Pak. J. Bot., 34(4): 405-410, 2002.

MITOTIC APPARATUS MOVEMENT AND CELL WALL PATTERNING IN ROOT APICAL MERISTEM OF *ALLIUM CEPA*

B. SH. BEHBOODI* AND L. SAMADI

Department of Biology,
Faculty of Science, University of Tehran, Tehran,
Islamic Republic of Iran.

Abstract

The positioning of cell wall in a dividing cell is very crucial for organ development. It is determined by the cortical information of the cytoskeleton in G2 phase called Preprophase Band (PPB). The cell plate is also perpendicular to the Mitotic Apparatus (MA). Thus coordination of PPB, the MA orientation and the position of the aforesaid future cell wall is of critical importance in these cells. Cells with oblique MAs and their position in the apical meristem was studied by cytological techniques. The role of microfilaments in cell plate formation is described by application of Cytochalasin B. The oblique MAs have a developmental role in the formation of long cortex cells and the central cylinder. Actin filaments are responsible for organization of the cell wall position.

Introduction

The form of any organ in plants is determined by its predetermined division plans in dividing cells and the growth direction growing cells acquire as a result. At no stage of plant development the cells crawl over one another. The walls of adjacent cells are glued together by a middle lamella, made mainly of pectic substances (Varner & Lin, 1989). The rate of cell division in an enlarging organ may be different from adjacent cell layers. Thus the cell wall patterning has an important developmental role in morphogenesis and organogenesis. During cell division, as the daughter nuclei take up their new position, a cell plate arises *de novo* between them. The site of the future cell plate is appointed prior to division. The cortex contains "information" that determines final cell wall position. (Liu & Palevitz, 1992). A group of microtubules, called Preprophase Band (PPB), mark the cell plan position in G2 phase. In addition to microtubules, PPB is composed of microfilaments and other proteins (Ding *et al.*1991).

The cell plate is oriented perpendicular to the mitotic apparatus (MA) which is responsible for separating the duplicated chromosomes. In some cells of onion root apical meristem, the metaphase MAs are obliquely oriented (Palevitz, 1988). At first glance, the abnormal configuration of MA would seem to present significant developmental problems. But these oblique MAs are at odds with the predetermined site of cell plate. Therefore, these cells are suitable to study the coincidence of PPB, MA alignment and cell plate position. In the present study we have applied the inhibitor of microfilament associations (Cytochalasin B) to consider their role in cell wall patterning.

*E-mail: behboodi@khayam.ut.ac.ir

Materials and Methods

Cytochalasins, a group of fungal metabolites, bind to the barbed end of actin filaments and inhibit both the association and dissociation of actin subunits at Key-Word: Mitotic Apparatus (MA), Apical meristem, Cytochalasin B, Cell wall patterning.this end (Cooper, 1987). Onion growing roots were germinated in 0.2, 0.5, 5, 10 μg/ml concentrations of Cytochalasin B for 24, 48 and 72 hours. A comparable set of control roots were germinated in distilled water and treated roots were fixed using 3% glutaraldehyde in phosphate buffer, pH 7.4 for 4 hours. Fixed roots were dehydrated through a graded ethanol series (25, 50, 75, 100, 100%, each step 1h). Dehydrated roots were then taken though a graded toluene series (25, 50, 75, 100% in ethanol, 1h each step). Finally tissues were embedded in paraffin by a paraffin series (25, 50, 75, 100% in toluene, each step 2h at 57°C). They were then sectioned on a microtome of 7 μm thicknesses, mounted on slides, deparaffinized and mordanted in 5% ferric ammonium sulfate for 2h. They were then stained by Regaud Heamatoxylin dye (Jensen, 1962). Slides were observed under the Zizze III microscope and photographed.

Results and Discussion

In root apex of *Allium cepa*, oblique MAs are noticed in Metaphase and Anaphase (Fig. 1-a, b two oblique divisions in metaphase). These divisions are generally observed in the initial cells of the central cylinder and cortex where 47% of oblique division are in the cortex and 39% are in the central cylinder. These oblique MAs still move chromosomes to the poles during anaphase. Therefore, at late anaphase separated chromosomes are in two opposite corners of the cell (Fig. 1-d, e, f). Since, the cell plate is perpendicular to mitotic spindles; the MA must move and settle in the correct position.

In Cytochalasin B treatments, as well, the telophasic cell plates are correctly oriented. Thus microfilaments are not involved in MA movement. Cytochalasin B has heterogeneous effects on cells. In 0.2 and 0.5 μg/ml doses there are no observable changes in cell division and cell wall patterning. In 5 and 10 μg/ml concentrations various effects of Cytochalasin B were observed. In some cells, the fragmoplast is not formed in telophase (Fig. 2-a, b, c). Therefore, these cells are binucleated and cell wall has not been organized (Fig. 2-d). In others, cell wall position changed and propelled to a corner of the cells (Fig. 2-e). Thus, the cells which are derived from these division, are asymmetric (Fig. 2-f). In these doses, aberrant mitotic divisions were also observed (Fig. 3).

With regards to the function of PPB microfilaments, there is a possibility that actin filaments guide the arrangement of cortical microtubules into PPB which would still be present in the absence after Colchicine treatment. This indicates that PPB microtubules are responsible for actin aggregation and not vice versa (Liu & Palevitz, 1992). The other probability is that actin microfilaments have no specific function and their presence in the PPB is a consequence of the aggregation of PPB microtubules (Palevitz, 1987). Our observation shows that PPB microfilaments organize cell plate and cell wall patterning. In cytochalasin-treated cells the future

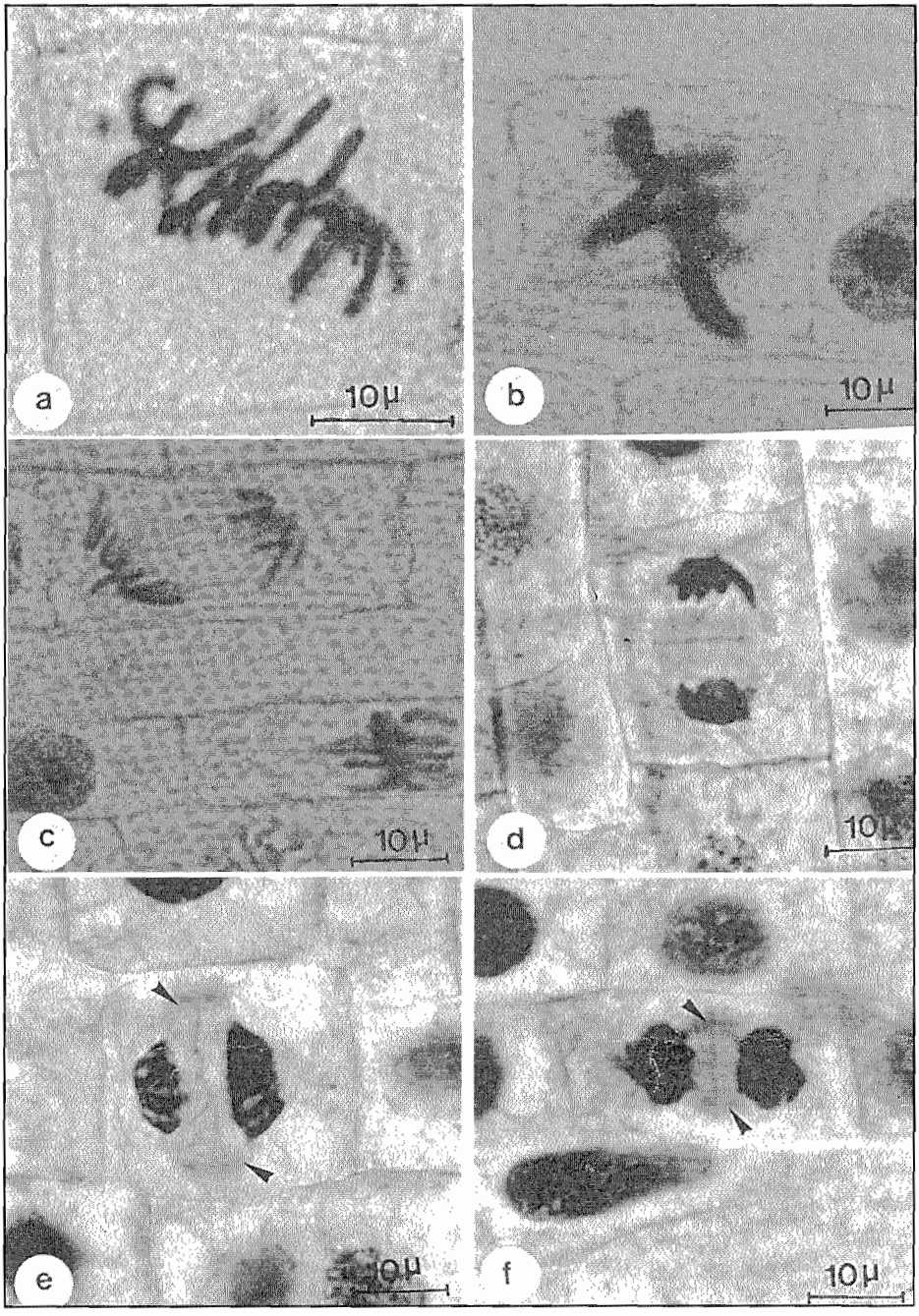

Fig. 1. Oblique mitotic apparatus in normal root apical meristem. a,b-Oblique MA in Metaphasic cells. c-Oblique MA in a Anaphasic cell. Chromosomes are in two opposite corners of the cell. d, e, f- Telophase. Fragmoplast is formed in the mid zone of cell. Arrows show fragmoplasts and intermediate filaments in its two ends.

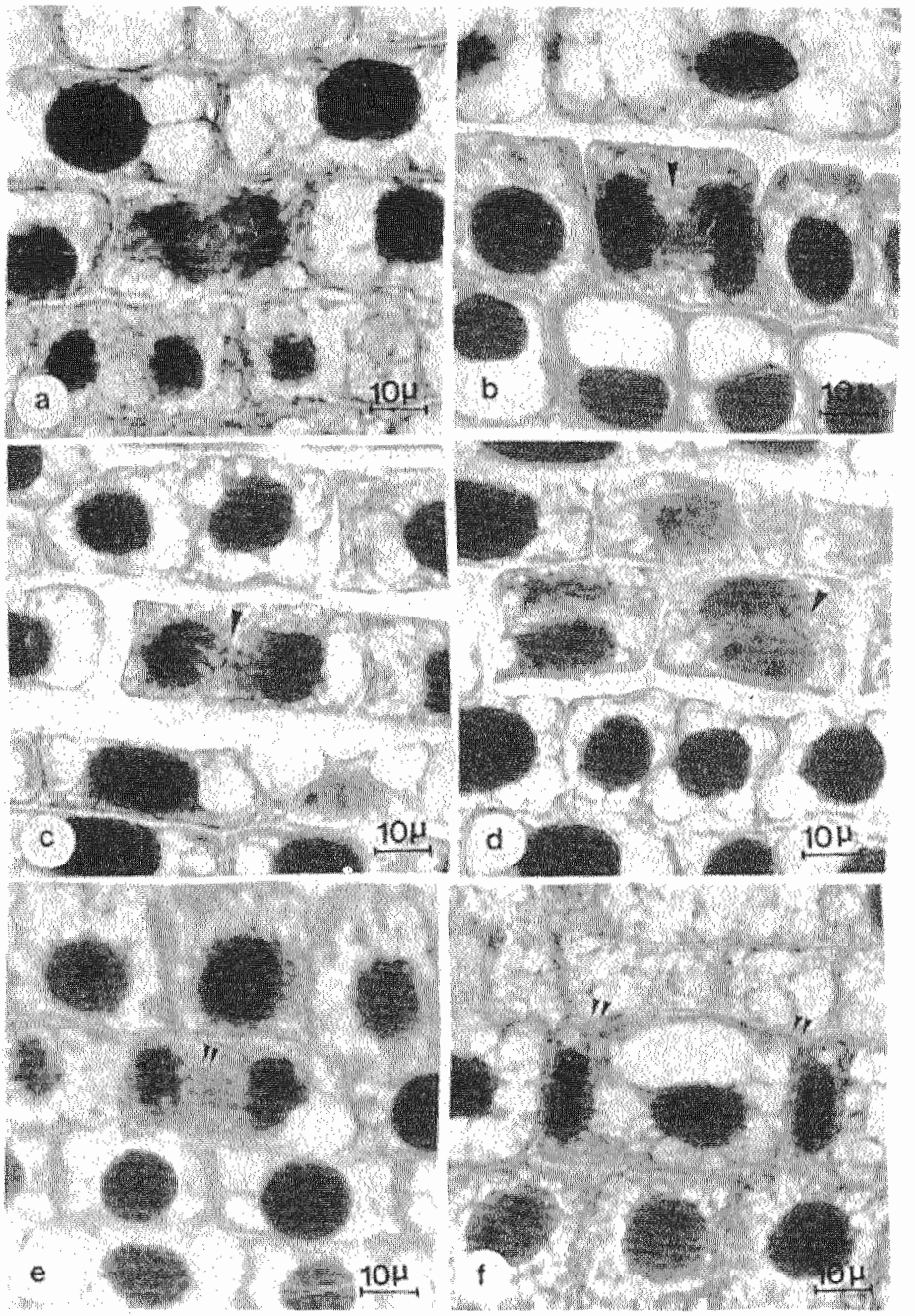

Fig. 2. Cytochalasin B treatment in 5-10 μg/ml concentrations. a- Late anaphase. In cytochalasin treated cells anaphase is normal. b,c- The fragmoplast is not formed in telophase (arrow). d- A binucleated cell (arrow). e- Change in cell wall position and its propelling to a corner of the cell (double arrows). f- The asymmetric divided cell derived from "e".

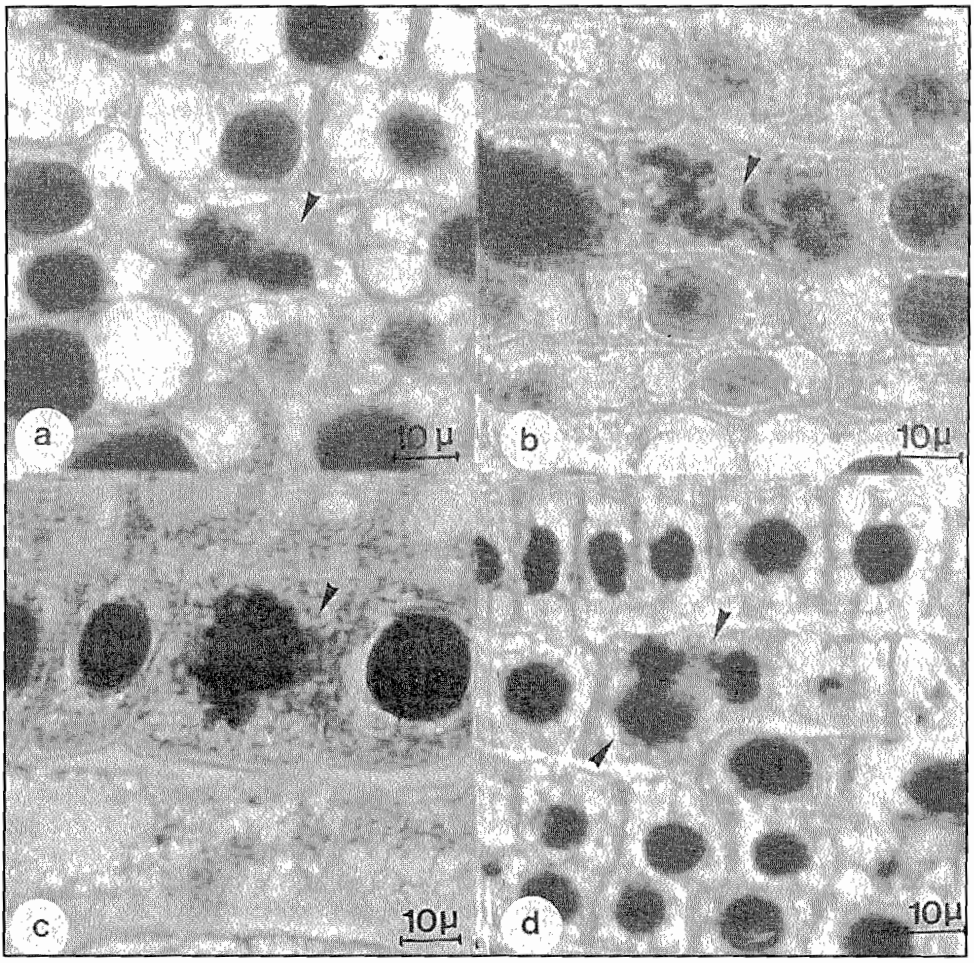

Fig. 3. a,b,c,d- Aberrant mitotic divisions after cytochalasin B treatment. The mitotic step can not be recognized.

cell wall is not placed at predetermined position. Traas and their colleagues have shown that there are actin filaments in and around the spindles (Traas *et al.*, 1987) in carrot cells. Recently, the role of F-actin as a key component in maintaining the geometry of mitosis has been reported (Lloyd, 1991; 1999).

The oblique aligned MAs must move and settle in their correct site. But microfilaments are not involved in MA movement, because our results show that in cytochalasin treated cells, all telophasic MAs are correctly oriented. Perhaps, intermediate filaments are responsible for this process which have been recognized in baundles of 10nm microfilaments associated with the nucleus (Ross *et al.*, 1991). These filaments may having a function in MA movement and plasticity. Why are the MAs in these cells oblique? There seems to be a prominent role of cell shape in MA orientation of root cells (Oud & Nanninga, 1992). It seems that mechanical pressure and space limitation is also the cause for the MA to be oblique.

In any case, MA morphology and movement may be programmed after a special manner. Results of Cande (1990) have shown that the MA morphology and alignment have a genetic basis. We consider that these oblique MAs produce new direction of growth. It seems that there is a median mode from longitudinal to radial growth. The long cortex cells and the central cylinder are produced in this way. The movement of MA causes the PPB effect on future cell wall position. Thus it has a critical role in cell wall patterning and organ development.

References

Cande, W.Z. 1990. Centrosomes: Composition and reproduction. *Curr. Opin. Cell Biol.*, 2: 301-305.

Cooper, J.A. 1987. Effects of cytochalasin and Phalloidin on actin. *J. Cell Biol.*, 105: 1473-1478.

Ding, B., R. Turgeon and M.V. Parthasarthy. 1991. Microfilaments in preprophase hand of freeze substituted tobacco root cells. *Protoplasma*, 165: 209-211.

Jensen, W.A. 1962. *Botanical Histochemistry*. Freeman and Co., pp. 91-93.

Liu, B. and B.A. Palevitz. 1992. Organization of cortical microfilaments in dividing cells. *Cell Motil. Cytoskel.*, 23: 252-264.

Lloyd, C.W. 1991. Cytoskeletal elements of the phragmosome establish the division plane in vacuolated plant cells. Academic Press N.Y. pp. 245-257.

Lloyd, C. 1999. How I learned to love a carrot: the role of the cytoskeleton in shaping plant cells. *BioEssay*, 21: 1061-1068.

Oud, J.L. and N. Nanninga. 1992. Cell shape, chromosome orientation and the position of the planes of division in *Vicia faba* root cortex cells. *J. Cell Sci.*, 103: 847-855.

Palevitz, B.A. 1987. Actin in preprophase band of Allium cepa. *J. Cell Biol.*, 104: 1515-1519.

Palevitz, B.A. 1988. Microtubular fir-tree in mitotic spindle of onion roots. *Protoplasma*, 142: 47-78.

Ross, J.H.E., A. Hutchings, G.W. Butcher, E.B. Lane and C.W. Lloyd. 1991. The intermediate filament-related system of higher plant cells shares an epitope with cytokerstin. *J. Cell Sci.* 99: 91-98.

Traas, J.A., J.H. Doonan, D.J. Raelins, P.J. Shaw, J. Watts and C.W. Lloyd. 1987. An actin network is present in the cytoplasm throughout the cell cycle of carrot cells and associated with the dividing nucleus. *J. Cell. Biol.*, 105: 387-395.

Varner, J.E. and L.S. Lin. 1989. Plant cell wall architecture. *Cell*, 56: 231-239.

(Received for publication 10 August 2001)