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Cytoskeletal aspects of nuclear migration during tip-growth in the fern *Adiantum* protonemal cell

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Summary. In the tip-growing protonemal cell, the nucleus migrates with the tip as it grows, keeping a constant distance between them. Cytoskeletal control of this nuclear migration was analyzed in *Adiantum capillus-veneris*. Using rhodamine-phalloidin (Rh-Phal), tubulin antibodies and confocal laser scanning microscopy, we found the presence of microtubule (MT) and microfilament (MF) strands connecting the cell nucleus to the cortex of the growing apex. The strands come from the apical end of the spindle-shaped nucleus and run through the endoplasm, arriving at the apical cortex, where a circular arrangement of MTs and MFs is present. Strands of MFs and MTs were also found to emanate from the proximal end of the nucleus and extend towards the cortex of the basal part of the cell. Double staining of MTs and MFs revealed a co-localization of these cytoskeletal elements. When MF strands were disrupted by cytochalasin B (CB), tip-growth ceased and nuclear movement stopped. After the application of colchicine, MT structures disappeared, tip-growth was largely inhibited, and the nucleus moved towards the basal part of the cell. When both CB and colchicine were applied to the cell, no basipetal migration of cell nucleus was observed. These results suggest that the MT strands between the apex and the nucleus may have a role in the anchorage of the cell nucleus to the tip during tip-growth, and that the MF strands may be important for basipetal movement of the nucleus. When the nucleus was dislocated basipetally by centrifugation, cytoskeletal strands between the cell apex and the nucleus were still observed, and by acropetal movement the nucleus resumed its previous position. The acropetal movement of the nucleus was inhibited by the application of both CB and colchicine but not by CB alone nor by colchicine alone, indicating that both cytoskeletal elements are involved in the forward movement of cell nucleus.

Keywords: *Adiantum capillus-veneris*; Microfilament; Microtubule; Nuclear migration; Protonema; Tip-growth.

Abbreviations: CB cytochalasin B; DAPI 4′,6-diamino-2-phenylindole; DMSO dimethylsulfoxide; PIPES piperazine-N,N′-bis(2-ethane-sulfonic acid); EGTA ethyleneglycol-bis-(β-aminoethyl-ether)-N,N,N′,N′-tetraacetic acid; MBS *m*-maleimidobenzoic acid N-hydroxysuccinimide ester; MF microfilament; MT microtubule; PMSF phenylmethylsulfonyl fluoride; PSM polyoxyethylene sorbitan monolaurate; Rh-Phal rhodamine-labeled phalloidin.

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Introduction

For many tip-growing cells, the nucleus moves in coordination with tip-growth, resulting in a constant distance between the tip and nucleus. These tip-growing cells thus offer a useful experimental system for the analysis of intracellular motility of the nucleus. As the nucleus moves much slower than the cytoplasmic streaming, this system is complementary to that of characean cells in which many smaller organelles are included in the rapid flow of the cytoplasm, and a great deal has been discovered on the involvement of actomyosin and on its regulation (Shimmen 1992). In this context, several studies have been done on nuclear migration in tip-growing cells, showing significant role of microtubules (MTs) in the migration of the nucleus (see reviews by Schnepf 1986, Nagai 1993, Williamson 1993). However, the participation of cytoskeletal elements in the movement of the nucleus is still unclear in terms of the roles of MTs and microfilaments (MFs). Here, we chose a protonemal cell of *Adiantum* at haplophase as an experimental system. Under red light, filamentous fern protonemata grow at the apex without cell division, showing typical tip-growth. As the protonemal cell grows, the nucleus moves with the cell apex keeping a constant distance of about 60–70 μm between the tip and the nucleus

(Wada et al. 1980). We analyzed the intracellular organization of MFs and MTs and examined the effects of cytoskeletal inhibitors on nuclear movement.

Materials and methods

Plant material and aseptic culture

Spores of *Adiantum capillus-veneris* L. were collected in the summer of 1986 and were stored at 5 °C in the dark until use. They were sterilized for 7–8 min with a 0.5% solution of sodium hypochlorite (10-fold diluted Antiformin; Wako Pure Chemical Industries, Ltd., Osaka) which contained 0.1% Triton X-100, and were sown in a line on a membrane placed on a cover slip. The membrane was made from 0.05% gelatin and 0.5% agar. Spores were covered with another agar-gelatin membrane and were placed at the bottom of a petri dish that contained 10% modified Murashige and Skoog's mineral salt solution (Kadota and Furuya 1977). The spores were cultured for 7 or 8 days at 25 °C under continuous red light of ca. 0.5 W/m^2 which was applied horizontally. Red light was obtained from a fluorescent lamp (FL40SD or FL10D; Toshiba Lighting and Technology Corp., Tokyo) with a 3 mm thick, red, acrylic plastic plate (Shinkolite A, #102; Mitsubishi Rayon Co., Ltd., Tokyo).
Unless otherwise stated, all the experimental and staining procedures described below were done at 25 °C.

Analyses of tip-growth and nuclear migration

Protonemata in a petri dish were placed on the stage of an inverted microscope (TMD, Nikon, Tokyo), maintaining the horizontal illumination with red light. The progression of tip-growth and nuclear migration in individual protonemal cells was monitored and recorded continuously under infrared light with the aid of an infrared light-sensitive video camera (C-2400-07ER; Hamamatsu Photonics K. K., Hamamatsu, Japan) coupled with a time-lapse video recorder (AG-6720; Matsushita Electric Co. Ltd., Kadoma, Japan). Infrared light was obtained by passing light from the light source of the microscope through an infrared light-transmitting filter (IR85; Hoya Corp., Akishima, Japan). Images were printed out with a video copy processor (SCT-P66; Mitsubishi Electric Co. Ltd., Tokyo). Positions of cell tip and nucleus (center of nucleus) in the images were determined by digitizer (KD3200; Graphtec Co., Tokyo) connected to a personal computer and using custom-made software. Changes in positions during various treatments were plotted against time for each individual protonemal cell.

Drug treatments

Drugs were added to the medium and the protonemata were cultured for further periods of time under red light. Colchicine and cytochalasin B (CB) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), respectively, and used at a concentration of 5 mM and of 104 µM (50 µg/ml), respectively. CB was applied in 0.5% dimethylsulfoxide (DMSO).

Cell centrifugation

Centrifugation of protonemata was conducted according to the methods described by Wada et al. (1983). Briefly, protonemal cells were transferred into a custom-made centrifuge cuvette (25 mm in diameter, 5 mm in thickness) and centrifuged basipetally at 510 **g** for 15 min in a centrifuge (05PR-2; Nissei Sangyo Co., Ltd., Tokyo) with a swinging bucket rotor (G assembly). After centrifugation, cells were transferred back into a petri dish containing culture medium with or without drugs.

Staining procedure

Microfilament staining

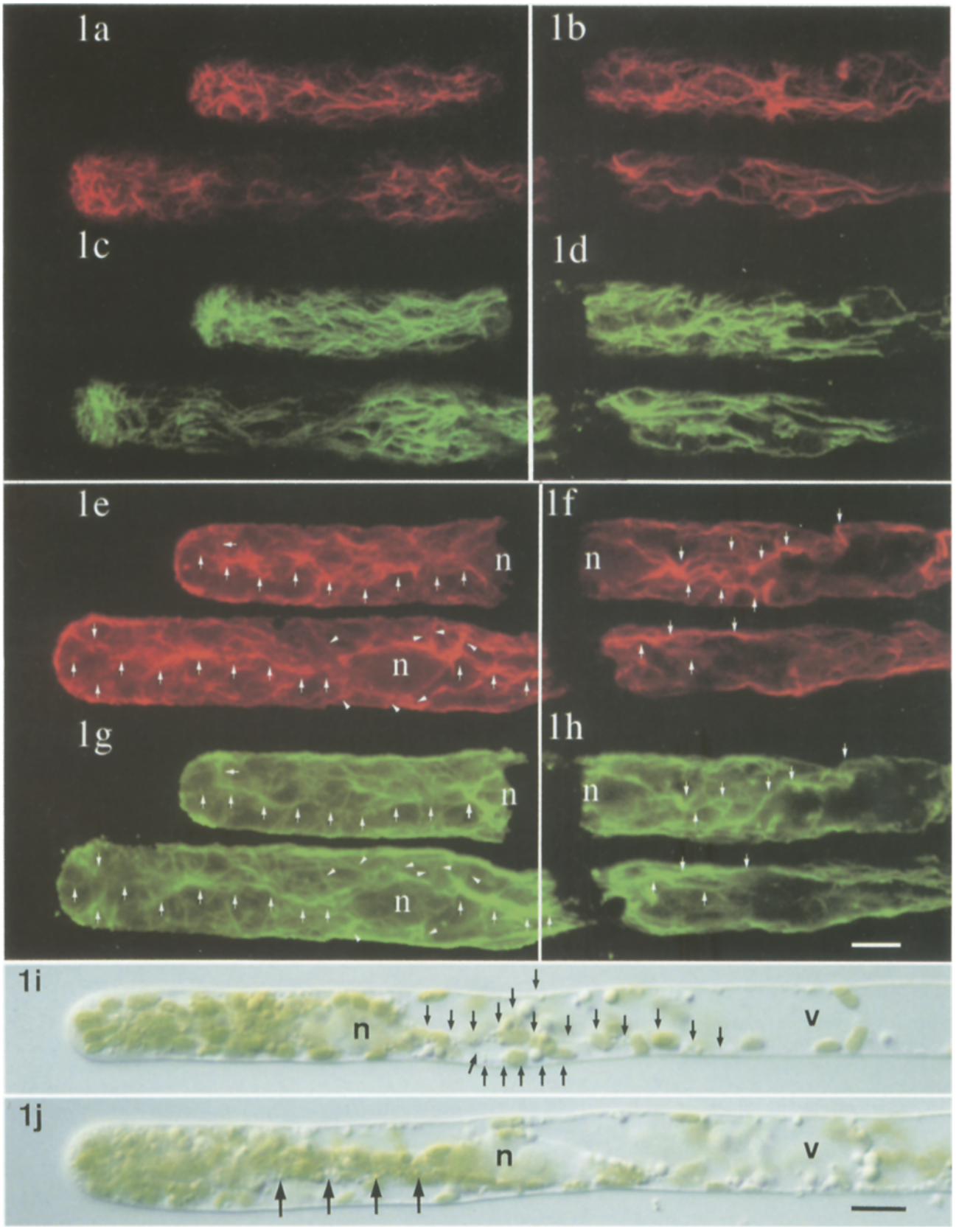
Modified methods after Kadota and Wada (1989) were employed for MF staining with Rh-Phal (Molecular Probes Inc., Eugene, OR, U.S.A.). Cells were prefixed for 30 min with 300 µM *m*-maleimidobenzoic acid N-hydroxysuccinimide ester (MBS) and 1.5% dimethylsulfoxide (DMSO) in buffer. The final concentration of DMSO was 2.5% because the buffer itself contained 1% DMSO as described below. The composition of the buffer was 20 mM piperazine-N,N′-bis(2-ethane-sulfonic acid) (PIPES), 5 mM ethyleneglycol-bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 5 mM $MgCl_2$, 1% DMSO and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.0 (adjusted with KOH). Cells were further fixed for 30 min with 1.2% formaldehyde in the buffer solution and rinsed in the buffer solution. Cells were stained for 3–6 h with 0.33 µM Rh-Phal and 0.15% Triton X-100 in the buffer solution.

Microtubule staining

Modified methods after Murata et al. (1987) were employed for immunostaining of MTs. Cells were fixed for 30 min with 3.5% formaldehyde in PIPES buffer (as above) and rinsed in the buffer solution. Fixed cells were cut transversely with a razor blade and then treated overnight with a 1% Nonidet P-40 in the buffer. After rinsing twice and washing once for 10 min in the phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, pH 7.3 adjusted with NaOH), a mixture (1:1) of 500-fold diluted mouse monoclonal antibodies against α-tubulin and against β-tubulin from chicken (N356 and N357; Amersham Japan Co., Tokyo) was applied to the cells in PBS for 4 h. Cells were rinsed and washed for 10 min with 0.05% polyoxyethylene sorbitan monolaurate (PSM; equivalent to Tween 20) in PBS, and then washed for 10 min with PBS. Cells were treated for 4 h with 10-fold diluted fluorescein-labeled secondary antibody (N1031; Amersham Japan Co.) in PBS.

Double-staining of MFs and MTs

Modified methods after Kadota and Wada (1992a) were used. Cells were prefixed in the same way as for MF staining and were further fixed for 30 min with 7% formaldehyde, 300 µM MBS and 1.5% DMSO in the PIPES buffer. After rinsing in the buffer solution, cells were cut transversely with a razor blade and stained overnight at ca. 5 °C with 0.33 µM Rh-Phal and 0.15% Triton X-100 in the buffer solution. After rinsing twice and washing once for 10 min with 0.05% PSM in PBS, a mixture (1:1) of 1000-fold diluted mouse monoclonal antibodies against α-tubulin and against β-tubulin from chicken was applied to the cells overnight at ca. 5 °C in PBS that contained 0.33 µM Rh-Phal and 0.05% PSM. Cells were rinsed twice and washed once for 10 min with 0.05% PSM in PBS. Cells were then treated overnight at ca. 5 °C or for 4 h at 25 °C with 10-fold diluted fluorescein-labeled secondary antibody, 0.33 µM Rh-Phal and 0.05% PSM in PBS.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DNA staining

To observe nuclei after the above-described preparations, DNA was stained with 0.1 μg/ml 4′,6-diamino-2-phenylindole (DAPI) which was included in the mounting medium described below.

Fluorescence microscopy

Cells were mounted with 0.1% *p*-phenylendiamine in PBS (pH 9, adjusted with NaOH) and observed under either confocal laser scanning microscope (LSM410; Carl Zeiss, Oberkochen, Federal Republic of Germany) or conventional epifluorescence microscope (Axioplan; Carl Zeiss). In the former system, excitation wavelength for fluorescein and for rhodamine were 488 and 543 nm, respectively. Dichroic beam splitters used were FT488/543 (DBS1) and FT560 (DBS2). Barrier filters employed were BP515-565 and BP575-640 for fluorescein and rhodamine emission, respectively. Size of pinhole was 20. Images were acquired through a × 63 Plan Apochromat objective (NA 1.40) at a resolution of 512 × 512 pixels and the number of frames averaged was 8. For the latter, filter sets of UV G365, G H546 and B450-490SB were used for DAPI, rhodamine and fluorescein fluorescence, respectively. Objective lens used was a ×100 Plan Neofluor (NA 1.30). An additional barrier filter (a custom-made interference filter having a peak at 608 nm and a half-band width of 51 nm; Carl Zeiss) was used to eliminate the red fluorescence from chlorophyll.

Results

Microfilament and microtubule organization in tip-growing protonemal cells

A circular arrangement of MFs and MTs was clearly seen in the cortex of the subapical region (Fig. 1 a–d). In the endoplasm, strands of co-localized MTs and MFs were found connecting the spindle-shaped nucleus to the apical cortex (Fig. 1 e–h). MF and MT strands were found to emanate from the apical end of the nucleus, running through the endoplasm, and connecting at the apical cortex in the region of the circular array. Many fine filaments were observed to branch from the strands to the cell periphery. Strands of MFs and MTs were also found to emanate from the proximal end of the nucleus and extend towards the cortex of basal part of the cell. The nucleus also appeared to be surrounded by MFs and MTs. Further, some MF and MT filaments appeared to connect the cell nucleus with the nearby cell cortex. Structures corresponding to the MF and MT strands extending from the nucleus could be observed even in living protonemal cells under Nomarski DIC conditions (Fig. 1 i, j). Cytoplasmic strands extending from the proximal end of the nucleus were frequently seen (Fig. 1 i), and a structure corresponding to the strands from the apical end of the nucleus could be also observed in a few cells (Fig. 1 j).

Effects of cytochalasin B and/or colchicine on the MFs and MTs

Cytoskeletal organization after treatment for 2 h with CB and/or colchicine are presented in Figs. 2–5 and Table 1. When colchicine was applied, MT filaments were disrupted and only a few fragments were seen (Fig. 3). MF filaments did not appear to be disturbed by the treatment. CB disrupted the MF organization but no effect was seen on MT organization except for the disappearance of the circular arrangement (Fig. 4). When both drugs were included in the culture medium, both elements of cytoskeleton were disrupted (Fig. 5).

Table 1. Effects of colchicine (5 mM) and/or CB (104 μM) on MF and MT structures

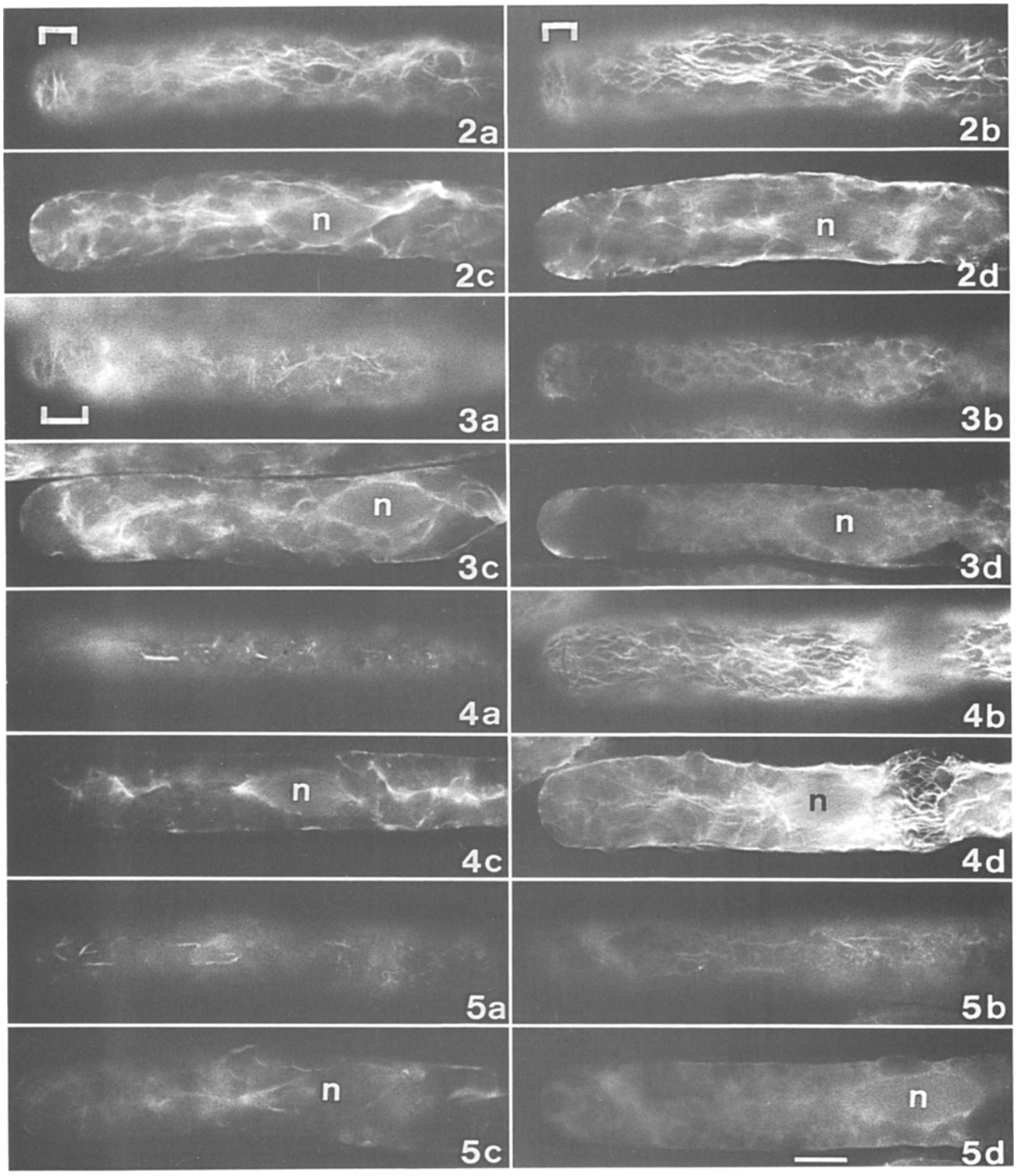
Treatment	Circular array (%)		Strands (%)	
	MF	MT	MF	MT
Control	96.0	99.3	99.5	95.3
DMSO	89.3	97.9	98.0	98.4
CB	0.5	32.1	11.2	98.2
Colchicine	84.6	3.9	95.6	0.0
CB + colchicine	1.0	0.0	10.1	0.0

Drugs were applied under red light for 2 h and the presence of each structure is assessed after staining. The presence of transversely aligned filaments at the cortex of the subapical region of the cell was used for the criterion for the circular array. Also the presence of continuous strands between the cell apex and the nucleus was used for the criterion for the strands. CB was applied with 0.5% DMSO, and DMSO alone control was also presented. More than 100 protonemata were counted for each treatment

Fig. 1 a–j. Confocal laser scanning images of MFs (**a**, **b**, **e**, and **f**) and MTs (**c**, **d**, **g**, and **h**) double-stained in tip-growing protonemal cells of *Adiantum capillus-veneris* and Nomarski DIC images (**i** and **j**) of living protonemal cells. Cells stained were cut transversely at the middle part. Several optical sections (each separated by 0.75 μm in the Z-axis) around the cell cortex and those around the middle of the cell are combined and shown in **a–d** and in **e–h**, respectively. It is evident that strands of MFs and MTs connect the nucleus to the apical cortex around circular arrays (arrows). Strands are also seen to extend from the proximal end of the nucleus to the cortex of basal part of the cell (arrows). Further, some MF and MT filaments appeared to connect the cell nucleus to the nearby cell cortex (arrowheads). Numbers of sections combined are 5 in **a** and **c**, 4 in **b** and **d**, 7 in **e** and **g**, and 6 in **f** and **h**. Structures corresponding to the strands can be observed also in living cells (**i** and **j**). Small arrows in **i** show the strands emanating from the proximal end of the nucleus. Large arrows in **j** show a structure extending from the apical end of the nucleus towards the cell apex, which may correspond to the cytoskeletal strands emanating from the apical end of the nucleus. Many chloroplasts, oil drops and smaller vesicles are seen to reside on the structures. Bars in h and j: 10 μm. *n* Cell nucleus; *v* vacuole

Migration of the nucleus during tip-growth of protonema under red light and the effects of CB and/or colchicine

Tip-growth and nuclear migration under red light was analyzed every 1 h in each individual protonemal cell. In control cells, the nucleus migrated with the apex during cell growth, resulting in a constant distance between the tip and nucleus (Fig. 6). When CB was applied, cells showed cessation of nuclear movement as well as that of tip-growth (Fig. 7). Colchicine treatment largely inhibited tip growth (Fig. 8). Acropetal movement of the nucleus was inhibited, and the treat-



ment induced basipetal migration of the nucleus (Fig. 8 and Table 2). When cells were treated by both CB and colchicine, cessation of nuclear movement was observed as well as that of tip-growth. However, no basipetal migration of the nucleus was observed in this case (Fig. 9 and Table 2).

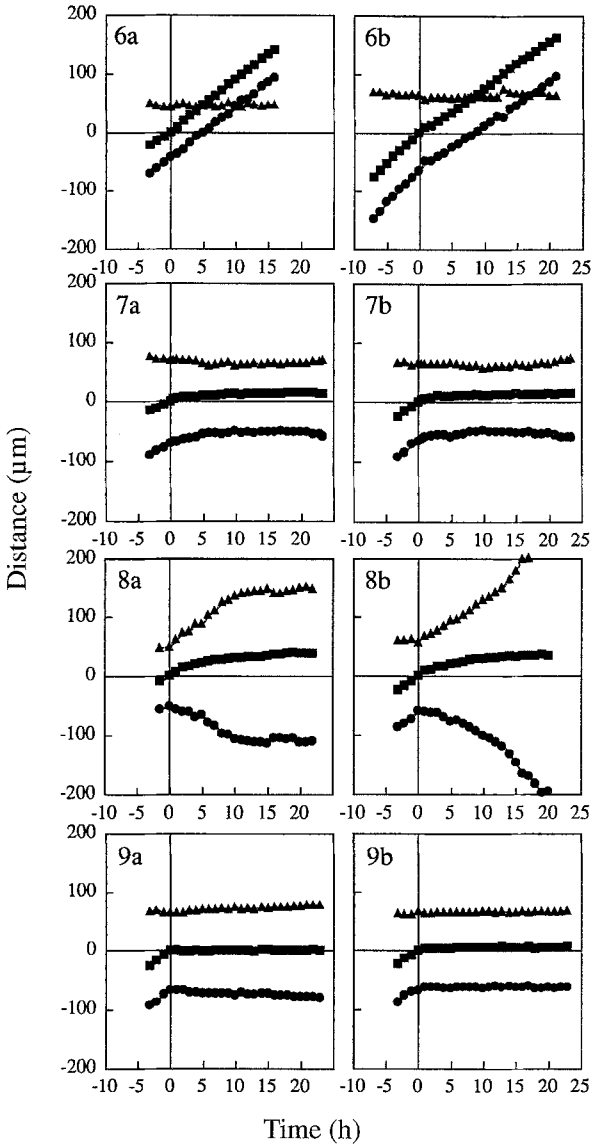


Table 2. Effect of cytoskeletal inhibitors on the intracellular position of the nucleus

Treatment	Position of nuclei (distance from tip, μm)		n
	at 0 h	at 15 h	
Control	60.0 ± 4.0	62.4 ± 4.0	6
DMSO	59.9 ± 4.4	63.9 ± 3.4	4
CB	64.9 ± 2.0	60.7 ± 2.9	7
Colchicine	63.6 ± 3.6	115.7 ± 12.1	6
Colchicine + DMSO	57.0 ± 2.4	133.8 ± 14.8	5
Colchicine + CB	69.9 ± 2.7	76.8 ± 2.9	7

The distance between the center of the nucleus and the tip of the protonema was measured before (at 0 h) and after drug treatment for 15 h (at 15 h). Data were obtained from continuous observation of individual cells under each condition (Figs. 6–9) and are indicated by an average ± standard error

Acropetal movement of cell nucleus induced by basipetal centrifugation and the effects of CB and/or of colchicine on the movement

The nucleus in the growing protonemal cell was dislocated basipetally by 26.8 ± 12.4 μm (average ± standard deviation; n = 55) by centrifugal treatment (510 **g**, 15 min), and the movement of the nucleus was examined thereafter. When protonemal cells were fixed immediately after basipetal centrifugation

Figs. 6–9. Tip-growth and nuclear migration in an individual protonemal cell of *Adiantum capillus-veneris* under red light in the absence or presence of CB and/or colchicine. Drug was applied at 0 h. ■ Tip; ● nucleus; ▲ distance between tip and nucleus

Fig. 6 a, b. In the absence of drugs. **b** DMSO was applied at 0 h for the control of CB application (Fig. 7)

Fig. 7 a, b. In the presence of CB (104 μM). **a** and **b** Two individual cells

Fig. 8 a, b. In the presence of colchicine (5 mM). **b** DMSO (0.5%) also applied together with colchicine as a control of the treatment with both CB and colchicine (Fig. 9)

Fig. 9 a, b. In the presence of both colchicine (5 mM) and CB (104 μM). **a** and **b** Two individual cells

Figs. 2–5. Effects of colchicine and/or CB on MF (**a** and **c**) and MT (**b** and **d**) structures in protonemal cells of *Adiantum capillus-veneris*. Drugs were applied for 2 h. **a** and **b** Focus on the upper cell surface. **c** and **d** Focus close to the middle of the cell. The bracket indicates a subapical circular arrangement of MFs or MTs. Bar in 5 d: 10 μm. *n* Cell nucleus

Fig. 2 a–d . Control cell without drug treatment (double-staining of MF and MT)

Fig. 3 a–d. Cells treated with colchicine (5 mM). MT structures are disrupted

Fig. 4 a–d. Cells treated with CB (104μM). MF structures are disrupted but fragments are still seen. Note the MT circular array is also lost by the treatment

Fig. 5 a–d. Cells treated with both colchicine (5 mM) and CB (104 μM). Both MF and MT structures are broken and only a few fragments are seen

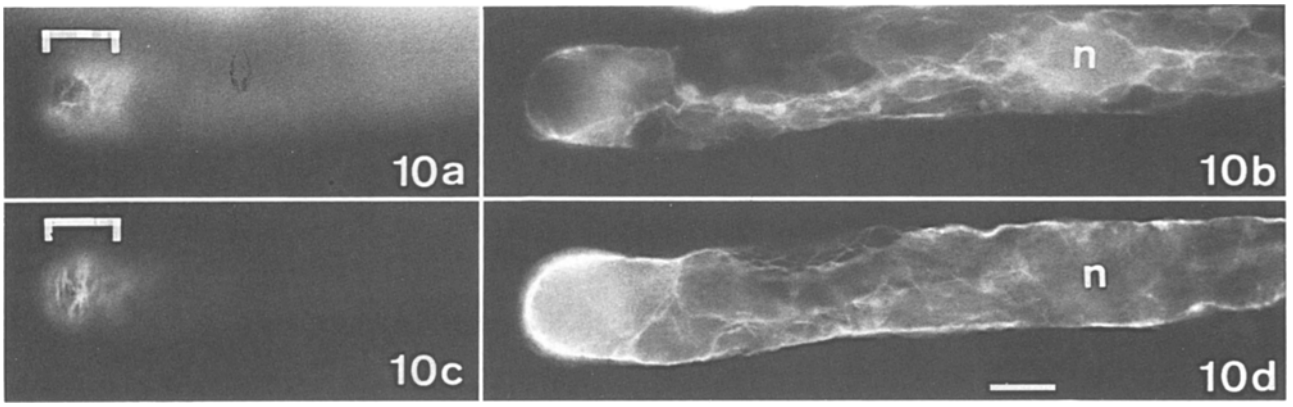


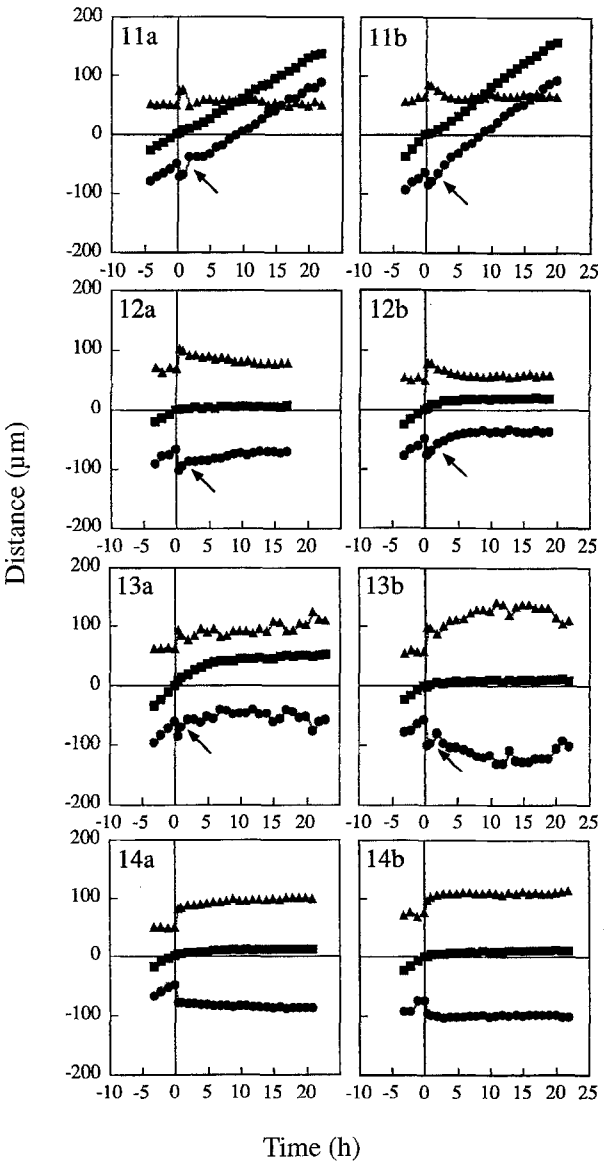
Fig. 10 a–d. Double-staining of MFs (**a** and **b**) and MTs (**c** and **d**) in basipetally centrifuged protonemal cells of *Adiantum capillus-veneris*. **a** and **b** Focus on the upper cell surface. **c** and **d** Focus close to the middle of the cell. Bar in d: 10 µm. *n* Cell nucleus. It is evident that strands of MFs and MTs still connect the nucleus to the apical cortex around the circular arrays

Table 3. Effect of cytoskeletal inhibitors on the acropetal migration of the nucleus after basipetal centrifugation

Treatment	Acropetal migration of nuclei (µm)	n
Control	20.1 ± 3.4	6
DMSO	17.5 ± 3.0	8
CB	15.2 ± 2.9	10
Colchicine	18.0 ± 4.2	10
Colchicine + DMSO	12.9 ± 3.2	5
Colchicine + CB	4.3 ± 1.2	16

Protonemal cells were centrifuged basipetally at 510 **g** for 15 min and the cell nucleus was dislocated basipetally. The drugs were applied immediately after centrifugation. Acropetal movement of the nucleus within 2.5 h after displacement was measured. Data were obtained from continuous observation of individual cells under each condition (Figs. 11–14) and are indicated by an average ± standard error

n Number of cells examined



Figs. 11–14. Tip-growth and nuclear migration in the basipetally centrifuged protonemal cells of *Adiantum capillus-veneris* under red light in the absence or presence of CB and/or colchicine. Protonemal cells were centrifuged basipetally at 510 **g** for 15 min at 0 h. Drugs were applied immediately after centrifugation. The first time point after centrifugation is 0.5 h after the beginning of centrifugal treatment. ■ Tip; ● nucleus; ▲ distance between tip and nucleus. Arrows indicate the acropetal migration of the nucleus after basipetal centrifugation

Fig. 11 a, b. In the absence of drugs. **b** DMSO (0.5%) applied after centrifugation as a control of CB application (Fig. 12)

Fig. 12 a, b. In the presence of CB (104 µM). **a** and **b** Two individual cells

Fig. 13 a, b. In the presence of colchicine (5 mM). **b** DMSO (0.5%) also applied together with colchicine as a control of the treatment with both CB and colchicine (Fig. 14)

Fig. 14 a, b. In the presence of both colchicine (5 mM) and CB (104 µM). **a** and **b** Two individual cells

and stained for MF and MT, the MT and MF strands connecting the nucleus to the apex were still observed (Fig. 10). Basipetal dislocation of the nucleus had only a slight effect on tip-growth and the cells kept growing after the treatment (Fig. 11). The displaced nuclei showed a movement back to their previous positions; that is, the nuclei migrated acropetally to resume their previous distance from the cell tip in a few hours (Fig. 11).

To examine the contribution of the cytoskeleton in the acropetal movement of the nucleus, CB and colchicine were applied after basipetal centrifugation. The movement was not suppressed either by CB alone or by colchicine alone (Figs. 12 and 13) but was inhibited by the application of both CB and colchicine (Fig. 14 and Table 3). The behavior of the nucleus in the later period was the same as in non-centrifuged cells. Nuclei stopped movement under CB application (Fig. 12), and basipetal migration was observed in the presence of colchicine (Fig. 13). The basipetal movement was again suppressed by the simultaneous application of CB (Fig. 14).

Discussion

Involvement of MTs rather than MFs in nuclear migration has been reported in the literature for many tip-growing cells, except for pollen tubes (Williamson 1992). In pollen tubes, the elimination of MTs affects tip-growth and nuclear movement much less than MFs (Franke et al. 1972, Heslop-Harrison et al. 1988). The association of MFs with the vegetative nucleus was observed in fluorescence and electron microscopy (Tiwari and Polito 1988a, b; Heslop-Harrison and Heslop-Harrison 1989). In other tip-growing cells, however, the available evidence indicates the common contribution of MTs in nuclear movement. Branch formation in moss protonemata is associated with the migration of the nucleus to the site of branching. MTs appear to connect the nucleus to the branch initial, and the migration is inhibited by MT-depolymerizing agents (Schmiedel and Schnepf 1979, Conrad et al. 1986, Doonan et al. 1986, Doonan 1991). In fungal hyphae, MT-dependent translocation of the nucleus is supported by pharmacological, morphological and genetic studies (Heath 1982, Herr and Heath 1982, Oakley and Morris 1980, McKerracher and Heath 1985). In germinating zygotes of fucoid algae, MTs extend from the nucleus to the emerging tip of the rhizoid, while MF staining by a phalloidin-derivative is restricted to the cortex of the tip (Brawley and Robinson 1985, Kropf et al. 1989, Kropf 1992). Involvement of MTs in nuclear movement during germination has also been suggested for fern spores (Vogelmann et al. 1981). In growing root hair cells, Lloyd et al. (1987) clearly showed that MT strands connect the advancing nucleus to the cortex of the growing apex. While they also revealed the association of MF strands with the nucleus, the connection of MF strands in the growing apex remains obscure (Lloyd et al. 1987, Ridge 1992). In *Adiantum* protonemal cells, the involvement of MTs in the positioning of the nucleus during tip-growth has been implicated by Mineyuki and Furuya (1985). They reported a change in the centrifugability of the nucleus in the presence of MT- but not MF-depolymerizing drugs.

For the endoplasmic cytoskeletal architecture of the fern protonemata of *Adiantum capillus-veneris* (Fig. 1), we here revealed the presence of co-localized MF and MT strands which anchor the advancing cell nucleus to the cortex of growing apex, where the presence of circular arrays of cortical cytoskeleton have already been demonstrated (Murata et al. 1987, Kadota and Wada 1992b). An MT-dependent junction between plasma membrane and cell wall ("subapical junction") has also been shown by the same workers (Kagawa et al. 1992). Both strands appear to emanate from the apical end of the nucleus. While the extension of MT filaments from the apical end of the nucleus has been detected by electron microscopy (Wada and O'Brien 1975), their connection and co-localization with MF is first presented in this study. Further, we clarify the presence of MF and MT strands which emanate from the proximal end of nucleus and extend towards the cortex in the vacuolated basal part of the cell. Moreover, staining of the cytoskeleton was found along the entire boundary of nucleus. Thus, it is evident that the advancing nucleus in the protonemal cell is suspended with a network of fine and bundled MF and MT filaments. The evidence indicates that not only the structure in front of the advancing cell nucleus but also that of proximal region may have significant role in nucleus positioning and migration. In this context, the works of McKerracher and Heath (1986) are of relevance. They examined the effects of UV irradiation of the area anterior to or posterior to the cell nucleus on nuclear movement and showed that irradiation at each area has effects as follows: UV damage of the posterior region accelerated nuclear velocity whereas UV damage of the anterior part

caused the nucleus to stop or move backwards. In the present work, drug studies revealed that there was no interdependency between the MT and MF organization in the strands; that is, if one element of the strands was disrupted by depolymerizing agent, the organization of the other element remained undisturbed (Figs. 3 and 4 and Table 1). This is in contrast to the situation of the circular array at the subapical cortex, where the organization of MTs was shown to depend on MF structure (Figs. 4 b and Table 1) (Kadota and Wada 1992b).

From the observation of tip-growth and nuclear behavior in each individual protonemal cell under the presence of cytoskeleton-depolymerizing drugs, MT structures are inferred to have an essential role in the maintenance of nuclear positioning. Under colchicine treatment, nuclear movement was uncoupled from the tip and basipetal migration was induced (Fig. 8 and Table 2). This basipetal migration did not occur when the cell was exposed to both colchicine and CB (Fig. 9 and Table 2), and there is an apparent dependence on MFs for basipetal migration of the nucleus. Similar effects of drugs on nuclear movement were reported in root hairs with the same conclusions drawn (Lloyd et al. 1987); the same cytoskeletal control exists in these tip-growing cells as in *Adiantum* for nuclear positioning. In the case of the root hair cell, the significance of MFs in the acropetal movement of nucleus was obscure because the cytochalasin treatment stopped tip-growth as well as nuclear movement. The same effect of CB on growth and nuclear movement was also observed in this study of *Adiantum* protonemata (Fig. 7). In these cases, as discussed by Williamson (1993), it is difficult to segregate the indirect effect of CB on nuclear movement through an inhibition of tip-growth, from the direct inhibition of the motile system by CB. In the present study, however, drug effects were further investigated on the acropetal migration of the nucleus in centrifuged cells. After basipetal centrifugation, acropetal migration of the nucleus occurred and the nucleus resumed its previous position (Fig. 11). The movement was blocked only in the presence of both CB and colchicine (Figs. 12–14 and Table 3). The evidence indicates that both MT and MF strands function in the acropetal movement of the nucleus and that when one element of the strands is lost the nucleus can move along the remaining cytoskeletal element, demonstrating a redundancy of motility machinery. While we do not know whether or not the acropetal movement of the nucleus after basipetal centrifugation is the same kind of movement seen under normal conditions, the results at least indicate the significance of both the MT and MF cytoskeleton in the acropetal nuclear migration.

In conclusion, the present study shows that the cytoskeleton is organized around the advancing nucleus in tip-growing fern protonemal cells, and revealed the presence of MFs and MTs as co-localized strands connecting the nucleus to both the growing apex and the basal cortex. Both cytoskeletal elements appear to be involved in nuclear movement during tip-growth. While the involvement of MTs in this movement has been analyzed in the literature, much less attention has been given to the participation of MFs except in pollen tubes. More work is needed to clarify the role of MFs in nuclear movement and positioning in tip-growing cells.

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