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BRIEF COMMUNICATION

Microtubule organization during successive microsporogenesis in *Allium cepa* and simultaneous cytokinesis in *Nicotiana tabacum*

Y.N. ZHANG, D.M. WEI, Y.Y. SONG, L. CHEN, X.Y. ZHU and H.Q. TIAN*

School of Life Science, Xiamen University, Xiamen 361005, P.R. China

Abstract

Microtubule cytoskeleton organization during microspore mother cell (MMC) meiosis in *Allium cepa* L. and microsporogenesis in *Nicotiana tabacum* L. was examined. The MMC microtubules (MTs) were short and well dispersed in the cytoplasm of both taxa. As the MMCs of both species entered metaphase of meiosis I, the MTs constructed a spindle that facilitated the chromosomes to orient in the meridian plane. At anaphase of meiosis I, the spindle MTs differentiated into two types: one MT type became short, pulled the chromosomes toward the two poles, and was designated as centromere MTs; the second type of MT connected the two poles, and was designated as pole MTs. In *A. cepa*, where successive cytokinesis was observed, pole MTs assumed a tubbish shape. Some new short MTs aggregated in the meridian plane and constricted to form a phragmoplast, which developed into a cell plate, divided the cytoplasm into two parts and produced a dyad. However, in tobacco, a phragmoplast was not generated in anaphase of meiosis I and II and cytokinesis did not occur. The spindle MTs depolymerized and reorganized the radial arrangement of MTs from the nucleate surface to the periplasm during anaphase. Following telophase of meiosis II, the cytoplasm produced centripetal furrows, which met in the center of the cell and divided it into four parts, serving as a form of cytokinesis. In this process, MTs appeared to bear no relationship to cytokinesis.

Additional key words: onion, phragmoplast, spindle, tobacco.

In higher plant sexual reproduction, two types of cytokinesis have been characterized during microsporogenesis. In the successive type, following meiosis I, a cell wall is formed separating the two nuclei to produce a dyad. Then both dyad cells begin to meiosis II. In the simultaneous type, following meiosis I, cytokinesis does not occur. Cell walls are formed by the cytoplasm producing furrows centripetally, which meet in the center of the cell. Four cells are the product following the second meiotic division. Brown and Lemmon (1996) studied microtubule configuration during microsporegenesis in orchid. Recently, several works studied abnormal changes of spindle microtubule during microsporogenesis in hybrids and triploids (Sidorchuk *et al*. 2008, Wang *et al.* 2010, Zhang *et al*. 2010). In present study, we examined MT configuration during meiosis in *N. tabacum*, a simultaneous cytokinesis type, and *A. cepa*, a successive cytokinesis type to explore the relationship between cytokinesis type and MT configuration.

Allium cepa L. and *Nicotiana tabacum* L. were grown in the Botany Garden of Xiamen University. Small anthers at meiosis stage were collected and fixed in a solution containing 4 % polyformaldehyde resolved in PIPES buffer (50 mmol PIPES, 5 mmol EGTA, 2 mmol $MgSO_4 \cdot 7\ H_2O$, 5 % mannitol, 10 % dimethyl sulfoxide (DMSO) and 1 % *Triton X-100*, pH 7.0). Anthers were fixed for 1 h, washed 3-times for 5 min each, placed on a slide coated with poly-L-lysine and squeezed to release microspore mother cells (MMCs) from the anthers. The slides were left undisturbed for 30 min as MMCs adhered to the slide. MMCs have a thick callous wall. Therefore, slides were incubated in 1 % snailase in PIPES buffer for 10 - 15 min to digest the callous wall. Following

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Abbreviations: MMC - microspore mother cell; MT - microtubule.
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* Corresponding author; fax: (+86) 592 2181015, e-mail: hqtian@xmu.edu.cu

enzymatic treatment, slides were washed 3-times with PIPES buffer (5 min each), then incubated for 1 h in 1 % bovine albumin buffered PIPES. MMCs were washed 3-times using PBS buffer to prepare for immunological action. PBS buffer contained 138 mmol NaCl, 2.68 mmol KCl, 1.47 mmol KH_2PO_4, and 8.1 mmol Na_2HPO_4. MMCs were first incubated for 1 h in the antibody against β-tubulin (*Sigma*, St. Louis, USA). Following incubation, MMCs were washed in PBS buffer 3-times for 10 min each. MMCs were incubated for 1 h in the secondary antibody labeled with fluorescein isothiocyanate (FITC). Following immunological reaction, the slides were washed in PBS buffer 3-times for 10 min each and stained using 1 μg dm^{-3} 4',6-diamidine-2'-phenylindole (DAPI) for 5 min to indicate chromatin state. Stained MMCs were observed using *Leica* confocal laser scanning microscopy (CLSM). The 488 nm excitation wavelength was used for FITC and 364 for DAPI.

A thick callous wall surrounded the MMC during the microspore mother cell stage in tobacco (Fig. 1*a*), which is characteristic of this cell type. The chromatin in the nucleus displayed a blue fluorescence after DAPI staining and was condensing into chromosomes, which is a developmental indicator of MMCs (Fig. 1*b*). The cytoplasmic MTs displayed reticulate structure and no specific distribution (Fig. 1*c*). When MMCs entered metaphase of meiosis I, all cellular MTs constructed a spindle to control chromosomal arrangement in the meridian plane, and MT proteins were not labeled in the cytoplasm (Fig. 1*d*). In anaphase of meiosis I, some spindle MTs shortened to pull the chromosomes toward

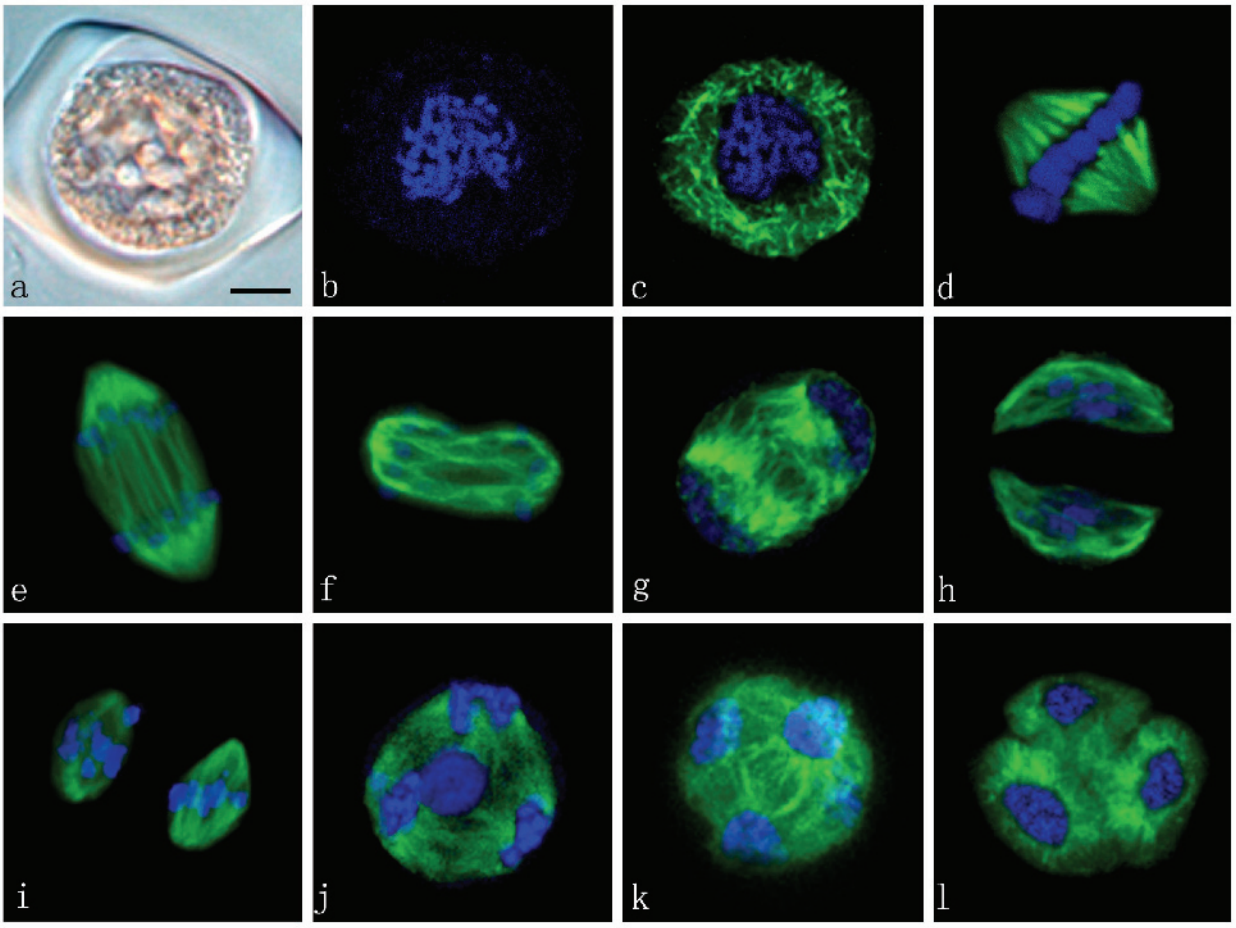

Fig. 1. Microtubule configuration during microsporogenesis of *Nicotiana tabacum* L. (*bar* = 10 μm): *a* - microspore mother cell (MMC); *b* - chromosomes in the nucleus of a MMC; *c* - MT distribution in a MMC; *d* - spindle MTs in metaphase of meiosis I in a MMC; *e* - the spindle centromere MTs are shortening to pull the chromosomes to the two poles, some polar MTs connect to the two polarities; *f* - the polar MTs between the two poles in the MMC in meiosis I anaphase have increased; *g* - the MTs between the two nuclei break in the middle during telophase of meiosis I and are concentrated towards the surface of the nucleus; *h* - two nuclei and the cytoplasm are distributed in the same cell following the completion of meiosis I; *i* - two spindles are located in the same cytoplasm in meiosis II metaphase; *j* - the spindle MTs depolymerized and the MTs are aggregated toward the surface of the nucleus; *k* - the MTs increase in number and connect among the four nuclei; *l* - the MMC begins cytokinesis following telophase of meiosis II by centripetal expansion and invagination of the plasma membrane to form a marginal furrow that separates the MMC cytoplasm.

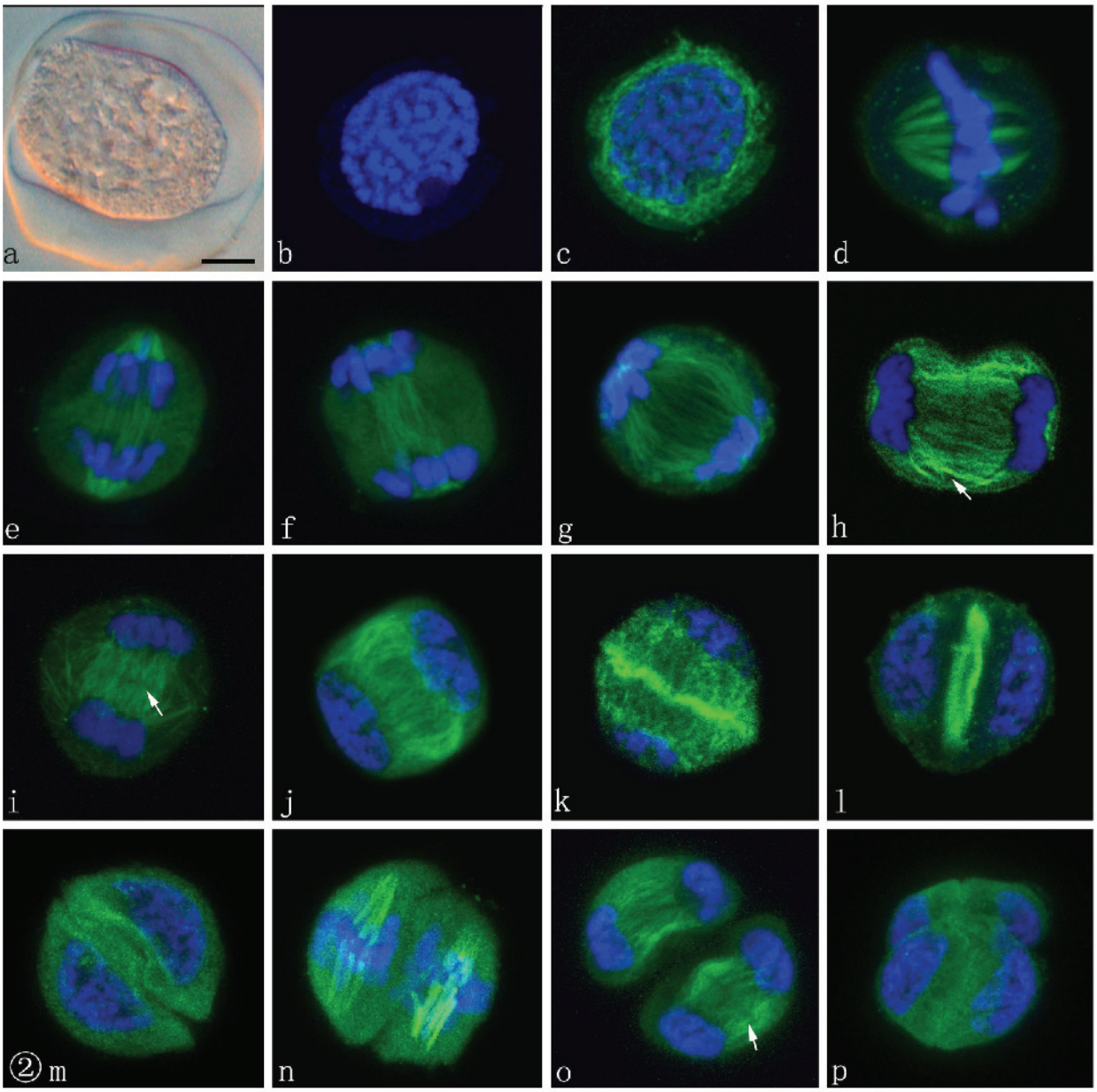

Fig. 2. Microtubule configurations during microsporogenesis of *Allium cepa* (*bar* = 10 μm): *a* - a microspore mother cell (MMC); *b* - chromosomes in the nucleus of a MMC; *c* - MT distribution in a MMC; *d* - spindle MTs in metaphase of meiosis I in a MMC; *e* - the spindle centromere MTs are shortening to pull the chromosomes to the two poles, some polar MTs connect to the two polarities; *f* - the centromere MTs depolymerize and only pole microtubules connect the two poles; *g* - the pole MTs assume a tubby shape to distribute peritropously; *h* - the tubbish distribution of MTs displays fewer microtubules in the center of the spindle; *i* - some newly formed MTs appear in the middle of the tubbish MTs; *j* - the newly formed MTs increase in number and construct a phragmoplast; *k* - the MTs between the two nuclei aggregate toward the meridian plane; *l* - the MT distribution assumes an orbicular configuration; the central area may be developing a cell plate; *m* - MTs in both dyad cells depolymerize to form microtubule proteins, which scatter in the cytoplasm; *n* - some MT proteins assemble into a spindle in each dyad cell in metaphase of meiosis II; *o* - the MTs in two dyad cells display a tubbish distribution in anaphase of meiosis II; *p* - the MTs depolymerize to form microtubule protein dispersing in the microspore cytoplasm.

the two poles, and were designated centromere MTs, which displayed intensified fluorescence and a cap-like structure. Some spindle MTs connected the two poles and were designated pole MTs (Fig. 1*e*). After both chromosome sets reached two poles, the centromere MTs were no longer evident and the pole MTs thickened and enlarged (Fig. 1*f*). The MTs ruptured from the middle, and shortened toward the nucleus during telophase of meiosis I. Subsequently, the MTs on the surface of the nucleus displayed increased fluorescence (Fig. 1*g*).

Tobacco meiosis is the simultaneous type, which is characterized by the absence of cell wall formation between the two nuclei and dyad development. The MTs re-oriented at 90° to produce two parallel long axels in the two cytoplasmic areas (Fig. 1*h*). At metaphase of meiosis II in the MMCs, two parallel spindles were formed in the cells (Fig. 1*i*). After both chromosome sets reached the poles, the spindle MTs depolymerized. Some new MTs reorganized on the surface of each chromosome set and displayed radial arrangement (Fig. 1*j*). The cellular MTs increased in number and connected between the four newly formed tetrahedrally arranged nuclei (Fig. 1*k*). Following the second nuclear meiotic division, MMCs initiated cell wall formation and cytokinesis at the MMC surface between the two nuclei, where the plasma membrane divided centripetally to form a marginal furrow that separated the MMC cytoplasm (Fig. 1*l*). During cytokinesis, a special MT structure was not evident at the front end of the division furrow. At the completion of MMC meiosis, the four microspores were entirely separated by a callous wall forming a tetrad. The MTs in each microspore were distributed radially from the nuclear surface to the periplasm.

A thick callous wall was observed surrounding the MMCs during the microspore mother cell stage in *A. cepa*, similar to that of tobacco (Fig. 2*a*). In prophase of meiosis I, the chromatin had condensed, transformed into chromosomes and exhibited blue fluorescence due to DAPI labeling (Fig. 2*b*). The MTs in the MMC cytoplasm were randomly distributed and the MT fluorescence on the nucleate surface was prominent, displaying radially from the nucleus towards the cell periplasm (Fig. 2*c*). MMCs entered metaphase of meiosis I and subsequently the MT proteins constructed a spindle, which showed conspicuous fluorescence. MT proteins, in addition to spindle MTs, remained in the cytoplasm, and the fluorescence appeared weak (Fig. 2*d*). In anaphase of meiosis I, the spindle MTs were divided into two parts: short MTs (centromere MTs), which pulled two sets of chromosomes toward two opposing poles, and long MTs (pole MTs), which connected two poles of the spindle. The centromere MTs shortened into a cap-like structure and the fluorescence was no longer evident (Fig. 2*e*). The pole MTs disappeared later than the centromere MTs (Fig. 2*f*). Some chambered MTs were produced from the two poles and formed a tubbish MT structure, which was distributed as a round cage (Fig. 2*g*). The MTs connecting the two nuclei were broken in the middle section, and displayed a dark line without MT fluorescence (Fig. 2*h*). Following anaphase, in the meridian plane formation region, newly formed MTs appeared (Fig. 2*i*) and increased in number (Fig. 2*j*). The cells subsequently entered telophase of meiosis I and the chromosomes condensed. A nucleus formed and the MTs between the two nuclei concentrated on the meridian plane to form a light line (Fig. 2*k*). This bright plane became an acentric ring-like structure, however MTs in the center were not observed where a cell plate would later develop (Fig. 2*l*). Cytokinesis in MMCs in *A. cepa* resulted in the formation of two dyad cells. In both dyad cells, the MT structure disappeared and the MT proteins dispersed in the cytoplasm (Fig. 2*m*). In metaphase of meiosis II, MT proteins in the both dyad cells constructed a spindle in each cell, which oriented the chromosomes on the meridian plane (Fig. 2*n*). The spindle depolymerized and again formed a tubbish microtubule structure in anaphase of meiosis II, similar to meiosis I. The MTs connecting the two nuclei were broken in the middle, and displayed a dark line (Fig. 2*o*). Following cytokinesis of meiosis II, four tetrad microspores were produced. Nearly all of the MTs depolymerized and only the MT proteins dispersed in the cytoplasm (Fig. 2*p*).

In higher plants, two types of meiotic divisions have been characterized in microsporogenesis. The successive type of cytokinesis of MMC is similar to the somatic mitosis, which is controlled by MTs. The simultaneous type of cytokinesis of MMC does not form a cell wall after the first nuclear meiotic division. In tobacco (simultaneous type), the microspore mother cell does not form a phragmoplast in anaphase of meiosis I and II. Therefore, cytokinesis does not occur after each nucleate division. When the second nucleate meiosis division is complete, the plasma membrane invaginates to produce a furrow that separates the cytoplasm. During this process, the MTs do not exhibit a special structure, indicating the absence of a relationship between the MTs and this specialized form of cytokinesis. The key difference between the two types is that MTs may or may not construct a phragmoplast during anaphase of meiosis I and II. Therefore, phragmoplast formation is a regulation point for the two types of meiosis.

Three MT configurations are described during cell division: *1*) before cell division, MTs accumulate on the surface of nucleus, which may orient the nucleus and serve a function in the breakdown of the nuclear membrane; *2*) spindle MTs control chromosomal movement and divide the chromosome equally into two parts; and *3*) phragmoplast MTs during anaphase (only in plants) are related to cytokinesis and equally divide a cell into two parts to form two cells. Several papers have been published describing the former two MT types. However, the origins of the phragmoplast MTs have not yet been elucidated. Generally, the phragmoplast is a cytoplasmic dense area in the spindle containing microtubules, actin filaments, endoplasmic reticulum (ER), and cell plate forming vesicles and forms in anaphase during common somatic division (Staehelin and Hepler 1996, Seguí-Simarro *et al*. 2004, Jürgens 2005). However, in median plane of the tobacco BY-2 suspension cell at late telophase, microtubules are absent from the central part of the phragmoplast (Esseling-Ozdoba *et al*. 2008). Shamina *et al*. (2007) studied cytoskeletal rearrangements during meiotic anaphase in a number of mono-cotyledonous plant species and found that central spindle

fibers that move centrifugally, along with newly-formed MTs, were the basis of phragmoplast formation and function in MMCs of monocotyledonous plant species with successive cytokinesis stages. In the present study, at meiosis I anaphase of *A. cepa*, the spindle MTs differentiated into two types: one the centromere MTs, which pulled chromosomes to move toward the two poles, and the other the pole MTs, which connected the two poles of the spindle to create a spaced structure. The pole MTs assumed a tubbish shape and newly formed MTs converged in the center to form a phragmoplast. Therefore, the phragmoplast MTs do not originate from the pole MTs, but are newly formed, which differs from studies in wheat (Shamina *et al*. 2007). Phragmoplast MTs may be derived from depolymerized centromere MT proteins.

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