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Effect of vinblastin and cytochalasin B on cell division in *Oedogonium capilliforme*

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Effects of vinblastin and cytochalasin B on the progress of individual stages of cell division in *Oedogonium capilliforme* were examined by light and electron microscopy. Vinblastin at a concentration of 100 μg/m*l* strongly inhibited the progress of mitosis and the formation of the cytoplasmic septum, processes in which microtubules were involved. It also inhibited the division of chloroplasts, but it did not inhibit the opening and the stretching of the rings of cell walls. Cytochalasin B at 100 μg/m*l* did not inhibit the progress of mitosis, the formation of the cytoplasmic septum, or the division of chloroplasts, but it did inhibit the elongation of the new lateral cell wall and the fusion of vesicles that probably contain substances necessary for the synthesis of new cell walls. The involvement of microtubules and microfilaments at various stages of cell division is discussed.

Key Index Words: cell division—cytochalasin B—Oedogonium—*microfilament—microtubule—vinblastin.*

Cell division in *Oedogonium*, a green alga, provides an unusual example of the formation of a new cell wall. A wall-ring appears at the top of the cell before mitosis, and it splits circularly and is pulled longitudinally to form the new cell wall after mitosis. Accordingly, many reports have been published on details of nuclear and cell division in this interesting alga in the past one hundred years (KLEBAHN 1892, VAN WISSELINGH 1908, UEDA 1960). Electron microscopic observations by PICKETT-HEAPS and FOWKE (1969, 1970 a, b) revealed the ultrastructural details of the formation and splitting of the cell-wall ring, of the process of mitosis, and of the formation of the septum.

The cell division in *Oedogonium* occurs as an integrated sequence of individual phenomena: ring formation, mitosis, septum formation, ring splitting, chloroplast division, and new wall formation. Movements associated with the individual phenomena should be driven by specific forces. In general, various cellular movements are driven by either microtubules or microfilaments, or by a combination of both. No reports have been published that have concentrated on the nature of the driving forces of the phenomena and movements that occur during cell division in *Oedogonium*.

We have examined the effects of vinblastin and of cytochalasin B upon various phenomena during cell division in *Oedogonium*. Vinblastin destroys microtubules and cytochalasin B destroys microfilaments. The driving forces involved in cell division are discussed with reference to our results.

Materials and Methods

Oedogonium capilliforme was cultured in ICHIMURA's C medium (1971) with a daily cycle of 13 hours of illumination under fluorescent light (2,000 lux) and 11 hours of darkness, at 20°C. Cells in division were transferred into media that contained vinblastin or cytochalasin B and were examined under a light microscope equipped with Nomarsky apparatus at hourly intervals after transfer. Vinblastin was dissolved in the culture medium at a concentration of 100 μg/m*l*. Cytochalasin B was first dissolv-

ed in dimethyl sulfoxide (2 mg/100 μl), and then diluted to 200, 100, or 50 μg/ml with 20 mM Hepes buffer (pH 7.4) that contained 20 mM KCl, and 0.1 mM $CaCl_2$. Cellulose was detected by fluorescence microscopy (Olympus, Tokyo, Japan; type BH2 RFA, with a violet exciter filter) in cells mounted in an aqueous solution of 25 μg/ml fluostain-1 (Dojin Chem.).

For electron microscopy, cells were fixed for 3 hours with 4% glutaraldehyde dissolved in phosphate buffer (pH 7.4) at room temperature. They were washed with water and postfixed for 12 hours with 1% osmium tetroxide at 4°C. After washing with water, cells were treated with 0.7% uranyl acetate, dehydrated with acetone, and embedded in SPURR's resin. Ultrathin sections were stained with lead citrate and examined with a Hitachi H700-S transmission electron microscope.

Results

1. *The process of cell division*

The first visible sign that indicated the start of cell division was the formation of the cell-wall ring at the top of the cell (Fig. 1A). The nucleus then divided into two (Fig. 1B). A thin sheet of cytoplasm appeared between the closely situated daughter nuclei (Fig. 1C), and this sheet developed until it traversed the centrally located large vacuole, finally dividing the cell into two (Fig. 1D). This cytoplasmic septum developed later into the lateral cell wall. After the daughter nuclei had moved apart from each other, chloroplasts (dotted regions in Fig. 1) divided around the septum (Fig. 1E). At the same time, the wall ring splitted circularly and was pulled longitudinally (Fig. 1F). Ring substances turned into new cell-wall substances which covered the plasma membrane previously located inside the ring. The new cell wall stretched to the same length as the average length of a cell. During the stretching of the new cell wall, the position of the septum shifted to the boundary between the old and the new cell walls. Chloroplasts

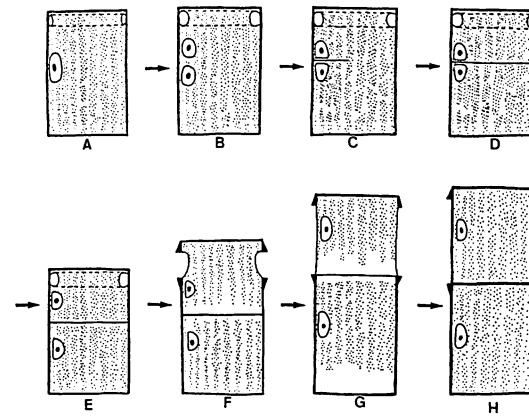


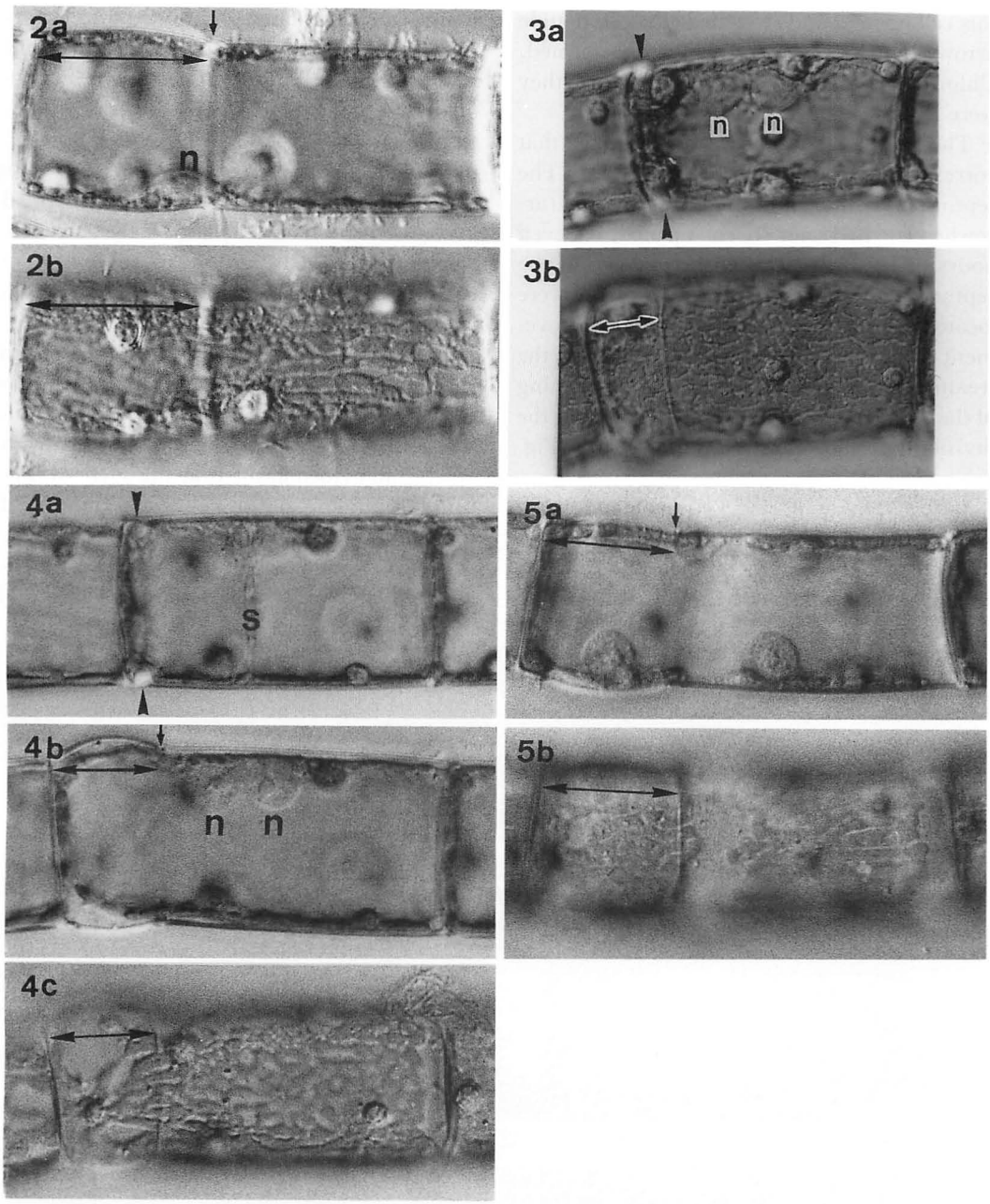
Fig. 1. Progress of cell division. A: Formation of the cell-wall ring before mitosis. B: Formation of two daughter nuclei after mitosis. C: Beginning of formation of the cytoplasmic septum. D: Completion of the cytoplasmic septum. E: Division of chloroplasts. F: Opening of the ring. G: stretching of the new cell wall. H: Completion of the daughter cells.

which were located in the region covered by the old cell wall also shifted slightly towards the new cell, so that a chloroplast-free region appeared at the bottom of the old cell (Fig. 1G). Later, the new cell wall thickened, the chloroplasts in both cells increased in size, and the chloroplast-free region disappeared (Fig. 1H).

Among these successive stages of cell division, the stages at which the division of chloroplasts and the movement of chloroplasts occur (Fig. 1E-G) are described for the first time here.

2. *Effects of vinblastin on cell division*

When cells at an early stage of cell division, corresponding to Figure 1A, were treated with 100 μg/ml vinblastin, the cell-wall rings opened and stretched as usual but nuclear division was inhibited (Fig. 2). No septum was formed and no division of chloroplasts occurred. The left portion of the cell in Figure 2 (indicated by a double arrow) was formed by the opening and the stretching of the ring during the treatment with vinblastin for 10 hours. A central vacuole and a nucleus were visible. The boundary between the new and the old cell walls was so strongly enhanced, as shown in Figure 2a (small arrow), as to suggest the misinterpretation that this cell had been



Figs. 2–5. Cells treated with 100 μg/m*l* vinblastin. ×830.

Fig. 2. A cell treated for 10 hours from a stage before mitosis. a: center view. b: surface view. Fig. 3. A cell treated at the end of mitosis. a: immediately after treatment. b: 7 hours after treatment. Fig. 4. A cell treated after completion of the cytoplasmic septum. a: immediate after treatment. b and c: 7 hours after treatment; center and surface view, respectively. Fig. 5. A cell treated for 7 hours after division of chloroplasts. a: center view. b: surface view. n: nuclei. s: septa. double arrows: regions of new cell wall. arrowheads: rings. small arrows: boundaries between the old and the new cell wall.

divided by a septum.

The cell shown in Figure 3a had just finished nuclear division and two daughter nuclei (n) had been formed. The wall ring (arrowheads) had not opened. After treatment with vinblastin for 7 hours, the wall ring of

this cell opened and stretched (Fig. 3b double arrow) but no septum was formed. Chloroplasts remained undivided as they were at the beginning of the treatment.

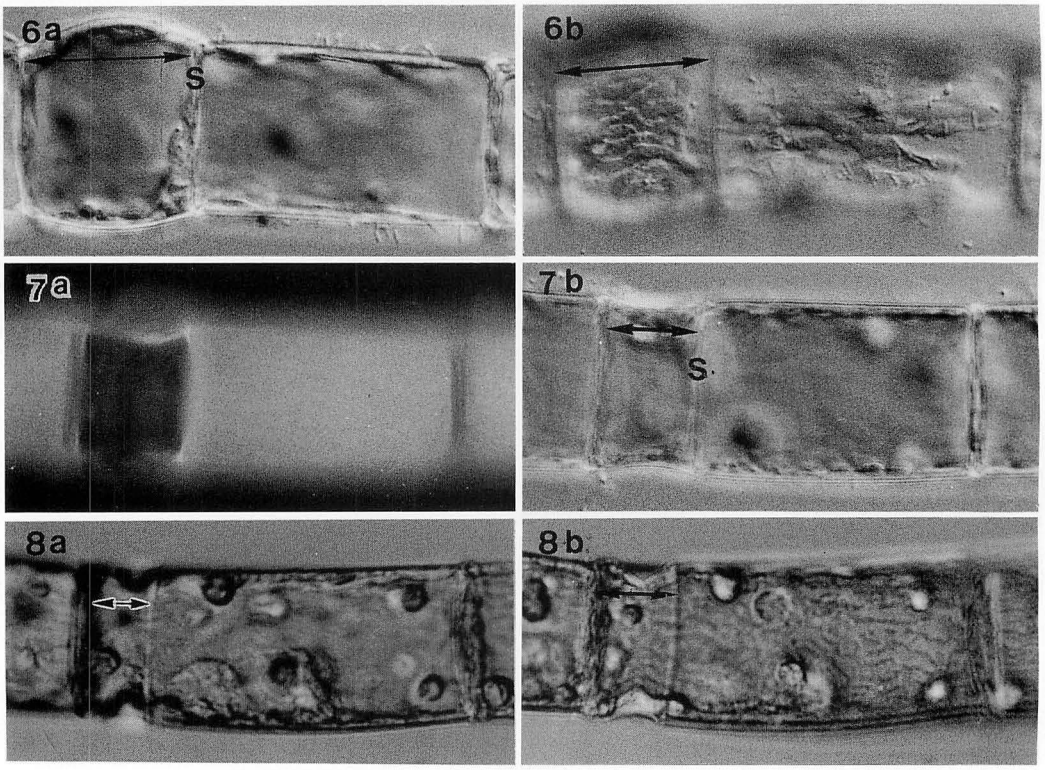
The cell in Figure 4a was at the stage that corresponded to the cell in Figure 1D. The septum (s) had been formed, but the ring (arrowheads) had not been opened. Seven hours of treatment caused breakdown of the septum (Fig. 4b). Two daughter nuclei were located close to each other without any movement from their initial site at the start of the treatment. The opening and the stretching of the ring was evident (double arrow) but the division of chloroplasts did not occur (Fig. 4c).

When cells at a stage equivalent to that shown in Figure 1E, where chloroplasts had divided and the daughter nuclei had moved apart, were treated with vinblastin for 7 hours, the ring opened, the septum disappeared and the chloroplast-free region at the middle of the cell did not decrease as a result of the stopping of a volume-increase of the chloroplasts (Fig. 5).

3. Effects of cytochalasin B on cell division

The treatment of cells with cytochalasin B at a concentration of 50 μg/m*l* had a relatively small effect on cell division. The division of the nucleus advanced normally, the septum was formed, and the chloroplasts divided. However, the rate of stretching of the opened ring decreased to about half of that in untreated cells.

The cell in Figure 6 was treated with 100 μg/m*l* cytochalasin B at the stage of septum formation. In this cell, the formation of the septum was completed (Fig. 6a, s), the ring opened, and the new cell wall was stretched (double arrow). Chloroplasts divided and



Figs. 6–7. Cells treated with 100 μg/m*l* cytochalasin B for 8 hours. ×830.

Fig. 6. A cell in which treatment started during septum formation. a: center view. b: surface view. Fig. 7. A cell treated from mitotic metaphase onwards. a: observed with a fluorescence microscope after staining with fluostain-1. b: center view. Fig. 8. A cell treated with 200 μg/m*l* cytochalasin B from mitotic metaphase onwards. ×830. a: center view. b: surface view. s: septa. double arrows: regions of new cell wall.

the septum migrated to the boundary between the old and the new cell walls (Fig. 6b). The new wall of daughter cells (left-side cell in Fig. 6) that had developed in cytochalasin B frequently had a convex curvature. Cells with such walls tended to rupture later. Concave new walls were formed when cells were treated with cytochalasin B before septum formation (Fig. 7). Figure 7a is a fluorescence micrograph of a cell treated with 100 μg/m*l* cytochalasin B, from the stage of mitotic metaphase, for 8 hours and then stained with fluostain-1. Old cell walls radiated fluorescence, while new walls did not radiate fluorescence. This difference suggests that synthesis of the cell wall is inhibited by cytochalasin B. The septum was formed and the chloroplasts divided in the cell (Fig. 7b).

Treatment with 200 μg/m*l* cytochalasin B strongly inhibited the progress of cell division at almost all stages; division of the nucleus, septum formation, and wall stretching ceased within 10 minutes. Only the opening of the ring was not inhibited. The cell in Figure 8 had been treated with 200 μg/m*l* cytochalasin B for 8 hours at a mitotic stage. The ring of this cell was opened but stretched to a lesser extent than normal (double arrows).

4. Ultrastructure of dividing cells

Since PICKETT-HEAPS and FOWKE (1969, 1970a, b) have clarified details of the ultrastructure of the mitotic process and of the opening of the ring, descriptions are restricted here to the processes of formation of the septum and the cell wall which were affected by treatment with vinblastin and cytochalasin B.

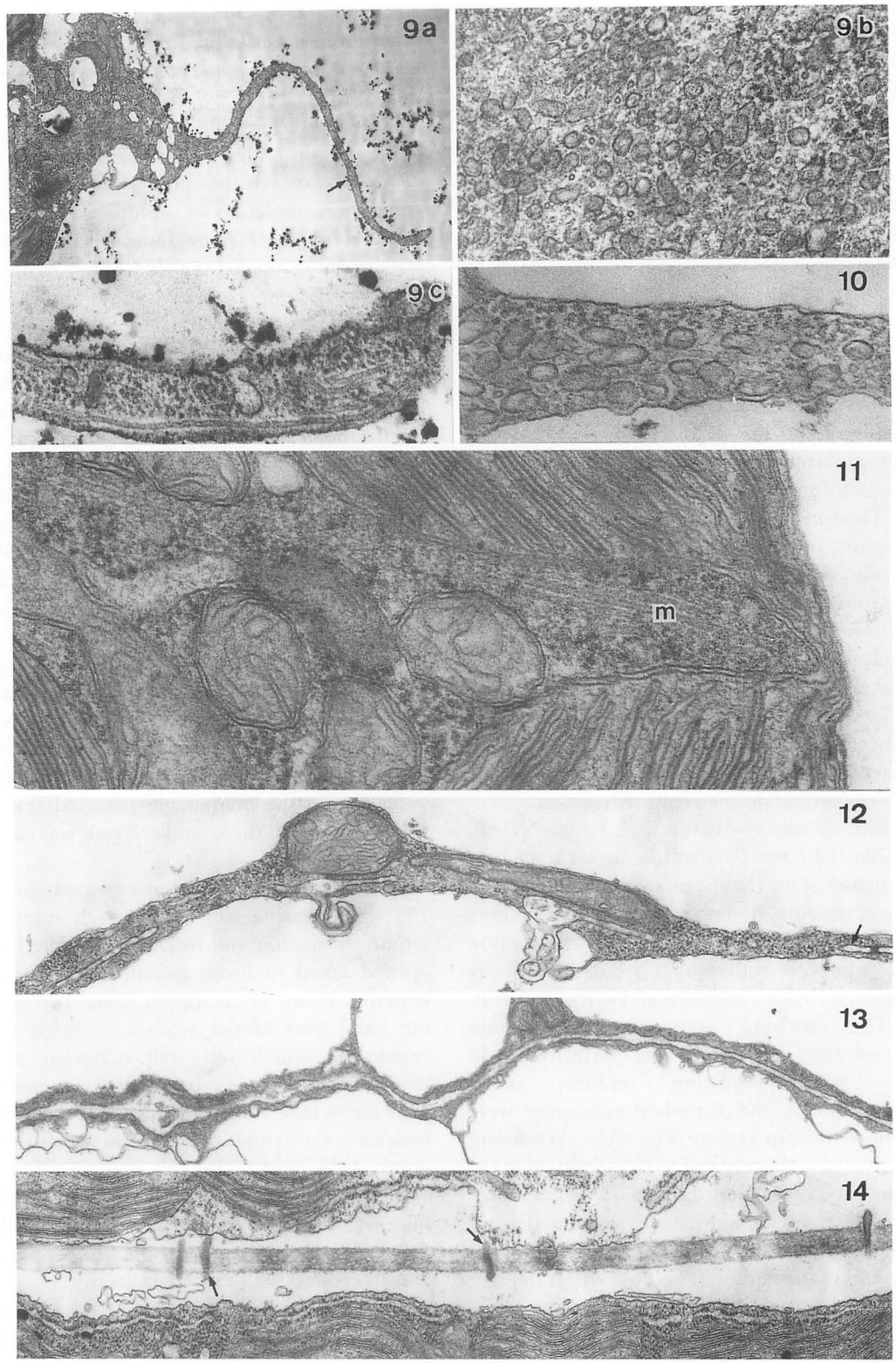
The growing septum was a thin cytoplasmic membrane (Fig. 9a arrow). In the young growing septum, many microtubules and abundant ribosomes were seen at the tip region (Fig. 9c). We found few vesicles, in contrast to the observations of PICKETT-HEAPS and FOWKE (1969, 1970b). Vesicles which might have originated from dictyosomes were distributed at the basal regions of the septum (Fig. 9b). After the septum had covered more than half of the cross-sectional area of the cell, these vesicles became visible in the septum. They increased in number, and they, in addition to microtubules and ribosomes, occupied most of the septum which had completed the separation of the cell into two parts (Fig. 10).

Microtubules extended from the periphery of the cell, through a region near to the center of the septum, to the other side of the cell. After the completion of septation, terminals of microtubules further approached the cell wall, pushing against the chloroplast to make narrow cavities. Figure 11 shows the cavity of a chloroplast, which is so deep that the envelope on one side of the chloroplast almost reaches the envelope on the other side which is in contact with the cell wall. Many microtubules, which are parallel to the plane of the cavity, are evident in Figure 11 (m).

Vesicles in the septum began to fuse to each other to make flat sheets (Fig. 12). These sheets extended their surface area by continuous fusion with vesicles and finally became a flat sheet that divided the septum into two (Fig. 13). Fusion of vesicles started at the periphery of the cell and advanced towards the center of the septum. The lumen of the flat sheet was transparent at the earlier stages of its formation, and later, gradually increased in electron density by taking up vesicles. At the final stage, plasmodesmata were formed and the lateral cell wall was completed (Fig. 14).

Cells which had been treated with 100 μg/m*l* cytochalasin B at an early stage of septum formation and in which the ring had opened failed to form a complete cell wall within 8 hours of treatment (Fig. 15). At the basal part of the septum, a large flat vesicle, in which cell wall materials and plasmodesmata were included, was seen. This flat vesicle was not continuous with the vesicles in the cytoplasmic septum which were oriented randomly (Fig. 15b arrows). These vesicles probably contained cell wall substances. There were many mitochondria, ER, and small vacuoles in the cytoplasmic septum of cells treated with cytochalasin B.

Figure 16 shows part of a cell that had been treated with cytochalasin B for 8 hours after mitosis. The stretching of the new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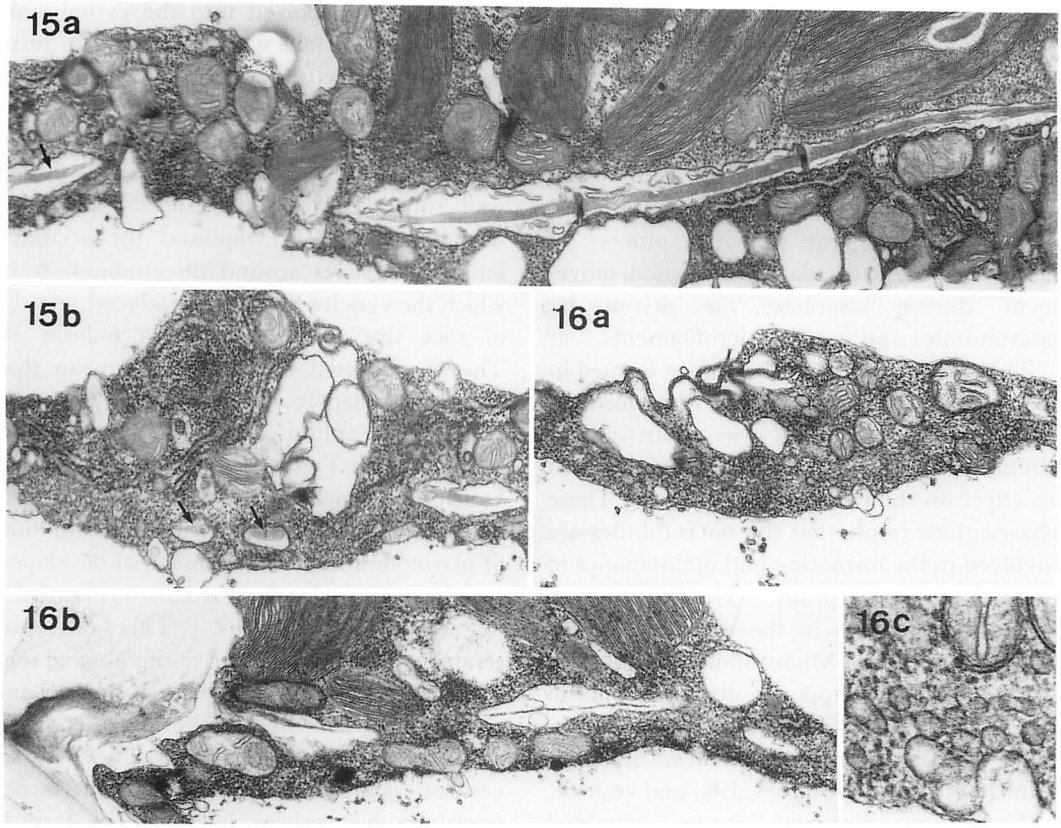

Fig. 15. A cell treated with cytochalasin B for 8 hours from an early stage of septum formation. a: basal part of the septum. ×16,000. b: central part of the septum. ×16,000.

Fig. 16. A cell treated with cytochalasin B for 8 hours from mitosis. a: central part of the septum. ×16,000. b: basal part of the septum. ×16,000. c: Portion of Fig. 16a is enlarged. ×53,000.

longitudinal cell wall in this cell did not extend as far as that of the cell in Figure 15. The central part and the basal part of the cytoplasmic septum are shown in Figure 16a and b, respectively. In the basal region, vesicles contained few wall substances, and in the middle region no such vesicles were seen. These observations indicate that the cell in Figure 16 was less actively involved in the formation of lateral cell wall than was the cell in Figure 15. In both cells, small vesicles, as shown in Figures 9b and 10, were seen in the cytoplasmic septum (Fig. 16c).

Discussion

The progress of mitosis in *Oedogonium* was sharply interrupted by vinblastin. This result is in complete accord with many reports of the inhibitory effect of anti-tubulin drugs on the progress of mitosis (Dustin 1984). By contrast, cytochalasin B at a concentration of less than 100 μg/m*l* did not inhibit the progress of mitosis. Similar results have been

Figs. 9–14. Portions of untreated cells.

Fig. 9. Beginning of the formation of the cytoplasmic septum. a: at low magnification. ×6,000. b: vesicles at the basal region of the septum. ×45,000. c: microtubules and ribosomes at the tip of the septum. ×45,000. Fig. 10. Vesicles in the completed septum. ×68,000. Fig. 11. Microtubules that terminate in a concavity of the chloroplast. ×60,000. Fig. 12. Flat vesicles in the septum. ×24,000. Fig. 13. Separation of two cells by a flat sheet. ×24,000. Fig. 14. Young lateral wall with plasmodesmata (arrows). ×19,000. m: microtubules.

reported in *Cyanidium* by MITA and KUROIWA (1988), who found that mitosis proceeded in the presence of 20 μg/m*l* cytochalasin B. GOTO and UEDA (1988) could not detect microfilaments in the mitotic spindle in *Spirogyra* by fluorescence microscopy using cells stained with rhodamine-phalloidin. All these results suggest that the process of mitosis, and in particular chromosomal movement during anaphase, is driven by microtubules and not by microfilaments.

The cytoplasmic septum was not formed in the presence of vinblastin and, when formed, the cytoplasmic septum was destroyed by vinblastin. Cytochalasin B did not have such an effect on the cytoplasmic septum. These observations imply that the microtubules are involved in the formation and maintenance of the cytoplasmic septum. The presence of many microtubules in the septum supports this hypothesis. Microtubules should be strong enough to support a disk of cytoplasm 30 μm in diameter and 0.3 μm in thickness that contains many organelles, such as mitochondria, dictyosomes, ER, and vesicles.

The young, growing septum contained mainly microtubules and ribosomes, and the vesicles were transported into the growing septum at later stages. Thus, the transport system of vesicles need not be formed or need not be activated at the early stages of septum formation. Two types of transport systems for vesicles are known, operated by microtubules and by microfilaments (FRANKE *et al.* 1972, Nagai and HAYAMA 1979, DUSTIN 1984, SCHLIWA 1985). The presence of vesicles in the septum that has developed in the presence of cytochalasin B may indicate that the microfilaments are not involved in vesicle transport in the septum of *Oedogonium*. Microtubules are probably deeply involved in the migration of these vesicles. The septum, therefore, grows at early stages by the lateral growth of microtubules accompanied by the ground cytoplasm and ribosomes, and grows at later stages by the accumulation of various components of the cytoplasm many of which are transported by a microtubular system after its activation.

Vesicles transported into the cytoplasmic septum did not fuse with each other to form flat sheets in cells treated with cytochalasin B. Cytochalasin B seems to inhibit the fusion of vesicles. The inhibition of fusion of vesicles may also prevent the supply of cell wall substances to flat vesicles. MOLLENHAUER *et al.* (1976) reported the accumulation of vesicles around dictyosomes, from which the vesicles had been produced, in cells of root tips treated with cytochalasin B. They interpreted their result to mean that both the transport system and the fusion of vesicles were inhibited in this case.

The cell in Figure 15a had a large fused vesicle in which wall substances had accumulated at high levels, with the formation of plasmodesmata. This image of developed vesicles may be understood in terms of the following considerations. This cell was treated with cytochalasin B at the stage of septum development, and vesicles would have partially fused at the periphery of the cell at the moment of treatment. These fused vesicles could develop somewhat, incorporating other vesicles, before cytochalasin B reached them and inhibited completely the fusion of vesicles.

Chloroplasts in *Oedogonium* were divided at the basal part of the septum. Recently, the involvement of microfilaments in the division of chloroplasts has been described (MITA and KUROIWA 1988, OROSS and POSSINGHAM 1989). MITA and KUROIWA reported that the division of chloroplasts in *Cyanidium* is inhibited by cytochalasin B but not by antitubulin drugs and they suggested that F-actin is deeply involved in the division of chloroplasts. By contrast, the division of chloroplasts in *Oedogonium* was inhibited by vinblastin and not by cytochalasin B. The difference in behavior between the chloroplasts of two species may derive from differences in the mode of division of the two types of chloroplast. In *Cyanidium*, chloroplasts are divided at their middles by furrowing, while in *Oedogonium* they are so strongly compressed by microtubules towards the cell wall that they are probably pinched off into two

daughter chloroplasts between the microtubules and the cell wall. If the microtubules are destroyed by vinblastin, chloroplasts are not compressed by microtubules and are not divided into two.

Rings of the cell wall could be opened after inhibition of both the progress of mitosis and formation of the septum by vinblastin. The opening of rings and the subsequent elongation of the new cell wall, in spite of the inhibition of the two processes by long-term treatment with vinblastin is surprising. The opening of the rings is, therefore, assumed to be an independent phenomenon which is not affected by the destruction of microtubules.

The elongation of the new cell wall may result from the longitudinal elongation of the protoplasm. The absence of an inhibitory effect of vinblastin on the elongation and the decrease in the rate of elongation by cytochalasin B suggest that microfilaments are involved in this elongation. Microfilaments that are longitudinally oriented may be involved. If such microfilaments are elongated in such a manner as to pull the protoplasm upwards, then the new cell wall would elongate. Accompanying the pulling upward of the protoplasm, the upward shifting of the positions of the septum and the chloroplasts might occur, as shown in Figures 1F and 1G.

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中川満代・野口哲子・植田勝巳：*Oedogonium capilliforme* の細胞分裂におよぼすビンブラスチンとサイトカラシン B の影響

光学顕微鏡と電子顕微鏡を使用して *Oedogonium capilliforme* の細胞分裂に及ぼすビンブラスチンとサイトカラシン B の影響について研究を行った。ビンブラスチンは 100 μg/ml の濃度において微小管の関与する核分裂の進行及び細胞質性隔壁の形成を阻害した。また，葉緑体分裂も阻害するが，細胞壁リングの開裂と伸長は阻害しなかった。サイトカラシン B は 100 μg/ml の濃度では核分裂の進行，細胞質性隔壁の形成，葉緑体分裂などを阻害しなかったが，細胞壁形成に関与する物質を含むと考えられる小胞の融合を阻害し新しい細胞壁の伸長を阻害した。これらのデータから，微小管や微繊維と各細胞分裂期の進行との関わりについて考察がおこなわれた。(630 奈良市北魚屋西町　奈良女子大学理学部生物学教室)