Experimental obliteration of the preprophase band alters the site of cell division, cell plate orientation and phragmoplast expansion in *Adiantum* protonemata

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Summary

Division sites in higher plant cells are known to be determined before mitosis, and cell plates are precisely inserted into the predetermined division sites at the end of cytokinesis by unknown mechanisms. When apically growing protonemal cells of the fern, *Adiantum capillus-veneris* L., grown under red light are transferred to the dark, apical growth ceases and the protonemal cells then divide. However, this mitotic event can be influenced by subsequent exposure to light. If red-light pre-cultured protonemata are incubated in the dark and then transferred back to red light (after 28–36 h), apical growth resumes and the nuclei migrate toward the growing tips; interestingly, mitosis still occurs, although in an altered fashion. In the re-irradiated cells, timing of the premitotic nuclear positioning is delayed markedly and irregularly oriented cell plates are frequently observed. Re-irradiation with red light also causes an increase in cells without preprophase bands (PPBs) at prophase and the irregular expansion of the phragmoplast at late telophase, while early phragmoplast microtubule (MT) organization takes place normally. These data suggest the indirect involvement of PPBs in the guidance of phragmoplast expansion.

Key words: *Adiantum* protonemata, cell plate, cytokinesis, red light, microtubules, photomorphogenesis, plant cell division, preprophase band, tip growth.

Introduction

Plant cell walls prevent cell migration in most tissue and therefore the control of the position and the orientation of new cell plates are prerequisites for normal plant morphogenesis. The division site, the position where a cell plate fuses to the parental cell wall at the end of cytokinesis, is predetermined before mitosis (Ôta, 1961). A preprophase band (PPB) of microtubules (MTs) begins to form at G_2 phase (Mineyuki *et al.* 1988) as a broad MT band, then it becomes narrow in order to predict the final position of the division site (Mineyuki *et al.* 1989; Mineyuki and Palevitz, 1990). However, the PPB disappears before metaphase and cell plates are inserted at the end of cytokinesis without PPBs. The mechanisms by which a new cell plate fuses to the former PPB site are unknown. Undefined factors that guide phragmoplast expansion and support cell plate maturation are presumed to accumulate during the time in which the PPB is present and presumably remain there until the stage of cell plate formation (Packard and Stack, 1976; Mineyuki and Gunning, 1990). In the largely vacuolated cells, actin filaments are reported to be present and may connect the edge of the phragmoplast with the PPB site, and are thought to be involved in the guidance of phragmoplast expansion (Kakimoto and Shibaoka, 1987; Trass *et al.* 1987; Lloyd and Trass, 1988).

Fern gametophytes are known to be valuable experimental systems for photomorphogenesis studies (Wada and Kadota, 1989). *Adiantum* protonemata grow under red light with a very low rate of cell division (Wada and Furuya, 1970). Cell cycling of the protonemata is arrested at G_1 phase (Miyata *et al.* 1979). When these protonemata are transferred to blue light, or to the dark, cell division occurs synchronously (Wada and Furuya, 1972). MT arrangements throughout the cell cycle in the protonemata have been well studied (Wada *et al.* 1980; Murata and Wada, 1989). Different types of MT systems are involved in the anchoring of the nucleus. In apically growing protonemata maintained under red light, cortical MTs form a circular band in the subapical part of the protonemal cell while some other MTs run near the leading edge of the nucleus to the subapical part of the protonema (Wada and O'Brien, 1975; Murata *et al.* 1987). It has been suggested that these MTs anchor the nucleus (Mineyuki and Furuya, 1985). In the prophase stage of dark-induced cell division, a PPB of MTs forms surrounding the nucleus. In addition, cytoplasmic MTs are also seen between the perinuclear region and the PPB (Wada *et al.* 1980; Mineyuki and Furuya, 1986; Murata and Wada,

1989). Presumably, the nucleus is anchored by these MTs (Mineyuki and Furuya, 1986).

If the nuclear position could be changed experimentally after premitotic nuclear positioning, the role of MTs in nuclear positioning, or their effect on the site of cell division might be deduced. In preliminary work, we found that protonemal cells re-irradiated with red light, after dark incubation for 28–36 h, resumed growth and subsequently the nucleus moved slightly toward the tip of the cell prior to cell division. In this work, we examined the effect of re-migration of the nucleus, induced by red-light treatment, upon the formation of the PPB and subsequent phragmoplast development. A preliminary account of this work has appeared in abstract form (Mineyuki, 1987).

Materials and methods

Plant material and culture methods were the same as described elsewhere (Mineyuki and Furuya, 1980; Murata and Wada, 1989). Briefly, spores of *Adiantum capillus-veneris* L., sterilized with sodium hypochlorite, were inoculated onto the inner surface of an empty plastic cuvette or sown between agar–gelatin thin films and then submerged in modified (Wada and Furuya, 1970) Murashige and Skoog's medium. After incubation for 1 day in the dark, the spores were cultured under red light (0.5 W m^{-2}) for 6 days and the resulting protonemata were transferred into the dark to induce cell division. In some experiments the undivided protonemata were transferred from the dark to red light after an appropriate period of a dark incubation. The temperature was kept at approximately 25 °C throughout the culture. Constant red light (0.5 W m^{-2}) was obtained by passing light from a white fluorescent lamp (FL40SD/NL or FL20SD, Toshiba Corp., Tokyo, Japan) through a red acrylic-resin filter (Shinkolite 102, Mitsubishi Rayon Co., Tokyo, Japan).

The positions of the nucleus and protonemal tip in each cell were observed continuously under infrared light with a microscope using an infrared viewer, or by using a video camera equipped with an infrared-sensitive tube (Mineyuki and Furuya, 1980). In some cases, protonemata were photographed under infrared illumination with infrared film (HIE 135–20, Eastman Kodak Co. Ltd., Rochester, USA) or under red light with panchromatic film (Neopan F, Fuji Photo Film Co., Tokyo, Japan). The infrared light was provided by a tungsten lamp with an infrared filter (IR85, Hoya Glass Co. Ltd, Tokyo).

MTs were observed using anti-tubulin immunofluorescence microscopy and the nuclei were visualized by the fluorescence of DAPI (4',6'-diamidino-2-phenylindole) as described previously (Murata and Wada, 1989).

Results

Timing of red-light treatment

Red-light treatment affects the site of cell division induced in the dark. In order to examine this effect in more detail it was necessary to identify the timing of red-light re-irradiation that caused maximum aberration of cell plate formation. This was done by illuminating the protonemata incubated in the dark with red light at various times. When the dark incubation period was too short (i.e. protonemata were still in G_1 phase), division did not occur (Wada *et al.* 1984). When the dark period was more than 40 h, apical growth resumed after the end of cell plate formation in most protonemata. Thus, the protonemata were treated with red light at 28, 32 and 36 h after the onset of dark incubation and the division sites in these cells were compared (Fig. 1, Table 1). Dark incubation for 28 h showed the greatest effect on the shift of division site; approximately 40 μm towards the cell tip compared with

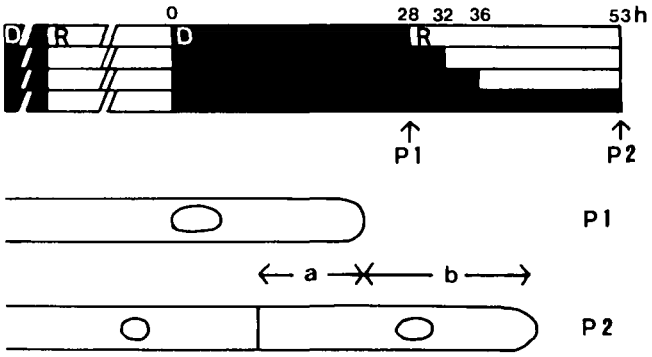

Fig. 1. Schematic representation of methods of red light treatment and the measurement of the position of cell plate and growth increment (Table 1). Spores were kept in the dark (D) for 1 day, then precultured for 6 days under red light (R) and the resulting protonemata were transferred to the dark. These protonemata were transferred to red light after 28, 32 or 36 h dark incubation, respectively. The first photograph (P1) was taken using infrared film at 28th h after transferring protonema to the dark; then another photograph (P2) was taken 25 h later. The position of cell plate (a) and growth increment (b), shown in Table 1, were determined using these photographs.

Table 1. *Effect of red light on the resumption of apical growth and the position of the cell plate in dark-induced cell division in* Adiantum *protonemata*

Timing of red-light treatment (h in the dark)	Position of cell plate (μm beneath the protonemal tip at 28th h)	Growth increment (μm)	Number of cells tested
28	35.4±3.4*	87.0±5.1*	56
32	58.5±4.2*	39.9±4.3*	43
36	63.6±3.1*	36.9±4.0*	39
Dark	74.4±2.2	1.2±0.5	39

Methods for the determination of the position of the cell plate and the growth increment are shown in Fig. 1.
*Significantly different from dark control ($P<0.005$).

the dark control. Thus, in all the experiments described below, re-irradiation with red light began 28 h after dark incubation. Under these conditions, cell plate formation took place in about 85 % of protonemata (Fig. 2, Table 3, below) and the timing of cell plate formation was slightly early (Fig. 2).

Apical growth, nuclear migration and premitotic nuclear positioning

The apical growth and the nuclear behavior in protonemata treated with, or without, red light were examined using a time-lapse video system. The protonemata had nearly ceased growing by 28 h after transfer to the dark (Fig. 3A). In one half of these cells, the apical part of the protonema was fully vacuolated.

The protonemata were re-irradiated with red light to induce apical growth again. Timing of the resumption of apical growth varied from cell to cell. In the majority of cells, especially in cells with vacuolated tips, apical growth began approximately 6 h after the onset of red-light irradiation (Fig. 3A). However, in protonemata with non-vacuolated tips (Fig. 4), the apical growth resumed soon after the red-light irradiation (Fig. 3B).

Nuclei that were located 60–90 μm from the tip at 28 h (Fig. 3; see also Mineyuki and Furuya, 1980) began to

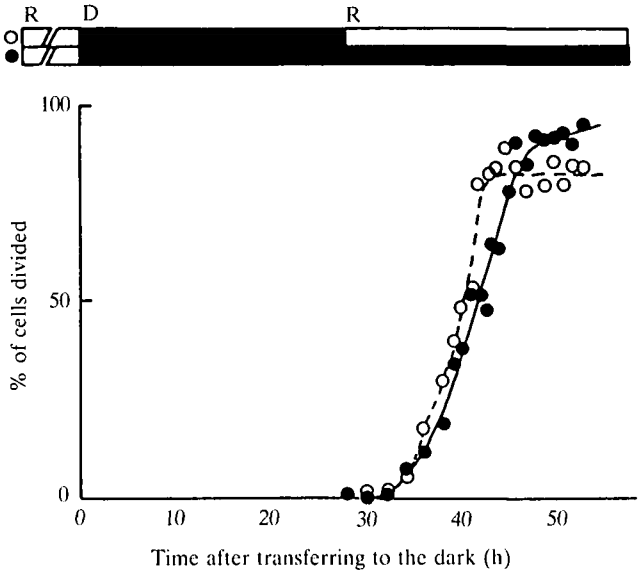

Fig. 2. Effect of red light on the timing of the dark-induced cell division in *Adiantum* protonemata. Protonemata grown under red light were transferred to the dark (●) or incubated in the dark for 28 h then under red light (○). Percentages of divided cells were then calculated. Each point was obtained by counting 100 cells. R (□), red light conditions. D (■), dark conditions.

Table 2. *Effect of red light on the timing of premitotic nuclear positioning in dark-induced cell division in* Adiantum *protonemata*

Light treatment	Timing of the end of the nuclear migration (h before the end of cytokinesis)	Number of cells observed
Dark	4.2±0.3	7
Red	2.9±0.2*	23

For the red-light treatment, protonemata were transferred to red light 28th h after the onset of dark incubation to induce cell division. The length of time from the end of nuclear migration until the completion of cytokinesis was determined according to the method described by Mineyuki and Furuya (1980).

*Significantly different from the dark control ($P<0.05$).

Table 3. *Effect of red light on cell plate orientation in dark-induced cell division in* Adiantum *protonemata*

Light treatment	% of cell plate formation	% of oblique cell plate
Dark	96.7±0.3	16.7±3.3
Red	85.3±0.9*	45.3±1.9*

For the red-light treatment, protonemata were transferred to red light 28th h after the onset of dark incubation to induce cell division. Data were obtained from 3 separate experiments in which approximately 100 protonemata were counted.

*Significantly different from the dark control ($P<0.005$).

migrate apically a few hours after red-light irradiation. In the majority of cells, some backward movement of nuclei was also observed prior to cell plate formation (Fig. 3A,B). When nuclei entered mitosis, the cytoplasm in the tip migrated again to the nuclear region and the rate of the apical growth decreased; presumably because the protonemal tip became vacuolated (Fig. 3A,B, Fig. 4).

A correlation was also noted between renewed growth and the division site. The greater the growth increment before mitosis, the larger the shift of division site. Soon after the cytokinesis, the cytoplasm again accumulated in the apical region and apical growth resumed thereafter at the normal rate ($6\,\mu m\,h^{-1}$ in Fig. 3A).

Although nuclei in protonemata grown under dark conditions stopped migrating 4.2 h (late G_2 or early prophase) before the end of the cytokinesis (Fig. 3C: see discussion by Mineyuki and Furuya, 1980), the time to the end of migration was significantly longer under red light illumination (Fig. 3A,B; Table 2).

Cell plate formation

The shape of the cell plates was examined in the red-light-treated cells after cytokinesis (Table 3). In these cells, obliquely oriented cell plates (Fig. 5B) were frequently seen. Conversely, most cell plates in control cells were perpendicular to the growing axis (Fig. 5A).

MT organization

MT organization at prophase was examined in the cells treated with red light for 10 h after 28 h incubation in the dark. Only one half of the red-light-treated prophase cells had a PPB (Table 4), although 84% of the prophase cells incubated for 38 h in the dark possessed a PPB (Fig. 6A,B). In the other half, no cortical MT bands were detectable even in the apical region or in the region where the nucleus was located before red light irradiation, although the cells always contained prominent perinuclear MTs and many cytoplasmic MTs between the nuclear region and the protonemal tip (Fig. 6D,E).

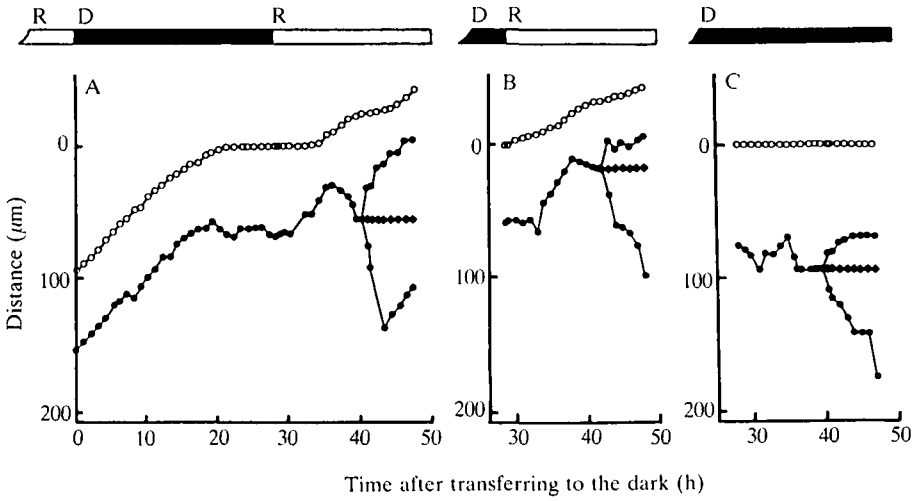

Fig. 3. Effect of red light on apical growth and nuclear migration in the dark-induced cell division in *Adiantum* protonemata pre-cultured under red light. The positions of the nucleus (●), cell plate (◆) and protonemal tip (○) were continuously observed and recorded in protonemata that were transferred to red light after 28 h dark incubation (A,B) or kept in the dark (C). Horizontal axes indicate the time after the onset of dark incubation. Vertical axes show distance along the filamentous cell axis relative to the position of the protonemal tip at 28th h in the dark. R (□), red light conditions. D (■), dark conditions.

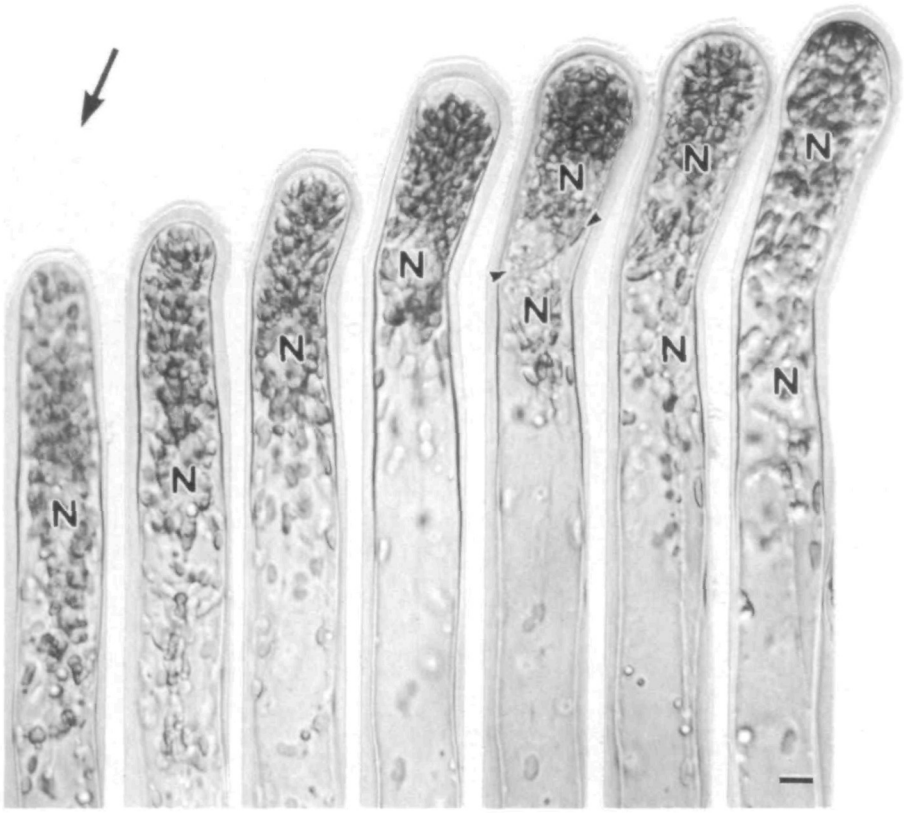

Fig. 4. Differential interference contrast views of cell division and apical growth in the red-light-treated *Adiantum* protonemata. A red-light-grown protonema was cultured for 28 h in the dark, then the cell was transferred to red light again. Photographs were taken every few 1 h intervals under the red light conditions. Red light for preculture was directed from the top and that for red-light treatment after 28 h dark incubation was from the direction indicated by an arrow. Pictures are arranged from left to right according to the time schedule of 1, 5, 9, 13, 16, 19, 21 h after the onset of the red-light treatment. Arrowheads show cell plate. N, nucleus. Bar, 10 μm.

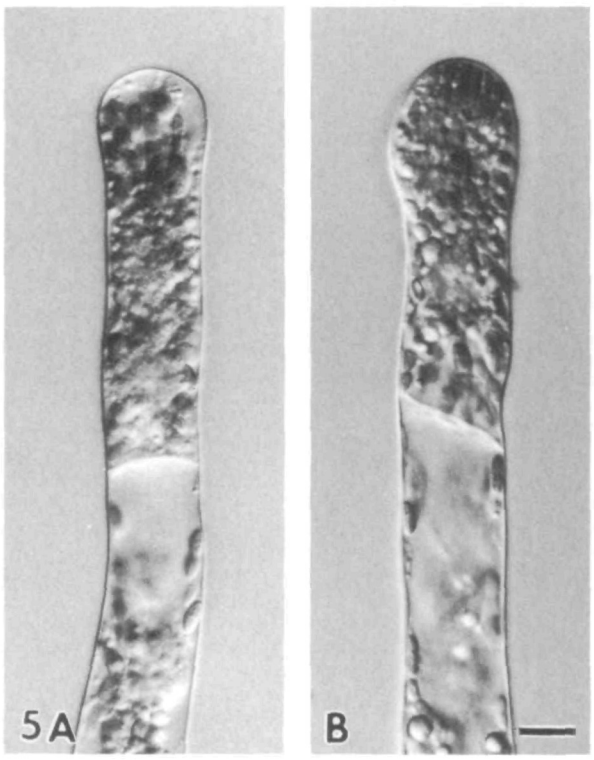

Fig. 5. Views of differential interference contrast of divided *Adiantum* protonemal cells treated with (B) or without (A) red light after 28 h incubation in the dark. Bar, 10 μm.

Table 4. *Effect of red light on PPB formation in dark-induced cell division in* Adiantum *protonemata*

Light treatment	% of prophase cells without PPB	Number of prophase cells observed
Dark	15.9*	63
Red	45.9*	74

Protonemata were observed 38 h after the induction of cell division by transferring from red light to the dark. For the red-light treatment, protonemata were transferred to red light 28th h after the onset of dark incubation to induce cell division.

* Significant difference by χ^2-test ($P<0.05$).

MT organization in phragmoplasts was next examined in the red-light-treated protonemata in telophase. At early telophase, when new cell plates appeared between the daughter nuclei, the MT configuration in the phragmoplasts was normal (Fig. 7A). In phragmoplasts seen in later stages of cell plate formation, however, the marginal part of the phragmoplast was often oriented irregularly, suggesting a lack of guidance for normal phragmoplast expansion.

Discussion

Premitotic nuclear positioning

Premitotic nuclear migration and anchoring are thought to be essential processes for determining the site of division in large or vacuolated cells (Sinnott and Bloch, 1941; Venverloo *et al.* 1980; Mineyuki and Furuya, 1980). In dark-induced cell division in *Adiantum* protonema, a nucleus moves approximately 30 μm toward the base after

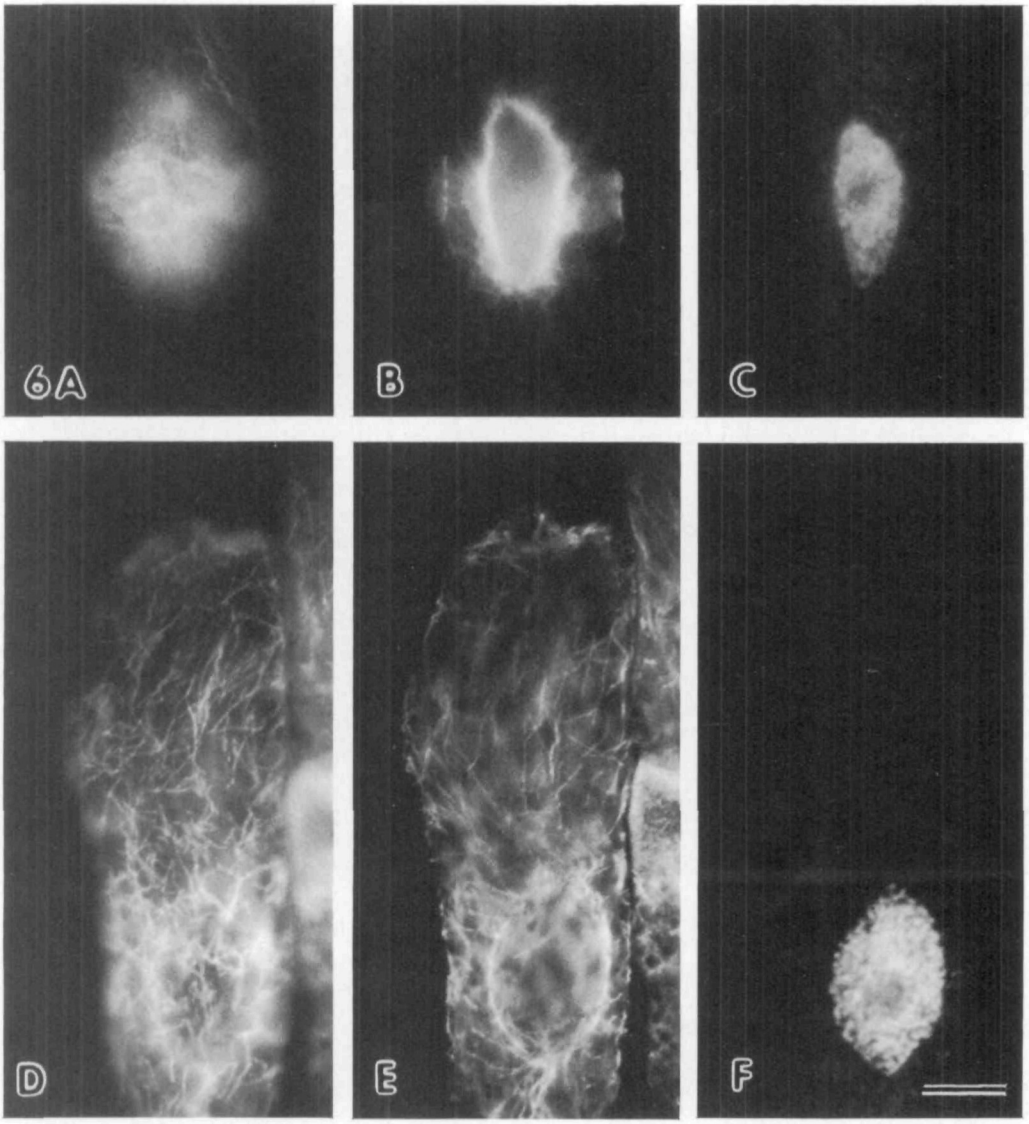

Fig. 6. Surface (A,D) and mid (B,E) optical sections of tubulin immunofluorescence, and nuclei stained with DAPI (C,F) in prophase cells of *Adiantum* protonemata treated with (D–F) or without (A–C) red light. A–C and D–F show the same cell, respectively. (A–C) A control cell that was incubated in the dark for 38 h. (D–F) A red-light-treated cell that was incubated under red light for 10 h after 28 h incubation in the dark. Bar, 10 μm.

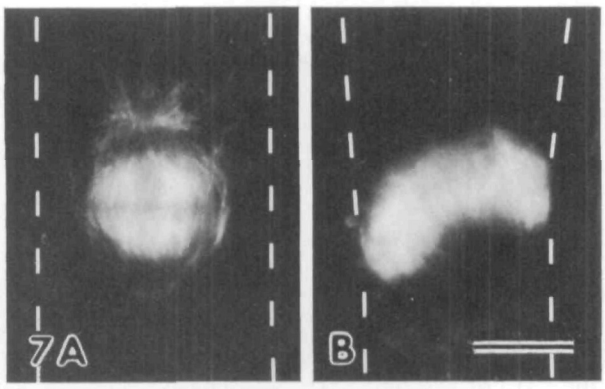

Fig. 7. Tubulin immunofluorescence in red-light-treated *Adiantum* protonemata showing a development of phragmoplasts. White broken lines indicate cell outlines. A. Early telophase. B. Late telophase. Bar, 10 μm.

the cessation of apical growth, then premitotic nuclear positioning occurs and cell division takes place there (Mineyuki and Furuya, 1980). The division site is not invariant and can be shifted by experimental manipulations such as blue-light irradiation, or cell centrifugation during G_1 phase. Under these conditions, nuclei always cease migrating at late G_2 irrespective of the position of the nucleus (Mineyuki and Furuya, 1980; Furuya, 1984). However, the present results show that red-light treatment during late S to G_2 (estimated from the data of Miyata *et al.* 1979) causes not only a shift in the division site, but also a delay in premitotic nuclear positioning. The lack of PPBs in these cells suggests that PPB is important for nuclear positioning and provides further support for the idea that PPB MTs, perinuclear MTs and cytoplasmic MTs linking the perinuclear region with the PPB are involved in premitotic nuclear anchoring at the division site in *Adiantum* protonemata (Mineyuki and Furuya, 1986).

Microtubule organization under red light

The present results can be compared with those from related experiments using centrifugation in *Adiantum* protonemata. When the nucleus in *Adiantum* protonema is moved away from the PPB site by centrifugation during PPB formation, a PPB forms at the normal site and another cortical MT band develops around the displaced nucleus (Murata and Wada, 1991). Similar double MT band formation has also been reported in the asymmetrical division of onion epidermis (Mineyuki *et al.* 1991). In this experiment, however, no PPB developed, either around the nucleus that had been caused to migrate from the normal PPB site by re-irradiation with red light or in the region where PPB would form if the protonema were not treated with red light. Red light interferes with PPB development. It is not clear how red light inhibits cortical MT band organization. An essential difference between the effects of centrifugation and red light treatment in *Adiantum* protonemata is the direction of nuclear movement, basipetal in the former and acropetal in the latter. Centrifugal treatment simply removes the nucleus from the original position but does not induce the tip growth; red light treatment, however, causes the resumption of tip growth.

MT systems that are quite different from those of preprophase cells are involved in tip growth (Schmiedel and Schnepf, 1980; Mineyuki and Furuya, 1985; Doonan *et al.* 1985; Lloyd *et al.* 1987; Wacker *et al.* 1988; Murata and Wada, 1989). In tip-growing *Adiantum* protonemata under red light, in which the cell cycle is arrested at early G_1 phase (Miyata *et al.* 1979), a cortical MT band in the subapical dome region and cytoplasmic MTs between the subapical dome region and the nucleus have been observed (Wada and O'Brien, 1975; Murata *et al.* 1987). When *Adiantum* protonemata in G_1 phase are treated with red light, protonemata re-adjust their cell cycle to early G_1 phase and stop there until the next mitotic induction (Wada *et al.* 1984); in the present work, however, protonemata, re-irradiated with red light during the S to G_2 phase, appear to progress in their cell cycle, without returning to G_1 phase. Thus, tip-growing protonemata in prophase observed in this study may be in a transient state, from the mitotic state to a tip-growing state in early G_1 phase. There is a possibility that red light treatment, which causes the onset of MT arrangement in G_1 phase, somehow interferes with the ability of the *Adiantum* protonemata to develop PPBs. If this is true, why isn't the cortical MT band in the subapical dome region observed in the red-light-treated prophase cells? Competition for tubulins between preprophase MT systems and the MT system for tip growth may be the reason why only perinuclear and cytoplasmic MT systems remain and cortical MT bands cannot develop.

PPB and cell plate orientation

Red-light treatment caused both a shift in division site and a change in cell plate orientation (perpendicular to oblique). Similar results are obtained by experimental manipulation with centrifugal treatment at the presumptive late G_2 phase (Murata and Wada, 1991). In these cases, nuclei were displaced by the experimental treatments, and the PPB was not seen near the displaced nuclei. These data suggest indirect involvement of the PPB in cell plate orientation. Irregularly oriented cell plates are also reported to occur following colchicine treatment during G_2 phase, in which PPB MTs are presumed to be destroyed (although MTs were not examined in these experiments; Venverloo and Libbenga, 1987; Mineyuki, 1987).

In the cell division of a guard mother cell of *Allium*, the PPB and cell plate are formed longitudinally, positioned parallel to the long axis of the cotyledon (although the metaphase spindle is oblique; Palevitz and Hepler, 1974*a*). Cell plate formation in the guard mother cell is composed of two separate phases: (1) part of the cell plate appears in the midst of an obliquely oriented phragmoplast; (2) the cell plate reorients to the former PPB site. Cytochalasin or blue light inhibits only the reorientation process (Palevitz and Hepler, 1974*b*, Palevitz, 1980, 1986). In cytochalasin B-treated *Tradescantia* stamen hair cells (Mineyuki and Gunning, 1990) and in red-light-treated *Adiantum* protonemata (this study), the appearance of the phragmoplast between daughter nuclei looks normal, but the phragmoplast expansion toward the correct division site is affected by the treatment. Unknown factors that are thought to accumulate at the PPB site (Mineyuki and Gunning, 1990) and cytochalasin-sensitive elements, presumably actin (see Lloyd and Trass, 1988), may be involved in the final process of cell plate formation, i.e. phragmoplast expansion to the correct division site.

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References

Doonan, J., Cove, D. J. and Lloyd, C. W. (1985). Immunofluorescence microscopy of microtubules in intact cell linages of the moss, *Physcomitrella patens*. I. Normal and CIPC-treated tip cells. *J. Cell Sci.* **75**, 131–147.

Furuya, M. (1984). Cell division patterns in multicellular plants. *A. Rev. Pl. Physiol.* **35**, 349–373.

Kakimoto, T. and Shibaoka, H. (1987). Actin filaments and microtubules in the preprophase band and phragmoplast of tobacco cells. *Protoplasma* **140**, 151–156.

Lloyd, C. W., Pearce, K. J., Ridge, R. W., Rawlins, D. J. and Shaw, P. J. (1987). Microtubules are involved in the coupled migration of the nucleus with the tip of legume root hairs, but F-actin is necessary for basipetal migration. *Cell Motil. Cytoskel.* **8**, 27–36.

Lloyd, C. W. and Trass, J. A. (1988). The role of F-actin in determining the division plane of carrot suspension cells. Drug studies. *Development* **102**, 211–221.

Mineyuki, Y. (1987). Cell division and the site of cell plate formation: involvement of PPB sites in cell plate maturation. In *XIV Int. Botanical Congress Abstract* 4-09c-1 (ed. W. Greuter, B. Zimmer and H.-D. Behnke), p. 206. Berlin.

Mineyuki, Y. and Furuya, M. (1980). Effect of centrifugation on the development and timing of premitotic positioning of nucleus in *Adiantum* protonemata. *Dev. Growth Differ.* **22**, 867–874.

Mineyuki, Y. and Furuya, M. (1985). Involvement of microtubules on nuclear positioning during apical growth in *Adiantum* protonemata. *Pl. Cell Physiol.* **26**, 627–634.

Mineyuki, Y. and Furuya, M. (1986). Involvement of colchicine-sensitive cytoplasmic element in premitotic nuclear positioning of *Adiantum* protonemata. *Protoplasma* **130**, 83–90.

Mineyuki, Y. and Gunning, B. E. S. (1990). A role for preprophase bands of microtubules in maturation of new cell walls, and a general proposal on the function of preprophase band sites in cell division in higher plants. *J. Cell Sci.* **97**, 527–537.

Mineyuki, Y., Marc, J. and Palevitz, B. A. (1989). Development of the preprophase band from random cytoplasmic microtubules in guard mother cells of *Allium*. *Planta* **174**, 518–526.

Mineyuki, Y., Marc, J. and Palevitz, B. A. (1991). Relationship between the preprophase band, nucleus and spindle in dividing *Allium* cotyledon cells. *J. Pl. Physiol.* (in press).

MINEYUKI, Y. AND PALEVITZ, B. A. (1990). Relationship between preprophase band organization, F-actin and the division site in *Allium*. Fluorescence and morphometric studies on cytochalasin-treated cells. *J. Cell Sci.* **97**, 283–295.

MINEYUKI, Y., WICK, S. M. AND GUNNING, B. E. S. (1988). Preprophase bands of microtubules and the cell cycle: kinetics and experimental uncoupling of their formation from the nuclear cycle in onion root tip cells. *Planta* **174**, 518–526.

MIYATA, M., WADA, M. AND FURUYA, M. (1979). Effects of phytochrome and blue-near ultraviolet light-absorbing pigment on duration of component phases of the cell cycle in *Adiantum* gametophytes. *Dev. Growth Differ.* **21**, 577–584.

MURATA, T., KADOTA, A., HOGETSU, T. AND WADA, M. (1987). Circular arrangement of cortical microtubules around the subapical part of a tip-growing fern protonema. *Protoplasma* **141**, 135–138.

MURATA, T. AND WADA, M. (1989). Re-organization of microtubules during preprophase band development in *Adiantum* protonemata. *Protoplasma* **151**, 73–80.

MURATA, T. AND WADA, M. (1991). Effects of centrifugation on preprophase band formation in *Adiantum* protonemata. *Planta* **183**, 391–398.

ÔTA, T. (1961). The role of cytoplasm in cytokinesis of plant cells. *Cytologia* **26**, 428–447.

PACKARD, M. J. AND STACK, S. M. (1976). The preprophase band: possible involvement in the formation of the cell wall. *J. Cell Sci.* **22**, 403–411.

PALEVITZ, B. A. (1980). Comparative effects of phalloidin and cytochalasin B on motility and morphogenesis in *Allium*. *Can. J. Bot.* **58**, 773–785.

PALEVITZ, B. A. (1986). Division plane determination in guard mother cells of *Allium*: video time-lapse analysis of nuclear movements and phragmoplast rotation in the cortex. *Devl Biology* **117**, 644–654.

PALEVITZ, B. A. AND HEPLER, P. K. (1974*a*). The control of the plane of division during stomatal differentiation in *Allium* I. Spindle reorientation. *Chromosoma* **46**, 297–326.

PALEVITZ, B. A. AND HEPLER, P. K. (1974*b*). The control of the plane of division during stomatal differentiation in *Allium* II. Drug studies. *Chromosoma* **46**, 327–341.

SCHMIEDEL, G. AND SCHNEPF, E. (1980). Polarity and growth of caulonema tip cells of the moss *Funaria hygrometrica*. *Planta* **147**, 405–413.

SINNOTT, E. AND BLOCH, R. (1941). Division in vacuolate plant cells. *Am. J. Bot.* **28**, 225–232.

TRASS, J. A., DOONAN, J. H., RAWLINS, D. J., SHAW, P. J., WATTS, J. AND LLOYD, C. W. (1987). An actin network is present in the cytoplasm throughout the cell cycle of carrot cells and associates with the dividing nucleus. *J. Cell Biol.* **105**, 387–395.

VENVERLOO, C. J., HOVENKAMP, P. H., WEEDA, A. J. AND LIBBENGA, K. R. (1980). Cell division in *Nautilocalyx* explants. I. Phragmosome, preprophase band and plane of cell division. *Z. PflPhysiol.* **100**, 161–174.

VENVERLOO, C. J. AND LIBBENGA, K. R. (1987). Regulation of the plane of cell division in vacuolated cells. I. The function of nuclear positioning and phragmosome formation. *J. Pl. Physiol.* **131**, 267–284.

WACKER, I., QUADER, H. AND SCHNEPF, E. (1988). Influence of the herbicide oryzalin on cytoskeleton and growth of *Funaria hygrometrica* protonemata. *Protoplasma* **142**, 55–67.

WADA, M. AND FURUYA, M. (1970). Photocontrol of the orientation of cell division in *Adiantum*. I. Effects of the dark and red periods in the apical cells of gametophytes. *Dev. Growth Differ.* **12**, 109–118.

WADA, M. AND FURUYA, M. (1972). Phytochrome action on the timing of cell division in *Adiantum* gametophytes. *Pl. Physiol.* **49**, 110–113.

WADA, M., HAYAMI, J. AND KADOTA, A. (1984). Returning dark-induced cell cycle to the beginning of G1 phase by red light irradiation in fern *Adiantum* protonemata. *Pl. Cell Physiol.* **25**, 1053–1058.

WADA, M. AND KADOTA, A. (1989). Photomorphogenesis in lower green plants. *A. Rev. Pl. Physiol. molec. Biol.* **40**, 169–191.

WADA, M., MINEYUKI, Y., KADOTA, A. AND FURUYA, M. (1980). The change of nuclear position and distribution of circumferentially aligned cortical microtubules during the progression of cell cycle in *Adiantum* protonemata. *Bot. Mag. Tokyo* **93**, 237–245.

WADA, M. AND O'BRIEN, T. P. (1975). Observations on the structure of the protonema of *Adiantum capillus-veneris* L. undergoing cell division following white-light irradiation. *Planta* **126**, 213–227.

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