Journal of Biological Research-Thessaloniki **10**: 139 – 147, 2008
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

The role of new microtubule assembly and MAP65 in microtubule bundle formation in pavement cells of *Asplenium nidus*

EMMANUEL PANTERIS* and DEBORA S. KARALI

Department of Botany, *School of Biology*, *Aristotle University*, *Thessaloniki*, *541 24*, *Greece*

Received: 8 April 2008 Accepted after revision: 9 September 2008

The morphogenesis of *Asplenium nidus* pavement (epidermal) cells is achieved by the organization of microtubule bundles under the anticlinal walls and of radial arrays at the bundle ends under the periclinal walls. These microtubule arrays dictate the local cell wall reinforcement, which, in turn, results in a differential growth pattern of pavement cells that attain a sinuous anticlinal contour. In order to investigate whether the microtubule bundles are formed by lateral bundling of pre-existing cortical microtubules or by the initiation of new ones, young leaves of *A. nidus* were treated with taxol, CIPC (chloropropham), colchicine, and cytochalasin-B. Microtubule organization and pavement cell morphogenesis in untreated as well as in drug-treated leaves were examined by immunofluorescence microscopy. In taxol- and CIPC-treated cells, the microtubules failed to form bundles. In cells that were at the proper developmental stage during recovery from colchicine treatment, the nascent microtubules appeared in the pattern of bundles. In cells treated with cytochalasin-B, which lacked actin filaments, typical microtubule bundles were organized. According to these observations, microtubule bundle organization and maintenance seem to require a continuous dynamic assembly of new microtubules. In untreated cells, proteins of the MAP65 family were found to co-localize at the microtubule bundles, suggesting a role for it in the above processes.

Key words: *Asplenium nidus*, cell morphogenesis, microtubules, MAP65, pavement cells.

INTRODUCTION

The morphogenesis of lobed plant cells, such as mesophyll and pavement (epidermal) cells, is achieved by a mechanism relying on microtubule bundle organization (reviewed by Panteris & Galatis, 2005). Especially for pavement cells, anticlinal microtubule bundles and radial arrays that line the periclinal walls are the key instruments for the achievement of a sinuous anticlinal contour. The above microtubule arrays control the local reinforcement of the pavement cell walls, the result of which is a sophisticated growth pattern. The outcome of this mechanism is the sinuous pavement cell morphogenesis (Panteris & Galatis, 2005). The above morphogenetic mechanism is universal among several plant species of various taxa, including ferns, dicots, and monocots (Panteris *et al*., 1994).

As far as the mechanism of microtubule bundling is concerned, there are two possible alternatives: the microtubule bundles are formed either by lateral association of pre-existing cortical microtubules or by the assembly of new microtubules. Bundle organization via new microtubule assembly in pavement cells is supported by recent data on microtubule nucleation activity at the cell cortex, as well as on the various molecular factors that affect cortical microtubule organization (reviewed by Panteris & Galatis, 2005). Contrary to the above, the observation that actin filaments may contribute to microtubule bundling in mesophyll cells (Wernicke & Jung, 1992) supports the view that the bundles are formed by intact microtubule translocation. This is similar to the mechanism of preprophase microtubule band narrowing, which has been found to be controlled by actin filaments

* Corresponding author: tel.: +30 2310 998908, fax: +30 2310 998389, e-mail: epanter@bio.auth.gr

(Eleftheriou & Palevitz, 1992). A similar option could also be supported for the microtubule bundles of pavement cells.

According to data concerning the preprophase microtubule band, several authors have supported the view of formation via translocation of pre-existing microtubules, while others the view of new microtubule assembly (see references in Panteris *et al*., 1995). Experimental studies, in which microtubules were stabilized with taxol, revealed that preprophase band initiation and maturation/narrowing depend on new microtubule assembly rather than on rearrangement of pre-existing microtubules (Panteris *et al*., 1995). However, there is no similar experimental confirmation about the mechanism of microtubule bundle organization in pavement cells at the moment.

Another factor that participates in microtubule bundle formation and/or stabilization has recently been found to be MAP65-1. It is found on cortical microtubules and the preprophase microtubule band (Smertenko *et al*., 2000, 2004; Chang *et al*., 2005; Mao *et al*., 2005) and its over-expression results in cortical microtubule bundling (Mao *et al*., 2005, 2006).

In order to reveal the mechanism of microtubule bundle organization in protodermal cells of *Asplenium nidus*, we applied treatments with taxol (a microtubule stabilizing drug), CIPC (chloropropham, a disrupter of microtubules and/or microtubule organizing centers), colchicine (an anti-microtubule drug) and cytochalasin-B (a disrupter of actin filaments). Further, we investigated the possible presence of MAP65-1 on the microtubule bundles. Our observations support the view that microtubule bundle organization in pavement cells depends on new microtubule assembly and that MAP65-1 may play a role in this process.

MATERIALS AND METHODS

Plant material and drug treatments

Asplenium nidus L. (a tropical fern) plants were grown in the laboratory at room temperature. The leaf epidermis of this plant shows a highly regular developmental gradation, which allows monitoring of every morphogenetic stage of the pavement cells. Apical leaf segments were cut and placed on cotton moistened with drug solutions in half-strength Hoagland nutrient medium. Taxol (50 μM) was applied for four days, CIPC (25 mM) for seven days, colchicine (2 mM) for five days followed by four days in Hoagland, and cytochalasin-B (100 μM) for two days. For control, leaf segments were placed on cotton moistened with half-strength Hoagland solution for the relevant time. All experiments were conducted at room temperature and the chemicals and reagents were purchased from Sigma, unless otherwise stated.

Western blotting of MAP65

For Western immunoblotting of putative MAP65 isoforms in *A. nidus*, young untreated leaves were extracted in a homogenization buffer consisting of 100 mM HEPES, 150 mM KCl, 250 mM sucrose, 0.1% w/v SDS, 1% w/v sodium deoxycholate, 10 mM dithiothreitol, and a commercial protease inhibitor cocktail (EDTA-free Complete™, Roche). The resulting homogenate was cleared by centrifugation (5600 g, 10 min) at 4°C. The protein concentration of the supernatant was estimated according to the method of Bradford, diluted with Laemmli sample buffer and electrophoresed according to standard procedures. The proteins were subsequently transferred to nitrocellulose membranes (Protran Schleicher & Schuell) using a semi-dry flatbed blotter (Transblot SD, BioRad), then blocked for 1 hr at room temperature with 5% BSA in Tris buffered saline supplemented with 0.05% Tween-20 (TBST) and incubated with a rabbit antibody raised against the conserved MAP65 peptide CEEESWLEDYNRD at a 1:1000 dilution overnight at 4°C. The membranes were then washed in TBST, blocked in 5% BSA and incubated at room temperature with horseradish peroxidase (HRP)-coupled anti-rabbit IgGs diluted in TBST supplemented with 1% BSA (Dako) at 1:5000. HRP activity was visualized with the ECL chemiluminescence reagent (Amersham, UK) by exposure on BioMax XR film.

Tubulin and MAP65 immunofluorescence and F-actin staining with phalloidin

Untreated as well as drug-treated leaves of *A. nidus* were prepared for immunofluorescence microscopy as follows: leaf segments (approx. 2 mm^2) were fixed for 20 min in 1% paraformaldehyde (PFA) in PEM (50 mM PIPES, 5 mM EGTA, 5 mM $MgSO_4$, pH 6.8) followed by 1 hr in 4% PFA in PEM. Triton X-100 (0.5%) and dimethylsulfoxide (DMSO, 1%) were added in the fixatives. After rinsing the leaf segments three times with PEM the middle lamellae were digested with 1-2% pectinase in PEM for 2 hrs, again followed by three rinses in PEM. The leaf segments were then forced several times through a Pasteur pi-

pette to release the cells. Aliquots of all suspensions were laid on poly-l-lysine-coated microslides and left to dry. In some samples, a second digestion with 2% cellulase was carried out. Afterwards, the cells were extracted with 1% Triton X-100 and 5% DMSO in PEM for 1 hr and then thoroughly washed.

Incubations with antibodies were performed overnight as follows: i) with a monoclonal rat antibody against yeast α-tubulin (YOL 1/34, Serotec) diluted 1:80 in PEM, and ii) after washing in PEM, with FITC-conjugated anti-rat IgG diluted 1:80 in the same buffer. For MAP65 localization, the samples were incubated overnight with a rabbit anti-MAP65 antibody (see above) diluted 1:100 in PEM. After washing in PEM, the samples were incubated with TRITC-conjugated anti-rabbit IgG diluted 1:80 in PEM. The cell walls were then stained with Calcofluor White M2R. Finally, the coverslips were carefully washed with PEM and mounted in a mixture of PEM-glycerol (1:2) containing 0.5% p-phenylenediamine to prevent fading of fluorescence.

For double labeling of F-actin and microtubules, the above procedure was followed with some additional steps. Small epidermal sheets were carefully removed and incubated in 300 μM MBS (m-maleimidobenzoyl-N-hydroxysuccinimide ester) in PEM, in which 0.1% (v/v) Triton X-100 and 2% (v/v) DMSO were added, for 30 min in the dark to stabilize the actin filaments. Afterwards, fixation in 1% and 4% PFA was carried out. Before extraction, a mild cell wall digestion for 10 min with 1% (w/v) pectinase, and 1% (w/v) cellulase in PEM was included. In the 4% (w/v) PFA as well as the enzyme solutions, 1% (v/v; diluted from a stock solution of 300 units in 1.5 ml methanol) Alexa-Fluor 568 (Molecular Probes) phalloidin was added. The epidermal sheets were incubated overnight with rat anti-α-tubulin antibody (YOL 1/34, Serotec) 1:80 in PEM. After washing with PEM, the epidermal sheets were incubated with FITC-anti-rat 1:80 in the same buffer with 10% (v/v) Alexa-Fluor 568 phalloidin overnight at 37°C. The epidermal sheets were then washed with the same buffer and mounted in the previously mentioned anti-fade solution.

The preparations were examined with a Zeiss IM35 microscope equipped with epi-fluorescence and photographed with Kodak T-Max 100 film rated at ISO 400. Some samples were examined with a Confocal Laser Scanning Microscope (CLSM, Nikon D-Eclipse C1).

RESULTS

Microtubule bundle organization in untreated cells

After cell division cycles were completed, leaf protodermal cells of *A. nidus* exhibited cortical microtubules already starting to form bundles, while the anticlinal walls remained more or less straight (Fig. 1). As protodermal cells kept on growing and their anticlinal walls started to become wavy, the microtubule bundles still persisted (Fig. 2A, D). MAP65 staining was found to co-localize with the above microtubule bundles (Fig. 2). The specificity of the anti-MAP65-1 antibody for possible MAP65 homologs of *A. nidus* was confirmed by Western blotting (Fig. 3).

Inhibitor-treated cells

Leaf protodermal cells, which were treated for four days with 50 μM taxol, developmentally similar to protodermal cells of untreated leaves, exhibited a uniform distribution of the cortical microtubules without any signs of bundle formation (Fig. 4A, B; cf Figs 1, 2) or thickening of the cell wall (Fig. 4C).

Protodermal cells treated for seven days with 25 mM CIPC possessed fewer microtubules than untreated cells (Fig. 5A-D). These microtubules were shorter than those of the untreated cells and ran-

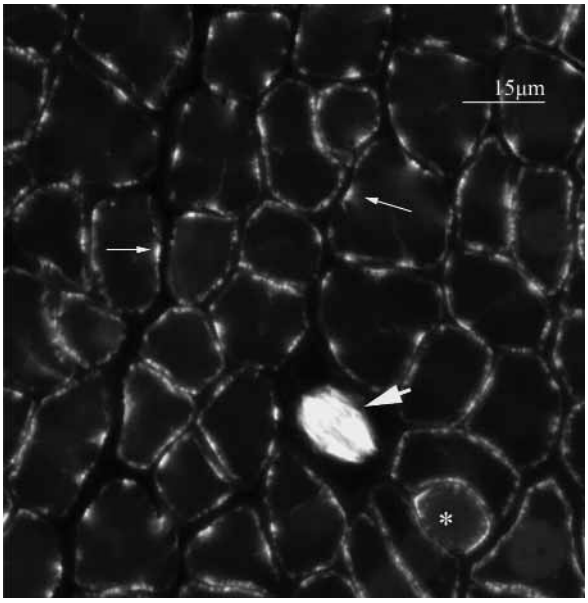

FIG. 1. Tubulin immunofluorescence of protodermal cells. In this central CLSM section as well as in all following figures, the cells are shown at top view. In this protodermal area, cell divisions (arrowhead) are about to cease, while microtubule bundles start to organize (arrows). The star notes a guard cell mother cell.

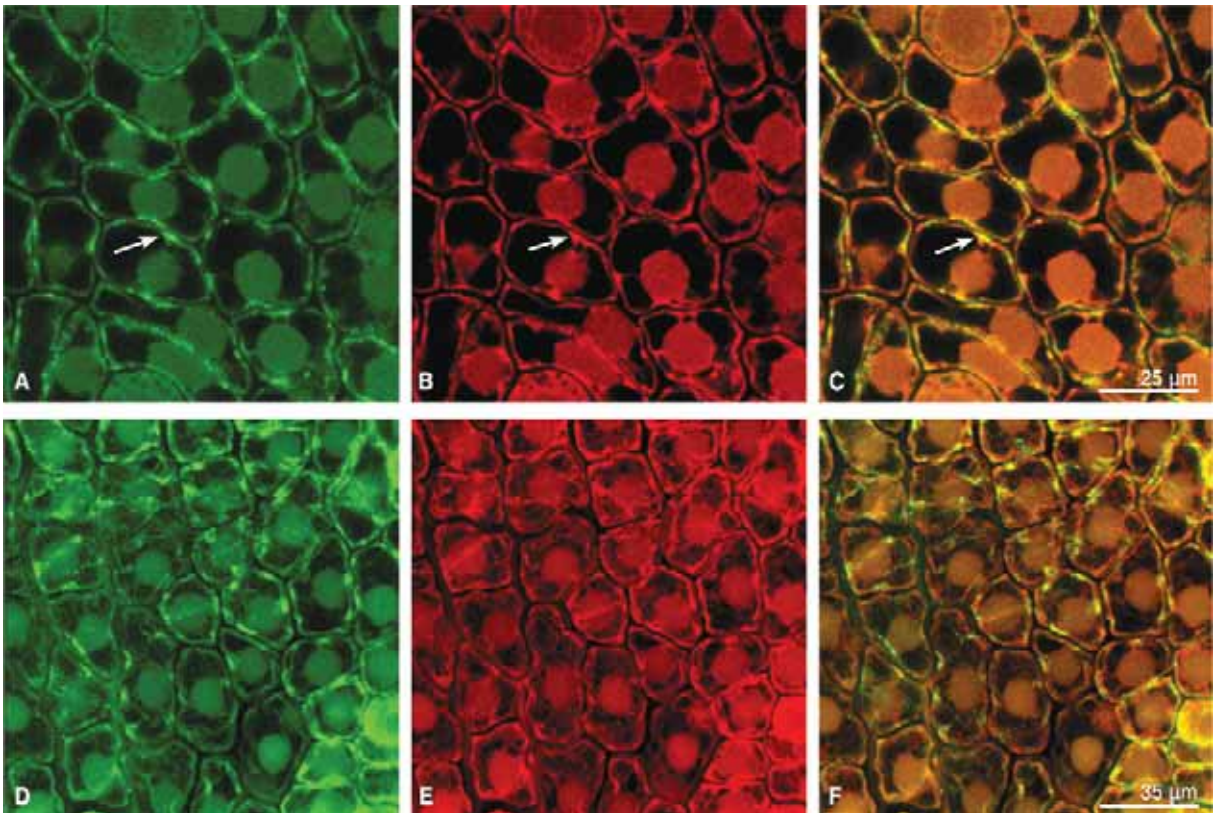

FIG. 2. CLSM micrographs of protodermal cells after immunostaining of tubulin (green signal in A, D) and MAP65 (red signal in B, E). In C, F a merge of the two stainings is depicted (the overlapping signal appears in yellow). A-C: Central optical CLSM section of protodermal cells, exhibiting microtubule bundles (arrow in A) under the anticlinal walls. The signal of MAP65 follows that of tubulin (arrow in B, see also merge in C). D-F: Projection of 30 optical CLSM sections of protodermal cells. Note that MAP65 signal (E) co-localizes with that of tubulin (D, see also merge in F).

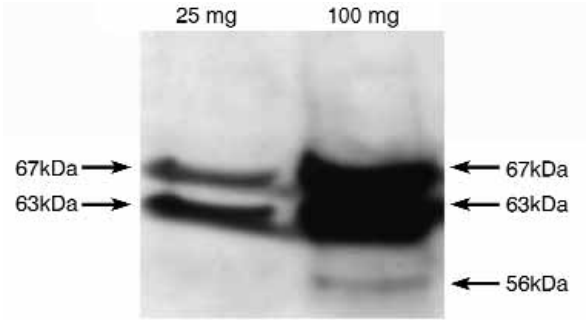

FIG. 3. Western blot of MAP65 proteins in *Asplenium nidus* protein extract. Two prominent bands at 63 and 67kDa can be seen at 25 mg protein sample. In 100 mg, a third band at 56kDa also appears, probably representing a low abundance MAP65 isoform or a degradation product.

domly arranged (Fig. 5A, C; cf Figs 1-2). No microtubule bundles could be observed in cells developmentally similar to untreated cells that possessed prominent microtubule bundles (Fig. 5B, D; cf Fig. 1). Certain CIPC-treated cells had already achieved slight anticlinal wall waviness (Fig. 5E) due to microtubule bundles organized before the onset of treatment, but no bundles were observable in these cells (Fig. 5C, D).

Microtubules were completely absent from protodermal cells treated with colchicine (2 mM) for five days (data not shown). In cells left to recover after this treatment for four days, new microtubules became organized (Fig. 6A, B). In protodermal cells

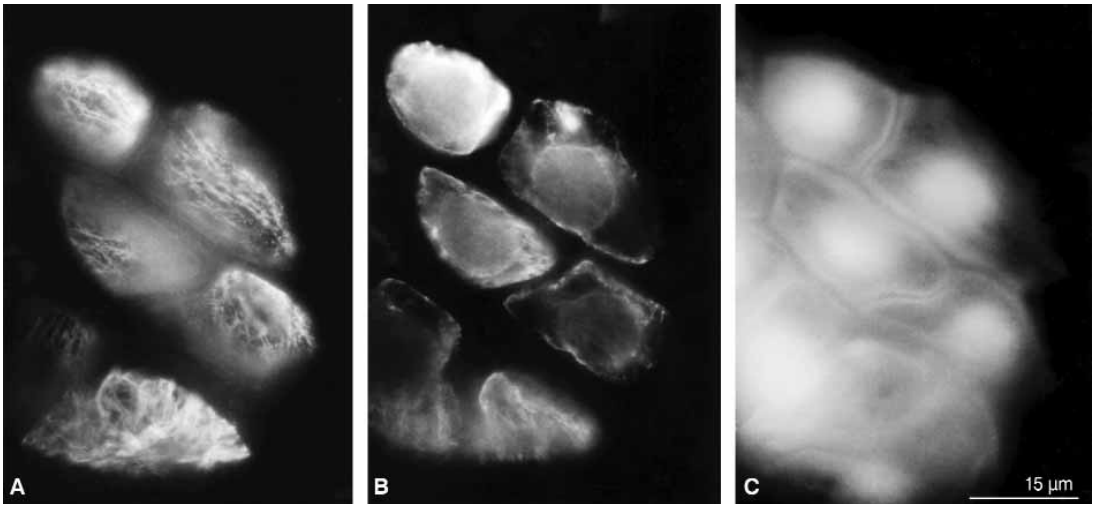

FIG. 4. Tubulin immunofluorescence (A, B) and staining with Calcofluor White M2R (C) of taxol-affected protodermal *Asplenium nidus* cells at top view. Numerous microtubules line the external periclinal (A) and anticlinal (B) walls, not organized in bundles. Local thickenings cannot be observed at the cell walls (C).

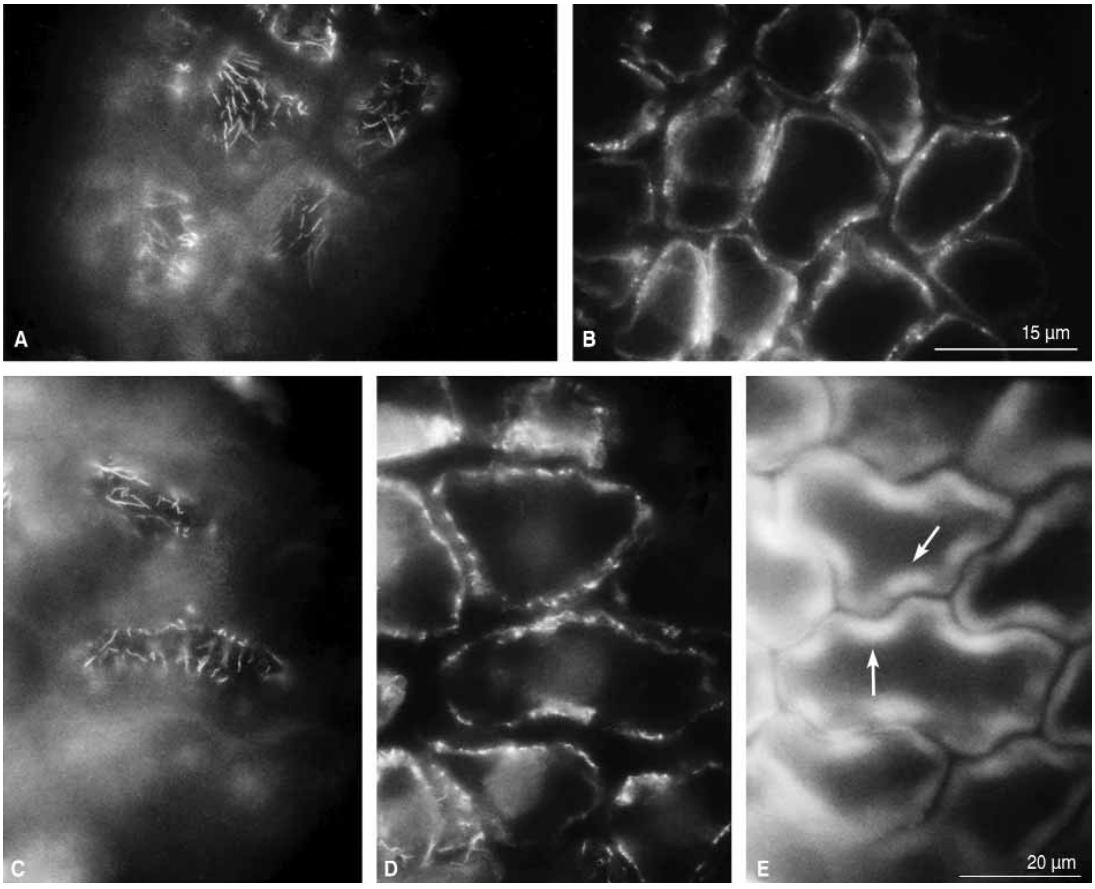

FIG. 5. CIPC-treated protodermal cells after tubulin immunostaining (A-D) and Calcofluor White M2R staining (E). External (A) and central (B) optical sections of protodermal cells with short branched microtubules, not arranged in bundles. C-E: Epidermal cells that had achieved a wavy anticlinal contour (E) before CIPC treatment. Although wall thickenings (arrows in E) had been deposited over the previously existing microtubule bundles and radial arrays, short randomly arranged microtubules line the external periclinal wall (C) and anticlinal walls (D) after the treatment.

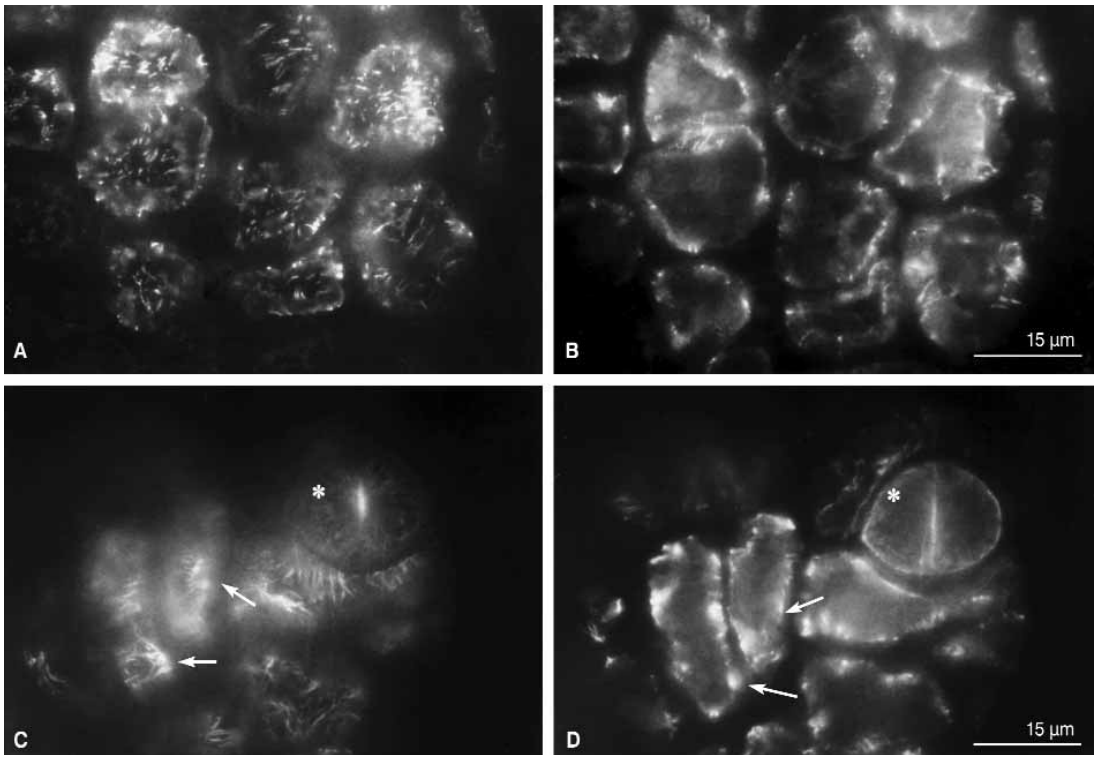

FIG. 6. Tubulin immunostaining of protodermal cells recovering after colchicine treatment. In some protodermal areas, short microtubules can be observed to reappear at external (A) and central (B) optical sections. In other protodermal areas, almost normal microtubules can be seen, organized in radial arrays (arrows in C) under the external periclinal wall (C) and in bundles under the anticlinal walls (arrows in D). The star in C, D notes a young stoma.

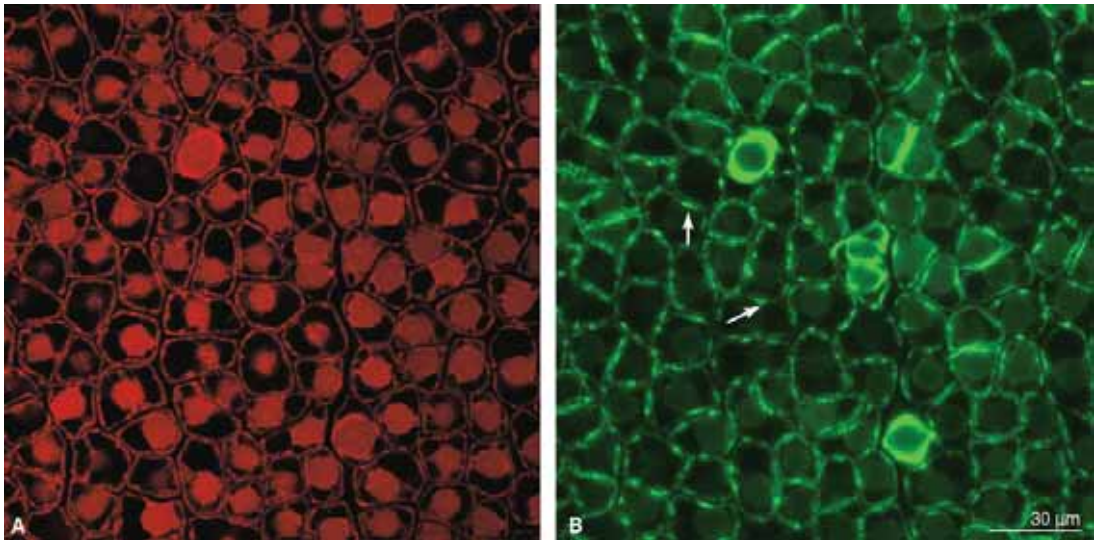

FIG. 7. Central CLSM section of protodermal area treated with cytochalasin-B and double-stained for F-actin (A) and microtubules (B). A: No actin filaments can be observed with phalloidin staining. B: In the same F-actin-free cells, the cortical microtubules are organized in bundles (arrows) under the anticlinal walls.

similar in size to the untreated cells with initiating bundles, the new microtubules were assembled *in situ* in bundles under the anticlinal walls (Fig. 6C, D; cf Figs 1-2).

Protodermal cells treated for two days with 100 μM cytochalasin-B, were totally devoid of actin filaments (Fig. 7A). In these cells, cortical microtubule bundles similar to those of the untreated cells could be observed (Fig. 7B; cf Figs 1-2).

DISCUSSION

Microtubule bundle organization depends on dynamic tubulin turnover

According to our results, leaf protodermal cells of *A. nidus* treated with taxol fail to organize microtubule bundles. The effect of taxol on the protodermal cells is similar to that in the preprophase/prophase cells of *Triticum turgidum* (Panteris *et al*., 1995). In both cases, taxol-induced stabilization of cortical microtubules delays or even arrests the transition from uniform arrangement to the bundle pattern. Taxol prevents tubulin turnover and microtubule disassembly but not the translocation/bunching of intact microtubules. Consequently, if microtubule bundle formation depended on the latter process, taxol should not inhibit it. On the contrary, the effect of taxol shows that, similarly to the preprophase microtubule band (Panteris *et al*., 1995), the anticlinal microtubule bundles of *A. nidus* protodermal cells are organized by the disassembly of pre-existing microtubules and the assembly of new ones.

The fact that cytochalasin-B treatment does not prevent microtubule bundle formation supports further the above conclusion. This drug disrupts actin filaments, which have been correlated with translocation of microtubules in the case of the preprophase band (Eleftheriou & Palevitz, 1992) and microtubules of mesophyll cells (Wernicke & Jung, 1992). As the microtubule bundles in the cells studied are organized in the absence of actin filaments, it seems that lateral translocation is not a part of the bundle formation process. On the other hand, F-actin is not involved in microtubule array organization by new microtubule assembly. In accordance, the observation of microtubule bundles, in cytochalasin-B-treated protodermal cells of *A. nidus*, substantiates further that these bundles are organized by new microtubule assembly.

The effect of CIPC on the protodermal cells might be explained by two alternatives. According to the first, the possible direct effect of the drug on the microtubules, as mentioned by some authors (Hoffman & Vaughn, 1994; Eleftheriou & Bekiari, 2000), influences their number, size and distribution, so that microtubule bundles are not organized. The other alternative might be the indirect action of CIPC through disruption of the possible cortical microtubule organizing centers (MTOCs, Clayton & Lloyd, 1984; Lloyd & Traas, 1988; Hoffman & Vaughn, 1994; Yemets *et al*., 2008). According to the latter alternative, cortical MTOC activation is necessary for microtubule bundle formation, as shown for *Adiantum capillus-veneris* mesophyll and epidermal cells (Panteris *et al*., 1993a, b). Further, this is supported by the formation of microtubules in a bundle-like pattern during the recovery from the colchicine treatment (see Fig. 6C, D). It seems that there is no need of pre-existing random cortical microtubules for bundle formation. On the contrary, the tubulin dimers, which are gradually released during recovery from colchicine, are readily recruited in nascent microtubule bundles. As it has been shown in other plant species, microtubules assemble readily from free tubulin heterodimers after anti-microtubule drug treatments at sites, where putative MTOCs function (Cleary & Hardham, 1988; Falconer *et al*., 1988; Galatis & Apostolakos, 1991).

Apart from the initial microtubule bundle assembly, the fact that CIPC treatment results in a disassembly of the already organized microtubule bundles (Fig. 5C-E), supports the view that the maintenance of the bundles also depends on dynamic microtubule turnover. The dynamic behaviour of cortical microtubules, which is required for such an organization mechanism, has already been confirmed (Shaw *et al*., 2003; Tian *et al*., 2004). Molecular factors implicated in cortical microtubule dynamics, such as the microtubule-severing protein katanin (Bichet *et al*., 2001; Burk *et al*., 2001; McClinton *et al*., 2001; Stoppin-Mellet *et al*., 2002; Webb *et al*., 2002), the microtubule nucleating large γ-tubulin complexes (Dryková *et al*., 2003) and the EB1 centrosomal homologue (Chan *et al*., 2003), may function as major co-ordinators of microtubule bundle organization and maintenance. In addition, the differential expression of tubulin genes, as it has been shown to occur during the development of *Hordeum vulgare* mesophyll cells (Hellman & Wernicke, 1998; Schröder *et al*., 2001), further supports that microtubule bundles are organized by new microtubule assembly.

MAP65 participates in microtubule bundle organization

Among the nine members of the MAP65 family of *Arabidopsis thaliana* (Hussey *et al*., 2002), MAP65-1 has been found on cortical microtubules, both interphase ones and those of the preprophase band (reviewed by Hamada, 2007). MAP65 proteins have been shown to form cross-bridges between parallel cortical microtubules (Chan *et al*., 1999), while the over-expression of MAP65-1 results in extra microtubule bundle organization (Mao *et al*., 2005, 2006). In accordance with this, the localization of MAP65 proteins on microtubule bundles in leaf protodermal cells of *A. nidus* may suggest a role for this protein in bundle formation. Although the number of MAP65 genes and isoforms in the above plant is unknown, the applied antibody recognizes the highly conserved peptide CEEESWLEDYNR (Mao *et al*., 2005) and stains MAP65 proteins both by Western blotting (Fig. 3) and immunofluorescence (Fig. 2). To our knowledge, this is the first report of MAP65 presence and possible role in ferns.

MAP65-1 has been also proposed to participate in microtubule assembly by direct binding to tubulin heterodimers (Li *et al*., 2007). As new microtubule assembly is required for microtubule bundle organization, the presence of MAP65 on the above arrays might also reflect its participation in bundle formation from the very early stages of their initiation. It might be proposed that MAP65 plays one role during the assembly of the microtubule bundles and another in maintaining the bundles by cross-bridging the developing microtubules.

ACKNOWLEDGEMENTS

The authors thank Dr. George Komis (Department of Botany, Faculty of Biology, University of Athens) for his assistance in performing the Western blots of this study. The anti-MAP65 antibody was a gift from Prof. Clive W Lloyd (John Innes Institute, Norwich, UK). Access to the Nikon D-Eclipse C1 CLSM of the Faculty of Veterinary Medicine of our University was kindly provided by Dr. Anastasia Tsingotjidou.

REFERENCES

Bichet A, Desnos T, Turner S, Grandjean O, Höfte H, 2001. BOTERO1 is required for normal orientation of cortical microtubules and anisotropic cell expansion in *Arabidopsis*. *Plant journal*, 25: 137-148.

Burk DH, Liu B, Zhong R, Morrison WH, Ye Z-H, 2001. A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *The plant cell*, 13: 807-827.

Chan J, Jensen CC, Jensen LC, Bush M, Lloyd CW, 1999. The 65-kDa carrot microtubule-associated protein forms regularly arranged filamentous cross-bridges between microtubules. *Proceedings of the national academy of sciences USA*, 96: 14931-14936.

Chan J, Calder GM, Doonan JH, Lloyd CW, 2003. EB 1 reveals mobile microtubule nucleation sites in *Arabidopsis*. *Nature cell biology*, 5: 967-971.

Chang HY, Smertenko AP, Igarashi H, Dixon DP, Hussey PJ, 2005. Dynamic interaction of NtMAP65-1a with microtubules *in vivo*. *Journal of cell science*, 118: 3195-3201.

Clayton L, Lloyd CW, 1984. The relationship between the division plane and spindle geometry in *Allium* cells treated with CIPC and griseofulvin: an anti-tubulin study. *European journal of cell biology*, 34: 248-253.

Cleary AL, Hardham AR, 1988. Depolymerization of microtubule arrays in root tip cells by oryzalin and their recovery with modified nucleation patterns. *Canadian journal of botany*, 66: 2353-2366.

Dryková D, Cenklová V, Sulimenko V, Volc J, Dráber P, Binarová P, 2003. Plant γ-tubulin interacts with $\alpha\beta$-tubulin dimers and forms membrane-associated complexes. *The plant cell*, 15: 465-480.

Eleftheriou EP, Palevitz BA, 1992. The effect of cytochalasin D on preprophase band organization in root tip cells of *Allium*. *Journal of cell science*, 103: 989-998.

Eleftheriou EP, Bekiari E, 2000. Ultrastructural effects of the herbicide chloropropham (CIPC) in root tip cells of wheat. *Plant soil*, 226: 11-19.

Falconer MM, Donaldson G, Seagull RW, 1988. MTOCs in higher plant cells: an immunofluorescent study of microtubule assembly sites following depolymerisation by APM. *Protoplasma*, 144: 46-55.

Galatis B, Apostolakos P, 1991. Patterns of microtubule reappearance in root tip cells of *Vigna sinensis* recovering from a colchicine treatment. *Protoplasma*, 160: 131-143.

Hamada T, 2007. Microtubule-associated proteins in higher plants. *Journal of plant research*, 120: 79-98.

Hellmann A, Wernicke W, 1998. Changes in tubulin protein expression accompany reorganization of microtubular arrays during cell shaping in barley leaves. *Planta*, 204: 220-225.

Hoffman JC, Vaughn KC, 1994. Mitotic disrupter herbicides act by a single mechanism but vary in efficacy. *Protoplasma*, 179: 16-25.

Hussey PJ, Hawkins TJ, Igarashi H, Kaloriti D, Smertenko A, 2002. The plant cytoskeleton: recent advances in the study of the plant microtubule-associated proteins MAP-65, MAP-190 and the *Xenopus* MAP215-

like protein, MOR1. *Plant molecular biology*, 50: 915-924.

Li H, Yuan M, Mao T, 2007. At-MAP65-1 binds to tubulin dimers to promote tubulin assembly. *Journal of biochemistry and molecular biology*, 40: 218-225.

Lloyd CW, Traas JA, 1988. The role of F-actin in determining the division plane of carrot suspension cells. Drug studies. *Development*, 102: 211-221.

Mao G, Chan J, Calder G, Doonan JH, Lloyd CW, 2005. Modulated targeting of GFP-AtMAP65-1 to central spindle microtubules during division. *Plant journal*, 43: 469-478.

Mao G, Buschmann H, Doonan JH, Lloyd CW, 2006. The role of MAP65-1 in microtubule bundling during *Zinnia* tracheary element formation. *Journal of cell science*, 119: 753-758.

McClinton RS, Chandler JS, Callis J, 2001. cDNA isolation, characterization, and protein intracellular localization of a katanin-like p60 subunit from *Arabidopsis thaliana*. *Protoplasma*, 216: 181-233.

Panteris E, Galatis B, 2005. The morphogenesis of lobed plant cells in the mesophyll and epidermis: organization and distinct roles of cortical microtubules and actin filaments. *New phytologist*, 167: 721-732.

Panteris E, Apostolakos P, Galatis B, 1993a. Microtubule organization, mesophyll cell morphogenesis and intercellular space formation in *Adiantum capillus veneris* leaflets. *Protoplasma*, 172: 97-110.

Panteris E, Apostolakos P, Galatis B, 1993b. Microtubule organization and cell morphogenesis in two semilobed cell types of *Adiantum capillus-veneris* L. leaflets. *New phytologist*, 125: 509-520.

Panteris E, Apostolakos P, Galatis B, 1994. Sinuous ordinary epidermal cells: behind several patterns of waviness, a common morphogenetic mechanism. *New phytologist*, 127: 771-780.

Panteris E, Apostolakos P, Galatis B, 1995. The effect of taxol on *Triticum* preprophase root cells: preprophase microtubule band organization seems to depend on new microtubule assembly. *Protoplasma*, 186: 72-78.

Schröder J, Stenger H, Wernicke W, 2001. α-Tubulin genes are differentially expressed during leaf cell development in barley (*Hordeum vulgare* L.) *Plant molecular biology*, 45: 723-730.

Shaw SL, Kamyar R, Ehrhardt DW, 2003 Sustained microtubule treadmilling in *Arabidopsis* cortical arrays. *Science*, 300: 1715-1718.

Smertenko A, Saleh N, Igarashi H, Mori H, Hauser-Hahn I, Jiang CJ, Sonobe S, Lloyd CW, Hussey PJ, 2000. A new class of microtubule-associated proteins in plants. *Nature cell biology*, 2: 750-753.

Smertenko AP, Chang H-Y, Wagner V, Kaloriti D, Fenyk S, Sonobe S, Lloyd C, Hauser M-T, Hussey PJ, 2004. The *Arabidopsis* microtubule-associated protein AtMAP65-1: molecular analysis of its microtubule bundling activity. *The plant cell*, 16: 2035-2047.

Stoppin-Mellet V, Gaillard J, Vantard M, 2002. Functional evidence for *in vitro* microtubule severing by the plant katanin homolog. *Biochemistry journal*, 365: 337-342.

Tian G-W, Smith D, Gluck S, Baskin TI, 2004. Higher plant cortical microtubule array analyzed *in vitro* in the presence of the cell wall. *Cell motility and the cytoskeleton*, 57: 26-36.

Webb M, Jouannic S, Foreman J, Linstead P, Dolan L, 2002. Cell specification in the *Arabidopsis* root epidermis requires the activity of *ECTOPIC ROOT HAIR 3* – a katanin-p60 protein. *Development*, 129: 123-131.

Wernicke W, Jung G, 1992. Role of cytoskeleton in cell shaping of developing mesophyll of wheat (*Triticum aestivum* L.). *European journal of cell biology*, 57: 88-94.

Yemets A, Stelmach O, Blume YB, 2008. Effects of the herbicide isopropyl-N-phenyl carbamate on microtubules and MTOCs in lines of *Nicotiana sylvestris* resistant and sensitive to its action. *Cell biology international*, 32: 623-629.