A new type of microtubular cytoskeleton in microsporogenesis of *Lavatera thuringiaca* L.

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**Summary.** In *Lavatera thuringiaca*, kariokinesis and simultaneous cytokinesis during the meiotic division of microsporogenesis follow a procedure similar to that which takes place in the majority of members of the class Angiospermae. However, chondriokinesis occurs in a unique way found only in species from the family Malvaceae. Chondriokinesis in such species is well documented, but the relationship between the tubulin cytoskeleton and rearrangement of cell organelles during meiosis in *L. thuringiaca* has not been precisely defined so far. In this study, the microtubular cytoskeleton was investigated in dividing microsporocytes of *L. thuringiaca* by immunofluorescence. We observed that, during prophase I and II, changes in microtubular cytoskeleton configurations have unique features, which have not been described for other plant species. At the end of prophase I, organelles (mostly plastids and mitochondria) form a compact envelope around the nucleus, and the subsequent phases of kariokinesis take place within this arrangement. At this point of cell division, microtubules surround the organelle envelope and separate it from the peripheral cytoplasm, which is devoid of plastids and mitochondria. In telophase I, two newly formed nuclei are tightly surrounded by the cell organelle envelopes, and these are separated by the phragmoplast. Later, when the phragmoplast disappears, cell organelles still surround the nuclei but also move a little, starting to occupy the place of the disappearing phragmoplast. After the breakup of tetrads, the radial microtubule system is well developed, and cell organelles can still be observed as a dense envelope around the nuclei. At a very late stage of sporoderm development, the radial microtubule system disappears, and cell organelles become gradually scattered in the cytoplasm of the microspores. Using colchicines, specific inhibitors of microtubule formation, we investigated the relationship between the tubulin cytoskeleton and the distribution of cell organelles. Our analysis demonstrates that impairment of microtubule organization, which constitutes only a single component of the cytoskeleton, is enough to disturb typical chondriokinesis in *L. thuringiaca*. This indicates that microtubules (independent of microfilaments) are responsible for the reorganization of cell organelles during meiotic division.

**Keywords:** *Lavatera thuringiaca*; Microsporogenesis; Microtubular cytoskeleton; Organelle; Colchicine.

**Introduction**

During sporogenesis and microsporogenesis in meiotic cells, regular movements of organelles take place in addition to kariokinesis and rearrangements of cytoplasmic components. Cytoplasm rearrangements and the distribution of organelles into progeny cells are believed to be as precise as the division of chromosomes in kariokinesis (Geneves 1967, 1971). Several reports have shown specific relationships between organelle movement and changes in the configuration of microtubules (MTs) and microfilaments (MFs) during meiosis (Wolniak 1976; Brown and Lemmon 1988b, c, 1989, 1990a, b, 1991a, 1996; van Lammeren et al. 1989; Tanaka 1991; Rodkiewicz et al. 1992; Shimamura et al. 1998, 2004).

During the meiotic division in sporogenesis, microsporogenesis, and megasporogenesis, the cytoskeleton undergoes a significant rearrangement. So far, four configurations of MTs have been described: the endoplasmic system, which can be reticulate or radial, meiotic spindles, the phragmoplast, and the radial MT system (RMS) surrounding the nucleus (van Lammeren et al. 1985, 1989; Hogan 1987; Brown and Lemmon 1988a–c, 1991a, 2000; Busby and Gunning 1988; Traas et al. 1989; Shimamura et al. 2004; Bohdanowicz et al. 2005). The tubulin and actin cytoskeleton is a highly labile structure. The meiotic transformation of the cytoskeleton is usually determined by the position of microtubule-organizing centers (MTOCs), which in turn respond to cell cycle and polarity signals (Pickett-Heaps et al. 1999). In plant cells, MTOCs
are associated with endomembranes, such as the plasmalemma, the endoplasmic reticulum, or nuclear and plastid envelopes (Vaughn and Harper 1998, Shimamura et al. 2004, Brown et al. 2004).

In many plant species, similar changes in the configuration of the microtubular cytoskeleton occur during sporogenesis and microsporogenesis with simultaneous cytokinesis (Rodkiewicz et al. 1992). Before meiosis and at the beginning of prophase I, the microtubular system is found in the cortical cytoplasm. In prometaphase, these endoplasmic MTs disappear and are absent until the end of meiosis. Subsequently, in late prophase I, polymerization of the kariokinetic spindle takes place, and MTOCs are predominantly associated with the nuclear envelope at this time. However, in monoplastidic species, cytoskeleton configurations mostly depend on the position of the plastid, which functions similarly to the centrosome in animal cells (Brown and Lemmon 1985, 1989, 1990a, b, 1991b, c; Shimamura et al. 2004).

During telophase I, after movement of chromosomes toward the poles, the RMS is formed, which initiates the typical phragmoplast. After formation of the phragmoplast, cell organelles move to the equatorial plate. Before the second meiotic division, the RMS and the phragmoplast disappear (Sheldon and Hawes 1988, Traas et al. 1989, Rodkiewicz et al. 1992). Subsequently, in metaphase II, two meiotic spindles are formed, usually oriented perpendicularly to each other, and in species with simultaneous cytokinesis they are separated by a layer of organelles (Rodkiewicz and Duda 1988, Brown and Lemmon 1996, Tchórzewska et al. 1996). After the second meiotic division, the RMSs are formed around the four nuclei (Hogan 1987; Brown and Lemmon 1988c, 1996; Traas et al. 1989; Tanaka 1991). Polymerization of phragmoplast MTs starts from the nuclei, moving towards the equatorial plane of the cell and then centrifugally in the direction of the mother cell wall. The microspores have a tetrahedral arrangement, with a quadrupolar phragmoplast formed between sister and nonsister nuclei.

*Lavatera thuringiaca* belongs to the family Malvaceae. Meiosis in the microsporogenesis of *L. thuringiaca* is similar to that found in the majority of plants with simultaneous cytokinesis, but the movement of organelles during meiosis is very specific and unique. This characteristic rearrangement of organelles has been described in other species belonging to the family Malvaceae: *Gossypium barbadense* (Denham 1924), *Malva silvestris* (Rodkiewicz and Duda 1988, Rodkiewicz et al. 1988, Kudlicka and Rodkiewicz 1990), and *Lavatera trimestris* (Longly and Waterkeyn 1979, Kudlicka and Rodkiewicz 1990).

In this study, we analyzed the microtubular-cytoskeleton changes during microsporogenesis in *L. thuringiaca*. We observed that the changes in the organization of the MTs differ significantly from those in other seed plants. Simultaneously, the organelle movement during the meiotic division was investigated. Additionally, using colchicine, which induces depolymerization of all categories of MTs, we were able to investigate the relationship between the tubulin cytoskeleton and the rearrangement of cell organelles.

**Material and methods**

**Plant material**

Differently sized anthers of *Lavatera thuringiaca* plants were collected from the Botanical Garden of Maria Curie-Skłodowska University in Lublin.

**Immunofluorescence microscopy**

Pieces of anthers were fixed for 24 h in 4% paraformaldehyde and 0.25% glutaraldehyde in MT stabilizing buffer (MSB) (Baluska and Barlow 1993), pH 6.9, at room temperature. They were then rinsed in MSB buffer, dehydrated, embedded in polyethylene glycol and sectioned according to the method of van Lammeren et al. (1985). Sections, 2 μm thick, were mounted on slides coated with 2% organosilicon (Sigma) and the slides were rinsed 3 times for 5 min each in phosphate-buffered saline (PBS). Next, they were treated with 0.1 M NaH2Cit in PBS, washed twice for 5 min in PBS and blocked with 0.1% bovine serum albumin (BSA) in PBS for 30 min. Subsequently, the slides were incubated in a moist chamber for 60 min at 37 °C with monoclonal anti-mouse α-tubulin (Sigma) diluted 1:200 in PBS. After washing with 0.1% BSA in PBS (3 times for 15 min), incubation with a secondary antibody was carried out for 60 min at 37°C. The secondary antibody, conjugated with fluorescein isothiocyanate (Sigma), was diluted 1:200 in PBS with 0.1% BSA. In order to stain DNA in the nuclei and organelles, 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) was added to the sections. The sections were observed with a fluorescence microscope (Nikon Optiphot II). Images of microspores were recorded on Kodak TMAX-400 film.

**Treatment with colchicine**

Differently sized flower buds of *L. thuringiaca* plants were treated with 0.2% colchicine (Sigma) for 48 h in a water chamber. Afterwards, the anthers were prepared for observation under the fluorescence microscope as described above.

**Electron microscopy**

For transmission electron microscopy (TEM), anthers were fixed in 2.5% paraformaldehyde and 2.5% glutaraldehyde in PBS buffer (pH 6.9). The specimens were washed three times in PBS and postfixed in 2% osmium tetroxide. The anthers were then dehydrated in a graded ethanol series, diluted with PBS buffer, and embedded in London Resin White Medium (Sigma). Ultrathin sections (60 nm) were stained with uranyl acetate (5 min) and lead citrate (10 min). The sections were observed with a JEM 100B transmission electron microscope.

**Results**

During meiosis in microsporogenesis of *L. thuringiaca*, organelles, mainly plastids and mitochondria, aggregate