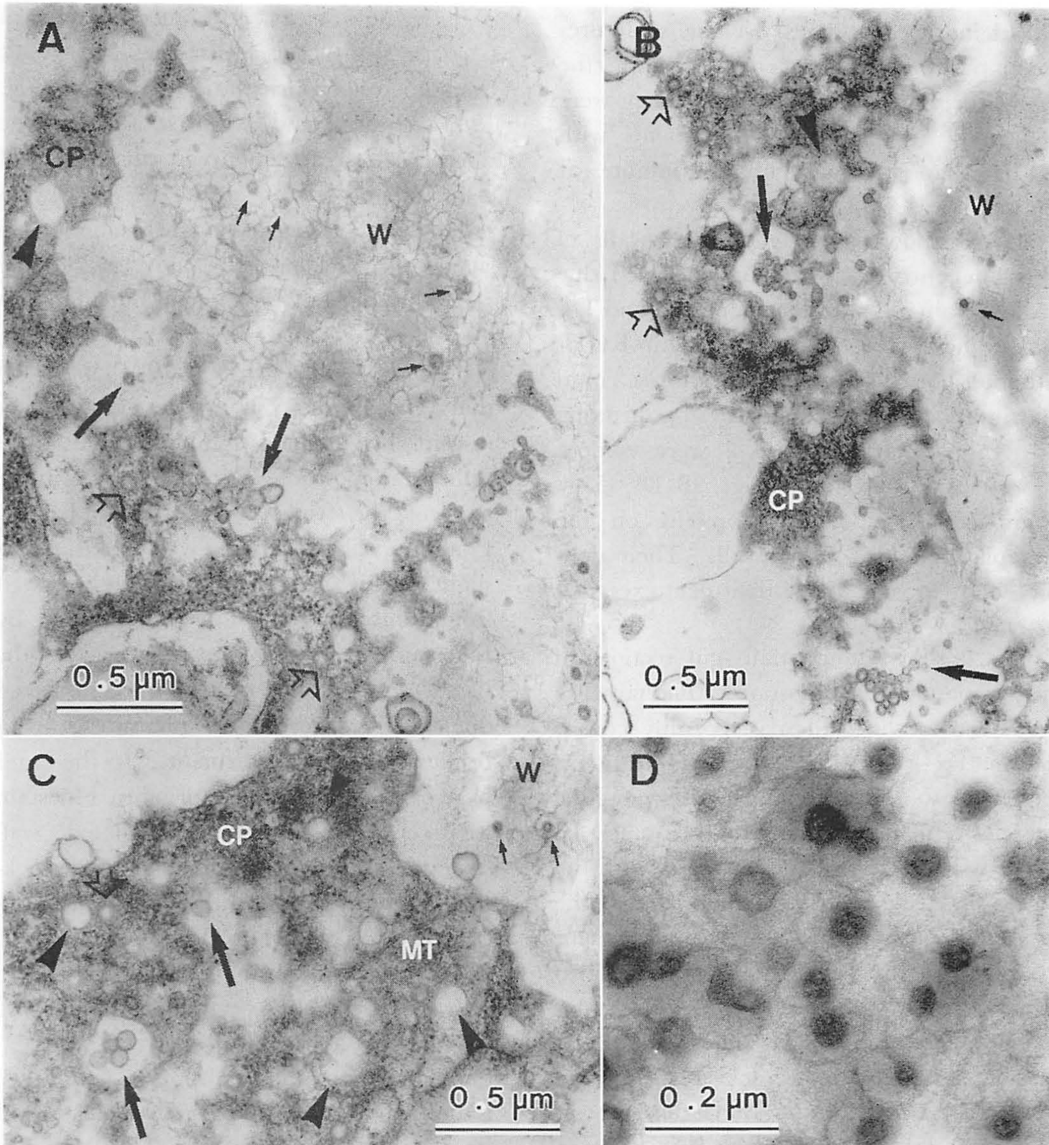

Fig. 4. Tangential section of *Chaetomorpha* cells treated with Fluostain. A–C, sections showing release of many osmiophilic vesicles; D, higher magnification of osmiophilic vesicles within the wall; Large arrows, osmiophilic vesicles in cytoplasmic vacuoles; small arrows, osmiophilic vesicles in the wall; open arrows, vesicles in the cytoplasm; arrowheads, cytoplasmic vacuoles; CP, cytoplasm; W, cell wall; MT, microtubule.

present (Fig. 2B). In the colchicine treated cells, these matrix substances were observed to be situated in the same part (Fig. 2C and D) and to contain microfibrils with ordered shift in orientation. Numerous protruding structures, 60 ± 5 nm in diameter, could also be seen within the thickened walls of both Fluostain and colchicine treated cells. These structures may possibly have accumulated in the walls during cell modification. They were removed by prolonged treatment with KOH-ethanol for more than 7 minutes at 25°C.

Fig. 3 shows cross sections of walls of cells in control (A) and in expanded due to Fluostain (B) and colchicine (C and D). Since they were not treated with KOH-ethanol nor shadowed, microfibrils could hardly be seen but the protruding structures shown in Fig. 2 were evident as osmiophilic vesicles (Fig. 3B–D), 55 ± 5 nm in diameter and present on the cytoplasmic side of the wall. Their density following colchicine treatment exceeded that with Fluostain.

Fig. 4 shows tangentially-cut sections of cells expanded by Fluostain. The network structure in the wall may possibly consist of matrix polysaccharides and proteins adhering to the microfibrils. Osmiophilic vesicles (small arrows) could usually be seen within walls surrounded by the network. As can be seen in Fig. 4D, the vesicular outlines are obviously biomembranes. In the peripheral cytoplasm, two kinds of vesicles, osmiophilic and osmiophobic, were observed. The former was present within cytoplasmic vacuoles (large arrows in Fig. 4C) and the latter only in the cytoplasm (open arrows in Fig. 4 A and B). Vesicles in vacuoles were almost similar in size to those in the cell wall. Vacuoles with (large arrows in Fig. 4) and without (arrowheads in Fig. 4) vesicles were frequently observed to open to the cell wall and vesicles appearing on the verge of being released to the wall were observed (large arrows in Fig. 4A and B).

Discussion

It is now clear that modification of *Chaetomorpha* thallus cells does not result from microfibril orientation (MIZUTA *et al.* 1986, OKUDA and MIZUTA 1987). The microfibril arrangement in the walls of cells expanded by colchicine is always ordered although the cells are spherical in shape. In the present study, it became clear that not only colchicine but Fluostain as well caused *Chaetomorpha* thallus cells to expand. QUADER *et al.* (1983) reported that Calcofluor White, the same fluorescent brightener as Fluostain, inhibits cellulose deposition in vivo. In the inside part of the walls of cells expanded by Fluostain, fibril density was noted to be slight. This may possibly have resulted from inhibition of cellulose deposition by Fluostain. However, expansion of the *Chaetomorpha* thallus cells is not considered due to the scarcity of microfibrils as a result of Fluostain treatment. In the outer part of the wall formed prior to Fluostain treatment, a cross polylamellated wall structure was clearly evident with a thickness essentially the same as that of a cylindrical cell wall (Fig. 2A and B). It was also noted that the expansion of *Chaetomorpha* cells occurred only in a certain part of the wall (Fig. 1B and C). Should expansion occur due to overall reduction in wall strength, the cell would be spherical as a whole. Thus, modification of the *Chaetomorpha* cell by the action of Fluostain is considered to occur through local loosening of the wall. This mechanism would also apply to the action of

colchicine (OKUDA and MIZUTA 1987).

Within the wall matrix of cells expanded by Fluostain and colchicine, numerous vesicles were observed (Fig. 2) and they were removed by prolonged treatment with KOH-ethanol solution. These vesicles were osmiophilic and enclosed by biomembranes. Structures similar to these were present in cytoplasmic vacuoles. There were indications that the vesicles in the vacuoles were released to walls along with other vacuolar constituents (Fig. 4). It thus appears possible that they are released from the cytoplasmic vacuoles and then transferred to the wall matrix. Similar type of vesicles have also been observed in *Chaetomorpha brachygona* (CHAN *et al.* 1978) and a possible relation between those and the deposition of acid polysaccharides has been suggested (ROBINSON and PRESTON 1971). In *C. moniligera,* however, vesicles accumulated within the wall were osmiophilic and there were numerous indications of the exocytosis of osmiophobic vesicles and vacuoles (Fig. 4). Since osmiophilic vesicles accumulate and matrix substances are deposited actively within the wall during cell modification, the vesicles may contain certain elements related to the destruction and reconstruction of matrix polysaccharides and structural proteins.

Abnormal cell expansion and the deposition of wall substances in higher plants have been found when treated with colchicine or coumarin, the latter a known inhibitor of cellulose deposition (ITOH 1976a, b). However, cell shape modification and abnormal wall thickening by Fluostain have so far been unknown phenomena. It appears certain that colchicine and Fluostain enhance both matrix deposition and release of cytoplasmic vesicles into *Chaetomorpha* cell walls. That only a certain area of the wall of each cell undergoes expansion may mean that the cytoplasm of this particular area has higher activity to produce substances that cause wall loosening. The detailed clarification of the functions of these vesicles will require additional research and analysis by biochemical methods.

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奥田一雄・水田　俊：タマジュズモ細胞の形の変化に伴う小胞の細胞壁への蓄積

タマジュズモの培養細胞は，ほぼ円柱形を保ちながら主として伸長生長する。この円柱形細胞をコルヒチン (COL) あるいはフルオスティン (FLU) を含む培地で培養すると，細胞下部の局部的な拡大生長が引き起こされ，細胞はつぼ状の形に変化する。このような変化を誘導あるいは促進する COL 及び FLU の細胞壁形態に及ぼす効果を電子顕微鏡で調べ，以下の結果を得た。1. COL 及び FLU は，細胞壁の肥厚を促進する。2. 肥厚した細胞壁の内側に，形態的に無構造の壁マトリックスが沈着する。 3. 壁マトリックス中のセルロースミクロフィブリルは，COL 処理細胞では密度が低下し，FLU 処理細胞ではほとんど見られない。4. 壁マトリックス中には多くの小胞が見られる。5. これらの小胞は，分泌小胞のエクソサイトシスによって細胞壁に放出される。これらの結果より，タマジュズモにおける細胞の形の変化と細胞壁形態の変化との関連性を考察した。（780 高知市曙町2-5-1　高知大学理学部生物学教室）