Protoplasma 130, 83–90 (1986)

PROTOPLASMA

Involvement of Colchicine-Sensitive Cytoplasmic Element in Premitotic Nuclear Positioning of *Adiantum* Protonemata

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Received March 26, 1985
Accepted September 20, 1985

Summary

When the red-light grown protonema of *Adiantum capillus-veneris* was transferred to the dark, the nucleus ceased its migration ca. 5 hours before cell plate formation (MINEYUKI and FURUYA 1980). To see whether the nucleus was held by some cytoplasmic structure during nuclear positioning, protonemata were treated with various centrifugal forces at different stages of the cell cycle. Nuclei of G_1 phase were easily displaced by centrifugation at $360 \times g$ for 15 minutes, but those of G_2 or M phase were not displaced by it, suggesting that the nuclei were held by some cytoplasmic elements in G_2 or M phase. This nuclear anchoring was not detectable in protonemata that were treated with 5 mM colchicine. With this treatment, the nucleus did not stop its migration at late G_2 and moved even in prophase. And the retardation of organelle movement which was observed in cytoplasm on the lateral side of the nucleus after the cessation of premitotic nuclear migration (MINEYUKI and FURUYA 1984) was not observed in the presence of colchicine. Thus the nuclei appear to be held by colchicine-sensitive structure in cytoplasm between the lateral surface of the nucleus and cell wall during the premitotic nuclear positioning. Electron micrographs showing cytoplasmic microtubules were consistent with the idea.

Keywords: *Adiantum;* Fern protonemata; Microtubules; Organelle movement; Premitotic nuclear positioning.

Abbreviations: PPN, Premitotic positioning of the nucleus; L region, Cytoplasm between the lateral surface of the nucleus and cell wall (see MINEYUKI *et al.* 1984).

1. Introduction

When cell division is induced in the dark in the apical cell of an *Adiantum* protonema that has been grown under continuous red light, the nucleus ceases its migration ca. 5 hours before cell plate formation (late G_2 phase) and stays there until karyokinesis. This timing of the cessation of nuclear migration is not influenced by centrifugation that changes the position of cell plate formation, indicating that the nucleus ceases its migration at late G_2 phase irrespective of the nuclear position (MINEYUKI and FURUYA 1980). Although the movement of organelles only on the lateral side of the nucleus slows down after the cessation of the premitotic nuclear migration (MINEYUKI *et al.* 1984), it is uncertain whether or not the nucleus is anchored by some cytoplasmic structure after the cessation of its migration. Nuclear anchoring is reportedly associated with microtubules in *Micrasterias* (KIERMAYER 1968), *Acetabularia* (WOODCOCK 1971) and a protozoan (LANNERS 1980). Involvement of microtubules in the nuclear positioning or migration is also suggested in the apical growing stage of the *Adiantum* protonemata (MINEYUKI and FURUYA 1985) and in other tip growing cells such as hyphae of fungi and moss caulonemata (SIEVERS and SCHNEPF 1981). However, there has been no evidence on the anchoring of nuclei and the involvement of the cytoskeleton in premitotic positioning of nuclei (PPN) in *Adiantum* protonemata.

The experiment in this report was carried out to examine whether or not nuclei were held by cytoplasmic elements after the cessation of the nuclear migration, and to see if the phenomenon of the retardation of organelle movement which was reported previously (MINEYUKI *et al.* 1984) was correlated with the cessation

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of the premitotic nuclear migration, then to see if any cytoskeleton were involved in anchoring of the nucleus.

2. Materials and Methods

2.1. Plant Material

Spores of the fern *Adiantum capillus-veneris* L. were collected in the summer of 1977 in a green house of the Botanic Gardens, Faculty of Science, University of Tokyo, Koishikawa, Tokyo. The spores were filtered through a fine mesh screen, then stored in a plastic container in a cold room at ca. 5 °C. Stored spores were used throughout this study.

2.2. Aseptic Culture

Aseptic culture of *Adiantum* spores was carried out according to the method described previously (MINEYUKI and FURUYA 1985). Briefly the spores, sterilized by the technique of ITO (1969) with diluted "Purelox" (4–6% aqueous solution of sodium hypochlorite, Oyalox Co., Tokyo), were aseptically inoculated on the surface of the MURASHIGE and SKOOG's (1962) medium modified by WADA and FURUYA (1970) in a Petri dish or the spores were inoculated on the inner surface of an empty plastic cuvette (Kartell, Italy) about 3 cm below the top and the cuvette was filled with the medium.

The spores were imbibed for 1 day in the dark, after which they were cultured for 6 days at 25 °C under continuous red light of ca. 0.5 Wm^{-2}. Consequently, protonemata grew horizontally at the apex towards the red light source at a very low rate of cell division, so that the ptononemata were in a single-celled filamentous form (WADA and FURUYA 1970). Cell division was induced by transferring the samples to darkness from continuous red light (WADA and FURUYA 1972). According to MIYATA *et al.* (1979), protonemata incubated in darkness for 20 hours were estimated to be in late G_1 phase, and those incubated for 32–40 hours mainly in G_2 or M phase.

For the study of organelle movement, the filamentous protonemata were placed on a slide, covered with a cover slip, then sealed with silicone rubber (Dow Corning 3140 RTV Coating, Dow Corning Co., U.S.A.).

2.3. Centrifugal Treatment

Filamentous protonemata grown in the above described plastic cuvettes were centrifuged basipetally for 15 minutes at 25 °C according to MINEYUKI and FURUYA (1980). The position of the nucleus in each single-celled protonema was observed with a microscope (Nikon SBR-Ke, Nippon Kogaku K.K., Tokyo) using an infrared viewer (FJW Industries, U.S.A.), the light provided by a tungsten lamp with an infrared filter (IR 85, Hoya Glass Co. Ltd., Tokyo). Protonemata were photographed with infrared film (HIE 135-20, Eastman Kodak Co. Ltd., U.S.A.), and the positions of nucleus and cell plate were photomicrographically determined with an ocular micrometer.

2.4. Treatment with Anti-Cytoskeleton Drugs

The solution of colchicine (Nakarai Chemicals Ltd., Kyoto) was sterilized by passage through a membrane filter (HA, 0.45 µm, Millipore Co., U.S.A.). Cytochalasin B (Sigma Chemical Company, U.S.A.) was dissolved in dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries Ltd.). These solutions were added to the basal medium to give the desired concentration, and were exchanged for the basal medium in the cuvettes keeping protonemata on the inner surface of the cuvettes.

2.5. Analysis of the Location of Particles

The movement of individual large, round particles was followed under Nomarski optics focused on the surface-longitudinal plane of a protonema and was recorded using the time-lapse video system (FURUYA *et al.* 1980). The locus of each particle was analyzed using a position analyser (VPA1000, For-A Co. Ltd., Tokyo), which was connected between the video tape recorder and video monitor so that coordinates and a cursor were superimposed on the images stored on the video tape (MINEYUKI *et al.* 1984). Data were processed by a microcomputer (Packett II Hy Personal Computer DDC7706C, Anritsu Denki K.K., Tokyo) and the result was printed out by a thermoprinter.

The position of particles (x, y) was determined at 18 seconds intervals for 100 points using a cursor and coordinates for determination of the diffusion coefficients. Coordinates in protonemata of VTR images were determined so that the x-axis of the coordinates was parallel to the longitudinal axis of protonemata and the y-axis was perpendicular to this. Only organelles on the surface-longitudinal plane, which was the nearest to the x—y plane, were used for the estimation of the diffusion coefficients to minimize the errors (Fig. 3). The displacement of particles (Δx, Δy) in 18, 36, 54, 72, 90, and 108 seconds intervals was calculated, then the mean square displacement ($\langle \Delta x^2 \rangle$, $\langle \Delta y^2 \rangle$) gave the diffusion coefficients by calculation, using the following formula (EINSTEIN 1905): $\langle \Delta x^2 \rangle = 2 D_x t$, and $\langle \Delta y^2 \rangle = 2 D_y t$, where D_x, D_y were the diffusion coefficients and t was the time of intervals measured between two points. The diffusion coefficients (D_x, D_y) were separately determined in each component axis.

2.6. Electron Microscopy

For electron microscopy, protonemata in agar blocks were fixed with 3.3% glutaraldehyde in GEMP-buffer [1 mM GTP (Yamasa Shoyu Co. Ltd., Chiba), 1 mM EGTA (Dojindo Laboratories, Kumamoto), 1 mM $MgCl_2$, 0.1 M sodium-phosphate buffer, pH 6.8] at 25 °C in darkness, washed with GEMP-buffer twice, postfixed in 2% OsO_4 at 0 °C, dehydrated with a series of ethanol, and embedded in Spurr's resin (1969). Thin sections were made with an ultramicrotome (OmU 2, Reichert, Austria), stained with uranyl acetate and lead citrate, and examined with an electron microscope (JEM 100-C at 100 kV, JEOL, Tokyo).

3. Results

3.1. Effects of Centrifugal Forces on Nuclear Displacement at Various Stages of Cell Cycle

Protonemata growing under red light were transferred to darkness so that the cell cycle synchronously progressed from early G_1 to M for 2 days. Samples taken at various stages were centrifuged basipetally at 110 or 360 × g for 15 minutes. The percent of protonemata showing displaced nuclei was determined after centrifugation (Fig. 1).

Nuclei were not displaced by basipetal centrifugation at 110 × g for 15 minutes in most protonemata that were growing under red light, whereas they became displaceable in more than half of the protonemata that

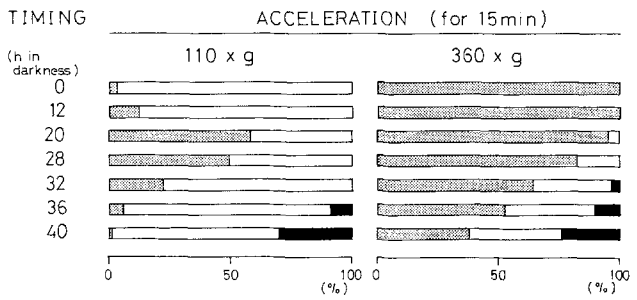

Fig. 1. Effect of centrifugation on nuclear displacement at different stages of cell cycle in filamentous protonemata of *Adiantum*. Protonemata were centrifuged at 110 × g (left) or 360 × g (right) for 15 minutes after various periods of dark incubation. ▨ Percentage of protonemata whose nuclei were displaced, □ Percentage of protonemata whose nuclei were not displaced, ■ Percentage of protonemata whose cell plates were formed. Each data was obtained from ca. 60 protonemata

were centrifuged at 20 hours after the transfer to darkness, *i.e.*, at late G_1 phase. The percent of protonemata with displaced nuclei decreased in the case of centrifugation at 32–40 hours after the transfer to darkness, *i.e.*, at G_2 or M, and in some of these protonemata nuclei were not displaced even by basipetal centrifugation at 360 × g for 15 minutes. It became clear that the magnitude of centrifugal force for displacement of nuclei varies depending upon the stage of the cell cycle.

Table 1. *Effects of colchicine and cytochalasin B on nuclear positioning in Adiantum protonemata*

Timing (hours in darkness)	Chemical treatment	% of protonemata whose nuclei were not displaced	
		exp. 1	exp. 2
32	Nil	15.7 (51)[a]	23.7 (76)[a]
	Colchicine[b]	3.4 (89)	9.2 (76)
	DMSO[c]	11.1 (99)	28.6 (56)
	Cytochalasin B[d]	13.6 (59)	26.7 (90)
36	Nil	17.0 (47)	26.5 (83)
	Colchicine	9.2 (76)	16.5 (85)
	DMSO	27.9 (61)	31.6 (76)
	Cytochalasin B	27.9 (61)	36.7 (49)
40	Nil	35.0 (60)	29.6 (71)
	Colchicine	13.4 (82)	8.6 (70)
	DMSO	30.0 (40)	26.0 (77)
	Cytochalasin B	23.3 (86)	31.5 (73)

[a] Number of protonemata counted.
[b] 5 mM colchicine in the medium.
[c] 0.5% dimethyl sulfoxide in the medium.
[d] 0.1 mM cytochalasine B and 0.5% DMSO in the medium.

3.2. Effect of Colchicine and Cytochalasin B on Nuclear Positioning in G_2 and M Phases

Protonemata grown under red light and then incubated for 30–38 hours in darkness were transferred to media with or without 5 mM colchicine, or to media containing 0.5% DMSO with or without 0.1 mM cytochalasin B and kept for 2 hours. Then they were centrifuged at 360 × g for 15 minutes, and the percent of protonemata whose nuclei were not displaced was determined thereafter (Tab. 1).

The transfer to medium with DMSO and with or without cytochalasin B, or without DMSO did not result in any significant difference in the percent of protonemata whose nuclei were not displaced. However, the percent of nondisplaced nuclei in those protonemata treated with 5 mM colchicine was much less than in those without it, suggesting that colchicine affected some cytoplasmic elements supporting the nucleus of G_2 or M phase in protonemata.

3.3. Effect of Colchicine and Cytochalasin B on Cell Plate Formation and on Nuclear Migration in G_2 and M Phases

Protonemata incubated for 28 hours in darkness were transferred to medium with or without 5 mM colchicine, or to one containing 0.5% DMSO with or without 0.1 mM cytochalasin B, and incubated in darkness thereafter. Then, the effect of colchicine and cytochalasin B on the timing of cell plate formation was microscopically measured. The timing of cell plate formation in protonemata treated with 5 mM colchicine was ca. 1 hour behind those without colchicine. Cytochalasin B, however, had no effect on the timing of cell plate formation.

In other experiments, protonemata incubated for 28 hours in darkness were transferred to medium containing 5 mM colchicine, or to that containing 0.5% DMSO with 0.1 mM cytochalasin B. The effects of these drugs on nuclear migration were monitored using time-lapse video system (Fig. 2). In this experiment, distruption of mitosis by colchicine was also anticipated, because colchicine is known to affect mitosis widely in plant and animal cells (D'AMATO 1959, DUSTIN 1978), and indeed colchicine delayed the timing of cell plate formation for 1 hour in the previous experiment. So, not only the timing of premitotic positioning of the nucleus (PPN) but also the timing of the cessation and resumption of organelle movement around the nucleus were observed. Because timing of the cessation and resumption of organelle movement closely corresponds with timing of

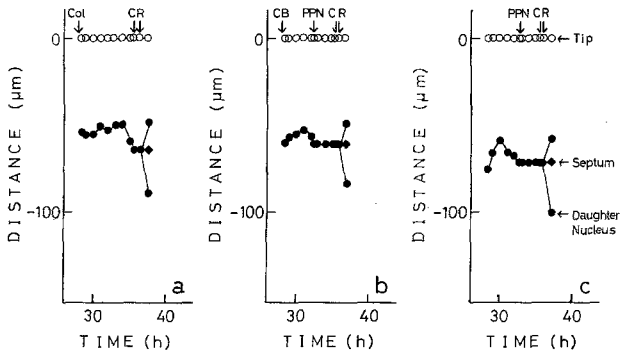

Fig. 2. Effect of colchicine and cytochalasin B on the nuclear positioning in G_2 and M phase in *Adiantum* protonemata. The position of nucleus (●) and protonemal tip (○) were observed and recorded after transferring protonemata to medium in the presence of 5 mM colchicine (*a*) or 0.1 mM cytochalasin B in 0.5% DMSO (*b*), or in the absence of colchicine and cytochalasin B (*c*). Horizontal axes show time after the transfer of protonemata from continuous red light to darkness. Vertical axes show the distance along the filamentous cell axis. *PPN* Time of the premitotic positioning of the nucleus; *C* Time of the cessation of organelle movement around the nucleus; *R* Time of the resumption of organelle movement around the nucleus

the beginning of metaphase and end of metaphase, respectively (MINEYUKI *et al.* 1983). The duration between the cessation of organelle movement and the resumption of organelle movement in the absence of colchicine was 25.6 ± 1.1 minute (mean ± standard error obtained from 10 protonemata), and the PPN took place 3.3 hours before the cessation of organelle movement. In the presence of 5 mM colchicine, however, the duration was 56.8 ± 9.7 minutes (22 protonemata). Although 9 of 36 colchicine-treated protonemata showed normal PPN, thirteen clearly showed nuclear migration even in prophase (Fig. 2 *a*), suggesting that colchicine affected PPN.

In protonemata treated with cytochalasin B, PPN took place ca. 5 hours before cell plate formation and the duration between the cessation and resumption of organelle movement was ca. 20 minutes (Fig. 2 *b*), indicating that the cytochalasin B had no effect on PPN and mitosis.

3.4. Effect of Colchicine on Brownian Motion-Like Organelle Movement on Lateral Side of Nucleus

Protonemata incubated for 28 hours in darkness after the transfer from red light were placed in medium containing 5 mM colchicine and incubated in darkness so that the nucleus moved even in prophase (see, Fig. 2 *a*). Organelle movement in the surface-longitudinal focusing plane in the protonema was recorded using the time-lapse video system. Movement of large, round particles in the cytoplasm between the lateral surface of the nucleus and cell wall (L region) was compared with their Brownian motion-like movement in the absence of colchicine.

Large, round particles in late G_2 and prophase protonemata in the presence of 5 mM colchicine showed undirectional Brownian motion-like movement. So, the diffusion coefficients were chosen as a parameter of the particle movement, and these values for colchicine treated protonema in G_2 phase and prophase were compared with non-treated protonema (Fig. 3). As the movement was so slow, positions of particles were measured at 100 points at 18 seconds intervals to determine the diffusion coefficients. Although the diffusion coefficients in a protonema without colchicine decreased after cessation of nuclear migration, the

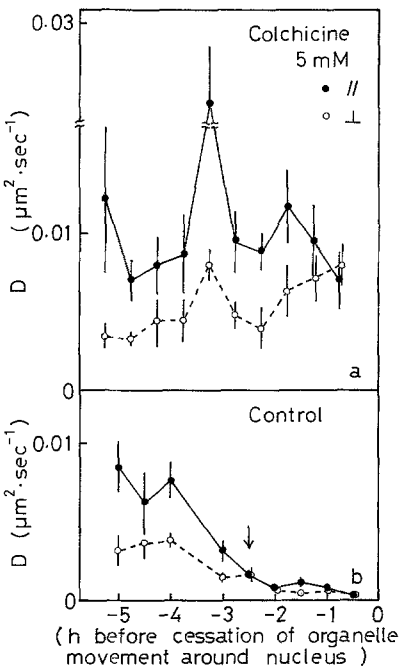

Fig. 3. Time course study on diffusion coefficients of large, round particles' movement on the lateral surface of the nucleus in *Adiantum* protonemata in the presence (*a*) or absence (*b*) of 5 mM colchicine. PPN did not take place in (*a*) but the PPN occurred ca. 2.5 hours before the cessation of the organelle movement in (*b*). (Arrow indicates the timing of the PPN.) ●: x-component (parallel to the growing axis of the protonema) of the organelle movement. ○: y-component (perpendicular to the growing axis of the protonema) of the organelle movement. Vertical axes show the diffusion coefficients. Horizontal axes show time before the cessation of the organelle movement. Each point shows a mean and standard error obtained from ca. four large, round particles the diameter of which were varied from 1.7 to 2.3 μm

Table 2. *Distribution of microtubules in the L region of Adiantum protonemata in G_2 and prophase in the presence or absence of 5 mM colchicine*

Cell no.	Nuclear stage	Number of microtubules					Number of sections
		Type 1	Type 2	Type 3	Others	Total	
(Without colchicine)							
32–6	G2	2.7 ± 0.5	0.9 ± 0.3	0.2 ± 0.1	1.7 ± 0.7	5.5 ± 0.9	10
32–7	G2	11.6 ± 1.5	1.1 ± 0.3	0.1 ± 0.1	2.8 ± 0.5	15.6 ± 1.7	9
36–1	G2	1.5 ± 1.5	0	0	1.0 ± 0.8	2.5 ± 2.5	2
36–4	G2	1	2	0	9	12	1
32–2	GP	3	0	0	1	4	1
36–2	GP	1	0	0	0	1	1
34–3	P	0	2	3	7	12	1
32–5	P	3.2 ± 0.6	0.7 ± 0.2	0.2 ± 0.2	2.7 ± 1.2	6.7 ± 1.4	6
42–1	P	2.3 ± 0.3	1.2 ± 0.3	1.5 ± 0.5	33.1 ± 3.5	38.3 ± 3.9	11
34–2	P	0	8	5	7	20	1
32–1	P	2.5 ± 0.5	4.0 ± 2.0	8.0 ± 1.6	10.0 ± 3.0	24.5 ± 3.5	2
42–4	P	0	14.3 ± 2.1	6.9 ± 1.3	35.7 ± 3.5	58.0 ± 5.3	8
(6 hours incubation with colchicine)							
34–5	GP	0	0	0	0	0	6
34–4	GP	0	0	0	0	0	2
34–3	GP	0.3 ± 0.2	0	0	0.4 ± 0.4	0.7 ± 0.6	7
34–2	P	0.2 ± 0.1	1.6 ± 0.4	0.2 ± 0.1	4.7 ± 0.9	6.5 ± 1.1	11
34–1	P	1.3 ± 0.3	1.5 ± 0.3	0	0.3 ± 0.3	3.0 ± 0.4	4
(8 hours incubation with colchicine)							
36–2	GP	1.7 ± 1.2	1	0	0	2.7 ± 1.2	3
36–6	GP	0.3 ± 0.2	2.6 ± 0.7	0.4 ± 0.3	8.3 ± 2.2	11.6 ± 2.8	7
36–4	GP	10.0 ± 2.7	0.5 ± 0.3	2.1 ± 1.6	11.1 ± 2.3	23.5 ± 4.7	4
36–3	P	3.0 ± 0.6	0	0	0	3.0 ± 0.6	3
36–5	P	1.6 ± 0.4	5.0 ± 0.7	0.6 ± 0.2	2.0 ± 0.3	11.4 ± 2.0	5
36–7	P	0.7 ± 0.7	0.4 ± 0.2	0	0.6 ± 0.3	1.7 ± 0.9	7

Type 1: Cortical microtubules lying parallel to the growing axis; Type 2: Circumferentially aligned cortical microtubules lying perpendicular to the growing axis; Type 3: Microtubules lying between perinuclear region and cortical region of the cell; Others: Microtubules lying in the perinuclear region. 1, 2, 3 correspond to numbers in Fig. 4, respectively.
G2: Chromosomes were not condensed; GP: Condensation of chromosomes was not prominent; P: Condensation of chromosomes was prominent. Protonemata were arranged according to the condensation rate of chromosomes.

diffusion coefficients in a protonema with 5 mM colchicine did not decrease even in prophase, and the nucleus also moved in prophase, indicating interplay of PPN and the retardation of Brownian motion-like organelle movement in the L region.

3.5. Distribution of Microtubules on the Lateral Side of the Nucleus

Protonemata were incubated in darkness for 32–42 hours so that nuclei of the protonemata stayed in G_2 or M phase, and the distribution of microtubules in the L region were observed with electron microscopy.

In protonemata in which the condensation of chromosomes was not detectable or was not prominent, the number of microtubules in the L region was smaller than that in prophase protonemata (Tab. 2). Although there was no prominent change in the number of cortical microtubules parallel to the growing axis (1 in Fig. 4) between the prophase stage and the former stages (Tab. 2), circumferentially aligned cortical microtubules lying perpendicular to the growing axis (2 in Fig. 4) increased in number in prophase (Tab. 2). Microtubules oriented between the perinuclear region and the cortical region of the cell were detectable in prophase protonemata. These microtubules often lay between oil droplets and other organelles (3 in Fig. 4).

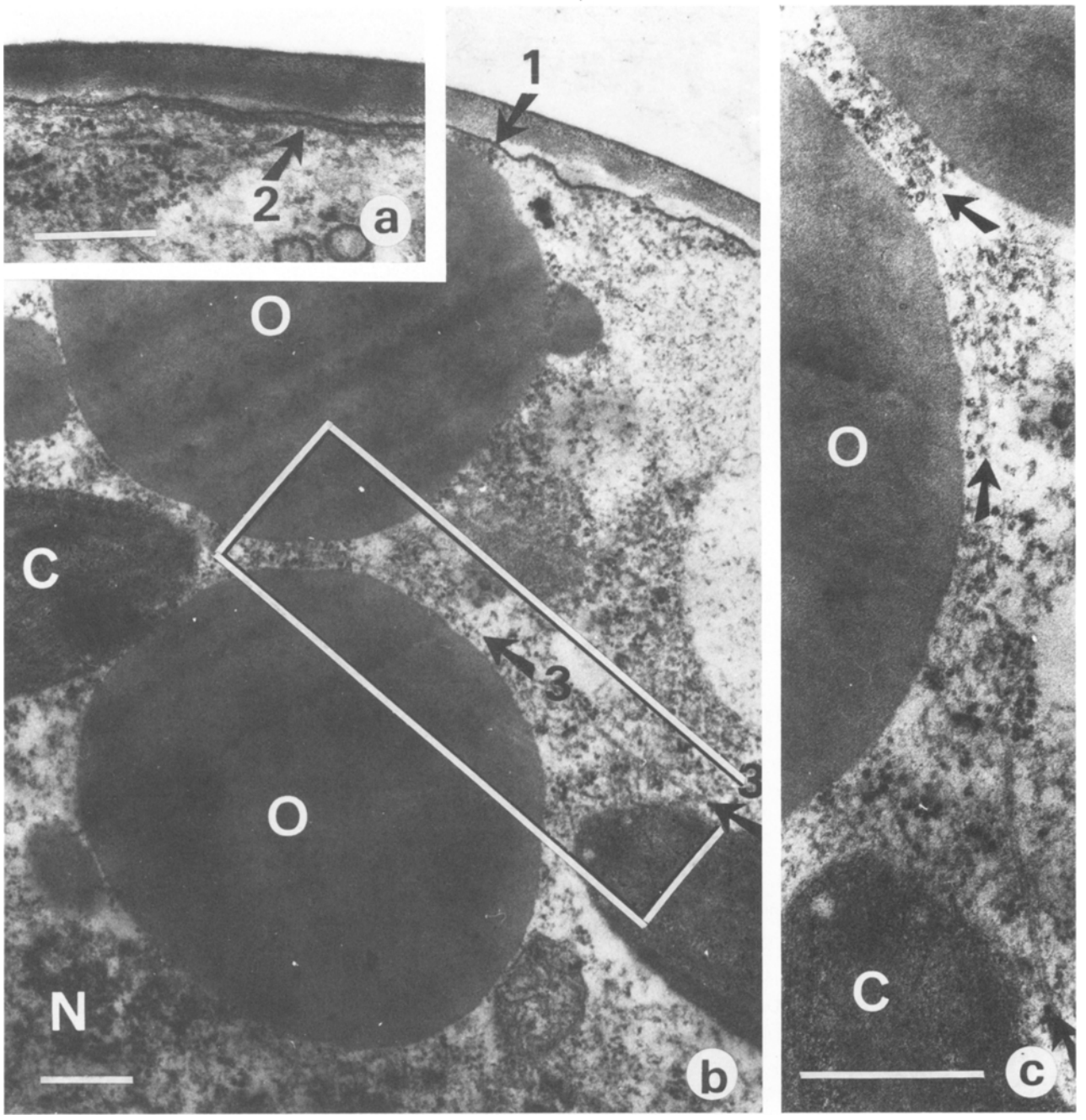

Fig. 4. Cross-section of the lateral side of a prophase nucleus of an *Adiantum* protonema. (*c*) is an enlargement of the rectangular region in (*b*). Arrows show microtubules. *N* nucleus; *O* oil droplet; *C* chloroplast. Bar = 0.5 μm

Finally, protonemata were transferred to the medium with 5 mM colchicine after incubation in darkness for 28 hours, incubated in darkness for a further 6 or 8 hours and fixed. Microtubules in protonemata incubated with 5 mM colchicine for 6 hours were less in number than those without colchicine, but when the incubation time increased, the number of microtubules was increased again (Tab. 2).

4. Discussion

Two different types of nuclear positioning are detectable in *Adiantum* protonemata. In nuclear positioning during apical growth which is observed in early G_1 phase, nuclei migrate ahead keeping constant distance from tip, but in PPN, nuclei are kept at a particular position and they do not move any more. Tip growth is not observed in PPN (MINEYUKI and FURUYA 1980). In

nuclear positioning during apical growth, nuclei are known to be displaced by the basipetal centrifugation at 360 × g for 15 minutes but not at 110 × g (MINEYUKI and FURUYA 1985). If nuclei cease their migration because of the cessation of apical growth and the nuclear supporting mechanism during apical growth is still maintained in PPN, the same result as the nuclear displacement during apical growth is anticipated from the centrifugation experiment. However, if the nuclear supporting mechanism during apical growth is disappeared and nuclei are not anchored by some cytoplasmic structure after the cessation of nuclear migration, it would be displaced by basipetal centrifugation. In contrast, if nuclei are held by some cytoplasmic structure, nuclei might not be displaced by the basipetal centrifugation even at a stronger acceleration. The centrifugation experiment with acceleration at 360 × g (Fig. 1) suggests a mechanism of supporting the nucleus against the centrifugal force in G_2 or M phase, so that the nuclei are held more firmly than during apical growth under red light.

Electron microscopy has demonstrated a significant participation of microtubules in the nuclear anchoring of the non-migrating nuclei in algae (KIERMAYER 1968, WOODCOCK 1971) and a protozoan (LANNERS 1980) and in the nuclear positioning of various kinds of tip growing cells (SIEVERS and SCHNEPF 1981). The fact that colchicine interferes with support of the nucleus but cytochalasin B does not (Tab. 1) shows the possibility that a microtubule system is involved in nuclear anchoring in G_2 or M phase.

If, as suggested above, microtubules are involved in nuclear anchoring in PPN, colchicine might also affect the PPN. The fact that nuclei in protonemata exposed to 5 mM colchicine continued to move even in prophase (Fig. 2) indicates that nuclear anchoring by a colchicine-sensitive cytoplasmic structure is responsible for the cessation of the nuclear migration at prophase. Nuclei have been suggested to be anchored in the L region after the cessation of nuclear migration from the analysis of Brownian motion-like organelle movement in *Adiantum* protonemata (MINEYUKI *et al.* 1984). On the other hand, colchicine has been known to lower cytoplasmic viscosity in onion root cells (NORTHEN 1950), *Spirogyra* (YAMAHA and UEDA 1940), eggs of *Arbacia* (BEAMS and EVANS 1940, WILBUR 1940) and mammalian tumor cells (NISHIMURA and BAUM 1957), especially in mitotic cells. The result of colchicine treatment on the Brownian motion-like organelle movement in *Adiantum* (Fig. 3) indicates that colchicine inhibits an increment of apparent cytoplasmic viscosity in L region which normally accompanies cessation of nuclear migration (MINEYUKI *et al.* 1984). This confirms the idea that the cytoplasm in the L region play an important role in the nuclear anchoring.

Candidates for the colchicine-sensitive cytoplasmic structure in the L region of *Adiantum* protonemata may be the preprophase band of microtubules (WADA *et al.* 1980) and microtubules radiating from the perinuclear region to the cell cortex (Fig. 4 *c*). The latter type of microtubule was recently reported using immunofluorescence techniques in *Haemanthus* endosperm (DE MEY *et al.* 1982) and in root meristems of *Allium* (TIWARI *et al.* 1984, WICK and DUNIEC 1983). In cells with a preprophase band, these radial microtubules were reported to connect the band and the nuclear envelope (BURGESS 1970, TIWARI *et al.* 1984, WICK and DUNIEC 1983, 1984). Involvement of the preprophase band in premitotic nuclear positioning has been proposed by BURGESS and NORTHCOTE (1967), but the observation on subsidiary cell formation in *Commelina* (PICKETT-HEAPS 1969) showed that the preprophase band was not engaged in leading or controlling premitotic nuclear migration. Thus one function of the preprophase band may be to anchor the nucleus at the "right" position in prophase.

ÔTA (1961) suggested from the results of centrifugal experiments during mitosis that some cytoplasmic structures might function in the determination of the direction of the mitotic axis. Cytoplasm in the L region, which is where the phragmosome lies in large vacuolated cells (SINNOTT and BLOCH 1940, 1941, JONES *et al.* 1960, VENVERLOO *et al.* 1980) and where the preprophase band and its associated cytoplasmic microtubules exist in most meristematic cells of higher plants (PICKETT-HEAPS and NORTHCOTE 1966 a, 1966 b, GUNNING 1982) may have some role in premitotic nuclear positioning. Although VENVERLOO *et al.* (1980) could not observe microtubules that run from the nucleus to the cell periphery in phragmosomes, GOOSEN-DE-ROO *et al.* (1984) recently succeeded in observing such microtubules in the phragmosome region. Although this region of the *Adiantum* protonemata is so narrow that phragmosomes could not be identified there (MINEYUKI *et al.* 1984), microtubules that may function in the anchoring of the nucleus were able to be observed in the L region in this study.

Acknowledgements

We are grateful to Dr. S. M. WICK for her careful reading of the manuscript. This research was supported in part by research grants from the Ministry of Education, Culture and Science of Japan to MASAKI FURUYA.

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