Journal of Biological Research **2**: 3 – 19, 2004
J. Biol. Res. is available online at http://www.jbr.gr

An inhibitor of the ATP-dependent endoplasmic reticulum Ca^{2+}-pump affects spindle organization in dividing cells of the angiosperm *Triticum turgidum* but not in species of gymnosperms and pteridophytes

MICHAEL ZACHARIADIS[1], HARTMUT QUADER[2], BASIL GALATIS[1] and PANAGIOTIS APOSTOLAKOS[1*]

[1] *Department of Botany, Faculty of Biology, University of Athens, Athens 157 84, Greece*
[2] *Institute of General Botany, University of Hamburg, D-22609 Hamburg, Germany*

Received: 14 July 2004 Accepted after revision: 10 September 2004

The effects of the mycotoxin cyclopiazonic acid (CPA) on the organization of the microtubule (Mt) cytoskeleton were investigated in dividing cells of different taxa of higher plants. CPA is a specific inhibitor of endoplasmic reticulum (ER) Ca^{2+}-ATPase, which greatly affects the cytosolic Ca^{2+} concentration.
In the CPA-affected cells of the angiosperm *Triticum turgidum* the maturation of the Mt preprophase band (PPB) is delayed, while the organization of the prophase spindle is disturbed or inhibited. During metaphase, the organization of the bipolar metaphase spindle and the arrangement of the chromosomes are perturbed. In contrast, in the CPA- affected dividing cells of the gymnosperm *Pinus brutia* and the pteridophytes *Asplenium nidus* and *Adiantum capillus-veneris*, the organization of the Mt cytoskeleton is not detectably disturbed. However, CPA affected the progress of the cell cycle in all the plants studied as well as other Ca^{2+}-dependent processes like cell plate development.
These observations suggest that: (a) the ER is involved in establishing cytosolic Ca^{2+}-gradients, which control Mt organization in mitotic cells of the angiosperm but not of the gymnosperm and the pteridophyte species examined and (b) in the spindle poles of *P. brutia*, *A. nidus* and *A. capillus-veneris*, microtubule organizing centres (MTOCs) may function, i.e. they display centrosomal properties.

Key words: Cyclopiazonic acid, cytosolic calcium, microtubule, phragmoplast formation, spindle formation.

INTRODUCTION

In dividing animal cells possessing centrioles, the mitotic spindle Mts are nucleated in the pericentriolar region of the centrosome, while in those that lack centrioles, they appear on the surface of the prometaphase/metaphase chromosomes (Merdes & Cleveland, 1997; Walczak *et al.,* 1998). Similarly, in dividing cells of higher plants, which lack centrioles, the mitotic and meiotic spindles seem to be formed *de novo* at prometaphase/metaphase from Mts nucleated in close vicinity to the chromosome surface (Kubiak *et al.,* 1986; Wang *et al.,* 1991; Binarová *et al.,* 1998, 2000; Chan & Cande, 1998; Franklin & Cande, 1999; Zachariadis *et al.,* 2000). MAPs and motor proteins are probably involved in Mt re-organization during spindle formation in animal (Merdes & Cleveland, 1997; Walczak *et al.,* 1998; Compton, 1998) and higher plant cells (Canaday *et al.,* 2000; Vantard *et al.,* 2000; Reddy, 2001; Kumagai & Hasezawa, 2001; Schmit, 2002).

Two C-terminal kinesin-like proteins (KCBP and Kat-A) and one N-terminal kinesin-like protein (TKRP-125) from plants co-localize with Mt arrays during cell division in higher plants (see reviews by

* Corresponding author: tel.: +30 210 7274628, fax: +30 210 7274702, e-mail: papostol@biol.uoa.gr

Reddy, 2001; Hepler *et al.,* 2002). The KCBP, a kinesin-like calmodulin binding protein that has been identified in plants only (Reddy, 2001), seems to be of particular importance for the organization of the mitotic Mt arrays. The Ca^{2+}/calmodulin complex controls the binding of this motor protein to Mts (Reddy, 2001). In *Tradescantia*, the KCBP acts differentially during the cell cycle and thus it may control the organization of the different Mt arrays in dividing cells (Vos *et al.,* 2000; Reddy, 2001; Hepler *et al.,* 2002). This differential KCBP activity is probably regulated by particular cytosolic Ca^{2+} gradients (Vos *et al.,* 2000; Hepler *et al.,* 2002), which are involved in Mt organization during plant cell division (Hepler, 1989, 1992; Wolniak, 1991). Most likely, the ability of the ER to sequester and release Ca^{2+} may play a role in forming a Ca^{2+} signal in dividing cells via distinct Ca^{2+} concentration changes (Hepler & Wolniak, 1984; Hepler, 1989, 1992).

The existing information on the mechanism of the mitotic spindle formation and on the key role of the cytosolic Ca^{2+} in the control of cell division has been exclusively derived from angiosperm plants (Hepler, 1989, 1992; Wolniak, 1991; Reddy, 2001; Hepler *et al.,* 2002). In the present work we investigated whether ER involving cytosolic Ca^{2+} gradients play a similar role in the organization of Mt arrays in dividing cells of other groups of higher plants. In particular, we studied the effects of the mycotoxin cyclopiazonic acid (CPA) on the organization of Mts in dividing cells of the angiosperm *Triticum turgidum*, the gymnosperm *Pinus brutia* and the pteridophytes *Asplenium nidus* and *Adiantum capillus-veneris*.

In animal and plant cells, CPA inhibits Ca^{2+} uptake into the ER lumen (Goeger *et al.,* 1988; Seidler *et al.,* 1989; Hsieh *et al.,* 1991). In *Nicotiana tabacum* suspension cells, CPA induces an elevation of the cytosolic Ca^{2+} concentration (Rengel & Zhang, 2003), while in growing pollen tubes, CPA induces a distinct elevation of the cytosolic Ca^{2+} concentration in the tip region (Novoa *et al.,* 1992). It also affects certain Ca^{2+}-dependent processes, like organelle movement and ER organization (Quader & Bechter, 1996; Quader *et al.,* 1996). CPA has also been used to investigate the role of cytosolic Ca^{2+} on membrane flow (Busch & Sievers, 1993) and on the gravitropic response (Sievers & Busch, 1992) in plants. Considering all the above studies it is expected that the cytosolic Ca^{2+} concentration, which is controlled by the ER, will be also disturbed in dividing cells treated with CPA.

MATERIALS AND METHODS

The following plants were used as experimental material: (a) Two to three day-old seedlings of the angiosperm *Triticum turgidum* var. *durum* Raddi grown in the darkness at 25°C. (b) Seedlings of the gymnosperm *Pinus brutia* Ten. grown for 20 days at 20°C in light. (c) Young leaves of the pteridophyte *Asplenium nidus* L. and root-tips of the pteridophyte *Adiantum capillus-veneris* L. grown under laboratory conditions.

Root-tips of *T. turgidum*, *P. brutia* and *A. capillus-veneris* and excised developing leaves of *A. nidus* were placed on cotton moistened with 10 or 20 μM CPA in tris/maleate buffer (5mM), pH 5.0, for the following time periods: root-tips of *T. turgidum* for 2, 3 and 6 h, *P. brutia* for 3 and 6 h, *A. capillus-veneris* for 6 h and the leaves of *A. nidus* for 6, 12, 24 and 48 h. Control samples were only placed in tris/maleate buffer, pH 5.0.

Mt immunolocalization and DNA staining in all the plant species examined was carried out according to the protocols given by Zachariadis *et al.* (2000, 2001, 2003). For Mt immunolocalization, the following monoclonal antibodies were used: the anti-a-tubulin clone YOL 1/34 (Harlan Seralab) in *T. turgidum*, *P. brutia* and *A. capillus-veneris* and the anti-a-tubulin clone DM1A (Sigma) in *T. turgidum* and *A. nidus*.

The immunofluorescent specimens were examined with a Zeiss Axioplan epifluorescence microscope and with a confocal-laser-scanning microscope (TCS-4D; Leica Microsystems, Bensheim, Germany). Mt immunolabeling in *T. turgidum*, *P. brutia* and *A. capillus-veneris* was carried out on squashed material, while in *A. nidus* on thin hand-made sections.

For electron microscopy examination, the samples were prefixed with 3% glutaraldehyde and 1% tannic acid in 50 mM cacodylate buffer pH 6.8 for 2 h, post-fixed with 1% OsO_4 in the same buffer for 6 h, dehydrated in an acetone series and embedded in Spurr's resin mixture according to the standard protocol. Thin sections were stained with uranyl acetate and lead citrate and examined with either a Philips 300 or a Philips 420 transmission electron microscopes.

For each plant and treatment, the number of cells in the different stages of the cell cycle was calculated. Three or more measurements were carried out for each case (*T. turgidum*: control, 6 measurements;

2 h CPA-treatment, 4 measurements; 6 h CPA-treatment, 3 measurements; *P. brutia*: control and 6 h CPA-treatment, 3 measurements; *A. nidus*: control, 8 measurements; 12 h CPA-treatment, 7 measurements). Descriptive statistics based on the above data were calculated using Graphpad Prism 4 (GraphPad Software, Inc.). For the comparison of affected vs. control cells, for each cell-cycle phase unpaired *t* test for two populations was used, assuming equal variances. For the statistical analyses, a P value of less than 0.05 was considered significant.

RESULTS

Triticum turgidum

General remarks

In control root-tips of *T. turgidum*, under the growth conditions used, 28% of the cells were in mitosis or cytokinesis. In Fig. 1, the distribution of the cells in the various cell-cycle stages is shown in relation to the total number of cells counted (5004 cells; Fig. 1A) and to the number of dividing cells only (1401 cells; Fig. 1B). The identification of the cell-cycle stages was based on chromatin and Mt organization. Mt organization in control dividing root-tip cells of *T. turgidum* has been previously described (Zachariadis *et al.,* 2000; Frantzios *et al.,* 2000, 2001) and is similar to that described for other *Triticum* species (Gunning & Steer, 1996).

In the CPA-affected root-tips, the progress of the cell cycle and the Mt organization is disturbed. The distribution of the root-tip cells after CPA treatment for 2 and 6 h in the various cell-cycle stages is depicted in Fig. 1 in relation to the total number of cells counted (Fig. 1A) and to the number of dividing cells only (Fig. 1B). For the 6-hour CPA-treatment, 1415 cells were counted from which 344 were at mitosis or cytokinesis, while for the 2-hour treatment, 373 mitotic and cytokinetic cells were counted. Examination of the histograms shown in Fig. 1 reveals that in CPA-treated root-tips, the course of the cell cycle is greatly disturbed. Statistically significant differences ($P<0.05$) were found in all stages of cell division after a 2-hour CPA-treatment but only in preprophase, prophase, and prometaphase/metaphase after a 6-hour treatment.

Study of the histograms in Fig. 1 shows that: (a) after a 6-hour CPA-treatment, the percentage of the cells in interphase remains relatively constant in relation to the control (Fig. 1A), a fact suggesting that the entry to and exit of the cells from cell division is not significantly affected. (b) CPA-treatment causes a great decrease in the percentage of cells in preprophase and an increase in prophase and metaphase (Fig. 1A,B), a fact suggesting acceleration of preprophase and lengthening of prophase and metaphase. The accumulation of cells in prophase is probably due to the delay of nuclear envelope breakdown at the prophase/prometaphase transition, which is controlled by the cytosolic Ca^{+2} concentration (Wolniak, 1991; Hepler, 1992). The accumulation of cells in metaphase is probably caused by the aberrations in the organization of the metaphase spindle and in the arrangement of the chromosomes, which are described in detail later.

Mt organization

In several CPA-treated preprophase/prophase cells, PPB maturation was delayed or inhibited (Fig. 1B). There were cells showing an advanced prophase chromatin condensation and a PPB at a young or developing stage of organization (Fig. 2A-C; cf Fig. 2E-H). CLSM examination revealed that these cells displayed numerous Mts traversing the cortical cytoplasm far from the PPB region as well as endoplasmic Mts (Fig. 3B; cf Fig. 3A). The organization of the prophase spindle was also affected in the CPA-treated cells. In a significant number of cells displaying a prophasic chromatin condensation, the number of the perinuclear Mts was drastically reduced (Fig. 2A-C; cf 2E-H). CLSM examination showed that in these cells the perinuclear Mts were aligned in various orientations (Fig. 3B; cf Fig. 3A) and at the end of prophase they failed to form a bipolar prophase spindle (Fig. 2B; cf. Fig. 2E, G and Fig. 3D; cf Fig. 3C). In the same cells, numerous Mts were localized in some distance from the nucleus, a feature not found in untreated prophase cells (Fig. 3B, D; cf Fig. 3C). The recognition of the preprophase and prophase cells during CLSM examination was based on the organization of the PPB, since in root-tips of *T. turgidum* the simultaneous localization of Mts and chromatin was not technically possible. Some CPA-treated prophase cells lacked perinuclear Mts and, consequently, prophase spindle formation was completely inhibited (Fig. 2D; cf Fig. 2E-H). The number of CPA-treated cells displaying disturbed prophase spindle increases during treatment (Fig. 1B). After 2 h treatment, the number of these cells reaches the 1/3, while after 6 h it increases to about 2/3 of the

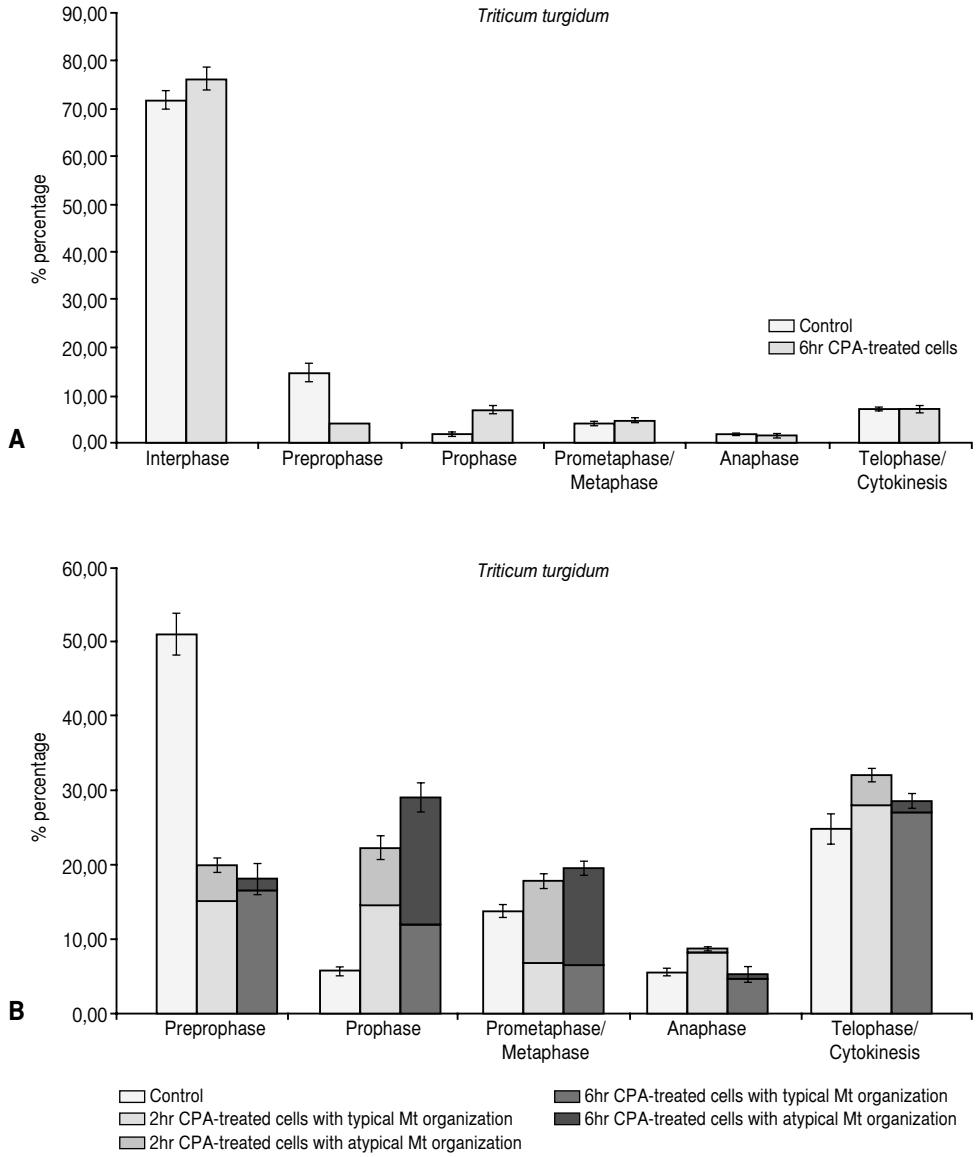

FIG. 1. Histograms showing the percentages of root-tip cells at different stages of the cell cycle in control and CPA-treated seedlings of *T. turgidum,* expressed in relation to the total number of root-tip cells counted (A) and to the number of dividing cells only (B). In Fig. 1B the columns of the CPA-treated cells consist of two parts showing the cells with typical or atypical Mt organization. In the cells with the atypical Mt organization are included:
Preprophase: cells with numerous endoplasmic Mts.
Prophase: cells in which the PPB maturation and/or the organization of the prophase spindle has been disturbed.
Prometaphase/metaphase: cells in which the metaphase spindle organization and the arrangement of the chromosomes has been disturbed.
Anaphase: cells in which the poleward movement of chromosomes has been disturbed.
Telophase/cytokinesis: cells in which the organization and the lateral expansion of the phragmoplast delay.
All the control dividing root-tip cells have typical Mt organization.
Error bars correspond to standard errors.

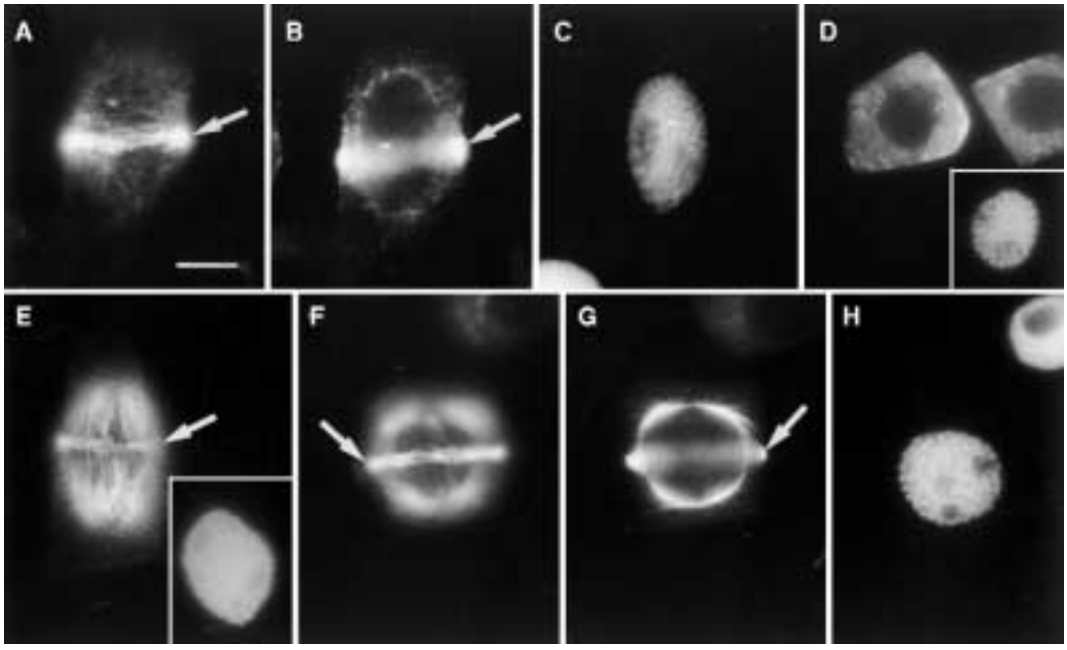

FIG. 2. Dividing CPA-affected (A-D) and control (E-H) root-tip cells of *T. turgidum* viewed with a fluorescent microscope after tubulin immunolabeling. Bar = 10 μm for all figures.

A, B: Optical sections through the surface (A) and median region (B) of a prophase cell. The arrows point to the developing PPB. Treatment with CPA 10 μM, 2 h.

C: The prophase nucleus of the cell shown in A, B after Hoechst 33258 staining.

D: A prophase cell lacking a prophase spindle. Treatment with CPA 10 μM, 6 h. *Inset* The nucleus after Hoechst 33258 staining.

E-G: Optical sections through the surface (E, F) and the median region (G) of two late prophase control cells. Note the bipolar organization of the prophase spindle. The arrows point to the mature PPB. *Inset in E* The nucleus after Hoechst 33258 staining.

H: The nucleus of the cell shown in F, G, after Hoechst 33258 staining.

treated prophase cells (Fig. 1B).

In about the 2/3 of the CPA-treated prometaphase/metaphase cells examined, atypical spindles were assembled (Fig. 1B), consisting of kinetochore Mt-bundles (K-Mt bundles), which were connected by numerous other intervening Mts (Fig. 3F, G). These spindles did not assume bipolarity and displayed more Mts than those in the untreated cells (Fig. 3F, G; cf Fig. 3E).

Most of the affected anaphase cells possessed a spindle of typical organization (Fig. 1B). However, in a few of them the shortening of the K-Mt bundles did not seem to proceed synchronously (Fig. 3I; cf Fig. 3H). Moreover, in the latter cells , the K-Mt bundles did not converge on the spindle poles, i.e. the anaphase spindle did not become fusiform (Fig. 3I). These aberrations disturbed the anaphase chromosome movement (Fig. 4A). In addition, the interzonal region showed more Mts than that in untreated anaphase cells (Fig. 3I; cf. Fig. 3H). They were arranged in well-organized parallel Mt-bundles, forming a Mt-system traversing the whole interzonal region (Fig. 3I).

The CPA-treated telophase/cytokinetic cells did not show significant aberrations in the phragmoplast organization (Fig. 5B; cf. Fig. 5A). However, in some cells the transformation of the interzonal Mt-system into a phragmoplast as well as the lateral phragmoplast/cell plate expansion was delayed (Fig. 1B). For example, the cytokinetic cell shown in Fig. 4B, C, retains the "coalescent" interzonal Mt system. Moreover, the phragmoplast of the cell shown in Fig. 5B is restricted to the space between the daughter nuclei, although the latter have already progressed to the end of cytokinesis, a fact shown by the numerous Mts surrounding them. In contrast, in the untreated cytokinetic cell shown in Fig. 5A, the phragmoplast is at the same developmental stage as that of the cell shown in Fig. 5B, but there are few Mts around the daughter nuclei.

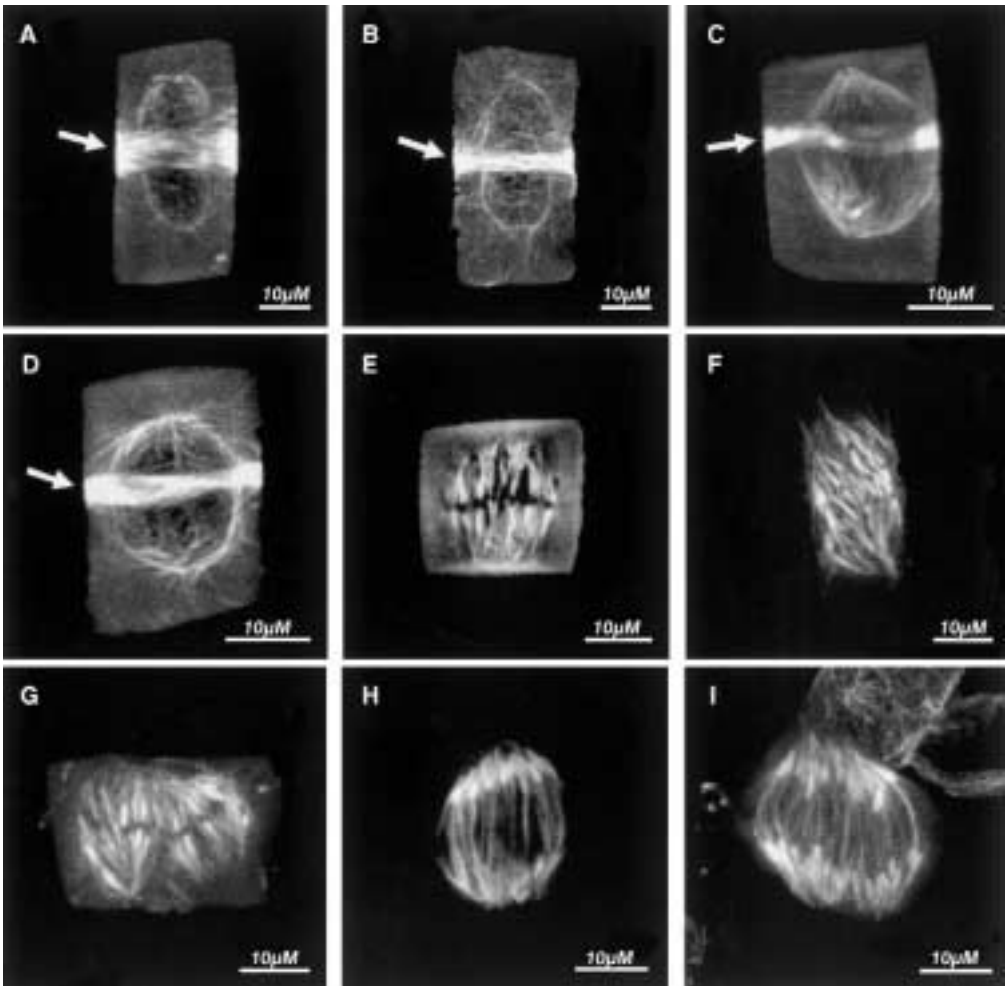

FIG. 3. Control (A, C, E, H) and CPA-affected (B, D, F,G, I) dividing root-tip cells of *T. turgidum* as they appear in the CLSM after tubulin immunolabeling. Treatment with CPA 10 μM, 3 h.

A: A control preprophase cell with a developing PPB (arrow).

B: A CPA-affected prophase cell with a PPB (arrow) at an advanced developing stage of organization. Numerous Mts traverse the cytoplasm in various directions, while few Mts are localized at the nuclear surface. The latter do not form a bipolar system.

C: A late prophase control cell with a mature PPB (arrow) and a bipolar prophase spindle.

D: A late prophase CPA-affected cell displaying a mature PPB (arrow). The perinuclear Mts do not form a bipolar spindle. Numerous other Mts are found far from the nucleus.

E: Metaphase spindle of a control cell.

F,G: Metaphase CPA-affected cells exhibiting atypical metaphase spindles. Numerous Mts are localized between the K-Mt bundles.

H, I: Anaphase spindle of a control (H) and a CPA-affected (I) cell.

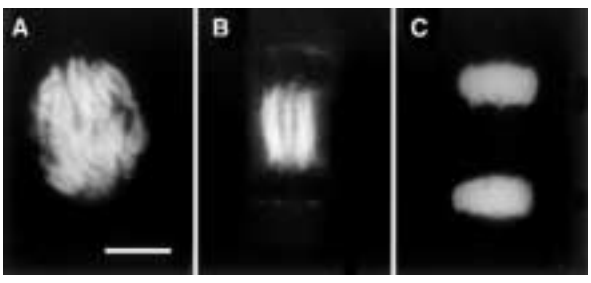

FIG. 4. Anaphase (A) and cytokinetic (B, C) CPA-affected root-tip cells of *T. turgidum*, as they appear in fluorescent microscope after tubulin immunolabeling and DNA staining with Hoechst 33258. Bar = 10 μM for all figures.

A: The segregation of the chromosomes in this cell has been disturbed. Treatment with CPA 10 μM, 2 h.

B: A cytokinetic cell retaining the interzonal Mt-system, although the daughter nuclei are in an advanced cytokinetic stage. Treatment with CPA 10 μM, 6 h.

C: The daughter nuclei of the cytokinetic cell shown in B.

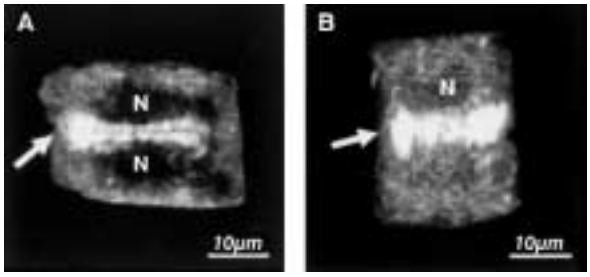

FIG. 5. Control (A) and CPA-affected (B) cytokinetic root-tip cells of *T. turgidum* as they appear in the CLSM after tubulin immunolabeling. The arrow in each cell points to the phragmoplast , which is restricted to the space between the daughter nuclei (N). In the CPA-affected cytokinetic cell (B), numerous Mts are found on the surface of the daughter nuclei, as well as in the rest of the cytoplasm. Treatment with CPA 10 μM, 3h.

Pinus brutia, Asplenium nidus, Adiantum capillus-veneris

General remarks

CPA did not significantly affect Mt organization in dividing cells of *P. brutia*, *A. nidus* and *A. capillus-veneris*. The entrance of CPA into the tissues of these plants was deduced from the disturbance of the cell cycle in them (Fig. 6). The distribution of dividing cells at the various cell division stages in control and in treated with CPA for 6 hours root-tips of *P. brutia* is illustrated in Fig. 6A, while that of dividing cells in control and in 12-hour CPA-treated leaves of *A. nidus* is shown in Fig. 6B.

In *P. brutia*, 406 control and 223 CPA-treated dividing cells were counted. The statistical analysis showed that in the 6-hour CPA-treated root-tips, significant differences ($P<0.05$) in relation to the control material were found in prophase and in prometaphase/metaphase. Specifically, there is a notable increase in the prophasic cell population, while the prometaphasic/metaphasic one decreases (Fig. 6A). These differences suggest that a lengthening of prophase and/or a remarkable delay in prophase/ prometaphase transition take place. In *A. nidus*, 681 control and 517 CPA-treated dividing cells were counted. Statistically significant differences ($P< 0.05$) between treated and control leaves were found in prophase and in telophase/cytokinesis (Fig. 6B). The remarkable decrease of the prophasic cell population in combination with the corresponding increase of the telophasic/cytokinetic one suggests that there is a significant delay in both entry to and exit of the cells from the cell division. In addition, the maintenance during CPA treatment of the percentage of metaphase and anaphase cells at the level of that of untreated leaves (Fig. 6B), suggests that there is also a retardation but not a blockage of mitosis.

The above data suggest that the CPA treatment, most likely, affected the cytosolic Ca^{2+} concentration in the gymnosperm and the pteridophytes examined. This view is further strengthened by the fact that in these plants the CPA treatment also disturbs cell plate development, a process that is Ca^{2+}- dependent (Verma, 2001). Many CPA-treated cytokinetic cells of *P. brutia* and *A. nidus* displayed undulated cell plates with local swollen regions (Fig. 7B, D cf. 7A, C). In addition, few CPA-treated post-cytokinetic cells of *A. nidus* showed perforated young daughter cell walls (Fig. 7E, F) and ER portions trapped into the daughter nuclei (Fig. 7E). Similar phenomena have not been observed in untreated dividing cells.

Pinus brutia: Mt organization

Mt organization in untreated dividing root-tip cells of *P. brutia* has recently been described (Zachariadis *et al.,* 2003) and is similar to that observed previously in other gymnosperms (Wang *et al.,* 1991; Fowke, 1993; Gilmer *et al.,* 1999). In CPA-treated preprophase/prophase cells, the PPB and the prophase spindle organization were not affected (Fig. 8B, D; cf Fig. 8A, C). The treated prometaphase cells, similarly to the untreated ones, had well-organized Mt-bundles. They traversed the perinuclear cytoplasm in parallel to the spindle axis (Fig. 8F-H; cf Fig. 8E). These Mt-bundles entered the nucleus through gaps of the nuclear envelope (Fig. 8F-H) and terminated on chromosomes as in the untreated cells (Fig. 9 A, B). The metaphase/anaphase spindle and the interzonal Mt system of CPA-treated cells showed the typical organization (Fig. 8J, L, N; cf Fig. 8I, K, M). In telophase/cytokinetic cells, the phragmoplast displayed an organization similar to that of the untreated cells (Fig. 8P; cf 8O) but delayed in reaching the cell periphery (Fig. 8P, Q).

Asplenium nidus: Mt organization

Mt organization in CPA-treated dividing leaf cells of *A. nidus* resembles that of untreated cells (Zachariadis *et al.,* 2003). Similarly to the latter cells, all the

preprophase/prophase CPA-treated cells exhibited PPBs and well-organized prophase spindles (Fig. 10D, E; cf Fig. 10A, B). In untreated and CPA-treated cells, the late prophase spindle consisted of two half-spindles, each one bearing Mt–bundles converging on the poles (Fig. 10B, E). At this stage, almost the whole Mt population was localized at the spindle poles, where large amounts of ER were also accumulated (Zachariadis *et al.,* 2003).

In the CPA-treated cells, the prophase spindle retains its organization at the onset of prometaphase and consists of two separate half-spindles (Fig. 11A). These emanated from the poles and are attached to the nuclear envelope (Fig. 11A, B), which showed invaginations. The Mt bundles of the spindle enter the nucleus through nuclear envelope gaps (Fig. 11B). In both untreated and CPA-treated cells, the metaphase spindle was barrel-shaped and consisted mainly of K-Mt bundles (Fig. 10F; cf Fig. 10C). Similarly to the untreated cells, the anaphase spindle in CPA-treated cells consisted of two independent half-spindles assembled from the shortening K-Mt bundles as well as other Mt bundles intervening between the anaphase chromosome arms (Fig. 10J; cf Fig. 10G). In both cases, the intervening Mt bundles increased in length and number in late anaphase and formed a well-organized interzonal Mt system, made of two independent sub-systems contacting each other at the equa-

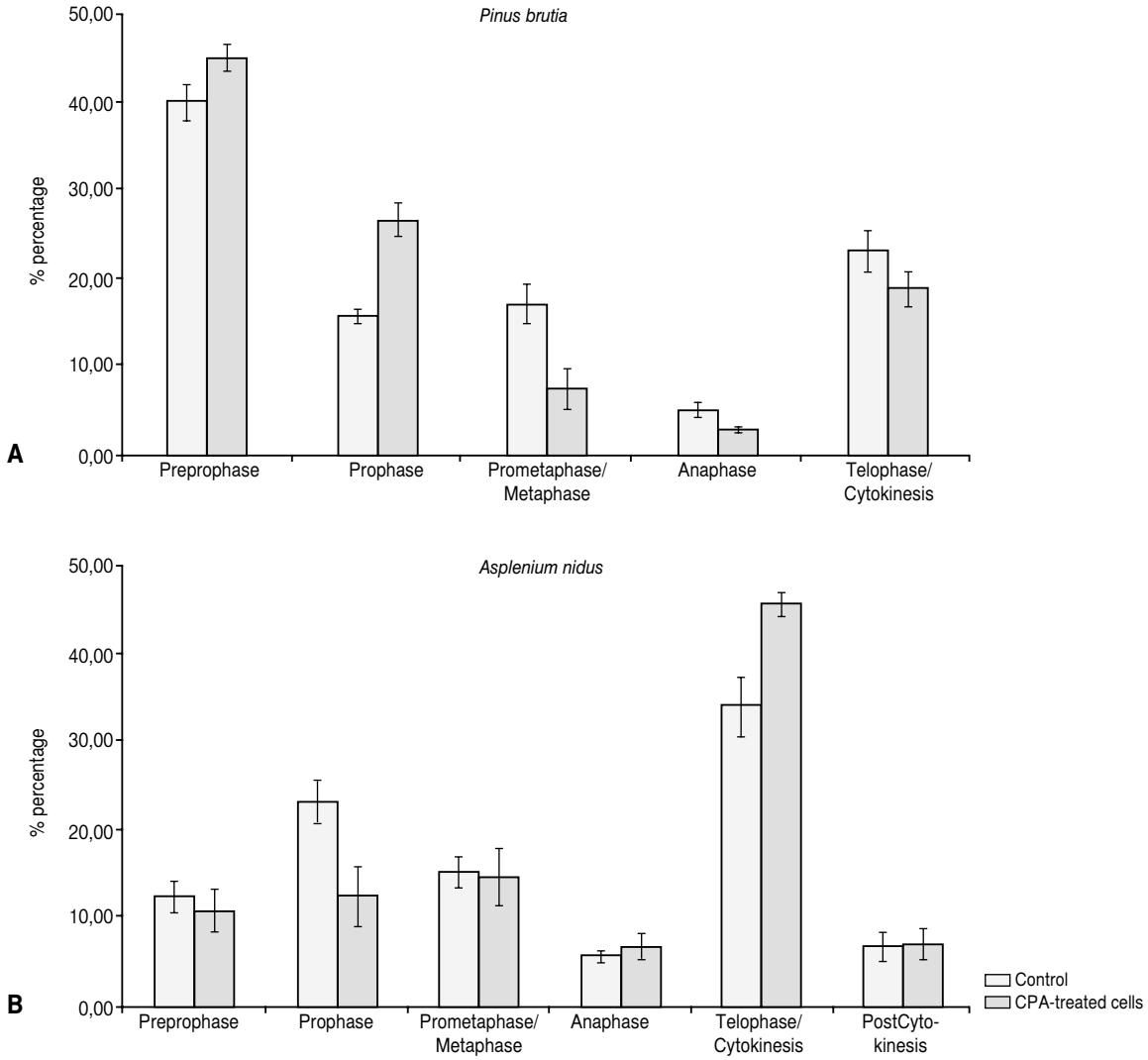

FIG. 6. Histograms showing the percentages of cells at different stages of cell division in control and CPA-treated root-tips of *P. brutia* (A) and in control and CPA-treated leaves of *A. nidus* (B). The percentages are expressed in relation to the number of dividing cells counted in each plant. Treatment with CPA 10 μM 6 h in *P. brutia* and 12 h in *A. nidus*. Error bars correspond to standard errors.

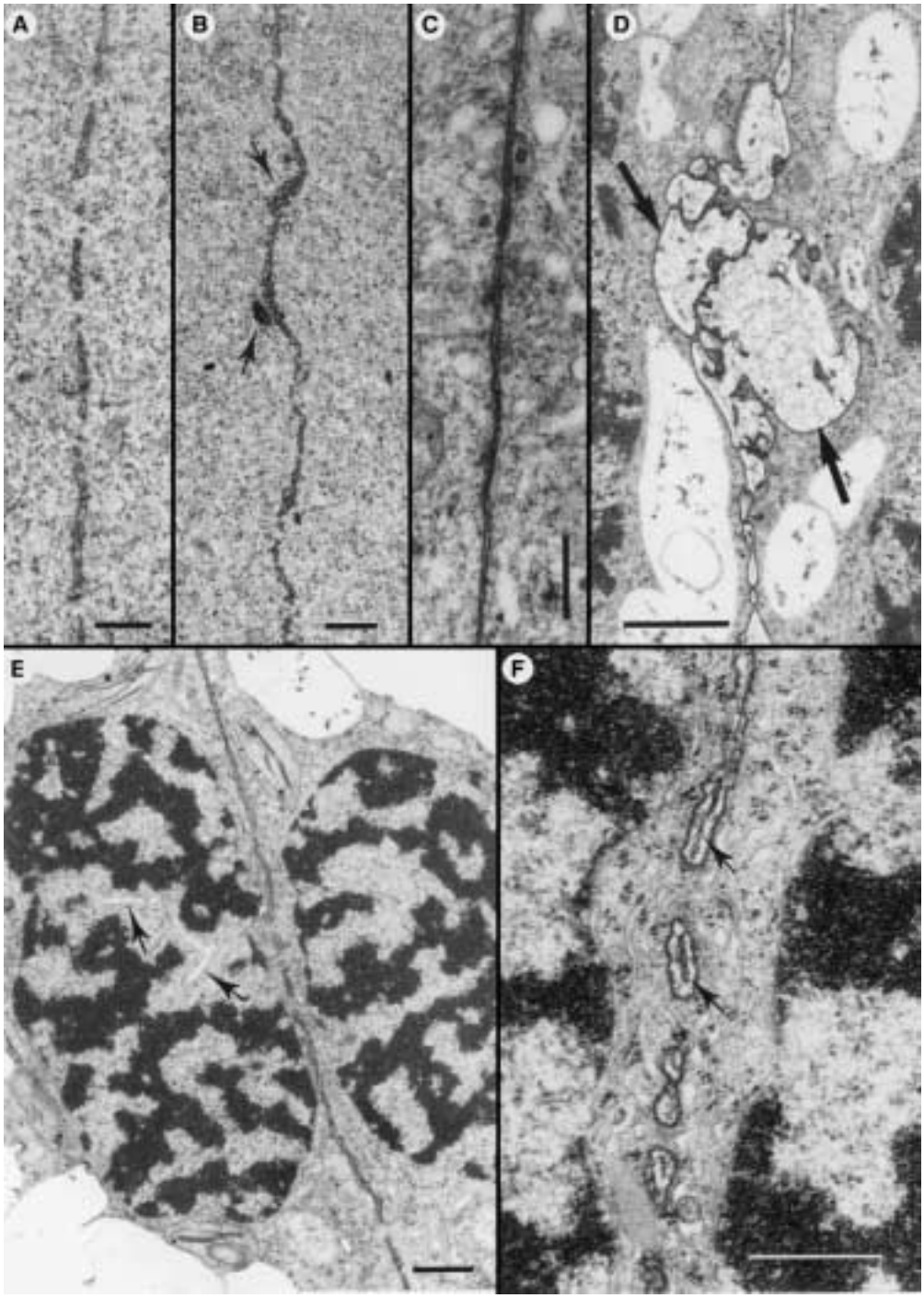

FIG. 7. Electron micrographs of control (A,C) and CPA-affected (B, D, E, F) cytokinetic cells of *P. brutia* (A, B) and *A. nidus* (C-F).

A, B: Cell plate regions taken from a control (A) and a CPA-affected (B) cytokinetic root-tip cell of *P. brutia*. The arrows in B show swollen cell plate regions. Treatment with CPA 10 μM, 6 h. Bars = 1 μm

C: Cell plate region taken from a control dividing leaf cell of *A. nidus.* Bar = 0.5 μm

D: The central region of an atypical cell plate from a CPA-affected *A. nidus* cytokinetic cell. The arrows point to intensely swollen cell plate regions. Treatment with CPA 20 μM, 48 h. Bar = 1 μm

E: Daughter nucleus of a CPA-affected post-cytokinetic cell of *A. nidus.* In the nucleoplasm, ER portions (arrows) have been trapped. Treatment with CPA 20 μM, 24 h. Bar = 1 μm

F: Region of the CPA-affected cell shown in E at a higher magnification. The daughter cell wall displays many perforations. The arrows indicate the middle lamella. Treatment with CPA 20 μM, 24 h. Bar = 0.5 μm

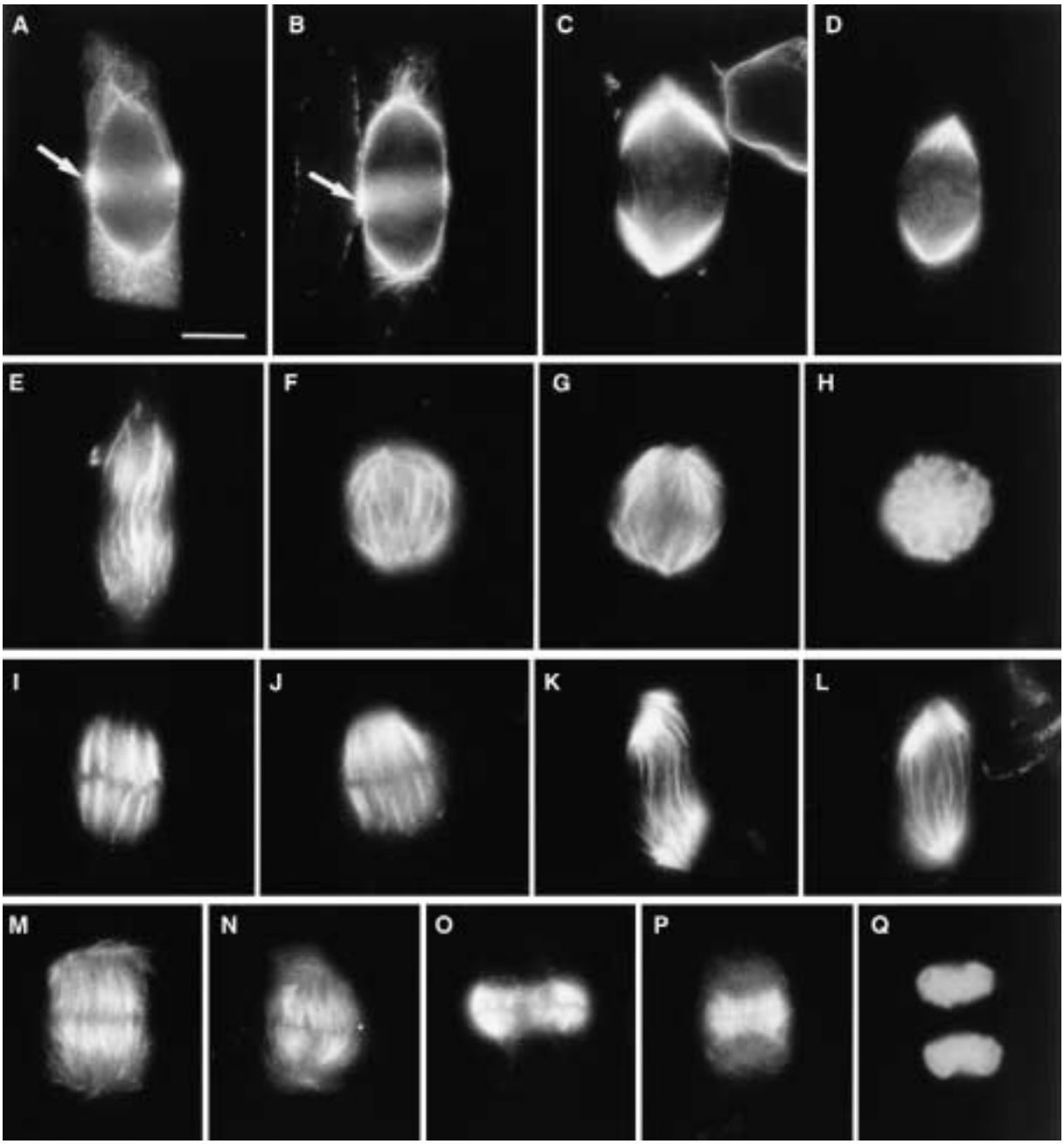

FIG. 8. Dividing control (A,C, E, I, K, M, O) and CPA-treated (B, D, F, G, H, J, L, N, P, Q) root-tip cells of *P. brutia* viewed with a fluorescent microscope after tubulin immunolabeling. Treatment with CPA 10 μM, 3 h. Bar = 10 μm for all figures.

A, B: Control (A) and CPA-treated (B) preprophase cells displaying perinuclear Mts and a PPB (arrows).

C, D: Prophase spindle in a control (C) and a CPA-treated (D) cell.

E: Mt organization in a prometaphase control cell.

F, G: Optical sections through the surface (F) and the median (G) region of the nucleus of a CPA-treated prometaphase cell. Mt bundles are localized at the nuclear surface as well as among chromosomes.

H: The prometaphase nucleus of the cell shown in F, G after DNA staining with 0.3 μg/ml propidium iodide.

I, J: Metaphase spindle in a control (I) and a CPA-treated (J) cell.

K, L: Anaphase spindle in a control (K) and a CPA-treated (L) cell.

M,N: Interzonal Mt-system in a control (M) and a CPA-treated (N) late anaphase cell.

O: Phragmoplast from a control cytokinetic cell.

P: A late-cytokinetic CPA-treated cell with a young phragmoplast.

Q: The daughter nuclei of the cell shown in P, after DNA staining with 0.3 μg/ml propidium iodide.

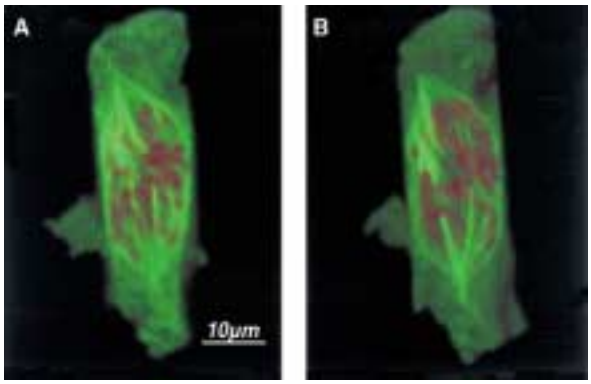

FIG. 9. A control prometaphase cell of *P. brutia* as it appears in CLSM after tubulin immunolabeling and DNA staining with 0.3 μg/ml propidium iodide. Single sections through different planes of the nucleus. The Mts are shown with a green colour while the chromosomes with red. It is clear that Mt bundles emerging from the spindle poles are connected with the chromosomes.

torial plane (Fig. 10K; cf Fig. 10H). During telophase, the interzonal Mt system transformed into a phragmoplast resembling that of the untreated cells (Fig. 10L; cf Fig. 10I). However, its expansion towards the cell periphery appears to be delayed. In the CPA-treated cell shown in Fig. 10L the phragmoplast has not reached the cell periphery, although the daughter nuclei are at an advanced cytokinetic stage (Fig. 10L; cf Fig. 10I).

Adiantum capillus-veneris: Mt organization

Similarly to the leaf cells of *A. nidus*, the Mt organization in dividing CPA-treated root-tip cells of *A. capillus-veneris* resembled that of the untreated cells (Panteris *et al.,* 1991, 2000). Fig. 12 illustrates a prophase (Fig. 12B), a prometaphase (Fig. 12D) and a metaphase (Fig.12F) CPA-treated cell displaying a prophase, prometaphase and metaphase spindle, respectively. The organization of these Mt arrays is similar to that of the respective spindles of the untreated cells (Fig. 12B, D, F; cf Fig. 12A, C, E). It should be also noted that, similarly to the leaf cells of *A. nidus*, the control and affected root-tip cells of *A. capillus-veneris* have prometaphase spindles consisted of two separate half-spindles (Fig. 12C, D).

DISCUSSION

General remarks

The main finding of this study is that the mechanisms, which control Mt organization in dividing vegetative cells of the angiosperm *T. turgidum*, differ from those in the gymnosperm *P. brutia* and the pteridophytes *A. nidus* and *A. capillus-veneris* in the following aspects: (a) the bipolar organization of the prophase and the metaphase/anaphase spindles in the angiosperm is controlled by gradients of cytosolic Ca^{2+}, which involves the ER, a phenomenon that does not seem to happen in the other plants. (b) In the gymnosperm and pteridophyte species examined, the Mts of the prophase spindle enter the nucleoplasm and contribute to metaphase spindle formation, a phenomenon not observed in the angiosperm *T. turgidum*. (c) The mode of the interzonal Mt-system reorganization, which leads to phragmoplast formation in the angiosperm, differs from that of the gymnosperm and the pteridophyte species studied.

Prophase spindle formation

In angiosperms, the prophase spindle Mts are nucleated on the surface of the preprophase/prophase nucleus (Vaughn & Harper, 1998; Canaday *et al.,* 2000; Vantard *et al.,* 2000; Kumagai & Hasezawa, 2001; Schmit, 2002). These Mts form the prophase spindle by self-organizing processes (Bajer & Molé-Bajer, 1986; Gunning, 1992; Smirnova & Bajer, 1998), probably involving motor proteins and MAPs (Vantard *et al.,* 2000; Reddy, 2001).

The inhibition of prophase spindle organization in CPA-treated root-tip cells of *T. turgidum* is probably due to the inability of the perinuclear Mts to form a bipolar structure, whereas many Mts are located at some distance from the nucleus (Fig. 3B, D). The findings of this work favour the hypothesis that in angiosperm prophase cells the cytosolic Ca^{2+} controls the bipolar organization of the Mts and their localization exclusively in the perinuclear cytoplasm. Since the only Ca^{2+}-dependent motor protein found in dividing cells of angiosperms so far is the KCBP (see Introduction), this is probably involved in prophase spindle formation (Smirnova *et al.,* 1998; Vos *et al.,* 2000; Reddy, 2001).

A number of CPA-treated prophase cells of *T. turgidum* lack perinuclear Mts, a phenomenon also observed in ethidium bromide treated cells (Zachariadis *et al.,* 2000). This has been attributed to inhibi-

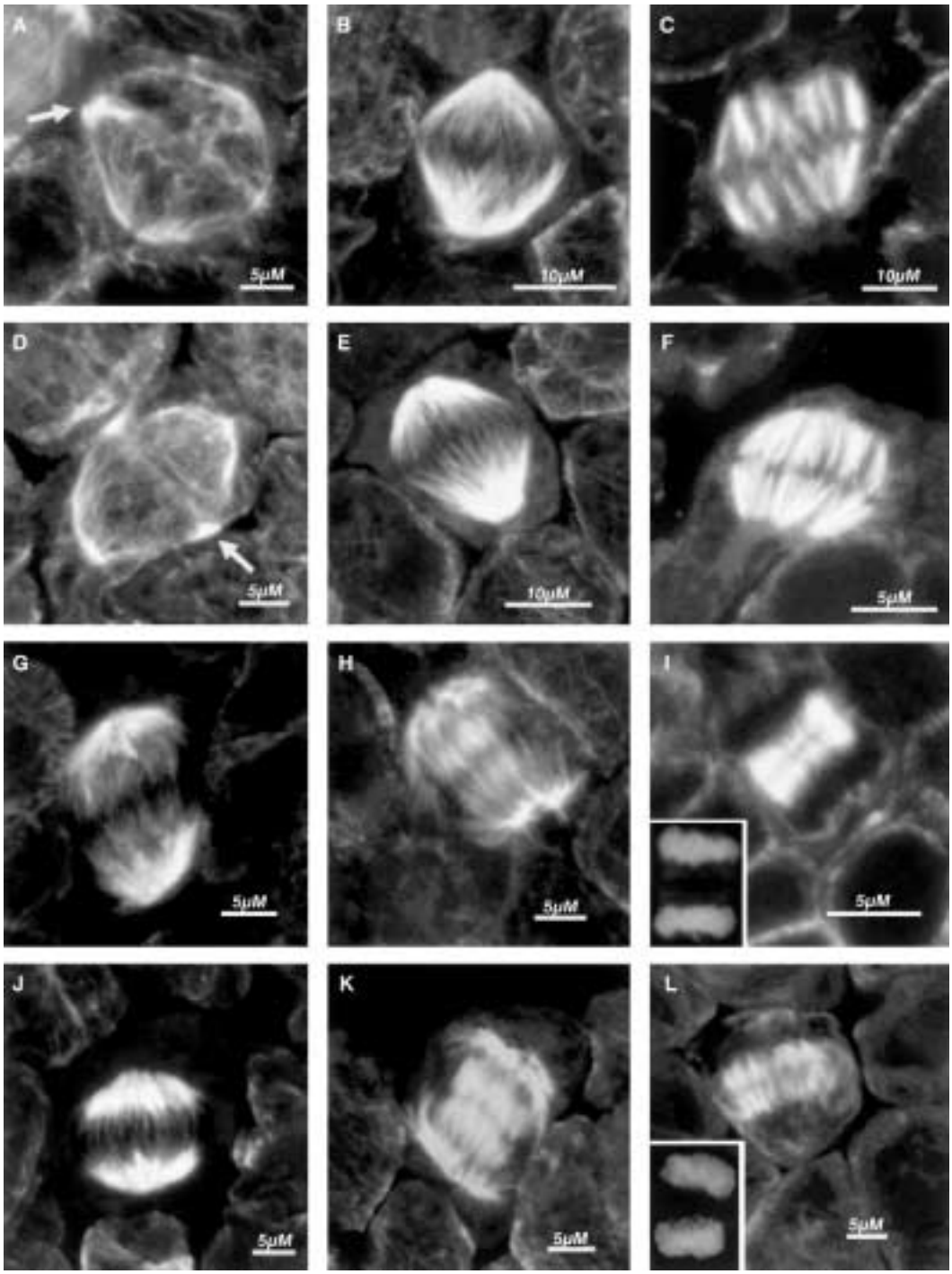

FIG. 10. Dividing control (A-C, G-I) and CPA-treated (D-F, J-L) leaf cells of *A. nidus* as they appear in CLSM after tubulin immunolabeling. Treatment with CPA10 μM, 6 h.

A, D: Preprophase cells (A: control, D: CPA-treated) encompassing perinuclear Mts and a PPB (arrows).

B,E: Late prophase cells (B: control , E: CPA-treated) displaying a well-organized prophase spindle.

C, F: Metaphase spindle from a control (C) and a CPA-treated (F) cell.

G, J: Early anaphase spindle from a control (G) and a CPA-treated (J) cell.

H, K: Late anaphase/early telophase cells (H: control, K: CPA-treated) having a well-organized interzonal Mt system.

I: A control telophase cell with a young phragmoplast. *Inset* The daughter nuclei, after DNA staining with 0.3 μg/ml propidium iodide.

L: A late cytokinetic CPA-treated cell displaying a phragmoplast localized between the daughter nuclei. *Inset* The daughter nuclei after DNA staining with 0.3 μg/ml propidium iodide.

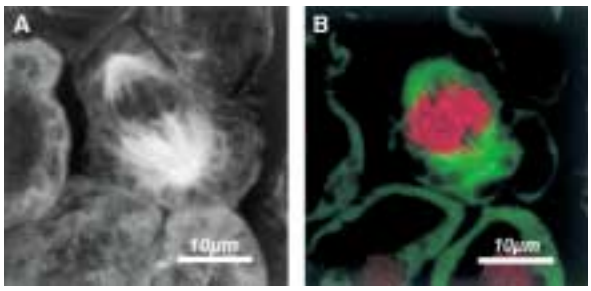

FIG. 11. **A:** A prometaphase CPA-treated leaf cell of *A. nidus* as it appears in CLSM after tubulin immunolabeling and DNA staining with 0.3 μg/ml propidium iodide. The spindle retains its prophasic organization (compare with Fig. 10E). Its Mt-bundles enter the nucleoplasm. Treatment with CPA 1*0* μM, 6 h.

B: A single CLSM section through the mid-plane of the prometaphase nucleus of the cell shown in A. The Mts are shown with a green colour, while the chromosomes with red. It is clear that Mt bundles emerging from the spindle poles are connected with the chromosomes.

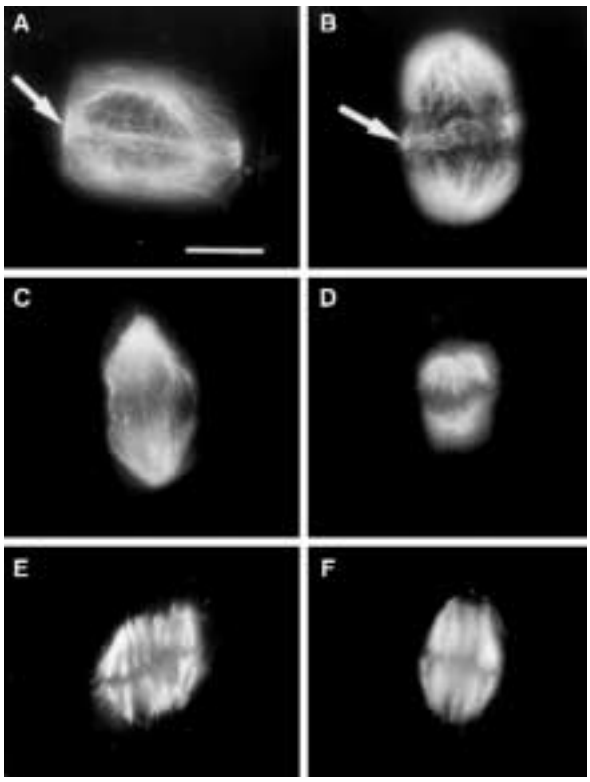

FIG. 12. Dividing control (A, C, E) and CPA-treated (B, D, F) root-tip cells of *A. capillus-veneris* viewed with a fluorescent microscope after tubulin immunolabeling. Treatment with CPA 10 μM, 3 h. Bar = 10 μm for all figures.

A, B: Prophase cells (A: control, B: CPA-treated) displaying a well-organized prophase spindle and a mature PPB (arrows).

C, D: Prometaphase spindles from a control (C) and a CPA-treated (D) cell, consisted of two half-spindles.

E, F: Metaphase spindles from a control (E) and a CPA-treated (F) cell.

tion or delay of the nuclear envelope disorganization and/or disturbance of its function (Zachariadis *et al.,* 2000). CPA may block the ability of the nuclear envelope to sequester Ca^{2+} from the perinuclear cytoplasm and/or may interfere with the transport processes occurring through it. The maintenance of relatively higher concentrations of Ca^{2+} in the perinuclear cytoplasm may explain the inhibition of perinuclear Mt formation.

Baluška *et al.* (1997) have suggested that in preprophase/prophase angiosperm cells intranuclear factors migrate to the perinuclear cytoplasm, where they activate the MTOCs responsible for the formation of the prophase spindle. This hypothesis is further supported by the localization of: (a) the mitosis-specific MPM-2 phosphoepitope, which is related to the centrosomal activity and function of MTOCs (Verde *et al.,* 1990; Vaughn & Harper, 1998; Conicella *et al.,* 2003), in the nucleoplasm of the G_2 nuclei of *Vicia faba* (Binarov˘ *et al.,* 1993). (b) γ-Tubulin and the Spc98p orthologues, which are involved in Mt nucleation in the nucleoplasm of *Vicia faba* and BY-2 suspension culture cells, respectively (Binarov˘ *et al.,* 2000; Erhardt *et al.,* 2001). The disturbance of the nuclear envelope disintegration in the CPA-treated cells (this work), and in the ethidium bromide-treated cells (Zachariadis *et al.,* 2000) may also prevent the movement of the above putative intranu-

clear factors to the perinuclear cytoplasm and, consequently, prevent the activation of the perinuclear MTOCs.

In contrast to *T. turgidum,* the prophase spindle organization of *P. brutia*, *A. nidus*, *A. capillus-veneris*, as well of the liverwort *Lunularia cruciata* (unpublished data), was not affected by CPA. Therefore, in these plants, prophase spindle organization seems to be a process not sensitive of the ER-controlled Ca^{2+} gradients. In the above plants, MTOCs probably function in the spindle poles nucleating and organizing the Mts of the mitotic spindle. In other words, the spindle poles seem to have centrosomal properties. These MTOCs do not appear sensitive to the ER-controlled Ca^{2+} gradients. Among the higher plants, distinct MTOCs have only been found in the spindle poles of liverworts (Apostolakos & Galatis, 1998). The persistence of the centrosomal activities in bryophyte and pteridophyte spindle poles may be related to the fact that they contribute to the formation of flagella during gametogenesis (Vaughn & Harper, 1998). In addition, two groups of gymnosperms, the Cycadophyta and Gingophyta, display motile male gametes (Raven *et al.,* 1986). In contrast, the absence of flagella from the angiosperm gametes may have led to the loss of any remnant centrosomal properties from their spindle poles (see also Fowke, 1993).

Metaphase spindle formation

The disturbance of the ER-dependent cytosolic Ca^{2+} gradients by CPA causes the formation of an atypical prometaphase/metaphase spindle in the angiosperm *T. turgidum*, which apparently consists of more Mts compared to untreated cells and does not assume a bipolar organization (Fig. 3F,G; cf Fig. 3E). These effects are not induced in the examined gymnosperm and pteridophyte cells. The apparent increase of the Mt number in the atypical metaphase spindle of *T. turgidum* supports the view that the local ER-dependent Ca^{2+} gradients control Mt polymerization/depolymerization in mitotic cells of angiosperms (Hepler & Wolniak, 1984; Hepler, 1989, 1992; Wolniak, 1991). In addition, the exclusive presence of numerous Mts within the atypical metaphase spindle suggests that Mt polymerization occurs in the immediate vicinity of prometaphase/metaphase chromosomes (Kubiak *et al.,* 1986; Wang *et al.,* 1991; Binarová *et al.,* 1998, 2000; Chan & Cande, 1998; Franklin & Cande 1999; Zachariadis *et al.,* 2000). Finally, the inability of the metaphase spindle of the CPA-treated *T. turgidum* cells to assume bipolarity implies that the mitotic spindle morphogenesis in angiosperms is Ca^{2+}-dependent and probably Ca^{2+}-dependent proteins, like the KCBP, play a predominant role (Vos *et al.,* 2000; Reddy, 2001).

In *T. turgidum* (Zachariadis *et al.,* 2000, this work) as well as in other angiosperms (Kubiak *et al.,* 1986; Wang *et al.,* 1991), the prophase spindle seems to be disorganized at the prophase/prometaphase transition, an observation not confirmed in the gymnosperm *P. brutia* and the pteridophytes *A. nidus* and *A. capillus-veneris*. In these plants, the prophase spindle persists at the onset of prometaphase and its Mt-bundles enter the nucleoplasm, thus contributing to metaphase spindle formation (Figs 9A, B, 11A, B, 12C, D). The prophase spindles of vegetative cells of other gymnosperms (Wang *et al.,* 1991; Fowke, 1993), pteridophytes (Burgess, 1970), as well as of bryophytes (Brown & Lemmon 1988, 1990) show similar properties. The metaphase spindle of the CPA-treated cells of *P. brutia*, *A. nidus* and *A. capillus-veneris* retains its bipolar structure. This phenomenon has been also observed in CPA treated cells of the liverwort *Lunularia cruciata* (unpublished data), a fact further supporting the view that some centrosomal properties still reside in the spindle poles of these plants.

Anaphase spindle formation

The delay of metaphase/anaphase transition in CPA-treated *T. turgidum* cells and the disturbance of the K-Mt bundle shortening during anaphase are probably due to the fact that cytosolic Ca^{2+} gradients control these processes in angiosperms (Hepler, 1989, 1992; Wolniak, 1991). In addition, the inability of the K-Mt bundles to converge on the poles of the anaphase spindle in CPA-treated cells of *T. turgidum* favours the view of Smirnova *et al.* (1998) and Reddy (2001) that Ca^{2+}-dependent motor proteins, such as the KCBP, may control this process. This phenomenon was not observed in the other plants examined here, a fact also suggesting that the spindle poles of these plants have MTOC properties, which are not affected by the CPA.

The delay of the anaphase in the CPA-treated cells revealed some structural differences between the plants studied regarding the anaphase spindle. In untreated and CPA-treated anaphase cells of *T. turgidum* and early anaphase cells of *P. brutia* the in-

terzonal Mts overlap each other and form a continuous system (Figs 3H, I, 8K, L) as has been described for *Allium* (Palevitz, 1988), *Picea* and *Pinus* (Wang *et al.,* 1991; Fowke, 1993). Our data support Palevitz's (1988) hypothesis that this interzonal Mt system originates from the elongation of the Mt-bundles, which traverse the mid-region of the metaphase spindle. In *A. nidus* and *A. capillus-veneris*, the interzonal Mt system is made of two not overlapping each other Mt sub-systems (Fig. 10H, K), as happens in the late anaphase *P. brutia* cells (Fig. 8M, N), an observation suggesting a different mode of interzonal Mt system formation. In these plants, the interzonal Mt arrays seem to emerge from the polar regions and gradually elongate towards the equatorial plane of the anaphase spindle. This mode of interzonal Mt system formation is similar to that in endosperm cells of *Haemanthus* (Smirnova & Bajer, 1994), in the liverwort *Reboulia* (Brown & Lemmon, 1990) and in the hornwort *Pheoceros* (Brown & Lemmon, 1988).

Phragmoplast formation

The mechanism of phragmoplast formation in *T. turgidum* root-tip cells is apparently similar to that observed in living angiosperm cells. Studies employing injection of exogenous fluorescently-labeled tubulin into living cells of *Tradescantia* showed that during the anaphase to telophase transition, the interzonal Mt bundles "coalesce" laterally to form an elongated Mt bundle in the middle of the cell, as happens and in *T. turgidum* root-tip cells (Fig. 4B). This Mt bundle then shortens and begins to expand laterally, giving rise to the phragmoplast (Zhang *et al.,* 1990; Hepler *et al.,* 1993). The Mt bundles of the interzonal system re-organize quite differently during phragmoplast formation in the gymnosperm *P. brutia* and the pteridophytes *A. nidus* and *A. capillus-veneris*, because they cover the whole space between the daughter chromosome groups during anaphase/telophase transition without lateral "coalescence" (Figs 8M, N, 10H, K; see also Fig. 7g in Panteris *et al.,* 1991). Finally, the phragmoplast replaces them in exactly the same area (Fig. 8O, P; cf. 8M, N and Fig. 10I, L; cf Fig. 10H, K). The lateral "coalescence" of the interzonal Mts during anaphase/telophase transition does not occur in the liverworts *Reboulia* (Brown & Lemmon, 1990) and *Lunularia cruciata* (unpublished data), in the hornwort *Pheoceros* (Brown & Lemmon, 1988) and in the endosperm cells of *Haemanthus* (Smirnova & Bajer, 1995).

ACKNOWLEDGEMENTS

This work was supported by a grant from the Greek-German Technology Program (GR1-045-099). The Greek State Scholarship Foundation awarded Michael Zachariadis a scholarship.

REFERENCES

Apostolakos P, Galatis B, 1998. Microtubules and gametophyte morphogenesis in the liverwort *Marchantia paleacea* Bert. In: Bates JW, Ashton NW, Duckett JD, eds. *Bryology for the twenty-first century*. Maney Publishing, Leeds: 205-221.

Bajer AS, Molè-Bajer J, 1986. Reorganization of microtubules in endosperm cells and cell fragments of the higher plant *Haemanthus* in vivo. *Journal of cell biology*, 102: 263- 281.

Baluška F, Volkmann D, Barlow PW, 1997. Nuclear components with microtubule-organizing properties in multicellular eukaryotes: functional and evolutionary considerations. *International review of cytology*, 175: 91-135.

Binarovˇ P, Cihalikova J, Doležel J, 1993. Localization of MPM-2 recognized phosphoproteins and tubulin during cell cycle progression in synchronized *Vicia faba* root meristem cells. *Cell biology international*, 9: 847-856.

Binarovˇ P, Dolezel J, Drabor P, Heberle-Bors E, Strand M, Bögre L, 1998. Treatment of *Vicia faba* root-tip cells with specific inhibitors to cyclin-dependent kinases leads to abnormal spindle formation. *The plant journal* 16: 697-707.

Binarovˇ P, Ceyklovˇ V, Hause B, Kubˇtovˇ E, Lysˇk M, Doležel J, Bögre L, Draber P, 2000. Nuclear γ-tubulin during acentriolar plant mitosis. *Plant cell*, 12: 433-442.

Brown RC, Lemmon BE, 1988. Preprophase microtubule systems and development of the mitotic spindle in hornworts (Bryophyta). *Protoplasma*, 143: 11-21.

Brown RC, Lemmon BE, 1990. Polar organizers mark division axis prior to preprophase band formation in mitosis of the hepatic *Reboulia hemisphaerica* (Bryophyta). *Protoplasma*, 156: 74-84.

Burgess J, 1970. Interactions between microtubules and the nuclear envelope during mitosis in a fern. *Protoplasma*, 71: 77-89.

Busch MB, Sievers A, 1993. Membrane traffic form endoplasmic reticulum to the Golgi apparatus is disturbed by an inhibitor of the Ca^{2+}-ATPase in the ER. *Protoplasma*, 177: 23-31.

Canaday J, Stoppin-Mellet V, Mutterer J, Lambert AM, Schmit A-C, 2000. Higher plant cells: gamma-tubulin and microtubule nucleation in the absence of centrosomes. *Microscopy research and technique*, 49: 487-495.

Chan A, Cande WZ, 1998. Maize meiotic spindles assemble around chromatin and do not require paired chromosomes. *Journal of cell science*, 111: 3507-3515.

Compton DA, 1998. Focusing on spindle poles. *Journal of cell science*, 111: 1477-1481.

Conicella C, Cremona G, Cammareri M, Errico A, Monti LM, 2003. Phosphorylation dynamics in male meiosis: MPM-2 colocalization with microtubule cytoskeleton. *Cell biology international*, 27: 183-184.

Erhardt M, Campagne S, Canaday J, Stoppin-Mellet V, Lambert AM, Schmit AC, 2001. Higher plant Spc98p orthologues and gamma-tubulin localize at microtubule nucleation sites *in vivo* and are involved in nucleation *in vitro. Biology of the cell*, 93: 241.

Fowke LC, 1993. Microtubules in dividing root cells of the conifer *Pinus radiata* and the cycad *Zamia furfuracea*. *Cell biology international*, 17: 143-151.

Frantzios G, Galatis B, Apostolakos P, 2000. Aluminium effects on microtubule organization in dividing root-tip cells of *Triticum turgidum*. I. Mitotic cells. *New phytologist*, 145: 211-224.

Frantzios G, Galatis B, Apostolakos P, 2001. Aluminium effects on microtubule organization in dividing root-tip cells of *Triticum turgidum*. II Cytokinetic cells. *Journal of plant research*, 114: 157-170.

Franklin AE, Cande WZ, 1999. Nuclear organization and chromosome segregation. *Plant cell*, 11: 523-534.

Gilmer S, Clay P, McRae TH, Fowke LC, 1999. Acetylated tubulin is found in all microtubule arrays of two species of pine. *Protoplasma*, 207: 174-185.

Goeger DE, Rieley RT, Dorner JW, Cole RJ, 1988. Cyclopiazonic acid inhibition of the Ca^{2+}-transport ATPase in rat skeletal muscle sarcoplasmic reticulum vesicles. *Biochemistry and pharmacology*, 37: 978-981.

Gunning BES, 1992. Use of confocal microscopy to examine transitions between successive microtubule arrays in the plant cell division cycle. In: Shibaoka H, ed. *Proceedings of the international symposium on the cellular basis of growth and development in plants*. Osaka, November 26-28, 1992. Osaka University, Osaka: 145-155.

Gunning BES, Steer MW, 1996. *Plant cell biology. Structure and function*. Jones and Bartlett Publishers, Boston.

Hepler PK, 1989. Membranes in the mitotic apparatus. In: Hyams J S, Brinkley BR, eds. *Mitosis: Molecules and mechanisms*. Academic Press Inc, San Diego: 241-271.

Hepler PK, 1992. Calcium and mitosis. *International review of cytology*, 138: 239-268.

Hepler PK, Wolniak SM, 1984. Membranes in the mitotic apparatus: Their structure and function. *International review of cytology*, 9: 169-238.

Hepler PK, Cleary AL, Gunning BES, Wadsworth P, Wasteneys GO, Zhang DH, 1993. Cytoskeletal dynamics in living plant cells. *Cell biology international*, 17: 127-142.

Hepler PK, Valster A, Molchan T, Vos JW, 2002. Roles for kinesin and myosin during cytokinesis. *Philosophical transactions of the royal society of London series B, biological sciences*, 357: 761-766.

Hsieh WL, Pierce WS, Sze H, 1991. Calcium-pumping ATPases in vesicles from carrot cells. *Plant physiology*, 97: 1535-1544.

Kubiak J, DeBrabander M, De Mey J, Tarkowska JA, 1986. Origin of the mitotic spindle in onion root cells. *Protoplasma*, 130: 51-56.

Kumagai F, Hasezawa S, 2001. Dynamic organization of microtubules and microfilaments during cell cycle progression in higher plant cells. *Plant biology*, 3: 4-16.

Merdes A, Cleveland DW, 1997. Pathways of spindle pole formation: different mechanisms; conserved components. *Journal of cell biology*, 138: 953-956.

Novoa J, Quader H, Reiss H-D, 1992. Effect of Ca^{2+}-ATPase inhibitors on pollen tube growth and intracellular Ca^{2+}-distribution. *The histochemical journal*, 24: 513-514.

Palevitz BA, 1988. Microtubular fir-trees in mitotic spindles of onion roots. *Protoplasma*, 142: 74-78.

Panteris E, Galatis B, Apostolakos P, 1991. Patterns of cortical and perinuclear microtubule organization in meristematic root cells of *Adiantum capillus veneris*. *Protoplasma*, 165: 173-188.

Panteris E, Apostolakos P, Gräf R, Galatis B, 2000. Gamma-tubulin colocalizes with microtubule arrays and tubulin paracrystals in dividing vegetative cells of higher plants. *Protoplasma*, 210: 179-187.

Quader H, Bechtler Ch, 1996. The calcium transport inhibitor cyclopiazonic acid reversibly affects the shape of the endoplasmic reticulum in onion epidermal cells. *Mitteilungen aus dem institut fur allgemeine botanik Hamburg*, 26: 191-199.

Quader H, Fast H, Manske H, 1996. Formation and disintegration of lamellar cisternae of the endoplasmic reticulum: regulatory aspects. In: Moller IM, Brodelius P, eds. *Plant membrane biology*. Clarendon Press, Oxford: 184-197.

Raven PH, Evert RF, Eichorn SF, 1986. *Biology of plants.* 4th edition. Worth Publishers, Inc. New York.

Reddy ASN, 2001. Molecular motors and their functions in plants. *International review of cytology*, 204: 97-178.

Rengel Z, Zhang W-H, 2003. Role of dynamics of intercellular calcium in aluminium toxicity syndrome. *New phytologist*, 159: 295-314.

Schmit A-C, 2002. Acentrosomal microtubule nucleation in higher plants. *International review of cytology*, 220: 257-289.

Seidler NW, Jona I, Vegh M, Martonosi A, 1989. Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *Journal of biological*

chemistry, 264: 17816-17823.

Sievers A, Bunch MB, 1992. An inhibitor of the Ca^{2+}-ATPase in the sarcoplasmic and endoplasmic reticula inhibits transduction of the gravity stimulus in cress roots. *Planta*, 88: 619-622.

Smirnova EA, Bajer AS, 1994. Microtubule converging centers and reorganization of the interphase cytoskeleton and the mitotic spindle in higher plants *Haemanthus*. *Cell motility and the cytoskeleton*, 27: 213-233.

Smirnova EA, Bajer AS, 1995. Antibody against phosphorylated proteins (MPM-2) recognizes mitotic microtubules in endosperm of *Haemanthus*. *Cell motility and the cytoskeleton*, 31: 34-44.

Smirnova EA, Bajer AS, 1998. Early stages of spindle formation and independence of chromosome and microtubule cycles in *Haemanthus* endosperm. *Cell motility and the cytoskeleton*, 40: 22-37.

Smirnova EA, Reddy ASN, Bowser J, Bajer AS, 1998. Minus end-directed kinesin-like motor protein, KCBP, localizes to anaphase spindle poles in *Haemanthus* endosperm. *Cell motility and the cytoskeleton*, 41: 271-280.

Vantard M, Cowling R, Delichere C, 2000. Cell cycle regulation of the microtubular cytoskeleton. *Plant molecular biology* 43: 691-703.

Vaughn KC, Harper JDI, 1998. Microtubule-organizing centers and nucleating sites in land plants. *International review of cytology*, 181: 75-149.

Verde F, Labbe J-C, Doree M, Karsenti E, 1990. Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of *Xenopus* eggs. *Nature*, 343: 233-238.

Verma DPS, 2001. Cytokinesis and building of the cell plate in plants. *Annual review of plant physiology and plant molecular biology*, 52: 751-784.

Vos JW, Safadi F, Reddy ASN, Hepler PK, 2000. The kinesin-like calmodulin binding protein is differentially involved in cell division. *Plant cell*, 12: 979-990.

Walczak CE, Vermos I, Mitchison TJ, Karsenti E, Heald R, 1998. A model for the proposed roles of different microtubule-based motor proteins in establishing spindle bipolarity. *Current biology*, 8: 903-913.

Wang H, Cutler AJ, Fowke, LC, 1991. Microtubule organization in cultured soybean and black spruce cells: Interphase-mitosis transition and spindle morphology. *Protoplasma*, 162: 46-54.

Wolniak SM, 1991. Patterns of regulation during mitosis. In: Lloyd CW, ed. *The cytoskeletal basis of plant growth and form*. Academic Press, London: 209-226.

Zachariadis M, Galatis B, Apostolakos P, 2000. Study of mitosis in root-tips of *Triticum turgidum* treated with the DNA intercalating agent ethidium bromide. *Protoplasma*, 211: 151-164.

Zachariadis M, Quader H, Galatis B, Apostolakos P, 2001. Endoplasmic reticulum preprophase band in dividing root-tip cells of *Pinus brutia*. *Planta*, 213: 824-827.

Zachariadis M, Quader H, Galatis B, Apostolakos P, 2003. Organization of the endoplasmic reticulum in dividing cells of the gymnosperms *Pinus brutia* and *Pinus nigra* and of the pterophyte *Asplenium nidus*. *Cell biology international*, 27: 31-40.

Zhang D, Wadsworth P, Hepler PK, 1990. Microtubule dynamics in living dividing plant cells: confocal imaging of microinjected fluorescent brain tubulin. *Proceedings of the national academy of sciences (USA)*, 87: 8820-8824.