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Review

Microtubule dynamics in plants

Valya N. Vassileva[1], Yuka Fujii[2], Robert W. Ridge[2]*

[1] Academic Metodi Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Academic Georgi Bonchev Street, Block 21, 1113 Sofia, Bulgaria; [2] Biology Department, Division of Natural Sciences, International Christian University, Mitaka, Tokyo 181-8585, Japan
* E-mail: rwr@icu.ac.jp

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Abstract Plant cells form a highly dynamic microtubule network organized into different arrays that are essential for many important cellular activities. A key feature of microtubules is their dynamic behaviour in which individual microtubules stochastically switch between periods of growth and disassembly. This feature contributes substantially to the rapid reorganization of the microtubule system in response to environmental and developmental stimuli. In this review we highlight recent developments on the dynamic behaviour of microtubules in plant cells and discuss some of the mechanisms responsible for the regulation of microtubule turnover. We also include an outline of the contribution of the dynamics toward the overall arrangement of the plant microtubule network. Special attention is given to the spatiotemporal changes in the microtubule configuration and dynamics during the establishment of the relationships between plants and other organisms.

Key words: Microtubules, mycorrhizal fungi, pathogenesis, symbiosis.

Microtubules are polarized polymers of α- and β-tubulin heterodimers, which are involved in the control of various aspects of plant morphogenesis and development, including cell division, intracellular transport, organization and motility. Plant microtubules are arranged into four different basic structures—the cortical interphase array, the preprophase band (PPB), the phragmoplast, and the mitotic spindle; the first three are unique to plant cells. In addition, some interphase plant cells possess endoplasmic microtubules (Lloyd et al. 1987; Sieberer et al. 2002). Each of these structures has its own function that varies according to the particular stage of the cell cycle (Williamson 1991; Goddard et al. 1994; Dhonukshe et al. 2005).

Microtubule polymerization and disassembly dynamics

As in other eukaryotes, the specific functions of microtubules in plant cells are highly dependent on their dynamic behaviour, characterized by periods of growth by polymerization and shrinkage by depolymerization with stochastic transitions between these two phases. The transition from growth to disassembly phase is termed a 'catastrophe', and the transition from disassembly to growth phase, a 'rescue' (Walker et al. 1988). This complicated behaviour of microtubules in living cells, known as dynamic instability (first described by Mitchison and Kirschner, 1984), is believed to be a function of the hydrolysis of GTP bound to β-tubulin. During microtubule polymerization, GTP-tubulin subunits add to the growing microtubule end with a following hydrolysis to GDP. As a result, the tip of the polymer only remains in GTP form, forming a stable (stabilising) GTP cap; the microtubule body is composed of GDP-tubulin. If hydrolysis exceeds the addition of GTP-tubulin, the microtubule loses its GTP-cap, undergoes a catastrophe and quickly depolymerizes (Walker et al. 1989). The relative rates of polymerization and depolymerization, combined with the catastrophe and rescue frequency, are termed basic dynamic instability parameters, and determine the nature of the microtubule network in the cell (Chan et al. 2003; Dhonukshe and Gadella 2003; Vos et al. 2003).

Another key feature of microtubules is their intrinsic structural polarity that arises from the head-to-tail association of α/β tubulin heterodimers in the microtubule lattice. As a consequence of this polarity, the rate at which polymerization and disassembly takes place at opposite microtubule ends is different. The end that grows and depolymerizes faster is known as the plus end, and the opposite more stable end is called the minus end (Figure 1). In most eukaryotes, the minus end is usually anchored at the microtubule-organizing centre (MTOC) where nucleation occurs (Figure 1, asterisks), whereas the plus end is free and displays dynamic

Abbreviations: MAPs, microtubule-associated proteins; MTOC, microtubule-organizing centre; PPB, preprophase band.

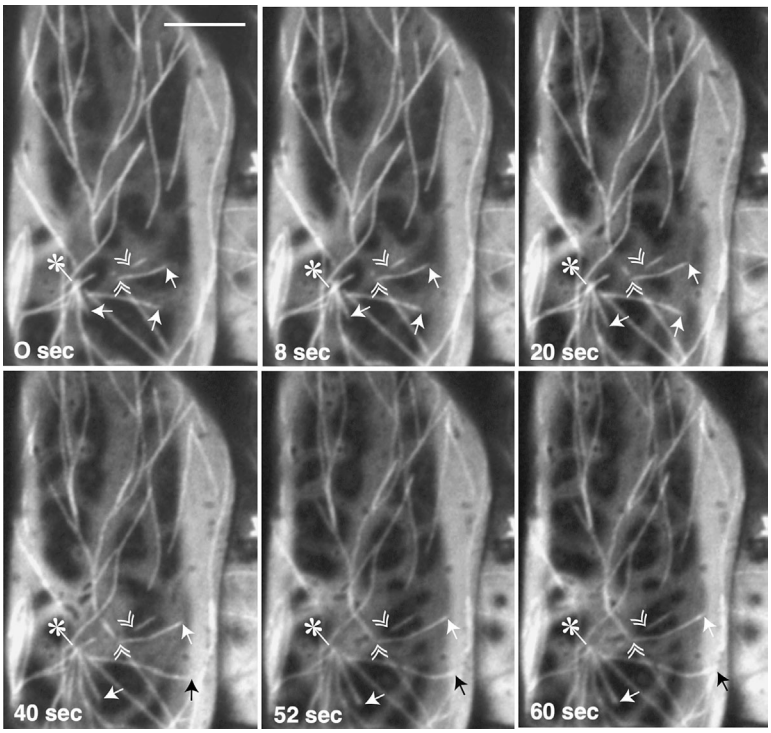

Figure 1. Time series of *in vivo* dynamic instability behaviour of cortical microtubules in root hairs of *Lotus japonicus*. Confocal laser images. Asterisks points to the site of microtubule nucleation, arrows—to growing plus ends of microtubules, double arrowheads show stable minus ends. Bar=3 μm. Unpublished data.

instability behaviour (Wade and Hyman 1997; Erhardt et al. 2002; Schmit 2002). This dynamic model was recently observed in root hairs of transgenic lines of *Lotus japonicus*, where the majority of the microtubules displayed stable minus ends and only plus polymer ends contributed to overall microtubule dynamicity (Figure 1; Vassileva et al. 2005). If the minus end is not anchored at the MTOC, the both microtubule ends exhibit growing and shortening events, a behaviour termed 'treadmilling' (originally termed by Margolis and Wilson, 1978). Treadmilling microtubules typically show addition of subunits to their plus end and loss of subunits from the opposite minus end. Unlike animal cells, higher plant cells do not possess centriole-based microtubule organizing centres; rather, microtubule nucleation in plant cells is related to the nuclear surface and multiple sites distributed throughout the cortex (Schmit 2002; Shaw et al. 2003; Dixit and Cyr 2004a). Shaw et al. (2003) have revealed that cortical microtubules in *Arabidopsis* plants are initiated at the cell cortex, after which they detach from the nucleation sites and undergo slow depolymerization at their minus ends. At the same time, the plus ends are dynamic and exhibit periods of growth and disassembly, resulting in sustained migration across the cortex by a 'hybrid treadmilling' mechanism. The coexistence of dynamic instability and treadmilling behaviour is also observed for microtubules in *Arabidopsis* root hairs, whose plus ends exhibit dynamic instability behaviour, while the opposite minus ends are stable or show slow depolymerization (Van Bruaene et al. 2004).

Early investigations of the mechanisms of microtubule dynamics through traditional immuno-cytochemical methods provide limited information, as they were performed on fixed cells and only static localization of the protein could obtained (Lloyd 1987; Goddard et al. 1994). Further experiments by microinjection of tubulin conjugated with fluorescent dyes, such as rhodamine or fluorescein, has allowed kinetic analysis of the microtubule network for up to several hours (Wymer et al. 1997; Himmelspach et al. 1999). The employment of GFP as a marker for exogenous gene expression has led to real-time observations of the dynamic behaviour of the plant cell cytoskeleton and has provided a powerful tool for a better understanding of its mechanisms (Haseloff and Amos 1995; Haseloff et al. 1997).

Generally, the dynamic organization of microtubules is achieved by polymerization of new microtubules, polymerization of existing microtubules to a new direction, microtubule reorientation, transport of microtubules to another position, and axial and lateral sliding of microtubules (Chan et al. 2003; Dhonukshe and Gadella 2003; Shaw et al. 2003; Vos et al. 2003). According to Shaw et al. (2003) the apparent microtubule dynamicity in *Arabidopsis* is a result mainly from polymerization and depolymerization at the

microtubule ends and not from translocation of the intact microtubule polymer. Changing a particular parameter of individual microtubule dynamic instability drives the turnover kinetics of the microtubule population, and determines polymer increase or loss. Based on analysis of the basic parameters of dynamic instability obtained for microtubules in *Arabidopsis* hypocotyl cells, Shaw et al. (2003) measured an increase in the total polymer level. Other quantitative *in vivo* studies of plant microtubule dynamics displayed different dynamic instability parameters of microtubules during the cell cycle. Over the transition from interphase to PPB formation, the microtubule growth rate and catastrophe frequency doubles, but the shrinkage rate and rescue frequency remains constant, which makes microtubules shorter and more dynamic (Dhonukshe and Gadella 2003). Similarly, Vos et al. (2003) observed an increase of microtubule dynamics during PPB formation, related to the higher growth rates and higher frequency of catastrophe and rescue events. These changes, together with the relatively low microtubule dynamics in PPB (Hush et al. 1994), could explain the accumulation of microtubules to the forming PPB and their disappearance from the cortex by a "search-and-capture" mechanism (Vos et al. 2003). This mechanism allows translocation of the highly dynamic microtubule population to the forming PPB, and its possible stabilization through bundling or cross-linking of microtubules. Thus, the dynamic instability parameters of plant microtubules are important determinants of the transition of interphase microtubules to the PPB.

Self-organization of microtubules and regulation of microtubule dynamics

The stochastic dynamics of microtubules can be modified and regulated at many levels, by which their specific arrangement into ordered arrays is achieved. The self-organization processes in the microtubule population might be partly responsible for some modifications of its stochastic behaviour (Hashimoto 2003). Recent studies by Dixit and Cyr (2004b) demonstrated that dynamic turnover of cortical microtubules is modified by intermicrotubule interactions that occur when the growing microtubule plus ends encounter previously existing cortical microtubules. The outcome of these interactions depends on the angle at which microtubules encounter each other. Specifically, shallow-angle encounters promote microtubule coalignment and bundling, whereas steep-angle encounters promote microtubule disassembly. Based on these results, the authors propose that these simple intermicrotubule interactions define to a high extent the emergence of coaligned microtubule groups from a randomly arranged microtubule population.

In the direct regulation of cellular microtubule dynamics, a variety of proteins termed microtubule-associated proteins (MAPs) (Hashimoto 2003; Van Damme et al. 2004) and their regulatory kinases and phosphatases (Sedbrook 2004) are involved. They usually bind to the microtubule lattice and influence microtubule behaviour during the cell cycle. Some of them have been identified to increase microtubule polymerization and stability by promoting bundling and cross-linking adjacent microtubules (Chan et al. 1999; Whittington et al. 2001; Yasuhara et al. 2002; Smertenko et al. 2004; Van Damme et al. 2004; Wicker-Planquart et al. 2004). This provokes a decrease in the frequency of microtubule catastrophe events (Caudron et al. 2000). Other MAPs function to destabilize and trigger the reorganization of microtubules (Dhonukshe et al. 2003; Gardiner et al. 2003). Thus, MAPs interact with microtubules, thereby affecting their dynamics, which leads to the control of microtubule length during different stages of the cell cycle (Hirokawa 1994; Desai and Mitchison 1997). In most cases, this is governed by the phosphorylation of MAPs that alters their affinity for microtubules. Phosphorylated MAPs are unable to bind to microtubules, which promotes microtubule disassembly (Hush et al. 1996). Treatment with inhibitors of protein phosphatases and kinases disorganizes cortical microtubules (Mizuno 1994; Baskin and Wilson 1997).

A wide range of environmental and developmental factors are known to affect the dynamics and architecture of microtubules in the cell (Desai and Mitchison 1997; Takemoto and Hardham 2004). Dynamic instability behaviour enables microtubules to be highly sensitive to light, gravity, exogenous forces, hormones, and toxicity (Williamson 1991; Ishida and Katsumi 1992; Nick 1998; Blancaflor 2002; Poschenrieder et al. 2004). Observations of gravity-induced changes of microtubules in epidermal cells of maize coleoptiles, injected with rhodamine-labeled tubulin, indicate that microtubules reorient from transverse to longitudinal by passing through a random phase before they align into increasingly parallel arrays (Himmelspach et al. 1999; Lloyd et al. 2000). From their *in vivo* studies, the authors conclude that the emergence of random microtubule arrays is a general feature of transition from transverse to longitudinal. Similar dynamic responses of microtubules to gravitropic stimulus were observed in hypocotyl cells of transgenic lines of *L. japonicus* stably expressing a fusion of green fluorescent protein and tubulin-α 6 (GFP-TUA6) from *A. thaliana* (Ueda et al. 1999) (see Figure 2A–K, unpublished data). Analysis of microtubule dynamics in GFP-TUA6-labeled *Lotus* hypocotyl cells over a period of 10 h showed that horizontal placement of the hypocotyl triggered the reorientation of cortical microtubules from randomly

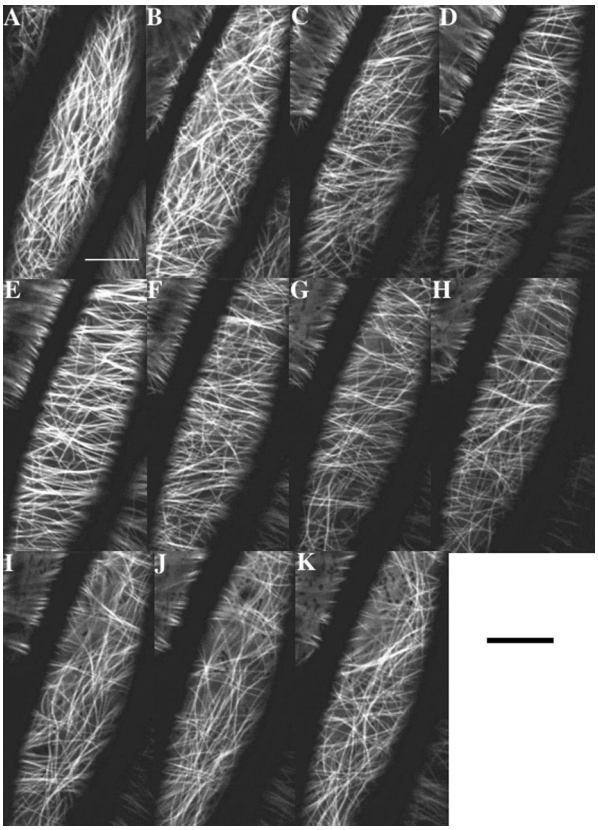

Figure 2. Gravity-induced microtubule reorientation in living *Lotus japonicus* hypocotyl cells. The plant was placed at 90 degrees to the gravity vector on a microscope stage. Confocal laser images were taken of the same hypocotyl cell at one hour intervals (A = 0 h). The majority of microtubules had clearly moved to transverse orientation by 3–4 h after induction (D, E), but gradually returned to random orientation by 9–10 h (J, K). Bar = 20 μm. Unpublished data.

organized patterns (Figure 2A, B) through a transitional stage with partly transverse (Figure 2C, D) to dominant transverse orientation by 4 h (Figure 2E and 2F), then microtubules began to reorient back toward transitional stage Figure 2G, H) to randomness Figure 2I–K). Two models have been proposed to explain how microtubules perceive and rearrange in response to gravity change (Himmelspach et al. 1999). The first is related to movement or sliding of individual microtubules, the second one includes selective stabilization or disassembly of microtubules based on the high microtubule dynamics. The overall reorientation process could include both models. Microtubule dynamic rearrangement during gravistimulation could merely be a response to the mechanical strain of bending (Hejnowicz et al. 2000). However, a study by Himmelspach and Nick (2001) revealed that gravity-induced microtubule reorientation in maize coleoptiles can be uncoupled from gravity bending. Preventing the upward bending and growth of coleoptiles by attaching them to microscope slides showed that growth inhibition without gravity stimulus does not provoke microtubules to reorient. Therefore, microtubules could directly respond to gravity, and reorientation during gravity change is not simply a consequence of mechanical bending; microtubules are intracellular gravity sensors and transducers.

Microtubule dynamics in plant-microbe interactions—pathogenesis and symbiosis

The microtubular cytoskeleton is believed to play a prominent role in signal transduction and regulation of the relationships between plants and other organisms that could be potential pathogens or partners (Timmers et al. 1998; Blancaflor et al. 2001; Takemoto et al. 2003; Takemoto and Hardham 2004; Vassileva et al. 2005; Weerasinghe et al. 2005). The spatiotemporal changes in plant microtubule configuration and dynamics during fungal and oomycete pathogen penetration and during the establishment of mutualistic associations with mycorrhizal fungi and rhizobia are very complex and variable.

Interactions with fungal and oomycete pathogens

The response of the microtubule cytoskeleton to pathogen attack varies depending on the interacting species. Reorientation and loss of microtubules is observed in the early stages of the incompatible interaction of soybean hypocotyls with the specific pathogen *Phytophthora sojae*, but not with compatible interactions (Cahill et al. 2002). Moreover, treatment of hypocotyls with the microtubule depolymerizing agent oryzalin prior to inoculation does not alter the compatible response, but leads to breakdown of the incompatible response. To answer the question if there is a relationship between microtubule configuration and oomycete pathogen infection, Takemoto et al. (2003) visualized epidermal cell structure after attack by nonhost, incompatible and compatible oomycete pathogens in cotyledons of GFP-TUA6 labeled *Arabidopsis* plants. In some epidermal cells there is no visible change in microtubule organization, but in others, microtubules show a tendency for circumferential alignment around the penetration site. The most obvious change for the all three pathogen interactions is the appearance of strong but diffuse fluorescence around the site of infection. This type of fluorescence lasts throughout the whole period of observation (up to 36 h after inoculation) and could be explained by the presence of monomeric or dimeric GFP-tubulin forms. The accumulation of the GFP-tubulin subunits at the penetration site may due to increased tubulin synthesis, or to selective microtubule depolymerization. A number of studies have suggested that microtubule

depolymerization provides a mechanism for the mobilization of the plant defense response against pathogen attacks (Kobayashi et al. 1994; Kobayashi et al. 1997; Binet et al. 2001). Although the precise biological significance of microtubule remodelling during pathogen infection is still poorly understood, the variable behaviour of microtubules during pathogen infection implies that the microtubule network is not directly involved in the defense response of plants.

Interactions with mycorrhizal fungi and symbiotic bacteria

Despite the miscellaneous information available on the plant microtubule behaviour during the establishment and progression of various mycorrhizal associations, there is no doubt that the microtubule cytoskeleton of both plant and fungal partners undergo dynamic changes (Matsubara et al. 1999; Timonen and Peterson 2002). The different categories of mycorrhizas are based primarily on the fungal partner in the association and the types of mycorrhizal structures formed. An important feature of mycorrhizas is the interface involved in nutrient exchange between the symbionts (Peterson and Massicotte 2004). The development of exchange interfaces in ectomycorrhizas, ectendomycorrhizas, and monotropoid mycorrhizas involve dramatic changes in the organization of microtubules (Kuga-Uetake et al. 2004). In arbuscular and orchid mycorrhizae, plant microtubules and actin microfilaments depolymerize almost completely in the plant cell cortex, and polymerize close to the surface of the hyphal masses, trunk hyphae or arbuscules (Matsubara et al. 1999; Blancaflor et al. 2001; Armstrong and Peterson 2002; Takemoto and Hardham 2004). Genre and Bonfante (1998) have hypothesised that cytoskeleton reorganisation during the symbiosis may influence the nutrient exchanges between symbiotic partners by controlling the transport mechanisms related to membrane repositioning and cell wall deposition. The study by Ditengou et al. (2003) shows that hypaphorine, the major indolic alkaloid compound delivered from ectomycorrhizal fungus *Pisolithus tinctorius* upon formation of mycorrhiza with *Eucalyptus* roots, significantly affects microtubule arrangement in the elongated root hairs of the host plant. In the subapical region of hypaphorine-treated root hairs, a highly fluorescent patch of aggregated microtubules usually appears, possibly as a consequence of bundling of the actin filaments. It is proposed that this hypaphorine-induced aggregation of microtubules and actin filaments could stabilize the structure of cytoskeletal elements, which may hinder the vesicle delivery and release in root hairs necessary for their elongation. Since hypaphorine is an IAA antagonist, and restoration of hypaphorine-induced aggregation of actin filament can be obtained by exogenous IAA application, it is suggested that endogenous IAA is necessary to maintain normal arrangement of cytoskeletal elements in elongating root hairs. Exogenous auxin is also known to stimulate root hair elongation (Katsumi et al. 2000). On the other hand, the study by Sampson and Heath (2005), where microtubule dynamics and subunit distribution were analysed in a strain of *Aspergillus nidulans* expressing GFPα-tubulin, suggest substantial independence between microtubules and hyphal tip growth. It was proposed that the population of dynamic apical microtubules enhance migration of the 'cytomatrix', thus ensuring that organelles and proteins maintain proximity to the constantly elongating hyphal tip (Sampson and Heath 2005).

Some reports show that in many ecto- and endomycorrhizal symbioses the level of plant tubulin expression and the contribution of plant α-, β-, and γ-tubulins to the total protein pool are higher compared to those in uncolonized systems (Bonfante et al. 1996; Timonen et al. 1996; Timonen and Peterson 2002; Timonen and Smith 2005). The gene from *Eucalyptus globulus*, encoding an α-tubulin (*EgTubA1*), shows enhanced expression under ectomycorrhiza colonization (Carnero Diaz et al. 1996). It was also observed that in transgenic tobacco roots, the promoter of the maize α-tubulin gene *Tub3a* is specifically activated in mycorrhizal cells (Bonfante et al. 1996). However, whether the observed changes in microtubular gene expression are a cause or a consequence of mycorrhizal root morphogenesis still remains to be established.

Using a γ-tubulin antibody, Genre and Bonfante (1999) have revealed MTOCs along the nuclear envelope and along the host membrane that surrounds the plant/fungus interface (perifungal membrane) in tobacco arbuscular mycorrhizas. The γ-tubulin is a microtubule-nucleating protein localized basically in MTOCs (Shimamura et al. 2004). Its presence at the perifungal membrane implies microtubule nucleation there.

During the establishment of the symbiotic association of legume plants with rhizobia, the microtubule cytoskeleton assists in the infection process, as well as in the differentiation and maintenance of root nodules (Ridge 1992; Timmers et al. 1998; Timmers et al. 1999; Catoira et al. 2001; Weerasinghe et al. 2003; Weerasinghe et al. 2005). Generally, microtubules are abundant at the site of infection thread initiation and surround the infection thread during its development (Ridge and Rolfe 1985). They connect the host cell nucleus to both the root hair tip and the growing infection thread tip and maintain it in a subapical position (Timmers et al. 1999; Sieberer et al. 2002; Takemoto and Hardham 2004). An immunochemistry study showed that application of *Rhizobium meliloti* Nod

factors caused rapid and dynamic changes in the pattern of microtubules in root hairs of *Medicago sativa* (Weerasinghe et al. 2003). The authors have observed a transient depolymerization of microtubular cytoskeleton and recovery thereafter in response to inoculation with purified *R. meliloti* Nod factors. Newly infected cells of *Pisum sativum* contain randomly oriented fragmented cortical and cytoplasmic microtubules exhibiting diffuse fluorescence (Davidson and Newcomb 2001). When the infected cells are fully developed, an extensive network of long cortical microtubules with random orientation and some parallel bundles is formed. The same authors assume that the cortical microtubules may be responsible for maintaining mitochondria and plastids in the periphery of nodule cells.

A recent study has revealed in details *in vivo* microtubule behaviour during the establishment and progression of the *Rhizobium*-legume symbiosis (Vassileva et al. 2005). Time-lapse confocal imaging of transgenic lines of the model legume *L. japonicus* that stably express a GFP-TUA6, has shown that the introduction of *Mesorhizobium loti* Nod factors directly affects some of the parameters of microtubule dynamic instability in root hairs, and thus, overall microtubule dynamics. In emerging and growing root hairs, the inoculation lowers microtubule polymerization rates by more than 45% and the frequency of rescue events about 60%, so that cortical microtubules become less dynamic at a specific time after symbiotic inoculation. The first visible response to inoculation are root hair deformations that occur mainly in the most infectible zone containing emerging and growing root hairs. Since these deformations usually follow the Nod factor-induced decrease in microtubule dynamics, it is possible that the less dynamic cortical microtubules are prerequisite for the change of the growth polarity of root hairs. The visualization of microtubule arrangement after application of purified *M. loti* Nod factors reveals an increase in the population of the short microtubules with a larger deviation from the main root hair axis compared to the controls (Figure 3). Quantification of the microtubule length and organization at defined distances from the root hair tip shows that in the base part of the growing root hairs, the length of microtubules is reduced significantly and their axial orientation is replaced by random orientation (Vassileva et al. 2005). Weerasinghe et al. (2005) also dynamically profiled alterations in the cytoskeleton of living *L. japonicus* root hairs, which precede their deformation induced by *M. loti* Nod factors. They observed quick disintegration of the fine endoplasmic microtubules, followed by repolymerization from the very tip of the root hair, and increase of their number at 5–10 min. Cortical microtubules show a gradual increase in intensity and become more fragmented.

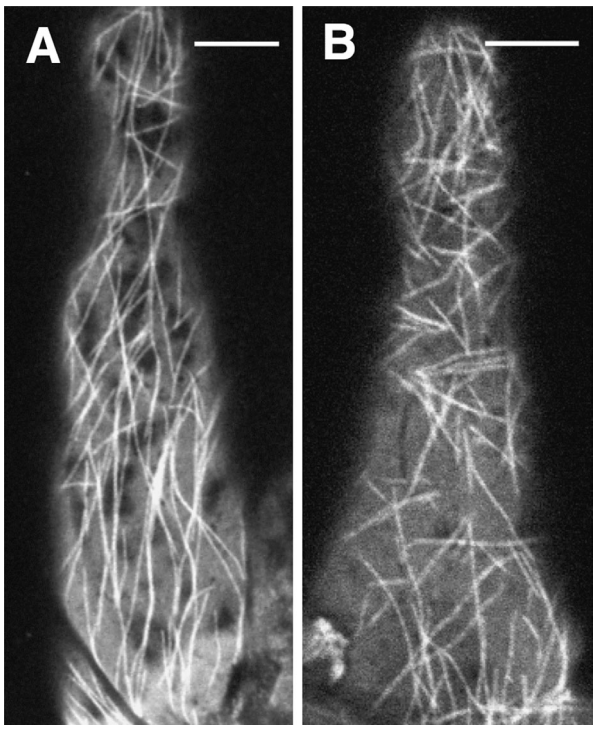

Figure 3. Cortical microtubule arrangement in growing root hairs of *L. japonicus*: A) control root hair cell; B) root hair cell inoculated with *Mezorhizobium loti* Nod factors. Confocal laser images. Bar=8 μm. Unpublished data.

In summary, higher plant cells possess a very dynamic microtubule system that performs a variety of tasks during cell division and differentiation. This system also provides a mechanism by which plant cells can rapidly recognize any intracellular and extracellular signals, and respond to them by quick reorganization. In the last several years it has become clear that the microtubule system together with actin microfilaments are going to be major topics of research in studies of plant signal perception and transduction. Future studies should emphasize the accurate elucidation of the specialized features and functions of microtubule dynamics as a regulatory switch in different cellular processes, and how they themselves are affected by the resulting microtubule dynamics.

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