Planta (1991) 183:391–398

Planta

Effects of centrifugation on preprophase-band formation in *Adiantum* protonemata

Takashi Murata and Masamitsu Wada

Department of Biology, Faculty of Science, Tokyo Metropolitan University, Fukazawa, Tokyo 158, Japan

Received 25 April; accepted 9 October 1990

Abstract. Protonemata of *Adiantum capillus-veneris* L., grown in red light and consisting of a single elongate cell, were centrifuged at various times after or just before the induction of synchronous cell division by irradiation with blue light (BL), and formation and location of preprophase bands (PPBs) of microtubules (MTs) were observed by immunofluorescence microscopy. The cells divided approx. 14–20 h after the onset of BL irradiation, irrespective of the timing of centrifugation. Basipetal centrifugation caused basipetal displacement (about 100 μm) of the nucleus. The nucleus did not return to its original position and cell division occurred at the new nuclear position. A PPB was formed in the region of the displaced nucleus except in some cells which presumably had been centrifuged just before cell division. Microtubule behavior in the apical region, where cell division occurred in non-centrifuged cells, depended on the time of centrifugation relative to the cell cycle. When protonemata were centrifuged before PPB formation, no PPB was formed in the apical region of most cells but, in some cells, another MT band indistinguishable from a PPB (we consider it PPB) was formed at this site after the displacement of the nucleus. When protonemata were centrifuged during or after PPB formation, the PPB, which had already formed in the apical region, did not disappear until the sedimented nucleus entered telophase or early interphase, while a new PPB was formed in most cells in the region of the displaced nucleus. These cells had thus two PPBs: one in the apical region, and another in the region where the displaced nucleus was located. Double centrifugation to displace the nucleus twice showed that once a PPB formed, irrespective of its intracellular position, it remained after the displacement of the nucleus. However, no PPBs were observed in the region where the nucleus was located after the first centrifugation if the nucleus was again displaced before a PPB had formed in this region. The positioning of the cell plate was also examined. When centrifugation was performed just before cell division, PPB formation around the displaced nucleus decreased and oblique cell plates were observed at an increased frequency. In conclusion, preprophase nuclei may have the ability to induce PPB formation in the neighboring cell cortex, and PPBs, in turn, may have a role in governing the orientation of cell plates.

Key words: *Adiantum* – Cell division – Microtubule – Preprophase band – Protonema (fern)

Abbreviations: BL = blue light; DAPI = 4′,6′-diamidino-2-phenylindole; MT = microtubule; PPB = preprophase band

Introduction

Spatial regulation of cell division is essential for morphogenesis in plants, in which morphogenetic cell movements cannot take place because of the presence of rigid cell walls. The preprophase band (PPB) of microtubules (MTs) is considered to play an important role in the determination of the division site (Gunning 1982). Immunofluorescence microscopy has recently made it possible to follow the process of PPB development in various cell types in detail (Wick and Duniec 1983, 1984; Simmonds 1986; Brown and Lemmon 1988; Cho and Wick 1989; Mineyuki et al. 1989; see Lloyd 1987 for review). However, both the function of the PPB and the mechanism by which its location is regulated are still unknown.

The technique of cell centrifugation has been used to study the role of nucleus and cytoplasm in the determination of division sites (e.g., Ôta 1961; Schmiedel and Schnepf 1980; Galatis et al. 1984) but, as pointed out by Gunning (1982), the effect of centrifugation on PPB formation in relation to the cell cycle, has not been investigated. To study the effect of centrifugation at specific times during the cell cycle, the timing of cell division should be synchronous and predictable, and a long cell is also desirable so that the nucleus can be displaced over a sufficiently large distance. Protonemata of *Adiantum* grow under red light without cell division, producing

an elongate cell, while blue light (BL) induces synchronous cell division in the apical region of the cell (Miyata et al. 1979; Murata and Wada 1989). In the present paper, we report the results of studies on the effect of centrifugation on PPB formation in synchronously dividing *Adiantum* protonemal cells, and also on the positioning and orientation of the cell plate.

Material and methods

Plant material. Culture methods for *Adiantum capillus-veneris* L. protonemata were as described in Murata and Wada (1989) except for the length of the culture period under red light and the fluence of BL. Briefly, spores of *Adiantum capillus-veneris* L. were sown between thin gelatin-agar films, and cultured in modified Murashige and Skoog's mineral salt solution for 1 d in the dark and then for 6 d under red light (approx. 0.5 $W \cdot m^{-2}$). The resulting protonemata were transferred to BL (approx. 2 $W \cdot m^{-2}$) to induce cell division. When L-shaped protonemata were desired, the direction of red light was changed by approx. 70–90° 12–20 h before the transfer to BL. All experiments were performed at 25° C.

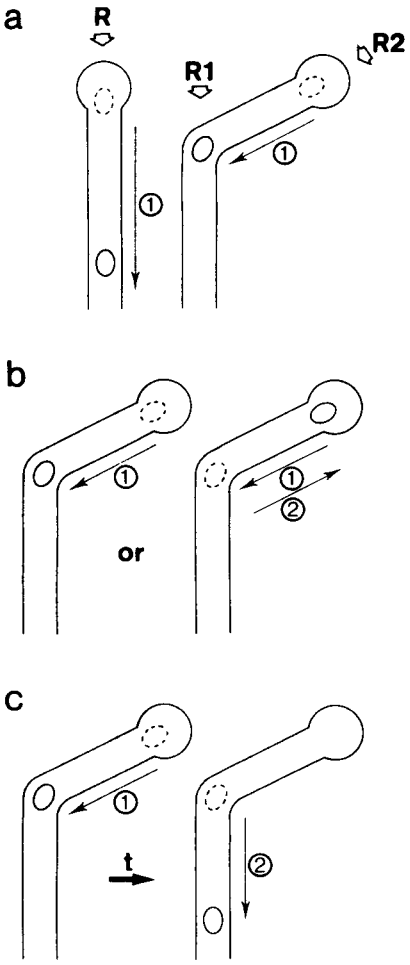


Fig. 1a–c. Schematic representation of methods of centrifugation. *Ellipses* in the protonemata show the position of the nucleus. *Thin arrows* show direction of centrifugal force. *Open arrows* in **a** show direction of red light during pre-culture period. Blue light is applied perpendicular to the page. **a** Single centrifugation for analysis of PPB formation. A linear (*left*) or bent (*right*) protonema is centrifuged at 2800·*g* for 15 min. **b** Centrifugation for analysis of orientation of the cell plate. A protonema is centrifuged at 2800·*g* for 15 min as in the right-hand protonema of **a** (*left*), and in some cases re-centrifuged immediately at 2800·*g* for 15 min (*right*). **c** Double centrifugation for analysis of PPB formation. A protonema is centrifuged at 2100·*g* for 15 min, and after scheduled time intervals (*t*) re-centrifuged at 2800·*g* for 15 min

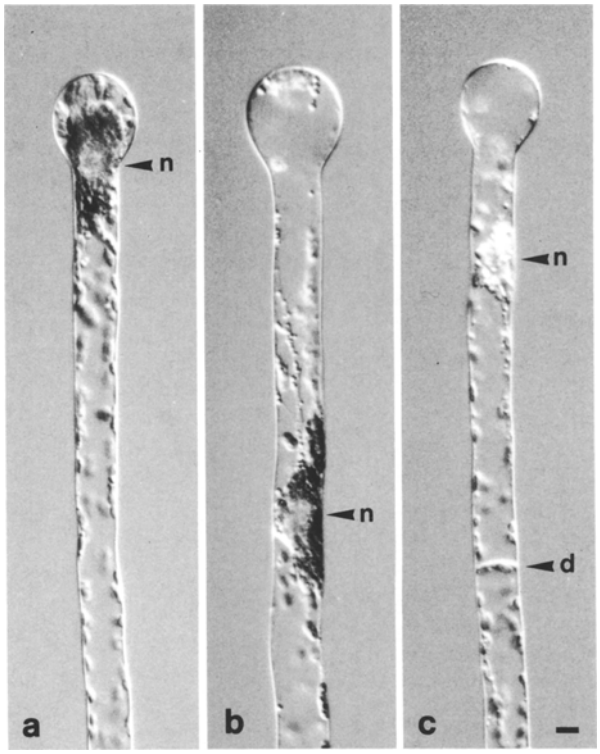


Fig. 2a–c. Photomicrographs showing the effects of centrifugation on organelle distribution and cell division in protonemal cells of *Adiantum capillus-veneris* L. Protonemata were centrifuged (2800·*g*, 15 min) 10 h after the onset of BL. Nomarski optics. *n* = position of the nucleus. **a** Before centrifugation. **b** Just after centrifugation. **c** 9 h after centrifugation, showing cell division (marked with *d*). Bar = 10 μm; ×310

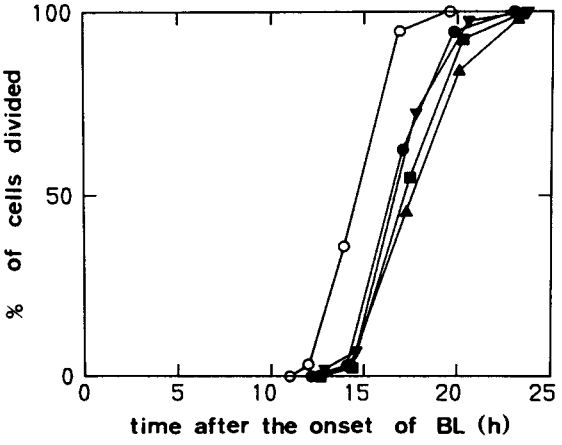


Fig. 3. Time-courses of cell division in protonemal cells of *Adiantum capillus-veneris* L. under BL. The protonemata were centrifuged just before BL irradiation (●–●) or 4 (▲–▲), 8 (■–■), 12 (▼–▼) h after BL irradiation; ○–○ non-centrifuged cells. Average from three or four dishes in which 50 cells each were counted. Standard errors are less than 9.0%

Centrifugation. Centrifugation was performed according to Wada et al. (1983). The linear or L-shaped protonemata were transferred into a custom-made centrifuge cuvette under appropriate (blue or red) light, and centrifuged basipetally at 2800·*g* for 15 min in the dark at 25° C (Fig. 1a). In some cases, protonemata were subsequently centrifuged acropetally at 2800·*g* for 15 min immediately after the first centrifugation (Fig. 1b). In these double-centrifugation experiments, L-shaped protonemata were centrifuged first at 2100·*g*, and after an interval of 3.5–8.5 h at 2800·*g*. Centrifugal forces in both centrifugations were applied toward the cell base (Fig. 1c). The centrifuged protonemata were cultured in their cuvettes under BL, without further transfer. Culturing in the cuvette without centrifugation affected neither the timing and position of cell division nor the MT arrangement.

Immunofluorescence microscopy. The methods used for immunofluorescence microscopy were the same as described in Murata and Wada (1989). Briefly, the protonemata were fixed in a solution of 8% paraformaldehyde, 0.2% picric acid, 1% dimethylsulfoxide, 5 mM ethyleneglycol-bis-(α-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 0.5 mM phenylmethylsulfonyl fluoride dissolved in 0.1 M sodium-phosphate buffer (pH 7.0). Protonemal cells were cut with a small piece of a razor blade, made permeable with a detergent solution, and treated with anti-tubulin antibody followed by a fluorescein-linked second antibody. The cells were mounted with mounting medium containing 4′,6′-diamidino-2-phenylindole (DAPI), and observed using an epifluorescence microscope (Axioplan; Carl Zeiss, Oberkochen, Germany).

Results

Effects of centrifugation on position and timing of cell division. Without centrifugation and before BL irradiation, the nucleus of the protonemal cell is located approx. 60 μm from the tip (Wada et al. 1980) and under BL divides approx. 30–40 μm from the tip (Murata and Wada 1989), i.e. cell division occurs in the apical region

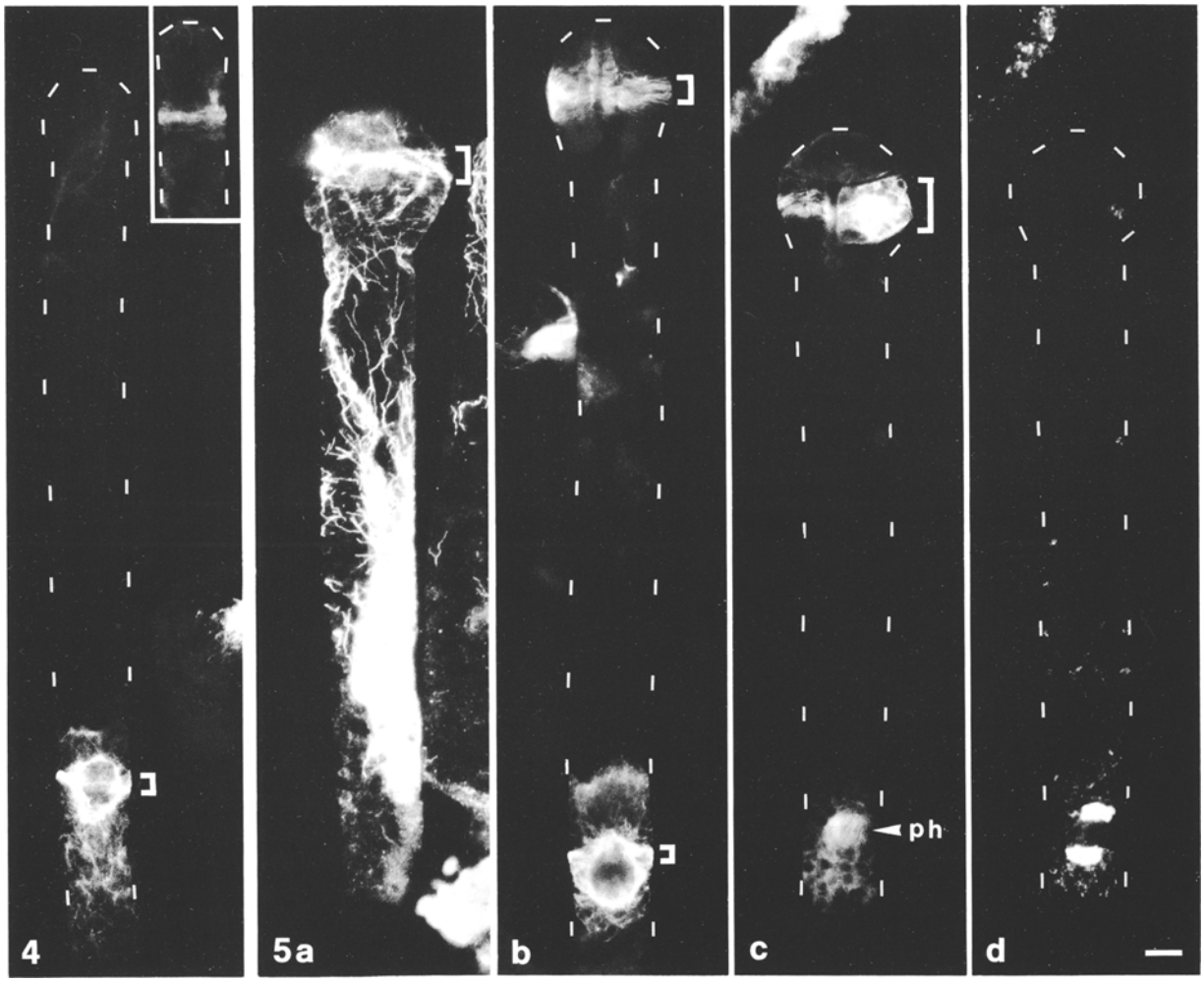


Fig. 4. Fluorescence micrographs showing the distribution of PPBs (14 h after the onset of BL) in protonemal cells of *Adiantum capillus-veneris* L. centrifuged before PPB formation. The protonema was centrifuged (2800·*g*, 15 min) 7 h after the onset of BL. A PPB is marked with a *bracket*. *Inset* shows a PPB formed in the apical region of a cell centrifuged just before BL irradiation. *Dotted lines* show the outline of cells. ×455

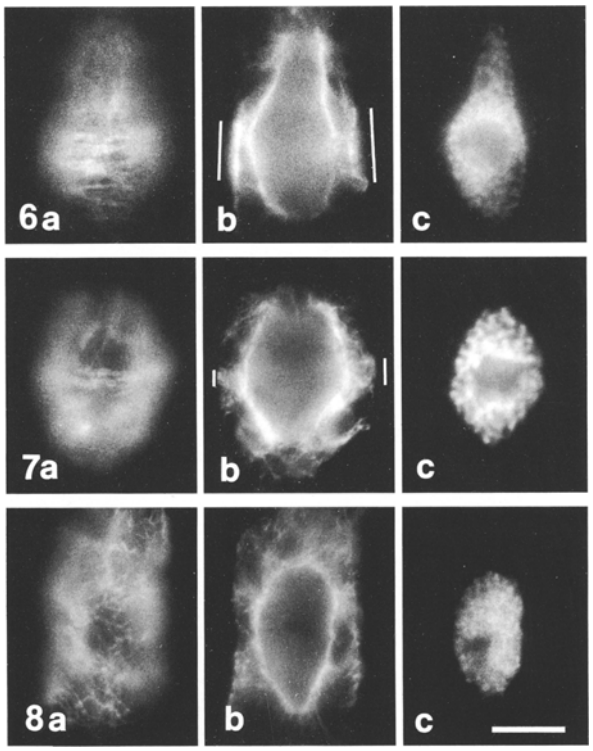
Fig. 5a–d. Fluorescence micrographs showing the pattern of PPBs in protonemal cells of *Adiantum capillus-veneris* centrifuged (2800·*g*, 15 min) after PPB formation. Protonemata were centrifuged 11–12 h after the onset of BL. Preprophase bands are marked with *brackets*; *dotted lines* show the outline of cells. **a** Just after the centrifugation; **b–d** 14 h after the onset of BL (approx. 3–4 h after centrifugation); **b** two PPBs formed in a single cell; **c** coexistence of a PPB and a phragmoplast (*ph*); **d** DAPI image of the same region as in **c**, showing telophase nuclei. Bar = 10 μm; ×455

of the long filamentous cell, near the site occupied by the nucleus before division. The nucleus is surrounded by apical cytoplasm which contains many chloroplasts, oil droplets and mitochondria (Wada and O'Brien 1975). When protonemata were centrifuged basipetally at 2800·*g* at various times just before or after transfer to BL, the nucleus and most organelles except the oil droplets were displaced basipetally (Fig. 2a, b). The amount of displacement of the nucleus varied from cell to cell and was also dependent on the timing of centrifugation. To obtain a population of protonemata with as uniformly displaced nuclei as possible for quantitative analysis, protonemata were made to bend at approx. 150–200 μm below the tip by changing the direction of the red light (see *Material and methods*), and centrifuged along the new axis of growth. The organelles were then displaced to the bend but not further downwards (see Fig. 9b).

After centrifugation was stopped, the nucleus migrated several tens of a micrometer toward the tip or base. When bent protonemata were used, most nuclei were located at the bend just after centrifugation so that subsequent nuclear migration, after centrifugation, could be easily measured. The magnitude of this migration varied but was generally greater in cells centrifuged earlier in the cell cycle (i.e., sooner after transfer to BL). No nucleus returned to its original position. Cell division occurred at the position occupied by the displaced nucleus. The orientation of the cell plate will be described in a later section.

Examination of the effect of centrifugation on the timing of cell division (Fig. 3) showed that, as described previously (Murata and Wada 1989), non-centrifuged cells divided 11–18 h after the transfer to BL; centrifuged cells divided approx. 14–20 h after the transfer, irrespective of the timing of centrifugation.

Formation of PPBs. Without centrifugation, a PPB starts to develop in the apical region approx. 9 h after the transfer of the protonema to BL (Murata and Wada 1989). When protonemata were centrifuged before PPB formation (between just before and 7 h after transfer to BL) and fixed 14 h after the transfer, a PPB was found at the new nuclear region (Fig. 4). In some cells (approx. 10–20%; Table 1), an MT band indistinguishable from a PPB was also found in the apical region, i.e. the site where PPBs develop in non-centrifuged cells (Fig. 4, inset) even though the nucleus was no longer present in that region.



Figs. 6a–c to 8a–c. High-magnification micrographs of the nuclear region of centrifuged protonemal cells of *Adiantum capillus-veneris*. Microtubules in cell periphery (**a**) and in the plane of the nucleus (**b**); DAPI image (**c**) shows prophase nuclei. Bar = 10 μm; ×950

Fig. 6a–c. Well-developed PPB in a cell centrifuged before PPB formation. In **b**, a PPB is seen in cross-section (*white lines*)
Fig. 7a–c. Faint PPB in a cell centrifuged after PPB formation. A PPB is also seen in cross-section (**b**, *white lines*)
Fig. 8a–c. No PPB formed around the displaced nucleus in this cell centrifuged after formation of the apical PPB

Table 1. The distribution of PPBs in the *Adiantum* protonema filament in relation to the timing of a single centrifugation (2800·*g*, 15 min)

Timing of centrifugation after the onset of BL irradiation (h)	Location(s) of PPB(s) after centrifugation (%)[a]				Cells with PPBs present at the time of centrifugation (%)[c]
	Apical region only	Both regions	Nuclear region only	Not determined[b]	
0[d]	0	12.7	74.5	12.8	–
4	0	22.4	69.0	8.6	–
7	0	23.2	58.9	17.9	2.9
10	1.9	59.3	16.7	22.1	40.9
12	21.9	53.1	0	25.0	88.9

[a] Preprophase bands were observed 14 h after the onset of BL irradiation, and 32–95 cells were examined for each time point
[b] Cells before PPB formation or after prophase
[c] Number of cells examined was 35–66. A PPB was present in the apical region only; no MT bands were observed in the cylindrical region
[d] Just before the onset of BL irradiation

Table 2. Relationship between timing of centrifugation (2800·*g*, 15 min) and frequency of PPB formation in *Adiantum* protonema cells. The percentage of PPBs in the nuclear region of prophase cells was calculated. Number of cells observed shown in parentheses

Treatment	Prophase cells without PPB (%)	
	Expt. 1[a]	Expt. 2[b]
No centrifugation	–	0 (48)
Centrifugation at 7 h	0 (40)	2.0 (48)
Centrifugation at 12 h	40.6 (32)	14.3 (21)

[a] Cells were fixed 14 h after the onset of BL irradiation
[b] Fixation was at 13 and 15 h in non-centrifuged and centrifuged cells, respectively

Table 3. The frequency of PPBs in the apical region of centrifuged *Adiantum* protonema cells in relation to the stage of mitosis. Protonemata were centrifuged (2800·*g*, 15 min) after 12 h and fixed after 14–15.5 h of BL. Non-centrifuged cells were fixed after 13.5–14 h. Number of cells observed shown in parentheses

Stages	Cells with PPB in the apical region (%)	
	Non-centrifuged cells	Centrifuged cells
Prophase	100 (135)	96.4 (83)
Prometaphase	62.9 (35)	100 (30)
Metaphase	0 (56)	90.3 (31)
Anaphase	–	76.2 (21)
Telophase	–	50.0 (52)
Early interphase[a]	–	2.2 (45)

[a] Counted were cells in which the phragmoplast had disappeared and recovery of the interphase MT array was not completed

When protonemata were centrifuged after the start of PPB formation (9–12 h after transfer to BL), the PPB that had been partially or fully formed in the apical region was present until the early interphase of the next cell cycle (Fig. 5b–d) although interphase MTs which are found adjacent to the PPB (Fig. 5a) at an early stage of PPB development (Murata and Wada 1989) were disrupted by the late prophase (Fig. 5b). Close to the displaced nucleus, a new PPB was formed in many cells but it was thinner and fainter than that formed in cells centrifuged before PPB formation (Figs. 6, 7). Consequently, many cells had two PPBs ("Both regions" in Table 1): the old PPB in the apical region, and a newly formed PPB around the displaced nucleus, although sometimes slightly above the equatorial plane of the latter (Fig. 5b). In some cells, however, no new PPB was formed even when the cells entered prophase (Fig. 8, Table 2), and the frequency of such cells was the greater the later the timing of centrifugation (shown as "Apical region only" in Table 1).

The timing of PPB disruption in centrifuged cells was quantitatively studied (Table 3). In non-centrifuged cells, PPBs disintegrated during prometaphase. In centrifuged cells, PPBs at the new nuclear region disintegrated also, at least until metaphase (data not shown). However, the PPBs in the apical region persisted in metaphase and only then began to disintegrate, 50% of the apical PPBs persisting during telophase, but disintegrating by the early interphase of the next cell cycle.

Double-centrifugation experiments. The above experiments show that pre-existing PPBs in the apical region remain stable after centrifugation. To test the possibility that the normal division site (i.e. the apical region) is especially stable for PPBs, we performed double-centrifugation experiments using L-shaped protonemata, obtained by changing of direction of red light (see *Material and methods*; Fig. 9). To displace the nucleus stepwise, the protonemata were centrifuged twice (Figs. 1c, 9), first 4 h (2100·*g*, 15 min; Fig. 9b) and then 7.5–12.5 h (2800·*g*, 15 min; Fig. 9c) after transfer to BL. Protonemal cells began to divide approx. 14 h after the onset of BL, although the precise time course of cell division

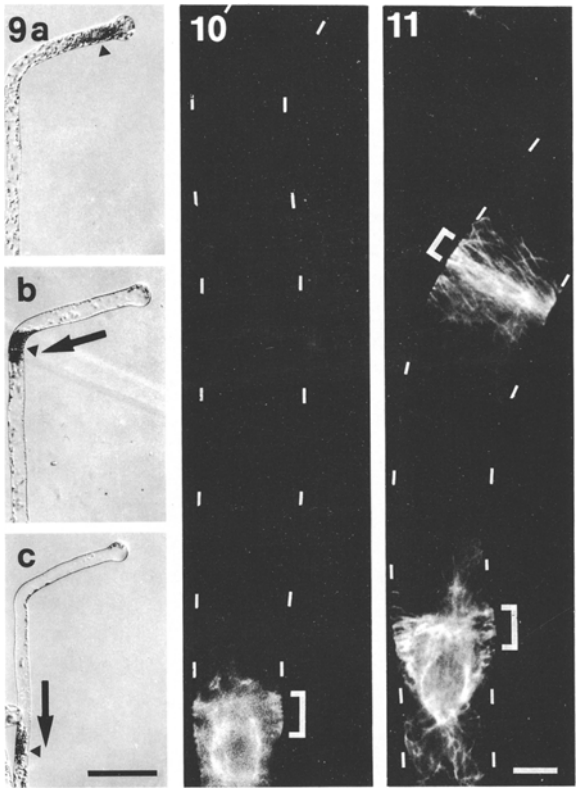


Fig. 9a–c. Photomicrographs showing bent protonemata and organelle sedimentation by double centrifugation in *Adiantum capillus-veneris*. *Arrows* show the direction of centrifugal force; *small arrowheads* indicate nuclear position. Nomarski optics. **a** Before centrifugation; **b** just after the first centrifugation; **c** just after the second centrifugation. Bar = 100 μm; × 90

Figs. 10, 11. Effects of double centrifugation on PPB formation in protonemal cells of *Adiantum capillus-veneris*. Bent protonemata were centrifuged 4 and 8.5 (**Fig. 10**) or 4 and 12.5 (**Fig. 11**) h after the onset of BL, and fixed at 14.5 h. The region between the bend and the nucleus is shown. A PPB (marked with a *bracket*) is present only in the nuclear region in Fig. 10, but a second PPB is present also in the bend region of Fig. 11. *Dotted lines* indicate cell outlines. Bar = 10 μm; × 530

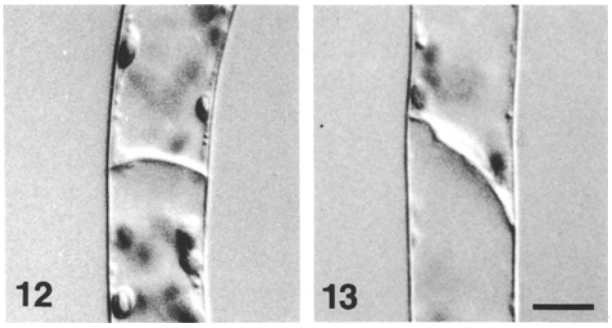
Table 4. The distribution of PPBs in *Adiantum* protonema cells in relation to the timing of a second centrifugation (2800·*g*, 15 min). First centrifugation (2100·*g*, 15 min) was performed 4 h after the onset of BL

Timing of 2nd centrifugation after BL irradiation (h)	Location(s) of PPB(s) after the 2nd centrifugation (%)[a]				Cells with PPBs present at the time of centrifugation (%)[c]
	Bending region only	Both regions	Later nuclear region only	Not determined[b]	
7.5	0	4.5	73.1	22.4	10.9
10	0	26.5	67.3	6.2	61.1
12.5	30.2	53.5	7.0	9.3	77.0

[a] Preprophase bands were observed 14.5 h after the onset of BL irradiation; 43–67 cells examined. Formation of a MT band in the apical region is disregarded in this table
[b] Cells before PPB formation or after prophase
[c] The percent of cells with a PPB at the nuclear region (bending region) was counted just before the second centrifugation; 108–128 cells examined

was not determined. The first centrifugation (at 4 h) moved the nucleus to the bend. A PPB which had been formed in the position where the nucleus came to rest after the first centrifugation (bending region) remained intact after the nucleus was further displaced by the second centrifugation (Fig. 11). In contrast to the case of single centrifugations, the frequency of PPB appearance in the bending region where the nucleus was located after the first centrifugation did not increase after the second centrifugation (Table 4, Fig. 10), indicating that further PPBs did not form in the bending region after displacement of the nucleus. The PPB in the apical region was, however, observed irrespective of the timing of the second centrifugation (data not shown).

Orientation of the cell plate. The orientation of the cell plate in centrifuged cells was also examined. In protonemata centrifuged 12 h after the onset of BL, in some of which no PPB had been formed around the nucleus (Table 3), the frequency with which an oblique cell plate was formed (Figs. 12, 13) increased greatly. The increase was far greater than that in protonemata centrifuged 7 h after the onset of BL (Table 5) in which a PPB had been formed in most prophase cells (Table 3). Acropetal centrifugation following a first, basipetal centrifugation to return the cytoplasm and the nucleus to their former positions (Fig. 1b) greatly decreased the percentage of oblique cell plates (Table 5), indicating that centrifugal damage had little effect on the orientation of the cell plate and that formation of a PPB is essential for normal (transverse) cell-plate orientation in *Adiantum* protonemata.



Figs. 12, 13. Cell-plate arrangement in a centrifuged protonemal cell of *Adiantum capillus-veneris*. Protonemata were centrifuged 12 h, and observed 19 h after the onset of BL. Nomarski optics. Bar = 10 μm; ×770. **Fig. 12.** Transverse cell plate. **Fig. 13.** Oblique cell plate

Table 5. Effect of centrifugation (2800·*g*, 15 min) on the orientation of the cell plate in *Adiantum* protonema cells. Protonemata were centrifuged 7 or 12 h after the onset of BL. Mean ± SD from three or four Petri dishes in which 50 cells each were counted

Treatment	Oblique cell plate (%)	
	Expt. 1	Expt. 2
No centrifugation[a]	–	2.7 ± 2.3
Centrifugation at 7 h[b]	1.3 ± 2.3	10.5 ± 7.0
Centrifugation at 12 h[b]	48.5 ± 5.3	40.0 ± 4.3
Centrifugation at 12 h (basipetal→acropetal)[b,c]	6.0 ± 5.3	6.5 ± 2.5

[a] Counted 22–23 h after the onset of BL irradiation
[b] Counted 24–27 h after the onset of BL irradiation
[c] Cells were centrifuged twice; first basipetally and then acropetally

Discussion

The role of the nucleus and-or associated cytoplasm in determination of PPB sites. It has been generally believed that the position of the nucleus does not determine the position of the PPB. In asymmetric division in *Azolla* root cells, a PPB is formed first and the nucleus reaches this site later (Gunning et al. 1978). In the mother cells of the stomatal subsidiary cells of *Triticum*, sedimentation of both the nucleus and the cytoplasm by continuous centrifugation does not change the PPB site (Galatis et al. 1984). However, in these studies PPB positioning was examined in relatively small cells. In the asymmetric divisions in *Azolla* roots, the nucleus migrates only approx. 6 μm, a distance equivalent to its diameter. In the case of subsidiary-cell mother cell of *Triticum*, the distance between the PPB site and the displaced nucleus is less than 20 μm. In our material the nucleus was displaced approx. 100 μm or more from the original division site, and did not return to its original position after

centrifugation. In about 10–20% of the protonemata centrifuged prior to PPB formation, a PPB did form in the apical region after the nucleus was displaced. Thus, we can not exclude the possibility that some unknown factor remained in the apical region after centrifugation and that this factor had the ability to initiate the formation of a PPB in the absence of a nearby nucleus. However, in most protonemata centrifuged prior to PPB formation, no PPB formed in the apical region where the nucleus resided prior to centrifugation. An alternative PPB was formed near the region of the sedimented nucleus and cytoplasm. These results demonstrate that in *Adiantum* protonemal cells the positioning of PPBs is influenced by the position of the nucleus and-or the cytoplasm closely associated with it.

The timing of determination of PPB sites. Preprophase bands are known to appear in the G2 phase of the cell cycle or later (Mineyuki et al. 1988; Simmonds 1986). However, the timing of the determination of the PPB position has not been studied because it is experimentally difficult to induce a PPB at any desired site in a cell or to inhibit it. In the present study of the single-celled protonemal cells of *Adiantum*, we were able to analyse this timing, making use of the fact that the position of cell division in these protonemata is influenced by the position of the nucleus.

When protonemal cells were centrifuged before PPB formation, PPBs appeared in most cells only around the sedimented nucleus in its new position (Table 1), indicating that "determination" of PPB sites before the start of PPB development in the cortical cytoplasm does not generally occur. However, in some cells, a PPB appeared in the apical region of the cell even when the cell was centrifuged before induction of cell division. Its formation indicates that at least in these exceptional cases the determination of PPB sites can occur at an earlier stage of the cell cycle, and once PPB formation is triggered, a PPB can develop without close proximity of the nucleus.

Two possibilities can be envisaged for the formation of two PPBs in a centrifuged cell. The first possibility is that the nucleus continually sends out a signal to form a PPB in its neighboring cortical cytoplasm until PPB formation is completed and centrifugation is performed during this period. The second possibility is that the nucleus can trigger PPB formation only during a very short period of its nuclear cycle; in other words, the nucleus is needed only for the initiation of PPB formation, and the PPB continues to develop independently of the nucleus. In this case, the two PPBs formed in a centrifuged cell indicate that the nuclear capacity to initiate PPB formation was resumed when the nucleus had been displaced. In further work it will be important to determine the time at which the nuclei lose the capacity to form a PPB, in order to distinguish between these possibilities. The decrease of PPB formation at the new nuclear region in cells centrifuged 12 h after the onset of BL (i.e. in a late stage of PPB development) seen in Table 2, can be explained in at least two ways: (i) The nuclei and-or co-sedimented cytoplasm do not have the ability to induce the onset of PPB formation in neighbouring cortical cytoplasm at later stages of PPB development (i.e., late prophase); (ii) there is not sufficient time for the formation of a PPB.

Preprophase-band stability. Our results from single and double centrifugation of protonemata indicate that PPBs that had been formed prior to centrifugation remain intact after the nucleus has been displaced. In fact, if the nucleus was displaced, PPBs persisted later into mitosis than in control protonemata. Thus, while nuclei during PPB formation seem to be able to induce the formation of PPBs, close proximity of a nucleus is not required for PPB stability. It is noteworthy that this observation held true in the double-centrifugation experiments, in which a PPB was first induced to form at the bent region where the nucleus came to rest after the first centrifugation, and then persisted after the nucleus had been displaced by the second one. This indicates that no elements are also exclusively found in the cortical cytoplasm or plasma membrane in the apical region, where the nucleus normally resides, that are required for PPB stability. The finding that PPBs persisted longer into mitosis (anaphase or telophase, versus prometaphase in controls) if the nucleus was displaced may indicate that the timing of PPB disruption is in some way regulated by the nucleus or some other organelles that sediment with the nucleus (also Y. Mineyuki and B.A. Palevitz, University of Georgia, Athens, USA, personal communication). Such a mechanism might be expected if the hypothesis is correct that tubulin molecules from PPBs are incorporated into the mitotic spindle (Pickett-Heaps 1969; Wick and Duniec 1984).

Role of PPBs in cell-plate formation. When *Adiantum* protonema cells were centrifuged at later stages of PPB development they divided without PPB formation in the area of the sedimented nucleus, indicating that the development of mitotic spindles and cell plates can proceed without an associated PPB. An oblique orientation of the cell plate was also observed more frequently when centrifugation was performed at later times. Thus, we suggest that while PPBs may not be required for mitosis or cytokinesis to occur, they may have a role in regulating the orientation of the cell plate.

This work was supported in part by Grant-in-Aid for General Scientific Research (No. 01540567) from the Ministry of Education, Science and Culture in Japan. The authors are grateful to Dr. T.H. Giddings Jr. (University of Colorado, Boulder, USA) for critical reading of the manuscript.

References

Brown, R.C., Lemmon, B.E. (1988) Preprophasic microtubule systems and development of the mitotic spindle in hornworts (Bryophyta). Protoplasma **143**, 11–21

Cho, S.-O., Wick, S.M. (1989) Microtubule orientation during stomatal differentiation in grasses. J. Cell Sci. **92**, 581–594

Galatis, B., Apostolakos, P., Katsaros, C. (1984) Experimental studies on the function of cortical cytoplasmic zone of the preprophase microtubule band. Protoplasma **122**, 11–26

Gunning, B.E.S. (1982) The cytokinetic apparatus: Its development and spatial regulation. In: The cytoskeleton in plant growth and development, pp. 229–292, Lloyd, C.W., ed. Academic Press, London

Gunning, B.E.S., Hardham, A.R., Hughes, J.E. (1978) Pre-prophase bands of microtubules in all categories of formative and proliferative cell division in *Azolla* roots. Planta **143**, 145–160

Lloyd, C.W. (1987) The plant cytoskeleton: The impact of fluorescence microscopy. Annu. Rev. Plant Physiol. **38**, 119–139

Mineyuki, Y., Wick, S.M., Gunning, B.E.S. (1988) Preprophase bands of microtubules and cell cycle: Kinetics and experimental uncoupling of their formation from the nuclear cycle in onion root-tip cells. Planta **174**, 518–526

Mineyuki, Y., Marc, J., Palevitz, B.A. (1989) Development of the preprophase band from random cytoplasmic microtubules in guard mother cells of *Allium cepa* L. Planta **178**, 291–296

Miyata, M., Wada, M., 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. Develop. Growth Different. **21**, 577–584

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

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

Pickett-Heaps, J.D. (1969) Preprophase microtubules and stomatal differentiation; Some effects of centrifugation on symmetrical and asymmetrical cell division. J. Ultrastruct. Res. **27**, 24–44

Schmiedel, G., Schnepf, E. (1980) Polarity and growth of caulonema tip cells of moss *Funaria hygrometrica*. Planta **147**, 405–413

Simmonds, D.H. (1986) Prophase bands of microtubules occur in protoplast cultures of *Vicia hajastana* Grossh. Planta **167**, 469–472

Wada, M., 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

Wada, M., Mineyuki, Y., Kadota, A., 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., Kadota, A., Furuya, M. (1983) Intracellular localization and dichroic orientation of phytochrome in plasma membrane and/or ectoplasm of a centrifuged protonema of fern *Adiantum capillus-veneris* L. Plant Cell Physiol. **24**, 1441–1447

Wick, S.M., Duniec, J. (1983) Immunofluorescence microscopy of tubulin and microtubule arrays in plant cells. I. Preprophase band development and concomitant appearance of nuclear envelope-associated tubulin. J. Cell Biol. **97**, 235–243

Wick, S.M., Duniec, J. (1984) Immunofluorescence microscopy of tubulin and microtubule arrays in plant cells. II. Transition between the pre-prophase band and the mitotic spindle. Protoplasma **122**, 45–55