Tansley review

Microtubules guide root hair tip growth

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Key words: *Arabidopsis thaliana*, cell polarity, cytoskeleton, *Medicago truncatula*, microtubules (MTs), root hair, tip growth.

Summary

The ability to establish cell polarity is crucial to form and function of an individual cell. Polarity underlies critical processes during cell development, such as cell growth, cell division, cell differentiation and cell signalling. Interphase cytoplasmic microtubules in tip-growing fission yeast cells have been shown to play a particularly important role in regulating cell polarity. By placing proteins that serve as spatial cues in the cell cortex of the expanding tip, microtubules determine the site where excocytosis, and therefore growth, takes place. Transport and the targeting of exocytotic vesicles to the very tip depend on the actin cytoskeleton. Recently, endoplasmic microtubules have been identified in tip-growing root hairs, which are an experimental system for plant cell growth. Here, we review the data that demonstrate involvement of microtubules in hair elongation and polarity of the model plants *Medicago truncatula* and *Arabidopsis thaliana*. Differences and similarities between the microtubule organization and function in these two species are discussed and we compare the observations in root hairs with the microtubule-based polarity mechanism in fission yeast.

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I. Introduction

Root hairs are lateral extensions of root epidermal cells. They elongate as cylindrical, uniaxilar structures, which reach an enormous length compared with their width. Root hairs enlarge the surface of the roots, and by this they support the absorption of water and nutrients (for a review, see Gilroy & Jones, 2000). Especially under low nutrient availability, root

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hairs facilitate an increased uptake of nutrients (phosphorus uptake in *Arabidopsis thaliana*: Bates & Lynch, 2000). Further, root hairs may serve in the anchoring of plants into the soil (Peterson & Farquhar, 1996; Gilroy & Jones, 2000), although Bailey *et al.* (2002) have shown in a mutant study that lateral roots, rather than root hairs, are crucial for anchorage. Finally, root hairs are sites for interaction of host plants with a range of soil-living symbiotic microorganisms (Clarkson, 1985; Dolan *et al.*, 1994; Peterson & Farquhar, 1996; Ridge, 1996) such as nitrogen-fixing rhizobacteria that infect legume roots (Mylona *et al.*, 1995; Long, 1996).

The shape of root hairs is produced by a very special form of polarized growth, called tip growth. In tip-growing cells, the actual growth process is restricted to the very tip (Derksen & Emons, 1990; Shaw *et al.*, 2000; Dumais *et al.*, 2004), also known as the apex or apical dome. Tip growth is a precisely orchestrated process characterized by the maintenance of a cylindrical shape with a more or less constant diameter and a uniaxial extension pattern (Shaw *et al.*, 2000). Tip-growing root hairs have a highly polarized cytoarchitecture. The tip growth machinery localizes in abundance in the apex and subapex of an elongating hair and consists of a tip-focused gradient of calcium ions, actin filaments, microtubules (MTs), Golgi-derived and endocytotic vesicles, Golgi bodies, endoplasmic reticulum, mitochondria, the nucleus, and the exo-/endocytosis molecules such as snare proteins and clathrin. The growth mechanism primarily consists of the fusion of vesicle membranes with the plasma membrane and exocytosis of cell wall material at the hair tip (Miller *et al.*, 1997; Wasteneys & Galway, 2003), leading to an irreversible increase in cell surface. The cytoskeleton has been identified to be a keyplayer in the tip growth process.

Comprehensive reviews that emphasize to a large extent the actin cytoskeleton in tip-growing plant cells have recently been published (Geitmann & Emons, 2000; Hepler *et al.*, 2001; Wasteneys & Galway, 2003). Although recent reviews by Grierson & Ketelaar (2004) and Hussey *et al.* (2004) give a good and detailed overview on the MT cytoskeleton in tip-growing plant cells, recent work (*Medicago truncatula*: Vos *et al.*, 2003; Sieberer *et al.*, 2005; *Medicago sativa*: Weerashinge *et al.*, 2003; *Arabidopsis thaliana*: Van Bruaene *et al.*, 2004) allows us now to understand better the role MTs play in tip-growing root hairs. In addition, one may gain insight in MT functioning by studying another tip-growing organism: fission yeast. A MT-based cell polarity mechanism in fission yeast has been identified; here, we will discuss this mechanism and the possibility that MTs perform a homologous function in setting up cell polarity in root hairs.

II. Root hair tip growth

Root hair development exhibits two fascinating aspects of the regulation of positional information during plant cell growth. (1) Before bulge formation starts, the root epidermal cell that will give rise to the root hair must break its symmetric growth pattern and (2) initiate cell growth at a very confined area. Root hairs emerge as bulges from root epidermal cells, the so-called trichoblasts, when intercalary cell elongation of these cells ceases (*Arabidopsis*: Sugimoto *et al.*, 2000; *Medicago*: Sieberer *et al.*, 2002). Unlike *Medicago*, where all epidermal root cells are trichoblasts (Sieberer & Emons, 2000), *Arabidopsis*, in addition to trichoblasts, has atrichoblasts, epidermal cells that do not produce root hairs (Dolan *et al.*, 1994). Studies on the cytoskeleton (actin: Miller *et al.*, 1999; actin and MTs: Baluška *et al.*, 2000), ultrastructure (*Vicia sativa*: Miller *et al.*, 2000) and cytoplasmic calcium gradients in *Arabidopsis* (Wymer *et al.*, 1997) support the conclusion that bulge formation is a distinctly different process than tip growth (for a review, see Grierson & Ketelaar, 2003).

Once tip growth has initiated, a cylindrically shaped tube develops with a more or less constant diameter (*Medicago*: approx. 10–14 μm; *Arabidopsis*: approx. 6–10 μm), which elongates uniaxially, perpendicular to the root axis, until the mature length of the hair has been reached (*Medicago*: up to 1 mm; *Arabidopsis*: can exceed 1 mm). When grown in liquid medium, root hair growth occurs with a growth rate of 0.8–1.4 μm min^{-1} in *Medicago* (Sieberer & Emons, 2000) and 0.93–2 μm min^{-1} in *Arabidopsis* (Wymer *et al.*, 1997; Bibikova *et al.*, 1999; Ketelaar *et al.*, 2002). Recently, Care & Crush (2004) reported pronounced pulsatile tip growth in young *Trifolium repens* root hairs, which is similar to the growth pattern in pollen tubes. Root hair diameter and growth rate rely on the precisely orchestrated exocytosis process of tip growth. This growth pattern reflects the underlying polarity of the cytoarchitecture. The subapex of tip-growing root hairs is filled with dense cytoplasm, whereas the basal part of the hair contains an enlarging vacuole, surrounded by a thin layer of cortical cytoplasm (*Arabidopsis*: Galway *et al.*, 1997; *V. sativa*: De Ruijter *et al.*, 1998; *M. truncatula*: Sieberer & Emons, 2000).

For a better understanding of the role MTs play in cell polarity and tip growth, we give a short description of common and different cytoarchitectural features in elongating root hairs of the model species for root hair growth: the legumes *Medicago* and *Vicia*, and *Arabidopsis*. Common to all tip-growing root hairs is, as stated above, the accumulation of cytoplasm in the subapical region. The subapical cytoplasm contains a high number of organelles such as endoplasmic reticulum, mitochondria, plastids and Golgi bodies (*V. sativa*: Miller *et al.*, 2000). The nucleus is positioned at the base of the subapical, cytoplasmic dense area and follows the growing hair tip at a fixed distance, which is 30–40 μm in *Medicago* (Sieberer & Emons, 2000; Sieberer *et al.*, 2002) and 40–75 μm in *Arabidopsis* (Ketelaar *et al.*, 2002). The cytoplasm in the extreme apex is devoid of large organelles but packed with vesicles, as transmission electron microscopic images reveal (*Equisetum hyemale*: Emons, 1987, *Vicia hirsuta*: Ridge, 1988; Ridge, 1992; *V. villosa*: Sherrier & van den Bosch, 1994, *Arabidopsis*: Galway *et al.*, 1997, *V. sativa*: Miller *et al.*, 2000).

A category of these vesicles are Golgi-derived and contain cell wall matrix material that will be deposited in the expanding cell wall upon exocytosis. Other vesicles are of endocytotic origin (Emons & Traas, 1986). In *Medicago* and *Vicia*, the cytoplasmic dense subapical region is more compact than in *Arabidopsis*: Not only is the tip–nucleus distance shorter, but also the cytoplasm is only penetrated temporarily by thin extensions of the central vacuole, whereas in *Arabidopsis* these extensions are bigger and occupy the subapical region for a longer time. The significance of these differences is not known.

III. Microtubule organization and dynamics in elongating root hairs

During the transition from a bulge into an elongating root hair, the very tip of the bulge progressively loses detectable CMTs (*M. truncatula*: Sieberer *et al.*, 2002). At this developmental stage, cytoplasm is accumulating at the tip of the bulge and forms a short cytoplasmic dense region. Endoplasmic MTs (EMTs) start to appear within this region (*Medicago*: Sieberer *et al.*, 2002; *Arabidopsis*: van Bruaene *et al.*, 2004). An indication that MTs could be involved in the initiation of tip growth comes from transgenic *Arabidopsis* lines with reduced α-tubulin expression (Bao *et al.*, 2001). Occasionally, multiple root hairs emerge from a single swelling in these lines. Although this is likely to be caused by a defective initiation of tip growth, alternatively bulge formation or tip growth itself may be defective, as all these defects could cause identical phenotypes.

Cortical MTs (CMTs) are present in all stages of root hair growth in all species examined (for a review, see Ketelaar & Emons, 2000, 2001). In this respect, root hairs are not different from other interphase plant cells. CMTs appear as dense arrays in growing root hairs and are oriented either net-axially (more or less longitudinally; *E. hyemale*: Emons & Wolters-Arts, 1983; Traas *et al.*, 1985; *Medicago*: Sieberer *et al.*, 2002; *M. sativa*: Weerasinghe *et al.*, 2003; *Arabidopsis*: van Bruaene *et al.*, 2004) or helically (*Allium cepa*: Lloyd, 1983; Traas *et al.*, 1985). Whether or not CMTs reach the very tip – the site of actual cell growth – in elongating root hairs has been under debate for a long time. CMTs have not been detected in root hair apices with immunocytochemistry after chemical fixation (*M. sativa*: Weerasinghe *et al.*, 2003) and freeze fixation/freeze substitution (*M. truncatula*: Sieberer *et al.*, 2002), and have not been detected with *in vivo* decoration by green fluorescent protein (GFP), either fused to the MT binding domain of mouse Microtubule Associated Protein (MAP) 4 (GFP-MBD; Marc *et al.*, 1998) (*M. truncatula*: Sieberer *et al.*, 2002; *Arabidopsis*: van Bruaene *et al.*, 2004) or to α-tubulin 6 (GFP-TUA6) of *Arabidopsis* (*Lotus japonicus*: Vassileva *et al.*, 2005). However, CMTs have been found in the extreme tips of elongating root hairs in transmission electron microscopy (TEM) images after freeze fixation/freeze substitution (*E. hyemale*: Emons, 1989, *Arabidopsis*: Galway *et al.*, 1997). There are several explanations for these contradicting observations, as follows. (1) CMTs are highly dynamic at the very tip and may therefore be difficult to visualize in a still image of a 4D confocal microscopy scan. (2) CMTs may be present in the tip region but not be fully accessible for antibodies or the GFP-fusion proteins because CMTs at this site might be decorated with MAPs. (3) The CMTs in the TEM images may be freeze fixation or freeze substitution artefacts. Even though it seems unlikely, the absence of CMTs in the extreme apex may be explained by the tip-focused Ca^{2+} gradient in growing root hairs (*Arabidopsis*: Schiefelbein *et al.*, 1992; Bibikova *et al.*, 1997; Wymer *et al.*, 1997; *V. sativa*: de Ruijter *et al.*, 1998; *Phaseolus vulgaris*: Cárdenas *et al.*, 1999; *M. sativa*: Felle *et al.*, 1999a,b). MTs are sensitive to elevated Ca^{2+} level concentrations (Cyr, 1991, 1994), and the high Ca^{2+} concentration in the apex of growing root hairs may cause depolymerization or inhibit polymerization. As the organization of putative CMTs in the apex of growing root hairs may give us indications about their function in tip growth, clarification of their organization by improved live-cell imaging techniques and detailed transmission electron microscopic analysis will be of interest.

After growth arrest, root hairs of *Medicago* and *Arabidopsis* show a decrease in CMT density. In fully grown root hairs, CMTs are either longitudinally (*Medicago*: Sieberer *et al.*, 2002; *Arabidopsis*: van Bruaene *et al.*, 2004) or helically (*Arabidopsis*: van Bruaene *et al.*, 2004) oriented and appear to be longer and better aligned than CMTs in growing hairs (Van Bruaene *et al.*, 2004). After growth arrest, CMTs reach the tip and converge there.

EMTs, MTs that are not located in the cell cortex, are atypical for interphase plant cells. However, recently they have been shown to be present in the subapical cytoplasmically dense region in elongating root hairs. Root hairs acquire EMTs when tip growth begins and retain these EMTs until growth stops (*M. truncatula*: Sieberer *et al.*, 2002; *A. thaliana*: van Bruaene *et al.*, 2004). In *Medicago* and *Arabidopsis*, EMTs were observed around and close to the nucleus in a high density (Sieberer *et al.*, 2002; van Bruaene *et al.*, 2004). Bakhuizen (1988) showed in a TEM study on growing *V. sativa* and *Pisum sativum* root hairs that EMTs are present in the vicinity of the nuclear envelope, and that bundles of EMTs run axially towards the hair tip. Van Bruaene *et al.* (2004) described in young tip-growing *Arabidopsis* hairs distinct sites in the vicinity of the surface of the nucleus where EMTs appear. They hypothesize that these locations are MT nucleation sites, and that in elongating *Arabidopsis* root hairs EMTs may be involved in the transport of new MT nucleation complexes from the nucleus to other places in the root hair. In tip-growing root hairs of the legumes *M. truncatula* (Sieberer *et al.*, 2002; see also Fig. 1a), *M. sativa* (Weerasinghe *et al.*, 2003), *V. hirsuta* (Lloyd *et al.*, 1987) and *L. japonicus* (Fig. 1b), the EMTs are organized into a vast 3D array that spreads throughout the entire subapex between the nucleus and the very tip (Fig. 1a,b). EMTs in the lower part of the subapical cytoplasmic dense region occur in a higher density and deviate more from the long axis of the

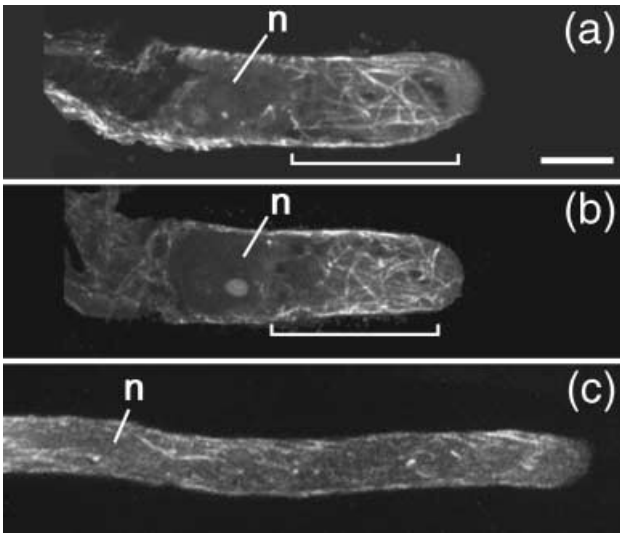

Fig. 1 Endoplasmic microtubules (MTs) in tip-growing root hairs. (a) *Medicago truncatula*; (b) *Lotus japonicus*; and (c) *Arabidopsis thaliana*. Brackets in (a) and (b) indicate the subapical region containing a dense array of endoplasmic MTs (EMTs). EMTs in the subapex of *Arabidopsis* root hairs do not form a vast array (c). MTs were visualized with a confocal laser scanning microscope in scanning steps of 1 μm after rapid freeze fixation / freeze substitution and immunocytochemistry. All images are projections of three median sections. Magnification is the same in all images. Bar, 10 μm; n, nucleus.

hair than the EMTs close to the root hair tip (Sieberer *et al.*, 2002). Some EMTs are reaching into the very tip (*M. truncatula*: Sieberer *et al.*, 2002), where MT ends make contact with the plasma membrane (*V. sativa*: Bakhuizen, 1988). In contrast to legumes, EMTs in growing *Arabidopsis* root hairs do not form a dense array in the subapex, but consist, at most, of a few bundles of EMTs between hair tip and the nucleus, as studies with GFP-MBD decorated MTs revealed (Van Bruaene *et al.*, 2004). With immunocytochemistry after rapid freeze fixation, EMTs could either not be detected in *Arabidopsis* hairs (Ketelaar *et al.*, 2002) or this method gave a much weaker signal on EMTs than in legume root hairs (Fig. 1c). The absence/low density of EMTs in these samples may indicate that immunocytochemistry is not sensitive enough to detect EMTs for the reasons given above. Alternatively, this may indicate that expression of GFP-MBD in *Arabidopsis* induces polymerization/bundling of EMTs. TEM analysis of EMTs may clarify these contradicting results.

MTs in elongating and fully grown root hairs of both species, *Medicago* and *Arabidopsis*, are highly dynamic. The EMTs in elongating root hairs especially are constantly changing their orientation and configuration, whereas CMTs in the root hair shank appear to be less dynamic (Van Bruaene *et al.*, 2004). Experiments with low concentrations of the MT depolymerizing drug oryzalin showed that EMTs are less stable than CMTs (*M. truncatula*: Sieberer *et al.*, 2002). Real-time 4D confocal microscopy revealed that EMT dynamics are caused by alternating phases of MT growth and shrinkage (*Medicago*: Vos *et al.*, 2003; *Arabidopsis*: van Bruaene *et al.*, 2004). Moreover, in *Medicago* EMTs were observed to grow from the subapical region into the very tip of the root hair (Vos *et al.*, 2003), where they remain only for a few seconds, after which they shrink again. Because of the high density and the unsynchronized dynamics of EMTs in *Medicago* root hairs, quantitative analysis proved difficult to be performed. Future studies of 4D confocal microscopy images should give more insights on EMT dynamics in these root hairs. A quantitative analysis of CMT dynamics in fully grown root hairs of *Arabidopsis* (Van Bruaene *et al.*, 2004) suggests a process called hybrid tread milling with dynamic instability at the MT plus ends that mainly point towards the hair tip, and slow depolymerization at the MT minus ends. This is similar to what recently has been described in *Arabidopsis* epidermal cells (Shaw *et al.*, 2003) and in tobacco bright yellow 2 cells (Vos *et al.*, 2004). Van Bruaene *et al.* (2004) further suggest that this process would allow MTs in root hairs first to nucleate and then to get ordered: upon contact with other MTs, MTs undergo part depolymerization followed by polymerization/reorientation, finally resulting in the alignment of CMTs. A similar observation has been made by Dixit & Cyr (2004a,b) in tobacco bright yellow 2 cells. In elongating *Arabidopsis* root hairs, dispersed nucleation sites of MTs have been identified in the subapical cytoplasmic dense region and the cortex (Van Bruaene *et al.*, 2004). Although MT density is too high to allow quantification of MT behaviours in these regions of elongating hairs with current methods, one can imagine a similar MT organizing mechanism in these hairs as described for fully grown hairs (Van Bruaene *et al.*, 2004). It is likely that MT dynamics are at least partially under control of MAPs; however, the regulation of MT dynamics by specific proteins has not been studied in root hairs.

IV. Microtubules and their role in root hair cell polarity

Root hairs of both species, *Medicago* and *Arabidopsis*, do have subapical EMTs during tip growth, although with differences in patterning and abundance. Do these differences reflect a difference in function of the EMTs? Experiments with the MT depolymerizing drug oryzalin show that EMTs in elongating *Medicago* root hairs contribute to cell polarity. Upon application of 1 μM oryzalin, EMTs, but not CMTs, disappear within minutes, the polar distribution of the cytoplasm in the subapex of the hairs is lost, and the nucleus–tip distance increases over time (Sieberer *et al.*, 2002). However, the smooth region (i.e. the vesicle-rich zone) at the very tip remains present and even increases in length over time; consequently, tip growth continues, although the growth rate is reduced by approx. 60% (Sieberer *et al.*, 2002). *M. truncatula* root hairs treated with oryzalin do not reach their mature length. *Arabidopsis* root hairs treated with up to 10 μM oryzalin continue

to grow at a normal growth rate, but in a wavy pattern (Bibikova *et al.*, 1999; Ketelaar *et al.*, 2002), which has not been described for *Medicago* (Sieberer *et al.*, 2002; Weerashinge *et al.*, 2003). It is not clear whether the absence of EMTs, CMTs or both is responsible for this wavy growth pattern. The nucleus–tip distance in *Arabidopsis* remains the same as without oryzalin treatment. Nuclear movement in *Arabidopsis* has been demonstrated to exclusively depend on the actin cytoskeleton (Ketelaar *et al.*, 2002; Van Bruaene *et al.*, 2003), whereas in elongating *Medicago* root hairs the EMTs clearly are needed in keeping the nucleus at a position of 30–40 μm to the tip, but perhaps not for nuclear movement per se, which depends on an intact actin cytoskeleton (*V. hirsuta*: Lloyd *et al.*, 1987; *M. truncatula*: Sieberer *et al.*, 2002).

Paclitaxel (taxol) is an anticancer drug that binds to MTs rather than to tubulin dimers, thereby inhibiting MT dynamics, eventually leading in a dose dependent manner to stabilization and bundling of MTs (for a review, see Blagosklonny & Fojo, 1999). Paclitaxel has also been shown to affect MTs in plant cells (Collings *et al.*, 1998). In elongating *Medicago* and *Arabidopsis* root hairs (*M. truncatula*: Sieberer *et al.*, 2002; *A. thaliana*: Bibikova *et al.*, 1997), paclitaxel does not obviously affect polar distribution of the cytoplasm in the subapex. However, the effect of paclitaxel on tip growth is, similar to that of oryzalin, different in the two species. In *Medicago*, tip growth continues without a deviation from the orginal growth axis, but growth rate drops in a similar manner as after oryzalin treatment (Sieberer *et al.*, 2002). Tip growth in paclitaxel-treated *Arabidopsis* root hairs continues in a wavy pattern with even steeper deviations from the straight growth axis than in oryzalin treated hairs, and without a significant drop in growth rate (Bibikova *et al.*, 1999; Ketelaar *et al.*, 2002). Could there be functional differences of the EMT array that cause the different response of the root hair on MT depolymerization or stabilization?

In *Arabidopsis*, MTs control growth direction, because inhibition of MT dynamics, both by depolymerization and stabilization, causes a wavy growth phenotype. Although MTs maintain the uniaxial expansion direction, there is no major physiological significance of altering growth direction. In legumes, however, the vast EMT array in elongating root hairs may have a specific role in the early interactions between legumes and soil-living rhizobacteria. Legume roots infected with rhizobia form new plant organs, the root nodules, where rhizobia fix atmospheric nitrogen and make it accessible for the plant. One of the first steps in the plant–bacteria interaction is the curling of root hairs around the bacteria (Kijne, 1992; Hadri & Bisseling, 1998; Esseling *et al.*, 2004). Root hair curling has been described as iterative growth reorientation (Esseling *et al.*, 2003).

MTs in legume root hairs respond to rhizobacterial signal molecules, the nodulation factors or Nod factors (*L. japonicus*: Vassileva *et al.*, 2005; Weerasinghe *et al.*, 2005; *Medicago*: Weerasinghe *et al.*, 2003; Sieberer *et al.*, 2005). Results from *L. japonicus* (Weerasinghe *et al.*, 2005) and *M. truncatula* (Sieberer *et al.*, 2005) show that Nod factors cause a subtle and short-termed shortening of the EMT array in elongating hairs. Vassileva *et al.* (2005) describe CMTs in elongating hairs to be less dynamic at a specific window after Nod factor treatment, but found no evidence of CMT depolymerization after Nod factor treatment, which is similar to observations made in *M. truncatula* (Sieberer *et al.*, 2005).

Growth-arresting hairs respond to globally applied Nod factors with root hair deformation, that is swelling of the hair tips (i.e. isodiametric growth) after which tip growth resumes, albeit in a deviant orientation. Oryzalin causes a deformation phenotype similar to Nod factors in growth-arresting root hairs; combined with Nod factors, it increases Nod-factor-induced growth deviation, whereas taxol suppresses this. Interestingly, the root hair deformation phenotype caused by Nod factors in *Medicago* root hairs can be mimicked by depolymerization and recovery of filamentous actin in *Arabidopsis* hairs where MTs have been experimentally ablated with oryzalin (Ketelaar *et al.*, 2003). EMTs are also affected by Nod factors in growth-arresting *Medicago* hairs. The short EMT array in these hairs completely disappears within minutes after application, but reappears after 20–30 min. The Nod-factor-induced depolymerization of the EMTs correlates with a loss of polar cytoarchitecture and straight growth directionality, whereas their site of reappearance predicted the new direction of hair elongation. These findings indicate that in addition to the actin cytoskeleton (*M. sativa*: Allen *et al.*, 1994; *P. vulgaris*: Cárdenas *et al.*, 1998, 2003; *V. sativa*: Ridge, 1992; De Ruijter *et al.*, 1998; Miller *et al.*, 1999) the MT cytoskeleton in *Medicago* root hairs is also a target of Nod factor signalling. These observations indicate that, both in legumes and in *Arabidopsis*, MTs are involved in determining growth directionality, possibly by defining in a yet unknown manner the place where exocytosis takes place. In doing so, MTs could steer polar cell expansion without interfering with actin-based tip growth itself. The major difference between *Arabidopsis* and legume species is that MTs just fine-tune the growth direction in *Arabidopsis* (and perhaps also in other nonlegumes), whereas they are an essential element in the initial stages of symbiosis between legumes and rhizobacteria. It is tempting to speculate that the specific function of EMTs in the symbiotic process may explain the more extended EMT array in legumes compared with *Arabidopsis*, and that the differential response of legumes and *Arabidopsis* root hairs to disturbance of MTs could be caused by the evolution of the EMT array to facilitate symbiosis. However, there may be specific properties of the EMT array for different species, independent of whether they are legumes or not.

How could MTs control the direction of tip growth? Bibikova *et al.* (1999) showed that in *Arabidopsis* taxol-stabilized MTs lose the ability to restrict tip growth to only one site by applying an intracellular artificial Ca^{2+} gradient (local uncaging of a caged Ca^{2+} ionophore). In untreated hairs, local

uncaging of Ca^{2+} led only to a transient and limited reorientation towards the artificial Ca^{2+} gradient, whereas root hairs with taxol-stabilized MTs showed a pronounced redirection of growth (Bibikova *et al.*, 1999). In *Medicago* root hairs, Nod factor treatment leads to elevated cytosolic Ca^{2+} levels (Allen *et al.*, 1994; de Ruijter *et al.*, 1998; Cárdenas *et al.*, 1999; Felle *et al.*, 1999a,b), which in turn lead to several downstream events affecting the cytoskeleton (for a review, see Lhuissier *et al.*, 2001). Nod factors may affect MTs in *Medicago* root hairs via transiently increased Ca^{2+} levels, because MT depolymerization can be triggered by elevated cytoplasmic Ca^{2+} levels (Cyr, 1991, 1994). Additional evidence that elevated calcium levels affect MTs is available from studies on the *Arabidopsis* ton mutant, which has high calcium-channel activity. CMTs of this mutant are disorganized (Thion *et al.*, 1998). These findings point towards an interaction between MTs and the tip-focused cellular machinery that maintains a high Ca^{2+} gradient at the root hair tip. A possible candidate involved in transferring the Nod factor signal to MTs may be phospholipase D (PLD), because activation of PLD causes MT disruption (Dhonukshe & Gadella, 2003; Gardiner *et al.*, 2003) and PLD is involved in Nod-factor-induced root hair deformation (Den Hartog *et al.*, 2001).

V. Microtubules and their putative role in targeting polarity markers to the very tip of elongating root hairs: what we might learn from *Schizosaccharomyces pombe*

Interesting observations concerning the role of MTs in the tip growth process were made in the tip-growing fission yeast *Schizosaccharomyces pombe*. Similarly to root hairs, the direction of polar growth in *S. pombe* is dependent on MTs. From the nucleus, which is in the middle of the yeast cell, three to four bundles of dynamic MTs radiate with their plus ends toward both tips of the cells. Mutations in the MAPs Tip1p, Tea1p, Tea2p and Mal3p lead to short MTs and bent/wavy cells or cells with multiple tips (Beinhauer *et al.*, 1997; Browning *et al.*, 2000; Brunner & Nurse, 2000; Behrens & Nurse, 2002). From a combination of mutant analysis and GFP techniques, it is clear that it is the linear transport and the delivery of the MAPs via the MTs toward the cell tips which is the crucial process in setting up the cells' polarity (Fig. 2). Tea2p is a kinesin-like motor protein that, along the MTs, interacts with at least the Clip170 homolog Tip1p (Busch & Brunner, 2004) and Tea1p, and moves toward the growing MT plus ends. Mal3p is an End Binding (EB1) homolog that labels the MTs and also moves, independent of Tea2p, to the MT plus ends. Once there, Mal3p directly interacts with Tip1p via a CAP-Gly domain containing region and it associates with Tea2p; therefore, Tea2p and Tea1p stay associated with the growing MT plus end. When the plus ends with the proteins reach the tip of the cell via MT polymerization, Mal3p plus end labelling of the MTs disappears (Busch & Brunner, 2004), the MTs undergo catastrophe and Tip1p, Tea1p and Tea2p stay in the growing tip. For Tea1p, it has been observed that it attaches to the membrane-associated protein Mod5p (Morphology defective 5; Snaith & Sawin, 2003) and forms a complex with Bud6p and For3p, a formin that assembles actin cables (Feierbach *et al.*, 2004). This could be a direct link between the polarity complex and actin polymerization, and thus the location of exocytosis and direction of growth.

A homology search for the proteins involved in setting up polarity in *S. pombe* reveals that the *Arabidopsis* genome

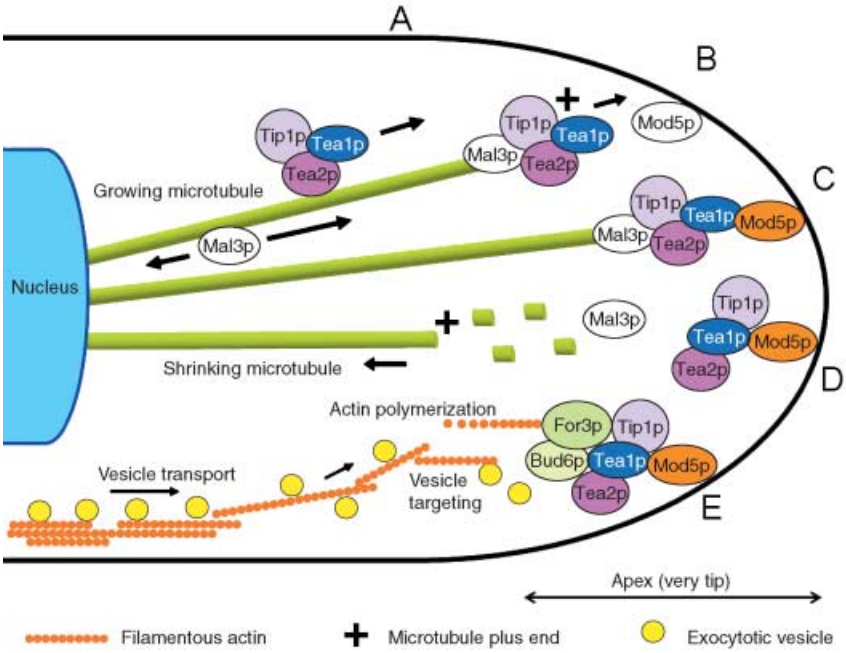

Fig. 2 Scheme of microtubule (MT) regulation of tip growth in *Schizosaccharomyces pombe*. A, The kelch-repeat protein Tea1p interacts with the kinesin-like motor protein Tea2p and the Clip170 homolog Tip1p, thereby moving towards the growing MT plus end. The EB1 homolog Mal3p moves independently of Tea2p to the MT plus end. A protein complex of Mal3p, Tip1p, Tea2p, and Tea1p associates with the growing MT plus end and gets delivered to cell tip by MT growth (B and C). D, Tip1p, Tea1p, and Tea2p may remain at the tip after Mal3p dissociation and MT depolymerization. Tea1p attaches to the membrane-associated protein Mod5p and (E) forms a complex with Bud6p and For3p, a formin that assembles actin filaments. The actin filaments facilitate the transport and targeting of exocytotic vesicles to the cell tip.

contains 3 EB1 homologs At3g47690 (AtEB1a), At5g62500 (AtEB1b) and At5g67270 (AtEB1c). In addition, expressed sequence tags (ESTs) of EB1 homologs have been found in *M. truncatula* (*Medicago truncatula* genome project: http://www.medicago.org). GFP-AtEB1 has been shown to decorate the plus ends of growing MTs in *Arabidopsis* (Chan *et al.*, 2003; Mathur *et al.*, 2003). Other proteins from the cell polarity pathway in which EB1 is active in fission yeast may be present in the *Arabidopsis* genome, but are difficult to identify, either due to large gene families in *Arabidopsis* or due to low homology between plant and fission yeast proteins. Even though not all proteins in the EB1 pathway may be present in plants, EB1 homologs have been found in plant cells and their localization to MT plus ends is similar to that in fission yeast. Therefore, EB1 proteins may perform the same function in plants as in *S. pombe*. Recently, Dhonukshe *et al.* (2005) described MT plus ends to be essential for intracellular polarization during division-plane establishment in plant cells, and suggest that in mitotic plant cells EMT plus ends may act as cell shape/polarity sensing and orienting machines by their sustained cortical targeting. Because growing *M. truncatula* root hairs have an array of highly dynamic EMTs (Sieberer *et al.*, 2002), which can grow from the subapical region into the very root hair tip (Vos *et al.*, 2003) and determine root hair elongation direction (Sieberer *et al.*, 2005), linear transport of polarity markers via MTs like in *S. pombe* could exist in plant root hairs.

VI. Conclusion

MTs are essential in determining the direction of root hair growth. In legumes, they determine the growth direction of a new outgrowth in growth terminating root hairs, treated with Nod factors. Because an essential element of the legume rhizobacterium symbiosis is the entrapment of bacteria in a root hair pocket, formed by iterative changes in growth direction, it is likely that MTs are important in the initiation of symbiosis. Interesting work on fission yeast shows that a MT-based polarity mechanism is present in this organism. One of the main proteins in this mechanism is EB1, of which homologs are present in plants. Future research in our exciting field will reveal if there is a MT-based polarity mechanism in plants as in fission yeast and identify the proteins that are involved in this mechanism.

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