Chapter 17
Visualization of Plant Microtubules

Elisa Graña

1 Introduction

Microtubules (MTs) are highly dynamic components of the cell cytoskeleton that are involved in many important processes such as cell division (chromosome movement, formation of preprophase band, phragmoplast, cortical band before preprophase, etc.), cellular transport (endocytosis, exocytosis, organelle movement: nuclei, chloroplasts, amyloplasts, etc.), and growth and differentiation (transport of cellulose precursors to the cell wall to form cellulose microfibrils, transition from division to expansion, stomata movement, etc.) (Marc 1997; Wasteneys 2004; Alberts 2008; Nick 2008a; Celler et al. 2016).

Microtubules were first clearly described by Ledbetter and Porter in 1963, who also named them as microtubules. At that time, they were still trying to find out the best fixing method to preserve their experimental samples in TEM (transmission electron microscopy), by testing different combinations of fixatives. Ledbetter and Potter described MTs after examining cortex cells from *Phleum pretense*, *Spirodela oligorrhiza* and *Juniperus chinensis* in interphase. Microtubules were parallel to each other, clustered in small groups of 5–6 units and circumferentially arranged to the long axis of the cells, like 'hoops around a barrel'. These authors observed also MTs as hollow cylinders in cross-sections. As well, MTs were also seen in dividing cells (specifically in telophase), although authors recognized that was much more difficult to find them in dividing cells. Ledbetter and Porter stated that 'thin lines oriented normal to the cell plate representing the tubules, are apparent in the interzone of the spindle'. Curiously, in that work they also inferred a possible relation between microtubules and cellulose microfibrils: 'the tubules or sub-units of them act as primers for cellulose deposition'.

E. Graña (✉)
Plant Ecophysiology Laboratory, Department of Plant Biology and Soil Science, University of Vigo, Vigo, Spain
e-mail: eli-grana@uvigo.es

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Nowadays, we know that MTs are formed thanks to the binding of certain globular proteins, α- and β-tubulin, which associate to form heterodimers that form linear rows of tubulin dimmers named protofilaments. One single microtubule consists of the union of 13 of these protofilaments (Hyams and Lloyd 1994; Kwiatkowska 2006; Donhauser et al. 2010). Its polymerization needs the presence of a chaperonin, which folds α- and β-tubulin (Gao et al. 1993). In addition to the structural proteins tubulins, MTs are accompanied by another kind of proteins, known as microtubule-associated proteins or MAPs, whose function is related to the organization of MTs (Mao et al. 2005).

Microtubules are defined as semi-rigid and polarized structures, with a plus (+) or growing end, where polarization predominates over depolarization; and a minus (−) or depolymerizing end (Alberts 2008; Guo et al. 2009). Microtubules are continuously being assembled and disassembled, in a property that is called *dynamic instability* (Mitchison and Kirschner 1984). Generally, the rate of assembly and disassembly is balanced, but in case that polymerization stops, microtubule is disassembled and it may disappear (Alberts 2008). It is specifically the dynamic nature of MTs the one responsible for their flexibility to reorganize themselves into diverse arrays, allowing changes in growth depending on different environmental or chemical signals (Chen et al. 2014). One of the most relevant MAPs is the motor protein kinesin, which joins MTs with the help of adenosine triphosphate (ATP). This joint allows kinesins to transport traffic vesicles, organelles and another proteins from minus to plus microtubule ends (Reddy 2001).

As MTs are direct or indirect targets of numerous signaling pathways, and also participate in signal transduction itself (Nick 2008b), their configuration is variable depending on the needs of the cell (Goddard et al. 1994). In fact, Yuan et al. (1994) described the microtubule dynamics like ‘an adaptation whereby sessile plants can continually and rapidly alter their direction of cell expansion in response to external and internal stimuli’.

Wasteneys (2004) postulates that one of MTs main roles is the modulation of signaling pathways that allow them to face environmental changes, being able to act as ‘sensors or transducers’ for inputs that regulate plant growth (Landrein and Hamant 2013; Nick 2013; Bhaskara et al. 2016). Different signaling molecules could directly or indirectly bind to microtubules thanks to protein complexes, and could be freed to the cytoplasm and be activated once microtubules run into depolymerization (Wasteneys 2004). Different signal triggers described to induce cytoskeleton rearrangement are osmotic stress, cold, exposure to heavy metals, pathogens, hormones, gravity, light, or high molecular weight molecules such as PEG (Westeneys 2004; Wang et al. 2011; Mei et al. 2012; Chen et al. 2014). Interestingly, many of these signals converge in auxin.

Wasteneys also described MTs to be ‘heavily congested places’: nucleotides, ions and specially proteins (MAPs and their regulatory elements, motor proteins), could use them as the central place from where assembly, coupling and stability is regulated and organized.

Assembly starts on the microtubules organizing centers, also known as MTOCs. The minus end is associated with the MTOC, and microtubule grows to the plus

direction. In animal cells, MTOCs are well identified as centrosomes, but in plants the assembly and organization sites have been under controversy, and it is thought that are located at the nuclear surface. As γ-tubulin predominates in the MTOCs from animal cells, many studies have focused on finding γ-tubulin clusters in plant cells, but no conclusive results were obtained (Goddard et al. 1994; Liu et al. 1994; Vaughn and Harper 1998).

The regulation of MTOCs involves assembling, dynamics, interactions with other cell elements, and association with motor and structural proteins (Marc 1997). In this way, the MTs associated proteins, or MAPs, cooperate with the tubulin dimmers assembly, join adjacent MTs or link MTs to other cellular structures (Marc 1997).

2 Typical Microtubule Arrangement

Cortical microtubules can be typically found arranged in three different conformations: transverse, oblique and longitudinal respect to the direction of cell growth. In interphase root tip cells, cortical MTs have been reported to be transverse (Collings and Wasteneys 2005), since transversal orientation is established early in the meristematic cells (Panteris et al. 2013). This orientation is constant in the cortex, in the endodermis and also in the stele (Panteris et al. 2013).

Microtubule reorientation starts when cells stop growing. MTs are found arranged in an oblique way, until they reach the longitudinal orientation, typical of elongation zone. Suppression of cell growth is traditionally related to longitudinal alignment (Panteris et al. 2013; Chen et al. 2014) (see Fig. 17.1).

3 Why Study Microtubules?

Studying these cytoskeleton components is not only useful for understanding the mechanisms of cellular organization, but for understanding the response of cells to different stimuli that are known to change the microtubule array configuration. The knowledge of the microtubule dynamics opens the door to novel technical applications. For example, some studies have been focused to improve the quality of wood and its products, based on the role of MTs in orientation and organization of cellulose microfibrils during the formation of the secondary cell wall, determining the mechanical properties of the wood (Funada 2008). On the other hand, several compounds have been described as typical antimicrotubule drugs: oryzalin, trifularin, colchicines, paclitaxel (better known as taxol), etc. Their antimicrotubule effects can be included in two groups: **mitotic disrupters** and **microtubule assembly inhibitors**. Both have been further studied due to their huge practical use. Besides being used as anticancer o anthelmintics agents (Jordan et al. 1998), these

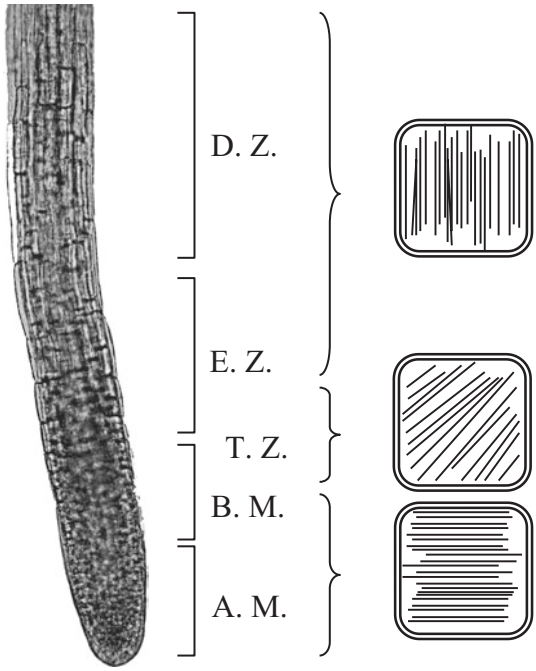

Fig. 17.1 Typical microtubule arrangement, depending on different root zones (*A.M.* apical meristem, *B.M.* basal meristems, *T.Z.* transition zone, *E.Z.* elongation zone, *D.Z.* differentiation zone)

compounds are source, for example, of potential herbicides, also known as anticytoskeletal herbicides.

One of the most studied microtubule assembly inhibitors is colchicine. This drug inhibits the assembly of new tubulin dimmers by blocking the union to the microtubule cap, preventing their polymerization by joining and blocking the binding sites of tubulin (Dayan et al. 2010; Oliva et al. 2002). Consequently, colchicine induces loss of MTs during cell division, leading to mitotic aberrations like blocked metaphases or polymorphic nuclei (Bartels and Hilton 1973; Vaughn et al. 1987).

Compounds with similar activities are podophyllotoxin (which inhibits the microtubule assembly, resulting in mitotic arrest at prometaphase, thereby decreasing the number of cells entering mitosis) (Vaughn and Vaughan 1988); dithiopyr (its target are the MAP proteins, avoiding the stabilization of the MTs involved in cell division) (Senseman 2007); or trifluralin (both dithiopyr and trifluralin cause irregularly formed nuclei, branched and undulating phragmoplast and incomplete and reticulate cell walls) (Lehnen and Vaughn 1991). Mitotic disrupters cause easily identifiable effects on plants. The most typical macroscopic effect is the ‘club root’ morphology, which is due to isodiametric cell growth in the elongation zone (Vaughn 2006). As result, roots appear massively thickened and distorted, accompanied by a decrease in hair root density. Besides, plant growth is generally retarded.

Among the microscopic effects, the most representative are branched and abnormally oriented cell plates, which do not divide the daughter cytoplasms in a proper way. Abnormalities in the movement of chromosomes have also been observed.

As result, it is possible to find multiple nuclei or small nuclear fragments, since the new nuclear envelopes are reformed around disoriented chromosomes (Vaughn 2006). This effect is due to an irregular organization of the spindle microtubules, appearing in clusters radiating from the same center. As consequence, abnormal anaphases are formed, known as 'star anaphases' (Lehnen et al. 1990).

Well-known mitotic disrupters are artemisin (Dayan et al. 1999), terbutol (Lehnen et al. 1990), or oryzalin, which induces the loss of cortical and spindle microtubules, causing mitotic aberrations as lobed nuclei and multinuclei cells (Bartels and Hilton 1973). It has been observed that oryzalin binds the tubulin dimer, co-polymerizing with free tubulin and decreasing the microtubule assembly. As well, left-handed helical growth in *Arabidopsis thaliana* oryzalin-treated seedlings has been also described (Nakamura et al. 2004).

In this chapter two different protocols to visualize plant MTs, one by immunostaining (using fluorescence or confocal microscopy) and other by transmission electron microscopy (TEM), are in detail described.

4 Immunostaining of Plant Microtubules

This method is carried out in two consecutive days and is based on plant tissue fixation, cell wall digestion and immunolocalization with specific antibodies. It has been specifically developed to observe cortical microtubules from *Arabidopsis thaliana* radicles, and it is based on the protocol of Holzinger and collaborators (2009), with some modifications. The steps to follow are detailed below, and are also summarized in Fig. 17.2. Examples of microtubule immunolabeling can be seen on Fig. 17.3.

Day 1

1. Roots from *Arabidopsis thaliana* seedlings are fixed for 45 min at room temperature in freshly prepared buffer containing 0.5% glutaraldehyde and 1.5% formaldehyde prepared in microtubule-stabilizing buffer or MSB (50 mM PIPES, 2 mM EGTA, 2 nM $MnSO_4$, pH 7.2) with 0.1% Triton X-100.
2. Wash samples in MSB buffer with 0.1% Triton X-100, for 20 min.
3. Wash samples again in MSB buffer until no foaming.
4. Chop the plant material with a razor blade to approximately 3 mm length.
5. Digest the cell walls to allow the entry of the antibodies in the cytoplasm, with MSB containing 1% cellulase and 1% pectolyase Y-23, pH 5.5. Digest at room temperature for 30 min.
6. Rinse in MSB, pH 7.2.
7. Permeabilize the root samples in methanol at −20 °C for 10 min.
8. Rehydrate samples by washes with PBS buffer, pH 7.4.
9. Incubate with 1 mg mL^{-1} $Na_2B_4O_7$ in PBS buffer for 20 min. The goal of this step is to reduce aldehyde-induced autofluorescence: $Na_2B_4O_7$ blocks free aldehyde groups.

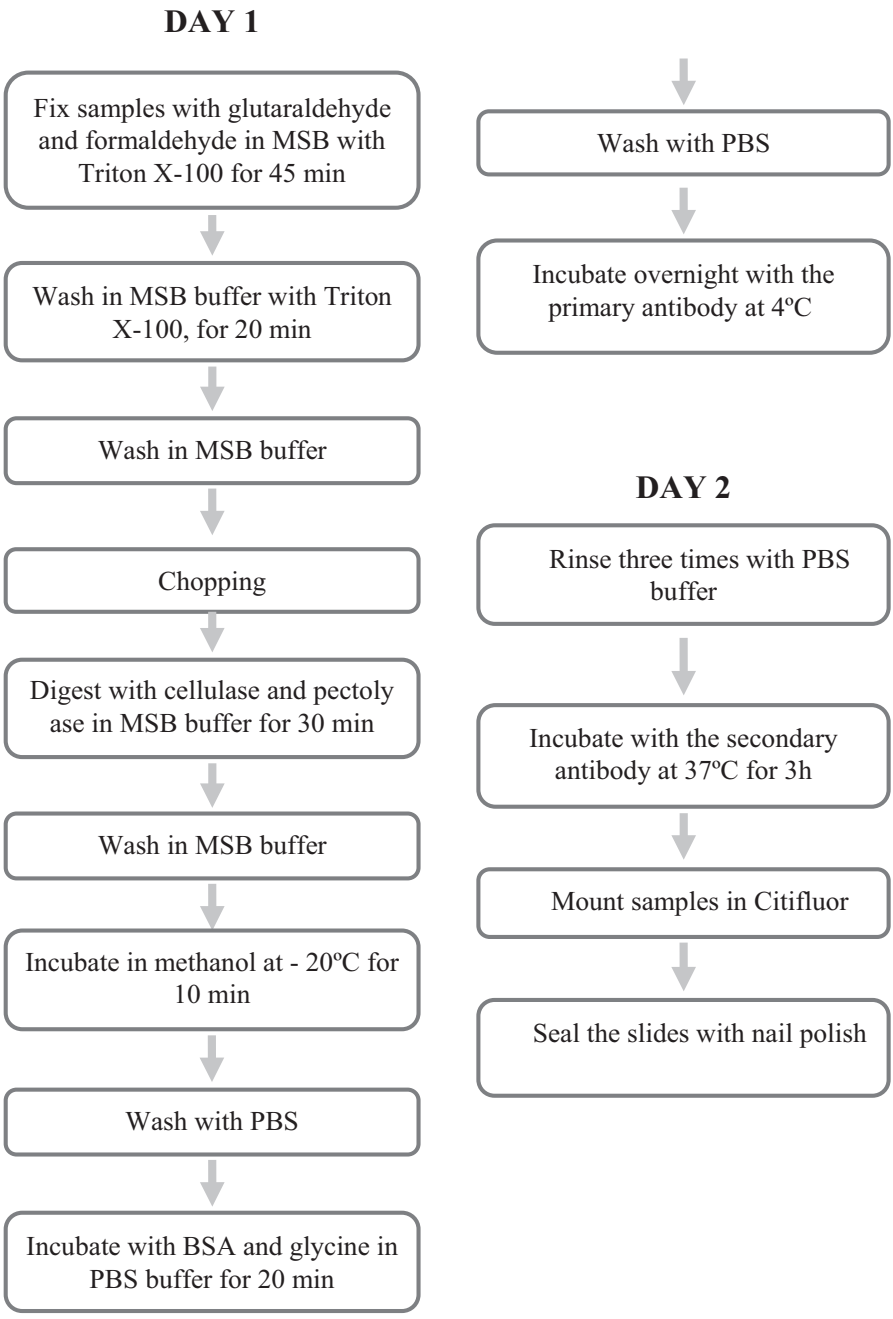

Fig. 17.2 Summarized scheme of microtubule immunolabeling procedure

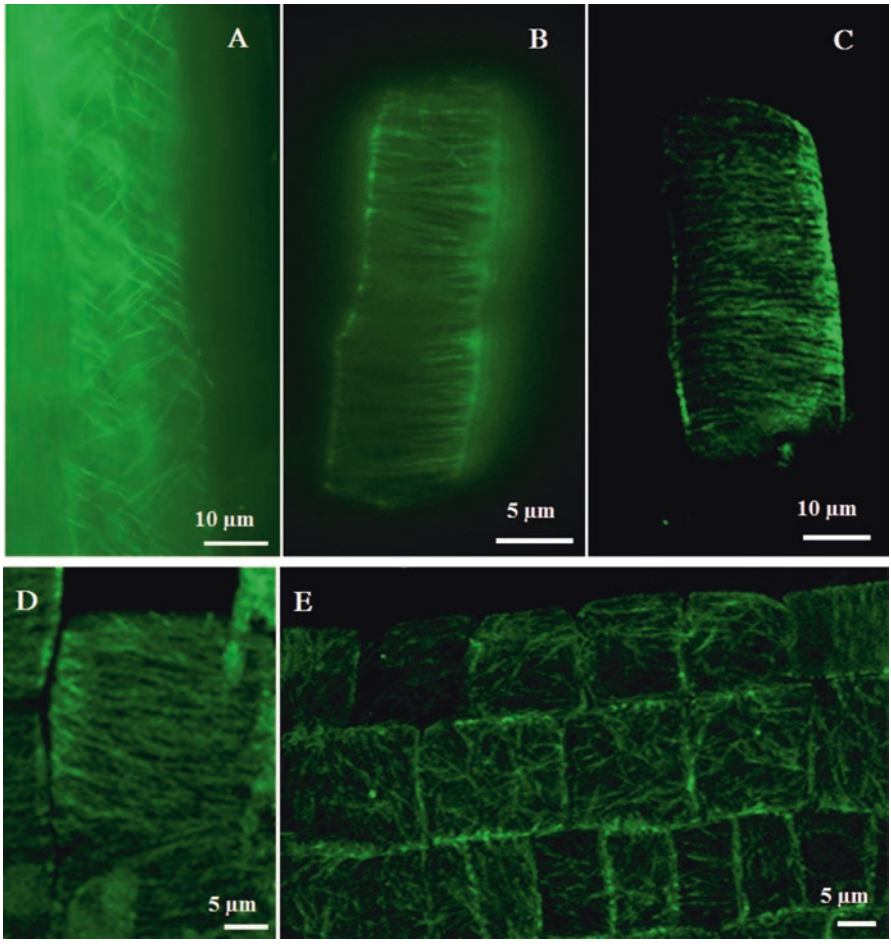

Fig. 17.3 Immunofluorescence of plant microtubules under fluorescence (**a**, **b**) or confocal microscopy (**c**–**e**; Z-stack images). Images (**a**, **d**) show obliquely oriented microtubules at transition zone. Images (**b**, **c**) show single cells with microtubules oriented 90° respect to the cell elongation plane. Image (**e**) shows erratically organized microtubules after farnesene treatment, a sesquiterpene known to cause microtubular alterations. (Araniti et al. 2016)

10. Wash plant material in PBS buffer.
11. Incubate with 1% BSA (bovine serum albumin) and 50 mM glycine in PBS buffer for 20 min.
12. Wash in PBS buffer.
13. Incubate overnight with the primary antibody (Sigma B512 anti-α tubulin; 1:1000 prepared in PBS buffer).

Day 2

14. Rinse three times with PBS buffer to remove properly the primary antibody. This prevents false positives.
15. Incubate samples with the secondary antibody (Alexa 488-conjugated goat anti-mouse IgG; 1:200 in PBS buffer) at 37 °C for 3 h.
16. Mount samples in Citifluor AF1 antifade agent to protect samples from the incident light, and seal covers to slides with nail polish.
17. Generate excitation at 488 nm and collect emission at 515 nm.

4.1 Tricks and Recommendations

- MSB buffer, without Triton, can be prepared in advanced and stored at 4 °C (Step 1).
- It is especially important that Step 1 is carried out at room temperature (including working buffers), since microtubules depolymerize in cold and nothing would be seen in the preparation.
- In order to facilitate samples management, steps 1–3 must be done in 2 mL eppendorfs; step 4 in 9 cm diameter Petri dishes; and steps 5–15 in six-well plates, using one well per step and treatment.
- Successive washing steps cause material loss in a very easy way. I strongly recommend collecting the chopped plant material using filters typical for cell and/or nuclei isolation protocols (Steps 5–15, see Fig. 17.2).
- If the immunostaining fails, I recommend increasing the amount of cellulose, since antibodies cannot access into the cell when cellulose microfibrils are very tight.

5 Visualization of Microtubules by TEM

The visualization of MTs by Transmission Electron Microscopy (TEM) gives additional information about microtubule arrangement; such as distance between microtubules, distance between microtubules and the cell wall, or number of microtubule clusters. Any variation of these parameters could be indicating abnormalities on microtubule organization due to plant stress (Araniti et al. 2016).

TEM visualization is especially useful for studying individual MTs, but also when other cellular structures need to also be visualized (Celler et al. 2016), which is specially interesting when the plant response to biotic and/or abiotic stress is being studied. So we will be able to see whether effects on MTs are also related to effects on cell wall disposition, presence of multi-nucleated cells, nuclei morphology or tissue organization (Araniti et al. 2016). Moreover, the high resolution of this technique allows to have a static picture of microtubule arrangement but gives also

essential information about the presence of cross-linking proteins or about the interaction of MTs with other cellular structures and endomembrane components in roots under stress conditions (Celler et al. 2016).

5.1 TEM Procedure

Microtubules visualization is conducted according to Holzinger et al. (2007) with approximately 25–50 root tips per sample. This protocol has been improved together with the members of the Central Research Services of the University of Vigo (CACTI).

1. Cut the tissue in 1–2 mm
When working with thin root tips, cuts are done with a sharp blade on agar or a wax plate directly in the fixative to avoid further damages to the roots
2. Fix the tissue in 50 mM sodium cacodylate buffer (pH 7.0) containing 2.5% glutaraldehyde fixative at room temperature for 2 h
3. Wash 2 times (1 h each) with 50 mM cacodylate buffer (pH 7.0) at 4 °C
4. Immerse samples in 50 mM sodium cacodylate buffer (pH 7.0) with 1% osmium tetroxide at 4 °C for 12 h
5. Wash 2 times (1 h each) with 50 mM cacodylate buffer (pH 7.0) at 4 °C
6. Perform sample dehydration in increasing ethanol dilutions (at 4 °C): 10%, 20%, 40%, 60%[a], 80%, 90%, 95%, 100%, each for 20 min, except the last one for 40 min; and lately in propylene oxide (2×15 min)
7. Infiltrate the sample in Spurr's resin at 4 °C as follows:
Spurr: propylene oxide (1:3 v/v) (3×2 h)
Spurr: propylene oxide (1:1 v/v) (3×2 h)[a]
Spurr: propylene oxide (3:1 v/v) (2×2 h plus 1×3 h)
8. Embed the sample in 100% Spurr's resin for 2×3 h and left it overnight at room temperature
9. Embed the sample again in 100% resin (2×3 h)
10. Place the sample in molds with pure resin to allow polymerization at 60 °C for 2–3 days
11. Prepare semithin sections (0.7 μm) for light microscopy and ultrathin sections (50–70 nm) for electron microscopy
12. Contrast the sections as follows:
Uranyl acetate for 30 min
Wash with Milli-Q water for 2 min
Lead citrate for 12 min
Wash with Milli-Q water for 2 min
13. Assemble ultrathin sections on copper grids of 100/200 mesh and examine by TEM using a JEOL JEM-1010 transmission electron microscope (at 100 kV) (Peabody, MA, USA) equipped with a CCD Orius-Digital Montage Plug-in camera (Gatan Inc., Gatan, CA, USA) and Gatan Digital Micrograph software (Gatan Inc.)

[a]It can stay overnight

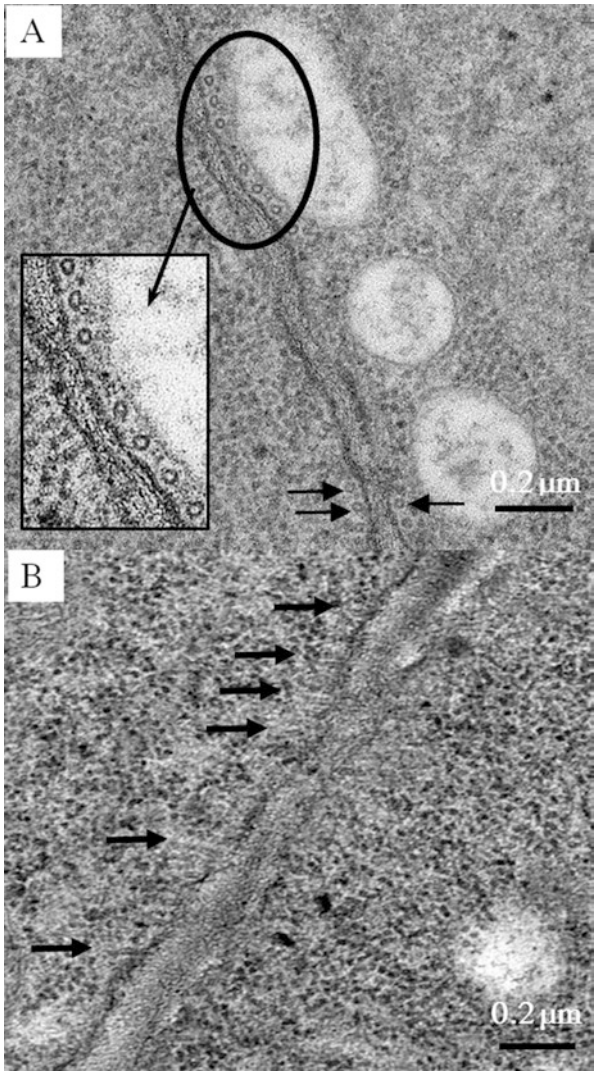

Fig. 17.4 Images show microtubules in (**a**) transversal section; and (**b**) longitudinal section. Arrows indicate microtubule localization

In so prepared ultra-thin sections, microtubules can be seen by TEM as small circles, aligned close to the cell wall in transversal sections (Fig. 17.4a) or as straight sticks in longitudinal sections (Fig. 17.4b).

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References

Alberts B (2008) Molecular biology of the cell, 5th edn. Garland Science, New York

Araniti F, Graña E, Krasuska U, Bogatek R, Reigosa MJ, Abenavoli MR, Sánchez-Moreiras AM (2016) Loss of gravitropism in farnesene-treated *Arabidopsis* is due to microtubule malformations related to hormonal and ROS unbalance. PLoS One 11(8):e0160202

Bartels PG, Hilton JL (1973) Comparison of trifularin, oryzalin, pronamide, prophan and colchicines treatments on microtubules. Pestic Biochem Physiol 3:462–472

Bhaskara GB, Wen T-N, Nguyen TT, Versules PE (2016) Protein phosphatase 2Cs and microtubule-associated stress protein 1 control microtubule stability, plant growth, and drought response. Plant Cell 29:169–191

Celler K, Fujita M, Kawamura E, Ambrose C, Herburger K, Holzinger A, Wasteneys GO (2016) Microtubules in plant cells: strategies and methods for immunofluorescence, transmission electron microscopy and live cell imaging. In: Gavin RH (ed) Cytoskeleton: methods and protocols, methods in molecular biology, vol 1365. pp 155–184

Chen X, Grandont L, Li H, Hauschild R, Paque S, Abuzeineh A, Rakusová H, Benkova E, Perrot-Rechenmann C, Friml J (2014) Inhibition of cell expansion by rapid ABP-1 mediated auxin effect on microtubules. Nature 516:90–93

Collings DA, Wasteneys GO (2005) Actin microfilament and microtubule distribution in the expanding root of *Arabidopsis thaliana*. Can J Bot 83:579–590

Dayan FE, Hernandez A, Allen SN, Moraes RM, Vroman JA, Avery MA, Duke SO (1999) Comparative phytotoxicity of artemisin and several sesquiterpene analogues. Phytochemistry 50(607):614

Dayan FE, Duke SO, Grossmann K (2010) Herbicides as probes in plant biology. Weed Sci 58(3):340–350

Donhauser ZJ, Jobs WB, Binka EC (2010) Mechanics of microtubules: effects of protofilament orientation. Biophys J 99(5):1668–1675

Funada R (2008) Microtubules and the control of wood formation. In: Nick P (ed) Plant microtubules. Plant cell monographs, vol 11. Springer, Berlin, pp 83–119

Gao Y, Valnberg IE, Chow RL, Cowan NJ (1993) Two cofactors and cytoplasmic chaperonin are required for the folding of α- and β-tubulin. Mol Cell Biol 13:2478–2485

Goddard RH, Wick SM, Silflow CD, Snustad DP (1994) Microtubule components of the plant cell cytoskeleton. Plant Physiol 104:1–6

Guo L, Ho CK, Kong Z, Lee YR, Qian Q, Liu B (2009) Evaluating the microtubule cytoskeleton and its interacting proteins in monocots by mining the rice genome. Ann Bot 103:387–402

Holzinger A, Wasteneys G, Lütz C (2007) Investigating cytoskeletal function in chloroplast protrusion formation in the arctic-alpine plant *Oxyria digyna*. Plant Biol 9(3):400–410

Holzinger A, Kawamura E, Wasteneys GO (2009) Strategies for imaging microtubules in plant cells. Methods Mol Biol 586:243–262

Hyams JS, Lloyd CW (1994) Microtubules. Wiley, New York

Jordan A, Hadfield JA, Lawrence NJ, McGown AT (1998) Tubulin as a target for anticancer drugs: agents which interact with the mitotic spindle. Med Res Rev 18:259–296

Kwiatkowska D (2006) Flower primordium formation at the *Arabidopsis* shoot apex: quantitative analysis of surface geometry and growth. J Exp Bot 57:571–580

Landrein B, Hamant O (2013) How mechanical stress controls microtubule behavior and morphogenesis in plants: history, experiments and revisited theories. Plant J 75:324–338

Ledbetter MC, Porter KR (1963) A 'microtubule' in plant cell fine structure. J Cell Biol 19:239–250

Lehnen LP Jr, Vaughn KC (1991) Immunofluorescence and electron microscopic investigations of the effects of ditiopyr on onion root tips. Pestic Biochem Physiol 40(1):58–67

Lehnen LP Jr, Vaughan MA, Vaughn KC (1990) Terbutol affects spindle microtubule organizing centers. J Exp Bot 41(5):537–546

Liu B, Joshi HC, Wilson TJ, Silflow CD, Palevitz BA, Snustad DP (1994) γ-tubulin in *Arabidopsis*: gene sequence, immunoblot, and immunofluorescence studies. Plant Cell 6:303–314

Mao T, Jin L, Li H, Liu B, Yuan M (2005) Two microtubule-associated proteins of the *Arabidopsis* MAP65 family function differently on microtubules. Plant Physiol 138:654–662

Marc J (1997) Microtubule-organizing center in plants. Trends Plant Sci 2(6):223–230

Mei Y, Gao HB, Yuan M, Xue HW (2012) The *Arabidopsis* ARCP protein, CSI1, which is required for microtubule stability, is necessary for root and anther development. Plant Cell 24:1066–1080

Mitchison TJ, Kirschner MW (1984) Dynamic instability of microtubule growth. Nature 312:237–242

Nakamura M, Naoi K, Shoji T, Hashimoto T (2004) Low concentrations of propyzamide and oryzalin alter microtubule dynamics in *Arabidopsis* epidermal cells. Plant Cell Physiol 45(9):1330–1334

Nick P (2008a) Control of cell axis. In: Nick P (ed) Plant microtubules. Plant cell monographs, vol 11. Springer, Berlin, pp 3–46

Nick P (2008b) Microtubules as sensors for abiotic stimuli. In: Nick P (ed) Plant microtubules. Plant cell monographs, vol 11. Springer, Berlin, pp 175–203

Nick P (2013) Microtubules, signalling and abiotic stress. Plant J 75:309–323

Oliva A, Moraes RM, Watson SB, Duke SO, Dayan FE (2002) Aryltetralin lignans inhibit plant growth by affecting the formation of mitotic microtubular organizing centers. Pestic Biochem Physiol 72:45–54

Panteris E, Adamakis I-DS, Daras G, Hatzopoulos P, Rigas S (2013) Differential responsiveness of cortical microtubule orientation to suppression of cell expansion among the developmental zones of *Arabidopsis thaliana* root apex. PLoS One 8(12):e 82442

Reddy AS (2001) Molecular motors and their functions in plants. Int Rev Cytol 204:97–178

Senseman SA (2007) In: Senseman SA (ed) Herbicide handbook, 9th edn. Weed Science Society of America, Lawrence

Vaughn KC (2006) The abnormal cell plates formed after microtubule disrupter herbicide treatment are enriched in callose. Pestic Biochem Physiol 84:63–71

Vaughn KC, Harper JDI (1998) Microtubule organizing centers and nucleating sites in land plants. Int Rev Cytol 181:75–149

Vaughn KC, Vaughan MA (1988) Mitotic disrupters from higher plants. In: Cutler HG (ed) Biologically active natural products: potential use in agriculture. ACS symposium series 380, Washington, DC. pp 273–293

Vaughn KC, Marks MD, Weeks DP (1987) A dinitroaniline-resistant mutant of *Eleusine indica* exhibits cross-resistance and supersensitivity to antimicrotubule herbicides and drugs. Plant Physiol 83:956–964

Wang SH, Kurepa J, Hashimoto T, Smalle JA (2011) Salt stress-induced disassembly of *Arabidopsis* cortical microtubule arrays involves 26S proteasome dependent degradation of SPIRAL1. Plant Cell 23:3412–3427

Wasteneys GO (2004) Progress in understanding the role of microtubules in plant cells. Curr Opin Plant Biol 7(6):651–660

Yuan M, Shaw PJ, Warn RM, Lloyd CW (1994) Dynamic reorientation of cortical microtubules, from transverse to longitudinal, in living plant cells. Proc Natl Acad Sci U S A 91:6050–6053